



(51) International Patent Classification:
Not classified

(21) International Application Number:
PCT/US2024/033648

(22) International Filing Date:
12 June 2024 (12.06.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/472,440 12 June 2023 (12.06.2023) US

(71) Applicant: **RENSSELAER POLYTECHNIC INSTITUTE** [US/US]; 110 8th Street, J Building, Troy, NY 12180 (US).

(72) Inventors; and

(71) Applicants: **BELFORT, Georges** [US/US]; 3 Main Entry Road, North Falmouth, MA 02556 (US). **KARANDE, Pankaj, Sakharam** [US/US]; 198 Pawling Avenue, Suite

10, Troy, NY 12180 (US). **HU, Mengyang** [US/US]; 2431 21st Street, Apt. 5, Troy, NY 12180 (US).

(72) Inventor: **XIA, Wenjun**; 3 25th Street, Apt. 2, Troy, NY 12180 (US).

(74) Agent: **GANGEMI, Anthony, P.**; Barclay Damon LLP, 545 Long Wharf Drive, 9th Floor, New Haven, CT 06511 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(54) Title: PEPTIDE LIGANDS FOR AFFINITY CAPTURE OF NUCLEIC ACIDS AND METHODS OF MAKING SAID LIGANDS

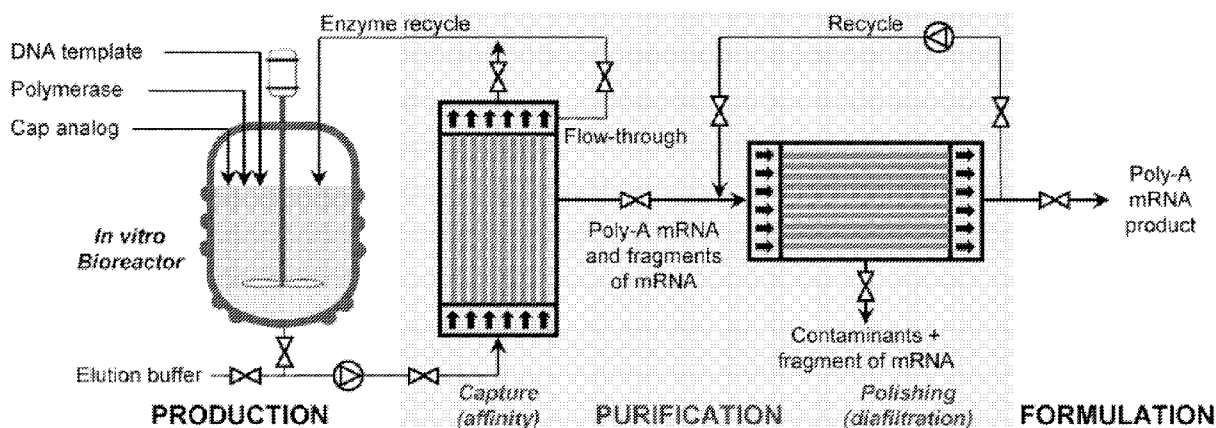


FIG. 1

(57) Abstract: Materials, methods, and systems for affinity membrane purification of nucleic acid products advantageously (i) reduce the residence times of the recovery process, (ii) allow continuous processing, (iii) retain a higher percentage of nucleic acids having the target structure, and (iv) reduce the "footprint" of the equipment. Systems include one or more bioreactors to synthesize nucleic acids. One or more affinity membrane separators modified by the inclusion of a peptide obtained using rational design, in silico optimization, and/or combinatorial screening. One or more diafiltration modules include flat sheet microporous membranes, cellulose hollow fiber microporous affinity membranes, or combinations thereof.

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

Published:

- *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

PEPTIDE LIGANDS FOR AFFINITY CAPTURE OF NUCLEIC ACIDS AND METHODS OF MAKING SAID LIGANDS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/472,440, filed June 12, 2023, which is incorporated by reference as if disclosed herein in its entirety.

BACKGROUND

[0002] Many opportunities for developing therapeutic nucleic acids, including DNA and single stranded ribonucleic acids (ss-mRNA), are currently being pursued. The development of a generalizable fast, effective, scalable, and economic purification platform will contribute to their technical and economic success.

[0003] The landscape of nucleic acid-based therapeutics and vaccines has experienced a transformative surge, propelled notably by the triumph of mRNA vaccines in combatting the COVID-19 pandemic. This success has ignited substantial advancements in both pre-clinical and clinical arenas, elevating mRNA as a potent modality for therapeutic interventions across various medical domains, including cancer and infectious diseases.

[0004] Integral to the synthesis of exogenous mRNA is the in vitro transcription process, a pivotal method wherein a linear DNA template undergoes transcription into mRNA using a single-subunit phage RNA polymerase, with the T7 phage polymerase predominantly employed. In the intricate landscape of in vitro transcription (IVT) mRNA production, the quality of the generated messenger RNA (mRNA) stands as a pivotal determinant for therapeutic and research applications. Among the challenges encountered in this process, the presence of double-stranded RNA (dsRNA) emerges as a critical concern.

[0005] Double stranded RNA, an inadvertent byproduct of IVT reactions, possesses the potential to trigger undesirable cellular responses, including the activation of innate immune pathways. Moreover, dsRNA can impede the efficient translation of mRNA and diminish its overall therapeutic efficacy. Consequently, the meticulous removal of dsRNA from the final

mRNA product becomes imperative to meet the stringent requirements for safety and efficacy in therapeutic applications.

[0006] Addressing this imperative, contemporary efforts are directed toward 1) upstream process: improvement of manufacturing process with reduced dsRNA byproduct formation; 2) downstream process: the development and optimization of dsRNA removal methods in IVT mRNA production. Reported dsRNA byproduct reduction methods including reducing divalent magnesium during IVT which also caused a drastic loss in yield, employment of competing oligonucleotides, co-tether DNA promoter and RNA polymerase, high temperature, and employment of chaotropic agents. For downstream removal of dsRNA, current strategies encompass a spectrum of purification techniques, ranging from enzymatic digestion, selective precipitation and cellulose-based purification to chromatographic methods. Reverse-phase high-pressure liquid chromatography (HPLC) is by far the most effective method for dsRNA removal. However, it has significant drawbacks of high cost, low yield, toxic chemicals (acetonitrile) and tendency of decreased integrity for long mRNA. A cost-effective, generalizable, fast, effective, scalable and translatable purification platform for dsRNA removal is therefore of great interest.

[0007] Affinity ligands are commonly employed for purification processes such as affinity chromatography. Typically, natural ligands including enzyme substrates and antigens are utilized as adsorbents. One famous example would be Protein A for monoclonal antibody purification. Natural ligands stand out for their easy accessibility and high affinity. However, they are also expensive to produce, difficult to elute from, and suffer from ligand leakage. Biomimetic affinity ligands such as peptides therefore attracted significant attention in recent years due to their cost-effectiveness, high specificity, low immune response and high chemical stability.

[0008] Therefore, identifying peptide affinity ligands selective for dsRNA in mRNA purification is of great interest. Similar contamination challenges exist in DNA production, such as the removal of undesired isoforms of plasmid DNA (pDNA). We aim to address these purification challenges by designing novel peptide affinity ligands that can selectively target specific species, whether for the purification product or contaminants like dsRNA.

[0009] Continuous purification of mRNA will speed-up manufacturing and reduce costs of production. Replacing resin-based chromatography (diffusive, slow and hence long residence

time), with adsorptive membranes (convective, fast and hence short residence time) for purification of mRNA products will (i) reduce the residence times of the recovery process, t_R , (ii) allow continuous processing, (iii) retain a higher percentage of folded mRNA due to the reduced treatment residence time, t_R , and (iv) significantly reduce the “footprint” of the equipment. Although, commercial affinity chromatography (Thermo Fisher) and affinity polymer monoliths (Sartorius) are alternate technologies for mRNA purification, neither are conducive to continuous operation and both suffer from pressure drop and mass transfer limitations (with larger t_R which is detrimental to mRNA stability).

[0010] Since current methods for purifying mRNA from in vitro transcription (IVT) of a DNA template at large scale involve relatively slow chromatographic techniques (e.g., liquid chromatography (LC), gel filtration chromatography, anion exchange chromatography, and affinity chromatography) with a filtration step, it seems reasonable to consider a fast, scalable and attractive technology like high surface area hollow fiber synthetic membranes in affinity and tangential flow modes.

[0011] A design strategy for binding selectively to ssRNA in a solution or attached to a surface is to use complementary binding (also called hybridization) of bases, like adenine (A) to thymine (T) or guanine (G) to cytosine (C). Since natural mRNA molecules have tails comprising a string of As (called oligo-dA_m, where m varies from 20 to 150) at the 3' end of the molecule, a complementary string of Ts (called oligo-dT_n where n varies from 15 to 60 and, named the “ligand” here) can be used to bind to the oligo-dA_m. This will allow selective affinity capture of a desired mRNA (such as an oligo-dA₂₀ or COVID-19 vaccine mRNA molecule from an in vitro transcription (IVT) reaction mixture) in solution or on a surface. Immobilizing oligo dT_n on microporous polar membranes (i.e., regenerated cellulose) in order to capture the oligo dA_m tail of mRNA is an attractive approach that is currently being pursued. However, it poses several complications including how to graft oligo dT_n at desirable densities, what is a desirable density, what value of n is used for a given value of m, what substrate is used, a source of the chemistry used for the immobilizations, etc.

[0012] Many naturally occurring proteins bind to nucleic acids with different selectivity. However, the challenges in protein isolation, production, and stability deter characterization and

applicability of such ligands for mRNA targeting. The generation of synthetic anti-mRNA antibodies (Fab fragments), together with their biochemical characterization has proved useful. Yet, as with native ligands, factors including production, size, and stability can limit the versatility of antibodies and even Fabs in applications; these factors comparatively promote the versatility of low molecular weight ligands.

