



(51) International Patent Classification:

C07K 14/00 (2006.01) C07K 19/00 (2006.01)

(21) International Application Number:

PCT/US2021/034104

(22) International Filing Date:

25 May 2021 (25.05.2021)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/030,836	27 May 2020 (27.05.2020)	US
63/051,549	14 July 2020 (14.07.2020)	US
63/067,643	19 August 2020 (19.08.2020)	US

(71) Applicant: UNIVERSITY OF WASHINGTON

[US/US]; 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US).

(72) Inventors: QUIJANO RUBIO, Alfredo; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US). PARK, Jooyoung; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US). YEH, Hsien-Wei; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US). BAKER, David; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US). CAO, Longxing; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US). COVENTRY, Brian; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US). GORESHNIK, Inna; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US). KOZODOY, Lisa; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US). STEWART, Lance, Joseph; c/o University of Washington, 4545 Roosevelt Way NE, Suite 400, Seattle, WA 98105-4721 (US).

(74) Agent: HARPER, David, S.; McDonnell Boehnen Hulbert & Berghoff LLP, 300 South Wacker Drive, Chicago, IL 60606 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD,

ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available):

ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- without international search report and to be republished upon receipt of that report (Rule 48.2(g))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: MODULAR AND GENERALIZABLE BIOSENSOR PLATFORM BASED ON DE NOVO DESIGNED PROTEIN SWITCHES

(57) Abstract: The disclosure provides cage proteins comprising a helical bundle, wherein the cage protein comprises a structural region and a latch region, wherein the latch region comprises one or more target binding polypeptide, wherein the cage protein further comprises a first reporter protein domain, wherein the first reporter protein domain undergoes a detectable change in reporting activity when bound to a second split reporter protein domain, and wherein the structural region interacts with the latch region to prevent solution access to the one or more target binding polypeptide.



5 **Modular and generalizable biosensor platform based on de novo designed protein switches**

Cross reference

 This application claims priority to U.S. Provisional Patent Application Serial Nos.
10 63/030,836 filed May 27, 2020; 63/051,549 filed July 14, 2020 and 63/067,643 filed August
 19, 2020, each incorporated by reference herein in its entirety.

Federal Funding Statement

 This invention was made with government support under Grant no. FA8750-17-C-
15 0219 awarded by the Defense Advanced Research Project Agency (DARPA). The
 government has certain rights in the invention.

Sequence Listing Statement:

 A computer readable form of the Sequence Listing is filed with this application by
20 electronic submission and is incorporated into this application by reference in its entirety. The
 Sequence Listing is contained in the file created on May 25, 2021 having the file name “20-
 1075-WO_Sequence-Listing_ST25.txt” and is 32,910 kb in size.

25 **Background**

 Sensor proteins have emerged as an active area of research. Traditional ELISA
 methods require multiple liquid-handling steps, preventing its use at the bedside. Lateral flow
 immunochromatographic assays are fast and cheap, but they have limited sensitivity,
 reproducibility, and poor quantitative performance. ELISA and lateral flow also require two
30 binding modules for the target being sensed, one for capture and the other for readout. One
 main hurdle of protein sensor construction is finding analyte binding domains that undergo
 sufficient conformational changes. The most commonly used binding domains (e.g.,
 antibodies) undergo only minor structural changes of the loops upon ligand binding.
 Coupling an appropriate reporter with optimal geometry to amplify the conformational
35 change is also key to a successful biosensor. However, computationally designing small
 molecule binding sites into protein interfaces and generating semisynthetic protein sensors
 are both quite challenging problems currently. Therefore, generalized approaches for

designing biosensors with a simple and robust computational protocol
empirical optimization are needed.

5 Summary

In one aspect, the disclosure provides cage proteins comprising a helical bundle, wherein the cage protein comprises a structural region and a latch region, wherein the latch region comprises one or more target binding polypeptide, wherein the cage protein further comprises a first reporter protein domain, wherein the first reporter protein domain undergoes
10 a detectable change in reporting activity when bound to a second split reporter protein domain, and wherein the structural region interacts with the latch region to prevent solution access to the one or more target binding polypeptide. In one embodiment, the cage protein further comprises the second reporter protein domain, wherein one of the first reporter protein domain and the second reporter domain is present in the latch region and the other is present
15 in the structural region, wherein an interaction of the first reporter protein domain and the second reporter protein domain is diminished in the presence of target to which the one or more target binding polypeptide binds. In another embodiment, the second reporter protein domain is not present in the cage protein. In another embodiment, the first reporter protein domain, and the second reporter domain when present, comprise a reporter protein domain
20 selected from the group consisting of luciferase (including but not limited to firefly, Renilla, and Gaussia luciferase), bioluminescence resonance energy transfer (BRET) reporters, bimolecular fluorescence complementation (BiFC) reporters, fluorescence resonance energy transfer (FRET) reporters, colorimetry reporters (including but not limited to β -lactamase, β -galactosidase, and horseradish peroxidase), cell survival reporters (including but not limited
25 to dihydrofolate reductase), electrochemical reporters (including but not limited to APEX2), radioactive reporters (including but not limited to thymidine kinase), and molecular barcode reporters (including but not limited to TEV protease). In one embodiment, the one or more target binding polypeptide is capable of binding to a target including but not limited to an antibody, a toxin, a diagnostic biomarker, a viral particle, a disease biomarker, a metabolite
30 or a biochemical analyte.

In another aspect, the disclosure provides key proteins capable of binding to the structural region of a cage protein of any embodiment of the disclosure that does not include the second reporter protein domain, wherein binding of the key protein to the cage protein only occurs in the presence of a target to which the cage protein one or more target binding

polypeptide can bind, wherein the key protein comprises a second repc
wherein interaction of the key protein second reporter protein domain and the cage protein
first reporter protein domain causes a detectable change in reporting activity from the first
reporter protein domain. In various embodiments, the second reporter protein domain
5 comprises a reporter protein domain selected from the group consisting of luciferase
(including but not limited to firefly, Renilla, and Gaussia luciferase), bioluminescence
resonance energy transfer (BRET) reporters, bimolecular fluorescence complementation
(BiFC) reporters, fluorescence resonance energy transfer (FRET) reporters, colorimetry
reporters (including but not limited to β -lactamase, β -galactosidase, and horseradish
10 peroxidase), cell survival reporters (including but not limited to dihydrofolate reductase),
electrochemical reporters (including but not limited to APEX2), radioactive reporters
(including but not limited to thymidine kinase), and molecular barcode reporters (including
but not limited to TEV protease).

In another aspect, the disclosure provides biosensors, comprising

15 (a) the cage protein of embodiment of the disclosure wherein the cage does not
include the second reporter protein domain; and

(b) the key protein of any embodiment of the disclosure;

wherein the key protein can only bind to the cage protein in the presence of a target to
which the cage protein one or more target binding polypeptide can bind; and wherein binding
20 of the first reporter protein domain of the cage protein to the second reporter protein domain
of the key protein causes a detectable change in reporting activity from the first reporter
protein domain.

In a further aspect, the disclosure provides methods for detecting a target, comprising

25 (a) contacting the cage protein of any embodiment of the disclosure where the
cage protein comprises the second reporter protein domain, or the biosensor of any
embodiment of the disclosure with a biological sample under conditions to promote binding
of the cage protein one or more target binding polypeptide to a target present in the biological
sample, causing a detectable change in reporting activity from the first reporter protein
domain; and

30 (b) detecting the change in reporting activity from the first reporter protein
domain, wherein the change in reporting activity identifies the sample as containing the
target.

In further aspects, the disclosure provides methods for designing a biosensor, cage
protein, or key protein comprising the steps of any method described herein, nucleic acids

encoding the cage protein or key protein of any embodiment of the disclosure, vectors comprising the nucleic acid of embodiment of the disclosure operatively linked to a suitable control element, such as a promoter, cells (such as recombinant cells) comprising the cage protein, key protein, composition, nucleic acid, or expression vector of any embodiment of the disclosure, pharmaceutical compositions comprising the cage protein, key protein, composition, nucleic acid, expression vector, or cell of any embodiment of the disclosure, and a pharmaceutically acceptable carrier, an epitope comprising or consisting of the amino acid sequence of SEQ ID NO: 27384, and methods detecting Troponin I in a sample, comprising contacting a biological sample with the epitope under conditions suitable to promote binding of Troponin I in the sample to the epitope to form a binding complex, and detecting binding complexes that demonstrate presence of Troponin I in the sample.

Figure Legends

Figure 1(a-f). De novo design of multi state allosteric biosensors. a, Sensor schematic. The biosensor consists of two protein components: lucCage and lucKey, which exist in a closed (Off) and open state (On). The closed form of lucCage (left) cannot bind to lucKey, thus, preventing the split luciferase SmBit fragment from interacting with LgBit. The open form (right) can bind both target and key, and allows SmBit to combine with LgBit on lucKey to reconstitute luciferase activity. **b**, Thermodynamics of biosensor activation. The free energy cost ΔG_{open} of the transition from closed cage (species 1) to open cage (species 2) disfavors association of key (species 5) and reconstitution of luciferase activity (species 6) in the absence of target. In the presence of the target, the combined free energies of target binding (2 $\rightarrow$ 3; ΔG_{LT}), key binding (3 $\rightarrow$ 4; ΔG_{CK}), and SmBit-LgBit association (4 $\rightarrow$ 7; ΔG_{R}) overcome the unfavorable ΔG_{open} , driving opening of the lucCage and reconstitution of luciferase activity. **c**, Biosensor design strategy based on thermodynamics. For each biosensor, the designable parameters are ΔG_{open} and ΔG_{CK} ; ΔG_{R} is the same for all targets, and ΔG_{LT} is pre-specified for each target. For sensitive but low background analyte detection, ΔG_{open} and ΔG_{CK} must be designed such that the closed state (species 1) is substantially lower in free energy than the open state (species 6) in the absence of target, but higher in free energy than the open state in the presence of target (species 7). **d-f**, Numerical simulations of the coupled equilibria shown in **b** for different values of **(d)** K_{open} , **(e)** K_{LT} , and **(f)** $[\text{lucKey}]_{\text{tot}}$ and $[\text{lucCage}]_{\text{tot}}$. K_{open} , K_{LT} , K_{CK} were set to 1×10^{-3} , 1 nM, and 10 nM respectively, and the concentration of the sensor components to 10:100 nM (lucCage:lucKey) except where

explicitly indicated. **d**, Increasing ΔG_{open} shifts response to higher anal
The sensor limit of detection is approximately $0.1 \times K_{\text{LT}}$; the driving force for opening the
switch becomes too weak below this concentration. **f**, The effective target detection range can
be tuned by changing the sensor component concentrations.

Figure 2(a-d). Design and characterization of *de novo* biosensors incorporating small proteins as sensing domains. **a**, General strategy and structural validation for caging small protein domains into LOCKR switches. Left: design model of the *de novo* binder HB1.9549.2 bound to the stem region of influenza hemagglutinin (HA, ribbon representation)¹⁵. Right: crystal structure of sCageHA_267_1S, comprising HB1.9549.2 grafted into a shortened and stabilized version of the LOCKR switch (sCage, ribbon representation). Middle: All residues of HB1.9549.2 involved in binding to HA (top) except for F273 are buried in the closed state of the switch (bottom) to block its interaction. The labels indicate the same set of amino acids in the two panels (F2 in the top panel corresponds to F273 in the lower panel). **b-d**, Functional characterization of 3 allosteric biosensors: lucCageBot (detection of botulinum neurotoxin B (BoNT/B)), lucCageProA (detection of Fc domain), and lucCageHer2 (detection of Her2 receptor). Left: structural models of the indicated biosensors (ribbon representation) incorporating a *de novo* designed binder for BoNT/B (Bot.671.2), the C domain of the generic antibody binding protein Protein A (SpaC) and a Her2-binding affibody respectively, grafted into lucCage comprising a caged SmBiT fragment. Middle: kinetic measurement of luminescence intensity upon addition of 50 nM of analyte (BoNT/B, IgG Fc, or Her2) to a mixture of 10 nM of each lucCage and 10 nM of lucKey. Right: detection over a wide range of analyte concentrations by changing the biosensor concentration (50, 5 and 1 nM lucCage and lucKey; cyan, magenta and black lines respectively).

Figure 3(a-h). Design and characterization of biosensors for cardiac troponin I and for an anti-HBV antibody. **a**, Design of lucCageTrop, a sensor for cardiac Troponin I. Left: Structure of cardiac troponin (PDB ID: 4Y99); Right: Design model of lucCageTrop, the cTnI sensor in the closed state containing segments of cTnT and cTnC. **b**, Left: Kinetics of luminescence increase upon addition of 1 nM cTnI to 0.1 nM lucCageTrop sensor + 0.1 nM of lucKey. Right: A wide analyte (cTnI) detection range can be achieved by changing the concentration of the sensor components (lines). The grey area indicates the cTnI concentration range relevant to the diagnosis of acute myocardial infarction (AMI); the dotted line indicates clinical AMI cut-off defined by W.H.O. (0.6 ng/mL, 25 pM). **c**, Design models of lucCageHBV and lucCageHBV α , containing SmBiT, and one or two tandem antigenic

epitopes from the Hepatitis B Virus (HBV) PreS1 protein, respectively (two epitope copies) has higher affinity for the anti-HBV antibody HzK κ 121-3.2 (K_d = 0.68 nM) than lucCageHBV (one epitope copy) (K_d = 20 nM) as demonstrated by biolayer interferometry. **e**, Left: Kinetics of bioluminescence signal increase upon addition of 10n anti-HBV antibody to 1nM lucCageHBV α + 1nM lucKey. Right: By varying the concentrations of the sensor components, sensitive anti-HBV antibody detection can be achieved over a wide concentration range. **f**, Schematic of the detection mechanism for HBV protein PreS1 using lucCageHBV. **g**, Kinetics of bioluminescence following addition of the anti-HBV antibody (step 1) and subsequently PreS1 (step 2). The bioluminescence decreases upon PreS1 addition as PreS1 competes with the sensor for the antibody. **h**, Sensitive detection of PreS1 can be achieved over the relevant post-HBV infection concentration levels (grey area). The sensor is pre-mixed with the anti-HBV antibody; the PreS1 detection range can be tuned by varying the concentration of antibody (indicated by colored labels).

Figure 4(a-d). Design of biosensors for detection of anti-SARS-CoV-2 antibodies

and SARS-CoV-2 RBD. a, SARS-CoV-2 viral structure representation showing the major structural proteins: Envelope protein (E), membrane protein (M), nucleocapsid protein (N), and the Spike protein (S) containing the receptor-binding domain (RBD). Linear epitopes for the M and N proteins were selected based on published immunogenicity data. **b**, Left panel: structural model of lucCageSARS2-M. Two copies of the SARS-CoV-2 Membrane protein a.a. 1-17 epitope are grafted into lucCage connected with a flexible spacer. Middle panel: kinetics of luminescent activation of lucCageSARS2-M (50 nM) + lucKey (50nM) upon addition of anti-SARS-CoV-1 Membrane protein rabbit polyclonal antibodies at 100 nM (ProSci, 3527). These antibodies, originally raised against a peptide corresponding to 13 amino acids near the amino-terminus of SARS-CoV Matrix protein, cross-react with residues 1-17 of the SARS-CoV-2 Membrane protein. Right panel: response of lucCageSARS2-M (5 nM) + lucKey (5nM) to varying concentrations of target anti-M pAb. **c**, Left panel: structural model of lucCageSARS2-N. Two copies of the SARS-CoV-2 Nucleocapsid protein 369-382 epitope are grafted into lucCage connected with a flexible spacer. Middle panel: kinetics of luminescent activation of lucCageSARS2-N (50 nM) + lucKey (50nM) upon addition of 100 nM anti-SARS-CoV-1-N mouse monoclonal antibody (clone 18F629.1). This antibody originally raised against residues 354-385 of the SARS-CoV-1 Nucleocapsid protein cross-reacts with residues 369-382 of the SARS-CoV-2 Nucleocapsid protein. Right panel: response of lucCageSARS2-N (50 nM) + lucKey (50nM) to varying concentration of target (anti-N mAb). **d**, Functional characterization of lucCageRBD, a SARS-CoV-2 RBD sensor.

Left panel: structural model of lucCageRBD showing the LCB1 binder comprising a caged SmBiT fragment. Second panel: kinetic measurement of luminescence intensity upon addition of 16.7 nM of RBD to a mixture of 1 nM of lucCageRBD and 1 nM of lucKey. Third panel: detection over a wide range of analyte concentrations by changing the biosensor concentration (10 and 1 nM lucCage and lucKey). Right panel: Limit of detection (LOD) determination of lucCageRBD and lucKey at 1 nM each for detection of RBD in solution. LOD was determined to be 15 pM.

Figure 5. Biosensor specificity. Each sensor at 1 nM was incubated with 50 nM of its cognate target (black lines) and the targets for the other biosensors (grey lines). Targets are Bcl-2, BoNT/B, human IgG Fc, Her2, cardiac Troponin I, anti-HBV antibody (HzKR127-3.2), anti-SARS-CoV-1-M polyclonal antibody and SARS-CoV-2 RBD. All experiments were performed in triplicate, representative data are shown, and data are presented as mean values +/- s.d.

Figure 6(a-g). Determination of the optimal SmBit position in lucCage and characterization of lucCageBim, a Bcl-2 biosensor. **a**, Protein models showing the different threading positions of SmBiT and the Bim peptide on the latch helix of the *de novo* LOCKR switch. **b**, Experimental screening of 11 *de novo* Bcl-2 sensors. Eleven variants were generated by combining the SmBit and Bim positions in (a) and characterized by activation of their luminescence upon addition of Bcl-2. Luminescence measurements were performed with each design (20 nM) and lucKey (20 nM) in the presence or absence of Bcl-2 (200 nM). SmBiT312-Bim339 (hence referred to as lucCageBim) was selected for posterior characterization due to its higher brightness, dynamic range and stability. **c-g**, Characterization of lucCageBim. **c**, Structural design model in ribbon representation. **d**, Blow-up showing the predicted interface of SmBiT and Cage. **e**, Blow-up showing the predicted interface of Bim and Cage. **f**, Kinetic luminescence measurements upon addition of Bcl-2 (200 nM) to a mix of lucCageBim (20 nM) and lucKey (20 nM). **g**, Tunable sensitivity of lucCageBim to Bcl-2 by changing the concentrations of sensor (lucCageBim and lucKey) components (curves).

Figure 7(a-d). Functional screening of sCageHA designs and crystal structure of sCageHA_267-1S. **a**, Structural models of sCageHA designs with the embedded *de novo* binder HB1.9549.2. The HB1.9549.2 protein was grafted into a parental six-helix bundle (sCage) at different positions along the latch helix including three consecutive glycine residues. The black arrows indicate the additionally introduced single V255S (1S) or double V255S/I270S (2S) mutation(s) on the latch. **b**, Experimental validation of five sCageHA

designs binding to HA in the presence or absence of the key by biolayer
 concentration of the sCages and the key were 1 μ M and 2 μ M, respectively. sCageHA_267-
 1S exhibited the highest fold of activation. **c**, Structural comparison showing the flexible
 nature of sCage to enable caging of HB1.9549.2. The structural model of sCage and the
 5 crystal structure of sCageHA_267-1S are superposed, and a narrow section (black box) is
 shown in an orthogonal view for detail. The N-terminal helix of HB1.9549.2 is displaced
 from the latch helix (α 6) by 3.2 Å (middle panel) with a concomitant displacement of α 5 and
 partial disruption of a hydrogen-bond network involving Q16 and N214 of sCage (right
 panels). **d**, A blow-up view of the intramolecular interactions of sCageHA_267-1S. The HA-
 10 binding residues are highlighted. Both the N-terminal helix (α 1) and the following helix (α 2)
 of HB1.9549.2 interact with the cage. The intramolecular interactions are all hydrophobic.
 The bulky hydrophobic side chain of F285 tightly abuts against the backbone atoms of α 5 of
 sCage, which is unlikely to happen without a bending of α 5. Unfavorable interactions are also
 found: F273 is solvent-exposed, and the Y287 hydroxyl group is buried in the apolar
 15 environment. The rightmost panel shows the quality of the electron density map.

Figure 8(a-d). Design and characterization of a Botulinum neurotoxin B sensor.

a, Structural models of the botulinum neurotoxin B (BoNT/B) sensor designs showing the
 different threading positions of Bot.0671.2 (PDB ID: 5VID) on the latch of lucCage. The
 SmBit peptide is shown in ribbon representation. I328S and L345S indicate mutations
 20 introduced to tune the latch-cage interface (1S=I328S, 2S=I328S/L345S)², and “GGG”
 indicates the presence of three consecutive glycine residues between the latch and the grafted
 protein. The black box shows a close-up view of the interface of Cage and Bot.0671.2 in the
 349_2S design. **b**, Experimental screening of 9 *de novo* BoNT/B sensors. Luminescence
 measurements were performed for each design (20 nM) and lucKey (20 nM) in the presence
 25 or absence of the BoNT/B protein (200 nM). The luminescence values for each design were
 normalized to 100 in the absence of BoNT/B. Design 349_2S was selected as the best
 candidate due to high sensitivity and stability, and was named lucCageBot. **c**, Determination
 of lucCageBot sensitivity. Bioluminescence was measured over 6000 s in the presence of
 serially diluted BoNT/B protein. From top to bottom - lucCageBot:lucKey concentration
 30 (nM) = 50:5, 5:5, 1:10, 0.5:0.5. **d**, Limit of detection (LOD) calculations for the sensor at
 different concentrations. From top to bottom - lucCageBot:lucKey concentration (nM) =
 50:5, 5:5, 1:10, 0.5:0.5. Error bars represent SD.

Figure 9 (a-d). Design and characterization of an Fc domain sensor. **a**, Structural
 models of the Fc sensor designs showing the different threading positions of the *S. aureus*

Protein A domain C (PDB ID: 4WWI) on the latch of lucCage. The Sn in ribbon representation. I328S and L345S indicate mutations introduced to tune the latch-cage interface, (1S=I328S, 2S=I328S/L345S)², and “GGG” indicates the presence of three consecutive glycine residues between the latch and the grafted protein. **b**, Experimental screening of 6 *de novo* Fc domain sensors. Luminescence measurements were performed for each design (20 nM) and lucKey (20 nM) in the presence or absence of recombinant human IgG1 Fc (200 nM). The luminescence values were normalized to 100 in the absence of Fc. Design 351_2S was selected as the best candidate due to high sensitivity and stability, and was named lucCageProA. **c**, Determination of lucCageProA's sensitivity. Bioluminescence was measured over 6000 s in the presence of serially diluted Fc protein. From top to bottom - lucCageBot:lucKey concentration (nM) = 50:5, 5:5, 1:10, 0.5:0.5. **d**, Limit of detection (LOD) calculations for the sensor at different concentrations. From top to bottom - lucCageBot:lucKey concentration (nM) = 50:5, 5:5, 1:10, 0.5:0.5. Error bars represent SD.

Figure 10(a-d). Design and characterization of a Her2 sensor. **a**, Structural models of the Her2 sensor designs showing the different threading positions of the Her2 affibody protein (PDB ID: 3MZW) on the latch of lucCage. The SmBit peptide is shown in ribbon representation. I328S and L345S indicate mutations introduced to tune the latch-cage interface, (1S=I328S, 2S=I328S/L345S)², and “GGG” indicates the presence of three consecutive glycine residues between the latch and the grafted protein. The black boxes show a blow-up view of the interface of Cage and the Her2 affibody in the 354_2S design. **b**, Experimental screening of 7 *de novo* Her2 sensors. Luminescence measurements were taken for each design (20 nM) and lucKey (20 nM) in the presence or absence of the ectodomain of Her2 (200 nM). The luminescence values were normalized to 100 in the absence of Her2 ectodomain. Design 354_2S was selected as the best candidate due to high sensitivity and stability, and was named lucCageHer2. **c**, Determination of lucCageHer2's sensitivity. Bioluminescence was measured over 6000 s in the presence of serially diluted Her2 ectodomain protein. From top to bottom - lucCageBot:lucKey concentration (nM) = 50:5, 5:5, 1:10, 0.5:0.5. **d**, Limit of detection (LOD) calculations for the sensor at different concentrations. From top to bottom - lucCageBot:lucKey concentration (nM) = 50:5, 5:5, 1:10, 0.5:0.5. Error bars represent SD.

Figure 11(a-f). Design, selection, and engineering of lucCageTrop for cardiac Troponin I detection. **a**, Experimental screening of designed sensors for cardiac Troponin I (cTnI). Fragments of cardiac Troponin T, namely cTnTf1-f6, were computationally grafted into lucCage at different positions of the latch. All designs were produced in *E. coli* and

experimentally screened at 20 nM and 20 nM lucKey for an increase in the presence of cTnI (100 nM). The luminescence values were normalized to 100 in the absence of cTnI. Design 336-cTnTf6-K342A was selected as the best candidate (named lucCageTrop627) based on its sensitivity, activation fold-change, and stability.

5 cTnTf1:226-EDQLREKAKELWQTI-240 (SEQ ID NO:27385)

cTnTf2:226-EDQLREKAKELWQTIYN-242 (SEQ ID NO:27386)

cTnTf3:226-EDQLREKAKELWQTIYNLEAE-246 (SEQ ID NO:27387)

cTnTf4:226-EDQLREKAKELWQTIYNLEAEKFD-249 (SEQ ID NO:27388)

cTnTf5:226-EDQLREKAKELWQTIYNLEAEKFDLQE-252 (SEQ ID NO:27389)

10 cTnTf6:226-EDQLREKAKELWQTIYNLEAEKFDLQEKFKQQYEINVLRNRINDNQ-272 (SEQ ID NO:27390) **b**, Models of lucCageTrop627 and lucCageTrop, an improved version by fusion

of cardiac Troponin C (cTnC) at the C-terminus of lucCageTrop627. The models are shown in ribbon representation comprising SmBit a fragment of cTnT (PDB ID: 4Y99), and cTnC (PDB ID: 4Y99). The black box shows a close-up view of the interface of Cage and cTnT in

15 the lucCageTrop design. **c**, The binding affinity of lucCageTrop627 and lucCageTrop to cTnI was measured by biolayer interferometry. lucCageTrop showed 7-fold higher affinity to cTnI than lucCageTrop627. **d**, Comparison of bioluminescence kinetics between lucCageTrop627 (top) and lucCageTrop (bottom) in the presence of serially diluted cTnI. Higher binding

affinity leads to improved dynamic range and sensitivity of the sensor. **e**, Determination of

20 lucCageTrop's sensitivity. Bioluminescence was measured over 6000 s in the presence of serially diluted cTnI. From top to bottom - lucCageTrop:lucKey concentration (nM) = 1:10, 1:1, 0.5:0.5, 0.1:0.1. **f**, Limit of detection (LOD) calculations for the sensor at different

concentrations. From top to bottom - lucCageTrop:lucKey concentration (nM) = 1:10, 1:1, 0.5:0.5, 0.1:0.1. Error bars represent SD.

25 **Figure 12(a-f). Design and characterization of an anti-HBV antibody sensor. a**,

The energy-minimized models of lucCage designs are shown with the threaded segments of SmBit and the antigenic motif of PreS, respectively. The black box shows a blown-up view

of the cage-motif interface of the HBV344 design. **b**, Experimental screening of all designs performed by monitoring the luminescence of each lucCage (20 nM) and lucKey (20 nM) in

30 the presence or absence of the anti-HBV antibody HzKR127-3.2 (100 nM). The

luminescence values were normalized to 100 in the absence of anti-HBV. The design

HBV344 was selected due to its better performance and was named lucCageHBV. **c,d**,

Determination of lucCageHBV sensitivity. Bioluminescence was measured over 6000 s in the presence of serially diluted HzKR127-3.2. From top to bottom - lucCageHBV:lucKey

35 concentration (nM) = 50:5, 5:5, 1:1. The maximum values of the curves in **c**, are used to

obtain the curves in **d**. **e**, Limit of detection (LOD) calculations for the concentrations. From top to bottom - lucCageHBV:lucKey concentration (nM) = 50:5, 5:5, 1:1. **f**, Luminescence kinetics after the addition of the antibody (anti-HBV, first arrow). From top to bottom - anti-HBV antibody concentrations = 100, 50, 12.5 nM. At 6000 s, different concentrations of the PreS1 domain were injected into the wells, and the decreased luminescence signals were used to detect PreS1. Error bars represent SD.

Figure 13(a-d). Experimental characterization of lucCageHBV α for improved detection of an anti-HBV antibody.

a, Structural model of lucCageHBV α with a blow-up detail of the predicted interface between the PreS1 epitope and lucCage. The design

comprises two copies of the epitope PreS1 (a.a. 35-46)

GANSNNPDWDFNGGSGGGSSGFGANSNNPDWDFNPN (SEQ ID NO:27630), spaced by a flexible linker to enable bivalent interaction with the antibody. The SmBit peptide is shown in ribbon representation. **b**, Determination of lucCageHBV α detection sensitivity to the presence of the antibody HzKR127-3.2 (anti-HBV). Bioluminescence was measured over 6000 s in the

presence of serially diluted HzKR127-3.2. From top to bottom - lucCageHBV α :lucKey concentration (nM) = 50:5, 5:5, 1:10, 0.5:0.5. **c**, The linear region of a calibration curve was used to determine the limit of detection (LOD) and the dynamic range of antibody detection.

d, Bioluminescence images acquired with a BioRad ChemiDoc imaging system. From top to bottom - lucCageHBV α :lucKey concentration (nM) = 50:5, 5:5, 1:10. Changes in

bioluminescence intensity levels were detected as a function of the concentration of HzKR127-3.2.

Figure 14(a-d). Design and characterization of sensors for anti-SARS-CoV-2 antibodies. **a-b**, Experimental screening of *de novo* sensors for antibodies against the SARS-CoV-2 membrane protein (**a**), and the nucleocapsid protein (**b**). Selected epitopes of the

membrane protein (M1, M3 and M4;

M1_1-31:MADSNGTITVEELKKLLEQWNLVIGFLFTWI (SEQ ID NO:27659);

M3_1-17:MADSNGTITVEELKKLLE (SEQ ID NO:27660);

M4_8-24:ITVEELKKLLEQWNLVI (SEQ ID NO:27661)) and the nucleocapsid protein

(N6 single (PKDKKKKKADETQALPQRQKK; SEQ ID NO:27662) and N62 single

(KKDKKKKKADETQAL; SEQ ID NO:27663) were computationally grafted into lucCage at different positions of the latch. Each design comprised two tandem copies of each epitope, separated by a flexible linker, to take advantage of the bivalent binding of antibodies. All designs were experimentally screened for increase in luminescence at 20nM of each lucCage design and 20nM of lucKey in the presence of anti-M rabbit polyclonal antibodies (ProSci,

3527) (a) or anti-N mouse monoclonal antibody at 100nM (clone 18F6 luminescence values were normalized to 100 in the absence of antibodies. Designs M3_1-17_334 and N62_369-382_340 were selected as the best candidates due to high sensitivity and stability, and were named lucCageSARS2-M and ucCageSARS2-N respectively. **c**, Left panel: structural model of lucCageSARS2-M, showing a blow-up of the predicted interface between the M3 epitope and lucCage. Middle panel: determination of lucCageSARS2-M (MADSNGTITVEELKKLLEGGSGGMADSNGTITVEELKKLLE (SEQ ID NO:27392)) sensitivity to anti-M pAb. Bioluminescence was measured over 4000 s in the presence of serially diluted anti-M pAb. From top to bottom - lucCageSARS2-M:lucKey concentration (nM) = 50:50, 5:5. Right panel: limit of detection (LOD) calculations for the sensor at different concentrations. **d**, Left panel: structural model of lucCageSARS2-N, showing a blow-up of the predicted interface between the N62 epitope and lucCage. Middle panel: determination of lucCageSARS2-N (KKDKKKKADETQALGGSGGKKDKKKKADETQAL; SEQ ID NO:27548) sensitivity to anti-N mAb. Bioluminescence was measured over 4000 s for lucCageSARS2-N + lucKey at 50 nM in the presence of serially diluted anti-N antibody. Right panel: LOD calculations for the sensor. Error bars represent SD.

Figure 15(a-e). **a**, Experimental screening of *de novo* sensors for the receptor-binding domain (RBD) of the SARS-CoV-2 Spike protein. All designs were experimentally screened for increase in luminescence at 20 nM of each lucCage design and 20 nM of lucKey in the presence of 200 nM RBD. The luminescence values were normalized to 100 in the absence of RBD. Design lucCageRBDdelta4_348 was selected as the best candidate due to high sensitivity and stability, and was named lucCageRBD. **b**, Structural model of lucCageRBD composed of the LCB1 binder grafted into lucCage comprising a caged SmBiT fragment. The black boxes show a blow-up view of the interface of Cage and LCB1 binder in the lucCageRBD design. **c**, Determination of lucCageRBD's sensitivity. Bioluminescence was measured over 10000 s in the presence of serially diluted RBD protein. From top to bottom - lucCageRBD:lucKey concentration (nM) = 1:1, 1:10, 10:10. **d**, Limit of detection (LOD) calculations for the sensor at different concentrations. From top to bottom - lucCageRBD:lucKey concentration (nM) = 1:1, 1:10, 10:10. **e**, Bioluminescence images acquired with a BioRad ChemiDoc imaging system. Changes in bioluminescence intensity levels were detected as a function of the concentration of RBD with lucCageRBD at 1nM and lucKey at 10 nM.

Figure 16. General principle of LOCKR-based biosensor and expanding readouts by various split protein assembly.

Figure 17 (a-c). (a) Schematic diagram, emission spectrum, and changes of BRET ratios of intermolecular HBV antibody BRET sensor (S0512). (b) Schematic diagram, emission spectrum, and standard curve of intramolecular HBV antibody BRET sensor (B0622). The linker optimization was performed for optimal BRET efficiency. (c) Emission spectrum and dose-dependent changes of BRET ratios of B0622_6 to the presence of HBV antibody (DFISREVSKEELIKENMRSK is SEQ ID NO:27655; DFISREELIKENMRSK is SEQ ID NO: 27656; DFISRELIKENMRSK is SEQ ID NO: 27657; and DFISREKENMRSK is SEQ ID NO: 27658). 2 nM of sensor concentration and 20, 5, 0 nM (left to right) of MBP_Key were used.

Figure 18. Schematic diagram, the hydrolysis mechanism of Nitrocefin (colorimetric substrate), and the dose-dependent changes of β -lactamase activities to human cardiac Troponin I (cTnI) for colorimetric Troponin I sensor (LacATrop). β -lactamase activities were monitored at OD490. The initial rate of β -lactamase in each cTnI was calculated as β -lactamase activities. Photo below showed the dose-dependent color changed in solution from yellow to reddish in the presence of cTnI.

Figure 19(a-d). CoV LOCKR Diagnostic. **A.** The strategy for both negative and positive controls is illustrated. The negative control will receive an added excess of synthetic linear peptide epitope to occupy all epitope binding sites on available antibodies. The positive control sample will contain lucCage-ProA / lucKey components to measure the presence of IgG or IgM antibodies wherein the Latch component of the lucCage contains the Fc domain antibody binding Protein A. **B.** Functional positive control lucCage-ProA component (have already been identified (and are capable of detecting polyclonal rabbit IgG antibodies (middle panel) together with a lucKey within minutes after addition vs. buffer containing only LucKey (black line) in the presence of Nano-Glo® reagents (Promega). The right panel demonstrates the sensitivity of the system for as little as 10 nM of IgG, with normalized luminescence at different concentrations of sensor (lucCage + lucKey) at 1, 10, and 5 nM, incubated with different concentrations of IgG. **C.** Evaluation of LOCKR Biosensor Specificity. Sensors at 10 nM (LucCageSARS2-N at 50nM) were incubated with 50 nM of cognate target, the targets for the other biosensors or buffer. Strong responses were observed only for the cognate targets. **D.** POCD CoV LOCKR Device. The device—pre-filled in a sterile package (left)—includes in one channel the (+) positive control lucCage-ProA / lucKey reagents which are designed to activate upon binding IgG, (s) the test sample lucCage-Coronavirus-Epitope / lucKey reagents, and (-) the negative control reagents which are lucCage-Coronavirus-Epitope / lucKey plus excess peptide epitope [~ 1 mM].

Figure 20(a-c). CoV LOCKR Diagnostic. Designed LOCKR

provide a kinetic “all in solution” assay to detect the presence of epitope-specific antibodies.

A. At the start, lucCage-Epitope and lucKey proteins are present in solution that is dark in the

“OFF” state. **B.** Upon addition of a fluid containing antibodies capable of binding to the

5 epitope of interest the Latch binding interface of the lucCage is exposed allowing the lucKey domain to bind, positioning the fused large bit of split luciferase to bind to the small bit of split luciferase. This results in reconstitution of luciferase luminescence (“ON”). **C.** Addition

of recombinant antigen containing the Epitope of interest will shift the equilibrium of antibody binding from the Latch to the antigen, causing less reconstitution of split luciferase
10 activity, resulting in a dim light emittance (“DIM”).

Figure 21. Indirect Detection. The sensor platforms of the disclosure can be repurposed to accommodate an "indirect detection" approach, in which the split reporter protein (intermolecular or intramolecular embodiments; an intermolecular embodiment is shown in **Figure 21**) is reconstituted by pre-incubation of the biosensor with the target
15 (exemplified by an anti-HBV antibody) for the target binding polypeptide, resulting in fluorescence activation in this example. The activated biosensor is then incubated with a sample to detect the presence of an antigen to which the antibody binds (in this example Hepatitis B virus antigen (PreS1)), resulting in binding of the antibody to the antigen, loss of interaction between the split reporter protein components, and reduction/elimination of
20 reporting activity (in this case, loss of fluorescence activity).

Figure 22. Control Samples for CoV LOCKR Diagnostic. **A.** The strategy for both negative and positive controls is illustrated. The negative control will receive an added excess of synthetic linear peptide epitope to occupy all epitope binding sites on available antibodies in the sample. While the positive control sample will contain lucCage-ProA / lucKey
25 components to measure the presence of IgG or IgM antibodies wherein the Latch component of the lucCage contains the Fc domain antibody binding protein Protein A. **B.** Functional positive control lucCage-ProA component have already been identified (middle panel) and are capable of detecting polyclonal rabbit IgG antibodies together with a lucKey within minutes after addition vs. buffer containing only LucKey (black line) in the presence of
30 Nano-Glo® reagents (Promega). The right panel demonstrates the sensitivity of the system for as little as 10 nM of IgG, with normalized luminescence at different concentrations of sensor (lucCage + lucKey) at 1, 10, and 5 nM, incubated with different concentrations of IgG.

Detailed Description

All references cited are herein incorporated by reference in their application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: Molecular Cloning: A Laboratory Manual (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), Gene Expression Technology (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, CA), “Guide to Protein Purification” in Methods in Enzymology (M.P. Deutscher, ed., (1990) Academic Press, Inc.); PCR Protocols: A Guide to Methods and Applications (Innis, et al. 1990. Academic Press, San Diego, CA), Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed. (R.I. Freshney. 1987. Liss, Inc. New York, NY), Gene Transfer and Expression Protocols, pp. 109-128, ed. E.J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, TX).

As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise.

As used herein, the amino acid residues are abbreviated as follows: alanine (Ala; A), asparagine (Asn; N), aspartic acid (Asp; D), arginine (Arg; R), cysteine (Cys; C), glutamic acid (Glu; E), glutamine (Gln; Q), glycine (Gly; G), histidine (His; H), isoleucine (Ile; I), leucine (Leu; L), lysine (Lys; K), methionine (Met; M), phenylalanine (Phe; F), proline (Pro; P), serine (Ser; S), threonine (Thr; T), tryptophan (Trp; W), tyrosine (Tyr; Y), and valine (Val; V).

In all embodiments of polypeptides disclosed herein, any N-terminal methionine residues are optional (i.e.: may be present or may be absent).

All embodiments of any aspect of the disclosure can be used in combination, unless the context clearly dictates otherwise.

Unless the context clearly requires otherwise, throughout the description and the claims, the words ‘comprise’, ‘comprising’, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”. Words using the singular or plural number also include the plural and singular number, respectively. Additionally, the words “herein,” “above,” and “below” and words of similar import, when used in this application, shall refer to this application as a whole and not to any particular portions of the application.

In a first aspect, the disclosure provides cage proteins comprising a helical bundle, wherein the cage protein comprises a structural region and a latch region, wherein the latch region comprises one or more target binding polypeptide, wherein the cage protein further

comprises a first reporter protein domain, wherein the first reporter protein domain provides a detectable change in reporting activity when bound to a second reporter protein domain, and wherein the structural region interacts with the latch region to prevent solution access to the one or more target binding polypeptide.

5 Cage proteins and their use in protein switches are generally described in US patent application publication number US20200239524, incorporated by reference herein in its entirety. The present disclosure provides a significant improvement to such cage proteins and proteins switches by incorporating reporters and one or more target binding polypeptide, permitting use as a modular and generalizable biosensor platform that can enable a wide
10 range of readouts for different sensing purposes as disclosed herein.

The cage polypeptide comprises a latch region and a structural region (i.e.: the remainder of the cage polypeptide that is not the latch region). The latch region may be present near either terminus of the cage polypeptide. In one embodiment, the latch region is placed at the C-terminal helix. In various embodiments, the latch region may comprise a part
15 or all of a single alpha helix in the cage polypeptide at the N-terminal or C-terminal portions. In various other embodiments, the latch region may comprise a part or all of a first, second, third, fourth, fifth, sixth, or seventh alpha helix in the cage polypeptide. In other embodiments, the latch region may comprise all or part of two or more different alpha helices in the cage polypeptide; for example, a C-terminal part of one alpha helix and an N-terminal
20 portion of the next alpha helix, all of two consecutive alpha helices, etc.

The examples provide extensive details on exemplary cage proteins and reporting activities. Any suitable reporting protein domains may be used that involves two separate protein components (for example, BRET and FRET formats, as described herein), or
25 reporting proteins that can be split into two (or more) protein domains and its activity can be reconstituted when the two (or more) split protein domains are joined.

The detectable change may be any increase or a decrease in the relevant reporting activity, as deemed suitable for an intended purpose. Various non-limiting embodiments of detectable changes in reporting activity that can be utilized are described below when
30 discussing the biosensors of the disclosure, and in the examples.

In one embodiment, the cage protein further comprises the second reporter protein domain, wherein one of the first reporter protein domain and the second reporter domain is present in the latch region and the other is present in the structural region, wherein an interaction of the first reporter protein domain and the second reporter protein domain is

diminished in the presence of target to which the one or more target binds.

In another embodiment, the second reporter protein domain is not present in the cage protein and is present in another component (i.e.: the “key”, described below), or may be present elsewhere.

In one embodiment, cage protein the helical bundle comprises between 2-9, 2-8, 2-7, 3-9, 3-8, 3-7, 4-9, 4-8, 4-7, 5-9, 5-8, 5-7, 6-9, 6-8, 6-7, 2-6, 3-6, 4-6, 5-6, 2-5, 3-5, 4-5, 2-4, 3-4, 2-3, 2, 3, 4, 5, 6, 7, 8, or 9 alpha helices.

In another embodiment, each helix in the structural region of the cage protein may independently be between 18-60, 18-55, 18-50, 18-45, 22-60, 22-55, 22-50, 22-45, 25-60, 25-55, 25-50, 25-45, 28-60, 28-55, 28-50, 28-45, 32-60, 32-55, 32-50, 32-45, 35-60, 35-55, 35-50, 35-45, 38-60, 38-55, 38-50, 38-45, 40-60, 40-58, 40-55, 40-50, or 40-45 amino acids in length.

In another embodiment, the latch region may be extended in the designs of the present disclosure due to presence of the one or more target binding polypeptide within the latch region, and thus an alpha helix/alpha helices in the latch region may be significantly longer than in the structural region, limited only by the length of the target binding polypeptide present in the latch.

In any of these embodiments, adjacent alpha helices in the cage protein may optionally be linked by amino acid linkers. Amino acid linkers connecting each alpha helix can be of any suitable length or amino acid composition as appropriate for an intended use. In one non-limiting embodiment, each amino acid linker is independently between 2 and 10 amino acids in length, not including any further functional sequences that may be fused to the linker. In various non-limiting embodiments, each amino acid linker is independently 3-10, 4-10, 5-10, 6-10, 7-10, 8-10, 9-10, 2-9, 3-9, 4-9, 5-9, 6-9, 7-9, 8-9, 2-8, 3-8, 4-8, 5-8, 6-8, 7-8, 2-7, 3-7, 4-7, 5-7, 6-7, 2-6, 3-6, 4-6, 5-6, 2-5, 3-5, 4-5, 2-4, 3-4, 2-3, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids in length. In all embodiments, the linkers may be structured or flexible (e.g. poly-GS). These linkers may encode further functional sequences, as deemed appropriate for an intended use.

The latch region may be present at any suitable location on the cage protein as deemed appropriate for an intended purpose. In one embodiment, the latch region is at the C-terminus of the cage protein. In another embodiment, the latch region may be at the N-terminus of the cage protein.

Similarly, the first reporter protein domain may be present at or within the cage protein as deemed appropriate for an intended purpose. In one embodiment, the first reporter protein domain is present in the latch region. In one embodiment, the first reporter protein domain is at the C-terminus of the latch region or within 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid of the C-terminus of the latch region. In another embodiment, the first reporter protein domain is at or within 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid of the N-terminus of the latch region.

In another embodiment, the second reporter protein may be present in the cage protein; in this embodiment, the second reporter protein domain may be present in the structural region. In one such embodiment, the second reporter protein may be present at the N-terminus of the structural region, or may be within 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid of the N-terminus of the structural region.

The cage protein comprises one or more (i.e., 1, 2, 3, etc.) target binding polypeptides. In one embodiment, the cage protein comprises one target binding polypeptide. In another embodiment, the cage protein comprises two target binding polypeptides. In one embodiment, the one or more target binding polypeptide and the first reporter protein domain are separated by at least 10 amino acids in the latch region of the cage protein. In another embodiment, the one or more target binding polypeptide is at or within 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid of the C-terminus of the latch region.

Any suitable reporting protein domains may be used that involves two separate protein components (for example, BRET and FRET formats, as described herein), or reporting proteins that can be split into two (or more) protein domains and its activity can be reconstituted when the two (or more) split protein domains are joined. In one embodiment, the first reporter protein domain, and the second reporter domain when present in the cage protein, comprise reporter protein domains selected from the group consisting of luciferase (including but not limited to firefly, Renilla, and Gaussia luciferase), bioluminescence resonance energy transfer (BRET) reporters, bimolecular fluorescence complementation (BiFC) reporters, fluorescence resonance energy transfer (FRET) reporters, colorimetry reporters (including but not limited to β -lactamase, β -galactosidase, and horseradish peroxidase), cell survival reporters (including but not limited to dihydrofolate reductase), electrochemical reporters (including but not limited to APEX2), radioactive reporters (including but not limited to thymidine kinase), and molecular barcode reporters (including but not limited to TEV protease).

In one embodiment, the cage protein does not include the secor
one such embodiment, the first reporter protein domain comprises:

(a) an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:

5 27359 and 27664-27672: VTGYRLFEEIL (SmBit) (SEQ ID NO:27359), VTGYRLFEEKIL (SEQ ID NO:27664), VTGYRLFEEKIS (SEQ ID NO:27665), VSGWRLFKKIS (SEQ ID NO:27666), VEGYRLFEEKIS (SEQ ID NO:27667), VTGYRLFEEKES (SEQ ID NO:27668), VTGWRLFEEKIL (SEQ ID NO:27669), VTGWRLFKEIL (SEQ ID NO:27670), VTGYRLFKEIL (SEQ ID NO:27671), LAGWRLFKKIS (SEQ ID NO:27672);

(b) an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 27360-27361:

15 VFAHPETL VKVKDAEDQLGA RVGYIELDLN SGKILESFRP EERFPMMSTF KVLICGAVLS RVDAGQEQLG RRIHYSQNDL VEYSPVTEKH LTDGMTVREL CSAAITMSDN TAANLLLTII GGPKELTAFI HNMGDHTVRL DRWEPELNEA IPNDERDTT PAAMATTLRK LLTGNGR (split β -lactamase A; SEQ ID NO:27360) and

20 LLTLASRQQLIDWME ADKVAGPLL R SALPAGWFIA DKSGAGERGS RGIIAALGPD GKPSRIVVIY TTGSQATMDE RNRQIAEIGA SLIKHW (Split beta lactamase B; SEQ ID NO:27361);

(c) an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:27362-27378, wherein underlined residues are amino acid linkers or other optional residues that may be present or absent, and when present may be any amino acid sequence, and wherein any N-terminal methionine residues may be present or absent:

30 VFTLEDFVGDWRQTAGYNLSQVLEQGGVSSLFQNLGVSVTPIQIRIVLSGENGLKIDIHVIIPYEGLSG DQMGQIEKIFKVVPVDNHHFKVILHYGTLVIDGVTPNMDYFGRPYEGIAVFDGKKITVTGTLWNGN KIIDERLINPDGSLLFRVTINGVTGWRLHERILA (TeLuc; SEQ ID NO:27362) (full luminescent or fluorescent protein that can be used to create FRET and/or BRET sensors);

35 LIK ENMRSKLYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV EGGPLPFAFD ILATHFMYGS KVFIIKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ DTSIQDGEI YNVKVRGVNF PANGPVMQKK TLGWEPTSTET MYPADGGLEG RCDKALKLVG GGHLHVNFKT TYKSKKPVKM PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSNLGGMD ELYK (CyOFF variant; SEQ ID NO:27363) (full luminescent or fluorescent protein that can be used to create FRET and/or BRET sensors);

40 VSKGEELIK ENMRSKLYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV EGGPLPFAFD ILATHFMYGS KVFIIKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ DTSIQDGEI YNVKVRGVNF PANGPVMQKK TLGWEPTSTET MYPADGGLEG RCDKALKLVG GGHLHVNFKT TYKSKKPVKM

PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSNLGGMD ELYK (CyOFP v2
NO:27364) (full luminescent or fluorescent protein that can be used
BRET sensors);

- 5 EELIK ENMRSKLYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV EGGPLPFAFD ILATHFMYGS
KVFIKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ DTSIQDGELI YNVKVRGVNF
PANGPVMQKK TLGWEPSTET MYPADGGLEG RCDKALKLVG GGHLHVNFKT TYKSKKPVKM
PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSNLGGMD ELYK (CuOFP variant; SEQ ID
NO:27365) (full luminescent or fluorescent protein that can be used to create FRET and/or
10 BRET sensors);

- KVFTLGDVFGDWRQTAGYNQAQVLEQGGLTSLFQNLGVSVTPIQIRIVLSGENGLKIDIHV
IIPYEGLSCDQMAQIEKIFKVVPVDDHHFKAILHYGTLVIDGVTNPMIDYFGQPYEGIA
KFDGKKITVTGTLWNGNTIIDERLINPDGSLFRVTINGVTGWRHERILA (LumiLuc; SEQ ID
15 NO:27366) (full luminescent or fluorescent protein that can be used to create FRET and/or
BRET sensors);

- MVSKGEEDNM ASLPATHELH IFGSINGVDF DMVGQGTGNP NDGYEELNLK STKGDLQFSP W
ILVPHIGYG FHQYLPYPDG MSPFQAAMVD GSGYQVHRTM QFEDGASLTV NYRYTYEGSH IKG
EAQVKGT GFPADGPVMT NSLTAADWCR SKKTYPNDKT IISTFKWSYT TGNGKRYRST ARTTY
20 TFAKP MAANYLKNQP MYVFRKTELK HSKTELNFKE WQKAFTDVMG MDELYK
(mNeonGreen; SEQ ID NO:27367) (full luminescent or fluorescent protein that can
be used to create FRET and/or BRET sensors);

- MVSKGEAVIK EFMRFKVHME GSMNGHEFEI EGEGERPYE GTQTAKLKVT KGGPLPFSWD
ILSPQFMYGS RAFIKHPADI PDYKQSFPE GFKWERVMNF EDGAVTVTQ DTSLEDGTL
25 I YKVKLRGTNF PPDGPVMQKK TMGWEASTER LYPEDGVLKG DIKMALRLKD GGRYLADF
KT TYKAKKPVQM PGAYNVDRKL DITSHNEDYT VVEQYERSEG RHSTGGMDEL YK
(mScarlet-i; SEQ ID NO:27368) (full luminescent or fluorescent protein that can be
used to create FRET and/or BRET sensors);

- SGKSYPTVSADYQKAVEKAKKRLGGFIAEKRCAPLMLRLAWHSAGTFDKRTKTGGPFGTIRYPaelah
30 SANSGLDI AVRLLLEPLKAEPFILSYADFYQLAGVVAVEVTGGPEVPFHPGREDKPELPPEGRLPDATK
GSDHLRDVFGKAMGLTDQDIALSGGHTLGAAHKERSGFEGPWT SNPLVFDNSY FTELLSGEKEGGG
SGGGGS (APEX2-1-200; SEQ ID NO:27369);

GGGGSGGGGS GLLQLPSDKALLSDPVFRPLVDKYAADEDAFFADYAEAHQKLSELGFADA
(APEX2-201-250; SEQ ID NO:27370);

- 35 MGSHHHHHHSGSENLYFQSGGS
VRPLNCIVA VSQNMGIGKN GDLWPPLRN ESKYFQRMFT TSSVEGKQNL
VIMGRKTWFS IPEKNRPLKD RINIVLSREL KEPPRGHFL AKSLDDALRL
IEQPELGGGGSGGGGS (DHFR_A (1-105); SEQ ID NO:27371);
- 40 SGSGDPDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERLIREAAAASEKISRE
GGGGSGGGGS ASKV DMVWIVGGSS VYQEAMNQPGLRLFVTRIM QEFESDTFFP
EIDLKGYKLL PEYPGVLSEV QEEKGIKYKF EVYEKKD (DHFR_B (106-186); SEQ ID
NO:27372);

- 45 QLTPTFYDNSCPNVSNIVRDIIIVNELRSDPRIAASILRLHFHDCFVNGCDASILLDNTTSFRTEKDAF
GNANSARGFSVIDR
MKAAVESACPGTVSCADLLTIAAQQSVTLAGGPSWRVPLGRRDSLQAFLLANANLPAPFFTLPLQKLD

SFRNVGLNRSSDLVALSGGHTFGKSQCRFIMDRLYNFSNTGLPDPTLNTTYI
 (sHRPa is the large split HRP fragment. It consists
 1-213 of horseradish peroxidase (HRP) with the following 4
 mutations: T21I, P78S, R93G, N175S) (SEQ ID NO:27373);

5 NLSALVDFDLRTPTIFDNKYYVNLEEQKGLIQSDQELFSSPDATDTIPLVRSFANSTQTFNFAFVEAM
 DRMGNIPTLTGTQGQIRRNCRVNSNGGSGS (sHRPb is the small split HRP
 fragment. It consists of amino acids 214-308 of horseradish
 peroxidase (HRP) with the following 2 mutations: N255D, L299R) (SEQ
 ID NO:27374);

10 GESLFKGPRDYNPISSTICHLTNE SDGHTTSLYGIGFGPFIIITNKHLFRNNGTLLVQSLHGVFKVKN
 TTTLQQHLIDGRDMIIRMPKDFPPFPQKLKFREPQREERICLVTTNFQTGGGSGGGGS (N_Tev
 (1-118) (SEQ ID NO:27375);

GGGSGGGGSKSMSSMVSDTSCTFPSSDGIFWKHWIQTKDGQCGSPLVSTRDGFIVGIHSASNFTNTN
 NYFTSVPKNFMELLTNQEAQQWVSGWRLNADSVLWGGHKVFMDKP C_Tev (119-221) (SEQ
 15 ID NO:27376);

MASYPCHQHA SAFDQAARSR GHSNRRTALR PRRQQEATEV RLEQKMPTLL
 RVIIDGPHGM GKTTTTQLLV ALGSRDDIVY VPEPMTYWQV LGASETIANI
 YTTQHRLDQG EISAGDAVV MTSAQITMGM PYAVTDAVLA PHIGGEAGSS
 HAPPPALTLI FDRHPIAALL CYPARYLMG SMTPQAVLAF VALIPPTLP
 20 TNIVLGALPE DRHIDRLAKR QRPGERLDA MLAAIRRVYG LLANTVRYLQ
 GGGSWREDWG QLSGT GGGSGGGGS (thymidine kinase_TK_A (1-265) (SEQ ID
 NO: 27377); and/or

GGGSGGGGS AVPPQ GAEPQSNAGP RPHIGDTLFT LFRAPPELLAP
 NGDLYNVFAW ALDVLAKRLR PMHVFILDYD QSPAGCRDAL LQLTSGMVQT
 25 HVTTPGSIPT ICDLARTFAR EMGEAN (thymidine kinase_TK_B (266-376) (SEQ
 ID NO: 27378)

This embodiment of the cage protein comprising a reporter protein domain will
 interact with the second biosensor component “key” protein (discussed below) comprising a
 second reporter domain in presence of a target analyte.

