US010385115B2

(12) **United States Patent**
Chilkoti et al.

(10) **Patent No.: US 10,385,115 B2**
(45) **Date of Patent: Aug. 20, 2019**

(54) **FIBRONECTIN TYPE III DOMAIN-BASED FUSION PROTEINS**

(71) Applicant: **Duke University**, Durham, NC (US)

(72) Inventors: **Ashutosh Chilkoti**, Durham, NC (US); **Mandana Manzari**, Durham, NC (US); **Mareva Fevre**, San Jose, CA (US)

(73) Assignee: **Duke University**, Durham, NC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 15 days.

(21) Appl. No.: **15/561,799**

(22) PCT Filed: **Mar. 25, 2016**

(86) PCT No.: **PCT/US2016/024202**
§ 371 (c)(1),
(2) Date: **Sep. 26, 2017**

(87) PCT Pub. No.: **WO2016/154530**
PCT Pub. Date: **Sep. 29, 2016**

(65) **Prior Publication Data**

US 2018/0258157 A1 Sep. 13, 2018

Related U.S. Application Data

(60) Provisional application No. 62/138,847, filed on Mar. 26, 2015.

(51) **Int. Cl.**

A61K 38/16	(2006.01)
A61K 38/39	(2006.01)
C07K 14/62	(2006.01)
C07K 14/78	(2006.01)
A61P 35/00	(2006.01)
A61K 9/00	(2006.01)
A61K 38/00	(2006.01)

(52) **U.S. Cl.**
CPC ***C07K 14/78*** (2013.01); ***A61K 9/0019*** (2013.01); ***A61P 35/00*** (2018.01); *A61K 38/00* (2013.01); *C07K 2319/00* (2013.01); *C07K 2319/01* (2013.01); *C07K 2319/74* (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,554,101	A	11/1985	Hopp
4,976,734	A	12/1990	Urry et al.
5,250,516	A	10/1993	Urry
5,336,256	A	8/1994	Urry
5,578,577	A	11/1996	Ching et al.
5,580,859	A	12/1996	Felgner et al.
5,676,646	A	10/1997	Hofmann et al.
5,679,647	A	10/1997	Carson et al.
5,702,359	A	12/1997	Hofmann et al.
5,703,055	A	12/1997	Felgner et al.
6,068,650	A	5/2000	Hofmann et al.
6,096,020	A	8/2000	Hofmann
6,120,493	A	9/2000	Hofmann
6,150,148	A	11/2000	Nanda et al.
6,181,964	B1	1/2001	Hofmann et al.
6,192,270	B1	2/2001	Hofmann et al.
6,207,749	B1	3/2001	Mayes et al.
6,208,893	B1	3/2001	Hofmann
6,216,034	B1	4/2001	Hofmann et al.
6,233,482	B1	5/2001	Hofmann et al.
6,241,701	B1	6/2001	Hofmann
6,245,515	B1	6/2001	Vogelstein et al.
6,296,831	B1	10/2001	Weller et al.
6,302,874	B1	10/2001	Zhang et al.
6,623,950	B1	9/2003	Osten et al.
6,660,247	B1	12/2003	Gutowska et al.
6,841,617	B2	1/2005	Jeong et al.
6,852,834	B2	2/2005	Chilkoti
6,869,588	B2	3/2005	Weller et al.
7,033,571	B2	4/2006	Gutowska et al.
7,087,244	B2	8/2006	Jeong et al.
7,429,458	B2	9/2008	Chilkoti
7,664,545	B2	2/2010	Westersten et al.
7,674,882	B2	3/2010	Kaplan et al.
8,129,330	B2	3/2012	Martinez et al.

(Continued)

FOREIGN PATENT DOCUMENTS

CA	2327325	A1	11/1999
CA	2423488	A1	4/2002

(Continued)

OTHER PUBLICATIONS

Abbruzzese et al., "A phase I clinical, plasma, and cellular pharmacology study of gemcitabine," J. Clin. Oncol. 1991, 3, 491-498.
Aladini et al., "Chemical Synthesis and Characterization of Elastin-Like Polypeptides (ELPs) With Variable Guest Residues," J Pept Sci, 2016, 22(5):334-342.
Alconcel et al., "FDA-approved poly(ethylene glycol)-protein conjugate drugs," Polym. Chem. 2, 2011, 1442-1448.
Alley et al., "Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay," Cancer Res., 1988, 48, 589-601.
Aluri et al., "Elastin-like peptide amphiphiles Form nanofibers with tunable length," Biomacromolecules, 2012, 13, 2645-2654.

(Continued)

Primary Examiner — Christine J Saoud
Assistant Examiner — Jon M Lockard
(74) *Attorney, Agent, or Firm* — Michael Best & Friedrich LLP

(57) **ABSTRACT**

Provided herein are fusion proteins including at least one binding polypeptide and at least one unstructured polypeptide. The fusion protein may further include at least one linker. Further provided are methods for determining the presence of a target in a sample, methods of treating a disease, methods of diagnosing a disease in a subject, and methods of determining the effectiveness of a treatment for a disease in a subject. The methods may include administering to the subject an effective amount of the fusion protein.

15 Claims, 6 Drawing Sheets

Specification includes a Sequence Listing.

(56) **References Cited**

U.S. PATENT DOCUMENTS

8,283,125	B2	10/2012	Cebolla Ramirez et al.
8,470,967	B2	6/2013	Chilkoti et al.
8,497,356	B2	7/2013	Chilkoti et al.
8,912,310	B2	12/2014	Chilkoti et al.
9,127,047	B2	9/2015	Chilkoti
9,592,303	B2	3/2017	Chilkoti et al.
9,771,396	B2	9/2017	Chilkoti et al.
2002/0052443	A1	5/2002	Greenwald et al.
2002/0146794	A1	10/2002	Tomycz
2003/0175290	A1	9/2003	Renner et al.
2003/0225251	A1	12/2003	Sallberg et al.
2003/0233675	A1	12/2003	Cao et al.
2004/0053976	A1	3/2004	Martinez et al.
2004/0101852	A1	5/2004	Bennett et al.
2005/0255554	A1	11/2005	Chilkoti
2005/0288229	A1	12/2005	Sindrey et al.
2006/0025524	A1	2/2006	Schneider et al.
2006/0034796	A1	2/2006	Ashwell et al.
2007/0087114	A1	4/2007	Chilkoti et al.
2007/0117173	A1	5/2007	Levison et al.
2008/0181861	A1	7/2008	Jiang et al.
2009/0098652	A1	4/2009	Stupp et al.
2010/0015070	A1	1/2010	Bollschweiler et al.
2010/0048473	A1	2/2010	Chaikof et al.
2010/0241054	A1	9/2010	Dacey et al.
2010/0311059	A1	12/2010	Didion et al.
2010/0325765	P1	12/2010	Pait et al.
2011/0119778	A1	5/2011	Liss
2011/0207673	A1	8/2011	Chilkoti et al.
2011/0294189	A1	12/2011	Chilkoti et al.
2011/0305718	A1	12/2011	Mugica et al.
2012/0208742	A1	8/2012	Primiano et al.
2013/0079277	A1	3/2013	Chilkoti
2013/0079280	A1	3/2013	Baca et al.
2013/0096058	A1	4/2013	Baca et al.
2013/0102993	A1	4/2013	Kim et al.
2013/0157889	A1	6/2013	Chilkoti et al.
2013/0165389	A1	6/2013	Schellenberger et al.
2013/0172274	A1	7/2013	Chilkoti
2013/0330335	A1	12/2013	Bremel et al.
2014/0024600	A1	1/2014	Chilkoti et al.
2014/0163201	A1	6/2014	Winter et al.
2014/0294932	A1	10/2014	Kim et al.
2015/0094270	A1	4/2015	Harris et al.
2015/0112022	A1	4/2015	Chilkoti et al.
2016/0120952	A1	5/2016	Chilkoti
2016/0220727	A1	8/2016	Lu et al.
2016/0271262	A1	9/2016	Lopez et al.
2016/0303091	A1	10/2016	Wang
2016/0355802	A1	12/2016	Isaacs et al.

FOREIGN PATENT DOCUMENTS

WO	WO 2003/040165	A2	10/2002
WO	WO 2006/004778	A2	1/2006
WO	WO 2007/073486	A2	6/2007
WO	WO 2007/108013	A2	9/2007
WO	WO 2007/134245	A2	11/2007
WO	WO 2009/067584	A1	5/2009
WO	WO 2011/123813	A2	10/2011
WO	WO 2013/065009	A1	5/2013
WO	WO 2014/194244	A1	12/2014
WO	WO 2015/130846	A2	9/2015
WO	WO 2016/065273	A1	4/2016
WO	WO 2016/154530	A1	9/2016
WO	WO 2017/015132	A1	1/2017
WO	WO 2017/024182	A1	2/2017
WO	WO 2017/112825	A2	6/2017
WO	WO 2017/112826	A2	6/2017

OTHER PUBLICATIONS

Amiram et al., "A depot-forming glucagon-like peptide-1 fusion protein reduces blood glucose for five days with a single injection," J. Control. Release, 2013, 172, 144-151.

Amiram et al., "Injectable protease-operated depots of glucagon-like peptide-1 provide extended and tunable glucose control," Proc. Natl. Acad. Sci. 110, 2013, 2792-2797.

Antos et al., "Lipid Modification of Proteins through Sortase-Catalyzed Transpeptidation," J. Am. Chem. Soc. 2008, 130, 16338-16343.

Antos et al., "Site-Specific N- and C-Terminal Labeling of a Single Polypeptide Using Sortases of Different Specificity," J. Am. Chem. Soc. 2009, 131, 10800-10801.

Arias et al., "Superior preclinical efficacy of gemcitabine developed as chitosan nanoparticulate system," Biomacromolecules 2011, 12, 97-104.

Armstrong et al., "Antibody against poly(ethylene glycol) adversely affects PEG-asparaginase therapy in acute lymphoblastic leukemia patients," Cancer 110, 2007, 103-111.

Armstrong et al., "The Hydrodynamic Radii of Macromolecules and Their Effect on Red Blood Cell Aggregation," Biophys. J., 2004, 87, 4259-4270.

Arnida et al., "Geometry and surface characteristics of gold nanoparticles influence their biodistribution and uptake by macrophages," Eur J Pharm Biopharm, 2011, 77, 417-423.

Averick et al., "ATRP under biologically relevant conditions: grafting from a protein," ACS Macro. Lett. 1, 2012, 6-10.

Averick et al., "Protein-polymer hybrids: conducting ARGET ATRP from a genetically encoded cleavable ATRP initiator," Eur. Polym. J. 49, 2013, 2919-2924.

Awai et al., "Studies of the metabolism of I-131-labeled human transferrin," J. Lab. Clin. Med. 61, 1963, 363-396.

Axup et al., "Synthesis of site-specific antibody-drug conjugates using unnatural amino acids," Proc Natl Acad Sci USA, 2012, 109(40):16101-16106.

Baggio et al., "A recombinant human glucagon-like peptide (GLP)-1-albumin protein (Albugon) mimics peptidergic activation of GLP-1 receptor-dependent pathways coupled with satiety, gastrointestinal motility, and glucose homeostasis," Diabetes 53, 2004, 2492-2500.

Banga et al., "Parenteral controlled delivery and parmacokinetics of therapeutic peptides and proteins," (CRC Press, Boca Raton, FL, 2005).

Bansal et al., "PEGylation improves pharmacokinetic profile, liver uptake and efficacy of Interferon gamma in liver fibrosis," J. Control. Release 2011, 154, 233-240.

Bedford et al., "WW domain-mediated interactions reveal a spliceosome-associated protein that binds a third class of proline-rich motif: The proline glycine and methionine-rich motif," PNAS, 1998, 95: 10602-10607.

Bellucci et al., "A noncanonical function of sortase enables site-specific conjugation of small molecules to lysine residues in proteins," Angew. Chem. Int. Ed. 54, 2015, 441-445.

Bellucci et al., "Three-in-One Chromatography-Free Purification, Tag Removal, and Site-Specific Modification of Recombinant Fusion Proteins Using Sortase A and Elastin-like Polypeptides," Angewandte Chemie International Edition, 2013, 52(13):3703-3708.

Bender et al., "Synthesis, Crystallization, and Biological Evaluation of an Orally Active Prodrug of Gemcitabine," J. Med. Chem. 2009, 52, 6958-6961.

Berisio et al., "Imino Acids and Collagen Triple Helix Stability: Characterization of Collagen-like Polypeptides Containing Hyp-Hyp-Gly Seqeucne Repeats," JACS, 2004, 126: 11402-11403.

Berndt et al., "Synthetic lipidation of peptides and amino acids: Monolayer structure and properties," J. Am. Chem. Soc., 1995, 117, 9515-9522.

Bessa et al., "Thermoresponsive self-assembled elastin-based nanoparticles for delivery of BMPs," Journal of Controlled Release, 2010, 142, 312-318.

Bhattacharyya et al., "A paclitaxel-loaded recombinant polypeptide nanoparticle outperforms Abraxane in multiple murine cancer models," Nat. Commun. 2015, 6, 7939.

Bidwell et al., "Development of elastin-like polypeptide for thermally targeted delivery of doxorubicin," Biochemical Pharmacology, 2007, 73(5):620-631.

(56) **References Cited**

OTHER PUBLICATIONS

Boekhorst et al., "Genome-wide detection and analysis of cell wall-bound proteins with LPxTG-like sorting motifs," J. Bacteriol. 187, 2005, 4928-4934.
Bond, "Exenatide (Byetta) as a novel treatment option for type 2 diabetes mellitus," Proc. (Bayl. Univ. Med. Cent.) 19, 2006, 281-284.
Bontempo et al., "Streptavidin as a macroinitiator for polymerization: in situ protein-polymer conjugate formation," J. Am. Chem. Soc., 2005, 6508-6509.
Boyer et al., "Well-Defined Protein-Polymer Conjugates via in Situ RAFT Polymerization," J. Am. Chem. Soc. 2007, 129, 7145-7154.
Broyer et al., "Emerging synthetic approaches for protein-polymer conjugations," Chem. Commun. 2011, 47, 2212.
Brusa et al., "Antitumor activity and pharmacokinetics of liposomes containing lipophilic gemcitabine prodrugs," Anticancer Res. 2007, 27, 195-199.
Cai et al., "Long-acting preparations of exenatide," Drug Des. Dev. Ther. 7, 2013, 963-970.
Caliceti et al., "Pharmacokinetic and biodistribution properties of poly(ethylene glycol)-protein conjugates," Adv. Drug Deliv. Rev, 2003, 55, 1261-1277.
Campbell et al., "Pegylated peptides V. Carboxy-terminal PEGlyted analogs of growth hormone-releasing factor (GRF) display enhanced duration of biological activity in vivo," J. Peptide Res., 1997, 49:527-537.
Carrico et al., "Introducing genetically encoded aldehydes into proteins," Nat Chem Biol, 2007, 3(6):321-322.
Chen et al., "Anti-hypervariable region antibody induced by a defined peptide: An approach for studying the structural correlates of idiotypes," PNAS, 1984, 81:1784-1788.
Chen et al., "Bioinspired Modular Synthesis of Elastin-Mimic Polymers to Probe the Mechanism of Elastin Elasticity," J. Am. Chem. Soc., 2009, 132(13):4577-4579.
Chen et al., "Site-specific labeling of cell surface proteins with biophysical probes using biotin ligase," Nat Methods, 2005, 2(2):99-104.
Chilkoti et al., "Design of thermally responsive, recombinant polypeptide carriers for targeted drug delivery," Advance Drug Delivery Reviews, 2002, 54:1093-1111.
Chilkoti et al., "Targeted drug delivery by thermally responsive polymers," Advanced Drug Delivery Reviews, 2002, 54:613-630.
Chithrani et al., "Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells," Nano Lett, 2006, 6, 662-668.
Chitkara et al., "Self-Assembling, Amphiphilic Polymer-Gemcitabine Conjugate Shows Enhanced Antitumor Efficacy Against Human Pancreatic Adenocarcinoma," Bioconjug. Chem. 2013, 24, 1161-1173.
Cho et al., "Effects of hofmeister anions on the phase transition temperature of elastin-like polypeptides," J. Phys. Chem. B., 2008, 112, 13765-13771.
Cho et al., "Therapeutic nanoparticles for drug delivery in cancer," Clin. Cancer Res., 2008, 14, 1310-1316.
Chow et al., "Peptide-based biopolymers in biomedicine and biotechnology," Mater. Sci. Eng. R Reports, 2008, 62, 125-155.
Chow et al., "Ultra-High Expression of a Thermally Responsive Recombinant Fusion Protein in *E. coli*," Biotechnology Progress, 2006, 22(3):638-646.
Christensen et al., "Fusion order controls expression level and activity of elastin-like polypeptide fusion proteins," Protein Science, 2009, 18:1377-1387.
Coin et al., "Solid-phase peptide synthesis: from standard procedures to the synthesis of difficult sequences," Nat. Protoc., 2007, 2, 3247.
Craik et al., "The future of peptide-based drugs," Chemical biology & drug design 81, 2013, 136-147.
Cui et al., "Amino acid sequence in constitutionally isomeric tetrapeptide amphiphiles dictates architecture of one-dimensional nanostructures," J. Am. Chem. Soc., 2014, 136, 12461-12468.
Cui et al., "Self-assembly of peptide amphiphiles: from molecules to nanostructures to biomaterials," Biopolymers, 2010, 94, 1-18.
Dalla Poza et al., "Targeting gemcitabine containing liposomes to CD44 expressing pancreatic adenocarcinoma cells causes an increase in the antitumoral activity," Biochim. Biophys. Acta, 2013, 1828, 1396-1404.
Dasgupta et al., "Isopeptide Ligation Catalyzed by Quintessential Sortase A: Mechanistic Cues From Cyclic and Branched Oligomers of Indolicidin," The Journal of Biological Chemistry, 2011, vol. 286, No. 27, pp. 23996-24006, Supplemental Information.
De et al., "Temperature-Regulated Activity of Responsive Polymer-Protein Conjugates Prepared by Grafting-from via RAFT Polymerization," J. Am. Chem. Soc. 2008, 130, 11288-11289.
Dennis et al., "Co-Translational Myristoylation Alters the Quaternary Structure of HIV-1 Nef in Solution," Proteins: Structure, Function, and Bioinformatics, 2005, 60:658-669.
Depp et al., "Native protein-initiated ATRP: A viable and potentially superior alternative to PEGylation for stabilizing biologics," Acta Biomater. 2009, 5, 560-569.
Diehl et al., "A Good Practice Guide to the Administration of Substances and Removal of Blood Including Routes and Volumes," J Appl Toxicol, 2001, 21, 15-23.
Donnelly et al., "DNA Vaccines," Ann. Rev. Immunol., 1997, 15, 617-648.
Dreher et al., "Evaluation of an elastin-like polypeptide-doxorubicin conjugate for cancer therapy," J. of Controlled Release, 2003, 91:31-43.
Dreher et al., "Temperature triggered self-assembly of polypeptides into multivalent spherical micelles," J. Am. Chem. Soc. 2008, 130, 687-694.
Dreher, M. R. PhD. Thesis, Duke University, Durham, NC, Apr. 2006.
Dreis et al., "Preparation, Characterisation and Maintenance of Drug Efficacy of Doxorubicin-Loaded Human Serum Albumin (HSA) Nanoparticles," Int. J. Pharm., 2007, 341, 207-214.
Drucker et al., "The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes," Lancet 368, 2006, 1696-1705.
Drucker, "Glucagon-like peptides," Diabetes 47, 1998, 159-169.
Du et al., "Tailor-made dual pH-sensitive polymer-doxorubicin nanoparticles for efficient anticancer drug delivery," J. Am. Chem. Soc., 2011, 133, 17560-17563.
Duan et al., "Fibronectin type III domain based monobody with high activity," Biochemistry, 2007 46(44):12656-12664.
Dubey et al., "Development and evaluation of folate functionalized albumin nanoparticles for targeted delivery of gemcitabine," Int J Pharm., 2015, 492(1-2):80-91.
Duncan, R. "Polymer conjugates as anticancer nanomedicines," Nat. Rev. Cancer 2006, 6, 688-701.
Duronio et al., "Protein N-myristoylation in *Escherichia coli*: Reconstitution of a eukaryotic protein modification in bacteria," Proc. Nail. Acad. Sci. USA, 1990, vol. 87, pp. 1506-1510.
Dyrberg et al., "Peptide as Atigens," J. Exp. Med., 1986, 164:1344-1349.
Eisenhaber et al., "Prediction of lipid posttranslational modifications and localization signals from protein sequences: Big-PI, NMT and PTS1," Nucleic Acids Res., 2003, 31, 3631-3634.
Etrych et al., "HPMA Copolymer Conjugates of Paclitaxe; and Docetaxel with pH-Controlled Drug Release," Molecular Pharmaceutics, 2010, 7(4):1015-1026.
Farazi et al., "Structures of Saccharomyces cerevisiae N-myristoyltransferase with bound myristoylCoA and peptide provide insights about substrate recognition and catalysis," Biochemistry, 2001, 40, 6335-6343.
Feng et al., "Protein resistant surfaces: comparison of acrylate graft polymers bearing oligo-ethylene oxide and phosphorylcholine side chains," Biointerphases, 2006, 1 (1), 50.
Fluegel et al., "Chain stiffness of elastin-like polypeptides," Biomacromolecules 2010, 11, 3216-3218.
Friedman et al., "Directed Evolution to Low Nanomolar Affinity of a Tumor-Targeting Epidermal Growth Factor Receptor-Binding Affibody Molecule," J. Mol. Biol., 2008, 376, 1388-1402.

(56) **References Cited**

OTHER PUBLICATIONS

Furgeson et al., "Structural optimization of a "smart" doxorubicin-polypeptide conjugate for thermally targeted delivery to solid tumors," Journal of Controlled Release, 2006, 110:362-369.
Gaberc-Porekar et al., "Obstacles and pitfalls in the PEGylation of therapeutic proteins," Curr. Opin. Drug Discov. Devel. 11, 2008, 242-250.
Gabizon et al., "Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes," Cancer Res. 1994, 54, 987-992.
Ganson et al., "Control of hyperuricemia in subjects with refractory gout, and induction of antibody against poly(ethylene glycol) (PEG), in a phase I trial of subcutaneous PEGylated urate oxidase," Arthritis Res. Ther. 8, 2006, R12-R22.
Ganson et al., "Pre-existing anti-PEG antibody linked to first-exposure allergic reactions to Pegnivacogin, a PEGylated RNA aptamer," J. Allergy Clin. Immunology, (2015).
Gao et al., "In situ growth of a PEG-like polymer from the C terminus of an intein fusion protein improves pharmacokinetics and tumor accumulation" PNAS Early Edition, 2010, vol. 107, 1-6.
Gao et al., "In situ growth of a PEG-like polymer from the C terminus of an intein fusion protein improves pharmacokinetics and tumor accumulation," Proc. Natl. Acad. Sci. 107, 2010, 16432-16437.
Gao et al., "In situ growth of a stoichiometric PEG-like conjugate at a protein's N-terminus with significantly improved pharmacokinetics," Proc. Natl. Acad. Sci., 2009,15231-15236.
Garanger et al., "Structural Evolution of a Stimulus-Responsive Diblock Polypeptide Micelle by Temperature Tunable Compaction of its Core," Macromolecules, 2015, 48, 6617-6627.
Garay et al., "Antibodies against polyethylene glycol in healthy subjects and in patients treated with PEG-conjugated agents," Expert Opinion. Drug Deliv. 9, 2012, 1319-1323.
Gauthier et al., "Peptide/protein-polymer conjugates: synthetic strategies and design concepts," Chem. Commun., 2008, 2591-2611.
Ge et al., "Self-Cleavable Stimulus Responsive Tags for Protein Purification without Chromatography" J. Am. Chem. Soc., 2005, 127: 11228-11229.
Genbank Accession NM_001182082.1 (2017).
Gibson et al., "Enzymatic assembly of DNA molecules up to several hundred kilobases," Nat Methods, 2009, 6, 343-345.
Gluck et al., "Single Vector System for Efficient N-myristoylation of Recombinant Proteins in *E. coli*," Plos One. 2010, 5(4) e100881.
Göke et al., "Exendin-4 is a high potency agonis and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting b-cells," J. Biol. Chem. 268, 1993, 19650-19655.
Gordon et al., "Protein N-myristoylation," J. Biol. Chem., 1991, 266, 8647-8650.
Goutelle et al., "The Hill equation: a review of its capabilities in pharmacological modelling. Fundam," Clin. Pharmacol. 22, 2008, 633-648.
Green et al., "Novel dipeptidyl peptidase IV resistant analogues of glucagon-like peptide-1(7-36)amide have preserved biological activities in vitro conferring improved glucose-lowering action in vivo" J. of Mol. Endocrin, 2003, 31(3): 529-540.
Greenfield, "Using circular dichroism spectra to estimate protein secondary structure," Nat. Protoc., 2006, 1(6):2876-90.
Grover et al., "Protein-Polymer Conjugates: Synthetic Approaches by Controlled Radical Polymerizations & Interesting Applications", Curr Opin Chem Bioi., Dec. 2010; 14(6): 818-827.
Haider et al., "Genetically engineered polymers: Status and prospects for controlled release," J. Control. Release, 2004, 95, 1-26.
Hamidi et al., "Pharmacokinetic Consequences of Pegylation," Drug Deliv. 2006, 13, 399-409.
Hamley, "Self-assembly of amphiphilic peptides," Soft Matter, 2011, 7, 4122.
Harries et al., "Nanoparticle Albumin-Bound Paclitaxel for Metastatic Breast Cancer," J. Clin. Oncol., 2005, 23(31):7768-7771.
Harris et al., "Pegylation," Clinical Pharmacokinetics, 2001, 40(7):539-551.
Hartgerink et al., "Self-assembly and mineralization of peptide-amphiphile nanofibers," Science, 2001, 294, 1684-8.
Hassouneh et al., "Elastin-like Polypeptide Diblock Copolymers Self-Assemble into Weak Micelles," Macromolecules, 2015, 48, 4183-4195.
Hassouneh et al., "Elastin-Like Polypeptides as a Purification Tag for Recombinant Proteins," Curr Protoc Protein Sci., 2010, Chapter 6. Unit 6.11. 10.1002/0471140864.ps0611s61.
Hassouneh et al., "Fusions of elastin-like polypeptides to pharmaceutical proteins," Methods Enzymol., 2012, 502, 215-37.
Hassouneh et al., "Unexpected Multivalent Display of Proteins by Temperature Triggered Self-assembly of Elastin-like Polypeptide Block Copolymers," Biomacromolecules, 2012, vol. 13, Issue 4, pp. 1598-1605.
He et al., "Comparative genomics of elastin: Sequence analysis of a highly repetitive protein," Matrix Biology, 2007, 26:524-540.
He et al., "Improving protein resistance of α-Al2O3 membranes by modification with POEGMA brushes," Applied Surface Science, 2011, 258 (3), 1038-1044.
Heagerty et al., Biometrics, "Time-dependent ROC curves for censored survival data and a diagnostic marker," 2000, 56(2):337-44.
Heal et al., "N-Myristoyl transferase-mediated protein labelling in vivo," Org. Biomol. Cham., 2008, 6(13):2308-2315.
Heal et al., "Site-specific N-terminal labelling of proteins in vitro and in vivo using N-myristoyl transferase and bioorthogonal ligation chemistry," Chem. Commun., 2008, 3, 480-482.
Heredia et al., "In Situ Preparation of Protein-"Smart" Polymer Conjugates with Retention of Bioactivity," J. Am. Chem. Soc. 2005, 127, 16955-16960.
Hershfield et al., "Induced and pre-existing anti-polyethylene glycol antibody in a trial of every 3-week dosing of pegloticase for refractory gout, including in organ transplant recipients," Arthritis Res. Ther. 16, 2014, R63.
Ho et al., "Chemoenzymatic Labeling of Proteins for Imaging in Bacterial Cells," J. Am. Chem. Soc., 2016, 138(46):15098-15101.
Hober et al., "Protein A chromatography for antibody purification," Journal of Chromatography B 848, 2007, pp. 40-47.
Hochkoeppler, "Expanding the landscape of recombinant protein production in *Escherichia coli*," Biotechnol. Lett., 2013, 35, 1971-1981.
Holehouse et al., "CIDER: Classification of Intrinsically Disordered Ensemble Regions," Biophysical Journal, 2015, vol. 108, Issue 2, Supplement 1, p. 228a.
Ilangovan et al., "Structure of sortase, the transpeptidase that anchors proteins to the cell wall of *Staphylococcus aureus*," Proc. Natl. Acad. Sci. 98, 2001, 6056-6061.
Inostroza-Brito et al., "Co-assembly, spatiotemporal control and morphogenesis of a hybrid protein-peptide system," Nat. Chem., 2015, 7, 1-8.
Ishida et al., "Accelerated blood clearance (ABC) phenomenon upon repeated injection of PEGylated liposomes," International Journal of Pharmaceutics, 2008, 354(1-2):56-62.
Jakubowski et al., "Activators regenerated by electron transfer for atom-transfer radical polymerization of (meth)acrylates and related block copolymers," Angew. Chem. Int. Ed., 2006, 4482-4486.
Janes et al., "Chitosan nanoparticles as delivery systems for doxorubicin," J. Control Release, 2001, 73, 255-267.
Jia et al., "Preparation, physicochemical characterization and cytotoxicity in vitro of gemcitabine-loaded PEG-PDLLA nanovesicles," World J. Gastroenterol. 2010, 16(8):1008-1013.
Jiang et al., "The internal structure of self-assembled peptide amphiphiles nanofibers," Soft Matter, 2007, 3, 454.
Jin et al., "Protein-resistant polyurethane prepared by surface-initiated atom transfer radical graft polymerization (ATRgP) of water-soluble polymers: effects of main chain and side chain lengths of grafts," Colloids and surfaces. B, Biointerfaces, 2009, 70 (1), 53-9.
Junutula et al., "Site-specific conjugation of a cytotoxic drug to an antibody improves the therapeutic index," Nat Biotechnol, 2008, 26(8):925-932.

