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(54) **Title:** ORTHOGONAL PROTEIN HETERODIMERS

(57) **Abstract:** Disclosed herein are designed heterodimer proteins, monomeric polypeptides capable of forming heterodimer proteins, protein scaffolds including such polypeptides, and methods for using the heterodimer proteins and subunit polypeptides for designing logic gates.

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ORTHOGONAL PROTEIN HETERODIMERS

Cross Reference

This application claims priority to U.S. Provisional Patent Application Serial Nos. 62/755,264 filed November 2, 2018 and 62/904,800 filed September 24, 2019, each incorporated by reference herein in their entirety.

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This invention was made with government support under Grant No. GM103533 awarded by the National Institutes of Health. The government has certain rights in the invention

Background

Heterodimeric interaction specificity between two DNA strands, and between protein and DNA, is often achieved by varying side chains or bases coming off the protein or DNA backbone—for example, the bases participating in Watson-Crick base pairing in the double helix, or the side chains of protein contacting DNA in TALEN-DNA complexes. This modularity enables the generation of an essentially unlimited number of orthogonal DNA-DNA and protein-DNA heterodimers. In contrast, protein-protein interaction specificity is often achieved through backbone shape complementarity, which is less modular and hence harder to generalize.

Summary

In one aspect, the disclosure provides designed heterodimer proteins, comprising:

(a) a monomer A polypeptide, wherein the monomer A polypeptide is a non-naturally occurring polypeptide comprising 1-5 alpha helices connected by amino acid linkers; and

(b) a monomer B polypeptide, wherein the monomer B polypeptide is a non-naturally occurring polypeptide comprising 1-5 alpha helices connected by amino acid linkers,

wherein monomer A and monomer B non-covalently interact to form the designed heterodimer protein. In one embodiment,

(i) monomer A comprises a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an odd-numbered SEQ ID NO selected from the group consisting of selected from the group SEQ ID NOS: 1-290; and

(ii) monomer B comprises a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an even-numbered SEQ ID NO selected from the group consisting of selected from the group SEQ ID NOS: 1-290, wherein the even-numbered SEQ ID NO is the binding partner of the odd-numbered SEQ ID NO. in step (i).

In another aspect, the disclosure provides non-naturally occurring polypeptide comprising a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-290.

In another aspect, the disclosure provides non-naturally occurring polypeptide comprising a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494.

In another aspect, the disclosure provides proteins comprising 2, 3, 4, or more non-naturally occurring polypeptides having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-290, wherein the 2, 3, 4, or more naturally occurring polypeptides are covalently linked. In one embodiment, each of the 2, 3, 4, or more non-naturally occurring polypeptides are different. In another embodiment, each of the 2, 3, 4, or more non-naturally occurring polypeptides are present in a fusion protein.

In another aspect, the disclosure provides proteins comprising 2, 3, 4, or more non-naturally occurring polypeptides having at least 70%, 75%, 80%, 85%, 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-290, 1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494 wherein the 2, 3, 4, or more naturally occurring polypeptides are covalently linked. In one embodiment, each of the 2, 3, 4, or more non-naturally occurring polypeptides are different. In another embodiment, each of the 2, 3, 4, or more non-naturally occurring polypeptides are present in a fusion protein. In each of these aspects, amino acid changes from the reference amino acid sequence may be conservative amino acid substitutions. In another embodiment, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions are invariant compared to the reference amino acid sequence.

In another aspect, the disclosure provides protein scaffolds, comprising

a) a first designed component comprised of any number of monomer A polypeptides and/or monomer B polypeptides, each from different heterodimers, connected into a single component by amino acid linkers.

b) a second designed component, comprising corresponding monomers for each monomer A and/or monomer B in the first designed component one;

wherein the first and second designed components interact to form the protein scaffold, and wherein each monomer A only interacts in the scaffold with its monomer B mate.

In another aspect, the disclosure provides methods of forming the designed heterodimer protein of any embodiment of the disclosure, comprising:

a) providing two of the monomers as unlinked monomers;

b) providing the other two monomers as linked monomers;

whereby the unlinked monomers associate with their respective monomer of the same heterodimer, and not with any of the other monomers.

In another aspect, the disclosure provides designed heterodimer proteins, comprising:

a) asymmetric buried hydrogen bond networks incorporated into regularly repeating backbone structures; and

b) helix hairpin helix monomers wherein the supercoil phases of the helices are fixed at 0, 90, 180, or 270 degrees and the supercoil twist (ω0) and helical twist (ω1) are held constant for either a two layer left handed super coil (ω0=-2.85 and ω1=102.85), or a 5 layer untwisted bundle (ω0=0 and ω1=100)

In another aspect, the disclosure provides fusion proteins comprising a polypeptide of the formula X-B-Z, wherein:

(a) the X domain is a non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices, wherein the X domain is capable of non-covalently binding to a first target;

(b) the Z domain is a non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices, wherein the Z domain is capable of non-covalently binding to either (i) a second target that differs from the first target, or (ii) a different non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices; and

(c) the B domain is an amino acid linker;

wherein a combined number of alpha helices from the X domain and the Z domain is 4, 5, or 6; and

wherein the X domain and the Z domain interact at a binding interface, wherein the binding interface comprises a hydrogen bond network in which at least one side chain in each alpha helix hydrogen of the X domain bonds with a side chain in an alpha helix in the Z domain, and wherein the binding interface comprises a plurality of hydrophobic residues.

In another aspect, the disclosure provides kits or compositions, comprising at least two fusion proteins comprising the formula X-B-Z, wherein

the B domain in each fusion protein is independently a polypeptide linker;

the X domain in each fusion protein comprises a first non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices;

the Z domain in each fusion protein comprises a second non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices, wherein a combined number of alpha helices from the X domain and the Z domain in each individual fusion protein is 4, 5, or 6; wherein the X domain and the Z domain interact at a binding interface, wherein the binding interface comprises a hydrogen bond network in which at least one side chain in each X domain alpha helix bonds with a side chain in an alpha helix in the Z domain; wherein

the X domain in a first fusion protein is capable of non-covalently binding to a first target;

the Z domain in a second fusion protein is capable of non-covalently binding to a second target; and

the X domains and Z domains in each individual fusion protein that are not capable of non-covalently binding to the first target or the second target are capable of non-covalently binding to an X or a Z domain of a different fusion protein in the plurality of fusion proteins.

In one embodiment of the fusion proteins, kits, or compositions, each X domain and each Z domain comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide comprising the amino acid sequence selected from SEQ ID NO:1-290, with the proviso that the X domain and the Z domain do not do not form a heterodimer (a-b) pair. In another embodiment at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions of each X domain and each Z domain are invariant compared to the reference amino acid sequence.

In one aspect, the disclosure provides methods, comprising:

(i) contacting a fusion protein according to any embodiment of the disclosure with a biological sample under conditions to promote non-covalent binding of the fusion protein with first target and second target present in the sample, and

(ii) detecting non-covalent binding of the one or more fusion proteins to the first target and/or the second target in the biological sample.

In one embodiment, the method comprises detecting cooperative non-covalently binding of the one or more fusion proteins to the first target and the second target in the biological sample. In another embodiment, the method comprises detecting non-covalent binding of the one or more fusion proteins to the first target or the second target in the biological sample.

In another aspect, the disclosure provides methods for target detection, comprising

(a) contacting a biological sample with at least two fusion proteins, wherein each of the at least two fusion proteins comprises the formula X-B-Z, wherein

each B is independently a polypeptide linker;

each X domain comprises a first non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices;

each Z domain comprises a second non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices, wherein a combined number of alpha helices from the X domain and the Z domain in each individual fusion protein is 4, 5, or 6; wherein the X domain and the Z domain interact at a binding interface, wherein the binding interface comprises a hydrogen

bond network in which at least one side chain in each X domain alpha helix bonds with a side chain in an alpha helix in the Z domain; wherein

the X domain in a first fusion protein is capable of non-covalently binding to a first target;

the Z domain in a second fusion protein is capable of non-covalently binding to a second target; and

the X domains and Z domains in each individual fusion protein that are not capable of non-covalently binding to the first target or the second target are capable of non-covalently binding to an X or a Z domain of a different fusion protein in the plurality of fusion proteins;

(b) detecting non-covalent binding of the two or more fusion proteins to the first target and/or the second target in the biological sample.

In one aspect, the disclosure provides compositions comprising

(a) a first polypeptide comprising 2 alpha helices, wherein the first polypeptide is capable of non-covalently binding a first target; and

(b) a second polypeptide comprising 2 alpha helices, wherein the first polypeptide is capable of non-covalently binding to the second polypeptide, and wherein the second polypeptide is capable of non-covalently binding a second target that differs from the first target; wherein:

(i) a binding affinity of the first polypeptide for the first target is approximately equal to a binding affinity of the second polypeptide for the second target; and

(ii) the binding affinity of the first polypeptide for the first target and the binding affinity of the second polypeptide for the second target are greater than the binding affinity of the first target and the second target for each other.

In one aspect, the disclosure provides compositions comprising

(a) a first polypeptide comprising 2 alpha helices, wherein the first polypeptide is capable of non-covalently binding a first target; and

(b) a second polypeptide comprising 2 alpha helices, wherein the first polypeptide is capable of non-covalently binding to the second polypeptide, and wherein the second polypeptide is capable of non-covalently binding a second target that differs from the first target; wherein:

(i) a binding affinity of the first polypeptide for the second polypeptide is greater than a binding affinity of the second polypeptide for the second target;

(ii) a binding affinity of the first polypeptide for the first target is approximately equal to a binding affinity of the second polypeptide for the second target; and

(iii) the binding affinity of the first polypeptide for the first target and the binding affinity of the second polypeptide for the second target are greater than the binding affinity of the first target and the second target for each other.

In another aspect, the disclosure provides compositions comprising

(a) a first polypeptide comprising 4 alpha helices, wherein the first polypeptide is capable of non-covalently binding a first target; and

(b) a second polypeptide comprising 4 alpha helices, wherein the second polypeptide is capable of non-covalently binding a second target that differs from the first target; wherein:

(i) a binding affinity of the first target for the second target is greater than a binding affinity of the first polypeptide for the first target;

(ii) a binding affinity of the first polypeptide for the first target is approximately equal to a binding affinity of the second polypeptide for the second target; and

(iii) the sum of the binding affinity of (A) the first polypeptide for the first target and (B) the binding affinity of the second polypeptide for the second target, is greater than the binding affinity of the first target and the second target.

In various embodiments for each composition of the disclosure, the composition may further comprise the first target and the second target, and the first target and/or the second target further may comprise one or more effector polypeptide domains. In one embodiment, the first polypeptide and/or the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-290, or the group consisting of SEQ ID NOS:1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494. In another embodiment, the first target and/or the second target each comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-290, or the group consisting of SEQ ID NOS:1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, with the proviso that the first target forms a

heterodimer (a-b) pair with the first polypeptide, and the second target forms a heterodimer (a-b) pair with the second polypeptide. In another embodiment, the compositions are contacted with a biological sample and binding is detected, such as detecting an output signal caused by actions of effector polypeptides upon binding.

The disclosure also provides nucleic acids encoding the polypeptides, proteins, and fusion proteins of the disclosure; expression vectors comprising the nucleic acids operatively linked to a promoter; and host cells comprising the nucleic acids, expression vectors, and/or polypeptides, proteins, fusion proteins, scaffolds, and designed heterodimer pairs of the disclosure.

Description of the Figures

FIGs. 1A to 1H shows modular heterodimer design. FIG. 1A shows individual helix generation: the helical phase ($\Delta\phi_1$), supercoil radius (R) and offset along the Z-axis (Z offset) were exhaustively sampled; a total of 11 free parameters since there is no z offset for the first helix. FIG. 1B shows top-down view of the parallel twisted backbone. FIG. 1C shows representative hydrogen bond networks identified using HBNET^{TM}. FIG. 1D shows matches of multiple HBNET^{TM} containing heptads to a single full length backbone. FIG. 1E shows addition of loops to connect the 4 helices into two helix hairpins. FIGs. 1F, 1G, and 1H show SEC trace, CD spectra and (inset) temperature melt, and SAXS (black, experimental SAXS data; red, spectra computed from the designed backbones) profile of the design DHD37_ABXB. Experiments were performed once.

FIGs. 2A to 2F show structural characterization of designed heterodimers. FIGs. 2A-2D show crystal structures superimposed on design models with monomers; cross-sections on backbones (left) indicate locations of designed hydrogen-bond networks (middle panels). Solid and dashed boxes compare networks in design model and crystal structure. Black boxes compare overall hydrophobic packing. FIG. 2A shows DHD_131, 2.4 Å resolution with 1.0 Å Cα RMSD. FIG. 2B shows DHD37_1:234, 3.3 Å resolution with 1.4 Å RMSD. FIG. 2C shows DHD_127, 1.8 Å resolution with 1.7 Å RMSD. FIG. 2D shows DHD_15, 3.4 Å resolution with 0.9 Å RMSD; hydrogen bond networks were not well resolved. FIGs. 2E-2F show DHD_39 and DHD_120 backbones and designed hydrogen bond networks. Experimental SAXS data (black) are similar to spectra computed from the designed backbones.

FIGs. 3A to 3C shows new functionality from DHD combinations. FIG. 3A shows induced dimerizer formed from “b” component of DHD13_XAAA fused to “b” component

of DHD37_ABXB with an intervening flexible linker. The "a" components of the two heterodimers are brought into close proximity by the heterodimerizer. FIG. 3B shows Y2H data on 4 induced dimerization systems. For each pair of bars: left, without heterodimerizer fusion; right, with heterodimerizer fusion. Dashed line indicates background growth with unfused AD and DBD. Data are mean ± s.d. from 3 biological repeats. FIG. 3C shows 9_a, 13_XAAA_a and 37_ABXB_a were covalently linked to form a scaffold, recruiting 9_b (hexahistidine tagged), 13_XAAA_b and 37_ABXB_b.

FIGs. 4A to 4C show all-against-all orthogonality assessment. FIG. 4A shows Y2H for 21 heterodimers show heterodimer formation with little homodimer formation. First letter at bottom indicates monomer fused to AD, second letter, to DBD. FIG. 4B shows Y2H all by all testing of 9 pairs of heterodimers, colors indicate growth. Boxes indicate designed cognate heterodimer pairs, dashed black box indicates a set of 6 orthogonal heterodimers. FIG. 4C shows Off-target binding of DHD15_a and DHD13_XAAA_b, in the absence (left) or presence (right) of DHD15_b and DHD13_XAAA_a. Data are mean ± s.d.. Red dashed line indicates background growth with unfused AD and DBD.

FIGs. 5A to 5B show example HBNets resulting from the systematic search. FIG. 5A shows overlay of 50 backbones with different Crick parameters for each helix. FIG. 5B shows example hydrogen bond networks from the systematic search, each involving at least 4 residues and contacting all 4 helices.

FIGs. 6A to 6C show thermal and chemical denaturation of DHDs. FIGs. 6A and 6B show CD spectra for thermal denaturation of DHD_15 and DHD_20, respectively. Top, wavelength scan at 25°C, 75°C, 95°C, and final 25°C. Designs were alpha helical and stable up to 95°C. Bottom, CD temperature melts, monitoring absorption at 222 nm as temperature was increased from 25°C to 95°C. FIG. 6C shows GdnHCl denaturation of DHD_127 by CD monitoring absorption at 222 nm. All CD experiments were performed once.

FIG. 7A to 7B show backbone and hydrogen bond network permutations. FIG. 7A shows on a 2+2 backbone (left), two loops were designed to connect the 4 helices into a single monomer in 2 different ways (middle), after which 4 different cut points were introduced to generate 4 possible backbone permuted heterodimers of a single helix and a three helix bundle (3+1 heterodimers, right). For example, 2:134 refers to a heterodimer where the original helix 2 is a single helix, and helices 1, 3, and 4 were connected into a 3 helix bundle. FIG. 7B shows hydrogen bond network permutation. Each unique network was assigned a letter (Networks "A" and "B" in this case), and with the hydrophobic packing assigned X. The backbone on the left reads "ABXB", with its first heptad accommodates

network "A", its second and fourth heptad accommodate network "B", and its third heptad accommodates hydrophobic packing only.

FIGs. 8A to 8H show crystal structure of the domain swapped DHD_15 and biophysical characterization of higher order oligomers. FIG. 8A shows crystal structure of DHD_15 at pH 6.5, with 2.25 Å resolution. FIG. 8B shows superposition of design models in color onto both halves of the crystal structure in white, with backbone RMSD of 1.83 Å. FIGs. 8C to 8F show SEC traces of the induced dimerization DHD_9-13 fusion, DHD_15-37 fusion, DHD_13-37 fusion, and the scaffolding complex in FIG. 3C (the peak at around 15 mL corresponds to the fully assembled complex, followed by a peak representing excess of individual components). FIG. 8G shows CD thermal melt curves for the scaffolding complex in FIG. 3C. Wavelength scan was performed at 25°C, 75°C, 95°C, and final 25°C. Design was alpha helical and stable up to 95°C. FIG. 8H, CD chemical denaturation profile of the scaffolding complex in FIG. 3C. 2 (FIG. 8C to 8F) or 1 (FIG. 8G to 8H) biologically independent repeats were performed.

FIGs. 9A to 9G show Y2H all-against-all assay of 16 DHDs. FIG. 9A shows Y2H assay with cell growth on agar plates containing 100 mM 3-AT, lacking tryptophan, leucine and histidine. Plates were imaged at Day 5. White, no growth on agar plates; grey, weak growth forming non-circular colonies; black, strong growth. FIG. 9B shows Y2H result by growing yeast culture in liquid media containing 100 mM 3-AT, lacking tryptophan, leucine and histidine. OD 600 values were measured at Day 2 to evaluate cell growth. FIG. 9C shows an additional set of DHDs tested by Y2H showing improved orthogonality. FIG. 9D shows distribution of OD 600 values for non-cognate interactions in FIG. 9B, the majority of cells grew to OD 600 values less than 0.4, indicating weak interactions for non-cognate binding. FIG. 9E shows more buried bulky polar residues strongly correlates with design success. f, Successful designs tend to have bigger polar interface surface area. FIG. 9G shows designs with better hydrophobic packing (as reported by the ROSETTA™ filter value Average Degree on Ile, Leu and Val residues) tend to have a higher chance of being constitutive heterodimers. Two (FIGs. 9A to 9C) independent experiments were performed.

FIG. 10 shows hydrogen bond network sequence motifs of the set of 6 orthogonal pairs in Y2H experiments. Letters patches mark the location of hydrogen bond network forming residues on the backbones, and indicate residue identities.

FIGs. 11A to 11H show cooperativity of CIPHR logic gates. FIG. 11A shows backbone structure of *A:A'* heterodimer building block, with hydrogen bond network detail in inset. Bottom right, condensed representation used throughout figures. FIG. 11B shows

thermodynamic cycle describing the induced dimerization system. FIG. 11C shows simulation of the induced dimerization system under thermodynamic equilibrium. A and B' monomers were held constant while titrating in various initial amounts of the A'-B dimerizer proteins. If binding is not cooperative (small c), the final amount of trimeric complexes decreases when the dimerizer protein is in excess. FIG. 11D shows equilibrium denaturation experiments monitored by CD for designs with 6- and 12- amino acid (AA) linkers. Circles represent experimental data, and lines are fits to the 3-state unimolecular unfolding model. Design models are shown on the side. FIG. 11E shows experimental SAXS profile of *1'-2'* with a 6-residue linker (in black), fitted to the calculated profile of *1:1'* heterodimer. FIG. 11F shows an induced dimerization system using a 6-residue linker. FIG. 11G shows a two-input AND gate schematic. FIG. 11H shows a three-input AND gate.

FIGs. 12A to 12G show CIPHR two input logic gates. FIG. 12A shows CIPHR gates are built from DHDs (top) with monomers or covalently connected monomers as inputs (left); some gates utilize only the designed cognate interactions (left side of middle panel), while others take advantage of observed inter and intramolecular binding affinity hierarchies (right side of middle panel). FIGs. 12B and 12C show two-input AND (12B) and OR (12C) CIPHR logic gates based on orthogonal DHD interactions. FIGs. 12D to 12G show NOT (12D), NOR (12E), XNOR (12F), and NAND (12G) CIPHR logic gates made from multispecific and competitive protein binding. For each gate, black dots represent individual Y2H growth measurement corrected over background growth, with their average values shown in bars. * indicates no yeast growth over background. 0s and 1s in the middle and right blocks represent different input states and expected outputs, respectively. \

FIGs. 13A to 13E show three-input CIPHR logic gates. FIG. 13A shows schematic of a three-input AND gate. FIG. 13B shows schematic of a three-input OR gate. FIG. 13C shows Y2H results confirmed activation of the 3-input OR gate with either of the inputs. FIG. 13D shows schematic of a DNF gate. FIG. 13 E shows Y2H results confirmed proper activation of the gate. For each gate, black dots represent individual measurements corrected over background growth, with their average values shown in bars.

FIG. 14A shows molecular implementation of the cooperative induced dimerization system, binding only occurs when all three components are present. FIG. 14B shows size exclusion chromatography profiles of *1'-2'* variants with 0, 2, 6, 12, and 24 amino acids in the flexible linker connecting *1'* and *2*.

FIGs. 15A and 15B show binding affinity gradient from individual Y2H experiments. FIG. 15A shows the 8:8' heterodimer binds more tightly than the homodimers of its

monomers. FIG. 15B shows binding affinity gradient among the monomers of **1:1'**, **9:9'**, and **10:10'** pairs.

FIG. 16 shows exemplary heterodimer proteins comprising combinations of monomer A and monomer B.

Detailed Description

All references cited are herein incorporated by reference in their entirety. Within this 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. Deutshcer, 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).

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.

It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

The term "interface residue" or "interface position", as used herein, means amino acid residues or positions that are interacting between at least two monomers in heterodimer, heterotrimer, heterotetramer, etc. The interaction comprises a hydrogen bond network in which at least a hydrogen from an alpha helix in the first monomer binds to a side chain in an alpha helix in the second monomer. In some aspects, the interaction comprises at least one hydrogen bond, at least two hydrogen bonds, at least three hydrogen bonds, at least four hydrogen bonds, at least five hydrogen bonds, at least six hydrogen bonds, at least seven hydrogen bonds, at least eight hydrogen bonds, at least nine hydrogen bonds, and at least ten hydrogen bonds. In some aspects, the interface residue comprises hydrophobic residues.

The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While the specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize.

In a first aspect the disclosure provide designed heterodimer proteins, comprising:

(a) a monomer A polypeptide, wherein the monomer A polypeptide is a non-naturally occurring polypeptide comprising 1-5 alpha helices connected by amino acid linkers; and

(b) a monomer B polypeptide, wherein the monomer B polypeptide is a non-naturally occurring polypeptide comprising 1-5 alpha helices connected by amino acid linkers,

wherein monomer A and monomer B non-covalently interact to form the designed heterodimer protein.

The disclosure provides designed heterodimer proteins according to this aspect formed by the non-covalent interaction of two different alpha-helix-containing polypeptides (monomer A and monomer B).

By doubling the interaction surface area of protein coiled coils with an additional helix, and incorporating modular hydrogen bond networks, a wide range of heterodimeric interaction specificities can be achieved, as described herein. Millions of helical backbones with varying degrees of supercoiling around a central axis were generated and searched for those accommodating extensive hydrogen bond networks,

followed by connecting the helices with short loops and designing the remainder of the sequence. As disclosed in the examples that follow, designs expressed in *E coli* exclusively formed heterodimers, and crystal structures of exemplary designs fit the computational models and confirmed the designed hydrogen bond networks. Following mixing of independently expressed and purified heterodimer designs, the vast majority of the interactions observed by native mass spectrometry were between the designed cognate pairs. The large sets of orthogonal polypeptide heterodimers disclosed herein can be used, for example, to generate synthetic protein logic gates, transcriptional networks and other synthetic biology applications.

Heterodimers are generally more useful than homodimers in bioengineering because of their ability to bring together two different entities (often fusion proteins). A long standing challenge in the field has been to come up with a set of orthogonally interacting protein heterodimers -- monomers that selectively form cognate pairs and in the meantime avoid binding to other non-cognate monomers. Disclosed herein include such sets of orthogonal heterodimers, which can be programmably expanded into an even bigger set. The ability to bring together two different fusion proteins via genetically fused heterodimers allowed the design of protein-based logic gates, as also disclosed herein.

In one embodiment, monomer A and monomer B have their interaction specificity determined by at least one designed hydrogen bond network at the interface between monomer A and monomer B. In some aspects, (i) monomer A comprises 1 helix, and monomer B comprises 1 helix; (ii) monomer A comprises 1 helix and monomer B comprises 2 helices; (iii) monomer A comprises 1 helix and monomer B comprises 3 helices, (iv) monomer A comprises 1 helix and monomer B comprises 4 helices; or (v) monomer A comprises 1 helix and monomer B comprises 5 helices. In some aspects, (i) monomer A comprises 2 helices, and monomer B comprises 1 helix; (ii) monomer A comprises 2 helices and monomer B comprises 2 helices; (iii) monomer A comprises 2 helices and monomer B comprises 3 helices, (iv) monomer A comprises 2 helices and monomer B comprises 4 helices; or (v) monomer A comprises 2 helices and monomer B comprises 5 helices. In some aspects, (i) monomer A comprises 3 helices, and monomer B comprises 1 helix; (ii) monomer A comprises 3 helices and monomer B comprises 2 helices; (iii) monomer A comprises 3 helices and monomer B comprises 3 helices, (iv) monomer A comprises 3 helices and monomer B comprises 4 helices; or (v) monomer A comprises 3 helices and monomer B comprises 5 helices. In

some aspects, (i) monomer A comprises 4 helices, and monomer B comprises 1 helix; (ii) monomer A comprises 4 helices and monomer B comprises 2 helices; (iii) monomer A comprises 4 helices and monomer B comprises 3 helices, (iv) monomer A comprises 4 helices and monomer B comprises 4 helices; or (v) monomer A comprises 4 helices and monomer B comprises 5 helices. In some aspects, (i) monomer A comprises 5 helices, and monomer B comprises 1 helix; (ii) monomer A comprises 5 helices and monomer B comprises 2 helices; (iii) monomer A comprises 5 helices and monomer B comprises 3 helices, (iv) monomer A comprises 5 helices and monomer B comprises 4 helices; or (v) monomer A comprises 5 helices and monomer B comprises 5 helices.

Any suitable amino acid linkers can be used to separate the alpha helices in each monomer. The length and amino acid content may vary based on an intended use, and can be determined by one of skill in the art based on the teachings herein. The polypeptide monomers may include any other useful sequences, including detectable tags and purification tags. In one non-limiting embodiment, at least one of monomer A and monomer B comprises a hexahistidine tag.

In another embodiment, the disclosure provides heterodimers, comprising:

(a) a monomer A polypeptide, wherein the monomer A polypeptide is a non-naturally occurring polypeptide comprising 1-5 alpha helices, wherein adjacent alpha helices may optionally be connected by an amino acid linker; and

(b) a monomer B polypeptide, wherein the monomer B polypeptide is a non-naturally occurring polypeptide comprising 1-5 alpha helices, wherein adjacent alpha helices may optionally be connected by an amino acid linker;

wherein the monomer A polypeptide and the monomer B polypeptide non-covalently interact to form the designed heterodimer protein.

In one embodiment, the monomer A polypeptide and the monomer B polypeptide have their interaction specificity determined by at least one hydrogen bond network at the interface between the monomer A polypeptide and the monomer B polypeptide. In another embodiment,

(i) the monomer A polypeptide comprises 2 alpha helices, and the monomer B polypeptide comprises 3 alpha helices;

(ii) the monomer A polypeptide comprises 3 alpha helices and the monomer B polypeptide comprises 3 alpha helices;

(iii) the monomer A polypeptide comprises 3 alpha helices and the monomer B polypeptide comprises 4 alpha helices,

(iv) the monomer A polypeptide comprises 4 alpha helices and the monomer B polypeptide 3 alpha helices;

(v) the monomer A polypeptide comprises 4 alpha helices and the monomer B polypeptide comprises 4 alpha helices;

(vi) the monomer A polypeptide comprises 5 alpha helices and the monomer B polypeptide comprises 4 alpha helices;

(vii) the monomer A polypeptide comprises 4 alpha helices and the monomer B polypeptide comprises 5 alpha helices;

(viii) the monomer A polypeptide comprises 5 alpha helices and the monomer B polypeptide comprises 5 alpha helices;

(ix) the monomer A polypeptide comprises 2 alpha helices and the monomer B polypeptide comprises 2 alpha helices; or

(x) the monomer A polypeptide comprises 3 alpha helices and the monomer B polypeptide comprises 2 alpha helices.

In one embodiment of any of the above embodiments,

(i) monomer A comprises a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an odd-numbered SEQ ID NO selected from the group consisting of selected from the group SEQ ID NOS: 1-290; and

(ii) monomer B comprises a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an even-numbered SEQ ID NO selected from the group consisting of selected from the group SEQ ID NOS: 1-290, wherein the even-numbered SEQ ID NO is the binding partner of the odd-numbered SEQ ID NO. in step (i).

The amino acid sequences of SEQ ID NOS:1-290 are provided in Table 1A. The "binding partners" are sequentially numbered (and similarly named) as shown in the Table. For example, SEQ ID NO:1 (DHD9 A) and SEQ ID NO:2 (DHD9 B) are binding partners, so that if monomer A comprises the polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of SEQ ID NO:1, then monomer B comprises the polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along

the length of the amino acid sequence of SEQ ID NO:2. Similarly, SEQ ID NOS:3-4 are binding partners, SEQ ID NO:5-6 are binding partners….SEQ ID NOS:289-290 are binding partners. Those of skill in the art will clearly understand what is meant by binding partner based on the teachings herein.

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Table 1A

Design name	Oligomerization State	Chain	Design sequence
DHD9	Heterodimer	a	GSPKEEARELIRKQKELIKEQKKLIKEAKQKSDSRDAERIWKRSREINRES KKINKRIKELIKS **SEQ ID NO:1**
DHD9	Heterodimer	b	PKKEAEELAEESEELHDRSEKLHERAEQSSNSEEARKILEDIERISERIEE ISDRIERLLRS **SEQ ID NO:2**
DHD13_X AAA	Heterodimer	a	GTKEDILERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKL LEESLRLLKELLELSEESAQLLYEQR **SEQ ID NO:3**
DHD13_X AAA	Heterodimer	b	GTEKRLLEEAERAHREQKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKKI LEEIRELSKRSLELLREILYLSQEQKGSLVPR **SEQ ID NO:4**
DHD13_X AXA	Heterodimer	a	TKEDILERQRKIIERAQEIIRRQQEILEELERIIRKPGSSEEAMKRMLKLL EESLRLLKELLELLEESAQLLYEQR **SEQ ID NO:5**
DHD13_X AXA	Heterodimer	b	GSTEKRLLEEAERAHREAKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKK ILEEIRELSKRLLELLREILYLSQEQK **SEQ ID NO:6**
DHD13_X AAX	Heterodimer	a	TKEDILERARKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKLL EESLRLLKELLELSEELAQLLYEQR **SEQ ID NO:7**
DHD13_X AAX	Heterodimer	b	GSTEKRLLEEAERAIREQKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKK ILEEIRELSKRSLELLREILYLLQEQK **SEQ ID NO:8**
DHD13_2 :341	Heterodimer	a	TKEDILERQRKIIERAQEIHRRQQEILEELEYIIR **SEQ ID NO:9**
DHD13_2 :341	Heterodimer	b	MSEEAMKRMLKLLEESLRLLKELLELSEESAQLLYEQRKANNGSETEKRLL EEAERAHREQKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKKILEEIREL SKRSLELLREILYLSQEQK **SEQ ID NO:10**
DHD13_A AAA	Heterodimer	a	MTKEDILERQRKIIERAQEIHRRQQEILKEQEKIIRKPGSSEEAMKRSLKL IEESLRLLKELLELSEESAQLLYEQR **SEQ ID NO:11**
DHD13_A AAA	Heterodimer	b	GTEKRLLEEAERAHREQKEIIKKAQELHKELTKIHQQSGSSEEAKKRALKI SQEIRELSKRSLELLREILYLSQEQK **SEQ ID NO:12**
DHD13_B AAA	Heterodimer	a	TKEDILERQRKIIERAQEIHRRQQEILKRSEEIIRKPGSSEEALETLRELQ EESLRLLKELLELSEESAQLLYEQR **SEQ ID NO:13**
DHD13_B AAA	Heterodimer	b	GSTEKRLLEEAERAHREQKEIIKKAQELHRRTEEIIRQSGSSEEAKDELRR IQEEIRELSKRSLELLREILYLSQEQK **SEQ ID NO:14**
DHD13_4 :123	Heterodimer	a	TTKRYLEEAERAHREQKEIIKKAQELHRRLEEIVRQ **SEQ ID NO:15**
DHD13_4 :123	Heterodimer	b	GSSEEAKKEAKKILEEIRELSKRSLELLREILYLSQQVNDVDEKALERQRK IIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKLLEESLRLLKELL ELSEESAQLLYEAR **SEQ ID NO:16**
DHD13_1 :234	Heterodimer	a	EAMKRMLKLLEESLRLLKELLELSEESAQLLYEAR **SEQ ID NO:17**
DHD13_1 :234	Heterodimer	b	TTKRYLEEAERAHREQKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKKIL EEIRELSKRSLELLREILYLSQQVNDVDEKALERQRKIIERAQEIHRRQQE ILEELERIIRKPGS **SEQ ID NO:18**
DHD15	Heterodimer	a	TREELLRENIELAKEHIEIMREILELLQKMEELLEKARGADEDVAKTIKEL LRRLKEIIERNQRIAKEHEYIARERS **SEQ ID NO:19**
DHD15	Heterodimer	b	GTERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLKKARGADEKVLDELRK IIERIRELLDRSRKIHERSEEIAYKEE **SEQ ID NO:20**
DHD20	Heterodimer	a	GDRQELIRRNIELLKEHIKILEEISQLIEELSELLDKSSSEEVVKRYKKIL ERYKQLLRKSQEIHKESSEIAKKES **SEQ ID NO:21**

DHD20	Heterodimer	b	GDEQKLIERSQRMQKESLELLKEIIKILDTIEKLLDKPDSEELLDTIKKLH DTLKKIHDRNKKLLKEHEEILRQRSGSLVPR **SEQ ID NO:22**
DHD21	Heterodimer	a	DKEEEYKRLLDEIKEILKESKEVLKDSKRVLEDIKRKVPDDDLVKLLEKHV RLLEEHVKLLEQLIREAEKSSK **SEQ ID NO:23**
DHD21	Heterodimer	b	QGSSAEELLKKIKESEKKIRDSLRKIKEIIKKSRKEGVDDKQLDLIRKVVE SHRDLLRLHRDLLRLLREETS **SEQ ID NO:24**
DHD25	Heterodimer	a	DIDESIKEVEKLLEEVEQSLQKLDDSLKKLLEKVNQDPDVDDSVRKIVKRH VEILKRHEEVLKRLIEVVKEHTKTVK **SEQ ID NO:25**
DHD25	Heterodimer	b	GSDREEVHKEIVKLIREIIKIHKKILKIHEKIKNGEIDPSEILKLSEEIKK LTDTIIKIIEDLEQLTRDLRR **SEQ ID NO:26**
DHD27	Heterodimer	a	DRKEIVKRHQKVVELLKESSKLLRESSKLLQRLLDKTGDENLQKAVDDQDK AIKRQETAIRKSQEASKKLD **SEQ ID NO:27**
DHD27	Heterodimer	b	DNSEEIKKVAKTSREVAEYSERVAKENDKVVKTLEEGKIDESELLRLLEES IKIFDTALKLHEEAYKLHQDLVRKVS **SEQ ID NO:28**
DHD30	Heterodimer	a	DESEAASVAIESVQILVESVKLLEESVRILLDAVKKNGVEDLLRVAQRWEK LVDEWLKVVKRWLDNVRDIQR **SEQ ID NO:29**
DHD30	Heterodimer	b	GSDKAEEVEKSVRKIEESIKKIRKSIKKAEDAVQLLKEGKIDAKDFLRIVR EDLEVVKEDVEIVKEDVENVREFSS **SEQ ID NO:30**
DHD33	Heterodimer	a	SDKEVSDKLLKASKKLLKVSEELLEVVRRLLKALKDDELIKKIADLLRKII DKDKKFIRTSEEIVKESR **SEQ ID NO:31**
DHD33	Heterodimer	b	GSDLKEVLKTVEEAVKEIIKSSEELLQISRKILEISRVGVDEHEYISAIRE YLKALEKHIQILKKFIEILKELIRAVS **SEQ ID NO:32**
DHD34_XAAXA	Heterodimer	a	SKEEIDKIVKKHKKKIEEHKKKVDELKKLVEEHDKRVSQDKDDKVKKLSEE VKKIIKRLEEVSKRLEEVSKKLLKVISDKR **SEQ ID NO:33**
DHD34_XAAXA	Heterodimer	b	GSNDEELKKILETLDRILKKLDKILTRLIEVLKKSEDPNLDDKDYTELVKQ FIELIKKYEEVVKEYEEVVRQLIRLFS **SEQ ID NO:34**
DHD34_XAXXA	Heterodimer	a	SKEEIDKIVKKHKKKIEELKKLVDELKKLVEEHDKRVSQDKDDKVKKLSEE VKKIIKRVEEVAKRLEEVSKKLLKVISDKR **SEQ ID NO:35**
DHD34_XAXXA	Heterodimer	b	GSNDEELKKILETLDRILKKLEKILTRLIEVLKKSEDPNLDDKDYTELVKQ FIELIKKFEEVIKEYEEVVRQLIRLFS **SEQ ID NO:36**
DHD34_XAAAA	Heterodimer	a	SKEEIDKIVKKHKKKIEEHKKKVDEHKKLVEEHDKRVSQDKDDKVKKLSEE LKKISKRLEEVSKRLEEVSKKLLKVISDKR **SEQ ID NO:37**
DHD34_XAAAA	Heterodimer	b	GSNDEELKKILETLDRILKKLDKILTRLDEVLKKSEDPNLDDKDYTELVKQ YIELVKKYEEVVKEYEEVVRQLIRLFS **SEQ ID NO:38**
DHD36	Heterodimer	a	DHSRKLKEILDRLRKHVKRLKEHLDELRDLVRQVPEDKLLEHVVKLSDKIL QISERAVREFTKSVDKDS **SEQ ID NO:39**
DHD36	Heterodimer	b	GSDKKDELERILDEIRRLIERLDEILSRLNKLLELLKHGVPNAKEVVKDYI RLLKEYLELVKEFLKLVKRHADLVS **SEQ ID NO:40**
DHD37_ABXB	Heterodimer	a	DSDEHLKKLKTFLENLRRHLDRLDKHIKQLRDILSENPEDERVKDVIDLSE RSVRIVKTVIKIFEDSVRKKE **SEQ ID NO:41**
DHD37_ABXB	Heterodimer	b	GSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVE LLKRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:42**
DHD37_BBBB	Heterodimer	a	MDEEDHLKKLKTHLEKLERHLKLLEDHAKKLEDILKERPEDSAVKESIDEL RRSIELVRESIEIFRQSVEEEE **SEQ ID NO:43**
DHD37_BBBB	Heterodimer	b	GDVKELTKILDTLTKILETATKVIKDATKLLEEHRKSDKPDPRLIETHKKL VEEHETLVRQHKELAEEHLKRTR **SEQ ID NO:44**
DHD37_XBXB	Heterodimer	a	DSDEHLKKLKTFLENLRRHLDRLDKLLKELRDILSENPEDERVKDVIDELE RVIRIVKTVIKIFEDSVRKKE **SEQ ID NO:45**
DHD37_XBXB	Heterodimer	b	GSDDKELDKLLDTLEKILQTATKIIDDLNKVLEKLRRSERKDPKVIETVVE LLKRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:46**
DHD37_AXXB	Heterodimer	a	DSDEHLKKLKTFLENLRRLEDLLDKHIKQLRDILSENPEDERVKDVIDLSE RVVRTVKTVIKIFEDSVRKKE **SEQ ID NO:47**
DHD37_AXXB	Heterodimer	b	GSDDKELDKLLDTLEKILQTATKVVDDANKLLEKLRRSERKDPKVVETYVE LLKRLEKLIKELLEIAKTHAKKVE **SEQ ID NO:48**
DHD37_3:124	Heterodimer	a	DSDEHLKKLKTFLENLRRHLDRLDKHIKQLRDILSEN **SEQ ID NO:49**
DHD37_3:124	Heterodimer	b	EDERVKDVIDLSERSVRIVKTVIKIFEDSVRKLEKTKPDSKTAKELDKLLD TLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKEL

			LEIAKTHAKKVE **SEQ ID NO:50**
DHD37_1:234	Heterodimer	a	DSDEHLYKLKTFLENLRRHLDRLDKHIKQLRDILSENPEDERVKDAIDLSE RSVRIVKTVIKIFEDSVRKKEKRPIDKRDDKELDKLLDTLEKILQTATKII DDANKLLEYLRR **SEQ ID NO:51**
DHD37_1:234	Heterodimer	b	GDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:52**
DHD37_AXBB	Heterodimer	a	DSDEHLDRLDKHLKKLKTFLENLRRHIKQLRDILSENPEDERVKDVIDLSK TVIKIFEDSVRKKERSVRIVE **SEQ ID NO:53**
DHD37_AXBB	Heterodimer	b	GSDDKEATKIIDDLDKLLDTLEKILQTANKLLEKLRRSERKDPKVVETYVK AVKELLEIAKTHAELLKRHEKKVE **SEQ ID NO:54**
DHD37_XBBA	Heterodimer	a	DSDEHIKQLRDHLDRLDKHLKKLKTFLENLRRILSENPEDERVKTVIKIFE DSVRKKERSVRIVKDVIDLSE **SEQ ID NO:55**
DHD37_XBBA	Heterodimer	b	GSDDKEANKLLEKATKIIDDLDKLLDTLEKILQTLRRSERKDPKAVKELLE IAKTHAELLKRHEKVVETYVKKVE **SEQ ID NO:56**
DHD39	Heterodimer	a	DHSRKLEEILDRLRKHVKRLLEHLRELLSLVKENPEDKDLVEVLELSLAIL RRSLEAVEAFLKSVTKKDPDDEDLRRKADEIRKEVEEIKKSLAEVEKEIYK LK **SEQ ID NO:57**
DHD39	Heterodimer	b	GSSADDVLEDILKIIRELIEILDQILSLLNQLLKLLRHGVPNAKKVVEKYK EILELYLQLVSLFLKIVKTHADAVSGKIDKKAEEEIKKEEEKIKEKLRQAK DILKKLQEEIDKTR **SEQ ID NO:58**
DHD40	Heterodimer	a	DRDAHLYKLLTFLEQLVRHLDRLVKHITQLRDIVKKDPEDERAVDVIRQSV RSLEIVITVLKIFVDSVSDAARSKEAEKIVRKIRKEIDEIRQKLREIDKEV KKTTS **SEQ ID NO:59**
DHD40	Heterodimer	b	GSNDKVLDKILDILDRILRLATRVIDLANKLLQVKKKSTHKDPRIVETYKE LLKIHETAVRLLLELADLHRRLKSKDEEANKRVETELDRIRKKVKDIEDKV RKLEDKVRKTAS **SEQ ID NO:60**
DHD43	Heterodimer	a	NDLSKEVLKKLEKSVEELLRRVQKSVKEAQKRGLLSDELVDRHLKILNQLV KRHLELLQEVIKRSDKK **SEQ ID NO:61**
DHD43	Heterodimer	b	GSDEAVKRVVEKSLKILDEVIKKSLDILRELIELQIRHAKDDESVIRASKS ALKDAIEALKKSLDEIKKALKRSADEG **SEQ ID NO:62**
DHD65	Heterodimer	a	SSEEVVKVHEKVVKLHKEILELLKKIIKIHETAARDPDDKDSIKKLSDEIK KIVKRIEDISDQAKRESSDAQRKQS **SEQ ID NO:63**
DHD65	Heterodimer	b	DKEEESKELLKKLKEILKRSEELLEESKELLKLAKNGEIDESELADADRKL NKKHEKLVQDIQDLLREHERQDR **SEQ ID NO:64**
DHD70	Heterodimer	a	DEKKKIDKIVKETEDLLQKSEKLLQQSKEAVKRIRSQVKENEIVDRLLRIS EELLKISRRLVEISRRIASTLS **SEQ ID NO:65**
DHD70	Heterodimer	b	GSSKEEVIRLLKENVRLIKENLELLTRNLKLITDLVRGSNGSEEKIKTLKE LLKEYRELLKRYRKLVEDYKRLVDKHD **SEQ ID NO:66**
DHD88	Heterodimer	a	EIQELIKSSRRIIEESKELIKESEEVLRRIKEILDRIRNGVDNQEDLLREI LKLLTKNLKIIQRNLKLLQDNAEILKRLVS **SEQ ID NO:67**
DHD88	Heterodimer	b	GSYIEDVIKKILDVSRELIKLSRTIIKISEEINKQLQQGRDTKDLVKKYDE IIKKYTRIVQHYTELIKELQKLLS **SEQ ID NO:68**
DHD89	Heterodimer	a	SPTEEAIQLSQRVIELSKRVIELSKEILKLLKRVLDLLPDLDKNEEKRLDD YDKELKEYDKELKKYEKRLKDLAS **SEQ ID NO:69**
DHD89	Heterodimer	b	GSEEEEILKIQKELLRIQSEILDKQKKILDTLRSNGAVTEEVRSILEKVER LSEEAKELSKEAKELTKEVSKLIS **SEQ ID NO:70**
DHD90	Heterodimer	a	SPLKELNNQLLRLLRELVKVSKKIVDLSKTIIEVLKHTDLDPRLLDSLEKS QQELDKSQKELDKVVKELTKVNKKLQ **SEQ ID NO:71**
DHD90	Heterodimer	b	GSPLEDLVRKYDELVKTYEKLVEEFKKAVDKYDKAVKKAPVSKEATDSLDL IRKVLELLDRNLKLIKENAKLIKELLK **SEQ ID NO:72**
DHD91	Heterodimer	a	SPTRENEKVIKENEKVISDNERVLEEVVKVVETATDRKEIQDAVDEVRKSV DKLRDSVRKLEESVRTLD **SEQ ID NO:73**
DHD91	Heterodimer	b	GSPIKDISKRLLEISKRLVEISDRIVELLQRIADSKDPNKDLQKEVKDVLE EYKRLVREYREVVKEYEKVVS **SEQ ID NO:74**
DHD92	Heterodimer	a	DEDEHVKQLIKNADLLRKHAELLKELVKLFQEIASQIPDDRVAKKVTDVVD RIDKILKQTEKLVRRTKQILDYSR **SEQ ID NO:75**
DHD92	Heterodimer	b	GSNLEELVKLLKEVLEMHERLLRIHEDLVEAHKSNASDKESERKLKKSDKD

			IKESLKKIKSIIDQVRYIQS **SEQ ID NO:76**
DHD93	Heterodimer	a	PVEDIIEESLRLLEESLKLLNRILKLLEDSLRKLPRSEEWRQRLDEFRKKL EDWKEELERWIEDVRYKKT **SEQ ID NO:77**
DHD93	Heterodimer	b	GSDEDYESREIIDEIRKLLDRSKKIVHRSQRLVERVKSTPLSEDQEDLIRR HEETINRHRELVKELEKVLEDHERHIR **SEQ ID NO:78**
DHD94	Heterodimer	a	PEEDSRRVLERFVRVSREVLKVLEEFLRVSEELLREADRDRDRRLEEYERQ VDELREEIRRYKEEVDKFDKEVKYYKK **SEQ ID NO:79**
DHD94	Heterodimer	b	GSPEKDENRKLLDKVRKLVEKSRRLVEELRKLVDQSTKNGLIDEKALRKQQ EVLRKVEEVLEKQERVLRELEEISYRVI **SEQ ID NO:80**
DHD94_3 :214	Heterodimer	a	GSPERDENRKLLDKVRKLVEKSRRLVEELRKLVDQSTKN **SEQ ID NO:81**
DHD94_3 :214	Heterodimer	b	GSDEKALRKQQEVLRKVEEVLEKQERVLRELEEISYRVITRGEDHKAEEDS RRVLERFVRVSREVLKVLEEFLRVSEELLREADRDRDRRLEEYERQVDELR EEIRRYKEEVDKFDKEVKYYKK **SEQ ID NO:82**
DHD94_2 :143	Heterodimer	a	GSDRRLEEYERQVDELREEIRRYKEEVDKFDKEVKYYKK **SEQ ID NO:83**
DHD94_2 :143	Heterodimer	b	GSPERDENRKLLDKVRKLVEKSRRLVEELRKLVDQSTKNGLIDEKALRKQQ EVLRKVEEVLEKQERVLRELEEISYRVITRGEDHKAEEDSRRVLERFVRVS REVLKVLEEFLRVSEELLREADR **SEQ ID NO:84**
DHD95	Heterodimer	a	DLSEESKKFVEKVKKLEKESRELEKQVKKIEEDSRSVENDVQKEFLELLKR LLDIQKKVVEVLREVVKVQQYVDS **SEQ ID NO:85**
DHD95	Heterodimer	b	GSDSEYESRQVLRELDTVLKDSHTVLEALRQVIRDSQDVVSKSDEESRRVI DDLEKVIQDSKKVLDDIKRLIDKSKSIKS **SEQ ID NO:86**
DHD96	Heterodimer	a	NEDELLKLLTENLKLLDENLKLLRENLSLLRQANNITDKNRIREIVKQSKE IVKQSREILKQSKEIVERIKYIVS **SEQ ID NO:87**
DHD96	Heterodimer	b	GSSLYELTQRYEKLVQQYEELVKDYRRLVKKLEKLKRDNKPDKRLLKEIVD VIKKSVEIIDRSLKLLEESIKILEETD **SEQ ID NO:88**
DHD97	Heterodimer	a	SQERSLEILKRILDVLKESLEILKESLSILRQLASRIKNPNRKIEEILKES DKIIKESDKVLKEIEEVIRYSS **SEQ ID NO:89**
DHD97	Heterodimer	b	GSDIEYESKEILELIKELLKLSRELLKESRRALELVRKSRDDSIVEEVIQV HKKVLDIHKEVLKIVRKVVEVHRRVKS **SEQ ID NO:90**
DHD98	Heterodimer	a	SKKDESTKLERLAEKIDEITKRIEELVKDVKRKSSEGVDKDQQQKIDEVFQ KLLDLQREILEILDRILKVQQYILD **SEQ ID NO:91**
DHD98	Heterodimer	b	GSDLEYLNRRLLQLIKTLIDLNRHLLKLIDKLKKLNSREGDEEKIKEESKQ IQEQFKEIVERSKEIIKQIKEIIKRSQ **SEQ ID NO:92**
DHD99	Heterodimer	a	DFERSSRRLEKVVEDLRRSSDRLREVIDELRKSADEKDEDEDLRRARKEHR DLIEELKRALEKQEEIIKHLQELVYRQL **SEQ ID NO:93**
DHD99	Heterodimer	b	GSEESEEVRKVVERIKKISRELEEVVKELDRVSKEFDRHGETDEIVREHER IVEKLEEIVKKHTKIVEELAEIVYKQQ **SEQ ID NO:94**
DHD100	Heterodimer	a	SDDDSVRVLDEIVKILDESVKLLKESLKLLDDFLRTKPDDHLKEVVKESKK VVEQSKKVLDRIKKIIYESK **SEQ ID NO:95**
DHD100	Heterodimer	b	GSDLLYLSKELLKLVRELLKLSRELVELSRRLVNSTHKSPELVKKYDKLVK KYQDLLKKLADVADEYLRQRS **SEQ ID NO:96**
DHD101	Heterodimer	a	DEKDYHRRLIEHLEDLVRRHEELIKRQKKVVEELERRGLDERLRRVVDRFR RSSERWEEVIERFRQVVDKLRKSVE **SEQ ID NO:97**
DHD101	Heterodimer	b	GSDAYDLDRIVKEHRRLVEEQRELVEELEKLVRRQEDHRVDKKESHEILER LERIIRRSTRILTELEKLTDEFERRTR **SEQ ID NO:98**
DHD102	Heterodimer	a	DERYRAREHIRRVEEHTKRLRHILKRLREHEEKLRRELKPGDEITESVDRF KKIVDQFEESIKKFETVSEELRKSDS **SEQ ID NO:99**
DHD102	Heterodimer	b	GSDRQRILDRLDKILEKLDDILKKLKDILETLSKDDVSDRRHKDLVEKFRE LVDTHHKLVERYRELVYQNR **SEQ ID NO:100**
DHD102_ 1:243	Heterodimer	a	GSDEITESVDRFKKIVDQFEESIKKFETVSEELRKSIS **SEQ ID NO:101**
DHD102_ 1:243	Heterodimer	b	GSDPQRAADRLDKILEKLDDILKKLKDILETLSKDDVKDRRAKDLVEKFRE LVDTHHKLVERYRELVYTATAGSDLARELIRRVEEHTKRLRHILKRLREHE EKLRR **SEQ ID NO:102**
DHD103	Heterodimer	a	NADDQLATSIKKLEDSIDQLIKIVRKFEESVKKLQKHGVDQHHVEILRKIV