30

In another embodiment, the cage comprises the second reporter protein domain,
 wherein

(a) one of the first reporter protein domain and the second reporter protein domain
 comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%,
 35 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid
 sequence of SEQ ID NOS: 27359, and 27664-27672;

and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%,
 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence
 of SEQ ID NO: 27379, wherein the N-terminal methionine residue may be present or absent:

MVFTLEDFVGDWEQTAAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRIVRSGENALKII
 EVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTI
 LFRVTINS (LgBiT) (SEQ ID NO:27379);

(b) one of the first reporter protein domain and the second reporter protein domain
 5 comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,
 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:
 27360

VFAHPETL VKVKDAEDQLGA RVGYIELDLN SGKILESFRP EERFPMSTF KVLICGAVLS
 RVDAGQEQLG RRIHYSQNDL VEYSPVTEKH LTDGMTVREL CSAAITMSDN TAANLLTTI
 10 GGPKELTAFI HNMGDHVTRL DRWEPELNEA IPNDERDTT PAAMATTLRK LLTGNGR (split β -
 lactamase A) (SEQ ID NO: 27360),

and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%,
 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence
 of SEQ ID NO:27361:

15 LLTLASRQQLIDWME ADKVAGPLL SALPAGWFIA DKSGAGERGS RGIIAALGPD GKPSRIVVIY
 TTGSQATMDE RNRQIAEIGA SLIKHW (Split beta lactamase B) (SEQ ID NO:
 27361);

(c) one of the first reporter protein domain and the second reporter protein domain
 comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,
 20 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID
 NO:27362:

VFTLEDFVGDWRQTAGYNLSQVLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYEGLSG
 DQMGQIEKIFKVVYPVDNHHFKVILHYGTLVIDGVTPNMIDYFGRPYEGIAVFDGKKITVTGTLWNGN
 KIIDERLINPDGSLLFRVTINGVTGWRLHERILA (TeLuc) (SEQ ID NO:27362), (full

25 luminescent or fluorescent protein that can be used to create FRET and/or BRET sensors)

and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%,
 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence
 selected from the group consisting of SEQ ID NOS:27363-27365:

30 LIK ENMRSLKYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV EGGPLPFAFD ILATHFMYGS
 KVFIKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ DTSIQDGEI YNVKVRGVNF
 PANGPVMQKK TLGWEPSTET MYPADGGLEG RCDKALKLVG GGHLHVNFKT TYKSKKPVKM
 PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSNLGGMD ELYK (CyOFF variant) (SEQ ID
 NO:27363) (full luminescent or fluorescent protein that can be used to create FRET

35 and/or BRET sensors);

VSKGEELIK ENMRSKLYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV EGC
 KVFIKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ DTSLQDGELI YNVKVRGVNF
 PANGPVMQKK TLGWEPSTET MYPADGGLEG RCDKALKLVG GGHLHVNFKT TYKSKKPVKM
 5 PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSNLGGMD ELYK(CyOFP variant) (SEQ ID
 NO: 27364) (full luminescent or fluorescent protein that can be used to create FRET
 and/or BRET sensors); and

EELIK ENMRSKLYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV EGGPLPFAFD ILATHFMYGS
 10 KVFIKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ DTSLQDGELI YNVKVRGVNF
 PANGPVMQKK TLGWEPSTET MYPADGGLEG RCDKALKLVG GGHLHVNFKT TYKSKKPVKM
 PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSNLGGMD ELYK(CyOFP variant) (SEQ ID
 NO: 27365) (full luminescent or fluorescent protein that can be used to create FRET
 and/or BRET sensors);

15 (d) one of the first reporter protein domain and the second reporter protein domain
 comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,
 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:
 27366:

20 KVFTLGDFVGDWRQTAGYNQAQVLEQGGLTSLFQNLGVSVTPIQIRIVLSGENGLKIDIHV
 IIPYEGLSCDQMAQIEKIFKVVPVDDHHFKAILHYGTLVIDGVTNPMIDYFGQPYEGIA
 KFDGKKITVTGTLWNGNTIIDERLINPDGSLLFRTVINGVTGWRLHERILA (LemLuc) (SEQ ID
 NO: 27366) (full luminescent or fluorescent protein that can be used to create FRET and/or
 BRET sensors),

25 and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%,
 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence
 of SEQ ID NO: 27368, wherein the N-terminal methionine residue may be present or absent:
 MVSKGEAVIK EFMRFKVHME GSMNGHEFEI EGEGERPYE GTQTAKLKVT KGGPLPFSWD ILSPQFMYG
 S RAFIKHPADI PDYKQSFPE GFKWERVMNF EDGGAVTVTQ DTSLEDGTLI YKVKLRGTNF PPDGPVM
 30 QKK TMGWEASTER LYPEDGVLKG DIKMALRLKD GGRYLADFKT TYKAKKPVQM PGAYNVDRKL DITSH
 NEDYT VVEQYERSEG RHSTGGMDEL YK (mScarlet-i) (SEQ ID NO: 27368) (full
 luminescent or fluorescent protein that can be used to create FRET and/or BRET sensors);

(e) one of the first reporter protein domain and the second reporter protein domain
 comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,
 35 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID
 NO: 27367, wherein the N-terminal methionine residue may be present or absent:

MVSKGEEDNM ASLPATHELH IFGSINGVDF DMVGQGTGNP NDGYEELNLK S1
 G FHQYLPYPDG MSPFQAAMVD GSGYQVHRM QFEDGASLTV NYRYTYEGSH _
 VMT NSLTAADWCR SKKTPNDKT IISTFKWSYT TGNGKRYRST ARTTYTFAKP MAANYLKNQP MYVFR
 KTELK HSKTELNFK E WQKAFTDVMG MDELYK (mNeonGreen) (SEQ ID NO:27367), (full

- 5 luminescent or fluorescent protein that can be used to create FRET and/or BRET sensors),
 and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%,
 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence
 of SEQ ID NO:27368, wherein the N-terminal methionine residue may be present or absent:

10 MVSKGEAVIK EFMRFKVHME GSMNGHEFEI EGEGERPYE GTQTAKLKVT KGGPLPFSWD ILSPQFMYG
 S RAFIKHPADI PDYYKQSFPE GFKWERVMNF EDGGAVTVTQ DTSLEDGTLI YKVKLRGTNF PPDGPVM
 QKK TMGWEASTER LYPEDGVLKG DIKMALRLKD GGRYLADFKT TYKAKKPVQM PGAYNVDRKL DITSH
 NEDYT VVEQYERSEG RHSTGGMDEL YK (mScarlet-i) (SEQ ID NO: 27368) (full

luminescent or fluorescent protein that can be used to create FRET and/or BRET sensors);

- (f) one of the first reporter protein domain and the second reporter protein domain
 15 comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,
 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence SEQ ID NO:
 27369, wherein underlined residues are optional residues that may be present or absent, and
 when present may be any amino acid sequence

20 SGKSYPTVSADYQKAVEKAKKRLGGFIAEKRCAPLMLRLAWHSAGTFDKRKTGGPFGTIRYPaelahsANSGLD
 IAVRLLEPLKAFFPILSYADFYQLAGVVAVEVTGGPEVFPFHGREDKPELPPGRLPDATKGSdHLRDVFGKAMG
 LTDQDIVALSggHTLGAahKERSGFEGPWTsnPLVFDNSYfTEllsGEKEGGGGSGGGGS (APEX2-1-200)
 (SEQ ID NO: 27369) (split engineered variant of soybean ascorbate peroxidase protein
 for chemiluminescent and colorimetric detection system);

- and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%,
 25 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence
 of SEQ ID NO: 27370, wherein underlined residues are optional residues that may be present
 or absent, and when present may be any amino acid sequence

GGGGSGGGGS GLLQLPSDKALLSDPVFRPLVDKYAADEDAFFADYAEahQKLSELGFADA (APEX2-
 201-250) (SEQ ID NO: 27370) (split engineered variant of soybean ascorbate
 30 peroxidase protein for chemiluminescent and colorimetric detection system);

- (g) one of the first reporter protein domain and the second reporter protein domain
 comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,
 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:
 27371, wherein underlined residues are optional residues that may be present or absent, and
 35 when present may be any amino acid sequence

MGSHHHHHHSGSGSENLYFQSGSGS

VRPLNCIVA VSQNMIGIGKN GDLPPWPLRN ESKYFQRM TT TSSVEGKQNL

VIMGRK TWFS IPEKNRPLKD RINIVLSREL KEPPRG AHFL AKSLDDALRL

IEQPELGGGGSGGGGS (DHFR_A (1-105)); (SEQ ID NO: 27371) (split dihydrofolate

5 reductase protein reporter for cell survival or fluorescence)

and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27372, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence

10 SGSG DPDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERLIREAAAASEKISRE

GGGGSGGGGS ASKV DMVWIVGGSS VYQEAMNQPG HLRLFVTRIM QEFESDTFFP

EIDLGKYKLL PEYPGVLSEV QEEKGIKYKF EVYEKKD (DHFR_B (106-186)); (SEQ ID NO: 27372) (split dihydrofolate reductase protein reporter for cell survival or fluorescence);

(h) one of the first reporter protein domain and the second reporter protein domain
15 comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27373, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence

20 QLTPTFYDN SCPNVSNIVRDIIVNELRSDPRIAASILRLHFHDCFVNGCDASILLDNTTSFRTEKDAFGNANSA
RGFSVIDRMKAAVESACPGTVSCADLLTIAAQQSVTLAGGPSWRVPLGRRDSLQAFDLANANLPAPFFTLPLQLK
DSFRNVGLNRSSDLVALSGGHTFGKSQCRFIMDRLYNFSNTGLPDPTLNTTYLQTLRGLCPNLGGSGS (sHRPa
is the large split HRP fragment. It consists of amino acids 1-213 of
horseradish peroxidase (HRP) with the following 4 mutations: T21I, P78S,
R93G, N175S: plasmid 73147 (SEQ ID NO: 27373);

25 and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27374, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence

30 NLSALVDFDLRTPTIFDNKYVNLEEQKGLIQSDQELFSSPDATDTIPLVRSFANSTQTFNFAFVEAMDRMGNIT
PLTGTQGQIRNCRVNSNGGSGS (sHRPb is the small split HRP fragment. It
consists of amino acids 214-308 of horseradish peroxidase (HRP) with the
following 2 mutations: N255D, L299R: plasmid 73148) (SEQ ID NO: 27374);

(i) one of the first reporter protein domain and the second reporter protein domain
35 comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:

27375, wherein underlined residues are optional residues that may be present when present may be any amino acid sequence

GESLFKGPRDYNPISSSTICHLTNESDGHTTSLYGIGFGPFIIITNKHLFRNNGTLLVQSLHGVFKVKNNTTTLQQH
LIDGRDMIIRMPKDFPPFPQKLKFREPQREERICLVTTNFQTGGGGSGGGGSN_Tev (1-118) (SEQ

5 ID NO: 27375) (Split TEV protease);

and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27376, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence

10 GGGGSGGGGSKSMSSMVSDTSCTFPSSDGIFWKHWIQTkdGQCGSPLVSTRDGFIVGIHSASNFTNTNNYFTSV
PKNFMELLTNQEAQQWVSGWRLNADSVLWGGGHKVFMDKP (C_Tev (119-221)); (SEQ ID NO:
27376) (Split TEV protease);

(j) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,
15 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:
27377, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence, and wherein the N-terminal methionine residue may be present or absent:

MASYPCHQHA SAFDQAARSR GHSNRRRTALR PRRQQEATEV RLEQKMPTLL
20 RVIYIDGPHGM GKTTTTQLLV ALGSRDDIVY VPEPMTYWQV LGASETIANI
YTTQHRDLQD EISAGDAVV MTSAQITMGM PYAVTDAVLA PHIGGEAGSS
HAPPPALTLI FDRHPIAALL CYPAAARYLMG SMTPQAVLAF VALIPPTLP
TNIVLGALPE DRHIDRLAKR QRPGERLDLA MLAAIRRVYG LLANTVRYLQ
GGGSWREDWG QLSGT GGGGSGGGGS (thymidine kinase_TK_A (1-265)) (SEQ ID NO:
25 27377);

and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27378, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence

30 GGGGSGGGGS AVPPQ GAEPQSNAGP RPHIGDTLFT LFRAPPELLAP
NGDLYNVFAW ALDVLAKRLR PMHVFILDYD QSPAGCRDAL LQLTSGMVQT
HVTTPGSIPT ICDLARTFAR EMGEAN (thymidine kinase_TK_B (266-376)) (SEQ ID NO:
27378)

These embodiments of the cage protein comprising two reporter protein domains
35 interact with the second biosensor component “key” in presence of a target analyte. The

conformational change induced by this interaction enables the approximation for the two reporter proteins in the cage protein, allowing analyte quantification by measuring increase (or decrease) in reporter signal.

Any suitable target binding polypeptide that binds a target of interest may be used in the cage proteins of the disclosure as deemed appropriate for an intended use. As noted above, the cage protein may comprise 1, 2, 3, 4 or more target binding polypeptides, as exemplified herein. In one embodiment, the cage protein comprises 1 target binding polypeptide. In another embodiment, the cage protein comprises 2, 3, or 4 target binding polypeptides. In embodiments comprising 2 or more target binding polypeptides, each target binding polypeptide may be the same or may be different.

Similarly, the target of the one or more target binding polypeptides may be any target as suitable for an intended purpose for which one or more target binding polypeptides are available. In one non-limiting embodiment, the one or more target binding polypeptide is capable of binding to a target including but not limited to an antibody, a toxin, a diagnostic biomarker, a viral particle, a disease biomarker, a metabolite or a biochemical analyte of interest. In embodiments where there are 2 or more target binding polypeptides, each target binding polypeptide may bind the same target, or may independently bind to different targets. In embodiments where the 2 or more target binding polypeptides bind to the same target, they may bind to the same region of the target (for example, to add avidity to the interaction), or may bind to different regions of the target.

As will be understood by those of skill in the art, the one or more target binding polypeptides may comprise any type of polypeptide, including but not limited to de novo designed proteins, affibodies, affimers, ankyrin repeat proteins (naturally occurring or designed), nanobodies, etc.

In one embodiment, the one or more target binding polypeptide is capable of binding to an antibody target. In another embodiment, the one or more target binding polypeptide comprises one or more epitope recognized by antibodies against a viral target. In a further embodiment, the one or more target binding polypeptide comprises one or more epitope recognized by antibodies against SARS-Cov-2. In various other embodiments described herein, the one or more target binding polypeptide is capable of binding to a disease marker or toxin, Bcl-2, Her2 receptor, Botulinum neurotoxin B, cardiac Troponin I, albumin, epithelial growth factor receptor, prostate-specific membrane antigen (PSMA), citrullinated peptides, brain natriuretic peptides, or any other suitable target.

In various non-limiting embodiments, the one or more target bi comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:27380-27430.

5

Table 1. Exemplary target binding polypeptides

Biosensors	Sensing domain	Sensing domain sequence
lucCageBim	Bim	EIWIAQELRRIGDEFNAYYAAA (SEQ ID NO:27380)
lucCageBoT	Bot.0671.2	MFAELKAKFFLEIGDRDAARNALRKAGYSDEEAER IIRKYELE (SEQ ID NO:27381)
lucCageProA	Protein A domain C (SpaC)	EQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSK EILAEAKKLNDAAQAPK (SEQ ID NO:27382)
lucCagHer2	Her2 affibody	EMRNAYWEIALLPNLNNQKRAFIRSLYDDPSQSA NLLAEAKKLNDAAQAPK (SEQ ID NO:27383)
lucCageTrop	cTnI + cTnC	EDQLREKAKELWQTIYNLEAEKFDLQEKFKQQKYE INVLRNRINDNQKVSCTKDDSKGKSEELSDLFRM FDKNADGYIDLEELKIMLQATGETITEDDIEELMK DGDKNNDGRIDYDEFLEFMKGVE (SEQ ID NO:27384) - cTnTf1:226- EDQLREKAKELWQTI-240 (SEQ ID NO:27385) - cTnTf2:226- EDQLREKAKELWQTIYN-242 (SEQ ID NO:27386) - cTnTf3:226- EDQLREKAKELWQTIYNLEAE-246 (SEQ ID NO:27387) - cTnTf4:226- EDQLREKAKELWQTIYNLEAEKFD-249 (SEQ ID NO:27388) - cTnTf5:226- EDQLREKAKELWQTIYNLEAEKFDLQ E-252 (SEQ ID NO:27389) - cTnTf6:226- EDQLREKAKELWQTIYNLEAEKFDLQ EKFKQQKYEINVLRNRINDNQ-272 (SEQ ID NO:27390) - EDQLREAAKELWQTIYNLEAEKFDLQEKFKQQ KYEINVLRNRINDNQKVSCTKDDSKGKSEEL SDFRMDKNADGYIDLEELKIMLQATGETIT EDDIEELMKDGDKNNDGRIDYDEFLEFMKGVE (SEQ ID NO:27391)
lucCageSARS2-M	SARS-CoV-2 nucleocapsid protein (a.a. 369-382) 2x	MADSNGTITVEELKKLLEGGSGGMADSNGTITVEE LKKLLE (SEQ ID NO:27392) - MADSNGTITVEELKKLLEQWNLV IGFLFLTWIGGSGGMADSNGTIT VEELKKLLEQWNLVIGFLFTWI (SEQ ID NO:27393) - ITVEELKKLLEQWNLVIGGSGGI TVEELKKLLEQWNLVI (SEQ ID NO:27394)

lucCageSARS2-N	SARS-CoV-2 membrane protein (a.a. 1-17) 2x	N62:KKDKKKKADETQALGGSGGKKDKKKKADETQ AL (SEQ ID NO:27548 N6:PKDKKKKADETQALPQRQKKGGSGGPKDKK KKADETQALPQRQKK (SEQ ID NO:27547)
sCageHA	HB1.9549.2	TFACRIAARIAAEFGYSEEQIKELLKNAGCSEDEA RDAVEYLR (SEQ ID NO:27396)

>LCB1-1

DKEWILQKIYEIMRLLDDELGHAEASMRVSDLIYEFMKKGDERLLEEAERLLEEVEER (SEQ ID NO:27397)

>LCB1-2

5 DKEEILNKIYEIMRLLDDELGNAEASMRVSDLIILEFMKKGDERLLEEAERLLEEVEER (SEQ ID NO: 27398)

>LCB1-3

DKEWILQKIYEIMRLLDDELGHAEASMRVSDLIYEFMKQGDERLLEEAERLLEEVEER (SEQ ID NO: 27399)

>LCB1-4

10 DKENILQKIYEIMKTLTDQLGHAEASMQVSDLIYEFMKQGDERLLEEAERLLEEVEER (SEQ ID NO: 27400)

>LCB1-5

DKENILQKIYEIMKTLTDQLGHAEASMNVSVDLIYEFMKQGDERLLEEAERLLEEVEER (SEQ ID NO: 27401)

LCB1_v1.1_Cys

DKENILQKIYEIMKTLTDQLGHAEASMQVSDLIYEFMKQGDERLLEEAERLLEEVEERC (SEQ ID NO: 27402)

15 >LCB1_v1.2

DKENILQKIYEIMKTLTDQLGHAEASMYVSDLIYEFMKQGDERLLEEAERLLEEVEER (SEQ ID NO: 27403)

>LCB1_v1.3

DKENILQKIYEIMKTLEQLGHAEASMQVSDLIYEFMKQGDERLLEEAERLLEEVEER (SEQ ID NO: 27404)

>LCB1_v1.4

20 DKENILQKIYEIMKTLEQLGHAEASMQVSDLIYEFMKQGDENLLEEAQEQLLEVEER (SEQ ID NO: 27405)

>LCB1_v1.5 (LCB1_v1.3 with N-link Glycosylation)

DKENILQKIYEIMKTLEQLGHAEASMNVSVDLIYEFMKQGDERLLEEAERLLEEVEER (SEQ ID NO: 27406)

>LCB2-1

SDDEDSVRYLlyMAELRYEQGNPEKAKKILEMAEFIAKRNNNEELERLVREVKKRL (SEQ ID NO: 27407)

25 >LCB2-2

SDDEDAVRYLlyMAELLYKQGNPEEAKKLELAEFIAKRNNNEELERLVREVKKRL (SEQ ID NO: 27408)

>LCB3-1

NDEELHMLMTDLVYEALHFAKDEEIKKRVFTLFELADKAYKNNDRQKLEKVVEELKELLERLLS (SEQ ID
NO: 27409)

30 >LCB3-2

NDEELMLVTDLVAEALLFAKDEEIKKRVFTLFELADKAYKNNDRTLSKVSELKELLERLQ (SEQ ID NO:
27410)

>LCB3_v1.2

NDDELHMQMTDLVYEALHFAKDEEIQKHVFQOLFEEKATKAYKNKDRQKLEKVVEELKI
NO: 27411)

>LCB3-4

NDDELHMQMTDLVYEALHFAKDEEIQKHVFQOLFENATKAYKNKDRQKLEKVVEELKELLERLLS (SEQ ID
NO: 27412)

>LCB3_v1.1

NDDELHMQMTDLVYEALHFAKDEEFQKHVFQOLFEEKATKAYKNNDKDRQKLEKVVEELKELLERLLS (SEQ ID
NO: 27413)

>LCB3_v1.3

NDDELHMQMTDLVYEALHFAKDEEFQKHVFQOLFEEKATKAYKNKDRQKLEKVVEELKELLERLLS (SEQ ID
NO: 27414)

>LCB3_v1.4

NDDELHMQMTDLVYEALHFAKDEEFQKHVFQOLFEEKATKARKNKDRQKLEKVVEELKELLERLLS (SEQ ID
NO: 27415)

>LCB3_v1.5

NDDELHMQMTDLVYEALHFAKDEEMQKRVFQLFQADKAYKTKDRQKLEKVVEELKELLERLLS (SEQ ID
NO: 27416)

>LCB4-1

QREKRLKQLEMLLEYAIERNDP~~YLMFDVAVEMLR~~LAEENNDERIIERAKRILEEYE (SEQ ID NO: 27417)

>LCB4-2

DREERLKYLEMLLELAVERNDPYLIFDVAIELLRLAEENNDERIYERAKRILEEVE (SEQ ID NO: 27418)

>LCB5-1

SLEELKEQVKELKKELSPEMRRLIEEALRFLEE~~GNPAMAMMVLSDLVYQ~~LGDP~~PRVIDLYMLV~~TKT (SEQ ID
NO: 27419)

>LCB5-2

SLEEVKEILKELKKELSPEDRRLIEEALRLLEE~~GNPAMAS~~MLSDLVFLLGDPRVIELMLVTKT (SEQ ID
NO: 27420)

>LCB6-1

DRE~~QRLV~~RFLVRLASKFNLS~~PEQILQLF~~EVLEELLERGVSEEEIRKQLEEVAKELG (SEQ ID NO: 27421)

>LCB6-2

DRE~~QRLV~~RFLVRLASKFNLSMEQILILFDVLEELLERGVSEEEIRKILEEVAKEL (SEQ ID NO: 27422)

>LCB7-1

DDDIRYLIYMAKLRL~~EQ~~GNPEEA~~AEKVLEMA~~RFLAERLGMEELLKEVRELLRKIEELR (SEQ ID NO:
27423)

>LCB7-2

DDDVRYLIYMAKLILLEQGNPEEA~~AEKVLESARFAA~~ELLGNEELLKEVRELLRKIEELR (SEQ ID NO:
27424)

>LCB8-1

PIIELLREAKEKN~~DEF~~AI~~SDALYLVNELLQ~~RTGDPRL~~EEVLYLIW~~RALKEKDPRLLDRAIELFER (SEQ ID
NO: 27425)

>LCB8-2

PVTELLREAKEKNDPMAISDALFLVFELAQRTGDPRLLEEVLFLIWRALKEKDPRLLI

NO: 27426)

>AHB1-1

DEDLEELERLYRKAAEEVAKEAKDASRRGDDERAKEQMEERAMRLEFDQVFELAQELQEKQTDGNRQKATHLKD KAVKE

5 AADELYQ RVR

(SEQ ID NO: 27427)

>AHB1-2

DEDLEELERLYRKAAEEVAKEAEEASRRGDKERAKELLERLHFLDQVFELAQELQEKLTDEKRQKATHLKD KAVHE

AADELYQ RVR

10 (SEQ ID NO: 27428)

>AHB2-1

ELEERV MHLIDQVSELAHELLHKL TGEELQRATHFDKWANE AILELIKSDDEREIREIEEEEARRILEHLEELARK

(SEQ ID NO: 27429)

>AHB2-2_

15 ELEEQVMHVLIDQVSELAHELLHKL TGEELERAAYFNWWATEM MLELIKSDDEREIREIEEEEARRILEHLEELARK

(SEQ ID NO: 27430)

The polypeptides of SEQ ID NOS: 27397-27430 bind with high affinity to the SARS-CoV-2 Spike glycoprotein receptor binding domain (RBD). The polypeptides of SEQ ID NOS: 27397-27430 have been subjected to extensive mutational analysis, permitting determination of allowable substitutions at each residue within the polypeptide. Allowable substitutions are as shown in **Table 3** (The number denotes the residue number, and the letters denote the single letter amino acids that can be present at that residue).

Thus, in one embodiment, the one or more target binding polypeptide comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 27397-27430, or selected from SEQ ID NOS: 27397-27406, 27409-27416, 27427-27430. In another embodiment, amino acid substitutions relative to the reference target binding polypeptide amino acid sequence (i.e.: one of SEQ ID NOS: 27397-27430) are selected from the allowable amino acid substitutions provided in Table 1.

The residue numbers of the interface residues which are within 8Å to the RBD target are listed below in **Table 2**.

Table 2

'LCB1': [3, 6, 7, 10, 13, 17, 20, 22, 23, 25, 26, 29, 32, 33, 36],

'LCB2': [1, 2, 5, 6, 9, 12, 13, 16, 20, 32, 35, 39],

'LCB3': [1, 3, 4, 6, 7, 10, 11, 13, 14, 15, 18, 27, 30, 33, 34, 37],

'LCB4': [8, 11, 12, 15, 23, 24, 26, 27, 28, 30, 31, 34, 56],

'LCB5': [35, 37, 38, 40, 41, 44, 47, 48, 53, 56, 60, 63],

'LCB6': [3, 4, 7, 8, 11, 12, 14, 15, 21, 24, 25, 28, 31, 32, 35],

5 'LCB7': [2, 3, 6, 7, 9, 10, 13, 17, 29, 32, 33, 36],

'LCB8': [14, 15, 16, 19, 22, 23, 26, 29, 30, 38, 41, 42, 45],

'AHB1', [34, 38, 41, 45, 48, 49, 52, 63, 64, 67, 68, 70, 71, 74, 78, 81, 82, 85],

'AHB2', [4, 7, 11, 14, 15, 18, 21, 26, 29, 30, 33, 34, 36, 37, 40, 43, 44, 47, 48].

10 In another embodiment, interface residues are identical to those in the reference target binding polypeptide (i.e.: one of SEQ ID NOS:27397-27430 or are conservatively substituted relative to interface residues in the reference target binding polypeptide as detailed in **Table 2**).

Table 3

15	LCB1 (SEQ ID NOS: 27397-27406)
	1 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
	2 -- A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
	3 -- A, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, Y
	4 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
20	5 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
	6 -- A, C, I, L, M, Q, T, V
	7 -- A, C, D, E, F, G, H, M, N, P, Q, R, S, V, W, Y
	8 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
	9 -- C, I, L, M, N, Q, T, V
25	10 -- C, F, V, W, Y
	11 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
	12 -- A, C, D, H, I, L, M, N, S, T, V, Y
	13 -- C, I, M, Q
	14 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
30	15 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
	16 -- C, F, I, L, M, T, V
	17 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
	18 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
	19 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
35	20 -- A, C, D, E, F, G, H, K, L, M, N, Q, R, S, T, W
	21 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
	22 -- A, C, D, F, G, H, I, L, M, N, P, Q, S, T, V, W, Y
	23 -- C, E, M, N, P, Q, S, T, V

- 24 -- A,C,D,E,F,G,H,K,L,M,N,P,Q,R,S,T,V,W,Y
 25 -- A,C,G,M,N,Q,S,T,V
 26 -- M,N,V
 27 -- A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
 5 28 -- A,C,G,I,L,S,T,V
 29 -- A,C,S,V,W
 30 -- D
 31 -- A,C,D,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
 32 -- C,F,H,I,L,M,N,P,T,V
 10 33 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 34 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 35 -- A,C,D,F,H,M,Q,V,W,Y
 36 -- A,C,D,E,G,H,I,L,M,N,Q,R,S,T,V,W,Y
 37 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 15 38 -- A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
 39 -- A,C,D,E,F,G,H,K,L,M,N,P,Q,R,S,T,V,W,Y
 40 -- D,E,G,H,N,P,Q
 41 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 42 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 20 43 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 44 -- A,C,D,E,F,G,H,I,K,L,M,Q,R,S,V,W,Y
 45 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 46 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 47 -- A,C,G,P,S,T,V
 25 48 -- A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
 49 -- A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
 50 -- A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
 51 -- A,C,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
 52 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 30 53 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 54 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 55 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 56 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y

 35 LCB2 (SEQ ID NOS: 27407- 27408)
 1 -- A,C,D,E,G,N,P,S,T
 2 -- D,M,P,Q,Y
 3 -- A,D,E,N,Q
 4 -- C,D,E,V
 40 5 -- D
 6 -- A,C,D,E,G,N,Q,S,T,V

- 7 -- A, C, G, I, L, M, P, S, T, V
- 8 -- A, C, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
- 9 -- D, N, Y
- 10 -- I, L, T
- 5 11 -- C, E, G, I, L, M, W
- 12 -- F, H, Y
- 13 -- E, M, Q, R, V
- 14 -- A, C, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
- 15 -- A, C, D, E, G, H, I, K, L, M, N, Q, R, S, T, V
- 10 16 -- C, H, L, T
- 17 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 18 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
- 19 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 20 -- A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, Y
- 15 21 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 22 -- A, C, D, E, G, I, K, L, N, P, Q, R, S, T, V
- 23 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 24 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 25 -- A, C, E, G, H, I, K, N, P, Q, R, S, T, Y
- 20 26 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 27 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 28 -- H, K, R, T, Y
- 29 -- C, D, E, H, I, K, L, M, N, P, Q, R, S, T, V, Y
- 30 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, Y
- 25 31 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, Y
- 32 -- F, H, I, K, L, M, P, Q, R, Y
- 33 -- A, C, G, P, S, T
- 34 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
- 35 -- F, H, Y
- 30 36 -- A, C, E, H, I, L, M, S, V
- 37 -- A, C, E, G, H, L, M, Q, R, S, T, V, W
- 38 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 39 -- A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V
- 40 -- A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 35 41 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 42 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 43 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 44 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 45 -- A, C, E, F, I, L, M, P, S, T, V, W, Y
- 40 46 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 47 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y

48 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
49 -- A,C,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
50 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
51 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
5 52 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
53 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
54 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
55 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
56 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
10
LCB3 (SEQ ID NOS: 27409- 27416)
1 -- C,E,F,I,M,N,T,W
2 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
3 -- D,G,L,M,N,S,Y
15 4 -- A,C,E,F,H,K,Q,T
5 -- A,C,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
6 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
7 -- A,C,D,F,I,L,M,P,R,S,V,W
8 -- A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
20 9 -- A,C,E,F,G,H,I,L,M,N,Q,R,S,T,V,Y
10 -- A,C,F,G,H,K,M,N,Q,R,S,T,Y
11 -- D,F,H,L,M,N,Q
12 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
13 -- A,F,I,L,M,N,Q,S,T,V
25 14 -- A,C,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
15 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
16 -- A,C,D,E,F,G,H,I,L,M,N,P,Q,R,S,T,V,W,Y
17 -- A,C,D,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W
18 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
30 19 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
20 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
21 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
22 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
23 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
35 24 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
25 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
26 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
27 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
28 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
40 29 -- A,C,D,E,F,G,I,L,M,N,P,S,T,V,W,Y
30 -- C,E,F,H,L,N,S,W,Y

31 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 32 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 33 -- A,C,E,F,I,K,P,Q,S,V,W,Y
 34 -- A,D,E,F,G,H,M,N,P,Q,R,S,V,W,Y
 5 35 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 36 -- A,C,E,G,H,I,M,N,Q,S,T,V
 37 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 38 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 39 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 10 40 -- A,C,D,E,F,G,H,K,L,M,N,P,Q,R,S,T,V,W,Y
 41 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 42 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 43 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 44 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 15 45 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 46 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 47 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 48 -- A,C,E,F,G,I,K,L,M,N,P,Q,S,T,V,W
 49 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 20 50 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 51 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 52 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 53 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 54 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 25 55 -- A,C,E,F,G,H,I,K,L,M,N,Q,S,T,V,W,Y
 56 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 57 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 58 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 59 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 30 60 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 61 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 62 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 63 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 64 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 35
 LCB4 (SEQ ID NO: 27417- 27418)
 1 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 2 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 3 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 40 4 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 5 -- C,D,H,K,N,Q,R,Y

- 6 -- A, C, F, G, I, K, L, M, P, Q, R, S, T, V, Y
- 7 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 8 -- A, C, H, I, M, N, Q, R, S, T, V, Y
- 9 -- A, C, D, G, H, I, K, L, M, N, Q, R, S, T, V, Y
- 5 10 -- A, C, D, E, M, N, P, Q, S, T, V
- 11 -- C, D, G, H, I, K, L, M, N, P, R, S, T, V
- 12 -- F, G, I, L
- 13 -- F, I, L, M, S, V, Y
- 14 -- A, C, D, E, G, L, M, N, Q, R, S, T, V
- 10 15 -- C, E, F, G, H, I, L, M, S, V, W, Y
- 16 -- A, G, T, Y
- 17 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
- 18 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
- 19 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
- 15 20 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 21 -- C, D, Q, Y
- 22 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 23 -- E, F, H, Y
- 24 -- A, F, G, I, L, M, W
- 20 25 -- A, C, E, G, H, I, K, L, M, N, Q, R, S, T, V, Y
- 26 -- C, F, H, I, L, N, S, T, V, W
- 27 -- D, Q, W, Y
- 28 -- A, C, D, I, L, V, Y
- 29 -- A, C, E, G, K, L, N, Q, R, S, T
- 25 30 -- C, I, L, M, P, T, V
- 31 -- C, D, E
- 32 -- A, C, E, I, L, M, Q, S, T, V, Y
- 33 -- A, C, E, F, G, H, I, K, L, M, Q, R, S, T, V, Y
- 34 -- C, D, F, G, H, L, M, N, P, R, S, T, W, Y
- 30 35 -- A, C, E, F, G, H, I, K, L, N, P, R, T, V, W
- 36 -- A, C, G, S, T, V
- 37 -- A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, Y
- 38 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 39 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 35 40 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, Y
- 41 -- A, C, D, E, G, H, K, N, Q, S, W
- 42 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 43 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, Y
- 44 -- A, E, F, G, H, I, K, L, M, N, Q, R, S, T, V
- 40 45 -- A, C, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
- 46 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y

47 -- A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
48 -- A,C,M,S,T,V
49 -- A,H,I,K,L,M,N,Q,R,S,T,V,W,Y
50 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
5 51 -- A,F,I,K,L,M,R,T,V,W,Y
52 -- F,I,K,L,M,V
53 -- A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
54 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
55 -- A,C,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
10 56 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y

LCB5 (SEQ ID NO: 27419- 27420)

1 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
2 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
15 3 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
4 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
5 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
6 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
7 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
20 8 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
9 -- A,C,E,F,G,H,I,L,M,N,Q,S,T,V,W,Y
10 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
11 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
12 -- A,C,D,E,F,G,H,I,L,M,N,P,Q,R,S,T,V,W,Y
25 13 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
14 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
15 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
16 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
17 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
30 18 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
19 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
20 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
21 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
22 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
35 23 -- A,C,D,E,F,G,H,I,L,M,N,P,Q,R,S,T,W,Y
24 -- A,C,D,E,F,G,H,I,L,M,N,P,Q,S,T,V,W,Y
25 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
26 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
27 -- A,C,G,H,I,S,T,V
40 28 -- A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
29 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y

- 30 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 31 -- A, C, E, F, H, I, K, L, M, N, Q, S, T, V, W, Y
 32 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 33 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 5 34 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 35 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
 36 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 37 -- A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, Y
 38 -- A, C, D, E, G, I, L, M, N, P, Q, S, T, V, W
 10 39 -- A, C, F, G, L, M, N, S, T, V, W
 40 -- A, C, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, Y
 41 -- C, H, I, L, M, P, R
 42 -- A, C, E, G, H, I, L, M, P, T, V, Y
 43 -- C, I, L, M, Q, T, V
 15 44 -- A, C, D, F, G, H, I, M, S, T
 45 -- D, Y
 46 -- A, C, D, F, I, L, R, V
 47 -- C, E, G, I, V
 48 -- F, I, V, W, Y
 20 49 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
 50 -- A, C, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
 51 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 52 -- C, D, E, H, I, K, N, P, Q, R, S, T, Y
 53 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 25 54 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 55 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 56 -- F, I, L, M, T, V, W
 57 -- A, C, D, E, F, G, H, N, P, Q, R, S, T, W, Y
 58 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 30 59 -- A, C, F, I, L, M, T, V, Y
 60 -- C, F, M, N, V, Y
 61 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
 62 -- A, C, F, G, I, L, M, S, T, V, W
 63 -- A, C, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
 35 64 -- A, C, E, F, G, H, K, L, N, P, R, S, T, W, Y
 65 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y

LCB6 (SEQ ID NO: 27421- 27422)

- 1 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 40 2 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 3 -- E, W

- 4 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 5 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 6 -- F, L, M, R, S
- 7 -- H, T, V
- 5 8 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 9 -- F, M
- 10 -- A, K, L, W
- 11 -- D, E, G, V, Y
- 12 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 10 13 -- E, L
- 14 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 15 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 16 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 17 -- F, N, P, S
- 15 18 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 19 -- L, N, Q, V
- 20 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 21 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 22 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 20 23 -- C, D, P, Q, R, W
- 24 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 25 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 26 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 27 -- D, H, L, S, W
- 25 28 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 29 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 30 -- L, Q, V, W
- 31 -- I, K, L, S
- 32 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 30 33 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 34 -- A, F, L, T, V
- 35 -- C, D, G, H, K, L, N, T
- 36 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 37 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 35 38 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 39 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 40 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 41 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 42 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 40 43 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
- 44 -- F, I

45 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
46 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
47 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
48 -- L,Q,R,T
5 49 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
50 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
51 -- C,V,Y
52 -- A,E,H,K
53 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
10 54 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
55 -- C,F,H,L,P,W,Y
56 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y

LCB7 (SEQ ID NO: 27423- 27424)
15 1 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
2 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
3 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
4 -- I,T,V
5 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
20 6 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
7 -- L,P,Y
8 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
9 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
10 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
25 11 -- A
12 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
13 -- A,L,P
14 -- H,L,R,T,Y
15 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
30 16 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
17 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
18 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
19 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
20 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
35 21 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
22 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
23 -- A,S
24 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
25 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
40 26 -- C,G,S,V,Y
27 -- K,L,M,W

- 28 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 29 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 30 -- A, Y
 31 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 5 32 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 33 -- A, C, F, I, K, L, V, W
 34 -- A, H, L
 35 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 36 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 10 37 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 38 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 39 -- A, C, K, L, M, N
 40 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 41 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 15 42 -- A, C, D, L, V
 43 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 44 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 45 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 46 -- Q, S, V
 20 47 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 48 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 49 -- E, L
 50 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 51 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 25 52 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 53 -- I
 54 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 55 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 56 -- L, M, N, R
 30 57 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y

LCB8 (SEQ ID NO: 27425- 27426)

- 1 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 2 -- C, F, I, L, M, S, V, W, Y
 35 3 -- A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
 4 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
 5 -- A, C, F, G, I, K, L, M, Q, S, T, V, W, Y
 6 -- H, I, K, L, M
 7 -- A, H, I, K, L, M, N, P, Q, R, W, Y
 40 8 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
 9 -- A, C, F, G, I, L, M, S, Y

- 10 -- A, F, H, K, L, M, Q, R, S
- 11 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
- 12 -- A, C, D, E, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y
- 13 -- A, C, D, E, F, G, H, M, N, Q, S, W, Y
- 5 14 -- C, D, E, H, N, Q, S
- 15 -- A, D, E, F, H, I, L, M, N, P, Q, S, T, V, W, Y
- 16 -- C, F, M, N, R, Y
- 17 -- A, C, I, L, M, Q, R, V
- 18 -- A, C, F, H, I, L, M, T, V, Y
- 10 19 -- I, Q, S
- 20 -- D, N
- 21 -- A, C, G, S, V
- 22 -- A, C, I, L, M, V
- 23 -- C, F, R, T, W, Y
- 15 24 -- A, C, D, E, F, G, H, I, L, M, N, Q, R, S, T, V, W, Y
- 25 -- C, E, S, T, V, Y
- 26 -- A, C, D, E, F, G, H, N, Q, S, T
- 27 -- A, C, D, E, G, H, I, K, L, M, N, Q, R, S, T, V
- 28 -- C, E, F, G, H, I, K, L, M, Q, R, W, Y
- 20 29 -- A, C, F, G, H, I, K, L, M, N, Q, R, S, T, V, Y
- 30 -- A, C, E, G, H, K, M, N, P, Q, R, T
- 31 -- A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, Y
- 32 -- A, C, D, E, G, H, I, K, N, Q, R, S, T, W
- 33 -- A, C, E, G, H, K, M, N, P, Q, R, S, W, Y
- 25 34 -- C, D, E, F, H, M, N, W, Y
- 35 -- A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, Y
- 36 -- A, C, D, E, F, G, H, K, L, M, N, Q, R, S, T, V, W, Y
- 37 -- F, G, H, I, L, M, S, T, Y
- 38 -- D, E, H, Q, W, Y
- 30 39 -- C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, Y
- 40 -- A, C, E, G, H, I, K, M, P, V, Y
- 41 -- C, F, H, I, K, L, M, R, S, T, V
- 42 -- E, F, I, T, W, Y
- 43 -- A, C, D, E, F, H, I, L, M, N, Q, R, S, T, V, W, Y
- 35 44 -- C, G, I, K, L, M, T, V, Y
- 45 -- G, S, W, Y
- 46 -- C, I, K, L, M, N, Q, R, S, T
- 47 -- A, C, E, N, Q, S, T, V
- 48 -- C, D, E, F, H, I, L, M, W
- 40 49 -- C, D, F, H, K, L, M, N, Q, R, T
- 50 -- A, C, D, E, N, Y

- 51 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
52 -- A,C,D,E,G,H,K,L,M,N,Q,R,S,T
53 -- A,C,D,E,F,G,H,I,L,M,N,P,Q,S,T,V,W,Y
54 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
5 55 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,S,T,V,W,Y
56 -- C,I,L,M
57 -- A,C,D,E,G,I,N,Q,S,T
58 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
59 -- A,C,G,P,S
10 60 -- A,C,E,F,G,I,L,M,N,Q,S,T,V
61 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
62 -- A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
63 -- A,C,E,F,G,H,I,L,M,N,Q,S,T,V,W,Y
64 -- A,C,D,E,G,H,I,K,L,M,N,P,Q,S,T,V
15 65 -- A,C,D,E,G,H,I,K,L,M,N,P,Q,R,S,T,W,Y
- AHB1 (SEQ ID NOS: 27427- 27428)
- 20 1 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
2 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
3 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
4 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
5 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
6 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
25 7 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
8 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
9 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
10 -- A,C,F,H,I,K,L,M,N,Q,R,S,T,V,W,Y
11 -- F,N,Y
30 12 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
13 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
14 -- A,D,G
15 -- A,C,D,E,G,H,I,K,L,M,N,Q,R,S,T,V
16 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
35 17 -- A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
18 -- A,C,D,E,F,G,H,I,L,M,N,Q,S,T,V,W,Y
19 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
20 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
21 -- A,C,E,G,S,V
40 22 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
23 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y

24 -- A,C,D,E,F,H,K,L,M,N,Q,R,S,T,V,Y
25 -- A,C,D,F,G,H,L,M,N,Q,R,S,T,V,W,Y
26 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
27 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
5 28 -- A,C,D,E,F,G,H,K,L,M,N,P,Q,R,S,T,Y
29 -- A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
30 -- A,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
31 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
32 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
10 33 -- A,G,S
34 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
35 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
36 -- A,C,D,E,F,G,H,K,L,M,N,P,Q,R,S,T,V,W,Y
37 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
15 38 -- A,C,E,G,H,M,P,Q
39 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
40 -- A,C,D,E,G,K,N,Q,R,S,T
41 -- A,C,D,E,F,G,H,I,L,M,N,P,Q,S,T,V,W,Y
42 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
20 43 -- A,C,D,E,F,G,H,I,K,L,M,N,Q,S,T,V,W,Y
44 -- E,F,H,Q,S,W,Y
45 -- D,N
46 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
47 -- C,T,V
25 48 -- F,S,W,Y
49 -- A,C,D,E,F,G,H,I,K,L,M,N,Q,R,S,T,V,W,Y
50 -- A,C,F,H,I,K,L,M,N,Q,R,S,T,V,W,Y
51 -- A,D,G,H,N,S
52 -- H,K,Q,R
30 53 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
54 -- A,C,H,I,K,L,M,N,P,Q,R,S,T,V
55 -- A,C,E,G,H,K,N,Q,R,S,T
56 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
57 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
35 58 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
59 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
60 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
61 -- A,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
62 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
40 63 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
64 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y

65 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 66 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 67 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 68 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 5 69 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 70 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 71 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 72 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 73 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 10 74 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 75 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 76 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 77 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 78 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 15 79 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 80 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 81 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 82 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 83 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 20 84 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y
 85 -- A,C,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W,Y

AHB2 (SEQ ID NO: 27429- 27430)

25 1 -- C,G,A,V,F,Y,W,S,Q,D,E,R,K
 2 -- C,P,G,V,I,M,L,F,Y,W,S,N,Q,D,E,R,H
 3 -- C,G,A,V,I,F,S,T,D,E,K
 4 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
 5 -- C,P,G,A,V,M,L,Y,W,S,N,Q,D,E,R,K,H
 30 6 -- G,A,V,I,F,S,T,D,H
 7 -- C,P,G,V,I,M,L,F,W,S,T,N,Q,E,R,K,H
 8 -- C,P,G,A,V,M,L,Y,W,S,T,N,Q,D,E,R,K,H
 9 -- C,P,G,A,V,I,M,L,F,W,S,T,N,Q,D,E,R,K,H
 10 -- C,P,G,A,V,I,L,Y,W,S,T,N,E,R,K
 35 11 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,H
 12 -- C,P,G,A,V,I,L,F,Y,W,S,T,N,Q,D,E,R,K,H
 13 -- C,G,A,V,M,L,F,W,S,T,N,E,H
 14 -- C,P,G,A,V,I,Y,S,T,N,D,E,R,H
 15 -- C,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K
 40 16 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
 17 -- C,P,G,A,V,L,Y,W,S,T,Q,D,E,R

18 -- C,P,A,V,I,M,F,Y,N,Q,R,K,H
19 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
20 -- C,P,G,A,V,M,L,Y,W,N,Q,E,R,K,H
21 -- C,P,G,A,V,I,M,L,F,Y,W,S,N,Q,E,R,K,H
5 22 -- C,P,G,A,V,M,L,F,Y,S,T,N,Q,D,E,R,K,H
23 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,E,R,K
24 -- C,P,G,A,V,I,M,L,F,Y,W,S,Q,E,R,H
25 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,R,H
26 -- C,G,A,V,L,Y,S,N,D,R,K,H
10 27 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
28 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
29 -- C,P,G,V,I,M,L,F,Y,W,S,T,N,Q,D,R,K,H
30 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
31 -- C,G,A,V,I,M,L,F,Y,W,S,T,Q,D,E,R,K,H
15 32 -- P,G,A,V,I,L,W,S,T,D,R,H
33 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,E,R,K,H
34 -- C,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
35 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
36 -- C,P,G,A,V,I,L,F,Y,S,T,N,Q,D,E,R,H
20 37 -- C,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
38 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,Q,E,R,K
39 -- C,P,G,A,V,I,W,S,Q,E,R,H
40 -- C,P,G,A,V,I,L,Y,W,S,T,N,D,E,R,K,H
41 -- C,P,G,A,V,I,M,L,Y,W,S,T,N,Q,D,E,R,K,H
25 42 -- C,P,G,A,V,M,L,Y,W,S,T,N,Q,D,E,R,K,H
43 -- C,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
44 -- C,P,G,A,V,I,M,L,F,W,S,T,Q,D,E,R,H
45 -- C,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
46 -- C,P,G,A,V,I,M,L,F,S,T,Q,E,R,K
30 47 -- C,G,A,V,I,M,L,F,W,S,T,N,Q,D,E,R,H
48 -- C,P,G,A,V,I,M,L,F,Y,W,S,N,Q,E,R,K
49 -- C,P,G,A,V,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
50 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
51 -- C,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
35 52 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
53 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,D,E,R,K,H
54 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
55 -- C,P,G,A,V,I,M,L,F,Y,S,T,N,Q,D,E,R,K,H
56 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
40 57 -- C,P,G,A,V,I,M,L,F,Y,W,S,T,N,Q,D,E,R,K,H
58 -- C,G,A,V,I,M,L,F,Y,W,S,T,N,E,R,K,H

59 -- C, P, G, A, V, I, M, L, F, Y, W, S, T, N, Q, D, E, R, K, H
 60 -- C, G, A, V, I, M, L, F, Y, W, S, T, Q, D, E, R, K
 61 -- C, P, G, A, V, I, M, L, F, Y, W, S, N, Q, D, E, R, K, H
 62 -- C, G, A, V, L, S, T, N, D, E, K, H
 5 63 -- C, P, G, A, V, I, L, F, Y, W, S, T, N, Q, D, E, R, K, H
 64 -- C, P, G, A, V, I, M, L, F, Y, W, S, T, N, Q, D, E, R, H
 65 -- C, G, A, V, I, M, L, F, Y, S, T, N, R, K, H
 66 -- C, P, G, A, V, I, M, L, W, T, Q, E, R
 67 -- C, P, G, A, V, I, M, L, F, Y, W, S, T, N, Q, D, E, R, K, H
 10 68 -- C, P, G, A, V, I, L, F, Y, W, S, T, N, Q, D, E, R, H
 69 -- P, G, V, I, M, L, Y, W, S, T, Q, R, K
 70 -- C, P, G, A, V, I, M, L, F, Y, W, S, T, N, Q, D, E, R, K, H
 71 -- C, G, A, V, L, F, W, S, Q, D, E, R, K
 72 -- C, V, I, L, S
 15 73 -- P, G, A, V, S, T, E
 74 -- C, A, L, F, Y, S, T, R, H
 75 -- C, P, G, V, I, L, F, W, S, N, D, E, R, K

20 In one embodiment, the one or more target binding polypeptide comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:27397-27406 and 27431-27466.