[0013] Therefore, there remains a need in the art for improved materials, methods and systems for purification of nucleic acids.

SUMMARY

[0014] Aspects of the present disclosure are directed to identification of selective binding ligands and coupling these ligands to synthetic polymer filtration membranes to purify nucleic acids, e.g., by separating ds-mRNA and ss-mRNA. Some aspects of the present disclosure are directed to purifying labile ss-mRNA from ds-mRNA quickly, at high yield and purity, and in continuous scalable production mode utilizing the ligands. Some aspects of the present disclosure are directed to a process to rationally and/or combinatorially design, screen, test, and identify peptide ligands that bind selectively to ss-mRNA and not to ds-mRNA or ds-DNA or vice versa. Some aspects of the present disclosure are directed to a material, i.e., such binding ligand peptides. In some embodiments, the method includes identifying and emulating the binding domain of such proteins with small targeting agents, like peptides, for selective mRNA binding.

[0015] Some aspects of the disclosure are directed to systems for continuous affinity membrane manufacturing, comprising: one or more bioreactors including components sufficient to promote synthesis of a nucleic acid, the bioreactors including at least one inlet and at least one outlet; an outlet stream in fluid communication with the one or more bioreactors, the outlet stream including a concentration of nucleic acid; one or more affinity membrane separators in fluid communication with the outlet stream, the affinity membrane separators being modified by the inclusion of a peptide ligand comprising twenty or fewer residues; and one or more diafiltration modules in fluid communication with the affinity membrane separators.

[0016] Some aspects of the disclosure are directed to systems for continuous affinity membrane manufacturing, wherein the nucleic acid comprises ss-mRNA, ds-mRNA, DNA, or a derivative or conjugate thereof. In some embodiments, the nucleic acid comprises a non-natural nucleic acid, L-sugars, and/or synthetic nucleotides/nucleosides.

[0017] Some aspects of the disclosure are directed to systems for continuous affinity membrane manufacturing as, wherein the one or more affinity membrane separators comprises a flat sheet microporous membranes, a cellulose hollow fiber microporous affinity membranes, or a combination thereof. Some aspects of the disclosure are directed to systems for continuous affinity membrane manufacturing, wherein the affinity membrane separators have a total membrane surface area of about 4 m² or greater.

[0018] Some aspects of the disclosure are directed to, the system for continuous affinity membrane manufacturing is for purification of a ss-mRNA vaccine against coronaviruses, e.g., SARS-CoV-2.

[0019] Some aspects of the disclosure are directed to membranes for purification of nucleic acids comprising: a nucleic acid-binding peptide covalently bound to the membrane, wherein the membrane comprises a cellulose-based membrane or a hollow fiber membrane. Some aspects of the disclosure are directed to regenerated cellulose membranes. Some aspects of the disclosure are directed to nucleic acid-binding peptides covalently bound to the membrane via a peptide bond. Some aspects of the disclosure are directed to nucleic acid-binding peptides covalently bound to the membrane via a heterobifunctional crosslinker or a homobifunctional crosslinker.

[0020] Some aspects of the disclosure are directed to membranes comprising a ds-mRNA-binding peptide comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues, a ss-mRNA-binding peptide comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues, or a DNA-binding peptide comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues.

[0021] Some aspects of the disclosure are directed to method of making a membrane for purification of nucleic acids comprising: reacting a nucleic acid-binding peptide to the membrane, wherein the nucleic acid-binding peptide comprises 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues. Some aspects of the disclosure are directed to methods, wherein the step of

reacting comprises forming a peptide bond between the nucleic acid-binding peptide and the membrane. Some aspects of the disclosure are directed to methods, wherein forming the peptide bond is mediated by diisopropylcarbodiimide (DIC) and ethyl (hydroxyimino)cyanoacetate (Oxyma). Some aspects of the disclosure are directed to methods, wherein the nucleic acid comprises ss-mRNA, ds-mRNA, or DNA. Some aspects of the disclosure are directed to methods, wherein the one or more affinity membrane separators comprises a flat sheet microporous membranes, a cellulose hollow fiber microporous affinity membranes, or a combination thereof.

[0022] Some aspects of the disclosure are directed to methods of making a membrane for purification of nucleic acids comprising: reacting a functional group of a membrane with a first end of a crosslinker; and reacting a second end of the crosslinker with a nucleic acid-binding peptide that comprises 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues. Some aspects of the disclosure are directed to methods, wherein the membrane comprises a flat sheet microporous membranes, a cellulose hollow fiber microporous affinity membranes, or a combination thereof.

[0023] Some aspects of the disclosure are directed to methods of making a membrane for purification of nucleic acids comprising: reacting a carboxyl group of a regenerated cellulose membrane with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride to form an O-acylisourea; and reacting an N-hydroxysuccinimide-modified nucleic acid-binding peptide with the O-acylisourea, wherein the nucleic acid-binding peptide comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues.

[0024] Some aspects of the disclosure are directed to methods of making a membrane for purification of nucleic acids comprising: reacting a hollow fiber membrane with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride to form an O-acylisourea; and reacting an N-hydroxysuccinimide-modified nucleic acid-binding peptide with the O-acylisourea, wherein the nucleic acid-binding peptide comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues.

[0025] Some aspects of the disclosure are directed to methods of designing a nucleic acid-binding peptide comprising: selecting a protein design template based on one or more the following criteria: (i) a binding affinity to the nucleic acid; (ii) a binding selectivity to the nucleic acid versus a contaminant; (iii) a structural information; and (iv) a length of the protein design

template; and generating a library of peptide candidates comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues using linear epitope mapping of the protein design template.

[0026] Some aspects of the disclosure are directed to methods of identifying a nucleic acid-binding peptide comprising: binding the nucleic acid to a phage comprising the nucleic acid-binding peptide; amplifying an insert of the phage using PCR amplification; and sequencing the insert. Some aspects of the disclosure are directed to methods, wherein sequencing the insert comprises next-generation sequencing. Some aspects of the disclosure are directed to methods further comprising sequencing the phage genome. Some aspects of the disclosure are directed to methods further comprising confirming that the nucleic acid-binding peptide binds to the nucleic acid using an ELISA assay. Some aspects of the disclosure are directed to methods further comprising: synthesizing the nucleic acid-binding peptide on a cellulose disk; dissolving the cellulose disk; printing the dissolved cellulose disk on a slide; incubating a fluorescently labeled nucleic acid on the slide; washing the slide; and imaging a fluorescence of the slide.

[0027] Some aspects of the disclosure are directed to methods of elucidating a mechanism of binding between a nucleic acid and a nucleic acid-binding peptide comprising: preparing a structure of the nucleic acid and preparing a structure of the nucleic acid-binding peptide; generating a nucleic acid-peptide complex from the nucleic acid and nucleic acid-binding peptide structures; conducting molecular dynamics simulations on the nucleic acid-peptide complex; calculating a first binding free energy of the nucleic acid-binding peptide to the nucleic acid; calculating a second binding free energy of the nucleic acid-binding peptide to a competitor; and modifying the nucleic acid-binding peptide by stabilizing the peptide backbone or mutating a residue of the nucleic acid-binding peptide to obtain a modified nucleic acid-binding peptide that comprises a binding efficacy to the nucleic acid that is greater than a binding efficacy of the nucleic acid-binding peptide to the nucleic acid.

BRIEF DESCRIPTION OF THE DRAWINGS

[0001] The drawings show embodiments of the disclosed subject matter for the purpose of illustrating the invention. However, it should be understood that the present application is not limited to the precise arrangements and instrumentalities shown in the drawings, wherein:

[0002] FIG 1 is a scheme for a system for continuous affinity membrane manufacturing according to some embodiments of the present disclosure;

[0003] FIG 2 is a scheme for methods of identifying and designing peptide affinity ligands for nucleic acid purification according to some embodiments of the present disclosure;

[0004] FIG 3 is a scheme for methods of isolating inserts from whole phage vector using PCR amplification according to some embodiments of the present disclosure;

[0005] FIG 4 is a scheme for preparation of a Library for Next Generation Sequencing according to some embodiments of the present disclosure;

[0006] FIG 5 is a scheme for methods of processing sequencing result data according to some embodiments of the present disclosure;

[0007] FIG. 6 is a schematic of peptide library design and library screening according to some embodiments of the present disclosure; and

[0008] FIG. 7 is a schematic for peptide synthesis and rationally designed peptide screening according to some embodiments of the present disclosure.

DETAILED DESCRIPTION

[0028] Referring now to FIG. 1, some aspects of the disclosed subject matter are directed to an affinity membrane manufacturing process for the production of purified ss-mRNA vaccines and other products from IVT feed. In some embodiments, the system and method of FIG. 1 is operated continuously. In some embodiments, the system and method of FIG. 1 is used for the hollow fiber affinity membrane recovery of purified ss-mRNA from ds-mRNA. In some embodiments, the membrane of the system in FIG. 1, e.g., a commercial flat sheet microporous regenerated cellulose (RC) membrane, is modified with one or more selective affinity ligands for targeting ssRNA. In some embodiments, the ssRNA is an ss-mRNA vaccine. In some embodiments, the system is operated to maximize yield of product, e.g., ss-RNA. In some embodiments, the system is operated to maximize purity of product. In some embodiments, the system is operated to balance yield and purity of product. In some embodiments, the system

comprises a bioreactor for *in vitro* synthesis of mRNA vaccine, a modified hollow fiber cellulose affinity membrane separator, and a tangential flow hollow fiber filtration polishing step.