			EIFRQHIEKLKKHLEKLRYTSS **SEQ ID NO:103**
DHD103	Heterodimer	b	GSDKEYLVTEHEKLVREHEKIVSEIEKLVKKHEAGVDESELEEILKKVEKL LRKLDEILEQLTQLLRKTE **SEQ ID NO:104**
DHD103_ 1:423	Heterodimer	a	GSDQHVVEILRKIVEIFRQHIEKLKKHLEKLRYTSS **SEQ ID NO:105**
DHD103_ 1:423	Heterodimer	b	GSDAEYLVTEHEKLVREHEKIVSEIEKLVKKHEKGVDESELEEILKKVEKL LRKLDEILEQLTQLLRKAEKHIDKHSKAADQLATSIKKLEDSIDQLIKIVR KFEESVKKLQKH **SEQ ID NO:106**
DHD104	Heterodimer	a	DEDDDIRRVLDESRRVLEHSRRVLKRSEEVLEKASRKKEKDTEEIEKHLKR LREHAKKLEKHRRELDDFLYKEI **SEQ ID NO:107**
DHD104	Heterodimer	b	GSRDKYLLERLNDILKKLDEIVDKLSDILKRLKDVRHDDRLQELVERYKEI VKEYKRIVEEYEKLVREFEEQQR **SEQ ID NO:108**
DHD105	Heterodimer	a	DRDYEDKEFKKIIKELEDVQEELKKLQEKIKRFSSELEEPNELLKEQLKVN EEQLEVNKKILKILRDQLKQNE **SEQ ID NO:109**
DHD105	Heterodimer	b	GSDAEYKVRESVKRSKESVKHSEDVVDKLNKSVKLSESGHSDAEKASRELV KLVREVVELSREVIKLSEKVLRVIS **SEQ ID NO:110**
DHD106	Heterodimer	a	DLQYKQEKLIRHFDRVVREWDKLVRKFSKVLEKQKHESKDKELEEASRRVD ELIKRLREQLKRSKEILRRLKELSRKSS **SEQ ID NO:111**
DHD106	Heterodimer	b	GSDWEELLRRLEKVLQEYEEIVKELIDLIERLIKVSEDKSKDASEYKKLVT ELEKLISKLEEISKKLEELVKEYEYKTE **SEQ ID NO:112**
DHD107	Heterodimer	a	DAKDELEKSLQEIEESLKELKKLLEELDKSLRELTSQGRNKKLEEHIKKVQ KFIELVKKYIKAVQDYLKEVRYDNS **SEQ ID NO:113**
DHD107	Heterodimer	b	GSDKERAARATEEMVKLTKKLLKAVEDLVRDVRRLLKEGLISEKHARIAET ILEVFKKHAKIIKKHVDIVKYDES **SEQ ID NO:114**
DHD108	Heterodimer	a	GSPLKERLLEIQRDLDRVLEEVVERLLRIQERLDSVVERKPPDVHEEYKYI VDEIREIVERVVREYEEIVKRIDEEVR **SEQ ID NO:115**
DHD108	Heterodimer	b	GSEEDERIRYDLDRIRKDVRRKLEEIRQRVRELEKKLRDAGHRRDEKELLR ELIETSKDILRLVEELLKKIIDKSEDLLRKTE **SEQ ID NO:116**
DHD109	Heterodimer	a	GSDEEDYINENVEKDVRDIEDDVRRINERIRELLEKIRTEEVLQRVLEEHH ELVERVLRKLVEILRKHEEENR **SEQ ID NO:117**
DHD109	Heterodimer	b	GSDEEEYYKEKLHKLLREIEELLKHYRELVRRLEELVKRGELDKDTAAHIL ERLSELLERIIRRVAHTLRRLSEERR **SEQ ID NO:118**
DHD110	Heterodimer	a	GSDEDEISYDSKRRVEEIVRQAREKSEKSRKDIEDVAEVLRKGDVSEKEVV DELVKVLEEQVKVLREAVERLREVLKKQVDDVR **SEQ ID NO:119**
DHD110	Heterodimer	b	GSDIVELVDHLLKRSLKLLEELAELVRRLLEKSTELLKRRTEEHKEEVVEE SEYMVRELEERLRRVVDESEKLVRDADKHIR **SEQ ID NO:120**
DHD111	Heterodimer	a	GSKEKDIVKTLVDLLRENLETLERLIEEVVRLLKENVDVRDEGRDDKDSER ILRDIKRRIDEAAKESREIIERIEKEVEYRSR **SEQ ID NO:121**
DHD111	Heterodimer	b	GSPEVDVLRRIVREILKASEELLRLLRKLIDEALKLSERKRDSQEYREVVD RVKKELERLLDEYRKLVEELKEKLRYDTR **SEQ ID NO:122**
DHD112	Heterodimer	a	GSDKRYESEKLKRRLDEAVEKVREVVERVERESDRVLEEVRRRRESKEVVD KVIEDNDKALEDVLRVVDEVAKVVRDVVRENTR **SEQ ID NO:123**
DHD112	Heterodimer	b	GSPREYHSKDILRKVDEILERIRRHADRVKKKSERLKRENVDVNEHSKDVK RVIRELLELVKELLRLAKKHSDDQQE **SEQ ID NO:124**
DHD113	Heterodimer	a	GSDEDEILYHSERLLQKLKKELDDLKEKSRELLEELKKEDPDDRLIERIIR LHDEVLKDLDEVLKNILEVHREVLERLR **SEQ ID NO:125**
DHD113	Heterodimer	b	DKLDRLLKIHEEALRRAEELIKRLLDIHRRALDLARRGELDDYLLKESERE LREIIRRAREELKESRDRLEEISR **SEQ ID NO:126**
DHD114	Heterodimer	a	GSPKEELIRRVLEEVKRLNEKLLEIIRRAAELVKRANDELPETEKLREIDR ELEKKLKEIEDELRRIDKELDDALYEIED **SEQ ID NO:127**
DHD114	Heterodimer	b	GSPKLDKLRELLERNLEKLREILEEVLKILRTNLERVREDIRDEDVLQEYE RLIRKAEEDLRRVLKEYDDLLKKLVYELR **SEQ ID NO:128**
DHD115	Heterodimer	a	GSKEDESVKRAEEIVRTLLKLLEDSLREAERSLRDIKNGEDEHNLRRISEK LEELSKRITETIERLLRELQYTSR **SEQ ID NO:129**
DHD115	Heterodimer	b	GSPNQELLDRVRKILEDLLRLNEELVRLNKELLKRALEMRRKNRDSEEVLE RLAEEYRKRLEEYRRELEKLLEELEETIYRYKR **SEQ ID NO:130**
DHD116	Heterodimer	a	GSDESEEAQHEVEKVLDDIRRLSEHLQKRLEEVLEEVYELRREGSDRTEVV

			ELLKEVIREIVRVNREALERLLRVVEEAVKRNE **SEQ ID NO:131**
DHD116	Heterodimer	b	GSDEEELVETVKRIQKEILDRLTELAKLLVEIQREIKKLKDEGEDDKELKR LSDELEEKVRQVVEEIKRLSDELEETVEYVSR **SEQ ID NO:132**
DHD117	Heterodimer	a	GSDEEEEVVRRAEELVKEHEELIERVIRTHEELVYKLEDQGADKKLVDVLK RVVEESERVAREIVKVSRELIRLLEEASR **SEQ ID NO:133**
DHD117	Heterodimer	b	GSSKEEILKELEDLQRRLIEELKKLQERVVELLEELIKRLRDRGRDDKHLK RLVKEVRRLSEEVLRSIKEVSDRVRYQLR **SEQ ID NO:134**
DHD118	Heterodimer	a	GSDKEEESEYLLRDLVRLLEKVKEKIEEVNREVEKLLKKVKDGRLDRREVL REILRLNRELAEIIKEVVDRIRHVVERSER **SEQ ID NO:135**
DHD118	Heterodimer	b	GSDLHEVVYETKELLKRIEEVVEELRKKSEDIIRKAERGEISEDELKRLQE EIAREAKKLLDEIKRVLERHLEQTL **SEQ ID NO:136**
DHD119	Heterodimer	a	GSPVEEIIKEVVKRVIEVQEKVLRIISHAVKRVVEVQKKYDPGSEESNRVV EEVKKTIEDAIRESDEVVDEVVKRIQYTVR **SEQ ID NO:137**
DHD119	Heterodimer	b	GSPEQEIADRILTEIRESQKELERLARKILKLLDESQEKAKRGRLSEEESD ELLERIKKELDELLERSKELLKKIEYELR **SEQ ID NO:138**
DHD120	Heterodimer	a	GSDEDKEANRVLDEVLKTVRDLLETANEVLKEVLYRLKRTDDQEKVVRTLT EVLKEHLKLVEEIVRILDKVLKEHLETEK **SEQ ID NO:139**
DHD120	Heterodimer	b	GSPEDDVLRRLEEVSEKILRVAEDVARQLREVSEKITQGKVDRKEWEEDIK RLKRELEELLREWKEEIERLTYELR **SEQ ID NO:140**
DHD121	Heterodimer	a	GSRREEVVKRIRELLKRNKELIDRIRELLEENEYLDKDARDKDVLRRSVEL LEELVRILEESVELAKEIIKLLREVVE **SEQ ID NO:141**
DHD121	Heterodimer	b	GSDEKEDNRRLQHKIERILEKNEDLQRKLEEILELLERGEADEEKIDRLRK AVEDYRRVVEEIKEDVKRHKYTVR **SEQ ID NO:142**
DHD122	Heterodimer	a	GSDEKEEAKKASEESVRTVERILEELLKASEESVELLRRGEDAKDVVERSK EALKRVKELLDEVVKRSDEILKYIHN **SEQ ID NO:143**
DHD122	Heterodimer	b	GSDEKKLINEVVETQKRLIKEAAKRLSEVVRHQTELIRELREKNVDDKDVE KLLKESLDLAEEIVRRIKELLDESKKLVEYVSN **SEQ ID NO:144**
DHD123	Heterodimer	a	GSPDMDEVKRVLDELIEIQEEILREIKRVLEKLIKIQEDNGSEYESREVVR EIVEIARKLVERSRRVVKKITETLQ **SEQ ID NO:145**
DHD123	Heterodimer	b	GSDERYATREIVERIERIAREILKRTEEIVREVREVLSRDVDQEEVVRRLA DLLRESVELVQHLVRRVEELLQESVERKK **SEQ ID NO:146**
DHD124	Heterodimer	a	GSPEREALREVLEDLKRVTDRLRELVERVLEELKKVTDHVDSERILRESRR VLKELKDIIEEILRESEKVLEKLKYTED **SEQ ID NO:147**
DHD124	Heterodimer	b	GSPAREILEEVVKKHLEVVEDAARILEEIIREHEKAVREDRDKKELEEISR DLLRKAREALKKVKDISDDLSREIEYVAS **SEQ ID NO:148**
DHD125	Heterodimer	a	GSPVEEAIKKVIDDLRDVQRKIRELVEELIRLLEEVQRDNDKRESEYVVER VEEILRRITETSREVVRKAVEDLS **SEQ ID NO:149**
DHD125	Heterodimer	b	GSDSDEKAEYLLKEMERVVRESDEVVKKILRDLEEVLERLRRGEISEDDVT EILKELAERHIRAIEELVRRLRELLERHKR **SEQ ID NO:150**
DHD126	Heterodimer	a	GSPVEEVLKELSEVNERVRDIAREIIERLSEVNEEVKETDDEDELKKISKK VVDEVEDLLRKILEVSEEVVRRVEYHDR **SEQ ID NO:151**
DHD126	Heterodimer	b	GSPKEDILREVLRRHKEIVREIVRLVREAVETHLELVKRNSDDRDAQDVIR KLEEDLERLVRHAQEVIEEIFYRLH **SEQ ID NO:152**
DHD127	Heterodimer	a	GSPRSYLLKELADLSQHLVRLLERLVRESERVVEVLERGEVDEEELKRLED LHRELEKAVREVRETHREIRERSR **SEQ ID NO:153**
DHD127	Heterodimer	b	GSDREYIIKDILDSQEHLLRLIEELLETQKELLEILKRRPDSVERVRELVR RSKEIADEIRRQSDRNVRLLEEVSK **SEQ ID NO:154**
DHD128	Heterodimer	a	GSDEKDEIRHVIESVERLIEDIKRLLKTLRELAHDDSDKKTVKEVLDRVKE MIERHRRELEEHRKELERAEYEVR **SEQ ID NO:155**
DHD128	Heterodimer	b	GSESEDRIKELLKRHIELVERHEELLHEIKKLIDLEEKDDKDREEAVKRID DAIKESEEMLEESKEILEEIEYLNR **SEQ ID NO:156**
DHD129	Heterodimer	a	GSSLEDSVRLNDEVVKVVERVVRLNQEVVRLIKHATDVEDEETVKYVLERV REVLDESREVLKRVHELLEESERRLE **SEQ ID NO:157**
DHD129	Heterodimer	b	GSHEKDIVYKVEDLVRKSDRIAERAREIVKRSRDIMREIRKDKDNKKLSDD LLKVTRDLQRVVDELEELSRELLRVAEESRK **SEQ ID NO:158**
DHD130	Heterodimer	a	GSPELDEVKKLIDELKKSVERLEESIREVKESIKKLRKGDIDAEENIKLLK ENIKIVRENIKIIKEIIDVVQYVLR **SEQ ID NO:159**

DHD130	Heterodimer	b	GSDEEEIEELLRELEKLLKKSEEALEESKKLIDESEELLRRDRLDKEKHVR ASEEHVKLSEEHLRISREIVKILEKAVYSTR **SEQ ID NO:160**
DHD131	Heterodimer	a	GSDESDRIRKIVEESDEIVKESRKLAERARELIKESEDKRVSEERNERLLE ELLRILDENAELLKRNLELLKEVLYRTR **SEQ ID NO:161**
DHD131	Heterodimer	b	GSDEDDELERLLREYHRVLREYEKLLEELRRLYEEYKRGEVSEEESDRILR EIKEILDKSERLWDLSEEVWRTLLYQAE **SEQ ID NO:162**
DHD132	Heterodimer	a	GSDKKDASRRAIRVLHEFVRVSEEVLEVLRKSVESLKRLDVDEKIKRTHDR IEEELRRWKRELEELIERLREWEYHQD **SEQ ID NO:163**
DHD132	Heterodimer	b	GSDDEEEDKRLLEEVKRSLDTDERILEKLRHSLERQLEDVDKDEDSRRVLR ELDEITKRSREVVKRLRKLAYESK **SEQ ID NO:164**
DHD133	Heterodimer	a	GSDKEYKLDRILRRLDELIKQLSRILEEIERLVDELEREPLDDKEVQDVIE RIVELIDEHLELLKEYIKLLEEYIKTTK **SEQ ID NO:165**
DHD133	Heterodimer	b	GSPSKEYQEKSAERQKELLHEYEKLVRHLRELVEKLQRRELDKEEVLRRLV EILERLKDLHKKIEDAHRKNEEAHKENK **SEQ ID NO:166**
DHD134	Heterodimer	a	GSRDRKISEELIKALEDHIRMLEELIRAIEEHIKLAERGVDEKELRESLEE LKKIVDELEKSLEELRKLAERYKYETR **SEQ ID NO:167**
DHD134	Heterodimer	b	GSPKEESVEELKRVIDKHEEILRELKRVLEEHERVSHDEDENELRRSLERL KHILDRLHESLKELHELLKKNEYTER **SEQ ID NO:168**
DHD135	Heterodimer	a	GSDHEYWVKIVERILRVMEKHAEIVKKHLEIVERVVREGPSEDLRRKLKES LREIEESLRELKELLDELDELSEKTR **SEQ ID NO:169**
DHD135	Heterodimer	b	GSDEEYVTRSQRRLKRLLEEYIKVVEEHARLVERNERDDKELKRSIDELDK LTKELLELVKRYKELVDKTET **SEQ ID NO:170**
DHD136	Heterodimer	a	GSDKEEIVKLQDEVIKTLERHLDILRKHIDLLEKLKDHLSEELKERVDRSI KKLEESIKRLERIIEELQELAEYSL **SEQ ID NO:171**
DHD136	Heterodimer	b	GSREEELKESAEELERSVRELKKEADKYKEEVDRLHYRGKVDKDWVRVVEK LIKLVEEHLELIREHLELLKEERR **SEQ ID NO:172**
DHD137	Heterodimer	a	GSDMEYELKKSAEELRKSLEELKRILDELHKSLRELRRHGDDEEYVQTVEE LRKELEEHAKKLEEHLKELERVAT **SEQ ID NO:173**
DHD137	Heterodimer	b	PEYELKKSVDDLKRDVDRLVEEVEEVFELSKERLREDRKHLELVEEMVRLI EKHLELIKEHLKLADDHVR **SEQ ID NO:174**
DHD138	Heterodimer	a	GSREKDESKELNDEYKKLLEEYERLLRRSEELVKRAKGPRDEKELKRILEE NEDILRRTKEILERTKEISEEQKYRRR **SEQ ID NO:175**
DHD138	Heterodimer	b	GSDKDERQERLNEESDKSNEESERSNRESEELNRRARGPNDEKELQEILDR HLELLERNQRLLDENKEILRESQYLND **SEQ ID NO:176**
DHD139	Heterodimer	a	GSENKYILKEILKLLRENLKLLHDILRLLDENLEELEKHGAKDLDDYRRKI EEIRKKVEDYREKIEEIEKKVERDR **SEQ ID NO:177**
DHD139	Heterodimer	b	GSESEYTQEEILELLKESIKLLREILRLLEESEELWRRENTKSERSEEIKE RAKEAIKRSEEILERVKRLSDHSR **SEQ ID NO:178**
DHD140	Heterodimer	a	GSDEEEANYVSDKAVKIAEDVQELLKELLELSEVVRRGEVDEDEYDRVLRK LQEVMKEYEEVLKEYEEVSRKHE **SEQ ID NO:179**
DHD140	Heterodimer	b	GSPEKYLIKTQEELLRRHAEILEDLIRKVERQVDLRRKVDERDEDLKRELE RSLRELERLVRESSRLVEEIRELSKEIKR **SEQ ID NO:180**
DHD141	Heterodimer	a	GSDEEYELERISRESKELLERYKRLLREYQELLKELRHVKDLDRAVKIIHE LMRVSKELVEISHRLLELHERLVRRRK **SEQ ID NO:181**
DHD141	Heterodimer	b	GSEKEYIEKLSRKIEEDIRRSEERAKDSERLVRRLEELAKRKRLDLDDVLR VAEENLEILEDNLRILEEILKEQDKSNR **SEQ ID NO:182**
DHD142	Heterodimer	a	GSPHEEVVELHERVMEISERAVELIQRIIDIIRRIREDDKDIEKLVKTIRD LVREYEELHRELEEIDEEIYKKSE **SEQ ID NO:183**
DHD142	Heterodimer	b	GSDHEDVVRLHEDLVRKQEDARRVLEEIVRLAEEIVEVIKKDEKDKDRVTR LVEEIEKLVEEYKKKVDEMRKISDEIKYRSR **SEQ ID NO:184**
DHD143	Heterodimer	a	GSRAREVVKRAKRIIEEWQKILEEWRRILEEWRRLLEDERVDDRDNERIIR ENERVIRENEKIIRDVIRLLEELLYERR **SEQ ID NO:185**
DHD143	Heterodimer	b	GSREDEELEEEIDRIRQMVEEYEELVKEYEELTEKYKQGKVDKEESKKIIE KSERLLDLSQDAVRKVKEIIRRILYTNR **SEQ ID NO:186**
DHD144	Heterodimer	a	GSPKEEIVKLHDESAELHRRSVEVADEILKMHERSKDVDDERESRELSKEI ERLIREVEEVSKRIKRLSEEVEYLVR **SEQ ID NO:187**
DHD144	Heterodimer	b	GSPLEEILKIQRRINKIQDDINKILHEILRMQEKLNRSSDKDEVEESLRRI RELIKRIKDLSKEIEDLSREVKYRTT **SEQ ID NO:188**

DHD145	Heterodimer	a	GSPEDEHVYVVREIYEVLREHAEVLEENREVIERLLEAKKRGDKSEELVKE LKKSIDKLKEISRKLEEIVKELEKVSEKLK **SEQ ID NO:189**
DHD145	Heterodimer	b	GSDEDETSYRILELLREIVRASRELIRLSEELLEVARRDDKDETVLETLIR EYKELLDRYRRLIEELTRLVEEYEERSR **SEQ ID NO:190**
DHD146	Heterodimer	a	GSTQEEINRIQHEVLRIQEEIDEILRDIVEKLKAISRGELDHEVVKDVEDK VREALEKSEELLDKSRKVEYKSE **SEQ ID NO:191**
DHD146	Heterodimer	b	GSDEEELNRELLEKSKRLVDINRDIIRTAQELIEMLKDSKDGRVDEDTKRE LRDKLRKLEEKLERVREELRKYEELLRYVQR **SEQ ID NO:192**
DHD147	Heterodimer	a	GSDEKDRVYEILKEVQRLVKEYRDISKEIEDLVKHYEHITDDEAQEVSKEL IDKSLRASEIVRELIRLIKELLDELE **SEQ ID NO:193**
DHD147	Heterodimer	b	GSDEEDVLYHLRELLEELKRVSDDYERLVREIKETSERKDRDTKENKDMLD ELVKAHREQEKLLERLVRLLEELFERKR **SEQ ID NO:194**
DHD1	Heterodimer	a	PREQAIRISEEIIRISKKIIEILERTRSSTAREAMKWAKDSIRLAEESKYL LDK **SEQ ID NO:195**
DHD1	Heterodimer	b	IEDDVKKIQDSTKKAQKETIEALERSTSSTARKQMEEQKEQIRLQKEAMYL LKK **SEQ ID NO:196**
DHD2	Heterodimer	a	SREEIAKLQEEVIKLQRRVIELQKEVIELQRRAKELTSSYTKEILEIQRRI EEIQREIEEIQKRIEEIQEEIQRRT **SEQ ID NO:197**
DHD2	Heterodimer	b	SDEEIKRLSEEVIQLSRRVIKMSREAIKLSREVQKLTPSYQKRIKEIADRS IELARESIEIAKRSEKIAEESQRRT **SEQ ID NO:198**
DHD3	Heterodimer	a	PAKDEALKMANESLELAKKSARLIQESSSKEILERIEKIQRRIAELQDRIA YLIKK **SEQ ID NO:199**
DHD3	Heterodimer	b	PAKDEALRMIDESRELIKKSNELIQRSSSKEILERILEIQRKIAELQKRIQ YLLKS **SEQ ID NO:200**
DHD4	Heterodimer	a	TDEARYRSERIVKEAKRLLDEARRRSEKIVREAKQRSNSEDAKRIMEENLR ESEEAARRLREIIRRNLEESRETG **SEQ ID NO:201**
DHD4	Heterodimer	b	TREALEYQRKMAEEIEDLLREALRRQEEMVREAKQRSLSEEFKRIMERILE EQERVMRLAKEALERILEEQKRTG **SEQ ID NO:202**
DHD5	Heterodimer	a	SERTKREAKRSQEEILREAKEAMRRAKESQDHRQNRDGSNSEDLERLSQEQ KRELEEVERRLKELAREQKYKLEDS **SEQ ID NO:203**
DHD5	Heterodimer	b	SEDLKRILKEITERELKLMQDLMEILKKITEDENNLDSNNSEDLKRSIEKA RRILDEALRKLEESARRAKYIQEDN **SEQ ID NO:204**
DHD6	Heterodimer	a	TEDEIRESLKWLDEVLQELREIARESNEVLERNRQKSRSDKLREDIERYKK RMEEARKKLDDQLNKYKKRMDENRS **SEQ ID NO:205**
DHD6	Heterodimer	b	TEEELKESKKFAEDLARSARRALKESKRVLEEISQASRSKKLEEIVRRYKE QVKRWQDEWDERAREYRKRMKENRS **SEQ ID NO:206**
DHD7	Heterodimer	a	TKTEEIERLAREIKKLSEKVERLAQEIEELSRRVKEENSTDRELKEANREI ERAIREIEKANKRMEEALRRMKYNG **SEQ ID NO:207**
DHD7	Heterodimer	b	TKTEEHERLAREISKLADEHRKLAKIIEELARRIKEENLTDDELREAIRKI EDALRKNKEALKIMKEAAERNRYNT **SEQ ID NO:208**
DHD8	Heterodimer	a	TKKEESRELARESEELARESEKLARKSLELARRAESSGSEEEKRRIIDENR KIIERNREIIERNKEIIEYNKELIS **SEQ ID NO:209**
DHD8	Heterodimer	b	TKDEESLELNRESEELNRKSEELNRKSKELNDRAESSNSEEEEKEILREHK EILREHLEILRRHKEILRRHKYLTS **SEQ ID NO:210**
DHD16	Heterodimer	a	TREELLRENIELAKEHIEIMREILELLQKMEELLERQSSEDILEELRKIIE RIRELLDRSRKIHERSEEIAYKEE **SEQ ID NO:211**
DHD16	Heterodimer	b	SEDIAREIKELLRRLKEIIERNQRIAKEHEYIARERKKLDPSNEKERKLLE RSRRLQEESKRLLDEMAEIMRRIKKLLD **SEQ ID NO:212**
DHD18	Heterodimer	a	DRQKLIEENIKLLDKHIKILEEILRLLKKDIDLLKKSSSEEVLEELKKIHR RIDKLLDESKKIHKRSSEIVKKRS **SEQ ID NO:213**
DHD18	Heterodimer	b	DEQKLIETSQRLQEKSERLLEKFEQILREASDLYRKPDSEELLRRVEKLLR ELEKLIRENQDLARKHEKILRDQS **SEQ ID NO:214**
DHD19	Heterodimer	a	DRQELIRENIELLKKHIKIVKEIQKLIETFIELLKKSSSEEILRRLKKILK RIEKLYRESQEIHKRSEEIAKKRQ **SEQ ID NO:215**
DHD19	Heterodimer	b	DEERLIDKSRELQKESEELLKELLKIFKRIEELLEKPDSEELIREIKKLLE TLSEIHKRNEKLARTHEEILRQQS **SEQ ID NO:216**
DHD22	Heterodimer	a	STRDVQREIAKAFKKMADVQKKLAEEIKRHVKNVEKKNKDNDEYRKIATEL LKKATESQKKLKELLDRIRKSDS **SEQ ID NO:217**

DHD22	Heterodimer	b	DKDDRSTSLLKRVEKLIDESDRIIDKFTTLIELSRNGKIDDDQYKKELKEI LELLKKYDKHVKEVEELLKRLNS **SEQ ID NO:218**
DHD23	Heterodimer	a	SKRKALEVSERVVRISEKVVRVLDESSDLLKKSYDDSDKFAELIDRHEEKI KKWKKLIKEWLEIIQRHKS **SEQ ID NO:219**
DHD23	Heterodimer	b	SAEEFVKLSEEAVKRSKEILDIVRKQVKLVKAGVDKHEITDSLRKSEKLIE EHKELIKTHRDLLRREN **SEQ ID NO:220**
DHD24	Heterodimer	a	SSTEILKRFKRALRESEKIVKHSRRVLKIIREVLKQKPTQAVHDLVRIIET QVKALEEQLKVLKRIVEALERQS **SEQ ID NO:221**
DHD24	Heterodimer	b	DKQKEIKDILEKTRRIAEESRKIAEKFDEIIKRSTEGKIDESLTKELEELV KEVIKLSEDDARTSDDLVRKES **SEQ ID NO:222**
DHD26	Heterodimer	a	DEDESIKLTRKSIEETRKSLKIIKEVVELIREVLKHIKDLDKEIFERIDKI LDKYKKQVDTYDEILKEYEKKQR **SEQ ID NO:223**
DHD26	Heterodimer	b	SELDEQKELIKKQEKLIEEQQRLLSKIRRMFKERVKDQELLREIQKVLKRS QEIVETSKKILDRSDKTTE **SEQ ID NO:224**
DHD28	Heterodimer	a	DQKEINTRIVEKLERIFKKSKEIVRQSERVISTIEKKTEDERELDLLRRHV KIVREHLKLLEELLKIIKEVQKESE **SEQ ID NO:225**
DHD28	Heterodimer	b	DTEELVKRLNELLKELSKLVKEFIKILETYRKDQTKDTSKISERVDRILKT YEDLLQKYKEILEKIEKQLS **SEQ ID NO:226**
DHD29	Heterodimer	a	DYARLIDQAVEVTRKVVEVNVTVARVNDKFAKHLGDEELRRVSEHLKEVSK DLQEVAKKSKDAARQVK **SEQ ID NO:227**
DHD29	Heterodimer	b	DVSKVAEEYLQISKTLVDISRTLLEISERLVRLVRTVADDRSEVKKAIEDS IEVLKTSEEVVRQIKRASDKLVKAIS **SEQ ID NO:228**
DHD31	Heterodimer	a	DAKEIQRRVVEIQTEVVKLQKKAVDIIRKIIEAFNNSNIDQSLLEAAKEIV KEIDKLEKLTESLLEESKKLLKRSS **SEQ ID NO:229**
DHD31	Heterodimer	b	SAEEVVKLAKIFLELLRESIKLLKRSVDLLRKSSDPSLDKSEAEKVSREIE KVSDTSLKLSKKALDVVKRALKVAS **SEQ ID NO:230**
DHD32	Heterodimer	a	DEKDAARKARKVSEEAKEASKKIEKALEESKRILNTLKQKKDEQEVKVIKE HEDVLRQIEKIQKQVLEIQKEVAKLLESLD **SEQ ID NO:231**
DHD32	Heterodimer	b	SADDVARASEKVLRVARESAKAADKSLEVFKEVVKRGDKEAFLQVVKINEE VVKINITVIRILIEVSKTAT **SEQ ID NO:232**
DHD38	Heterodimer	a	DEYVKETLKQLREALASLREADKRITELVKEARKKPLSEAARKFAEAIVTH VKVVVEHVEVVLRHVEVLVEAKKNGVIDKSILDNALRIIENVIRLLSNVIR VVDEVLQDLD **SEQ ID NO:233**
DHD38	Heterodimer	b	DASDVIRRIHELFEEVHRLIEAVHRAIEDVAKAAQKKGLDESAVEILAELS KELAKLSRRLAEISREIQKVVTDPDDKEAVERLKEIIKEIKKQLDELRDRL RKLQDLLYKLK **SEQ ID NO:234**
DHD60	Heterodimer	a	SEDKAHHDIVRVLEELIKIHDELMKISEEILKATSDSTATDETKEELKRRS KEAQKKSDTLVKIVKELEKESRKAQS **SEQ ID NO:235**
DHD60	Heterodimer	b	DDEEKYRQIIREAQEISKTAKRILRDAQEISKRIRHQGVDRSEHQRLVDLL RELIKEHHKLLRRQQEADTRND **SEQ ID NO:236**
DHD63	Heterodimer	a	DRKDKARKASEKLEEVIQRWKTVADKWKKMVDLVSNGKLSQEEVARVTEEL LKIQTELAKLLEEHAKVLQESAS **SEQ ID NO:237**
DHD63	Heterodimer	b	SDEESIKTQSELIKTSEELLKDVKRIDEELQKLRDDPTLDESELKKRVKEW SDRVRKAKEISRKIQEIVKESKKRSS **SEQ ID NO:238**
DHD66	Heterodimer	a	DKDEELRKVIEKYREMVKEYRKVIREYEEVIKSSKTIDKSSLISLSRKMVE LSQRVIDVSDEVAKVLSRKQS **SEQ ID NO:239**
DHD66	Heterodimer	b	TDEERLKKQTKELKEQTKQLEKQKDLLEKISNGEISKDEIQEIIKESKKIA KESQKALDSSRKALEEVS **SEQ ID NO:240**
DHD67	Heterodimer	a	DEKEVSKEIIKVLKDIAKVQQKVIEVSQRLASVLRADDDNVVKRALEEYEK ILEELRELNKEIEKLTDKYRKVTS **SEQ ID NO:241**
DHD67	Heterodimer	b	DSDEQTKELEKLTELHKRHVEKLKKQTKESREVDSNKLWKSKDVKDKLSES EKELQKLSDQDKKAKDALESSRRKND **SEQ ID NO:242**
DHD69	Heterodimer	a	DAEEQLKLLTKLLRHQQRLLQLIKESLKLIEKIDQSSQENQDEIRKWREVT KKLRELIKTSEKLVRELEKSYKKSS **SEQ ID NO:243**
DHD69	Heterodimer	b	SLRDVVRRYQELVRRYDELIKTLTEILKKYQKKGAEDKDASTELVKAVRTS LKLSKELLKLNSELLKEDS **SEQ ID NO:244**
DHD71	Heterodimer	a	SKEELKRKLDELKKRSDTLKELSKKLKEISERNPDDKSVHRTIIRIHREFV KNHKEIVRVIEEIVSDKS **SEQ ID NO:245**

DHD71	Heterodimer	b	SKQDEHDRLLKIHDKLVKQHDELLKLLTKLSRAGDSVTKKKLEEILRKLQE VSKQLEESLKDADKVSKDIN **SEQ ID NO:246**
DHD72	Heterodimer	a	TVQSLLEQHVKIVKRSIEILERHTQILQDIARSQGVSKELEDVERQVKEYR KEVKKLEEDLRQLSRNSK **SEQ ID NO:247**
DHD72	Heterodimer	b	SDSDRIEKLIRESTELLKEQQKLAKRSRELAETVESLPLTEEYLKQQREHQ KKIEKLLKDSEKHLEELKRLVKSEK **SEQ ID NO:248**
DHD73	Heterodimer	a	DSEKRIEDILRTDLELAKRDAELVKEHIKLVKRIDLSEELKKQVEDVEKES KKLEDSSEKLVQKVRKRSS **SEQ ID NO:249**
DHD73	Heterodimer	b	DEEERAKDLRKYLEEQTQYYRTVTEHLRNLEKVVEELERRGKPSSELQQIL ERSQRIYKETTEIYDTSKKLIEELDKHHR **SEQ ID NO:250**
DHD148	Heterodimer	a	PLEDILKRHLDKVRELVRLSEEVNKLAKEVLDILKDKRVDEKELDKVLKEL EKVVEEYERAVKESRDLLRELRETTR **SEQ ID NO:251**
DHD148	Heterodimer	b	DKERLLEIHERIQKLLDRNLEIIERLLRLLREARDIKDDDKLDKVIKRLKE LSEESKDILDKIKELLKESEKELT **SEQ ID NO:252**
DHD149	Heterodimer	a	PEDEVIRVIEELLRIAAEVDEVHRRNVEVQEEASRVTDRERLERLNRESEE LIKRSRELIEEQRKLIERLERLAT **SEQ ID NO:253**
DHD149	Heterodimer	b	DLEELIKEYAEVVRRHHKAVRDLERLVRELANAKHASEEELKRIATEILRI VKELIRVQERLIKLSEDSNEESR **SEQ ID NO:254**
DHD150	Heterodimer	a	PTDEVIEVLKELLRIHRENLRVNEEIVEVNERASRVTDREELERLLRRSNE LIKRSRELNEESKKLIEKLERLAT **SEQ ID NO:255**
DHD150	Heterodimer	b	DNEEIIKEARRVVEEYKKAVDRLEELVRRAENAKHASEKELKDIVREILRI SKELNKVSERLIELWERSQERAR **SEQ ID NO:256**
DHD151	Heterodimer	a	PKEDIDRVSRELVRVHKELLEVLRKSTEIVEAVARNEKDERTIEEVLEEQE RAVRKLEEVSKKHKEAVKRLK **SEQ ID NO:257**
DHD151	Heterodimer	b	ELERLSEEIQKLSDRLIELIRRHSKVLEEIVRLLKHKDNDEREVRRLLKLL RDLTRRYEEVLRKVEEIVKRQEDESR **SEQ ID NO:258**
DHD152	Heterodimer	a	PEEDILRLLRKLVEVDKELLEVVRESTEVVRLVARNEKDVETVERVLRKQE EVVRKYERVSRELEEAVRRLK **SEQ ID NO:259**
DHD152	Heterodimer	b	ELKDLVEEIVKLSKENLKLWEDHSRVLEEIVRLLKHKDNDEREVRRLLKLL EDLTRRAEETSRRIEEIVKEAEDRAR **SEQ ID NO:260**
DHD153	Heterodimer	a	DEERELREVLRKHHRVVREWTKVVEELKRVVELLKRGETSEEDLLRVLKKL LEMDKRILEVNREVLRVLEKRLT **SEQ ID NO:261**
DHD153	Heterodimer	b	SLEEIIEELVELVRRSVEIAKESDEVARRIVESEDKKKELIDTLRDLHREW QEVTKRAEELVREAEKEVR **SEQ ID NO:262**
DHD154	Heterodimer	a	TAEELLEVHKKSDRVTKEHLRVSEEILKVVEVLTRGEVSSEVLKRVLRKLE ELTDKLRRVTEEQRRVVEKLN **SEQ ID NO:263**
DHD154	Heterodimer	b	DLEDLLRRLRRLVDEQRRLVEELERVSRRLEKAVRDNEDERELARLSREHS DIQDKHDKLAREILEVLKRLLERTE **SEQ ID NO:264**
DHD155	Heterodimer	a	PEDDVVRIIKEDLESNREVLREQKEIHRILELVTRGEVSEEAIDRVLKRQE DLLKKQKESTDKARKVVEERR **SEQ ID NO:265**
DHD155	Heterodimer	b	DEVRLITEWLKLSEESTRLLKELVELTRLLRNNVPNVEEILREHERISREL ERLSRRLKDLADKLERTRR **SEQ ID NO:266**
DHD156	Heterodimer	a	DEDEVVKVHEEHVKSHEEIHRSHEEVVRAAEEDKRDSRELRTLMEEHRKLL EENEKSIEEVKKIHERVKR **SEQ ID NO:267**
DHD156	Heterodimer	b	KKEELIDISKEVLDLDDEINKISKEILELIKKLLRLKEEGREDKDKAREVK RRIRELHRRIQELNKRLRELHKRVQETKR **SEQ ID NO:268**
DHD157	Heterodimer	a	PEEDIARRVEDLLRKSEELIKESEKILKESKRLLDRNDSDKRVLETNLRLI DKHTKLLERNLELLEELLKLAEDVAK **SEQ ID NO:269**
DHD157	Heterodimer	b	RFKDLSREYIEVVKRLLELSREALEVLREIKDTDKTDKKRIKELIDRLRKL IEEYKRIIDRLRKLSKDLEEEHR **SEQ ID NO:270**
DHD158	Heterodimer	a	DEEELVKILKELQRLSEESLEINKRLVEILRLLRRGEVPKEEVEKKLREIK KEQEKLDREHEKIKKRIEEITK **SEQ ID NO:271**
DHD158	Heterodimer	b	SLKEKILEIIERNMKLVELSNRSVEIVARILKGEKDDEETLERLLREWDKI TRDYEEIIKESRKLVKELEEEAK **SEQ ID NO:272**
DHD159	Heterodimer	a	SKTEILRKALEIHKEQIDIVRKLIELSEEVLKLVEESKEKNLEKLKRIDEE TDRLLERLDELHKRLTELAERLK **SEQ ID NO:273**
DHD159	Heterodimer	b	SDDEARKQLEEMKRRLREVEKKSKRVEERVRELERLVRENREDEDRVLKTL EDLLRENEKLVRTIERHVREQRELSKEVK **SEQ ID NO:274**

DHD160	Heterodimer	a	SEEELEKKADELRKLSEEWRKLQEEDKRLSEMVEKGELDLQEVDEHSLRVL ERATEVHRTVDKVIEEILRTTN **SEQ ID NO:275**
DHD160	Heterodimer	b	SEKERHRESQETQEEIRRTHEEIIRKLEEILRRAKAGELPEETLDRLRRIM ERLKELSERLDDLVRKLRDDHRREQK **SEQ ID NO:276**
DHD161	Heterodimer	a	SEKEILEELKRILKRVKDISDRLEELDKRTEEIARREPTKELVDELVKIHR DWLRLHEEILKLVDDALKKVEDATK **SEQ ID NO:277**
DHD161	Heterodimer	b	DLRELLELQREASRLHRELVKLLTELVKKLELIAKGEDIREEDLKRIKERL EEIKKRSKRIKEESDEIDKKTK **SEQ ID NO:278**
DHD162	Heterodimer	a	SERELQRELNKIVRRILEIHREVSELHQRAVKLIRENDNSEELEEISRRIE ELSKELEKLVREHDEIVKTIE **SEQ ID NO:279**
DHD162	Heterodimer	b	SEREKLDRNDEELKEINKRVEEIKERSDRITEAIEKNERSEEEIRRLSREQ NEALQRLLELHKKLVKLHRELLEDTR **SEQ ID NO:280**
DHD163	Heterodimer	a	DKEDVIRVHDEQHKLIEEQLELTRRIAELVREIAKNTASEEEIKEMLKEIK RLDDRSREIQDRLQKLLEEIRRKTK **SEQ ID NO:281**
DHD163	Heterodimer	b	TEEEIVELNKDIQRKSKEHIDLQNELVKKIERAIRENNITEELLEELERLL RESEKIVEEIRRITDKIRKDAK **SEQ ID NO:282**
DHD164	Heterodimer	a	SEKEILERLLRLSKEQNEISEEIHRLTERLVELKRRKDDDERLKRILDRQK RLVERAREISKEYEDLLRKLE **SEQ ID NO:283**
DHD164	Heterodimer	b	SMEELLRKNARLSRKQLKIIDEHLELSTKLTRGEAGDETLEEIERRSREML EEQRRVDEESKRIREKLK **SEQ ID NO:284**
DHD165	Heterodimer	a	SEEEIRDIVEKLLRTHEEVLKEIKKLLDDSERVRRRELDKKDLDRIQKEQR DIQEENKEKAKRFDELVKELKKAAK **SEQ ID NO:285**
DHD165	Heterodimer	b	SEEEHRRTMEKVEKEVRDIKRRSEEVKKKVKANTLSEEDLVRLLERLVEDH KRLQDLSQEIIERDEKATK **SEQ ID NO:286**
DHD166	Heterodimer	a	DEDELAKEIEDVQRRNKESQEEHDKSVKKLEAAERGEIDEDSLLRVLEEDI KVLEKDIEVLERSIEVIEKAE **SEQ ID NO:287**
DHD166	Heterodimer	b	SEKELIRRLLEQQRQHLRLSERLIELSRRLVEVVRKGKDNRDLLRELKKLS EEHKKHSKDDHEKVREIREREK **SEQ ID NO:288**
DHS 17	Heterodimer	a	DRKDLLKRNIKLLDRHLKILDTILKLLEKLSELLKKSSSEEVVKEYKKILD EIRKLLEESKEIHKESKEILERES **SEQ ID NO:289**
DHD17	Heterodimer	b	DEEKLIERSKRLQEESEQLLEKFEQILRELTELLEKPDSEELARKIKKLHD ELRKIIKRNQELIREHEEILRKRD **SEQ ID NO:290**

In one aspect, the monomer A polypeptide comprises a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an odd-numbered SEQ ID NO selected from the group consisting of selected from the group SEQ ID NOS: 1-290; wherein GlySer at amino acids 1 and 2 of SEQ ID NO: 1, 55, 81, 83, 101, 105, 115, 117, 119, 121, 123, 125, 127, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, or 193 are optional, e.g., GlySer at amino acids 1 and 2 of SEQ ID NO: 1, 55, 81, 83, 101, 105, 115, 117, 119, 121, 123, 125, 127, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, or 193 are not present, and

wherein the odd-numbered SEQ ID NO ("chain a") is the binding partner of the SEQ ID NO. ("chain b") in Tables 1A.

In another aspect, the monomer B polypeptide comprises a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an even-numbered SEQ ID NO selected from the group consisting of selected from the group SEQ ID NOS: 1-290, wherein GlySer at amino acids 1 and 2 of SEQ ID NO: 6, 8, 14, 16, 26, 30, 32, 34, 36, 38, 40, 42, 46, 48, 54, 56, 58, 60, 62, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 176, 178, 180, 182, 184, 186, 188, 190, 192, or 194 are optional, e.g., GlySer at amino acids 1 and 2 of SEQ ID NO: 6, 8, 14, 16, 26, 30, 32, 34, 36, 38, 40, 42, 46, 48, 54, 56, 58, 60, 62, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 176, 178, 180, 182, 184, 186, 188, 190, 192, or 194 are not present, wherein the even-numbered SEQ ID NO ("chain b") is the binding partner of the SEQ ID NO. ("chain a") in Table 1A.

In another embodiment of any of the above embodiments,

(i) monomer A comprises a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an odd-numbered SEQ ID NO selected from the group consisting of selected from the group SEQ ID NOS: 1-290, 331, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494; and

(ii) monomer B comprises a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an even-numbered SEQ ID NO selected from the group consisting of selected from the group SEQ ID NOS: 1-290, 331, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484,

486, 488, 490, 493, and 494, wherein the even-numbered SEQ ID NO is the binding partner of the odd-numbered SEQ ID NO. in step (i).

The amino acid sequences of SEQ ID NOS:1-290, 331, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494 are provided in Table 1B. The "binding partners" have similar design names as shown in Table 1B. For example, SEQ ID NO:1 (DHD9 A) and SEQ ID NO:2 (DHD9 B) are binding partners, and For example, SEQ ID NO:331 (DHD9 A) and SEQ ID NO:2 (DHD9 B) are binding partners, so that if monomer A comprises the polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:331, then monomer B comprises the polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of SEQ ID NO:2. Similarly, SEQ ID NOS:3-4 are binding partners, SEQ ID NO:5-6 and 5-332 are binding partners, etc. Those of skill in the art will clearly understand what is meant by binding partner based on the teachings herein.

Table 1B

Design name	Oligomerization State	Chain	Design sequence
DHD9	Heterodimer	a	GSPKEEARELIRKQKELIKEQKKLIKEAKQKSDSRDAERIWKRSREINRES KKINKRIKELIKS **SEQ ID NO:1** PKEEARELIRKQKELIKEQKKLIKEAKQKSDSRDAERIWKRSREINRESKK INKRIKELIKS **SEQ ID NO:331**
DHD9	Heterodimer	b	PKKEAEELAEESEELHDRSEKLHERAEQSSNSEEARKILEDIERISERIEE ISDRIERLLRS **SEQ ID NO:2**
DHD13_X AAA	Heterodimer	a	GTKEDILERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKL LEESLRLLKELLELSEESAQLLYEQR **SEQ ID NO:3**
DHD13_X AAA	Heterodimer	b	GTEKRLLEEAERAHREQKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKKI LEEIRELSKRSLELLREILYLSQEQKGSLVPR **SEQ ID NO:4**
DHD13_X AXA	Heterodimer	a	TKEDILERQRKIIERAQEIIRRQQEILEELERIIRKPGSSEEAMKRMLKLL EESLRLLKELLELLEESAQLLYEQR **SEQ ID NO:5**
DHD13_X AXA	Heterodimer	b	GSTEKRLLEEAERAHREAKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKK ILEEIRELSKRLLELLREILYLSQEQK **SEQ ID NO:6** TEKRLLEEAERAHREAKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKKIL EEIRELSKRLLELLREILYLSQEQK **SEQ ID NO:332**
DHD13_X AAX	Heterodimer	a	TKEDILERARKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKLL EESLRLLKELLELSEELAQLLYEQR **SEQ ID NO:7**
DHD13_X AAX	Heterodimer	b	GSTEKRLLEEAERAIREQKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKK ILEEIRELSKRSLELLREILYLLQEQK **SEQ ID NO:8** TEKRLLEEAERAIREQKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKKIL EEIRELSKRSLELLREILYLLQEQK **SEQ ID NO: 334**
DHD13_2	Heterodimer	a	TKEDILERQRKIIERAQEIHRRQQEILEELEYIIR **SEQ ID NO:9**

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DHD13_2:341	Heterodimer	b	MSEEAMKRMLKLLEESLRLLKELLELSEESAQLLYEQRKANNGSETEKRLLEEAERAHREQKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKKILEEIRELSKRSLELLREILYLSQEQK **SEQ ID NO:10**
DHD13_AAAA	Heterodimer	a	MTKEDILERQRKIIERAQEIHRRQQEILKEQEKIIRKPGSSEEAMKRSLKLIEESLRLLKELLELSEESAQLLYEQR **SEQ ID NO:11**
DHD13_AAAA	Heterodimer	b	GTEKRLLEEAERAHREQKEIIKKAQELHKELTKIHQQSGSSEEAKKRALKISQEIRELSKRSLELLREILYLSQEQK **SEQ ID NO:12**
DHD13_BAAA	Heterodimer	a	TKEDILERQRKIIERAQEIHRRQQEILKRSEEIIRKPGSSEEALETLRELQEESLRLLKELLELSEESAQLLYEQR **SEQ ID NO:13**
DHD13_BAAA	Heterodimer	b	GSTEKRLLEEAERAHREQKEIIKKAQELHRRTEEIIRQSGSSEEAKDELRRIQEEIRELSKRSLELLREILYLSQEQK **SEQ ID NO:14** TEKRLLEEAERAHREQKEIIKKAQELHRRTEEIIRQSGSSEEAKDELRRIQEEIRELSKRSLELLREILYLSQEQK **SEQ ID NO:336**
DHD13_4:123	Heterodimer	a	TTKRYLEEAERAHREQKEIIKKAQELHRRLEEIVRQ **SEQ ID NO:15**
DHD13_4:123	Heterodimer	b	GSSEEAKKEAKKILEEIRELSKRSLELLREILYLSQQVNDVDEKALERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKLLEESLRLLKELLELSEESAQLLYEAR **SEQ ID NO:16** SEEAKKEAKKILEEIRELSKRSLELLREILYLSQQVNDVDEKALERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKLLEESLRLLKELLELSEESAQLLYEAR **SEQ ID NO:338**
DHD13_1:234	Heterodimer	a	EAMKRMLKLLEESLRLLKELLELSEESAQLLYEAR **SEQ ID NO:17**
DHD13_1:234	Heterodimer	b	TTKRYLEEAERAHREQKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKKILEEIRELSKRSLELLREILYLSQQVNDVDEKALERQRKIIERAQEIHRRQQEILEELERIIRKPGS **SEQ ID NO:18**
DHD15	Heterodimer	a	TREELLRENIELAKEHIEIMREILELLQKMEELLEKARGADEDVAKTIKELLRRLKEIIERNQRIAKEHEYIARERS **SEQ ID NO:19**
DHD15	Heterodimer	b	GTERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLKKARGADEKVLDELRKIIERIRELLDRSRKIHERSEEIAYKEE **SEQ ID NO:20**
DHD20	Heterodimer	a	GDRQELIRRNIELLKEHIKILEEISQLIEELSELLDKSSSEEVVKRYKKILERYKQLLRKSQEIHKESSEIAKKES **SEQ ID NO:21**
DHD20	Heterodimer	b	GDEQKLIERSQRMQKESLELLKEIIKILDTIEKLLDKPDSEELLDTIKKLHDTLKKIHDRNKKLLKEHEEILRQRSGSLVPR **SEQ ID NO:22**
DHD21	Heterodimer	a	DKEEEYKRLLDEIKEILKESKEVLKDSKRVLEDIKRKVPDDDLVKLLEKHVRLLEEHVKLLEQLIREAEKSSK **SEQ ID NO:23**
DHD21	Heterodimer	b	QGSSAEELLKKIKESEKKIRDSLRKIKEIIKKSRKEGVDDKQLDLIRKVVESHRDLLRLHRDLLRLLREETS **SEQ ID NO:24**
DHD25	Heterodimer	a	DIDESIKEVEKLLEEVEQSLQKLDDSLKKLLEKVNQDPDVDDSVRKIVKRHVEILKRHEEVLKRLIEVVKEHTKTVK **SEQ ID NO:25**
DHD25	Heterodimer	b	GSDREEVHKEIVKLIREIIKIHKKILKIHEKIKNGEIDPSEILKLSEEIKKLTDTIIKIIEDLEQLTRDLRR **SEQ ID NO:26** DREEVHKEIVKLIREIIKIHKKILKIHEKIKNGEIDPSEILKLSEEIKKLTDTIIKIIEDLEQLTRDLRR **SEQ ID NO: 340**
DHD27	Heterodimer	a	DRKEIVKRHQKVVELLKESSKLLRESSKLLQRLLDKTGDENLQKAVDDQDKAIKRQETAIRKSQEASKKLD **SEQ ID NO:27**
DHD27	Heterodimer	b	DNSEEIKKVAKTSREVAEYSERVAKENDKVVKTLEEGKIDESELLRLLEESIKIFDTALKLHEEAYKLHQDLVRKVS **SEQ ID NO:28**
DHD30	Heterodimer	a	DESEAASVAIESVQILVESVKLLEESVRILLDAVKKNGVEDLLRVAQRWEKLVDEWLKVVKRWLDNVRDIQR **SEQ ID NO:29**
DHD30	Heterodimer	b	GSDKAEEVEKSVRKIEESIKKIRKSIKKAEDAVQLLKEGKIDAKDFLRIVREDLEVVKEDVEIVKEDVENVREFSS **SEQ ID NO:30** DKAEEVEKSVRKIEESIKKIRKSIKKAEDAVQLLKEGKIDAKDFLRIVREDLEVVKEDVEIVKEDVENVREFSS **SEQ ID NO: 342**
DHD33	Heterodimer	a	SDKEVSDKLLKASKKLLKVSEELLEVVRRLLKALKDDELIKKIADLLRKIIDKDKKFIRTSEEIVKESR **SEQ ID NO:31**