Table 4: Exemplary LCB1 variants

Name	Binder Protein
LCB1_4N	DKENILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKKGDERLLEEAERLLEEVEE (SEQ ID NO:27431)
LCB1_4K	DKEKILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKKGDERLLEEAERLLEEVEE (SEQ ID NO:27432)
LCB1_14K	DKEWILQKIYEIMKLLDELGHAEASMRVSDLIYEFMKKGDERLLEEAERLLEEVEE (SEQ ID NO:27433)
LCB1_15T	DKEWILQKIYEIMRTLDELGHAEASMRVSDLIYEFMKKGDERLLEEAERLLEEVEE (SEQ ID NO:27435)
LCB1_18Q	DKEWILQKIYEIMRLLDQLGHAEASMRVSDLIYEFMKKGDERLLEEAERLLEEVEE (SEQ ID NO:27436)
LCB1_18K	DKEWILQKIYEIMRLLDKLGHAASMRVSDLIYEFMKKGDERLLEEAERLLEEVEE (SEQ ID NO: 27437)
LCB1_27Q	DKEWILQKIYEIMRLLDELGHAEASMQVSDLIYEFMKKGDERLLEEAERLLEEVEE (SEQ ID NO: 27438)
LCB1_27Y	DKEWILQKIYEIMRLLDELGHAEASMYVSDLIYEFMKKGDERLLEEAERLLEEVEE (SEQ ID NO: 27439)
LCB1_17E	DKEWILQKIYEIMRLLLEELGHAEASMRVSDLIYEFMKKGDERLLEEAERLLEEVEE (SEQ ID NO: 27440)
LCB1_17R	DKEWILQKIYEIMRLLRELGHAEASMRVSDLIYEFMKKGDERLLEEAERLLEEVEE (SEQ ID NO: 27441)

LCB1_42N	DKEWILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKGDE (SEQ ID NO: 27442)
LCB1_49Q	DKEWILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKGDERLLEEAEQLLEEVER (SEQ ID NO: 27443)
LCB1_52Q	DKEWILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKGDERLLEEAEERLLQEVER (SEQ ID NO: 27444)
LCB1_32L	DKEWILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKGDERLLEEAEERLLEEVER (SEQ ID NO: 27445)
LCB1_28A	DKEWILQKIYEIMRLLDELGHAEASMRASDLIYEFMKGDERLLEEAEERLLEEVER (SEQ ID NO: 27446)
LCB1_v1.3_ACH1	DKENILQKIYEIMKTLEQLGHAEASMYVSDLIYEFMKGQDERLLEEAEERLLEEVER (SEQ ID NO: 27447)
LCB1_v1.3_ACH2	DKENILQKIYEIMKTLEQLGHAEASMQVSDLIYEFMKGQDENLLEEAEERLLEEVER (SEQ ID NO: 27448)
LCB1_v1.3_ACH3	DKENILQKIYEIMKTLEQLGHAEASMQVSDLIYEFMKGQDERLLEEAEQLLEEVER (SEQ ID NO: 27449)
LCB1_v1.3_ACH4	DKENILQKIYEIMKTLEQLGHAEASMYVSDLIYEFMKGQDENLLEEAEQLLEEVER (SEQ ID NO: 27450)
LCB1_v1.3_ACH5	DKENILQKIYEIMKTLEQLGHAEASMQVSDLIYEFMKGQDENLLEEAEQLLEEVER (SEQ ID NO: 27451)
LCB1_v1.3_1	DRENILQKIYEIMKELEKLGHAESMQVSDLIYEFMQDKDERLLEEAEERLLEEVKR (SEQ ID NO: 27452)
LCB1_v1.3_2	DRENILQKIYEIMKELRQLGHAEASMQVSDLIYEFMKTDKRLLLEEAEERLLEEVKR (SEQ ID NO: 27453)
LCB1_v1.3_3	DRENILQKIYEIMKTLRRLGHAEASMQVSDLIYEFMQDKDKRLLLEEAEERLLEEVQR (SEQ ID NO: 27454)
LCB1_v1.3_4	DKENVLQKIYEIMKELERLGHAESMQVSDLIYEFMKTDERLLEEAEERLLEEVKR (SEQ ID NO: 27455)
LCB1_v1.3_5	DRENILQKIYEIMKTLEKLGHAESMQASDLIYEFMKTDERLLEEAEERLLEEVQR (SEQ ID NO: 27456)
LCB1_v1.3_6	DKENILQKIYEIMKTLRALGHAEASMQVSDLIYEFMQTKDERLLEEAEERLLEEVKR (SEQ ID NO: 27457)
LCB1_v1.3_7	DKENVLQKIYEIMKTLEKLGHAESMQVSDLIYEFMQTKDKRLLLEEAEERLLEEVQR (SEQ ID NO: 27458)
LCB1_v1.3_15	DRENILQKIYEIMKELEKLGHAESMQVSDLIYEFMQDKDENLLEEAEERLLEEVKR (SEQ ID NO: 27459)
LCB1_v1.3_16	DRENILQKIYEIMKELRQLGHAEASMQVSDLIYEFMKTDKNLLLEEAEERLLEEVKR (SEQ ID NO: 27460)
LCB1_v1.3_17	DRENILQKIYEIMKTLRRLGHAEASMQVSDLIYEFMQDKDKNLLLEEAEERLLEEVQR (SEQ ID NO: 27461)
LCB1_v1.3_19	DRENILQKIYEIMKTLEKLGHAESMQASDLIYEFMKTDENLLEEAEERLLEEVQR (SEQ ID NO: 27462)
LCB1_v1.3_20	DKENILQKIYEIMKTLRALGHAEASMQVSDLIYEFMQTKDENLLEEAEERLLEEVKR (SEQ ID NO: 27463)
LCB1_v1.3_21	DKENVLQKIYEIMKTLEKLGHAESMQVSDLIYEFMQTKDKNLLLEEAEERLLEEVQR (SEQ ID NO: 27464)
LCB1_v2.2	DKENVLQKIYEIMKELERLGHAESMQVSDLIYEFMKTDENLLEEAEERLLEEVKR (SEQ ID NO: 27465)
LCB1_v2.2_ompT	DKENVLQKIYEIMKELERLGHAESMQVSDLIYEFMKTDENLLEEAEERLLEEVTR (SEQ ID NO: 27466)

In another embodiment, the one or more target binding polypeptide comprises an amino acid substitution relative to the amino acid sequence of SEQ ID NO: 27397 at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or all 18 residues selected from the group consisting of 2, 4, 5, 14, 15, 17, 18, 27, 28, 32, 37, 38, 39, 41, 42, 49, 52, and 55. In a further

embodiment, the substitutions in the one or more target binding polypeptide the substitutions listed in Table 5, either individually or in combinations in a given row.

Table 5. Exemplary LCB1 mutations

Name	Parent	Mutations from WT										
LCB1_1	LCB1	W4N	R14 K	L15 T	E18 Q	R27 Q	K38 Q					
LCB1_2	LCB1	W4N	R14 K	L15 T	E18 Q	R27 Y	K38 Q					
LCB1_3	LCB1	W4N	R14 K	L15 T	D17 E	E18 Q	R27 Q	K38 Q				
LCB1_4	LCB1	W4N	R14 K	L15 T	D17 E	E18 Q	R27 Q	K38 Q	R42 N	R49 Q	E52 Q	
LCB1_4N	LCB1	W4N										
LCB1_4K	LCB1	W4K										
LCB1_14K	LCB1	R14K										
LCB1_15T	LCB1	L15T										
LCB1_18Q	LCB1	E18Q										
LCB1_18K	LCB1	E18K										
LCB1_27Q	LCB1	R27Q										
LCB1_27Y	LCB1	R27Y										
LCB1_38Q	LCB1	K38Q										
LCB1_17E	LCB1	D17E										
LCB1_17R	LCB1	D17R										
LCB1_42N	LCB1	R42N										
LCB1_49Q	LCB1	R49Q										
LCB1_52Q	LCB1	E52Q										
LCB1_32L	LCB1	I32L										
LCB1_28A	LCB1	V28A										
LCB1_v1.3	LCB1	W4N	R14 K	L15 T	D17 E	E18 Q	R27 Q	K38 Q				
LCB1_v1.3_ACH 1	LCB1_v1.3	W4N	R14 K	L15 T	D17 E	E18 Q	R27 Y	K38 Q				
LCB1_v1.3_ACH 2	LCB1_v1.3	W4N	R14 K	L15 T	D17 E	E18 Q	R27 Q	K38 Q	R42 N			
LCB1_v1.3_ACH 3	LCB1_v1.3	W4N	R14 K	L15 T	D17 E	E18 Q	R27 Q	K38 Q	R49 Q			
LCB1_v1.3_ACH 4	LCB1_v1.3	W4N	R14 K	L15 T	D17 E	E18 Q	R27 Y	K38 Q	R42 N	R49 Q		
LCB1_v1.3_ACH 5	LCB1_v1.3	W4N	R14 K	L15 T	D17 E	E18 Q	R27 Q	K38 Q	R42 N	R49 Q		
LCB1_v1.3_1	LCB1_v1.3	K2R	W4N	R14 K	L15 E	D17 E	E18 K	R27 Q	K37 Q	K38 D	G39 K	E55 K
LCB1_v1.3_2	LCB1_v1.3	K2R	W4N	R14 K	L15 E	D17 R	E18 Q	R27 Q	K38 T	G39 K	E41 K	E55 K
LCB1_v1.3_3	LCB1_v1.3	K2R	W4N	R14 K	L15 T	D17 R	E18 R	R27 Q	K37 Q	K38 D	G39 K	E41 K
LCB1_v1.3_4	LCB1_v1.3	W4N	I5V	R14 K	L15 E	D17 E	E18 R	R27 Q	K38 T	G39 K	E55 K	
LCB1_v1.3_5	LCB1_v1.3	K2R	W4N	R14 K	L15 T	D17 E	E18 K	R27 Q	V28 A	K38 T	G39 K	E55 Q
LCB1_v1.3_6	LCB1_v1.3	W4N	R14 K	L15 T	D17 R	E18 A	R27 Q	K37 Q	K38 T	G39 K	E55 K	
LCB1_v1.3_7	LCB1_v1.3	W4N	I5V	R14 K	L15 T	D17 E	E18 K	R27 Q	K37 Q	K38 T	G39 K	E41 K
LCB1_v1.3_15	LCB1_v1.3	K2R	W4N	R14 K	L15 E	D17 E	E18 K	R27 Q	K37 Q	K38 D	G39 K	R42 N

LCB1_v1.3_16	LCB1_v1.3	K2R	W4N	R14 K	L15 E	D17 R	E18 Q	R27 Q				
LCB1_v1.3_17	LCB1_v1.3	K2R	W4N	R14 K	L15 T	D17 R	E18 R	R27 Q	Q	D	K	K
LCB1_v1.3_19	LCB1_v1.3	K2R	W4N	R14 K	L15 T	D17 E	E18 K	R27 Q	V28 A	K38 T	G39 K	R42 N
LCB1_v1.3_20	LCB1_v1.3	W4N	R14 K	L15 T	D17 R	E18 A	R27 Q	K37 Q	K38 T	G39 K	R42 N	E55 K
LCB1_v1.3_21	LCB1_v1.3	W4N	I5V	R14 K	L15 T	D17 E	E18 K	R27 Q	K37 Q	K38 T	G39 K	E41 K
LCB1_v2.2	LCB1_v1.3	W4N	I5V	R14 K	L15 E	D17 E	E18 R	R27 Q	K38 T	G39 K	R42 N	E55 K
LCB1_v2.2_omp T	LCB1_v1.3	W4N	I5V	R14 K	L15 E	D17 E	E18 R	R27 Q	K38 T	G39 K	R42 N	E55 T

In a further embodiment, the one or more target binding polypeptide comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:27409-27416 and 27467-27493.

Table 6. Exemplary LCB3 variants

Name	Binder Protein
LCB3_8Q	NDDELHMQMTDLVYEALHFAKDEEIKKRVFQLFELADKAYKNNDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27467)
LCB3_8T	NDDELHMTMTDLVYEALHFAKDEEIKKRVFQLFELADKAYKNNDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27468)
LCB3_19K	NDDELHMLMTDLVYEALHFAKDEEIKKRVFQLFELADKAYKNNDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27469)
LCB3_19I	NDDELHMLMTDLVYEALHIAKDEEIKKRVFQLFELADKAYKNNDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27470)
LCB3_25F	NDDELHMLMTDLVYEALHFAKDEEFKKRVFQLFELADKAYKNNDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27471)
LCB3_25M	NDDELHMLMTDLVYEALHFAKDEEMKKRVFQLFELADKAYKNNDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27472)
LCB3_26Q	NDDELHMLMTDLVYEALHFAKDEEIQKRVFQLFELADKAYKNNDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27473)
LCB3_28H	NDDELHMLMTDLVYEALHFAKDEEIKKHVFQLFELADKAYKNNDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27474)
LCB3_35K	NDDELHMLMTDLVYEALHFAKDEEIKKRVFQLFEKADKAYKNNDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27475)
LCB3_37T	NDDELHMLMTDLVYEALHFAKDEEIKKRVFQLFELATKAYKNNDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27476)
LCB3_40R	NDDELHMLMTDLVYEALHFAKDEEIKKRVFQLFELADKARKNNDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27477)
LCB3_43K	NDDELHMLMTDLVYEALHFAKDEEIKKRVFQLFELADKAYKNNDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27478)
LCB3_34G	NDDELHMLMTDLVYEALHFAKDEEIKKRVFQLFGLADKAYKNNDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27479)
LCB3_34Y	NDDELHMLMTDLVYEALHFAKDEEIKKRVFQLFYLDKAYKNNDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27480)
LCB3_34T	NDDELHMLMTDLVYEALHFAKDEEIKKRVFQLFTLADKAYKNNDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27481)
LCB3_49K	NDDELHMLMTDLVYEALHFAKDEEIKKRVFQLFELADKAYKNNDRQKLKKVVEELKELLE RLLS (SEQ ID NO: 27482)

LCB3_v1.2_ACH1	NDDELHMQMTDLVYEALHFAKDEEIQKHVFQLFGKATKAYKNKD RLLS (SEQ ID NO: 27483)
LCB3_v1.2_ACH2	NDDELHMQMTDLVYEALHFAKDEEIQKHVFQLFYKATKAYKNKDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27484)
LCB3_v2.2	NLDELHMQMTDLVYEALHFAKDEEFQKHVFQLFEKATKAYKNKDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27485)
LCB3_v1.3_2	NDDELHMQMTDLVYEALHFAKTEEFQKHVFQLFEKATKAYKNKDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27486)
LCB3_v1.3_3	NDDELHMQMTDLVYEALHFAKDEEFQKHVFQLFEKARKAYKNKDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27487)
LCB3_v1.3_4	NDDELHMQMTDLVWEALHFAKDEEFQKHVFQLFEKARKAYKNKDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27488)
LCB3_v1.3_5	NDDELHMQMTDLVWEALHFAKDEEFQKHVFQLFEKATKAYKNKDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27489)
LCB3_v1.3_6	NEDELHMQMTDLVWEALHFAKDEEFQKHVFQLFEKATKAYKNKDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27490)
LCB3_v1.3_7	NDDELHMQMTDLVWEALHFAKTEEFQKHVFQLFEKATKAYKNKDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27491)
LCB3_v1.3_15	NLDELHMQMTDLVYEALHFAKTEEFQKHVFQLFEKATKAYKNKDRQKLEKVVEELKELLE RLLS (SEQ ID NO: 27492)
LCB3_v2.3	NIDELLMQVTDLIYEALHFAKDEEFQKHAFQLFEKATKAYKNKDKQKLEKVVEELKELLE RILS (SEQ ID NO: 27493)

In one embodiment, the target binding comprises an amino acid substitution relative to the amino acid sequence of SEQ ID NO:27409 at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or all 20 residues selected from the group consisting 2, 6, 8, 9, 13, 14, 19, 22, 25, 26, 28, 29, 34, 35, 37, 40, 43, 45, 49, and 62. In another embodiment, the substitutions are selected from the substitutions listed in Table 7, either individually or in combinations in a given row.

Table 7. Exemplary LCB3 mutations

Name	Parent	Mutations from WT								
LCB3_1	LCB3	L8Q	I25F	K26Q	R28H	L35K	D37T			
LCB3_2	LCB3	L8Q	K26Q	R28H	L35K	D37T	N43K			
LCB3_3	LCB3	L8Q	I25F	K26Q	R28H	L35K	D37T	N43K		
LCB3_4	LCB3	L8Q	F19K	I25F	K26Q	R28H	L35K	D37T	Y40R	N43K
LCB3_8Q	LCB3	L8Q								
LCB3_8T	LCB3	L8T								
LCB3_19K	LCB3	F19K								
LCB3_19I	LCB3	F19I								
LCB3_25F	LCB3	I25F								
LCB3_25M	LCB3	I25M								
LCB3_26Q	LCB3	K26Q								
LCB3_28H	LCB3	R28H								

LCB3_35K	LCB3	L35K								
LCB3_37T	LCB3	D37T								
LCB3_40R	LCB3	Y40R								
LCB3_43K	LCB3	N43K								
LCB3_34G	LCB3	E34G								
LCB3_34Y	LCB3	E34Y								
LCB3_34T	LCB3	E34T								
LCB3_49K	LCB3	E49K								
LCB3_v1.2	LCB3	L8Q	K26Q	R28H	L35K	D37T	N43K			
LCB3_v1.2_ACH1	LCB3_v1.2	L8Q	K26Q	R28H	E34G	L35K	D37T	N43K		
LCB3_v1.2_ACH2	LCB3_v1.2	L8Q	K26Q	R28H	E34Y	L35K	D37T	N43K		
LCB3_v2.2	LCB3_v1.3	D2L	L8Q	I25F	K26Q	R28H	L35K	D37T	N43K	
LCB3_v1.3_2	LCB3_v1.3	L8Q	D22T	I25F	K26Q	R28H	L35K	D37T	N43K	
LCB3_v1.3_3	LCB3_v1.3	L8Q	I25F	K26Q	R28H	L35K	D37R	N43K		
LCB3_v1.3_4	LCB3_v1.3	L8Q	Y14W	I25F	K26Q	R28H	L35K	D37R	N43K	
LCB3_v1.3_5	LCB3_v1.3	L8Q	Y14W	I25F	K26Q	R28H	L35K	D37T	N43K	
LCB3_v1.3_6	LCB3_v1.3	D2E	L8Q	Y14W	I25F	K26Q	R28H	L35K	D37T	N43K
LCB3_v1.3_7	LCB3_v1.3	L8Q	Y14W	D22T	I25F	K26Q	R28H	L35K	D37T	N43K
LCB3_v1.3	LCB3_v1.2	L8Q	I25F	K26Q	R28H	L35K	D37T	N43K		
LCB3_v1.3_15	LCB3_v1.3	D2L	L8Q	D22T	I25F	K26Q	R28H	L35K	D37T	N43K
LCB3_v2.3	LCB1_v2.1	D2I	H6L	L8Q	M9V	V13I	I25F	K26Q	R28H	V29A, L35K, D37T, N43K, R45K, L62I

In one embodiment, the target binding comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 27427-27430 and 27494.

AHB2 ELEEQVMHVLQVSELAHELLHKLTGEEELERAAYFNWWATEMMLLELIKSDDEREIREIEEEAARILEH
v2 LEELART (SEQ ID NO: 27494)

In one such embodiment, the one or more target binding polypeptide comprises an amino acid substitution relative to the amino acid sequence of SEQ ID NO: 27430 at or both residues selected from the group consisting 63 and 75. In another embodiment, the substitutions comprise R63A and/or K75T.

In a further embodiment, the cage protein comprises the amino acid sequence that is 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of a cage polypeptide disclosed in US20200239524 (or WO2020/018935), not including optional amino acid residues and not including amino acid residues in the latch region. These cage protein amino acid sequences do not include the one or more target binding polypeptides or the first reporter protein domain (or the second reporter protein domain when present), which can thus be added to the cage proteins of this embodiment.

Exemplary such embodiment are SEQ ID NOS:1-49, 51-52, 54-59, 61, 65, 67-91, 92-2033, 2034-14317, 27094-27117, 27120-27125, 27,278 to 27,321, and cage polypeptides with an even-numbered SEQ ID NO between SEQ ID NOS: 27126 and 27276), Table 3 (Table 8 in the current application), and/or Table 4 (Table 9 in the current application) of a cage polypeptide disclosed in US20200239524, and reproduced herein and in the sequence listing.

In each embodiment, the N-terminal and/or C-terminal 60 amino acids of each cage protein may be optional, as the terminal 60 amino acid residues may comprise a latch region that can be modified, such as by replacing all or a portion of a latch with the one or more target binding polypeptide and the first reporter protein domain. In one embodiment, the N-terminal 60 amino acid residues are optional; in another embodiment, the C-terminal 60 amino acid residues are optional; in a further embodiment, each of the N-terminal 60 amino acid residues and the C-terminal 60 amino acid residues are optional. In one embodiment, these optional N-terminal and/or C-terminal 60 residues are not included in determining the percent sequence identity. In another embodiment, the optional residues may be included in determining percent sequence identity.

Table 8

Row number	Cage (column 1)	Key (column 2)
1	LOCKR_extend18 (SEQ ID NO:6), BimLOCKR_extend18 (SEQ ID NO:22), , lfix-long-Bim-t0 (SEQ ID NO: 54), lfix-long-GFP-t0 (SEQ ID NO: 55), lfix-short-BIM-t0 (SEQ ID NO: 56), lfix-short-GFP-t0 (SEQ ID NO: 57),	p18_MBP (SEQ ID NO:27020), p76-long (SEQ ID NO:27027), p76-short (SEQ ID NO:27028),
2	LOCKRb (SEQ ID NO:7),	key_b (SEQ ID NO:27022)
3	LOCKRc (SEQ ID NO:8),	key_c (SEQ ID NO:27023)

Table 9

Cage Name	Cage Sequence	Key Name	Key Sequence
2plus1_Cage_Cte_rm_2406	SEVDEVVKEVEDLVRNEELVEEVVRRVEKVVTDDRR LVEEVVREIRKIVKDVEDLARKLDKEELKRVLDEMRE RIERLLEKLRRHSKKLDDELKRLLEELREHSRRVEKR LEDLLKELRERGVDEKVLKLEKVIREVRESTRALR KVVEVIRRVREESERALRDLERVVKEVEKRMREAAAR (SEQ ID NO:27126)	2plus1_Key_Cte_rm_2406	EKVLKLEKVIREVRE RSTRALRKVEEVIRRV REESERALRDLERVV EVEKRMREAAAR (SEQ ID NO:27127)
2plus1_Cage_Cte_rm_5398	SVEELLRKLEEVLRKIREENERSLKELRDRAREIVKR NRETNRLEEEVIKELEKRLSGADKEKVEELVRRIRRI VERVVEEDRRTVEEIEKIAREVVKRDRDSADRVRRTV EDVLRKATGSEDIVRKIERIVETIEREVRESVKKVEE IARDIRRKVDES SVKNVEKLLRDVDKKARDRKK (SEQ ID NO:27128)	2plus1_Key_Cte_rm_5398	EDIVRKIERIVETIER EVRESVKKVEEIIARDI RRKVDES SVKNVEKLLR DVKKARDRKK (SEQ ID NO:27129)
2plus1_Cage_Cte_rm_5405	SESDDVIRKLELLEELRTHVEKSIRDRLRKILEDSTR HAKRSIEELERLLEEVRRKPGDEEVRTVEEISRRVA ENVKRLEDLYRRMEEEVKKNLDRLRKRVEDIIREVEE ARKKGVDEEKLKDLIRKLRDILRRAAEAHKKLIDDAR ESLERAKREHEKLIDRLKKILEELER (SEQ ID NO:27130)	2plus1_Key_Cte_rm_5405	EEKLKDILRKLRDILR RAAEAHKKLIDDARES LERAKREHEKLIDRLK KILEELER (SEQ ID NO:27131)
2plus1_Cage_Cte_rm_5406	DREREVKKRLDEVREIRERLLRRVEEESRRVAEEIIR LIEEVRRRNKKVTEEIRELLKGLKDKKEVRRVLERLR KLNAESDELLERILERLRLVEATNRLVKAIIEELRR LVEKIVREVPDSEELREELKKLERKIEKVAKEIHDHD KEVTERLEDLLRRITEHARKSDREIEETAR (SEQ ID NO:27132)	2plus1_Key_Cte_rm_5406	EELREELKKLERKIEK VAKEIHDHDKEVTERL EDLLRRITEHARKSDR EIEETAR (SEQ ID NO:27133)
2plus1_Cage_Cte_rm_5409	SEAEELLKRLEDRAEEILRRLEEILRTSRKLAEDVLR ELEKLLRESERRIREVLEELRGIKDKKELEDVIREVE KELDESLSERSRELLKDVLLKDDNLKESERLVEDIDR ELAKILEDLKKAGVPKEVVDEIKRIVDEVRERLKRIV DENAKIVEDARRALEKIVKENEEILRLKKELRELK (SEQ ID NO:27134)	2plus1_Key_Cte_rm_5409	KEVVDEIKRIVDEVRE RLKRIVDENAKIVEDA RRALEKIVKENEEILR RLKKELRELK (SEQ ID NO:27135)
2plus1_Cage_5414_GFP_11_Cterm	SEIEKILKEIEDLARRDEEVSKKIVEDIRRLAKEVED TSRDIVRKIEELAKRVLDRLRKDGSKKEEKEVREVV KTLEELVKDNHRLIRRAVEEMKRLVEENHRHSREVVK ELEDLVRELKRGSGSEDSESDHMLHEYVNAAGITSE KSNEEVKRTVEELVRRMEESNDRVRDLVRRVLEELKR AVD (SEQ ID NO:27140)	2plus1_Key_Cte_rm_5414	EDSERLVREVEDLVRR LVRRSEKSNEEVKRTV EELVRRMEESNDRVRD LVRRVLEELKRAVD (S EQ ID NO:27141)
2plus1_Cage_Cte_rm_5421	SVDEVLKEIEDALRRLKEEVERVLKENEDLRRLEEE VRRVLKEDEELLESKRGVGSDEVDRVDEIAKLSA EILEKVKKVVEKIRDSLETVKRRVDDVRRRLKELLDE IKRGSDEKAIRDVAKEIRDRLKELEEEIEEVTRNLK LLADVEEEIIRRVHEKTRRLLETVLRRAT (SEQ ID NO:27146)	2plus1_Key_Cte_rm_5421	EKAIRDVAKEIRDRLK ELEEEIEEVTRNLKL LADVEEEIIRRVHEKTR RLLETVLRRAT (SEQ ID NO:27147)
2plus1_Cage_Cte_rm_5432	DEIRKVVEITDILLKASNDKNRVVEEIRDLLRKS LADELVERLRALVEDLRRRIDKSGDKETAEDIVRRI EELKRILKEIEDLARRINREIERLVEEVERDNRDVNR AIEELLKDIARRGGSEDLKRVVEERAREVSRNEESMR RVKEDADVSEANKEVLDVRVEEVKRLIEEVRET (SEQ ID NO:27148)	2plus1_Key_Cte_rm_5432	SEDLKRVVEERAREVSR RNEESMRVKEDADV SEANKEVLDVRVEEVK RLIEEVRET (SEQ ID NO:27149)
2plus1_Cage_Cte_rm_5435	STAETVAEEVERVLKHSDDLKEVEDVNRVVEEIEKR VIRELEEEENERLVAEVRKGVKGEILAEIEKRLADNSE KVREVAERAKKLEENTARVKDILRESRKLVKDLLDE VRGTGSEEAAREIIKRLREVNRKTKELDELKHS VLERVKRLIDELRKHSSEEVLEDLRRRAK (SEQ ID NO:27150)	2plus1_Key_Cte_rm_5435	EAAAREIIKRLREVNR RTKEKLDELKHSSEV LVRVKRLIDELRKHS EVLEDLRRRAK (SEQ ID NO:27151)
2plus	SRVEEIIEDLRRLLLEEIRKENEDSIRRSKELLDRVKE	2plus1	EDKARKVAEVAEKVLR

1_Cage_Cte_rm_5439	INDTIIAELERLLKDIEKEVREKGESESEEVKKALRAV LEELEKLLRRVAEINEEVLRRNSKLVEEDERRNREVL KELARLVEELIREIGDEDKARKVAEVAEKVLRDIDKL DRESKEAFRATNEETIAKLDEDTARVAERVKKAIEDLA K (SEQ ID NO:27154)	Key_Cte_rm_5439	D N RVKKAIEDLAK (SEQ ID NO:27155)
2plus1_Cage_Cte_rm_5447	SEADDVLKKLAETVKRIIERLKKLTDDSRRLVEEVHR RNDKLSKESAEAVRKAEERGIDEKDVRKLLLEDLKKKS EEVAERNKRILDTLREISKRAEDEVKVLKELEKTLK ELEDRRPDSEELSAEVKKLLDEVKALARHKDENDKL LKEIEDSLRRHKEENDRLLEKLKESTR (SEQ ID NO:27156)	2plus1_Key_Cte_rm_5447	EELSAEVKKLLDEVK ALARHKDENDKLLKEI EDSLRRHKEENDRLLE KLKESTR (SEQ ID NO:27157)
2plus1_Cage_Cte_rm_5456	SAEELLREVAELVKRVDEDLRRLLEEVVASNEEVIRR LEEILKRIEENRKVVEELRRGGVSEDVRESKRLVD ESRRVIEKLVKESADSVERTRETVDRLREELKRLVEE IAKMVKGGSSEETVKRLLDELRELLERLKRRTIEELLK RNRDLLADAEEKARRLLEENRKLKKAARDTAT (SEQ ID NO:27158)	2plus1_Key_Cte_rm_5465	EETVKRLLDELRELLE RLKRRTIEELLKRNRL LADAEEKARRLLEENR KLLKAARDTAT (SEQ ID NO:27159)
2plus1_Cage_Nte_rm_2406	SEVDEVVKEVEDLVRNEELVEEVVRRVEKVVTDDDR LVEEVVREIRKIVKDVEDLARKLDKEELKRVLDRE RIERLLEKLRRHSKKLDDELKRLLEELREHSRRVEKR LEDLLKELRERGVDEKVLKLEKVIREVRESTRALR KVVEEVIRRVREESERALRDLERVVKEVKRMREAR (SEQ ID NO:27162)	2plus1_Key_Nte_rm_2406	SEVDEVVKEVEDLVR NEELVEEVVRRVEKVV TDDRRLVEEVVREIRK IVKDVEDLARK (SEQ ID NO:27163)
2plus1_Cage_Nte_rm_5406	DREREVKKRLDEVREIRERLLRRVEEESRRVAEEIRR LIEEVRRRNKKVTEEIRELLKGLKDKEEVRRVLERLR KLNAESDELLERILERLRLVEATNRLVKAIIEELRR LVEKIVREVPDSEELREELKKLERKIEKVAKIEHDHD KEVTERLEDLLRRITEHARKSDREIEETAR (SEQ ID NO:27164)	2plus1_Key_Nte_rm_5406	DREREVKKRLDEVRE IERLLRRVEEESRRVA EEIRRLIEEVRRRNKK VTEEIRELLKGL (SEQ ID NO:27165)
2plus1_Cage_Nte_rm_5409	SEAEELLKRLEDRAEEILRRLEEILRTSRKLAEDVLR ELEKLLRESERRIREVLEELRGIKDKKELEDVIREVE KELDESLSERSRELLKDVLLKLDNLKESERLVEDIDR ELAKILEDLKKAGVPKEVVDEIKRIVDEVRERLKRIV DENAKIVEDARRALEKIVKENEEILRRLKKELRELK (SEQ ID NO:27166)	2plus1_Key_Nte_rm_5409	SEAEELLKRLEDRAEE ILRRLEEILRTSRKLA EDVLRLEKLLRESER RIREVLEELRGI (SEQ ID NO:27167)
3plus_Cage_529_GFP11_Cterm	SEAEDLEELIKELAELLKDVIRKLEKINRRLVKILED IIRRLKEISKEAEEELRKGTVEDKDILRDLERRLREI LEESDRLLLEELKRRLEEILRKSKEELLRRLEEVLR KRAEEVKRSNLPKEELIKEIVKLLLEELLRVIEKILED NIRLLEELVEVIKEILEKHLRLLEELVRVIERILREV GKDKDEAERRDHMLHEYVNAAGITEEVKRRLEEEELT RLRETHKKIEKELREALKRVDRST (SEQ ID NO:27178)	3plus_Key_Cterm_529	KDEAERRRRELKDKLD RLREEHEEVKRRLEEE LTRLRETHKKIEKELR EALKRVDRST (SEQ ID NO:27179)
3plus1_Cage_Cte_rm_500	SEKEELKRLLDKLLKELKRLSDELKATIDKILKILKE VSEEVKRTADELLDAIRGGVDEEVLEIKREIEEIE KKLRKVNKEIEDEIREIKKKLDEVDKITKEVEKIKE ALDKGGVDAKEVIKALKEILKEHADVFEDVLRRLKEI IKRHRDVVKEVLEELRKILEKVAEVLKRQGRSEDEL KVEEDLKRLEDKLLKLLLEDYEKKVRELEETLDDLLRK YEETLRRLEKELEEAER (SEQ ID NO:27184)	3plus1_Key_Cte_rm_500	EDELKRVVEEDLKRLED KLKLLLEDYEKKVREL EETLDDLLRKYEETLR RLEKELEEAER (SEQ ID NO:27185)
3plus1_Cage_Cte_rm_510	SEKEELLKLIKRVIELLKRVLLEHLRLVEDVIRRLKE LLDSNEKIVREVIEDLKRLLDEVGRDKEELDRIKEKL EEVLEERYKRRLEEIKRDLERMLEDYKRELKRIEEDLR RVLEEVERIATRGEPAEALIDKLRLKILERALRELDK LSKKLDELKLVLEELEKSNEIDKLLKDVLRVVEEG GASEDLLRKAKKVITEVREKLKRNLEDVRRVIEDVKR KSARILEEARRLIEEVERELEKIRK (SEQ ID NO:27190)	3plus1_Key_Cte_rm_510	EDLLRKAKKVITEVRE KLKRNLEDVRRVIEDV KRKSARILEEARRLIE EVERELEKIRK (SEQ ID NO:27191)
3plus1_Cage_528	SEAEDLEELIKELAELLKDVIRKLEKINRRLVKILED IIRRLKEISKEAEEELRKGTVEDKDILRDLERRLREI LEESDRLLLEELKRRLEEILRKSKEELLRRLEEVLR EIL	3plus1_Key_Cte_rm_528	KDEAERRRRELKDKLD RLREEHEEVKRRLEEE LTRLRETHKKIEKELR

_GFP1 1_Cte rm	KRAEEVKRSNLPKEELIKEIVKLLLEELLRVIEKILED NIRLLEELVEVIKEILEKHLRLLEELVRVIERILREV GRDHMLVHEYVNAAGITLDRLEEEHVEVKRRLEEEELT RLRETHKKIEKELREALKRVDRST (SEQ ID NO:27192)		E I
3plus 1_Cag e_528 _GFP1 1_Cte rm	SEAEDLEELIKELAELLKDVIRKLEKINRRRLVKILED IIRRLKEISKEAEEELRKGTVEDKDILRDLERRLREI LEESDRLLLEELKRRLEELIRKSKELLRRLEEVLRIL KRAEEVKRSNLPKEELIKEIVKLLLEELLRVIEKILED NIRLLEELVEVIKEILEKHLRLLEELVRVIERILREV GKDKRDHMLVHEYVNAAGITLREEHVEVKRRLEEEELT RLRETHKKIEKELREALKRVDRST (SEQ ID NO:27194)	3plus1_ Key_Cte rm_528	KDEAERRRRRELKDKLD RLREEHVEVKRRLEEE LTRLRETHKKIEKELR EALKRVDRST (SEQ ID NO:27195)
3plus 1_Cag e_528 _GFP1 1_Cte rm	SEAEDLEELIKELAELLKDVIRKLEKINRRRLVKILED IIRRLKEISKEAEEELRKGTVEDKDILRDLERRLREI LEESDRLLLEELKRRLEELIRKSKELLRRLEEVLRIL KRAEEVKRSNLPKEELIKEIVKLLLEELLRVIEKILED NIRLLEELVEVIKEILEKHLRLLEELVRVIERILREV GKDKDEAERDMLVHEYVNAAGITHEEVKRRLEEEELT RLRETHKKIEKELREALKRVDRST (SEQ ID NO:27196)	3plus1_ Key_Cte rm_528	KDEAERRRRRELKDKLD RLREEHVEVKRRLEEE LTRLRETHKKIEKELR EALKRVDRST (SEQ ID NO:27197)
3plus 1_Cag e_529 _GFP1 1_Cte rm	SEAEDLEELIKELAELLKDVIRKLEKINRRRLVKILED IIRRLKEISKEAEEELRKGTVEDKDILRDLERRLREI LEESDRLLLEELKRRLEELIRKSKELLRRLEEVLRIL KRAEEVKRSNLPKEELIKEIVKLLLEELLRVIEKILED NIRLLEELVEVIKEILEKHLRLLEELVRVIERILREV GKRDHMLVHEYVNAAGITDRLREEHVEVKRRLEEEELT RLRETHKKIEKELREALKRVDRST (SEQ ID NO:27198)	3plus1_ Key_Cte rm_529	KDEAERRRRRELKDKLD RLREEHVEVKRRLEEE LTRLRETHKKIEKELR EALKRVDRST (SEQ ID NO:27199)
3plus 1_Cag e_529 _GFP1 1_Cte rm	SEAEDLEELIKELAELLKDVIRKLEKINRRRLVKILED IIRRLKEISKEAEEELRKGTVEDKDILRDLERRLREI LEESDRLLLEELKRRLEELIRKSKELLRRLEEVLRIL KRAEEVKRSNLPKEELIKEIVKLLLEELLRVIEKILED NIRLLEELVEVIKEILEKHLRLLEELVRVIERILREV GKDRDHMLVHEYVNAAGITRLREEHVEVKRRLEEEELT RLRETHKKIEKELREALKRVDRST (SEQ ID NO:27200)	3plus1_ Key_Cte rm_529	KDEAERRRRRELKDKLD RLREEHVEVKRRLEEE LTRLRETHKKIEKELR EALKRVDRST (SEQ ID NO:27201)
3plus 1_Cag e_529 _GFP1 1_Cte rm	SEAEDLEELIKELAELLKDVIRKLEKINRRRLVKILED IIRRLKEISKEAEEELRKGTVEDKDILRDLERRLREI LEESDRLLLEELKRRLEELIRKSKELLRRLEEVLRIL KRAEEVKRSNLPKEELIKEIVKLLLEELLRVIEKILED NIRLLEELVEVIKEILEKHLRLLEELVRVIERILREV GKDKDRDHMLVHEYVNAAGITREEHVEVKRRLEEEELT RLRETHKKIEKELREALKRVDRST (SEQ ID NO:27202)	3plus1_ Key_Cte rm_529	KDEAERRRRRELKDKLD RLREEHVEVKRRLEEE LTRLRETHKKIEKELR EALKRVDRST (SEQ ID NO:27203)
3plus 1_Cag e_534 _GFP1 1_Cte rm	DEDRIIEEIARLLEELLRELLELIKKLIETNRRRLNEE HERAVRELARLLEELLDRLVKKGISDEKLKRIRERLK RALDDLRLHREINKRLEDLVRELEKLVREILKELKD ALEELRRASARAGGEEVLRRLLEEIVKKLLDLVRRILE RLKEIHKDNVRLRLRELNERLTRIVEDLVRLIREILRE AGVDERDHMLVHEYVNAAGITIKRLHEDLERKLKKESE DELREIEARLEEKIRRLLEEKLERKRR (SEQ ID NO:27206)	3plus1_ Key_Cte rm_534	EKIAEEIERELEELRR MIKRLHEDLERKLKES EDELREIEARLEEKIR RLEEKLERKRR (SEQ ID NO:27207)
3plus 1_Cag e_534 _GFP1 1_Cte rm	DEDRIIEEIARLLEELLRELLELIKKLIETNRRRLNEE HERAVRELARLLEELLDRLVKKGISDEKLKRIRERLK RALDDLRLHREINKRLEDLVRELEKLVREILKELKD ALEELRRASARAGGEEVLRRLLEEIVKKLLDLVRRILE RLKEIHKDNVRLRLRELNERLTRIVEDLVRLIREILRE AGVDEKIRDHMLVHEYVNAAGITRLHEDLERKLKKESE DELREIEARLEEKIRRLLEEKLERKRR (SEQ ID NO:27208)	3plus1_ Key_Cte rm_534	EKIAEEIERELEELRR MIKRLHEDLERKLKES EDELREIEARLEEKIR RLEEKLERKRR (SEQ ID NO:27209)

3plus 1_Cag e_534 _GFP1 1_Cte rm	DEDRIIEEIIARLLEELLRELELELIKKLIETNRRRLNEE HERAVRELARLLEELLDRLVKKGISDEKLRIRERLKR RALDDLERLHREINKRLEDLVRELEKLVREILKELKD ALEELRRASARAGGEEVLRRLLEEIVKKLLDLVRRILE RLKEIHKDNVRLRLRELNERLTRIVEDLVRLIREILRE AGVDEKIAEEIERDHMVLEHYVNAAGITLERKRLKESE DELREIEARLEEKIRRLLEEKLERKRR (SEQ ID NO:27210)	3plus1_ Key_Cte rm_534	E M EDELREIEARLEEKIR RLEEKLERKRR (SEQ ID NO:27211)
3plus 1_Cag e_Cte rm_53 9	SEKEKLLKESEEEVRRRLRRTLEELLRKYREVLERLRK ELREIEERVDRDVRRLKEVLDRKGLDIDITIIKEVEDL LKTVDLRLRELLDKIRRLTKEAIEVVREIIRIVRHA ERVKDELKKEGGDKELDRVDRLIKENTRHLKEILDR IEDLVRRSEKKLRDIIREVRLIEELRKKAEI KKG DERLVKTLIEDVEAVIKRILELITRVAEDNERNVLERI IRELTDNLERHLKIVREIVK (SEQ ID NO:27212)	3plus1_ Key_Cte rm_539	ERLVKTLIEDVEAVIK RILELITRVAEDNERNV LERIIRELTDNLERHL KIVREIVK (SEQ ID NO:27213)
3plus 1_Cag e_Cte rm_54 8	DKAEVLREALKLLKDLLLEELIKIHEESLKRILDLIDT LVKVHEDALRALKELLERSGLDERELRKVERMATESL RTIAKLKEELRDLARRSLEKLREDLKRVDLTKRVEE KVRRTGPSEELIEELIRTIEKLLKEIVRINEEVKAV RELLKTLKLSEDVVRRIEELRKGGVPPEIDRELKR VVEELRRLHEEIKERLDDVARRSEELRRIKKLKEV VKEIRKKLK (SEQ ID NO:27214)	3plus1_ Key_Cte rm_548	EEIDRELKRVVEELRR LHEEIKERLDDVARRS EEELRRIKKLKEVVK EIRKKLK (SEQ ID NO:27215)
3plus 1_Cag e_Cte rm_55 6	SERELIERWLELHKEILRLIRELVERLLKLHREILDT IKKLIRELLELLEDIARKLGLDKEAKDELREIAKRVE DKLEKLERESRKVEEDLKRKLKELTDES DTVKRVDR VVRGTQSREEIAEELLRLDRKLLKAVEELLKEILDL NKKLLDDVRAILEETRRVLEKLLDRVRRGERTDDERR TLTELLKRMEDILEKVERTLKKLLDDSDARMAEEVKKT LKELLERSEKVAEDVRK (SEQ ID NO:27216)	3plus1_ Key_Cte rm_556	DDERRTLTELLKRMED ILEKVERTLKKLLDDSD ARMAEEVKKT LKELLE RSEKVAEDVRK (SEQ ID NO:27217)
3plus 1_Cag e_Cte rm_56 0	SKKELLEEVVRRATIELLKRHLEKLKRILEEIVRLLEE HLEKVERVLEAILSLDDLLRRGGDERAIRTLEDVKR RLREILERLADENAKAIKRLADLLDKLEKRNKEAIER LLEEILEELKRVRRDEELLRVLETLLKIIEDILRENTK VLEDLLRLVEEILEANLRVVEELLRLAREILTEIVGD EDKLKEIEDELRRLLLEELRRLDKAIKDRLRELKKDLD EANRIKETLKKLLREVEK (SEQ ID NO:27218)	3plus1_ Key_Cte rm_560	EDKLKEIEDELRRLL ELRRLDKAIKDRLREL KKDLDEANRIKETLK KLLREVEK (SEQ ID NO:27219)
3plus 1_Cag e_568 _GFP1 1_Cte rm	KEIEETLKELEDLNREMVTNRRVLEETRRLNKETVD RVKATLDELAKMLKKLVDDVRKGPTSEELKRLLAELE ELLARVVRVVEELLKKSTDLLERAVKDSADALRRSHE VLKEVASRVKRAKDEGLPREEVRLRLRELLERHAKVL KDIVRVSEKLLREHLKVLREIVEVLEELLERILKVIL DTTRDHMLHEYVNAAGITKRRLKEVIDRYEDELRLK RKEYKEKIDKYERKLEEIERRERT (SEQ ID NO:27220)	3plus1_ Key_Cte rm_568	KAVEELEKALEEIKRR LKEVIDRYEDELRLKLR KEYKEKIDKYERKLEE IERRERT (SEQ ID NO:27221)
3plus 1_Cag e_568 _GFP1 1_Cte rm	KEIEETLKELEDLNREMVTNRRVLEETRRLNKETVD RVKATLDELAKMLKKLVDDVRKGPTSEELKRLLAELE ELLARVVRVVEELLKKSTDLLERAVKDSADALRRSHE VLKEVASRVKRAKDEGLPREEVRLRLRELLERHAKVL KDIVRVSEKLLREHLKVLREIVEVLEELLERILKVIL DTTGGDRDHMLHEYVNAAGITLKEVIDRYEDELRLK RKEYKEKIDKYERKLEEIERRERT (SEQ ID NO:27222)	3plus1_ Key_Cte rm_568	KAVEELEKALEEIKRR LKEVIDRYEDELRLKLR KEYKEKIDKYERKLEE IERRERT (SEQ ID NO:27223)
3plus 1_Cag e_Cte rm_58 1	SALETVKKLLEDSSSEKIERIVEEDERVAKESSDRIR LVEEDKRVADEILDIEKIGDITDILLKLVEEWSRTSK KLLDDVLKLHKDWSDDSRLLLEEILRVHEELIRRVKE ILDREGKPEEVVRELEKVLKESLDTLEEIIIRRLDEAN AATVKRVADVIRELEDINRKVLEEIKRGSDDAEAVIK VIEKLIRANKRVWDALLKINEDLVRVNKT VWKELLRV NEKLARDLERVVK (SEQ ID NO:27226)	3plus1_ Key_Cte rm_581	AAVIKLVIEKLIRANK RVWDALLKINEDLVRV NKT VWKELLRVNEKLA RDLERVVK (SEQ ID NO:27227)
3plus 1_Cag	SKEEKLKDDVRAVLEDLDRVLKELEKLSNDRRELKR VLDRITDLHRRILDELKRGIGSEELLRRVEKVLKDNL	3plus1_ Key_Cte	SKAAEDILRVLEKLVK VSREAIKLILELSEHH

e_Cte rm_58 5	DLRKLVEEHKESSEDLKRVEDLVREIKEVLRKLLLE LEDGRGTDIRKIEEEIERLLRKIRKAVEESKDLNRRNS ERIEEVARRSEELARRLLKEIRERGDASKAAEDILRVL EKLVKVSREAIKLILELSEHHVRVSTRIARLLLDVAR KLAEVIKEAER (SEQ ID NO:27228)	rm_585	V K ID NO:27229)
3plus 1_Cag e_Cte rm_58 7	SEIEDVIRRLRKILEDLERVSEKLLREIKKILDEARR LNEEVIKEIKRVLEDAVRVFRDGSKSKEELAKLVEEL IRELAKLAKEVDEIHKRIVERLAKALVEDAERIHRKIV ETLEEIVRGVPSEELKRVVEAIVEVIKEHLKVLADVI RRIIKAIEENAETIKRVLEDIVRVLELVLRGESIED LVREVERLIKRIEDSLRELEKTVRELLKRIKEASDKV REDVDRLIKELKEAAD (SEQ ID NO:27230)	3plus1_ Key_Cte rm_587	IEDLVREVERLIKRIE DSLRELEKTVRELLKR IKEASDKVREDVDRLI KELKEAAD (SEQ ID NO:27231)
3plus 1_Cag e_Cte rm_60 5	SREELLDRILEAIAKILEDLKRLIDENLARLEEVVRE LERIIDRNKLKIREILDELKKGSGSEEILEKIKKVDK ELEDLIRRLKKLEDLIRETERRRLREILKRI RDLLKE VKDRDKDLERLLEVL EEVL RVIAELAKELLDSL RKVL KVVEEVLRLLEVNKEVLDVIRELAKDGGSD EII RKL DELLKEVEKVHKEVKDRIRKLL EDHKRSLDEVKKKLE RLLERAKEVVEREKK (SEQ ID NO:27232)	3plus1_ Key_Cte rm_605	DEIIRKLDELLEKEVEK VHKEVKDRIRKLL EDH KRSLDEVKKKLERLLE RAKEVVEREKK (SEQ ID NO:27233)
3plus 1_Cag e_Cte rm_60 7	SEREELLERIKELKRVKDKLDEDLKRLKEILEKLKE KARDLEELRRRIEEVREKLERTGRDELVKEVLDTV RRNLENLKRVLVEDILRKLEENVKNLTDLVREILKLIT ELIKRLEDGGLPKEVLDALRRVLEKLEELLREILERL KRSLEAVKRKIEELLKELEERSLDELRRALERIRKEIG DSETAVRAIIRVLEKHLEAVRRVLEELLKVLA EHLET VRELIERLKRVL EEAEI EVVERVAR (SEQ ID NO:27234)	3plus1_ Key_Cte rm_607	SETAVRAIIRVLEKHL EAVRRVLEELLKVLA E HLETVRELIERLKRVL EEAEI EVVERVAR (SEQ ID NO:27235)
3plus 1_Cag e_611 GFP1 1_Cte rm	SLEEITKRLLLEVEENLARHEEILRELLELAKRLAKE DRDILEEVLKLI EELLKLL EDNGSSEEDLKRL LKEVI EELRAVVVKRVKDKWDEVVKRIEDLVKKLKE LHDDTLR KLRELVRKIVTDI SESGGEAEKVVRVVEKILELVERL AKVVKESVEKLLEILRELA EVSKRVAEALLRLLEELV RVIRIKDERDHMVLHEYVNAAGITLLDELEEVHKKRVK KELEDII EENRRVVKRVRDELREIKRELDE (SEQ ID NO:27238)	3plus1_ Key_Cte rm_611	ERTLREVVRKVLEEAK RLLEDEEVEHKKRVKKE LEDII EENRRVVKRVR DELREIKRELDE (SEQ ID NO:27239)
3plus 1_Cag e_Cte rm_63 2	SEKELVDDIRRILEEILRLLRSLL EEVIRLLEENEKL VRRHLKTVIDILRRVAKLLDENGIRTDEADRVLERLE KAHRELLEDYKRALEKIKETLERVLREAE EVVKKIDD ALRKLGGSKVLRLLLEELLRLVEKIAEEIKRLLSEL VRVTEELVRTNKELLEEA VRVIRKEVGDDSLVREVEE LIKRLKHHIDDLLKTSRDLVKRVLDLVDEVVKRVEDL VERVKEKIDT (SEQ ID NO:27240)	3plus1_ Key_Cte rm_632	DSLREVEELIKRLEK HIDDLLKTSRDLVKRV LDLVDEVVKRVEDLVE RVKEKIDT (SEQ ID NO:27241)
3plus 1_Cag e_646 GFP1 1_Cte rm	DAEEVVKRLADVLRENDETIRKVVEDLVRIAEENDRL WKKLVEDIAEILRRIVELLRRGGVPEELLDRLAKVVK SIVEKA EKILERLNRVSKAIAEKLKTIVDELNEVSKE IVKRAEDILRK GKDKETVLRALRTL VKEYADLSKEVL ERVERIVREYVKLSDEVVKSLAEI VEELIRI IEDLLR KGNRDHMLHEYVNAAGITRKLLEDVKKASEDIVREV ERIVRELAKRSDEILKKLEDIVEKLRE (SEQ ID NO:27244)	3plus1_ Key_Cte rm_646	EDVKRALEELVSRLRK LLEDVKKASEDIVREV ERIVRELAKRSDEILK KLEDIVEKLRE (SEQ ID NO:27245)
3plus 1_Cag e_646 GFP1 1_Cte rm	DAEEVVKRLADVLRENDETIRKVVEDLVRIAEENDRL WKKLVEDIAEILRRIVELLRRGGVPEELLDRLAKVVK SIVEKA EKILERLNRVSKAIAEKLKTIVDELNEVSKE IVKRAEDILRK GKDKETVLRALRTL VKEYADLSKEVL ERVERIVREYVKLSDEVVKSLAEI VEELIRI IEDLLR KGNLDEDRDHMLHEYVNAAGITEDVKKASEDIVREV ERIVRELAKRSDEILKKLEDIVEKLRE (SEQ ID NO:27246)	3plus1_ Key_Cte rm_646	EDVKRALEELVSRLRK LLEDVKKASEDIVREV ERIVRELAKRSDEILK KLEDIVEKLRE (SEQ ID NO:27247)
3plus 1_Cag e_646	DAEEVVKRLADVLRENDETIRKVVEDLVRIAEENDRL WKKLVEDIAEILRRIVELLRRGGVPEELLDRLAKVVK SIVEKA EKILERLNRVSKAIAEKLKTIVDELNEVSKE	3plus1_ Key_Cte rm_646	EDVKRALEELVSRLRK LLEDVKKASEDIVREV ERIVRELAKRSDEILK