(56) **References Cited**

OTHER PUBLICATIONS

Kanoski et al., "The role of nausea in food intake and body weight suppression by peripheral GLP-1 receptor agonists, exendin-4 and liraglutide," Neuropharmacology 62, 2012, 1916-1927.
Katti et al., "Amino acid repeat patterns in protein sequences: Their diversity and structural-functional implications," Protein Science, 2000, 9: 1203-1209.
Keefe et al., "Poly(zwitterionic)protein conjugates offer increased stability without sacrificing binding affinity or bioactivity," Nat Chem, 2012, 4(1):59-63.
Keller et al., "Empirical Statistical Model to Estimate the Accuracy of Peptide Identifications Made by MS/MS and Database Search," Anal. Chem. 2002, 74, 5383-5392.
Kelly et al., "How to study proteins by circular dichroism," Biochim. Byophys. Acta—Proteins Proteomics, 2005, 1751(2):119-39.
Khandare et al., "Polymer-drug conjugates: Progress in polymeric drugs," Prog. Polym. Sci., 2005, vol. 31, pp. 359-397.
Kim et al., "Site-Specific PEGylated Exendin-4 Modified with a High Molecular Weight Trimeric PEG Reduces Steric Hindrance and Increases Type 2 Antidiabetic Therapeutic Effects," Bioconjugate Chem. 2012, 23, 2214-2220.
Kim et al., "Ultrasensitive Carbon nanotube-based biosensors using antibody-binding fragments," Analytical Biochemistry, 2008, 381, 193-198.
Knop et al., "Poly(ethylene glycol) in Drug Delivery: Pros and Cons as Well as Potential Alternatives," Angewandte Chemie International Edition, 2010, 49(36):6288-6308.
Knudsen, "Glucagon-like Peptide-1: The Basis of a New Class of Treatment for Type 2 Diabetes" J. Med. Chem, 2004, 47: 4128-4134.
Kobashigawa et al., "Attachment of an NMR-Invisible Solubility Enhancement Tag Using a Sortase-Mediated Protein Ligation Method," J Biomol NMR. Mar. 2009, vol. 43, No. 3; pp. 145-150.
Kontos et al., "Drug development: longer-lived proteins," Chemical Society Reviews, 2012, 41(7):2686-2695.
Kothare et al., "Pharmacokinetics, pharmacodynamics, tolerability, and safety of exenatide in Japanese patients with type 2 diabetes mellitus," J. Clin. Pharmacol. 48, 2008, 1389-1399.
Kramer et al., "Quantitative Side-Chain Modifications of Methionine-Containing Elastin-Like Polypeptides as a Versatile Tool to Tune Their Properties," ACS Macro Lett., 2015, 4(11):1283-1286.
Kruger et al., "Analysis of the Substrate Specificity of the *Staphylococcus aureus* Sortase Transpeptidase SrtA†," Biochemistry, 2004, 43, 1541-1551.
Kulkarni et al., "Bioorthogonal Chemoenzymatic Functionalization of Calmodulin for Bioconjugation Applications," Bioconjug. Chem., 2015, 26(10):2153-2160.
Kulkarni et al., "Selective functionalization of the protein N terminus with N-myristoyl transferase for bioconjugation in cell lysate," ChemBioChem, 2013, 14, 1958-1962.
Kumar et al., "N-Terminal Region of the Catalytic Domain of Human N-Myristoyltransferase 1 Acts as an Inhibitory Module," PLoS One, 2015, 10(5):e0127661.
Kyte et al., "A Simple Method for Displaying the Hydropathic Character of a Protein," J. Mol. Biol., 1982, 157:105-132.
Langer et al., "Designing materials for biology and medicine," Nature, 2004, 428, 487-92.
Le Droumaguet et al., "Recent advances in the design of bioconjugates from controlled/living radical polymerization," Polym. Chem. 2010, 1, 563-598.
Le Meins et al., "Hybrid polymer/lipid vesicles: State of the art and future perspectives," Mater. Today, 2013, 16, 397-402.
Leader et al., "Protein therapeutics: a summary and pharmacological clasification," Nat. Rev. Drug Discov. 7, 2008, 21-39.
Lee et al., "Atomistic molecular dynamics simulations of peptide amphiphile self-assembly into cylindrical nanofibers," J. Am. Chem. Soc., 2011, 133, 3677-3683.
Lee et al., "Theranostic nanoparticles with controlled release of gemcitabine for targeted therapy and MRI of pancreatic cancer," ACS Nano 2013, 7(3):2078-2089.
Lele et al., "Synthesis of uniform protein-polymer conjugates," Biomacromolecules 6, 2005, 3380-3387.
Lennen et al., "Membrane Stresses Induced by Overproduction of Free Fatty Acids in *Escherichia coli*," Appl Environ Microb., 2011, 77(22):8114-28.
Leung et al., "Bio-Click Chemistry: Enzymatic Functionalization of PEGylated Capsules for Targeting Applications**," Angew. Chem. Int. Ed. 2012, 51, 7132-7136.
Le Vine et al., "Thioflavine T interaction with synthetic Alzheimer's disease beta-amyloid peptides: detection of amyloid aggregation in solution," Protein Sci., 1993, 2, 404-10.
Li et al., "Molecular description of the lcst behavior of an elastin-like polypeptide," Biomacromolecules, 2014, 15, 3522-3530.
Li et al., "Protein adsorption on oligo(ethylene glycol)-terminated alkanethiolate self-assembled monolayers: The molecular basis for nonfouling behavior," The journal of physical chemistry. B, 2005, 109 (7), 2934-41.
Li et al., "Temperature-Triggered Phase Separation of a Hydrophilic Resilin-Like Polypeptide," Macramol. Rapid Commun., 2015, 36(1):90-95.
Liao et al., "Removal of N-terminal methionine from recombinant proteins by engineered *E. coli* methionine aminopeptidase," Prot. Sci. 13, 2004, 1802-1810.
Liechty et al., "Polymers for Drug Delivery Systems," Annual review of chemical and biomolecular engineering, 2010, 1:149-173.
Lim et al., "Improved Non-Chromatographic Purification of a Recombinant Protein by Cationic Elastin-like Polypeptides" Biomacromolecules, 2007, 8(5): 1417-1424.
Lim et al., "In situ cross-linking of elastin-like polypeptide block copolymers for tissue repair," Biomacromolecules, 2008, 9, 222-230.
Lim et al., "In vivo post-translational modifications of recombinant mussel adhesive protein in insect cells," Biotechnol. Prog., 2011, 27, 1390-1396.
Linder et al., "Lipid Modifications of G Protein Subunits," J. Biol. Chem., 1991, 266(7):4654-4659.
Ling et al., "Protein thioester synthesis enabled by sortase," J. Am Chem Soc, 2012, 134(26):10749-10752.
Liu et al., "Hydrophobic modifications of cationic polymers for gene delivery," Prog. In Polym. Sci., 2010, 35, 1144-1162.
Liu et al., "In Situ Formation of Protein-Polymer Conjugates through Reversible Addition Fragmentation Chain Transfer Polymerization**," Angew. Chem. Int. Ed. 2007, 46, 3099-3103.
Liu et al., "Injectable intratumoral depot of thermally responsive polypeptide-radionuclide conjugates delays tumor progression in a mouse model," J. Control Release, 2010, 144(1):2-9.
Livingstone, "Theoretical property predictions. Curr Top Med Chem FIELD Full Journal Title:Current topics in medicinal chemistry," Curr. Top. Med. Chem. 2003, 3, 1171-1192.
Lovshin et al., "Incretin-based therapies for type 2 diabetes mellitus," Nat. Rev. Endocrinol. 5, 2009, 262-269.
Luginbuhl et al., "One-week glucose control via zero-order release kinetics from an injectable depot of glucagon-like peptide-1 fused to a thermosensitive biopolymer," Nat. Biomed. Eng., 2017, 1, 0078.
Luginbuhl et al., "Recombinant Synthesis of Hybrid Lipid-Peptide Polymer Fusions that Self-Assemble and Encapsulate Hydrophobic Drugs," Angew Chem Int Ed Engl., 2017, 56: 13979-13984.
Lukyanov et al., "Micelles From Lipid Derivatives of Water-Soluble Polymers as Delivery Systems for Poorly Soluble Drugs," Adv. Drug Deliver. Rev., 2004, 56(9):1273-1289.
Lund et al., "Phase II study of gemcitabine (2',2'-difluorodeoxycytidine) in previously treated ovarian cancer patients," J. Natl. Cancer. Inst. 1994, 86(20):1530-1533.
Ma et al., "Non-fouling oligo(ethylene glycol)-functionalized polymer brushes synthesized by surface-initiated atom transfer radical polymerization," Advanced Materials 2004, 16 (4), 338.
Ma et al., "Protein-resistant polymer coatings on silicon oxide by surface-initiated atom transfer radical polymerization," Langmuir : the ACS journal of surfaces and colloids, 2006, 22 (8), 3751-6.

(56) **References Cited**

OTHER PUBLICATIONS

Ma et al., "Surface-Initiated Atom Transfer Radical Polymerization of Oligo(ethylene glycol) Methyl Methacrylate from a Mixed Self-Assembled Monolayer on Gold," Advanced Functional Materials, 2006, 16 (5), 640-648.
MacEwan et al., "Digital switching of local arginine density in a genetically encoded self-assembled polypeptide nanoparticle controls cellular uptake," Nano Lett., 2012, 12, 3322-3328.
MacEwan et al., "Elastin-like polypeptides: Biomedical applications of tunable biopolymers," Biopolymers, 2010, 94, 60-77.
MacEwan et al., "Non-chromatographic Purification of Recombinant Elastin-like Polypeptides and their Fusions with Peptides and Proteins from *Escherichia coli*," 2014, 88, p. e51583.
MacEwan et al., "Phase Behavior and Self-Assembly of Perfectly Sequence-Defined and Monodisperse Multiblock Copolypeptides," Biomacromolecules, 2017, 18(2):599-609.
Mack et al., "Antiobesity action of peripheral exenatide (exendin-4) in rodents: effects on food intake, body weight, metabolic status and side-effect measures," Int. J. Obes. 30, 2006, 1332-1340.
MacKay et al., "Self-assembling chimeric polypeptide-doxorubicin conjugate nanoparticles the abolish tumors after single injection," Nat Mater, 2009, 8(12):993-999.
Maeda et al., "Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review," J. Control. Release, 2000, 65(1-2)271-284.
Magnusson et al., "In Situ Growth of Side-Chain PEG Polymers from Functionalized Human Growth Hormone—A New Technique for Preparation of Enhanced Protein-Polymer Conjugates," Bioconjugate Chem. 21, 2010, 671-678.
Malik et al., "Recent advances in protein and peptide drug delivery systems," Curr. Drug Deliv. 2, 2007, 141-151.
Mann et al., "Proteomic analysis of post-translational modifications," Nat. Biotechnol., 2003, 21, 255-61.
Mao et al., "Sortase-mediated protein ligation: a new method for protein engineering," J. Am. Chem. Soc., 2004, 126(9):2670-2671.
Maraffini et al., "Sortases and the art of anchoring proteins to the envelopes of Gram-positive bacteria," Microbiol. Mol Biol Rev, 2006, 70(1):192-221.
Marr et al., "Effect of Temperature on the Composition of Fatty Acids in *Escherichia coli*," J Bacteriol., 1962, 84(6):1260-7.
Maskarinec et al., "Protein engineering approaches to biomaterials design," Curr. Opin. Biotechnol., 2005, 16, 422-426.
Massey et al., "Self-Assembly of a Novel Organometallic—Inorganic Block Copolymer in Solution and the Solid State: Nonintrusive Observation of Novel Wormlike Poly(ferrocenyldimethylsilane)-b-Poly(dimethylsiloxane) Micelles," J. Am. Chem. Soc. 1998, 120(37):9533-9540.
Mastria et al., "Doxorubicin-conjugated polypeptide nanoparticles inhibit metastasis in two murine models of carcinoma," J Control Release, 2015, 208:52-8.
Matsumura et al., "A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs," Cancer Res. 1986, 46, 6387-6392.
Matyjaszewski et al., "Atom transfer radical polymerization," Chem. Rev. 101, 2001, 2921-2990.
Matyjaszewski et al., "Macromolecular engineering by atom transfer radical polymerization," J. Am. Chem. Soc. 136, 2014, 6513-6533.
Maurer-Stroh et al., "N-terminal N-myristoylation of proteins: prediction of substrate proteins from amino acid sequence" J Mol Biol., 2002, 317(4):541-557.
Maurer-Stroh et al., "N-terminal N-myristoylation of proteins: Refinement of the sequence motif and its taxon-specific differences," J Mol Biol., 2002, 317(4):523-540.
Mayo et al., "Cell Adhesion Promoting Peptide GVKGDKGNPGWPGAP from the Collagen Type IV Triple Helix: Cis/Trans Proline-Induced Multiple 1H NMR Conformations and Evidence for a KG/PG Multiple Turn Repeat Motif in the All-Trans proline State," Biochemistry, 1991, 30: 8251-8267.
McDaniel et al., "A unified model for de novo design of elastin-like polypeptides with tunable inverse transition temperatures," Biomacromolecules, 2013, 14(8):2866-2872.
McDaniel et al., "Drug delivery to solid tumors by elastin-like polypeptides," Adc. Drug Deliver. Rev., 2010, 62(15):1456-1467.
McDaniel et al., "Noncanonical Self-Assembly of Highly Asymmetric Genetically Encoded Polypeptide Amphiphiles into Cylindrical Micelles," Nano Lett., 2014, 14(11):6590-6598.
McDaniel et al., "Self-assembly of thermally responsive nanoparticles of a genetically encoded peptide polymer by drug conjugation," Chem. Int. Ed. 2013, 52, 1683-1687.
McDaniel, J.R. et al., "Recursive Directional Ligation by Plasmid Reconstruction Allows Rapid and Seamless Cloning of Oligomeric Genes," Biomacromolecules, 2010, 11(4):944-952.
McHale et al., "Synthesis and in vitro evaluation of enzymatically cross-linked elastin-like polypeptide gels for cartilaginous tissue repair," Tissue Eng, 2005, 11, 1768-1779.
McIlhinney et al., "Characterization of a polyhistidine-tagged form of human myristoyl-CoA: protein N-myristoyltransferase produced in *Escherichia coli*," European Journal of Biochemistry, 1994, 222(1):137-146.
Mejuch et al., "Synthesis of lipidated proteins," Bioconjug. Chem. 27, 2016, 1771-1783.
Mero et al., "Transglutaminase-mediated PEGylation of proteins: direct identification of the sites protein medification by mass spectrometry using a novel monodisperse PEG," Bioconjug Chem, 2009, 20(2):384-389.
Meyer et al., "Genetically Encoded Synthesis of Protein-Based Polymers with Precisely Specified Molecular Weight and Sequence by Recursive Directional Ligation: Examples from the Elastin-like Polypeptide System," Biomacromolecules, 2002, 3:357-367.
Meyer et al., "Purification of recombinant proteins by fusion with thermally-responsive polypeptide," Nat. Biotechnol.,1999, 17(11):1112-1115.
Meyer et al., "Quantification of the effects of chain length and concentration on the thermal behavior of elastin-like polypeptides," Biomacromolecules, 2004, 5(3):846-51.
Meyer et al., "Targeting a Genetically Engineered Elastin-Like Polypeptide to Solid Tumors by Local Hyperthermia," Cancer Res., 2001, 61(4):1548-1554.
Mosbach et al., "Formation of proinsulin by immobilized Bacillus subtilis," Nature, 1983, 302, 543-545.
Muiznies et al., "Modulated growth, stability and interactions of liquid-like coacervate assemblies of elastin," Matrix Biology 36, 2014, pp. 39-50.
Muralidharan et al., "Protein Ligation: an Enabling Technology for the Biophysical Analysis of Proteins," Nature Methods, 2006, vol. 3, No. 6, pp. 429-438.
Nagarsekar et al., "Genetically Engineered Polymers for Drug Delivery," Journal of Drug Targeting, 1999, 7(1):11-32.
Nahire et al., "Multifunctional Polymersomes for Cytosolic Delivery of Gemcitabine and Doxorubicin to Cancer Cells," Biomaterials 2014, 35(24):6482-6497.
Nairn et al., "A Synthetic Resilin Is Largely Unstructured," Biophysical Journal, 2008, vol. 95 3358-3365.
Nakaoka et al., "Prolongation of the serum half-life period of superoxide dismutase by poly(ethylene glycol) modification," Journal of Controlled Release, 1997, 46(3):253-261.
Neidigh et al., "Exendin-4 and glucagon-like-peptide-q: NMR structural comparisons in the solution and micelle-associated states," Biochemistry 40, 2001, 13188-13200.
Newcomb et al., "Advances in cryogenic transmission electron microscopy for the characterization of dynamic self-assembling nanostructures," Current Opinion in Colloid and Interface Science, 2012, 17, 350-359.
Nicolas et al., "Fluorescently tagged polymer bioconjugates from protein derived macroinitiators," Chem. Commun. 2006, 45, 4697-4699.
Nielsen, "Incretin mimetics and DPP-IV inhibitors for the treatment of type 2 diabetes," Drug Discov. Today 10, 2005, 703-710.
Nucci et al., "The therapeutic value of poly(ethylene glycol)-modified proteins," Adv. Drug Deliv. Rev., 1991, 6(2):133-151.

(56) **References Cited**

OTHER PUBLICATIONS

Nuhn et al., "Secondary structure formation and LCST behavior of short elastin-like peptides," Biomacromolecules, 2008, 9, 2755-2763.
O'Day et al., "Therapeutic Protein-polymer Conjugates: Advancing beyond PEGylation," J. Am. Chem. Soc., 2014, vol. 136, pp. 14323-14332.
Ortony et al., "Internal dynamics of a supramolecular nanofibre," Nat. Mater., 2014, 13, 1-5.
Pace et al., "How to measure and predict the molar absorption coefficient of a protein" Protein Science 1995, 4: 2411-2423.
Palva et al., "Secretion of interferon by Bacillus subtilis," Gene, 1983, 22, 229-235.
Paolino et al., "Folate-targeted supramolecular vesicular aggregates as a new frontier for effective anticancer treatment in in vivo model," Eur. J. Pharm. Biopharm. 2012, 82(1):94-102.
Paolino et al., "Gemcitabine-loaded PEGylated unilamellar liposomes vs GEMZAR: biodistribution, pharmacokinetic features and in vivo antitumor activity," J. Control. Release 2010, 144(2):144-150.
Papa et al., "PEGylated Liposomal Gemcitabine: Insights Into a Potential Breast Cancer Therapeutic," Cell Oncol. (Dordr) 2013, 36(6):449-457.
Paramonov et al., "Self-assembly of peptide-amphiphile nanofibers: The roles of hydrogen bonding and amphiphilic packing," J. Am. Chem. Soc., 2006, 128, 7291-7298.
Park et al., "Formulation optimization and in vivo proof-of-concept study of thermosensitive liposomes balanced by phospholipid, elastin-like polypeptide, and cholesterol," PLoS One, 2014, 9: e103116, 13 pages.
Parkes et al. "Discovery and development of exenatide: the first antidiabetic agent to leverage the multiple benefits of the incretin hormone, GLP-1," Expert Opinion. Drug Deliv. 8, 2012, 219-244.
Parveen et al., "Nanomedicine," Clinical Pharmacokinetics, 2006, 45(10):965-988.
Peeler et al., "Genetically encoded initiator for polymer growth from proteins," J. Am. Chem. Soc. 132, 2010, 13575-13577.
Peters, "Serum albumin," Adv. Protein Chem. 37, 1985, 161-245.
Petitdemange et al., "Tuning Thermoresponsive Properties of Cationic Elastin-like Polypeptides by Varying Counterions and Side-Chains," Bioconjug. Chem., 2017, 28(5):1403-1412.
Pinkas et al., "Tunable, post-translational hydroxylation of collagen domains in *Escherichia coli*," ACS Chem. Biol., 2011, 6, 320-324.
Popp et al., "Site-specific labeling via sortase-mediated transpeptidation," Curr. Protoc. Protein Sci. 56, 2009, 15.13.1-15.13.9.
Popp et al., "Sortase-Catalyzed Transformations That Improve the Properties of Cytokines," PNAS, 2011, vol. 108, No. 8, pp. 3169-3174.
Pulaski et al., "Mouse 4T1 breast tumor model," Curr. Protoc. Immunol 2001, Chapter 20, Unit 20.2.
Qi et al., Dataset for a brush-polymer conjugate of exendin-4 reduces blood glucose for up to five days and eliminates poly(ethylene glycol) antigenicity. Figshare, 2016, <http://dx.doi.org/10.6084/m9.figshare.3976761> 28 pages.
Qi et al., "Growing polymers from peptides and proteins: a biomedical perspective," Polym. Chem. 5, 2014, 266-276.
Qi et al., "Protein-polymer conjugation—moving beyond PEGylation," Curr. Opin. Chem. Biol. 28, 2015, 181-193.
Qi et al., "Sortase-catalyzed initiator attachment enables high yield growth of a stealth polymer from the C terminus of a protein," Macromol. Rapid Commun. 34, 2013, 1256-1260.
Qiu et al., "Polymer Architecture and Drug Delivery," Pharmaceutical Research, 2006, 23(1):1-30.
Quiroz et al., "Sequence heuristics to encode phase behaviour in intrinsically disordered protein polymers," Nat. Mater., 2015, 14, 1164-1171.
Radivojac et al., "Intrinsic Disorder and Functional Proteomics," Biophysical Journal, 2007, vol. 92, Issue 5, pp. 1439-1456.
Rapaka et al., "Coacervation of Sequential Polypeptide Models of Tropoelastin," Int J Peptide Protein Res, 1978, 11: 97-108.
Rauscher et al. "Proline and Glycine Control Protein Self-Organization into Elastomeric or Amyloid Fibrils," Structure, 2006, 14:1667-1676.
Richards et al., "Engineered fibronectin type III domain with a RGDWE sequence binds with enhanced affinity and specificity to human avβ3 integrin," J Mol Biol, 2003, 326(5):1475-1488.
Riddles et al., "Ellman's reagent: 5,5'-dithiobis(2-nitrobenzoic acid)—a reexamination," Anal Biochem. 1979, 94(1):75-81.
Ritcher et al., "Antibodies against polyethylene glycol produced in animals by immunization with monomethoxy polyethylene glycol modified proteins," Int. Arch. Allergy Appl. Immunol. 70, 1983, 124-131.
Ritcher et al., "Polyethylene glycol reactive antibodies in man: titer distribution in allergic patients treated with monomethoxy polyethylene glycol modified allergens or placebo, and in healthy blood donors," Int. Arch. Allergy Appl. Immunol. 74, 1984, 36-39.
Rivory et al., "Effects of lipophilicity and protein binding on the hepatocellular uptake and hepatic disposition of two anthracyclines, doxorubicin and iododoxorubicin," Cancer Chemother Pharmacol, 1996, 38(5):439-445.
Roberts et al., "Elastin-like polypeptides as models of intrinsically disordered proteins," FEBS Lett., 2015, 589, 2477-2486.
Robinet et al. "Elastin-derived peptides enhance angiogenesis by promoting endothelial cell migration and tubulogenesis through upregulation of MTI-MMP," J. Cell Science, 2005, 118:343-356.
Römer et al., "The elaborate structure of spider silk: structure and function of a natural high performance fiber," Prion 2, 2008, 154-161.
Ruiz van Haperen et al., "Regulation of phosphorylation of deoxycytidine and 2',2'-difluorodeoxycytidine (gemcitabine); effects of cytidine 5'-triphosphate and uridine 5'-triphosphate in relation to chemosensitivity for 2',2'-difluorodeoxycytidine," Biochem. Pharmacol. 1996, 51(7):911-908.
Saifer et al., "Selectivity of binding of PEGs and PEG-like oligomers to anti-PEG antibodies induced by methoxyPEG-proteins," Molecular Immunology 57, 2014, 236-246.
Sandler et al., "Gemcitabine: Single-Agent and Combination Therapy in Non-Small Cell Lung Cancer," Oncologist 1999, 4(3)241-251.
Schellenberger et al., "A recombinant polypeptide extends the in vivo half-life of peptides and proteins in a tunable manner," Nat. Biotechnol. 27, 2009, 1186-1188.
Senin et al., "N-Myristoylation of recoverin enhances its efficiency as an inhibitor of rhodopsin kinase," Febs. Lett., 1995, 376, 87-90.
Senior et al., "Val-Gly-Val-Ala-Pro-Gly, a Repeating Peptide in Elastin, Is Chemotactic for Fibroblasts and Monocytes," The Journal of Cell Biology, 1984, 99: 870-874.
Serrano et al., "An infrared spectroscopic study of the conformational transition of elastin-like polypeptides," Biophys. J., 2007, 93, 2429-2435.
Shen et al., "Conjugation site modulates the in vivo stability and thearpeutic activity of antibody-drug conjugates," Nat Biotechnol, 2012, 30(2):184-189.
Sheparovych et al., "Stimuli-Responsive Properties of Peptide-Based Copolymers Studied via Directional Growth of Self-Assembled Patterns on Solid Substrate," Biomacromolecules, 2009, 10:1955-1961.
Sherman et al., "Next-Generation PEGylation Enables Reduced Immunoreactivity of PEG-Protein Conjugates," Drug and Development & Delivery, 2012, vol. 12, No. 5, 36-42.
Sherman et al., "Role of the Methoxy Group in Immune Responses to mPEG-Protein Conjugates," Bioconjugate Chemistry, 2012, 23, 485-499.
Shi et al., "Cell adhesion on a POEGMA-modified topographical surface," Langmuir: the ACS journal of surfaces and colloids, 2012, 28 (49), 17011-8.
Shimoboji et al., "Temperature-Induced Switching of Enzyme Activity with Smart Polymer-Enzyme Conjugates," Bioconjugate Chem. 2003, 14, 517-525.
Siegwart et al., "ATRP in the Design of Functional Materials for Biomedical Applications," Prog Polymer Science, 2012, vol. 37, No. 1, pp. 18-37.
Silva et al., "Selective differentiation of neural progenitor cells by high-epitope density nanofibers," Science, 2004, 303, 1352-5.

(56) **References Cited**

OTHER PUBLICATIONS

Simakova et al., "Aqueous ARGET ATRP," Macromolecules 45, 2012, 6371-6379.
Sorkin et al., "Signal transduction and endocytosis: close encounters of many kinds," Nat Rev Mol Cell Biol, 2002, 3(8):600-614.
Stefl et al., "RNA sequence-and shape-dependent recognition by proteins in the ribonucleoprotein particle" EMBO reports (2005) 6(1):33-38.
Sumerlin, "Proteins as Initiators of Controlled Radical Polymerization: Grafting-from via ATRP and RAFT," ACS Macro Lett. 2012, 1, 141-145.
Surwit et al., Diet-induced type II diabetes in C57BL/6J mice, Diabetes 37, 1988, 1163-1167.
Swee et al., "Sortase-mediated modification of αDEC205 affords optimization of antigen presentation and immunization against a set of viral epitopes," Proc Natl Acad Sci USA, 2013, 110(4):1428-1433.
Swers et al., Multivalent Scaffold Proteins as Superagonists of TRAIL Receptor 2-Induced Apoptosis, Mol Cancer Ther, 2013, 12, 1235-1244.
Tang et al., "Combinatorial codon scrambling enables scalable gene synthesis and amplification of repetitive proteins," Nature Mater., 2016, 15, 419-424.
Tantakitti et al., "Energy landscapes and functions of supramolecular systems," Nat. Mater., 2016, 15, 469-476.
Tedja et al., "Effect of TiO2 nanoparticle surface functionalization on protein adsorption, cellular uptake and cytotoxicity: the attachment of PEG comb polymers using catalytic chain transfer and thiol-ene chemistry," Polymer Chemistry, 2012, 3 (10), 2743-2751.
Thorens et al., "Cloning and functional expression of the human islet GLP-1 receptor: demonstration that Exendin-4 Is an agonist and Exendin-(9-39) an antagonist of the receptor," Diabetes 42, 1993, 1678-1682.
Tong et al., "Protein Modification with Amphiphilic Block Copoly(2-oxazoline)s as a New Plataform for Enhanced Cellular Delivery," Mol. Pharm., 2010, vol. 7, No. 4, pp. 984-992.
Ton-That et al., "Assembly of pili on the surface of Corynebacterium diptheriae," 2003, 50(4):1429-1438.
Ton-That et al., "Purification and characterization of sortase, the transpeptide that cleaves surface proteins of *Staphylococcus aureus* and the LPXTG motif," Proc Natl Acad Sci USA, 1999, 96(22):12424-12429.
Torchilin, "Recent advances with liposomes as pharmaceutical carriers," Nature Rev. Drug Discov. 2005, 4(2):145-160.
Towler et al., "Purification and Characterization of Yeast Myristoyl-Coa—Protein N-Myristoyltransferase," P Natl Acad Sci USA, 1987, 84(9):2708-12.
Trabbic-Carlson et al., "Expression and purification of recombinant proteins from *Escherichia coli*: Comparison of an elastin-like polypeptide fusion with an oligohistidine fusion" Protein Science, 2004, 13: 3274-3284.
Triola et al., "Chemical biology of lipidated proteins," ACS Chemical Biology, 2012, 7, 87-99.
Troyanskaya et al., "Nonparametric methods for identifying differentially expressed genes in microarray data," Bioinformatics, 2002, 18(11):1454-61.
Tsarevsky et al., "Deactivation efficiency and degree of control over polymerization in ATRP in protic solvents," Macromolecules 37, 2004, 9768-9778.
Tsume et al., "The development of orally administrable gemcitabine prodrugs with D-enantiomer amino acids: Enhanced membrane permeability and enzymatic stability," Eur. J. Pharm. Biopharm. 2014, 86(3):514-523.
Turunen et al., "Paclitaxel Succinate Analogs: Anionic Introduction as a Strategy to Impart Blood Brain Barrier Permeability," Bioorg Med Chem Lett, 2008, 18(22):5971-5974.
Urry et al., "Elastic protein-based polymers in soft tissue augmentation and generation," J. Biomater. Sci. Polym. Ed., 1998, 9, 1015-1048.
Urry et al., "Hydrophobicity Scale for Proteins Based on InverseTemperature Transitions," Biopolymers, 1992, 32:1243-1250.
Urry et al., "Physical chemistry of biological free energy transduction as demonstrated by elastic protein-based polymers," J. of Phys. Chem. B.,1997, 101, 11007-11028.
Urry et al., "Temperature of polypeptide inverse temperature transition depends on mean residue hydrophobicity," J. Am. Chem. Soc., 1991, 113(11):4346-4348.
Vasey et al., "Phase I clinical and Pharmacokinetic study of PK1 (N-(2-Hydroxypropyl)methacrylamide Copolymer Doxorubicin): First member of a New Class of Chemotherapeutic Agents-Drugs-Polymer Conjugates" Clinical Cancer Research, 1999, 5:83-94.
Vega et al., "Targeting Doxorubicin to Epidermal Growth Factor Receptors by Site-Specific Conjugation of C225 to Poly(L-Glutamic Acid) through a Polyethylene Glycol Spacer," Pharmaceutical Research, 2003, 20(5):826-832.
Veronese et al., "PEGylation, successful approach to drug delivery," Drug Discovery Today, 2005, 10(21):1451-1458.
Veronese, "Peptide and protein PEGylation: a review of problems and solutions," Biomaterials 22, 2001, 405-417.
Viegas et al., "Polyoxazoline: Chemistry, properties and applications," Bioconjugate Chem., 2011, vol. 22, pp. 976-986.
Voelker et al., "Alteration of the specificity and regulation of fatty acid synthesis of *Escherichia coli* by expression of a plant medium-chain acyl-acyl carrier protein thioesterase," J Bacteriol., 1994, 176(23):7320-7.
Vrignaud et al., "Strategies for the nanoencapsulation of hydrophilic molecules in polymer-based nanoparticles," Biomaterials 2011, 32(33):8593-8604.
Walczak, "Death Receptor—Ligand Systems in Cancer, Cell Death, and Inflammation," Cold Spring Harb. Perspect. Biol., 2013, 5, a008698.
Walsh et al., "Post-translational modifications in the context of therapeutic proteins," Nat. Biotechnol., 2006, 24, 1241-1252.
Walsh et al., "Posttranslationale Proteinmodifikation: die Chemie der Proteomdiversifizierung," Angew Chem, 2005, 117, 7508-7539.
Walsh et al., "Protein posttranslational modifications: The chemistry of proteome diversifications," Angew. Chem. Int. Ed., 2005, 44, 7342-7372.
Wang et al., "Enhanced Tumor Delivery of Gemcitabine via PEG-DSPE/TPGS Mixed Micelles," Mol. Pharm. 2014, 11, 1140-1150.
Wendt et al., "DNA-mediated Folding and Assembly of MyoD-E47 Heterodimers," Journal of Biol. Chem., 1998, 273(10):5735-5743.
Werle et al., "Strategies to improve plasma half life time of peptide and protein drugs," Amino Acids 30, 2006, 351-367.
Wienkers et al., "Predicting in vivo drug interactions from in vitro drug discovery data," Nat. Rev. Drug. Discov. 2005, 4(10):825-833.
Williamson et al., "Efficient N-terminal labeling of proteins by use of sortase," Angew Chem Int ed Engl, 2012, 51(37):9377-9380.
Winzell et al., "The high-fat diet-fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes," Diabetes 53, 2004, S215-S219.
Wold, "In vivo chemical modification of proteins," Annu. Rev. Med., 1981, 50, 783-814.
Wu et al., "Site-specific chemical modification of recombinant proteins produced in mammalian cells by using the genetically encoded aldehyde tag," Proc Natl Acad Sci USA, 2009, 106(9):3000-3005.
Wu et al., "Sortase A-Catalyzed Transpeptidation of Glycosylphosphatidylinositol Derivatives for Chemoenzymatic Synthesis of GPI-Anchored Proteins," J. Am. Chem. Soc. 2010, 132, 1567-1571.
Xavier et al., "HPLC Method for the Dosage of Paclitaxel in Copaiba Oil: Development, Validation, Application to the Determination of the Solubility and Partition Coefficients," Chromatographia, 2016, 79, 405-412.
Xu et al., "A quality by design (QbD) case study on liposomes containing hydrophilic API: II. Screening of critical variables, and establishment of design space at laboratory scale," Int. J. Pharm. 2012, 423(2):543-553.