DHD33	Heterodimer	b	GSDLKEVLKTVEEAVKEIIKSSEELLQISRKILEISRVGVDEHEYISAIRE YLKALEKHIQILKKFIEILKELIRAVS **SEQ ID NO:32** DLKEVLKTVEEAVKEIIKSSEELLQISRKILEISRVGVDEHEYISAIREYL KALEKHIQILKKFIEILKELIRAVS **SEQ ID NO:344**
DHD34_X AAXA	Heterodimer	a	SKEEIDKIVKKHKKKIEEHKKKVDELKKLVEEHDKRVSQDKDDKVKKLSEE VKKIIKRLEEVSKRLEEVSKKLLKVISDKR **SEQ ID NO:33**
DHD34_X AAXA	Heterodimer	b	GSNDEELKKILETLDRILKKLDKILTRLIEVLKKSEDPNLDDKDYTELVKQ FIELIKKYEEVVKEYEEVVRQLIRLFS **SEQ ID NO:34** NDEELKKILETLDRILKKLDKILTRLIEVLKKSEDPNLDDKDYTELVKQFI ELIKKYEEVVKEYEEVVRQLIRLFS **SEQ ID NO: 346**
DHD34_X AXXA	Heterodimer	a	SKEEIDKIVKKHKKKIEELKKLVDELKKLVEEHDKRVSQDKDDKVKKLSEE VKKIIKRVEEVAKRLEEVSKKLLKVISDKR **SEQ ID NO:35**
DHD34_X AXXA	Heterodimer	b	GSNDEELKKILETLDRILKKLEKILTRLIEVLKKSEDPNLDDKDYTELVKQ FIELIKKFEEVIKEYEEVVRQLIRLFS **SEQ ID NO:36** NDEELKKILETLDRILKKLEKILTRLIEVLKKSEDPNLDDKDYTELVKQFI ELIKKFEEVIKEYEEVVRQLIRLFS **SEQ ID NO: 348**
DHD34_X AAAA	Heterodimer	a	SKEEIDKIVKKHKKKIEEHKKKVDEHKKLVEEHDKRVSQDKDDKVKKLSEE LKKISKRLEEVSKRLEEVSKKLLKVISDKR **SEQ ID NO:37**
DHD34_X AAAA	Heterodimer	b	GSNDEELKKILETLDRILKKLDKILTRLDEVLKKSEDPNLDDKDYTELVKQ YIELVKKYEEVVKEYEEVVRQLIRLFS **SEQ ID NO:38** NDEELKKILETLDRILKKLDKILTRLDEVLKKSEDPNLDDKDYTELVKQYI ELVKKYEEVVKEYEEVVRQLIRLFS **SEQ ID NO: 418**
DHD36	Heterodimer	a	DHSRKLKEILDRLRKHVKRLKEHLDELRDLVRQVPEDKLLEHVVKLSDKIL QISERAVREFTKSVDKDS **SEQ ID NO:39**
DHD36	Heterodimer	b	GSDKKDELERILDEIRRLIERLDEILSRLNKLLELLKHGVPNAKEVVKDYI RLLKEYLELVKEFLKLVKRHADLVS **SEQ ID NO:40** DKKDELERILDEIRRLIERLDEILSRLNKLLELLKHGVPNAKEVVKDYIRL LKEYLELVKEFLKLVKRHADLVS **SEQ ID NO: 350**
DHD37_A BXB	Heterodimer	a	DSDEHLKKLKTFLENLRRHLDRLDKHIKQLRDILSENPEDERVKDVIDLSE RSVRIVKTVIKIFEDSVRKKE **SEQ ID NO:41**
DHD37_A BXB	Heterodimer	b	GSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVE LLKRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:42** DDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELL KRHEKAVKELLEIAKTHAKKVE **SEQ ID NO: 352**
DHD37_B BBB	Heterodimer	a	MDEEDHLKKLKTHLEKLERHLKLLEDHAKKLEDILKERPEDSAVKESIDEL RRSIELVRESIEIFRQSVEEEE **SEQ ID NO:43**
DHD37_B BBB	Heterodimer	b	GDVKELTKILDTLTKILETATKVIKDATKLLEEHRKSDKPDPRLIETHKKL VEEHETLVRQHKELAEEHLKRTR **SEQ ID NO:44**
DHD37_X BXB	Heterodimer	a	DSDEHLKKLKTFLENLRRHLDRLDKLLKELRDILSENPEDERVKDVIDELE RVIRIVKTVIKIFEDSVRKKE **SEQ ID NO:45**
DHD37_X BXB	Heterodimer	b	GSDDKELDKLLDTLEKILQTATKIIDDLNKVLEKLRRSERKDPKVIETVVE LLKRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:46** DDKELDKLLDTLEKILQTATKIIDDLNKVLEKLRRSERKDPKVIETVVELL KRHEKAVKELLEIAKTHAKKVE **SEQ ID NO: 354**
DHD37_A XXB	Heterodimer	a	DSDEHLKKLKTFLENLRRLEDLLDKHIKQLRDILSENPEDERVKDVIDLSE RVVRTVKTVIKIFEDSVRKKE **SEQ ID NO:47**
DHD37_A XXB	Heterodimer	b	GSDDKELDKLLDTLEKILQTATKVVDDANKLLEKLRRSERKDPKVVETYVE LLKRLEKLIKELLEIAKTHAKKVE **SEQ ID NO:48** DDKELDKLLDTLEKILQTATKVVDDANKLLEKLRRSERKDPKVVETYVELL KRLEKLIKELLEIAKTHAKKVE **SEQ ID NO: 356**
DHD37_3 :124	Heterodimer	a	DSDEHLKKLKTFLENLRRHLDRLDKHIKQLRDILSEN **SEQ ID NO:49**
DHD37_3 :124	Heterodimer	b	EDERVKDVIDLSERSVRIVKTVIKIFEDSVRKLEKTKPDSKTAKELDKLLD TLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKEL LEIAKTHAKKVE **SEQ ID NO:50**
DHD37_1 :234	Heterodimer	a	DSDEHLYKLKTFLENLRRHLDRLDKHIKQLRDILSENPEDERVKDAIDLSE RSVRIVKTVIKIFEDSVRKKEKRPIDKRDDKELDKLLDTLEKILQTATKII DDANKLLEYLRR **SEQ ID NO:51**

DHD37_1:234	Heterodimer	b	GDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:52**
DHD37_AXBB	Heterodimer	a	DSDEHLDRLDKHLKKLKTFLENLRRHIKQLRDILSENPEDERVKDVIDLSK TVIKIFEDSVRKKERSVRIVE **SEQ ID NO:53**
DHD37_AXBB	Heterodimer	b	GSDDKEATKIIDDLDKLLDTLEKILQTANKLLEKLRRSERKDPKVVETYVK AVKELLEIAKTHAELLKRHEKKVE **SEQ ID NO:54** DDKEATKIIDDLDKLLDTLEKILQTANKLLEKLRRSERKDPKVVETYVKAV KELLEIAKTHAELLKRHEKKVE **SEQ ID NO: 358**
DHD37_XBBA	Heterodimer	a	DSDEHIKQLRDHLDRLDKHLKKLKTFLENLRRILSENPEDERVKTVIKIFE DSVRKKERSVRIVKDVIDLSE **SEQ ID NO:55**
DHD37_XBBA	Heterodimer	b	GSDDKEANKLLEKATKIIDDLDKLLDTLEKILQTLRRSERKDPKAVKELLE IAKTHAELLKRHEKVVETYVKKVE **SEQ ID NO:56** DDKEANKLLEKATKIIDDLDKLLDTLEKILQTLRRSERKDPKAVKELLEIA KTHAELLKRHEKVVETYVKKVE **SEQ ID NO: 360**
DHD39	Heterodimer	a	DHSRKLEEILDRLRKHVKRLLEHLRELLSLVKENPEDKDLVEVLELSLAIL RRSLEAVEAFLKSVTKKDPDDEDLRRKADEIRKEVEEIKKSLAEVEKEIYK LK **SEQ ID NO:57**
DHD39	Heterodimer	b	GSSADDVLEDILKIIRELIEILDQILSLLNQLLKLLRHGVPNAKKVVEKYK EILELYLQLVSLFLKIVKTHADAVSGKIDKKAEEEIKKEEEKIKEKLRQAK DILKKLQEEIDKTR **SEQ ID NO:58** SADDVLEDILKIIRELIEILDQILSLLNQLLKLLRHGVPNAKKVVEKYKEI LELYLQLVSLFLKIVKTHADAVSGKIDKKAEEEIKKEEEKIKEKLRQAKDI LKKLQEEIDKTR **SEQ ID NO: 362**
DHD40	Heterodimer	a	DRDAHLYKLLTFLEQLVRHLDRLVKHITQLRDIVKKDPEDERAVDVIRQSV RSLEIVITVLKIFVDSVSDAARSKEAEKIVRKIRKEIDEIRQKLREIDKEV KKTTS **SEQ ID NO:59**
DHD40	Heterodimer	b	GSNDKVLDKILDILDRILRLATRVIDLANKLLQVKKKSTHKDPRIVETYKE LLKIHETAVRLLLELADLHRRLKSKDEEANKRVETELDRIRKKVKDIEDKV RKLEDKVRKTAS **SEQ ID NO:60** NDKVLDKILDILDRILRLATRVIDLANKLLQVKKKSTHKDPRIVETYKELL KIHETAVRLLLELADLHRRLKSKDEEANKRVETELDRIRKKVKDIEDKVRK LEDKVRKTAS **SEQ ID NO: 364**
DHD43	Heterodimer	a	NDLSKEVLKKLEKSVEELLRRVQKSVKEAQKRGLLSDELVDRHLKILNQLV KRHLELLQEVIKRSDKK **SEQ ID NO:61**
DHD43	Heterodimer	b	GSDEAVKRVVEKSLKILDEVIKKSLDILRELIELQIRHAKDDESVIRASKS ALKDAIEALKKSLDEIKKALKRSADEG **SEQ ID NO:62** DEAVKRVVEKSLKILDEVIKKSLDILRELIELQIRHAKDDESVIRASKSAL KDAIEALKKSLDEIKKALKRSADEG **SEQ ID NO: 366**
DHD65	Heterodimer	a	SSEEVVKVHEKVVKLHKEILELLKKIIKIHETAARDPDDKDSIKKLSDEIK KIVKRIEDISDQAKRESSDAQRKQS **SEQ ID NO:63**
DHD65	Heterodimer	b	DKEEESKELLKKLKEILKRSEELLEESKELLKLAKNGEIDESELADADRKL NKKHEKLVQDIQDLLREHERQDR **SEQ ID NO:64**
DHD70	Heterodimer	a	DEKKKIDKIVKETEDLLQKSEKLLQQSKEAVKRIRSQVKENEIVDRLLRIS EELLKISRRLVEISRRIASTLS **SEQ ID NO:65**
DHD70	Heterodimer	b	GSSKEEVIRLLKENVRLIKENLELLTRNLKLITDLVRGSNGSEEKIKTLKE LLKEYRELLKRYRKLVEDYKRLVDKHD **SEQ ID NO:66** SKEEVIRLLKENVRLIKENLELLTRNLKLITDLVRGSNGSEEKIKTLKELL KEYRELLKRYRKLVEDYKRLVDKHD **SEQ ID NO: 368**
DHD88	Heterodimer	a	EIQELIKSSRRIIEESKELIKESEEVLRRIKEILDRIRNGVDNQEDLLREI LKLLTKNLKIIQRNLKLLQDNAEILKRLVS **SEQ ID NO:67**
DHD88	Heterodimer	b	GSYIEDVIKKILDVSRELIKLSRTIIKISEEINKQLQQGRDTKDLVKKYDE IIKKYTRIVQHYTELIKELQKLLS **SEQ ID NO:68** YIEDVIKKILDVSRELIKLSRTIIKISEEINKQLQQGRDTKDLVKKYDEII KKYTRIVQHYTELIKELQKLLS **SEQ ID NO: 370**
DHD89	Heterodimer	a	SPTEEAIQLSQRVIELSKRVIELSKEILKLLKRVLDLLPDLDKNEEKRLDD YDKELKEYDKELKKYEKRLKDLAS **SEQ ID NO:69**
DHD89	Heterodimer	b	GSEEEEILKIQKELLRIQSEILDKQKKILDTLRSNGAVTEEVRSILEKVER LSEEAKELSKEAKELTKEVSKLIS **SEQ ID NO:70**

			EEEEILKIQKELLRIQSEILDKQKKILDTLRSNGAVTEEVRSILEKVERLS EEAKELSKEAKELTKEVSKLIS **SEQ ID NO: 372**
DHD90	Heterodimer	a	SPLKELNNQLLRLLRELVKVSKKIVDLSKTIIEVLKHTDLDPRLLDSLEKS QQELDKSQKELDKVVKELTKVNKKLQ **SEQ ID NO:71**
DHD90	Heterodimer	b	GSPLEDLVRKYDELVKTYEKLVEEFKKAVDKYDKAVKKAPVSKEATDSLDL IRKVLELLDRNLKLIKENAKLIKELLK **SEQ ID NO:72** PLEDLVRKYDELVKTYEKLVEEFKKAVDKYDKAVKKAPVSKEATDSLDLIR KVLELLDRNLKLIKENAKLIKELLK **SEQ ID NO: 374**
DHD91	Heterodimer	a	SPTRENEKVIKENEKVISDNERVLEEVVKVVETATDRKEIQDAVDEVRKSV DKLRDSVRKLEESVRTLD **SEQ ID NO:73**
DHD91	Heterodimer	b	GSPIKDISKRLLEISKRLVEISDRIVELLQRIADSKDPNKDLQKEVKDVLE EYKRLVREYREVVKEYEKVVS **SEQ ID NO:74** PIKDISKRLLEISKRLVEISDRIVELLQRIADSKDPNKDLQKEVKDVLEEY KRLVREYREVVKEYEKVVS **SEQ ID NO: 376**
DHD92	Heterodimer	a	DEDEHVKQLIKNADLLRKHAELLKELVKLFQEIASQIPDDRVAKKVTDVVD RIDKILKQTEKLVRRTKQILDYSR **SEQ ID NO:75**
DHD92	Heterodimer	b	GSNLEELVKLLKEVLEMHERLLRIHEDLVEAHKSNASDKESERKLKKSDKD IKESLKKIKSIIDQVRYIQS **SEQ ID NO:76** NLEELVKLLKEVLEMHERLLRIHEDLVEAHKSNASDKESERKLKKSDKDIK ESLKKIKSIIDQVRYIQS **SEQ ID NO: 378**
DHD93	Heterodimer	a	PVEDIIEESLRLLEESLKLLNRILKLLEDSLRKLPRSEEWRQRLDEFRKKL EDWKEELERWIEDVRYKKT **SEQ ID NO:77**
DHD93	Heterodimer	b	GSDEDYESREIIDEIRKLLDRSKKIVHRSQRLVERVKSTPLSEDQEDLIRR HEETINRHRELVKELEKVLEDHERHIR **SEQ ID NO:78** DEDYESREIIDEIRKLLDRSKKIVHRSQRLVERVKSTPLSEDQEDLIRRHE ETINRHRELVKELEKVLEDHERHIR **SEQ ID NO: 380**
DHD94	Heterodimer	a	PEEDSRRVLERFVRVSREVLKVLEEFLRVSEELLREADRDRDRRLEEYERQ VDELREEIRRYKEEVDKFDKEVKYYKK **SEQ ID NO:79**
DHD94	Heterodimer	b	GSPEKDENRKLLDKVRKLVEKSRRLVEELRKLVDQSTKNGLIDEKALRKQQ EVLRKVEEVLEKQERVLRELEEISYRVI **SEQ ID NO:80** PEKDENRKLLDKVRKLVEKSRRLVEELRKLVDQSTKNGLIDEKALRKQQEV LRKVEEVLEKQERVLRELEEISYRVI **SEQ ID NO: 382**
DHD94_3 :214	Heterodimer	a	GSPERDENRKLLDKVRKLVEKSRRLVEELRKLVDQSTKN **SEQ ID NO:81** PERDENRKLLDKVRKLVEKSRRLVEELRKLVDQSTKN **SEQ ID NO: 337**
DHD94_3 :214	Heterodimer	b	GSDEKALRKQQEVLRKVEEVLEKQERVLRELEEISYRVITRGEDHKAEEDS RRVLERFVRVSREVLKVLEEFLRVSEELLREADRDRDRRLEEYERQVDELR EEIRRYKEEVDKFDKEVKYYKK **SEQ ID NO:82** DEKALRKQQEVLRKVEEVLEKQERVLRELEEISYRVITRGEDHKAEEDSRR VLERFVRVSREVLKVLEEFLRVSEELLREADRDRDRRLEEYERQVDELREE IRRYKEEVDKFDKEVKYYKK **SEQ ID NO: 384**
DHD94_2 :143	Heterodimer	a	GSDRRLEEYERQVDELREEIRRYKEEVDKFDKEVKYYKK **SEQ ID NO:83** DRRLEEYERQVDELREEIRRYKEEVDKFDKEVKYYKK **SEQ ID NO: 339**
DHD94_2 :143	Heterodimer	b	GSPERDENRKLLDKVRKLVEKSRRLVEELRKLVDQSTKNGLIDEKALRKQQ EVLRKVEEVLEKQERVLRELEEISYRVITRGEDHKAEEDSRRVLERFVRVS REVLKVLEEFLRVSEELLREADR **SEQ ID NO:84** PERDENRKLLDKVRKLVEKSRRLVEELRKLVDQSTKNGLIDEKALRKQQEV LRKVEEVLEKQERVLRELEEISYRVITRGEDHKAEEDSRRVLERFVRVSRE VLKVLEEFLRVSEELLREADR **SEQ ID NO: 386**
DHD95	Heterodimer	a	DLSEESKKFVEKVKKLEKESRELEKQVKKIEEDSRSVENDVQKEFLELLKR LLDIQKKVVEVLREVVKVQQYVDS **SEQ ID NO:85**
DHD95	Heterodimer	b	GSDSEYESRQVLRELDTVLKDSHTVLEALRQVIRDSQDVVSKSDEESRRVI DDLEKVIQDSKKVLDDIKRLIDKSKSIKS **SEQ ID NO:86** DSEYESRQVLRELDTVLKDSHTVLEALRQVIRDSQDVVSKSDEESRRVIDD LEKVIQDSKKVLDDIKRLIDKSKSIKS **SEQ ID NO: 388**

DHD96	Heterodimer	a	NEDELLKLLTENLKLLDENLKLLRENLSLLRQANNITDKNRIREIVKQSKE IVKQSREILKQSKEIVERIKYIVS **SEQ ID NO:87**
DHD96	Heterodimer	b	GSSLYELTQRYEKLVQQYEELVKDYRRLVKKLEKLKRDNKPDKRLLKEIVD VIKKSVEIIDRSLKLLEESIKILEETD **SEQ ID NO:88** SLYELTQRYEKLVQQYEELVKDYRRLVKKLEKLKRDNKPDKRLLKEIVDVI KKSVEIIDRSLKLLEESIKILEETD **SEQ ID NO: 390**
DHD97	Heterodimer	a	SQERSLEILKRILDVLKESLEILKESLSILRQLASRIKNPNRKIEEILKES DKIIKESDKVLKEIEEVIRYSS **SEQ ID NO:89**
DHD97	Heterodimer	b	GSDIEYESKEILELIKELLKLSRELLKESRRALELVRKSRDDSIVEEVIQV HKKVLDIHKEVLKIVRKVVEVHRRVKS **SEQ ID NO:90** DIEYESKEILELIKELLKLSRELLKESRRALELVRKSRDDSIVEEVIQVHK KVLDIHKEVLKIVRKVVEVHRRVKS **SEQ ID NO: 392**
DHD98	Heterodimer	a	SKKDESTKLERLAEKIDEITKRIEELVKDVKRKSSEGVDKDQQQKIDEVFQ KLLDLQREILEILDRILKVQQYILD **SEQ ID NO:91**
DHD98	Heterodimer	b	GSDLEYLNRRLLQLIKTLIDLNRHLLKLIDKLKKLNSREGDEEKIKEESKQ IQEQFKEIVERSKEIIKQIKEIIKRSQ **SEQ ID NO:92** DLEYLNRRLLQLIKTLIDLNRHLLKLIDKLKKLNSREGDEEKIKEESKQIQ EQFKEIVERSKEIIKQIKEIIKRSQ **SEQ ID NO: 394**
DHD99	Heterodimer	a	DFERSSRRLEKVVEDLRRSSDRLREVIDELRKSADEKDEDEDLRRARKEHR DLIEELKRALEKQEEIIKHLQELVYRQL **SEQ ID NO:93**
DHD99	Heterodimer	b	GSEESEEVRKVVERIKKISRELEEVVKELDRVSKEFDRHGETDEIVREHER IVEKLEEIVKKHTKIVEELAEIVYKQQ **SEQ ID NO:94** EESEEVRKVVERIKKISRELEEVVKELDRVSKEFDRHGETDEIVREHERIV EKLEEIVKKHTKIVEELAEIVYKQQ **SEQ ID NO: 396**
DHD100	Heterodimer	a	SDDDSVRVLDEIVKILDESVKLLKESLKLLDDFLRTKPDDHLKEVVKESKK VVEQSKKVLDRIKKIIYESK **SEQ ID NO:95**
DHD100	Heterodimer	b	GSDLLYLSKELLKLVRELLKLSRELVELSRRLVNSTHKSPELVKKYDKLVK KYQDLLKKLADVADEYLRQRS **SEQ ID NO:96** DLLYLSKELLKLVRELLKLSRELVELSRRLVNSTHKSPELVKKYDKLVKKY QDLLKKLADVADEYLRQRS **SEQ ID NO: 398**
DHD101	Heterodimer	a	DEKDYHRRLIEHLEDLVRRHEELIKRQKKVVEELERRGLDERLRRVVDRFR RSSERWEEVIERFRQVVDKLRKSVE **SEQ ID NO:97**
DHD101	Heterodimer	b	GSDAYDLDRIVKEHRRLVEEQRELVEELEKLVRRQEDHRVDKKESHEILER LERIIRRSTRILTELEKLTDEFERRTR **SEQ ID NO:98** DAYDLDRIVKEHRRLVEEQRELVEELEKLVRRQEDHRVDKKESHEILERLE RIIRRSTRILTELEKLTDEFERRTR **SEQ ID NO: 400**
DHD102	Heterodimer	a	DERYRAREHIRRVEEHTKRLRHILKRLREHEEKLRRELKPGDEITESVDRF KKIVDQFEESIKKFETVSEELRKSDS **SEQ ID NO:99**
DHD102	Heterodimer	b	GSDRQRILDRLDKILEKLDDILKKLKDILETLSKDDVSDRRHKDLVEKFRE LVDTHHKLVERYRELVYQNR **SEQ ID NO:100** DRQRILDRLDKILEKLDDILKKLKDILETLSKDDVSDRRHKDLVEKFRELV DTHHKLVERYRELVYQNR **SEQ ID NO: 402**
DHD102_ 1:243	Heterodimer	a	GSDEITESVDRFKKIVDQFEESIKKFETVSEELRKSIS **SEQ ID NO:101** DEITESVDRFKKIVDQFEESIKKFETVSEELRKSIS **SEQ ID NO: 341**
DHD102_ 1:243	Heterodimer	b	GSDPQRAADRLDKILEKLDDILKKLKDILETLSKDDVKDRRAKDLVEKFRE LVDTHHKLVERYRELVYTATAGSDLARELIRRVEEHTKRLRHILKRLREHE EKLRR **SEQ ID NO:102** DPQRAADRLDKILEKLDDILKKLKDILETLSKDDVKDRRAKDLVEKFRELV DTHHKLVERYRELVYTATAGSDLARELIRRVEEHTKRLRHILKRLREHEEK LRR **SEQ ID NO: 404**
DHD103	Heterodimer	a	NADDQLATSIKKLEDSIDQLIKIVRKFEESVKKLQKHGVDQHHVEILRKIV EIFRQHIEKLKKHLEKLRYTSS **SEQ ID NO:103**
DHD103	Heterodimer	b	GSDKEYLVTEHEKLVREHEKIVSEIEKLVKKHEAGVDESELEEILKKVEKL LRKLDEILEQLTQLLRKTE **SEQ ID NO:104** DKEYLVTEHEKLVREHEKIVSEIEKLVKKHEAGVDESELEEILKKVEKLLR KLDEILEQLTQLLRKTE **SEQ ID NO: 406**
DHD103_	Heterodimer	a	GSDQHVVEILRKIVEIFRQHIEKLKKHLEKLRYTSS **SEQ ID NO:105**

1:423			DQHVVEILRKIVEIFRQHIEKLKKHLEKLRYTSS **SEQ ID NO: 343**
DHD103_1:423	Heterodimer	b	GSDAEYLVTEHEKLVREHEKIVSEIEKLVKKHEKGVDESELEEILKKVEKLLRKLDEILEQLTQLLRKAEKHIDKHSKAADQLATSIKKLEDSIDQLIKIVRKFEESVKKLQKH **SEQ ID NO:106** DAEYLVTEHEKLVREHEKIVSEIEKLVKKHEKGVDESELEEILKKVEKLLRKLDEILEQLTQLLRKAEKHIDKHSKAADQLATSIKKLEDSIDQLIKIVRKFEESVKKLQKH **SEQ ID NO: 408**
DHD104	Heterodimer	a	DEDDDIRRVLDESRRVLEHSRRVLKRSEEVLEKASRKKEKDTEEIEKHLKRLREHAKKLEKHRRELDDFLYKEI **SEQ ID NO:107**
DHD104	Heterodimer	b	GSRDKYLLERLNDILKKLDEIVDKLSDILKRLKDVRHDDRLQELVERYKEIVKEYKRIVEEYEKLVREFEEQQR **SEQ ID NO:108** RDKYLLERLNDILKKLDEIVDKLSDILKRLKDVRHDDRLQELVERYKEIVKEYKRIVEEYEKLVREFEEQQR **SEQ ID NO: 410**
DHD105	Heterodimer	a	DRDYEDKEFKKIIKELEDVQEELKKLQEKIKRFSSELEEPNELLKEQLKVNEEQLEVNKKILKILRDQLKQNE **SEQ ID NO:109**
DHD105	Heterodimer	b	GSDAEYKVRESVKRSKESVKHSEDVVDKLNKSVKLSESGHSDAEKASRELVKLVREVVELSREVIKLSEKVLRVIS **SEQ ID NO:110** DAEYKVRESVKRSKESVKHSEDVVDKLNKSVKLSESGHSDAEKASRELVKLVREVVELSREVIKLSEKVLRVIS **SEQ ID NO: 412**
DHD106	Heterodimer	a	DLQYKQEKLIRHFDRVVREWDKLVRKFSKVLEKQKHESKDKELEEASRRVDELIKRLREQLKRSKEILRRLKELSRKSS **SEQ ID NO:111**
DHD106	Heterodimer	b	GSDWEELLRRLEKVLQEYEEIVKELIDLIERLIKVSEDKSKDASEYKKLVTELEKLISKLEEISKKLEELVKEYEYKTE **SEQ ID NO:112** DWEELLRRLEKVLQEYEEIVKELIDLIERLIKVSEDKSKDASEYKKLVTELEKLISKLEEISKKLEELVKEYEYKTE **SEQ ID NO: 414**
DHD107	Heterodimer	a	DAKDELEKSLQEIEESLKELKKLLEELDKSLRELTSQGRNKKLEEHIKKVQKFIELVKKYIKAVQDYLKEVRYDNS **SEQ ID NO:113**
DHD107	Heterodimer	b	GSDKERAARATEEMVKLTKKLLKAVEDLVRDVRRLLKEGLISEKHARIAETILEVFKKHAKIIKKHVDIVKYDES **SEQ ID NO:114** DKERAARATEEMVKLTKKLLKAVEDLVRDVRRLLKEGLISEKHARIAETILEVFKKHAKIIKKHVDIVKYDES **SEQ ID NO: 416**
DHD108	Heterodimer	a	GSPLKERLLEIQRDLDRVLEEVVERLLRIQERLDSVVERKPPDVHEEYKYIVDEIREIVERVVREYEEIVKRIDEEVR **SEQ ID NO:115** PLKERLLEIQRDLDRVLEEVVERLLRIQERLDSVVERKPPDVHEEYKYIVDEIREIVERVVREYEEIVKRIDEEVR **SEQ ID NO: 459**
DHD108	Heterodimer	b	GSEEDERIRYDLDRIRKDVRRKLEEIRQRVRELEKKLRDAGHRRDEKELLRELIETSKDILRLVEELLKKIIDKSEDLLRKTE **SEQ ID NO:116** EEDERIRYDLDRIRKDVRRKLEEIRQRVRELEKKLRDAGHRRDEKELLRELIETSKDILRLVEELLKKIIDKSEDLLRKTE **SEQ ID NO: 420**
DHD109	Heterodimer	a	GSDEEDYINENVEKDVRDIEDDVRRINERIRELLEKIRTEEVLQRVLEEHHELVERVLRKLVEILRKHEEENR **SEQ ID NO:117** DEEDYINENVEKDVRDIEDDVRRINERIRELLEKIRTEEVLQRVLEEHHELVERVLRKLVEILRKHEEENR **SEQ ID NO: 345**
DHD109	Heterodimer	b	GSDEEEYYKEKLHKLLREIEELLKHYRELVRRLEELVKRGELDKDTAAHILERLSELLERIIRRVAHTLRRLSEERR **SEQ ID NO:118** DEEEYYKEKLHKLLREIEELLKHYRELVRRLEELVKRGELDKDTAAHILERLSELLERIIRRVAHTLRRLSEERR **SEQ ID NO: 422**
DHD110	Heterodimer	a	GSDEDEISYDSKRRVEEIVRQAREKSEKSRKDIEDVAEVLRKGDVSEKEVVDELVKVLEEQVKVLREAVERLREVLKKQVDDVR **SEQ ID NO:119** DEDEISYDSKRRVEEIVRQAREKSEKSRKDIEDVAEVLRKGDVSEKEVVDELVKVLEEQVKVLREAVERLREVLKKQVDDVR **SEQ ID NO: 347**
DHD110	Heterodimer	b	GSDIVELVDHLLKRSLKLLEELAELVRRLLEKSTELLKRRTEEHKEEVVEESEYMVRELEERLRRVVDESEKLVRDADKHIR **SEQ ID NO:120** DIVELVDHLLKRSLKLLEELAELVRRLLEKSTELLKRRTEEHKEEVVEESEYMVRELEERLRRVVDESEKLVRDADKHIR **SEQ ID NO: 424**
DHD111	Heterodimer	a	GSKEKDIVKTLVDLLRENLETLERLIEEVVRLLKENVDVRDEGRDDKDSERILRDIKRRIDEAAKESREIIERIEKEVEYRSR **SEQ ID NO:121** KEKDIVKTLVDLLRENLETLERLIEEVVRLLKENVDVRDEGRDDKDSERIL

			RDIKRRIDEAAKESREIIERIEKEVEYRSR **SEQ ID NO: 349**
DHD111	Heterodimer	b	GSPEVDVLRRIVREILKASEELLRLLRKLIDEALKLSERKRDSQEYREVVD RVKKELERLLDEYRKLVEELKEKLRYDTR **SEQ ID NO:122** PEVDVLRRIVREILKASEELLRLLRKLIDEALKLSERKRDSQEYREVVDRV KKELERLLDEYRKLVEELKEKLRYDTR **SEQ ID NO: 426**
DHD112	Heterodimer	a	GSDKRYESEKLKRRLDEAVEKVREVVERVERESDRVLEEVRRRRESKEVVD KVIEDNDKALEDVLRVVDEVAKVVRDVVRENTR **SEQ ID NO:123** DKRYESEKLKRRLDEAVEKVREVVERVERESDRVLEEVRRRRESKEVVDKV IEDNDKALEDVLRVVDEVAKVVRDVVRENTR **SEQ ID NO: 351**
DHD112	Heterodimer	b	GSPREYHSKDILRKVDEILERIRRHADRVKKKSERLKRENVDVNEHSKDVK RVIRELLELVKELLRLAKKHSDDQQE **SEQ ID NO:124** PREYHSKDILRKVDEILERIRRHADRVKKKSERLKRENVDVNEHSKDVKRV IRELLELVKELLRLAKKHSDDQQE **SEQ ID NO: 428**
DHD113	Heterodimer	a	GSDEDEILYHSERLLQKLKKELDDLKEKSRELLEELKKEDPDDRLIERIIR LHDEVLKDLDEVLKNILEVHREVLERLR **SEQ ID NO:125** DEDEILYHSERLLQKLKKELDDLKEKSRELLEELKKEDPDDRLIERIIRLH DEVLKDLDEVLKNILEVHREVLERLR **SEQ ID NO: 353**
DHD113	Heterodimer	b	DKLDRLLKIHEEALRRAEELIKRLLDIHRRALDLARRGELDDYLLKESERE LREIIRRAREELKESRDRLEEISR **SEQ ID NO:126**
DHD114	Heterodimer	a	GSPKEELIRRVLEEVKRLNEKLLEIIRRAAELVKRANDELPETEKLREIDR ELEKKLKEIEDELRRIDKELDDALYEIED **SEQ ID NO:127** PKEELIRRVLEEVKRLNEKLLEIIRRAAELVKRANDELPETEKLREIDREL EKKLKEIEDELRRIDKELDDALYEIED **SEQ ID NO: 355**
DHD114	Heterodimer	b	GSPKLDKLRELLERNLEKLREILEEVLKILRTNLERVREDIRDEDVLQEYE RLIRKAEEDLRRVLKEYDDLLKKLVYELR **SEQ ID NO:128** PKLDKLRELLERNLEKLREILEEVLKILRTNLERVREDIRDEDVLQEYERL IRKAEEDLRRVLKEYDDLLKKLVYELR **SEQ ID NO: 430**
DHD115	Heterodimer	a	GSKEDESVKRAEEIVRTLLKLLEDSLREAERSLRDIKNGEDEHNLRRISEK LEELSKRITETIERLLRELQYTSR **SEQ ID NO:129** KEDESVKRAEEIVRTLLKLLEDSLREAERSLRDIKNGEDEHNLRRISEKLE ELSKRITETIERLLRELQYTSR **SEQ ID NO: 357**
DHD115	Heterodimer	b	GSPNQELLDRVRKILEDLLRLNEELVRLNKELLKRALEMRRKNRDSEEVLE RLAEEYRKRLEEYRRELEKLLEELEETIYRYKR **SEQ ID NO:130** PNQELLDRVRKILEDLLRLNEELVRLNKELLKRALEMRRKNRDSEEVLERL AEEYRKRLEEYRRELEKLLEELEETIYRYKR **SEQ ID NO: 432**
DHD116	Heterodimer	a	GSDESEEAQHEVEKVLDDIRRLSEHLQKRLEEVLEEVYELRREGSDRTEVV ELLKEVIREIVRVNREALERLLRVVEEAVKRNE **SEQ ID NO:131** DESEEAQHEVEKVLDDIRRLSEHLQKRLEEVLEEVYELRREGSDRTEVVEL LKEVIREIVRVNREALERLLRVVEEAVKRNE **SEQ ID NO: 359**
DHD116	Heterodimer	b	GSDEEELVETVKRIQKEILDRLTELAKLLVEIQREIKKLKDEGEDDKELKR LSDELEEKVRQVVEEIKRLSDELEETVEYVSR **SEQ ID NO:132** DEEELVETVKRIQKEILDRLTELAKLLVEIQREIKKLKDEGEDDKELKRLS DELEEKVRQVVEEIKRLSDELEETVEYVSR **SEQ ID NO: 434**
DHD117	Heterodimer	a	GSDEEEEVVRRAEELVKEHEELIERVIRTHEELVYKLEDQGADKKLVDVLK RVVEESERVAREIVKVSRELIRLLEEASR **SEQ ID NO:133** DEEEEVVRRAEELVKEHEELIERVIRTHEELVYKLEDQGADKKLVDVLKRV VEESERVAREIVKVSRELIRLLEEASR **SEQ ID NO: 361**
DHD117	Heterodimer	b	GSSKEEILKELEDLQRRLIEELKKLQERVVELLEELIKRLRDRGRDDKHLK RLVKEVRRLSEEVLRSIKEVSDRVRYQLR **SEQ ID NO:134** SKEEILKELEDLQRRLIEELKKLQERVVELLEELIKRLRDRGRDDKHLKRL VKEVRRLSEEVLRSIKEVSDRVRYQLR **SEQ ID NO: 436**
DHD118	Heterodimer	a	GSDKEEESEYLLRDLVRLLEKVKEKIEEVNREVEKLLKKVKDGRLDRREVL REILRLNRELAEIIKEVVDRIRHVVERSER **SEQ ID NO:135** DKEEESEYLLRDLVRLLEKVKEKIEEVNREVEKLLKKVKDGRLDRREVLRE ILRLNRELAEIIKEVVDRIRHVVERSER **SEQ ID NO: 363**
DHD118	Heterodimer	b	GSDLHEVVYETKELLKRIEEVVEELRKKSEDIIRKAERGEISEDELKRLQE EIAREAKKLLDEIKRVLERHLEQTL **SEQ ID NO:136** DLHEVVYETKELLKRIEEVVEELRKKSEDIIRKAERGEISEDELKRLQEEI

			AREAKKLLDEIKRVLERHLEQTL **SEQ ID NO: 438**
DHD119	Heterodimer	a	GSPVEEIIKEVVKRVIEVQEKVLRIISHAVKRVVEVQKKYDPGSEESNRVV EEVKKTIEDAIRESDEVVDEVVKRIQYTVR **SEQ ID NO:137** PVEEIIKEVVKRVIEVQEKVLRIISHAVKRVVEVQKKYDPGSEESNRVVEE VKKTIEDAIRESDEVVDEVVKRIQYTVR **SEQ ID NO: 365**
DHD119	Heterodimer	b	GSPEQEIADRILTEIRESQKELERLARKILKLLDESQEKAKRGRLSEEESD ELLERIKKELDELLERSKELLKKIEYELR **SEQ ID NO:138** PEQEIADRILTEIRESQKELERLARKILKLLDESQEKAKRGRLSEEESDEL LERIKKELDELLERSKELLKKIEYELR **SEQ ID NO: 440**
DHD120	Heterodimer	a	GSDEDKEANRVLDEVLKTVRDLLETANEVLKEVLYRLKRTDDQEKVVRTLT EVLKEHLKLVEEIVRILDKVLKEHLETEK **SEQ ID NO:139** DEDKEANRVLDEVLKTVRDLLETANEVLKEVLYRLKRTDDQEKVVRTLTEV LKEHLKLVEEIVRILDKVLKEHLETEK **SEQ ID NO: 367**
DHD120	Heterodimer	b	GSPEDDVLRRLEEVSEKILRVAEDVARQLREVSEKITQGKVDRKEWEEDIK RLKRELEELLREWKEEIERLTYELR **SEQ ID NO:140** PEDDVLRRLEEVSEKILRVAEDVARQLREVSEKITQGKVDRKEWEEDIKRL KRELEELLREWKEEIERLTYELR **SEQ ID NO: 442**
DHD121	Heterodimer	a	GSRREEVVKRIRELLKRNKELIDRIRELLEENEYLDKDARDKDVLRRSVEL LEELVRILEESVELAKEIIKLLREVVE **SEQ ID NO:141** RREEVVKRIRELLKRNKELIDRIRELLEENEYLDKDARDKDVLRRSVELLE ELVRILEESVELAKEIIKLLREVVE **SEQ ID NO: 369**
DHD121	Heterodimer	b	GSDEKEDNRRLQHKIERILEKNEDLQRKLEEILELLERGEADEEKIDRLRK AVEDYRRVVEEIKEDVKRHKYTVR **SEQ ID NO:142** DEKEDNRRLQHKIERILEKNEDLQRKLEEILELLERGEADEEKIDRLRKAV EDYRRVVEEIKEDVKRHKYTVR **SEQ ID NO: 444**
DHD122	Heterodimer	a	GSDEKEEAKKASEESVRTVERILEELLKASEESVELLRRGEDAKDVVERSK EALKRVKELLDEVVKRSDEILKYIHN **SEQ ID NO:143** DEKEEAKKASEESVRTVERILEELLKASEESVELLRRGEDAKDVVERSKEA LKRVKELLDEVVKRSDEILKYIHN **SEQ ID NO: 371**
DHD122	Heterodimer	b	GSDEKKLINEVVETQKRLIKEAAKRLSEVVRHQTELIRELREKNVDDKDVE KLLKESLDLAEEIVRRIKELLDESKKLVEYVSN **SEQ ID NO:144** DEKKLINEVVETQKRLIKEAAKRLSEVVRHQTELIRELREKNVDDKDVEKL LKESLDLAEEIVRRIKELLDESKKLVEYVSN **SEQ ID NO: 446**
DHD123	Heterodimer	a	GSPDMDEVKRVLDELIEIQEEILREIKRVLEKLIKIQEDNGSEYESREVVR EIVEIARKLVERSRRVVKKITETLQ **SEQ ID NO:145** PDMDEVKRVLDELIEIQEEILREIKRVLEKLIKIQEDNGSEYESREVVREI VEIARKLVERSRRVVKKITETLQ **SEQ ID NO: 373**
DHD123	Heterodimer	b	GSDERYATREIVERIERIAREILKRTEEIVREVREVLSRDVDQEEVVRRLA DLLRESVELVQHLVRRVEELLQESVERKK **SEQ ID NO:146** DERYATREIVERIERIAREILKRTEEIVREVREVLSRDVDQEEVVRRLADL LRESVELVQHLVRRVEELLQESVERKK **SEQ ID NO: 448**
DHD124	Heterodimer	a	GSPEREALREVLEDLKRVTDRLRELVERVLEELKKVTDHVDSERILRESRR VLKELKDIIEEILRESEKVLEKLKYTED **SEQ ID NO:147** PEREALREVLEDLKRVTDRLRELVERVLEELKKVTDHVDSERILRESRRVL KELKDIIEEILRESEKVLEKLKYTED **SEQ ID NO: 375**
DHD124	Heterodimer	b	GSPAREILEEVVKKHLEVVEDAARILEEIIREHEKAVREDRDKKELEEISR DLLRKAREALKKVKDISDDLSREIEYVAS **SEQ ID NO:148** PAREILEEVVKKHLEVVEDAARILEEIIREHEKAVREDRDKKELEEISRDL LRKAREALKKVKDISDDLSREIEYVAS **SEQ ID NO: 450**
DHD125	Heterodimer	a	GSPVEEAIKKVIDDLRDVQRKIRELVEELIRLLEEVQRDNDKRESEYVVER VEEILRRITETSREVVRKAVEDLS **SEQ ID NO:149** PVEEAIKKVIDDLRDVQRKIRELVEELIRLLEEVQRDNDKRESEYVVERVE EILRRITETSREVVRKAVEDLS **SEQ ID NO: 377**
DHD125	Heterodimer	b	GSDSDEKAEYLLKEMERVVRESDEVVKKILRDLEEVLERLRRGEISEDDVT EILKELAERHIRAIEELVRRLRELLERHKR **SEQ ID NO:150** DSDEKAEYLLKEMERVVRESDEVVKKILRDLEEVLERLRRGEISEDDVTEI LKELAERHIRAIEELVRRLRELLERHKR **SEQ ID NO: 452**
DHD126	Heterodimer	a	GSPVEEVLKELSEVNERVRDIAREIIERLSEVNEEVKETDDEDELKKISKK

			VVDEVEDLLRKILEVSEEVVRRVEYHDR **SEQ ID NO:151** PVEEVLKELSEVNERVRDIAREIIERLSEVNEEVKETDDEDELKKISKKVV DEVEDLLRKILEVSEEVVRRVEYHDR **SEQ ID NO: 379**
DHD126	Heterodimer	b	GSPKEDILREVLRRHKEIVREIVRLVREAVETHLELVKRNSDDRDAQDVIR KLEEDLERLVRHAQEVIEEIFYRLH **SEQ ID NO:152** PKEDILREVLRRHKEIVREIVRLVREAVETHLELVKRNSDDRDAQDVIRKL EEDLERLVRHAQEVIEEIFYRLH **SEQ ID NO: 454**
DHD127	Heterodimer	a	GSPRSYLLKELADLSQHLVRLLERLVRESERVVEVLERGEVDEEELKRLED LHRELEKAVREVRETHREIRERSR **SEQ ID NO:153** PRSYLLKELADLSQHLVRLLERLVRESERVVEVLERGEVDEEELKRLEDLH RELEKAVREVRETHREIRERSR **SEQ ID NO: 381**
DHD127	Heterodimer	b	GSDREYIIKDILDSQEHLLRLIEELLETQKELLEILKRRPDSVERVRELVR RSKEIADEIRRQSDRNVRLLEEVSK **SEQ ID NO:154** DREYIIKDILDSQEHLLRLIEELLETQKELLEILKRRPDSVERVRELVRRS KEIADEIRRQSDRNVRLLEEVSK **SEQ ID NO: 456**
DHD128	Heterodimer	a	GSDEKDEIRHVIESVERLIEDIKRLLKTLRELAHDDSDKKTVKEVLDRVKE MIERHRRELEEHRKELERAEYEVR **SEQ ID NO:155** DEKDEIRHVIESVERLIEDIKRLLKTLRELAHDDSDKKTVKEVLDRVKEMI ERHRRELEEHRKELERAEYEVR **SEQ ID NO: 383**
DHD128	Heterodimer	b	GSESEDRIKELLKRHIELVERHEELLHEIKKLIDLEEKDDKDREEAVKRID DAIKESEEMLEESKEILEEIEYLNR **SEQ ID NO:156** ESEDRIKELLKRHIELVERHEELLHEIKKLIDLEEKDDKDREEAVKRIDDA IKESEEMLEESKEILEEIEYLNR **SEQ ID NO: 458**
DHD129	Heterodimer	a	GSSLEDSVRLNDEVVKVVERVVRLNQEVVRLIKHATDVEDEETVKYVLERV REVLDESREVLKRVHELLEESERRLE **SEQ ID NO:157** SLEDSVRLNDEVVKVVERVVRLNQEVVRLIKHATDVEDEETVKYVLERVRE VLDESREVLKRVHELLEESERRLE **SEQ ID NO: 385**
DHD129	Heterodimer	b	GSHEKDIVYKVEDLVRKSDRIAERAREIVKRSRDIMREIRKDKDNKKLSDD LLKVTRDLQRVVDELEELSRELLRVAEESRK **SEQ ID NO:158** HEKDIVYKVEDLVRKSDRIAERAREIVKRSRDIMREIRKDKDNKKLSDDLL KVTRDLQRVVDELEELSRELLRVAEESRK **SEQ ID NO: 460**
DHD130	Heterodimer	a	GSPELDEVKKLIDELKKSVERLEESIREVKESIKKLRKGDIDAEENIKLLK ENIKIVRENIKIIKEIIDVVQYVLR **SEQ ID NO:159** PELDEVKKLIDELKKSVERLEESIREVKESIKKLRKGDIDAEENIKLLKEN IKIVRENIKIIKEIIDVVQYVLR **SEQ ID NO: 387**
DHD130	Heterodimer	b	GSDEEEIEELLRELEKLLKKSEEALEESKKLIDESEELLRRDRLDKEKHVR ASEEHVKLSEEHLRISREIVKILEKAVYSTR **SEQ ID NO:160** DEEEIEELLRELEKLLKKSEEALEESKKLIDESEELLRRDRLDKEKHVRAS EEHVKLSEEHLRISREIVKILEKAVYSTR **SEQ ID NO: 462**
DHD131	Heterodimer	a	GSDESDRIRKIVEESDEIVKESRKLAERARELIKESEDKRVSEERNERLLE ELLRILDENAELLKRNLELLKEVLYRTR **SEQ ID NO:161** DESDRIRKIVEESDEIVKESRKLAERARELIKESEDKRVSEERNERLLEEL LRILDENAELLKRNLELLKEVLYRTR **SEQ ID NO: 389**
DHD131	Heterodimer	b	GSDEDDELERLLREYHRVLREYEKLLEELRRLYEEYKRGEVSEEESDRILR EIKEILDKSERLWDLSEEVWRTLLYQAE **SEQ ID NO:162** DEDDELERLLREYHRVLREYEKLLEELRRLYEEYKRGEVSEEESDRILREI KEILDKSERLWDLSEEVWRTLLYQAE **SEQ ID NO: 464**
DHD132	Heterodimer	a	GSDKKDASRRAIRVLHEFVRVSEEVLEVLRKSVESLKRLDVDEKIKRTHDR IEEELRRWKRELEELIERLREWEYHQD **SEQ ID NO:163** DKKDASRRAIRVLHEFVRVSEEVLEVLRKSVESLKRLDVDEKIKRTHDRIE EELRRWKRELEELIERLREWEYHQD **SEQ ID NO: 391**
DHD132	Heterodimer	b	GSDDEEEDKRLLEEVKRSLDTDERILEKLRHSLERQLEDVDKDEDSRRVLR ELDEITKRSREVVKRLRKLAYESK **SEQ ID NO:164** DDEEEDKRLLEEVKRSLDTDERILEKLRHSLERQLEDVDKDEDSRRVLREL DEITKRSREVVKRLRKLAYESK **SEQ ID NO: 466**
DHD133	Heterodimer	a	GSDKEYKLDRILRRLDELIKQLSRILEEIERLVDELEREPLDDKEVQDVIE RIVELIDEHLELLKEYIKLLEEYIKTTK **SEQ ID NO:165** DKEYKLDRILRRLDELIKQLSRILEEIERLVDELEREPLDDKEVQDVIERI

			VELIDEHLELLKEYIKLLEEYIKTTK **SEQ ID NO: 393**
DHD133	Heterodimer	b	GSPSKEYQEKSAERQKELLHEYEKLVRHLRELVEKLQRRELDKEEVLRRLV EILERLKDLHKKIEDAHRKNEEAHKENK **SEQ ID NO:166** PSKEYQEKSAERQKELLHEYEKLVRHLRELVEKLQRRELDKEEVLRRLVEI LERLKDLHKKIEDAHRKNEEAHKENK **SEQ ID NO: 468**
DHD134	Heterodimer	a	GSRDRKISEELIKALEDHIRMLEELIRAIEEHIKLAERGVDEKELRESLEE LKKIVDELEKSLEELRKLAERYKYETR **SEQ ID NO:167** RDRKISEELIKALEDHIRMLEELIRAIEEHIKLAERGVDEKELRESLEELK KIVDELEKSLEELRKLAERYKYETR **SEQ ID NO: 395**
DHD134	Heterodimer	b	GSPKEESVEELKRVIDKHEEILRELKRVLEEHERVSHDEDENELRRSLERL KHILDRLHESLKELHELLKKNEYTER **SEQ ID NO:168** PKEESVEELKRVIDKHEEILRELKRVLEEHERVSHDEDENELRRSLERLKH ILDRLHESLKELHELLKKNEYTER **SEQ ID NO: 470**
DHD135	Heterodimer	a	GSDHEYWVKIVERILRVMEKHAEIVKKHLEIVERVVREGPSEDLRRKLKES LREIEESLRELKELLDELDELSEKTR **SEQ ID NO:169** DHEYWVKIVERILRVMEKHAEIVKKHLEIVERVVREGPSEDLRRKLKESLR EIEESLRELKELLDELDELSEKTR **SEQ ID NO: 397**
DHD135	Heterodimer	b	GSDEEYVTRSQRRLKRLLEEYIKVVEEHARLVERNERDDKELKRSIDELDK LTKELLELVKRYKELVDKTET **SEQ ID NO:170** DEEYVTRSQRRLKRLLEEYIKVVEEHARLVERNERDDKELKRSIDELDKLT KELLELVKRYKELVDKTET **SEQ ID NO: 472**
DHD136	Heterodimer	a	GSDKEEIVKLQDEVIKTLERHLDILRKHIDLLEKLKDHLSEELKERVDRSI KKLEESIKRLERIIEELQELAEYSL **SEQ ID NO:171** DKEEIVKLQDEVIKTLERHLDILRKHIDLLEKLKDHLSEELKERVDRSIKK LEESIKRLERIIEELQELAEYSL **SEQ ID NO: 399**
DHD136	Heterodimer	b	GSREEELKESAEELERSVRELKKEADKYKEEVDRLHYRGKVDKDWVRVVEK LIKLVEEHLELIREHLELLKEERR **SEQ ID NO:172** REEELKESAEELERSVRELKKEADKYKEEVDRLHYRGKVDKDWVRVVEKLI KLVEEHLELIREHLELLKEERR **SEQ ID NO: 474**
DHD137	Heterodimer	a	GSDMEYELKKSAEELRKSLEELKRILDELHKSLRELRRHGDDEEYVQTVEE LRKELEEHAKKLEEHLKELERVAT **SEQ ID NO:173** DMEYELKKSAEELRKSLEELKRILDELHKSLRELRRHGDDEEYVQTVEELR KELEEHAKKLEEHLKELERVAT **SEQ ID NO: 401**
DHD137	Heterodimer	b	PEYELKKSVDDLKRDVDRLVEEVEEVFELSKERLREDRKHLELVEEMVRLI EKHLELIKEHLKLADDHVR **SEQ ID NO:174**
DHD138	Heterodimer	a	GSREKDESKELNDEYKKLLEEYERLLRRSEELVKRAKGPRDEKELKRILEE NEDILRRTKEILERTKEISEEQKYRRR **SEQ ID NO:175** REKDESKELNDEYKKLLEEYERLLRRSEELVKRAKGPRDEKELKRILEENE DILRRTKEILERTKEISEEQKYRRR **SEQ ID NO: 403**
DHD138	Heterodimer	b	GSDKDERQERLNEESDKSNEESERSNRESEELNRRARGPNDEKELQEILDR HLELLERNQRLLDENKEILRESQYLND **SEQ ID NO:176** DKDERQERLNEESDKSNEESERSNRESEELNRRARGPNDEKELQEILDRHL ELLERNQRLLDENKEILRESQYLND **SEQ ID NO: 476**
DHD139	Heterodimer	a	GSENKYILKEILKLLRENLKLLHDILRLLDENLEELEKHGAKDLDDYRRKI EEIRKKVEDYREKIEEIEKKVERDR **SEQ ID NO:177** ENKYILKEILKLLRENLKLLHDILRLLDENLEELEKHGAKDLDDYRRKIEE IRKKVEDYREKIEEIEKKVERDR **SEQ ID NO: 405**
DHD139	Heterodimer	b	GSESEYTQEEILELLKESIKLLREILRLLEESEELWRRENTKSERSEEIKE RAKEAIKRSEEILERVKRLSDHSR **SEQ ID NO:178** ESEYTQEEILELLKESIKLLREILRLLEESEELWRRENTKSERSEEIKERA KEAIKRSEEILERVKRLSDHSR **SEQ ID NO: 478**
DHD140	Heterodimer	a	GSDEEEANYVSDKAVKIAEDVQELLKELLELSEVVRRGEVDEDEYDRVLRK LQEVMKEYEEVLKEYEEVSRKHE **SEQ ID NO:179** DEEEANYVSDKAVKIAEDVQELLKELLELSEVVRRGEVDEDEYDRVLRKLQ EVMKEYEEVLKEYEEVSRKHE **SEQ ID NO: 407**
DHD140	Heterodimer	b	GSPEKYLIKTQEELLRRHAEILEDLIRKVERQVDLRRKVDERDEDLKRELE RSLRELERLVRESSRLVEEIRELSKEIKR **SEQ ID NO:180** PEKYLIKTQEELLRRHAEILEDLIRKVERQVDLRRKVDERDEDLKRELERS