_GFP1 1_Cte rm	IVKRAEDILRKGGDKETVLRALRTLIVKEYADLSKEVL ERVERIVREYVKLSDEVVKSLAEIVEELIRIIEEDLLR KGNLDEDVRDHMVLHEYVNAAGITDVKKASEDIVREV ERIVRELAKRSDEILKKLEDIVEKLRE (SEQ ID NO:27248)		K I
3plus 1_Cag e_647 _GFP1 1_Cte rm	DAEEVVKRLADVLRENDETIRKVVEDLVRIAEENDRL WKKLVEDIAEILRRIVELLRRGGVPEELLDRLAKVVK SIVEKAEKILERLNRVSKAIAEKLKTIVDELNEVSKE IVKRAEDILRKGGDKETVLRALRTLIVKEYADLSKEVL ERVERIVREYVKLSDEVVKSLAEIVEELIRIIEEDLLR KGNLRDHMVLHEYVNAAGITKLEDVKKASEDIVREV ERIVRELAKRSDEILKKLEDIVEKLRE (SEQ ID NO:27250)	3plus1_ Key_Cte rm_647	EDVKRALEELVSRLRK LLEDVKKASEDIVREV ERIVRELAKRSDEILK KLEDIVEKLRE (SEQ ID NO:27251)
3plus 1_Cag e_647 _GFP1 1_Cte rm	DAEEVVKRLADVLRENDETIRKVVEDLVRIAEENDRL WKKLVEDIAEILRRIVELLRRGGVPEELLDRLAKVVK SIVEKAEKILERLNRVSKAIAEKLKTIVDELNEVSKE IVKRAEDILRKGGDKETVLRALRTLIVKEYADLSKEVL ERVERIVREYVKLSDEVVKSLAEIVEELIRIIEEDLLR KGNLDEDVKRALERDHMVLHEYVNAAGITSEDIVREV ERIVRELAKRSDEILKKLEDIVEKLRE (SEQ ID NO:27252)	3plus1_ Key_Cte rm_647	EDVKRALEELVSRLRK LLEDVKKASEDIVREV ERIVRELAKRSDEILK KLEDIVEKLRE (SEQ ID NO:27253)
3plus 1_Cag e_Cte rm_64 7	DAEEVVKRLADVLRENDETIRKVVEDLVRIAEENDRL WKKLVEDIAEILRRIVELLRRGGVPEELLDRLAKVVK SIVEKAEKILERLNRVSKAIAEKLKTIVDELNEVSKE IVKRAEDILRKGGDKETVLRALRTLIVKEYADLSKEVL ERVERIVREYVKLSDEVVKSLAEIVEELIRIIEEDLLR KGNLDEDVKRALEELVSRLRKLEDVKKASEDIVREV ERIVRELAKRSDEILKKLEDIVEKLRE (SEQ ID NO:27254)	3plus1_ Key_Cte rm_647	EDVKRALEELVSRLRK LLEDVKKASEDIVREV ERIVRELAKRSDEILK KLEDIVEKLRE (SEQ ID NO:27255)
3plus 1_Cag e_Cte rm_65 3	DEEETLRRLLEKVELAKEYLDSKEVIDRTTKLLDE YLKTSKRIVDATVELLERGDLGPDELKRLAEELERS LRELEEEIKRLKRELEESLKKLKEIIDRLAEAAEKLL AVLKRGEGSEEEALRALASLVRELIEVLRENDERLRD VLRLIEALRKNNILERVLRKLVRAAEERGRDESSR EALEEARRRLEELLRELNEITKDLEAKLEKLLRDLNE LTKALEEELKRLLDLKKRTD (SEQ ID NO:27256)	3plus1_ Key_Cte rm_653	SREALEEARRRLEELL RELNEITKDLEAKLEK LLRDLNELTKALEEEL KRLLDLKKRTD (SEQ ID NO:27257)
3plus 1_Cag e_Cte rm_65 8	DEERIKTLEDINAKLVEDIKRILDKVAELNERLADA IRKILEETKRILEATTRKVRKDGEISEELLRRLEEKL RKLEDLERVLAHEHEDESRRILEEVERLLKRHADASK ELLDRAERSVARGVKSDEKELVDRLKKLIDDSLESVREL IERLKELLDRLVKSVEDLIRTIKELLDRLVEVLREGV SDKDTLRTVEKLVEDVKRRLDKLEDYKRLIEEVKKE LDKLLKEYEDALREIKKRIDE (SEQ ID NO:27258)	3plus1_ Key_Cte rm_658	KDTLRTVEKLVEDVKR RLDKLLEDYKRLIEEV KKELDKLLKEYEDALR EIKKRIDE (SEQ ID NO:27259)
3plus 1_Cag e_Nte rm_26 3	SLVDELRKSLERNVRVSEEVARRLKEALKRWVDVVRK VVEDLIRLNEDVVRVVEKVTVDESAIERVRRIEEELN RKLDVAVLKKNEDLVRRLTELLDKLLEENRRIVEELDE DLKRRGGTEEVIDTILELIERSIERLKRLLDELLRIV REALKDNKRVDENLKKLKEILDELKDGVEDEELKR VLEKAADLHRRLLKDRHRKLEDLERIIRELKKKLDEV VEENKRSVDELKR (SEQ ID NO:27262)	3plus1_ Key_Nte rm_263	SLVDELRKSLERNVRV SEEVARRLKEALKRWV DVVRKVVEDLIRLNED VVRVVEKV (SEQ ID NO:27263)
3plus 1_Cag e_647 _GFP1 1_Nte rm	DAEEVVKRLADVLRENDETIRKVVEDLVRIAEENDRL WRDHMVLHEYVNAAGITLLRRGGVPEELLDRLAKVVK SIVEKAEKILERLNRVSKAIAEKLKTIVDELNEVSKE IVKRAEDILRKGGDKETVLRALRTLIVKEYADLSKEVL ERVERIVREYVKLSDEVVKSLAEIVEELIRIIEEDLLR KGNLDEDVKRALEELVSRLRKLEDVKKASEDIVREV ERIVRELAKRSDEILKKLEDIVEKLRE (SEQ ID NO:27276)	3plus1_ Key_Nte rm_647	DAEEVVKRLADVLREN DETIRKVVEDLVRIAE ENDRLWKKLVEDIAEI LRRIVELLRRG (SEQ ID NO:27277)

In various specific embodiments, the cage proteins comprise an amino acid sequence at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, 99%, or 100% identical, not including optional amino acid residues, to the amino acid sequence of a cage protein selected from the group consisting of SEQ ID NOS: 27497-27620, wherein the N-terminal protein purification tag (MGSHHHHHHGGSGSENLYFQSGG (SEQ ID NO:27624); or MGSHHHHHHGGSENLYFQG (SEQ ID NO:27625); or GSHHHHHHGGSGSENLYFQG (SEQ ID NO:27626)) is optional, is not considered in the percent identity comparison, and can be present or absent. In one embodiment the N-terminal protein purification tag is absent.

Table 10. Amino acid sequences

(The sequences below contain a 6His-TEV tag for protein purification purposes MGSHHHHHHGGSGSENLYFQG (SEQ ID NO: 27495) or variant thereof. The amino acids N-terminal to the structural region are optional and are not considered in the percent identity comparison relevant to the claimed cage protein

(The structural region is in parenthesis) The region C-terminal to the parenthesis constitutes the latch region.

The SmBit sequence (VTGYRLFEEIL) (SEQ ID NO:27359) is underlined.

The sensing domains are in **bold**

lucCageBim variants (Bcl2 sensors)

- SmBit sequence: VTGYRLFEEIL (SEQ ID NO:27359)
- BIM sequence: **EIWIAQELRRIGDEFNAYYA** (SEQ ID NO:27496)

>nluc301_bim331

MGSHHHHHHGGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLEL
VYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
DELQKLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
EAERAIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL
RELLRALAQLQELNLDLLRLASEL) ggsVTGYRLFEEILRVKRESKRIVEDAERLs **REEIWIAQELRRIGDEFNAYYA**
YYAAASEKISRE (SEQ ID NO:27497)

>nluc308_bim331

MGSHHHHHHGGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLEL
VYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
DELQKLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
EAERAIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL
RELLRALAQLQELNLDLLRLASEL) TDPDEARVTGYRLFEEILRIVEDAERLs **REEIWIAQELRRIGDEFNAYYA**
AASEKISRE (SEQ ID NO: 27498)

>nluc312_bim331

MGSHHHHHHGGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLEL
VYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
DELQKLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
EAERAIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL
RELLRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLs **REEIWIAQELRRIGDEFNAYYA**
AASEKISRE (SEQ ID NO: 27499)

>nLuc315_bim331

MGSHHHHHHGGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRI
VYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
DELQKLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
5 EAERAIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL
RELLRALAQLQELNLDLLRLASEL) TDPDEARKAIARVKVTGYRLFEEILRLsRE**EIWIAQELRRIGDEFNAYYA**
AASEKISRE (SEQ ID NO: 27500)

>nLuc301_bim339

MGSHHHHHHGGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLEL
VYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
DELQKLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
10 EAERAIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL
RELLRALAQLQELNLDLLRLASEL) ggsVTGYRLFEEILRVKRESKRIVEDAERLsREAAAASEKIE**EIWIAQELR**
15 **RIGDEFNAYYAE** (SEQ ID NO: 27501)

>nLuc308_bim339

MGSHHHHHHGGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLEL
VYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
20 DELQKLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
EAERAIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL
RELLRALAQLQELNLDLLRLASEL) TDPDEARVTGYRLFEEILRIVEDAERLsREAAAASEKIE**EIWIAQELRRIG**
DEFNAYYAE (SEQ ID NO: 27502)

>nLuc312_bim339

MGSHHHHHHGGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLEL
VYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
DELQKLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
25 EAERAIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL
RELLRALAQLQELNLDLLRLASEL) TDPDEARKAIARVVTGYRLFEEILDAERLsREAAAASEKIE**EIWIAQELRRIG**
30 **DEFNAYYAE** (SEQ ID NO: 27503)

>nLuc315_bim339

MGSHHHHHHGGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLEL
VYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
35 DELQKLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
EAERAIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL
RELLRALAQLQELNLDLLRLASEL) TDPDEARKAIARVKVTGYRLFEEILRLsREAAAASEKIE**EIWIAQELRRIG**
40 **DEFNAYYAE** (SEQ ID NO: 27504)

>nLuc301_bim343

MGSHHHHHHGGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLEL
VYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
DELQKLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
45 EAERAIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL
RELLRALAQLQELNLDLLRLASEL) ggsVTGYRLFEEILRVKRESKRIVEDAERLsREAAAASEKISREAE**EIWIA**
QELRRIGDEFNAYYA (SEQ ID NO: 27505)

>nLuc308_bim343

MGSHHHHHHGGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLEL
VYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
DELQKLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
50 EAERAIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL
RELLRALAQLQELNLDLLRLASEL) TDPDEARVTGYRLFEEILRIVEDAERLsREAAAASEKISREAE**EIWIAQEL**
55 **RRIGDEFNAYYA** (SEQ ID NO: 27506)

>nLuc312_bim343

MGSHHHHHHGGSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLEL
VYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
60 DELQKLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
EAERAIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL

RELLRALAQLQELNLDLLRLASEL) TDPDEARKAIAVVTGYRLFEEILDDAERLsREA/
RRIGDEFNAYYA (SEQ ID NO: 27507)

>nluc315_bim343

5 **MGSHHHHHHSGSGSENLYFQSGG** (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLEL
 VYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
 DELQKLNLELAKLLLKAIAETQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
 EAERAIRAAKRESERIIEEARLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL
 RELLRALAQLQELNLDLLRLASEL) TDPDEARKAIAVVTGYRLFEEILRLsREAAAASEKISREAEIWIAQEL
 10 **RRIGDEFNAYYA** (SEQ ID NO: 27508)

lucCageTrop variants (cardiac Troponin I sensors)

- SmBit sequence: VTGYRLFEEIL (SEQ ID NO: 27359)

15 - Variants of cardiac troponin T (cTnT) used sequences:

- cTnTf1:226-**EDQLREKAKELWQTI**-240 (SEQ ID NO:27385)

- cTnTf2:226-**EDQLREKAKELWQTIYN**-242 (SEQ ID NO:27386)

- cTnTf3:226-**EDQLREKAKELWQTIYNLEAE**-246 (SEQ ID NO:27387)

- cTnTf4:226-**EDQLREKAKELWQTIYNLEAEKFD**-249 (SEQ ID NO:27388)

20 - cTnTf5:226-**EDQLREKAKELWQTIYNLEAEKFDLQE**-252 (SEQ ID NO:27389)

- cTnTf6:226- **EDQLREKAKELWQTIYNLEAEKFDLQEKFKQKYEINVLNRINDNQ**-272 (SEQ
 ID NO:27390)

-cTnC:

KVSKTKDDSKGKSEEELSDLFRMFMDKNADGYIDLEELKIMLQATGETITEDDIEELMKDGDKNNDG

25 **RIDYDEFLEFMKGVE** (SEQ ID NO:27627)

>336-cTnTf4-K342A (jp625_1fix_nluc312_cTnT336_K342A_359end)

30 **MGSHHHHHHSGSGSENLYFQSGG** (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLEL
 VYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
 DELQKLNLELAKLLLKAIAETQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
 EAERAIRAAKRESERIIEEARLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL
 RELLRALAQLQELNLDLLRLASEL) TDPDEARKAIAVVTGYRLFEEILDDAERLsREAAAA**EDQLREaAKELWQTI**
YNLEAEKFD (SEQ ID NO: 27509)

>336-cTnTf6-K342A (jp626_1fix-nluc312_cTnT336_K342A_362end)

35 **MGSHHHHHHSGSGSENLYFQSGG** (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLEL
 VYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
 DELQKLNLELAKLLLKAIAETQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
 EAERAIRAAKRESERIIEEARLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL
 RELLRALAQLQELNLDLLRLASEL) TDPDEARKAIAVVTGYRLFEEILDDAERLsREAAAA**EDQLREaAKELWQTI**
 40 **YNLEAEKFDLQE** (SEQ ID NO: 27510)

>336-cTnTf6-K342A (jp627_1fix-nluc312_cTnT336_K342A_0001_382end)

45 **MGSHHHHHHSGSGSENLYFQSGG** (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLEL
 VYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
 DELQKLNLELAKLLLKAIAETQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
 EAERAIRAAKRESERIIEEARLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL
 RELLRALAQLQELNLDLLRLASEL) TDPDEARKAIAVVTGYRLFEEILDDAERLsREAAAA**EDQLREaAKELWQTI**
YNLEAEKFDLQEKFKQKYEINVLNRINDNQ (SEQ ID NO: 27511)

50 >339-cTnTf3 (jp628_1fix-nluc312_cTnT339_359end)

MGSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAV
 ELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQK
 LNLELAKLLLKAIAETQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDAEARA
 IRAAKRESERIIEEARLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
 55 ALAQLQELNLDLLRLASEL) TDPDEARKAIAVVTGYRLFEEILDDAERLsREAAAASEK**EDQLREKAKELWQTIYN**
LEAE (SEQ ID NO: 27512)

>339-cTnTf5 (jp629_1fix-nluc312_cTnT339_0001_365end)

60 **MGSHHHHHHSGSENLYFQG** (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAV
 ELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQK
 LNLELAKLLLKAIAETQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDAEARA

IRAAKRESERIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLF
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARERLSREAAAASEI
LEAEKFDLQEKFKQKQKYEINVLRNRINDNQ (SEQ ID NO: 27513)

5 >339-cTnTf6 (jp630_1fix-nluc312_cTnT339_0001_385end)
MGSHHHHHHGSSENYFYQG(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAV
ELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQK
LNLELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERA
IRAAKRESERIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
10 ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARERLSREAAAASEKI**EDQLREKAKELWQTIYN**
LEAEKFDLQEKFKQKQKYEINVLRNRINDNQKFKQKQKYEINVLRNRINDNQ (SEQ ID NO: 27514)

>343-cTnTf2 (jp631_1fix-nluc312_cTnT343_359end)
MGSHHHHHHGSSENYFYQG(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAV
15 ELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQK
LNLELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERA
IRAAKRESERIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARERLSREAAAASEKISREA**EDQLREKAKELWQ**
20 **TIYN** (SEQ ID NO: 27515)

>343-cTnTf5 (jp632_1fix-nluc312_cTnT343_0001_369end)
MGSHHHHHHGSSENYFYQG(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAV
ELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQK
LNLELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERA
25 IRAAKRESERIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARERLSREAAAASEKISREA**EDQLREKAKELWQ**
TIYNLEAEKFDLQEKFKQKQKYEINVLRNRINDNQ (SEQ ID NO: 27516)

>343-cTnTf6 (jp633_1fix-nluc312_cTnT343_0001_389end)
MGSHHHHHHGSSENYFYQG(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAV
ELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQK
LNLELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERA
IRAAKRESERIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
30 ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARERLSREAAAASEKISREA
35 **EDQLREKAKELWQTIYNLEAEKFDLQEKFKQKQKYEINVLRNRINDNQKFKQKQKYEINVLRNRINDNQ** (SEQ ID
NO: 27517)

>345-cTnTf1 (jp634_1fix-nluc312_cTnT345_359end)
MGSHHHHHHGSSENYFYQG(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAV
40 ELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQK
LNLELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERA
IRAAKRESERIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARERLSREAAAASEKISREA**EDQLREKAKEL**
45 **WQTI** (SEQ ID NO: 27518)

>345-cTnTf5 (jp635_1fix-nluc312_cTnT345_0001_371end)
MGSHHHHHHGSSENYFYQG(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAV
ELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQK
LNLELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERA
50 IRAAKRESERIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARERLSREAAAASEKISREA**EDQLREKAKEL**
WQTIYNLEAEKFDLQEKFKQKQKYEINVLRNRINDNQ (SEQ ID NO: 27519)

>345-cTnTf6 (jp636_1fix-nluc312_cTnT345_0001_391end)
MGSHHHHHHGSSENYFYQG(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAV
ELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQK
LNLELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERA
IRAAKRESERIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
55 ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARERLSREAAAASEKISREA**EDQLREKAKEL**
60 **WQTIYNLEAEKFDLQEKFKQKQKYEINVLRNRINDNQKFKQKQKYEINVLRNRINDNQ** (SEQ ID NO: 27520)

>lucCageTrop
 MSHHHHHHSGSGSENLYFQGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRI
 VYLAVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDAL
 DELQKLNLELAKLLLKAIAETQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIID
 5 EAERAIRAAKRESERIEEARLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELL
 RELLRALAQLOELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEDQLREaAKELWQTI
 YNLEAEKFDLQEKFKQKYEINVLRNRINDNQKVSCTKDDSKGKSEEEELSDLFMRMFDKNADGYIDLEELKIMLQA
 TGETITEDDIEELMKDGDKNNDGRIDYDEFLEFMKGVE (SEQ ID NO: 27521)

10 **lucCageBot variants (Botulinum neurotoxin B sensors)**
 - Bot.0671.2 sequence: **MFAELKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE** (SEQ
 ID NO: 27381)

>BoNTB_338_1S
 15 GSHHHHHHSGSGSENLYFQGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
 ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEAR
 KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFLEA
 AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIDEAERAIRAAKRESERIEEA
 RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
 20 ALAQLOELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKMF
AE (SEQ ID NO: 27522)

> BoNTB_341_1S
 25 GSHHHHHHSGSGSENLYFQGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
 ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEAR
 KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFLEA
 AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIDEAERAIRAAKRESERIEEA
 RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
 30 ALAQLOELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISRM
FAELKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE* (SEQ ID NO: 27523)

>BoNTB_342_1S
 35 GSHHHHHHSGSGSENLYFQGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
 ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEAR
 KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFLEA
 AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIDEAERAIRAAKRESERIEEA
 RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
 40 ALAQLOELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISRE
MFAELKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE* (SEQ ID NO: 27524)

>BoNTB_345_1S
 45 GSHHHHHHSGSGSENLYFQGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
 ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEAR
 KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFLEA
 AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIDEAERAIRAAKRESERIEEA
 RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
 50 ALAQLOELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISRE
 AERM**FAELKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE*** (SEQ ID NO: 27525)

>BoNTB_348_2S
 55 GSHHHHHHSGSGSENLYFQGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
 ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEAR
 KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFLEA
 AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIDEAERAIRAAKRESERIEEA
 RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
 60 ALAQLOELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISRE
 AERSIRM**FAELKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE*** (SEQ ID NO: 27526)

>BoNTB_349_2S
 GSHHHHHHSGSGSENLYFQGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
 ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEAR

KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAI
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIDEAERAI RAAKRESERI
RRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISRE
AERSIRE**MFaelKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE*** (SEQ ID NO: 27527)

>BoNTB_352_2S
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIDEAERAI RAAKRESERIIEEA
RRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISRE
AERSIREAAA**MFaelKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE*** (SEQ ID NO: 27528)

>BoNTB_355_2S
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIDEAERAI RAAKRESERIIEEA
RRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISRE
AERSIREAAAASE**MFaelKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRKYELE*** (SEQ ID NO:
27529)

>BoNTB_GGG_2S
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIDEAERAI RAAKRESERIIEEA
RRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISRE
AERSIREAAAASEKISREGGG**MFaelKAKFFLEIGDRDAARNALRKAGYSDEEAERIIRK
YELE*** (SEQ ID NO: 27530)

>BoNTB_GGG_2S_fullBotBinder
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIDEAERAI RAAKRESERIIEEA
RRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISRE
AERSIREAAAASEKISREGGGSHMQ**MFaelKAKFFLEIGDRDAARNALRKAGYSDEEA
RIIRKYELE*** (SEQ ID NO: 27531)

lucCageProA variants (Fc domain biosensors)

- Staphylococcus aureus Protein A domain C (SpaC) sequence:
EQQNafYEILHLPLNLTeeQRNGFIQSLKDDPSVSKEILAEAKKLNDQAQPK (SEQ ID NO:27382)

>SpaC_360GGG
MGSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIDEAERAI RAAKRESERIIE
EARRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKIS
REAERSIREAAAASEKISREGGGFN**KEQQNafYEILHLPLNLTeeQRNGFIQSLKDDPSVSKEILAEAKKLNDQA
PK*** (SEQ ID NO: 27532)

>SpaC_354-2S
MGSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE

- AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKI
 ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAF
 EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
 EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
 5 LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKIS
 REAERSIREAAA**EQQNAFYEILHLPLNLTTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDQAQPK***
 (SEQ ID NO: 27533)
- >SpaC_351_2S
 10 **MGSHHHHHHSGSGSENLYFQG**(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
 AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
 ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFL
 EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
 EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
 15 LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKIS
 REAERSIREAA**EQQNAFYEILHLPLNLTTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDQAQPK***
 (SEQ ID NO: 27534)
- >SpaC_350_2S
 20 **MGSHHHHHHSGSGSENLYFQG**(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
 AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
 ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFL
 EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
 EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
 25 LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKIS
 REAERSIREA**EQQNAFYEILHLPLNLTTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDQAQPK***
 (SEQ ID NO: 27535)
- >SpaC_347_2S
 30 **MGSHHHHHHSGSGSENLYFQG**(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
 AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
 ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFL
 EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
 EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
 35 LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKIS
 REAERSI**EQQNAFYEILHLPLNLTTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDQAQPK***
 (SEQ ID NO: 27536)
- >SpaC_347_1S
 40 **MGSHHHHHHSGSGSENLYFQG**(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
 AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
 ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFL
 EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
 EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
 45 LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKIS
 REAERLI**EQQNAFYEILHLPLNLTTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDQAQPK***
 (SEQ ID NO: 27537)
- lucCageHer2 variants (Fc domain biosensors)**
- 50 - Her2 affibody sequence:
EMRNAYWEIALLPNLNNQOKRAFIRSLYDDPSQSANLLAEAKKLNDQAQPK (SEQ ID NO:27383)
- >AffiHer2_347_1S
 55 **MGSHHHHHHSGSGSENLYFQG**(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
 AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
 ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFL
 EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
 EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
 LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKIS
 60 REAERLI**EMRNAYWEIALLPNLNNQOKRAFIRSLYDDPSQSANLLAEAKKLNDQAQPK***
 (SEQ ID NO: 27538)

>AffiHer2_347_2S

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKIS
REAERSIREA**EMRNAYWEIALLPNLNNQQKRAFIRSLYDDPSQSANLLAEAKKLNDAPK***

(SEQ ID NO: 27539)

E

>AffiHer2_350_2S

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKIS
REAERSIREA**EMRNAYWEIALLPNLNNQQKRAFIRSLYDDPSQSANLLAEAKKLNDAPK***

(SEQ ID NO: 27540)

>AffiHer2_351_2S

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKIS
REAERSIREA**EMRNAYWEIALLPNLNNQQKRAFIRSLYDDPSQSANLLAEAKKLNDAPK***

(SEQ ID NO: 27541)

>AffiHer2_354-2S

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKIS
REAERSIREAAAA**SEEMRNAYWEIALLPNLNNQQKRAFIRSLYDDPSQSANLLAEAKKLNDAPK***

(SEQ ID NO: 27542)

>AffiHer2_360GGG

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKIS
REAERSIREAAAASEKISREGGVVDNKF**EMRNAYWEIALLPNLNNQQKRAFIRSLYDDPSQSANLLAEAKKLNDAPK***

(SEQ ID NO: 27543)

>AffiHer2_354-2S_2x1

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEE
ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKIS
REAERSIREAAAA**SEEMRNAYWEIALLPNLNNQQKRAFIRSLYDDPSQSANLLAEAKKLNDAPKGGGNKEMRNA**
YWEIALLPNLNNQQKRAFIRSLYDDPSQSANLLAEAKKLNDAPK* (SEQ ID NO: 27544)

>AffiHer2_354-2S_2x2

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
5 ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAASEKIS
10 REAERSIREAAAA**SEMRNAYWEIALLPNLNNQQKRAFIRSLYDDPSQSANLLAEAKKLNDQAQPKGGGNKEMRNA**
YWEIALLPNLNNQQKRAFIRSLYDDPSQSANLLAEAKKLNDQAQPK* (SEQ ID NO: 27545)

>AffiHer2_354-2S_3x

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLE
AIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEE
15 ARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFL
EAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIE
EARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAASEKIS
20 REAERSIREAAAA**SEMRNAYWEIALLPNLNNQQKRAFIRSLYDDPSQSANLLAEAKKLNDQAQPKGGGNKEMRNA**
YWEIALLPNLNNQQKRAFIRSLYDDPSQSANLLAEAKKLNDQAQPKGGGNKEMRNAYWEIALLPNLNNQQKRAFI
RSLYDDPSQSANLLAEAKKLNDQAQPK * (SEQ ID NO: 27546)

lucCageSARS2N variants (anti-SARS-CoV-2 Nucleocapsid protein antibodies sensors)

- SARS-Cov-2 Nucleocapsid protein epitope peptides used:
 - N6: **PKDKKKKKADETQALPQRQKGGSGGPKDKKKKKADETQALPQRQK** (SEQ ID NO:27547)
 - N62: **KKDKKKKKADETQALGGSGGKDKKKKKADETQAL** (SEQ ID NO:27548)

>lucCageSARS2-N6_368-388_339

GSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFLEA
35 AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAASEK**PKDKKKKKADETQALPQR**
QKGGSGGPKDKKKKKADETQALPQRQK* (SEQ ID NO: 27549)

>lucCageSARS2-N6_368-388_346

GSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
45 RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAASEKISRE
AERS**PKDKKKKKADETQALPQRQKGGSGGPKDKKKKKADETQALPQRQK*** (SEQ ID NO: 27550)

>lucCageSARS2-N6_368-388_353

GSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
55 ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAASEKISRE
AERSIREAAAA**PKKDKKKKKADETQALPQRQKGRADLRKTKRRKPTKPKHCRNVKKS** (SEQ ID NO: 27551)

>lucCageSARS2-N62_369-382_336

GSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRRAAKAFLEA

AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERI
 RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLR
 ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAA**SKDKKKKKADETQALGGSGGK**
KDKKKKADETQAL* (SEQ ID NO: 27552)

5

>lucCageSARS2-N62_369-382_340
 GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
 ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
 KAIRDAAEESRKILEEGSGSGSDALDELQKNLELAKLLLKAIAETQDLNLRRAAKAFLEA
 AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
 RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
 ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAA**SEKIKDKKKKKADETQALGGSGGK**
GGKKDKKKKADETQAL* (SEQ ID NO: 27553)

10

>lucCageSARS2-N62_369-382_343
 GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
 ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
 KAIRDAAEESRKILEEGSGSGSDALDELQKNLELAKLLLKAIAETQDLNLRRAAKAFLEA
 AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
 RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
 ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAA**SEKISREAKDKKKKKADETQALGGSGGK**
DKKKKKADETQAL* (SEQ ID NO: 27554)

15

20

>lucCageSARS2-N62_369-382_347
 GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
 ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
 KAIRDAAEESRKILEEGSGSGSDALDELQKNLELAKLLLKAIAETQDLNLRRAAKAFLEA
 AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
 RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
 ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAA**SEKISREAKDKKKKKADETQALGGSGGK**
DKKKKKADETQAL* (SEQ ID NO: 27555)

25

30

>lucCageSARS2-N62_369-382_350
 GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
 ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
 KAIRDAAEESRKILEEGSGSGSDALDELQKNLELAKLLLKAIAETQDLNLRRAAKAFLEA
 AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
 RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
 ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAA**SEKISREAKDKKKKKADETQALGGSGGK**
KDKKKKKADETQAL* (SEQ ID NO: 27556)

35

40

>lucCageSARS2-N62_369-382_354
 GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
 ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
 KAIRDAAEESRKILEEGSGSGSDALDELQKNLELAKLLLKAIAETQDLNLRRAAKAFLEA
 AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
 RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
 ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAA**SEKISREAKDKKKKKADETQALGGSGGK**
DKKKKKADETQALGGSGGKDKKKKKADETQAL* (SEQ ID NO: 27557)

45

50

lucCageSARS2M variants (anti-SARS-Cov-2 Membrane protein antibodies sensors)

- SARS-Cov-2 Membrane protein epitope peptides used:

- M1_1-31: **MADSNGTITVEELKKLLEQWNLVIGFLFTLWIGSGGMADSNGTITVEELKKLLEQWNLVIGFLFTWI**
 (SEQ ID NO: 27393)
- M3_1-17: **MADSNGTITVEELKKLLEGGSGGMADSNGTITVEELKKLLE** (SEQ ID NO: 27392)
- M4_8-24: **ITVEELKKLLEQWNLVIGSGGITVEELKKLLEQWNLVI** (SEQ ID NO: 27394)

55

>lucCageSARS2-M3_1-17_341
 GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
 ARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR

60

KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAI
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERI
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISR**MADSNGTITVEELKK**
5 **LLEGGSGGMADSNGTITVEELKKLLE*** (SEQ ID NO: 27558)

>lucCageSARS2-M3_1-17_343
GSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
10 KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISRE**MADSNGTITVEEL**
15 **KKLLEGGSGGMADSNGTITVEELKKLLE*** (SEQ ID NO: 27559)

>lucCageSARS2-M3_1-17_348
GSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
20 KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISRE**MADSNGTI**
25 **TVEELKKLLEGGSGGMADSNGTITVEELKKLLE*** (SEQ ID NO: 27560)

>lucCageSARS2-M3_1-17_350
GSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
30 KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISRE**MADSN**
35 **GTITVEELKKLLEGGSGGMADSNGTITVEELKKLLE*** (SEQ ID NO: 27561)

>lucCageSARS2-M4_8-24_334
GSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
35 KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
40 ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAI**TVEELKKLLEQWNLVIGGSGG**
45 **ITVEELKKLLEQWNLVI*** (SEQ ID NO: 27562)

>lucCageSARS2-M4_8-24_340
GSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
45 KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
50 ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKIS**ITVEELKKLLEQWNLV**
55 **IGGSGGITVEELKKLLEQWNLVI*** (SEQ ID NO: 27563)

>lucCageSARS2-M4_8-24_341
GSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAI
ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEAR
55 KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIIEEA
RRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
60 ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD AERLSREAAAASEKISR**ITVEELKKLLEQWNL**
65 **VIGGSGGITVEELKKLLEQWNLVI*** (SEQ ID NO: 27564)

>lucCageSARS2-M4_8-24_348

GSHHHHHHGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEAI
ARLQELNLELVYLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILF
KAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEA
AAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKRESERIEEA
5 RRLIEKAKEESERIEEGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLR
ALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAASEKISREASIRITVEELKK
LLEQWNLVIGSGGITVEELKKLLEQWNLVI* (SEQ ID NO: 27565)

>lucCageM3_334_SmBit_position301
10 GSHHHHHHSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELV
YLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALD
ELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDE
AERAIRAAKRESERIEEAARLIEKAKEESERIEEGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLR
ELLRALAQLQELNLDLLRLASEL) ggsVTGYRLFEEILRVKRESKRIVEDARLSREAAAMADSNGTITVEELKK
15 LLEGGSGGMADSNGTITVEELKKLLE (SEQ ID NO: 27566)

>lucCageM3_334_SmBit_position308
GSHHHHHHSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELV
YLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALD
20 ELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDE
AERAIRAAKRESERIEEAARLIEKAKEESERIEEGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLR
ELLRALAQLQELNLDLLRLASEL) TDPDEARVTGYRLFEEILRVKRESKRIVEDARLSREAAAMADSNGTITVEELKKLLE
GGSGGMADSNGTITVEELKKLLE (SEQ ID NO: 27567)

>lucCageM3_334_7loop
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIEEAARLIEKAKEESERIEEGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
30 RALAQLQELNLDLLRLASEL) TDGGSGGGPDEARKAIAVTGYRLFEEILDARLSREAAAMADSNGTITVEELK
KLLEGGSGGMADSNGTITVEELKKLLE (SEQ ID NO: 27568)

>lucCageM3_334_3loop
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
35 KLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIEEAARLIEKAKEESERIEEGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQLQELNLDLLRLASEL) TDGGGPDEARKAIAVTGYRLFEEILDARLSREAAAMADSNGTITVEELKKLLE
LSREAAAMADSNGTITVEELKKLLEGGSGGMADSNGTITVEELKKLLE (SEQ ID NO: 27569)
40

>lucCageM3_341_SmBit_position301
GSHHHHHHSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELV
YLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALD
45 ELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDE
AERAIRAAKRESERIEEAARLIEKAKEESERIEEGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLR
ELLRALAQLQELNLDLLRLASEL) ggsVTGYRLFEEILRVKRESKRIVEDARLSREAAAASEKISRMADSNGTI
TVEELKKLLEGGSGGMADSNGTITVEELKKLLE (SEQ ID NO: 27570)

>lucCageM3_341_SmBit_position308
GSHHHHHHSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELV
YLAVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALD
50 ELQKLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDE
AERAIRAAKRESERIEEAARLIEKAKEESERIEEGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLR
ELLRALAQLQELNLDLLRLASEL) TDPDEARVTGYRLFEEILRVKRESKRIVEDARLSREAAAASEKISRMADSNGTITVE
55 ELKKLLEGGSGGMADSNGTITVEELKKLLE (SEQ ID NO: 27571)

>lucCageM3_341_7loop
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
60 KLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER

AIRAAKRESERIIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELI
RALAQLQELNLDLLRLASEL) TDGGGSGGGPDEARKAIAVTGYRLFEEILDAERLSI
ITVEELKKLLEGGSGGMADSNGTITVEELKKLLE (SEQ ID NO: 27572)

5 >lucCageM3_341_3loop
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
10 RALAQLQELNLDLLRLASEL) TDGGGPDEARKAIAVTGYRLFEEILDAER
LSREAAAASEKISRMADSNGTITVEELKKLLEGGSGGMADSNGTITVEELKKLLE (SEQ ID NO: 27573)

>LUCCAGEM3_334_4copies
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
15 VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREAAAAMADSNGTITVEELKKL
20 EGGSGGMADSNGTITVEELKKLLEGGSGGMADSNGTITVEELKKLLEGGSGGMADSNGTITVEELKKLLE*
(SEQ ID NO: 27574)

>LUCCAGEM3_337_4copies
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
25 VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREAAAASEMADSNGTITVEELK
KLLEGGSGGMADSNGTITVEELKKLLEGGSGGMADSNGTITVEELKKLLEGGSGGMADSNGTITVEELKKLLE
30 (SEQ ID NO: 27575)

>LUCCAGEM3_341_4copies
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
35 AIRAAKRESERIIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREAAAASEKISRMADSNGTITV
EELKKLLEGGSGGMADSNGTITVEELKKLLEGGSGGMADSNGTITVEELKKLLEGGSGGMADSNGTITVEELKKL
LE (SEQ ID NO: 27576)

40 >LUCCAGEM3_348_4copies
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
45 RALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREAAAASEKISREASERSIRMAD
SNGTITVEELKKLLEGGSGGMADSNGTITVEELKKLLEGGSGGMADSNGTITVEELKKLLEGGSGGMADSNGTIT
VEELKKLLE (SEQ ID NO: 27577)

>LUCCAGEM3_334_2copiesnolinker
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
50 VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREAAAAMADSNGTITVEELKKL
55 EMADSNGTITVEELKKLLE (SEQ ID NO: 27578)

>LUCCAGEM3_337_2copiesnolinker
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
60 VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL

RALAQLOELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREA/
KLEEMADSNGTITVEELKKLLE (SEQ ID NO: 27579)

>LUCCAGEM3_341_2copiesnolinker

GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAI AETQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQLOELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREAAAASEKISRMADSNGTITV
EELKKLLEEMADSNGTITVEELKKLLE (SEQ ID NO: 27580)

>LUCCAGEM3_348_2copiesnolinker

GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAI AETQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQLOELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREAAAASEKISREAERSIRMAD
SNGTITVEELKKLLEEMADSNGTITVEELKKLLE (SEQ ID NO: 27581)

>LUCCAGEM3_334_4copies_linker

GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAI AETQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQLOELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREAAAMADSNGTITVEELKKLL
EGGSGGMADSNGTITVEELKKLLEGGSGGGSGGSGGSGGMADSNGTITVEELKKLLEGGSGGMADSNGTITVEEL
KKLLE (SEQ ID NO: 27582)

>LUCCAGEM3_337_4copies_linker

GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAI AETQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQLOELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREAAAASEMADSNGTITVEELK
KLEGGSGGMADSNGTITVEELKKLLEGGSGGGSGGSGGSGGMADSNGTITVEELKKLLEGGSGGMADSNGTITV
EELKKLLE (SEQ ID NO: 27583)

>LUCCAGEM3_341_4copies_linker

GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAI AETQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQLOELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREAAAASEKISRMADSNGTITV
EELKKLLEGGSGGMADSNGTITVEELKKLLEGGSGGGSGGSGGSGGMADSNGTITVEELKKLLEGGSGGMADSN
GTITVEELKKLLE (SEQ ID NO: 27584)

>LUCCAGEM3_348_4copies_linker

GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAI AETQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQLOELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREAAAASEKISREAERSIRMAD
SNGTITVEELKKLLEGGSGGGSGGSGGSGGMADSNGTITVEELKKLLEGGSGGMADSNGTITVEELKKLLEGGSG
GMADSNGTITVEELKKLLE (SEQ ID NO: 27585)

>LUCCAGEM3_334_2copies_linker_SpaC_Z

GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAI AETQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL

RALAQQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREAF
EGGSGGMADSNGTITVEELKKLLEGGGGSGGGSGGGSGGGSGGNKFNKEQQNAFYI
LKDDPSVSKEILAEAKKLNDQAAPKGGVDNKNFNKEQQNAFYEILHLPNLNEEQRNAFIQSLKDDPSQSANLLAEA
KKLNDQAAPK (SEQ ID NO: 27586)

>LUCCAGEM3_337_2copies_linker_SpaC_Z
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREAAAASEMADSNGTITVEELK
KLLEGGSGGMADSNGTITVEELKKLLEGGGGSGGGSGGGSGGGSGGGSGGNKFNKEQQNAFYEILHLPNLTEEQRNGF
IQSLKDDPSVSKEILAEAKKLNDQAAPKGGVDNKNFNKEQQNAFYEILHLPNLNEEQRNAFIQSLKDDPSQSANLL
AEAKKLNDQAAPK (SEQ ID NO: 27587)

>LUCCAGEM3_341_2copies_linker_SpaC_Z
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREAAAASEKISRMADSNGTITV
EELKKLLEGGSGGMADSNGTITVEELKKLLEGGGGSGGGSGGGSGGGSGGGSGGNKFNKEQQNAFYEILHLPNLTEEQ
RNGFIQSLKDDPSVSKEILAEAKKLNDQAAPKGGVDNKNFNKEQQNAFYEILHLPNLNEEQRNAFIQSLKDDPSQS
ANLLAEAKKLNDQAAPK (SEQ ID NO: 27588)

>LUCCAGEM3_348_2copies_linker_SpaC_Z
GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
KLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
AIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERDAERLSREAAAASEKISREAERSIRMAD
SNGTITVEELKKLLEGGSGGMADSNGTITVEELKKLLEGGGGSGGGSGGGSGGGSGGGSGGNKFNKEQQNAFYEILHL
PNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDQAAPKGGVDNKNFNKEQQNAFYEILHLPNLNEEQRNAFIQSL
KDDPSQSANLLAEAKKLNDQAAPK (SEQ ID NO: 27589)

lucCageRBD variants (SARS-CoV2 Spike Protein Receptor binding domain (RBD) biosensors)

- LCB1: **DKEWILQKIYEIMRLLDDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVEER** (SEQ ID NO: 27397)
- LCB1_delta4: **ILQKIYEIMRLLDDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVEER** (SEQ ID NO: 27590)

>lucCageRBD_336
MGSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYL
AVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDEL
QKLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDAE
RAIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAA**DKEWILQKIYEIMRLDDE**
LGHAESMRVSDLIYEFMKGDERLLEEAERLLEEVEER* (SEQ ID NO: 27591)

>lucCageRBD_340
MGSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYL
AVELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDEL
QKLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDAE
RAIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKIS**DKEWILQKIYEIMR**
LLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVEER* (SEQ ID NO: 27592)

>lucCageRBD_344

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAE/
AVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEARKAIRDAAEE:
QKLNLELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEI IDEAE
RAIRAAKRESERI IEEARRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
5 LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAEDKEWILQKIY
EIMRLLDELGHAEASMRVSDLIYEFMKKGDERLLEEAERLLEEVEVER* (SEQ ID NO: 27593)

>lucCageRBD_347

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYL
10 AVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDEL
QKLNLELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEI IDEAE
RAIRAAKRESERI IEEARRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAERSIDKEWILQ
15 KIYEIMRLLDELGHAEASMRVSDLIYEFMKKGDERLLEEAERLLEEVEVER* (SEQ ID NO: 27594)

>lucCageRBD_351

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYL
20 AVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDEL
QKLNLELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEI IDEAE
RAIRAAKRESERI IEEARRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAERSIREAADKE
25 WILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKKGDERLLEEAERLLEEVEVER* (SEQ ID NO: 27595)

>lucCageRBD_354

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYL
30 AVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDEL
QKLNLELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEI IDEAE
RAIRAAKRESERI IEEARRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAERSIREAAAAS
35 DKEWILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKKGDERLLEEAERLLEEVEVER* (SEQ ID NO:
27596)

>lucCageRBD_GGG_360

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYL
35 AVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDEL
QKLNLELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEI IDEAE
RAIRAAKRESERI IEEARRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAERSIREAAAAS
40 EKISREGGGDKEWILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKKGDERLLEEAERLLEEVEVER* (SEQ ID
NO: 27597)

>lucCageRBDdelta4_336

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYL
45 AVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDEL
QKLNLELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEI IDEAE
RAIRAAKRESERI IEEARRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASILQKIYEIMRLLDELGHA
EASMRVSDLIYEFMKKGDERLLEEAERLLEEVEVER* (SEQ ID NO: 27598)

>lucCageRBDdelta4_340

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYL
50 AVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDEL
QKLNLELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEI IDEAE
RAIRAAKRESERI IEEARRLIEKAKEESERI IREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
LRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISILQKIYEIMRLLDE
55 LGHAEASMRVSDLIYEFMKKGDERLLEEAERLLEEVEVER* (SEQ ID NO: 27599)

>lucCageRBDdelta4_344

MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYL
60 AVELTDPKRIRDEI KEVKDKSKEI IRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDEL
QKLNLELAKLLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEI IDEAE

RAIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELAREI
 LRALAQQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAAA:
LLDELGHAEASMRVSDLIYEFMKKGDERLLEEAEERLLEEVEVER* (SEQ ID NO: 21600)

5 >lucCageRBDdelta4_347
 MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYL
 AVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDEL
 QKLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDAE
 10 RAIIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
 LRALAQQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAAASEKISREAEERSI**ILQKIYE**
IMRLDELGHAEASMRVSDLIYEFMKKGDERLLEEAEERLLEEVEVER* (SEQ ID NO: 27601)

>lucCageRBDdelta4_348
 MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYL
 15 AVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDEL
 QKLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDAE
 RAIIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
 LRALAQQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAAASEKISREAEERSI**ILQKIY**
EIMRLDELGHAEASMRVSDLIYEFMKKGDERLLEEAEERLLEEVEVER* (SEQ ID NO: 27602)

20 >lucCageRBDdelta4_351
 MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYL
 AVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDEL
 25 QKLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDAE
 RAIIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
 LRALAQQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAAASEKISREAEERSI**REAAILQ**
KIYIEMRLDELGHAEASMRVSDLIYEFMKKGDERLLEEAEERLLEEVEVER* (SEQ ID NO: 27603)

>lucCageRBDdelta4_354
 30 MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYL
 AVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDEL
 QKLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDAE
 RAIIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
 LRALAQQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAAASEKISREAEERSI**REAAAA**
 35 **ILQKIYIEMRLDELGHAEASMRVSDLIYEFMKKGDERLLEEAEERLLEEVEVER*** (SEQ ID NO: 27604)

>lucCageRBDdelta4_357
 MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYL
 40 AVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDEL
 QKLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDAE
 RAIIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
 LRALAQQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAAASEKISREAEERSI**REAAAA**
 EK**ILQKIYIEMRLDELGHAEASMRVSDLIYEFMKKGDERLLEEAEERLLEEVEVER*** (SEQ ID NO: 27605)

45 >lucCageRBDdelta4_GGG_360
 MGSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYL
 AVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDEL
 50 QKLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDAE
 RAIIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLREL
 LRALAQQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAAASEKISREAEERSI**REAAAA**
 EKISREGGG**ILQKIYIEMRLDELGHAEASMRVSDLIYEFMKKGDERLLEEAEERLLEEVEVER*** (SEQ ID NO:
 27606)

55 >lucCageRBD_348_d4LCB1v1.3
 GSHHHHHHSGSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
 VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
 KLNLELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDAE
 AIRAAKRESERIEEARRLIEKAKEESERIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
 60 RALAQQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDARLSREAAAAASEKISREAEERSI**IRILQKIYE**
IMKTLEQLGHAEASMQVSDLIYEFMKQGDERLLEEAEERLLEEVEVER* (SEQ ID NO: 27607)

> lucCageRBD_delta4_348
 GSHHHHHHSGSGSENLYFQG(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
 VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
 5 KLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
 AIRAAKRESERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
 RALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAERSIRILQKIYE
 IMRLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVE (SEQ ID NO: 27608)

>lucCageRBD_smbit128
 GSHHHHHHSGSGSENLYFQG(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
 VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
 KLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
 10 AIRAAKRESERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
 15 RALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAERSIRILQKIYE
 IMRLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVE (SEQ ID NO: 27609)

>lucCageRBD_smbit99
 GSHHHHHHSGSGSENLYFQG(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
 20 VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
 KLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
 AIRAAKRESERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
 RALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLSREAAAASEKISREAERSIRILQKIYE
 25 IMRLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVE (SEQ ID NO: 27610)

>lucCageRBD_smbit86
 GSHHHHHHSGSGSENLYFQG(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
 VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
 KLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
 30 AIRAAKRESERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
 RALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGwRlFkIsDAERLSREAAAASEKISREAERSIRILQKIYE
 IMRLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVE (SEQ ID NO: 27611)

>lucCageRBD_smbit104
 GSHHHHHHSGSGSENLYFQG(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
 35 VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
 KLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
 AIRAAKRESERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
 RALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGyRlFkIsDAERLSREAAAASEKISREAERSIRILQKIYE
 40 IMRLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVE (SEQ ID NO: 27612)

>lucCageRBD_smbit101
 GSHHHHHHSGSGSENLYFQG(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
 VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
 45 KLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
 AIRAAKRESERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
 RALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRlFkEsDAERLSREAAAASEKISREAERSIRILQKIYE
 IMRLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVE (SEQ ID NO: 27613)

>lucCageRBD_smbit_Y315W_E320K
 GSHHHHHHSGSGSENLYFQG(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
 VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
 KLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
 AIRAAKRESERIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
 55 RALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGwRlFkILDAERLSREAAAASEKISREAERSIRILQKIYE
 IMRLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVE (SEQ ID NO: 27614)

>lucCageRBD_smbit_Y315W_E319K
 GSHHHHHHSGSGSENLYFQG(SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
 60 VELTDPKRIRDEI KEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
 KLNLELAKLLKAI AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER

AIRAAKRESERIIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELI
 RALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGwRLfKEILDAERLSREAAAAASI
 IMRLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVEE (SEQ ID NO: 27615)

- 5 >lucCageRBD_smbit_E319K
 GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
 VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
 KLNLELAKLLLKAIATQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
 AIRAAKRESERIIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
 10 RALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLfKEILDAERLSREAAAAASEKISREAERSIRILQKIYE
 IMRLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVEE (SEQ ID NO: 27616)
- >lucCageRBD_SmBit_position301
 GSHHHHHHSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELV
 15 YLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALD
 ELQKLNLELAKLLLKAIATQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDE
 AERAIRAAKRESERIIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLR
 ELLRALAQLQELNLDLLRLASEL) ggsVTGYRLFEEILRVKRESKRIVEDAERLSREAAAAASEKISREAERSIRI
 LQKIYEIMRLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVEE (SEQ ID NO: 27617)
- 20 >lucCageRBD_SmBit_position308
 GSHHHHHHSGSENLYFQSGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELV
 YLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALD
 ELQKLNLELAKLLLKAIATQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDE
 25 AERAIRAAKRESERIIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLR
 ELLRALAQLQELNLDLLRLASEL) TDPDEARVTGYRLFEEILRIVEDAERLSREAAAAASEKISREAERSIRILQK
 IYEIMRLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVEE (SEQ ID NO: 27618)
- >lucCageRBD_loop
 30 GSHHHHHHSGSENLYFQG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLA
 VELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQ
 KLNLELAKLLLKAIATQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAER
 AIRAAKRESERIIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
 RALAQLQELNLDLLRLASEL) TDGSGSGPDEARKAIAVTGYRLFEEILDAERLSREAAAAASEKISREAERSIRIL
 35 QKIYEIMRLDELGHAEASMRVSDLIYEFMKGDERLLEEAERLLEEVEE (SEQ ID NO: 27619)
- 40 LacATrop (split β -lactamase A in bold; underline cTnT and cTnC):
MGSHHHHHHSGSENLYFQG (SGGS**VFAHPETLVK VKDAEDQLGA RVGYIELDLN**
SGKILESFRP EERFPMMSTF KVLICGAVLS RVDAGQEQLG RRIHYSQNDL
VEYSPVTEKH LTDGMTVREL CSAAITMSDN TAANLLLT TI GGPKELTAFI
HNMGDHVT RL DRWEP E LNEA IPNDERD TTT PAAMATTLRK LLTG ENGR
 45 SGGGSGGGGSGGGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELT
 DPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN
 ELAKLLLKAIATQDLNLRRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRA
 AKRESERIIIEEARRLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALA
 QLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILDAERLIREAAAAASEDQLREAAKELWQTIYNLEAEKF
 50 DLQEKFKQKYEINVLNRNINDNQVSKTKDDSKGKSEEEISDLFRMFDKNADGYIDLEELKIMLQATGETITED
 DIEELMKDGDKNNDGRIDYDEFLEFMKGVE (SEQ ID NO: 27620)

- In another aspect, the disclosure provides key proteins capable of binding to the
 55 structural region of a cage protein of any embodiment or combination of embodiments
 disclosed herein that does not include the second reporter protein domain, wherein binding of
 the key protein to the cage protein only occurs in the presence of a target to which the cage

protein one or more target binding polypeptide can bind, wherein the key protein second reporter protein domain, wherein interaction of the key protein second reporter protein domain and the cage protein first reporter protein domain causes a detectable change in reporting activity from the first reporter protein domain.