(56) **References Cited**

OTHER PUBLICATIONS

Xu et al., "Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats," Diabetes 48, 1999, 2270-2276.
Xu et al., "Self-assembly behavior of peptide amphiphiles (PAs) with different length of hydrophobic alkyl tails," Colloids Surfaces B Biointerfaces, 2010, 81, 329-335.
Yang et al., "Poly(carboxybetaine) nanomaterials enable long circulation and prevent polymer-specific antibody production," Nano Today, 2014, 9(1):10-16.
Yoo et al., "A systemic Small RNA Signaling System in Plants" The Plant Cell (2004) vol. 16, pp. 1979-2000.
Yoo et al., "Biodegradable Nanoparticles Containing Doxorubicin-Plga Conjugate for Sustained Release," Pharm. Res., 1999, 16(7):1114-1118.
Youn et al., "Evaluation of therapeutic potentials of site-specific PEGylated glucagon-like peptide-1 isomers as a type 2 anti-diabetic treatment: Insulinotropic activity, glucose-stabilizing capability, and proteolytic stability" Biochem. Pharmacol, 2007, 73: 84-93.
Youn et al., "High-yield production of biologically active mono-PEGylated salmon calcitonin by site-specific PEGylation," J. Control. Release 117, 2007, 371-379.
Zhang et al., "A self-assembly pathway to aligned monodomain gels," Nat. Mater., 2010, 9, 594-601.
Zhang et al., "Nanoparticles in medicine: therapeutic applications and developments," Clin. Pharmacol. Ther. 2008, 83(5):761-769.
Zhang et al., "Shape Effects of Nanoparticles Conjugated with Cell-Penetrating Peptides (HIV Tat PTD) on CHO Cell Uptake," Bioconjugate Chem, 2008, 19(9):1880-1887.
Zhao et al., "Fluorescence probe techniques used to study micelle formation in water-soluble block copolymers," Langmuir 1990, 6(2):514-516.
Zimm, "Apparatus and Methods for Measurement and Interpretation of the Angular Variation of Light Scattering; Preliminary Results on Polystyrene Solutions," J. Chem. Phys. 1948, 16, 1099-1116.
Zong et al., "Crystal structures of *Staphylococcus aureus* sortase A and its substrate complex," J. Biol. Chem. 279, 2004, 31383-31389.
International Search Report and Written Opinion for Application No. PCT/US2008/084159 dated Feb. 27, 2009 (8 pages).
International Search Report and Written Opinion for Application No. PCT/US2016/024202 dated Aug. 26, 2016 (9 pages).
International Search Report and Written Opinion for Application No. PCT/US2016/045655 dated Dec. 2, 2016 (13 pages).
International Search Report and Written Opinion for Application No. PCT/US2016/068141 dated Jul. 19, 2017 (12 pages).
International Search Report and Written Opinion for Application No. PCT/US2016/068142 dated Jul. 19, 2017 (12 pages).
International Search Report and Written Opinion for Application No. PCT/US2018/032785 dated Sep. 25, 2018 (16 pages).
International Search Report and Written Opinion for Application No. PCT/US2017/035530 dated Aug. 23, 2017 (13 pages).
International Search Report and Written Opinion for Application No. PCT/US2017/052887 dated Jan. 26, 2018 (20 pages).
International Search Report and Written Opinion for Application No. PCT/US2017/051661 dated Jan. 2, 2018 (12 pages).
International Search Report and Written Opinion for Application No. PCT/US2018/013611 dated May 30, 2018 (18 pages).
United States Patent Office Action for U.S. Appl. No. 13/904,836 dated Mar. 27, 2014 (10 pages).
United States Patent Office Notice of Allowance for U.S. Appl. No. 13/904,836 dated Jul. 30, 2014 (6 pages).
United States Patent Office Action for U.S. Appl. No. 13/942,037 dated Jan. 15, 2016 (19 pages).
United States Patent Office Action for U.S. Appl. No. 13/942,037 dated Jun. 4, 2015 (33 pages).
United States Patent Office Action for U.S. Appl. No. 13/942,037 dated Nov. 28, 2016 (22 pages).
United States Patent Office Action for U.S. Appl. No. 13/942,037 dated Feb. 9, 2018 (29 pages).
United States Patent Office Notice of Allowance for U.S. Appl. No. 13/245,459 dated Feb. 27, 2013 (13 pages).
United States Patent Office Action for U.S. Appl. No. 14/572,391 dated Oct. 26, 2016 (11 pages).
United States Patent Office Notice of Allowance for U.S. Appl. No. 14/572,391 dated Jun. 16, 2017 (10 pages).
United States Patent Office Action for U.S. Appl. No. 15/387,536 dated Sep. 27, 2018 (11 pages).
United States Patent Office Action for U.S. Appl. No. 15/387,540 dated Sep. 27, 2018 (12 pages).
AACR, "AACR Cancer Progress Report 2016," Clin Cancer Res, 2016, 22, 143 pages.
Adams et al., "Safety and utilization of blood components as therapeutic delivery systems," Curr Pharm Biotechnol, 2003, 4(5): 275-82.
Adams et al., "Sustained release of antibiotics from injectable and thermally responsive polypeptide depots," J Biomed Mater Res B Appl Biomater, 2009, 90, 67-74.
Adamska et al., "Pancreatic ductal adenocarcinoma: Current and evolving therapies," J Mol Sci, 2017, 18, 1338-1380.
Adiseshaiah et al., "Nanomedicine strategies to overcome the pathophysiological barriers of pancreatic cancer," Nat Rev Clin Oncol, 2016, 13, 750-765.
Albanese et al., "The effect of nanoparticle size, shape, and surface chemistry on biological systems," Annu. Rev. Biomed. Eng., 2012, 14, 1-16.
Andersen et al., "Extending Half-life by Indirect Targeting of the Neonatal Fc Receptor (FcRn) Using a Minimal Albumin Binding Domain," Journal of Biological Chemistry, 2011, 286(7): p. 5234-5241.
Asai et al., "Protein polymer hydrogels by in situ, rapid and reversible self-gelation," Biomaterials, 2012, 33, 5451-5458.
Atun et al., "Expanding global access to radiotherapy," Lancet Oncol, 2015, 16, 1153-1186.
Awasthi et al., "Comparative benefits of Nab-paclitaxel over gemcitabine or polysorbate-based docetaxel in experimental pancreatic cancer," Carcinogenesis, 2013, 34, 2361-2369.
Awasthi et al., "Evaluation of combination treatment benefits of nab-paclitaxel in experimental pancreatic cancer," Journal of Clinical Oncology, 2012, 30, 170.
Azhdarinia et al., "Regional radiochemotherapy using in situ hydrogel," Pharm Res., 2005, 22, 776-783.
Bache et al., "Investigating the accuracy of microstereotactic-body-radiotherapy utilizing anatomically accurate 3D printed rodent-morphic dosimeters," Medical Physics, 2015, 42, 846-855.
Bailey et al., "Genomic analyses identify molecular subtypes of pancreatic cancer," Nature, 2016, 531, 47-52.
Bamford et al., "The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website," British Journal of Cancer, 2004, 91, 355-358.
Barbuti et al., "Paclitaxel through the ages of anticancer therapy: Exploring its role in chemoresistance and radiation therapy," Cancers, 2015, 7, 2360-2371.
Barnett et al., "Normal tissue reactions to radiotherapy: towards tailoring treatment dose by genotype," Nat Rev Cancer, 2009, 9, 134-142.
Barton et al., "Estimating the demand for radiotherapy form the evidence: A review of changes from 2003 to 2012," Radiother Oncol, 2014, 112, 140-144.
Baskar et al., "Cancer and Radiation Therapy: Current Advances and Future Directions," Int. J. Med. Sci., 2012, 9, 193-199.
Begg et al., "Strategies to improve radiotherapy with targeted drugs," Nat Rev Cancer, 2011, 11, 239-253.
Bernacki et al., "Length-dependent aggregation of uninterrupted polyalanine peptides," Biochemistry, 2011, 50, 9200-9211.
Blasko et al., "Brachytherapy for carcinoma of the prostate: Techniques, patient selection, and clinical outcomes," Seminars in Radiation Oncology, 2002, 12, 81-94.
Blasko et al., "The role of external beam radiotherapy with I-125/Pd-103 brachytherapy for prostate carcinoma," Radiother Oncol, 2000, 57, 273-278.

(56) **References Cited**

OTHER PUBLICATIONS

Blasko et al., "Transperineal percutaneous iodine-125 implantation for prostatic carcinoma using transrectal ultrasound and template guidance," Endocurietherapy/Hyperthermia Oncology, 1987, 3, 131-139.

Bley et al., "Microtubule stabilising agents and ionising radiation: Multiple exploitable mechanisms for combined treatment," Eur J Cancer, 2013, 49, 245-253.

Bocci et al., "The pharmacological bases of the antiangiogenic activity of paclitaxel," Angiogenesis, 2013, 16, 481-492.

Bochicchio et al., "Investigating by CD the molecular mechanism of elasticity of elastomeric proteins," Chirality, 2008, 20, 985-994.

Boldt, "Use of albumin: an update," Br J. Anaesth., 2010, 104 (3), 276-284.

Branco et al., "Self-assembling materials for therapeutic delivery," Acta Biomaterialia, 2009, 5(3): p. 817-831.

Burchard, "Light Scattering Techniques," Physical techniques for the study of food biopolymers, 1994, 151-213.

Burnouf, "Modern plasma fractionation," Transfus. Med. Rev., 2007, 21 (2), 101-117.

Cabral et al., "Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size," Nature Nanotechnology, 2011, 6, 815-823.

Callahan et al., "Triple stimulus-responsive polypeptide nanoparticles that enhance intratumoral spatial distribution," Nano Letters, 2012, 12, 2165-2170.

Camilloni et al., "Determination of secondary structure populations in disordered states of proteins using nuclear magnetic resonance chemical shifts," Biochemistry, 2012, 51, 2224-2231.

Cao et al., "Monitoring the effects of anti-angiogenesis on the radiation sensitivity of pancreatic cancer xenografts using dynamic contrast-enhanced computed tomography," Int J Radiation Oncol Biol Phys, 2014, 88, 412-418.

Cardenes et al., "Locally advanced pancreatic cancer: Current therapeutic approach," The Oncologist, 2006, 11, 612-623.

Cataldo et al., "Radiation-induced crosslinking of collagen gelatin into a stable hydrogel," Journal of Radioanalytical and Nuclear Chemistry, 2008, 275, 125-131.

Ceska et al., "A new and rapid method for the clinical determination of α-amylase activities in human serum and urine. Optimal conditions," Clinica Chimica Acta, 1969, 26, 437-444.

Chakrabartty et al., "Stability of α-Helices," Adv Protein Chem, 1995, 46, 141-176.

Chang et al., "Tumor-stroma interaction in orthotopic primary pancreatic cancer xenografts during hedgehog pathway inhibition," Int. J. Cancer, 2013, 133, 225-235.

Chaudhury et al., "The major histocompatibility complex-related Fc receptor for IgG (FcRn) binds albumin and prolongs its lifespan," J Exp Med, 2003, 197(3): p. 315-22.

Chen et al., "Anisotropic hydrogels fabricated with directional freezing and radiation-induced polymerization and crosslinking method," Materials Letters, 2012, 89, 104-107.

Chen et al., "Rheology of Soft Materials," Annual Review of Condensed Matter Physics, 2010, 1, 301-322.

Chen et al., "The use of self-adjuvanting nanofiber vaccines to elicit high-affinity B cell responses to peptide antigens without inflammation," Biomaterials 34, 2013, 8776-8785.

Cho et al., "Hydrogen bonding of β-turn structure is stabilized in D(2)O," J Am Chem Soc, 2009, 131, 15188-15193.

Choi et al., "Renal Clearance of Nanoparticles," Nature biotechnology, 2007, 25(10): p. 1165-1170.

Choy et al., "Investigation of taxol as a potential radiation sensitizer," Cancer, 1993, 71, 3774-3778.

Christensen et al., "Predicting Transition Temperatures of Elastin-Like Polypeptide Fusion Proteins," Biomacromolecules, 2013, 14(5): p. 1514-1519.

Cid-Arregui et al., "Perspectives in the treatment of pancreatic adenocarcinoma," World Journal of Gastroenterology, 2015, 21, 9297-9316.

Ciezki et al., "Brachytherapy or surgery? A composite view," Oncology, 2009, 23, 960-964.

Cima, "AVMA Guidelines for the Euthanasia of Animal: 2013 Edition," Journal of the American Veterinary Medical Association, 2013, 242, 102 pages.

Cirulis et al., "Viscoelastic properties and gelation of an elastin-like polypeptide," Journal of Rheology, 2009, 53, 1215-1228.

Clarke et al., "Tropoelastin massively associates during coacervation to form quantized protein spheres," Biochemistry, 2006, 45, 9989-9996.

Clavé et al., "Amylase, lipase, pancreatic isoamylase, and phospholipase A in diagnosis of acute pancreatitis," Clinical Chemistry, 1995, 41, 1129-1134.

Colomb et al., "Radiation-Convertible Polymers from Norbornenyl Derivatives. Crosslinking with Ionizing Radiation," Journal of Applied Polymer Science, 1970, 14, 1659-1670.

Conrad et al., "ELPylated anti-human TNF therapeutic single-domain antibodies for prevention of lethal septic shock," Plant Biotechnology Journal, 2011, 9, 22-31.

Darzynkiewicz et al., "DNA content measurement for DNA ploidy and cell cycle analysis," Current Protocols in Cytometry, 2001, 7.5.1-7.5.24.

De Simone et al., "Accurate random coil chemical shifts from an analysis of loop regions in native states of proteins," J Am Chem Soc, 2009, 131, 16332-16333.

Deer et al., "Phenotype and genotype of pancreatic cancer cell lines," Pancreas, 2010, 39, 425-435.

Dejana et al., "The role of adherens junctions and VE-cadherin in the control of vascular permeability," J Cell Sci, 2008, 121, 2115-2122.

Delaglio et al., "NMRPipe: A multidimensional spectral processing system based on UNIX pipes," Journal of Biomolecular NMR 6, 1995, 277-293.

DeLisser et al., "Vascular endothelial platelet endothelial cell adhesion molecule 1(PECAM-1) regulates advanced metastatic progression," PNAS, 2010, 107, 18616-18621.

Dennis et al., "Albumin binding as a general strategy for improving the pharmacokinetics of proteins," J Biol Chem, 2002, 277(38): p. 35035-43.

Deshayes et al., "Radium 223 dichloride for prostate cancer treatment," Drug Des Devel Ther, 2017, 11, 2643-2651.

Diana et al., "Prognostic role and correlation of CA9, CD31, CD68 and CD20 with the desmoplastic stroma in pancreatic ductal adenocarcinoma," Oncotarget, 2016, 7, 72819-72832.

Ding et al., "Mechanism for the alpha-helix to beta-hairpin transition," Proteins, 2003, 53, 220-228.

Dreher et al., "Thermal cycling enhances the accumulation of a temperature-sensitive biopolymer in solid tumors," Cancer Res, 2007, 67, 4418-4424.

Du et al., "Endoscope-assisted brachytherapy for pancreatic cancer: From tumor killing to pain relief and drainage," Journal of interventional gastroenterology, 2011, 1, 23-27.

Ducreux et al., "Radiation plus docetaxel and cisplatin in locally advanced pancreatic carcinoma: A non-comparative randomized phase II trial," Digestive and Liver Disease, 2014, 46, 950-955.

Duke University, "Gemcitabine/Nab-Paclitaxel With HIGRT in Resectable Pancreatic Cancer," Clinical Trial NCT02318095 <https://clinicaltrials.gov/ct2/show/NCT02318095> Accessed Jan. 11, 2017.

Duncan, "The dawning era of polymer therapeutics," Nature Reviews Drug Discovery, 2003, 2, 347-360.

Eisenhauer et al., "New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1)," Eur J Cancer, 2017, 45, 228-247.

Ellis, "Macromolecular crowding: obvious but underappreciated," Trends Biochem. Sci., 2001, 26 (10), 597-604.

Engin et al., "Thermoradiotherapy in the management of superficial malignant tumors," Clinical Cancer Research, 1995, 1, 139-145.

Erickson-Miller et al., "Differential toxicity of camptothecin, topotecan and 9-aminocamptothecin to human, canine, and murine myeloid progenitors (CFU-GM) in vitro," Cancer Chemother. Pharmacol., 1997, 39 (5), 467-472.

Falk et al., "Hyperthermia in oncology," Int J Hyperthermia, 2001, 17, 1-18.

(56) **References Cited**

OTHER PUBLICATIONS

Farmer et al., "Conformational behavior of chemically reactive alanine-rich repetitive protein polymers," Biomacromolecules, 2005, 6, 1531-1539.

Fernandez-Colino et al., "Amphiphilic Elastin-Like Block Co-Recombinamers Containing Leucine Zippers: Cooperative Interplay between Both Domains Results in Injectable and Stable Hydrogels," Biomacromolecules, 2015, 16, 3389-3398.

Free et al., "A Phase 1, multi-center, randomized, double-blind, placebo controlled study to evaluate the safety/tolerability, pharmacokinetic and hemodynamic response following single ascending subcutaneous doses of PB1046 (Vasomera) in subjects with essential hypertension," Circulation, 2018, 130:A19112.

Frilling et al., "Recommendations for management of patients with neuroendocrine liver metastases," The lancet oncology, 2014, 15, e8-21.

Fu et al., Recent Patents on Anti-Cancer Drug Discovery, 2009. 4(3): p. 262-272.

Fujiwara et al., "Modulating effect of the PI3-kinase inhibitor LY294002 on cisplatin in human pancreatic cancer cells," Journal of Experimental & Clinical Cancer Research, 2008, 27, 76.

Furumoto et al., "Effect of coupling of albumin onto surface of PEG liposome on its In vivo disposition," International Journal of Pharmaceutics, 2007, 329(1-2): p. 110-116.

Ghoorchian et al., "Molecular architecture influences the thermally induced aggregation behavior of elastin-like polypeptides," Biomacromolecules, 2011, 12, 4022-4029.

Gianni et al., "Nonlinear pharmacokinetics and metabolism of paclitaxel and its pharmacokinetic/pharmacodynamic relationships in humans," J. Clin. Oncol., 1995, 13 (1), 180-190.

Giberti et al., "Radical retropubic prostatectomy versus brachytherapy for low-risk prostatic cancer: a prospective study," World J Urol, 2009, 27, 607-612.

Glassman et al., "Toughening of Thermoresponsive Arrested Networks of Elastin-Like Polypeptides to Engineer Cytocompatible Tissue Scaffolds," Biomacromolecules, 2016, 17, 415-426.

Gosline et al., "Elastic proteins: biological roles and mechanical properties," Philos Trans R Soc Lond B Biol Sci, 2002, 357, 121-132.

Gottlieb et al., "NMR chemical shifts of common laboratory solvents as trace impurities," J. Org. Chem., 1997, 62, 7512-7515.

Greco et al., "The search for synergy: a critical review from a response surface perspective," Pharmacological Reviews, 1995, 24, 331-385.

Green et al., "Abraxane®, a novel Cremophor®-free, albumin-bound particle form of paclitaxel for the treatment of advanced non-small-cell lung cancer," Annals of Oncology, 2006, 17, 1263-1268.

Grimm et al., "Advances in Brachytherapy," Reviews in Urology, 2004, 6, S37-S48.

Güngör et al., "Pancreatic cancer," British Journal of Pharmacology, 2014, 171, 849-858.

Guo et al., "Nanoparticles escaping RES and endosome: challenges for siRNA delivery for cancer therapy," J. Nanomaterials, 2011, 2011: 1-12.

Gustafsson, "Nonlinear structured-illumination microscopy: wide-field fluorescence imaging with theoretically unlimited resolution," Proc Natl Acad Sci U S A, 2005, 102, 13081-13086.

Gustafsson, "Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy," Short Communication. Journal of Microscopy, 2000, 198, 82-87.

Guzman et al., "Leiodermatolide, a novel marine natural product, has potent cytotoxic and antimitotic activity against cancer cells, appears to affect microtubule dynamics, and exhibits antitumor activity," Int. J. Cancer, 2016, 139, 2116-2126.

Halozyme Therapeutics, "PEGPH2O Plus Nab-Paclitaxel Plus Gemcitabine Compared With Nab-Paclitaxel Plus Gemcitabine in Subjects With Stage IV Untreated Pancreatic Cancer (HALO-109-202)," Clinical Trial NCT01839487 <https://clinicaltrials.gov/ct2/show/study/NCT01839487> Accessed May 29, 2018.

Hamada et al., "Novel therapeutic strategies targeting tumor-stromal interactions in pancreatic cancer," Frontiers in Physiology, 2013, vol. 4, Article 331, 7 pages.

Han et al., "Survival of patients with advanced pancreatic cancer after iodine^{125} seeds implantation brachytherapy: A meta-analysis," Medicine, 2017, 96, e5719.

Harmon et al., "A Model for Hysteresis Observed in Phase Transitions of Thermally Responsive Instrinsically Disordered Protein Polymers," Biophysical Journal, 2017, 112(3):207a.

Hathout et al., "Analysis of seed loss and pulmonary seed migration in patients treated with virtual needle guidance and robotic seed delivery," American journal of clinical oncology, 2011, 34, 449-453.

Herrero-Vanrell et al., "Self-assembled particles of an elastin-like polymer as vehicles for controlled drug release," J Control Release, 2005, 102, 113-122.

Hidalgo, "Pancreatic Cancer," N Engl J Med, 2010, 362, 1605-1617.

Hingorani et al., "Phase lb Study of PEGylated Recombinant Human Hyaluronidase and Gemcitabine in Patients with Advanced Pancreatic Cancer," Clinical Cancer Research, 2016, 22, 2848-2854.

Ho et al., "Internal radiation therapy for patients with primary or metastatic hepatic cancer: a review," Cancer, 1998, 83, 1894-1907.

Holm et al., "Transperineal ^{125}iodine seed implantation in prostatic cancer guided by transrectal ultrasonography," The Journal of urology, 2002, 167, 985-988.

Hopp et al., "The effects of affinity and valency of an albumin-binding domain (ABD) on the half-life of a single-chain diabody-ABD fusion protein," Protein Engineering Design and Selection, 2010, 23(11): p. 827-834.

Hortobágyi, "Anthracyclines in the Treatment of Cancer," Drugs, 1997, vol. 54, No. 4, pp. 1-7.

Howell et al., "The MIRD Perspective 1999," J Nucl Med, 1999, 40, 3S-10S.

Hruby et al., "New bioerodable thermoresponsive polymers for possible radiotherapeutic applications," Journal of Controlled Release, 2007, 119, 25-33.

Hruby et al., "Thermoresponsive polymeric radionuclide delivery system—an injectable brachytherapy," Eur J Pharm Sci., 2011, 42, 484-488.

Hrycushko et al., "Direct intratumoral infusion of liposome encapsulated rhenium radionuclides for cancer therapy: effects of non-uniform intratumoral dose distribution," Med Phys, 2011, 38, 1339-1347.

Hu et al., "Design of tumor-homing and pH-responsive polypeptide-doxorubicin nanoparticles with enhanced anticancer efficacy and reduced side effects," Chemical Communications, 2015, 51, 11405-11408.

Huang et al., "Photodynamic Therapy Synergizes with Irinotecan to Overcome Compensatory Mechanisms and Improve Treatment Outcomes in Pancreatic Cancer," Cancer Research, 2016, 76, 1066-1077.

Huotari et al., "Endosome maturation," EMBO J, 2011, 30 (17), 3481-3500.

Hwang et al., "Caprolactonic poloxamer analog: PEG-PCL-PEG," Biomacromolecules, 2005, 6, 885-890.

Hwang et al., "Gene therapy for primary and metastatic pancreatic cancer with intraperitoneal retroviral vector bearing the wild-type p. 53 gene," Surgery, 1998, 124, 143-151.

Ibrahim et al., "Phase I and pharmacokinetic study of ABI-007, a Cremophor-free, protein-stabilized, nanoparticle formulation of paclitaxel," Clin. Cancer Res., 2002, 8 (5), 1038-1044.

Ito et al., "In vivo antitumor effect of the mTOR inhibitor CCI-779 and gemcitabine in xenograft models of human pancreatic cancer," International Journal of Cancer, 2006, 118, 2337-2343.

Jacob et al., "Human phagocytes employ the myeloperoxidase-hydrogen peroxide system to synthesize dityrosine, trityrosine, pulcherosine, and isodityrosine by a tyrosyl radical-dependent pathway," J. Biol. Chem., 1996, 271, 19950-19956.

Jain, "Barriers to Drug-Delivery in Solid Tumors," Sci Am, 1994, 271, 58-65.

(56) **References Cited**

OTHER PUBLICATIONS

Jenkins et al., In vivo monitoring of tumor relapse and metastasis using bioluminescent PC-3M-luc-C6 cells in murine models of human prostate cancer. Clinical & Experimental Metastasis, 2003, 20, 745-756.

Ji et al., "RGD-conjugated albumin nanoparticles as a novel delivery vehicle in pancreatic cancer therapy," Cancer Biology & Therapy, 2012, 13, 206-215.

Johansson et al., "Structure, Specificity, and Mode of Interaction for Bacterial Albumin-binding Modules," J. Biol. Chem. 2002, 277 (10), 8114-8120.

Johansson et al., "The GA module, a mobile albumin-binding bacterial domain, adopts a three-helix-bundle structure," FEBS Lett, 1995, 374(2): 257-261.

Jonsson et al., "Engineering of a femtomolar affinity binding protein to human serum albumin," Protein Engineering Design and Selection, 2008, 21(8): 515-527.

Kaighn et al., "Establishment and characterization of a human prostatic carcinoma cell line (PC-3)," Investigative urology, 1979, 17, 16-23.

Kaitin et al., "Pharmaceutical innovation in the 21st century: new drug approvals in the first decade, 2000-2009," Clin Pharmacol Ther, 2011, 89, 183-188.

Kamisawa et al., "Pancreatic cancer," Lancet, 2016, 388, 73-85.

Kanyama et al., "Usefulness of Repeated Direct Intratumoral Gene Transfer Using Hemagglutinating Virus of Japan-Liposome Method for Cytosine Deaminase Suicide Gene Therapy," Cancer Research, 2001, 61, 14-18.

Karamanolis et al., "Increased expression of VEGF and CD31 in postradiation rectal tissue: implications for radiation proctitis," Mediators Inflamm, 2013, 515048.

Karperien, A. FracLac for Image J, version 2.5 <http://rsb.info.nih.gov/ij/plugins/fraclac/FLHelp/Introduction.htm> 1999-2012.

Katakura, "Nuclear Data Sheets for A=125," Nuclear Data Sheets, 2011, 112, 495-705.

Kato et al., "Acidic extracellular microenvironment and cancer," Cancer Cell Int, 2013, 13, 89, 8 pages.

Keten et al., "Nanoconfinement controls stiffness, strength and mechanical toughness of β-sheet crystals in silk," Nat Mater, 2010, 9, 359-367.

Khanna et al., "The dog as a cancer model," Nat. Biotechnol., 2006, 24, 1065-1066.

Khazov et al., "Nuclear Data Sheets for A=131," Nuclear Data Sheets, 2006, 107, 2715-2930.

Kim et al., "Recombinant elastin-mimetic biomaterials: Emerging applications in medicine," Adv Drug Deliv Rev, 2010, 62, 1468-1478.

Kobayashi et al., "Summary of recombinant human serum albumin development," Biologicals, 2006, 34(1): 55-59.

Koehler et al., "Albumin affinity tags increase peptide half-life In vivo," Bioorganic & Medicinal Chemistry Letters, 2002, 12(20): 2883-2886.

Koong et al., "Phase II study to assess the efficacy of conventionally fractionated radiotherapy followed by a stereotactic radiosurgery boost in patients with locally advanced pancreatic cancer," Int J Radiation Oncol Biol Phys, 2005, 63, 320-323.

Kraulis et al., "The serum albumin-binding domain of streptococcal protein G is a three-helical bundle: a heteronuclear NMR study," FEBS letters, 1996, 378(2): p. 190-194.

Krause et al., "Structure and function of claudins," Biochmica et Biophysica Acta, 2008, 1778, 631-645.

Krempien et al., "Neoadjuvant chemoradiation in patients with pancreatic adenocarcinoma," HPB (Oxford), 2006, 8, 22-28.

Kupelian et al., "Radical prostatectomy, external beam radiotherapy <72 Gy, external beam radiotherapy > or =72 Gy, permanent seed implantation, or combined seeds/external beam radiotherapy for stage T1-T2 prostate cancer," International journal of radiation oncology, biology, physics, 2004, 58, 25-33.

Labelle et al., "Vascular endothelial cadherin promotes breast cancer progression via transforming growth factor β signaling," Cancer Res, 2008, 68, 1388-1397.

Lacroix et al., "Elucidating the folding problem of alpha-helices: local motifs, long-range electrostatics, ionic-strength dependence and prediction of NMR parameters," J Mol Biol, 1998, 284, 173-191.

Lee et al., "Immunohistochemical analysis of claudin expression in pancreatic cystic tumors," Oncology Reports, 2011, 25(4): 971-978.

Lee et al., "In vivo bioluminescent imaging of irradiated orthotopic pancreatic cancer xenografts in nonobese diabeti-sever combined immunodeficient mice: a novel method for targeting and assaying efficacy of ionizing radiation," Transl. Oncol., 2010, 3, 153-159.

Lee et al., "Mechanical properties of cross-linked syntheti elastomeric polypentapeptides," Macromolecules, 2001, 34, 5968-5974.

Lee et al., "Phase transition and elasticity of protein-based hydrogels," J. Biomater. Sci. Polymer Edn, 2001, 12, 229-242.

Levy et al., "Novel Exenatide Analogs with Peptidic Albumin Binding Domains: Potent Anti-Diabetic Agents with Extended Duration of Action," PLoS ONE, 2014, 9(2): e87704, 9 pages.

Li et al., "Elastin is an essential determinant of arterial morphogenesis," Nature, 1998, 393, 276-280.

Li et al., "Nanoparticles Evading the Reticuloendothelial System: Role of the Supported Bilayer," Biochim. Biophys. Acta, 2009, 1788 (10), 2259-2266.

Li et al., "Pancreatic cancer," Lancet, 2004, 363, 1049-1057.

Lillie et al., "The viscoelastic basis for the tensile strength of elastin," Int J Biol Macromol, 2002, 30, 119-127.

Lin et al., "Utility of immunohistochemistry in the pancreatobiliary tract," Arch Pathol Lab Med, 2015, 139, 24-38.

Litiere et al., "RECIST—learning from the past to build the future," Nat Rev Clin Oncol, 2017, 14, 187-192.

Liu et al., "Brachytherapy using injectable seeds that are self-assembled from genetically encoded polypeptides in situ," Cancer Res, 2012, 72, 5956-5965.

Liu et al., "Tracking the in vivo fate of recombinant polypeptides by isotopic labeling," Journal of Controlled Release, 2006, 114, 184-192.

Liu et al., "Tumor accumulation, degradation and pharmacokinetics of elastin-like polypeptides in nude mice," Journal of Controlled Release, 2006, 116, 170-178.

Ludden, "Nonlinear pharmacokinetics: clinical Implications," Clin. Pharmacokinet., 1991, 20 (6), 429-446.

Luo et al., "Noncovalent Modulation of the Inverse Temperature Transition and Self-Assembly of Elastin-b-Collagen-like Peptide Bioconjugates," J Am Chem Soc, 2015, 137, 15362-15365.

MacEwan et al., "Applications of elastin-like polypeptides in drug delivery," Journal of Controlled Release, 2014, 190: p. 314-330.

MacEwan et al., "Controlled apoptosis by a thermally toggled nanoscale amplifier of cellular uptake," Nano Letters, 2014, 14, 2058-2064.

Maitra et al., "Pancreatic Cancer," Annu Rev Pathol Mech Dis, 2008, 3, 157-188.

Manzoor et al., "Overcoming limitations in nanoparticle drug delivery: triggered, intravascular release to improve drug penetration into tumors," Cancer Res, 2012, 72, 5566-5575.

Mao et al., "DNA repair by nonhomologous end joining and homologous recombination during cell cycle in human cells," Cell cycle, 2008, 7, 2902-2906.

Mariam et al., "Albumin corona on nanoparticles—a strategic approach in drug delivery," Drug Deliv., 2016, 23 (8), 2668-2676.