			LRELERLVRESSRLVEEIRELSKEIKR **SEQ ID NO: 480**
DHD141	Heterodimer	a	GSDEEYELERISRESKELLERYKRLLREYQELLKELRHVKDLDRAVKIIHE LMRVSKELVEISHRLLELHERLVRRRK **SEQ ID NO:181** DEEYELERISRESKELLERYKRLLREYQELLKELRHVKDLDRAVKIIHELM RVSKELVEISHRLLELHERLVRRRK **SEQ ID NO: 409**
DHD141	Heterodimer	b	GSEKEYIEKLSRKIEEDIRRSEERAKDSERLVRRLEELAKRKRLDLDDVLR VAEENLEILEDNLRILEEILKEQDKSNR **SEQ ID NO:182** EKEYIEKLSRKIEEDIRRSEERAKDSERLVRRLEELAKRKRLDLDDVLRVA EENLEILEDNLRILEEILKEQDKSNR **SEQ ID NO: 482**
DHD142	Heterodimer	a	GSPHEEVVELHERVMEISERAVELIQRIIDIIRRIREDDKDIEKLVKTIRD LVREYEELHRELEEIDEEIYKKSE **SEQ ID NO:183** PHEEVVELHERVMEISERAVELIQRIIDIIRRIREDDKDIEKLVKTIRDLV REYEELHRELEEIDEEIYKKSE **SEQ ID NO: 411**
DHD142	Heterodimer	b	GSDHEDVVRLHEDLVRKQEDARRVLEEIVRLAEEIVEVIKKDEKDKDRVTR LVEEIEKLVEEYKKKVDEMRKISDEIKYRSR **SEQ ID NO:184** DHEDVVRLHEDLVRKQEDARRVLEEIVRLAEEIVEVIKKDEKDKDRVTRLV EEIEKLVEEYKKKVDEMRKISDEIKYRSR **SEQ ID NO: 484**
DHD143	Heterodimer	a	GSRAREVVKRAKRIIEEWQKILEEWRRILEEWRRLLEDERVDDRDNERIIR ENERVIRENEKIIRDVIRLLEELLYERR **SEQ ID NO:185** RAREVVKRAKRIIEEWQKILEEWRRILEEWRRLLEDERVDDRDNERIIREN ERVIRENEKIIRDVIRLLEELLYERR **SEQ ID NO: 413**
DHD143	Heterodimer	b	GSREDEELEEEIDRIRQMVEEYEELVKEYEELTEKYKQGKVDKEESKKIIE KSERLLDLSQDAVRKVKEIIRRILYTNR **SEQ ID NO:186** REDEELEEEIDRIRQMVEEYEELVKEYEELTEKYKQGKVDKEESKKIIEKS ERLLDLSQDAVRKVKEIIRRILYTNR **SEQ ID NO: 486**
DHD144	Heterodimer	a	GSPKEEIVKLHDESAELHRRSVEVADEILKMHERSKDVDDERESRELSKEI ERLIREVEEVSKRIKRLSEEVEYLVR **SEQ ID NO:187** PKEEIVKLHDESAELHRRSVEVADEILKMHERSKDVDDERESRELSKEIER LIREVEEVSKRIKRLSEEVEYLVR **SEQ ID NO: 415**
DHD144	Heterodimer	b	GSPLEEILKIQRRINKIQDDINKILHEILRMQEKLNRSSDKDEVEESLRRI RELIKRIKDLSKEIEDLSREVKYRTT **SEQ ID NO:188** PLEEILKIQRRINKIQDDINKILHEILRMQEKLNRSSDKDEVEESLRRIRE LIKRIKDLSKEIEDLSREVKYRTT **SEQ ID NO: 488**
DHD145	Heterodimer	a	GSPEDEHVYVVREIYEVLREHAEVLEENREVIERLLEAKKRGDKSEELVKE LKKSIDKLKEISRKLEEIVKELEKVSEKLK **SEQ ID NO:189** PEDEHVYVVREIYEVLREHAEVLEENREVIERLLEAKKRGDKSEELVKELK KSIDKLKEISRKLEEIVKELEKVSEKLK **SEQ ID NO: 417**
DHD145	Heterodimer	b	GSDEDETSYRILELLREIVRASRELIRLSEELLEVARRDDKDETVLETLIR EYKELLDRYRRLIEELTRLVEEYEERSR **SEQ ID NO:190** DEDETSYRILELLREIVRASRELIRLSEELLEVARRDDKDETVLETLIREY KELLDRYRRLIEELTRLVEEYEERSR **SEQ ID NO: 490**
DHD146	Heterodimer	a	GSTQEEINRIQHEVLRIQEEIDEILRDIVEKLKAISRGELDHEVVKDVEDK VREALEKSEELLDKSRKVEYKSE **SEQ ID NO:191** TQEEINRIQHEVLRIQEEIDEILRDIVEKLKAISRGELDHEVVKDVEDKVR EALEKSEELLDKSRKVEYKSE **SEQ ID NO: 419**
DHD146	Heterodimer	b	GSDEEELNRELLEKSKRLVDINRDIIRTAQELIEMLKDSKDGRVDEDTKRE LRDKLRKLEEKLERVREELRKYEELLRYVQR **SEQ ID NO:192** DEEELNRELLEKSKRLVDINRDIIRTAQELIEMLKDSKDGRVDEDTKRELR DKLRKLEEKLERVREELRKYEELLRYVQR **SEQ ID NO: 492**
DHD147	Heterodimer	a	GSDEKDRVYEILKEVQRLVKEYRDISKEIEDLVKHYEHITDDEAQEVSKEL IDKSLRASEIVRELIRLIKELLDELE **SEQ ID NO:193** DEKDRVYEILKEVQRLVKEYRDISKEIEDLVKHYEHITDDEAQEVSKELID KSLRASEIVRELIRLIKELLDELE **SEQ ID NO: 421**
DHD147	Heterodimer	b	GSDEEDVLYHLRELLEELKRVSDDYERLVREIKETSERKDRDTKENKDMLD ELVKAHREQEKLLERLVRLLEELFERKR **SEQ ID NO:194** DEEDVLYHLRELLEELKRVSDDYERLVREIKETSERKDRDTKENKDMLDEL VKAHREQEKLLERLVRLLEELFERKR **SEQ ID NO: 494**
DHD1	Heterodimer	a	PREQAIRISEEIIRISKKIIEILERTRSSTAREAMKWAKDSIRLAEESKYL

			LDK **SEQ ID NO:195**
DHD1	Heterodimer	b	IEDDVKKIQDSTKKAQKETIEALERSTSSTARKQMEEQKEQIRLQKEAMYL LKK **SEQ ID NO:196**
DHD2	Heterodimer	a	SREEIAKLQEEVIKLQRRVIELQKEVIELQRRAKELTSSYTKEILEIQRRI EEIQREIEEIQKRIEEIQEEIQRRT **SEQ ID NO:197**
DHD2	Heterodimer	b	SDEEIKRLSEEVIQLSRRVIKMSREAIKLSREVQKLTPSYQKRIKEIADRS IELARESIEIAKRSEKIAEESQRRT **SEQ ID NO:198**
DHD3	Heterodimer	a	PAKDEALKMANESLELAKKSARLIQESSSKEILERIEKIQRRIAELQDRIA YLIKK **SEQ ID NO:199**
DHD3	Heterodimer	b	PAKDEALRMIDESRELIKKSNELIQRSSSKEILERILEIQRKIAELQKRIQ YLLKS **SEQ ID NO:200**
DHD4	Heterodimer	a	TDEARYRSERIVKEAKRLLDEARRRSEKIVREAKQRSNSEDAKRIMEENLR ESEEAARRLREIIRRNLEESRETG **SEQ ID NO:201**
DHD4	Heterodimer	b	TREALEYQRKMAEEIEDLLREALRRQEEMVREAKQRSLSEEFKRIMERILE EQERVMRLAKEALERILEEQKRTG **SEQ ID NO:202**
DHD5	Heterodimer	a	SERTKREAKRSQEEILREAKEAMRRAKESQDHRQNRDGSNSEDLERLSQEQ KRELEEVERRLKELAREQKYKLEDS **SEQ ID NO:203**
DHD5	Heterodimer	b	SEDLKRILKEITERELKLMQDLMEILKKITEDENNLDSNNSEDLKRSIEKA RRILDEALRKLEESARRAKYIQEDN **SEQ ID NO:204**
DHD6	Heterodimer	a	TEDEIRESLKWLDEVLQELREIARESNEVLERNRQKSRSDKLREDIERYKK RMEEARKKLDDQLNKYKKRMDENRS **SEQ ID NO:205**
DHD6	Heterodimer	b	TEEELKESKKFAEDLARSARRALKESKRVLEEISQASRSKKLEEIVRRYKE QVKRWQDEWDERAREYRKRMKENRS **SEQ ID NO:206**
DHD7	Heterodimer	a	TKTEEIERLAREIKKLSEKVERLAQEIEELSRRVKEENSTDRELKEANREI ERAIREIEKANKRMEEALRRMKYNG **SEQ ID NO:207**
DHD7	Heterodimer	b	TKTEEHERLAREISKLADEHRKLAKIIEELARRIKEENLTDDELREAIRKI EDALRKNKEALKIMKEAAERNRYNT **SEQ ID NO:208**
DHD8	Heterodimer	a	TKKEESRELARESEELARESEKLARKSLELARRAESSGSEEEKRRIIDENR KIIERNREIIERNKEIIEYNKELIS **SEQ ID NO:209**
DHD8	Heterodimer	b	TKDEESLELNRESEELNRKSEELNRKSKELNDRAESSNSEEEEKEILREHK EILREHLEILRRHKEILRRHKYLTS **SEQ ID NO:210**
DHD16	Heterodimer	a	TREELLRENIELAKEHIEIMREILELLQKMEELLERQSSEDILEELRKIIE RIRELLDRSRKIHERSEEIAYKEE **SEQ ID NO:211**
DHD16	Heterodimer	b	SEDIAREIKELLRRLKEIIERNQRIAKEHEYIARERKKLDPSNEKERKLLE RSRRLQEESKRLLDEMAEIMRRIKKLLD **SEQ ID NO:212**
DHD18	Heterodimer	a	DRQKLIEENIKLLDKHIKILEEILRLLKKDIDLLKKSSSEEVLEELKKIHR RIDKLLDESKKIHKRSSEIVKKRS **SEQ ID NO:213**
DHD18	Heterodimer	b	DEQKLIETSQRLQEKSERLLEKFEQILREASDLYRKPDSEELLRRVEKLLR ELEKLIRENQDLARKHEKILRDQS **SEQ ID NO:214**
DHD19	Heterodimer	a	DRQELIRENIELLKKHIKIVKEIQKLIETFIELLKKSSSEEILRRLKKILK RIEKLYRESQEIHKRSEEIAKKRQ **SEQ ID NO:215**
DHD19	Heterodimer	b	DEERLIDKSRELQKESEELLKELLKIFKRIEELLEKPDSEELIREIKKLLE TLSEIHKRNEKLARTHEEILRQQS **SEQ ID NO:216**
DHD22	Heterodimer	a	STRDVQREIAKAFKKMADVQKKLAEEIKRHVKNVEKKNKDNDEYRKIATEL LKKATESQKKLKELLDRIRKSDS **SEQ ID NO:217**
DHD22	Heterodimer	b	DKDDRSTSLLKRVEKLIDESDRIIDKFTTLIELSRNGKIDDDQYKKELKEI LELLKKYDKHVKEVEELLKRLNS **SEQ ID NO:218**
DHD23	Heterodimer	a	SKRKALEVSERVVRISEKVVRVLDESSDLLKKSYDDSDKFAELIDRHEEKI KKWKKLIKEWLEIIQRHKS **SEQ ID NO:219**
DHD23	Heterodimer	b	SAEEFVKLSEEAVKRSKEILDIVRKQVKLVKAGVDKHEITDSLRKSEKLIE EHKELIKTHRDLLRREN **SEQ ID NO:220**
DHD24	Heterodimer	a	SSTEILKRFKRALRESEKIVKHSRRVLKIIREVLKQKPTQAVHDLVRIIET QVKALEEQLKVLKRIVEALERQS **SEQ ID NO:221**
DHD24	Heterodimer	b	DKQKEIKDILEKTRRIAEESRKIAEKFDEIIKRSTEGKIDESLTKELEELV KEVIKLSEDDARTSDDLVRKES **SEQ ID NO:222**
DHD26	Heterodimer	a	DEDESIKLTRKSIEETRKSLKIIKEVVELIREVLKHIKDLDKEIFERIDKI LDKYKKQVDTYDEILKEYEKKQR **SEQ ID NO:223**

DHD26	Heterodimer	b	SELDEQKELIKKQEKLIEEQQRLLSKIRRMFKERVKDQELLREIQKVLKRS QEIVETSKKILDRSDKTTE **SEQ ID NO:224**
DHD28	Heterodimer	a	DQKEINTRIVEKLERIFKKSKEIVRQSERVISTIEKKTEDERELDLLRRHV KIVREHLKLLEELLKIIKEVQKESE **SEQ ID NO:225**
DHD28	Heterodimer	b	DTEELVKRLNELLKELSKLVKEFIKILETYRKDQTKDTSKISERVDRILKT YEDLLQKYKEILEKIEKQLS **SEQ ID NO:226**
DHD29	Heterodimer	a	DYARLIDQAVEVTRKVVEVNVTVARVNDKFAKHLGDEELRRVSEHLKEVSK DLQEVAKKSKDAARQVK **SEQ ID NO:227**
DHD29	Heterodimer	b	DVSKVAEEYLQISKTLVDISRTLLEISERLVRLVRTVADDRSEVKKAIEDS IEVLKTSEEVVRQIKRASDKLVKAIS **SEQ ID NO:228**
DHD31	Heterodimer	a	DAKEIQRRVVEIQTEVVKLQKKAVDIIRKIIEAFNNSNIDQSLLEAAKEIV KEIDKLEKLTESLLEESKKLLKRSS **SEQ ID NO:229**
DHD31	Heterodimer	b	SAEEVVKLAKIFLELLRESIKLLKRSVDLLRKSSDPSLDKSEAEKVSREIE KVSDTSLKLSKKALDVVKRALKVAS **SEQ ID NO:230**
DHD32	Heterodimer	a	DEKDAARKARKVSEEAKEASKKIEKALEESKRILNTLKQKKDEQEVKVIKE HEDVLRQIEKIQKQVLEIQKEVAKLLESLD **SEQ ID NO:231**
DHD32	Heterodimer	b	SADDVARASEKVLRVARESAKAADKSLEVFKEVVKRGDKEAFLQVVKINEE VVKINITVIRILIEVSKTAT **SEQ ID NO:232**
DHD38	Heterodimer	a	DEYVKETLKQLREALASLREADKRITELVKEARKKPLSEAARKFAEAIVTH VKVVVEHVEVVLRHVEVLVEAKKNGVIDKSILDNALRIIENVIRLLSNVIR VVDEVLQDLD **SEQ ID NO:233**
DHD38	Heterodimer	b	DASDVIRRIHELFEEVHRLIEAVHRAIEDVAKAAQKKGLDESAVEILAELS KELAKLSRRLAEISREIQKVVTDPDDKEAVERLKEIIKEIKKQLDELRDRL RKLQDLLYKLK **SEQ ID NO:234**
DHD60	Heterodimer	a	SEDKAHHDIVRVLEELIKIHDELMKISEEILKATSDSTATDETKEELKRRS KEAQKKSDTLVKIVKELEKESRKAQS **SEQ ID NO:235**
DHD60	Heterodimer	b	DDEEKYRQIIREAQEISKTAKRILRDAQEISKRIRHQGVDRSEHQRLVDLL RELIKEHHKLLRRQQEADTRND **SEQ ID NO:236**
DHD63	Heterodimer	a	DRKDKARKASEKLEEVIQRWKTVADKWKKMVDLVSNGKLSQEEVARVTEEL LKIQTELAKLLEEHAKVLQESAS **SEQ ID NO:237**
DHD63	Heterodimer	b	SDEESIKTQSELIKTSEELLKDVKRIDEELQKLRDDPTLDESELKKRVKEW SDRVRKAKEISRKIQEIVKESKKRSS **SEQ ID NO:238**
DHD66	Heterodimer	a	DKDEELRKVIEKYREMVKEYRKVIREYEEVIKSSKTIDKSSLISLSRKMVE LSQRVIDVSDEVAKVLSRKQS **SEQ ID NO:239**
DHD66	Heterodimer	b	TDEERLKKQTKELKEQTKQLEKQKDLLEKISNGEISKDEIQEIIKESKKIA KESQKALDSSRKALEEVS **SEQ ID NO:240**
DHD67	Heterodimer	a	DEKEVSKEIIKVLKDIAKVQQKVIEVSQRLASVLRADDDNVVKRALEEYEK ILEELRELNKEIEKLTDKYRKVTS **SEQ ID NO:241**
DHD67	Heterodimer	b	DSDEQTKELEKLTELHKRHVEKLKKQTKESREVDSNKLWKSKDVKDKLSES EKELQKLSDQDKKAKDALESSRRKND **SEQ ID NO:242**
DHD69	Heterodimer	a	DAEEQLKLLTKLLRHQQRLLQLIKESLKLIEKIDQSSQENQDEIRKWREVT KKLRELIKTSEKLVRELEKSYKKSS **SEQ ID NO:243**
DHD69	Heterodimer	b	SLRDVVRRYQELVRRYDELIKTLTEILKKYQKKGAEDKDASTELVKAVRTS LKLSKELLKLNSELLKEDS **SEQ ID NO:244**
DHD71	Heterodimer	a	SKEELKRKLDELKKRSDTLKELSKKLKEISERNPDDKSVHRTIIRIHREFV KNHKEIVRVIEEIVSDKS **SEQ ID NO:245**
DHD71	Heterodimer	b	SKQDEHDRLLKIHDKLVKQHDELLKLLTKLSRAGDSVTKKKLEEILRKLQE VSKQLEESLKDADKVSKDIN **SEQ ID NO:246**
DHD72	Heterodimer	a	TVQSLLEQHVKIVKRSIEILERHTQILQDIARSQGVSKELEDVERQVKEYR KEVKKLEEDLRQLSRNSK **SEQ ID NO:247**
DHD72	Heterodimer	b	SDSDRIEKLIRESTELLKEQQKLAKRSRELAETVESLPLTEEYLKQQREHQ KKIEKLLKDSEKHLEELKRLVKSEK **SEQ ID NO:248**
DHD73	Heterodimer	a	DSEKRIEDILRTDLELAKRDAELVKEHIKLVKRIDLSEELKKQVEDVEKES KKLEDSSEKLVQKVRKRSS **SEQ ID NO:249**
DHD73	Heterodimer	b	DEEERAKDLRKYLEEQTQYYRTVTEHLRNLEKVVEELERRGKPSSELQQIL ERSQRIYKETTEIYDTSKKLIEELDKHHR **SEQ ID NO:250**
DHD148	Heterodimer	a	PLEDILKRHLDKVRELVRLSEEVNKLAKEVLDILKDKRVDEKELDKVLKEL EKVVEEYERAVKESRDLLRELRETTR **SEQ ID NO:251**

DHD148	Heterodimer	b	DKERLLEIHERIQKLLDRNLEIIERLLRLLREARDIKDDDKLDKVIKRLKE LSEESKDILDKIKELLKESEKELT **SEQ ID NO:252**
DHD149	Heterodimer	a	PEDEVIRVIEELLRIAAEVDEVHRRNVEVQEEASRVTDRERLERLNRESEE LIKRSRELIEEQRKLIERLERLAT **SEQ ID NO:253**
DHD149	Heterodimer	b	DLEELIKEYAEVVRRHHKAVRDLERLVRELANAKHASEEELKRIATEILRI VKELIRVQERLIKLSEDSNEESR **SEQ ID NO:254**
DHD150	Heterodimer	a	PTDEVIEVLKELLRIHRENLRVNEEIVEVNERASRVTDREELERLLRRSNE LIKRSRELNEESKKLIEKLERLAT **SEQ ID NO:255**
DHD150	Heterodimer	b	DNEEIIKEARRVVEEYKKAVDRLEELVRRAENAKHASEKELKDIVREILRI SKELNKVSERLIELWERSQERAR **SEQ ID NO:256**
DHD151	Heterodimer	a	PKEDIDRVSRELVRVHKELLEVLRKSTEIVEAVARNEKDERTIEEVLEEQE RAVRKLEEVSKKHKEAVKRLK **SEQ ID NO:257**
DHD151	Heterodimer	b	ELERLSEEIQKLSDRLIELIRRHSKVLEEIVRLLKHKDNDEREVRRLLKLL RDLTRRYEEVLRKVEEIVKRQEDESR **SEQ ID NO:258**
DHD152	Heterodimer	a	PEEDILRLLRKLVEVDKELLEVVRESTEVVRLVARNEKDVETVERVLRKQE EVVRKYERVSRELEEAVRRLK **SEQ ID NO:259**
DHD152	Heterodimer	b	ELKDLVEEIVKLSKENLKLWEDHSRVLEEIVRLLKHKDNDEREVRRLLKLL EDLTRRAEETSRRIEEIVKEAEDRAR **SEQ ID NO:260**
DHD153	Heterodimer	a	DEERELREVLRKHHRVVREWTKVVEELKRVVELLKRGETSEEDLLRVLKKL LEMDKRILEVNREVLRVLEKRLT **SEQ ID NO:261**
DHD153	Heterodimer	b	SLEEIIEELVELVRRSVEIAKESDEVARRIVESEDKKKELIDTLRDLHREW QEVTKRAEELVREAEKEVR **SEQ ID NO:262**
DHD154	Heterodimer	a	TAEELLEVHKKSDRVTKEHLRVSEEILKVVEVLTRGEVSSEVLKRVLRKLE ELTDKLRRVTEEQRRVVEKLN **SEQ ID NO:263**
DHD154	Heterodimer	b	DLEDLLRRLRRLVDEQRRLVEELERVSRRLEKAVRDNEDERELARLSREHS DIQDKHDKLAREILEVLKRLLERTE **SEQ ID NO:264**
DHD155	Heterodimer	a	PEDDVVRIIKEDLESNREVLREQKEIHRILELVTRGEVSEEAIDRVLKRQE DLLKKQKESTDKARKVVEERR **SEQ ID NO:265**
DHD155	Heterodimer	b	DEVRLITEWLKLSEESTRLLKELVELTRLLRNNVPNVEEILREHERISREL ERLSRRLKDLADKLERTRR **SEQ ID NO:266**
DHD156	Heterodimer	a	DEDEVVKVHEEHVKSHEEIHRSHEEVVRAAEEDKRDSRELRTLMEEHRKLL EENEKSIEEVKKIHERVKR **SEQ ID NO:267**
DHD156	Heterodimer	b	KKEELIDISKEVLDLDDEINKISKEILELIKKLLRLKEEGREDKDKAREVK RRIRELHRRIQELNKRLRELHKRVQETKR **SEQ ID NO:268**
DHD157	Heterodimer	a	PEEDIARRVEDLLRKSEELIKESEKILKESKRLLDRNDSDKRVLETNLRLI DKHTKLLERNLELLEELLKLAEDVAK **SEQ ID NO:269**
DHD157	Heterodimer	b	RFKDLSREYIEVVKRLLELSREALEVLREIKDTDKTDKKRIKELIDRLRKL IEEYKRIIDRLRKLSKDLEEEHR **SEQ ID NO:270**
DHD158	Heterodimer	a	DEEELVKILKELQRLSEESLEINKRLVEILRLLRRGEVPKEEVEKKLREIK KEQEKLDREHEKIKKRIEEITK **SEQ ID NO:271**
DHD158	Heterodimer	b	SLKEKILEIIERNMKLVELSNRSVEIVARILKGEKDDEETLERLLREWDKI TRDYEEIIKESRKLVKELEEEAK **SEQ ID NO:272**
DHD159	Heterodimer	a	SKTEILRKALEIHKEQIDIVRKLIELSEEVLKLVEESKEKNLEKLKRIDEE TDRLLERLDELHKRLTELAERLK **SEQ ID NO:273**
DHD159	Heterodimer	b	SDDEARKQLEEMKRRLREVEKKSKRVEERVRELERLVRENREDEDRVLKTL EDLLRENEKLVRTIERHVREQRELSKEVK **SEQ ID NO:274**
DHD160	Heterodimer	a	SEEELEKKADELRKLSEEWRKLQEEDKRLSEMVEKGELDLQEVDEHSLRVL ERATEVHRTVDKVIEEILRTTN **SEQ ID NO:275**
DHD160	Heterodimer	b	SEKERHRESQETQEEIRRTHEEIIRKLEEILRRAKAGELPEETLDRLRRIM ERLKELSERLDDLVRKLRDDHRREQK **SEQ ID NO:276**
DHD161	Heterodimer	a	SEKEILEELKRILKRVKDISDRLEELDKRTEEIARREPTKELVDELVKIHR DWLRLHEEILKLVDDALKKVEDATK **SEQ ID NO:277**
DHD161	Heterodimer	b	DLRELLELQREASRLHRELVKLLTELVKKLELIAKGEDIREEDLKRIKERL EEIKKRSKRIKEESDEIDKKTK **SEQ ID NO:278**
DHD162	Heterodimer	a	SERELQRELNKIVRRILEIHREVSELHQRAVKLIRENDNSEELEEISRRIE ELSKELEKLVREHDEIVKTIE **SEQ ID NO:279**
DHD162	Heterodimer	b	SEREKLDRNDEELKEINKRVEEIKERSDRITEAIEKNERSEEEIRRLSREQ NEALQRLLELHKKLVKLHRELLEDTR **SEQ ID NO:280**

DHD163	Heterodimer	a	DKEDVIRVHDEQHKLIEEQLELTRRIAELVREIAKNTASEEEIKEMLKEIK RLDDRSREIQDRLQKLLEEIRRKTK **SEQ ID NO:281**
DHD163	Heterodimer	b	TEEEIVELNKDIQRKSKEHIDLQNELVKKIERAIRENNITEELLEELERLL RESEKIVEEIRRITDKIRKDAK **SEQ ID NO:282**
DHD164	Heterodimer	a	SEKEILERLLRLSKEQNEISEEIHRLTERLVELKRRKDDDERLKRILDRQK RLVERAREISKEYEDLLRKLE **SEQ ID NO:283**
DHD164	Heterodimer	b	SMEELLRKNARLSRKQLKIIDEHLELSTKLTRGEAGDETLEEIERRSREML EEQRRVDEESKRIREKLK **SEQ ID NO:284**
DHD165	Heterodimer	a	SEEEIRDIVEKLLRTHEEVLKEIKKLLDDSERVRRRELDKKDLDRIQKEQR DIQEENKEKAKRFDELVKELKKAAK **SEQ ID NO:285**
DHD165	Heterodimer	b	SEEEHRRTMEKVEKEVRDIKRRSEEVKKKVKANTLSEEDLVRLLERLVEDH KRLQDLSQEIIERDEKATK **SEQ ID NO:286**
DHD166	Heterodimer	a	DEDELAKEIEDVQRRNKESQEEHDKSVKKLEAAERGEIDEDSLLRVLEEDI KVLEKDIEVLERSIEVIEKAE **SEQ ID NO:287**
DHD166	Heterodimer	b	SEKELIRRLLEQQRQHLRLSERLIELSRRLVEVVRKGKDNRDLLRELKKLS EEHKKHSKDDHEKVREIREREK **SEQ ID NO:288**
DHS 17	Heterodimer	a	DRKDLLKRNIKLLDRHLKILDTILKLLEKLSELLKKSSSEEVVKEYKKILD EIRKLLEESKEIHKESKEILERES **SEQ ID NO:289**
DHD17	Heterodimer	b	DEEKLIERSKRLQEESEQLLEKFEQILRELTELLEKPDSEELARKIKKLHD ELRKIIKRNQELIREHEEILRKRD **SEQ ID NO:290**

In some aspects, non-limiting examples of the monomer A polypeptide and monomer B polypeptide pairs are shown in FIG. 16.

In some aspects, the amino acid sequence of SEQ ID NOs: 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494 useful for the monomer A polypeptide or the monomer B polypeptide is not linked to GlySer at the N terminus of the sequence or does not comprise GlySer at the N terminus. In some aspects, the monomer A polypeptide and/or the monomer B polypeptide comprises at least one amino acid, at least two amino acids, at least three amino acids, at least four amino acids, at least five amino acids, at least six amino acids, at least seven amino acids, at least eight amino acids, at least nine amino acids, or at least ten amino acids at the N terminus or the C terminus of the amino acid sequence. In some aspects, the additional amino acids are not GlySer at the N terminus.

In some aspects, the protein of the present disclosure comprises a heterodimer comprising a monomer A polypeptide and a monomer B polypeptide, wherein the monomer A polypeptide comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence as set forth in SEQ ID NO: 331, 5, 7, 13, 15, 25, 29, 31, 33, 35, 37, 39, 41, 45, 47, 53, 55, 57, 59, 61, 65, 67, 69, 71, 73, 75, 77, 79, 337, 339, 85, 87, 89, 91, 93,

95, 97, 99, 341, 103, 343, 107, 109, 111, 113, 459, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, or 421 and the monomer B polypeptide comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence as set forth in SEQ ID NO: 2, 332, 334, 336, 338, 340, 342, 344, 346, 348, 418, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 420, 422, 424, 426, 428, 126, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 174, 476, 478, 480, 482, 484, 486, 488, 490, 492, or 494, respectively.

In some aspects, the protein of the present disclosure comprises a heterodimer comprising a monomer A polypeptide and a monomer B polypeptide, wherein the monomer A polypeptide comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of SEQ ID NO: 331, 5, 7, 13, 15, 25, 29, 31, 33, 35, 37, 39, 41, 45, 47, 53, 55, 57, 59, 61, 65, 67, 69, 71, 73, 75, 77, 79, 337, 339, 85, 87, 89, 91, 93, 95, 97, 99, 341, 103, 343, 107, 109, 111, 113, 459, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, or 421, wherein the amino acid sequence of the monomer A polypeptide does not comprise GlySer at the N terminus, and the monomer B polypeptide comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of SEQ ID NO: 2, 332, 334, 336, 338, 340, 342, 344, 346, 348, 418, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 420, 422, 424, 426, 428, 126, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 174, 476, 478, 480, 482, 484, 486, 488, 490, 492, or 494, respectively, wherein the amino acid sequence of the monomer B polypeptide does not comprise GlySer at the N terminus.

In some aspects, the protein of the present disclosure comprises a heterodimer comprising a monomer A polypeptide and a monomer B polypeptide, wherein the monomer A polypeptide consists of an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of

the amino acid sequence as set forth in SEQ ID NO: 331, 5, 7, 13, 15, 25, 29, 31, 33, 35, 37, 39, 41, 45, 47, 53, 55, 57, 59, 61, 65, 67, 69, 71, 73, 75, 77, 79, 337, 339, 85, 87, 89, 91, 93, 95, 97, 99, 341, 103, 343, 107, 109, 111, 113, 459, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, or 421 and the monomer B polypeptide consists of an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence as set forth in SEQ ID NO: 2, 332, 334, 336, 338, 340, 342, 344, 346, 348, 418, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 420, 422, 424, 426, 428, 126, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 174, 476, 478, 480, 482, 484, 486, 488, 490, 492, or 494, respectively.

In one embodiment of any of the above embodiments, amino acid changes from the reference amino acid sequence are conservative amino acid substitutions. As used herein, "conservative amino acid substitution" means an amino acid substitution that does not alter or substantially alter polypeptide function or other characteristics. A given amino acid can be replaced by a residue having similar physiochemical characteristics, e.g., substituting one aliphatic residue for another (such as Ile, Val, Leu, or Ala for one another), or substitution of one polar residue for another (such as between Lys and Arg; Glu and Asp; or Gln and Asn). Other such conservative substitutions, e.g., substitutions of entire regions having similar hydrophobicity characteristics, are well known. Polypeptides comprising conservative amino acid substitutions can be tested in any one of the assays described herein to confirm that a desired activity, e.g. antigen-binding activity and specificity of a native or reference polypeptide is retained.

Amino acids can be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in Biochemistry, second ed., pp. 73-75, Worth Publishers, New York (1975)): (1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M); (2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q); (3) acidic: Asp (D), Glu (E); (4) basic: Lys (K), Arg (R), His (H). Alternatively, naturally occurring residues can be divided into groups based on common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; (6) aromatic: Trp, Tyr, Phe. Non-conservative substitutions will entail

exchanging a member of one of these classes for another class. Particular conservative substitutions include, for example; Ala into Gly or into Ser; Arg into Lys; Asn into Gln or into H is; Asp into Glu; Cys into Ser; Gln into Asn; Glu into Asp; Gly into Ala or into Pro; His into Asn or into Gln; Ile into Leu or into Val; Leu into Ile or into Val; Lys into Arg, into Gln or into Glu; Met into Leu, into Tyr or into Ile; Phe into Met, into Leu or into Tyr; Ser into Thr; Thr into Ser; Trp into Tyr; Tyr into Trp; and/or Phe into Val, into Ile or into Leu.

In another embodiment of any of the above embodiments, amino acid residues at 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of defined interface positions are invariant compared to the reference amino acid sequence. Table 2 below provides the residue numbers within each A and B monomer that are present at the interface in the heterodimer. The position of interface residues are the same for A-B binding partners. Table 2 is organized by heterodimer design name (see the left-hand column in Tables 1A and 1B). Note that for purpose of defining the position of interface residues for each polypeptide in Table 1A and 1B, the "GS" residues at the amino terminus, if present, are not included.

Table 2. Interface residues by position number across both chains 'a' and 'b'

DHD_1
[5, 6, 8, 9, 12, 13, 16, 19, 20, 22, 23, 31, 34, 35, 38, 41, 42, 45, 48, 52, 55, 59, 63, 66, 70, 73, 74, 77, 80, 81, 85, 88, 89, 92, 95, 96, 99, 102, 103, 106]

DHD_2
[5, 6, 9, 12, 13, 16, 19, 20, 23, 26, 27, 30, 33, 36, 37, 38, 41, 44, 45, 48, 51, 55, 58, 62, 65, 69, 72, 73, 76, 81, 85, 88, 89, 92, 95, 96, 99, 102, 103, 106, 109, 110, 112, 114, 117, 120, 123, 124, 127, 128, 131, 134, 135, 137, 138, 141, 144, 145, 148, 149, 152]

DHD_3
[6, 7, 10, 11, 13, 14, 17, 20, 21, 24, 25, 28, 33, 36, 40, 43, 44, 47, 50, 51, 54, 62, 63, 66, 69, 73, 76, 77, 80, 81, 84, 89, 92, 93, 96, 99, 100, 103, 106, 107, 110, 112]

DHD_4
[1, 8, 11, 12, 15, 19, 22, 26, 29, 30, 33, 38, 39, 42, 45, 46, 49, 50, 53, 56, 57, 60, 63, 64, 67, 68, 71, 75, 76, 80, 83, 87, 90, 94, 98, 101, 105, 108, 113, 114, 117, 121, 124, 125, 128, 132, 135, 139, 142, 143, 146, 150]

DHD_5
[4, 8, 12, 15, 16, 19, 22, 23, 26, 30, 34, 38, 39, 44, 48, 51, 55, 58, 62, 66, 69, 73, 76, 80, 84, 87, 88, 92, 95, 96, 98, 99, 102, 105, 106, 110, 114, 115, 117, 120, 124, 127, 131, 135, 138, 142, 145, 149, 152]

DHD_6
[1, 5, 8, 9, 11, 12, 15, 16, 19, 22, 23, 26, 27, 30, 33, 35, 37, 42, 46, 49, 53, 56, 60, 64, 67, 71, 74, 77, 81, 84, 87,

88, 91, 92, 95, 99, 102, 105, 106, 109, 110, 111, 112, 113, 118, 122, 125, 129, 132, 133, 136, 140, 143, 147, 150]

DHD_7
[3, 6, 10, 13, 17, 20, 24, 27, 31, 34, 44, 47, 48, 51, 55, 58, 61, 62, 65, 69, 72, 75, 76, 79, 82, 86, 89, 90, 93, 96, 100, 103, 107, 110, 115, 120, 124, 127, 130, 131, 134, 138, 141, 145, 148, 151, 152]

DHD_8
[6, 10, 13, 17, 20, 24, 27, 28, 31, 34, 47, 50, 54, 57, 61, 64, 68, 71, 75, 76, 82, 83, 86, 89, 93, 96, 100, 103, 107, 110, 123, 126, 130, 133, 134, 137, 140, 144, 147, 150, 151, 152]

DHD_9
[12, 16, 19, 23, 26, 30, 33, 38, 39, 42, 46, 49, 52, 53, 56, 59, 60, 63, 67, 69, 88, 92, 95, 99, 102, 106, 109, 115, 122, 125, 129, 132, 136, 139, 143, 145]

DHD_16
[6, 9, 10, 13, 16, 17, 20, 23, 24, 27, 28, 30, 34, 43, 46, 50, 53, 57, 60, 64, 67, 71, 72, 5, 8, 12, 15, 19, 22, 23, 26, 29, 33, 41, 50, 57, 60, 64, 67, 68, 71, 74, 78]

DHD_18
[3, 6, 9, 10, 13, 16, 17, 20, 23, 24, 27, 31, 34, 38, 43, 46, 50, 53, 57, 60, 64, 67, 68, 71, 75, 78, 81, 85, 88, 91, 95, 98, 102, 105, 106, 109, 118, 121, 125, 128, 132, 135, 136, 139, 142, 146, 149, 150]

DHD_19
[3, 6, 9, 10, 13, 16, 17, 20, 23, 24, 27, 30, 31, 34, 38, 43, 46, 50, 53, 57, 60, 61, 64, 67, 71, 75, 81, 88, 91, 95, 98, 99, 102, 105, 109, 118, 121, 125, 128, 129, 132, 135, 139, 142, 146, 149, 150]

DHD_22
[1, 2, 5, 6, 9, 10, 12, 13, 16, 19, 20, 23, 27, 30, 31, 33, 34, 38, 41, 44, 47, 48, 51, 55, 58, 62, 65, 69, 72, 80, 81, 84, 87, 91, 94, 97, 98, 101, 102, 105, 108, 118, 122, 125, 126, 129, 132, 136, 139, 143, 146, 147, 148]

DHD_23
[1, 5, 6, 8, 9, 12, 15, 16, 19, 22, 23, 26, 29, 30, 33, 34, 40, 43, 47, 51, 54, 57, 58, 61, 64, 65, 68, 72, 75, 76, 79, 82, 83, 86, 89, 90, 93, 96, 97, 100, 104, 109, 110, 113, 116, 120, 123, 126, 127, 130, 134, 138]

DHD_24
[1, 5, 9, 12, 16, 19, 22, 26, 29, 30, 33, 40, 41, 42, 45, 48, 49, 51, 52, 55, 56, 59, 62, 63, 66, 69, 70, 73, 77, 80, 83, 84, 87, 91, 94, 98, 101, 105, 108, 109, 113, 118, 121, 125, 128, 129, 132, 136, 139, 142, 143, 147]

DHD_26
[5, 8, 9, 12, 16, 19, 22, 23, 26, 29, 30, 33, 37, 40, 44, 45, 48, 51, 52, 55, 58, 59, 62, 65, 66, 69, 73, 77, 80, 84, 87, 91, 94, 95, 98, 101, 105, 109, 112, 114, 115, 118, 119, 122, 125, 126, 129, 132, 136, 139, 143]

DHD_28
[2, 5, 6, 9, 13, 16, 20, 23, 26, 27, 30, 31, 33, 34, 46, 47, 50, 53, 54, 57, 60, 61, 64, 67, 68, 71, 75, 78, 82, 85, 86, 89, 92, 93, 96, 99, 100, 103, 106, 110, 114, 115, 118, 121, 125, 128, 132, 135, 139, 142, 146, 147]

DHD_29
[2, 5, 6, 8, 9, 10, 12, 13, 16, 19, 20, 22, 23, 26, 27, 30, 33, 34, 39, 42, 45, 46, 49, 53, 56, 60, 63, 66, 67, 70, 71, 74, 77, 78, 81, 84, 85, 88, 91, 92, 95, 98, 99, 102, 106, 112, 115, 116, 119, 120, 122, 123, 126, 129, 130, 133, 137, 140, 141, 144, 145]

DHD_31
[2, 5, 6, 9, 10, 12, 13, 16, 17, 19, 20, 23, 24, 26, 27, 30, 31, 33, 34, 37, 38, 39, 43, 46, 50, 54, 57, 60, 61, 64, 65, 68, 71, 75, 78, 81, 82, 85, 88, 89, 92, 95, 96, 99, 102, 103, 106, 109, 110, 119, 123, 126, 130, 133, 134, 137, 140, 141, 144, 147, 148, 151, 152]

DHD_32
[5, 6, 9, 12, 16, 19, 23, 26, 27, 30, 33, 34, 37, 44, 48, 49, 52, 55, 56, 58, 59, 62, 63, 65, 66, 69, 70, 73, 76, 77, 79, 80, 82, 83, 86, 87, 90, 93, 94, 97, 100, 101, 104, 107, 108, 111, 114, 115, 118, 123, 124, 127, 130, 133, 134, 137, 138, 140, 141, 144, 145, 147, 148, 151, 152]

DHD_38
[42, 43, 46, 49, 50, 52, 53, 56, 57, 60, 63, 64, 67, 70, 71, 74, 77, 79, 80, 84, 87, 88, 91, 94, 95, 98, 101, 102, 105, 109, 116, 117, 120, 123, 124, 127, 131, 134, 137, 138, 141, 144, 145, 149, 153, 158, 161, 162, 165, 168, 169, 172, 175, 176, 179, 182, 183, 185, 186, 187, 189]

DHD_39
[2, 6, 9, 13, 16, 20, 23, 27, 29, 30, 34, 40, 43, 46, 47, 50, 51, 54, 57, 58, 60, 61, 64, 110, 114, 117, 121, 124, 128, 131, 132, 135, 138, 145, 149, 152, 156, 159, 160, 163, 164, 166, 167, 170, 173, 174, 176, 177, 178]

DHD_40
[5, 9, 11, 12, 15, 16, 19, 23, 26, 29, 30, 33, 43, 46, 49, 50, 53, 56, 57, 59, 60, 63, 64, 67, 71, 108, 112, 116, 119, 123, 127, 130, 133, 134, 137, 138, 148, 151, 154, 158, 161, 165, 168, 169, 172, 175]

DHD_43
[3, 4, 7, 11, 14, 18, 22, 25, 29, 34, 35, 39, 43, 46, 47, 50, 51, 54, 57, 58, 61, 62, 65, 71, 72, 75, 76, 79, 82, 83, 86, 87, 90, 93, 94, 97, 98, 100, 101, 104, 111, 114, 115, 117, 118, 122, 125, 126, 129, 133, 136, 137, 140, 144]

DHD_60
[6, 7, 9, 10, 13, 16, 17, 20, 23, 24, 27, 30, 31, 34, 35, 51, 54, 55, 58, 61, 62, 65, 68, 72, 75, 76, 77, 83, 87, 90, 91, 93, 94, 97, 100, 101, 104, 105, 108, 111, 115, 116, 121, 122, 124, 125, 128, 131, 132, 135, 136, 138, 139, 142, 143, 149]

DHD_63
[6, 10, 13, 17, 20, 24, 27, 30, 31, 34, 41, 44, 45, 48, 51, 52, 55, 56, 58, 59, 62, 65, 66, 68, 69, 70, 72, 73, 74, 80, 83, 84, 87, 90, 93, 94, 97, 104, 105, 118, 122, 125, 126, 129, 132, 136, 139, 140, 143, 146, 150]

DHD_65
[2, 5, 6, 8, 9, 12, 13, 16, 19, 20, 23, 26, 27, 29, 30, 33, 34, 37, 43, 47, 50, 54, 57, 61, 64, 68, 71, 72, 75, 82, 85, 86, 89, 92, 93, 96, 99, 100, 103, 106, 107, 110, 118, 120, 121, 123, 127, 128, 131, 134, 135, 138, 139, 141, 142, 145, 148]

DHD_66
[6, 10, 13, 16, 17, 20, 24, 27, 31, 34, 42, 43, 46, 49, 50, 53, 54, 56, 57, 60, 63, 64, 67, 68, 71, 78, 81, 82, 85, 88, 89, 92, 95, 99, 102, 103, 107, 112, 113, 115, 116, 119, 123, 126, 127, 130, 133, 137, 140, 141]

DHD_67
[6, 9, 10, 13, 16, 17, 20, 21, 23, 24, 27, 28, 30, 31, 34, 36, 42, 46, 49, 52, 53, 56, 60, 63, 67, 70, 74, 77, 80, 81, 84, 87, 88, 91, 94, 95, 98, 101, 102, 105, 110, 114, 123, 130, 131, 133, 134, 140, 144, 147, 151]

DHD_69
[2, 5, 6, 9, 10, 12, 13, 16, 17, 19, 20, 23, 26, 27, 30, 33, 35, 44, 47, 51, 54, 58, 61, 65, 68, 72, 75, 76, 78, 82, 85,

86, 89, 92, 96, 99, 100, 102, 103, 106, 107, 111, 117, 118, 120, 121, 124, 127, 128, 131, 134, 135, 138, 139, 141, 142, 146]

DHD_70
[6, 9, 10, 13, 16, 17, 20, 23, 24, 26, 27, 30, 31, 34, 37, 38, 41, 43, 44, 47, 50, 51, 54, 57, 58, 61, 64, 65, 68, 71, 72, 73, 74, 78, 81, 82, 85, 88, 89, 92, 95, 96, 99, 102, 103, 106, 107, 109, 110, 111, 119, 120, 123, 124, 127, 130, 131, 134, 137, 138, 141, 144, 145, 148]

DHD_71
[9, 16, 19, 23, 30, 34, 40, 43, 44, 46, 47, 50, 51, 54, 57, 58, 61, 64, 65, 69, 72, 75, 78, 79, 82, 85, 86, 89, 92, 93, 96, 97, 99, 100, 103, 106, 111, 115, 118, 119, 121, 122, 125, 128, 129, 132, 136, 139, 140]

DHD_72
[1, 2, 5, 6, 8, 9, 12, 16, 19, 23, 26, 27, 30, 33, 34, 40, 43, 46, 47, 50, 54, 57, 63, 64, 67, 68, 75, 78, 79, 82, 85, 86, 89, 92, 93, 96, 99, 102, 103, 106, 108, 112, 115, 116, 119, 120, 123, 126, 130, 133, 137, 140, 143]

DHD_73
[2, 6, 9, 10, 12, 16, 17, 23, 24, 27, 30, 31, 34, 36, 37, 40, 43, 44, 47, 51, 54, 57, 58, 61, 65, 69, 79, 82, 86, 89, 92, 93, 96, 99, 100, 103, 104, 107, 117, 120, 124, 127, 131, 134, 137, 138, 141, 142, 145, 148, 149]

DHD_88
[2, 3, 6, 9, 13, 16, 20, 23, 27, 30, 34, 37, 48, 51, 52, 55, 56, 58, 59, 62, 63, 65, 66, 69, 70, 72, 73, 76, 79, 80, 81, 83, 87, 90, 91, 94, 97, 98, 101, 104, 105, 108, 111, 112, 115, 116, 121, 125, 128, 132, 135, 136, 139, 140, 142, 143, 146, 149, 150, 153, 154]

DHD_89
[1, 2, 5, 8, 9, 12, 15, 16, 19, 22, 23, 26, 29, 30, 33, 34, 36, 37, 38, 48, 51, 55, 58, 62, 65, 69, 72, 79, 82, 83, 86, 87, 89, 90, 93, 94, 97, 100, 101, 103, 104, 107, 108, 109, 110, 111, 114, 117, 118, 121, 124, 128, 131, 135, 138, 139, 142, 145, 146]

DHD_90
[1, 2, 5, 6, 8, 9, 12, 13, 16, 19, 20, 23, 26, 27, 29, 30, 33, 34, 37, 39, 43, 46, 47, 50, 54, 57, 61, 64, 68, 71, 75, 77, 78, 81, 82, 85, 88, 89, 91, 92, 95, 96, 99, 102, 103, 106, 109, 110, 113, 116, 119, 122, 125, 126, 129, 132, 133, 136, 139, 140, 143, 146, 147, 150, 151]

DHD_91
[2, 5, 9, 12, 16, 17, 19, 23, 26, 27, 30, 33, 39, 40, 43, 46, 50, 53, 57, 60, 64, 67, 70, 74, 77, 78, 81, 84, 85, 88, 91, 92, 95, 96, 98, 99, 101, 109, 112, 116, 119, 123, 126, 130, 133, 137, 138]

DHD_92
[5, 6, 8, 9, 12, 13, 15, 16, 19, 20, 22, 23, 26, 27, 29, 30, 33, 36, 37, 38, 42, 46, 49, 50, 53, 56, 57, 59, 60, 63, 67, 70, 71, 74, 77, 80, 81, 84, 87, 88, 91, 94, 95, 98, 101, 102, 105, 109, 110, 114, 118, 121, 125, 128, 129, 132, 135, 136, 139, 142, 143]

DHD_93
[2, 5, 6, 9, 10, 12, 13, 16, 19, 20, 23, 26, 27, 30, 34, 35, 37, 40, 44, 47, 51, 54, 58, 61, 65, 76, 79, 83, 86, 87, 90, 93, 94, 97, 100, 104, 107, 109, 113, 116, 117, 120, 123, 124, 127, 130, 131, 134, 137, 138, 141, 144, 145]

DHD_94
[1, 5, 8, 9, 12, 15, 16, 19, 22, 23, 26, 29, 30, 33, 37, 45, 48, 51, 52, 55, 59, 62, 66, 69, 73, 75, 76, 84, 87, 88, 91, 94, 98, 101, 102, 105, 108, 109, 111, 112, 115, 117, 118, 122, 126, 129, 130, 133, 136, 140, 143, 147, 150, 151, 154]

DHD_95
[2, 6, 9, 13, 16, 20, 23, 26, 27, 30, 34, 37, 39, 41, 42, 45, 48, 49, 52, 55, 56, 59, 62, 63, 66, 69, 70, 72, 73, 75, 81, 85, 88, 91, 92, 95, 96, 98, 99, 102, 105, 106, 109, 110, 113, 114, 120, 123, 124, 127, 130, 131, 134, 137, 138, 141, 145, 148, 151]

DHD_96
[1, 5, 6, 9, 10, 12, 13, 16, 19, 20, 23, 26, 27, 30, 33, 34, 42, 45, 46, 49, 52, 53, 56, 59, 60, 63, 66, 67, 70, 74, 75, 77, 81, 84, 88, 91, 95, 98, 102, 105, 114, 118, 119, 122, 123, 126, 129, 130, 132, 133, 136, 137, 139, 140, 143, 144, 146, 147, 150]

DHD_97
[1, 2, 5, 6, 8, 9, 12, 15, 16, 19, 20, 22, 23, 26, 27, 29, 30, 32, 33, 37, 39, 41, 44, 47, 51, 54, 58, 61, 65, 68, 71, 72, 79, 82, 83, 86, 89, 90, 93, 96, 97, 100, 103, 104, 107, 110, 115, 116, 119, 120, 121, 123, 126, 127, 130, 133, 134, 136, 137, 140, 141, 144, 147]

DHD_98
[6, 7, 9, 13, 16, 20, 23, 27, 30, 34, 35, 38, 43, 44, 50, 51, 53, 54, 57, 60, 61, 64, 67, 68, 71, 72, 74, 75, 78, 82, 85, 86, 89, 92, 93, 96, 99, 100, 103, 106, 110, 119, 123, 127, 130, 133, 134, 137, 141, 144, 148, 151, 152]

DHD_99
[2, 5, 6, 9, 12, 16, 19, 20, 23, 26, 30, 33, 34, 46, 50, 53, 57, 60, 64, 67, 70, 71, 74, 78, 82, 85, 89, 92, 96, 99, 103, 106, 110, 113, 123, 126, 130, 133, 137, 140, 141, 144, 147, 148, 151, 154, 155]

DHD_100
[1, 5, 8, 9, 12, 15, 16, 19, 22, 23, 26, 29, 30, 33, 38, 41, 42, 45, 49, 52, 55, 56, 59, 63, 66, 70, 73, 74, 77, 80, 81, 84, 87, 88, 91, 94, 95, 98, 101, 102, 104, 105, 106, 108, 109, 112, 115, 119, 122, 123, 126, 129, 130, 133, 136, 137]

DHD_101
[5, 6, 9, 12, 13, 16, 20, 23, 27, 30, 34, 39, 43, 46, 47, 50, 53, 54, 57, 60, 64, 67, 68, 71, 74, 75, 78, 79, 81, 84, 85, 88, 92, 95, 99, 102, 106, 109, 119, 120, 123, 126, 130, 133, 134, 137, 138, 140, 144, 147, 151]

DHD_102
[6, 9, 13, 16, 20, 22, 23, 27, 30, 34, 38, 44, 47, 51, 54, 55, 57, 58, 61, 65, 68, 69, 72, 75, 80, 83, 86, 90, 93, 97, 100, 104, 107, 108, 112, 113, 117, 121, 124, 128, 131, 132, 135, 138, 142, 145]

DHD_103
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DHD_104
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DHD_120
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DHD_122
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DHD13_AAAA
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DHD13_BAAA
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DHD13_XAAA
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DHD13_XAAX
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DHD34_XAAAA

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DHD34_XAAXA

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[2, 5, 9, 11, 12, 15, 16, 19, 23, 26, 29, 30, 33, 37, 43, 46, 49, 50, 53, 56, 57, 59, 60, 63, 64, 67, 77, 81, 84, 88, 91, 92, 95, 98, 99, 102, 105, 113, 116, 119, 120, 123, 126, 130, 133, 134, 137, 140, 141, 144]

DHD37_AXBB

[2, 5, 9, 12, 16, 18, 19, 22, 23, 26, 29, 30, 33, 37, 43, 46, 49, 50, 52, 53, 56, 57, 60, 67, 70, 71, 77, 78, 81, 84, 88, 91, 95, 96, 99, 102, 105, 113, 116, 119, 120, 123, 126, 127, 130, 133, 134, 137, 140, 144]

DHD37_AXXB

[2, 5, 9, 11, 12, 15, 16, 19, 22, 23, 26, 29, 30, 33, 37, 43, 46, 49, 50, 53, 56, 57, 59, 60, 63, 64, 67, 77, 81, 84, 88, 89, 92, 95, 98, 99, 102, 105, 113, 116, 119, 120, 123, 126, 130, 133, 134, 137, 140, 141, 144]

DHD37_BBBB

[5, 9, 12, 16, 19, 22, 23, 26, 30, 33, 42, 43, 46, 50, 53, 56, 57, 60, 63, 64, 66, 67, 74, 77, 78, 81, 84, 85, 88, 92, 95, 98, 99, 102, 105, 108, 111, 113, 116, 119, 123, 126, 130, 133, 137, 140, 141, 144]

DHD37_XBBA

[2, 5, 8, 9, 12, 16, 19, 23, 25, 26, 29, 30, 33, 37, 43, 45, 46, 49, 50, 53, 60, 63, 64, 67, 70, 71, 77, 78, 81, 84, 85, 88, 91, 95, 98, 102, 103, 105, 113, 116, 119, 120, 123, 126, 127, 130, 133, 137, 140, 141, 144]

DHD37_XBXB

[2, 5, 9, 11, 12, 15, 16, 19, 23, 26, 30, 33, 37, 43, 46, 50, 53, 56, 57, 59, 60, 63, 64, 67, 77, 81, 84, 88, 92, 95, 98, 99, 102, 105, 113, 116, 119, 120, 123, 126, 130, 133, 134, 137, 140, 141, 144]

XAAX

[3, 6, 9, 13, 16, 20, 23, 27, 30, 34, 43, 47, 50, 54, 57, 61, 64, 68, 71, 72, 3, 6, 9, 13, 16, 20, 23, 27, 30, 34, 43, 47, 50, 54, 57, 61, 64, 68, 71, 72]

XAXA
[3, 6, 9, 13, 16, 20, 23, 27, 30, 34, 43, 47, 50, 54, 57, 61, 64, 68, 71, 72, 3, 6, 9, 13, 16, 20, 23, 27, 30, 34, 43, 47, 50, 54, 57, 61, 64, 68, 71, 72]

In one embodiment, the monomer A polypeptide and the monomer B polypeptide have their interaction specificity determined by at least one designed hydrogen bond network at the interface between the monomer A and the monomer B. In some aspects, (i) monomer A comprises 1 helix, and monomer B comprises 1 helix; (ii) monomer A comprises 1 helix and monomer B comprises 2 helices; (iii) monomer A comprises 1 helix and monomer B comprises 3 helices, (iv) monomer A comprises 1 helix and monomer B comprises 4 helices; or (v) monomer A comprises 1 helix and monomer B comprises 5 helices, wherein the monomer A and the monomer B comprise a hydrogen bond network, e.g., hydrogen bonds that are capable of being formed by the interface residues according to Table 2. In some aspects, (i) monomer A comprises 2 helices, and monomer B comprises 1 helix; (ii) monomer A comprises 2 helices and monomer B comprises 2 helices; (iii) monomer A comprises 2 helices and monomer B comprises 3 helices, (iv) monomer A comprises 2 helices and monomer B comprises 4 helices; or (v) monomer A comprises 2 helices and monomer B comprises 5 helices, wherein the monomer A and the monomer B comprise a hydrogen bond network, e.g., hydrogen bonds that are capable of being formed by the interface residues according to Table 2. In some aspects, (i) monomer A comprises 3 helices, and monomer B comprises 1 helix; (ii) monomer A comprises 3 helices and monomer B comprises 2 helices; (iii) monomer A comprises 3 helices and monomer B comprises 3 helices, (iv) monomer A comprises 3 helices and monomer B comprises 4 helices; or (v) monomer A comprises 3 helices and monomer B comprises 5 helices, wherein the monomer A and the monomer B comprise a hydrogen bond network, e.g., hydrogen bonds that are capable of being formed by the interface residues according to Table 2. In some aspects, (i) monomer A comprises 4 helices, and monomer B comprises 1 helix; (ii) monomer A comprises 4 helices and monomer B comprises 2 helices; (iii) monomer A comprises 4 helices and monomer B comprises 3 helices, (iv) monomer A comprises 4 helices and monomer B comprises 4 helices; or (v) monomer A comprises 4 helices and monomer B comprises 5 helices, wherein the monomer A and the monomer B comprise a hydrogen bond network, e.g., hydrogen bonds that are capable of being formed by the interface residues according to Table 2. In some aspects, (i) monomer A comprises 5

helices, and monomer B comprises 1 helix; (ii) monomer A comprises 5 helices and monomer B comprises 2 helices; (iii) monomer A comprises 5 helices and monomer B comprises 3 helices, (iv) monomer A comprises 5 helices and monomer B comprises 4 helices; or (v) monomer A comprises 5 helices and monomer B comprises 5 helices, wherein the monomer A and the monomer B comprise a hydrogen bond network, e.g., hydrogen bonds that are capable of being formed by the interface residues according to Table 2.