As disclosed herein, the key proteins of this aspect can be used, for example, in conjunction with the cage polypeptides to displace the latch through competitive intermolecular binding that induces conformational change, leading to interaction of the key protein second reporter protein domain and the cage protein first reporter protein domain causes a detectable change in reporting activity from the first reporter protein domain.

In one embodiment, wherein the second reporter protein domain is at the N-terminus or the C-terminus of the key protein, or is within 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid of the N-terminus or the C-terminus of the key protein.

In another embodiment, the second reporter protein domain comprises a reporter protein domain selected from the group consisting of luciferase (including but not limited to firefly, Renilla, and Gaussia luciferase), bioluminescence resonance energy transfer (BRET) reporters, bimolecular fluorescence complementation (BiFC) reporters, fluorescence resonance energy transfer (FRET) reporters, colorimetry reporters (including but not limited to β -lactamase, β -galactosidase, and horseradish peroxidase), cell survival reporters (including but not limited to dihydrofolate reductase), electrochemical reporters (including but not limited to APEX2), radioactive reporters (including but not limited to thymidine kinase), and molecular barcode reporters (including but not limited to TEV protease). In various non-limiting embodiments, the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:27360-23379, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence, and wherein any N-terminal methionine residue may be present or absent.

In another embodiment, the key protein, not including the second reporter protein domain, comprises an amino acid sequence at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical, not including optional amino acid residues, to the amino acid sequence of a key polypeptide disclosed in US20200239524 (or WO2020/018935), or a key polypeptide selected from the group consisting of SEQ ID NOS:14318-26601, 26602-27015, 27016-

27050, 27,322 to 27,358, and key polypeptides with an odd-numbered SEQ ID NOS: 27127 and 27277), Table 3 (table 8 herein), and/or Table 4 (table 9 herein) or WO2020/018935.

In a further embodiment, the key protein comprises an amino acid sequence at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical, not including optional amino acid residues in parentheses, to the amino acid sequence of a key protein selected from the group consisting of SEQ ID NOS: 27621-27623, wherein residues in parentheses are optional and may be present or absent.

> lucKey: MGS-(His)6-TEV site-linker-LgBit-linker-latch sequence

(MGSHHHHHHSGSGSENLYFQG) SGMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQIRIVRSGE
NALKIDIHIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTNMLNYFGRPYEGIAVFDGKKI
TVTGTLWNGNKIIDERLITPDGSMLEFRVTINSGSGGGGGSGSGSGSDEARKAIARVKRESKRIVEDAERLIREA
AAASEKISREAERLIREAAAAASEKISRE (SEQ ID NO: 27621)

Key-2GGSGG-CyOFF (CyOFF sequence in bold/underline):

(M) DPDEARKAIARVKRESKRIVEDAERLIREAAAAASEKISREAERLIREAAAAASEKISREGGSGG **GGVSK**
GEELIK ENMRSKLYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV EGGPLPFAFD ILATHFMYGS
KVFIKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ DTSIQDGLI YNVKVRGVNF
PANGPVMQKK TLGWEPSTET MYPADGGLEG RCDKALKLVG GGHLHVNFKT TYKSKPKVKM
PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSNLGGGMD ELYK (SEQ ID NO: 27622)

Key-LacB (split β -lactamase B in bold/underline):

SGSGDPDEARKAIARVKRESKRIVEDAERLIREAAAAASEKISREAERLIREAAAAASEKISRESGGGGSGGGSGG
GG **LLTLASRQQLIDWME ADKVAGPLL R SALPAGWFIA DKSGAGERGS RGIIAALGPD GKPSRIVVIY**
TTGSQATMDE RNRQIAEIGA SLIKHW (SEQ ID NO: 27623)

In another aspect, the disclosure provides a biosensor, comprising (a) a cage protein of any embodiment or combination of embodiments herein, wherein the cage does not include the second reporter protein domain; and (b) the key protein of embodiment or combination of embodiments herein; wherein the key protein can only bind to the cage protein in the presence of a target to which the cage protein one or more target binding polypeptide can bind; and wherein binding of the first reporter protein domain of the cage protein to the second reporter protein domain of the key protein causes a detectable change in reporting activity from the first reporter protein domain.

As described herein the inventors have developed an inverted LOCKR system exemplified by a cage protein comprising a structural region and a latch region containing a first reporter protein domain and one or more target binding polypeptide (sometimes referred to as an analyte binding motif/target epitope in the examples), and a key protein which contains the second reporter protein domain linked to a key peptide. This system has at least

three important states (**Figure 1C**). *State 1* is a closed OFF state in which the latch region interacts with the target region, sterically occluding the one or more target binding polypeptide from binding its target and the first reporter protein domain from combining with the second reporter protein domain to reconstitute reporter protein activity. *States 2 or 3* are open states in which these binding interactions are not blocked, and the key protein can bind the cage protein structural domain. *State 7* is a stable ON state established when tri-molecular association of key protein with cage protein structural domain and the one or more target polypeptide with its target results in reconstitution of reporter protein activity. Mixing the cage protein with either a key protein or target alone is not sufficient to activate reporter activity. Both key protein and target together in the same solution with the cage protein results in reconstitution of reporter protein activity. Strong latch region-target interaction provides the driving force to populate the ON *State 7* (signal) over *State 6* (background). Further details are provided in the examples that follow.

As discussed above, the detectable change may be any increase or a decrease in the relevant reporting activity, as deemed suitable for an intended purpose. In various non-limiting embodiments, the detectable change in reporting activity may include, but is not limited to:

- The first reporter protein domain is a split fluorescent or luminescent protein domain that emits no fluorescence/luminescence, or detectably less fluorescence/luminescence then when bound to the second split reporter protein domain.
- The first and second reporter protein domains are BRET or FRET pairs that emit detectable signal at different wavelengths when bound to each other versus when not bound to each other.
- Cell survival selection by dihydrofolate reductase (DHFR) complementation in the presence of chosen target, when the first and second reporter protein domains reconstitute DHFR activity.
- Next generation sequencing as the readout to profile chemical or genetic perturbations on target-selective pathway when the first and second reporter protein domains reconstitute TEV protease activity for use as a molecular barcode.
- Positron emission tomography (PET) when the first and second reporter protein domains reconstitute thymidine kinase.

- Electrochemical readout when the first and second reporter protein domains reconstitute APEX2 activity.
- Colorimetry readout when the first and second reporter protein domains reconstitute beta-lactamase or horseradish peroxidase activity.

5

In various embodiments of the biosensor of the disclosure:

- (a) the first reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence SEQ ID NO: 27359, and 27664-27672

10

;

and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO 27379, wherein the N-terminal methionine residue may be present or absent

15

- (b) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27360, and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27361;

20

- (c) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27362, and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:27363-27365;

25

- (d) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27366, and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO 27368:

30

(e) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27367, wherein the N-terminal methionine residue may be present or absent, and the other reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27368, wherein the N-terminal methionine residue may be present or absent;

(f) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27369, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence; and the other reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27370, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence;

(g) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27371, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence, and the other reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27372, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence;

(h) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27373, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence, and the other reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27374, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence;

(i) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27375, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence, and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27376, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence;

(j) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27377, wherein the N-terminal methionine residue may be present or absent, and wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence, and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27378, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence.

In one specific embodiment of the biosensor, the cage protein comprises a cage protein comprising an amino acid sequence at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical, not including optional amino acid residues, to the amino acid sequence of a cage protein listed in Table 10, wherein the N-terminal protein purification tag

(MGSHHHHHHGSSENLYFQGG (SEQ ID NO:27624); or MGSHHHHHHGSSENLYFQG (SEQ ID NO:27625); or GSHHHHHHGSSENLYFQG (SEQ ID NO:27626)) is optional, and can be present or absent, and the key protein comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical, not including optional amino acid residues in parentheses, to the amino acid sequence of SEQ ID NO:27621.

> lucKey: MGS-(His)6-TEV site-linker-LgBit-linker-latch sequence

(MGSHHHHHHGSSENLYFQG) SGMVFTLEDVFGDWEQTAAYNLDQVLEQGGVSSLLQNLASVTPIQRIVRSGENALKIDIHIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKI

TVTGTLLWNGNKIIDERLITPDGSMLFRVTINSGGGGGGGGGSGGGSDEARKAIARV
AAASEKISREAERLIREAAAASEKISRE (SEQ ID NO: 27621)

In another specific embodiment of the biosensor, the cage protein and the key protein comprise a protein pair comprising:

5 (i) a cage protein comprising an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27620, wherein the residues in parentheses are optional and may be present or absent:

10 LacATrop (split β -lactamase A in bold; underline cTnT and cTnC):
(MGSHHHHHHGSSENLYFQG SGGs)**VFAHPETLVK VKDAEDQLGA RVGYIELDLN**
SGKILESFRP EERFPMSTF KVLICGAVLS RVDAGQEQLG RRIHYSQNDL
VEYSPVTEKH LTDGMTVREL CSAAITMSDN TAANLLLTIT GGPKELTAFI
HNMGDHVTRL DRWEPELNEA IPNDERDTT PAAMATTLRK LLTGENGR

15 SGGGSGGGGSGGGGSKEAAKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTD
PKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAEEESRKILEEGSGSGSDALDELQKLNLE
LAKLLLKAIATQDLNLRAAKAFLEAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAI RAA
KRESERIEEARLIEKAKEESERIEGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALAQ
LQELNLDLLRLASELTDPEARKAIAVTGYRLFEEILDAERLIREAAAASEDQLREAAKELWQTIYNLEAEKFDL

20 QEKFKQQKYEINVLNRINDNQKVSCTKDDSKGKSEELSDFRMFDKNADGYIDLEELKIMLQATGETITEDDI
EELMKDGDKNNDGRIDYDEFLEFMKGVE (SEQ ID NO:27620); and

(ii) a key protein comprising an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27361;

25 LLTLASRQQOLIDWME ADKVAGPLL R SALPAGWFIA DKSGAGERGS RGIIAALGPD GKPSRIVVIY
TTGSQATMDE RNRQIAEIGA SLIKHW (SEQ ID NO:27361)

In another aspect, the disclosure provides methods for detecting a target, comprising

(a) contacting the cage protein of any embodiment disclosed herein where the cage protein comprises the second reporter protein domain, or the biosensor of any embodiment herein with a biological sample under conditions to promote binding of the cage protein one or more target binding polypeptide to a target present in the biological sample, causing a detectable change in reporting activity from the first reporter protein domain; and

(b) detecting the change in reporting activity from the reporter protein domain, wherein the change in reporting activity identifies the sample as containing the target.

As described above, the inventors have developed an inverted LOCKR system exemplified by a cage protein comprising a structural region and a latch region containing a

first reporter protein domain and one or more target binding polypeptide to as an analyte binding motif/target epitope in the examples), and a key protein which contains the second reporter protein domain linked to a key peptide. As also discussed above, the detectable change may be any increase or a decrease in the relevant reporting activity, as deemed suitable for an intended purpose. Various non-limiting embodiments of the detectable change in reporting activity are described above, and methods for detecting such detectable changes are exemplified in detail in the examples that follow. Based on the teachings herein, those of skill in the art can determine the appropriate technique for measuring a detectable change of interest.

As exemplified in Figure 19 and discussed in example 3, the methods can accommodate an "indirect detection" approach, in which the reporter protein (intermolecular (second reporting domain in cage protein) or intramolecular (second reporter protein on key) embodiments; is reconstituted by pre-incubation of the biosensor with the target for the target binding polypeptide, resulting in restoration of reporter activity. The activated biosensor is then incubated with a sample to detect the presence of an target to which the one or more target binding polypeptide binds, resulting in binding of the target to the one or more target binding polypeptide, loss of interaction between the reporter protein components, and reduction/elimination of reporting activity.

Any suitable biological sample may be used, including but not limited to blood, serum, saliva, urine, semen, vaginal fluid, lymph, tissue fluid, digestive fluid, sweat, tears, nasal discharge, amniotic fluid, and breast milk.

Any target may be detected as deemed appropriate for an intended use and for which one or more target binding polypeptide is available for inclusion in the cage protein. In non-limiting embodiments, the target is selected from the group including but not limited to an antibody, a toxin, a diagnostic biomarker, a viral particle, or a disease biomarker. In one specific embodiment, the target is an antibody. In a further embodiment, the target comprises antibodies selective for a virus. In various such embodiments, the one or more target binding polypeptide may comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 27292-27394 and 27547-27548, and a polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 27397-27494. In these embodiments, the methods may be

used to detect the presence of antibodies against a SARS coronavirus, i
or SARS-CoV-2.

In various further embodiments, the cage polypeptide comprises the amino acid
sequence at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%,
5 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical, not including optional amino acid
residues, to the amino acid sequence of a cage protein listed in Table 10.

In another embodiment, the target is a disease marker or toxin. In one such
embodiment, the disease marker or toxin comprises Bcl-2, Her2 receptor, Botulinum
neurotoxin B, albumin, epithelial growth factor receptor, prostate-specific membrane antigen
10 (PSMA), citrullinated peptides, brain natriuretic peptides, and/or cardiac Troponin I. In
another embodiment, the one or more target binding polypeptide comprises an amino acid
sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%,
95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the
group consisting of SEQ ID NO: 27380-27390, wherein any N-terminal amino acid is
15 optional and may be present or absent.

In various further embodiments, the cage polypeptide comprises the amino acid
sequence at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%,
93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical, not including optional amino acid
residues, to the amino acid sequence of a cage protein listed in Table 10.

20 The disclosure also provides methods for designing/making a biosensor, cage protein,
or key protein comprising the steps of any method described herein, such as in the examples
that follow.

In another aspect, the disclosure provides nucleic acids encoding a cage protein, key
protein, or epitope of the disclosure. The nucleic acid sequence may comprise RNA (such as
25 mRNA) or DNA. Such nucleic acid sequences may comprise additional sequences useful for
promoting expression and/or purification of the encoded protein, including but not limited to
polyA sequences, modified Kozak sequences, and sequences encoding epitope tags, export
signals, and secretory signals, nuclear localization signals, and plasma membrane localization
signals. It will be apparent to those of skill in the art, based on the teachings herein, what
30 nucleic acid sequences will encode the proteins of the invention.

In another aspect, the disclosure provides expression vectors comprising the nucleic
acid of any embodiment or combination of embodiments of the disclosure operatively linked
to a suitable control sequence. "Expression vector" includes vectors that operatively link a
nucleic acid coding region or gene to any control sequences capable of effecting expression

of the gene product. "Control sequences" operably linked to the nucleic acid disclosure are nucleic acid sequences capable of effecting the expression of the nucleic acid molecules. The control sequences need not be contiguous with the nucleic acid sequences, so long as they function to direct the expression thereof. Thus, for example, intervening
5 untranslated yet transcribed sequences can be present between a promoter sequence and the nucleic acid sequences and the promoter sequence can still be considered "operably linked" to the coding sequence. Other such control sequences include, but are not limited to, polyadenylation signals, termination signals, and ribosome binding sites. Such expression vectors can be of any type known in the art, including but not limited to plasmid and viral-
10 based expression vectors. The control sequence used to drive expression of the disclosed nucleic acid sequences in a mammalian system may be constitutive (driven by any of a variety of promoters, including but not limited to, CMV, SV40, RSV, actin, EF) or inducible (driven by any of a number of inducible promoters including, but not limited to, tetracycline, ecdysone, steroid-responsive).

15 In one aspect, the present disclosure provides cells comprising the cage protein, key protein, epitope, biosensor, nucleic acid, and/or expression vector of any embodiment or combination of embodiments of the disclosure, wherein the cells can be either prokaryotic or eukaryotic, such as mammalian cells. In one embodiment the cells may be transiently or stably transfected with the nucleic acids or expression vectors of the disclosure. Such
20 transfection of expression vectors into prokaryotic and eukaryotic cells can be accomplished via any technique known in the art. A method of producing a polypeptide according to the invention is an additional part of the invention. The method comprises the steps of (a) culturing a host according to this aspect of the invention under conditions conducive to the expression of the polypeptide, and (b) optionally, recovering the expressed polypeptide.

25 In another aspect, the disclosure provides pharmaceutical compositions comprising
(a) the cage protein, key protein, biosensor, epitope, recombinant nucleic acid, expression vector, and/or the cell of any embodiment or combination of embodiments herein; and

(b) a pharmaceutically acceptable carrier.

30 The compositions may further comprise (a) a lyoprotectant; (b) a surfactant; (c) a bulking agent; (d) a tonicity adjusting agent; (e) a stabilizer; (f) a preservative and/or (g) a buffer. In some embodiments, the buffer in the pharmaceutical composition is a Tris buffer, a histidine buffer, a phosphate buffer, a citrate buffer or an acetate buffer. The composition may also include a lyoprotectant, e.g. sucrose, sorbitol or trehalose. In certain embodiments,

the composition includes a preservative e.g. benzalkonium chloride, benzalkonium chloride, chlorohexidine, phenol, m-cresol, benzyl alcohol, methylparaben, propylparaben, chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. In other embodiments, the composition includes a bulking agent, like glycine. In yet other embodiments, the composition includes a surfactant e.g., polysorbate-20, polysorbate-40, polysorbate- 60, polysorbate-65, polysorbate-80 polysorbate-85, poloxamer-188, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate, sorbitan trilaurate, sorbitan tristearate, sorbitan trioleate, or a combination thereof. The composition may also include a tonicity adjusting agent, e.g., a compound that renders the formulation substantially isotonic or isoosmotic with human blood. Exemplary tonicity adjusting agents include sucrose, sorbitol, glycine, methionine, mannitol, dextrose, inositol, sodium chloride, arginine and arginine hydrochloride. In other embodiments, the composition additionally includes a stabilizer, e.g., a molecule which substantially prevents or reduces chemical and/or physical instability of the nanostructure, in lyophilized or liquid form. Exemplary stabilizers include sucrose, sorbitol, glycine, inositol, sodium chloride, methionine, arginine, and arginine hydrochloride.

In a further aspect, the disclosure provide an epitope, comprising or consisting of the amino acid sequence of SEQ ID NO:27384

lucCageTrop

$$\text{cTnI} + \text{cTnC}$$

EDQLREKAKELWQTIYNLEAEKFDLQEKFKQKYEINVL
RNRINDNQVSKTKDDSGKSEEEELSDFRMFDKNADGY
IDLEELKIMLQATGETITEDDIEELMKDGDKNNDGRIDY
DEFLEFMKGVE (SEQ ID NO:27384)

The epitope can be used, for example, in the biosensors of the disclosure. In one aspect, the disclosure provides methods for detecting Troponin I in a sample, comprising contacting a biological sample with the epitope under conditions suitable to promote binding of Troponin I in the sample to the epitope to form a binding complex, and detecting binding complexes that demonstrate presence of Troponin I in the sample. All embodiments of biological samples and detection as disclosed herein can be used in these methods as well.

Examples

Here, we show that a very general class of allosteric protein-based biosensors can be created by inverting the flow of information through *de novo* designed protein switches in which binding of a peptide key triggers biological outputs of interest. Using broadly

applicable design principles, we allosterically couple binding of protein the reconstitution of luciferase activity and a bioluminescent readout through the association of designed lock and key proteins. Because the sensor is based purely on thermodynamic coupling of analyte binding to switch activation, only one target binding domain is required, which simplifies sensor design and allows direct readout in solution. We demonstrate the modularity of this platform by creating biosensors that, with little optimization, sensitively detect the anti-apoptosis protein Bcl-2, the hIgG1 Fc domain, the Her2 receptor, and Botulinum neurotoxin B, as well as biosensors for cardiac Troponin I and an anti-Hepatitis B virus (HBV) antibody that achieve the sub-nanomolar sensitivity necessary to detect clinically relevant concentrations of these molecules. We also use the approach to design sensors of antibodies against SARS-CoV-2 protein epitopes and of the receptor-binding domain (RBD) of the SARS-CoV-2 Spike protein. The latter, which incorporates a *de novo* designed RBD binder, has a limit of detection of 15pM with an up to seventeen fold increase in luminescence upon addition of RBD. The modularity and sensitivity of the platform enable the rapid construction of sensors for a wide range of analytes and highlights the power of *de novo* protein design to create multi-state protein systems with new and useful functions.

A protein biosensor can be constructed from a system with two nearly isoenergetic states - the equilibrium between which is modulated by the analyte being sensed. Desirable properties in such a sensor are (i) the analyte triggered conformational change should be independent of the details of the analyte (so the same overall system can be used to sense many different compounds) (ii) the system should be tunable so that analytes with different binding energies and relevant concentrations can be detected over a large dynamic range, and (iii) the conformational change should be coupled to a sensitive output. We hypothesized that these attributes could be attained by inverting the information flow in *de novo* designed protein switches in which binding to a target protein of interest is controlled by the presence of a peptide actuator. These switches consist of a constant “cage” region that sequesters a “latch” that binds the target of interest; addition of a peptide “key” displaces the latch from the cage leading to target binding and associated downstream events. However, from a thermodynamic viewpoint, the key and the target are equivalent: the binding of the two to the cage is thermodynamically coupled since the latch has to open, with free energy cost ΔG_{open} (Fig 1b), in order for either to bind. Hence, the free energy associated with binding both target and key is more favorable than the sum of the free energies of binding the two individually (Fig 1c). The difference between key and target is in their variability; the key is constant while the target can be any desired interaction. For an actuator, it is desirable to have

a constant input drive a wide range of customizable responses, and hence, the input was the (constant) key and the output was binding to a variety of targets associated with protein degradation, nuclear export, etc. We reasoned that the input to the system could be inverted to create biosensors with a constant readout -- addition of a (variable) target could induce binding of the (constant) key to the (constant) cage, and that this association could be coupled to an enzymatic readout. Such a system would satisfy properties (i) and (ii) above, as a wide range of binding activities can be caged, and since the switch is thermodynamically controlled, it is straightforward to adjust the relative energies of key and target binding to achieve activation at the relevant target concentrations. Because the key and the cage are always the same, the system is modular: the same molecular association can be coupled to the binding of many different targets.

To achieve property (iii), we reasoned that bioluminescence could provide a rapid and sensitive readout of analyte driven cage-key association, and explored the use of a reversible split luciferase complementation system. We developed a system consisting of two protein components: a 'lucCage' comprising a cage domain and a latch domain containing the short split luciferase fragment (SmBiT) and an analyte binding motif of choice; and a "lucKey", which comprises the larger split luciferase fragment (LgBit) and a key peptide (Fig. 1a). lucCage has two states: a closed state in which the cage domain binds the latch and sterically occludes the analyte binding motif from binding its target and SmBiT from combining with LgBit to reconstitute luciferase activity; and an open state in which these binding interactions are not blocked, and lucKey can bind the cage domain. Association of lucKey with lucCage results in the reconstitution of luciferase activity (Fig. 1a, right). The target may be viewed as allosterically regulating luciferase activity, since binding to the sensor is at a site distant from the enzyme active site.

The states of such a system are in thermodynamic equilibrium, with the tunable parameters ΔG_{open} and ΔG_{CK} governing the populations of the possible species, along with the free energy of association of the analyte to the binding domain ΔG_{LT} (Fig. 1b). To achieve high sensitivity, the closed state (species 1) must be substantially lower in free energy than the open state in the absence of target (species 6) to avoid background signal ($\Delta G_{1-6} > 0$), but higher in free energy than the open state in the presence of target (species 7, $\Delta G_{1-7} < 0$), so that target detection is energetically favorable (Fig. 1c). To guide the optimization of biosensor sensitivity, we simulated the dependence of the sensor system on ΔG_{open} (Fig. 1d), ΔG_{LT} (Fig. 1e), and the concentration of analyte and the sensor components (Fig. 1f) (See Supplementary Methods for details). As expected, the sensitivity of analyte

detection is a function of ΔG_{LT} , with a lower limit of roughly one-tenth binding (Fig. 1e; below this concentration, the free energy of binding is too small to open the switch). Hence sensing domains with high affinity to their target will yield more sensitive biosensors. The sensitivity of the system can be further tuned above this lower limit by varying the concentration of lucCage and lucKey, resulting in sensing systems responding to different target concentration ranges (Fig. 1f). Tuning the strength of the intramolecular cage-latch interaction (ΔG_{open}) affects the equilibrium population of the catalytically active species (species 6 and 7, Fig. 1d), which in turn affects the sensitivity: too tight interaction results in low signal in the presence of target, and too weak an interaction results in high background in the absence of target. Our design strategy aims to find this balance by designing sensors in the closed state (species 1) with a range of ΔG_{open} values: ΔG_{open} can be increased (decreased) by increasing (decreasing) the length of the latch helix and by introducing either favorable hydrophobic interactions or unfavorable steric clashes and buried polar atoms at the cage-latch interface; we employ both strategies to tune the sensors described below (ΔG_{CK} can also be tuned, but we did not find this necessary for the sensors described here).

To streamline the design of new sensors based on these principles, we developed a RosettaTM-based computational method for the incorporation of diverse sensing domains into the LOCKR switches called GraftSwitchMover. This method identifies the most suitable position for embedding a target binding peptide within the latch such that the resulting protein is stable in the closed state and the interactions with the target are blocked. This is done by maximizing favorable hydrophobic packing interactions between the peptide and the cage and minimizing the number of unfavorable buried hydrophilic residues. This method takes as input the 3-dimensional model of the switch, the sequence of a peptide that binds the target of interest, and a list of the residues in this peptide that interact with the target (interface residues), and returns a set of designs in which the binding of the peptide to the target is predicted to be blocked by association with the cage (See supplementary methods). The final set of designs covers a range of ΔG_{open} values (Fig. 1c), which can be further tuned through introducing destabilizing mutations in the latch: I328S ("1S") or I328S/L345S ("2S"). These designs are then experimentally characterized to find the most sensitive biosensors.

We first set out to test our hypothesis by grafting the SmBiT peptide and the Bim peptide in the closed state of the optimized asymmetric LOCKR switch described in Langan et al, 2020² (Fig. 6). SmBiT naturally adopts a β -strand conformation within the luciferase holoenzyme, but we assumed that it will adopt a helical secondary structure in the context of

the helical bundle scaffold, consistent with the observation that some p
adopt diverse secondary structures in a context-dependent manner. We sampled different
threadings for the two peptide sequences across the latch, built three-dimensional models,
selected the lowest energy solutions (3 positions for SmBiT, and 4 positions for the Bim
5 peptide) (Fig. 6a) and expressed twelve designs in *E. coli*. We mixed the designs with lucKey
in a 1:1 ratio, then added Bcl-2, which binds with nanomolar affinity to Bim, and monitored
luciferase activity (Fig. 6b). We found that upon the addition of Bcl-2 to a solution containing
the new Cage designs, lucKey, and furimazine substrate, there was a rapid increase in
luminescence (Fig. 6f), suggesting that the inverse LOCKR system can indeed function as a
10 biosensor. Further characterization of the best Bcl-2 sensor candidate, lucCageBim,
demonstrated that the analyte detection range could be tuned by varying the concentration of
the sensor (lucCage + lucKey) (Fig. 6g) as anticipated in our model simulations (Fig. 1f).
Experimental characterization of the different designs showed that inserting SmBiT into
position 312 of the LOCKR cage (SmBiT312) yielded the highest stability and brightness
15 (Fig. 6b), therefore we used this design, henceforward referred to as “lucCage”, as the base
scaffold for the biosensors described below.

To explore the versatility of our new biosensor platform, we next investigated the
incorporation of a range of binding modalities for analytes of interest within lucCage. First,
we set out to explore how to computationally cage target-binding proteins, rather than
20 peptides, in the closed state. We identified the primary interaction surface of the binding
protein to its target, extracted the main secondary structure elements involved in it to use
them in the computational protocol described above, and selected the best designs from the
many threadings generated. Then, we used RosettaTM Remodel to model the full-length
binding domain in the context of the switch and selected designs in which this interface was
25 buried against the cage with minimal steric clashes (See supplementary methods). As a test
case, we caged the *de novo* designed protein, HB1.9549.2, which binds to Influenza A H1
hemagglutinin (HA)¹⁵ into a shortened version of the LOCKR switch (sCage), optimized to
improve stability and facilitate crystallization efforts (Fig. 2a). Two of five designs were
functional, and bound HA in the presence but not the absence of key (Fig. 7b). The crystal
30 structure of the best design, sCageHA_267-1S, determined to 2.0 Å resolution (Table 11),
showed that all HA-binding residues except one (F273) interact with the cage domain
(blocking binding of the latch to the switch) as intended by design (Fig. 2a, Fig. 7a-c). With
this structural validation of the design concept in hand, we next sought to develop new
sensors using small proteins as sensing domains for the detection of botulinum neurotoxin,

the immunoglobulin Fc domain, and the Her2 receptor. To do so, we g
 designed binder for Botulinum neurotoxin B (BoNT/B)¹⁵, the C domain of the generic
 antibody binding protein Protein A¹⁶, and a Her2-binding affibody¹⁷, into lucCage. After
 screening a few designs for each target (Fig. 8-10), we obtained highly sensitive lucCages
 5 (lucCageBot, lucCageProA, and lucCageHer2) that can detect BoNT/B (Fig. 2b, Fig. 8), hIgG
 Fc domain (Fig. 2c, Fig. 9), and Her2 receptor (Fig. 2d; Fig. 10) respectively, demonstrating
 the modularity of the platform. The designed sensors responded within minutes upon adding
 the target, and their sensitivity could be tuned by changing the concentration of lucCage and
 lucKey (Fig. 2), as predicted by our model simulations (Fig. 1f). These sensors may be used
 10 in multiple applications, such as rapid and low-cost detection of highly toxic botulinum
 neurotoxins in the food industry, which currently relies heavily on live-animal bioassays, or
 detection of high serological levels of soluble Her2 (>15 ng/mL) associated with metastatic
 breast cancer, levels that could be detected with the current sensitivity of lucCageHer2.

We next designed sensors for additional targets relevant in clinical settings. Since
 15 bioluminescent sensors do not require light for excitation, highly sensitive and low
 background readout is more suited than fluorescence to directly measure analytes in
 biological media such as blood and serum for point-of-care applications. We first targeted
 cardiac troponin I (cTnI), which is the standard early diagnostic biomarker for acute
 myocardial infarction (AMI). We took advantage of the high-affinity interaction between
 20 cTnT, cTnC, and cTnI (Fig. 3a) and designed eleven biosensor candidates by inserting 6
 truncated cTnT sequences at different latch positions (Fig. 11a). The best candidate,
 lucCageTrop627, was able to detect cTnI but not at sufficiently low levels for clinical use
 (Fig. 11d). Because the rule-in and rule-out levels of cTnI assay for diagnosis of AMI in
 patients are in the low pM range, and because as noted above the limit of detection (LOD) of
 25 our sensor platform is about $0.1 \times K_d$ of the latch-target affinity (K_{LT}), we further increased
 the affinity of our sensor to cTnI by fusing cTnC to its terminus (Fig. 3a, Fig. 11b,c). The
 resulting sensor, lucCageTrop, has a single-digit pM LOD suitable for quantification of
 clinical samples (Fig. 3b, Fig. 11 e,f).

Detection of specific antibodies is important for monitoring the spread of a pathogen
 30 in a population (antibodies remain long after the pathogen has been eliminated), the success
 of vaccination, and levels of therapeutic antibodies. To adapt our system to be used in such
 antibody serological analyses, we sought to incorporate linear epitopes recognized by the
 antibodies of interest into lucCage, so that binding of an antibody would open the switch
 allowing lucKey binding and reconstitution of luciferase activity. We first developed a sensor

for anti-Hepatitis B virus (HBV) antibodies based on the crystal structure of the antibody (HzKR127) bound to a peptide from the PreS1 domain of the viral surface protein L²⁵. The best of 8 designs tested, lucCageHBV (HBV344), had a ~150% increase in luciferase activity upon addition of HzKR127-3.2, an improved version of HzKR127²⁶ (Fig. 12a,b). To further improve the dynamic range and LOD of lucCageHBV (~2 nM, Fig. 12c-e), we increased the latch-target affinity (K_{LT}) by introducing an additional copy of the peptide at the end of the latch to take advantage of the antibody bivalent interaction with its epitope (Fig. 3c,d). The resulting design, named lucCageHBV α , had a LOD of 260 pM and a dynamic range of 225% (Fig. 3e; Fig. 13a-c), with a luminescence intensity easily detectable with a camera (Fig. 13d). Hence the platform to detect specific antibodies with a LOD in the range for monitoring therapeutic antibodies. We next demonstrated the use of the lucCageHBV sensor to detect hepatitis B surface antigen (HBsAg). Since our sensors are under thermodynamic control, we hypothesized that the pre-assembly of sensor-antibody complex would re-equilibrate in the presence of the target HBsAg protein, PreS1, with antibody redistributing to bind free PreS1 instead of the epitope on lucCageHBV (Fig. 3f). Indeed, the luminescence of lucCageHBV plus HzKR127-3.2 mixture decreased shortly upon addition of the PreS1 domain (Fig. 3g); the sensitivity of this readout enabled quantification of PreS1 concentration in a clinically relevant range²⁸ (Fig. 3h, Fig. 12f). HBsAg seroclearance is one of the major biomarkers to monitor therapeutic progress following hepatitis diagnosis and vaccination efficacy, but current commercial HBsAg assays are unable to differentiate between the three HBsAg protein subtypes. Our PreS1 sensor (detecting HBsAg L antigen) shows that the system can achieve subtype-specific recognition.

The COVID-19 pandemic has showcased the urgent need for developing new diagnostic tools for tracking active infections by detecting the SARS-CoV-2 virus itself, and for detection of antiviral antibodies to evaluate the extent of the spread of the virus in the population and to identify individuals at lower risk of future infection. To design sensors for anti-SARS-CoV-2 antibodies, we first identified from the literature highly immunogenic linear epitopes in the SARS-CoV^{31,32} and SARS-CoV-2 proteomes^{33,34} that are not present in “common” strains of *coronaviridae* (i.e., HCoV-OC43, HCoV-HKU1, HCoV-229E, HCoV-NL63; we did not exclude reactivity against SARS-CoV or MERS as they are much less broadly distributed). Among these, we focused on two epitopes in the Membrane and Nucleocapsid proteins found to be recognized by SARS and COVID-19 patient sera for which cross-reactive animal-derived antibodies are commercially available (see Fig. 4 legend and Materials and methods for epitope and antibody description). We designed sensors for

each epitope (Fig. 14a,b) and identified designs that specifically respond to pure anti-M and anti-N protein antibodies (Fig. 4b,c). These sensors were fast (<5 minutes to reach full signal) and had a ~50-70% dynamic range in response to low nanomolar amounts of antibodies (Fig. 4b,c, Fig. 14c,d).

To create sensors capable of detecting SARS-CoV-2 viral particles directly, we integrated into the LucCage format a designed picomolar affinity binder to the receptor-binding domain (RBD) of the SARS-CoV-2 Spike protein named LCB1 (Fig. 4d). Of 13 candidates tested, the best, which we refer to as lucCageRBD, had minimal background, an outstanding dynamic range (1700%) easily detectable with a camera and low LOD (15 pM) (Fig. 4d, Fig. 15). The superior dynamic range and sensitivity of this sensor are consequences of the high affinity of LCB1 to RBD (K_{LT}), consistent with our thermodynamic model, highlighting the synergy of the LucCage sensor platform and *de novo* binder design.

Because of the modularity and engineerability of the LucCage system, it took only three weeks to design the SARS-CoV-2 antibody and RBD sensors, obtain synthetic genes, express and purify the proteins, and evaluate sensor performance.

To test the specificity of the biosensors developed in this work (excluding the indirect detection of PreS1 by lucCageHBV and lucCageRBD), we measured the activation kinetics of each in response to all the targets (Bcl-2, botulinum neurotoxin B, IgG Fc, Her2, cardiac Troponin I, the monoclonal anti-HBV antibody (HzKR127-3.2), the anti-SARS-CoV-1-M polyclonal antibody (clone 3527), the anti-SARS-CoV-1-N monoclonal antibody (clone 18F629.1), and PreS1). As shown in Fig. 5, each sensor responded rapidly and sensitively to its cognate target, but not to any of the others. A summary of each lucCage sensor characteristics and sensing domains used can be found in Table 12 and Table 13, respectively.

Most previous protein-based biosensor platforms depend on the specific geometry of a target-sensor interaction to trigger a conformational change in the reporter component and hence are specialized for a subset of detection challenges. Because of this target dependence, considerable optimization can be required to achieve high sensitivity detection of a new target. Our sensor platform is based on the thermodynamic coupling between defined closed and open states of the system, thus, its sensitivity depends on the free energy change upon the sensing domain binding to the target but not the specific geometry of the binding interaction. This enables the incorporation of various binding modalities, including small peptides, globular mini proteins, antibody epitopes and *de novo* designed binders, to generate sensitive sensors for a wide range of protein targets with little or no optimization. For point of care (POC) applications, our system has the advantages of being homogeneous, no-wash, all-in-

solution, a nearly instantaneous readout, and its quantification of lumir performed by means of inexpensive and accessible devices such as a cell phone camera. In hospital settings, the ability to predictably make a wide range of sensors under the same principle could enable quick readout of large numbers of different compounds using an array of hundreds of different sensors on, for example, a 384-well plate.

Up until recently, the focus of *de novo* protein design was on the design of proteins with new structures corresponding to single deep free energy minima; our results highlight the progress in the field which now enables more complex multistate systems to be readily generated. Our sensors are expressed at high levels in cells and are very stable, which considerably facilitates the further manufacturing process. The general “molecular device” architecture of our platform synergizes particularly well with complementary advances in the *de novo* design of high-affinity miniprotein binders, which can be designed with three dimensional structures readily compatible with the lucCage platform. LucCageRBD highlights the potential of this fully *de novo* approach, with a 1700% dynamic range and 15 pM LOD from a sensor coming straight out of the computer, without any experimental optimization.

References

1. Stein, V. & Alexandrov, K. Synthetic protein switches: design principles and applications. *Trends Biotechnol.* **33**, 101–110 (2015).
2. Langan, R. A. *et al.* De novo design of bioactive protein switches. *Nature* **572**, 205–210 (2019).
3. Adams, E. R. *et al.* Antibody testing for COVID-19: A report from the National COVID Scientific Advisory Panel. *medRxiv* 2020.04.15.20066407 (2020).
4. Yeh, H.-W. & Ai, H.-W. Development and Applications of Bioluminescent and Chemiluminescent Reporters and Biosensors. *Annu. Rev. Anal. Chem.* **12**, 129–150 (2019).
5. Greenwald, E. C., Mehta, S. & Zhang, J. Genetically Encoded Fluorescent Biosensors Illuminate the Spatiotemporal Regulation of Signaling Networks. *Chem. Rev.* **118**, 11707–11794 (2018).
6. Schena, A., Griss, R. & Johnsson, K. Modulating protein activity using tethered ligands with mutually exclusive binding sites. *Nat. Commun.* **6**, 7830 (2015).
7. Arts, R. *et al.* Semisynthetic Bioluminescent Sensor Proteins for Direct Detection of Antibodies and Small Molecules in Solution. *ACS Sens* **2**, 1730–1736 (2017).
8. Xue, L., Prifti, E. & Johnsson, K. A General Strategy for the Semisynthesis of

Ratiometric Fluorescent Sensor Proteins with Increased Dynamic

Soc. **138**, 5258–5261 (2016).

9. Guo, Z. *et al.* Generalizable Protein Biosensors Based on Synthetic Switch Modules. *J. Am. Chem. Soc.* **141**, 8128–8135 (2019).
- 5 10. Edwardraja, S. *et al.* Caged activators of artificial allosteric protein biosensors. *ACS Synth. Biol.* (2020) doi:10.1021/acssynbio.9b00500.
11. Ribeiro, L. F., Warren, T. D. & Ostermeier, M. Construction of Protein Switches by Domain Insertion and Directed Evolution. *Methods Mol. Biol.* **1596**, 43–55 (2017).
12. Dixon, A. S. *et al.* NanoLuc Complementation Reporter Optimized for Accurate
10 Measurement of Protein Interactions in Cells. *ACS Chem. Biol.* **11**, 400–408 (2016).
13. Minor, D. L., Jr & Kim, P. S. Context-dependent secondary structure formation of a designed protein sequence. *Nature* **380**, 730–734 (1996).
14. Huang, P.-S. *et al.* RosettaRemodel: a generalized framework for flexible backbone protein design. *PLoS One* **6**, e24109 (2011).
- 15 15. Chevalier, A. *et al.* Massively parallel de novo protein design for targeted therapeutics. *Nature* **550**, 74–79 (2017).
16. Deis, L. N. *et al.* Suppression of conformational heterogeneity at a protein-protein interface. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 9028–9033 (2015).
17. Eigenbrot, C., Ultsch, M., Dubnovitsky, A., Abrahmsén, L. & Härd, T. Structural basis
20 for high-affinity HER2 receptor binding by an engineered protein. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 15039–15044 (2010).
18. Hobbs, R. J., Thomas, C. A., Halliwell, J. & Gwenin, C. D. Rapid Detection of Botulinum Neurotoxins-A Review. *Toxins* **11**, (2019).
19. Perrier, A., Gligorov, J., Lefèvre, G. & Boissan, M. The extracellular domain of Her2 in
25 serum as a biomarker of breast cancer. *Lab. Invest.* **98**, 696–707 (2018).
20. Yu, Q. *et al.* Semisynthetic sensor proteins enable metabolic assays at the point of care. *Science* **361**, 1122–1126 (2018).
21. Rubini Gimenez, M. *et al.* One-hour rule-in and rule-out of acute myocardial infarction using high-sensitivity cardiac troponin I. *Am. J. Med.* **128**, 861–870.e4 (2015).
- 30 22. Collins, M. H. Serologic Tools and Strategies to Support Intervention Trials to Combat Zika Virus Infection and Disease. *Trop Med Infect Dis* **4**, (2019).
23. Pondé, R. A. de A. Expression and detection of anti-HBs antibodies after hepatitis B virus infection or vaccination in the context of protective immunity. *Arch. Virol.* **164**, 2645–2658 (2019).

24. van Rosmalen, M. *et al.* Dual-Color Bioluminescent Sensor Proteins for
Drug Monitoring of Antitumor Antibodies. *Anal. Chem.* **90**, 3592–3599 (2018).
25. Chi, S.-W. *et al.* Broadly neutralizing anti-hepatitis B virus antibody reveals a
complementarity determining region H3 lid-opening mechanism. *Proc. Natl. Acad. Sci.*
5 *U. S. A.* **104**, 9230–9235 (2007).
26. Kim, J. H. *et al.* Enhanced humanization and affinity maturation of neutralizing anti-
hepatitis B virus preS1 antibody based on antigen-antibody complex structure. *FEBS*
Lett. **589**, 193–200 (2015).
27. Ovacik, M. & Lin, K. Tutorial on Monoclonal Antibody Pharmacokinetics and Its
10 Considerations in Early Development. *Clin. Transl. Sci.* **11**, 540–552 (2018).
28. Locarnini, S. & Bowden, S. Hepatitis B surface antigen quantification: Not what it
seems on the surface. *Hepatology* vol. 56 411–414 (2012).
29. Cornberg, M. *et al.* The role of quantitative hepatitis B surface antigen revisited. *Journal*
of Hepatology vol. 66 398–411 (2017).
- 15 30. Perera, R. A. *et al.* Serological assays for severe acute respiratory syndrome coronavirus
2 (SARS-CoV-2), March 2020. *Euro Surveill.* **25**, (2020).
31. Chow, S. C. S. *et al.* Specific epitopes of the structural and hypothetical proteins elicit
variable humoral responses in SARS patients. *J. Clin. Pathol.* **59**, 468–476 (2006).
32. He, Y., Zhou, Y., Siddiqui, P., Niu, J. & Jiang, S. Identification of immunodominant
20 epitopes on the membrane protein of the severe acute respiratory syndrome-associated
coronavirus. *J. Clin. Microbiol.* **43**, 3718–3726 (2005).
33. Wang, H. *et al.* SARS-CoV-2 proteome microarray for mapping COVID-19 antibody
interactions at amino acid resolution. (2020) doi:10.1101/2020.03.26.994756.
34. Dahlke, C. *et al.* Distinct early IgA profile may determine severity of COVID-19
25 symptoms: an immunological case series. *medRxiv* 2020.04.14.20059733 (2020).
35. Yu, Q. *et al.* A biosensor for measuring NAD levels at the point of care. *Nature*
Metabolism vol. 1 1219–1225 (2019).
36. Arts, R. *et al.* Detection of Antibodies in Blood Plasma Using Bioluminescent Sensor
Proteins and a Smartphone. *Anal. Chem.* **88**, 4525–4532 (2016).
- 30 37. Tenda, K. *et al.* Paper-Based Antibody Detection Devices Using Bioluminescent BRET-
Switching Sensor Proteins. *Angewandte Chemie* vol. 130 15595–15599 (2018).
38. Adamson, H. *et al.* Affimer–Enzyme–Inhibitor Switch Sensor for Rapid Wash-free
Assays of Multimeric Proteins. *ACS Sens.* **4**, 3014–3022 (2019).
39. Schena, A., Griss, R. & Johnsson, K. Corrigendum: Modulating protein activity using

tethered ligands with mutually exclusive binding sites. *Nat. Comm*

40. Berger, S. *et al.* Computationally designed high specificity inhibitors delineate the roles of BCL2 family proteins in cancer. *Elife* **5**, (2016).
41. Jin, R., Rummel, A., Binz, T. & Brunger, A. T. Botulinum neurotoxin B recognizes its
5 protein receptor with high affinity and specificity. *Nature* **444**, 1092–1095 (2006).
42. Shen, A. *et al.* Mechanistic and structural insights into the proteolytic activation of *Vibrio cholerae* MARTX toxin. *Nat. Chem. Biol.* **5**, 469–478 (2009).
43. Otwinowski, Z. & Minor, W. [20] Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* **276**, 307–326 (1997).
- 10 44. Liebschner, D. *et al.* Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in Phenix. *Acta Crystallogr D Struct Biol* **75**, 861–877 (2019).
45. Potterton, L. *et al.* Developments in the CCP4 molecular-graphics project. *Acta Crystallogr. D Biol. Crystallogr.* **60**, 2288–2294 (2004).

15 **Methods**

Design of the sensor system: lucCage and lucKey

SmBit (VTGYRLFEEIL; SEQ ID NO: 27359) was grafted into the latch of the asymmetric LOCKR switch described in Langan et al, 2019 using GraftSwitchMover, a
20 RosettaScripts™-based protein design algorithm (See Supplementary Methods for details). The grafting sampling range was assigned between residues 300-330. The resulting designs were energy-minimized, visually inspected and selected for subsequent gene synthesis, protein production and biochemical analyses. The best SmBit position on the latch was experimentally determined to be an insertion at residue 312, as described in Fig. 6. lucKey
25 was assembled by genetically fusing the LgBit of NanoLuc¹² to the key peptide described in Langan et al, 2019. (See Table 10 for the full sequence list)

Computational grafting of sensing domains into lucCage

Peptides and epitopes: The amino acid sequence for each sensing domain was grafted
30 using Rosetta™ GraftSwitchMover into all α -helical registers between residues 325-360 of lucCage (See Supplementary Methods for details). The resulting lucCages were energy-minimized, visually inspected and typically less than ten designs were selected for subsequent protein production and biochemical characterization.

Protein domains: First, the main secondary structure elements 1
surface of the binding protein were identified, their amino acid sequence was extracted and
grafted into lucCage using theGraftSwitchMover as described above. Then, we used
RosettaTM Remodel¹⁴ to model the full-length binding domain in the context of the switch in
5 which this interface was buried against the cage (See Supplementary Methods for details).
The designs were energy-minimized and visually inspected for selection. Typically, less than
ten designs were selected for biochemical characterization.

Synthetic gene construction

10 The designed protein sequences were codon optimized for *E. coli* expression (IDT
codon optimization tool) and ordered as synthetic genes in pET21b+ or pET29b+ *E. coli*
expression vectors (IDT). The synthetic gene was inserted at the NdeI and XhoI sites of each
vector, including an N-terminal hexahistidine tag followed by a TEV protease cleavage site
and a stop codon was added at the C terminus.

General procedures for bacterial protein production and purification

15 The *E. coli* LEMO21(DE3) strain (NEB) was transformed with a pET21b+ or
pET29b+ plasmid encoding the synthesized gene of interest. Cells were grown for 24 hours
in LB media supplemented with carbenicillin or kanamycin. Cells were inoculated at a 1:50
20 mL ratio in the Studier TBM-5052 autoinduction media supplemented with carbenicillin or
kanamycin, grown at 37 °C for 2-4 hours, and then grown at 18 °C for an additional 18 h.
Cells were harvested by centrifugation at 4000g at 4 °C for 15 min and resuspended in 30 ml
lysis buffer (20 mM Tris-HCl pH 8.0, 300 mM NaCl, 30 mM imidazole, 1 mM PMSF, 0.02
mg/mL DNase). Cell resuspensions were lysed by sonication for 2.5 minutes (5 second
25 cycles). Lysates were clarified by centrifugation at 24,000g at 4 °C for 20 min and passed
through 2 ml of Ni-NTA nickel resin (Qiagen, 30250) pre-equilibrated with wash buffer, (20
mM Tris-HCl pH 8.0, 300 mM NaCl, 30 mM imidazole). The resin was washed twice with
10 column volumes (CV) of wash buffer, and then eluted with 3 CV of elution buffer (20 mM
Tris-HCl pH 8.0, 300 mM NaCl, 300 mM imidazole). The eluted proteins were concentrated
30 using Ultra-15 Centrifugal Filter Units (Amicon) and further purified by using a SuperdexTM
75 Increase 10/300 GL (GE Healthcare) size exclusion column in Tris Buffered Saline (TBS;
25 mM Tris-HCl pH 8.0, 150 mM NaCl). Fractions containing monomeric protein were
pooled, concentrated, and snap-frozen in liquid nitrogen and stored at -80 °C.

In vitro bioluminescence characterization

A Synergy™ Neo2 Microplate Reader (BioTek) was used for all in vitro bioluminescence measurements. Assays were performed in 1:1=HBS-EP:Nano-Glo assay buffer for anti-HBV and RBD sensors while 1:1=DPBS:Nano-Glo assay buffer was used for other sensors. 10X lucCage, 10X lucKey, and 10X target proteins of desired concentrations were first prepared from stock solutions. For each well of a white opaque 96-well plate, 10 µL of 10X lucCage, 10 µL of 10X lucKey, and 20 µL of buffer were mixed to reach the indicated concentration and ratio. The plate was centrifuged at $1000 \times g$ for 1 min and incubated at RT for additional 10 min. Then, 50 µL of 50X diluted furimazine (Nano-Glo™ luciferase assay reagent, Promega) was added to each well. Bioluminescence measurements in the absence of target were taken every 1 min post-injection (0.1 s integration and 10 s shaking during intervals). After ~15 min, 10 µL of serially diluted 10X target protein plus a blank was injected and bioluminescence kinetic acquisition continued for a total of 2 h. To derive EC₅₀ values from the bioluminescence-to-analyte plot, the top three peak bioluminescence intensities at individual analyte concentrations were averaged, subtracted from blank, and used to fit the sigmoidal 4PL curve. To calculate the LOD, the linear region of bioluminescence responses of sensors to its analyte was extracted and a linear regression curve was obtained. It was used to derive the standard deviation of the response (SD) and the slope of the calibration curve (S). The LOD was determined as $3 \times (SD/S)$. The experimental measurements were taken in triplicate and the mean values are shown where applicable. The results were successfully replicated using different batches of pure proteins on different days.