Marten et al., "A randomized multicentre phase II trial comparing adjuvant therapy in patients with interferon alpha-2b and 5-FU alone or in combination with either external radiation treatment and cisplatin (CapRI) or radiation alone regarding event-free survival—CapRI-2," BMC Cancer, 2009, 9, 1-8.

Matsumura, "Cancer stromal targeting (CAST) therapy," Advanced Drug Delivery Reviews, 2012, 64, 710-719.

McConkey et al., "Molecular Characterization of Pancreatic Cancer Cell Lines," Pancreatic Cancer, 2010, 457-469.

McDaniel et al., "Actively targeting solid tumours with thermoresponsive drug delivery systems that respond to mild hyperthermia," Int J Hyperthermia, 2013, 29, 501-510.

(56) **References Cited**

OTHER PUBLICATIONS

McDaniel et al., "Doxorubicin-conjugated chimeric polypeptide nanoparticles that respond to mild hyperthermia," Control. Release, 2012, 159 (3), 362-367.
McDaniel et al., "Rational design of "heat seeking" drug loaded polypeptide nanoparticles that thermally target solid tumors," Nano Letters, 2014, 14, 2890-2895.
Merrick et al., "Seed fixity in the prostate/periprostatic region following brachytherapy," International journal of radiation oncology, biology, physics, 2000, 46, 215-220.
Methods and Welfare Considerations in Behavioral Research with Animal. (2002).
Meyer et al., "Drug targeting using thermally responsive polymers and local hyperthermia," Journal of Controlled Release, 2001, 74, 213-224.
Miao et al., "Sequence and domain arrangements influence mechanical properties of elastin-like polymeric elastomers," Biopolymers, 2013, 99, 392-407.
Miao et al., "Structural determinants of cross-linking and hydrophobic domains for self-assembly of elastin-like polypeptides," Biochemistry, 2005, 44, 14367-14375.
Michl et al., "Current concepts and novel targets in advanced pancreatic cancer," Gut, 2013, 62, 317-326.
Micsonai et al. "Accurate secondary structure prediction and fold recognition for circular dichroism spectroscopy," Proc Natl Acad Sci U S A, 2015, 112, E3095-3103.
Milenic et al., "Antibody-targeted radiation cancer therapy," Nature Reviews Drug Discovery, 2004, 3, 488-498.
Miller et al., "Solubilized, Spaced Polyalanines: A Context-Free System for Determining Amino Acid α-Helix Propensities," Journal of the American Chemical Society, 2002, 124, 945-962.
Miyata et al., "Polymeric micelles for nano-scale drug delivery," Reaction & Functional Polymers, 2011, 71, 227-234.
Mjelle et al., "Cell cycle regulation of human DNA repair and chromatin remodeling genes," DNA Repair, 2015, 30, 53-67.
Morgan et al., "The combination of epidermal growth factor receptor inhibitors with gemcitabine and radiation in pancreatic cancer," Clin Cancer Res, 2008, 14, 5142-5149.
Muiznieks et al., "Structural changes and facilitated association of tropoelastin," Archives of Biochemistry and Biophysics, 2003, 410, 317-323.
Muñoz et al., "Elucidating the folding problem of helical peptides using empirical parameters," Nature Structural Biology, 1994, 1, 399-409.
Muñoz et al., "Elucidating the Folding Problem of Helical Peptides using Empirical Parameters. II†. Helix Macrodipole Effects and Rational Modification of the Helical Content of Natural Peptides," Journal of Molecular Biology, 1995, 245, 275-296.
Muñoz et al., "Elucidating the folding problem of helical peptides using empirical parameters. III. Temperature and pH dependence," J Mol Biol, 1995, 245, 297-308.
Murphy et al., "A dosimetric model of duodenal toxicity after stereotactic body radiotherapy for pancreatic cancer," Int J Radiation Oncology Biol Phys, 2010, 78, 1420-1426.
Na et al., "Thermoresponsive pore structure of biopolymer microspheres for a smart drug carrier," Langmuir, 2010, 26, 11165-11169.
Nettles et al., "Applications of elastin-like polypeptides in tissue engineering," Adv Drug Deliv Rev, 2010, 62, 1479-1485.
Nettles et al., "In situ crosslinking elastin-like polypeptide gels for application to articular cartilage repair in a goat osteochondral defect model," Tissue Eng Part A, 2008, 14, 1133-1140.
Newton et al., "Commissioning a small-field biological irradiator using point, 2D, and 3D dosimetry techniques," Medical Physics, 2011, 38, 6754-6762.
Nichols et al., "Claudin 4 protein expression in primary and metastatic pancreatic cancer," Am J Clin Pathol, 2004, 121, 226-230.
Nie, "Understanding and overcoming major barriers in cancer nanomedicine," Nanomedicine (Lond) 2010, 5 (4), 523-528.
Nilvebrant et al., "The albumin-binding domain as a scaffold for protein engineering," Computational and Structural Biotechnology Journal, 2013, 6: e201303009, 8 pages.
Ogawara et al., "Pre-coating with serum albumin reduces receptor-mediated hepatic disposition of polystyrene nanosphere: implications for rational design of nanoparticles," Journal of Controlled Release, 2004, 100(3): 451-455.
Pagani et al., "International guidelines for management of metastatic breast cancer: can metastatic breast cancer be cured?," Journal of the National Cancer Institute, 2010, 102, 456-463.
Palta et al., "Interim Acute Toxicity Analysis and Surgical Outcomes of Neoadjuvant Gemcitabine/nab-Paclitaxel and Hypofractionated Image Guided Intensity Modulated Radiation Therapy in Resectable and Borderline Resectable Pancreatic Cancer (ANCHOR) Study," International Journal of Radiation Oncology • Biology • Physics, 2016, 96, S204-S205.
Paoloni et al., "Translation of new cancer treatments from pet dogs to humans," Nat. Rev. Cancer 2008, 8 (2), 147-156.
Patil et al., "Cellular delivery of doxorubicin via pH-controlled hydrazone linkage using multifunctional nano vehicle based on poly(beta-I-malic acid)," Int J Mol Sci, 2012, 13, 11681-11693.
Phan et al., "Temperature-responsive self-assembly of charged and uncharged hydroxyethylcellulose-graft-poly(N-isopropylacrylamide) copolymer in aqueous solution," Colloid Polym. Sci., 2011, 289 (9), 993-1003.
Pliarchopoulou et al., "Pancreatic cancer: Current and future treatment strategies," Cancer Treatment Reviews, 2009, 35, 431-436.
Pometun et al., "Quantitative observation of backbone disorder in native elastin," J Biol Chem, 2004, 279, 7982-7987.
Potters et al., "12-year outcomes following permanent prostate brachytherapy in patients with clinically localized prostate cancer," The Journal of urology, 2005, 173, 1562-1566.
Potters et al., "Monotherapy for stage T1-T2 prostate cancer: radical prostatectomy, external beam radiotherapy, or permanent seed implantation," Radiotherapy and oncology: journal of the European Society for Therapeutic Radiology and Oncology, 2004, 71, 29-33.
Potters et al., "Potency after permanent prostate brachytherapy for localized prostate cancer," International journal of radiation oncology, biology, physics, 2001, 50(5): 1235-1242.
Prestwich et al., "Beta dose point kernels for radionuclides of potential use in radioimmunotherapy," J Nucl Med, 1989, 30, 1036-1046.
Privratsky et al., "PECAM-1: regulator of endothelial junctional integrity," Cell Tissue Res, 2014, 355, 607-619.
Prostate Seed Center, "Brachytherapy seed pre-plan rendering," <http://www.prostateseedcenter.com/dynamics-of-brachytherapy> webpage available as early as Aug. 30, 2012.
Provenzano et al., "Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma," Cancer cell, 2012, 21, 418-429.
Provenzano et al., "Hyaluronan, fluid pressure, and stromal resistance in pancreas cancer," Br J Cancer, 2013, 108, 1-8.
Qiu et al., "Development of Orthotopic Pancreatic Tumor Mouse Models," Methods Mol Biol, 2013, 980: 215-223.
Quarmby et al., "Irradiation induces upregulation of CD31 in human endothelial cells," Arterioscler Thromb Vasc Biol, 1999, 19, 588-597.
Quarmby et al., "Radiation-induced normal tissue injury: role of adhesion molecules in leukocyte-endothelial cell interactions," Int J Cancer, 1999, 82, 385-395.
Rabotyagova et al., "Protein-based block copolymers," Biomacromolecules, 2011, 12, 269-289.
Ragupathi et al., "Abstract A73: Antitumor activity of MVT-5873, a monoclonal antibody targeting sialyl Lewisa, alone and in combination with gemcitabine/nab-paclitaxel in a BxPC3 human pancreatic cancer xenograft model," Cancer Research, 2016, 76.
Rankine et al., "Investigating end-to-end accuracy of image guided radiation treatment delivery using a micro-irradiator," Physics in Medicine and Biology, 2013, 58, 7791-7801.
Rao et al., "Synthetic nanoparticles camouflaged with biometric erythrocyte membranes for reduced reticuloendothelial system uptake," Nanotechnology, 2016, 27 (8), 85106, 9 pages.

(56) **References Cited**

OTHER PUBLICATIONS

Ratner et al., "Radiation-grafted hydrogels for biomaterial applications as studied by the ESCA technique," Journal of Applied Polymer Science, 1978, 22, 643-664.
Ratner, "A pore way to heal and regenerate: 21st century thinking on biocompatibility," Regen Biomater, 2016, 3, 107-110.
Regier et al., American Heart Association 2014 Scientific Sessions, 2015, vol. 7, pp. 299-303.
Reguera et al., "Thermal Behavior and Kinetic Analysis of the Chain Unfolding and Refolding and of the Concomitant Nonpolar Solvation and Desolvation of Two Elastin-like Polymers," Macromolecules, 2003, 36, 8470-8476.
Ren et al., "Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide," Cell Stem Cell, 2008, 2(2): p. 141-150.
Ribeiro et al., "Influence of the amino-acid sequence on the inverse temperature transition of elastin-like polypeptides," Biophysical Journal, 2009, 97, 312-320.
Richards et al., "Man's best friend: what can pet dogs teach us about non-Hodgkin lymphoma?" Inmunol Rev., 2016, 263 (1), 173-191.
Rincon et al., "Biocompatibility of elastin-like polymer poly(VPAVG) microparticles: in vitro and in vivo studies," Journal of Biomedical Materials Research, 2005, 78A, 343-351.
Rios-Doria et al., "Doxil synergizes with cancer immunotherapies to enhance antitumor responses in syngeneic mouse models," Neoplasia, 2015, 17, 661-670.
Roopenian et al., "FcRn: the neonatal Fc receptor comes of age," Nat. Rev. Immunol., 2007, vol. 7, No. 9, 715-725.
Rosenberg et al., "Present and future innovations in radiation oncology," Surg Oncol Clin N Am, 2013, 22, 599-618.
Rozak et al., "G148-GA3: a streptococcal virulence module with atypical thermodynamics of folding optimally binds human serum albumin at physiological temperatures," Biochim Biophys Acta, 2005, 1753(2): p. 226-33.
Russo et al., "The role of neoadjuvant therapy in pancreatic cancer: a review," Future Oncol, 2016, 12, 669-685.
Ryerson et al., "Annual report to the nation on the status of cancer, 1975-2012, featuring the Increasing incidence of liver cancer," Cancer, 2016, 122, 1312-1337.
Saba et al., "A Comparative Oncology Study of Iniparib Defines Its Pharmacokinetic Profile and Biological Activity in a Naturally-Occurring Canine Cancer Model," PLoS One, 2016, 11(2): 1-11.
Safran et al., "Gemcitabine, paclitaxel, and radiation for locally advanced pancreatic cancer: A phase I trial," Int J Radiation Oncology Biol Phys, 2002, 54, 137-141.
Sagle et al., "Investigating the hydrogen-bonding model of urea denaturation," J Am Chem Soc, 2009, 131, 9304-9310.
Schaal et al., "Biopolymer β-brachytherapy delivered with concomitant paclitaxel outperforms traditional x-ray radiation to include complete regression in multiple pancreatic tumor xenograft models through synergistic modulation of the tumor microenvironment," Poster #5831, 2018.
Schaal et al., "Injectable polypeptide micelles that form radiation crosslinked hydrogels in situ for intratumoral radiotherapy," Journal of Controlled Release, 2016, 228, 58-66.
Schellenberg et al., "Gemcitabine chemotherapy and single-fraction stereotactic body radiotherapy for locally advanced pancreatic cancer," Int J Radiation Oncol Biol Phys, 2008, 72, 678-686.
Schellenberg et al., "Single-fraction stereotactic body radiation therapy and sequential gemcitabine for the treatment of locally advanced pancreatic cancer," Int J Radiation Oncology Biol Phys, 2011, 81, 181-188.
Schlaff et al., "Bringing the heavy: carbon ion therapy in the radiobiological clinical context," Radiation Oncology, 2014, 9, 1-18.
Schneider et al., "NIH Image to ImageJ: 25 years of image analysis," Nature Methods, 2012, 9, 671-675.
Shadwick, "Mechanical design in arteries," J Exp Biol, 1999, 202, 3305-3313.
Shang et al., "pH-Dependent Protein Conformational Changes in Albumin:Gold Nanoparticle Bioconjugates: A Spectroscopic Study," Langmuir, 2007, 23 (5), 2714-2721.
Shao et al., "Super-resolution 3D microscopy of live whole cells using structured illumination," Nat Methods, 2011, 8, 1044-1046.
Siegel et al., "Absorbed fractions for electrons and beta particles in spheres of various sizes," J Nucl Med, 1994, 35, 152-156.
Silberstein et al., "The SNM Practice Guideline for Therapy of Thyroid Disease with ^{131}I, 3.0," J Nucl Med, 2012, 53, 1-19.
Simnick et al., "In vivo tumor targeting by a NGR-decorated micelle of a recombinant diblock copolypeptide," J Control Release, 2011, 155, 144-151.
Simnick et al., "Morphing low-affinity ligands into high-avidity nanoparticles by thermally triggered self-assembly of a genetically encoded polymer," ACS Nano, 2010, 4, 2217-2227.
Sisson et al., "Radiation safety in the treatment of patients with thyroid diseases by radioiodine 131I: practice recommendations of the American Thyroid Association," Thyroid, 2011, 21, 335-346.
Sonawane et al., "Hydrazo linkages in pH responsive drug delivery systems," European Journal Pharmaceutical Sciences, 2017, 99, 45-65.
Sousa et al., "Production of a polar fish antimicrobial peptide in *Escherichia coli* using an ELP-intein tag," J Biotechnol, 2016, 234:83-89.
Sriraman et al., "Barriers to drug delivery in solid tumors," Tissue Barriers, 2014, 2, 2-10.
Stock et al., "Penile erectile function after permanent radioactive seed implantation for treatment of prostate cancer," The Journal of urology, 2001, 165, 436-439.
Stork et al., "A novel tri-functional antibody fusion protein with improved pharmacokinetic properties generated by fusing a bispecific single-chain diabody with an albumin-binding domain from streptococcal protein G," Protein Engineering Design and Selection, 2007, 20(11): p. 569-576.
Strohmaier et al., "Comparison of ^{60}Co and ^{192}Ir sources in HDR brachytherapy," J Contemp Brachyther, 2011, 3, 199-208.
Stutz et al., "Seed loss through the urinary tract after prostate brachytherapy: examining the role of cystoscopy and urine straining post implant," Medical physics, 2003, 30, 2695-2698.
Sugyo et al., "Evaluation of efficacy of radioimmunotherapy with 90Y-labeled fully human anti-transferring receptor monoclonal antibody in pancreatic cancer mouse models," PLoS One, 2015, 10, 1-17.
Sun et al., "Contributions of the extracellular and cytoplasmic domains of platelet-endothelial cell adhesion molecule-1 (PECAM-1/CD31) in regulating cell-cell localization," J. Cell Sci., 2000, 113, 1459-1469.
Sun et al., "Efficacy and safety of the hypoxia-activated prodrug TH-302 in combination with gemcitabine and nab-paclitaxel in human tumor xenograft models of pancreatic cancer," Cancer Biology & Therapy, 2015, 16, 438-449.
Sun et al., "EUS-guided interstitial brachytherapy of the pancreas: a feasibility study," Gastrointestinal Endoscopy, 2005, 62, 775-779.
Sunamura et al., "Gene Therapy for Pancreatic Cancer Targeting the Genomic Alterations of Tumor Suppressor Genes using Replication-selective Oncolytic Adenovirus," Human Cell, 2002, 15, 138-150.
Sussman et al., "Porous implants modulate healing and induce shifts in local macrophage polarization in the foreign body reaction," Ann Biomed Eng, 2014, 42, 1508-1516.
Takalkar et al., "Radium-223 dichloride bone-targeted alpha particle therapy for hormone-refractory breast cancer metastatic to bone," Exp Hematol Oncol, 2014, 8, 23.
Tallarida, "Quantitative methods for assessing drug synergism," Genes & Cancer, 2011, 2, 1003-1008.
Tamburro et al., "Dissection of human tropoelastin: exon-by-exon chemical synthesis and related conformational studies," Biochemistry, 2003, 42, 13347-13362.
Tamburro et al., "Localizing alpha-helices in human tropoelastin: assembly of the elastin "puzzle"," Biochemistry, 2006, 45, 9518-9530.
Tan et al., "Characterization of a new primary human pancreatic tumor line," Cancer investigation, 1986, 4, 15-23.

(56) **References Cited**

OTHER PUBLICATIONS

Tang et al., "Identification of PECAM-1 in solid tumor cells and its potential involvement in tumor cell adhesion to endothelium," J. Biol. Chem., 1993, 268, 22883-22894.
Teicher, "In vivo/ex vivo and in situ assays used in cancer research: a brief review," Toxicol. Pathol., 2009, 37 (1), 114-122.
Therasse et al., "New guidelines to evaluate the response to treatment in solid tumors," J Natl Cancer Inst, 2000, 92, 205-216.
Tompa et al., "Fuzzy complexes: polymorphism and structural disorder in protein-protein interactions," Trends Biochem Sci, 2008, 33, 2-8.
Trabbic-Carlson et al., "Effect of protein fusion on the transition temperature of an environmentally responsive elastin-like polypeptide: a role for surface hydrophobicity?," Protein Engineering Design and Selection, 2004, 17(1): 57-66.
Trakul et al., "Stereotactic body radiotherapy in the treatment of pancreatic cancer," Semin Radiat Oncol, 2014, 24, 140-147.
Trieu et al., "P0157 Preclinical evaluation of NBN-paclitaxel in pancreatic cancer xenograft models," Eur J Cancer, 2014, 50, e53.
Tu et al., "Stages in tropoelastin coalescence during synthetic elastin hydrogel formation," Micron, 2010, 41, 268-272.
Tward et al., "Survival of men with clinically localized prostate cancer treated with prostatectomy, brachytherapy, or no definitive treatment: impact of age at diagnosis," Cancer, 2006, 107, 2392-2400, doi:10.1002/cncr.22261.
Uchida et al., "Potential of adenovirus-mediated REIC/Dkk-3 gene therapy for use in the treatment of pancreatic cancer," Journal of Gastroenterology and Hepatology, 2014, 29, 973-983.
Urry et al., "Calculation of distorted circular dichroism curves for poly-L-glutamic acid suspensions," Arch Biochem Biophys, 1970, 137, 214-221.
Urry et al., "Coacervation of solubilized elastin effects a notable conformational change," Nature, 1969, 222, 795-796.
Urry et al., "Differential scatter of left and right circularly polarized light by optically active particulate systems," Proc Natl Acad Sci U S A, 1970, 65, 845-852.
Urry et al., "Distortions in circular dichroism patterns of particulate (or membranous) systems," Arch Biochem Biophys, 1968, 128, 802-807.
Urry et al., "Temperature dependence of length of elastin and its polypentapeptide," Biochem Biophys Res Commun, 1986, 141, 749-755.
Urry, "Protein elasticity based on conformations of sequential polypeptides: The biological elastic fiber," J Protein Chemistry, 1984, 3, 403-436.
Valkenburg et al., "Targeting the tumour stroma to improve cancer therapy," Nature Reviews Clinical Oncology, 2018, 15, 366-381.
Van der Lee et al., "Classification of intrinsically disordered regions and proteins," Chem Rev, 2014, 114, 6589-6631.
Van Roey et al., "Short linear motifs: ubiquitous and functionally diverse protein interaction modules directing cell regulation," Chem Rev, 2014, 114, 6733-6778.
Van Roy, "Beyond E-cadherin: roles of other cadherin superfamily members in cancer," Nat Rev Cancer, 2014, 14, 121-134.
Vicini et al., "An interinstitutional and interspecialty comparison of treatment outcome data for patients with prostate carcinoma based on predefined prognostic categories and minimum follow-up," Cancer, 2002, 95, 2126-2135.
Volkova et al., "Anthracycline Cardiotoxicity: Prevalence, Pathogenesis and Treatment," Curr. Cardiol. Rev., 2011, vol. 7, No. 4, pp. 214-220.
Vrhovski et al., "Biochemistry of tropoelastin," Eur J Biochem, 1998, 258, 1-18.
Wang et al., "Extending Half Life of H-Ferritin Nanoparticle by Fusing Albumin Binding Domain for Doxorubicin Encapsulation," Biomacromolecules, 2018, 12, 19(3):773-781.
Wang et al., "Quantitative Mapping of the Spatial Distribution of Nanoparticles in Endo-Lysosomes by Local pH," Nano Lett., 2017, 17(2): 1226-1232.
Wang et al., "Size and dynamics of caveolae studied using nanoparticles in living endothelial cells," ACS nano, 2009, 3(12): p. 4110-4116.
Waterman et al., "Edema associated with I-125 or Pd-103 prostate brachytherapy and its impact on post-implant dosimetry: an analysis based on serial CT acquisition," International journal of radiation oncology, biology, physics, 1998, 41, 1069-1077.
Wei et al., "Anticancer drug nanomicelles formed by self-assembling amphiphilic dendrimer to combat cancer drug resistance," Proceedings of the National Academy of Sciences of the United States of America, 2015, 112(10): 2978-2983.
Williams et al., "Targeted radionuclide therapy," Medical Physics, 2008, 35, 3062-3068.
Wood et al., "Experiences Using Chloramine-T and 1,3,4,6-Tetrachloro-3-Alpha,6-Alpha-Diphenylglycoluril (Iodogen) for Radioiodination of Materials for Radioimmunoassay," J Clin Chem Clin Bio, 1981, 19, 1051-1056.
Wright et al., "Self-assembly of block copolymers derived from elastin-mimetic polypeptide sequences," Advanced Drug Delivery Reviews, 2002, 54, 1057-1073.
Wright et al., "Thermoplastic elastomer hydrogels via self-assembly of an elastin-mimetic triblock polypeptide," Advanced Functional Materials, 2002, 12, 149-154.
Wust et al., "Hyperthermia in combined treatment of cancer," The Lancet Oncology, 2002, 3, 487-497.
Xia et al., "Tunable self-assembly of genetically engineered silk--elastin-like protein polymers," Biomacromolecules, 2011, 12, 3844-3850.
Xu et al., "Genetically engineered block copolymers: influence of the length and structure of the coiled-coil blocks on hydrogel self-assembly," Pharm Res, 2008, 25, 674-682.
Xu et al., "Role of pancreatic stellate cells in pancreatic cancer metastasis," Am J of Pathology, 2010, 177, 2585-2596.
Yates et al., "Contemporary management of patients with high-risk non-muscle-invasive bladder cancer who fail intravesical BCG therapy," World journal of urology, 2011, 29, 415-422.
Yeo et al., "Coacervation of tropoelastin," Adv Colloid Interface Sci, 2011, 167, 94-103.
Yokoe et al., "Albumin-conjugated PEG liposome enhances tumor distribution of liposomal doxorubicin in rats," International Journal of Pharmaceutics, 2008, 353(1-2): 28-34.
Yousefpour et al., "Co-opting biology to deliver drugs," Biotechnol Bioeng, 2014, 111(9): p. 1699-1716.
Yousefpour et al., "Genetically Encoding Albumin Binding into Chemotherapeutic-loaded Polypeptide Nanoparticles Enhances Their Antitumor Efficacy," Nano Lett. 2018, 18(12): 7784-7793.
Yu et al., "Effectiveness and security of CT-guided percutaneous implantation of (125)I seeds in pancreatic carcinoma," The British journal of radiology, 2014, 87, 20130642, 7 pages.
Zhang et al., "Novel agents for pancreatic ductal adenocarcinoma: emerging therapeutics and future directions," Jounral of Hematology & Oncology, 2018, 11:14, 17 pages.
Zhao et al., "A new Bliss Independence model to analyze drug combination data," J Biomol Screen, 2014, 19, 817-821.
Zini et al., "Contemporary management of adrenocortical carcinoma," European urology, 2011, 60, 1055-1065.
International Search Report and Written Opinion for Application No. PCT/US2018/040409 dated Nov. 5, 2018 (16 pages).
United States Patent Office Notice of Allowance for U.S. Appl. No. 15/387,536 dated Mar. 13, 2019 (13 pages).
United States Patent Office Notice of Allowance for U.S. Appl. No. 15/387,540 dated Apr. 17, 2019 (9 pages).

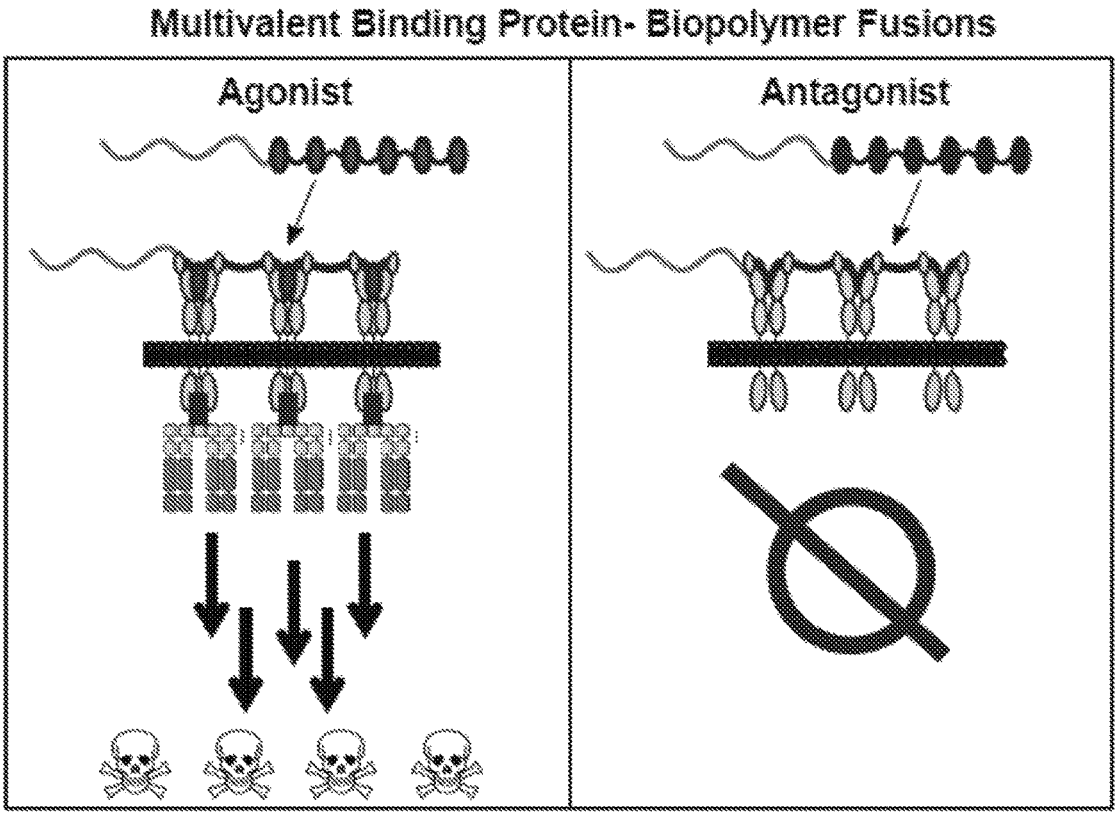

Multivalent Binding Protein- Biopolymer Fusions
Agonist
Antagonist

FIG. 1

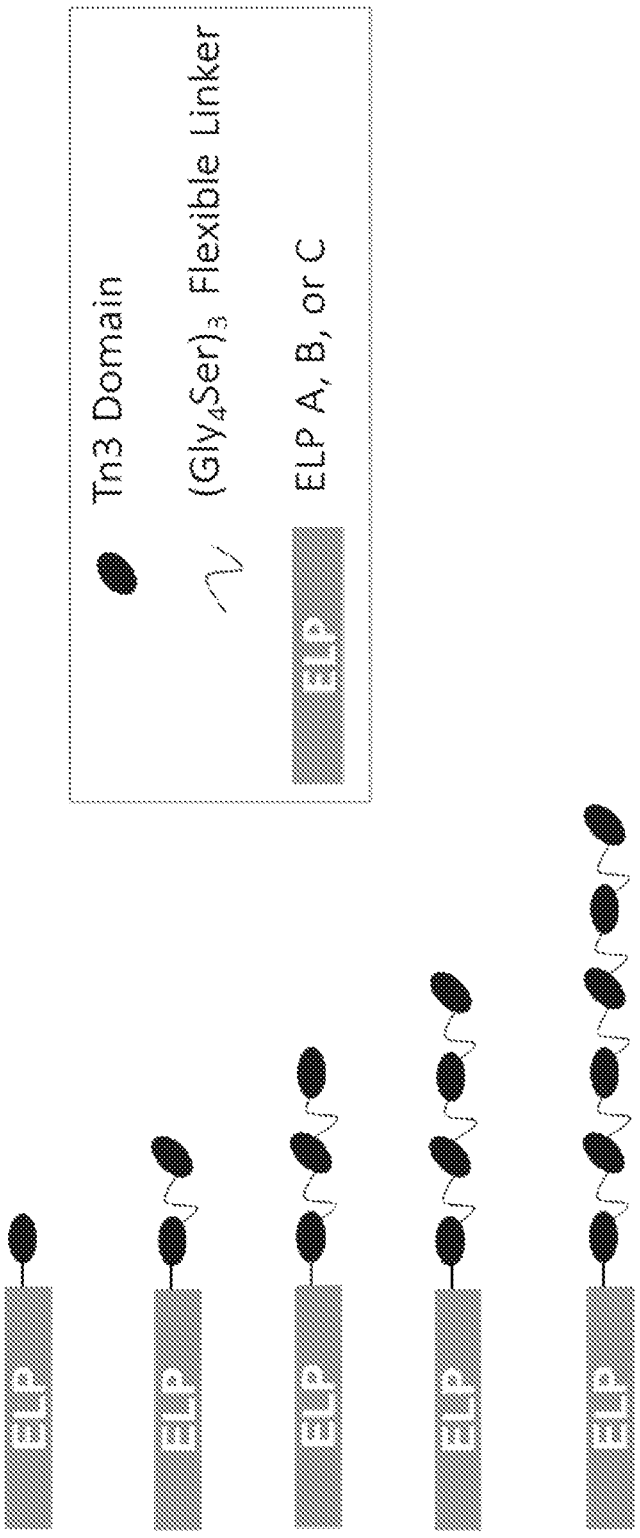
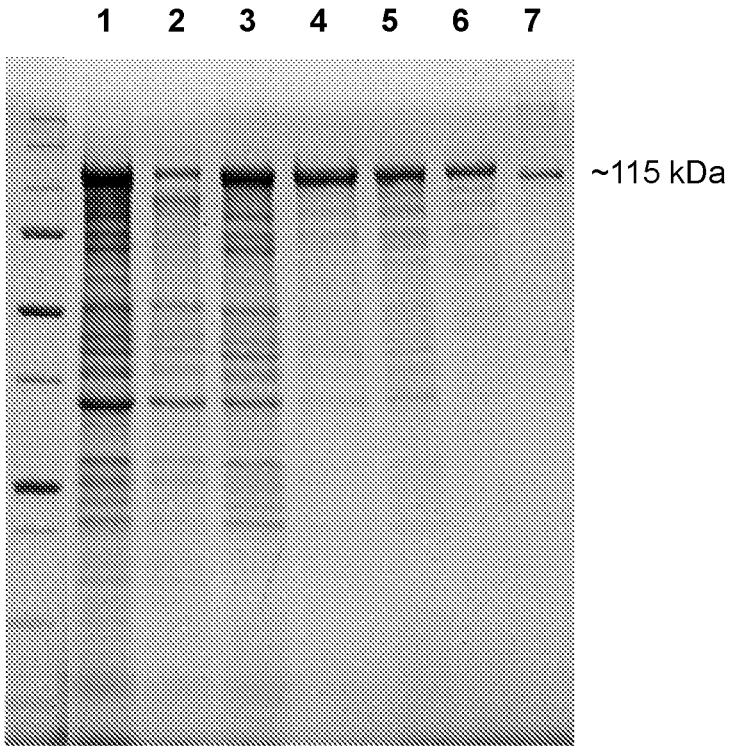