In a second aspect, the disclosure provides non-naturally occurring polypeptides comprising a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-290, or the group consisting of SEQ ID NOS:1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494. The amino acid sequences of SEQ ID NOS: 1-290 are provided in Table 1A, and the amino acid sequences of SEQ ID NOS: 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494 are provided in Table 1B, and can be used, for example, to generate the heterodimers of the disclosure.

In some aspects, the disclosure provides non-naturally occurring polypeptides comprising a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-290, wherein GlySer at amino acid positions 1 and 2 of SEQ ID NO: 1, 55, 81, 83, 101, 105, 115, 117, 119, 121, 123, 125, 127, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, or 193 are optional, e.g., not present. The amino acid sequences of SEQ ID NOS: 1-290 are provided in Table 1A, and can be used, for example, to generate the heterodimers of the disclosure.

In some aspects, the disclosure provides non-naturally occurring polypeptides comprising a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-290, wherein GlySer at amino acid positions 1 and 2 of SEQ ID NO: 6, 8, 14, 16, 26, 30, 32,

34, 36, 38, 40, 42, 46, 48, 54, 56, 58, 60, 62, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 176, 178, 180, 182, 184, 186, 188, 190, 192, or 194 are optional, e.g., not present.

In some aspects, the disclosure provides non-naturally occurring polypeptides comprising a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494. The amino acid sequences of SEQ ID NOS: 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494 are provided in Table 1B, and can be used, for example, to generate the heterodimers of the disclosure.

In some aspects, the disclosure provides non-naturally occurring polypeptides comprising a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, wherein the SEQ ID NOs: 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494 is not linked to GlySer at the immediate N terminus or the polypeptide does not comprise GlySer at the N terminus.

In some aspects, the disclosure provides non-naturally occurring polypeptides comprising a polypeptide consisting of an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494. In some aspects, the disclosure

provides non-naturally occurring polypeptides comprising a polypeptide consisting of the sequence of SEQ ID NOs: 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494.

In one embodiment, the amino acid changes from the reference amino acid sequence are conservative amino acid substitutions. In another embodiment, amino acid residues at 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of defined interface positions are invariant compared to the reference amino acid sequence. The defined interface residues are as provided in Table 2.

In a second aspect, the disclosure provides proteins comprising 2, 3, 4, or more non-naturally occurring polypeptides having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-290, wherein the 2, 3, 4, or more naturally occurring polypeptides are covalently linked. In some aspects, the disclosure provides proteins comprising 2, 3, 4, or more non-naturally occurring polypeptides having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-290 and 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, wherein the 2, 3, 4, or more naturally occurring polypeptides are covalently linked. In some aspects, the sequences of monomer A and monomer B listed herein can be modified (substituted) such that the resulting amino acid sequence maintains a hydrogen bond network of the original amino acid sequence as described in Tables 1A and 1B.

In this aspect, the proteins can be used to generate scaffolds that can be used for any suitable purpose including but not limited to those disclosed herein. In one embodiment, each of the 2, 3, 4, or more non-naturally occurring polypeptides are different. In another embodiment, the 2, 3, 4, or more non-naturally occurring polypeptides may include 2, 3, 4, or more identical polypeptides. In all embodiments, the 2, 3, 4, or more non-naturally occurring polypeptides may, for example, be covalently linked as part of a fusion protein. The 2, 3, 4, or more non-naturally

occurring polypeptides may each be separated by an amino acid linker. Any suitable amino acid linker may be used.

In some aspects, the linker is a flexible linker. In some aspects, the linker is a GS linker. In other aspects, the GS linker comprises (GGS)n, (GSEGS)n (SEQ ID NO:423) or (GGGS)n (SEQ ID NO:425), wherein n is an integer between 1 and 100. In some aspects, the linker comprises an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence as set forth in GSEGSGSEGSGS (SEQ ID NO:427) or GSEGSGSEGSGGS (SEQ ID NO:461). In some aspects, the linker comprises an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence as set forth in GSEGSGSEGS (SEQ ID NO:429). In some aspects, the linker comprises an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence as set forth in (GSEGS)n, wherein n is 1 to 10, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 (SEQ ID NO:423).

In one embodiment, each of the 2, 3, 4, or more non-naturally occurring polypeptides have at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an odd-numbered SEQ ID NO: selected from the group consisting of SEQ ID NOS:1-290. In one embodiment, each of the 2, 3, 4, or more non-naturally occurring polypeptides have at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an odd-numbered SEQ ID NO: selected from the group consisting of SEQ ID NOS:1-290, wherein GlySer at amino acid positions 1 and 2 of SEQ ID NO: 1, 55, 81, 83, 101, 105, 115, 117, 119, 121, 123, 125, 127, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, or 193 are optional, e.g., not present. In another embodiment, each of the 2, 3, 4, or more non-naturally occurring polypeptides have at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an even-numbered SEQ ID NO: selected from the group consisting of SEQ ID NOS:1-290. In another embodiment, each of the 2, 3, 4, or more non-naturally occurring polypeptides have at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an even-numbered SEQ ID NO: selected from the group consisting of SEQ ID NOS:1-290, wherein GlySer at amino acid positions 1 and 2 of SEQ ID NO: 6, 8, 14, 16, 26, 30, 32, 34, 36, 38, 40, 42, 46, 48, 54, 56, 58, 60, 62, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 176, 178, 180, 182, 184, 186, 188, 190, 192, or 194 are optional, e.g., not present. In a further embodiment, the 2, 3, 4, or more non-naturally occurring polypeptides include:

(a) polypeptides having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an odd-numbered SEQ ID NO: selected from the group consisting of SEQ ID NOS:1-290, or selected from the group consisting of SEQ ID NOS: 1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494; and

(b) polypeptides having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an even-numbered SEQ ID NO: selected from the group consisting of SEQ ID NOS:1-290, or selected from the group consisting of SEQ ID NOS: 1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494.

In some aspects, the 2, 3, 4, or more non-naturally occurring polypeptides include:

(a) a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of chain a in Table 1A and/or 1B; and

(b) a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of chain b in Table 1A and/or 1B.

In some aspects, the protein of the present disclosure comprises a heterotrimer. In some aspects, the heterotrimer comprises a monomer having an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,

99%, or 100% sequence identity along the length of the amino acid sequence described in Tables 1A and 1B, wherein the amino acid sequence forms a hydrogen bond network, e.g., hydrogen bond network formed by the interface residues according to Table 2. In some aspects, the heterotrimer of the present disclosure comprises at least two monomers, wherein each of the monomers comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence described in Tables 1A and 1B, wherein the amino acid sequence forms a hydrogen bond network, e.g., hydrogen bond network formed by the interface residues according to Table 2.

In some aspects, the heterotrimer of the present disclosure comprises at least three monomers, wherein each of the monomers comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence described in Tables 1A and 1B, wherein the amino acid sequence forms a hydrogen bond network, e.g., hydrogen bond network formed by the interface residues according to Table 2.

In some aspects, the heterotrimer of the present disclosure comprises at least one heterodimer, wherein the heterodimer comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence described in Tables 3 and 4, wherein the amino acid sequence forms a hydrogen bond network, e.g., hydrogen bond network formed by the interface residues according to Table 2.

In some aspects, the heterotrimer of the present disclosure comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence described in Tables 3 and 4, wherein the amino acid sequence forms a hydrogen bond network.

In some aspects, the protein of the present disclosure comprises a heterotetramer. In some aspects, the heterotetramer comprises a monomer having an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence described in Tables 1A and 1B, wherein the amino acid sequence forms a hydrogen bond network, e.g., hydrogen bond network formed by the interface residues according to Table 2. In some aspects, the heterotetramer of the present disclosure comprises at least two monomers, wherein each of the monomers comprises an amino

acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence described in Tables 1A and 1B, wherein the amino acid sequence forms a hydrogen bond network, e.g., hydrogen bond network formed by the interface residues according to Table 2.

In some aspects, the heterotetramer of the present disclosure comprises at least three monomers, wherein each of the monomers comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence described in Tables 1A and 1B, wherein the amino acid sequence forms a hydrogen bond network, e.g., hydrogen bond network formed by the interface residues according to Table 2.

In some aspects, the heterotetramer of the present disclosure comprises at least four monomers, wherein each of the monomers comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence described in Tables 1A and 1B, wherein the amino acid sequence forms a hydrogen bond network, e.g., hydrogen bond network formed by the interface residues according to Table 2.

In some aspects, the heterotetramer of the present disclosure comprises at least one heterodimer, wherein the heterodimer comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence described in Tables 1A and 1B, wherein the amino acid sequence forms a hydrogen bond network, e.g., hydrogen bond network formed by the interface residues according to Table 2.

In some aspects, the heterotetramer of the present disclosure comprises at least two heterodimers, wherein each of the two heterodimers comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence described in Tables 1A and 1B, wherein the amino acid sequence forms a hydrogen bond network, e.g., hydrogen bond network formed by the interface residues according to Table 2.

In some aspects, the heterotetramer of the present disclosure comprises at least one heterotrimer, wherein the heterotrimer comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or

100% sequence identity along the length of the amino acid sequence described in Tables 3 and 4, wherein the amino acid sequence forms a hydrogen bond network.

In another embodiment, the protein comprises the amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS:291, 294, 296, 299, and 302-305. The amino acid sequence of SEQ ID NOS:291, 294, 296, 299, and 302-305 is provided in Table 3. These are merely exemplary such proteins of this aspect of the disclosure, and those of skill in the art will understand that any suitable combination of the monomers of the disclosure can be used in generating the proteins of this aspect.

Table 3

Design name	Oligomerization State	Chain	Design Sequence
DHDSC_9-13-37	Heterotetramer (linker italicized and underlined)	9a-13a-37a	PKEEARELIRKQKELIKEQKKLIKEAKQKSDSRDAERIWKRSREINRESKK INKRIKELIKS*GSEGSGSEGSGS*TKEDILERQRKIIERAQEIHRRQQEILE ELERIIRKPGSSEEAMKRMLKLLEESLRLLKELLELSEESAQLLYEQR*GSE GSGSEGSGS*DSDEHLKKLKTFLENLRRHLDRLDKHIKQLRDILSENPEDER VKDVIDLSERSVRIVKTVIKIFEDSVRKKE **SEQ ID NO:291**
DHD9-13	Heterotrimer	9a-13a	PKEEARELIRKQKELIKEQKKLIKEAKQKSDSRDAERIWKRSREINRESKK INKRIKELIKSGSEGSGSEGSGSTKEDILERQRKIIERAQEIHRRQQEILE ELERIIRKPGSSEEAMKRMLKLLEESLRLLKELLELSEESAQLLYEQR **SEQ ID NO:294**
DHD15-37	Heterotrimer	15b-37a	TERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLDDPDSEDIAREIKELLR RLKEIIERNQRIAKEHEYIARERSGPGSGSEGSDSDEHLKKLKTFLENLRR HLDRLDKHIKQLRDILSENPEDERVKDVIDLSERSVRIVKTVIKIFEDSVR KKE **SEQ ID NO:296**
DHD13-37	Heterotrimer	13b-37b	TEKRLLEEAERAHREQKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKKIL EEIRELSKRSLELLREILYLSQEQKGSEGSGSEGSGSDDKELDKLLDTLEK ILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIA KTHAKKVE **SEQ ID NO:299**
OPHD_15-9	Heterotrimer	15b-9a	TERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLDDPDSEDIAREIKELLR RLKEIIERNQRIAKEHEYIARERSGSEGSGSEGSGSPKEEARELIRKQKEL IKEQKKLIKEAKQKSDSRDAERIWKRSREINRESKKINKRIKELIKS **SEQ ID NO:302**
OPHD_37-9	Heterotrimer	37a-9a	PKKEAEELAEESEELHDRSEKLHERAEQSSNSEEARKILEDIERISERIEE ISDRIERLLRSGSEGSGSEGSGSDDKELDKLLDTLEKILQTATKIIDDANK LLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:303**
OPHD_13-9	Heterotrimer	13a-9a	TKEDILERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKLL EESLRLLKELLELSEESAQLLYEQRGSEGSGSEGSGSPKEEARELIRKQKE LIKEQKKLIKEAKQKSDSRDAERIWKRSREINRESKKINKRIKELIKS **SEQ ID NO:304**
OPHD_9-37	Heterotrimer	9b-37a	PKKEAEELAEESEELHDRSEKLHERAEQSSNSEEARKILEDIERISERIEE ISDRIERLLRSGSEGSGSEGSDSDEHLKKLKTFLENLRRHLDRLDKHIKQL RDILSENPEDERVKDVIDLSERSVRIVKTVIKIFEDSVRKKE **SEQ ID NO:305**

In a third aspect, the disclosure provides protein scaffolds, comprising

a) a first designed component comprised of any number of monomer A polypeptides and/or monomer B polypeptides, each from different heterodimers, connected into a single component by amino acid linkers.

b) a second designed component, comprising corresponding monomers for each monomer A and/or monomer B in the first designed component one;

wherein the first and second designed components interact to form the protein scaffold, and wherein each monomer A only interacts in the scaffold with its monomer B binding partner. In one embodiment, the first designed component may comprise the protein of any embodiment or combination of embodiments disclosed herein, and/or the second designed component may comprise a plurality of individual polypeptides of embodiment or combination of embodiments disclosed herein. In non-limiting embodiments, the first designed component and the second designed component may comprise a set of three (Heterotrimer) or four (Heterotetramer) binding partners as shown in Table 4. As will be understood by those of skill in the art based on the teachings herein, heterotrimers of the disclosure (including but not limited to the exemplary heterotrimers shown in Table 4) include a first component fusion protein of two polypeptides of the disclosure, and the second component comprises two separate polypeptides that are binding partners of the two polypeptides in the fusion protein. For example, the DHD9-13 scaffold comprises a first designed component comprising the DHD9 A monomer covalently linked to the DHD13 A monomer, and the second designed component comprises individual DHD9 B and DHD13 B monomers. Different scaffolds are separated in the Table by a blank row. As will be understood by those of skill I the art, these are merely exemplary; the monomers in the first designed component may be linked in any order, and any monomers may be included in the designed components. As will be further understood by those of skill based on the teachings herein, heterotetramers (including but not limited to the exemplary heterotetramer shown in Table 4) include a first component fusion protein of three polypeptides of the disclosure, and the second component comprises three separate polypeptides that are binding partners of the three polypeptides in the fusion protein.

Table 4

DHD9-13	Heterotrimer	9a-13a	PKEEARELIRKQKELIKEQKKLIKEAKQKSDSRDAERIWKRSREINRESKK INKRIKELIKSGSEGSGSEGSGSTKEDILERQRKIIERAQEIHRRQQEILE

			ELERIIRKPGSSEEAMKRMLKLLEESLRLLKELLELSEESAQLLYEQR **SEQ ID NO:294**
DHD9-13	Heterotrimer	9b	PKKEAEELAEESEELHDRSEKLHERAEQSSNSEEARKILEDIERISERIEE ISDRIERLLRS **SEQ ID NO:2**
DHD9-13	Heterotrimer	13b	GSHHHHHHGSGSENLYFQGSTEKRLLEEAERAHREQKEIIKKAQELHRRLE EIVRQSGSSEEAKKEAKKILEEIRELSKRSLELLRELYLSQEQK **SEQ ID NO:295**
DHD9-13	Heterotrimer	13b	HHHHHHGSGSENLYFQGSTEKRLLEEAERAHREQKEIIKKAQELHRRLEEI VRQSGSSEEAKKEAKKILEEIRELSKRSLELLRELYLSQEQK **SEQ ID NO:431**
DHD15-37	Heterotrimer	15b-37a	TERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLDDPDSEDIAREIKELLR RLKEIIERNQRIAKEHEYIARERSGPGSGSEGSDSDEHLKKLKTFLENLRR HLDRLDKHIKQLRDILSENPEDERVKDVIDLSERSVRIVKTVIKIFEDSVR KKE **SEQ ID NO:296**
DHD15-37	Heterotrimer	15a	TREELLRENIELAKEHIEIMREILELLQKMEELLERQSSEDILEELRKIIE RIRELLDRSRKIHERSEEIAYKEE **SEQ ID NO:297**
DHD15-37	Heterotrimer	37b	GSHHHHHHGSGSENLYFQGSDDKELDKLLDTLEKILQTATKIIDDANKLLE KLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:298**
DHD15-37	Heterotrimer	37b	HHHHHHGSGSENLYFQGSDDKELDKLLDTLEKILQTATKIIDDANKLLEKL RRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:433**
DHD13-37	Heterotrimer	13b-37b	TEKRLLEEAERAHREQKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKKIL EEIRELSKRSLELLREILYLSQEQKGSEGSGSEGSGSDDKELDKLLDTLEK ILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIA KTHAKKVE **SEQ ID NO:299**
DHD13-37	Heterotrimer	13a	TKEDILERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKLL EESLRLLKELLELSEESAQLLYEQR **SEQ ID NO:300**
DHD13-37	Heterotrimer	37a	GSSHHHHHHSSGENLYFQGSDSDEHLKKLKTFLENLRRHLDRLDKHIKQLR DILSENPEDERVKDVIDLSERSVRIVKTVIKIFEDSVRKKE **SEQ ID NO:301**
DHD13-37	Heterotrimer	37a	SHHHHHHSSGENLYFQGSDSDEHLKKLKTFLENLRRHLDRLDKHIKQLRDI LSENPEDERVKDVIDLSERSVRIVKTVIKIFEDSVRKKE **SEQ ID NO:435**
OPHD_15-9	Heterotrimer	15b-9a	TERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLDDPDSEDIAREIKELLR RLKEIIERNQRIAKEHEYIARERSGSEGSGSEGSGSPKEEARELIRKQKEL IKEQKKLIKEAKQKSDSRDAERIWKRSREINRESKKINKRIKELIKS **SEQ ID NO:302**
OPHD_15-9	Heterotrimer	15a	TREELLRENIELAKEHIEIMREILELLQKMEELLEKARGADEDVAKTIKEL LRRLKEIIERNQRIAKEHEYIARERS **SEQ ID NO:19**
OPHD_15-9	Heterotrimer	9b	PKKEAEELAEESEELHDRSEKLHERAEQSSNSEEARKILEDIERISERIEE ISDRIERLLRS **SEQ ID NO:2**
OPHD_37-9	Heterotrimer	37a-9a	PKKEAEELAEESEELHDRSEKLHERAEQSSNSEEARKILEDIERISERIEE ISDRIERLLRSGSEGSGSEGSGSDDKELDKLLDTLEKILQTATKIIDDANK LLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:303**
OPHD_37-9	Heterotrimer	37b	GSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVE LLKRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:42**
OPHD_37-9	Heterotrimer	37b	DDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELL KRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:352**
OPHD_37-9	Heterotrimer	9b	PKKEAEELAEESEELHDRSEKLHERAEQSSNSEEARKILEDIERISERIEE ISDRIERLLRS **SEQ ID NO:2**

OPHD_13-9	Heterotrimer	13a-9a	TKEDILERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKLLEESLRLLKELLELSEESAQLLYEQRGSEGSGSEGSGSPKEEARELIRKQKELIKEQKKLIKEAKQKSDSRDAERIWKRSREINRESKKINKRIKELIKS **SEQ ID NO:304**
OPHD_13-9	Heterotrimer	13b	GTEKRLLEEAERAHREQKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKKILEEIRELSKRSLELLREILYLSQEQKGSLVPR **SEQ ID NO:4**
OPHD_13-9	Heterotrimer	9b	PKKEAEELAEESEELHDRSEKLHERAEQSSNSEEARKILEDIERISERIEEISDRIERLLRS **SEQ ID NO:2**
OPHD_9-37	Heterotrimer	9b-37a	PKKEAEELAEESEELHDRSEKLHERAEQSSNSEEARKILEDIERISERIEEISDRIERLLRSGSEGSGSEGSDSDEHLKKLKTFLENLRRHLDRLDKHIKQLRDILSENPEDERVKDVIDLSERSVRIVKTVIKIFEDSVRKKE **SEQ ID NO:305**
OPHD_9-37	Heterotrimer	9a	GSPKEEARELIRKQKELIKEQKKLIKEAKQKSDSRDAERIWKRSREINRESKKINKRIKELIKS **SEQ ID NO:1**
OPHD_9-37	Heterotrimer	9a	PKEEARELIRKQKELIKEQKKLIKEAKQKSDSRDAERIWKRSREINRESKKINKRIKELIKS **SEQ ID NO:331**
OPHD_9-37	Heterotrimer	37b	GSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:42**
OPHD_9-37	Heterotrimer	37b	DDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:352**
DHDSC_9-13-37	Heterotetramer	9a-13a-37a	PKEEARELIRKQKELIKEQKKLIKEAKQKSDSRDAERIWKRSREINRESKKINKRIKELIKSGSEGSGSEGSGSTKEDILERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKLLEESLRLLKELLELSEESAQLLYEQRGSEGSGSEGSGSDSDEHLKKLKTFLENLRRHLDRLDKHIKQLRDILSENPEDERVKDVIDLSERSVRIVKTVIKIFEDSVRKKE **SEQ ID NO:291**
DHDSC_9-13-37	Heterotetramer	9b	PKKEAEELAEESEELHDRSEKLHERAEQSSNSEEARKILEDIERISERIEEISDRIERLLRS **SEQ ID NO:2**
DHDSC_9-13-37	Heterotetramer	13b	TEKRLLEEAERAHREQKEIIKKAQELHRRLEEIVRQSGSSEEAKKEAKKILEEIRELSKRSLELLREILYLSQEQK **SEQ ID NO:292**
DHDSC_9-13-37	Heterotetramer	37b	GSSHHHHHHSSGENLYFQGSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:293**
DHDSC_9-13-37	Heterotetramer	37b	SHHHHHHSSGENLYFQGSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE **SEQ ID NO:437**

In these embodiments, the scaffold may be stable up to 95°C and has a guanidine denaturation midpoint of 4 M, as described in the examples that follow.

In some aspects, the heterotrimer or heterotetramer of the present disclosure does not comprise a His tag.

In another aspect, the disclosure provides protein scaffolds, comprising

(a) a fusion protein comprising of 2, 3, 4, or more polypeptides, wherein each polypeptide present in the fusion protein is a non-naturally occurring polypeptide comprises 1-5 alpha helices, wherein adjacent alpha helices may optionally be connected by an amino acid linker;

wherein each polypeptide in the fusion protein is capable of non-covalently interacting with a binding partner, and wherein the fusion protein does not comprise a binding partner for any polypeptide present in the fusion protein; and

(b) a binding partner for at least one of the polypeptides present in the fusion protein;

wherein the fusion protein and the binding partner non-covalently interact to form the protein scaffold, wherein an interaction specificity between the binding partner and the at least polypeptide in the fusion protein are determined by at least one hydrogen bond network at the interface between the binding partner and the at least one polypeptide.

Binding partners are polypeptides capable of forming heterodimers with a polypeptide present in the fusion protein, and are exemplified above with respect to SEQ ID NO:1-290. The binding partner for at least one polypeptide in the fusion protein may comprise a binding partner for 2, 3, 4, or all polypeptides in the fusion protein. As will be understood, when more than one binding partner is present, they are present as individual binding partner polypeptides, and not linked together.

The fusion protein may comprise 2, 3, 4, or more polypeptides. In certain embodiments, the fusion protein comprises at least 3 or 4 polypeptides in total. Exemplary embodiments of such fusion proteins are provided herein, for example in describing heterotrimer and heterotetramer embodiments in Table 4. The polypeptides in the fusion protein may all be the same, may all be different, or may include both identical and distinct polypeptides. In one specific embodiment, each polypeptide in the fusion protein is a different polypeptide.

In one embodiment,

(i) the fusion protein comprises 2, 3, 4, or more polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of SEQ ID NOS: 1-290, or SEQ ID NOS: 1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494; and

(ii) the binding partner comprises a binding partner as defined herein for each polypeptide in (i), wherein each binding partner has at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group SEQ ID NOS: 1-

290, or selected from the group consisting of SEQ ID NOS: 1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494. As described herein, the odd-numbered SEQ ID NOS: between SEQ ID NO:1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494 are noted as "A" monomers and the even-numbered SEQ ID NOS between SEQ ID NO:1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494 are the "B" monomers, with adjacent A and B monomers in Tables 1A and 1B capable of forming heterodimers as described in detail herein. Thus, for example, if the fusion protein included the polypeptide of SEQ ID NO:1, then binding partner may include SEQ ID NO:2, while if the fusion protein included the polypeptide of SEQ ID NO:2, then binding partner may include SEQ ID NO:1. The numerous combinations of fusion protein polypeptides and binding partners will be clear to those of skill in the art based on the teachings herein.

In one embodiment, amino acid changes in the fusion protein and the binding partner from the reference amino acid sequence are conservative amino acid substitutions. In another embodiment amino acid residues at 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of defined interface positions in the polypeptides in the fusion protein and the binding partner are invariant compared to the reference amino acid sequence. In a further embodiment, the at least one hydrogen bond network is asymmetric. In a further embodiment, the binding interface comprises at least 25% hydrophobic residues.. In another embodiment, the scaffold is stable up to 95°C and has a guanidine denaturation midpoint of 4 M.

In another embodiment, the disclosure provides methods of forming the designed heterodimer disclosed herein, comprising:

a) providing two of the monomers as unlinked monomers;

b) providing the other two monomers as linked monomers

whereby the unlinked monomers associate with their respective monomer of the same heterodimer, and not with any of the other monomers. Further details of this aspect are provided in the examples that follow.

In another embodiment, the disclosure provides a designed heterodimer protein comprising:

a) asymmetric buried hydrogen bond networks incorporated into regularly repeating backbone structures; and

b) helix hairpin helix monomers wherein the supercoil phases of the helices are fixed at 0, 90, 180, or 270 degrees and the supercoil twist (ω0) and helical twist (ω1) are held constant for either a two layer left handed super coil (ω0=-2.85 and ω1=102.85), or a 5 layer untwisted bundle (ω0=0 and ω1=100) 27. Further details of this aspect are provided in the examples that follow.

In another embodiment, the disclosure provides uses of the polypeptide, protein, heterodimer protein, protein scaffold , nucleic acid, expression vector, and/or cell of any embodiment or combination of embodiments for any suitable purposed, including but not limited to those disclosed herein such as designing protein logic gates

In a fourth aspect, the disclosure provides fusion proteins comprising a polypeptide of the formula X-B-Z, wherein:

(a) the X domain is a non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices, wherein the X domain is capable of non-covalently binding to a first target;

(b) the Z domain is a non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices, wherein the Z domain is capable of non-covalently binding to either (i) a second target that differs from the first target, or (ii) a different non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices; and

(c) the B domain is an amino acid linker;

wherein a combined number of alpha helices from the X domain and the Z domain is 4, 5, or 6; and

wherein the X domain and the Z domain interact at a binding interface, wherein the binding interface comprises a hydrogen bond network in which at least one side chain in each alpha helix hydrogen of the X domain bonds with a side chain in an alpha helix in the Z domain, and wherein the binding interface comprises a plurality of hydrophobic residues.

Each helix in the X domain H-bonds with at least one helix in the Z domain and each helix in the Z domain H-bonds with at least one helix in the X domain.

In a fifth aspect, the disclosure provides kits or compositions, comprising at least two fusion proteins comprising the formula X-B-Z, wherein

the B domain in each fusion protein is independently a polypeptide linker;

the X domain in each fusion protein comprises a first non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices;

the Z domain in each fusion protein comprises a second non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices, wherein a combined number of alpha helices from the X domain and the Z domain in each individual fusion protein is 4, 5, or 6; wherein the X domain and the Z domain interact at a binding interface, wherein the binding interface comprises a hydrogen bond network in which at least one side chain in each X domain alpha helix bonds with a side chain in an alpha helix in the Z domain; wherein

the X domain in a first fusion protein is capable of non-covalently binding to a first target;

the Z domain in a second fusion protein is capable of non-covalently binding to a second target; and

the X domains and Z domains in each individual fusion protein that are not capable of non-covalently binding to the first target or the second target are capable of non-covalently binding to an X or a Z domain of a different fusion protein in the plurality of fusion proteins.

The fusion proteins and kits can be used, for example, in the methods disclosed herein such as for logic gate construction, and for any other suitable use as will be appreciated by those of skill in the art based on the teachings herein. Specifically, fusion proteins can be used for designing 2-input AND and OR logic gates built from *de novo* designed proteins that regulate the association of arbitrary protein units ranging from split enzymes to transcriptional machinery in vitro, and in living cells. Binding interaction cooperativity makes the gates largely insensitive to stoichiometric imbalances in the inputs, and the modularity of the approach enables ready extension to 3-input OR, AND, and disjunctive normal form gates. The modularity and cooperativity of the control elements, coupled with the ability to de novo design an essentially unlimited number of protein components, enables design of sophisticated post-translational control logic over a wide range of biological functions.

In one embodiment, the Z domain is a non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices, wherein the Z domain is capable of non-covalently binding to a second target that differs from the first target, This embodiment is useful, for example, for generating single component dimerizers for use in AND/NOR gates. In another embodiment, the Z domain is a non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices, wherein the Z domain is capable of non-covalently binding to a different non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices. This embodiment is useful, for example, for generating 2 or 3-component dimerizers for use in AND/NOR gates.

The first targets and second targets may be any target suitable for an intended use. In non-limiting embodiments, the first target and/or the second target may comprise polypeptides or nucleic acids.

In one embodiment of the kit or composition,

(i) the first fusion protein has the formula X1-B1-Z1, wherein the X1 domain is capable of non-covalently binding to the first target; and

(ii) the second fusion protein has the formula X2-B2-Z2, wherein the Z2 domain is capable of non-covalently binding to the second target; and wherein the Z1 and X2 domains are capable of non-covalently binding to each other.

In another embodiment of the kit or composition,

(i) the first fusion protein has the formula X1-B1-Z1, wherein the X1 domain is capable of non-covalently binding to the first target; and

(ii) the second fusion protein has the formula X2-B2-Z2,

(iii) the at least two fusion proteins comprise a third fusion protein of formula X3-B3-Z3, wherein the Z3 domain is capable of non-covalently binding to the second target; wherein

(A) the Z1 and X2 domains are capable of non-covalently binding to each other; and

(B) the Z2 and X3 domains are capable of non-covalently binding to each other.

In one embodiment of the fusion protein or the kits or compositions, the binding interface comprises at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60% or greater hydrophobic residues. The B domain linker may be any suitable amino acid sequence, including but not limited to those described herein. In one embodiment, the B domain for each fusion protein is independently between 6-12, 6-11, 6-10, 7-12, 7-11, 7-10, 8-12, 8-11, 8-10, 9-12, 9-11, 9-10, 10-12, 10-11, 11-12, 6, 7, 8, 9, 10, 11, or 12 amino acids in length.

In another embodiment, the combined number of alpha helices from the X and Z domains in an individual fusion protein is 4. In a further embodiment, the X domain of each fusion protein has 2 alpha helices and the Z domain of each fusion protein has 2 alpha helices. In one embodiment, either the X domain or the Z domain of each fusion protein has 1 alpha helix and the other has 3 alpha helices.

In one embodiment, each X domain and each Z domain comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to SEQ ID NOS:1-290, or selected from the group consisting of SEQ ID NOS: 1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, with the proviso that the X domain and the Z domain do not form a heterodimer (a-b) pair. In one embodiment, each X domain and each Z domain comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to SEQ ID NOS:1-290 and 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, with the proviso that the X domain and the Z domain do not form a heterodimer (a-b) pair. In one non-limiting embodiment, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions as defined in Table 2 are invariant in the polypeptides relative to the reference polypeptide.

A different nomenclature is used in the examples that follow. Table 5 provides correspondence between the names used in the examples and in Tables 1A and 1B. The first column is the numbering used in the examples, while the second column lists the corresponding name in Tables 1A and 1B. For example, polypeptide 1 in the examples is DHD37_ABXB (a), 1' is DHD37_ABXB (b). Polypeptide 2 is DHD15 (a), 2' is DHD15 (b), and so on.

Table 5

1: DHD 37_ABXB

2: DHD 15

3: DHD 131

4: DHD 101

5: DHD 9

6: DHD 150

7: DHD 154

8: DHD 17

9: DHD 13_XAAA

10: DHD 39

11: DHD 155

In one embodiment, each fusion protein independently comprises a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide having the amino acid sequence of a sequence selected from the group consisting of SEQ ID NO: 302, 303, 306-326, 439, 441, 443, 445, 447, 449, 451, 453, 455, and 457:

2'-1'_2-residue_linker
GSTERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLDDPDSEDIAREIKELLRRLKEIIERNQRIAKEHEYIARE
RSAADDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKK
VE (SEQ ID NO: 306)

2'-1'_2-residue_linker
TERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLDDPDSEDIAREIKELLRRLKEIIERNQRIAKEHEYIARERS
AADDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE
(SEQ ID NO:439)

2'-1'_6-residue_linker
GSTERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLDDPDSEDIAREIKELLRRLKEIIERNQRIAKEHEYIARE
RSGGSGSPDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKT
HAKKVE (SEQ ID NO: 307)

2'-1'_6-residue_linker
TERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLDDPDSEDIAREIKELLRRLKEIIERNQRIAKEHEYIARERS
GGSGSPDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHA
KKVE (SEQ ID NO:441)

2'-1'_12-residue_linker
GSTERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLDDPDSEDIAREIKELLRRLKEIIERNQRIAKEHEYIARE
RSGGSGSPGGSGSPDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKEL
LEIAKTHAKKVE (SEQ ID NO: 308)

2'-1'_12-residue_linker
TERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLDDPDSEDIAREIKELLRRLKEIIERNQRIAKEHEYIARERS
GGSGSPGGSGSPDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLE
IAKTHAKKVE (SEQ ID NO:443)

2'-1'_24-residue_linker
GSTERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLDDPDSEDIAREIKELLRRLKEIIERNQRIAKEHEYIARE
RSGGSGSPGGSGSPGGSGSPGGSGSPDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVE
LLKRHEKAVKELLEIAKTHAKKVE (SEQ ID NO: 309)
2'-1'_24-residue_linker
TERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLDDPDSEDIAREIKELLRRLKEIIERNQRIAKEHEYIARERS
GGSGSPGGSGSPGGSGSPGGSGSPDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELL
KRHEKAVKELLEIAKTHAKKVE (SEQ ID NO:445)
11-7'
PEDDVVRIIKEDLESNREVLREQKEIHRILELVTRGEVSEEAIDRVLKRQEDLLKKQKESTDKARKVVEERRGSE
GSGSEGSDLEDLLRRLRRLVDEQRRLVEELERVSRRLEKAVRDNEDERELARLSREHSDIQDKHDKLAREILEVL
KRLLERTE (SEQ ID NO: 310)
1'-4'
GSDAYDLDRIVKEHRRLVEEQRELVEELEKLVRRQEDHRVDKKESHEILERLERIIRRSTRILTELEKLTDEFER
RTRGSEGSGSEGSGSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKE
LLEIAKTHAKKVE (SEQ ID NO: 311)
1'-4'
DAYDLDRIVKEHRRLVEEQRELVEELEKLVRRQEDHRVDKKESHEILERLERIIRRSTRILTELEKLTDEFERRT
RGSEGSGSEGSGSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELL
EIAKTHAKKVE (SEQ ID NO:447)
4-3'
GSDEDDELERLLREYHRVLREYEKLLEELRRLYEEYKRGEVSEEESDRILREIKEILDKSERLWDLSEEVWRTLL
YQAEGSEGSGSEGSDEKDYHRRLIEHLEDLVRRHEELIKRQKKVVEELERRGLDERLRRVVDRFRRSSERWEEVI
ERFRQVVDKLRKSVE (SEQ ID NO: 312)
4-3'
DEDDELERLLREYHRVLREYEKLLEELRRLYEEYKRGEVSEEESDRILREIKEILDKSERLWDLSEEVWRTLLYQ
AEGSEGSGSEGSDEKDYHRRLIEHLEDLVRRHEELIKRQKKVVEELERRGLDERLRRVVDRFRRSSERWEEVIER
FRQVVDKLRKSVE (SEQ ID NO:449)
3-2'*
GSTERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLKKARGADEKVLDELRKIIERIRELLDRSRKIHERSEEIA
YKEEGSEGSGSEGSGSDESDRIRKIVEESDEIVKESRKLAERARELIKESEDKRVSEERNERLLEELLRILDENA
ELLKRNLELLKEVLYRTR (SEQ ID NO: 313)
3-2'*
TERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLKKARGADEKVLDELRKIIERIRELLDRSRKIHERSEEIAYK
EEGSEGSGSEGSGSDESDRIRKIVEESDEIVKESRKLAERARELIKESEDKRVSEERNERLLEELLRILDENAEL
LKRNLELLKEVLYRTR (SEQ ID NO:451)
1'-3'
GSDEDDELERLLREYHRVLREYEKLLEELRRLYEEYKRGEVSEEESDRILREIKEILDKSERLWDLSEEVWRTLL
YQAEGSEGSGSEGSGSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVK
ELLEIAKTHAKKVE (SEQ ID NO: 314)
1'-3'

DEDDELERLLREYHRVLREYEKLLEELRRLYEEYKRGEVSEEESDRILREIKEILDKSERLWDLSEEVWRTLLYQ
AEGSEGSGSEGSGSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKEL
LEIAKTHAKKVE (SEQ ID NO:453)

1'-5

PKKEAEELAEESEELHDRSEKLHERAEQSSNSEEARKILEDIERISERIEEISDRIERLLRSGSEGSGSEGSGSD
DKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE
(SEQ ID NO: 303)

5'-2'

TERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLDDPDSEDIAREIKELLRRLKEIIERNQRIAKEHEYIARERS
GSEGSGSEGSGSPKEEARELIRKQKELIKEQKKLIKEAKQKSDSRDAERIWKRSREINRESKKINKRIKELIKS
(SEQ ID NO: 302)

1-6

DSDEHLKKLKTFLENLRRHLDRLDKHIKQLRDILSENPEDERVKDVIDLSERSVRIVKTVIKIFEDSVRKKEGSE
GSGSEGSGSEGSGSEGSGSEGSGSEGSPTDEVIEVLKELLRIHRENLRVNEEIVEVNERASRVTDREELERLLRR
SNELIKRSRELNEESKKLIEKLERLAT (SEQ ID NO: 315)

1'-7

DDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVEGS
EGSGSEGSTAEELLEVHKKSDRVTKEHLRVSEEILKVVEVLTRGEVSSEVLKRVLRKLEELTDKLRRVTEEQRRV
VEKLN (SEQ ID NO: 316)

6'-7

DNEEIIKEARRVVEEYKKAVDRLEELVRRAENAKHASEKELKDIVREILRISKELNKVSERLIELWERSQERARG
SEGSGSEGSTAEELLEVHKKSDRVTKEHLRVSEEILKVVEVLTRGEVSSEVLKRVLRKLEELTDKLRRVTEEQRR
VVEKLN (SEQ ID NO: 317)

1'-6-7

DDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVEGS
EGSGSEGSGSEGSGSEGSGSEGSGSEGSPTDEVIEVLKELLRIHRENLRVNEEIVEVNERASRVTDREELERLLR
RSNELIKRSRELNEESKKLIEKLERLATGSEGSGSEGSGSEGSGSEGSGSEGSGSEGSTAEELLEVHKKSDRVTK
EHLRVSEEILKVVEVLTRGEVSSEVLKRVLRKLEELTDKLRRVTEEQRRVVEKLN (SEQ ID NO: 318)

11-1

DSDEHLKKLKTFLENLRRHLDRLDKHIKQLRDILSENPEDERVKDVIDLSERSVRIVKTVIKIFEDSVRKKEGSE
GSGSEGSPEDDVVRIIKEDLESNREVLREQKEIHRILELVTRGEVSEEAIDRVLKRQEDLLKKQKESTDKARKVV
EERR (SEQ ID NO: 319)

11-6'

DNEEIIKEARRVVEEYKKAVDRLEELVRRAENAKHASEKELKDIVREILRISKELNKVSERLIELWERSQERARG
SEGSGSEGSPEDDVVRIIKEDLESNREVLREQKEIHRILELVTRGEVSEEAIDRVLKRQEDLLKKQKESTDKARK
VVEERR (SEQ ID NO: 320)

11-7'

DLEDLLRRLRRLVDEQRRLVEELERVSRRLEKAVRDNEDERELARLSREHSDIQDKHDKLAREILEVLKRLLERT
EGSEGSGSEGSGSEGSGSEGSGSEGSGSEGSPEDDVVRIIKEDLESNREVLREQKEIHRILELVTRGEVSEEAID
RVLKRQEDLLKKQKESTDKARKVVEERR (SEQ ID NO: 321)

1'-6

GSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE
GSEGSGSEGSPTDEVIEVLKELLRIHRENLRVNEEIVEVNERASRVTDREELERLLRRSNELIKRSRELNEESKK
LIEKLERLAT (SEQ ID NO: 322)

1'-6

DDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVEGS
EGSGSEGSPTDEVIEVLKELLRIHRENLRVNEEIVEVNERASRVTDREELERLLRRSNELIKRSRELNEESKKLI
EKLERLAT (SEQ ID NO:455)

7-1

DSDEHLKKLKTFLENLRRHLDRLDKHIKQLRDILSENPEDERVKDVIDLSERSVRIVKTVIKIFEDSVRKKEGSE
GSGSEGSTAEELLEVHKKSDRVTKEHLRVSEEILKVVEVLTRGEVSSEVLKRVLRKLEELTDKLRRVTEEQRRVV
EKLN (SEQ ID NO: 323)

4'-2'*

GTERKLLERSRRLQEESKRLLDEMAEIMRRIKKLLKKARGADEKVLDELRKIIERIRELLDRSRKIHERSEEIAY
KEEGSEGSGSEGSGSDAYDLDRIVKEHRRLVEEQRELVEELEKLVRRQEDHRVDKKESHEILERLERIIRRSTRI
LTELEKLTDEFERRTR (SEQ ID NO: 324)

2*-1'

TREELLRENIELAKEHIEIMREILELLQKMEELLEKARGADEDVAKTIKELLRRLKEIIERNQRIAKEHEYIARE
RSGSEGSGSEGSGSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKEL
LEIAKTHAKKVE (SEQ ID NO: 325)

1'-9

GSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE
GSEGSGSEGSGTKEDILERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKLLEESLRLLKELLEL
SEESAQLLYEQR (SEQ ID NO: 326)

1'-9

DDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVEGS
EGSGSEGSGTKEDILERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKLLEESLRLLKELLELSE
ESAQLLYEQR (SEQ ID NO:457)

In some aspects, each fusion protein independently comprises a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to, a polypeptide having the amino acid sequence of SEQ ID NO: 302, 303, 306-326, 439, 441, 443, 445, 447, 449, 451, 453, 455, and 457, wherein GlySer at amino acid residues 1 and 2 of any of 302, 303, 306-326, 439, 441, 443, 445, 447, 449, 451, 453, 455, and 457 are optional, e.g., not present.

In another embodiment, the kits or compositions further comprising the first target and the second target. In one embodiment, the first target and the second target each independently comprise a polypeptide of the formula X10-B10-Z10, wherein

(a) the X10 domain is a non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices;

(b) the Z10 domain is a non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices; and

(c) the B10 domain is an amino acid linker;

wherein the X domain and the Z domain interact at a target binding interface, wherein the target binding interface comprises a hydrogen bond network in which at least one side chain in each alpha helix hydrogen of the X domain bonds with a side chain in a different alpha helix in the Z domain, and wherein the target binding interface comprises a plurality of hydrophobic residues. In one embodiment, the target binding interface comprises at least 25% hydrophobic residues. In another embodiment, the B10 domain for the first target and the second target is independently between 6-12, 6-11, 6-10, 7-12, 7-11, 7-10, 8-12, 8-11, 8-10, 9-12, 9-11, 9-10, 10-12, 10-11, 11-12, 6, 7, 8, 9, 10, 11, or 12 amino acids in length. In another embodiment, the combined number of alpha helices from the X and Z domains in the first target and the second target protein is 4. In a further embodiment,

(a) the X10 domain of each of the first target and the second target has 2 alpha helices and the Z10 domain of each of the first target and the second target has 2 alpha helices; or

(b) either the X10 domain or the Z10 domain of each of the first target and the second target has 1 alpha helix and the other has 3 alpha helices. In one embodiment, each X10 domain and each Z10 domain comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide having the amino acid sequence selected from SEQ ID NOS:1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494 , with the proviso that the X10 domain forms a heterodimer (a-b) pair with the X domain of the fusion protein, and the Z10 domain forms a heterodimer (a-b) pair with the Z domain of the fusion protein. In one embodiment, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions of the first target and/or the second target are invariant compared to the reference polypeptide amino acid sequence (interface residues shown in Table 2).

In another embodiment, the first target and/or the second target further comprise one or more effector polypeptide domains linked to one or more of the X10 and/or Z10 domains, for example, wherein the one or more effector polypeptide domains may comprise a polypeptide including, but not limited to, nucleic acid binding proteins, transcription factors, receptor binding proteins, split enzymes, effectors of membrane receptors, etc.

In a sixth aspect, the disclosure provides methods, comprising:

(i) contacting the fusion protein of embodiment or combination of embodiments of the fifth or sixth aspects disclosed herein with a biological sample under conditions to promote non-covalent binding of the fusion protein with first target and second target present in the sample, and

(ii) detecting non-covalent binding of the one or more fusion proteins to the first target and/or the second target in the biological sample.

The detecting may comprise any suitable means for detecting binding, including but not limited to mass spectrometry, yeast-2-hybrid detection, functional assays, or any other suitable assay as will be clear to those of skill in the art based on the current disclosure. In one embodiment, the method comprises detecting cooperative non-covalent binding of the one or more fusion proteins to the first target and the second target in the biological sample. This embodiment comprises use of the fusion proteins in AND gate logic, as described in more detail in the examples that follow. As used herein, "cooperative" binding means binding the fusion protein cannot bind to the first target without also binding to the second target, and the fusion protein cannot bind to the second target without binding to the first target.

In another embodiment, the method comprises detecting non-covalent binding of the one or more fusion proteins to the first target or the second target in the biological sample. This embodiment comprises use of the fusion proteins in OR gate logic, as described in more detail in the examples that follow.

In another embodiment, the disclosure provides methods comprising:

(a) contacting a biological sample with at least two fusion proteins, wherein each of the at least two fusion proteins comprises the formula X-B-Z, wherein

each B is independently a polypeptide linker;

each X domain comprises a first non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices;

each Z domain comprises a second non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices, wherein a combined number of alpha helices from the X domain and the

Z domain in each individual fusion protein is 4, 5, or 6; wherein the X domain and the Z domain interact at a binding interface, wherein the binding interface comprises a hydrogen bond network in which at least one side chain in each X domain alpha helix bonds with a side chain in an alpha helix in the Z domain; wherein

the X domain in a first fusion protein is capable of non-covalently binding to a first target;

the Z domain in a second fusion protein is capable of non-covalently binding to a second target; and

the X domains and Z domains in each individual fusion protein that are not capable of non-covalently binding to the first target or the second target are capable of non-covalently binding to an X or a Z domain of a different fusion protein in the plurality of fusion proteins;

(b) detecting non-covalent binding of the two or more fusion proteins to the first target and/or the second target in the biological sample. This embodiment comprises use of the fusion proteins in 2 component AND or OR gate logic, as described in more detail in the examples that follow.

In one embodiment of the AND or OR gate logic, the detecting comprises detecting cooperative non-covalent binding of the two or more fusion proteins to the first target and the second target in the biological sample. In another embodiment,

(i) the first fusion protein has the formula X1-B1-Z1, wherein the X1 domain is capable of non-covalently binding to the first target; and

(ii) the second fusion protein has the formula X2-B2-Z2, wherein the Z2 domain is capable of non-covalently binding to the first target; and wherein the Z1 and X2 domains are capable of non-covalently binding to each other.

In a further embodiment,

(i) the first fusion protein has the formula X1-B1-Z1, wherein the X1 domain is capable of non-covalently binding to the first target; and

(ii) the second fusion protein has the formula X2-B2-Z2,

(iii) the at least two fusion proteins comprise a third fusion protein of formula X3-B3-Z3, wherein the Z3 domain is capable of non-covalently binding to the second target; wherein

(A) the Z1 and X2 domains are capable of non-covalently binding to each other; and

(B) the Z2 and X3 domains are capable of non-covalently binding to each other.

In another embodiment, the X domains, Y domains, B domains, and or fusion proteins are as recited in any embodiment or combination of embodiments disclosed herein, such as in the fourth and fifth aspects. In one embodiment, at least one of the fusion proteins comprises one or more effector polypeptide domains linked to one or more of the X and/or Z domains, and wherein the detecting step comprises detecting an output signal caused by binding the first target and/or the second target. In another embodiment, the detecting step comprises detecting an output signal from the one or more effector polypeptide caused by cooperative non-covalently binding of the first target and the second target. Such detection may be by any suitable means dependent in part on the output signal to be detected, including but not limited to those disclosed herein. The output signal to be detected may be any suitable output signal including but not limited to fluorescence activity, functional activity, etc.

Any suitable effector polypeptide domain may be employed as suitable for an intended use. In one embodiment, the one or more effector polypeptide domains may comprise a polypeptide including, but not limited to, nucleic acid binding proteins, transcription factors, receptor binding proteins, nucleic acid binding proteins, transcription factors, receptor binding proteins, split enzymes, effectors of membrane receptors, etc.

In a seventh aspect, the disclosure provides compositions comprising

(a) a first polypeptide comprising 2 alpha helices, wherein the first polypeptide is capable of non-covalently binding a first target; and

(b) a second polypeptide comprising 2 alpha helices, wherein the first polypeptide is capable of non-covalently binding to the second polypeptide, and wherein the second polypeptide is capable of non-covalently binding a second target that differs from the first target; wherein:

(i) a binding affinity of the first polypeptide for the first target is approximately equal to a binding affinity of the second polypeptide for the second target; and

(ii) the binding affinity of the first polypeptide for the first target and the binding affinity of the second polypeptide for the second target are greater than the binding affinity of the first target and the second target for each other.

Compositions of this seventh aspect can be used, for example, as NOR gates as described in detail in the examples that follow.

In one embodiment, the composition further comprises the first target and the second target. The first targets and second targets may be any target suitable for an intended use. In non-limiting embodiments, the first target and/or the second target may comprise

polypeptides or nucleic acids. In another embodiment, the first target and/or the second target further comprise one or more effector polypeptide domains. Any effector polypeptide domains may be used as suitable for an intended use. In one embodiment, the one or more effector polypeptide domains may comprise a polypeptide including, but not limited to, nucleic acid binding proteins, transcription factors, receptor binding proteins, split enzymes, effectors of membrane receptors, etc. In another embodiment, the first polypeptide and/or the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NOS:1-290 and 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, as listed in Tables 1A and 1B. In one embodiment, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions of the first polypeptide and/or the second polypeptide are invariant compared to the reference polypeptide amino acid sequence (interface residues shown in Table 2).

In one non-limiting and exemplary embodiment,

(a) the first polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to one having the amino acid sequence of SEQ ID NO:3

9
GTKEDILERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKLLEESLRLLKELLELSEESAQLLYE
QR (SEQ ID NO: 3); and

(b) the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to one having the amino acid sequence of SEQ ID NO:58.

10′
GSSADDVLEDILKIIRELIEILDQILSLLNQLLKLLRHGVPNAKKVVEKYKEILELYLQLVSLFLKIVKTHADAV
SGKIDKKAEEEIKKEEEKIKEKLRQAKDILKKLQEEIDKTR (SEQ ID NO: 58)

In one non-limiting and exemplary embodiment,

(a) the first polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to one having the amino acid sequence of SEQ ID NO:3

9

GTKEDILERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKLLEESLRLLKELLELSEESAQLLYE QR (SEQ ID NO: 3); and

(b) the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to one having the amino acid sequence of SEQ ID NO:362.