[0029] Surprisingly, relatively short peptides (having less than or equal to about 20 amino acid residues) derived from mRNA-binding proteins selectively bind to nucleic acids (NDA, ds-mRNA, and/or ss-mRNA). In some embodiments, interactions between peptides and representative mRNA targets are computationally and/or theoretically probed to further develop affinity and selectivity.

[0030] In some embodiments, systems for continuous affinity membrane manufacturing, comprise: one or more bioreactors including components sufficient to promote synthesis of a nucleic acid, the bioreactors including at least one inlet and at least one outlet; an outlet stream in fluid communication with the one or more bioreactors, the outlet stream including a concentration of nucleic acid; one or more affinity membrane separators in fluid communication with the outlet stream, the affinity membrane separators being modified by the inclusion of a peptide ligand comprising twenty or fewer residues; and one or more diafiltration modules in fluid communication with the affinity membrane separators.

[0031] In some embodiments, the nucleic acid comprises ss-mRNA, ds-mRNA, DNA, or a derivative or conjugate thereof. In some embodiments, the nucleic acid comprises a non-natural nucleic acid, L-sugars, and/or synthetic nucleotides/nucleosides.

[0032] In some embodiments, the one or more affinity membrane separators comprises a flat sheet microporous membranes, a cellulose hollow fiber microporous affinity membranes, or a combination thereof. In some embodiments, the affinity membrane separators have a total membrane surface area of about 4 m² or greater.

[0033] Some aspects of the disclosure are directed to, the system for continuous affinity membrane manufacturing is for purification of a ss-mRNA vaccine against coronaviruses, e.g., SARS-CoV-2.

[0034] In some embodiments, membranes for purification of nucleic acids comprise: a nucleic acid-binding peptide covalently bound to the membrane, wherein the membrane comprises a cellulose-based membrane or a hollow fiber membrane. In some embodiments, the

membrane comprises a regenerated cellulose membrane. In some embodiments, nucleic acid-binding peptides covalently bond to the membrane via a peptide bond. In some embodiments, nucleic acid-binding peptides covalently bond to the membrane via a heterobifunctional crosslinker or a homobifunctional crosslinker.

[0035] In some embodiments, membranes comprise a ds-mRNA-binding peptide comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues, a ss-mRNA-binding peptide comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues, or a DNA-binding peptide comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues.

[0036] In some embodiments, methods of making a membrane for purification of nucleic acids comprise: reacting a nucleic acid-binding peptide to the membrane, wherein the nucleic acid-binding peptide comprises 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues. In some embodiments, the step of reacting comprises forming a peptide bond between the nucleic acid-binding peptide and the membrane. In some embodiments, forming the peptide bond is mediated by diisopropylcarbodiimide (DIC) and ethyl (hydroxyimino)cyanoacetate (Oxyma). In some embodiments, the nucleic acid comprises ss-mRNA, ds-mRNA, or DNA. In some embodiments, the one or more affinity membrane separators comprises a flat sheet microporous membranes, a cellulose hollow fiber microporous affinity membranes, or a combination thereof.

[0037] In some embodiments, methods of making a membrane for purification of nucleic acids comprise: reacting a functional group of a membrane with a first end of a crosslinker; and reacting a second end of the crosslinker with a nucleic acid-binding peptide that comprises 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues. In some embodiments, the membrane comprises a flat sheet microporous membranes, a cellulose hollow fiber microporous affinity membranes, or a combination thereof.

[0038] In some embodiments, methods of making a membrane for purification of nucleic acids comprise: reacting a carboxyl group of a regenerated cellulose membrane with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride to form an O-acylisourea; and reacting an N-hydroxysuccinimide-modified nucleic acid-binding peptide with the O-acylisourea, wherein the nucleic acid-binding peptide comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues.

[0039] In some embodiments, methods of making a membrane for purification of nucleic acids comprise: reacting a hollow fiber membrane with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride to form an O-acylisourea; and reacting an N-hydroxysuccinimide-modified nucleic acid-binding peptide with the O-acylisourea, wherein the nucleic acid-binding peptide comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues.

[0040] In some embodiments, methods of designing a nucleic acid-binding peptide comprise: selecting a protein design template based on one or more the following criteria: (i) a binding affinity to the nucleic acid; (ii) a binding selectivity to the nucleic acid versus a contaminant; (iii) a structural information; and (iv) a length of the protein design template; and generating a library of peptide candidates comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues using linear epitope mapping of the protein design template.

[0041] In some embodiments, methods of identifying a nucleic acid-binding peptide comprise: binding the nucleic acid to a phage comprising the nucleic acid-binding peptide; amplifying an insert of the phage using PCR amplification; and sequencing the insert. In some embodiments, sequencing the insert comprises next-generation sequencing. In some embodiments, methods further comprise sequencing the phage genome. In some embodiments, methods further comprise confirming that the nucleic acid-binding peptide binds to the nucleic acid using an ELISA assay. In some embodiments, methods further comprise: synthesizing the nucleic acid-binding peptide on a cellulose disk; dissolving the cellulose disk; printing the dissolved cellulose disk on a slide; incubating a fluorescently labeled nucleic acid on the slide; washing the slide; and imaging a fluorescence of the slide.

[0042] In some embodiments, methods of elucidating a mechanism of binding between a nucleic acid and a nucleic acid-binding peptide comprising: preparing a structure of the nucleic acid and preparing a structure of the nucleic acid-binding peptide; generating a nucleic acid-peptide complex from the nucleic acid and nucleic acid-binding peptide structures; conducting molecular dynamics simulations on the nucleic acid-peptide complex; calculating a first binding free energy of the nucleic acid-binding peptide to the nucleic acid; calculating a second binding free energy of the nucleic acid-binding peptide to a competitor; and modifying the nucleic acid-binding peptide by stabilizing the peptide backbone or mutating a residue of the nucleic acid-

binding peptide to obtain a modified nucleic acid-binding peptide that comprises a binding efficacy to the nucleic acid that is greater than a binding efficacy of the nucleic acid-binding peptide to the nucleic acid.

[0043] In some embodiments, the design of peptide affinity ligands for biologic purification including phage display and rational design can be done using the strategies shown in FIG. 2. In some embodiments, a rational and focused approach was based on mimicking the active binding pocket of naturally occurring target specific binding proteins. In some embodiments, protein design templates were selected based on one or more of the following criteria: (i) binding affinity to the target; (ii) binding selectivity/specificity to the target versus the unwanted species; (iii) structural information available; and (iv) length/size of the protein. In some embodiments, protein domains were identified as mimicking targets, and a library of 16-mer peptide candidates was generated using linear epitope mapping of the target proteins. In some embodiments, particular focus was placed on the peptides that cover the key interacting regions of the protein.

[0044] Referring to FIG. 2, approaches employed to identify and design peptide affinity ligands for nucleic acid purification comprise rational design based on known binders of ds-RNA that are available from nature. In some embodiments, the power of numbers can be used in combinatorial screening. In some embodiments, the benefit of numbers and a broad and randomized search space can be obtained. In some embodiments, the benefit of evolution and a narrow but focused search space can be obtained. In some embodiments these approaches can be used individually or in combination for a comprehensive strategy. In some embodiments, a short peptide sequence that is 12-mer long can have a potential search space of 10^{15} represented by the grey box which is a million times larger compared to the starting library for combinatorial screening. In some embodiments, the goal then comprises using sequence and composition information from these approaches as input for computational methods to scan the search space that experiments missed.

[0045] *Phage display selection of target selective peptides*

[0046] In some embodiments, phage display can be used to identify peptides that bind selectively to one target over the other. In some embodiments, a randomized Ph.D.-12™ library (New England Biolabs Inc., Ipswich, MA, USA) with a complexity of 10^9 and a concentration of

2×10^{13} pfu/mL can be used. In some embodiments, five repeat units of 12mer random peptides can be fused to pIII coat protein of M13 phage for selection. In some embodiments, phage display selection process can be carried out in two main steps, negative selection and positive selection. In some embodiments, prior to the selection process, four rounds of negative selection can be carried out to remove bead/tube binding phages. In some embodiments, for each round of negative selection, of hydrophilic streptavidin magnetic beads (New England Biolabs Inc., Ipswich, MA, USA) can be washed three times with 200 μ l binding buffer. In some embodiments, the phage library (10 μ l, 10^{11} pfu) can be suspended in binding buffer, added to the washed beads, incubated for about 30 minutes at room temperature, and the supernatant passed onto the next round of negative selection. In some embodiments, bead/tube bound phages can remain in the tube while the supernatant that is depleted of tube/bead bound phages can be passed onto the target panning process. In some embodiments, for the positive selection, ~ 2 μ g of biotinylated target can be suspended in binding buffer, added to about 55 μ l washed streptavidin magnetic beads and incubated for about 30 minutes at room temperature. In some embodiments, the target immobilized beads can then be washed three times with binding buffer. In some embodiments, the prescreened phage library can be added to the beads and incubated at room temperature for about 30 minutes. In some embodiments, the beads can be washed with about 200 μ l binding buffer. In some embodiments, bound phages can be eluted using about 0.2M Glycine-HCl, pH2.2. In some embodiments, the eluate can be immediately neutralized with Tris-HCl, pH9. In some embodiments, the eluted phage pool can be tittered, submitted for Sanger sequencing, and amplified for the next round of selection. In some embodiments, the DNA can be extracted for each selected library from each round of panning for Next Generation Sequencing.

[0047] In some embodiments, starting from the second round of selection, competitors can be added to increase the selectivity of the selection process to the target. In some embodiments, competitor can be added to the selected phage pool and preincubated for about 30 minutes at room temperature prior to allow binding in free solution. In some embodiments, the competitor-phage mixture can be then added to target immobilized beads and the same panning protocol can be carried out after. In some embodiments, the stringency of selection can be increased for each round of panning by increasing the detergent concentration, number of washing steps, and/or the amount of competitors added.