Tn3 Domain
$(Gly_4Ser)_3$ Flexible Linker
ELP A, B, or C
ELP
ELP
ELP
ELP
ELP
ELP

FIG. 2



MW of ELP A $(VPGVG)_{120}$= 50 kDa
+
MW of 6 Tn3 repeats = 60 kDa

FIG. 3

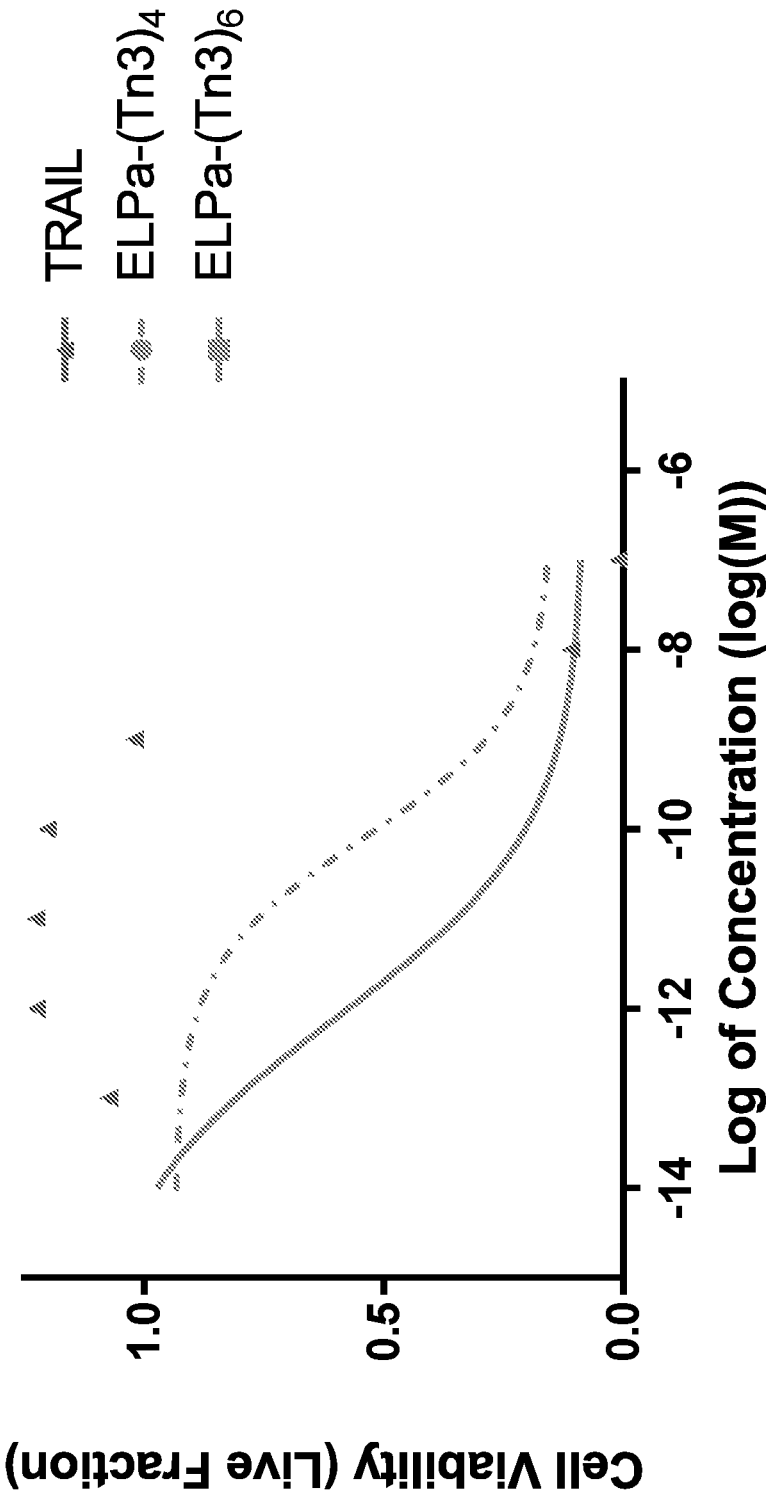

TRAIL
ELPa-(Tn3)4
ELPa-(Tn3)6
Cell Viability (Live Fraction)
1.0
0.5
0.0
-14
-12
-10
-8
-6
Log of Concentration (log(M))

FIG. 4

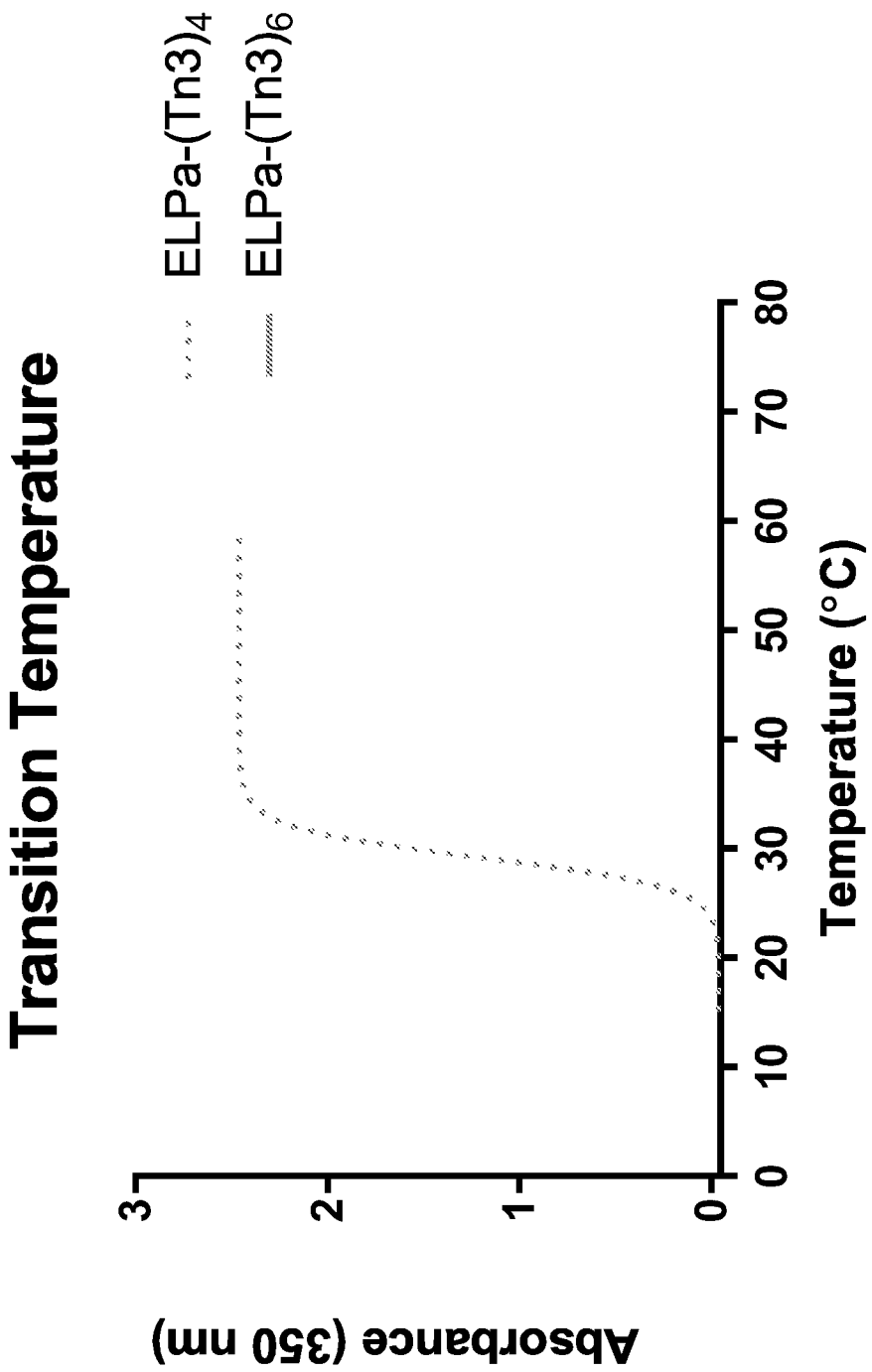

Transition Temperature
ELPa-(Tn3)4
ELPa-(Tn3)6
Absorbance (350 nm)
0
1
2
3
0
10
20
30
40
50
60
70
80
Temperature (°C)

FIG. 5

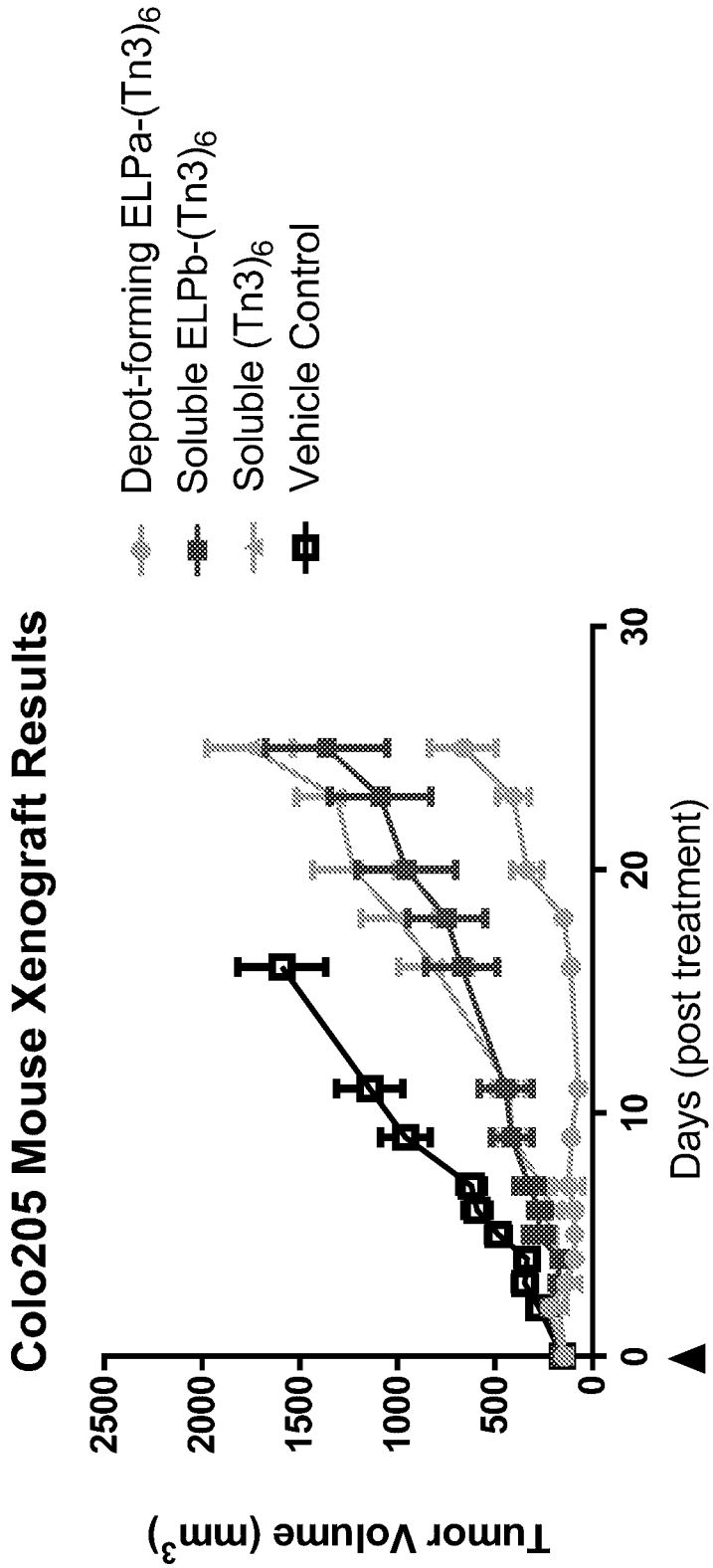

Colo205 Mouse Xenograft Results
Depot-forming ELPa-(Tn3)6
Soluble ELPb-(Tn3)6
Soluble (Tn3)6
Vehicle Control
Tumor Volume (mm3)
2500
2000
1500
1000
500
0
0
10
20
30
Days (post treatment)

FIG. 6

FIBRONECTIN TYPE III DOMAIN-BASED FUSION PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present patent application is a national stage filing under 35 U.S.C. § 371 of International Application No. PCT/US2016/024202, filed on Mar. 25, 2016, which claims the benefit of United States Provisional Application No. 62/138,847, filed Mar. 26, 2015, the content of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

The sequence listing is filed with the application in electronic format only and is incorporated by reference herein. The sequence listing text file "028193-9193-US01_As_Filed_Sequence_Listing.txt", was created on Aug. 15, 2017, and is 46,836 bytes in size.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with government support under grant RO1 EB007205, 2032358, and 2032363 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD

The disclosure relates to antibody mimetics and, more particularly, to fusions of unstructured polypeptides and multivalent proteins that specifically bind a target. The multivalent proteins can bind a target such as a cell surface receptor, for example, and thereby affect cellular physiology. The unstructured polypeptide component can render the fusion protein environmentally responsive, and thereby expand the scope of drug delivery options.

INTRODUCTION

Proteins can be powerful therapeutic agents when engineered for affinity, specificity, and selectivity for a clinical target. Their complexity, versatility, tolerability, and diversity often make them superior alternatives to small molecule drugs, and the long half-life, specificity, and selectivity of some proteins make them attractive for some therapies. Biotechnological advances have enabled the engineering of proteins with specific properties and the manipulation of existing proteins for maximum therapeutic potential. Although protein engineering allows for the development of potent therapeutics targeted toward a protein or receptor of interest, the body has many mechanisms with which to clear such protein therapies. Thus, delivery is a critical issue for effectively translating a protein therapeutic to the clinic. There is a need for reliable and broadly applicable protein delivery solutions.

SUMMARY

In an aspect, provided herein are fusion proteins. The fusion protein may include at least one binding polypeptide and at least one unstructured polypeptide. In some embodiments, the fusion protein comprises a plurality of unstructured polypeptides. In some embodiments, the fusion protein comprises a plurality of binding polypeptides. In some embodiments, the fusion protein further includes a linker positioned between at least two adjacent binding polypeptides. In some embodiments, the fusion protein further includes a linker positioned between at least two adjacent unstructured polypeptides. In some embodiments, the linker comprises at least one glycine and at least one serine. In some embodiments, the linker comprises an amino acid sequence consisting of SEQ ID NO: 3 ($(Gly_4Ser)_3$). In some embodiments, the linker comprises an amino acid sequence consisting of SEQ ID NO: 4. In some embodiments, the plurality of binding polypeptides forms an oligomer. In some embodiments, the binding polypeptide binds a target. In some embodiments, the fusion protein binds more than one target. In some embodiments, the at least one binding polypeptide comprises a Fibronectin type III (FnIII) domain. In some embodiments, the FnIII domain binds TNF-related apoptosis-inducing ligand receptor 2 (TRAILR-2). In some embodiments, the at least one binding polypeptide comprises at least one amino acid sequence of consisting of SEQ ID NO: 17 (RGDS). In some embodiments, the at least one binding polypeptide comprises a plurality of amino acid sequences consisting of SEQ ID NO: 17 (RGDS). In some embodiments, the at least one unstructured polypeptide comprises at least one PG motif comprising an amino acid sequence selected from PG, $P(X)_nG$ (SEQ ID NO: 18), and $(U)_mP(X)_nG(Z)_p$ (SEQ ID NO: 20), or a combination thereof, wherein m, n, and p are independently an integer from 1 to 15, and wherein U, X, and Z are independently any amino acid. In some embodiments, the at least one unstructured polypeptide includes a thermally responsive polypeptide. In some embodiments, the thermally responsive polypeptide comprises an elastin-like polypeptide (ELP). In some embodiments, the at least one unstructured polypeptide includes an amino acid sequence consisting of $(VPGXG)_n$ (SEQ ID NO: 19), wherein X is any amino acid except proline and n is an integer greater than or equal to 1. In some embodiments, n is 60, 120, or 180. In some embodiments, X is valine. In some embodiments, the fusion protein further includes at least one linker positioned between the at least one binding polypeptide and the at least one unstructured polypeptide. In some embodiments, the fusion protein includes a plurality of linkers between the at least one binding polypeptide and the at least one unstructured polypeptide. In some embodiments, the at least one binding polypeptide is positioned N-terminal to the at least one unstructured polypeptide. In some embodiments, the at least one binding polypeptide is positioned C-terminal to the at least one unstructured polypeptide. In some embodiments, the at least one unstructured polypeptide has a LCST between about 0° C. and about 100° C. In some embodiments, the at least one unstructured polypeptide has a UCST between about 0° C. and about 100° C.

In another aspect, provided herein are methods for treating a disease in a subject in need thereof. The method may include administering to the subject an effective amount of the fusion protein as described herein. In some embodiments, the fusion protein is administered in a controlled release formulation. In some embodiments, the fusion protein forms a depot upon administration to the subject. In some embodiments, the fusion protein is administered intravenously, intraarterially, or intraperitoneally to the subject. In some embodiments, the disease includes cancer. In some embodiments, the fusion protein is administered intratumorally. In some embodiments, the cancer is colorectal adenocarcinoma. In some embodiments, the at least one binding polypeptide includes an FnIII domain or a plurality of FnIII domains, and the disease is a disease associated with

TRAILR-2. In some embodiments, the disease is a disease associated with a target of the at least one binding polypeptide.

In another aspect, provided herein are multivalent fusion proteins. The multivalent fusion protein may include at least one Fibronectin type III (FnIII) domain and at least one elastin-like polypeptide (ELP). In some embodiments, the FnIII domain binds TNF-related apoptosis-inducing ligand receptor 2 (TRAILR-2). In some embodiments, the at least one ELP includes an amino acid sequence consisting of $(VPGXG)_n$ (SEQ ID NO: 19), wherein X is any amino acid except proline and n is an integer greater than or equal to 1. In some embodiments, n is 60, 120, or 180. In some embodiments, X is valine. In some embodiments, the at least one FnIII domain includes an amino acid sequence consisting of SEQ ID NO: 1. In some embodiments, the multivalent fusion protein includes a plurality of FnIII domains. In some embodiments, the multivalent fusion protein includes 2, 4, or 6 FnIII domains. In some embodiments, the multivalent fusion protein further includes a linker positioned between at least two adjacent FnIII domains. In some embodiments, the linker includes at least one glycine and at least one serine. In some embodiments, the linker includes an amino acid sequence consisting of SEQ ID NO: 3 $((Gly_4Ser)_3)$. In some embodiments, the linker includes an amino acid sequence consisting of SEQ ID NO: 4.

In another aspect, provided herein are methods for treating a disease associated with TNF-related apoptosis-inducing ligand receptor 2 (TRAILR-2) in a subject in need thereof. The methods may include administering to the subject an effective amount of the multivalent fusion protein as detailed herein. In some embodiments, the disease includes cancer. In some embodiments, the cancer includes colorectal adenocarcinoma. In some embodiments, the multivalent fusion protein is administered intravenously, intraarterially, or intraperitoneally to the subject. In some embodiments, the multivalent fusion protein is administered intratumorally. In some embodiments, the multivalent fusion protein forms a depot upon administration to the subject. In some embodiments, the multivalent fusion protein is administered in a controlled release formulation.

In another aspect, provided herein are methods of diagnosing a disease in a subject. The method may include contacting a sample from the subject with a fusion protein as detailed herein, and detecting binding of the fusion protein to a target to determine presence of the target in the sample, wherein the presence of the target in the sample indicates the disease in the subject. In some embodiments, the disease is selected from cancer, metabolic disease, autoimmune disease, cardiovascular disease, and orthopedic disorder.

In another aspect, provided herein are methods of determining the presence of a target in a sample. The method may include contacting the sample with a fusion protein as detailed herein under conditions to allow a complex to form between the fusion protein and the target in the sample, and detecting the presence of the complex, wherein presence of the complex is indicative of the target in the sample. In some embodiments, the sample is obtained from a subject and the method further includes diagnosing a disease, prognosticating, or assessing the efficacy of a treatment of the subject. In some embodiments, the method further includes assessing the efficacy of a treatment of the subject, and the method further includes modifying the treatment of the subject as needed to improve efficacy.

In another aspect, provided herein are methods of determining the effectiveness of a treatment for a disease in a subject in need thereof. The method may include contacting a sample from the subject with a fusion protein as described herein under conditions to allow a complex to form between the fusion protein and a target in the sample, determining the level of the complex in the sample, wherein the level of the complex is indicative of the level of the target in the sample, and comparing the level of the target in the sample to a control level of the target, wherein if the level of the target is different from the control level, then the treatment is determined to be effective or ineffective in treating the disease. In some embodiments, the method further includes modifying the treatment or administering a different treatment to the subject when the treatment is determined to be ineffective in treating the disease.

In another aspect, provided herein are methods of diagnosing a disease in a subject. The method may include contacting a sample from the subject with a fusion protein as described herein, determining the level of a target in the sample, and comparing the level of the target in the sample to a control level of the target, wherein a level of the target different from the control level indicates disease in the subject.

In some embodiments, the control level corresponds to the level in the subject at a time point before or during the period when the subject has begun treatment, and the sample is taken from the subject at a later time point. In some embodiments, the sample is taken from the subject at a time point during the period when the subject is undergoing treatment, and the control level corresponds to a disease-free level or to the level at a time point before the period when the subject has begun treatment.

In some embodiments, the fusion protein is labeled with a reporter. In some embodiments, the disease is selected from cancer, metabolic disease, autoimmune disease, cardiovascular disease, and orthopedic disorder.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a schematic illustrating the architecture of the protein biopolymer fusions. The multivalent protein drugs can act as agonists to amplify receptor signaling or as antagonists to inhibit ligand binding and prevent receptor signaling.

FIG. **2** is a schematic of the multivalent TRAILR-2 agonist-ELP constructs. The Tn3-ELP fusions were constructed to express ELPs at the N-terminus (shown) or C-terminus (not shown). Each Tn3 unit had a molecular weight of approximately 10 kDa, and the molecular weight of the ELPs varied.

FIG. **3** shows SDS-PAGE analysis of the ELP-TRAILR-2 agonist fusion protein at various steps in the purification process. Lanes: 1: Cell lysate; 2: hot spin 1 supernatant; 3: hot spin 1 pellet; 4: cold spin 2 supernatant; 5: hot spin 2 pellet; 6: purified product (cold spin 3 supernatant); 7: purified product (cold spin 3 supernatant). Samples in lanes 1-6 contained reducing agent; lane 7 did not.

FIG. **4** is a graph showing that tetravalent TRAILR-2-ELPa-$(Tn3)_4$ fusions inhibited cell viability of Colo205 human colorectal adenocarcinoma cells and outperformed TRAIL. Hexavalent TRAILR-2-ELPa-$(Tn3)_6$ fusions exhibited potent activation of apoptosis as well. Presence of ELP did not affect the potency of the drug.

FIG. **5** is a graph showing the transition temperatures. The transition temperature of the 6 repeat agonist ELP fusion was 29.2° C., and the transition temperature of the 4 repeat agonist ELP fusion was 27.9° C. This range was appropriate for s.c./intratumoral injections in mouse Colo205 xenograft models.

FIG. **6** is a graph showing the changes in tumor volume in Colo205 colorectal cancer xenograft models in response to multivalent TRAILR-2 specific ELP fusions. Tumors in mice treated with depot-forming ELPa-$(Tn3)_6$ fusions underwent partial regression and delayed growth.

DETAILED DESCRIPTION

Provided herein are compositions and methods for delivering protein therapeutics to a subject. The compositions and methods include a fusion protein. The fusion protein may include a binding polypeptide fused to an unstructured polypeptide. In some embodiments, the unstructured polypeptide may include a thermally responsive protein polymer, which may facilitate slow release from a gel-like depot. The use of protein drugs, particularly antibodies, has led to many successful treatments. The long half-life, specificity, and selectivity of engineered antibodies make them excellent for some therapies. The limitations of architecture, valency, potency, aggregation, and manufacturing cost of antibodies can be major hindrances in translation to the clinic. The compositions and methods detailed herein may overcome these limitations and facilitate the use of protein therapeutics for clinical use. The fusion proteins may allow for the treatment of disease by effectively delivering binding polypeptides so they may associate with their target to treat the disease. The fusion proteins may also be used to detect a target, detect or diagnose disease, and/or determine the efficacy of a treatment.

1. Definitions

The terms "comprise(s)," "include(s)," "having," "has," "can," "contain(s)," and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms "a," "and," and "the" include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments "comprising," "consisting of," and "consisting essentially of," the embodiments or elements presented herein, whether explicitly set forth or not.

For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

The term "about" as used herein as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain aspects, the term "about" refers to a range of values that fall within 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

"Affinity" refers to the binding strength of a binding polypeptide to its target (i.e., binding partner).

"Agonist" refers to an entity that binds to a receptor and activates the receptor to produce a biological response. An "antagonist" blocks or inhibits the action or signaling of the agonist. An "inverse agonist" causes an action opposite to that of the agonist. The activities of agonists, antagonists, and inverse agonists may be determined in vitro, in situ, in vivo, or a combination thereof.

"Amino acid" as used herein refers to naturally occurring and non-natural synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code. Amino acids can be referred to herein by either their commonly known three-letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Amino acids include the side chain and polypeptide backbone portions.

As used herein, the term "biomarker" refers to a naturally occurring biological molecule present in a subject at varying concentrations that is useful in identifying and/or classifying a disease or a condition. The biomarker can include genes, proteins, polynucleotides, nucleic acids, ribonucleic acids, polypeptides, or other biological molecules used as an indicator or marker for disease. In some embodiments, the biomarker comprises a disease marker. For example, the biomarker can be a gene that is upregulated or downregulated in a subject that has a disease. As another example, the biomarker can be a polypeptide whose level is increased or decreased in a subject that has a disease or risk of developing a disease. In some embodiments, the biomarker comprises a small molecule. In some embodiments, the biomarker comprises a polypeptide.

The terms "control," "reference level," and "reference" are used herein interchangeably. The reference level may be a predetermined value or range, which is employed as a benchmark against which to assess the measured result. "Control group" as used herein refers to a group of control subjects. The predetermined level may be a cutoff value from a control group. The predetermined level may be an average from a control group. Cutoff values (or predetermined cutoff values) may be determined by Adaptive Index Model (AIM) methodology. Cutoff values (or predetermined cutoff values) may be determined by a receiver operating curve (ROC) analysis from biological samples of the patient group. ROC analysis, as generally known in the biological arts, is a determination of the ability of a test to discriminate one condition from another, e.g., to determine the performance of each marker in identifying a patient having CRC. A description of ROC analysis is provided in P. J. Heagerty et al. (*Biometrics* 2000, 56, 337-44), the disclosure of which is hereby incorporated by reference in its entirety. Alternatively, cutoff values may be determined by a quartile analysis of biological samples of a patient group. For example, a cutoff value may be determined by selecting a value that corresponds to any value in the 25th-75th percentile range, preferably a value that corresponds to the 25th percentile, the 50th percentile or the 75th percentile, and more preferably the 75th percentile. Such statistical analyses may be performed using any method known in the art and can be implemented through any number of commercially available software packages (e.g., from Analyse-it Software Ltd., Leeds, UK; StataCorp LP, College Station, Tex.; SAS Institute Inc., Cary, N.C.). The healthy or normal levels or ranges

for a target or for a protein activity may be defined in accordance with standard practice.

The term "expression vector" indicates a plasmid, a virus or another medium, known in the art, into which a nucleic acid sequence for encoding a desired protein can be inserted or introduced.

The term "host cell" is a cell that is susceptible to transformation, transfection, transduction, conjugation, and the like with a nucleic acid construct or expression vector. Host cells can be derived from plants, bacteria, yeast, fungi, insects, animals, etc. In some embodiments, the host cell includes *Escherichia coli*.

"Polymer" as used herein is intended to encompass a homopolymer, heteropolymer, block polymer, co-polymer, ter-polymer, etc., and blends, combinations and mixtures thereof. Examples of polymers include, but are not limited to, functionalized polymers, such as a polymer comprising 5-vinyltetrazole monomer units and having a molecular weight distribution less than 2.0. The polymer may be or contain one or more of a star block copolymer, a linear polymer, a branched polymer, a hyperbranched polymer, a dendritic polymer, a comb polymer, a graft polymer, a brush polymer, a bottle-brush copolymer and a crosslinked structure, such as a block copolymer comprising a block of 5-vinyltetrazole monomer units. Polymers include, without limitation, polyesters, poly(meth)acrylamides, poly(meth) acrylates, polyethers, polystyrenes, polynorbornenes and monomers that have unsaturated bonds. For example, amphiphilic comb polymers are described in U.S. Patent Application Publication No. 2007/0087114 and in U.S. Pat. No. 6,207,749 to Mayes et al., the disclosure of each of which is herein incorporated by reference in its entirety. The amphiphilic comb-type polymers may be present in the form of copolymers, containing a backbone formed of a hydrophobic, water-insoluble polymer and side chains formed of short, hydrophilic non-cell binding polymers. Examples of other polymers include, but are not limited to, polyalkylenes such as polyethylene and polypropylene; polychloroprene; polyvinyl ethers; such as poly(vinyl acetate); polyvinyl halides such as poly(vinyl chloride); polysiloxanes; polystyrenes; polyurethanes; polyacrylates; such as poly(methyl (meth)acrylate), poly(ethyl (meth)acrylate), poly(n-butyl (meth)acrylate), poly(isobutyl (meth)acrylate), poly(tert-butyl (meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl (meth)acrylate), poly(lauryl (meth)acrylate), poly (phenyl (meth)acrylate), poly(methyl acrylate), poly (isopropyl acrylate), poly(isobutyl acrylate), and poly (octadecyl acrylate); polyacrylamides such as poly (acrylamide), poly(methacrylamide), poly(ethyl acrylamide), poly(ethyl methacrylamide), poly(N-isopropyl acrylamide), poly(n, iso, and tert-butyl acrylamide); and copolymers and mixtures thereof. These polymers may include useful derivatives, including polymers having substitutions, additions of chemical groups, for example, alkyl groups, alkylene groups, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art. The polymers may include zwitterionic polymers such as, for example, polyphosphorycholine, polycarboxybetaine, and polysulfobetaine. The polymers may have side chains of betaine, carboxybetaine, sulfobetaine, oligoethylene glycol (OEG), sarcosine or polyethyleneglycol (PEG). For example, poly(oligoethyleneglycol methacrylate) (poly (OEGMA)) may be used. Poly(OEGMA) may be hydrophilic, water-soluble, non-fouling, non-toxic and non-immunogenic due to the OEG side chains.

"Polynucleotide" as used herein can be single stranded or double stranded, or can contain portions of both double stranded and single stranded sequence. The polynucleotide can be nucleic acid, natural or synthetic, DNA, genomic DNA, cDNA, RNA, or a hybrid, where the polynucleotide can contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine, and isoguanine. Polynucleotides can be obtained by chemical synthesis methods or by recombinant methods.

A "peptide" or "polypeptide" is a linked sequence of two or more amino acids linked by peptide bonds. The polypeptide can be natural, synthetic, or a modification or combination of natural and synthetic. Peptides and polypeptides include proteins such as binding proteins, receptors, and antibodies. The terms "polypeptide", "protein," and "peptide" are used interchangeably herein. "Primary structure" refers to the amino acid sequence of a particular peptide. "Secondary structure" refers to locally ordered, three dimensional structures within a polypeptide. These structures are commonly known as domains, e.g., enzymatic domains, extracellular domains, transmembrane domains, pore domains, and cytoplasmic tail domains. Domains are portions of a polypeptide that form a compact unit of the polypeptide and are typically 15 to 350 amino acids long. Exemplary domains include domains with enzymatic activity or ligand binding activity. Typical domains are made up of sections of lesser organization such as stretches of beta-sheet and alpha-helices. "Tertiary structure" refers to the complete three dimensional structure of a polypeptide monomer. "Quaternary structure" refers to the three dimensional structure formed by the noncovalent association of independent tertiary units.