Biolayer interferometry (BLI)

Protein-protein interactions were measured by using an Octet® RED96 System (ForteBio) using streptavidin-coated biosensors (ForteBio). Each well contained 200 µL of solution, and the assay buffer was HBS-EP+ Buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.05% v/v Surfactant P20, 0.5% non-fat dry milk). The biosensor tips were loaded with analyte peptide/protein at 20 µg/mL for 300 s (threshold of 0.5 nm response), incubated in HBS-EP+ Buffer for 60 s to acquire the baseline measurement, dipped into the solution containing Cage and/or Key for 600 s (association step) and dipped into the HBS-EP+ Buffer for 600 s (dissociation steps). The binding data were analyzed with the ForteBio Data Analysis Software version 9.0.0.10.

Design and characterization of lucCageBim

The Bim peptide sequence (EIWIAQELRRIGDEFNAYYAAA) was threaded into the lucCage scaffold as described in the “Design of sensing domains into lucCage” section. The selected designs were expressed in *E. coli*, purified and characterized for luminescence activation. The bioluminescence detection signal was measured for each design lucCage at 20 nM mixed with lucKey at 20 nM, in the presence or absence of target Bcl-2 protein at 200nM. Bcl-2 was expressed as described somewhere else ⁴⁰.

Design and characterization of lucCageHer2, lucCageProA, lucCageBot and lucCageRBD

The main binding motifs of the Bot.0671.2 de novo binder, *S. aureus* Protein A domain C (SpaC), the Her2 affibody and the *de novo* RBD binder LCB1 were threaded into lucCage as described in the “Design of sensing domains into lucCage” section (See Table 13 for sequences of sensing domains). The selected designs were expressed in *E. coli*, purified and characterized for luminescence activation. The bioluminescence detection signal was measured for each design lucCage at 20 nM mixed with lucKey at 20 nM, in the presence or absence of 200nM target protein. The target proteins used were: Botulinum Neurotoxin B HcB expressed as previously described ⁴¹, human IgG1 Fc-HisTag (AcroBiosystems, Cat. No. IG1-H5225) and human Her2-HisTag (AcroBiosystems, Cat. No. HE2-H5225).

Design and characterization of lucCageTrop

The cardiac Troponin T (cTnT) binding motif (EDQLREKAKELWQTIYNLEAEKFDLQEKFKQKYEINVLRNRINDNQ; SEQ ID NO: 27390) was split into fragments of different length (see Fig. 11) and threaded into the lucCage scaffold as described in the “Design of sensing domains into lucCage” section. The selected designs were expressed in *E. coli*, purified and characterized for luminescence activation. The bioluminescence detection signal was measured for each design lucCage at 20 nM mixed with lucKey at 20 nM in the presence or absence of 100 nM cardiac Troponin I (Genscript, Cat. No. Z03320-50). Subsequently, lucCageTrop, an improved version by fusion to cardiac Troponin C (cTnC), was created by genetically fusing the following sequence to the C terminus of lucCageTrop627 (KVSCTKDDSKGKSEEELSDLFRMFDKNADGYIDLEELKIMLQATGETITEDDIEELM KDGDKNNDGRIDYDEFLEFMKGVE; SEQ ID NO: 27627).

Design and characterization of lucCageHBV and lucCageHBV α

The binding motif (GANSNNPDWDFN (SEQ ID NO: 27629)) was threaded into the lucCage scaffold at every position after residues 330 using the Rosetta™ GraftSwitchMover. Following the Rosetta™ FastRelax protocol, eight designs were selected for protein production. Bioluminescence was measured with the designed lucCages (20 nM) and lucKey (20 nM) in the presence or absence of the anti-HVB antibody HzKR127-3.2 (100 nM) to select lucCageHBV. Subsequently, lucCageHBV α was constructed by genetically fusing a sequence containing a second antigenic motif (GGSGGGSSGFGANSNNPDWDFNPN; SEQ ID No:27628) to lucCageHBV.

10 Design and characterization of lucCageSARS2-M and lucCageSARS2-N

Antigenic epitopes of the SARS-CoV-2 membrane protein (a.a. 1-31, 1-17 and 8-24) and the nucleocapsid protein (a.a. 368-388 and 369-382) were computationally grafted into lucCage as described in the “Design of sensing domains into lucCage” section. The selected designs were expressed in *E. coli*, purified and characterized for luminescence activation. All designs at 50nM were mixed with 50nM lucKey and experimentally screened for an increase in luminescence in the presence of rabbit anti-SARS-CoV Membrane polyclonal antibodies (ProSci, Cat. No.: 3527) at 100nM or mouse anti-SARS-CoV Nucleocapsid monoclonal antibody (clone 18F629.1, NovusBio Cat. No. NBP2-24745) at 100 nM.

20 Design and characterization of sCageHA variants

HB1.9549.2 was embedded into the parental six-helix bundle for sCage design at different positions along the latch helix of the scaffold. To promote more favorable intramolecular interactions, three consecutive residues on the latch were intentionally substituted with glycine to allow for conformational freedom. The five designs were produced in *E. coli*. Biolayer interferometry analysis was performed with purified Cages (1 μ M) and biotinylated Influenza A H1 hemagglutinin (HA)¹⁵ loaded onto streptavidin-coated biosensor tips (ForteBio) in the presence or absence of the key (2 μ M) using an Octet™ instrument (ForteBio).

30 Production and purification of HzKR127-3.2

The synthetic V_H and V_L DNA fragments were subcloned into the pdCMV-dhfrC-cA10A3 plasmid containing the human C γ 1 and C κ DNA sequences. The vector was introduced into HEK 293T cells using Lipofectamine™ (Invitrogen), and the cells were grown in FreeStyle™ 293 (GIBCO) in 5% CO₂ in a 37 °C humidified incubator. The culture

supernatant was loaded onto a protein A-SepharoseTM column (Millipore). The antibody was eluted by the addition of 0.2 M glycine-HCl (pH 2.7), followed by immediate neutralization with 1 M Tris-HCl (pH 8.0). The solution was dialyzed against 10 mM HEPES-NaOH (pH 7.4), and the purity of the protein was analyzed by SDS-PAGE.

5

Production and purification of the PreS1 domain

The DNA fragment encoding the PreS1 domain (residues 1-56) was cloned into the pGEX-2T (GE Healthcare) plasmid, and the protein was produced in the *E. coli* BL21(DE3) strain (NEB) at 18 °C as a fusion protein with glutathion-S-transferase (GST) at the N-terminus. The cell lysates were prepared in a buffer solution (25 mM Tris-HCl pH 8.0, 300 mM NaCl), and clarified supernatant was loaded onto GSTBindTM Resin (Novagen). The GST-PreS1 domain was eluted with the same buffer containing additional 10 mM reduced glutathione, further purified using a SuperdexTM 75 Increase 10/300 GL (GE Healthcare) size exclusion column, and concentrated to 34 µM.

15

Production of sCageHA_267-1S and its variants

sCageHA_267-1S and sCageHA_267-1S(E99Y/T144Y) were expressed at 18 °C in the *E. coli* LEMO21(DE3) strain (NEB) as a fusion protein containing a (His)₁₀-tagged cysteine protease domain (CPD) derived from *Vibrio cholerae*⁴² at the C-terminus. The protein was purified using HisPurTM nickel resin (Thermo), a HiTrapTM Q anion exchange column (GE Healthcare) and a HiLoad 26/60 SuperdexTM 75 gel filtration column (GE Healthcare). For Selenomethionine (SeMet)-labeling, an I30M mutation was introduced additionally to generate a sCageHA_267-1S(E99Y/T144Y/I30M) variant. This protein was expressed in the *E. coli* B834 (DE3) RIL strain (Novagen) in the minimal media containing SeMet, and purified according to the same procedure for purifying the other variants.

25

Crystallization and structure determination of sCageHA_267-1S

Two point mutations (Glu99Tyr and Thr144Tyr) were introduced in an attempt to induce favorable crystal packing interactions. Good-quality single crystals of sCageHA_267-1S(E99Y/T144Y/I30M) were obtained in a hanging-drop vapor-diffusion setting by micro-seeding in a solution containing 11% (v/v) ethanol, 0.25 M NaCl, 0.1 M TrisHCl (pH 8.5). The crystals required strict maintenance of the temperature at 25 °C. For cryoprotection, the crystals were soaked briefly in the crystallization solution supplemented with 15% 2,3-butanediol and flash-cooled in the liquid nitrogen. A single-wavelength anomalous dispersion

30

(SAD) data set was collected at the Se absorption peak and processed \ positions and initial electron density map were calculated using the AutoSol module in PHENIX⁴⁴. The model building and structure refinement were performed by using COOT⁴⁵ and PHENIX.

5

Supplementary Information

Supplementary discussion:

Our generalized protein sensory system based on a *de novo* switch relies on the thermodynamic coupling (see Fig. 1a-c) between a defined close state (K_{open}) and a defined open state (K_{LT} and K_{CK}). With our system), the target specificity to arbitrary targets can be achieved not only by incorporating known binding domains but also de novo binders where we have full control over protein fold and geometry. Because there is no flexible or semi-flexible linker in our system and we are capable of designing different types of interaction to cage binding domains, the conformational change is thus decoupled from the binder-target interaction, which makes this system more structurally predictable at open state. A newly developed GraftSwitchMover in RosettaTM allows sensor design in one step, bypassing the need with the other formats to empirically re-engineer sensor configuration. The intermolecular association of the LucKey with the open form of the sensor generates the luminescent signal, providing an additional tunable parameter K_{CK} that can be optimized along with K_{open} to maximize sensor dynamic range, analytical range, specificity, and sensitivity.

10
15
20

Supplementary methods

1. Thermodynamic model

25

The equilibrium constants were defined as K_{open} for latch opening (Equation 1), K_{CK} for the dissociation constant of the lucCage and lucKey (Equation 2 and 3), and K_{LT} for the dissociation constant of the latch and target (Equation 4 and 5). K_{R} describes the equilibrium of the reconstituted luciferase, which is determined by the reported dissociation constant of the NanoBit system ($190 \mu\text{M}$ ¹⁹) and the effective local concentration (C_{eff}) of split counterparts (Equation 6 and 7). We set C_{eff} to 1 mM here as the literature suggested high micromolar to low millimolar range for intramolecular interaction partners²⁰, and our modular switch should span much shorter distance than flexible linkers. The total amount of each component is constant, so Equations 8, 9, and 10 were introduced. Given four equilibrium constants (K_{open} , K_{CK} , K_{LT} , and K_{R}) and three total concentrations ($[\text{lucCage}]_{\text{total}}$,

30

[lucKey]_{total}, and [target]_{total}), python module sympy.solve was used to solve the equations numerically and find the concentration of each species at equilibrium. The total concentration of luminescent species **6** and **7** was extracted from the solution, divided by [lucCage]_{total}, and plotted for corresponding figures with various K_{open} for **Fig. 1d**, K_{LT} for **Fig. 1e**, and [lucCage]_{total}, [lucKey]_{total} for **Fig. 1f**. Numbers for **Fig. 1f** are normalized between 0-1.

Equation 1:

$$K_{open} = \frac{[2]}{[1]}$$

Equation 2:

$$K_{CK} = \frac{[2] \times [\text{lucKey}]_{free}}{[5]}$$

Equation 3:

$$K_{CK} = \frac{[3] \times [\text{lucKey}]_{free}}{[4]}$$

Equation 4:

$$K_{LT} = \frac{[6] \times [\text{target}]_{free}}{[7]}$$

Equation 5:

$$K_{LT} = \frac{[2] \times [\text{target}]_{free}}{[3]}$$

Equation 6:

$$K_R = \frac{190 \mu\text{M}}{C_{eff}} = \frac{[5]}{[6]}$$

Equation 7:

$$K_R = \frac{[4]}{[7]}$$

Equation 8:

$$[\text{lucCage}]_{total} = [1] + [2] + [3] + [4] + [5] + [6] + [7]$$

Equation 9:

$$[\text{lucKey}]_{total} = [\text{lucKey}]_{free} + [4] + [5] + [6] + [7]$$

Equation 10:

$$[\text{target}]_{total} = [\text{target}]_{free} + [3] + [4] + [7]$$

2. Computational grafting of sensing domains into lucCage

The structural models of the lucCage sensors were created by grafting each sensing domain onto the latch of the lucCage scaffold (See Table 13). The design was performed using a RosettaScriptsTM protocol, (*GraftSwitch_relax.xml*, See code availability) to thread a list of sensing domains with annotated interface residues (*sensing_domains.fasta*, See Code Availability) into the model of lucCage (*lucCage.pdb*, See Code Availability). A bash script (*run_GraftSwitch.sh*, See Code Availability) was used to call RosettaScriptsTM. This protocol uses two successive RosettaTM movers: (i) GraftSwitchMover to thread the desired sensing domain sequence into a defined region of the lucCage latch (amino acids 325-359) and to select designs with the defined “important residues” buried in the cage/latch interface; (ii) and MultiplePoseMover to relax (FastRelax to find the lowest energy structure given the mutations from the previous mover.), filter and score each output model resulting from the

previous mover. The resulting designs were further evaluated by eye or done by selecting designs showing favorable hydrophobic packing interactions between the newly threaded sequence and the cage and discarding designs with unfavorable buried hydrophilic residues that could destabilize the closed state of the sensor (unless these residues were annotated as “important residues”).

For grafting mini-protein binders with a pre-defined tertiary structure (i.e., Bot.671.2, SpaC, and the Her2 affibody) we first identified the primary interaction surface of the binding protein to its target and identified the main secondary structure elements involved in it. We added the amino acid sequence of these elements in the *sensing_domains.fasta* file to use them in the protocol described above. The outputs were lucCage design models with the grafted interface element. Then, we used RosettaTM Remodel domain insertion²¹ to model the full-length sensing domain in the context of the switch (*remodel_domain_insertion.sh*, See Code Availability), followed by Relax to find the lowest energy structure (*relax.sh*, See Code Availability). Finally, the best designs were selected by eye in PyMol 2.0.

Supplementary Information references

1. Bahadır, E. B. & Sezgintürk, M. K. Lateral flow assays: Principles, designs and labels. *Trends Analyt. Chem.* **82**, 286–306 (2016).
2. Yeh, H.-W. & Ai, H.-W. Development and Applications of Bioluminescent and Chemiluminescent Reporters and Biosensors. *Annu. Rev. Anal. Chem.* **12**, 129–150 (2019).
3. Greenwald, E. C., Mehta, S. & Zhang, J. Genetically Encoded Fluorescent Biosensors Illuminate the Spatiotemporal Regulation of Signaling Networks. *Chem. Rev.* **118**, 11707–11794 (2018).
4. Glasgow, A. A. *et al.* Computational design of a modular protein sense-response system. *Science* **366**, 1024–1028 (2019).
5. Guo, Z. *et al.* Generalizable Protein Biosensors Based on Synthetic Switch Modules. *J. Am. Chem. Soc.* **141**, 8128–8135 (2019).
6. Yu, Q. *et al.* Semisynthetic sensor proteins enable metabolic assays at the point of care. *Science* **361**, 1122–1126 (2018).
7. van Rosmalen, M. *et al.* Dual-Color Bioluminescent Sensor Proteins for Therapeutic Drug Monitoring of Antitumor Antibodies. *Anal. Chem.* **90**, 3592–3599 (2018).
8. Adamson, H. *et al.* Affimer–Enzyme–Inhibitor Switch Sensor for Rapid Wash-free Assays of Multimeric Proteins. *ACS Sens.* **4**, 3014–3022 (2019).
9. Tenda, K. *et al.* Paper-Based Antibody Detection Devices Using Bioluminescent

BRET-Switching Sensor Proteins. *Angewandte Chemie* vol. 130 1

10. Griss, R. *et al.* Bioluminescent sensor proteins for point-of-care therapeutic drug monitoring. *Nat. Chem. Biol.* **10**, 598–603 (2014).

11. Arts, R. *et al.* Detection of Antibodies in Blood Plasma Using Bioluminescent
5 Sensor Proteins and a Smartphone. *Anal. Chem.* **88**, 4525–4532 (2016).

12. Lopez-Ruiz, N. *et al.* Smartphone-based simultaneous pH and nitrite colorimetric determination for paper microfluidic devices. *Anal. Chem.* **86**, 9554–9562 (2014).

13. Leippe, D. M. *et al.* A bioluminescent assay for the sensitive detection of
10 proteases. *Biotechniques* **51**, 105–110 (2011).

14. Troy, T., Jekic-McMullen, D., Sambucetti, L. & Rice, B. Quantitative comparison of the sensitivity of detection of fluorescent and bioluminescent reporters in animal models. *Mol. Imaging* **3**, 9–23 (2004).

15. Yeh, H.-W., Wu, T., Chen, M. & Ai, H.-W. Identification of Factors Complicating Bioluminescence Imaging. *Biochemistry* **58**, 1689–1697 (2019).

16. Yeh, H.-W. *et al.* ATP-Independent Bioluminescent Reporter Variants To Improve in Vivo Imaging. *ACS Chem. Biol.* **14**, 959–965 (2019).

17. Edwardraja, S. *et al.* Caged activators of artificial allosteric protein biosensors. *ACS Synth. Biol.* (2020) doi:10.1021/acssynbio.9b00500.

18. Langan, R. A. *et al.* De novo design of bioactive protein switches. *Nature* **572**, 205–210 (2019).

19. Dixon, A. S. *et al.* NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions in Cells. *ACS Chem. Biol.* **11**, 400–408 (2016).

20. Krishnamurthy, V. M., Semetey, V., Bracher, P. J., Shen, N. & Whitesides, G. M. Dependence of Effective Molarity on Linker Length for an Intramolecular Protein–Ligand System. *Journal of the American Chemical Society* vol. 129 1312–1320 (2007).

21. Huang, P.-S. *et al.* RosettaRemodel: a generalized framework for flexible
30 backbone protein design. *PLoS One* **6**, e24109 (2011).

Table 11. X-ray data collection and structure refinement statistics

Data Collection	SelMET-sCageHA_267-1S(E99Y/T144Y/I30M)
Space group	C2
Unit cell dimensions	
a, b, c (Å)	178.993 60.127 71.799
a, β, γ (°)	90, 112.463, 90
Wavelength (Å)	0.9794
Resolution (Å)	50-2.03 (2.03-2.00) ^a
R_{sym}	6.6 (16.5) ^a
$I/\sigma(I)$	24.0 (3.5) ^a
Completeness (%), >1σ	70.6 (33.8) ^a
Redundancy	2.5 (1.6) ^a
Refinement	
Resolution (Å)	46.09-1.99 (2.06-1.99) ^a
No. of reflections	37603
$R_{\text{work}} / R_{\text{free}}$	0.2078/0.2515
R.m.s deviations	
bond (Å) / angle (°)	0.007/0.910
Average B-values (Å ²)	38.19
Ramachandran plot (%)	
Favored / Additional allowed	97.7/2.3
Generously allowed	0.0

^aThe numbers in parentheses are the statistics from the highest resolution shell.

Table 12. Summary of biosensors in this work

Biosensor	Analytical target	Dynamic range ^a	LOD (nM)	Detection range (nM)
lucCageBim	Bcl-2	200%	0.2	0.2-12.5
lucCageBoT	Botulinum neurotoxin B	130%	0.4	0.4-50
lucCageProA	Fc domain	350%	0.39	0.39-12.5
lucCagHer2	Her2 receptor	380%	0.23	0.23-25
lucCageTrop	Troponin I	250%	0.009	0.009-0.3
lucCageHBV	Anti-HBV antibody (HzKR127-3.2)	98%	2	2-100
lucCageHBV α	Anti-HBV antibody (HzKR127-3.2)	215%	0.26	0.26-100
lucCageHBV+ HzKR127-3.2	PreS1	80%	0.6	0.6-100
lucCageSARS2-M	anti-SARS-Cov-M	50%	2.9	2.9-250
lucCageSARS2-N	anti-SARS-Cov-NP	70%	3.0	3.0-100
lucCageRBD	SARS-CoV-2 RBD	1700%	0.015	0.015-6

^aDefined as intensimetric change ($\Delta E/E_{\min}$) of total bioluminescence intensity. ΔE is the maximal change in total bioluminescence emission at saturated target concentration and $E_{\min}$ is the emission in the absence of the analytical target.

Table 13. List of sensing domains used in this work

Biosensors	Sensing domain	Sensing domain sequence
lucCageBim	Bim	EIWIAQELRRIGDEFNAYYAAA (SEQ ID NO:27380)
lucCageBoT	Bot.0671.2	MFAELKAKFFLEIGDRDAARNALRKAGYSDEEAER IIRKYELE (SEQ ID NO:27381)
lucCageProA	Protein A domain C (SpaC)	EQQNAFYIEILHLPNLTEEQRNGFIQSLKDDPSVSK EILAEAKKLNDQAAPK (SEQ ID NO:27382)
lucCagHer2	Her2 affibody	EMRNAYWEIALLPNLNNQQKRAFIRSLYDDPSQSA NLLAEAKKLNDQAAPK (SEQ ID NO:27383)
lucCageTrop	cTnI + cTnC	EDQLREKAKELWQTIYNLEAEKFDLQEKFKQQKYE INVLNRINDNQKVSCTKDDSKGKSEELSDLFMR FDKNADGYIDLEELKIMLQATGETITEDDIEELMK DGDKNNDGRIDYDEFLEFMKGVE (SEQ ID NO:27384)

		NO:27384)
lucCageHBV	preS1 (a.a. 35-46)	GANSNNPDWDFN (SEQ ID NO:27629)
lucCageHBV α	preS1 (a.a. 35-46) 2x	GANSNNPDWDFNGGSGGGSSGFGANSNNPDWDFNP N (SEQ ID NO:27630)
lucCageSARS2-M	SARS-CoV-2 nucleocapsid protein (a.a. 369- 382) 2x	MADSNGTITVEELKKLLEGGSGGMADSNGTITVEE LKKLLE (SEQ ID NO:27392)
lucCageSARS2-N	SARS-CoV-2 membrane protein (a.a. 1-17) 2x	KKDKKKKADETQALGGSGGKKDKKKKADETQAL (SEQ ID No:27548)
lucCageRBD	LCB1_delta4	ILQKIYEIMRLLELGHAEASMRVSDLIYEFMKKG DERLLEEAERLLEEVEVER (SEQ ID NO:27590)

Example 2

Expanding the universal readouts for LOCKR-based biosensors

5 The abovementioned sensor platform can be repurposed to accommodate almost all split reporters where one complementary reporter fragment is genetically fused onto the N-terminal of the cage and the other fragment to the C-terminal of the latch (intramolecular) or key (intermolecular). Various types of split-protein pairs or RET pairs (Figure 16) can enable a wide range of readouts, such as bioluminescence (firefly¹, Renilla², and Gaussia³ luciferase), bioluminescence resonance energy transfer⁴⁻⁶ (BRET), bimolecular fluorescence complementation^{7,8} (BiFC), fluorescence resonance energy transfer (FRET), colorimetry (β -lactamase⁹, β -galactosidase¹⁰, and horseradish peroxidase¹¹), cell survival (dihydrofolate reductase¹²), electrochemical (APEX2¹³), radioactive (thymidine kinase¹⁴), and molecular barcode reporter (TEV protease¹⁵).

15 The de novo switch platforms of the disclosure can be generalizable and customized to detect arbitrary targets of interest, but can also be reprogrammed with a wide range of readouts for different sensing purposes. For cellular imaging, sensors with BiFC or FRET readout can provide excellent spatiotemporal resolution to monitoring the dynamic of intracellular target. In the broad synthetic biology field, the sensors can, for example, 1) facilitate multiplex cell-based assays that use genetic biosensors for drug discovery; 2) profile chemical or genetic perturbations on target-selective pathway using molecular barcodes (TEV protease) with next-generation sequencing (NGS) as the readout technology; and 3) conduct cell survival selection by dihydrofolate reductase (DHFR) complementation in the presence of chosen target. For in vivo imaging, the biological activities and protein targets can be
25 monitored by split-luminescent proteins or by positron emission tomography (PET) with

split-thymidine kinase, which allow for imaging in deep tissue. For point-of-care applications, colorimetry readout provides the most convenient setup since no instrument is required for signal acquisition. Besides, an electrochemical readout is readily compatible with the most successful POC device – glucometer, which can read the electrochemical signal for the detection of low-abundance target. Overall, we anticipate that the combination of our de novo sensor design, binder design, and split-protein reassembly can lead to a veritable explosion of applications with user-defined inputs.

To provide proof of concept, we designed an intermolecular BRET sensor (S0512) to detect HBV antibody where teLuc was genetically fused to the cage and CyOFP was tethered to the C-terminal of the key (Figure 17A). Two copies of epitope sequences were threaded on the latch. In the presence of HBV antibody, ~5% ratiometric change (580/450 nm) was observed with a limit of detection ~11 nM. Meanwhile, we also design an intramolecular BRET sensor (S0622) containing teLuc (BRET donor) and CyOFP (BRET acceptor) on the N- and C- terminal of cage. The design leads to high initial BRET efficiency. In the presence of HBV antibody, the antibody-latch driving force will break the interaction of cage-latch and then increase the distance of BRET donor and acceptor, leading to a decrease in BRET efficiency (Figure 17B). The limit of detection of S0622 was determined to be ~11 nM with ~20% 450/580 nm ratiometric change. To improve the ratiometric change, we optimized the linker length between key and CyOFP. B0622_6 showed the highest initial BRET efficiency. Up to ~207% 450/580 nm ratiometric change was observed while the sensor retained low nM sensitivity (Figure 17C). Again, the dynamic range and sensitivity of sensor can be modulated by the key concentration, which is one of the tunable factors in our modular sensor platform.

To expand the readout for point-of-care application, we utilized the split β -lactamase to report the assembly of cage and key upon the actuation. Reconstituted β -lactamase is able to catalyze the hydrolysis of a colorimetric substrate – Nitrocefin, thereby giving reddish product (OD 490). This colorimetry readout is advantageous over optical readout for point-of-care applications because the color change can be directly distinguished by human eyes. Compare to flash type bioluminescence, which generally shows the bursting emission causing a significant complexity on time-dependent signal acquisition, the resultant colorimetric product accumulates in solution overtime. Therefore, it is an end-point assay (more active β -lactamase reaches to the end-point faster). Notably, β -lactamase can remain active in biological fluid e.g., serum and urine¹⁹. The critical design insight here is to lower the background activity as much as possible to reduce the chance of false positives. We

demonstrate the conversion of lucCageTrop to LacATrop by simply m fusion and a Key-LacB fusion (Figure 18). The β -lactamase activities were turned on in the presence of human cardiac Troponin I (cTnI). Good standard curves were obtained with low nM sensitivity and the color change from yellow to red can be easily determined by human eyes.

Design sequence:

S0512 (teLuc sequence in bold font; underline HBV epitopes):

MGSHHHHHHSGSGSENLYFQGS**GGVFTLEDFVGDWRQT**AGYNLS**QVLEQGGVSSSLFQNLGVSVTP**IQRI
 10 **VLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVPVDNHHFKVILHYGTLVIDGVT**PNMIDYFGR
PYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLLFRVTINGVTGWRLHERILAGS (SELARKLL
 EASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDD
 AAKESEKILEEAREAISGSGSELAKLLKAIETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTD
 PATIREALEHAKRRSKEIIDEAERAIRAAKRESERIEEARRLIEKSGSGSELARELLRAHAQLQRL
 15 NLELLRELLRALAQLQELNLDLLRLASEL) TDPDEARKAANSNNPDWDFIVEDAERLIREAAAAANSN
NPDWDFLIR (SEQ ID NO:27651)

Key-2GGSGG-CyOFP (CyOFP sequence in bold font):

MDPDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERLIREAAAASEKISREGSGG
 20 **GGVSK GEELIK ENMRSKLYLE GSVNGHQFC THEGEGKPYE GKQTNRIKVV EGGPLPFAFD**
ILATHFMYGS KVFIKYPADL **PDYFKQSFPE GFTW**ERVMVF **EDGGVLTATQ DTS**LQDGELI
YNVKVRGVNF PANGPVMQKK TLGWEPSTET **MYPADGGLEG RCDKALKLVG GGHLHVNFKT**
TYKSKPKVKM PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSNLGGGMD **ELYK** (SEQ ID
 NO: 27622)

B0622 (teLuc sequence in bold font; CyOFP sequence bold and underlined; underline HBV epitopes):

MGSHHHHHHSGSGSENLYFQGS**GGVFTLEDFVGDWRQT**AGYNLS**QVLEQGGVSSSLFQNLGVSVTP**IQRI
VLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVPVDNHHFKVILHYGTLVIDGVTPNMIDYFGR
 30 **PYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLLFRV**TINGVTGWRLHERILAGS (SKEAAKKL
 QDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSK
 EIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLNLELAKLLLKAI
 AETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKR
 ESERIEEARRLIEKAKEESERIEGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
 35 RALAQLQELNLDLLRLASEL) TDPDEARKAANSNNPDWDFIVEDAERLIREAAAASEKISREAERLAN
SNNPDWDFISRE **VSKGEELIK ENMRSKLYLE GSVNGHQFC THEGEGKPYE GKQTNRIKVV**
EGGPLPFAFD ILATHFMYGS KVFIKYPADL **PDYFKQSFPE GFTW**ERVMVF **EDGGVLTATQ**

DTSLQDGELI YNVKVRGVNF PANGPVMQKK TLGWEPSTET MYPADGGI

GGHLHVNFKT TYKSKKPVKM PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSLGGM

ELYK (SEQ ID NO:27652)

5 B0622_4:

MGSHHHHHHSGSGSENLYFQSGGGVFTLEDFVGDWRQTAGYNLSQVLEQGGVSSLFQNLGVSVTPIQRI
VLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVPVDNHHFKVILHYGTLVIDGVTPNMIDYFGR
PYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLFRVTINGVTGWRLHERILAGS (SKEAAKKL
QDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSK
10 EIIRRAEKEIDDAAKESKKILEEARKAIRDAAESRKILEEGSGSGSDALDELQKLNLELAKLLLKAI
AETQDLNLRAAKAFLEAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKR
ESERIIEEARLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQLQELNLDLLRLASEL) TDPDEARKAANSNNPDWDFIVEDAERLIREAAAASEKISREAERLAN
SNNPDWDFISRE EELIK ENMRSKLYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV

15 EGGPLPFAFD ILATHFMYGS KVFYKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ
DTSLQDGELI YNVKVRGVNF PANGPVMQKK TLGWEPSTET MYPADGGLEG RCDKALKLVG
GGHLHVNFKT TYKSKKPVKM PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSLGGM
ELYK (SEQ ID NO:27653)

20 B0622_6:

MGSHHHHHHSGSGSENLYFQSGGGVFTLEDFVGDWRQTAGYNLSQVLEQGGVSSLFQNLGVSVTPIQRI
VLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVPVDNHHFKVILHYGTLVIDGVTPNMIDYFGR
PYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLFRVTINGVTGWRLHERILAGS (SKEAAKKL
QDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSK
25 EIIRRAEKEIDDAAKESKKILEEARKAIRDAAESRKILEEGSGSGSDALDELQKLNLELAKLLLKAI
AETQDLNLRAAKAFLEAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRAAKR
ESERIIEEARLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELL
RALAQLQELNLDLLRLASEL) TDPDEARKAANSNNPDWDFIVEDAERLIREAAAASEKISREAERLAN
SNNPDWDFISRE LIK ENMRSKLYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV

30 EGGPLPFAFD ILATHFMYGS KVFYKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ
DTSLQDGELI YNVKVRGVNF PANGPVMQKK TLGWEPSTET MYPADGGLEG RCDKALKLVG
GGHLHVNFKT TYKSKKPVKM PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSLGGM
ELYK (SEQ ID NO:27654)

35 Key-LacB (split β -lactamase B in bold):

SGSGDPDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERLIREAAAASEKISRESGGGGG
GGGGSGGGG **LLTLASRQQLIDWME ADKVAGPLLR SALPAGWFIA DKSGAGERGS**

RGIIAALGPD GKPSRIVVIY TTGSQATMDE RNRQIAEIGA SLIKHW

27623)

LacATrop (split β -lactamase A in bold; underline cTnT and cTnC):

5 MGSHHHHHHGSGSENLYFQGS~~SGGS~~**VFAHPETLVK VKDAEDQLGA** RVGYIELDLN
SGKILESFRP EERFPMSTF KVLLCGAVLS RVDAG**QEQLG** RRIHYSQNDL
VEYSPVTEKH LTDGMTVREL CSAAITMSDN TAANLLLT**TI** GGP**KELTAFL**
HNMGDHVTRL DRWEPELNEA IPNDERDTT**PAAMATTLRK** LLTG**ENGR**
SGGGGSGGGGSGGGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELV
10 YLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSG
SGSDALDELQKLNLELAKLLKAI**AETQDLN**LRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIRRA
LEHAKRRSKEIIDEAERAIRAAKRESERIIEEARLIEKAKEESERIIREGSGSGDPDIKKLQDLNIE
LARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEILD
AERLIREAAA**SEDQLREAAKELWQTIYNLEAEKFDLQEKFKQKYEINVLNRINDNQKVS**KT**KDDS**
15 KGKSEELSDLFRMFDKNADGYIDLEELKIMLQATGETITEDDIEELMKDGDKNNDGRIDYDEFLEFM
KGVE (SEQ ID NO:27620)

References:

- (1) Luker, K. E.; Smith, M. C.; Luker, G. D.; Gammon, S. T.; Piwnica-Worms, H.; Piwnica-Worms, D. *Proc Natl Acad Sci U S A* **2004**, *101*, 12288-12293.
- (2) Kaihara, A.; Kawai, Y.; Sato, M.; Ozawa, T.; Umezawa, Y. *Anal Chem* **2003**, *75*, 4176-4181.
- (3) Remy, I.; Michnick, S. W. *Nat Methods* **2006**, *3*, 977-979.
- (4) Chu, J.; Oh, Y.; Sens, A.; Ataie, N.; Dana, H.; Macklin, J. J.; Laviv, T.; Welf, E. S.; Dean, K. M.; Zhang, F.; Kim, B. B.; Tang, C. T.; Hu, M.; Baird, M. A.; Davidson, M. W.; Kay, M. A.; Fiolka, R.; Yasuda, R.; Kim, D. S.; Ng, H. L., et al. *Nat Biotechnol* **2016**, *34*, 760-767.
- (5) Yeh, H. W.; Karmach, O.; Ji, A.; Carter, D.; Martins-Green, M. M.; Ai, H. W. *Nat Methods* **2017**, *14*, 971-974.
- (6) Yeh, H. W.; Xiong, Y.; Wu, T.; Chen, M.; Ji, A.; Li, X.; Ai, H. W. *Acs Chem Biol* **2019**, *14*, 959-965.
- (7) Zhou, J.; Lin, J.; Zhou, C.; Deng, X.; Xia, B. *Acta Biochim Biophys Sin (Shanghai)* **2011**, *43*, 239-244.
- (8) Ohashi, K.; Kiuchi, T.; Shoji, K.; Sampei, K.; Mizuno, K. *Biotechniques* **2012**, *52*, 45-50.
- (9) Galarneau, A.; Primeau, M.; Trudeau, L. E.; Michnick, S. W. *Nat Biotechnol* **2002**, *20*, 619-622.
- (10) Wehrman, T. S.; Casipit, C. L.; Gewertz, N. M.; Blau, H. M. *Nat Methods* **2005**, *2*, 521-527.
- (11) Martell, J. D.; Yamagata, M.; Deerinck, T. J.; Phan, S.; Kwa, C. G.; Ellisman, M. H.; Sanes, J. R.; Ting, A. Y. *Nat Biotechnol* **2016**, *34*, 774-780.
- (12) Remy, I.; Michnick, S. W. *Proc Natl Acad Sci U S A* **1999**, *96*, 5394-5399.

(13) Han, Y.; Branon, T. C.; Martell, J. D.; Boassa, D.; Shechner, D.; Ellism; *Chem Biol* **2019**, *14*, 619-635.

(14) Massoud, T. F.; Paulmurugan, R.; Gambhir, S. S. *Nat Med* **2010**, *16*, 921-926.

(15) Wehr, M. C.; Holder, M. V.; Gailite, I.; Saunders, R. E.; Maile, T. M.; Ciirdaeva, E.; Instrell, R.; Jiang, M.; Howell, M.; Rossner, M. J.; Tapon, N. *Nat Cell Biol* **2013**, *15*, 61-U132.

(16) Landry, C. R.; Levy, E. D.; Abd Rabbo, D.; Tarassov, K.; Michnick, S. W. *Cell* **2013**, *155*, 983-989.

(17) Bowes, J.; Brown, A. J.; Hamon, J.; Jarolimek, W.; Sridhar, A.; Waldron, G.; Whitebread, S. *Nat Rev Drug Discov* **2012**, *11*, 909-922.

(18) Geyer, P. E.; Holdt, L. M.; Teupser, D.; Mann, M. *Mol Syst Biol* **2017**, *13*, 942.

(19) Adamson, H.; Ajayi, M. O.; Campbell, E.; Brachi, E.; Tiede, C.; Tang, A. A.; Adams, T. L.; Ford, R.; Davidson, A.; Johnson, M.; McPherson, M. J.; Tomlinson, D. C.; Jeuken, L. J. C. *ACS Sens* **2019**, *4*, 3014-3022.

Example 3

As exemplified in **Figure 20**, (Panel A) the above-mentioned sensor platform can be repurposed to accommodate an "indirect detection" approach, in which the split reporter protein (intermolecular or intramolecular embodiments; an intermolecular embodiment is shown below) is reconstituted by pre-incubation of the biosensor with the target (exemplified by an antibody) for the target binding polypeptide, resulting in luminescence activation in this example. The activated biosensor is then incubated with a sample to detect the presence of an antigen to which the antibody binds, resulting in binding of the antibody to the antigen, loss of interaction between the split reporter protein components, and reduction/elimination of reporting activity (in this case, loss of luminescence activity). As will be clear based on the disclosure herein, this embodiment can be used for indirect detection of any analyte of interest. This approach is not limited to using antibodies and their cognate antigens. In another embodiment (Panel B) the split reporter protein (intermolecular or intramolecular embodiments; an intermolecular embodiment is shown below) is reconstituted by pre-incubation of the biosensor with the target (exemplified by the SARS-CoV-2 Spike protein) for the target binding polypeptide, resulting in luminescence activation in this example. The activated biosensor is then incubated with a sample to detect the presence of an inhibitor (exemplified by LCB1 inhibitor) to which the Spike binds, resulting in binding of the antibody to the antigen, loss of interaction between the split reporter protein components, and reduction/elimination of reporting activity (in this case, loss of luminescence activity). This approach can be used for detection of an inhibitor, but also as a tool to evaluate the inhibitory

potency of multiple variants. As will be clear based on the disclosure I embodiment can be used for indirect detection of any analyte of interest. Another example is shown in **Figure 21**.

5 Exemplary uses

Diagnostic sensors herein (lucCageBim, lucCageBot, lucCageTrop, lucCageProA, lucCageHer2, lucCageHBV, lucCageSARS2-M, lucCageSARS2-N) and measured the activation kinetics of each in response to all of their targets (Bcl-2, botulinum neurotoxin B, cardiac Troponin I, IgG Fc, Her2, anti-HBV (HzKR127-3.2), the anti-SARS-M polyclonal antibody (3527,)), the anti-SARS-N monoclonal antibody (18F629.1)). Each sensor responded rapidly and sensitively to its cognate target, but not to any others (**Figure 19C**). Through SeroNet, the best CoV LOCKR Diagnostics can be formatted into various POCDs (**Figure 19D**).

LOCKR Diagnostic combinations that activate chemiluminescence in the presence of anti-coronavirus "anti-epitope" specific antibodies from drop of blood or serum, and that can be turned off by addition of an antigen that contains the epitope of interest are exemplified in **Figure 22**.

Example 4.

SARS -CoV-2 infection is thought to often start in the nose, with virus replicating there for several before spreading to the broader respiratory system. Delivery of a high concentration of a viral inhibitor into the nose and into the respiratory system generally could therefore potentially provide prophylactic protection, and therapeutic efficacy early in infection, and could be particularly useful for health care workers and others coming into frequent contact with infected individuals. A number of monoclonal antibodies are in development as systemic SARS-CoV-2 therapeutics, but these compounds are not ideal for intranasal delivery as antibodies are large and often not extremely stable molecules, and the density of binding sites is low (two per 150Kd antibody); the Fc domain provides little added benefit. More desirable would be protein inhibitory with the very high affinity for the virus of the monoclonals, but with higher stability and very much smaller size to maximize the density of inhibitory domains and enable direct delivery into the respiratory system through nebulization.

We set out to *de novo* design high affinity binders to the RBD that compete with Ace2 binding. We explored two strategies: first we attempted to scaffold the alpha helix in Ace2

that makes the majority of the interactions with the RBD in a small des
 makes additional interactions with the RBD to attain higher affinity, and second, we sought to
 design binders completely from scratch that do not incorporate any known binding interaction
 with the RBD. An advantage of the second approach is that the range of possibilities for
 5 design is much larger, and so potentially higher affinity binding modes can be identified. For
 the first approach, we used the Rosetta™ blue print builder to generate small proteins which
 incorporate the Ace2 helix and for the second approach, RIF docking and design using large
 miniprotein libraries. The designs interact with distinct regions of the RBD surface
 surrounding the Ace2 binding sites. Designs for approach 1, and approach 2, were encoded
 10 in long oligonucleotides, and screened for binding to fluorescently tagged RBD on the yeast
 cell surface. Deep sequencing identified 3 Ace2 helix scaffolded designs (approach 1), and
 150 de novo interface designs (approach 2) that were clearly enriched following FACS
 sorting for RBD binding. Designs were expressed in *E. coli* and purified, and many were
 found to be have soluble expression and to bind RBD in biolayer interferometry experiments
 15 and could effectively compete with ACE-2 for binding to RBD (example shown in Figure 2).
 Based on BLI data the RBD binding affinities of minibinders are: LCB1 <1nM, LCB3
 <1nM. The affinities of LCB2, LCB4, LCB5, LCB6, LCB7, LCB8 range from 1~20nM,
 with relative strength of different binders being LCB4 > LCB2 > LCB9 = LCB5 > LCB6 >
 LCB7.

20 To determine whether the designs binding the RBD through the designed interfaces,
 site saturation libraries in which every residue in each design was substituted with each of the
 20 amino acids one at a time were constructed, and subjected to FACS sorting for RBD
 binding. Deep sequencing showed that the binding interface residues and protein core
 residues were conserved in many of the designs for which such site saturation libraries
 25 (SSM's) were constructed (SSMs were used to define allowable positions for amino acid
 changes in **Table 3**). For most of the designs, a small number of substitutions were enriched
 in the FACS sorting, suggesting they increase binding affinity for RBD. For the highest
 affinity of the approach 1 designs, and 8 of the approach 2 designs, combinatorial libraries
 incorporating these substitutions were constructed and again screened for binding with
 30 FACS; because of the very high binding affinity the concentrations used in the sorting were
 as low as 20pM. Each library converged on a small number of closely related sequences, and
 for each design, one of the optimized variants was expressed in *E. coli* and purified.

The binding of the 8 optimized designs with different binding modes to RBD was
 investigated by biolayer interferometry. For a number of the designs, the K_d's ranged from

1-20nM, and for the remainder, the K_d 's were below 1nM, too strong t
with this technique. Circular dichroism spectra of the designs were consistent with the design
models, and the designs retained full binding activity after a number of days at room
temperature.

5 We investigated the ability of the designs to block infection of human cells by live
virus. 100 FFU of SARS-CoV-2 was added to $2.5-3 \times 10^4$ vero cells in the presence of
varying amounts of the designed binders. We observed potent inhibition of infection for all
of the designs with IC_{50} 's ranging from 1 nM to 0.02 nM.

The designed binders have several advantages over antibodies as potential
10 therapeutics. Together, they span a range of binding modes, and in combination viral escape
would be quite unlikely. The retention of activity after extended time at elevated
temperatures suggests they would not require a cold chain. The designs are 20 fold smaller
than a full antibody molecule, and hence in an equal mass have 20 fold more potential
neutralizing sites, increasing the potential efficacy of a locally administered drug. The cost of
15 goods and the ability to scale to very high production should be lower for the much simpler
miniproteins, which unlike antibodies, do not require expression in mammalian cells for
proper folding. The small size and high stability should make them amenable to direct
delivery into the respiratory system by nebulization. Immunogenicity is a potential problem
with any foreign molecule, but for previously characterized small de novo designed proteins
20 little or no immune response has been observed, perhaps because the high solubility and
stability together with the small size makes presentation on dendritic cells less likely.

References

1. Yuan M, Wu NC, Zhu X, Lee CD, So RTY, Lv H, Mok CKP, Wilson IA: **A highly**
25 **conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-**
CoV. *Science* 2020, **368**(6491):630-633.
2. Case JB, Rothlauf PW, Chen RE, Liu Z, Zhao H, Kim AS, Bloyet L-M, Zeng Q, Tahan S,
Droit L *et al*: **Neutralizing antibody and soluble ACE2 inhibition of a replication-**
competent VSV-SARS-CoV-2 and a clinical isolate of SARS-CoV-2. *bioRxiv*
30 2020:2020.2005.2018.102038.

We claim

1. A cage protein comprising a helical bundle, wherein the cage protein comprises a structural region and a latch region, wherein the latch region comprises one or more target binding polypeptide, wherein the cage protein further comprises a first reporter protein domain, wherein the first reporter protein domain undergoes a detectable change in reporting activity when bound to a second split reporter protein domain, and wherein the structural region interacts with the latch region to prevent solution access to the one or more target binding polypeptide.
2. The cage protein of claim 1, further comprising the second reporter protein domain, wherein one of the first reporter protein domain and the second reporter domain is present in the latch region and the other is present in the structural region, wherein an interaction of the first reporter protein domain and the second reporter protein domain is diminished in the presence of target to which the one or more target binding polypeptide binds.
3. The cage protein of claim 1, wherein the second reporter protein domain is not present in the cage protein.
4. The cage protein of any one of claims 1-3, wherein the helical bundle comprises between 2-9, 2-8, 2-7, 3-9, 3-8, 3-7, 4-9, 4-8, 4-7, 5-9, 5-8, 5-7, 6-9, 6-8, 6-7, 2-6, 3-6, 4-6, 5-6, 2-5, 3-5, 4-5, 2-4, 3-4, 2-3, 2, 3, 4, 5, 6, 7, 8, or 9 alpha helices.
5. The cage protein of any one of claims 1-4, wherein each helix in the structural region is independently between 18-60, 18-55, 18-50, 18-45, 22-60, 22-55, 22-50, 22-45, 25-60, 25-55, 25-50, 25-45, 28-60, 28-55, 28-50, 28-45, 32-60, 32-55, 32-50, 32-45, 35-60, 35-55, 35-50, 35-45, 38-60, 38-55, 38-50, 38-45, 40-60, 40-58, 40-55, 40-50, or 40-45 amino acids in length.
6. The cage protein of any one of claims 1-5, comprising amino acid linkers connecting adjacent alpha helices.
7. The cage protein of claim 6, wherein the amino acid linkers are independently between 2 and 10 amino acids in length, not including any further functional sequences that may be fused to the linker, or independently 3-10, 4-10, 5-10, 6-10, 7-10, 8-10, 9-10, 2-9, 3-

9, 4-9, 5-9, 6-9, 7-9, 8-9, 2-8, 3-8, 4-8, 5-8, 6-8, 7-8, 2-7, 3-7, 4-7, 5-7, 6-7, 2-6, 3-6, 4-6, 5-6, 2-5, 3-5, 4-5, 2-4, 3-4, 2-3, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids in length.

8. The cage protein of any one of claims 1-7, wherein the latch region is at the C-terminus of the cage protein. In other embodiments, the latch region may be at the N-terminus of the cage protein

9. The cage protein of any one of claims 1-8, wherein the first reporter protein domain is present in the latch region.

10. The cage protein of claim 9, wherein the second reporter protein, when present, is present in the structural region.

11. The cage protein of claim 10, wherein the second reporter protein is at the N-terminus of the structural region, or is within 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid of the N-terminus of the structural region.

12. The cage protein of claim 9, wherein the one or more target binding polypeptide and the first reporter protein domain are separated by at least 10 amino acids in the latch region.

13. The cage protein of any one of claims 1-12, wherein the one or more target binding polypeptide is at or within 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid of the C-terminus of the latch region.

14. The cage protein of any one of claims 1-12, wherein the first reporter protein domain is at the C-terminus of the latch region or within 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid of the C-terminus of the latch region, or wherein the first reporter protein domain is at the N-terminus of the latch region or within 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid of the N-terminus of the latch region.

15. The cage protein of any one of claims 1-14, wherein the first reporter protein domain, and the second reporter domain when present, comprise reporter protein domains selected from the group consisting of luciferase (including but not limited to firefly, Renilla, and

Gaussia luciferase), bioluminescence resonance energy transfer (BRET) reporters, bimolecular fluorescence complementation (BiFC) reporters, fluorescence resonance energy transfer (FRET) reporters, colorimetry reporters (including but not limited to β -lactamase, β -galactosidase, and horseradish peroxidase), cell survival reporters (including but not limited to dihydrofolate reductase), electrochemical reporters (including but not limited to APEX2), radioactive reporters (including but not limited to thymidine kinase), and molecular barcode reporters (including but not limited to TEV protease).

16. The cage protein of any one of claims 1-15 wherein the latch region is at the C-terminus of the cage protein.

17. The cage protein of any one of claims 1-15, wherein the latch region is at the N-terminus of the cage protein.

18. The cage protein of any one of claims 1-8, wherein the first reporter protein domain is present in the latch region.

19. The cage protein of claim 18, wherein the second reporter protein, when present, is present in the structural region.

20. The cage protein of claim 19, wherein the second reporter protein is at the N-terminus of the structural region, or is within 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid of the N-terminus of the structural region.

21. The cage protein of claim 18, wherein the one or more target binding polypeptide and the first reporter protein domain are separated by at least 10 amino acids in the latch region.

22. The cage protein of any one of claims 1-21, wherein the one or more target binding polypeptide is at or within 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid of the C-terminus of the latch region.

23. The cage protein of any one of claims 1-21, wherein the first reporter protein domain is at the C-terminus of the latch region.

24. The cage protein of any one of claims 1-23, wherein the first reporter protein domain, and the second reporter domain when present, comprise a split reporter protein domain from a reporter selected from the group consisting of luciferase (including but not limited to firefly, Renilla, and Gaussia luciferase), bimolecular fluorescence complementation (BiFC) reporters, colorimetry reporters (including but not limited to β -lactamase, β -galactosidase, and horseradish peroxidase), cell survival reporters (including but not limited to dihydrofolate reductase), electrochemical reporters (including but not limited to APEX2), radioactive reporters (including but not limited to thymidine kinase), and molecular barcode reporters (including but not limited to TEV protease).