[0048] *Sanger sequencing*

[0049] In some embodiments, at the end of each round of panning, the eluted phages can be tittered, and about ten random plaques can be picked and submitted for Sanger sequencing.

[0050] *DNA extraction and PCR amplification of insert*

[0051] In some embodiments, the DNA of each round of panning selected phage library can be extracted following the standard NEB protocol. In some embodiments, about 100 µl of amplified phage library ($\sim 10^{12}$ pfu) can be precipitated by adding about 40µl of 20%PEG/2.5MNaCl solution and incubated at about 4°C overnight. In some embodiments, the single stranded DNA from phage can be extracted using NaI buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 4 M sodium iodide (NaI)] and ethanol. AMPure XP beads (Beckman Coulter, Inc) can be used to remove unwanted salt and protein present after the crude extraction.

[0052] In some embodiments, the polymerase chain reaction (PCR) can be used to amplify the 36nt (12 amino acid) insert by using the forward primer 5'-5'- NKK NKK ACT ATC TAT TCT CAC TCT- 3' and reverse primer 5' - TTC GGC CGA ACC TCC ACC -3'. In some embodiments, the primers anneal right before and after the insert as shown in FIG. 3. In some embodiments, the total amplicon has 78 bp in length. Q5 high fidelity master mix (New England Biolabs Inc., Ipswich, MA, USA) can be used for PCR amplification. In some embodiments, PCR conditions listed in **Table 1** can be used. In some embodiments, PCR products can be purified using AMPure XP beads.

[0053] **Table 1. Exemplary PCR cycling condition for insert amplification**

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
35 Cycles	98°C	10 seconds
	63°C	20 seconds
	72°C	20 seconds
Final Extension	72°C	5 minutes

Hold	4°C	
------	-----	--

[0054] Referring to FIG. 3, in some embodiments, insert isolation from whole phage vector can be done using PCR amplification. In some embodiments, forward and reverse primers to 12 AA (36 bp) insert region in M13KE vector can be aligned. In some embodiments, a PCR product of 78 bp can be generated.

[0055] *Illumina next generation sequencing*

[0056] In some embodiments, the amplified double stranded DNA (90bp) obtained from the PCR amplification can be prepared for Next Generation sequencing (NGS) following NEB NGS library preparation manual (NEBNext® Ultra™ II DNA Library Prep Kit for Illumina®) as demonstrated in FIG. 4. In some embodiments, a unique barcode can be added to each DNA library for sequence analysis later. In some embodiments, gel electrophoresis (Agilent 2100 Bioanalyzer), Qubit® DNA HS (High Sensitivity) assay and dsDNA nanodrop measurements can be performed to confirm the integrity and size and concentration of the PCR product. In some embodiments, mid-output Illumina NextSeq kit can be employed for the sequencing process. In some embodiments, a total of about 180 million reads can be collected for about 5 libraries (Library 1: 1st panning, library 2: 2nd panning, library 3: 3rd panning, library 4: 4th panning, library 5: repeated 4th panning). In some embodiments, the raw reads can be processed and translated to peptide sequences following steps shown in FIG. 5.

[0057] Referring to FIG. 5, in some embodiments, sequencing result data can be processed using the steps shown. In some embodiments, data processing can be performed using MATLAB scripts as indicated in FIG. 5.

[0058] *ELISA confirmation of selected phage library's binding and binding selectivity to the target*

[0059] In some embodiments, in the ELISA assay, individual wells can be separately immobilized with target, competitor, and a blank control in 0.1M NaHCO₃ at pH 8.6. In some embodiments, following immobilization, the excess target solution can be removed, and the wells can be washed about three times with 0.05% Tween20 binding buffer. In some

embodiments, subsequently, 10^{11} pfu of the third-round selected phage library can be introduced into the initial wells, while serial dilutions of the selected phage library can be added to the subsequent wells to confirm target selectivity. In some embodiments, the phage library can undergo a one-hour incubation with the immobilized target at room temperature, followed by three washes with binding buffer (0.05% Tween20). In some embodiments, a 100 μ L volume of a 1:1000 dilution of M13 antibody can be added to each well and incubated for an additional hour. In some embodiments, afterward, the wells can be washed thrice with binding buffer. In some embodiments, subsequently, a 100 μ L volume of a 1:5000 dilution of anti-IgG antibody-HRP can be added to each well for one hour, followed by another three washes with binding buffer. In some embodiments, 75 μ L of TMB substrate can be added to each well for colorimetric development, and the reaction can be halted by introducing 75 μ L of stop solution. In some embodiments, the binding events can be quantified by measuring the absorbance at 450 nm. In some embodiments, triplicate wells can be employed for each target and each phage dilution to provide standard deviation values.

[0060] *High-throughput microarray screening of peptide-target interactions*

[0061] In some embodiments, candidate peptide libraries can be constructed using rational design strategy and from phage display were both synthesized and screened for binding to the target using a high-throughput microarray screening platform. In some embodiments, the peptides can be synthesized directly on Fmoc-beta-Alanine modified cellulose disks using SPOT synthesis employing standard solid phase peptide synthesis chemistry with diisopropylcarbodiimide (DIC) and ethyl (hydroxyimino)cyanoacetate (Oxyma). In some embodiments, the sidechain protection groups of the peptides can then be removed, and the peptide-cellulose conjugates can then be dissolved in DMSO and printed onto microarray slides. In some embodiments, the peptide printed microarray slides can be screened with fluorescently labeled target and competitor separately by one hour room temperature incubation. In some embodiments, following the one hour incubation, the slides can be washed three times with binding buffer and scanned using a flatbed scanner. In some embodiments, the images can be scanned with respective excitation/emission wavelength. In some embodiments, the images can be processed using ImageJ to extract the fluorescent intensity of each individual peptide spot.

[0062] *Computational approaches to investigate the selective binding mechanism of the peptide to the target*

[0063] In some embodiments, to elucidate the selective binding mechanisms of peptides to their corresponding targets, a series of computational techniques can be employed, including molecular docking and dynamics simulations. In some embodiments, the process can commence with the independent preparation of the peptide and target structures. In some embodiments, the peptide conformations can be predicted utilizing the PEP-FOLD 4 online server, while the target structures can be retrieved from the Protein Data Bank's experimentally resolved crystal structures or constructed using the MOE platform. In some embodiments, an initial peptide-target complex can be generated using docking servers such as ClusPro, with the optimal binding pose selected based on a ranking score derived from a shape complementarity scoring function. In some embodiments, this complex can be subsequently prepared for molecular dynamics (MD) simulations using the CHARMM-GUI server, and simulations can be conducted over a 100 ns period using GROMACS. In some embodiments, post-simulation, the molecular mechanics/Poisson-Boltzmann (Generalized-Born) surface area method (gmx_MMPBSA) can be applied to calculate the binding free energy to both the target and a designated competitor. In some embodiments, the optimal binding poses can be extracted using VMD and MDanalysis tools. In some embodiments, further refinement involved a mutational analysis of the peptide, including alanine scanning via gmx_MMPBSA, aimed at optimizing the peptide's selective binding affinity relative to the competitor. In some embodiments, AI-assisted tools such as RoseTTAFold All atom can be implemented to confirm and refine the binding selectivity of the peptide by obtaining detailed structures of the binding complexes and analyzing the predicted Local Distance Difference Test (pLDDT) and Per-residue Average Error (PAE) scores. In some embodiments, techniques like RFDiffusion and ProteinMPNN can further refine the peptide design by stabilizing the peptide backbone and modifying specific amino acids to enhance binding efficacy.

[0064] An exemplary library screening approach is shown in FIG. 6 (Database of protein targets graphic reproduced from Bradley M. Lunde, Claire Moore & Gabriele Varani, *RNA-binding proteins: modular design for efficient function*, 8 NAT REV MOL CELL BIOL 479–490, Fig. 3 (Jun. 2007)). In some embodiments, peptides with high affinity towards ss-mRNA and ds-

mRNA are identified using phage panning. Exemplary processes include incubating a phage library with support beads and retain supernatant for negative selection against support (e.g., 1 round). The process can also include negative selection against non-preferred target by challenging the supernatant from the previous step with bead-immobilized ss-mRNA or ds-mRNA individually. The process can further include positive selection (e.g., 5 rounds) by challenging the supernatant from either of the last two steps with bead-immobilized complementary ss-mRNA or ds-mRNA and performing biopanning (e.g., 1 round of selection followed by 2-3 rounds of enrichment each).

[0065] Rational ligand design from RNA binding proteins (RBPs) can be used to design a peptide library from known RBPs. In some embodiments, available databases are scanned. For example, Blast mRNA sequence against known RNA binding sequences in RBP databases and identify the best matching sequences (e.g., the best, top three, top five etc. matches) and their RBPs. In some embodiments, the peptide library is designed by examining the active sites of RBPs involved in binding to mRNA. The process can also include creating an epitope map of the RBP binding region and further optimization to (a) maximize contact regions/residues, (b) enhance physico-chemical-geometric-topographical binding structures, and/or (c) incorporate mRNA specific binding features based on existing knowledge.