10′

SADDVLEDILKIIRELIEILDQILSLLNQLLKLLRHGVPNAKKVVEKYKEILELYLQLVSLFLKIVKTHADAVSG KIDKKAEEEIKKEEEKIKEKLRQAKDILKKLQEEIDKTR (SEQ ID NO:362)

In another embodiment, the first target and/or the second target each comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-290, with the proviso that the first target forms a heterodimer (a-b) pair with the first polypeptide, and the second target forms a heterodimer (a-b) pair with the second polypeptide. In another embodiment, the first target and/or the second target each comprises a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-290 and 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, with the proviso that the first target forms a heterodimer (a-b) pair with the first polypeptide, and the second target forms a heterodimer (a-b) pair with the second polypeptide. Heterodimer A-B pairs among the polypeptides of SEQ ID NOS:1-290 and 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494 are described at length above (See also FIG. 16).

In one embodiment, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions of the first target and/or the second target are invariant compared to the reference polypeptide amino acid sequence (interface residues shown in Table 2).

The compositions of this seventh aspect can be used for any suitable purpose, including in designing NOR logic gates. In one embodiment, the disclosure provides methods comprising

(a) contacting a biological sample with the composition of any embodiment or combination of embodiments of the seventh aspect of the disclosure; and

(b) detecting binding, of the first polypeptide to the first target and binding of the second polypeptide to the second target in the sample, such as detecting an output signal caused by actions of effector polypeptides upon binding. Additional details of the use of the compositions of the seventh aspect of the disclosure in NOR logic gates re described in detail in the examples that follow.

In an eighth aspect, the disclosure provides compositions comprising:

(a) a first polypeptide comprising 2 alpha helices, wherein the first polypeptide is capable of non-covalently binding a first target; and

(b) a second polypeptide comprising 2 alpha helices, wherein the first polypeptide is capable of non-covalently binding to the second polypeptide, and wherein the second polypeptide is capable of non-covalently binding a second target that differs from the first target; wherein:

(i) a binding affinity of the first polypeptide for the second polypeptide is greater than a binding affinity of the second polypeptide for the second target;

(ii) a binding affinity of the first polypeptide for the first target is approximately equal to a binding affinity of the second polypeptide for the second target; and

(iii) the binding affinity of the first polypeptide for the first target and the binding affinity of the second polypeptide for the second target are greater than the binding affinity of the first target and the second target for each other.

Compositions of this eighth aspect can be used, for example, as XNOR gates as described in detail in the examples that follow. In one embodiment, the composition further comprises the first target and the second target. The first targets and second targets may be any target suitable for an intended use. In non-limiting embodiments, the first target and/or

the second target may comprise polypeptides or nucleic acids. In another embodiment, the first target and/or the second target further comprise one or more effector polypeptide domains. Any effector polypeptide domains may be used as suitable for an intended use. In one embodiment, the one or more effector polypeptide domains may comprise a polypeptide including, but not limited to, nucleic acid binding proteins, transcription factors, receptor binding proteins, split enzymes, effectors of membrane receptors, etc. In another embodiment, the first polypeptide and/or the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NOS:1-290 and 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, as listed in Tables 1A and 1B. In one embodiment, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions of the first polypeptide and/or the second polypeptide are compared to the reference polypeptide amino acid sequence (interface residues shown in Table 2).

In one non-limiting and exemplary embodiment,

(a) the first polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to one having the amino acid sequence of SEQ ID NO:3

9
GTKEDILERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKLLEESLRLLKELLELSEESAQLLYE
QR (SEQ ID NO: 3); and

(b) the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to one having the amino acid sequence of SEQ ID NO:42.

1′ (b)
GSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE
(SEQ ID NO: 42)

In one non-limiting and exemplary embodiment,

(a) the first polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to one having the amino acid sequence of SEQ ID NO:3

9
GTKEDILERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKLLEESLRLLKELLELSEESAQLLYE
QR (SEQ ID NO: 3); and

(b) the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to one having the amino acid sequence of SEQ ID NO:352.

1′ (b)
DDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE
(SEQ ID NO:352)

In another embodiment, the first target and/or the second target each comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-290 and 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, with the proviso that the first target forms a heterodimer (a-b) pair with the first polypeptide, and the second target forms a heterodimer (a-b) pair with the second polypeptide. Heterodimer A-B pairs among the polypeptides of SEQ ID NOS:1-290 and 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494 are described at length above (See also FIG. 16). In one embodiment, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions of the first target and/or the second target are invariant compared to the reference polypeptide amino acid sequence (interface residues shown in Table 2).

The compositions of this eighth aspect can be used for any suitable purpose, including in designing XNOR logic gates. In one embodiment, the disclosure provides methods comprising

(a) contacting a biological sample with the composition of any one of claims 50-56; and

(b) detecting binding interactions between the first polypeptide and the first target. the second polypeptide and the second target, the first polypeptide and the second polypeptide, and the first target and the second target in the sample, such as detecting an output signal caused by actions of effector polypeptides upon binding. Additional details of the use of the compositions of the eighth aspect of the disclosure in XNOR logic gates re described in detail in the examples that follow.

In a ninth aspect, the disclosure provides compositions comprising:

(a) a first polypeptide comprising 4 alpha helices, wherein the first polypeptide is capable of non-covalently binding a first target; and

(b) a second polypeptide comprising 4 alpha helices, wherein the second polypeptide is capable of non-covalently binding a second target that differs from the first target; wherein:

(i) a binding affinity of the first target for the second target is greater than a binding affinity of the first polypeptide for the first target;

(ii) a binding affinity of the first polypeptide for the first target is approximately equal to a binding affinity of the second polypeptide for the second target; and

(iii) the sum of the binding affinity of (A) the first polypeptide for the first target and (B) the binding affinity of the second polypeptide for the second target, is greater than the binding affinity of the first target and the second target.

Compositions of this ninth aspect can be used, for example, as NAND gates as described in detail in the examples that follow. In one embodiment, the composition further comprises the first target and the second target. The first targets and second targets may be any target suitable for an intended use. In non-limiting embodiments, the first target and/or the second target may comprise polypeptides or nucleic acids. In another embodiment, the first target and/or the second target further comprise one or more effector polypeptide domains. Any effector polypeptide domains may be used as suitable for an intended use. In one embodiment, the one or more effector polypeptide domains may comprise a polypeptide including, but not limited to, nucleic acid binding proteins, transcription factors, receptor

binding proteins, split enzymes, effectors of membrane receptors, etc. In another embodiment, the first polypeptide and/or the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NOS:1-290 and 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, as listed in Tables 1A and 1B. In one embodiment, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions of the first polypeptide and/or the second polypeptide are invariant compared to the reference polypeptide amino acid sequence (interface residues shown in Table 2).

In one non-limiting and exemplary embodiment,

(a) the first polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to one having the amino acid sequence of SEQ ID NO:42

1′ (b)

GSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE (SEQ ID NO: 42)

(b) the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to one having the amino acid sequence of SEQ ID NO: 57.

10

DHSRKLEEILDRLRKHVKRLLEHLRELLSLVKENPEDKDLVEVLELSLAILRRSLEAVEAFLKSVTKKDPDDEDL RRKADEIRKEVEEIKKSLAEVEKEIYKLK (SEQ ID NO: 57)

In one non-limiting and exemplary embodiment,

(a) the first polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to one having the amino acid sequence of SEQ ID NO:352

1′ (b)

DDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKVVETYVELLKRHEKAVKELLEIAKTHAKKVE (SEQ ID NO:352)

(b) the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to one having the amino acid sequence of SEQ ID NO: 57.

10

DHSRKLEEILDRLRKHVKRLLEHLRELLSLVKENPEDKDLVEVLELSLAILRRSLEAVEAFLKSVTKKDPDDEDL RRKADEIRKEVEEIKKSLAEVEKEIYKLK (SEQ ID NO: 57)

In another embodiment, the first target and/or the second target each comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:1-290 and 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, with the proviso that the first target forms a heterodimer (a-b) pair with the first polypeptide, and the second target forms a heterodimer (a-b) pair with the second polypeptide. Heterodimer A-B pairs among the polypeptides of SEQ ID NOS:1-290 and 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494 are described at length above (See also FIG. 16). In one embodiment, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions of the first target and/or the second target are compared to the reference polypeptide amino acid sequence (interface residues shown in Table 2).

The compositions of this ninth aspect can be used for any suitable purpose, including in designing NAND logic gates. In one embodiment, the disclosure provides methods comprising

(a) contacting a biological sample with the composition of any one of claims 60-66; and

(b) detecting binding interactions between the first polypeptide and the first target. the second polypeptide and the second target, and the first target and the second target in the

sample, such as detecting an output signal caused by actions of effector polypeptides upon binding.

As used throughout the present application, the term "polypeptide" is used in its broadest sense to refer to a sequence of subunit amino acids. The polypeptides of the invention may comprise L-amino acids, D-amino acids (which are resistant to L-amino acid-specific proteases in vivo), or a combination of D- and L-amino acids. The polypeptides described herein may be chemically synthesized or recombinantly expressed. The polypeptides may be linked to other compounds to promote an increased half-life in vivo, such as by PEGylation, HESylation, PASylation, glycosylation, or may be produced as an Fc-fusion or in deimmunized variants. Such linkage can be covalent or non-covalent as is understood by those of skill in the art.

As will be understood by those of skill in the art, the polypeptides of the invention may include additional residues at the N-terminus, C-terminus, or both that are not present in the polypeptides of the invention; these additional residues are not included in determining the percent identity of the polypeptides of the invention relative to the reference polypeptide.

As noted above, the polypeptides of the invention may include additional residues at the N-terminus, C-terminus, or both. Such residues may be any residues suitable for an intended use, including but not limited to detection tags (i.e.: fluorescent proteins, antibody epitope tags, etc.), linkers, therapeutic agents, ligands suitable for purposes of purification (His tags, etc.), ligands to drive localization, and peptide domains that add functionality to the polypeptides.

In a tenth aspect, the disclosure provides nucleic acids encoding the polypeptide, protein, fusion protein, scaffold, or design component of any embodiment or combination of embodiments disclosed herein. The nucleic acid sequence may comprise single stranded or double stranded RNA or DNA in genomic or cDNA form, or DNA-RNA hybrids, each of which may include chemically or biochemically modified, non-natural, or derivatized nucleotide bases. Such nucleic acid sequences may comprise additional sequences useful for promoting expression and/or purification of the encoded polypeptide, 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 nucleic acid sequences will encode the polypeptides of the disclosure.

In an eleventh aspect, the disclosure provides expression vector comprising one or more nucleic acids of the disclosure operatively linked to a 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 sequences of the 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 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, including but not limited plasmid and viral-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). The expression vector must be replicable in the host organisms either as an episome or by integration into host chromosomal DNA. In various embodiments, the expression vector may comprise a plasmid, viral-based vector, or any other suitable expression vector.

In a twelfth aspect, the disclosure provides cells comprising one or more nucleic acid, expression vector, polypeptide, protein, heterodimer protein, and/or protein scaffold of any embodiment or combination of embodiments disclosed herein. Nucleic acids or expression vectors may be episomal or chromosomally integrated. Any suitable cell type may be used, such prokaryotic or eukaryotic cells. The cells can be transiently or stably engineered to incorporate the expression vector of the disclosure, using techniques including but not limited to bacterial transformations, calcium phosphate co-precipitation, electroporation, or liposome mediated-, DEAE dextran mediated-, polycationic mediated-, or viral mediated transfection

In addition, the disclosure provides methods of producing a polypeptide, fusion protein, protein, heterodimer, etc. (collectively referred to as polypeptide) disclosed herein. In one embodiment, the method comprises the steps of (a) culturing a host according to this aspect of the disclosure under conditions conducive to the expression of the polypeptide, and (b) optionally, recovering the expressed polypeptide. The expressed polypeptide can be

recovered from the cell free extract or recovered from the culture medium. In another embodiment, the method comprises chemically synthesizing the polypeptides.

Example 1 Design of orthogonal protein heterodimers

Abstract: Here we demonstrate that heterodimeric interaction specificity can be achieved using extensive and modular buried hydrogen bond networks. We used the Crick generating equations to produce millions of four helix backbones with varying degrees of supercoiling around a central axis, identified those accommodating extensive hydrogen bond networks, and designed connected pairs of helices with short loops and optimize the remainder of the sequence. 65 of 97 such designs expressed in *E. coli* formed constitutive heterodimers, and crystal structures of four designs were in close agreement with the computational models and confirmed the designed hydrogen bond networks. In cells, a set of six heterodimers were found to be fully orthogonal, and in vitro, following mixing of 32 chains from sixteen heterodimer designs, denaturation in 5M GdnHCl and reannealing, the vast majority of the interactions were between the designed cognate pairs. The ability to design orthogonal protein heterodimers enables sophisticated protein based control logic for synthetic biology, and illustrates that nature has not fully explored the possibilities for programmable biomolecular interaction modalities. Hydrogen bond networks, including modular hydrogen bond networks are described in published patent application number WO2017173356, incorporated by reference herein.

Orthogonal sets of protein-protein and protein-peptide interactions play important roles in biological systems. Creation of new specificities by sequence redesign has been difficult, often resulting in promiscuous binding We hypothesized that large sets of designed heterodimers could be generated by incorporating asymmetric buried hydrogen bond networks into regularly repeating backbone structures. We generated helical bundle heterodimers in which each monomer is a helix-turn-helix starting from four-helix backbones. For each of the four helices, we exhaustively sampled the helical phase ($\Delta\phi_1$), supercoil radius (R) and offset along the Z-axis (Z offset) (FIG.. 1A), restricting the supercoil phases of the helices to 0, 90, 180 and 270 degrees, and the supercoil twist (ω_0) and helical twist (ω_1) to the ideal values for either a two layer left handed super coil (ω_0=-2.85 and ω_1=102.85), or a 5 layer untwisted bundle (ω_0=0 and ω_1=100) (Fig. 5A-B). This yielded 27 million untwisted and 60 million left-handed supercoiled backbones for both parallel and antiparallel orientations of opposing helices (Fig. 1B).

To identify the modular hydrogen bond network equivalents to DNA base pairs, we used ROSETTA™ HBNET [21] to design buried hydrogen bond networks in the central repeat units of each backbone, and obtained 2251 hydrogen bond networks involving at least 4 side chain residues with all heavy-atom donors and acceptors participating in hydrogen bonds, and connecting all 4 helices (Fig. 1c; Fig. 6, Table 6). We then identified all of the geometrically compatible placements of these hydrogen bond networks in each backbone (Fig. 1d), selected backbones accommodating at least two networks, and connected pairs of helices with short loops (Fig. 1e). Low energy sequences were identified using ROSETTADESIGN™ [22] calculations in which the hydrogen bond networks were held fixed. Designs with fully satisfied hydrogen bond networks and tight hydrophobic packing were selected for experimental characterization, excluding those with networks with C2 symmetry to disfavor homodimerization of monomers. Designed heterodimers (DHDs) are referred to by numbers with monomers labeled a or b; for example, DHD15_a refers to monomer "a" of design DHD15.

Table 6: The frequency of observing each hydrogen bond networks during the systematic search.

HBNet composition	Frequency	Percentage (%)
(S/T)2Q1Y1	13954	3.87071362
(S/T)3Q1	9959	2.76253669
(S/T)2D1H1	8452	2.34450849
(S/T)1Q2Y1	7603	2.10900356
(S/T)1D1Q1Y1	7359	2.04132016
(S/T)3D1	6332	1.75643963
(S/T)3D1Q1	5525	1.53258512
(S/T)1D1Q3	5071	1.40664962
(S/T)1D1Q2	5062	1.4041531
(S/T)2N1Y1	5046	1.39971484
(S/T)1N1Q1Y1	4921	1.36504097
(S/T)2H2	4683	1.29902192
(S/T)2H1Q1	4572	1.26823152
(S/T)3H1	3955	1.09708129
(S/T)2D1Q2	3946	1.09458477
(S/T)1D1N1Q2	3862	1.07128393
(S/T)3N1	3783	1.04937005
(S/T)2D1Y1	3762	1.04354484
(S/T)2D1Q1	3669	1.01774747

(S/T)1D1H1Q1	3653	1.01330922
(S/T)1D1Q1W1	3409	0.94562582
(S/T)1D1Q2Y1	3342	0.92704063
(S/T)2Q3	3111	0.86296331
(S/T)2D1N1Q1	2999	0.83189552
(S/T)2Q2Y1	2850	0.79056427
(S/T)1D1W1Y1	2849	0.79028688
(S/T)2N1Q2	2741	0.76032865
(S/T)2D1Q1Y1	2723	0.75533562
(S/T)1D1N1Q1Y1	2684	0.74451737
(S/T)2Q1W1	2641	0.73258956
(S/T)2H1N1Q1	2591	0.71872001
(S/T)2Q2	2582	0.71622349
(S/T)2N1Q1	2554	0.70845654
(S/T)2D1W1	2467	0.68432353
(S/T)2H1N1	2377	0.65935834
(S/T)4Q1	2305	0.63938619
(S/T)1N1Q2Y1	2296	0.63688967
(S/T)1D2Q2	2285	0.63383837
(S/T)2D1H1Q1	2276	0.63134185
(S/T)2D1Q1W1	2267	0.62884533
(S/T)1H1Q1Y1	2222	0.61636274
(S/T)1D1N1Q1	2207	0.61220187
(S/T)2H1Y1	2150	0.59639059
(S/T)1D1N1Y1	2109	0.58501756
(S/T)1Q1Y2	1962	0.54424109
(S/T)1H1Q2	1957	0.54285413
(S/T)1Q1W1Y1	1954	0.54202196
(S/T)2N1Q1Y1	1935	0.53675153
(S/T)3H1Q1	1901	0.52732024
(S/T)1D1H1W1	1879	0.52121764

94 of the 97 selected designs were well-expressed in *E. coli* with both monomers co-purifying by Ni-affinity chromatography (only one monomer contains a hexahistidine tag). For 85/94, the dominant species observed in size exclusion chromatography (SEC) had the expected size (Fig. 1f). Three designs characterized by CD spectroscopy were found to be all alpha helical and stable at 95°C (Fig. 1g, Fig. 6). Sequences and other information on the designs are provided in Tables 1A-B (above).

We explored the extent to which the heterodimer set could be expanded by permuting the hydrogen bond networks in the different helical repeat units, and by permuting the backbone connectivity. Assigning each unique network a letter, DHD37_XBBA indicates a

variant where the second, third and fourth repeat units have hydrogen bond networks B, B, and A, and the first heptad has exclusively hydrophobic residues in the core, while DHD103_1:423 indicates a heterodimer where one monomer consists of the first helix of DHD103 and the other monomer consists of helices 2 through 4 (Fig. 7). 13 of 14 hydrogen bond network permuted variants and 9 of 10 "3+1" backbone-permuted heterodimers (generated from five starting "2+2" heterodimers) ran as single peaks on SEC.

SAXS spectra collected for 44 designs were consistent with the design models (Fig. 1h, Fig. 2f-h,). The X-ray crystal structures of DHD131, DHD37_1:234, DHD127 and DHD15 had backbone Cα atom RMSDs to the design models ranging from 0.95 to 1.7 Å. The extensive five-residue buried hydrogen bond network of DHD131 (involving two serines, an asparagine, a tyrosine, and a tryptophan) is nearly identical in the crystal structure, with an additional water molecule bridging the interactions (Fig. 2a). The two designed hydrogen bond networks in DHD37_1:234, which contain buried histidine and tyrosine aromatic side chains sterically disfavoring homodimers, are in close agreement with the crystal structure (Fig. 2b). In DHD127, the histidines in the two hydrogen bond networks adopt a rotamer different from the design model (Fig. 2c), making a hydrogen bond with a water molecule. A crystal structure of DHD15 at pH 7.0 is similar to the design model (Fig. 2d), while a structure at pH 6.5 is of a domain-swapped, hetero-tetramer conformation.

We built three induced dimerization systems by fusing one monomer each from two different heterodimers via a flexible linker, and testing whether the remaining two monomers from each pair could be brought together by the fusion (Fig. 3a). In each case, the three components co-purified by Ni-NTA chromatography (one monomer has a hexahistidine tag); In yeast two-hybrid assays (Y2H) with monomers from two different heterodimers fused to the DNA binding domain (DBD) and transcriptional activation domain (AD), expression of the heterodimerizer fusion as a separate polypeptide chain increased signal significantly over background (Fig. 3b).

We covalently linked the monomer chain "a" subunits of 3 DHDs via flexible linkers (FIG. 3C), and co-expressed this "scaffold" and the 3 separate chain "b" monomers, one with a hexahistidine tag, in *E. coli*. The scaffold plus monomer assembly is stable at 95°C and has a guanidine denaturation midpoint of 4 M (FIG. 9).

By generating interfaces with many polar groups which are energetically costly to bury without geometrically matched hydrogen bonding interactions, our design protocol implicitly disfavors non-cognate interactions (explicit negative design to disfavor non-cognate interactions is computationally intractable given the very large number of possible

off-target binding modes). For 24 designs, strong interactions were observed by Y2H with the two partners fused to DBD and AD, but not when either partner was fused to both domains; the designed heterodimers, but not the homodimers, form in cells (Fig. 4A). The 24 monomers in 12 of these designs were crossed in an all-by-all Y2H experiment; interactions were observed for all cognate pairs, and 27 of the 552 possible non-cognate interactions (Fig. 9). Orthogonality was higher for an 8 DHD subset: of 240 possible non-cognate interactions, only 4 were observed (Fig. 4B; the interacting polar residues are depicted schematically in Fig. 10). Co-expression of unfused monomers eliminated off-target interactions (Fig. 4C); the cognate interactions are evidently stronger than the non-cognate interactions.

Our results demonstrate that the domain of unbounded sets of orthogonal heterodimeric biomolecules constructed from a single repeating backbone is not limited to nucleic acids. Interaction specificity arises from extensive buried hydrogen bond networks such as the fully connected TYR-SER-TRP-ASN-SER (SEQ ID NO:333) crystallographically confirmed network in Fig. 2a, and heterogeneity in the size of the residues at the designed interface (Fig. 9d-i), analogous to the contribution of steric effects to Watson-Crick base pairing specificity. Our large set of orthogonal interactions, together with the retention of specificity in the fused monomer systems (the induced dimerizer and scaffold of Fig. 3), and the interaction strength hierarchy illustrated by the cognate interaction competition experiment (Fig. 4c), can be used, by way of non-limiting example, to prepare protein based cellular control circuits with faster response times and better integration with signaling inputs and outputs than current nucleic acid based circuitry.

Methods for Example 1

Computational Design

1. Systematic sampling of parametric helical backbones

We used a generalization of the Crick coiled-coil parameters [5] to independently sample all four helices of the heterodimers supercoiled around the same axis. The supercoil twist (ω_0) and helical twist (ω_1) were coupled and ideal values were used [20] with ω_0 and ω_1 held constant among the helices. A left-handed supercoil results from ω_0=-2.85 and ω_1=102.85, and a straight bundle with no supercoiling from ω_0=0 and ω_1=100. The supercoil phases ($\Delta\phi_0$) for the helices were fixed at 0°, 90°, 180° and 270°, respectively. The offset along the Z-axis (Z offset) for the first helix was fixed to 0 as a reference point, with the rest of the helices independently sampling from -1.51 Å to 1.51 Å, with a step size of 1.51 Å. All helices sampled helical phases ($\Delta\phi_1$) independently, from 0° to 90°, with a step size of 10°.

Two of the helices with a $\Delta\phi_0$ separation of 180° sampled the radius from Z-axis (R) from 5 Å to 8 Å, while the other two sampled from 7 Å to 10 Å, all with a step size of 1 Å. Each helix is set to have 35 residues to accommodate 5 heptad repeats. After removing redundant sample points from the overlapping regions of radii sampling, the supercoiled helical bundles contained more than 60 million unique backbones, and the straight helical bundles contained more than 27 million unique backbones.

2. HBNet Search

For each parametrically generated backbone, HBNet™ [21] was used to search the middle heptad for hydrogen bond networks that connect all four helices, contain at least four side chains contributing hydrogen bonds, have all heavy atom donors and acceptors satisfied, and span the intermolecular interface. Symmetry was not enforced during the HBNet™ search. For buried interface positions, only non-charged polar amino acids were considered; for residues that were at the boundary between protein core and surface, all polar amino acids were considered. A subsequent Rosetta™ design calculation was performed to optimize hydrophobic packing, with atom pair restraints from HBNet™ being put on the newly identified hydrogen bond networks. Finally, a minimization step and side chain repacking step was performed without atom pair restraints on hydrogen bonding residues to evaluate how well the networks remained intact in the absence of the constraints. Designs with at most 5 alanines in the middle heptad and no buried unsatisfied polar heavy atoms were selected for downstream design.

3. Generating combinations of HBNets™ with heptad stacking

The purpose of this step is to identify five-heptad backbones (full backbones) that can accommodate at least 2 HBNets™. Instead of generating one-heptad backbones and full backbones separately, searching for HBNets™ in the one-heptad backbones and aligning them to all full backbones, we reasoned the heptad stacking method remains the same if one simply searches for HBNets™ in the middle heptad on all full backbones, extracts the middle heptads, and aligns them to all full backbones. We therefore extracted the middle heptads containing HBNets™, generated all variants of chain ordering, and did pairwise alignment of middle heptads to full backbones using TMalign [30]. All alignments with root mean square deviation (RMSD) less than 0.3 were identified and full backbones that can accommodate at least 2 middle heptads were selected for final design.

4. Connecting parametric helical backbones

Helical backbones are connected with short 2-5 residue loops such that the RMSD of each loop is less than 0.4 RMSD to a nine residues stretch in a native protein. Distance and directionality between helices limit what loops can connect, as such, our closure extends and shrinks helices by up to 3 residues. We then superimpose all short loops from the PDB onto the first and last two helical residues. The loops with the lowest stub-RMSD are minimized using the Rosetta™ score function onto the helical endpoints to ensure a near perfect closure. Loop quality is assessed by measuring the distance in RMSD to the closest nine stretch in the PDB. The loop with the lowest RMSD is returned as the solution. We repeat this procedure to connect all helices and report the solution with the lowest RMSD.

5. Design calculations

Backbones were regularized using Cartesian space minimization in Rosetta™ to alleviate any torsional strain introduced by heptad stacking. Two consecutive Rosetta™ packing rounds were performed with increasing weight on the repulsive energy to optimize hydrophobic packing, while constraining the hydrogen bond network residues. A FastDesign step was subsequently used within a generic Monte Carlo mover to optimize secondary structure shape complementarity, while allowing at most 8% alanine, 3 methionine and 3 phenylalanine in the protein core. The last step of minimization and side chain repacking to identify the movement of HBNets without atom pair constraints is the same as what was described in Step 2.

6. Selection criteria and metrics used to evaluate designs

Designs were selected based on the following criteria: change in polar surface area upon binding (dSASA_polar) greater than 800 Å; secondary structure shape complementarity (ss_sc) score greater than 0.65; holes score around HBNets less than -1.4; no buried unsatisfied heavy atoms; at least one buried bulky polar side chains per monomer. Selected designs were then visually inspected for good packing of hydrophobic side chains, especially the interdigitation of isoleucine, leucine and valine. Surface tyrosines were added at non-interfering positions to aid protein concentration measurement by recording OD280. Surface charge residues for a few of the designs were redesigned to shift the theoretical isoelectric point away from buffer pH.

RMSD calculations

Crystal structures and the corresponding design models were superimposed with TMalign using all heavy atoms. From this alignment, RMSD was calculated across all alpha-carbon atoms, and also across heavy atoms of the hydrogen bond network residues.

Logistic Regression

Designs were first scored with various filters in Rosetta™ with the filter values reported. Experimental results and Rosetta™ filter values were used as input to a logistic regression method [31] to find correlations between computational metrics and experimental observations.

Visualization and figures

All structural images for figures were generated using PyMOL [32].

Buffer and media recipe

TBM-5052: 1.2% [wt/vol] tryptone, 2.4% [wt/vol] yeast extract, 0.5% [wt/vol] glycerol, 0.05% [wt/vol] D-glucose, 0.2% [wt/vol] D-lactose, 25 mM Na2HPO4, 25 mM KH2PO4, 50 mM NH4Cl, 5 mM Na2SO4, 2 mM MgSO4, 10 μM FeCl3, 4 μM CaCl2, 2 μM MnCl2, 2 μM ZnSO4, 400 nM CoCl2, 400 nM NiCl2, 400 nM CuCl2, 400 nM Na2MoO4, 400 nM Na2SeO3, 400 nM H3BO3

Lysis buffer: 20 mM Tris, 300 mM NaCl, 20 mM Imidazole, pH 8.0 at room temperature

Wash buffer: 20 mM Tris, 300mM NaCl, 30 mM Imidazole, pH 8.0 at room temperature

Elution buffer: 20 mM Tris, 300 mM NaCl, 250 mM Imidazole, pH 8.0 at room temperature

Buffer W: 100 mM Tris-HCl pH 8.0, 150 mM NaCl and 1 mM EDTA

Buffer E: Buffer W containing 2.5 mM D-desthiobiotin

TBS buffer: 20 mM Tris pH 8.0, 100 mM NaCl

Construction of synthetic genes

For the expression of heterodimers, both monomers were encoded in the same plasmid, separated by a ribosome binding sequence (GAAGGAGATATCATC; SEQ ID NO:327). Synthetic genes were ordered from Genscript Inc. (Piscataway, N.J., USA) and delivered in pET21-NESG *E. coli* expression vector, inserted between the NdeI and XhoI

sites. For the pET21-NESG constructs, a hexahistidine tag and a tobacco etch virus (TEV) protease cleavage site (GSSHHHHHHSSGENLYFQGS; SEQ ID NO:328) were added in frame at the N-terminus of the second monomer. A stop codon was introduced at the 3' end of the second monomer to stop expression of the C-terminal hexahistidine tag in the vector. For purification with Strep-tactin resin, a streptavidin tag (SAWSHPQFEKGGGSGGGSGGSAWSHPQFEKSGENLYFQGS; SEQ ID NO:329) coding sequence was cloned in frame 5' of the first monomer sequence.

For the co-expression of 3 and 4 proteins from the same plasmid (induced dimerization and synthetic scaffold designs), synthetic genes were cloned in the pRSFDuet-1 expression vector. The first (in the case of 3 proteins) or first two (in the case of 4 proteins) genes were cloned between NcoI and HindIII sites, with a ribosome binding site separating the 2 proteins in the latter case. The last two genes were cloned between NdeI and XhoI sites, separated by a ribosome binding site. A hexahistidine tag and a TEV protease cleavage site coding sequence were cloned in frame 5' of the last gene.

Genes for yeast-two-hybrid (Y2H) studies were cloned into plasmids bearing the GAL4 transcription activation domain (poAD) and the GAL4 DNA-binding domain (poDBD).

Protein expression

Plasmids were transformed into chemically competent *E. coli* expression strains BL21(DE3)Star (Invitrogen) or Lemo21™ (DE3) (New England Biolabs) for protein expression. Single colonies were picked from agar plates following transformation and growth overnight, and 5 ml starter cultures were grown at 37°C in Luria-Bertani (LB) medium containing 100 µg/mL carbenicillin (for pET21-NESG vectors) or kanamycin (for pRSFDuet-1 vectors) with shaking at 225 rpm for 18 hours at 37°C. Starter cultures were diluted into 500 ml TBM-5052 containing 100 µg/mL carbenicillin or kanamycin, and incubated with shaking at 225 rpm for 24 hours at 37°C.

For expression of $^{13}C^{15}N$- or ^{15}N-labeled protein, the plasmids were transformed into the Lemo21™ (DE3) *E. coli* expression strain and plated on M9/glucose plates containing 50 µg/mL carbenicillin. For the starter culture, a single colony was used for inoculation of 50 mL LB medium with 50 µg/mL carbenicillin in a 250 mL baffled flask, and incubated with shaking at 225 rpm for 18 hours at 37°C. 10 mL of the starter culture was then transferred to a 2 L baffled flask containing 500 mL of Terrific Broth™ (Difco), with 25 mM Na2HPO4, 25 mM KH2PO4, 50 mM NH4Cl, 5 mM Na2SO4, and 100 µg/mL carbenicillin. The culture

was grown at 37°C to an OD600 of approximately 1.0, then centrifuged at 5000 rcf for 15 minutes to pellet the cells. The Terrific Broth™ medium was removed, and the cells were washed briefly with 30 mL of phosphate buffered saline (PBS). The cells were then transferred to a fresh 2 L baffled flask containing 500 mL of labeled media (25 mM Na2HPO4, 25 mM KH2PO4, 50 mM 15NH4Cl, 5 mM Na2SO4, 0.2% (w/v) ^{13}C glucose), and 100 µg/mL carbenicillin. The cells were allowed to grow at 37°C for 2 hours, before IPTG (Carbosynth) was added to 1mM and the temperature was reduced to 18°C. The labeled glucose and NH4Cl were obtained from Cambridge Isotopes.

Affinity purification

Cells were harvested by centrifugation for 15 minutes at 5000 rcf at 4°C and resuspended in 20 ml lysis buffer. Lysozyme, DNAse, and EDTA-free cocktail protease inhibitor (Roche) were added to the resuspended cell pellet before sonication at 70% power for 5 minutes. For Immobilized metal affinity chromatography (IMAC), lysates were clarified by centrifugation at 4°C and 18,000 rpm for at least 30 minutes and applied to Ni-NTA (Qiagen) columns pre-equilibrated with lysis buffer. The column was washed two times with 5 column volumes (CV) of wash buffer, followed by 5 CV of elution buffer. For Strep tag purification, elution fractions from IMAC were applied to Strep-Tactin® Superflow resin (IBA) pre-equilibrated in Buffer W. The column was washed with 5 CV Buffer W, before applying 3 CV Buffer E to elute proteins off the column. Mass and purity of eluted proteins were confirmed using electrospray ionization mass spectrometry (ESI-MS) on a Thermo Scientific TSQ Quantum Access mass spectrometer.

Size-exclusion chromatography (SEC)

N-terminal hexahistidine tags and streptavidin tags were cleaved with TEV protease overnight at room temperature, at a ratio of 1 mg TEV for 100 mg of protein. Prior to addition of TEV, buffer was exchanged into lysis buffer. After TEV cleavage, sample was passed over an additional Ni-NTA column and washed with 1.5 CV of lysis buffer, flow through were collected and further purified by SEC using a Superdex™ 75 10/300 increase column (GE Healthcare) in TBS buffer.

Circular dichroism (CD) measurements

CD wavelength scans (260 to 195 nm) and temperature melts (25 to 95°C) were performed using an AVIV model 420 CD spectrometer. Temperature melts were carried out

at a heating rate of 4°C/min and monitored by the change in ellipticity at 222 nm; protein samples were diluted to 0.25 mg/mL in PBS pH 7.4 in a 0.1 cm cuvette. Guanidinium chloride (GdmCl) titrations were performed on the same spectrometer with automated titration apparatus in PBS pH 7.4 at 25°C, with a protein concentration of 0.025 mg/mL in a 1 cm cuvette with stir bar. Each titration consisted of at least 40 evenly distributed GdmCl concentration points with one minute mixing time for each step. Titrant solution consisted of the same concentration of protein in PBS + GdmCl.

Crystallization of protein samples

Purified protein samples were concentrated to approximately 20 mg/ml in 25 mM Tris pH 8.0 and 150 mM NaCl. Samples were screened with a 5-position deck Mosquito™ crystal (ttplabtech) with an active humidity chamber, utilizing the following crystallization screens: JCSG+™ (Qiagen), Crystal Screen™ (Hampton Research), PEG/Ion™ (Hampton Research), PEGRx HT™ (Hampton Research), Index™ (Hampton Research) and Morpheus™ (Molecular Dimensions). The optimal conditions for crystallization of the different designs were found as follows: OPHD_37_N3C1, 0.15 M potassium bromide and 30% w/v polyethylene glycol monomethyl ether 2000; OPHD_127, 0.12 M ethylene glycols, 0.1 M buffer system 3 pH 8,5, and 50% v/v precipitate mix 1 from the Morpheus screen; OPHD_15, 0.2 M Ammonium sulfate, 0.1 M BIS-TRIS pH 6.5, 18% v/v Polyethylene glycol 400; OPHD_15, 0.1 M Imidazole pH 7.0, and 25% v/v Polyethylene glycol monomethyl ether 550; OPHD_131, 0.2 M Ammonium acetate, 0.1 M HEPES pH 7.5, 25% w/v Polyethylene glycol 3,350. Crystals were obtained after 1 to 14 days by the hanging drop vapor diffusion method with the drops consisting of a 1:1, 2:1 and 1:2 mixture of protein solution and reservoir solution.

X-ray data collection and structure determination

The crystals of the designed proteins were looped and placed in the corresponding reservoir solution, containing 20% (v/v) glycerol if the reservoir solution did not contain cryoprotectant, and flash-frozen in liquid nitrogen. The X-ray data sets were collected at the Advanced Light Source at Lawrence Berkeley National Laboratory with beamlines 8.2.1 and 8.2.2. Data sets were indexed and scaled using either XDS [33] or HKL2000 [34]. Initial models were generated by the molecular-replacement method with the program PHASER [35] within the Phenix™ software suite [36], using the design models as the initial search models. Efforts were made to reduce model bias through refinement with simulated annealing using

Phenix.refine™ [37], or, if the resolution was sufficient, by using Phenix.autobuild™ [38] with rebuild-in-place set to false, simulated annealing and prime-and-switch phasing. Iterative rounds of manual building in COOT [39] and refinement in Phenix™ were used to produce the final models. Due to the high degree of self-similarity inherit in coiled-coil-like proteins, datasets for the reported structures suffered from a high degree of pseudo translational non-crystallographic symmetry, as report by Phenix.Xtriage™, which complicated structure refinement and may explain the higher than expected R values reported. RMSDs of bond lengths, angles and dihedrals from ideal geometries were calculated with Phenix™ [36]. The overall quality of all final models was assessed using the program MOLPROBITY™ [40].

Small Angle X-ray Scattering (SAXS)

Samples were purified by SEC in 25 mM Tris pH 8.0, 150 mM NaCl and 2% glycerol; fractions preceding the void volume of the column were used as blanks for buffer subtraction. Scattering measurements were performed at the SIBYLS™ 12.3.1 beamline at the Advanced Light Source. The X-ray wavelength (λ) was 1.27 Å, and the sample-to-detector distance was 1.5 m, corresponding to a scattering vector q ($q = 4\pi \sin\theta/\lambda$, where 2θ is the scattering angle) range of 0.01 to 0.3 $Å^{-1}$. A series of exposures, in equal sub-second time slices, were taken of each well: 0.3 second exposures for 10 seconds resulting in 32 frames per sample. For each sample, data was collected for two different concentrations to test for concentration-dependent effects; "low" concentration samples ranged from 2-3 mg/mL and "high" concentration samples ranged from 5-7 mg/mL. Data was processed using the SAXS FrameSlice™ online serve and analyzed using the ScÅtter™ software package [41,42]. FoXS™ [43,44] was used to compare design models to experimental scattering profiles and calculate quality of fit (χ) values.

Yeast two-hybrid assay

For each pair of binders tested, chemically competent cells of yeast strain PJ69-4a (MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4(deleted) gal80(deleted) LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ) were transformed with the appropriate pair of plasmids containing DNA binding domain or activation domains, using the LiAc/SS carrier DNA/PEG method [45]. In the case of induced dimerization, the heterodimerizer was cloned downstream of one of the "monomer proteins", separated by a p2a and nuclear locolization sequence (GSGATNFSLLKQAGDVEENPGPGDKAELIPEPPKKKRKVELGTA; SEQ ID NO:330). The p2a sequence ensures translational cleavage to make the heterodimerizer a

separate protein from the "monomer protein". The selection of transformed yeast cells was performed in synthetic dropout (SDO) media lacking tryptophan and leucine for 48 hours with shaking at 1000 rpm at 30°C. The resulting culture was diluted 1:100 and grown for 16 hours in fresh SDO media lacking tryptophan and leucine, before transferring to a 96 well plate and diluted 1:100 into SDO media containing 100 mM 3-Amino-1,2,4-triazole (3-AT), lacking tryptophan, leucine and histidine (5 mM 3-AT in the case of induced dimerization). The culture was incubated with shaking at 1000 rpm at 30°C. Since bringing the DNA binding domain and the transcription activation domain into proximity is necessary for the growth of yeast cells in media lacking histidine, binding of two proteins was indicated by the growth of yeast cells [46,47]. The optical density of yeast cells was recorded after 48 hours. For Y2H assay on agar plates, the 1:100 diluted overnight culture was transferred onto Nunc™ OmniTray™ (Thermo Fisher) using a 96 Solid Pin Multi-Blot Replicator (V&P Scientific), with the agar lacking tryptophan, leucine and histidine, and containing 100 mM 3-AT. The plates were imaged daily until Day 5 to monitor the sizes of colonies. Images were analyzed by the ColonyArea [48] package on ImageJ.

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Example 2. Orthogonal protein heterodimers for designing modular protein logic gates

Abstract: The de novo design of modular protein logic for regulating protein function at the post-transcriptional level is a challenge for computational protein design and could have wide ranging applications in synthetic biology. Here we describe the design of 2-input AND, OR, NAND, NOR, XNOR, and NOT gates built from *de novo* designed proteins that regulate the association of arbitrary protein units ranging from split enzymes to transcriptional machinery in vitro, and in living cells. Binding interaction cooperativity makes the gates largely insensitive to stoichiometric imbalances in the inputs, and the modularity of the approach enables ready extension to 3-input OR, AND, and disjunctive normal form gates. The modularity and cooperativity of the control elements, coupled with the ability to de novo design an essentially unlimited number of protein components, should enable design of sophisticated post-translational control logic over a wide range of biological functions.

Introduction

The ability to *de novo* design protein-based logic gates with modular control of arbitrary protein-protein interactions could open the door to the tunable design of novel bio-orthogonal functionalities.

In principle, it should be possible to design a wide range of logic gates *de novo* using a set of orthogonal heterodimeric molecules. For example, given hypothetical heterodimer pairs ***A:A'***, ***B:B'***, and ***C:C'*** , an AND gate modulating the association of ***A*** with ***C'*** can be constructed by genetically fusing ***A'*** and ***B***, and ***B'*** and ***C***: association occurs only in the presence of both ***A'-B***, and ***B'-C*** (here and below "***:***" denotes noncovalent interaction, and "***-***", genetic fusion via flexible linkers). Several building block properties are desirable for constructing such associative logic gates. First, there should be many mutually orthogonal heterodimeric pairs, so that gate complexity is not limited by the number of individual elements. Second, the building blocks should be modular and similar in structure so that differences in building block shape and other properties do not have to be considered when constructing the gates. Third, single building blocks should be able to bind to multiple partners with different, tunable affinities, allowing inputs to perform negation operations by disrupting pre-existing lower affinity interactions. Fourth, the interactions should be cooperative so gate activation is not sensitive to stoichiometric imbalances in the inputs. In

the above AND gate, for example, if the interactions are not cooperative, a large excess of ***A'-B*** will pull the equilibrium towards partially assembled complexes (***A'-B*** with either ***A*** or ***B'-C*** but not both), which will disrupt gate activation.

Here, we explored the possibility of designing logic gates satisfying all four of the above criteria using *de novo* designed protein heterodimers with hydrogen bond network-mediated specificity (*34*). Sets of 6 (in vivo) and 15 (in vitro) mutually orthogonal designed heterodimers (DHDs, hereafter referred to by numbers, e.g. *1* and *1'* form one cognate pair. with hydrogen bond network (see Fig. 11A inset for example) mediated specificity are available for logic gate construction, satisfying condition 1 (orthogonality). The heterodimeric interfaces all share the same four helix bundle topology (Fig. 11A), satisfying condition 2 (modularity). The shared interaction interface allows a limited amount of cross talk between pairs, leading to a hierarchy of binding affinities, satisfying condition 3 (multiple binding specificities). Inspired by cooperatively activatable systems in nature (*35*, *36*), we sought to achieve condition 4 (cooperativity) by constructing the monomer fusions (***A'-B*** and ***B'-C*** in the above example) in such a way that the interaction surfaces (with ***A*** and ***C'***) are buried within the fusions. The free energy required to expose these buried interfaces would oppose gate activation, and we reasoned that the system could be tuned so that only the binding energy provided by both interactions would be sufficient to overcome this barrier, thus ensuring cooperative gate activation (Fig. 11B). If condition 2 (modularity) holds, then a single scheme for ensuring cooperativity could in principle work for a wide range of gate configurations.

Design of cooperativity

To explore the design of cooperative building blocks, we focused on the simple system ***A*** + ***A'-B*** + ***B'*** (we refer to this as induced dimerization below, ***A*** and ***B'*** as the monomers, and ***A'-B*** as the dimerizer). If binding is not cooperative, the amount of the trimeric complex decreases when ***A'-B*** is in stoichiometric excess relative to ***A*** and ***B'***: the formation of intermediate dimeric species of the linker protein binding to either of the monomers competes with formation of trimeric complexes. On the contrary, if binding is cooperative such that no binding to either monomer occurs in the absence of the other, the amount of trimeric complex formed becomes insensitive to an excess of the dimerizer. A simple thermodynamic model of the effect of binding cooperativity on the stoichiometric response of such induced dimerization systems (Fig. 11B, supplemental materials modeling section) shows that as the binding cooperativity decreases, there is a corresponding decrease

in the final concentration of full trimeric complexes at high dimerizer concentrations (Fig. 11C).

We hypothesized that a folded four helix bundle like state of the ***A'-B*** dimerizer could oppose binding to either ***A*** or ***B'***, as the relatively hydrophobic interacting surfaces would likely be sequestered within the folded structure (Fig. 14A). We tested different flexible linker lengths connecting ***A'*** with ***B*** using heterodimers ***1:1'*** and ***2:2'*** as a model system. All designs were found to be folded and stable in circular dichroism (CD) guanidine hydrochloride (GdnHCl) denaturation experiments, with unfolding free energies greater than 13 kcal/mol (Fig. 11D, Table 10). Although ***1'-2'*** dimerizer constructs with short linkers of 0 and 2 residues, or with a very long 24 residue linker could be purified as monomers (Fig. 14B), they were prone to aggregation. In contrast, designs with 6 and 12 residue linkers remained largely monomeric (data not shown). Small angle x-ray scattering (SAXS) experiments (*37*) indicate their hydrodynamic radii are close to those of folded four-helix bundle DHDs (Fig. 11E). Linkers in this length range likely allow the two monomers (***1'*** and ***2'***) to fold back on each other such that the largely hydrophobic interaction surfaces are buried against each other; such a structure would have to partially unfold for ***1'-2'*** to interact with either ***1*** or ***2*** with free energy cost ΔG_{open} (Fig. 11B), the magnitude of which determines the extent of cooperativity for the gate. We selected a linker length of 6- or 12- residues for all of the following experiments.

We studied the cooperativity of the induced dimerizer system *in vitro* using native mass spectrometry (Fig. 11F). ***1***, ***2*** and ***1'-2'*** were separately expressed in *E.coli* and purified. We first measured the extent to which ***1*** activates the binding of ***2*** to ***1'-2'***. At 10 μM each of **2** and **1'-2'**, the fraction of ***2*** in complex with ***1'-2'*** increased from 3% to 100% upon addition of 20 μM ***1*** (data not shown); a fold increase comparable with naturally occurring allosteric systems (*35*). To assess how this activation of binding influences the sensitivity of binding to stoichiometric imbalance, 10 μM ***1*** and ***2*** were titrated with increasing concentrations of ***1'-2'*** (Fig. 11F), and the species formed determined by nMS. The heterotrimeric ***1:1'-2':2*** complex was observed over a wide range of ***1'-2'*** concentrations (data not shown). Even in the presence of a 6 fold excess of ***1'-2'*** , there was no decrease in the amount of ***1:1'-2':2*** formed, and neither ***1:1'-2'*** or ***1'-2':2*** were observed (data not shown). We define cooperativity as the ratio of the affinities in the presence and absence of the other monomer, which in our model directly relates to the free energy of opening of the dimerizer ($c = e^{\Delta G_{open}/RT}$, see supplementary materials). Matching the thermodynamic model to native MS

data (data not shown) produces an estimated *c* value of 991,000, which corresponds to ΔG_{open} of 7 kcal/mol. This is about half the measured unfolding free energy of *1'-2'*, suggesting that binding may not require complete unfolding of the four helix bundle state of the dimerizer.

With linker units displaying cooperative binding, we reasoned that the lack of dependence on stoichiometric excesses of one of the components should extend to more complex gates. Using nMS, we investigated the cooperativity of a 2-input AND gate constructed from the two inputs *1'-3'* and *3-2'*, and monomers *1* and *2* brought together by the two inputs (Fig. 11G). As the concentration of the 2 inputs was increased, the amount of heterotetrameric complex plateaued at a stoichiometry of 2:1, and then remained constant up to a molar ratio of 6:1. Very little partial complexes (heterotrimers and heterodimers) were observed, further indicating high cooperativity (data not shown). We constructed a 3-input AND gate from *1'-4'*, *4-3'*, and *3-2'*, which together should control the association of *1* and *2* (Fig. 11H). Similar to the 2-input AND gate, the amount of full, pentameric complexes only decreased slightly at greater than stoichiometric concentrations of inputs with no detectable competing tetrameric complexes (data not shown).

Modular logic gate construction

We explored the modular combination of DHDs to generate a range of 2-input Cooperatively Inducible Protein HeterodimeR (CIPHR) logic gates. Monomers from individual DHDs were linked to effector proteins of interest via genetic fusion, whose colocalization or dissociation is dependent upon the inputs. Taking advantage of previously measured all-by-all specificity matrices (*34*), two modes of interactions were explored: cognate binding between designed protein pairs, or competitive binding involving multispecific interactions. The choice of effector proteins is independent from the input proteins, allowing diverse functional outputs (Fig. 12A).

We used a variant of the yeast-two-hybrid (Y2H) assay to characterize the behavior of the designed logic gates, using a setup similar to previously described yeast-four-hybrid systems (*38*, *39*). To construct an AND gate, we fused *2* to the Gal4 activation domain (AD), and *1* to the Gal4 DNA binding domain (DBD). The colocalization of AD and DBD, and resulting induction of transcription of the *His3* gene, is dependent upon the expression of both input proteins (*1'-5*, *5'-2'*). Growth in media lacking histidine required expression of both inputs (Fig. 12B). An OR gate was similarly constructed by linking the *1-6* fusion to the AD and *7'* to the DBD. Expression of either of the inputs *1'-7* or *6'-7* results in growth by driving association of AD with DBD (Fig. 12C).

We explored the construction of additional boolean logic gates by exploiting binding affinity hierarchies identified in all by all Y2H experiments (*34*). ***8*** not only interacts with ***8'*** but also forms homodimers (Fig. 15A); hence ***8'*** must outcompete ***8*** homodimers to form the heterodimer. We constructed a NOT gate by fusing ***8*** to both AD or DBD; yeast cells stopped growing in the presence of co-expressed ***8'*** input protein (Fig. 12D). Based on the affinity hierarchy ***9:9'*** ≈ ***10:10'*** > ***9:10'*** (Fig. 15B), we constructed a NOR gate in which ***9*** was fused to the AD, ***10'*** to the DBD, with ***9'*** and ***10*** the two inputs. Either or both of the inputs outcompete the ***9:10'*** interaction and hinder yeast growth (Fig. 12E). Based on the affinity hierarchy ***9':1'*** > ***9:9'*** ≈ ***1:1'*** > ***9:1*** (Fig. 15B), an XNOR gate was constructed by fusing ***9*** to AD, ***1*** to DBD, and using ***9'*** and ***1'*** as the two inputs: the presence of either outcompetes the ***9:1*** binding and blocks growth, but when both are expressed they instead interact with each other and growth is observed (Fig. 12F). Similarly, a NAND gate was designed based on the interaction hierarchy ***1':10'*** > ***1:1'*** ≈ ***10:10'*** > ***1:10*** (Fig. 15B). Neither ***1*** nor ***10*** alone can outcompete the ***1':10'*** binding and hence growth occurs, but when both are expressed, the free energy of formation of both ***1:1'*** and ***10:10'*** outweighs that of ***1':10'*** and growth is blocked (Fig. 12G).

3 input CIPHR logic gates

We constructed a 3-input AND gate (Fig. 10M) in which monomers ***1*** and ***2*** are brought into proximity by the three inputs ***1'-4'***, ***4-3'***, and ***3-2'***. We experimentally tested all eight possible input combinations (Fig. 13A), quantifying all complexes using nMS with both ***1*** and ***2*** present. Consistent with proper function of a 3-input AND gate, ***1*** and ***2*** only showed significant co-assembly when all three inputs are present (data not shown).

To test the modularity of CIPHR logic gates, we designed two different 3-input CIPHR logic gates using the same 4 pairs of DHDs and tested them via Y2H. To make a 3-input OR gate, ***1'-6-7*** was fused to AD, and ***11'*** to DBD. Either one of the 3 inputs (***11-1***, ***11-6'***, ***11-7'***) is able to bring AD to DBD via their linked proteins (Fig. 13B). Y2H results confirmed the correct behavior of this logic gate in cells: any of the input proteins induces cell growth (Fig. 13C). We constructed a CIPHR disjunctive normal form (DNF, [***A*** AND ***B***] OR ***C***) gate by fusing ***1'-6*** to AD, ***11'*** to DBD with inputs ***11-7'***, ***7-1***, or ***11-6'*** (Fig. 13D). In Y2H experiments, the DNF gate functioned as designed, with low yeast growth levels when no input or only one of the ***11-7'*** and ***7-1*** input proteins are present, and high yeast growth levels otherwise (Fig. 13E).

References for Example 2

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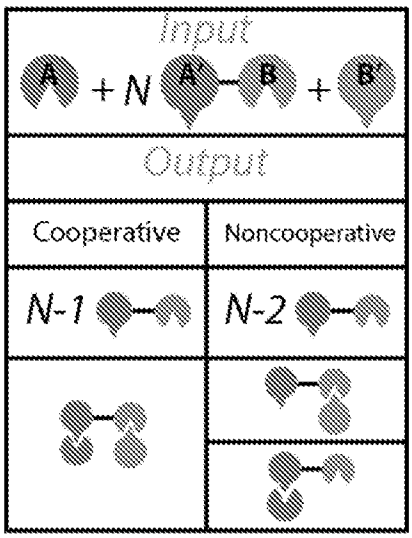
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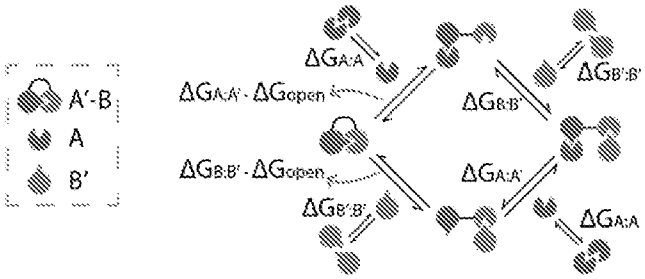
Table 7. Thermodynamic Modeling of Cooperativity



Referring to Table 7, for an induced dimerization system involving proteins A, A'-B, and B', a stoichiometric excess (N) of the A'-B protein results in partially assembled dimeric complexes if the binding is non-cooperative, but fully assembled trimeric complexes if the binding is cooperative.