"Reporter," "reporter group," "label," and "detectable label" are used interchangeably herein. The reporter is capable of generating a detectable signal. The label can produce a signal that is detectable by visual or instrumental means. A variety of reporter groups can be used, differing in the physical nature of signal transduction (e.g., fluorescence, electrochemical, nuclear magnetic resonance (NMR), and electron paramagnetic resonance (EPR)) and in the chemical nature of the reporter group. Various reporters include signal-producing substances, such as chromagens, fluorescent compounds, chemiluminescent compounds, radioactive compounds, and the like. In some embodiments, the reporter comprises a radiolabel. Reporters may include moieties that produce light, e.g., acridinium compounds, and moieties that produce fluorescence, e.g., fluorescein. In some embodiments, the signal from the reporter is a fluorescent signal. The reporter may comprise a fluorophore. Examples of fluorophores include, but are not limited to, acrylodan (6-acryloyl-2-dimethylaminonaphthalene), badan (6-bromoacetyl-2-dimethylamino-naphthalene), rhodamine, naphthalene, danzyl aziridine, 4-[N-[(2-iodoacetoxy)ethyl]-N-methylamino]-7-nitrobenz-2-oxa-1,3-diazole ester (IANBDE), 4-[N-[(2-iodoacetoxy)ethyl]-N-methylamino-7-nitrobenz-2-oxa-1,3-diazole (IANBDA), fluorescein, dipyrromethenboron difluoride (BODIPY), 4-nitrobenzo[c][1,2,5]oxadiazole (NBD), Alexa fluorescent dyes, and derivatives thereof. Fluorescein derivatives may include, for example, 5-fluorescein, 6-carboxyfluorescein, 3'6-carboxyfluorescein, 5(6)-carboxyfluorescein, 6-hexachlorofluorescein, 6-tetrachlorofluorescein, fluorescein, and isothiocyanate.

"Sample" or "test sample" as used herein can mean any sample in which the presence and/or level of a target is to be detected or determined. Samples may include liquids, solutions, emulsions, or suspensions. Samples may include a medical sample. Samples may include any biological fluid

or tissue, such as blood, whole blood, fractions of blood such as plasma and serum, muscle, interstitial fluid, sweat, saliva, urine, tears, synovial fluid, bone marrow, cerebrospinal fluid, nasal secretions, sputum, amniotic fluid, bronchoalveolar lavage fluid, gastric lavage, emesis, fecal matter, lung tissue, peripheral blood mononuclear cells, total white blood cells, lymph node cells, spleen cells, tonsil cells, cancer cells, tumor cells, bile, digestive fluid, skin, or combinations thereof. In some embodiments, the sample comprises an aliquot. In other embodiments, the sample comprises a biological fluid. Samples can be obtained by any means known in the art. The sample can be used directly as obtained from a patient or can be pre-treated, such as by filtration, distillation, extraction, concentration, centrifugation, inactivation of interfering components, addition of reagents, and the like, to modify the character of the sample in some manner as discussed herein or otherwise as is known in the art.

The term "sensitivity" as used herein refers to the number of true positives divided by the number of true positives plus the number of false negatives, where sensitivity ("sens") may be within the range of 0<sens<1. Ideally, method embodiments herein have the number of false negatives equaling zero or close to equaling zero, so that no subject is wrongly identified as not having a disease when they indeed have the disease. Conversely, an assessment often is made of the ability of a prediction algorithm to classify negatives correctly, a complementary measurement to sensitivity.

The term "specificity" as used herein refers to the number of true negatives divided by the number of true negatives plus the number of false positives, where specificity ("spec") may be within the range of 0<spec<1. Ideally, the methods described herein have the number of false positives equaling zero or close to equaling zero, so that no subject is wrongly identified as having a disease when they do not in fact have disease. Hence, a method that has both sensitivity and specificity equaling one, or 100%, is preferred.

By "specifically binds," it is generally meant that a polypeptide binds to a target when it binds to that target more readily than it would bind to a random, unrelated target.

"Subject" as used herein can mean a mammal that wants or is in need of the herein described fusion proteins. The subject may be a human or a non-human animal. The subject may be a mammal. The mammal may be a primate or a non-primate. The mammal can be a primate such as a human; a non-primate such as, for example, dog, cat, horse, cow, pig, mouse, rat, camel, llama, goat, rabbit, sheep, hamster, and guinea pig; or non-human primate such as, for example, monkey, chimpanzee, gorilla, orangutan, and gibbon. The subject may be of any age or stage of development, such as, for example, an adult, an adolescent, or an infant.

"Transition" or "phase transition" refers to the aggregation of the thermally responsive polypeptides. Phase transition occurs sharply and reversibly at a specific temperature called the lower critical solution temperature (LCST) or the inverse transition temperature T_t. Below the transition temperature, the thermally responsive polypeptide (or a polypeptide comprising a thermally responsive polypeptide) is highly soluble. Upon heating past the transition temperature, the thermally responsive polypeptides hydrophobically collapse and aggregate, forming a separate, gel-like phase. "Inverse transition cycling" refers to a protein purification method for thermally responsive polypeptides (or a polypeptide comprising a thermally responsive polypeptide). The protein purification method may involve the use of thermally responsive polypeptide's reversible phase transition behavior to cycle the solution through soluble and insoluble phases, thereby removing contaminants.

"Treatment" or "treating," when referring to protection of a subject from a disease, means preventing, suppressing, repressing, ameliorating, or completely eliminating the disease. Preventing the disease involves administering a composition of the present invention to a subject prior to onset of the disease. Suppressing the disease involves administering a composition of the present invention to a subject after induction of the disease but before its clinical appearance. Repressing or ameliorating the disease involves administering a composition of the present invention to a subject after clinical appearance of the disease.

"Substantially identical" can mean that a first and second amino acid sequence are at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% over a region of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100 amino acids.

"Valency" as used herein refers to the potential binding units or binding sites. The term "multivalent" refers to multiple potential binding units. The terms "multimeric" and "multivalent" are used interchangeably herein.

"Variant" used herein with respect to a polynucleotide means (i) a portion or fragment of a referenced nucleotide sequence; (ii) the complement of a referenced nucleotide sequence or portion thereof; (iii) a polynucleotide that is substantially identical to a referenced polynucleotide or the complement thereof; or (iv) a polynucleotide that hybridizes under stringent conditions to the referenced polynucleotide, complement thereof, or a sequences substantially identical thereto.

A "variant" can further be defined as a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. Representative examples of "biological activity" include the ability to be bound by a specific antibody or polypeptide or to promote an immune response. Variant can mean a substantially identical sequence. Variant can mean a functional fragment thereof. Variant can also mean multiple copies of a polypeptide. The multiple copies can be in tandem or separated by a linker. Variant can also mean a polypeptide with an amino acid sequence that is substantially identical to a referenced polypeptide with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids. See Kyte et al., *J. Mol. Biol.* 1982, 157, 105-132. The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indices of ±2 are substituted. The hydrophobicity of amino acids can also be used to reveal substitutions that would result in polypeptides retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a polypeptide permits calculation of the greatest local average hydrophilicity of that polypeptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity, as discussed in U.S. Pat. No. 4,554,101, which is fully incorporated herein by reference. Substitution of amino

acids having similar hydrophilicity values can result in polypeptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions can be performed with amino acids having hydrophilicity values within ±2 of each other. Both the hydrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties.

A variant can be a polynucleotide sequence that is substantially identical over the full length of the full gene sequence or a fragment thereof. The polynucleotide sequence can be 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the full length of the gene sequence or a fragment thereof. A variant can be an amino acid sequence that is substantially identical over the full length of the amino acid sequence or fragment thereof. The amino acid sequence can be 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the full length of the amino acid sequence or a fragment thereof.

2. Fusion Protein

The fusion protein includes at least one binding polypeptide and at least one unstructured polypeptide. The fusion protein may further include at least one linker.

In some embodiments, the fusion protein includes more than one binding polypeptide. The fusion protein may include at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 binding polypeptides. The fusion protein may include less than 30, less than 25, or less than 20 binding polypeptides. The fusion protein may include between 1 and 30, between 1 and 20, or between 1 and 10 binding polypeptides. In such embodiments, the binding polypeptides may be the same or different from one another. In some embodiments, the fusion protein includes more than one binding polypeptide positioned in tandem to one another. In some embodiments, the fusion protein includes 2 to 6 binding polypeptides. In some embodiments, the fusion protein includes two binding polypeptides. In some embodiments, the fusion protein includes three binding polypeptides. In some embodiments, the fusion protein includes four binding polypeptides. In some embodiments, the fusion protein includes five binding polypeptides. In some embodiments, the fusion protein includes six binding polypeptides.

In some embodiments, the fusion protein includes more than one unstructured polypeptide. The fusion protein may include at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 unstructured polypeptides. The fusion protein may include less than 30, less than 25, or less than 20 unstructured polypeptides. The fusion protein may include between 1 and 30, between 1 and 20, or between 1 and 10 unstructured polypeptides. In such embodiments, the unstructured polypeptides may be the same or different from one another. In some embodiments, the fusion protein includes more than one unstructured polypeptide positioned in tandem to one another.

In some embodiments, the fusion protein may be arranged as a modular linear polypeptide. For example, the modular linear polypeptide may be arranged in one of the following structures: $[\text{binding polypeptide}]_m$-$[\text{linker}]_k$-[unstructured polypeptide]; [unstructured polypeptide]-$[\text{linker}]_k$-$[\text{binding polypeptide}]_m$; $[\text{binding polypeptide}]_m$-$[\text{linker}]_k$-[unstructured polypeptide]-$[\text{binding polypeptide}]_m$-$[\text{linker}]_k$-[unstructured polypeptide]; or [unstructured polypeptide]-$[\text{binding polypeptide}]_m$-$[\text{linker}]_k$-[unstructured polypeptide]-$[\text{binding polypeptide}]_m$, in which k and m are each independently an integer greater than or equal to 1. In some embodiments, m is an integer less than or equal to 20. In some embodiments, m is an integer equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some embodiments, k is an integer less than or equal to 10. In some embodiments, k is an integer equal to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the at least one binding polypeptide is positioned N-terminal to the at least one unstructured polypeptide. In some embodiments, the at least one binding polypeptide is positioned C-terminal to the at least one unstructured polypeptide.

The fusion protein may be expressed recombinantly in a host cell according to one of skill in the art. The fusion protein may be purified by any means known to one of skill in the art. For example, the fusion protein may be purified using chromatography, such as liquid chromatography, size exclusion chromatography, or affinity chromatography, or a combination thereof. In some embodiments, the fusion protein is purified without chromatography. In some embodiments, the fusion protein is purified using inverse transition cycling.

In some embodiments, the fusion protein comprises a plurality of binding polypeptides comprising Tn3 domains (SEQ ID NO: 1 or 2), linked to one another with flexible glycine serine linkers (SEQ ID NO: 3), and an unstructured polypeptide comprising elastin-like polypeptide (FIG. **1**).

a. Binding Polypeptide

The binding polypeptide may comprise any polypeptide that is capable of binding at least one target. The binding polypeptide may bind at least one target. "Target" may be an entity capable of being bound by the binding polypeptide. Targets may include, for example, another polypeptide, a cell surface receptor, a carbohydrate, an antibody, a small molecule, or a combination thereof. The target may be a biomarker. The target may be activated through agonism or blocked through antagonism. The binding polypeptide may specifically bind the target. By binding target, the binding polypeptide may act as a targeting moiety, an agonist, an antagonist, or a combination thereof. In some embodiments, the binding polypeptide domain binds TRAILR-2. "TRAIL receptor 2" or "TRAILR-2" refers to the TNF-Related Apoptosis-Inducing Ligand (TRAIL) Receptor 2 protein. Upon binding TRAIL or other agonists, TRAILR-2 activates apoptosis, or programmed cell death, in tumor cells. In some embodiments, the binding polypeptide domain binds epidermal growth factor receptor (EGFR). Upon binding epidermal growth factor (EGF) and other growth factor ligands, EGFR activates signal transduction pathways that promote cell proliferation.

The binding polypeptide may be a monomer that binds to a target. The monomer may bind one or more targets. The binding polypeptide may form an oligomer. The binding polypeptide may form an oligomer with the same or different binding polypeptides. The oligomer may bind to a target. The oligomer may bind one or more targets. One or more monomers within an oligomer may bind one or more targets. In some embodiments, the fusion protein is multivalent. In

some embodiments, the fusion protein binds multiple targets. In some embodiments, the activity of the binding polypeptide alone is the same as the activity of the binding protein when part of a fusion protein.

In some embodiments, the binding polypeptide comprises an amino acid sequence consisting of Arg-Gly-Asp-Ser (RGDS; SEQ ID NO: 17). In some embodiments, the binding polypeptide comprises a plurality of amino acid sequences consisting of SEQ ID NO: 17. The amino acid sequence of SEQ ID NO: 17 may be present anywhere within the binding polypeptide. In some embodiments, the amino acid sequence of SEQ ID NO: 17 may be repeated in tandem within the binding polypeptide.

In some embodiments, the binding polypeptide comprises one or more scaffold proteins. As used herein, "scaffold protein" refers to one or more polypeptide domains with relatively stable and defined three-dimensional structures. Scaffold proteins may further have the capacity for affinity engineering. In some embodiments, the scaffold protein has been engineered to bind a particular target. The scaffold proteins may be the same or different.

In some embodiments, the scaffold protein comprises a fibronectin domain. Fibronectin is a high-molecular weight glycoprotein of the extracellular matrix that binds to membrane-spanning receptor proteins called integrins. Fibronectin binds extracellular matrix components such as collagen, fibrin, and heparan sulfate proteoglycans. Human fibronectin exists as a protein dimer, comprising two nearly identical polypeptide chains linked by a pair of C-terminal disulfide bonds. Each human fibronectin subunit contains three domains: type I, II, and III. Fibronectin type III (FnIII) refers to the third of the three types of internal repeats in human fibronectin. This domain is often referred to as a scaffold protein because it contains three CDR-like (complementarity determining region) loops that can be engineered to bind a protein of interest using molecular biology techniques. In some embodiments, the fibronectin domain comprises Tn3. "Tn3" or "Tn3 scaffold" refers to an FnIII domain from human tenascin C. Tn3 may comprise an amino acid sequence consisting of SEQ ID NO: 1 or 2. In some embodiments, Tn3 binds TRAIL receptor 2. The binding polypeptide may comprise one or more scaffold proteins further selected from, for example, alphahelical Z domain of protein A, anti-EGFR binding protein, DARPINS, knottins, and scFvs.

b. Unstructured Polypeptide

The unstructured polypeptide may comprise any polypeptide that has minimal or no secondary structure as observed by CD, being soluble at a temperature below its lower critical solution temperature (LCST) and/or at a temperature above its upper critical solution temperature (UCST), and comprising a repeated amino acid sequence. LCST is the temperature below which the polypeptide is miscible. UCST is the temperature above which the polypeptide is miscible. In some embodiments, the unstructured polypeptide has only UCST behavior. In some embodiments, the unstructured polypeptide has only LCST behavior. In some embodiments, the unstructured polypeptide has both UCST and LCST behavior. The unstructured polypeptide may comprise a repeated sequence of amino acids. The unstructured polypeptide may have a LCST between about 0° C. and about 100° C., between about 10° C. and about 50° C., or between about 20° C. and about 42° C. The unstructured polypeptide may have a UCST between about 0° C. and about 100° C., between about 10° C. and about 50° C., or between about 20° C. and about 42° C. In some embodiments, the unstructured polypeptide has a transition temperature between room temperature (about 25° C.) and body temperature (about 37° C.). In some embodiments, a fusion protein comprising one or more thermally responsive polypeptides has a transition temperature between room temperature (about 25° C.) and body temperature (about 37° C.). In some embodiments, the unstructured polypeptide has no LCST or UCST behavior. The unstructured polypeptide may have its LCST or UCST below body temperature or above body temperature at the concentration at which the fusion protein is administered to a subject.

In some embodiments, the unstructured polypeptide comprises an amino acid sequence that is rich in proline and glycine. In some embodiments, the unstructured polypeptide comprises a PG motif. In some embodiments, the unstructured polypeptide comprises a plurality of or repeated PG motifs. A PG motif comprises an amino acid sequence selected from PG, $P(X)_nG$ (SEQ ID NO: 18), and $(U)_mP(X)_nG(Z)_p$ (SEQ ID NO: 20), or a combination thereof, wherein m, n, and p are independently an integer from 1 to 15, and wherein U, X, and Z are independently any amino acid. $P(X)_nG$ may include PXG, PXXG, PXXXG, PXXXXG, PXXXXXG, PXXXXXXG, PXXXXXXXG, PXXXXXXXXXG, PXXXXXXXXXXG, PXXXXXXXXXG, PXXXXXXXXXXXXXG, PXXXXXXXXXXXXXXXG, and/or PXXXXXXXXXXXXXXXXG. The unstructured polypeptide may further include additional amino acids at the C-terminal and/or N-terminal end of the PG motif. These amino acids surrounding the PG motif may also be part of the overall repeated motif. The amino acids that surround the PG motif may balance the overall hydrophobicity and/or charge so as to control the LCST or UCST behavior of the unstructured polypeptide.

In some embodiments, the unstructured polypeptide comprises one or more thermally responsive polypeptides. Thermally responsive polypeptides may include, for example, elastin-like polypeptides (ELP). "ELP" refers to a polypeptide comprising the pentapeptide repeat sequence $(VPGXG)_n$, wherein X is any amino acid except proline and n is an integer greater than or equal to 1 (SEQ ID NO: 19). The unstructured polypeptide may comprise an amino acid sequence consisting of $(VPGXG)_n$. In some embodiments, X is not proline. In some embodiments, n is 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, or 300. In some embodiments, n may be less than 500, less than 400, less than 300, less than 200, or less than 100. In some embodiments, n may be between 1 and 500, between 1 and 400, between 1 and 300, or between 1 and 200. In some embodiments, n is 60, 120, or 180. ELP may be expressed recombinantly.

Thermally responsive polypeptides, for example, ELP, may have a phase transition. The thermally responsive polypeptide may impart a phase transition characteristic to the unstructured polypeptide and/or fusion protein. "Phase transition" or "transition" may refer to the aggregation of the thermally responsive polypeptide, which occurs sharply and reversibly at a specific temperature called the lower critical solution temperature (LCST) or the inverse transition temperature (Tt). Below the transition temperature (LCST or Tt), the thermally responsive polypeptides (or polypeptides comprising a thermally responsive polypeptide) may be highly soluble. Upon heating above the transition temperature, thermally responsive polypeptides hydrophobically may collapse and aggregate, forming a separate, gel-like phase.

In other embodiments, the thermally responsive polypeptide comprises a resilin-like polypeptide (RLP). RLPs are derived from Rec1-resilin. Rec1-resilin is environmentally responsive and exhibits a dual phase transition behavior. The thermally responsive RLPs can have LCST and UCST (Li et. al, *Macromol. Rapid Commun.* 2015, 36, 90-95.) Additional examples of suitable thermally responsive polypeptides are described in U.S. Patent Application Publication Nos. US2012/0121709, filed May 17, 2012, and US2015/0112022, filed Apr. 23, 2015, each of which is incorporated herein by reference.

The thermally responsive polypeptides can phase transition at a variety of temperatures and concentrations. Thermally responsive polypeptides, for example, ELP, may not affect the binding or potency of the binding polypeptides. Thermally responsive polypeptides may allow the fusion protein to be tuned by a user to any number of desired transition temperatures, molecular weights, and formats.

Thermally responsive polypeptides may exhibit inverse phase transition behavior and thus, the fusion protein comprising the thermally responsive polypeptide may exhibit inverse phase transition behavior. Inverse phase transition behavior may be used to form drug depots within a tissue of a subject for controlled (slow) release of the fusion protein. Inverse phase transition behavior may also enable purification of the fusion protein using inverse transition cycling, thereby eliminating the need for chromatography.

c. Linker

In some embodiments, the fusion protein further includes at least one linker. In some embodiments, the fusion protein includes more than one linker. In such embodiments, the linkers may be the same or different from one another. The fusion protein may include at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, or at least 100 linkers. The fusion protein may include less than 500, less than 400, less than 300, or less than 200 linkers. The fusion protein may include between 1 and 1000, between 10 and 900, between 10 and 800, or between 5 and 500 linkers.

The linker may be positioned in between a binding polypeptide and an unstructured polypeptide, in between binding polypeptides, in between unstructured polypeptides, or a combination thereof. Multiple linkers may be positioned adjacent to one another. Multiple linkers may be positioned adjacent to one another and in between the binding polypeptide and the unstructured polypeptide.

The linker may be a polypeptide of any amino acid sequence and length. The linker may act as a spacer peptide. The linker may occur between polypeptide domains. The linker may sufficiently separate the binding domains of the binding polypeptide while preserving the activity of the binding domains. In some embodiments, the linker comprises charged amino acids. In some embodiments, the linker is flexible. In some embodiments, the linker comprises at least one glycine and at least one serine. In some embodiments, the linker comprises an amino acid sequence consisting of $(Gly_4Ser)_3$ (SEQ ID NO: 3). In some embodiments, the linker comprises at least one proline. In some embodiments, the linker comprises an amino acid sequence consisting of SEQ ID NO: 4.

3. Polynucleotides

Further provided are polynucleotides encoding the fusion proteins detailed herein. A vector may include the polynucleotide encoding the fusion proteins detailed herein. To obtain expression of a polypeptide, one typically subclones the polynucleotide encoding the polypeptide into an expression vector that contains a promoter to direct transcription, a transcription/translation terminator, and if for a nucleic acid encoding a protein, a ribosome binding site for translational initiation. An example of a vector is pet24 (SEQ ID NO: 12). Suitable bacterial promoters are well known in the art. Further provided is a host cell transformed or transfected with an expression vector comprising a polynucleotide encoding a fusion protein as detailed herein. Bacterial expression systems for expressing the protein are available in, e.g., *E. coli, Bacillus* sp., and *Salmonella* (Paiva et al., *Gene* 1983, 22, 229-235; Mosbach et al., *Nature* 1983, 302, 543-545). Kits for such expression systems are commercially available. Eukaryotic expression systems for mammalian cells, yeast, and insect cells are well known in the art and are also commercially available. Retroviral expression systems can be used in the present invention. In some embodiments, the fusion protein comprises a polypeptide comprising an amino acid sequence of any one of SEQ ID NOs: 1-11 and 17-19. In some embodiments, the fusion protein comprises a polypeptide encoded by a polynucleotide sequence of any one of SEQ ID NOs: 13-14.

4. Administration

The fusion proteins as detailed above can be formulated in accordance with standard techniques well known to those skilled in the pharmaceutical art. Such compositions comprising a fusion protein can be administered in dosages and by techniques well known to those skilled in the medical arts taking into consideration such factors as the age, sex, weight, and condition of the particular subject, and the route of administration.

The fusion protein can be administered prophylactically or therapeutically. In prophylactic administration, the fusion protein can be administered in an amount sufficient to induce a response. In therapeutic applications, the fusion proteins are administered to a subject in need thereof in an amount sufficient to elicit a therapeutic effect. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, e.g., the particular composition of the fusion protein regimen administered, the manner of administration, the stage and severity of the disease, the general state of health of the patient, and the judgment of the prescribing physician.

The fusion protein can be administered by methods well known in the art as described in Donnelly et al. (*Ann. Rev. Immunol.* 1997, 15, 617-648); Felgner et al. (U.S. Pat. No. 5,580,859, issued Dec. 3, 1996); Felgner (U.S. Pat. No. 5,703,055, issued Dec. 30, 1997); and Carson et al. (U.S. Pat. No. 5,679,647, issued Oct. 21, 1997), the contents of all of which are incorporated herein by reference in their entirety. The fusion protein can be complexed to particles or beads that can be administered to an individual, for example, using a vaccine gun. One skilled in the art would know that the choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the route of administration.

The fusion proteins can be delivered via a variety of routes. Typical delivery routes include parenteral administration, e.g., intradermal, intramuscular or subcutaneous delivery. Other routes include oral administration, intranasal, intravaginal, transdermal, intravenous, intraarterial, intratumoral, intraperitoneal, and epidermal routes. In some

embodiments, the fusion protein is administered intravenously, intraarterially, or intraperitoneally to the subject.

The fusion protein can be a liquid preparation such as a suspension, syrup, or elixir. The fusion protein can be incorporated into liposomes, microspheres, or other polymer matrices (such as by a method described in Feigner et al., U.S. Pat. No. 5,703,055; Gregoriadis, Liposome Technology, Vols. I to III (2nd ed. 1993), the contents of which are incorporated herein by reference in their entirety). Liposomes can consist of phospholipids or other lipids, and can be nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

The fusion protein may be used as a vaccine. The vaccine can be administered via electroporation, such as by a method described in U.S. Pat. No. 7,664,545, the contents of which are incorporated herein by reference. The electroporation can be by a method and/or apparatus described in U.S. Pat. Nos. 6,302,874; 5,676,646; 6,241,701; 6,233,482; 6,216,034; 6,208,893; 6,192,270; 6,181,964; 6,150,148; 6,120,493; 6,096,020; 6,068,650; and 5,702,359, the contents of which are incorporated herein by reference in their entirety. The electroporation can be carried out via a minimally invasive device.

In some embodiments, the fusion protein is administered in a controlled release formulation. In some embodiments, the fusion protein comprises one or more thermally responsive polypeptides, the thermally responsive polypeptide having a transition temperature such that the fusion protein remains soluble prior to administration and such that the fusion protein transitions upon administration to a gel-like depot in the subject. In some embodiments, the fusion protein comprises one or more thermally responsive polypeptides, the thermally responsive polypeptide having a transition temperature such that the fusion protein remains soluble at room temperature and such that the fusion protein transitions upon administration to a gel-like depot in the subject. For example, in some embodiments, the fusion protein comprises one or more thermally responsive polypeptides, the thermally responsive polypeptide having a transition temperature between room temperature (about 25° C.) and body temperature (about 37° C.), whereby the fusion protein can be administered to form a depot. As used herein, "depot" refers to a gel-like composition comprising a fusion protein that releases the fusion protein over time. In some embodiments, the fusion protein can be injected subcutaneously or intratumorally to form a depot (coacervate). The depot may provide controlled (slow) release of the fusion protein. The depot may provide slow release of the fusion protein into the circulation or the tumor, for example. In some embodiments, the fusion protein may be released from the depot over a period of at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 1 week, at least about 1.5 weeks, at least about 2 weeks, at least about 2.5 weeks, at least about 3.5 weeks, at least about 4 weeks, or at least about 1 month.

5. Detection

As used herein, the term "detect" or "determine the presence of" refers to the qualitative measurement of undetectable, low, normal, or high concentrations of one or more fusion proteins, targets, or fusion proteins bound to target. Detection may include in vitro, ex vivo, or in vivo detection. Detection may include detecting the presence of one or more fusion proteins or targets versus the absence of the one or more fusion proteins or targets. Detection may also include quantification of the level of one or more fusion proteins or targets. The term "quantify" or "quantification" may be used interchangeably, and may refer to a process of determining the quantity or abundance of a substance (e.g., fusion protein or target), whether relative or absolute. Any suitable method of detection falls within the general scope of the present disclosure. In some embodiments, the fusion protein comprises a reporter attached thereto for detection. In some embodiments, the fusion protein is labeled with a reporter. In some embodiments, detection of fusion protein bound to target may be determined by methods including but not limited to, band intensity on a Western blot, flow cytometry, radiolabel imaging, cell binding assays, activity assays, SPR, immunoassay, or by various other methods known in the art.

In some embodiments, including those wherein the fusion protein is an antibody mimic for binding and/or detecting a target, any immunoassay may be utilized. The immunoassay may be an enzyme-linked immunoassay (ELISA), radioimmunoassay (RIA), a competitive inhibition assay, such as forward or reverse competitive inhibition assays, a fluorescence polarization assay, or a competitive binding assay, for example. The ELISA may be a sandwich ELISA. Specific immunological binding of the fusion protein to the target can be detected via direct labels, attached to the fusion protein or via indirect labels, such as alkaline phosphatase or horseradish peroxidase. The use of immobilized fusion proteins may be incorporated into the immunoassay. The fusion proteins may be immobilized onto a variety of supports, such as magnetic or chromatographic matrix particles, the surface of an assay plate (such as microtiter wells), pieces of a solid substrate material, and the like. An assay strip can be prepared by coating the fusion protein or plurality of fusion proteins in an array on a solid support. This strip can then be dipped into the test biological sample and then processed quickly through washes and detection steps to generate a measurable signal, such as a colored spot.

6. Methods

a. Methods of Treating a Disease

The present invention is directed to a method of treating a disease in a subject in need thereof. The method may comprise administering to the subject an effective amount of the fusion protein as described herein. The disease may be selected from cancer, metabolic disease, autoimmune disease, cardiovascular disease, and orthopedic disorders. In some embodiments, the disease is a disease associated with a target of the at least one binding polypeptide.

Metabolic disease may occur when abnormal chemical reactions in the body alter the normal metabolic process. Metabolic diseases may include, for example, insulin resistance, non-alcoholic fatty liver diseases, type 2 diabetes, insulin resistance diseases, cardiovascular diseases, arteriosclerosis, lipid-related metabolic disorders, hyperglycemia, hyperinsulinemia, hyperlipidemia, and glucose metabolic disorders.

Autoimmune diseases arise from an abnormal immune response of the body against substances and tissues normally present in the body. Autoimmune diseases may include, but are not limited to, lupus, rheumatoid arthritis, multiple sclerosis, insulin dependent diabetes mellitis, myasthenia gravis, Grave's disease, autoimmune hemolytic anemia, autoimmune thrombocytopenia purpura, Goodpasture's syndrome, pemphigus vulgaris, acute rheumatic fever, post-streptococcal glomerulonephritis, polyarteritis nodosa, myocarditis, psoriasis, Celiac disease, Crohn's disease, ulcerative colitis, and fibromyalgia.

Cardiovascular disease is a class of diseases that involve the heart or blood vessels. Cardiovascular diseases may include, for example, coronary artery diseases (CAD) such

as angina and myocardial infarction (heart attack), stroke, hypertensive heart disease, rheumatic heart disease, cardiomyopathy, heart arrhythmia, congenital heart disease, valvular heart disease, carditis, aortic aneurysms, peripheral artery disease, and venous thrombosis.

Orthopedic disorders or musculoskeletal disorders are injuries or pain in the body's joints, ligaments, muscles, nerves, tendons, and structures that support limbs, neck, and back. Orthopedic disorders may include degenerative diseases and inflammatory conditions that cause pain and impair normal activities. Orthopedic disorders may include, for example, carpal tunnel syndrome, epicondylitis, and tendinitis.

Cancers may include, but are not limited to, breast cancer, colorectal cancer, colon cancer, lung cancer, prostate cancer, testicular cancer, brain cancer, skin cancer, rectal cancer, gastric cancer, esophageal cancer, sarcomas, tracheal cancer, head and neck cancer, pancreatic cancer, liver cancer, ovarian cancer, lymphoid cancer, cervical cancer, vulvar cancer, melanoma, mesothelioma, renal cancer, bladder cancer, thyroid cancer, bone cancers, carcinomas, sarcomas, and soft tissue cancers. In some embodiments, the cancer is colorectal cancer. In some embodiments, the cancer is colorectal adenocarcinoma.

One application of protein therapeutics is cancer treatment. In specific embodiments, the present invention provides a method for using scaffold proteins in developing antibody mimetics for oncological targets of interest. With the emergence of scaffold protein engineering come the possibilities for designing potent protein drugs that are unhindered by steric and architectural limitations. Although potent protein drugs can be invaluable for diagnostics or treatments, successful delivery to the target region can pose a great challenge.