[0066] Microarray screening for ligand discovery can be used to synthesize and screen a peptide library to identify candidates with high affinity & high selectivity towards ss-mRNA and ds-mRNA. In some embodiments, binding affinity is quantified by synthesizing peptides identified from the peptide library, fabricating peptide microarrays, screening against soluble labeled ss-mRNA and ds-mRNA, and quantifying binding affinity. The process can also include quantifying binding selectivity for pure ss-mRNA versus pure ds-mRNA, selecting peptides with high binding affinity from the previous step and quantifying affinity in presence of soluble labeled ss-mRNA and soluble labeled ds-mRNA independently or simultaneously with orthogonal labels. The process can further include quantifying binding selectivity in feedstock conditions by challenging high affinity and high selectivity peptides from the previous step against both ss-mRNA and ds-mRNA independently in target-containing and/or depleted feedstock to quantify selectivity for ss-mRNA vs. ds-mRNA.

[0067] In some embodiments, the affinity ligands, e.g., peptides, are attached to flat sheet RC membranes using standard EDC-NHS coupling chemistry. In some embodiments, a plurality of affinity ligands are attached. In some embodiments, acylate oligo-dT (IDT, Coralville, Iowa) is grafted as a control. In some embodiments, the membranes modified with affinity ligand are tested against the oligo-dT control and an unmodified RC membrane as a negative control to demonstrate the efficacy of selective binding between ss-mRNA and ds-mRNA of the affinity ligand. ss-mRNA integrity, yield, and throughput can then be characterized using solution depletion and fluorescence and UV260/280 spectroscopy.

[0068] In some embodiments, membranes for purification of ss-mRNA from ds-mRNA comprise a ds-mRNA-binding peptide covalently bound to the membrane. In some embodiments, the membrane comprises a cellulose-based membrane or a hollow fiber membrane. In some embodiments, the cellulose-based membrane comprises a regenerated cellulose membrane. In some embodiments, the ds-mRNA-binding peptide is covalently bound to the membrane via a peptide bond, e.g., using EDC-NHS coupling chemistry. Although other crosslinking bonds are contemplated, e.g., amine to sulfhydryl. In some embodiments, the ds-mRNA-binding peptide is covalently bound to the membrane via a heterobifunctional crosslinker that is suitable for the solubility of the peptide, mRNAs, and membrane functional groups, e.g., heterobifunctional crosslinkers available from ThermoFisher Scientific and G-Biosciences, including 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), SMCC, sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC), succinimidyl 3-(2-pyridyldithio)propionate (SPDP), sulfosuccinimidyl 6-(3'-(2-pyridyldithio)propionamido)hexanoate (sulfo-LC-SPDP), and Pierce™ branded reagents. In some embodiments, the ds-mRNA-binding peptide is covalently bound to the membrane via a homobifunctional crosslinker, e.g., homobifunctional crosslinkers available from ThermoFisher Scientific, including disuccinimidyl glutarate (DSG), disuccinimidyl suberate (DSS), bis(sulfosuccinimidyl)suberate (BS3), PEGylated bis(sulfosuccinimidyl)suberate (BS(PEG)9), dithiobis(succinimidyl propionate) (DSP), 3,3'-dithiobis(sulfosuccinimidyl propionate) (DTSSP), ethylene glycol bis(succinimidyl succinate) (EGS), SMCC, sulfo-SMCC, SPDP, and LC-SPDP.

[0069] In some embodiments, ds-mRNA-binding peptide comprises a double stranded RNA binding domain-derived peptide, a flock house virus B2 protein-derived peptide, a tomato

aspermy 2b protein-derived peptide, an antimicrobial peptide, a toll like receptor 3-derived peptide, an arginine rich motif-derived peptide identified using the peptide screening methods described above.

[0070] In some embodiments, methods of making a membrane for purification of ss-mRNA comprises: reacting a carboxyl group of a regenerated cellulose membrane with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride to form an O-acylisourea; and reacting an N-hydroxysuccinimide-modified ds-mRNA-binding peptide with the O-acylisourea. In some embodiments, ds-mRNA binding peptide is derived from a double stranded RNA binding domain, a flock house virus B2 protein, a tomato aspermy 2b protein, an antimicrobial peptide, a toll like receptor 3 protein, or an arginine rich motif peptide.

[0071] In some embodiments, methods of making a membrane for purification of ss-mRNA comprises: reacting a hollow fiber membrane with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride to form an O-acylisourea; and reacting an N-hydroxysuccinimide-modified ds-mRNA-binding peptide with the O-acylisourea. In some embodiments, the ds-mRNA binding peptide is derived from a double stranded RNA binding domain, a flock house virus B2 protein, a tomato aspermy 2b protein, an antimicrobial peptide, a toll like receptor 3 protein, or an arginine rich motif peptide.

[0072] Some aspects of the disclosure are directed to systems for continuous affinity membrane manufacturing, comprising: one or more bioreactors including components sufficient to promote synthesis of ss-mRNA, the bioreactors including at least one inlet and at least one outlet; an outlet stream in fluid communication with the one or more bioreactors, the outlet stream including a concentration of ss-mRNA; one or more affinity membrane separators in fluid communication with the outlet stream, the affinity membrane separators being modified by the inclusion of a peptide derived from a double stranded RNA binding domain, a flock house virus B2 protein, a tomato aspermy 2b protein, an antimicrobial peptide, a toll like receptor 3 protein, or an arginine rich motif peptide; and one or more diafiltration modules in fluid communication with the affinity membrane separators. In some embodiments, the one or more affinity membrane separators include flat sheet microporous membranes, cellulose hollow fiber microporous affinity membranes, or combinations thereof, wherein the affinity membrane

separators have a total membrane surface area of about 4 m² or greater. In some embodiments, the ss-mRNA is a vaccine against coronaviruses, e.g., SARS-CoV-2, flu, respiratory syncytial virus (RSV), human metapneumovirus (HMPV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus (HSV), varicella-zoster virus (VZV), human immunodeficiency viruses (HIV), norovirus, Lyme disease, zika virus, Mpox, and other viruses. It should be appreciated that the materials, methods, and systems of the present disclosure can be used to discover and manufacture m-RNA-based vaccines and therapeutics for the treatment other diseases including cardiovascular diseases, pulmonary diseases, autoimmune diseases, cancer, and other diseases and/or morbidities.

[0073] In some embodiments, all products of mRNA are purified after synthesis at scale using a cell-free bioreactor called an *in vitro* transcription (IVT) bioreactor. In some embodiments, the systems and methods of the present disclosure are used for purification of all mRNA products. Referring now to FIG. 2, in some embodiments, one or more affinity ligands are applied to a laboratory RC hollow fiber module, e.g., (0.0001 m², Planova 75N, Asahi Kasei Medical). In some embodiments, modified membranes plus the unmodified RC membrane (negative control 2) are tested for efficacy of selective binding between ss-mRNA and ds-mRNA. In some embodiments, ss-mRNA integrity, yield, and throughput are characterized. In some embodiments, the performance (mRNA integrity, yield and throughput) is tested with a IVT feed.

[0074] Systems and methods of the present disclosure are advantageous in that they disclose peptide ligands that bind selectively to different classes of nucleic acids mRNA. This is useful, e.g., for purification of mRNA production and commercialization, as well as methods of identifying those ligands. Today, besides purifying mRNA vaccines, many mRNA products are being developed at Moderna (and Pfizer, BioNTech and Greenlight Biosciences among many others) for treatment of many diseases and agricultural applications. See <https://www.modernatx.com/research/product-pipeline>. The opportunities are enormous.

[0075] Besides such advantages for the latter ligands, the identification of small targeting agents, like peptides, for mRNA can add to basic knowledge of mRNA molecular interactions, especially when rational design strategies are incorporated in ligand development. Furthermore,

ligand design with a focus on elucidating binding characteristics can provide information for iterative design and future targeting efforts.

[0076] The advantages of cellulose substrate can be combined with conjugated ligands of the present disclosure. Peptides can be synthesized in a very high throughput manner. Binding domains can be mimicked or used to perform focused and rational mutations to test the effect of such modifications, also in a high throughput manner. Then, use of membrane surface grafting can be used to attach the selective binding peptides to microporous hydrophilic surfaces using grafting methods, e.g., standard EDC-NHS coupling.

EXAMPLES

[0077] To design dsRNA specific binding peptides for dsRNA capture and removal consistent with the embodiments discussed above, library of around 400 peptides have been designed from linear epitope mapping with peptides 16 residues in length overlapping by 14 residues. The linear epitope mapping strategy is used to cover the entire binding surface of the naturally occurring dsRNA binding proteins. A total of 358 peptides of the peptide library were synthesized and screened using microarray screening. The screening set-up is shown in FIG. 7.

[0078] Referring to FIG. 7, peptide synthesis and screening for rationally designed peptides was performed consistent with the embodiments discussed above. Step 1: peptides were synthesized onto the Fmoc -beta-alanine modified cellulose support using the standard solid phase peptide synthesis strategy; Step2: the peptide-cellulose conjugates were dissolved in DMSO and printed onto the microarray slides in duplicates; Step 3: dye and dsRNA were premixed to allow the dye-dsRNA conjugate to form prior to screening; Step 4: the dye-dsRNA conjugate were then added to the peptide printed microarray slides and incubated for 1 hour at room temperature. After incubation, the slides were washed with PBS (pH7.5) for three minutes on the shaker for three times; Step 4: the dried slides were then scanned using a flatbed scanner for microarray screening result.

[0079] In some embodiments, the top binding peptides would be stapled for its alpha helical structure to examine the structure impact on the dsRNA binding. Alpha helical structure was frequently observed at the binding region of the dsRNA binding proteins including this design

template proteins. Hydrocarbon stapling strategy (see e.g., Yang, S. W. et al. Structure of arabidopsis Hyponastic Leaves 1 and its molecular implications for miRNA Processing. Structure 18, 594-605 (2010)) would be utilized to staple the helical structure at the back of the binding interface. Three to four stapling locations would be tested for each of the peptides to determine the best stapling location.