We model the cooperatively induced dimerization system at thermodynamic equilibrium. Shown below (Table 8), assuming a 'closed' state for A'-B, where the binding interfaces are buried within the four-helix bundle, the binding of A'-B to either A or B' helix hairpins needs to overcome an energy barrier of transitioning from the 'closed' to 'open' state (ΔG_{open}). Therefore the free energy of binding between A'-B to A or B' can be expressed as $\Delta G_{A:A'}$ - ΔG_{open} and $\Delta G_{B:B'}$ - ΔG_{open}, respectively, where $\Delta G_{A:A'}$ and $\Delta G_{B:B'}$ represent the free energy of binding between the cognate pairs in the absence of the fusion. Once the A:A'-B or A-B':B complexes form, subsequent binding can be simply represented by the binding between cognate heterodimers: $\Delta G_{A:A'}$ or $\Delta G_{B:B'}$. We also observed the presence of $(A)_2$ and $(B')_2$ homodimers, therefore added free energy terms describing such processes into the model ($\Delta G_{A:A}$ or $\Delta G_{B':B'}$).

Table 8



ΔG relates to equilibrium constants by ΔG=-RTln$K$, and we further consider the system in terms of K. We make the simplifying assumption that the affinity of A'-B to either A or B' is identical (K_1 = [A:A'-B]/([A][A'-B]) = [A'-B:B']/([B][A'-B]). Finally, we define the cooperativity of the system, c, as the ratio between the equilibrium constants in the presence or absence of the other partner ($c=K_{B:B'}/K_1=K_{A:A'}/K_1$). For an entirely non-cooperative process (c=1), $K_{B:B'}=K_1$ and $K_{A:A'}=K_1$ *i.e.*, the first binding event does not affect the affinity of the subsequent binding event.

Since $K_1=\exp(-(\Delta G_{A:A'} - \Delta G_{open})/RT)$, rewriting the equation for c in terms of free energies leads to $c=\exp(\Delta G_{open})/RT$. Therefore, the extent of cooperativity is solely determined by the magnitude of the free energy required to partially unfold/expose the buried binding interfaces of the dimerizer A'-B.

We note that explicitly incorporating the equilibrium constants for homodimerization ($K_{A:A}$ and $K_{B':B'}$) only affect the absolute position of each equilibrium, but does not affect the magnitude of the cooperativity (see Table 9). Indeed, taking A as an example, the binding to the closed state becomes K_1 * $K_{A:A}$, and the binding to the open state becomes $K_{A:A'}$ * $K_{A:A}$. Because $K_{A:A}$ is present in both the numerator and the denominator, they cancel out, and c remains purely defined by the relative magnitudes of K_1 and $K_{A:A'}$.

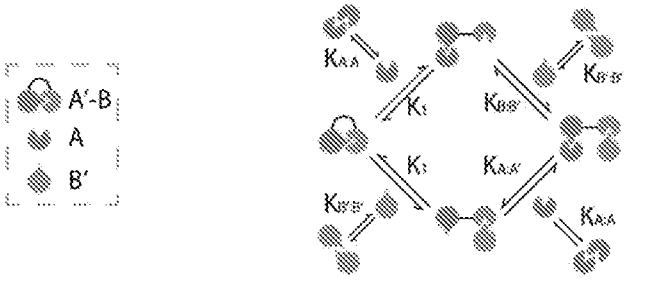


Table 9

We solved the following system of equations in *Mathematica* to simulate the amount of A:A'-B:B' at equilibrium as a function of the initial concentration of A'-B:

$$K_{A:A} = \frac{[A_2]}{[A][A]}$$

$$K_{B\prime:B\prime} = \frac{[B'_2]}{[B'][B']}$$

$$K_1 = \frac{[A:A'-B]}{[A][A'-B]}$$

$$K_1 = \frac{[B':A'-B]}{[B'][A'-B]}$$

$$K_{A:A\prime} = \frac{[A:A'-B:B']}{[A][A'-B:B']}$$

$$K_{B:B\prime} = \frac{[A:A'-B:B']}{[B'][A:A'-B]}$$

$$[A]_{tot} = 2*[A_2] + [A] + [A:A'-B] + [A:A'-B:B']$$

$$[B']_{tot} = 2*[B'_2] + [B'] + [A'-B:B'] + [A:A'-B:B']$$

$$[A'-B]_{tot} = [A'-B:B'] + [A:A'-B'] + [A:A'-B:B']$$

We knew from previous native MS titration experiments that the equilibrium dissociation constants of cognate designed heterodimers (DHDs) is in the ~10 nM range (*1*), therefore $K_{A:A'} = K_{B:B'} = 0.1$ nM^{-1}. Varying values of K_1 (and hence the cooperativity factor, $c = K_{A:A'}/K_1$) showed different responses of the amount of A:A'-B:B' at equilibrium as a function of the initial concentration of A'-B, as shown in Fig. 12C.

We experimentally estimated K_1 using native MS experiments. Mixing 10 μM of *1* and *1'-2'* resulted in no detectable amount of the *1:1'-2'* complex, suggesting very weak binding. The sensitivity of native MS places a lower-bound on the concentration of species that can be detected (0.0375 μM,). Using this value, a lower-bound for the affinity of *1:1'-2'* can be estimated ($1/K_1 \geq 2.65$ mM). This is close to the value of 9.91 mM obtained by calculating the affinity based on the *c* value of 991,000 reported in FIG. 13H.

This thermodynamic modeling demonstrates that binding cooperativity can be achieved for an induced dimerization system through occlusion of the binding interfaces. We achieved this by fusing hairpins via a flexible linker, rationalizing that the spontaneous folding of these constructs would bury the interaction interfaces on the inside of a four helical bundle like topology. Formation of these structures is corroborated by: *i*) SAXS profiles that

are consistent with DHDs structures, *ii*) *m*-values from chemical denaturation experiments consistent with $\Delta SASA$ for the unfolding of DHD topologies, and *iii*) $\Delta G_{open} < \Delta G_{folding}$, suggesting that exposing the binding interfaces requires partial unfolding of these fused constructs, but does not exceed the folding free energy of these proteins (a physically unrealistic scenario).

Materials and Methods

Buffer and media recipe

TBM-5052: 1.2% [wt/vol] tryptone, 2.4% [wt/vol] yeast extract, 0.5% [wt/vol] glycerol, 0.05% [wt/vol] D-glucose, 0.2% [wt/vol] D-lactose, 25 mM Na2HPO4, 25 mM KH2PO4, 50 mM NH4Cl, 5 mM Na2SO4, 2 mM MgSO4, 10 μM FeCl3, 4 μM CaCl2, 2 μM MnCl2, 2 μM ZnSO4, 400 nM CoCl2, 400 nM NiCl2, 400 nM CuCl2, 400 nM Na2MoO4, 400 nM Na2SeO3, 400 nM H3BO3.

Lysis buffer: 20 mM Tris, 300 mM NaCl, 20 mM Imidazole, pH 8.0 at room temperature.

Wash buffer: 20 mM Tris, 300mM NaCl, 30 mM Imidazole, pH 8.0 at room temperature.

Elution buffer: 20 mM Tris, 300 mM NaCl, 250 mM Imidazole, pH 8.0 at room temperature.

TBS buffer: 20 mM Tris pH 8.0, 100 mM NaCl.

YPAD buffer: Peptone 20 g/L, yeast extract 10 g/L, Adenine hemisulfate 10 μg/L, dextrose (20 g/L).

C-Trp-Ura-Leu-His+Adenine: hemisulfate+Glucose.

Yeast nitrogen base w/o amino acids (6.7 g/L), synthetic DO media (-Leu/-His/-Trp/-Ura) (1.4 g/L), dextrose (20 g/L), adenine hemisulfate (10 μg/L).

Construction of synthetic genes

For the expression of proteins in *E.coli*, synthetic genes were ordered from Genscript Inc. (Piscataway, N.J., USA) and delivered in pET21-NESG *E. coli* expression vector, inserted between the NdeI and XhoI sites. For each expression construct, a hexahistidine tag followed by a tobacco etch virus (TEV) protease cleavage site (GSSHHHHHHSSGENLYFQGS) (SEQ ID NO:328) were added in frame at the N-terminus of the protein. A stop codon was introduced at the 3' end of the protein coding sequence to prevent expression of the C-terminal hexahistidine tag in the vector.

Genes for yeast-two-hybrid (Y2H) studies were cloned into plasmids bearing the GAL4 DNA-binding domain (poDBD) and the GAL4 transcription activation domain (poAD) (*2*). Input proteins were cloned into plasmids V510 (uracil auxotrophic selection marker) and MX1 (bleomycin selection marker). Genes were expressed under the control of ADH1 promoters.

Protein expression

Plasmids were transformed into chemically competent *E. coli* expression strain Lemo21™(DE3) (New England Biolabs) for protein expression. Following transformation and overnight growth, single colonies were picked from agar plates into 5 ml Luria-Bertani (LB) medium containing 100 µg/mL carbenicillin (for pET21-NESG vectors) with shaking at 225 rpm for 18 hours at 37°C. Proteins were expressed using the autoinduction method (*7*): starter cultures were further diluted into 500 ml TBM-5052 containing 100 µg/mL carbenicillin, and incubated with shaking at 225 rpm for 24 hours at 37°C.

Affinity purification

E. coli cells were harvested by centrifugation at 5000 rcf for 15 minutes at 4°C and the pellet resuspended in 18 ml lysis buffer. EDTA-free cocktail protease inhibitor (Roche), lysozyme, and DNAse were added to the resuspended cell pellet, followed by cell lysis via sonication at 70% power for 5 minutes. Lysates were clarified by centrifugation at 4°C and 18,000 rpm for 45 minutes and applied to columns containing Ni-NTA (Qiagen) resin pre-equilibrated with lysis buffer. The column was washed two times with 5 column volumes (CV) of wash buffer, followed by 5 CV of elution buffer for protein elution.

Size-exclusion chromatography (SEC)

Eluted proteins were buffer exchanged into lysis buffer. N-terminal hexahistidine tags were removed with TEV protease cleavage overnight at room temperature, at a ratio of 1 mg TEV for 100 mg of protein. After TEV cleavage, sample was passed over a fresh Ni-NTA column and washed with 1.5 CV of lysis buffer, collecting flow through. The resulting proteins were purified by SEC using a Superdex™ 75 10/300 increase column (GE Healthcare) in TBS buffer.

Circular dichroism (CD) measurements

Circular dichroism (CD) wavelength scans (260 - 195 nm) and temperature melts (25 - 95 °C) were performed using an AVIV ™ model 420 CD spectrometer, with protein samples diluted to 0.25 mg/ml in PBS pH 7.4 in a 0.1-cm cuvette. Temperature melts were carried out at a heating rate of 4 °C/min and monitored by the change in ellipticity at 222 nm.

GdmCl titrations were performed on a JASCO ™ model J-1500 with automated titration apparatus in PBS pH 7.4 at 25 °C, with protein concentrations between 0.08 mg/ml to 0.025 mg/ml in a 1-cm cuvette with stir bar. Each titration consisted of at least 34 evenly distributed GdmCl concentration points up to 7.4 M with 30 seconds mixing time for each step. Titrant solution consisted of the same concentration of protein in PBS and GdmCl.

CD data analysis and model fitting

Folding free energies were obtained by fitting equilibrium denaturation data. Fused hairpin constructs had biphasic unfolding transitions, indicating the existence of an intermediate on their respective energy landscapes. Since native MS showed that Linker 0, Linker 2, Linker 6, and Linker 12 were almost exclusively monomeric in buffer (data not shown), it was concluded that these intermediates were partially folded monomeric species. Thus, the chemical denaturation data of these proteins was fitted to a unimolecular 3-state model:

$$N \Leftrightarrow I \Leftrightarrow D$$

where N represents the fully folded state, I a partially folded intermediate, and D the denatured state. The fraction of each species can be written as a function of $K_1 = [I]/[N]$ and $K_2 = [D]/[I]$, the equilibrium constants for the first and second transitions respectively:

$$f_N = (1 + K_1 + K_1 \cdot K_2)^{-1}$$
$$f_I = (1 + K_2 + \frac{1}{K_1})^{-1}$$
$$f_D = (1 + \frac{1}{K_2} + \frac{1}{K_1 \cdot K_2})^{-1}$$

In the context of equilibrium chemical denaturation experiments, the free energy of unfolding is a linear function of denaturant concentration:

$$\Delta G_{[den]} = \Delta G_{buffer} - m \cdot [den]$$

where $\Delta G_{[den]}$ represents the free energy of the system at a given concentration of denaturant, ΔG_{buffer} is the corresponding free energy change in the absence of denaturant, and m is a constant of proportionality that relates to the change in solvent-accessible surface area upon unfolding ($\Delta SASA$). Thus, the effect of denaturant on the equilibrium constant relating to each transition can be written as a function of its free energy difference in buffer, and a specific *m*-value:

$$K_1 = exp(\frac{m_1 \cdot [den] - \Delta G_1}{R \cdot T})$$
$$K_2 = exp(\frac{m_2 \cdot [den] - \Delta G_2}{R \cdot T})$$

By combining these expressions with the definitions for f_N, f_I, f_D, the fractional distribution of each species can be expressed as a function of denaturant concentration, and the free energy change corresponding to each transition (in buffer). Finally, for an ensemble spectroscopic technique such as CD, the observed signal (the dependent variable) as a function of denaturant concentration (the independent variable) can be expressed as a linear combination of the spectroscopic signals corresponding to each species, weighed by their fractional contribution to the ensemble:

$$MRE_{222nm} = f_N \cdot MRE_N + f_I \cdot MRE_I + f_D \cdot MRE_D$$

Where MRE_N, MRE_I, MRE_D represent the spectroscopic signatures (baselines) for the native, intermediate, and denatured states respectively. This equation was used to fit chemical denaturation data for the different linker proteins, and the fitted parameters are reported in Table 10. For Linker 24 in buffer, native MS revealed a significant proportion of dimer (data not shown). Therefore, this model is not entirely appropriate for describing the unfolding, and the fitted values for this construct should be interpreted with care. Nevertheless, denaturation performed at different concentrations of protein revealed that the position of the second transition was concentration-independent, and thus unimolecular. For this event, the model holds.

The total *m*-values for these linked hairpins were found to be around 3 kcal mol^{-1} M^{-1}. It has been shown that *m*-values correlate with $\Delta SASA$ of unfolding (*8*). For the folded state, *SASA* was estimated from the structures of DHDs (*1*) using PyMOL ™ to be 8800 $Å^2$. For the unfolded state, *SASA* was estimated using ProtSA ™ (*9*, *10*), and is about 20,000 $Å^2$. Thus,

ΔSASA for the unimolecular unfolding of a fused hairpin should be around 11,000 Å^2, which would have a predicted *m*-value of 3.3. This number is in close agreement with the fitted parameters reported here, in line with the notion that the folded state for these linker proteins has a four helix bundle topology.

Small Angle X-ray Scattering (SAXS)

Protein samples were purified by SEC in 25 mM Tris pH 8.0, 150 mM NaCl and 2% glycerol; elution fractions preceding the void volume of the column were used as blanks for buffer subtraction. Scattering measurements were performed at the SIBYLS ™ 12.3.1 beamline at the Advanced Light Source. The sample-to-detector distance was 1.5 m, and the X-ray wavelength (λ) was 1.27 Å, corresponding to a scattering vector q ($q = 4\pi \sin\theta/\lambda$, where 2θ is the scattering angle) range of 0.01 to 0.3 Å^{-1}. A series of exposures were taken of each well, in equal sub-second time slices: 0.3-s exposures for 10 s resulting in 32 frames per sample. For each sample, data were collected for two different concentrations to test for concentration-dependent effects; 'low' concentration samples ranged at 2.5 mg/ml and 'high' concentration samples at 5 mg/ml. Data were processed using the SAXS FrameSlice ™ online serve and analyzed using the ScÅtter ™ software package (*11*, *12*). The FoXS ™ online server (*13*, *14*) was used to compare experimental scattering profiles to design models and calculate quality of fit (χ) values.

Yeast two-hybrid assay for logic gates

Chemically competent cells of yeast strain PJ69-4a (MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4(deleted) gal80(deleted) LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ) were transformed with the appropriate pair of plasmids containing DNA binding domains (DBD) or activation domains (AD), using the LiAc/SS carrier DNA/PEG method (*15*). For two input CIPHR logic gates, genes encoding the input proteins (together with selection markers) were genetically integrated into either or both of the Ura3 locus (uracil auxotrophic selection marker) or the YCR043 locus (bleomycin selection marker). In the case of three input CIPHR logic gates, genes encoding two input proteins were genetically integrated as described, with the additional input cloned downstream of either the AD or DBD plasmid, separated by a p2a and nuclear localization sequence (GSGATNFSLLKQAGDVEENPGPGDKAELIPEPPKKKRKVELGTA; SEQ ID NO:330). The p2a sequence ensures translational cleavage to make the additional input protein a separate protein. The selection of transformed yeast cells was performed in synthetic dropout (SDO) medium lacking tryptophan and leucine for 48 h with shaking at 1,000 r.p.m. at 30 °C.

The resulting culture was diluted 1:100 and grown for 16 h in fresh SDO medium lacking tryptophan and leucine, before being diluted 1:100 in fresh SDO medium lacking tryptophan, leucine and histidine. The culture was incubated with shaking at 1,000 r.p.m. at 30 °C. As it is necessary to bring the DBD and the transcription activation domain into proximity for the growth of yeast cells in medium lacking histidine, successful activation of logic gates was indicated by the growth of yeast cells (*16*, *17*). The optical density of yeast cells was recorded at 24 h, 48 h, and 72 h.

Table 10. Fitted parameters for equilibrium chemical denaturation. Errors represent fitting errors.

	Linker 0	Linker 2	Linker 6	Linker 12	Linker 24
$\Delta G_1^{(N \Leftrightarrow I)}$ (kcal mol^{-1})	3.6 (±0.4)	3.5 (±0.2)	3.5 (±0.2)	2.7 (±0.1)	3.7 (±0.3)
$\Delta G_2^{(I \Leftrightarrow D)}$ (kcal mol^{-1})	9.8 (±0.6)	10.7 (±0.4)	12.2 (±0.4)	10.6 (±0.5)	10.4 (±0.8)
$\Delta G_{tot}^{(N \Leftrightarrow D)}$ (kcal mol^{-1})	13.5 (±0.7)	14.1 (±0.4)	15.7 (±0.5)	13.3 (±0.5)	14.1 (±0.8)
m_1 (kcal mol^{-1} M^{-1})	1.1 (±0.2)	1.0 (±0.1)	0.9 (±0.1)	0.75 (±0.05)	1.1 (±0.1)
m_2 (kcal mol^{-1} M^{-1})	1.8 (±0.1)	1.97 (±0.07)	2.22 (±0.08)	1.96 (±0.08)	2.0 (±0.1)
m_{tot} (kcal mol^{-1} M^{-1})	2.9 (±0.2)	3.0 (±0.1)	3.1 (±0.1)	2.71 (±0.09)	3.1 (±0.2)
MRE_N (deg cm^2 dmol^{-1})	-23,574 (±114)	-27,561 (±84)	-24,712 (±63)	-33,849 (±131)	-26,438 (±123)
MRE_I (deg cm^2 dmol^{-1})	-16,330 (±749)	-18,139 (±540)	-14,779 (±710)	-17,362 (±1,158)	-15,567 (±914)
MRE_D (deg cm^2 dmol^{-1})	-525 (±107)	-785 (±82)	-937 (±68)	-1,104 (±99)	-1,125 (±133)

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We claim

1. A designed heterodimer protein, comprising:

(a) a monomer A polypeptide, wherein the monomer A polypeptide is a non-naturally occurring polypeptide comprising 1-5 alpha helices connected by amino acid linkers; and

(b) a monomer B polypeptide, wherein the monomer B polypeptide is a non-naturally occurring polypeptide comprising 1-5 alpha helices connected by amino acid linkers,

wherein monomer A and monomer B non-covalently interact to form the designed heterodimer protein.

2. The designed heterodimer protein of claim 1 wherein monomer A and monomer B have their interaction specificity determined by at least one designed hydrogen bond network at the interface between monomer A and monomer B.

3. The designed heterodimer protein of any one of claims 1-2, wherein at least one of monomer A and monomer B comprises a hexahistidine tag, and/or wherein
(i) monomer A comprises 2 alpha helices, and monomer B comprises 3 alpha helices;
(ii) monomer A comprises 3 alpha helices and monomer B comprises 3 alpha helices;
(iii) monomer A comprises 3 alpha helices and monomer B comprises 4 alpha helices,
(iv) monomer A comprises 4 alpha helices and monomer B comprises 3 alpha helices;
(v) monomer A comprises 4 alpha helices and monomer B comprises 4 alpha helices;
(vi) monomer A comprises 5 alpha helices and monomer B comprises 4 alpha helices;
(vii) monomer A comprises 4 alpha helices and monomer B comprises 5 alpha helices;
(viii) monomer A comprises 5 alpha helices and monomer B comprises 5 alpha helices;
(ix) monomer A comprises 2 alpha helices and monomer B comprises 2 alpha helices;
or (x) monomer A comprises 3 alpha helices and monomer B comprises 2 alpha helices.

4. The designed heterodimer protein of any one of claims 1-3, wherein:

(i) monomer A comprises a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an odd-numbered SEQ ID NO

selected from the group consisting of selected from the group SEQ ID NOS: 1-290; and

(ii) monomer B comprises a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an even-numbered SEQ ID NO selected from the group consisting of selected from the group SEQ ID NOS: 1-290, wherein the even-numbered SEQ ID NO is the binding partner of the odd-numbered SEQ ID NO. in step (i).

5. The designed heterodimer protein of claim 4, wherein amino acid changes from the reference amino acid sequence are conservative amino acid substitutions.

6. The designed heterodimer protein of claim 4 or 5, wherein at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of defined interface positions are invariant compared to the reference amino acid sequence.

7. A non-naturally occurring polypeptide comprising a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-290.

8. The non-naturally occurring polypeptide of claim 8, wherein amino acid changes from the reference amino acid sequence are conservative amino acid substitutions.

9. The non-naturally occurring polypeptide of claim 7 or 8, wherein at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions are invariant compared to the reference amino acid sequence.

10. A protein comprising 2, 3, 4, or more non-naturally occurring polypeptides having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS: 1-290, wherein the 2, 3, 4, or more naturally occurring polypeptides are covalently linked.

11. The protein of claim 10, wherein each of the 2, 3, 4, or more non-naturally occurring polypeptides are different.

12. The protein of claim 10 or 11, wherein each of the 2, 3, 4, or more non-naturally occurring polypeptides are present in a fusion protein.

13. The protein of any one of claims 10-12, wherein each of the 2, 3, 4, or more non-naturally occurring polypeptides have at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an odd-numbered SEQ ID NO: selected from the group consisting of SEQ ID NOS:1-290.

14. The protein of any one of claims 10-12, wherein each of the 2, 3, 4, or more non-naturally occurring polypeptides have at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an even-numbered SEQ ID NO: selected from the group consisting of SEQ ID NOS:1-290.

15. The protein of any one of claims 10-12, wherein the 2, 3, 4, or more non-naturally occurring polypeptides include:

(a) polypeptides having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an odd-numbered SEQ ID NO: selected from the group consisting of SEQ ID NOS:1-290; and

(b) polypeptides having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an even-numbered SEQ ID NO: selected from the group consisting of SEQ ID NOS:1-290.

16. The protein of any one of claims 10-15, comprising the amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group consisting of SEQ ID NOS:291, 294, 296, 299, and 302-305.

17. The protein of claim any one of claims 10-16, wherein at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions are invariant compared to the reference amino acid sequence.

18. A protein scaffold, comprising

a) a first designed component comprised of any number of monomer A polypeptides and/or monomer B polypeptides, each from different heterodimers, connected into a single component by amino acid linkers.

b) a second designed component, comprising corresponding monomers for each monomer A and/or monomer B in the first designed component one;

wherein the first and second designed components interact to form the protein scaffold, and wherein each monomer A only interacts in the scaffold with its monomer B mate.

19. The protein scaffold of claim 18, wherein the first designed component comprises the protein of any one of claims 10-17, and the second designed component comprises a plurality of individual polypeptides of any one of claims 7-9.

20. The protein scaffold of claim 18 or 19, wherein any of the monomers in the second designed component comprises a linker.

21. The protein scaffold of any one of claims 18-20, wherein the scaffold is stable up to 95°C and has a guanidine denaturation midpoint of 4 M.

22. A method of forming the designed heterodimer protein of claims 1-6, comprising:

a) providing two of the monomers as unlinked monomers;

b) providing the other two monomers as linked monomers

whereby the unlinked monomers associate with their respective monomer of the same heterodimer, and not with any of the other monomers.

21. A designed heterodimer protein comprising:

a) asymmetic buried hydrogen bond networks incorporated into regularly repeating backbone structures; and

b) helix hairpin helix monomers wherein the supercoil phases of the helices are fixed at 0, 90, 180, or 270 degrees and the supercoil twist (ω0) and helical twist (ω1) are held constant for either a two layer left handed super coil (ω0=-2.85 and ω1=102.85), or a 5 layer untwisted bundle (ω0=0 and ω1=100)

22. A nucleic acid encoding the polypeptide of claims 7-9 or the protein of any one of claims 10-17.

23. An expression vector comprising the nucleic acid of claim 22 operatively linked to a promoter.

24. A cell comprising the nucleic acid of claim 22, the expression vector of claim 23, and/or the polypeptide, protein, heterodimer protein, and/or protein scaffold of any claim herein.

25. Use of the polypeptide, protein, heterodimer protein, protein scaffold , nucleic acid, expression vector, and/or cell of any preceding claim for any suitable purposed, including but not limited to those disclosed herein such as designing protein logic gates.

26. A fusion protein comprising a polypeptide of the formula X-B-Z, wherein:

(a) the X domain is a non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices, wherein the X domain is capable of non-covalently binding to a first target;

(b) the Z domain is a non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices, wherein the Z domain is capable of non-covalently binding to either (i) a second target that differs from the first target, or (ii) a different non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices; and

(c) the B domain is an amino acid linker;

wherein a combined number of alpha helices from the X domain and the Z domain is 4, 5, or 6; and

wherein the X domain and the Z domain interact at a binding interface, wherein the binding interface comprises a hydrogen bond network in which at least one side chain in each alpha helix hydrogen of the X domain bonds with a side chain in an alpha helix in the Z domain, and wherein the binding interface comprises a plurality of hydrophobic residues.

27. A kit or composition, comprising at least two fusion proteins comprising the formula X-B-Z, wherein

the B domain in each fusion protein is independently a polypeptide linker;

the X domain in each fusion protein comprises a first non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices;

the Z domain in each fusion protein comprises a second non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices, wherein a combined number of alpha helices from the X domain and the Z domain in each individual fusion protein is 4, 5, or 6; wherein the X domain and the Z domain interact at a binding interface, wherein the binding interface comprises a hydrogen bond network in which at least one side chain in each X domain alpha helix bonds with a side chain in an alpha helix in the Z domain; wherein

the X domain in a first fusion protein is capable of non-covalently binding to a first target;

the Z domain in a second fusion protein is capable of non-covalently binding to a second target; and

the X domains and Z domains in each individual fusion protein that are not capable of non-covalently binding to the first target or the second target are capable of non-covalently binding to an X or a Z domain of a different fusion protein in the plurality of fusion proteins.

28. The kit or composition of claim 27, wherein

(i) the first fusion protein has the formula X1-B1-Z1, wherein the X1 domain is capable of non-covalently binding to the first target; and

(ii) the second fusion protein has the formula X2-B2-Z2, wherein the Z2 domain is capable of non-covalently binding to the second target; and wherein the Z1 and X2 domains are capable of non-covalently binding to each other.

29. The kit or composition of claim 27, wherein:

(i) the first fusion protein has the formula X1-B1-Z1, wherein the X1 domain is capable of non-covalently binding to the first target; and

(ii) the second fusion protein has the formula X2-B2-Z2,

(iii) the at least two fusion proteins comprise a third fusion protein of formula X3-B3-Z3, wherein the Z3 domain is capable of non-covalently binding to the second target; wherein

(A) the Z1 and X2 domains are capable of non-covalently binding to each other; and

(B) the Z2 and X3 domains are capable of non-covalently binding to each other.

30. The fusion protein of claim 26 or the kit or compositions of claims 27-29, wherein the binding interface comprises at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60% or greater hydrophobic residues.

31. The fusion protein, kit, or composition of any one of claims 26-30, wherein the B domain for each fusion protein is independently between 6-12, 6-11, 6-10, 7-12, 7-11, 7-10, 8-12, 8-11, 8-10, 9-12, 9-11, 9-10, 10-12, 10-11, 11-12, 6, 7, 8, 9, 10, 11, or 12 amino acids in length.

32. The fusion protein, kit, or composition of any one of claims 26-31, wherein the combined number of alpha helices from the X and Z domains in an individual fusion protein is 4.

33. The fusion protein, kit, or composition of any one of claims 26-32, wherein the X domain of each fusion protein has 2 alpha helices and the Z domain of each fusion protein has 2 alpha helices.

34. The fusion protein, kit, or composition of any one of claims 26-33, wherein either the X domain or the Z domain of each fusion protein has 1 alpha helix and the other has 3 alpha helices.

35. The fusion protein, kit, or composition of any one of claims 26-34, wherein each X domain and each Z domain comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%,

80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide comprising the amino acid sequence selected from SEQ ID NO:1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, with the proviso that the X domain and the Z domain do not do not form a heterodimer (a-b) pair.

36. The fusion protein, kit, or composition of claim 35, wherein at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions of each X domain and each Z domain are invariant compared to the reference amino acid sequence.

37. The fusion protein, kit, or composition of any one of claims 26-36, wherein each fusion protein independently comprises a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO: 302, 303, 306-326, 439, 441, 443, 445, 447, 449, 451, 453, 455, and 457.

38. The kit or composition of any one of claims 27-37, further comprising the first target and the second target.

39. The kit or composition of claim 38, wherein the first target and the second target each independently comprise a polypeptide of the formula X10-B10-Z10, wherein

(a) the X10 domain is a non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices;

(b) the Z10 domain is a non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices; and

(c) the B10 domain is an amino acid linker;

wherein the X domain and the Z domain interact at a target binding interface, wherein the target binding interface comprises a hydrogen bond network in which at least one side chain in each alpha helix hydrogen of the X domain bonds with a side chain in a different

alpha helix in the Z domain, and wherein the target binding interface comprises a plurality of hydrophobic residues.

40. The kit or composition of claim 39, wherein the target binding interface comprises at least 25% hydrophobic residues.

41. The kit, or composition of any one of claims 39-40, wherein the B10 domain for the first target and the second target is independently between 6-12, 6-11, 6-10, 7-12, 7-11, 7-10, 8-12, 8-11, 8-10, 9-12, 9-11, 9-10, 10-12, 10-11, 11-12, 6, 7, 8, 9, 10, 11, or 12 amino acids in length.

42. The kit, or composition of any one of claims 38-41, wherein the combined number of alpha helices from the X and Z domains in the first target and the second target protein is 4.

43. The kit, or composition of any one of claims 39-42, wherein

(a) the X10 domain of each of the first target and the second target has 2 alpha helices and the Z10 domain of each of the first target and the second target has 2 alpha helices; or

(b) either the X10 domain or the Z10 domain of each of the first target and the second target has 1 alpha helix and the other has 3 alpha helices

44. The kit, or composition of any one of claims 39-43, wherein each X10 domain and each Z10 domain comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, with the proviso that the X10 domain forms a heterodimer (a-b) pair with the X domain of the fusion protein, and the Z10 domain forms a heterodimer (a-b) pair with the Z domain of the fusion protein.

45. The fusion protein, kit, or composition of claim 44, wherein at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%,

95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions of each X domain and each Z domain are invariant compared to the reference polypeptide amino acid sequence.

46. The kit, or composition of any one of claims 39-45, wherein the first target and/or the second target further comprise one or more effector polypeptide domains linked to one or more of the X10 and/or Z10 domains, for example, wherein the one or more effector polypeptide domains may optionally comprise a polypeptide including, but not limited to, nucleic acid binding proteins, transcription factors, receptor binding proteins, split enzymes, effectors of membrane receptors, etc.

47. Use of the fusion proteins, kits, or compositions of any one of claims 26-46 for any purpose as described herein.

48. A method, comprising:

(i) contacting a fusion protein according to any one of claims 26-37 with a biological sample under conditions to promote non-covalent binding of the fusion protein with first target and second target present in the sample, and

(ii) detecting non-covalent binding of the one or more fusion proteins to the first target and/or the second target in the biological sample.

49. The method of claim 48, wherein the method comprises detecting cooperative non-covalently binding of the one or more fusion proteins to the first target and the second target in the biological sample.

50. The method of claim 48, wherein the method comprises detecting non-covalent binding of the one or more fusion proteins to the first target or the second target in the biological sample.

51. A method for target detection, comprising

(a) contacting a biological sample with at least two fusion proteins, wherein each of the at least two fusion proteins comprises the formula X-B-Z, wherein

each B is independently a polypeptide linker;

each X domain comprises a first non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices;

each Z domain comprises a second non-naturally occurring polypeptide comprising 1, 2, or 3 alpha helices, wherein a combined number of alpha helices from the X domain and the Z domain in each individual fusion protein is 4, 5, or 6; wherein the X domain and the Z domain interact at a binding interface, wherein the binding interface comprises a hydrogen bond network in which at least one side chain in each X domain alpha helix bonds with a side chain in an alpha helix in the Z domain; wherein

the X domain in a first fusion protein is capable of non-covalently binding to a first target;

the Z domain in a second fusion protein is capable of non-covalently binding to a second target; and

the X domains and Z domains in each individual fusion protein that are not capable of non-covalently binding to the first target or the second target are capable of non-covalently binding to an X or a Z domain of a different fusion protein in the plurality of fusion proteins;

(b) detecting non-covalent binding of the two or more fusion proteins to the first target and/or the second target in the biological sample.

52. The method of claim 51, wherein the detecting comprises detecting cooperative non-covalent binding of the two or more fusion proteins to the first target and the second target in the biological sample.

53. The method of claim 51 or 52, wherein:

(i) the first fusion protein has the formula X1-B1-Z1, wherein the X1 domain is capable of non-covalently binding to the first target; and

(ii) the second fusion protein has the formula X2-B2-Z2, wherein the Z2 domain is capable of non-covalently binding to the first target; and wherein the Z1 and X2 domains are capable of non-covalently binding to each other.

54. The method of claim 51 or 52, wherein:

(i) the first fusion protein has the formula X1-B1-Z1, wherein the X1 domain is capable of non-covalently binding to the first target; and

(ii) the second fusion protein has the formula X2-B2-Z2,

(iii) the at least two fusion proteins comprise a third fusion protein of formula X3-B3-Z3, wherein the Z3 domain is capable of non-covalently binding to the second target; wherein

(A) the Z1 and X2 domains are capable of non-covalently binding to each other; and

(B) the Z2 and X3 domains are capable of non-covalently binding to each other.

55. The method of any one of claims 51-54, wherein the X domains, Y domains, B domains, and or fusion proteins are as recited in any one of claims 26-37.

56. The method of any one of claims 48-55, wherein at least one of the fusion proteins comprises one or more effector polypeptide domains linked to one or more of the X and/or Z domains, and wherein the detecting step comprises detecting an output signal caused by binding the first target and/or the second target.

57. The method of claim 56, wherein the detecting step comprises detecting an output signal from the one or more effector polypeptide caused by cooperative non-covalently binding of the first target and the second target.

58. The method of claim 56 or 57, wherein the one or more effector polypeptide domains may comprise a polypeptide including, but not limited to, nucleic acid binding proteins, transcription factors, receptor binding proteins, nucleic acid binding proteins, transcription factors, receptor binding proteins, split enzymes, effectors of membrane receptors, etc..

59. The method of any one of claims 56-58, wherein the output signal may include, but is not limited to fluorescence activity, functional activity, etc.

60. The fusion protein, kit or composition of any one of claims 26-46, or the method of any one of claims 48-59, wherein the first target and/or the second target comprise polypeptides or nucleic acids.

61. A composition comprising

(a) a first polypeptide comprising 2 alpha helices, wherein the first polypeptide is capable of non-covalently binding a first target; and

(b) a second polypeptide comprising 2 alpha helices, wherein the first polypeptide is capable of non-covalently binding to the second polypeptide, and wherein the second polypeptide is capable of non-covalently binding a second target that differs from the first target; wherein:

(i) a binding affinity of the first polypeptide for the first target is approximately equal to a binding affinity of the second polypeptide for the second target; and

(ii) the binding affinity of the first polypeptide for the first target and the binding affinity of the second polypeptide for the second target are greater than the binding affinity of the first target and the second target for each other.

62. The composition of claim 61, further comprising the first target and the second target.

63. The composition of claim 62, wherein the first target and/or the second target further comprise one or more effector polypeptide domains.

64. The composition of claim 63, wherein the one or more effector polypeptide domains may comprise a polypeptide including, but not limited to, nucleic acid binding proteins, transcription factors, receptor binding proteins, split enzymes, effectors of membrane receptors, etc.

65. The composition of any one of claims 61-64, wherein the first polypeptide and/or the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494.

66. The composition of claim 65, wherein at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions of the first

polypeptide and/or the second polypeptide are invariant compared to the reference polypeptide amino acid sequence

67. The composition of any one of claims 61-66, wherein

(a) the first polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to the amino acid sequence of SEQ ID NO:3; and

(b) the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to the amino acid sequence of SEQ ID NO:58.

68. The composition of any one of claims 61-67, wherein the first target and/or the second target each comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, with the proviso that the first target forms a heterodimer (a-b) pair with the first polypeptide, and the second target forms a heterodimer (a-b) pair with the second polypeptide.

69. The composition of claim 46, wherein at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions of the first target and/or the second target are invariant compared to the reference polypeptide amino acid sequence.

70. A method, comprising:

(a) contacting a biological sample with the composition of any one of claims 61-69; and

(b) detecting binding, of the first polypeptide to the first target and binding of the second polypeptide to the second target in the sample, such as detecting an output signal caused by actions of effector polypeptides upon binding.

71. A composition comprising

(a) a first polypeptide comprising 2 alpha helices, wherein the first polypeptide is capable of non-covalently binding a first target; and

(b) a second polypeptide comprising 2 alpha helices, wherein the first polypeptide is capable of non-covalently binding to the second polypeptide, and wherein the second polypeptide is capable of non-covalently binding a second target that differs from the first target; wherein:

(i) a binding affinity of the first polypeptide for the second polypeptide is greater than a binding affinity of the second polypeptide for the second target;

(ii) a binding affinity of the first polypeptide for the first target is approximately equal to a binding affinity of the second polypeptide for the second target; and

(iii) the binding affinity of the first polypeptide for the first target and the binding affinity of the second polypeptide for the second target are greater than the binding affinity of the first target and the second target for each other.

72. The composition of claim 71, further comprising the first target and the second target.

73. The composition of claim 72, wherein the first target and/or the second target further comprise one or more effector polypeptide domains.

74. The composition of claim 73, wherein the one or more effector polypeptide domains may comprise a polypeptide including, but not limited to, nucleic acid binding proteins, transcription factors, receptor binding proteins, split enzymes, effectors of membrane receptors, etc.

75. The composition of any one of claims 71-74, wherein the first polypeptide and/or the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide

comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494.

76. The composition of claim 75, wherein at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions of the first polypeptide and/or the second polypeptide are invariant compared to the reference polypeptide amino acid sequence.

77. The composition of any one of claims 71-76, wherein

(a) the first polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited the amino acid sequence of SEQ ID NO:3； and

(b) the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to the amino acid sequence of SEQ ID NO:42.

78. The composition of any one of claims 71-77, wherein the first target and/or the second target each comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, with the proviso that the first target forms a heterodimer (a-b) pair with the first polypeptide, and the second target forms a heterodimer (a-b) pair with the second polypeptide.

79. The composition of claim 78, wherein at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98%, 99%, or 100% of amino acid residues at defined interface positions of the first target and/or the second target are invariant compared to the reference polypeptide amino acid sequence.

80. A method, comprising:

(a) contacting a biological sample with the composition of any one of claims 71-79; and

(b) detecting binding interactions between the first polypeptide and the first target. the second polypeptide and the second target, the first polypeptide and the second polypeptide, and the first target and the second target in the sample, such as detecting an output signal caused by actions of effector polypeptides upon binding.

81. A composition comprising

(a) a first polypeptide comprising 4 alpha helices, wherein the first polypeptide is capable of non-covalently binding a first target; and

(b) a second polypeptide comprising 4 alpha helices, wherein the second polypeptide is capable of non-covalently binding a second target that differs from the first target; wherein:

(i) a binding affinity of the first target for the second target is greater than a binding affinity of the first polypeptide for the first target;

(ii) a binding affinity of the first polypeptide for the first target is approximately equal to a binding affinity of the second polypeptide for the second target; and

(iii) the sum of the binding affinity of (A) the first polypeptide for the first target and (B) the binding affinity of the second polypeptide for the second target, is greater than the binding affinity of the first target and the second target.

82. The composition of claim 81, further comprising the first target and the second target.

83. The composition of claim 82, wherein the first target and/or the second target further comprise one or more effector polypeptide domains.

84. The composition of claim 83, wherein the one or more effector polypeptide domains may comprise a polypeptide including, but not limited to, nucleic acid binding proteins,

transcription factors, receptor binding proteins, split enzymes, effectors of membrane receptors, etc.

85. The composition of any one of claims 81-84, wherein the first polypeptide and/or the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494.

86. The composition of claim 85, wherein at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions of the first polypeptide and/or the second polypeptide are invariant compared to the reference polypeptide amino acid sequence.

87. The composition of any one of claims 81-86, wherein

(a) the first polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited the amino acid sequence of SEQ ID NO: 42； and

(b) the second polypeptide comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to the amino acid sequence of SEQ ID NO:57.

88. The composition of any one of claims 81-87, wherein the first target and/or the second target each comprise a polypeptide that is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the full length of a polypeptide selected from the group including, but not limited to a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448,

450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, with the proviso that the first target forms a heterodimer (a-b) pair with the first polypeptide, and the second target forms a heterodimer (a-b) pair with the second polypeptide.

89. The composition of claim 88, wherein at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of amino acid residues at defined interface positions of the first target and/or the second target are invariant compared to the reference polypeptide amino acid sequence.

90. A method, comprising:

(a) contacting a biological sample with the composition of any one of claims 81-88; and

(b) detecting binding interactions between the first polypeptide and the first target. the second polypeptide and the second target, and the first target and the second target in the sample, such as detecting an output signal caused by actions of effector polypeptides upon binding.

91. A nucleic acid encoding the fusion protein of any one of claims 26 and 30-37.

92. An expression vector comprising the nucleic acid of claim 91 operatively linked to a promoter.

93. A host cell comprising the nucleic acid of claim 91 or the expression vector of claim 92.

94. The host cell of claim 93, wherein the nucleic acid or the expression vector is integrated into a host cell chromosome.

95. The host cell of claim 93, wherein the nucleic acid or the expression vector is episomal.

96. A heterodimer, comprising:

(a) a monomer A polypeptide, wherein the monomer A polypeptide is a non-naturally occurring polypeptide comprising 1-5 alpha helices, wherein adjacent alpha helices may optionally be connected by an amino acid linker; and

(b) a monomer B polypeptide, wherein the monomer B polypeptide is a non-naturally occurring polypeptide comprising 1-5 alpha helices, wherein adjacent alpha helices may optionally be connected by an amino acid linker;

wherein the monomer A polypeptide and the monomer B polypeptide non-covalently interact to form the designed heterodimer protein.

97. The heterodimer of claim 96 wherein the monomer A polypeptide and the monomer B polypeptide have their interaction specificity determined by at least one hydrogen bond network at the interface between the monomer A polypeptide and the monomer B polypeptide.

98. The heterodimer of claim 96 or 97, wherein

(i) the monomer A polypeptide comprises 2 alpha helices, and the monomer B polypeptide comprises 3 alpha helices;

(ii) the monomer A polypeptide comprises 3 alpha helices and the monomer B polypeptide comprises 3 alpha helices;

(iii) the monomer A polypeptide comprises 3 alpha helices and the monomer B polypeptide comprises 4 alpha helices,

(iv) the monomer A polypeptide comprises 4 alpha helices and the monomer B polypeptide 3 alpha helices;

(v) the monomer A polypeptide comprises 4 alpha helices and the monomer B polypeptide comprises 4 alpha helices;

(vi) the monomer A polypeptide comprises 5 alpha helices and the monomer B polypeptide comprises 4 alpha helices;

(vii) the monomer A polypeptide comprises 4 alpha helices and the monomer B polypeptide comprises 5 alpha helices;

(viii) the monomer A polypeptide comprises 5 alpha helices and the monomer B polypeptide comprises 5 alpha helices;

(ix) the monomer A polypeptide comprises 2 alpha helices and the monomer B polypeptide comprises 2 alpha helices; or

(x) the monomer A polypeptide comprises 3 alpha helices and the monomer B polypeptide comprises 2 alpha helices.

99. The heterodimer of any one of claims 96-98, wherein:

(i) the monomer A polypeptide comprises the amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an odd-numbered SEQ ID NO selected from the group consisting of selected from the group SEQ ID NOS: 1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494; and

(ii) the monomer B polypeptide the amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of an even-numbered SEQ ID NO selected from the group consisting of selected from the group SEQ ID NOS: 1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494, wherein the even-numbered SEQ ID NO is the binding partner of the odd-numbered SEQ ID NO. in step (i).

100. The heterodimer of claim 99, wherein amino acid changes from the reference amino acid sequence are conservative amino acid substitutions.

101. The heterodimer of claim 99 or 100, wherein amino acid residues at 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of defined interface positions are invariant compared to the reference amino acid sequence.

102. A protein scaffold, comprising

(a) a fusion protein comprising of 2, 3, 4, or more polypeptides, wherein each polypeptide present in the fusion protein is a non-naturally occurring polypeptide comprises 1-5 alpha helices, wherein adjacent alpha helices may optionally be connected by an amino acid linker;

wherein each polypeptide in the fusion protein is capable of non-covalently interacting with a binding partner, and wherein the fusion protein does not comprise a binding partner for any polypeptide present in the fusion protein; and

(b) a binding partner for at least one of the polypeptides present in the fusion protein;

wherein the fusion protein and the binding partner non-covalently interact to form the protein scaffold, wherein an interaction specificity between the binding partner and the at least polypeptide in the fusion protein are determined by at least one hydrogen bond network at the interface between the binding partner and the at least one polypeptide.

103. The protein scaffold of claim 102, wherein the binding partner for at least one of the polypeptides present in the fusion protein comprises a binding partner for each polypeptide present in the fusion protein.

104. The protein scaffold of claim 102 or 103, wherein the fusion protein comprises at least 3 or 4 polypeptides in total.

105. The protein scaffold of any one of claims 102-104, wherein each polypeptide in the fusion protein are different polypeptides.

106. The protein scaffold of any one of claims 102-105, wherein the fusion protein comprises the protein of any one of claims 20-26, and the binding partner comprises a plurality of individual polypeptides of any one of claims 10-12.

107. The protein scaffold of any one of claims 102-106, wherein

(i) the fusion protein comprises 2, 3, 4, or more polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence of SEQ ID NOS: 1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494; and

(ii) the binding partner comprises a binding partner as defined herein for each polypeptide in (i), wherein each binding partner has at least 70%, 75%, 80%, 85%,

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity along the length of the amino acid sequence selected from the group SEQ ID NOS: 1-290, 331, 332, 334, 336-422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458-460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 493, and 494.

108. The protein scaffold of claim 107, wherein amino acid changes in the fusion protein and the binding partner from the reference amino acid sequence are conservative amino acid substitutions.

109. The protein scaffold of claim 107 or 108, wherein amino acid residues at 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of defined interface positions in the polypeptides in the fusion protein and the binding partner are invariant compared to the reference amino acid sequence.

110. The protein scaffold any one of claims 102-109, wherein the at least one least one hydrogen bond network is assymetric.

111. The protein scaffold any one of claims 102-111, wherein the binding interface comprises at least 25% hydrophobic residues..

112. The protein scaffold any one of claims 102-111, wherein the scaffold is stable up to 95°C and has a guanidine denaturation midpoint of 4 M.

113. The heterodimer protein of any one of claims 96 to 101 or protein scaffold of any one of claims 102 to 112, wherein the amino acid linker comprises (GSEGS)n (SEQ ID NO:423), wherein n is between 1 and 10.

114. The heterodimer protein or protein scaffold of claim 113, wherein the amino acid linker comprises GSEGSGSEGSG (SEQ ID NO:429) or GSEGSGSEGSGGS (SEQ ID NO:461).

115. The protein scaffold of any one of claims 102 to 114, wherein the hydrogen bond network comprises one or more hydrogen bonds.

116. The protein scaffold of any one of claims 102 to 115, wherein the hydrogen bond network is formed between the interface residues according to Table 2.