25. The cage protein of any one of claims 1-24, wherein the cage does not include the second split reporter domain, wherein the first split reporter protein domain comprises:

(a) an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27359 and 27664-27672;

(b) an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 27360-27361:

VFAHPETL VKVKDAEDQLGA RVGYIELDLN SGKILESFRP EERFPMMSTF KVLICGAVLS
RVDAGQEQLG RRIHYSQNDL VEYSPVTEKH LTDGMTVREL CSAAITMSDN TAANLLLTII
GGPKELTAFL HNMGDHVTRL DRWEPELNEA IPNDERDTT PAAMATTLRK LLTGENGR
(split β -lactamase A; SEQ ID NO:27360) and

LLTLASRQQLIDWME ADKVAGPLLR SALPAGWFIA DKSGAGERGS RGIIAALGPD
GKPSRIVVIY TTGSQATMDE RNRQIAEIGA SLIKHW (Split beta lactamase B; SEQ ID
NO:27361);

(c) an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:27362-27378, wherein underlined residues are amino acid linkers or other optional residues that may be present or absent, and when present may be any amino acid sequence, and wherein any N-terminal methionine residues may be present or absent:

VFTLEDFVGDWRQTAGYNLSQVLEQGGVSSLFQNLGVSVTPIQIRIVLSGENGLKIDIHVIIPYEGLSG
DQMGQIEKIFKVVYPVDNHHFKVILHYGTLVIDGVTPNMDYFGRPYEGIAVFDGKKITVTGTLWNGN
KIIDERLINPDGSLFRVTINGVTGWRLHERILA (TeLuc; SEQ ID NO:27362) (full
luminescent or fluorescent protein that can be used to create FRET and/or BRET sensors);

- LIK ENMRSKLYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV EGGPLPFAFD ILATHFMYGS
KVFYKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ DTSIQDGELI YNVKVRGVNF
PANGPVMQKK TLGWEPSTET MYPADGGLEG RCDKALKLVG GGHLHVNFKT TYKSKKPVKM
5 PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSLNKGMD ELYK (CyOFF variant; SEQ ID
NO:27363) (full luminescent or fluorescent protein that can be used to create FRET and/or
BRET sensors);
- VSKGEELIK ENMRSKLYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV EGGPLPFAFD ILATHFMYGS
10 KVFYKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ DTSIQDGELI YNVKVRGVNF
PANGPVMQKK TLGWEPSTET MYPADGGLEG RCDKALKLVG GGHLHVNFKT TYKSKKPVKM
PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSLNKGMD ELYK (CyOFF variant SEQ ID
NO:27364) (full luminescent or fluorescent protein that can be used to create FRET and/or
BRET sensors);
- EELIK ENMRSKLYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV EGGPLPFAFD ILATHFMYGS
KVFYKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ DTSIQDGELI YNVKVRGVNF
PANGPVMQKK TLGWEPSTET MYPADGGLEG RCDKALKLVG GGHLHVNFKT TYKSKKPVKM
15 PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSLNKGMD ELYK (CuOFF variant; SEQ ID
20 NO:27365) (full luminescent or fluorescent protein that can be used to create FRET and/or
BRET sensors);
- KVFTLGDVFGDWRQTAGYNQAQVLEQGGTSLFQNLGVSVTPIQRIVLSGENGLKIDIHV
IIPYEGLSCDQMAQIEKIFKVVYPVDDHHFKAILHYGTLVIDGVTNPMIDYFGQPYEGIA
25 KFDGKKITVTGTLWNGNTIIDERLINPDGSLFRVTINGVTGWRHERILA (LumiLuc; SEQ ID
NO:27366) (full luminescent or fluorescent protein that can be used to create FRET and/or
BRET sensors);
- MVSKGEEDNM ASLPATHELH IFGSINGVDF DMVGQGTGNP NDGYEELNLK STKGDLQFSP W
ILVPHIGYG FHQYLPYPDG MSPFQAAMVD GSGYQVHRM QFEDGASLTV NYRYTYEGSH IKG
30 EAQVKGT GFPADGPVMT NSLTAADWCR SKKTYPNDKT IISTFKWSYT TGNGKRYRST ARTTY
TFAKP MAANYLKNQP MYVFRKTELK HSKTELNFKE WQKAFTDVMG MDELYK
(mNeonGreen; SEQ ID NO:27367) (full luminescent or fluorescent protein that can
be used to create FRET and/or BRET sensors);
- MVSKGEAVIK EFMRFKVHME GSMNGHEFEI EGEGERPYE GTQTAKLKVT KGGPLPFSWD
35 ILSPQFMYGS RAFIKHPADI PDYKQSFPE GFKWERVMNF EDGAVTVTQ DTSLEDGTL
I YKVKLRGTNF PPDGPVMQKK TMGWEASTER LYPEDGVLKG DIKMALRLKD GGRYLADF
KT TYKAKKPVQM PGAYNVDRKL DITSHNEDYT VVEQYERSEG RHSTGGMDEL YK
(mScarlet-i; SEQ ID NO:27368) (full luminescent or fluorescent protein that can be
used to create FRET and/or BRET sensors);
- SGKSYPTVSADYQKAVEKAKKRLGGFIAEKRCAPLMLRLAWHSAGTFDKRKTGGPFGTIRYPaelah
40 SANGLDIAVRLLEPLKAEFPILSYADFYQLAGVAVEVTGGPEVFPFHGREDKPELPPEGRLPDATK
GSDHLRDVFGKAMGLTDQDIVALSGGHTLGAHAKERSGFEGPWTSNPLVFDNSYFTELLSKEKEGGG
SGGGGS (APEX2-1-200; SEQ ID NO:27369);
- GGGGSGGGGS GLLQLPSDKALLSDPVFRPLVDKYAADEDAFFADYAEAHQKLSELGFADA
45 (APEX2-201-250; SEQ ID NO:27370);
- MGSHHHHHHSGSGSENLYFQSGGS

VRPLNCIVA VSQNMIGIGKN GDLWPPLRN ESKYFQRM TT TSSVEGKQNL
 VIMGRKTWFS IPEKNRPLKD RINIVLSREL KEPPRG AHFL AKSLDDALRL
 IEQPELGGGGSGGGGS (DHFR_A (1-105); SEQ ID NO:27371);

5 SGSGDPDEARKAIARVKRESKRIVEDAERLIREAAAAASEKISREAERLIREAAAAASEKISRE
 GGGGSGGGGS ASKV DMVWIVGGSS VYQEAMNQPG HLRLFVTRIM QEFESDTFFP
 EIDLGKYKLL PEYPGVLSEV QEEKGIKYKF EVYEK KD (DHFR_B (106-186); SEQ ID
 NO:27372);

10 QLTPTFYDN SCPNVSNIVRDIIVNELRSDPRIAASILRLHFHDCFVNGCDASILLDNTTSFRTEKDAF
 GNANSARGFSVIDR
 MKAAVESACPGTVSCADLLTIAAQQSVTLAGGPSWRVPLGRRDSLQAFDLANANLPAPFFTLPLQKD
 SFRNVGLNRSSDLVALSGGHTFGKSQCRFIMDRLYNFSNTGLPDPTLNTTYLQTLRGLCPLNGGSGS
 (sHRPa is the large split HRP fragment. It consists of amino acids
 1-213 of horseradish peroxidase (HRP) with the following 4
 15 mutations: T21I, P78S, R93G, N175S) (SEQ ID NO:27373);

NLSALVDFDLRTPTIFDNKYVNL EEQKGLIQSDQELFSSPDATDTIPLVRSFANSTQTFNFAFVEAM
 DRMGNITPLTGTQGQIRRNCRVNSNGGSGS (sHRPb is the small split HRP
 fragment. It consists of amino acids 214-308 of horseradish
 peroxidase (HRP) with the following 2 mutations: N255D, L299R) (SEQ
 20 ID NO:27374);

GESLFGKPRDYNPISSTICHLTNE SDGHTTSLYGIGFGPFITNKHLFRRNNGTLLVQSLHGVFKVKN
 TTTLQQHLIDGRDMIIRMPKDFPFPQKLKFREPQREERICLVTTNFQTGGGGSGGGGS (N_Tev
 (1-118) (SEQ ID NO:27375);

25 GGGGSGGGGSKSMSSMVSDTSC TFPSSDGIFWKHWIQTKDGQCGSPLVSTRDGFIVGIHSASNFTNTN
 NYFTSVPKNFMELLTNQEAQQWVSGWRLNADSVLWGGHKVFM D KP C_Tev (119-221) (SEQ
 ID NO:27376);

30 MASYPCHQHA SAFDQAARSR GHSNRRTALR PRRQQEATEV RLEQKMPTLL
 RVYIDGPHGM GKT TTTTQLLV ALGSRDDIVY VPEPMTYWQV LGASETIANI
 YTTQHRLDQG EISAGDAVV MTSAQITMG M PYAVTDAVLA PHIGGEAGSS
 HAPPPALTLI FDRHP IAALL CYP AARYLMG SMTPQAVLAF VALIPPTLP G
 TNIVLGALPE DRHIDRLAKR QRPGERLDLA MLAAIRRVYG LLANTVRYLQ
 GGGSWREDWG QLSGT GGGGSGGGGS (thymidine kinase_TK_A (1-265) (SEQ ID
 NO: 27377); and/or

35 GGGGSGGGGS AVPPQ GAEPQSNAGP RPHIGDTLFT LFRAPPELLAP
 NGDLYNVFAW ALDVLAKRLR PMHVFILDYD QSPAGCRDAL LQLTSGMVQT
 HVTTPGSIPT ICDLARTFAR EMGEAN (thymidine kinase_TK_B (266-376) (SEQ
 ID NO: 27378).

26. The cage protein of any one of claims 1-24, wherein the cage comprises the second
 40 split reporter protein domain, wherein

- (a) one of the first reporter protein domain and the second reporter protein
 domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%,

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NOS: 27359, and 27664-27672;

and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence

5 of SEQ ID NO: 27379, wherein the N-terminal methionine residue may be present or absent:
MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRIVRSGENALKIDIHIIIPYEGLSADQMAQIE
EVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLITPDGSM
LFRVTINS (LgBiT) (SEQ ID NO: 27379);

(b) one of the first reporter protein domain and the second reporter protein domain
10 comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,
94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:
27360

VFAHPETL VKVKDAEDQLGA RVGYIELDLN SGKILESFRP EERFPMSTF KVLICGAVLS
RVDAGQEQLG RRIHYSQNDL VEYSPVTEKH LTDGMTVREL CSAAITMSDN TAANLLLTII
15 GGPKELTAFI HNMGDHVTIRL DRWEPELNEA IPNDERDTT PAAMATTLRK LLTGNGR (split β -
lactamase A) (SEQ ID NO: 27360),

and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%,
91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence
of SEQ ID NO: 27361:

20 LLTLASRQQLIDWME ADKVAGPLLK SALPAGWFIA DKSGAGERGS RGIIAALGPD GKPSRIVVIY
TTGSQATMDE RNRQIAEIGA SLIKHW (Split beta lactamase B) (SEQ ID NO:
27361);

(c) one of the first reporter protein domain and the second reporter protein domain
comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,
25 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID
NO: 27362:

VFTLEDFVGDWRQTAGYNLSQVLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYEGLSG
DQMGQIEKIFKVVYPVDNHHFKVILHYGTLVIDGVTPNMIDYFGRPYEGIAVFDGKKITVTGTLWNGN
KIIDERLINPDGSLLFRVTINGVTGWRLHERILA (TeLuc) (SEQ ID NO: 27362), (full

30 luminescent or fluorescent protein that can be used to create FRET and/or BRET sensors)

and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%,
91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence
selected from the group consisting of SEQ ID NOS: 27363-27365:

LIK ENMRSKLYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV EGGPLPFAFD ILATHFMYGS
 KVFIKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ DTSIQDGEI YNVKVRGVNF
 PANGPVMQKK TLGWEPTSTET MYPADGGLEG RCDKALKLVG GGHLHVNFKT TYKSKKPVKM
 PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSNLGGMD ELYK (CyOFP variant) (SEQ ID

- 5 NO: 27363) (full luminescent or fluorescent protein that can be used to create FRET and/or BRET sensors);

VSKGEELIK ENMRSKLYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV EGGPLPFAFD ILATHFMYGS
 KVFIKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ DTSIQDGEI YNVKVRGVNF
 10 PANGPVMQKK TLGWEPTSTET MYPADGGLEG RCDKALKLVG GGHLHVNFKT TYKSKKPVKM
 PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSNLGGMD ELYK(CyOFP variant) (SEQ ID

- NO: 27364) (full luminescent or fluorescent protein that can be used to create FRET and/or BRET sensors); and

EELIK ENMRSKLYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV EGGPLPFAFD ILATHFMYGS
 KVFIKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ DTSIQDGEI YNVKVRGVNF
 PANGPVMQKK TLGWEPTSTET MYPADGGLEG RCDKALKLVG GGHLHVNFKT TYKSKKPVKM
 PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSNLGGMD ELYK(CyOFP variant) (SEQ ID

- 15 NO: 27365) (full luminescent or fluorescent protein that can be used to create FRET and/or BRET sensors);

(d) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:

- 25 27366:

KVFTLGDFVGDWRQTAGYNQAQVLEQGGLTSLFQNLGVSVTPIQRIVLSGENGLKIDIHV
 IIPYEGLSCDQMAQIEKIFKVVYPVDDHHFKAILHYGTLVIDGVTNPMIDYFGQPYEGIA
 KFDGKKITVTGTLWNGNTIIDERLINPDGSLLFRTVINGVTGWRLHERILA (LemiLuc) (SEQ ID

- NO: 27366) (full luminescent or fluorescent protein that can be used to create FRET and/or BRET sensors),

and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27368, wherein the N-terminal methionine residue may be present or absent:

MVSKGEAVIK EFMRFKVHME GSMNGHEFEI EGEGERPYE GTQTAKLKVT KGGPLPFSWD ILSPQFMYG
 35 S RAFIKHPADI PDYKQSFPE GFKWERVMNF EDGGAVTVTQ DTSLEDGTI YKVKLRGTNF PPDGPVM
 QKK TMGWEASTER LYPEDGVKLG DIKMALRLKD GGRLADFKT TYKAKKPVQM PGAYNVDRKL DITSH

NEDYT VVEQYERSEG RHSTGGMDEL YK (mScarlet-i) (SEQ ID NO:27368) (full luminescent or fluorescent protein that can be used to create FRET and/or BRET sensors);

(e) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,

5 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27367, wherein the N-terminal methionine residue may be present or absent:

MVSKGEEDNM ASLPATHELH IFGSINGVDF DMVGQGTGNP NDGYEELNLK STKGDLQFSP WILVPHIGY
G FHQYLPYPDG MSPFQAAMVD GSGYQVHRTM QFEDGASLTV NYRYTYEGSH IKGEAQVKGT GFPADGP
VMT NSLTAADWCR SKKTPNDKT IISTFKWSYT TGNGKRYRST ARTTYTFAKP MAANYLKNQP MYVFR

10 KTELK HSKTELNFKKE WQKAFTDVMG MDELYK (mNeonGreen) (SEQ ID NO:27367), (full luminescent or fluorescent protein that can be used to create FRET and/or BRET sensors),

and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27368, wherein the N-terminal methionine residue may be present or absent:

15 MVSKGEAVIK EFMRFKVHME GSMNGHEFEI EGEGERPYE GTQTAKLKVT KGGPLPFSWD ILSPQFMYG
S RAFIKHPADI PDYYKQSFPE GFKWERVMNF EDGGAVTVTQ DTSLEDGTLI YKVKLRGTNF PPDGPVM
QKK TMGWEASTER LYPEDGVLKG DIKMALRLKD GGRYLADFKE TYKAKKPVQM PGAYNVDRKL DITSH
NEDYT VVEQYERSEG RHSTGGMDEL YK (mScarlet-i) (SEQ ID NO: 27368) (full

luminescent or fluorescent protein that can be used to create FRET and/or BRET sensors);

20 (f) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence SEQ ID NO: 27369, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence

25 SGKSYPTVSADYQKAVEKAKKRLGGFIAEKRCAPLMRLAWHSAGTFDKRTKTGGPFGTIRYPAELAHSANSGLD
IAVRLLEPLKAEFPILSYADFYQLAGVVAVEVTGGPEVPFHPGREDKPELPPEGRLPDATKGS DHLRDVFGKAMG
LTDQDIVALS GGGHTLGA AHKERSGFEGPWT SNPLVFDNSYFTELL SGEKEGGGGSGGGGS (APEX2-1-200)
(SEQ ID NO: 27369) (split engineered variant of soybean ascorbate peroxidase protein for chemiluminescent and colorimetric detection system);

30 and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27370, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence

GGGSGGGGS GLLQLPSDKALLSDPVFRPLVDKYAADEDAFFADYAEAHQKLSELGFADA (APEX2-201-250) (SEQ ID NO: 27370) (split engineered variant of soybean ascorbate peroxidase protein for chemiluminescent and colorimetric detection system);

(g) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27371, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence

MGSHHHHHHGSSENLYFQSGGS

VRPLNCIVA VSQNMGIGKN GDLWPFPPLRN ESKYFQRMFT TSSVEGKQNL
VIMGRKWTWFS IPEKNRPLKD RINIVLSREL KEPPRGAHFL AKSLDDALRL
IEQPELGGGSGGGGS (DHFR_A (1-105)); (SEQ ID NO: 27371) (split dihydrofolate reductase protein reporter for cell survival or fluorescence)

and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27372, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence

SGSG DPDEARKAIARVKRESKRIVEDAERLIREAAAAASEKISREAEERLIREAAAAASEKISRE
GGGSGGGGS ASKV DMVWIVGGSS VYQEA MNQPG HLRLFVTRIM QEFESDTFFP

EIDLKGYKLL PEYPGVLSEV QEEKGIKYKF EVYEKKD (DHFR_B (106-186)); (SEQ ID NO: 27372) (split dihydrofolate reductase protein reporter for cell survival or fluorescence);

(h) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27373, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence

QLTPTFYDN SCPNVSNIVRDIIVNELRSDPRIAASILRLHFHDCFVNGCDASILLDNTTSFRTEKDAFGNANSA
RGFSVIDRMKAAVESACPGTVSCADLLTIAAQQSVTLAGGPSWRVPLGRRDSLQAFLLDLANANLPAPFFFTLPQLK
DSFRNVGLNRSSDLVALSGGHTFGKSQCRFIMDRLYNFSNTGLPDPTLNTTYLQTLRGLCPLNGGSGS (sHRPa
is the large split HRP fragment. It consists of amino acids 1-213 of horseradish peroxidase (HRP) with the following 4 mutations: T21I, P78S, R93G, N175S: plasmid 73147 (SEQ ID NO: 27373);

and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence

of SEQ ID NO:27374, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence

NLSALVDFDLRTPTIFDNKYYVNL EEQKGLIQSDQELFSSPDATDTIPLVRSFANSTQTFFNAFVEAMDRMGNIT
PLTGTQGQIRRNCRVVNSNGGSGS (sHRPb is the small split HRP fragment. It

consists of amino acids 214-308 of horseradish peroxidase (HRP) with the following 2 mutations: N255D, L299R: plasmid 73148) (SEQ ID NO: 27374);

(i) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:

27375, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence

GESLFKGP DYNPISSTICHLTNESDGHTTSLYGIGFGPFITNKHLFRRNNGTLLVQSLHG VFVKVNTTTTLQQH
LIDGRDMI IIRMPKDFPPFPQKLKFREPQREERICLVTTNFQTGGGSGGGGSN_Tev (1-118) (SEQ
ID NO: 27375) (Split TEV protease);

and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27376, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence

GGGSGGGGSKSMSSMVSDTSCTFPSSDGIFWKHWIQTKDQGCGSPLVSTRDGFIVGIHSASNFTNTNNYFTSV
PKNFMELLTNQEAQQWVSGWRLNADSVLWGGHKVFMDKP (C_Tev (119-221)); (SEQ ID NO:
27376) (Split TEV protease);

(j) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:

27377, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence, and wherein the N-terminal methionine residue may be present or absent:

MASYPCHQHA SAFDQAARSR GHSNRRTALR PRRQQEATEV RLEQKMPTLL
RVYIDGPHGM GKTTTTQLLV ALGSRDDIVY VPEPMTYWQV LGASETIANI
YTTQHRLDQG EISAGDAVV MTSAQITMGM PYAVTDAVLA PHIGGEAGSS
HAPPPALTLI FDRHPAALL CYPAAARYLMG SMTPQAVLAF VALIPPTLP
TNIVLGALPE DRHIDRLAKR QRPGERLDLA MLAAIRRVYG LLANTVRYLQ

GGGSWREDWG QLSGT GGGSGGGGS (thymidine kinase_TK_A (1-265)) (SEQ ID NO:
27377);

and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27378, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence

5 GGGGSGGGGS AVPPQ GAEPQSNAGP RPHIGDTLFT LFRAPPELLAP
 NGDLYNVFAW ALDVLAKRLR PMHVFILDYD QSPAGCRDAL LQLTSGMVQT
 HVTTPGSIPT ICDLARTFAR EMGEAN (thymidine kinase_TK_B (266-376) (SEQ ID NO:
 27378) .

10 27. The cage protein of any one of claims 1-26, wherein the one or more target binding polypeptide is capable of binding to a target including but not limited to an antibody, a toxin, a diagnostic biomarker, a viral particle, a disease biomarker, a metabolite or a biochemical analyte.

15 28. The cage protein of any one of claims 1-26, wherein the one or more target binding polypeptide is capable of binding to an antibody target.

29. The cage protein of claim 28, wherein the one or more target binding polypeptide comprises one or more epitope recognized by antibodies against a viral target.

20

30. The cage protein of claim 28 or 29, wherein the one or more target binding polypeptide comprises one or more epitope recognized by antibodies against SARS-Cov-2.

25 31. The cage protein of any one of claims 1-26, wherein the one or more target binding polypeptide is capable of binding to a disease marker or toxin.

32. The cage protein of any one of claims 1-26, wherein the one or more target binding polypeptide is capable of binding to Bcl-2, Her2 receptor, Botulinum neurotoxin B, albumin, epithelial growth factor receptor, prostate-specific membrane antigen (PSMA), citrullinated peptides, brain natriuretic peptides, and/or cardiac Troponin I.

30

33. The cage protein of any one of claims 1-32, wherein the one or more target binding polypeptide comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%,

80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 27380-27430.

34. The cage protein of claim 33, wherein the one or more target binding polypeptide comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:27397-27430, or selected from SEQ ID NOS:27397-27406, 27409-27416, and 27427-27430.

35. The cage protein of claim 34, wherein amino acid substitutions relative to the reference target binding polypeptide amino acid are selected from the allowable amino acid substitutions provided in Table 3

36. The cage protein of claim 34 or 35, wherein interface residues are identical to those in the reference target binding polypeptide or are conservatively substituted relative to interface residues in the reference target binding polypeptide as detailed in Table 2.

37. The cage protein of any one of claims 34-36, wherein the one or more target binding polypeptide comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 27397-27406 and 27431-27466.

38. The cage protein of claim 37, wherein the one or more target binding polypeptide comprises an amino acid substitution relative to the amino acid sequence of SEQ ID NO:27397 at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or all 18 residues selected from the group consisting of 2, 4, 5, 14, 15, 17, 18, 27, 28, 32, 37, 38, 39, 41, 42, 49, 52, and 55.

39. The cage protein of claim 38, wherein the substitutions in the one or more target binding polypeptide are selected from the substitutions listed in Table 5, either individually or in combinations in a given row.

40 The cage protein of any one of claims 34-36, wherein the one or more target binding polypeptide comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 27409-27416 and
5 27467-27493.

41. The cage protein of claim 40, wherein the target binding comprises an amino acid substitution relative to the amino acid sequence of SEQ ID NO:27409 at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or all 20 residues selected from the group consisting
10 2, 6, 8, 9, 13, 14, 19, 22, 25, 26, 28, 29, 34, 35, 37, 40, 43, 45, 49, and 62.

42. The cage protein of claim 41, wherein the substitutions are selected from the substitutions listed in Table 7, either individually or in combinations in a given row.

15 43. The cage protein of any one of claims 34-36, wherein the target binding comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 27427-27430 and 27494.

20 44. The cage protein of claim 44, wherein the one or more target binding polypeptide comprises an amino acid substitution relative to the amino acid sequence of SEQ ID NO:27429 at or both residues selected from the group consisting 63 and 75.

45. The cage protein of claim 44, wherein the substitutions comprise R63A and/or K75T.

25 46. The cage protein of any one of claims 1-45, comprising the amino acid sequence at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of a cage protein selected from the group consisting of SEQ ID NOS:1-49, 51-52, 54-59, 61, 65, 67-91, 92 -
30 2033, 2034-14317, 27094-27117, 27120-27125, 27,278 to 27,321, even-numbered SEQ ID NOS between SEQ ID NOS: 27126 and 27276, and cage proteins listed in Tables 8 and 9, not including optional amino acid residues (, and not including amino acid residues in the latch region, and wherein the N-terminal and/or C-terminal 60 amino acids of each cage protein may be optional

47. The cage protein of any one of claims 1-47, comprising the amino acid sequence at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical, not including optional amino acid residues, to the amino acid sequence of a cage protein selected from the consisting of SEQ ID NOS: 27497-27620, wherein the N-terminal protein purification tag (MGSHHHHHHSGSGSENLYFQSGG (SEQ ID NO:27624); or MGSHHHHHHSGSENLYFQG (SEQ ID NO:27625); or GSHHHHHHSGSGSENLYFQG (SEQ ID NO:27626)) is optional, are not considered in the percent identity comparison, and can be present or absent and preferably are absent.

48. A key protein capable of binding to the structural region of a cage protein of any one of claims 1-47 that does not include the second reporter protein domain, wherein binding of the key protein to the cage protein only occurs in the presence of a target to which the cage protein one or more target binding polypeptide can bind, wherein the key protein comprises a second reporter protein domain, wherein interaction of the key protein second reporter protein domain and the cage protein first reporter protein domain causes a detectable change in reporting activity from the first reporter protein domain .

49. The key protein of claim 48, wherein the second reporter protein domain is at the N-terminus or the C-terminus of the key protein, or is within 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid of the N-terminus or the C-terminus of the key protein.

50. The key protein of any one of claims 48-49, wherein the second reporter protein domain comprises a reporter protein domain selected from the group consisting of luciferase (including but not limited to firefly, Renilla, and Gaussia luciferase), bioluminescence resonance energy transfer (BRET) reporters, bimolecular fluorescence complementation (BiFC) reporters, fluorescence resonance energy transfer (FRET) reporters, colorimetry reporters (including but not limited to β -lactamase, β -galactosidase, and horseradish peroxidase), cell survival reporters (including but not limited to dihydrofolate reductase), electrochemical reporters (including but not limited to APEX2), radioactive reporters (including but not limited to thymidine kinase), and molecular barcode reporters (including but not limited to TEV protease).

51. The key protein of any one of claims 48-50, wherein the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:27360-23379, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence, and wherein any N-terminal methionine residue may be present or absent.

52. The key protein of any one of claims 48-51, wherein the key protein, not including the second split reporter protein domain, comprises an amino acid sequence at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical, not including optional amino acid residues, to the amino acid sequence selected from the group consisting of SEQ ID NOS:14318-26601, 26602-27015, 27016-27050, 27,322 to 27,358, and key polypeptides with an odd-numbered SEQ ID NO between SEQ ID NOS: 27127 and 27277), key proteins in Table 8, and key proteins in Table 9.

53. The key protein of any one of claims 48-52, wherein the key protein comprises an amino acid sequence at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical, not including optional amino acid residues in parentheses, to the amino acid sequence of a key protein selected from the group consisting of SEQ ID NOS: 27621-27623, wherein residues in parentheses are optional and may be present or absent.

> lucKey: MGS-(His)6-TEV site-linker-LgBit-linker-latch sequence
(MGSHHHHHHSGSENLYFQG) SGMVFTLEDVFGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRIVRSGE
NALKIDIHIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTNMLNYFGRPYEGIAVFDGKKI
TVTGTTLWNGNKIIDERLITPDGSMLFRVTINSGSGGGSGGGSGGSDEARKAIARVKRESKRIVEDAERLIREA
AAASEKISREAERLIREAAAASEKISRE (SEQ ID NO:27621)

Key-2GGSGG-CyOFP (CyOFP sequence in bold/underline):
(M) DPDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERLIREAAAASEKISREGGSGG **GGVSK**
GEELIK ENMRSKLYLE GSVNGHQFKC THEGEGKPYE GKQTNRIKVV EGGPLPFAFD ILATHFMYGS
KVFIKYPADL PDYFKQSFPE GFTWERVMVF EDGGVLTATQ DTSLQDGELI YNVKVRGVNF
PANGPVMQKK TLGWEPSTET MYPADGGLEG RCDKALKLVG GGHLHVNFKT TYKSKKPVKM
PGVHYVDRRL ERIKEADNET YVEQYEHAVA RYSNLGGGM ELYK (SEQ ID NO: 27622)

Key-LacB (split β -lactamase B in bold/underline):
SGSGDPDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERLIREAAAASEKISRESGGGGSGGGSGG
GG **LLTLASRQQLIDWME ADKVAGPLLR SALPAGWFIA DKSGAGERGS RGIIAALGPD GKPSRIVVIY**
TTGSQATMDE RNRQIAEIGA SLIKHW (SEQ ID NO: 27623)

54. A biosensor, comprising

(a) the cage protein of any one of claims 1-47 wherein the cage does not include the second reporter protein domain; and

(b) the key protein of any one of claims 48-53;

wherein the key protein can only bind to the cage protein in the presence of a target to which the cage protein one or more target binding polypeptide can bind; and wherein binding of the first reporter protein domain of the cage protein to the second reporter protein domain of the key protein causes a detectable change in reporting activity from the first reporter protein domain.

55. The biosensor of claim 54, wherein

(a) the first reporter protein domain comprises an amino acid sequence at least

70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or

100% identical to the amino acid sequence SEQ ID NO: 27359, and 27664-27672;

and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO 27379, wherein the N-terminal methionine residue may be present or absent

(b) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27360, and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27361;

(c) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27362, and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS:27363-27365;

(d) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27366, and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO 27368:

5 (e) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27367, wherein the N-terminal methionine residue may be present or absent, and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,
10 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO 27368, wherein the N-terminal methionine residue may be present or absent;

 (f) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID
15 NO:27369, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence; and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid of SEQ ID NO:27370, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid
20 sequence;

 (g) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID
NO:27371, wherein underlined residues are optional residues that may be present or absent,
25 and when present may be any amino acid sequence, and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO: 27372, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence;

30 (h) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27373, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence, and the other comprises an amino acid

sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27374, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence;

(i) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27375, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence, and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27376, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence;

(j) one of the first reporter protein domain and the second reporter protein domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27377, wherein the N-terminal methionine residue may be present or absent, and wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence, and the other comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27378, wherein underlined residues are optional residues that may be present or absent, and when present may be any amino acid sequence.

56. The biosensor of claim 54, wherein the cage protein comprises a cage protein of claim 47 and the key protein comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical, not including optional amino acid residues in parentheses, to the amino acid sequence of SEQ ID NO: 27621.

> lucKey: MGS-(His)6-TEV site-linker-LgBit-linker-latch sequence
 (MGSHHHHHGSGSENLYFQG) SGMVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQIRIVRSGE
 NALKIDIHIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKI
 TVTGTLWNGNKIIDERLITPDGSMLEFRVTINSGGSGGGSGGGSGGSDEARKAIARVKRESKRIVEDAERLIREA
 AAASEKISREAERLIREAAAASEKISRE

57. The biosensor of claim 54, wherein the cage protein and the key protein comprise a protein pair comprising

(i) a cage protein comprising an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27620, wherein the residues in parentheses are optional and may be present or absent:

LacATrop (split β -lactamase A in bold; underline cTnT and cTnC):

(MGSHHHHHGSGSENLYFQG SGGG)**VFAHPETLVK VKDAEDQLGA RVGYIELDLN**

SGKILESFRP EERFPMMSTF KVLICGAVLS RVDAGQEQLG RRIHYSQNDL

VEYSPVTEKH LTDGMTVREL CSAAITMSDN TAANLLLT TI GGPKELTAFL

HNMGDHVTRL DRWEPELNEA IPNDERDTT PAAMATT LRK LLTGNGR

SGGGGSGGGGSGGGG (SKEAAKKLQDLNIELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELT

DPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESKKILEEARKAIRDAAEESRKILEEGSGSGSDALDELQKLN

ELAKLLKAIATQDLNLRAAKAFLEAAKLQELNIRAVELLVKLTDPATIRRALEHAKRRSKEIIDEAERAIRA

AKRESERIEEARLIEKAKEESERIIREGSGSGDPDIKKLQDLNIELARELLRAHAQLQRLNLELLRELLRALA

QLQELNLDLLRLASEL) TDPDEARKAIAVTGYRLFEEIILDAERLIREAAAASEDQLREAAKELWQTIYNLEAEKF

DLQEKFKQKYEINVLNRNNDNQKVSTKDDSKGKSEEE^{SD}LSDFRMFDKNADGYIDLEELKIMLQATGETITED

DIEELMKDGDKNNDGRIDYDEFLEFMKGVE; and

(ii) a key protein comprising an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% identical to the amino acid sequence of SEQ ID NO:27631.

LLTLASRQQLIDWME ADKVAGPLL R SALPAGWFIA DKSGAGERGS RGIIAALGPD GKPSRIVVIY

TTGSQATMDE RNRQIAEIGA SLIKHW (SEQ ID NO:27361)

58. A method for detecting a target, comprising

(a) contacting the cage protein of any one of claims 47 where the cage protein comprises the second reporter protein domain, or the biosensor of any one of claims 40-43 with a biological sample under conditions to promote binding of the cage protein one or more target binding polypeptide to a target present in the biological sample, causing a detectable change in reporting activity from the first reporter protein domain; and

(b) detecting the change in reporting activity from the first reporter protein domain, wherein the change in reporting activity identifies the sample as containing the target.

59. The method of claim 58, wherein the target is selected from the group including but not limited to an antibody, a toxin, a diagnostic biomarker, a viral particle, a disease biomarker, a metabolite or a biochemical analyte.

5 60. The method of any one of claims 58-59, wherein the target is an antibody.

61. The method of claim 60, wherein the target comprises antibodies selective for a virus.

62. The method of claim 58, wherein the one or more target binding polypeptide
10 comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 27292-27394 and 27547-27548, and a polypeptide comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NO: 27397-27494.

15 63. The method of any one of claims 58-62, wherein the cage polypeptide comprises the cage protein of claim 47.

64. The method of any one of claims 58-59, wherein the target is a disease marker or
20 toxin.

65. The method of claim 64, wherein the disease marker or toxin comprises Bcl-2, Her2 receptor, Botulinum neurotoxin B, albumin, epithelial growth factor receptor, prostate-specific membrane antigen (PSMA), citrullinated peptides, brain natriuretic peptides, and/or
25 cardiac Troponin I.

66. The method of claim 64 or 65, wherein the one or more target binding polypeptide comprises an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid
30 sequence selected from the group consisting of SEQ ID NO: 27380-27390, wherein any N-terminal amino acid is optional and may be present or absent.

67. The method of any one of claims 64-66, wherein the cage protein comprises the cage
35 protein of claim 47.

68. A method for designing a biosensor, cage protein, or key protein comprising the steps of any method described herein.

5

69. A nucleic acid encoding the cage protein or key protein of any of the preceding claims.

70. An expression vector comprising the nucleic acid of claim 69 operatively linked to a suitable control element, such as a promoter.

10

71. A cell comprising the cage protein, key protein, composition, nucleic acid, or expression vector of any preceding claim.

72. A pharmaceutical composition comprising the cage protein, key protein, composition, nucleic acid, expression vector, or cell of any preceding claim, and a pharmaceutically acceptable carrier.

15

73. An epitope, comprising or consisting of the amino acid sequence of SEQ ID NO:

20 27384

74. A method for detecting Troponin I in a sample, comprising contacting a biological sample with the epitope of claim 73 under conditions suitable to promote binding of Troponin I in the sample to the epitope to form a binding complex, and detecting binding complexes that demonstrate presence of Troponin I in the sample.

25

1/23

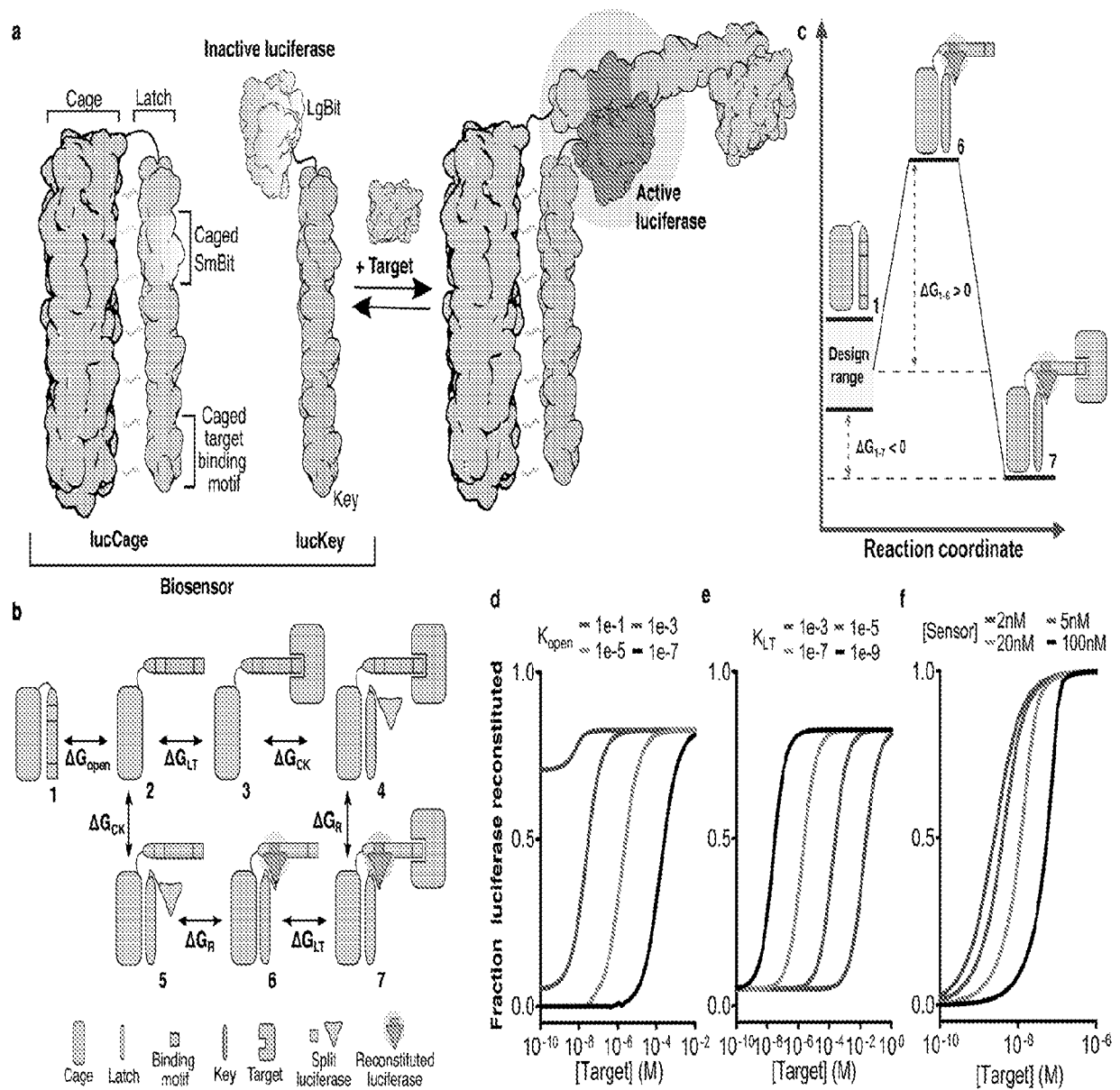


Figure 1

2/23

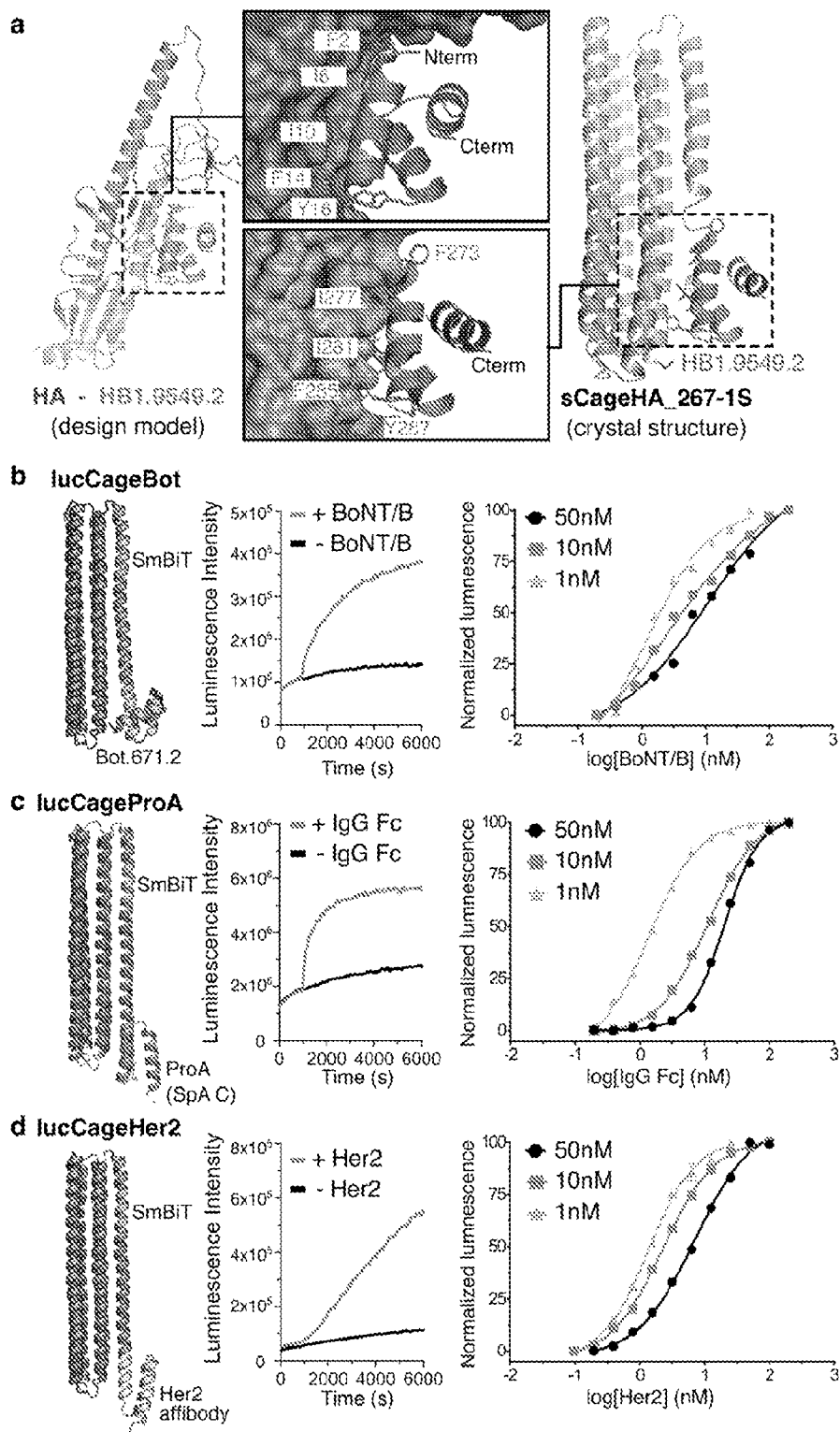


Figure 2

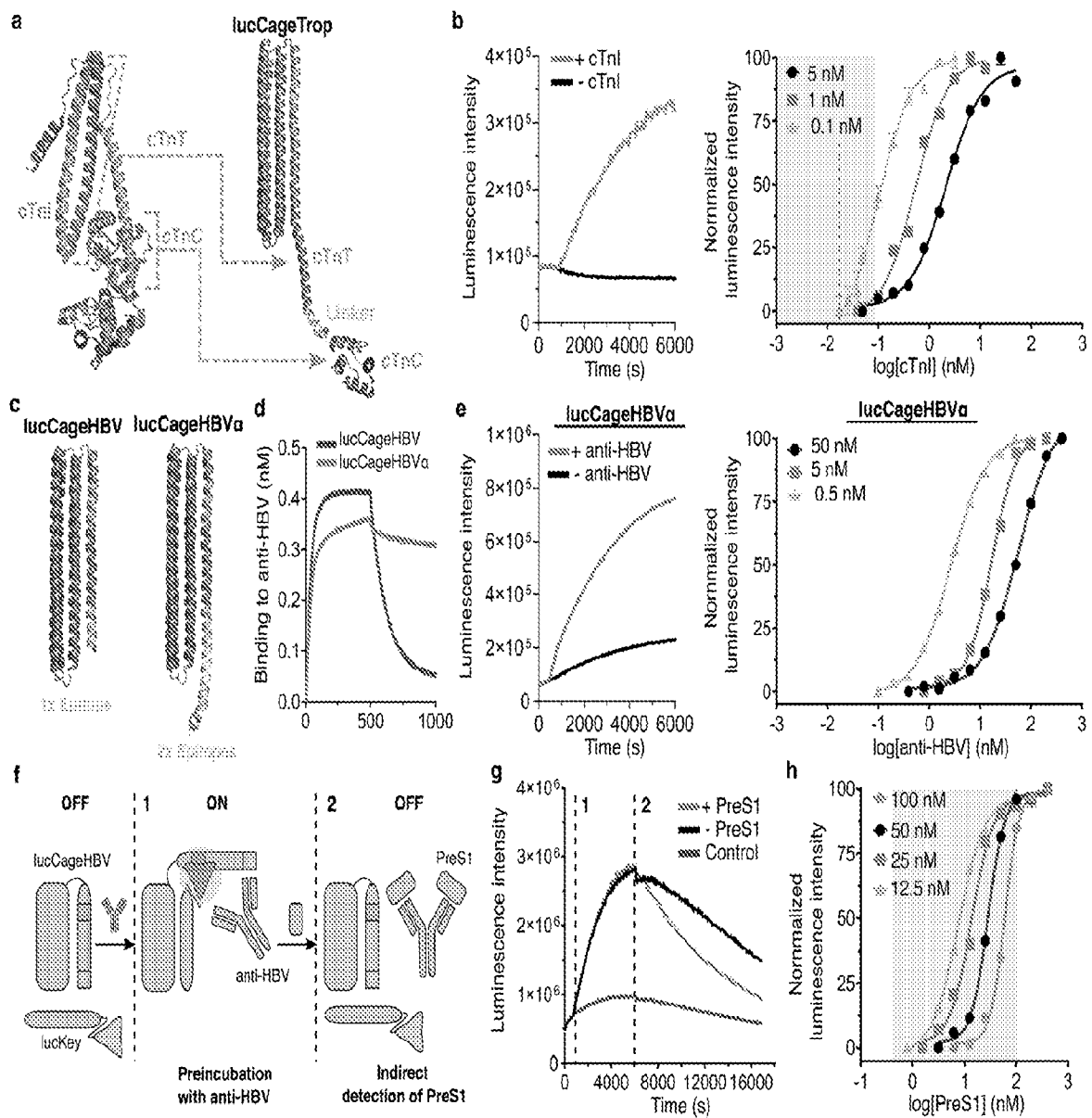


Figure 3

4/23

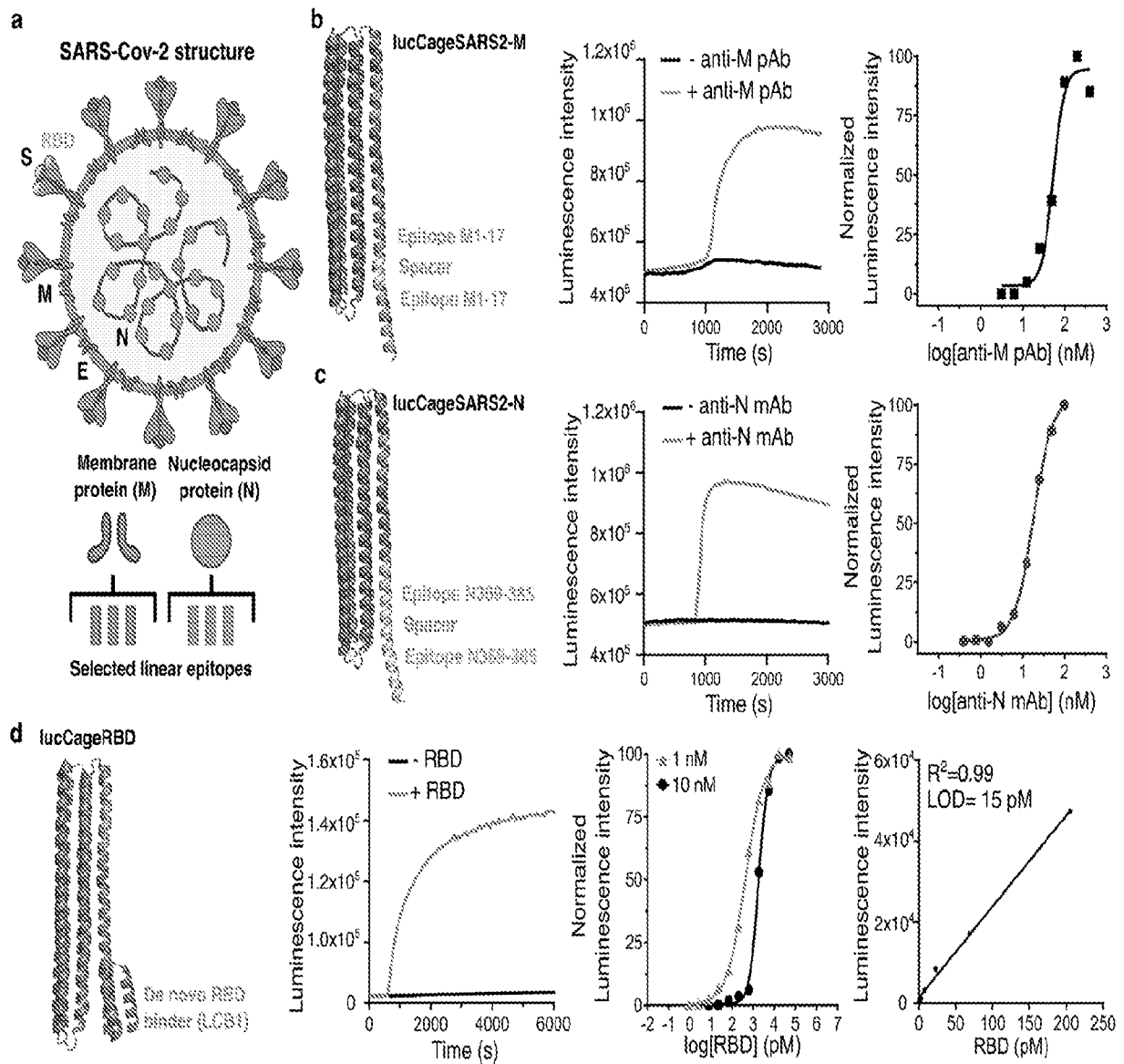
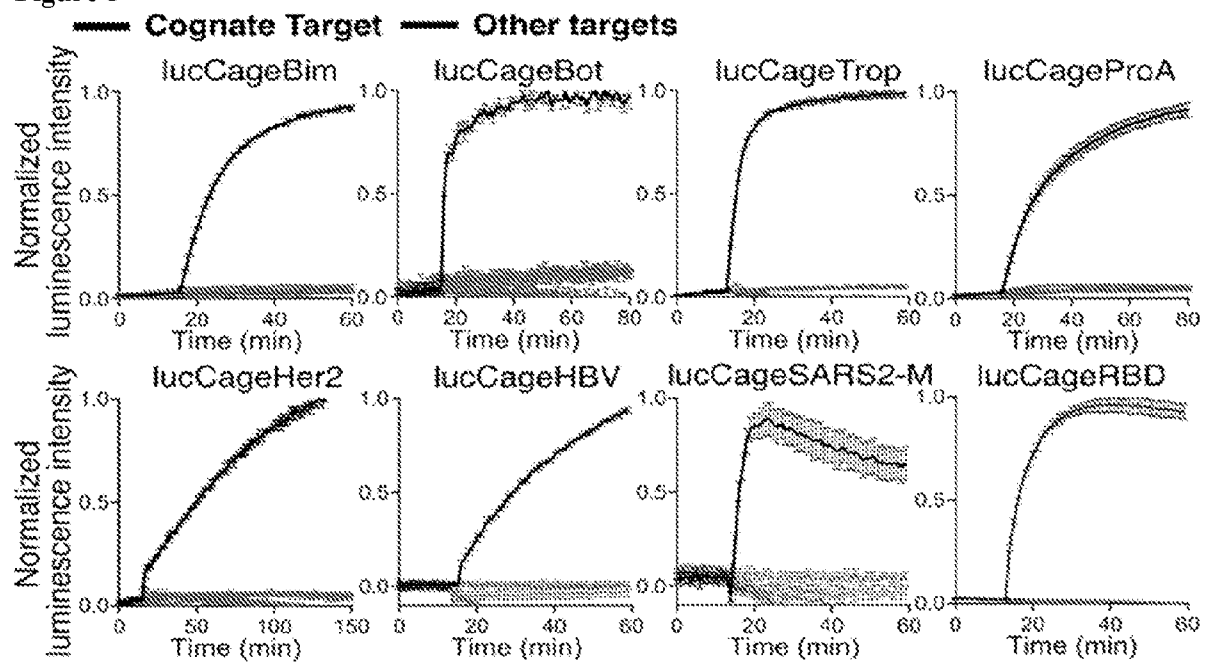