[0080] Although the invention has been described and illustrated with respect to exemplary embodiments thereof, it should be understood by those skilled in the art that the foregoing and various other changes, omissions and additions can be made therein and thereto, without parting from the spirit and scope of the present invention.

CLAIMS

What is claimed is:

1. A system for continuous affinity membrane manufacturing, comprising:
 - one or more bioreactors including components sufficient to promote synthesis of a nucleic acid, the bioreactors including at least one inlet and at least one outlet;
 - an outlet stream in fluid communication with the one or more bioreactors, the outlet stream including a concentration of nucleic acid;
 - one or more affinity membrane separators in fluid communication with the outlet stream, the affinity membrane separators being modified by the inclusion of a peptide ligand comprising twenty or fewer residues; and
 - one or more diafiltration modules in fluid communication with the affinity membrane separators.
2. The system for continuous affinity membrane manufacturing of claim 1, wherein the nucleic acid comprises ss-mRNA.
3. The system for continuous affinity membrane manufacturing of claim 1, wherein the nucleic acid comprises ds-mRNA.
4. The system for continuous affinity membrane manufacturing of claim 1, wherein the nucleic acid comprises DNA.
5. The system for continuous affinity membrane manufacturing as in any one of the preceding claims, wherein the one or more affinity membrane separators comprises a flat sheet microporous membranes, a cellulose hollow fiber microporous affinity membranes, or a combination thereof.
6. The system for continuous affinity membrane manufacturing as in any one of the preceding claims, wherein the affinity membrane separators have a total membrane surface area of about 4 m² or greater.

7. The system for continuous affinity membrane manufacturing of claim 2, wherein the ss-mRNA is a vaccine against coronaviruses, e.g., SARS-CoV-2.
8. A membrane for purification of nucleic acids comprising:
 - a nucleic acid-binding peptide covalently bound to the membrane,
 - wherein the membrane comprises a cellulose-based membrane or a hollow fiber membrane.
9. The membrane of claim 8, wherein the membrane comprises the cellulose-based membrane and comprises a regenerated cellulose membrane.
10. The membrane as in either claim 8 or 9, wherein the nucleic acid-binding peptide is covalently bound to the membrane via a peptide bond.
11. The membrane as in either claim 8 or 9, wherein the nucleic acid-binding peptide is covalently bound to the membrane via a heterobifunctional crosslinker.
12. The membrane as in either claim 8 or 9, wherein the nucleic acid-binding peptide is covalently bound to the membrane via a homobifunctional crosslinker.
13. The membrane as in any one of claims 8-12, wherein the nucleic acid-binding peptide comprises a ds-mRNA-binding peptide comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues.
14. The membrane as in any one of the claims 8-12, wherein the nucleic acid-binding peptide comprises a ss-mRNA-binding peptide comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues.
15. The membrane as in any one of the claims 8-12, wherein the nucleic acid-binding peptide comprises a DNA-binding peptide comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues.
16. A method of making a membrane for purification of nucleic acids comprising:

reacting a nucleic acid-binding peptide to the membrane,

wherein the nucleic acid-binding peptide comprises 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues.

17. The method of claim 16, wherein the step of reacting comprises forming a peptide bond between the nucleic acid-binding peptide and the membrane.
18. The method of claim 17, wherein forming the peptide bond is mediated by diisopropylcarbodiimide (DIC) and ethyl (hydroxyimino)cyanoacetate (Oxyma).
19. The method as in any one of claims 16-18, wherein the nucleic acid comprises ss-mRNA.
20. The method as in any one of claims 16-18, wherein the nucleic acid comprises ds-mRNA.
21. The method as in any one of claims 16-18, wherein the nucleic acid comprises DNA.
22. The method as in any one of claims 16-21, wherein the one or more affinity membrane separators comprises a flat sheet microporous membranes, a cellulose hollow fiber microporous affinity membranes, or a combination thereof.
23. A method of making a membrane for purification of nucleic acids comprising:

reacting a functional group of a membrane with a first end of a crosslinker; and

reacting a second end of the crosslinker with a nucleic acid-binding peptide that comprises 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues.
24. The method of claim 23, wherein the membrane comprises a flat sheet microporous membranes, a cellulose hollow fiber microporous affinity membranes, or a combination thereof.
25. A method of making a membrane for purification of nucleic acids comprising:

reacting a carboxyl group of a regenerated cellulose membrane with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride to form an O-acylisourea; and

reacting an N-hydroxysuccinimide-modified nucleic acid-binding peptide with the O-acylisourea,

wherein the nucleic acid-binding peptide comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues.

26. A method of making a membrane for purification of nucleic acids comprising:

reacting a hollow fiber membrane with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride to form an O-acylisourea; and

reacting an N-hydroxysuccinimide-modified nucleic acid-binding peptide with the O-acylisourea,

wherein the nucleic acid-binding peptide comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues.

27. A method of designing a nucleic acid-binding peptide comprising:

selecting a protein design template based on one or more the following criteria: (i) a binding affinity to the nucleic acid; (ii) a binding selectivity to the nucleic acid versus a contaminant; (iii) a structural information; and (iv) a length of the protein design template; and

generating a library of peptide candidates comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 residues using linear epitope mapping of the protein design template.

28. A method of identifying a nucleic acid-binding peptide comprising:

binding the nucleic acid to a phage comprising the nucleic acid-binding peptide;

amplifying an insert of the phage using PCR amplification; and

sequencing the insert.

29. The method of claim 28, wherein sequencing the insert comprises next-generation sequencing.
30. The method as in claim 28 or 28 further comprising sequencing the phage genome.
31. The method as in any one of claims 28-30 further comprising confirming that the nucleic acid-binding peptide binds to the nucleic acid using an ELISA assay.
32. The method as in any one of claims 27-31 further comprising:
- synthesizing the nucleic acid-binding peptide on a cellulose disk;
 - dissolving the cellulose disk;
 - printing the dissolved cellulose disk on a slide;
 - incubating a fluorescently labeled nucleic acid on the slide;
 - washing the slide; and
 - imaging a fluorescence of the slide.
33. A method of elucidating a mechanism of binding between a nucleic acid and a nucleic acid-binding peptide comprising:
- preparing a structure of the nucleic acid and preparing a structure of the nucleic acid-binding peptide;
 - generating a nucleic acid-peptide complex from the nucleic acid and nucleic acid-binding peptide structures;
 - conducting molecular dynamics simulations on the nucleic acid-peptide complex;
 - calculating a first binding free energy of the nucleic acid-binding peptide to the nucleic acid;

calculating a second binding free energy of the nucleic acid-binding peptide to a competitor; and

modifying the nucleic acid-binding peptide by stabilizing the peptide backbone or mutating a residue of the nucleic acid-binding peptide to obtain a modified nucleic acid-binding peptide that comprises a binding efficacy to the nucleic acid that is greater than a binding efficacy of the nucleic acid-binding peptide to the nucleic acid.