TNF-related apoptosis-inducing ligand receptor 2 (TRAILR-2, also called R5) activates the extrinsic death pathway in a range of human cancer cells (Walczak, et al. *Cold Spring Harb. Perspect. Biol.,* 2013, 5, a008698). TRAILR-2 may be targeted using its natural ligand, TNF-related apoptosis-inducing ligand (TRAIL, also called Apo2L), and other agonists. TRAIL is a homotrimer. TRAIL and other TRAILR-2 agonists may trigger programmed cell death (apoptosis). TRAIL and other TRAILR-2 agonists may have significant anti-tumor activity. However, TRAIL and other TRAILR-2 agonists have not been developed as a clinically efficacious treatment. A possible shortcoming of current TRAIL and other TRAILR-2 agonist therapies may be related to their limited valency. Upon binding of TRAILR-2 to homotrimeric TRAIL, the TRAILR-2 receptor trimerizes and subsequently initiates apoptotic cell death. However, current anti-TRAILR-2 mAbs are only bivalent. Indeed, higher order antibody crosslinking may be required for effective receptor engagement, clustering, and a robust anti-tumor response. Fusion proteins, as detailed herein, that bind multiple TRAILR-2 receptors may provide multivalent agonists capable of forming higher order complexes to treat cancer. FnIII domain has been engineered to have high affinity binding to TRAILR-2. Fusion proteins, as detailed herein, comprising FnIII domains flexible peptide linkers may be used as pro-apoptotic anti-cancer therapeutics. The increased molecular weight and controlled release of the fusion proteins, relative to a binding polypeptide alone, along with the unperturbed potency of the binding polypeptide, may provide a clinically viable option for patients with tumors expressing functional target protein (e.g. TRAILR-2).

In other aspects, provided are methods for treating a disease associated with TNF-related apoptosis-inducing ligand receptor 2 (TRAILR-2) in a subject in need thereof. The method may include administering to the subject an effective amount of a fusion protein as described herein.

b. Methods of Diagnosing a Disease

Provided herein are methods of diagnosing a disease. The methods may include administering to the subject a fusion protein as described herein, and detecting binding of the fusion protein to a target to determine presence of the target in the subject. The presence of the target may indicate the disease in the subject. In other embodiments, the methods may include contacting a sample from the subject with a fusion protein as described herein, determining the level of a target in the sample, and comparing the level of the target in the sample to a control level of the target, wherein a level of the target different from the control level indicates disease in the subject. In some embodiments, the disease is selected from cancer, metabolic disease, autoimmune disease, cardiovascular disease, and orthopedic disorders, as detailed above. In some embodiments, the target comprises a disease marker or biomarker. In some embodiments, the fusion protein may act as an antibody mimic for binding and/or detecting a target.

c. Methods of Determining the Presences of a Target

Provided herein are methods of determining the presence of a target in a sample. The methods may include contacting the sample with a fusion protein as described herein under conditions to allow a complex to form between the fusion protein and the target in the sample, and detecting the presence of the complex. Presence of the complex may be indicative of the target in the sample. In some embodiments, the fusion protein is labeled with a reporter for detection.

In some embodiments, the sample is obtained from a subject and the method further includes diagnosing, prognosticating, or assessing the efficacy of a treatment of the subject. When the method includes assessing the efficacy of a treatment of the subject, then the method may further include modifying the treatment of the subject as needed to improve efficacy.

d. Methods of Determining the Effectiveness of a Treatment

Provided herein are methods of determining the effectiveness of a treatment for a disease in a subject in need thereof. The methods may include contacting a sample from the subject with a fusion protein as detailed herein under conditions to allow a complex to form between the fusion protein and a target in the sample, determining the level of the complex in the sample, wherein the level of the complex is indicative of the level of the target in the sample, and comparing the level of the target in the sample to a control level of the target, wherein if the level of the target is different from the control level, then the treatment is determined to be effective or ineffective in treating the disease.

Time points may include prior to onset of disease, prior to administration of a therapy, various time points during administration of a therapy, and after a therapy has concluded, or a combination thereof. Upon administration of the fusion protein to the subject, the fusion protein may bind a target, wherein the presence of the target indicates the presence of the disease in the subject at the various time points. In some embodiments, the target comprises a disease marker or biomarker. In some embodiments, the fusion protein may act as an antibody mimic for binding and/or detecting a target. Comparison of the binding of the fusion protein to the target at various time points may indicate whether the disease has progressed, whether the diseased has

advanced, whether a therapy is working to treat or prevent the disease, or a combination thereof.

In some embodiments, the control level corresponds to the level in the subject at a time point before or during the period when the subject has begun treatment, and the sample is taken from the subject at a later time point. In some embodiments, the sample is taken from the subject at a time point during the period when the subject is undergoing treatment, and the control level corresponds to a disease-free level or to the level at a time point before the period when the subject has begun treatment. In some embodiments, the method further includes modifying the treatment or administering a different treatment to the subject when the treatment is determined to be ineffective in treating the disease.

7. Examples

Example 1

Design of Multivalent Protein-ELP Fusions

The fusion proteins included two parts (FIG. **1**): (i) a multivalent targeting component (e.g., TRAILR-2 agonist or EGFR antagonist) protein in which one or more scaffold protein units (e.g., SEQ ID NO: 1 and 2 or 5) are linked by glycine-serine flexible (e.g., SEQ ID NO: 3) or structured proline-containing linkers (e.g., SEQ ID NO: 4); and (ii) an elastin-like-polypeptide connected to the multivalent protein (e.g., SEQ ID NO: 7-9).

The fusion of (i) to (ii) was at the N- or C-terminus or (ii) was interspersed among (i).

Example 2

Design and Preparation of Multivalent Protein-ELP Expression Constructs

The DNA encoding the TRAILR-2-specific Tn3 unit (SEQ ID NO: 13; Swers et al., Mol. Cancer Ther., 2013, 12, 1235-1244) and the EGFR-specific domain (SEQ ID NO: 14; Friedman, et al., J. Mol. Biol. 2008, 376, 1388-1402) were purchased as double-stranded DNA "G-blocks" from Integrated DNA Technologies (Coralville, Iowa). The Tn3 G-block (SEQ ID NO: 13) was amplified using primers "Tn3For" and "Tn3Rev" primers (SEQ ID NO: 15 and 16, respectively). The gene was purchased with a $(Gly_4Ser)_3$ linker (SEQ ID NO: 3) at the C-terminus and designed with restriction sites compatible with recursive directional (RDL) ligation for seamless cloning of oligomeric genes. The EGFR-binding G-block (SEQ ID NO: 5) was purchased such that it could be inserted into the vector (SEQ ID NO: 12) using Gibson Assembly. The G-block contained 40-50 nucleic acid bases identical to those in the vector.

Enzymes used were from New England Biolabs (Ipswich, Mass.) The amplified Tn3 domain PCR product was purified using the Qiagen (Germantown, Md.) PCR cleanup kit and digested with BseRI for insertion into a BseRI/CIP digested pET-24(+) vector modified for RDL (McDaniel et al. Biomacromolecules, 2010, 11, 944-952). The insert and vector were agarose gel-purified and ligated with QuickLigase to clone the single unit construct. This was followed by digestion of the single unit construct (Tn3 in pET24(+)) with BseRI/CIP and ligation with BseRI-digested insert (Tn3 unit) to clone 2, 4, and 6 Tn3 repeats (written as $(Tn3)_2$, $(Tn3)_4$, $(Tn3)_6$) in the pET-24(+) vector. For cloning the FnIII domain, the G-block was inserted into the BseRI digested/CIP treated pET-24(+) RDL vector using the Gibson Assembly Master Mix (New England Biolabs; Ipswich, Mass.). Subcloning efficiency EB5α cells from EdgeBio (Gaithersburg, Md.) were used for cloning steps.

Once the multivalent Tn3 genes were obtained, the gene for ELP was recombinantly fused to the $(Tn3)_6$ using RDL. The RDL ligation method for this particular vector called for digestion of the oligomerized Tn3 in modified pET24(+) (SEQ ID NO: 12) with AcuI and BglI, and digestion of ELP (SEQ ID NO: 7-9) in pET24(+) with BseRI and BglI. The digested fragments of DNA were separated using agarose gel electrophoresis, and the DNA bands at the appropriate molecular weights were excised and gel-purified. The resulting fragments were ligated using QuickLigase and successful clones were obtained. The restriction digest scheme mentioned refers to fusion of ELP to the C-terminus of the multivalent agonist, but in some embodiments, the scheme was flipped if N-terminal fusion was desired. In other embodiments, ELP(s) were interspersed between Tn3 repeats with this cloning method. In still other embodiments, an eight-repeat histidine tag (SEQ ID NO: 6) was recombinantly included at the C-terminus for purification and/or analysis purposes. All gene sequences were verified by direct DNA sequencing (Eton Bioscience Inc., Durham, N.C.) prior to expression.

Example 3

Expression and Purification of Multivalent TRAILR-2 Agonist-ELP Fusion Proteins

The multivalent ELP-$(Tn3)_6$ fusion constructs (SEQ ID NO: 10 and 11; FIG. **2**) were transformed into BL21(DE3) cells (EMD/Novagen, Gibbstown, N.J.) for expression. Transformants were grown in Terrific Broth (TB) containing 45 μg/mL kanamycin and incubated overnight at 37° C. with shaking. Overnight cultures were diluted 1 to 40 into TB containing 45 μg/mL kanamycin and incubated at 37° C. with shaking for 5-8 hours. Protein expression was then induced by addition of IPTG to 1 mM, and incubation was resumed at 37° C. with shaking. In a specific embodiment, the Tn3-ELP fusion proteins were purified from the cell lysate using inverse transition cycling (ITC) as previously described (Christensen et al., *Protein Science* 2009, 18, 1377-1387; Hassouneh et al., *Methods Enzymol.* 2012, 502, 215-37). In another embodiments, C-terminally His_8-tagged ELP-Tn3 fusion proteins were purified from the periplasmic extract using immobilized metal affinity chromatography (IMAC; e.g., HisPur Ni-NTA resin from ThermoFisher Scientific, Pierce, Rockford, Ill.).

All purified proteins were analyzed by SDS-PAGE on Biorad Mini-PROTEAN TGX Tris-HCl Stain-Free (FIG. **3**) or Biorad 4-20% ReadyGel Tris-HCl protein gels for correct molecular weight bands. The protein bands on the latter gel type were visualized with EZBlue Coomassie Brilliant Blue G-250 colloidal protein stain (Sigma Aldrich). Endotoxin was removed from purified protein using an Acrodisc unit with a Mustang E membrane (Pall Corporation, Port Washington, N.Y.).

Example 4

In Vitro Testing of Fusion Protein Activity

To demonstrate that the multivalent ELP-$(Tn3)_6$ fusion proteins (SEQ ID NO: 10 and 11) could kill cancer cell lines with the same potency as the non-ELP agonists, the fusions were tested on Colo205 colorectal adenocarcinoma cells. A cell viability assay was performed to calculate an EC_{50} for

the various multivalent fusion proteins (FIG. **4**). The EC_{50} values were comparable to those reported by others for the multivalent agonists.

The cell viability assay was carried out as follows. The Colo205 cells were plated in 96 well plates at a density of 10,000 cells/well in 90 μL of complete media (RPMI 1640+10% FBS+5% HEPES+5% Sodium Pyruvate+P/S) and incubated for 5-4 hours at 37° C. with 5% CO_2. The cells were then treated with 10 μL 20 mM Tris 300 mM L-arginine pH 7 containing a serial dilution of a specific multivalent Tn3-ELP fusion protein or the vehicle control. The treatments were done in triplicate to account for technical variability. After 24-48 hours, the Promega CellTiter 96 Aqueous One Solution Reagent G3581 kit was used according to manufacturer's instructions to assay the number of viable cells using a colorimetric formazan assay method. The inhibition of cell viability was determined using measurements of the absorbance at 490, which is the maximum absorbance wavelength of the formazan product. The dose response curves were generated by plotting inhibition versus compound concentration. The dose response curve was approximated from the scatter plot using a four-parameter logistic model calculation in GraphPad Prism (La Jolla, Calif.), and EC_{50} was calculated as the concentration of Tn3-ELP required to kill 50% of the Colo205 cells. Fusion of ELPs to the multivalent TRAILR-2 specific Tn3 did not impact their potency (TABLE 1).

TABLE 1

EC_{50} values for various fusion proteins.

Fusion Protein	EC_{50}
TRAIL	2700 pM
$(Tn3)_4$	40 pM
ELPa-$(Tn3)_4$	80 pM
$(Tn3)_6$	1.6 pM
ELPa-$(Tn3)_6$	0.78 pM

Example 5

Spectrophotometry for Analysis of Fusion Protein Inverse Transition Temperature (T_t)

To evaluate the Tt of the fusion proteins, the optical density of the protein solution was monitored at 350 nm (OD350) as a function of temperature. The solution (10-100 μM in 20 mM Tris 300 mM L-arginine, pH 7) was heated at a rate of 1° C./minute using the Cary 300 UV-visible spectrophotometer equipped with a multicell thermoelectric temperature controller (Varian Instruments, Walnut Creek, Calif.). A sharp transition was indicated by the sudden increase in absorbance, and the inflection point of the absorbance versus temperature curve was used to calculate the Tt.

The derivative of the absorbance at 350 nm was calculated with respect to temperature, and the Tt (temperature at maximal turbidity gradient) was obtained. An example set of curves is provided in FIG. **5**. The most potent fusions were the 6-repeat Tn3 domain-ELP (SEQ ID NO: 10 and 11, respectively) were chosen for testing in vivo. The hydrophilic ELPb (SEQ ID NO: 8) had a Tt much higher than body temperature; this biopolymer was chosen for fusion to the bioactive protein as a size control. The hydrophobic ELPa (SEQ ID NO: 7) transitioned at 28° C. (see FIG. **5**) and formed a gel-like depot upon injection into the mouse.

Example 6

Determination of Therapeutic Efficacy In Vivo

Having successfully produced multivalent TRAILR-2 specific ELP-(Tn3)6 fusions that transition to form gel-like depots between room temperature and body temperature, we tested their therapeutic efficacy in a Colo205 colorectal adenocarcinoma mouse xenograft model. One million Colo205 cells (expressing TRAILR-2) were injected subcutaneously into the right flanks of five cohorts of female athymic nude mice. After two weeks, tumors had grown to a volume of approximately 150 mm^3, at which a point a single intratumoral injection of 20 mM Tris 300 mM L-arginine pH 7 (vehicle), TRAIL (not shown), depot-forming ELPa-$(Tn3)_6$ fusion, soluble ELPb-$(Tn3)_6$ fusion, or soluble $(Tn3)_6$ was administered. Throughout the experiment, mice were monitored for overall health and activity in accordance with the Duke University Institutional Animal Care & Use Committee. The mice in all treatment groups were dosed at 3.7 $\mu g/mm^3$ of protein drug and tumor volume was monitored with a digital caliper using the formula:

$$\text{Volume}=0.5\times\text{Length}\times(\text{Width})^2$$

As shown in FIG. **6**, the depot-forming ELPa-$(Tn3)_6$ fusion led to partial tumor regression and slower tumor growth when compared to all other groups. There is a therapeutic advantage of using the depot to release the protein-biopolymer fusion slowly over a longer period of time. This depot approach may be extended to improve the drug delivery of protein-drug conjugates. Also, additional combinations of bioactive multispecific protein-biopolymer fusions can be developed using the methods described herein. The protein architecture, flexibility of the design, and potent therapeutic efficacy make these modular fusions a potential platform for protein delivery.

* * *

The foregoing description of the specific aspects will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific aspects, without undue experimentation, without departing from the general concept of the present disclosure. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed aspects, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

The breadth and scope of the present disclosure should not be limited by any of the above-described exemplary aspects, but should be defined only in accordance with the following claims and their equivalents.

All publications, patents, patent applications, and/or other documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, and/or other document were individually indicated to be incorporated by reference for all purposes.

For reasons of completeness, various aspects of the invention are set out in the following numbered clauses:

Clause 1. A fusion protein comprising at least one binding polypeptide and at least one unstructured polypeptide.

Clause 2. The fusion protein of clause 1, wherein the fusion protein comprises a plurality of unstructured polypeptides.

Clause 3. The fusion protein of any one of the preceding clauses, wherein the fusion protein comprises a plurality of binding polypeptides.

Clause 4. The fusion protein of clause 3, further comprising a linker positioned between at least two adjacent binding polypeptides.

Clause 5. The fusion protein of clause 2, further comprising a linker positioned between at least two adjacent unstructured polypeptides.

Clause 6. The fusion protein of any one of clauses 4-5, wherein the linker comprises at least one glycine and at least one serine.

Clause 7. The fusion protein of clause 6, wherein the linker comprises an amino acid sequence consisting of SEQ ID NO: 3 ($(Gly_4Ser)_3$).

Clause 8. The fusion protein of any one of clauses 4-5, wherein the linker comprises an amino acid sequence consisting of SEQ ID NO: 4.

Clause 9. The fusion protein of any one of clauses 3-8, wherein the plurality of binding polypeptides forms an oligomer.

Clause 10. The fusion protein of any one of clauses 3-9, wherein the binding polypeptide binds a target, and wherein the fusion protein binds more than one target.

Clause 11. The fusion protein of any one of the preceding clauses, wherein the at least one binding polypeptide comprises a Fibronectin type III (FnIII) domain.

Clause 12. The fusion protein of clause 11, wherein the FnIII domain binds TNF-related apoptosis-inducing ligand receptor 2 (TRAILR-2).

Clause 13. The fusion protein of any one of the preceding clauses, wherein the at least one binding polypeptide comprises at least one amino acid sequence of consisting of SEQ ID NO: 17 (RGDS).

Clause 14. The fusion protein of clause 13, wherein the at least one binding polypeptide comprises a plurality of amino acid sequences consisting of SEQ ID NO: 17 (RGDS).

Clause 15. The fusion protein of any one of the preceding clauses, wherein the at least one unstructured polypeptide comprises at least one PG motif comprising an amino acid sequence selected from PG, $P(X)_nG$ (SEQ ID NO: 18), and $(U)_mP(X)_nG(Z)_p$ (SEQ ID NO: 20), or a combination thereof, wherein m, n, and p are independently an integer from 1 to 15, and wherein U, X, and Z are independently any amino acid.

Clause 16. The fusion protein of any one of the preceding clauses, wherein the at least one unstructured polypeptide comprises a thermally responsive polypeptide.

Clause 17. The fusion protein of clause 16, wherein the thermally responsive polypeptide comprises an elastin-like polypeptide (ELP).

Clause 18. The fusion protein of any one of the preceding clauses, wherein the at least one unstructured polypeptide comprises an amino acid sequence consisting of $(VPGXG)_n$ (SEQ ID NO: 19), wherein X is any amino acid except proline and n is an integer greater than or equal to 1.

Clause 19. The fusion protein of clause 18, wherein n is 60, 120, or 180.

Clause 20. The fusion protein of clause 18, wherein X is valine.

Clause 21. The fusion protein of any one of the preceding clauses, further comprising at least one linker positioned between the at least one binding polypeptide and the at least one unstructured polypeptide.

Clause 22. The fusion protein of clause 21, wherein the fusion protein comprises a plurality of linkers between the at least one binding polypeptide and the at least one unstructured polypeptide.

Clause 23. The fusion protein of any one of the preceding clauses, wherein the at least one binding polypeptide is positioned N-terminal to the at least one unstructured polypeptide.

Clause 24. The fusion protein of any one of clauses 1-23 wherein the at least one binding polypeptide is positioned C-terminal to the at least one unstructured polypeptide.

Clause 25. The fusion protein of any one of the preceding clauses, wherein the at least one unstructured polypeptide has a LCST between about 0° C. and about 100° C.

Clause 26. The fusion protein of any one of the preceding clauses, wherein the at least one unstructured polypeptide has a UCST between about 0° C. and about 100° C.

Clause 27. A method for treating a disease in a subject in need thereof, the method comprising administering to the subject an effective amount of the fusion protein according to any one of the preceding clauses.

Clause 28. The method of clause 27, wherein the fusion protein is administered in a controlled release formulation.

Clause 29. The method of clause 27, wherein the fusion protein forms a depot upon administration to the subject.

Clause 30. The method of any one of clauses 27-28, wherein the fusion protein is administered intravenously, intraarterially, or intraperitoneally to the subject.

Clause 31. The method of any one of clauses 27-30, wherein the disease comprises cancer.

Clause 32. The method of clause 31, wherein the fusion protein is administered intratumorally.

Clause 33. The method of any one of clauses 27-32, wherein the cancer is colorectal adenocarcinoma.

Clause 34. The method of any one of clauses 27-33, wherein the at least one binding polypeptide comprises an FnIII domain or a plurality of FnIII domains, and wherein the disease is a disease associated with TRAILR-2.

Clause 35. The method of any one of clauses 27-34, wherein the disease is a disease associated with a target of the at least one binding polypeptide.

Clause 36. A multivalent fusion protein comprising at least one Fibronectin type III (FnIII) domain and at least one elastin-like polypeptide (ELP), wherein the FnIII domain binds TNF-related apoptosis-inducing ligand receptor 2 (TRAILR-2).

Clause 37. The multivalent fusion protein of clause 36, wherein the at least one ELP comprises an amino acid sequence consisting of $(VPGXG)_n$ (SEQ ID NO: 19), wherein X is any amino acid except proline and n is an integer greater than or equal to 1.

Clause 38. The multivalent fusion protein of clause 37, wherein n is 60, 120, or 180.

Clause 39. The multivalent fusion protein of clause 37, wherein X is valine.

Clause 40. The multivalent fusion protein of any one of clauses 36-39, wherein the at least one FnIII domain comprises an amino acid sequence consisting of SEQ ID NO: 1.

Clause 41. The multivalent fusion protein of any one of clauses 36-40, wherein the multivalent fusion protein comprises a plurality of FnIII domains.

Clause 42. The multivalent fusion protein of clause 41, wherein the multivalent fusion protein comprises 2, 4, or 6 FnIII domains.

Clause 43. The multivalent fusion protein of clause 41 or 42, wherein the multivalent fusion protein further comprises a linker positioned between at least two adjacent FnIII domains.

Clause 44. The multivalent fusion protein of clause 43, wherein the linker comprises at least one glycine and at least one serine.

Clause 45. The multivalent fusion protein of clause 44, wherein the linker comprises an amino acid sequence consisting of SEQ ID NO: 3 ($(Gly_4Ser)_3$).

Clause 46. The multivalent fusion protein of clause 43, wherein the linker comprises an amino acid sequence consisting of SEQ ID NO: 4.

Clause 47. A method for treating a disease associated with TNF-related apoptosis-inducing ligand receptor 2 (TRAILR-2) in a subject in need thereof, the method comprising administering to the subject an effective amount of the multivalent fusion protein of any one of clauses 36-46.

Clause 48. The method of clause 47, wherein the disease comprises cancer.

Clause 49. The method of clause 48, wherein the cancer comprises colorectal adenocarcinoma.

Clause 50. The method of any one of clauses 47-49, wherein the multivalent fusion protein is administered intravenously, intraarterially, or intraperitoneally to the subject.

Clause 51. The method of any one of clauses 48-49, wherein the multivalent fusion protein is administered intratumorally.

Clause 52. The method of any one of clauses 47-51, wherein the multivalent fusion protein forms a depot upon administration to the subject.

Clause 53. The method of any one of clauses 47-51, wherein the multivalent fusion protein is administered in a controlled release formulation.

Clause 54. A method of diagnosing a disease in a subject, the method comprising contacting a sample from the subject with the fusion protein according to any one of clauses 1-26; and detecting binding of the fusion protein to a target to determine presence of the target in the sample, wherein the presence of the target in the sample indicates the disease in the subject.

Clause 55. The method of clause 54, wherein the disease is selected from cancer, metabolic disease, autoimmune disease, cardiovascular disease, and orthopedic disorder.

Clause 56. A method of determining the presence of a target in a sample, the method comprising contacting the sample with the fusion protein of any one of clauses 1-26 under conditions to allow a complex to form between the fusion protein and the target in the sample; and detecting the presence of the complex, wherein presence of the complex is indicative of the target in the sample.

Clause 57. The method of clause 56, wherein the sample is obtained from a subject and the method further comprises diagnosing a disease, prognosticating, or assessing the efficacy of a treatment of the subject.

Clause 58. The method of clause 57, wherein when the method further comprises assessing the efficacy of a treatment of the subject, then the method further comprises modifying the treatment of the subject as needed to improve efficacy.

Clause 59. A method of determining the effectiveness of a treatment for a disease in a subject in need thereof, the method comprising contacting a sample from the subject with the fusion protein of any one of clauses 1-26 under conditions to allow a complex to form between the fusion protein and a target in the sample; determining the level of the complex in the sample, wherein the level of the complex is indicative of the level of the target in the sample; and comparing the level of the target in the sample to a control level of the target, wherein if the level of the target is different from the control level, then the treatment is determined to be effective or ineffective in treating the disease.

Clause 60. A method of diagnosing a disease in a subject, the method comprising: contacting a sample from the subject with the fusion protein of any one of clauses 1-26; determining the level of a target in the sample; and comparing the level of the target in the sample to a control level of the target, wherein a level of the target different from the control level indicates disease in the subject.

Clause 61. The method of clause 59 or 60, wherein the control level corresponds to the level in the subject at a time point before or during the period when the subject has begun treatment, and wherein the sample is taken from the subject at a later time point.

Clause 62. The method of clause 59 or 60, wherein the sample is taken from the subject at a time point during the period when the subject is undergoing treatment, and wherein the control level corresponds to a disease-free level or to the level at a time point before the period when the subject has begun treatment.

Clause 63. The method of any one of clauses 59 and 61-62, the method further comprising modifying the treatment or administering a different treatment to the subject when the treatment is determined to be ineffective in treating the disease.

Clause 64. The method of any one of clauses 54-63, wherein the fusion protein is labeled with a reporter.

Clause 65. The method of any one of clauses 54-64, wherein the disease is selected from cancer, metabolic disease, autoimmune disease, cardiovascular disease, and orthopedic disorder.

SEQUENCES

SEQ ID NO: 1
TRAILR2-Specific Tn3, polypeptide
GAIEVKDVTDTTALITWAKPWVDPPPLWGCELTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPDT
EYEVSLICFDPYGMRSKPAKETFTT

SEQ ID NO: 2
TRAILR2-Specific Tn3 Sequence without Cysteines, polypeptide
GAIEVKDVTDTTALITWAKPWVDPPPLWGIELTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPDTE
YEVSLISFDPYGMRSKPAKETFTT

SEQ ID NO: 3
Flexible GlySer Linker, polypeptide
GGGGSGGGGSGGGGS

-continued

SEQUENCES

SEQ ID NO: 4
Proline-Containing Linker, polypeptide
PQPQPKPQPKPEPEPQPQG

SEQ ID NO: 5
EGFR-Binding Domain, polypeptide
GVDNKFNKEMWAAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAQAPKG

SEQ ID NO: 6
His-8 Tag, polypeptide
HHHHHHHH

SEQ ID NO: 7
ELP A, polypeptide
VGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVP
GVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGV
PGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVG
VPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGV
GVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPG
VGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVP
GVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGV
PGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVG
VPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGV
GVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPG
VGVPG

SEQ ID NO: 8
ELP B, polypeptide
VPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPG
AGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGV
PGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGG
GVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVP
GGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAG
VPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPG
AGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGV
PGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGG
GVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVP
GGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAG
VPGGGVPGAGVPGGGVPGAG

SEQ ID NO: 9
ELP C, polypeptide
VGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVP
GVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGV
PGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVG
VPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGV
GVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPG
VGVPG

SEQ ID NO: 10
ELPa-(Tn3)$_6$, polypeptide
VGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVP
GVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGV
PGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVG
VPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGV
GVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPG
VGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGV
PGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVG
VPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGV
GVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPG
VGVPGVGVPGAIEVKDVTDTTALITWAKPWVDPPPLWGCELTYGIKDVPGDRTTIDLQQKHTA
YSIGNLKPDTEYEVSLICFDPYGMRSKPAKETFTTGGGGSGGGGSGGGGSGAIEVKDVTDTTA
LITWAKPWVDPPPLWGCELTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPDTEYEVSLICFDPYG
MRSKPAKETFTTGGGGSGGGGSGGGGSGAIEVKDVTDTTALITWAKPWVDPPPLWGCELTY
GIKDVPGDRTTIDLQQKHTAYSIGNLKPDTEYEVSLICFDPYGMRSKPAKETFTTGGGGSGGG
GSGGGGSGAIEVKDVTDTTALITWAKPWVDPPPLWGCELTYGIKDVPGDRTTIDLQQKHTAYSI
GNLKPDTEYEVSLICFDPYGMRSKPAKETFTTGGGGSGGGGSGGGGSGAIEVKDVTDTTALIT
WAKPWVDPPPLWGCELTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPDTEYEVSLICFDPYGMR
SKPAKETFTTGGGGSGGGGSGGGGSGAIEVKDVTDTTALITWAKPWVDPPPLWGCELTYGIK
DVPGDRTTIDLQQKHTAYSIGNLKPDTEYEVSLICFDPYGMRSKPAKETFTTGGGGSGGGGSG
GGGS

SEQ ID NO: 11
ELPb-(Tn3)$_6$, polypeptide
VPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPG
AGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGV

-continued

SEQUENCES

PGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGG
GVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVP
GGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAG
VPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPG
AGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGV
PGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGG
GVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVP
GGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAGVPGGGVPGAG
VPGGGVPGAGVPGGGVPGAGAIEVKDVTDTTALITWAKPWVDPPPLWGCELTYGIKDVPGDR
TTIDLQQKHTAYSIGNLKPDTEYEVSLICFDPYGMRSKPAKETFTTGGGGSGGGGSGGGGSGA
IEVKDVTDTTALITWAKPWVDPPPLWGCELTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPDTEYE
VSLICFDPYGMRSKPAKETFTTGGGGSGGGGSGGGGSGAIEVKDVTDTTALITWAKPWVDPP
PLWGCELTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPDTEYEVSLICFDPYGMRSKPAKETFTT
GGGGSGGGGSGGGGSGAIEVKDVTDTTALITWAKPWVDPPPLWGCELTYGIKDVPGDRTTID
LQQKHTAYSIGNLKPDTEYEVSLICFDPYGMRSKPAKETFTTGGGGSGGGGSGGGGSGAIEVK
DVTDTTALITWAKPWVDPPPLWGCELTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPDTEYEVSLI
CFDPYGMRSKPAKETFTTGGGGSGGGGSGGGGSGAIEVKDVTDTTALITWAKPWVDPPPLW
GCELTYGIKDVPGDRTTIDLQQKHTAYSIGNLKPDTEYEVSLICFDPYGMRSKPAKETFTTGGG
GSGGGGSGGGGS

SEQ ID NO: 12
pet24 Vector, polynucleotide
TGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGC
GCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTT
CCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAG
GGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTC
ACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTC
TTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTT
GATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAA
ATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTCAGGTGGCACTTTTCGGGGAA
ATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGA
ATTAATTCTTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTA
TCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTT
CCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAA
CCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGAC
TGAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAG
CCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTGCGC
CTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGC
AACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTT
CTAATACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGG
AGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTG
ACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGG
CGCATCGGGCTTCCCATACAATCGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGA
GCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTAGAGCAAGA
CGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTT
TTATTGTTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGT
AGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAA
CAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTT
TCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCG
TAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCC
TGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGAC
GATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCC
AGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGC
GCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAAC
AGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGG
GTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCT
ATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCT
CACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTG
AGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAG
CGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCAT
ATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATACACTCC
GCTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACACCCGCCAACACCCGCTGACG
CGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCG
GGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGGCAGCTGCGGT
AAAGCTCATCAGCGTGGTCGTGAAGCGATTCACAGATGTCTGCCTGTTCATCCGCGTCCA
GCTCGTTGAGTTTCTCCAGAAGCGTTAATGTCTGGCTTCTGATAAAGCGGGCCATGTTAAG
GGCGGTTTTTTCCTGTTTGGTCACTGATGCCTCCGTGTAAGGGGGATTTCTGTTCATGGGG
GTAATGATACCGATGAAACGAGAGAGGATGCTCACGATACGGGTTACTGATGATGAACATG
CCCGGTTACTGGAACGTTGTGAGGGTAAACAACTGGCGGTATGGATGCGGCGGGACCAG
AGAAAAATCACTCAGGGTCAATGCCAGCGCTTCGTTAATACAGATGTAGGTGTTCCACAGG
GTAGCCAGCAGCATCCTGCGATGCAGATCCGGAACATAATGGTGCAGGGCGCTGACTTCC
GCGTTTCCAGACTTTACGAAACACGGAAACCGAAGACCATTCATGTTGTTGCTCAGGTCGC
AGACGTTTTGCAGCAGCAGTCGCTTCACGTTCGCTCGCGTATCGGTGATTCATTCTGCTAA
CCAGTAAGGCAACCCCGCCAGCCTAGCCGGGTCCTCAACGACAGGAGCACGATCATGCG
CACCCGTGGGGCCGCCATGCCGGCGATAATGGCCTGCTTCTCGCCGAAACGTTTGGTGG
CGGGACCAGTGACGAAGGCTTGAGCGAGGGCGTGCAAGATTCCGAATACCGCAAGCGAC
AGGCCGATCATCGTCGCGCTCCAGCGAAAGCGGTCCTCGCCGAAAATGACCCAGAGCGC