Figure 4

5/23

Figure 5



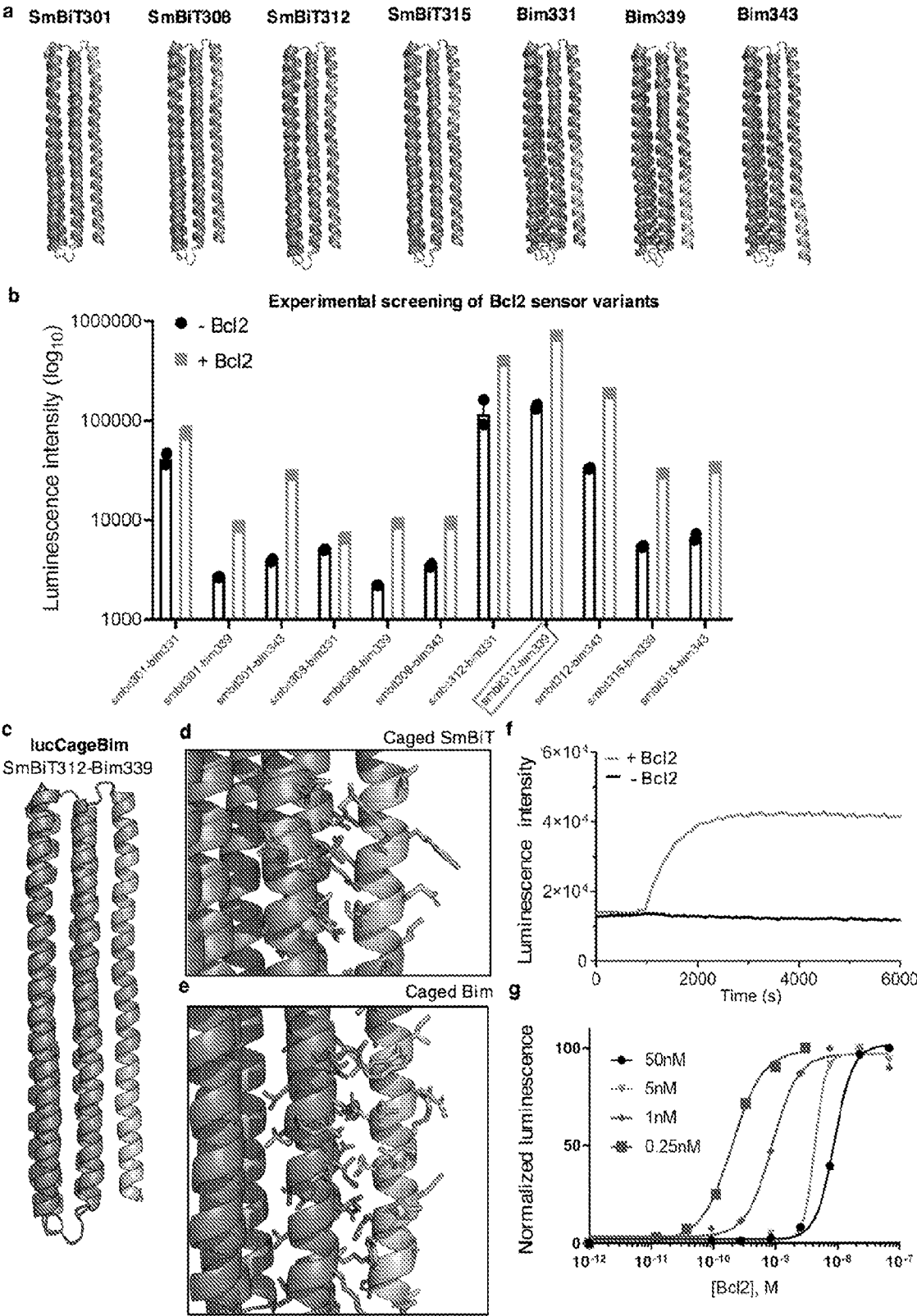


Figure 6

7/23

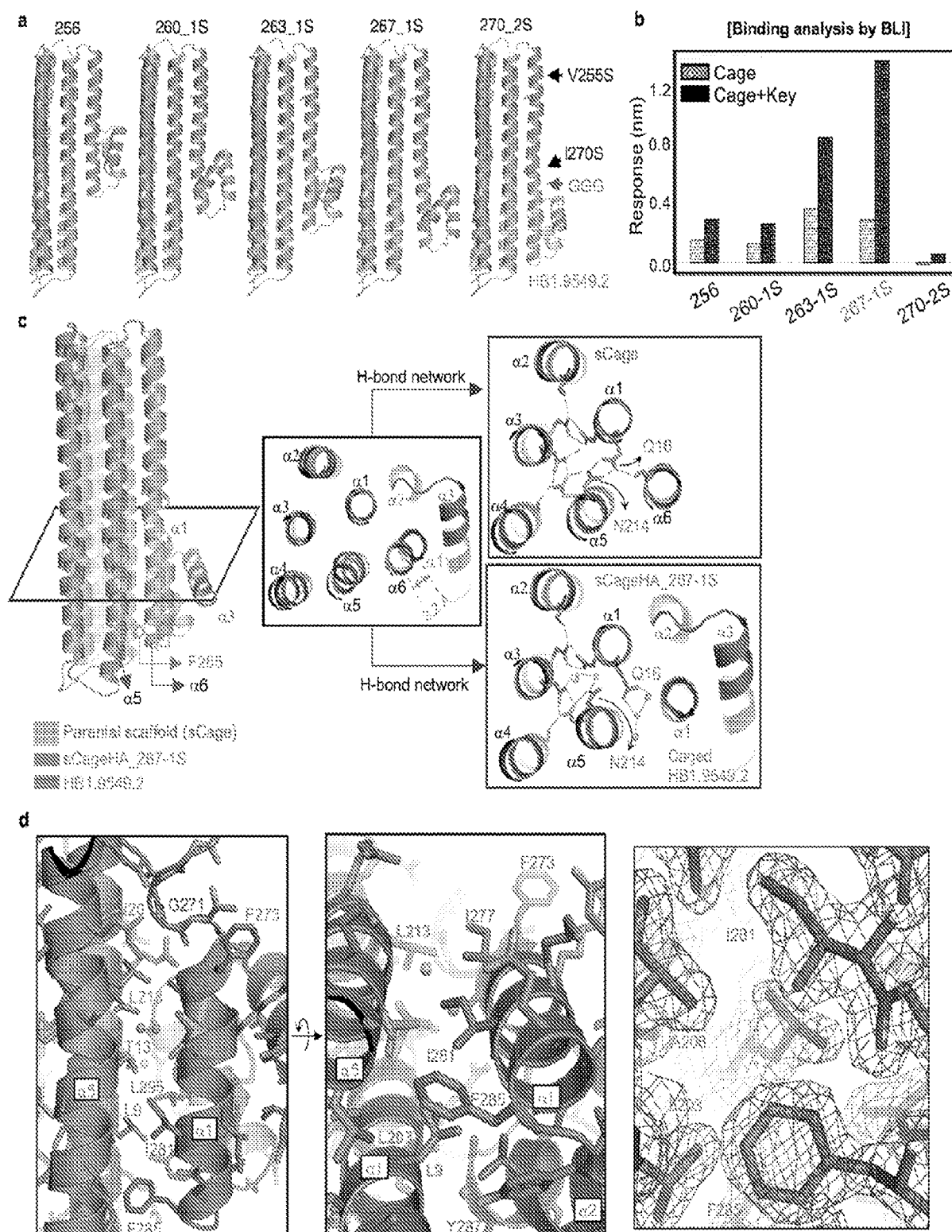


Figure 7

8/23

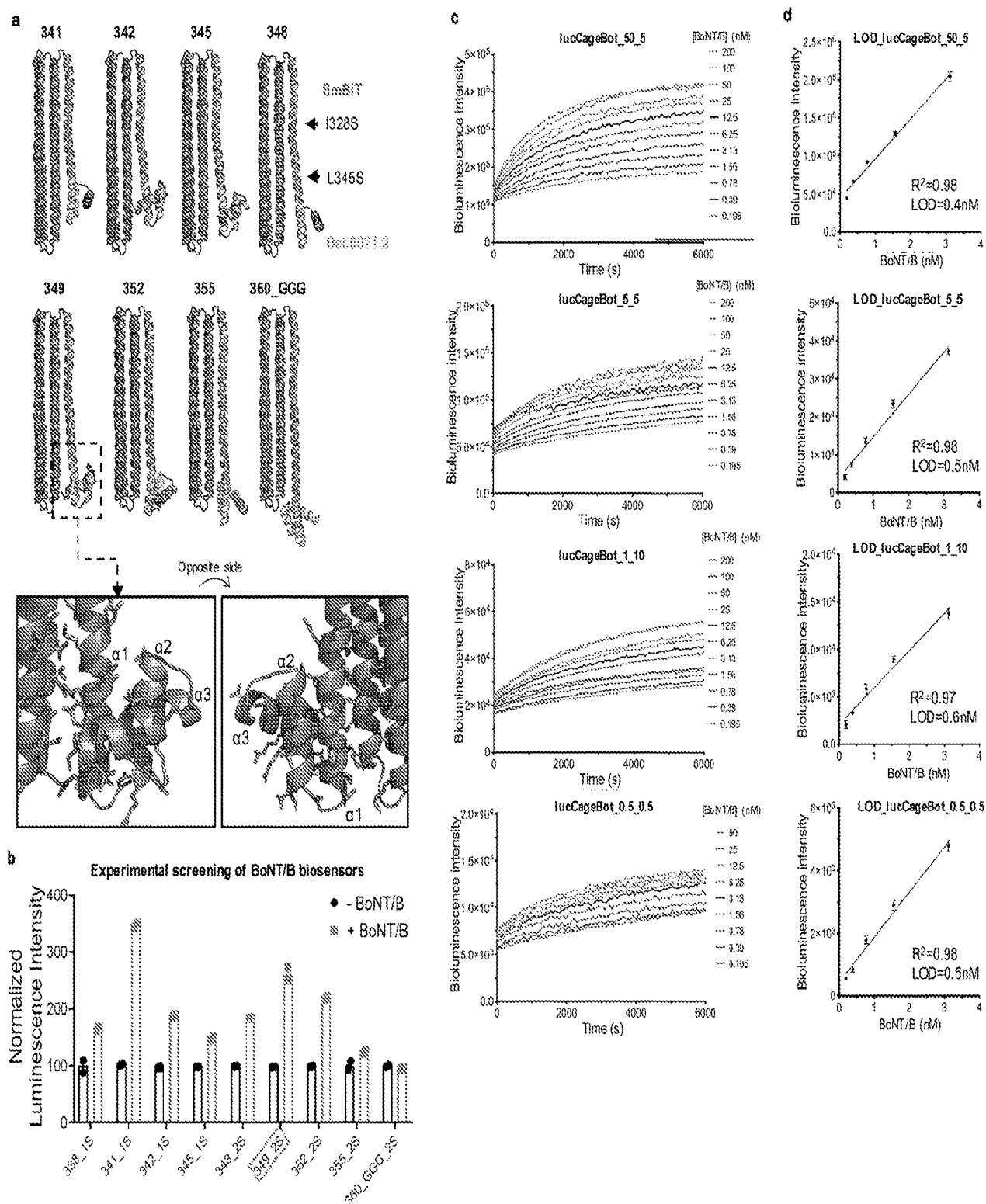


Figure 8

9/23

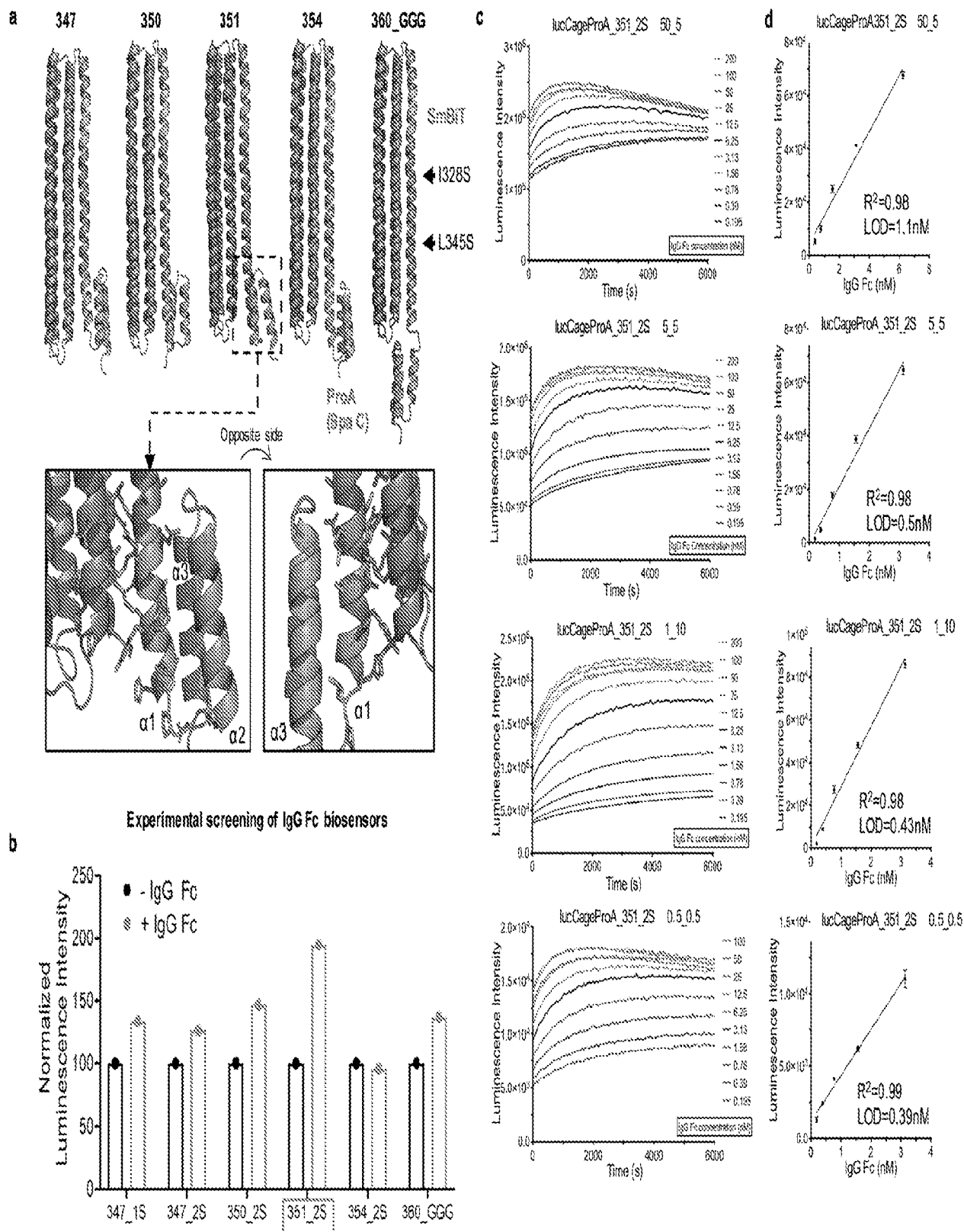


Figure 9

10/23

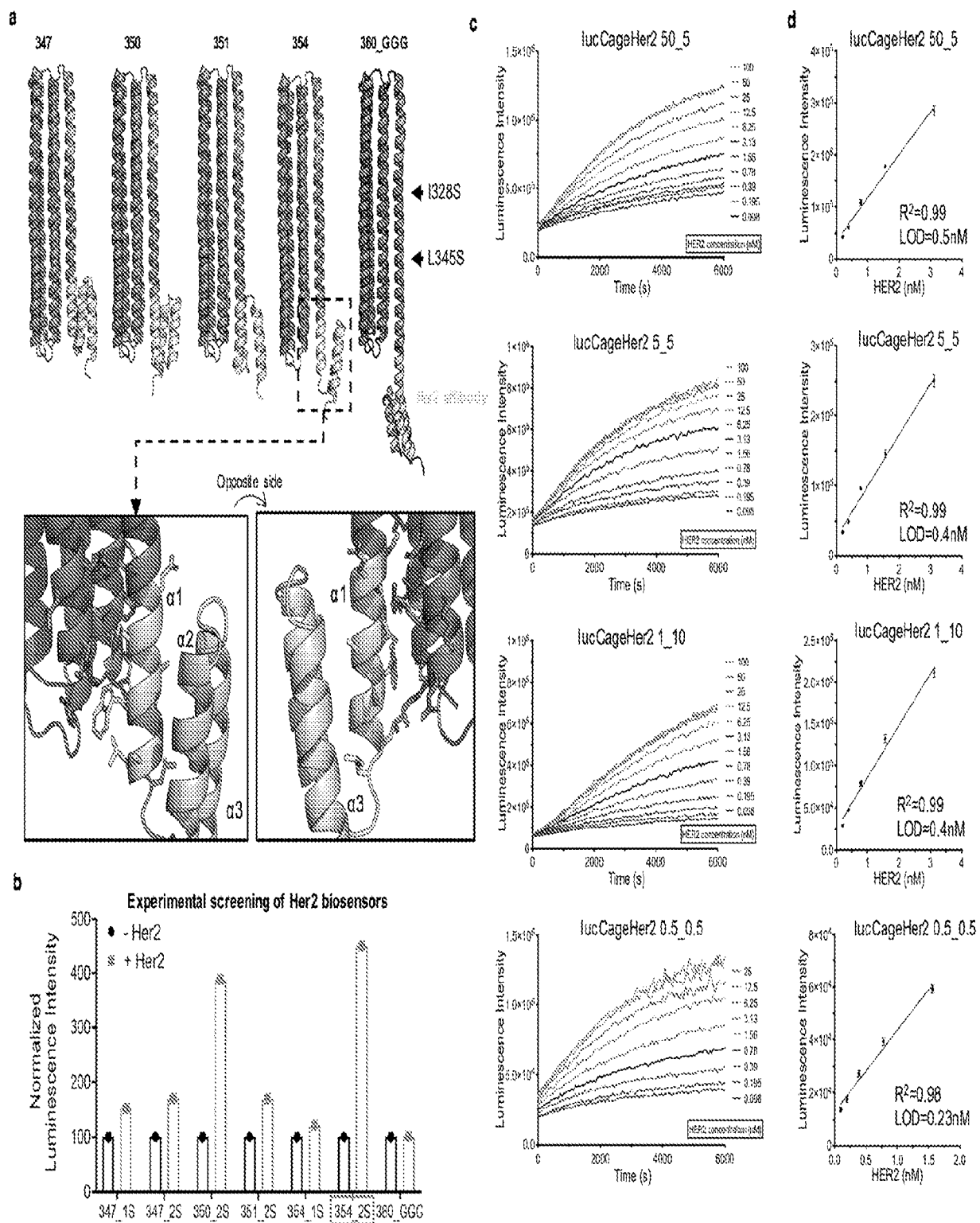


Figure 10

11/23

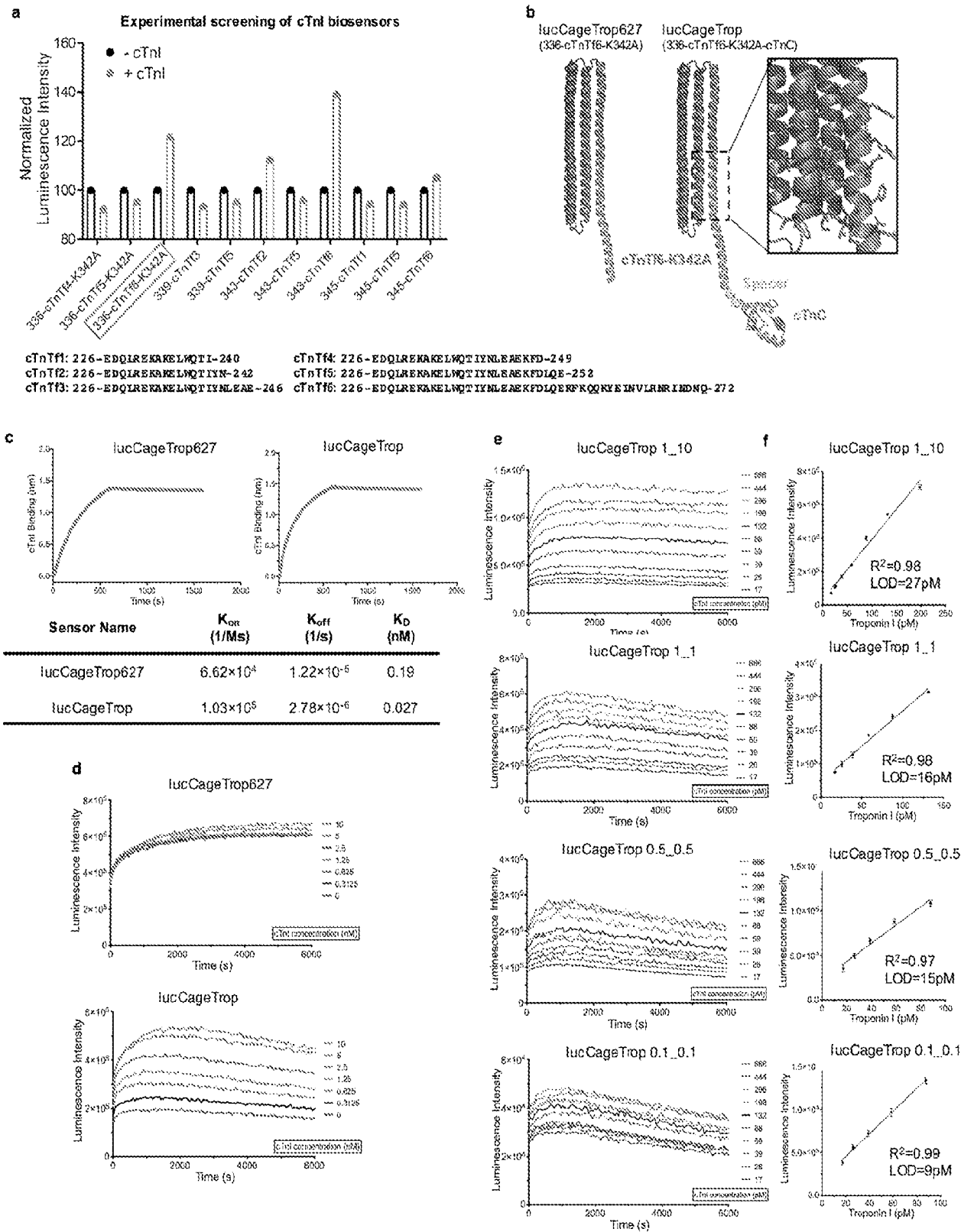


Figure 11

12/23

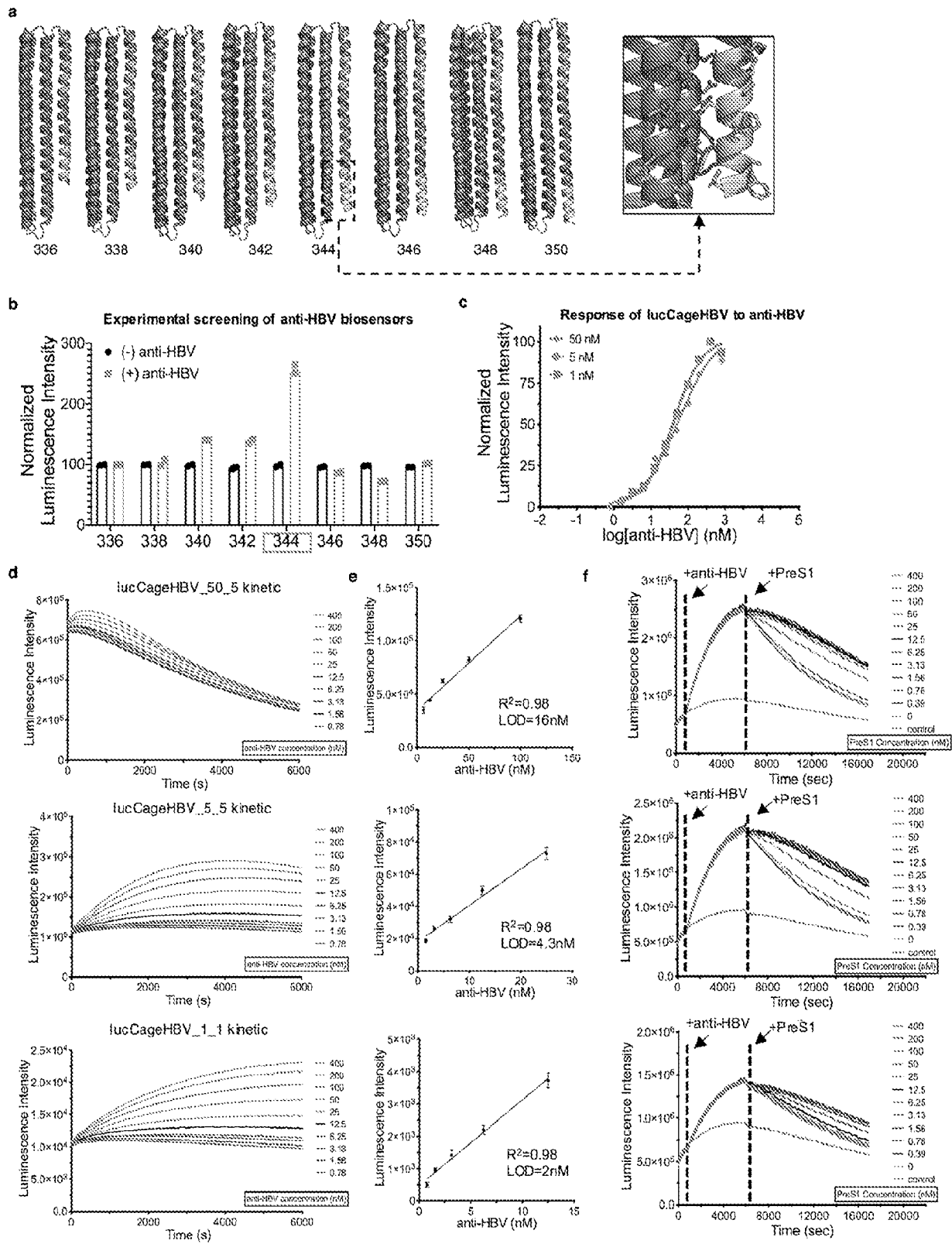


Figure 12

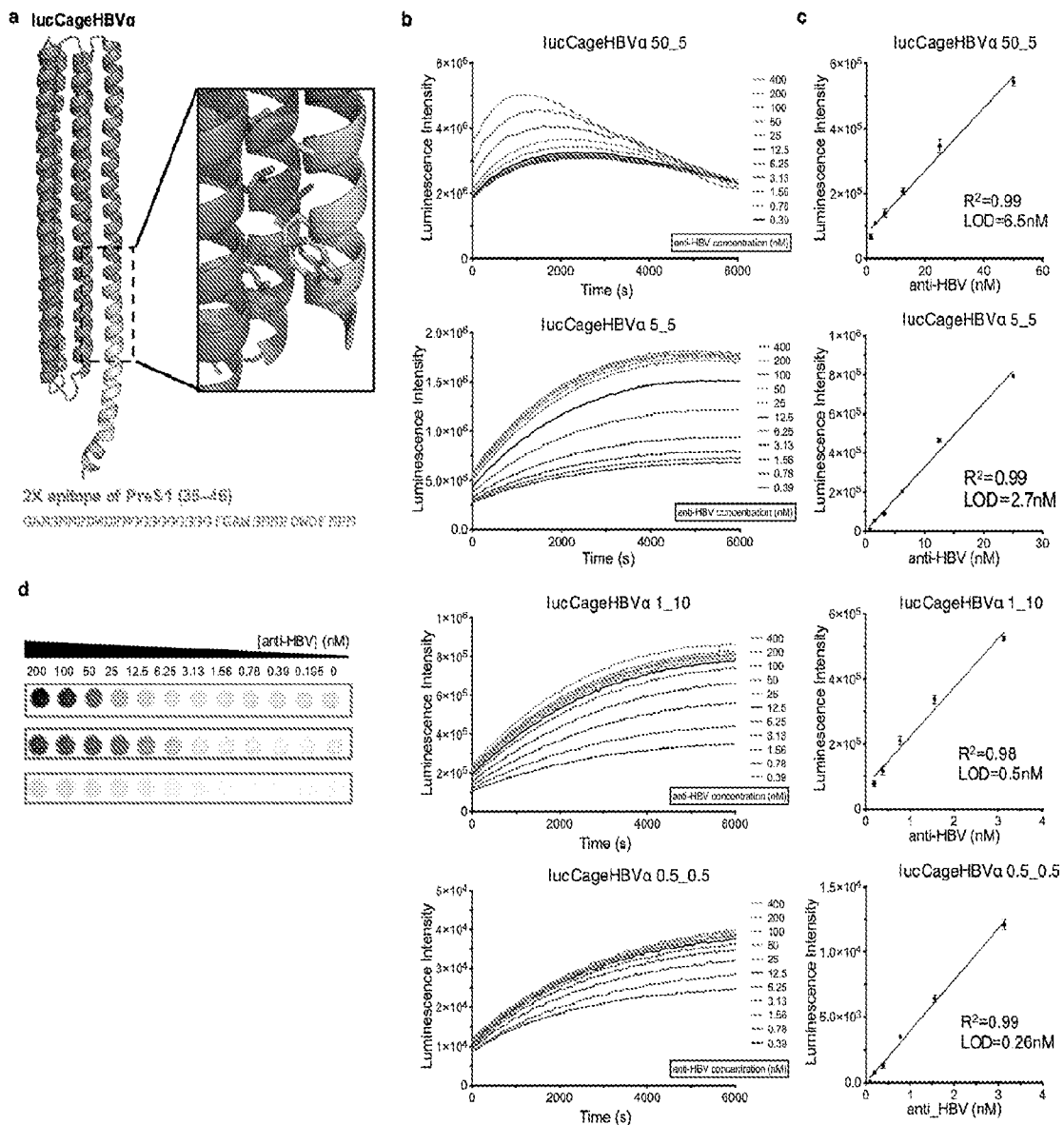


Figure 13

14/23

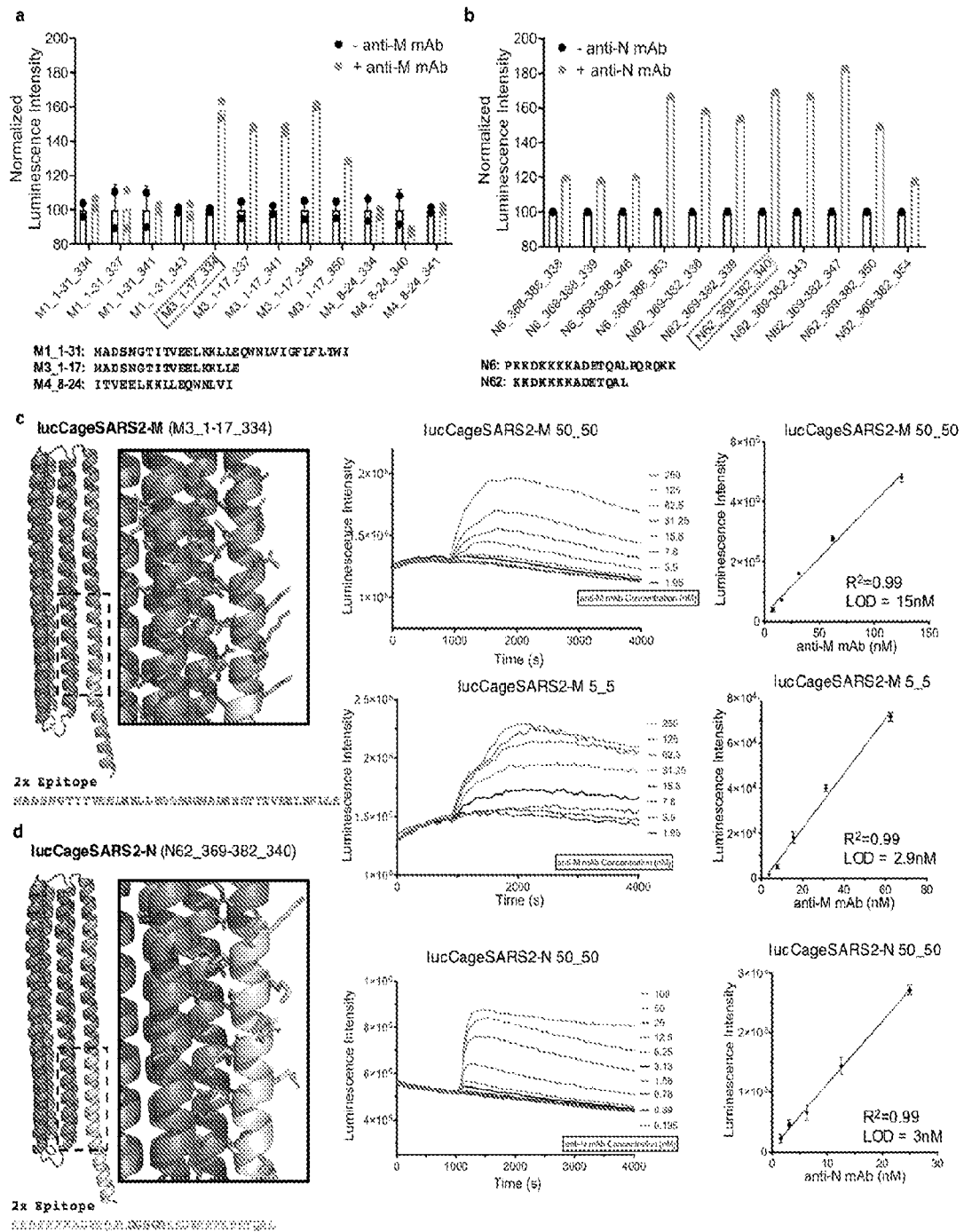


Figure 14

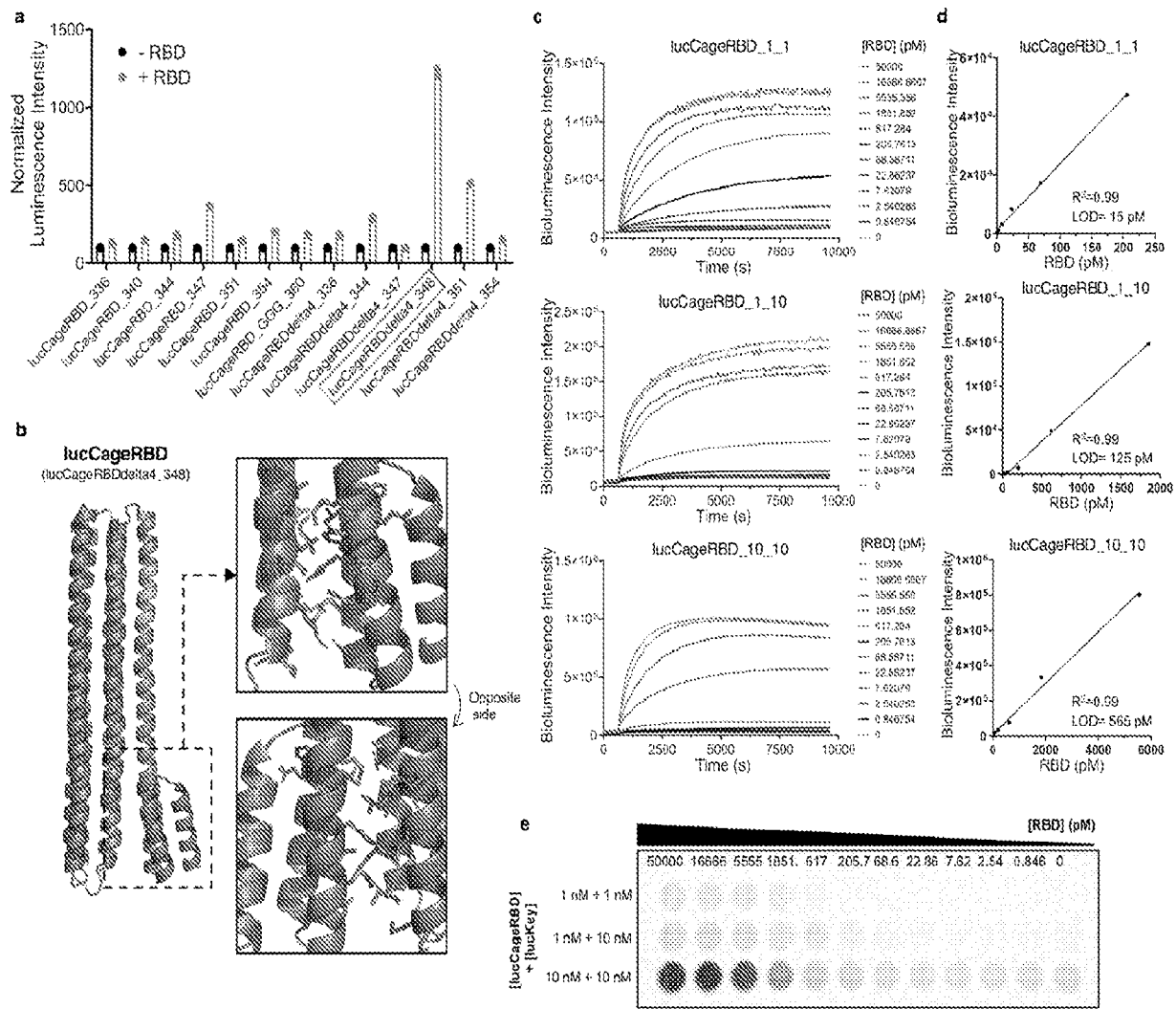


Figure 15

16/23

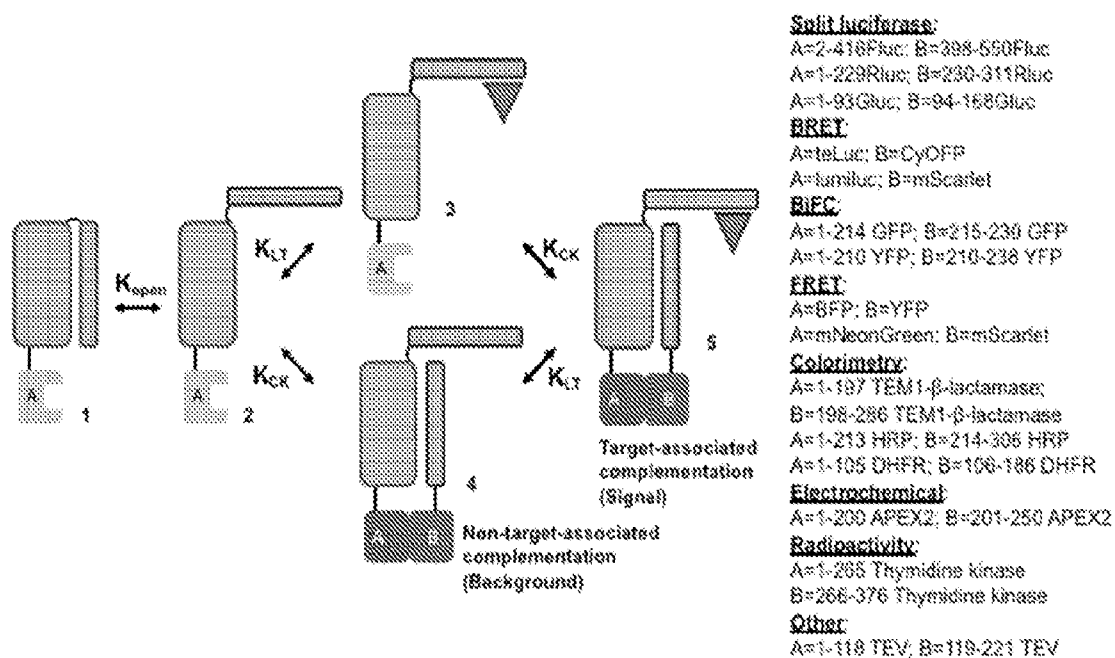
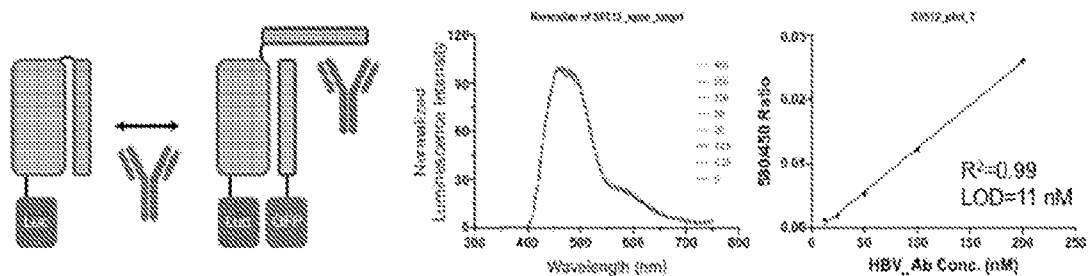


Figure 16

17/23

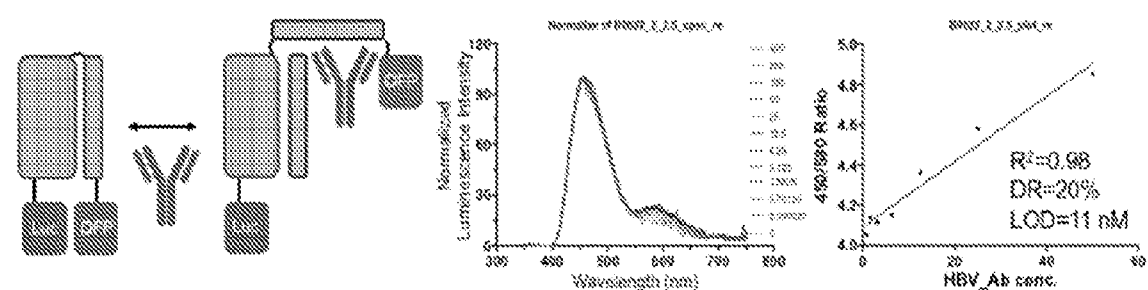
Figure 17

(A)

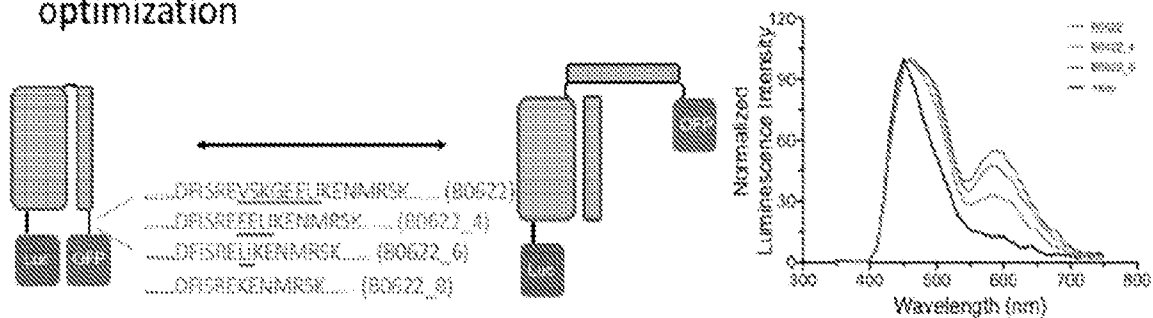


(B)

BRET HBV Ab sensor - intramolecular



optimization



18/23

(C)

BRET HBV Ab sensor – intramolecular

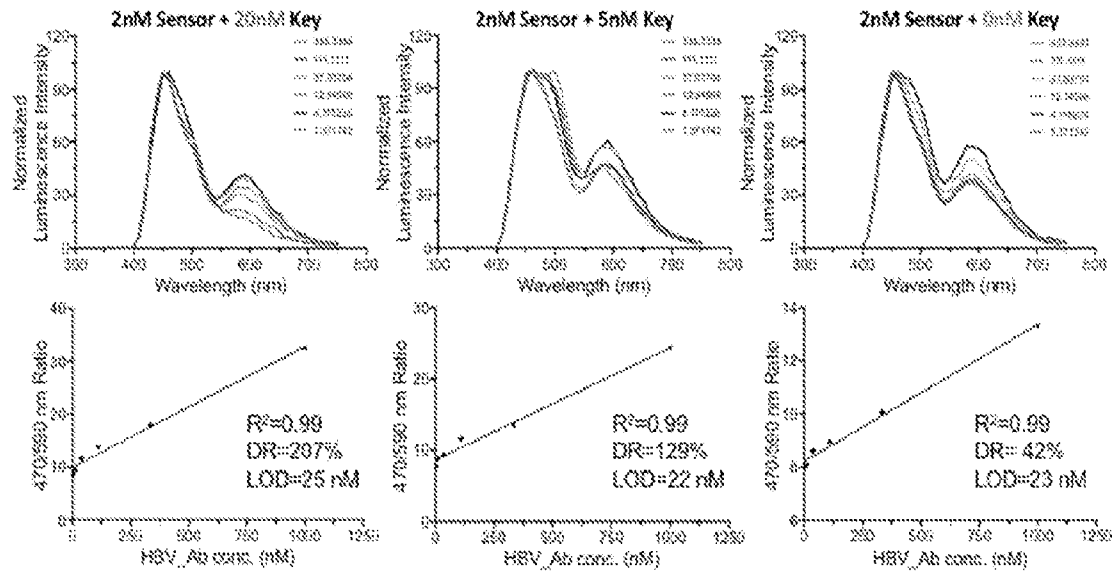


Figure 17 (Cont.)

19/23

Colorimetric Troponin I sensor

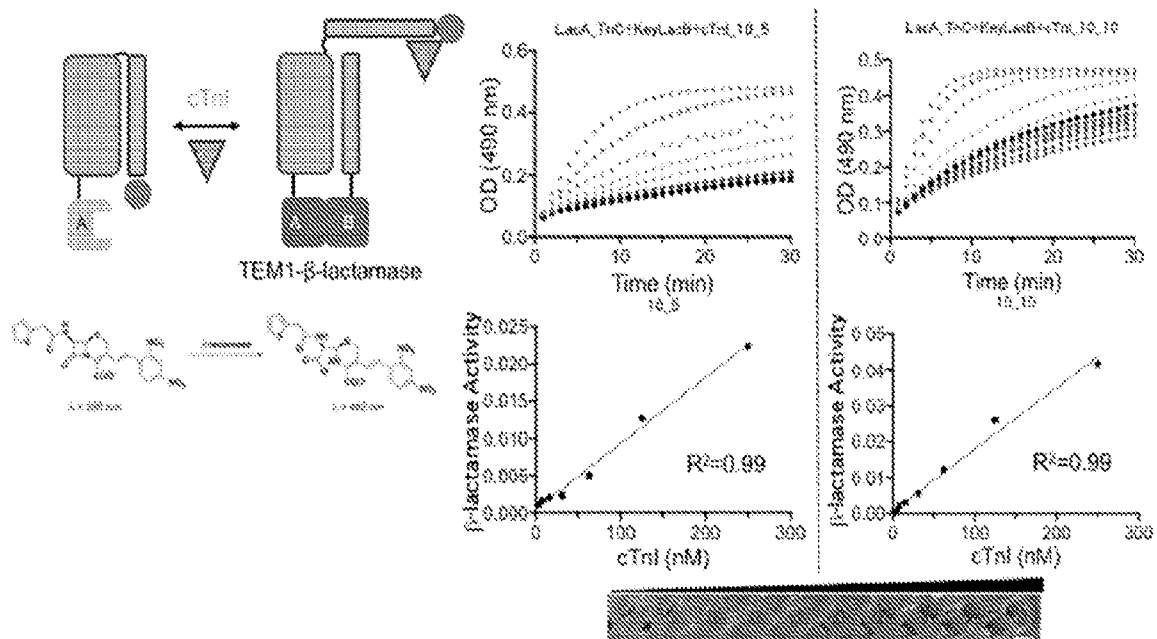


Figure 18

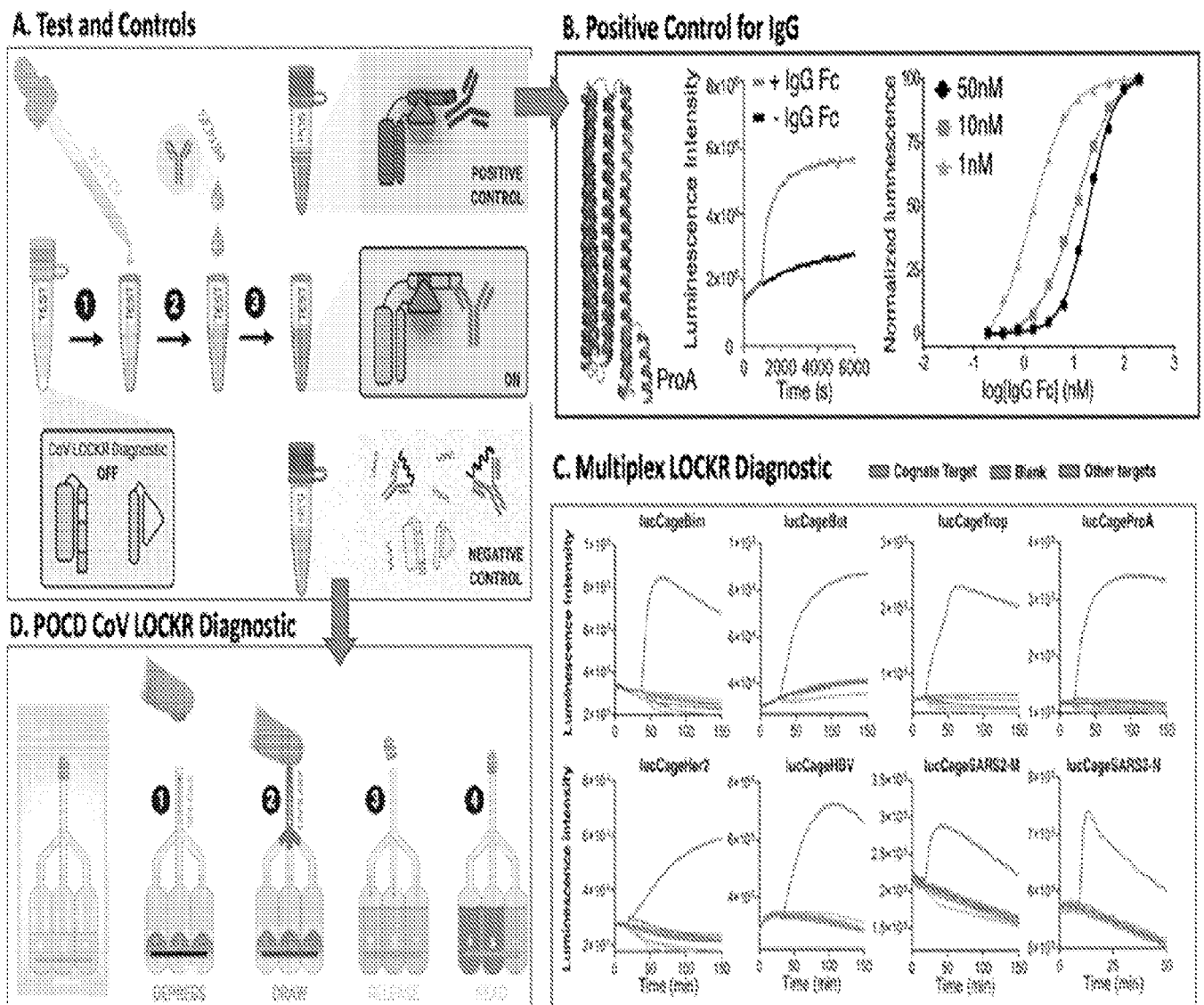


Figure 19

21/23

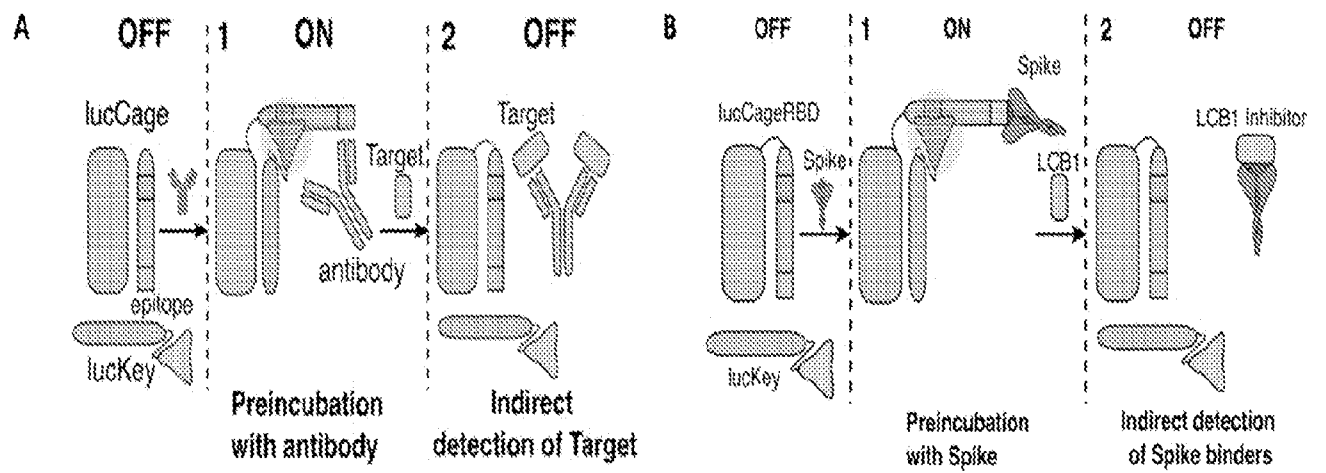


Figure 20

22/23

Figure 21

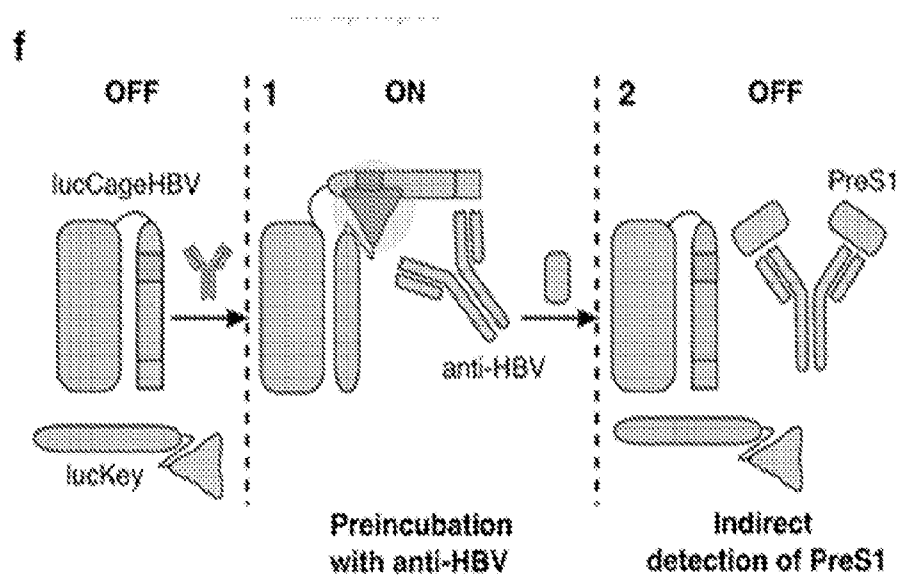


Figure 22