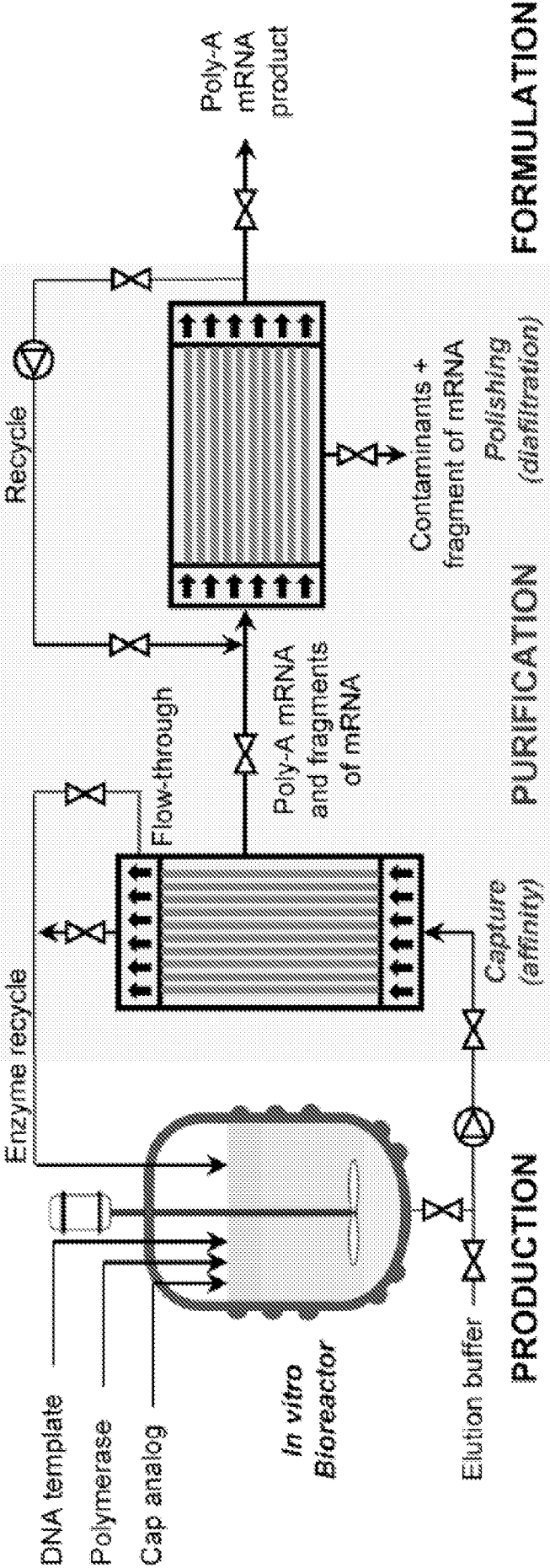
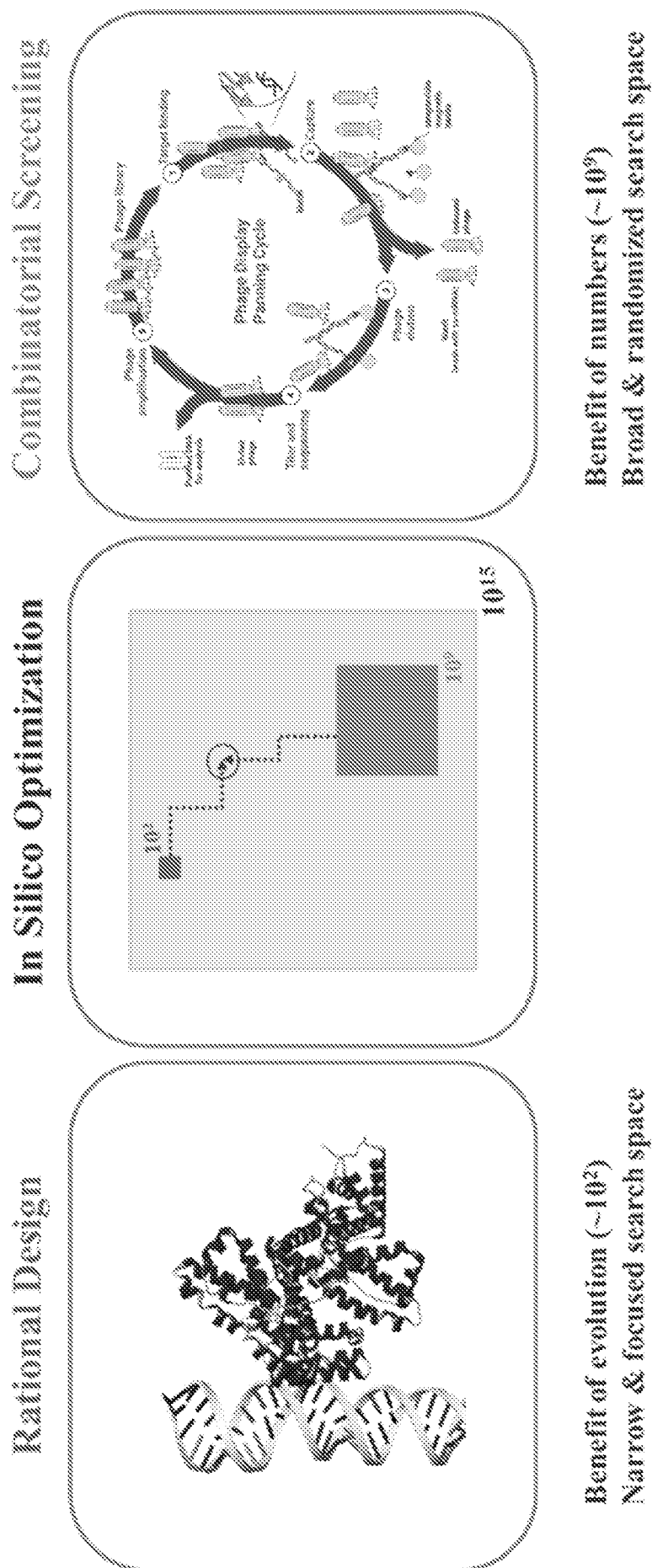