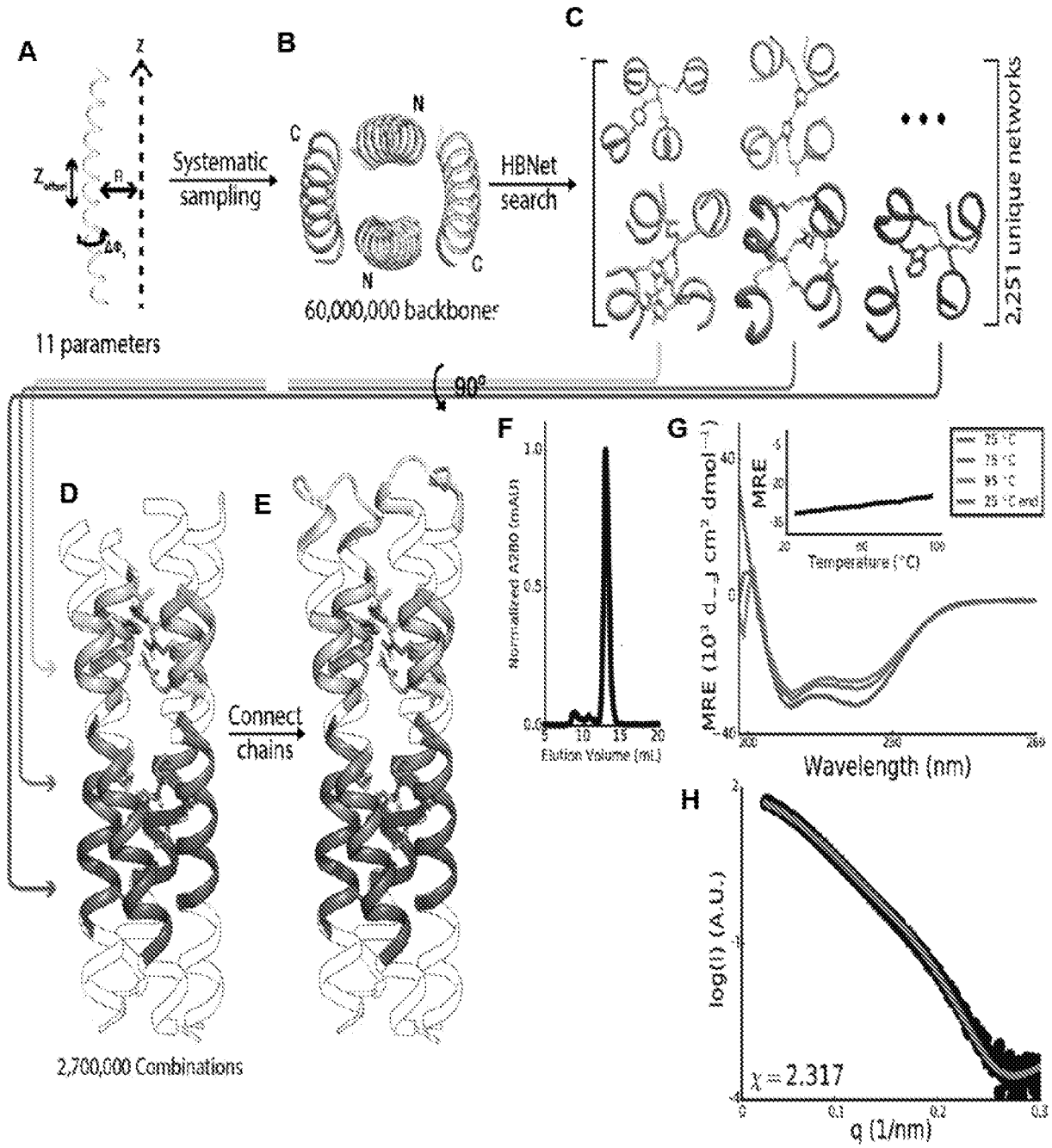

A
B
C
Systematic sampling
HBNet search
11 parameters
60,000,000 backbones
2,251 unique networks
90°
D
E
Connect chains
2,700,000 Combinations
F
Normalized A280 (mAU)
Elution Volume (mL)
G
MRE
Temperature (°C)
MRE (10³ deg cm² dmol⁻¹)
Wavelength (nm)
H
log(I) (A.U.)
χ = 2.317
q (1/nm)

FIGURE 1A – 1H

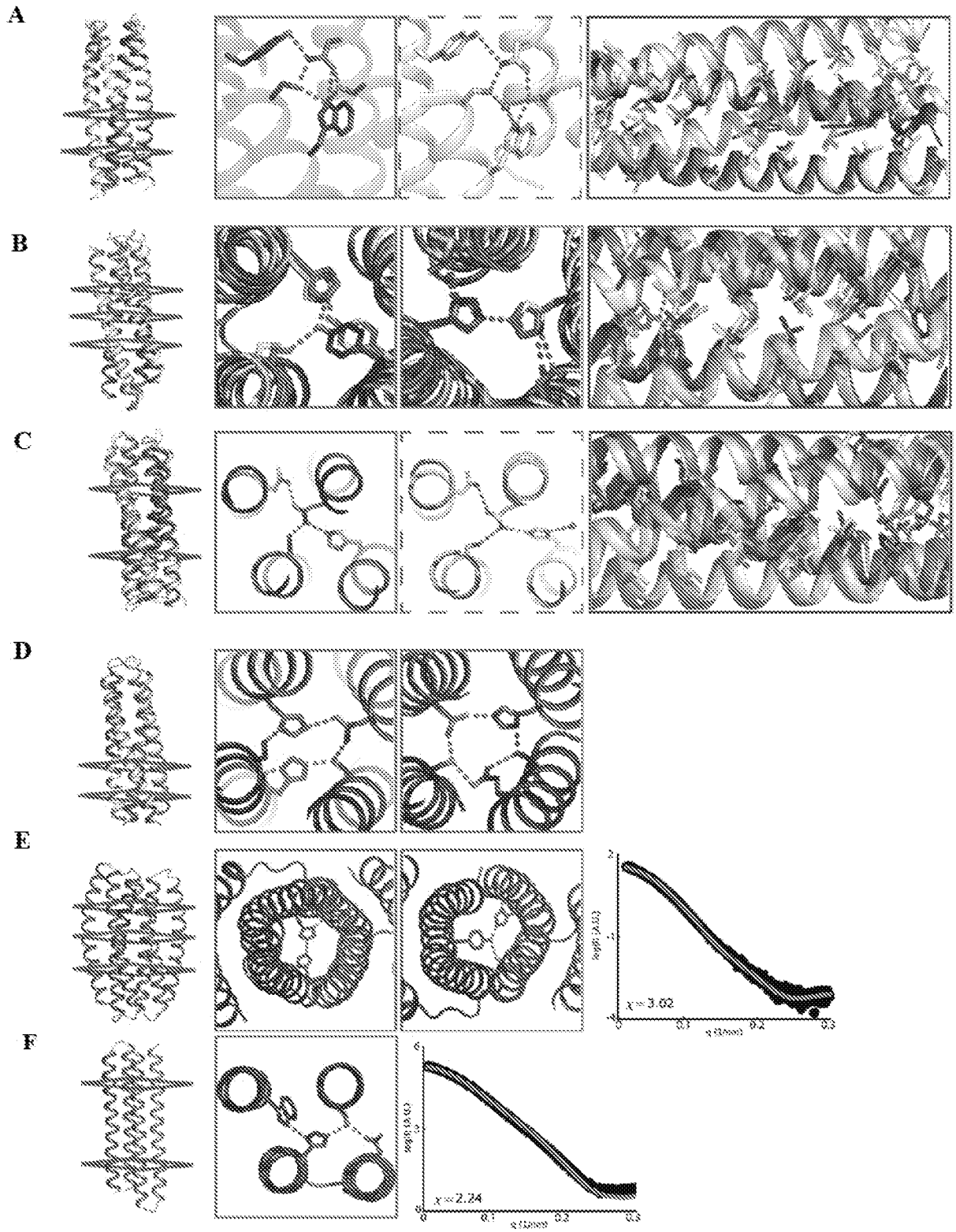


FIGURE 2A – 2F

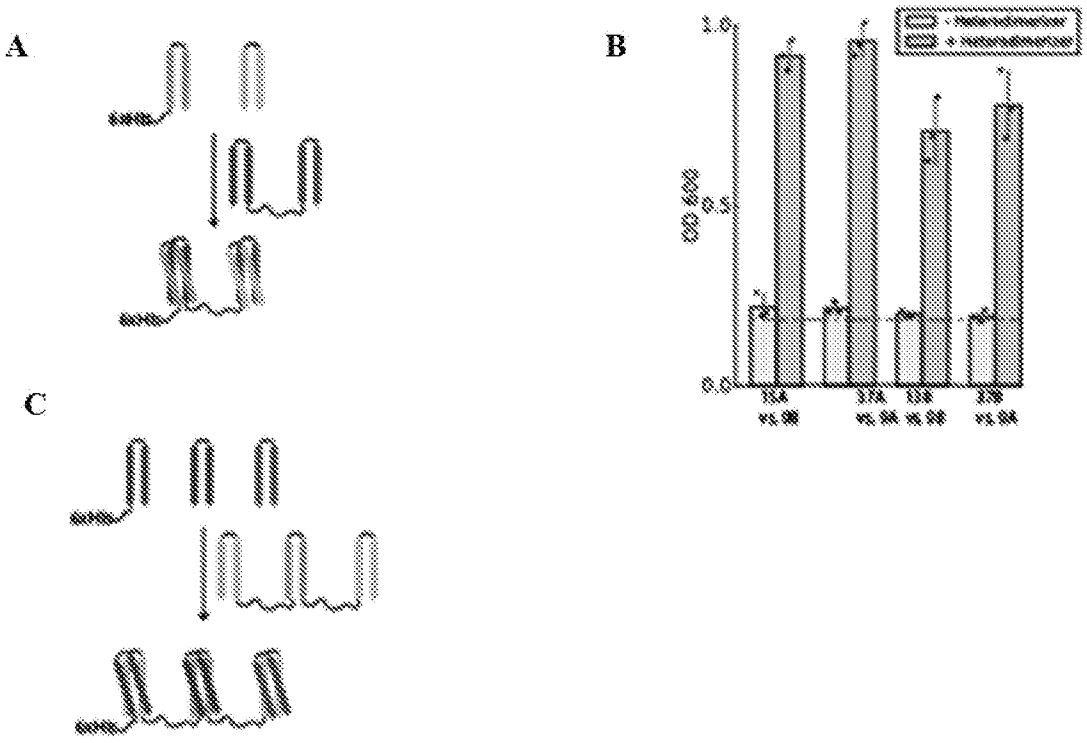


FIGURE 3A – 3C

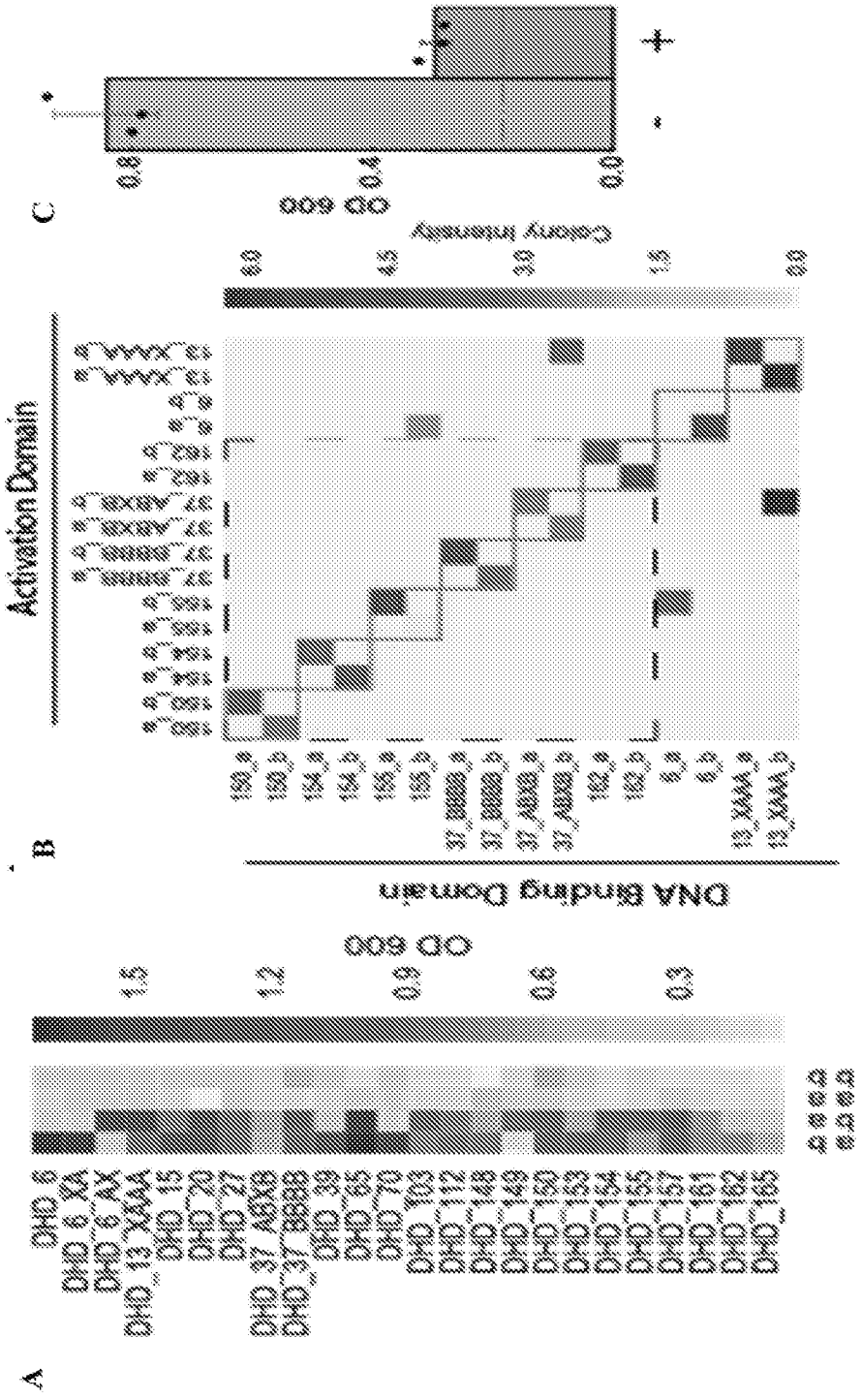
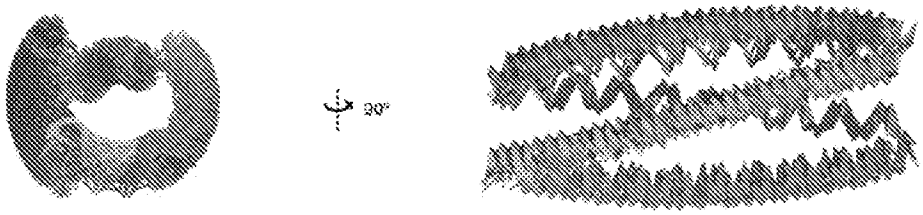

A
DHD_6
DHD_6_XA
DHD_6_AX
DHD_13_XAAA
DHD_15
DHD_20
DHD_27
DHD_37_AGXB
DHD_37_BBBB
DHD_38
DHD_65
DHD_70
DHD_103
DHD_112
DHD_148
DHD_149
DHD_150
DHD_153
DHD_154
DHD_155
DHD_157
DHD_161
DHD_162
DHD_165
OD 600
1.5
1.2
0.9
0.6
0.3
B
DNA Binding Domain
Activation Domain
C
OD 600
0.8
0.4
0.0
Colony Intensity
6.0
4.5
3.0
1.5
0.0

FIGURE 4A – 4C

A



B

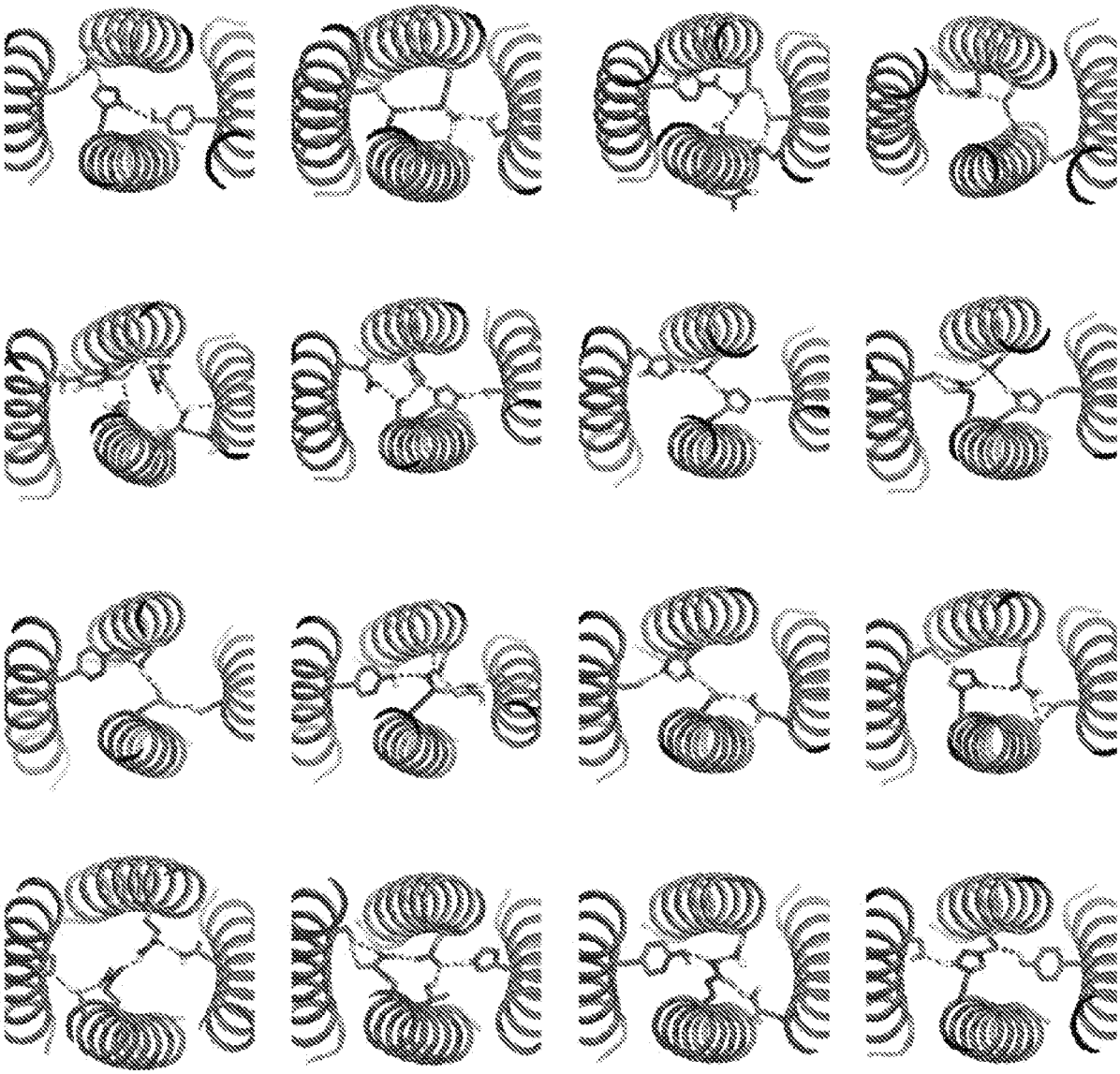


FIGURE 5A – 5B

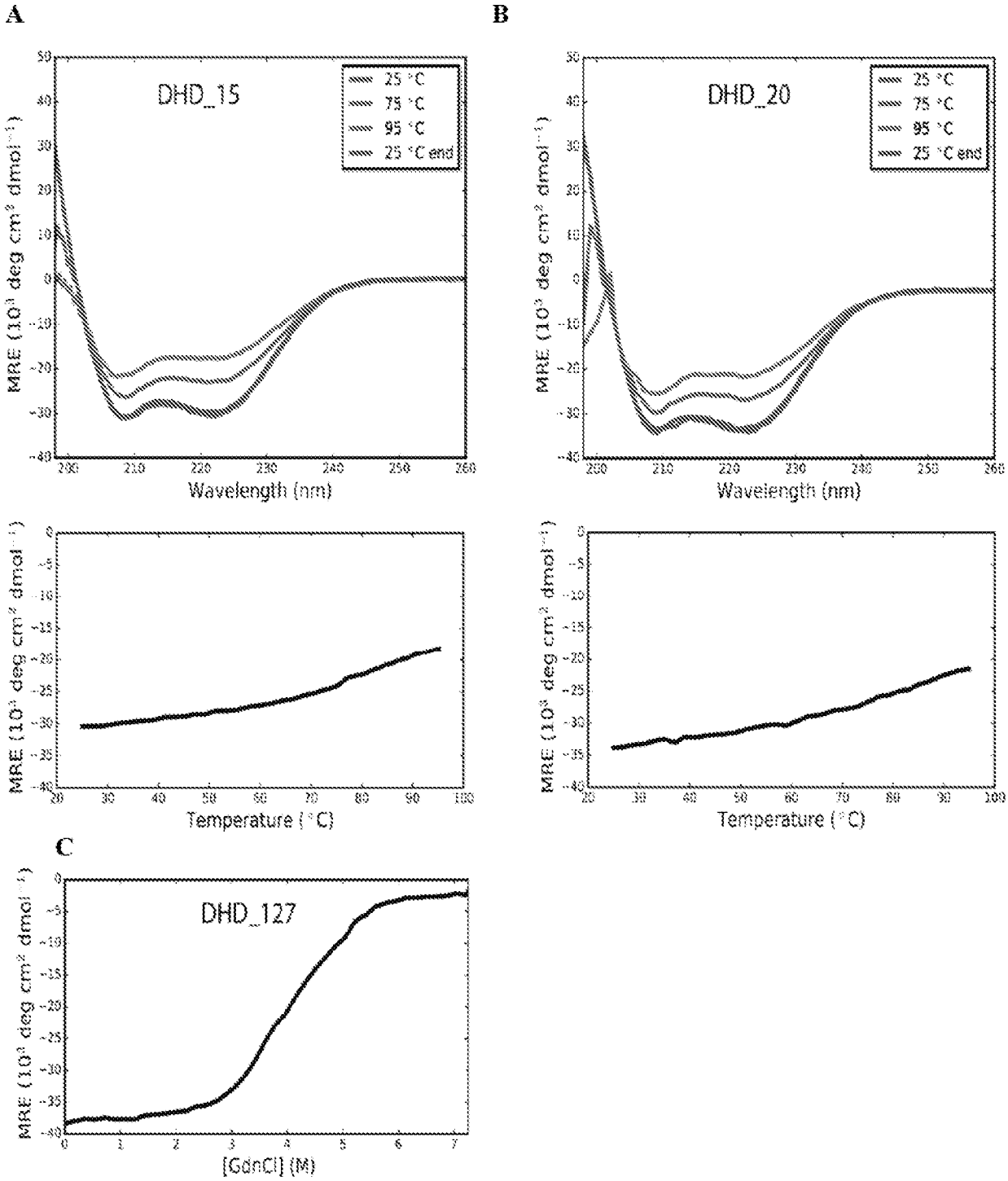

A
B
DHD_15
DHD_20
25 °C
75 °C
95 °C
25 °C end
MRE (10³ deg cm² dmol⁻¹)
Wavelength (nm)
Temperature (°C)
C
DHD_127
[GdnCl] (M)

FIGURE 6A – 6C

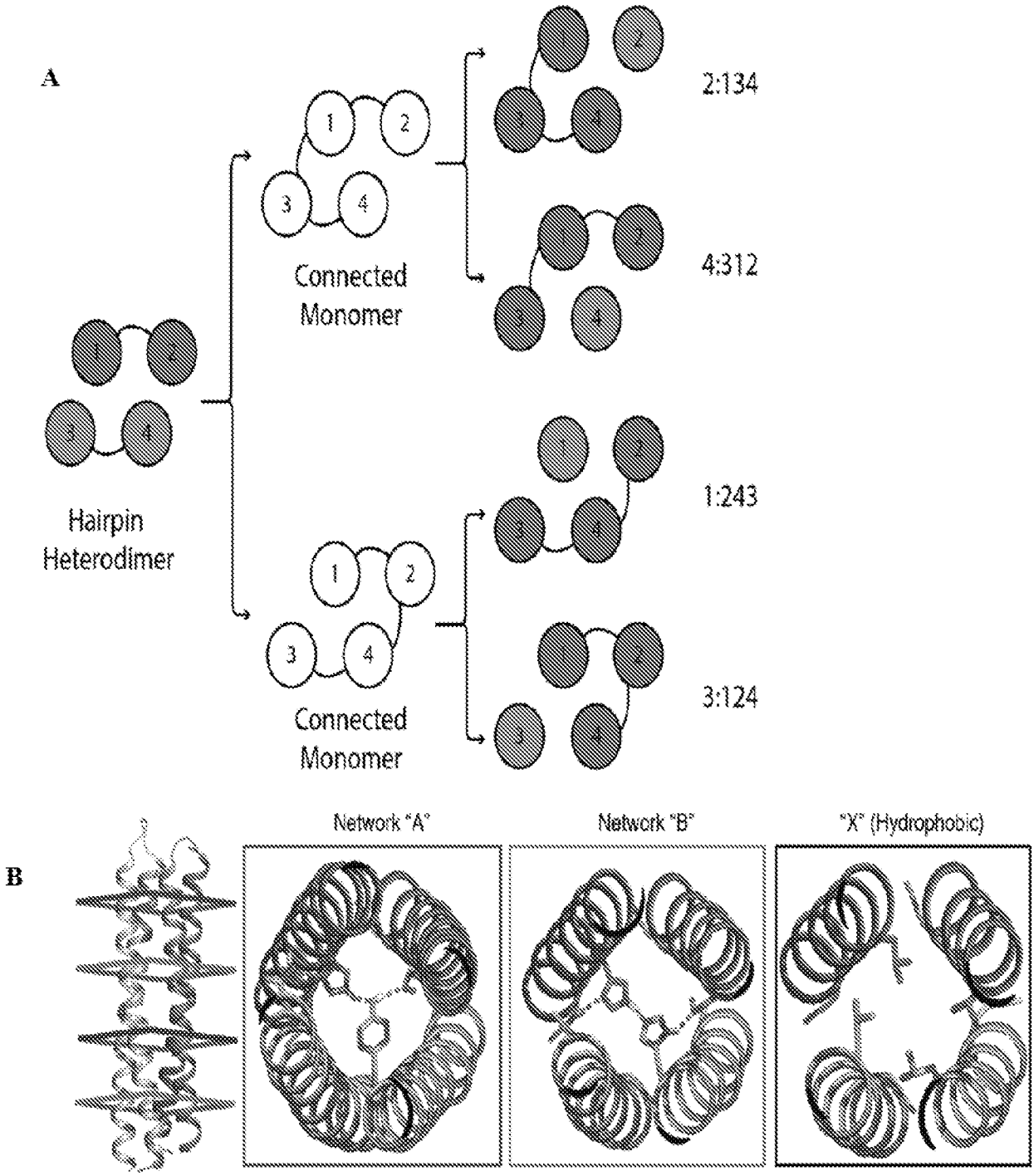

A
Hairpin Heterodimer
Connected Monomer
Connected Monomer
2:134
4:312
1:243
3:124
B
Network "A"
Network "B"
"X" (Hydrophobic)

FIGURE 7A – 7B

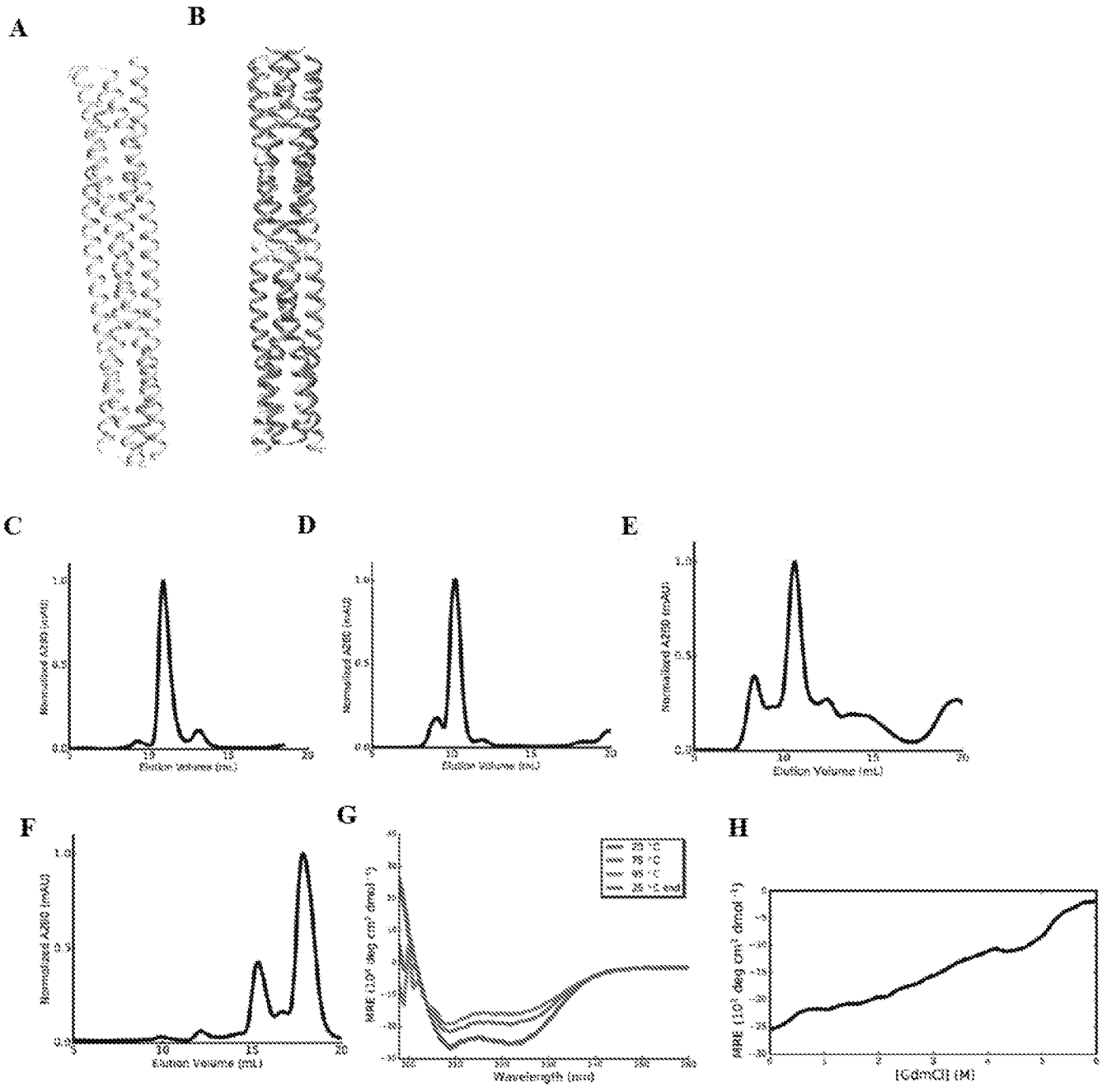

A
B
C
D
E
F
G
H
Normalized A280 (mAU)
Elution Volume (mL)
MRE (10³ deg cm² dmol⁻¹)
Wavelength (nm)
[GdmCl] (M)

FIGURE 8A – 8H

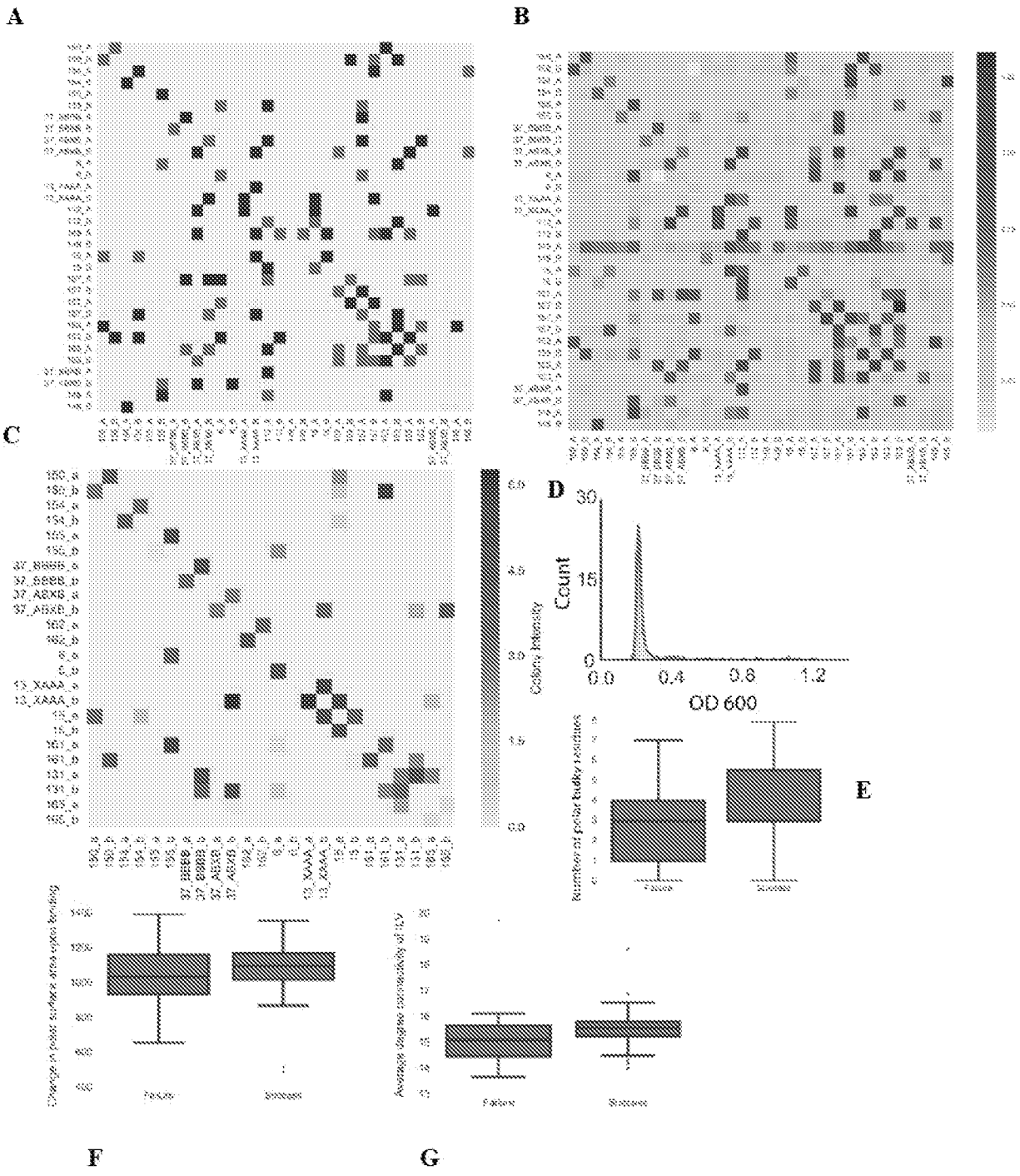


FIGURE 9A – 9G

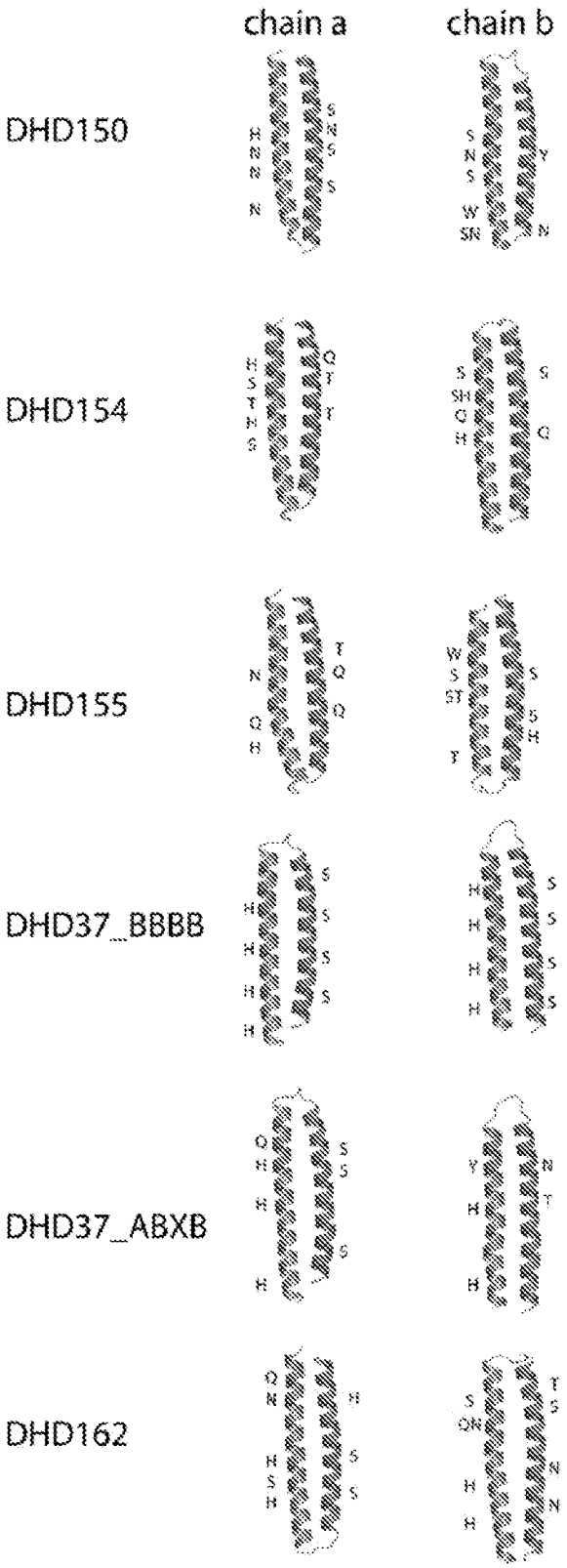

chain a
chain b
DHD150
DHD154
DHD155
DHD37_BBBB
DHD37_ABXB
DHD162

FIGURE 10

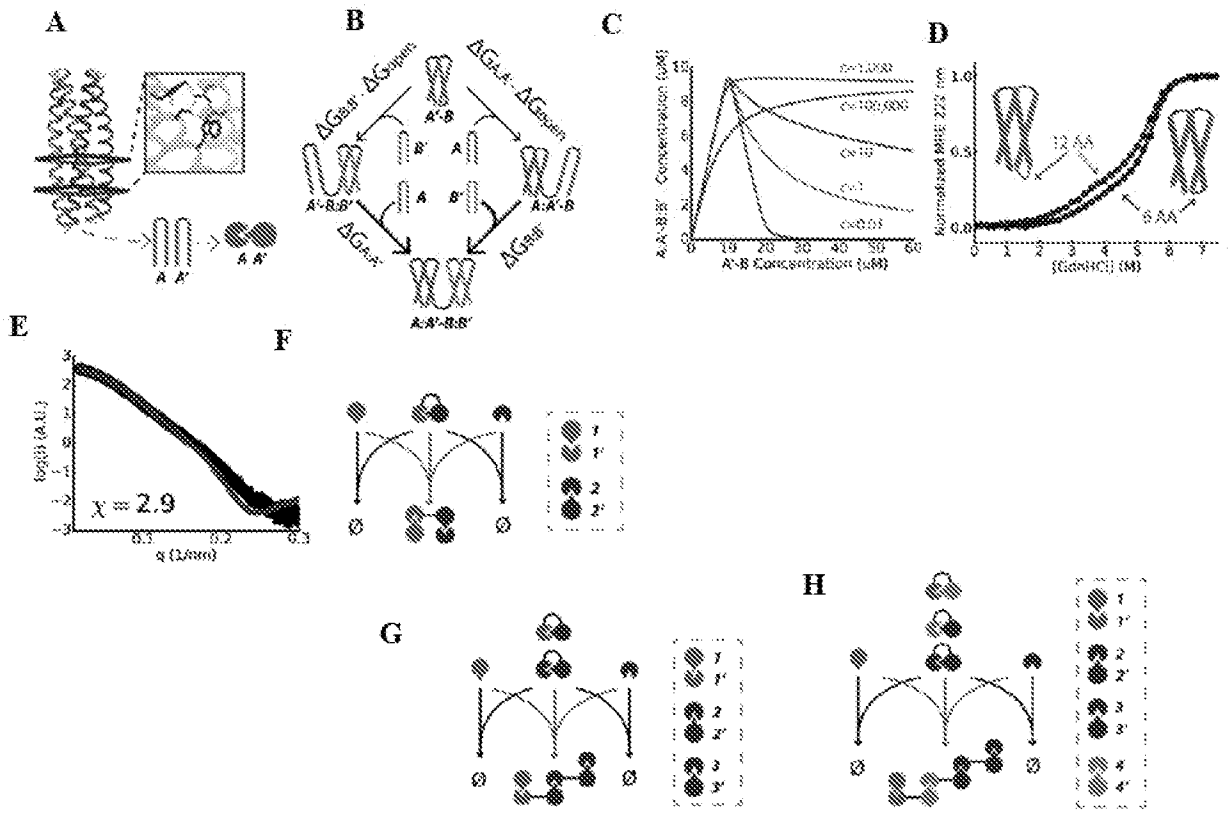


FIGURE 11A – 11H

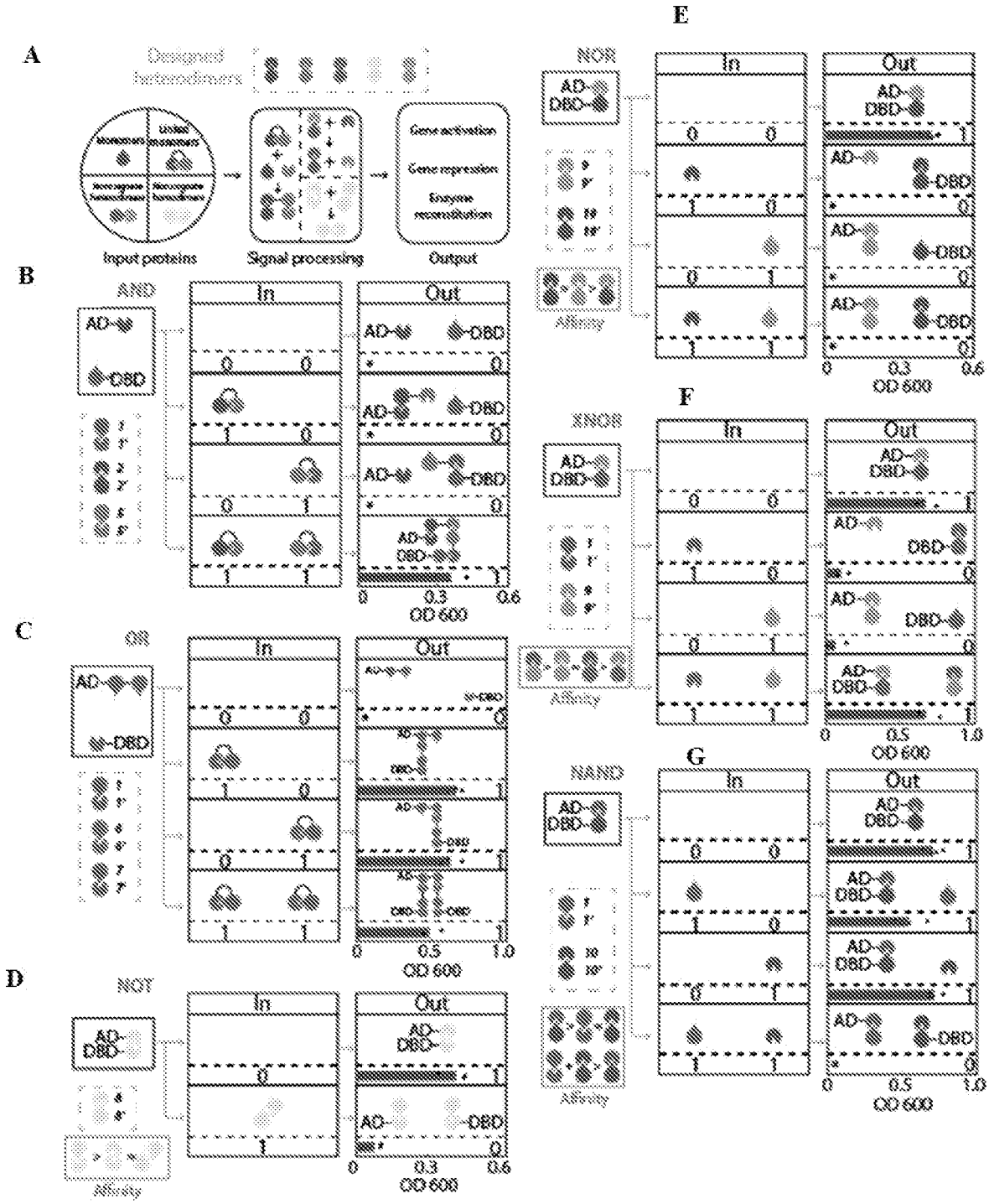


FIGURE 12A – 12G

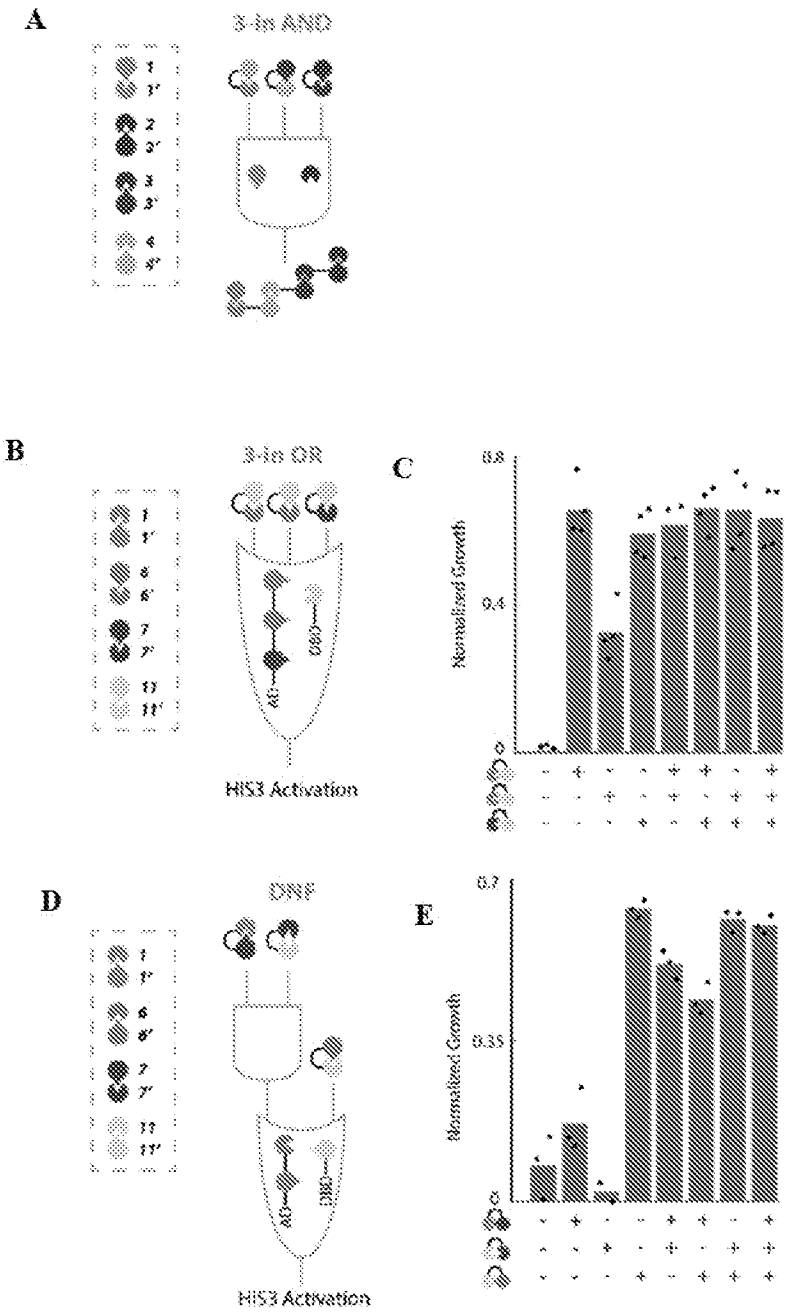
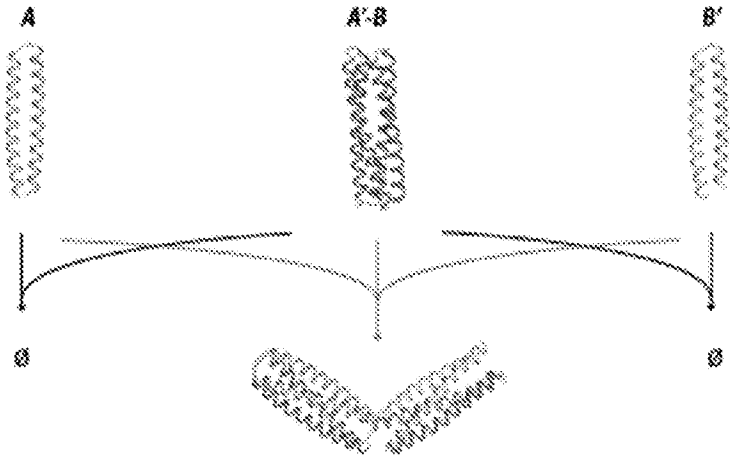


FIGURE 13A – 13E

A



B

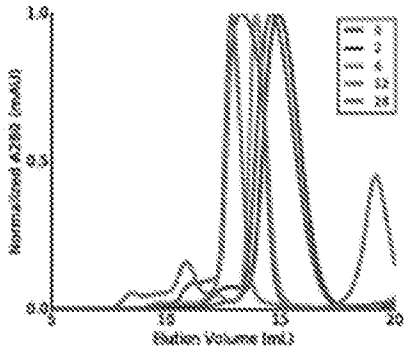


FIGURE 14A – 14B

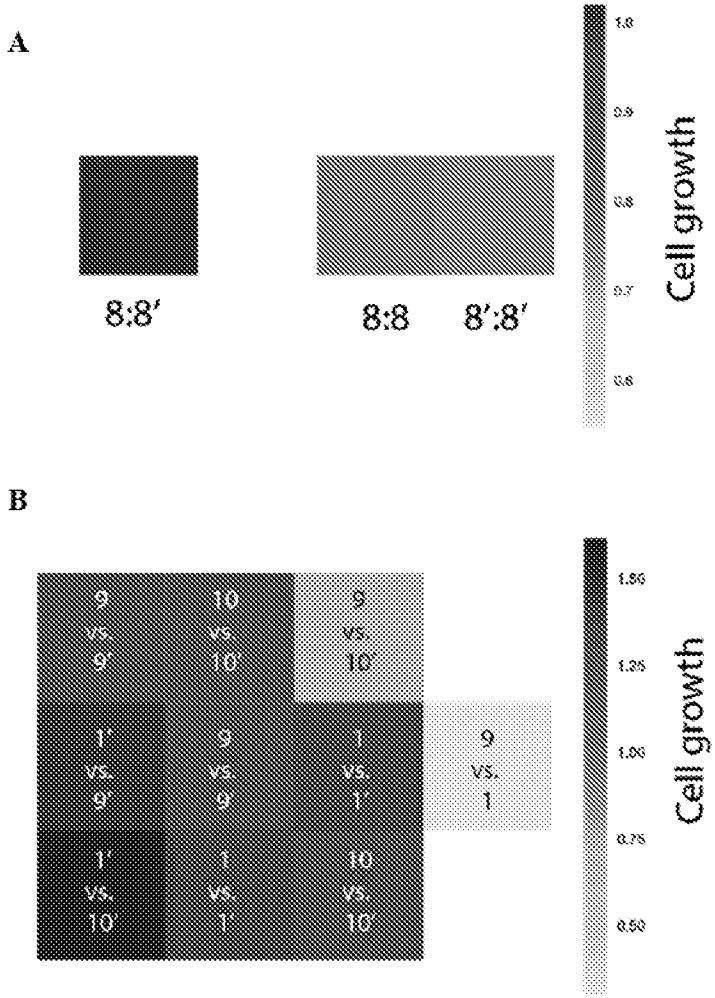


FIGURE 15A – 15B

Monomer A SEQ ID No.	Monomer B SEQ ID NO:
1	2
331	2
3	4
5	6
5	332
7	8
7	334
9	10
11	12
13	14
13	336
15	16
15	338
17	18
19	20
21	22
23	24
25	26
25	340
27	28
29	30
29	342
31	32
31	344
33	34
33	346
35	36
35	348
37	38
37	418
39	40
39	350
41	42
41	352
43	44
45	46
45	354
47	48
47	356
49	50
51	52
53	54
53	358

Monomer A SEQ ID No.	Monomer B SEQ ID NO:
55	56
55	360
57	58
57	362
59	60
59	364
61	62
61	366
63	64
65	66
65	368
67	68
67	370
69	70
69	372
71	72
71	374
73	74
73	376
75	76
75	378
77	78
77	380
79	80
79	382
81	82
337	384
83	84
339	386
85	86
85	388
87	88
87	390
89	90
89	392
91	92
91	394
93	94
93	396
95	96
95	398
97	98
97	400

Monomer A SEQ ID No.	Monomer B SEQ ID NO:
99	100
99	402
101	102
341	404
103	104
103	406
105	106
343	408
107	108
107	410
109	110
109	412
111	112
111	424
113	114
113	416
115	116
459	420
117	118
345	422
119	120
347	424
121	122
349	426
123	124
351	428
125	126
353	126
127	128
355	430
129	130
357	432
131	132
359	434
133	134
361	436
135	136
363	438
137	138
365	440
139	140
367	442
141	142

FIGURE 16

Monomer A SEQ ID No.	Monomer B SEQ ID NO:
369	444
143	144
371	446
145	146
373	448
147	148
375	450
149	150
377	452
151	152
379	454
153	154
381	456
155	156
383	458
157	158
385	460
159	160
387	462
161	162
389	464
163	164
391	466
165	166
393	468
167	168
395	470
169	170
397	472
171	172
399	474
173	174
401	174
175	176
403	476
177	178
405	478
179	180
407	480
181	182
409	482
183	184

Monomer A SEQ ID No.	Monomer B SEQ ID NO:
411	484
185	186
413	486
187	188
415	488
189	190
417	490
191	192
419	492
193	194
421	494
195	196
197	198
199	200
201	202
203	204
205	206
207	208
209	210
211	212
213	214
215	216
217	218
219	220
221	222
223	224
225	226
227	228
229	230
231	232
233	234
235	236
237	238
239	240
241	242
243	244
245	246
247	248
249	250
251	252
253	254
255	256
257	258

Monomer A SEQ ID No.	Monomer B SEQ ID NO:
259	260
261	262
263	264
265	266
267	268
269	270
271	272
273	274
275	276
277	278
279	280
281	282
283	284
285	286
287	288
289	290

FIGURE 16 (cont.)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/059654

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/435
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/030780 A2 (COMPLIX NV [BE]; DESMET JOHAN [BE]; LASTERS IGNACE [BE]) 12 March 2009 (2009-03-12) abstract page 15, line 16 - page 16, line 26 page 23 - page 26 page 32 - page 34 claims 1-17 ----- -/--	1-116

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 December 2019	09/03/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Keller, Yves

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97/12988 A1 (PENCE INC [CA]; HOUSTON MICHAEL E JR [CA] ET AL.) 10 April 1997 (1997-04-10) abstract page 3 - page 4 figure 3D figure 4A page 14 - page 16 figures 1-25 figures 1A-1F	1-116
X	O'SHEA E K ET AL: "Peptide 'Velcro': Design of a heterodimeric coiled coil", CURRENT BIOLOGY, CURRENT SCIENCE, GB, vol. 3, no. 10, 1 October 1993 (1993-10-01), pages 658-667, XP024248213, ISSN: 0960-9822, DOI: 10.1016/0960-9822(93)90063-T [retrieved on 1993-10-01] the whole document	1-116
X	HELENA GRADISAR ET AL: "De novo design of orthogonal peptide pairs forming parallel coiled-coil heterodimers", JOURNAL OF PEPTIDE SCIENCE, vol. 17, no. 2, 28 December 2010 (2010-12-28), pages 100-106, XP055066656, ISSN: 1075-2617, DOI: 10.1002/psc.1331 the whole document	1-116
X	CROOKS RICHARD O ET AL: "Deriving Heterospecific Self-Assembling Protein-Protein Interactions Using a Computational Interactome Screen", JOURNAL OF MOLECULAR BIOLOGY, ACADEMIC PRESS, UNITED KINGDOM, vol. 428, no. 2, 2 December 2015 (2015-12-02), pages 385-398, XP029407028, ISSN: 0022-2836, DOI: 10.1016/J.JMB.2015.11.022 the whole document	1-116
X	AARON W. REINKE ET AL: "A Synthetic Coiled-Coil Interactome Provides Heterospecific Modules for Molecular Engineering", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 132, no. 17, 5 May 2010 (2010-05-05), pages 6025-6031, XP055652786, ISSN: 0002-7863, DOI: 10.1021/ja907617a the whole document	1-116

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	AMANDA L. EDWARDS ET AL: "Challenges in Targeting a Basic Helix-Loop-Helix Transcription Factor with Hydrocarbon-Stapled Peptides", ACS CHEMICAL BIOLOGY, vol. 11, no. 11, 19 September 2016 (2016-09-19), pages 3146-3153, XP055373864, ISSN: 1554-8929, DOI: 10.1021/acschembio.6b00465 -----	
T	HELENA GRADISAR ET AL: "Design of a single-chain polypeptide tetrahedron assembled from coiled-coil segments", NATURE CHEMICAL BIOLOGY, vol. 9, no. 6, 28 April 2013 (2013-04-28), pages 362-366, XP055293813, Basingstoke ISSN: 1552-4450, DOI: 10.1038/nchembio.1248 the whole document -----	
X,P	CHEN ZIBO ET AL: "Programmable design of orthogonal protein heterodimers", NATURE, MACMILLAN JOURNALS LTD., ETC.¦, LONDON, vol. 565, no. 7737, 19 December 2018 (2018-12-19), pages 106-111, XP036664328, ISSN: 0028-0836, DOI: 10.1038/S41586-018-0802-Y [retrieved on 2018-12-19] the whole document -----	1-116

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/059654

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-116(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-116(partially)

A heterodimer protein, comprising:(a) a monomer A polypeptide, wherein the monomer A polypeptide comprising 1-5 alpha helices and(b) a monomer B polypeptide, wherein the monomer B polypeptide 1-5 alpha helices,wherein monomer A and monomer B non-covalently interact to form the designed heterodimer protein and wherein the alpha helice of monomer A is SEQ ID. No. 1 (as well as subject matter to the monomer A as defined above (SEQ ID No 1))

2-290. claims: 1-116(partially)

A heterodimer protein, comprising:(a) a monomer A polypeptide, wherein the monomer A polypeptide comprising 1-5 alpha helices and(b) a monomer B polypeptide, wherein the monomer B polypeptide 1-5 alpha helices,wherein monomer A and monomer B non-covalently interact to form the designed heterodimer protein and wherein the alpha helice of monomer A is SEQ ID. No. 2-290 (as well as subject matter to the monomer A as defined above (SEQ ID No 2-290)) each SEQ ID No representing a single invention

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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