FIG. 1



THE

In Slice Optimization

~~CONFIDENTIAL SECRET~~

Benefit of evolution ($\sim 10^2$)
Narrow & focused search space

Benefit of numbers ($\sim 10^9$)
Broad & randomized search space

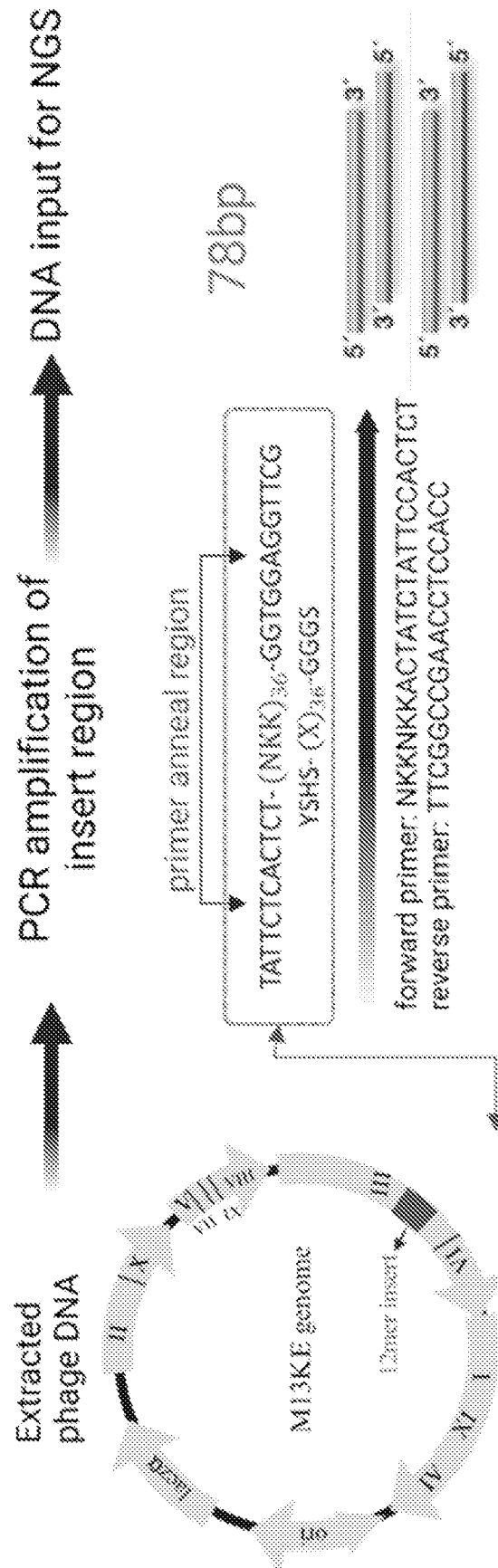


Fig. 3

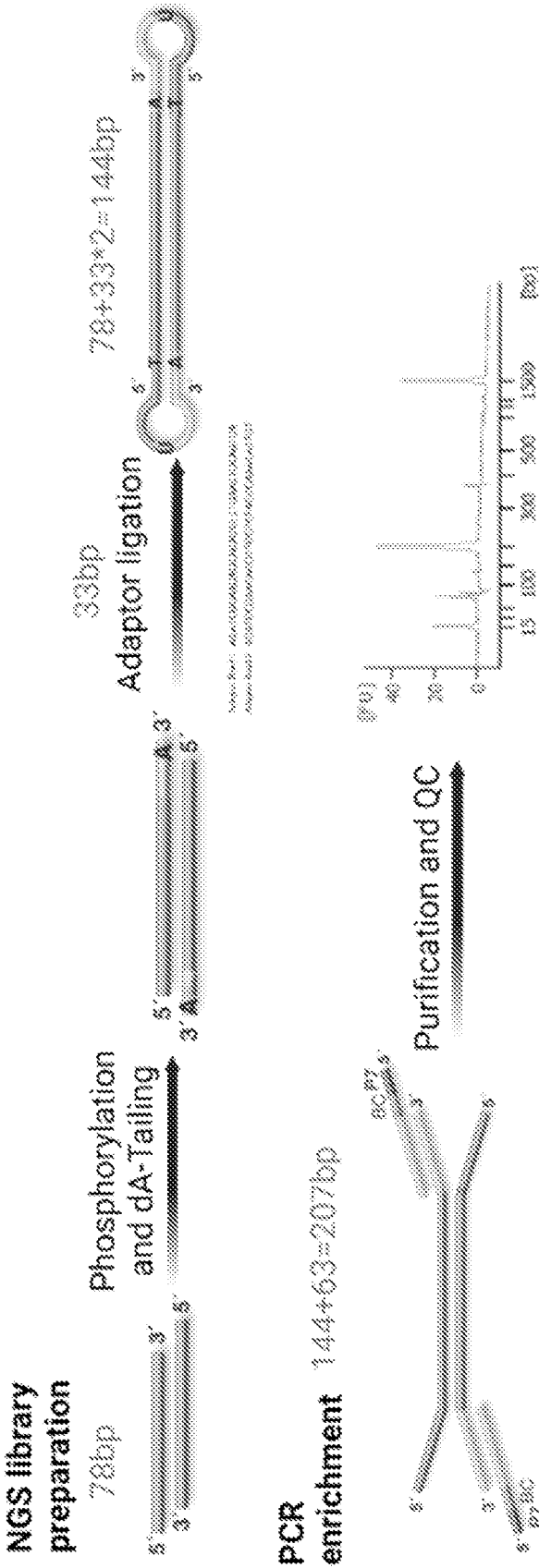


FIG. 4

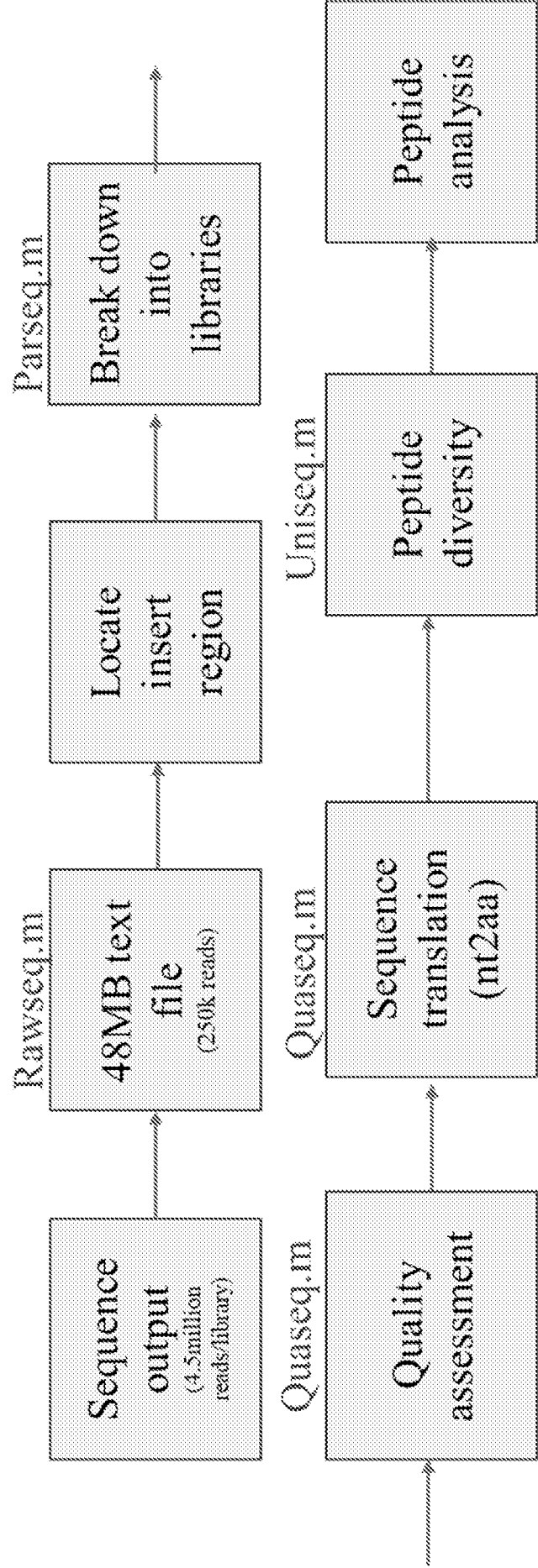
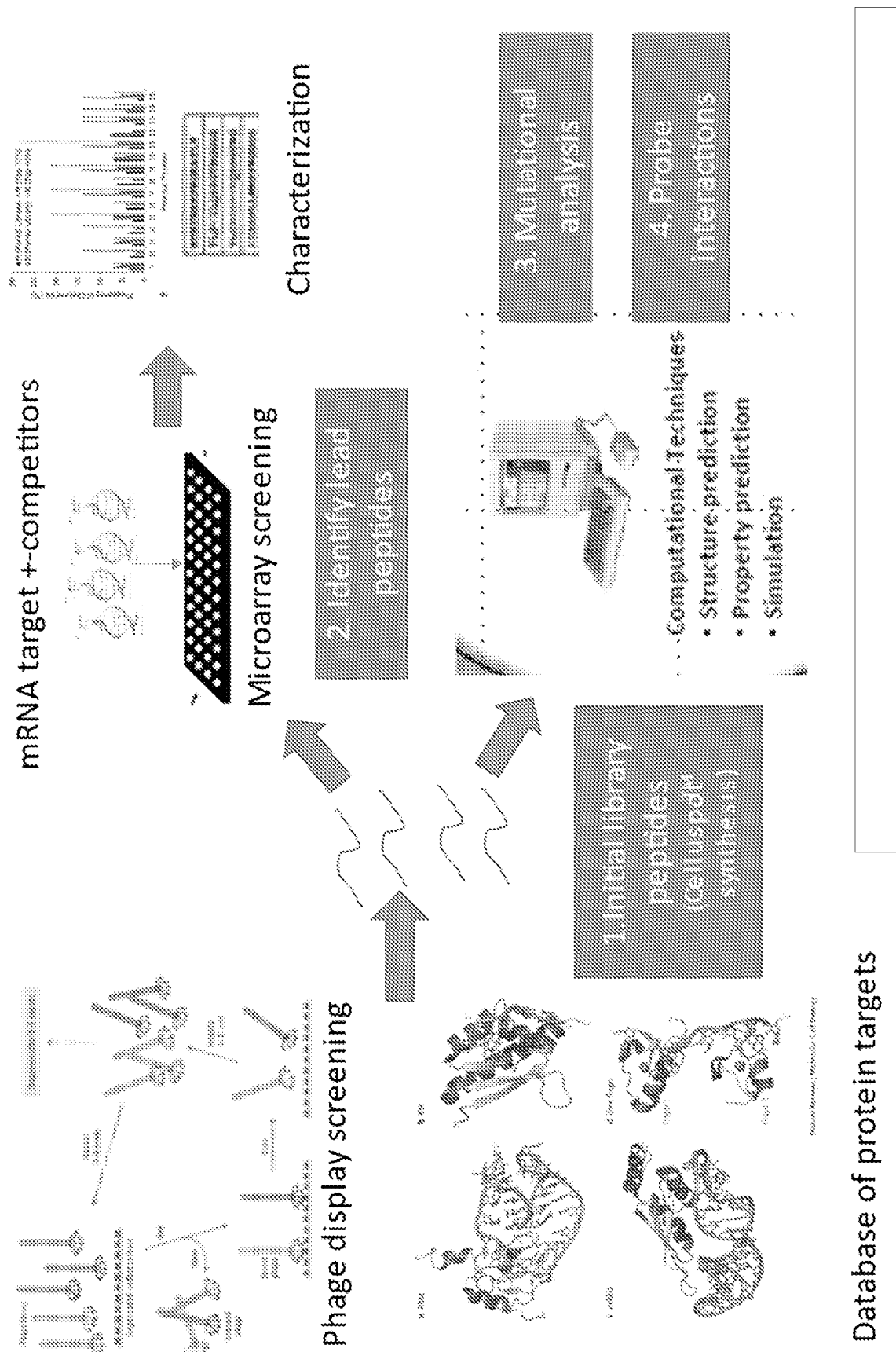


FIG. 5



F/G.6

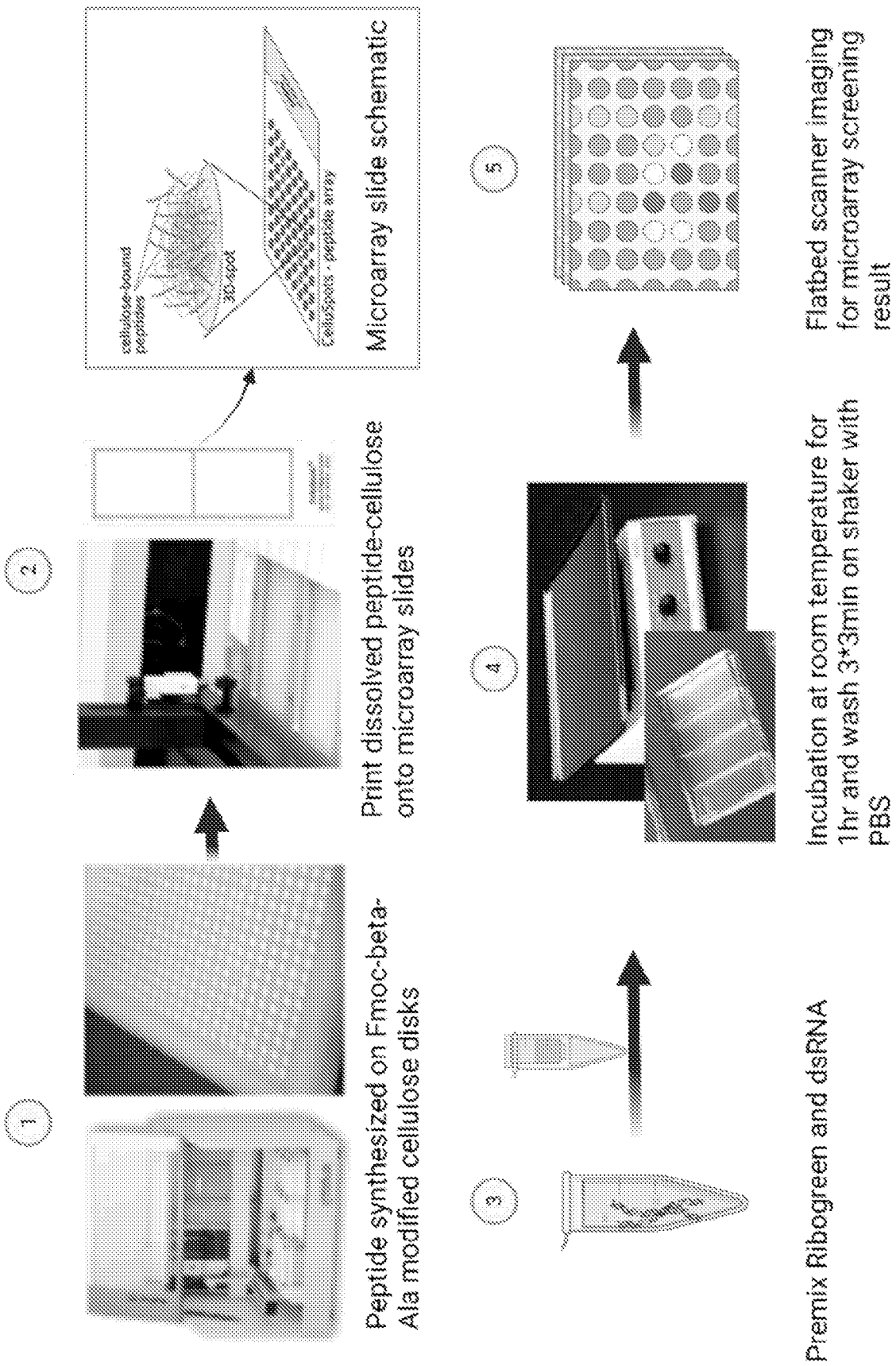


FIG. 7