-continued

SEQUENCES

TGCCGGCACCTGTCCTACGAGTTGCATGATAAAGAAGACAGTCATAAGTGCGGCGACGAT
AGTCATGCCCCGCGCCCACCGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGCATCG
GTCGAGATCCCGGTGCCTAATGAGTGAGCTAACTTACATTAATTGCGTTGCGCTCACTGCC
CGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGG
GAGAGGCGGTTTGCGTATTGGGCGCCAGGGTGGTTTTTCTTTTCACCAGTGAGACGGGCA
ACAGCTGATTGCCCTTCACCGCCTGGCCCTGAGAGAGTTGCAGCAAGCGGTCCACGCTGG
TTTGCCCCAGCAGGCGAAAATCCTGTTTGATGGTGGTTAACGGCGGGATATAACATGAGCT
GTCTTCGGTATCGTCGTATCCCACTACCGAGATATCCGCACCAACGCGCAGCCCGGACTC
GGTAATGGCGCGCATTGCGCCCAGCGCCATCTGATCGTTGGCAACCAGCATCGCAGTGG
GAACGATGCCCTCATTCAGCATTTGCATGGTTTGTTGAAAACCGGACATGGCACTCCAGTC
GCCTTCCCGTTCCGCTATCGGCTGAATTTGATTGCGAGTGAGATATTTATGCCAGCCAGCC
AGACGCAGACGCGCCGAGACAGAACTTAATGGGCCCGCTAACAGCGCGATTTGCTGGTGA
CCCAATGCGACCAGATGCTCCACGCCCAGTCGCGTACCGTCTTCATGGGAGAAAATAATA
CTGTTGATGGGTGTCTGGTCAGAGACATCAAGAAATAACGCCGGAACATTAGTGCAGGCA
GCTTCCACAGCAATGGCATCCTGGTCATCCAGCGGATAGTTAATGATCAGCCCACTGACG
CGTTGCGCGAGAAGATTGTGCACCGCCGCTTTACAGGCTTCGACGCCGCTTCGTTCTACC
ATCGACACCACCACGCTGGCACCCAGTTGATCGGCGCGAGATTTAATCGCCGCGACAATT
TGCGACGGCGCGTGCAGGGCCAGACTGGAGGTGGCAACGCCAATCAGCAACGACTGTTT
GCCCGCCAGTTGTTGTGCCACGCGGTTGGGAATGTAATTCAGCTCCGCCATCGCCGCTTC
CACTTTTTCCCGCGTTTTCGCAGAAACGTGGCTGGCCTGGTTCACCACGCGGGAAACGGT
CTGATAAGAGACACCGGCATACTCTGCGACATCGTATAACGTTACTGGTTTCACATTCACC
ACCCTGAATTGACTCTCTTCCGGGCGCTATCATGCCATACCGCGAAAGGTTTTGCGCCATT
CGATGGTGTCCGGGATCTCGACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCCC
AGTAGTAGGTTGAGGCCGTTGAGCACCGCCGCCGCAAGGAATGGTGCATGCAAGGAGAT
GGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAACAAGCGC
TCATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGC
CAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGGATC
GAGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAA
TTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGGAGTACATATGGGCTGATGATA
ATGATCTTCAGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACC
ACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTG
CTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGG
GTTTTTTGCTGAAAGGAGGAACTATATCCGGAT

SEQ ID NO: 13
Tn3 G-block, polynucleotide
TAAGAAGGAGGAGTACATATGGGCGCTATCGAAGTTAAAGACGTTACCGACACCACCGCT
CTGATCACCTGGGCTAAACCGTGGGTTGACCCGCCGCCGCTGTGGGGTTGCGAACTGAC
CTACGGTATCAAAGACGTTCCGGGTGACCGTACCACCATCGACCTGCAGCAGAAACACAC
CGCTTACTCTATCGGTAACCTGAAACCGGACACCGAATACGAAGTTTCTCTGATCTGCTTC
GACCCGTACGGTATGCGTTCTAAACCGGCTAAAGAAACCTTCACCACCGGTGGTGGTGGT
TCTGGTGGTGGTGGTTCTGGTGGTGGTGGTTCTGGCATATGTACTCCTCCTTA

SEQ ID NO: 14
EGFR Binding Domain G-block, polynucleotide
AGAAATAATTTTGTTTAACTTTAAGAAGGAGGAGTACATATGGGCGTTGATAACAAATTCAA
TAAAGAAATGTGGGCAGCCTGGGAAGAAATTCGTAACCTGCCGAACCTGAATGGTTGGCA
AATGACCGCCTTCATTGCGAGCCTGGTGGATGATCCGAGCCAAAGCGCTAATCTGCTGGC
GGAAGCGAAAAAACTGAACGACGCCCAAGCCCCGAAAGGCTGATAATAATGATCTTCAGG
ATCCGAATTCGAGCTCCGTC

SEQ ID NO: 15
Tn3 Forward Amplification Primer, polynucleotide
TAAGAAGGAGGAGTACATATGGGCGC

SEQ ID NO: 16
Tn3 Reverse Amplification Primer, polynucleotide
TAAGGAGGAGTACATATGCCAGAACCAC

SEQ ID NO: 17
Linker, polypeptide
RGDS

SEQ ID NO: 18
A PG motif, polypeptide, wherein X is any amino acid and n is an integer from 1 to 15
$P(X)_nG$

SEQ ID NO: 19
ELP repeat, polypeptide, wherein X is any amino acid except proline and n is an integer greater than or equal to 1
$(VPGXG)_n$

SEQ ID NO: 20
A PG motif, polypeptide, wherein U, X, and Z are independently any amino acid and m, n, and p are independently an integer from 1 to 15
$(U)_mP(X)_nG(Z)_p$

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 20

<210> SEQ ID NO 1
<211> LENGTH: 89
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 1

Gly Ala Ile Glu Val Lys Asp Val Thr Asp Thr Thr Ala Leu Ile Thr
1 5 10 15

Trp Ala Lys Pro Trp Val Asp Pro Pro Pro Leu Trp Gly Cys Glu Leu
20 25 30

Thr Tyr Gly Ile Lys Asp Val Pro Gly Asp Arg Thr Thr Ile Asp Leu
35 40 45

Gln Gln Lys His Thr Ala Tyr Ser Ile Gly Asn Leu Lys Pro Asp Thr
50 55 60

Glu Tyr Glu Val Ser Leu Ile Cys Phe Asp Pro Tyr Gly Met Arg Ser
65 70 75 80

Lys Pro Ala Lys Glu Thr Phe Thr Thr
85

<210> SEQ ID NO 2
<211> LENGTH: 89
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 2

Gly Ala Ile Glu Val Lys Asp Val Thr Asp Thr Thr Ala Leu Ile Thr
1 5 10 15

Trp Ala Lys Pro Trp Val Asp Pro Pro Pro Leu Trp Gly Ile Glu Leu
20 25 30

Thr Tyr Gly Ile Lys Asp Val Pro Gly Asp Arg Thr Thr Ile Asp Leu
35 40 45

Gln Gln Lys His Thr Ala Tyr Ser Ile Gly Asn Leu Lys Pro Asp Thr
50 55 60

Glu Tyr Glu Val Ser Leu Ile Ser Phe Asp Pro Tyr Gly Met Arg Ser
65 70 75 80

Lys Pro Ala Lys Glu Thr Phe Thr Thr
85

<210> SEQ ID NO 3
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 3

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> SEQ ID NO 4
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

-continued

<400> SEQUENCE: 4

Pro Gln Pro Gln Pro Lys Pro Gln Pro Lys Pro Glu Pro Glu Pro Gln
1 5 10 15

Pro Gln Gly

<210> SEQ ID NO 5
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 5

Gly Val Asp Asn Lys Phe Asn Lys Glu Met Trp Ala Ala Trp Glu Glu
1 5 10 15

Ile Arg Asn Leu Pro Asn Leu Asn Gly Trp Gln Met Thr Ala Phe Ile
20 25 30

Ala Ser Leu Val Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu
35 40 45

Ala Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys Gly
50 55 60

<210> SEQ ID NO 6
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 6

His His His His His His His His
1 5

<210> SEQ ID NO 7
<211> LENGTH: 600
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 7

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
1 5 10 15

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
20 25 30

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
35 40 45

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
50 55 60

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
65 70 75 80

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
85 90 95

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
100 105 110

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
115 120 125

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
130 135 140

-continued

```
Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
145                 150                 155                 160

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
                165                 170                 175

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            180                 185                 190

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        195                 200                 205

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
    210                 215                 220

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
225                 230                 235                 240

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
                245                 250                 255

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            260                 265                 270

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        275                 280                 285

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
    290                 295                 300

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
305                 310                 315                 320

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
                325                 330                 335

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            340                 345                 350

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        355                 360                 365

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
    370                 375                 380

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
385                 390                 395                 400

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
                405                 410                 415

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            420                 425                 430

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        435                 440                 445

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
    450                 455                 460

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
465                 470                 475                 480

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
                485                 490                 495

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            500                 505                 510

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        515                 520                 525

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
    530                 535                 540

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
545                 550                 555                 560

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
```

-continued

```
               565                 570                 575

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            580                 585                 590

Val Pro Gly Val Gly Val Pro Gly
        595                 600


<210> SEQ ID NO 8
<211> LENGTH: 600
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 8

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
1               5                   10                  15

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            20                  25                  30

Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly
        35                  40                  45

Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly
    50                  55                  60

Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly
65                  70                  75                  80

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
                85                  90                  95

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            100                 105                 110

Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly
        115                 120                 125

Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly
    130                 135                 140

Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly
145                 150                 155                 160

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
                165                 170                 175

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            180                 185                 190

Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly
        195                 200                 205

Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly
    210                 215                 220

Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly
225                 230                 235                 240

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
                245                 250                 255

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            260                 265                 270

Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly
        275                 280                 285

Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly
    290                 295                 300

Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly
305                 310                 315                 320

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
```

-continued

```
                325                 330                 335

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            340                 345                 350

Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly
        355                 360                 365

Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly
    370                 375                 380

Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly
385                 390                 395                 400

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
                405                 410                 415

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            420                 425                 430

Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly
        435                 440                 445

Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly
    450                 455                 460

Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly
465                 470                 475                 480

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
                485                 490                 495

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            500                 505                 510

Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly
        515                 520                 525

Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly
    530                 535                 540

Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly
545                 550                 555                 560

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
                565                 570                 575

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            580                 585                 590

Gly Gly Gly Val Pro Gly Ala Gly
        595                 600


<210> SEQ ID NO 9
<211> LENGTH: 300
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 9

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
1               5                   10                  15

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            20                  25                  30

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        35                  40                  45

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
    50                  55                  60

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
65                  70                  75                  80

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
```

-continued

```
                85                  90                  95

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            100                 105                 110

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        115                 120                 125

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
    130                 135                 140

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
145                 150                 155                 160

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
                165                 170                 175

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            180                 185                 190

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        195                 200                 205

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
    210                 215                 220

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
225                 230                 235                 240

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
                245                 250                 255

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            260                 265                 270

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        275                 280                 285

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
    290                 295                 300


<210> SEQ ID NO 10
<211> LENGTH: 1223
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 10

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
1               5                   10                  15

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            20                  25                  30

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        35                  40                  45

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
    50                  55                  60

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
65                  70                  75                  80

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
                85                  90                  95

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            100                 105                 110

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        115                 120                 125

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
    130                 135                 140

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
```

-continued

```
145                 150                 155                 160

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
                165                 170                 175

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            180                 185                 190

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        195                 200                 205

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
    210                 215                 220

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
225                 230                 235                 240

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
                245                 250                 255

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            260                 265                 270

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        275                 280                 285

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
    290                 295                 300

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
305                 310                 315                 320

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
                325                 330                 335

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            340                 345                 350

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        355                 360                 365

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
    370                 375                 380

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
385                 390                 395                 400

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
                405                 410                 415

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            420                 425                 430

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        435                 440                 445

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
    450                 455                 460

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
465                 470                 475                 480

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
                485                 490                 495

Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            500                 505                 510

Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val
        515                 520                 525

Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro
    530                 535                 540

Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly
545                 550                 555                 560

Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val
                565                 570                 575
```

-continued

```
Gly Val Pro Gly Val Gly Val Pro Gly Val Gly Val Pro Gly Val Gly
            580                 585                 590

Val Pro Gly Val Gly Val Pro Gly Ala Ile Glu Val Lys Asp Val Thr
        595                 600                 605

Asp Thr Thr Ala Leu Ile Thr Trp Ala Lys Pro Trp Val Asp Pro Pro
    610                 615                 620

Pro Leu Trp Gly Cys Glu Leu Thr Tyr Gly Ile Lys Asp Val Pro Gly
625                 630                 635                 640

Asp Arg Thr Thr Ile Asp Leu Gln Gln Lys His Thr Ala Tyr Ser Ile
                645                 650                 655

Gly Asn Leu Lys Pro Asp Thr Glu Tyr Glu Val Ser Leu Ile Cys Phe
            660                 665                 670

Asp Pro Tyr Gly Met Arg Ser Lys Pro Ala Lys Glu Thr Phe Thr Thr
        675                 680                 685

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
    690                 695                 700

Ala Ile Glu Val Lys Asp Val Thr Asp Thr Thr Ala Leu Ile Thr Trp
705                 710                 715                 720

Ala Lys Pro Trp Val Asp Pro Pro Pro Leu Trp Gly Cys Glu Leu Thr
                725                 730                 735

Tyr Gly Ile Lys Asp Val Pro Gly Asp Arg Thr Thr Ile Asp Leu Gln
            740                 745                 750

Gln Lys His Thr Ala Tyr Ser Ile Gly Asn Leu Lys Pro Asp Thr Glu
        755                 760                 765

Tyr Glu Val Ser Leu Ile Cys Phe Asp Pro Tyr Gly Met Arg Ser Lys
    770                 775                 780

Pro Ala Lys Glu Thr Phe Thr Thr Gly Gly Gly Gly Ser Gly Gly Gly
785                 790                 795                 800

Gly Ser Gly Gly Gly Gly Ser Gly Ala Ile Glu Val Lys Asp Val Thr
                805                 810                 815

Asp Thr Thr Ala Leu Ile Thr Trp Ala Lys Pro Trp Val Asp Pro Pro
            820                 825                 830

Pro Leu Trp Gly Cys Glu Leu Thr Tyr Gly Ile Lys Asp Val Pro Gly
        835                 840                 845

Asp Arg Thr Thr Ile Asp Leu Gln Gln Lys His Thr Ala Tyr Ser Ile
    850                 855                 860

Gly Asn Leu Lys Pro Asp Thr Glu Tyr Glu Val Ser Leu Ile Cys Phe
865                 870                 875                 880

Asp Pro Tyr Gly Met Arg Ser Lys Pro Ala Lys Glu Thr Phe Thr Thr
                885                 890                 895

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
            900                 905                 910

Ala Ile Glu Val Lys Asp Val Thr Asp Thr Thr Ala Leu Ile Thr Trp
        915                 920                 925

Ala Lys Pro Trp Val Asp Pro Pro Pro Leu Trp Gly Cys Glu Leu Thr
    930                 935                 940

Tyr Gly Ile Lys Asp Val Pro Gly Asp Arg Thr Thr Ile Asp Leu Gln
945                 950                 955                 960

Gln Lys His Thr Ala Tyr Ser Ile Gly Asn Leu Lys Pro Asp Thr Glu
                965                 970                 975

Tyr Glu Val Ser Leu Ile Cys Phe Asp Pro Tyr Gly Met Arg Ser Lys
            980                 985                 990
```

-continued

```
Pro Ala Lys Glu Thr Phe Thr Thr  Gly Gly Gly Gly Ser  Gly Gly Gly
        995                1000                1005

Gly Ser  Gly Gly Gly Gly Ser  Gly Ala Ile Glu Val  Lys Asp Val
    1010                1015                1020

Thr Asp  Thr Thr Ala Leu Ile  Thr Trp Ala Lys Pro  Trp Val Asp
    1025                1030                1035

Pro Pro  Pro Leu Trp Gly Cys  Glu Leu Thr Tyr Gly  Ile Lys Asp
    1040                1045                1050

Val Pro  Gly Asp Arg Thr Thr  Ile Asp Leu Gln Gln  Lys His Thr
    1055                1060                1065

Ala Tyr  Ser Ile Gly Asn Leu  Lys Pro Asp Thr Glu  Tyr Glu Val
    1070                1075                1080

Ser Leu  Ile Cys Phe Asp Pro  Tyr Gly Met Arg Ser  Lys Pro Ala
    1085                1090                1095

Lys Glu  Thr Phe Thr Thr Gly  Gly Gly Gly Ser Gly  Gly Gly Gly
    1100                1105                1110

Ser Gly  Gly Gly Gly Ser Gly  Ala Ile Glu Val Lys  Asp Val Thr
    1115                1120                1125

Asp Thr  Thr Ala Leu Ile Thr  Trp Ala Lys Pro Trp  Val Asp Pro
    1130                1135                1140

Pro Pro  Leu Trp Gly Cys Glu  Leu Thr Tyr Gly Ile  Lys Asp Val
    1145                1150                1155

Pro Gly  Asp Arg Thr Thr Ile  Asp Leu Gln Gln Lys  His Thr Ala
    1160                1165                1170

Tyr Ser  Ile Gly Asn Leu Lys  Pro Asp Thr Glu Tyr  Glu Val Ser
    1175                1180                1185

Leu Ile  Cys Phe Asp Pro Tyr  Gly Met Arg Ser Lys  Pro Ala Lys
    1190                1195                1200

Glu Thr  Phe Thr Thr Gly Gly  Gly Gly Ser Gly Gly  Gly Gly Ser
    1205                1210                1215

Gly Gly  Gly Gly Ser
    1220


<210> SEQ ID NO 11
<211> LENGTH: 1223
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 11

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
1               5                  10                  15

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            20                  25                  30

Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly
        35                  40                  45

Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly
    50                  55                  60

Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly
65                  70                  75                  80

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
                85                  90                  95

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            100                 105                 110
```

-continued

```
Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly
        115                 120                 125

Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly
    130                 135                 140

Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly
145                 150                 155                 160

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
                165                 170                 175

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            180                 185                 190

Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly
        195                 200                 205

Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly
    210                 215                 220

Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly
225                 230                 235                 240

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
                245                 250                 255

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            260                 265                 270

Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly
        275                 280                 285

Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly
    290                 295                 300

Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly
305                 310                 315                 320

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
                325                 330                 335

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            340                 345                 350

Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly
        355                 360                 365

Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly
    370                 375                 380

Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly
385                 390                 395                 400

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
                405                 410                 415

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            420                 425                 430

Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly
        435                 440                 445

Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly
    450                 455                 460

Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly
465                 470                 475                 480

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
                485                 490                 495

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            500                 505                 510

Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly
        515                 520                 525

Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly
```

-continued

```
    530                 535                 540

Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly
545                 550                 555                 560

Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro Gly Gly Gly Val
                565                 570                 575

Pro Gly Ala Gly Val Pro Gly Gly Gly Val Pro Gly Ala Gly Val Pro
            580                 585                 590

Gly Gly Gly Val Pro Gly Ala Gly Ala Ile Glu Val Lys Asp Val Thr
        595                 600                 605

Asp Thr Thr Ala Leu Ile Thr Trp Ala Lys Pro Trp Val Asp Pro Pro
    610                 615                 620

Pro Leu Trp Gly Cys Glu Leu Thr Tyr Gly Ile Lys Asp Val Pro Gly
625                 630                 635                 640

Asp Arg Thr Thr Ile Asp Leu Gln Gln Lys His Thr Ala Tyr Ser Ile
                645                 650                 655

Gly Asn Leu Lys Pro Asp Thr Glu Tyr Glu Val Ser Leu Ile Cys Phe
            660                 665                 670

Asp Pro Tyr Gly Met Arg Ser Lys Pro Ala Lys Glu Thr Phe Thr Thr
        675                 680                 685

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
    690                 695                 700

Ala Ile Glu Val Lys Asp Val Thr Asp Thr Thr Ala Leu Ile Thr Trp
705                 710                 715                 720

Ala Lys Pro Trp Val Asp Pro Pro Pro Leu Trp Gly Cys Glu Leu Thr
                725                 730                 735

Tyr Gly Ile Lys Asp Val Pro Gly Asp Arg Thr Thr Ile Asp Leu Gln
            740                 745                 750

Gln Lys His Thr Ala Tyr Ser Ile Gly Asn Leu Lys Pro Asp Thr Glu
        755                 760                 765

Tyr Glu Val Ser Leu Ile Cys Phe Asp Pro Tyr Gly Met Arg Ser Lys
    770                 775                 780

Pro Ala Lys Glu Thr Phe Thr Thr Gly Gly Gly Gly Ser Gly Gly Gly
785                 790                 795                 800

Gly Ser Gly Gly Gly Gly Ser Gly Ala Ile Glu Val Lys Asp Val Thr
                805                 810                 815

Asp Thr Thr Ala Leu Ile Thr Trp Ala Lys Pro Trp Val Asp Pro Pro
            820                 825                 830

Pro Leu Trp Gly Cys Glu Leu Thr Tyr Gly Ile Lys Asp Val Pro Gly
        835                 840                 845

Asp Arg Thr Thr Ile Asp Leu Gln Gln Lys His Thr Ala Tyr Ser Ile
    850                 855                 860

Gly Asn Leu Lys Pro Asp Thr Glu Tyr Glu Val Ser Leu Ile Cys Phe
865                 870                 875                 880

Asp Pro Tyr Gly Met Arg Ser Lys Pro Ala Lys Glu Thr Phe Thr Thr
                885                 890                 895

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
            900                 905                 910

Ala Ile Glu Val Lys Asp Val Thr Asp Thr Thr Ala Leu Ile Thr Trp
        915                 920                 925

Ala Lys Pro Trp Val Asp Pro Pro Pro Leu Trp Gly Cys Glu Leu Thr
    930                 935                 940

Tyr Gly Ile Lys Asp Val Pro Gly Asp Arg Thr Thr Ile Asp Leu Gln
945                 950                 955                 960
```

```
Gln Lys His Thr Ala Tyr Ser Ile Gly Asn Leu Lys Pro Asp Thr Glu
                965                 970                 975

Tyr Glu Val Ser Leu Ile Cys Phe Asp Pro Tyr Gly Met Arg Ser Lys
            980                 985                 990

Pro Ala Lys Glu Thr Phe Thr Thr  Gly Gly Gly Gly Ser  Gly Gly Gly
        995                 1000                1005

Gly Ser  Gly Gly Gly Gly Ser  Gly Ala Ile Glu Val  Lys Asp Val
    1010                1015                1020

Thr Asp  Thr Thr Ala Leu Ile  Thr Trp Ala Lys Pro  Trp Val Asp
    1025                1030                1035

Pro Pro  Pro Leu Trp Gly Cys  Glu Leu Thr Tyr Gly  Ile Lys Asp
    1040                1045                1050

Val Pro  Gly Asp Arg Thr Thr  Ile Asp Leu Gln Gln  Lys His Thr
    1055                1060                1065

Ala Tyr  Ser Ile Gly Asn Leu  Lys Pro Asp Thr Glu  Tyr Glu Val
    1070                1075                1080

Ser Leu  Ile Cys Phe Asp Pro  Tyr Gly Met Arg Ser  Lys Pro Ala
    1085                1090                1095

Lys Glu  Thr Phe Thr Thr Gly  Gly Gly Gly Ser Gly  Gly Gly Gly
    1100                1105                1110

Ser Gly  Gly Gly Gly Ser Gly  Ala Ile Glu Val Lys  Asp Val Thr
    1115                1120                1125

Asp Thr  Thr Ala Leu Ile Thr  Trp Ala Lys Pro Trp  Val Asp Pro
    1130                1135                1140

Pro Pro  Leu Trp Gly Cys Glu  Leu Thr Tyr Gly Ile  Lys Asp Val
    1145                1150                1155

Pro Gly  Asp Arg Thr Thr Ile  Asp Leu Gln Gln Lys  His Thr Ala
    1160                1165                1170

Tyr Ser  Ile Gly Asn Leu Lys  Pro Asp Thr Glu Tyr  Glu Val Ser
    1175                1180                1185

Leu Ile  Cys Phe Asp Pro Tyr  Gly Met Arg Ser Lys  Pro Ala Lys
    1190                1195                1200

Glu Thr  Phe Thr Thr Gly Gly  Gly Gly Ser Gly Gly  Gly Gly Ser
    1205                1210                1215

Gly Gly  Gly Gly Ser
    1220


<210> SEQ ID NO 12
<211> LENGTH: 5298
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 12

tggcgaatgg gacgcgccct gtagcggcgc attaagcgcg gcgggtgtgg tggttacgcg     60

cagcgtgacc gctacacttg ccagcgccct agcgcccgct cctttcgctt tcttcccttc    120

ctttctcgcc acgttcgccg gctttccccg tcaagctcta aatcgggggc tccctttagg    180

gttccgattt agtgctttac ggcacctcga ccccaaaaaa cttgattagg gtgatggttc    240

acgtagtggg ccatcgccct gatagacggt ttttcgccct ttgacgttgg agtccacgtt    300

ctttaatagt ggactcttgt tccaaactgg aacaacactc aaccctatct cggtctattc    360

ttttgattta taagggattt tgccgatttc ggcctattgg ttaaaaaatg agctgattta    420
```

-continued

acaaaaattt aacgcgaatt ttaacaaaat attaacgttt acaatttcag gtggcacttt 480

tcggggaaat gtgcgcggaa cccctatttg tttatttttc taaatacatt caaatatgta 540

tccgctcatg aattaattct tagaaaaact catcgagcat caaatgaaac tgcaatttat 600

tcatatcagg attatcaata ccatattttt gaaaaagccg tttctgtaat gaaggagaaa 660

actcaccgag gcagttccat aggatggcaa gatcctggta tcggtctgcg attccgactc 720

gtccaacatc aatacaacct attaatttcc cctcgtcaaa aataaggtta tcaagtgaga 780

aatcaccatg agtgacgact gaatccggtg agaatggcaa aagtttatgc atttctttcc 840

agacttgttc aacaggccag ccattacgct cgtcatcaaa atcactcgca tcaaccaaac 900

cgttattcat tcgtgattgc gcctgagcga gacgaaatac gcgatcgctg ttaaaaggac 960

aattacaaac aggaatcgaa tgcaaccggc gcaggaacac tgccagcgca tcaacaatat 1020

tttcacctga atcaggatat tcttctaata cctggaatgc tgttttcccg gggatcgcag 1080

tggtgagtaa ccatgcatca tcaggagtac ggataaaatg cttgatggtc ggaagaggca 1140

taaattccgt cagccagttt agtctgacca tctcatctgt aacatcattg gcaacgctac 1200

ctttgccatg tttcagaaac aactctggcg catcgggctt cccatacaat cgatagattg 1260

tcgcacctga ttgcccgaca ttatcgcgag cccatttata cccatataaa tcagcatcca 1320

tgttggaatt taatcgcggc ctagagcaag acgtttcccg ttgaatatgg ctcataacac 1380

cccttgtatt actgtttatg taagcagaca gttttattgt tcatgaccaa aatcccttaa 1440

cgtgagtttt cgttccactg agcgtcagac cccgtagaaa agatcaaagg atcttcttga 1500

gatccttttt ttctgcgcgt aatctgctgc ttgcaaacaa aaaaaccacc gctaccagcg 1560

gtggtttgtt tgccggatca agagctacca actcttttte cgaaggtaac tggcttcagc 1620

agagcgcaga taccaaatac tgtccttcta gtgtagccgt agttaggcca ccacttcaag 1680

aactctgtag caccgcctac atacctcgct ctgctaatcc tgttaccagt ggctgctgcc 1740

agtggcgata agtcgtgtct taccgggttg gactcaagac gatagttacc ggataaggcg 1800

cagcggtcgg gctgaacggg gggttcgtgc acacagccca gcttggagcg aacgacctac 1860

accgaactga gatacctaca gcgtgagcta tgagaaagcg ccacgcttcc cgaagggaga 1920

aaggcggaca ggtatccggt aagcggcagg gtcggaacag gagagcgcac gagggagctt 1980

ccaggggaa acgcctggta tctttatagt cctgtcgggt ttcgccacct ctgacttgag 2040

cgtcgatttt tgtgatgctc gtcaggggg cggagcctat ggaaaaacgc cagcaacgcg 2100

gccttttac ggttcctggc cttttgctgg ccttttgctc acatgttctt tcctgcgtta 2160

tcccctgatt ctgtggataa ccgtattacc gcctttgagt gagctgatac cgctcgccgc 2220

agccgaacga ccgagcgcag cgagtcagtg agcgaggaag cggaagagcg cctgatgcgg 2280

tattttctcc ttacgcatct gtgcggtatt tcacaccgca tatatggtgc actctcagta 2340

caatctgctc tgatgccgca tagttaagcc agtatacact ccgctatcgc tacgtgactg 2400

ggtcatggct gcgccccgac acccgccaac acccgctgac gcgccctgac gggcttgtct 2460

gctcccggca tccgcttaca gacaagctgt gaccgtctcc gggagctgca tgtgtcagag 2520

gttttcaccg tcatcaccga aacgcgcgag gcagctgcgg taaagctcat cagcgtggtc 2580

gtgaagcgat tcacagatgt ctgcctgttc atccgcgtcc agctcgttga gtttctccag 2640

aagcgttaat gtctggcttc tgataaagcg ggccatgtta agggcggttt tttcctgttt 2700

ggtcactgat gcctccgtgt aagggggatt tctgttcatg ggggtaatga taccgatgaa 2760

acgagagagg atgctcacga tacgggttac tgatgatgaa catgcccggt tactggaacg 2820

-continued

ttgtgagggt aaacaactgg cggtatggat gcggcgggac cagagaaaaa tcactcaggg 2880

tcaatgccag cgcttcgtta atacagatgt aggtgttcca cagggtagcc agcagcatcc 2940

tgcgatgcag atccggaaca taatggtgca gggcgctgac ttccgcgttt ccagacttta 3000

cgaaacacgg aaaccgaaga ccattcatgt tgttgctcag gtcgcagacg ttttgcagca 3060

gcagtcgctt cacgttcgct cgcgtatcgg tgattcattc tgctaaccag taaggcaacc 3120

ccgccagcct agccgggtcc tcaacgacag gagcacgatc atgcgcaccc gtggggccgc 3180

catgccggcg ataatggcct gcttctcgcc gaaacgtttg gtggcgggac cagtgacgaa 3240

ggcttgagcg agggcgtgca agattccgaa taccgcaagc gacaggccga tcatcgtcgc 3300

gctccagcga aagcggtcct cgccgaaaat gacccagagc gctgccggca cctgtcctac 3360

gagttgcatg ataaagaaga cagtcataag tgcggcgacg atagtcatgc cccgcgccca 3420

ccggaaggag ctgactgggt tgaaggctct caagggcatc ggtcgagatc ccggtgccta 3480

atgagtgagc taacttacat taattgcgtt gcgctcactg cccgctttcc agtcgggaaa 3540

cctgtcgtgc cagctgcatt aatgaatcgg ccaacgcgcg gggagaggcg gtttgcgtat 3600

tgggcgccag ggtggttttt cttttcacca gtgagacggg caacagctga ttgcccttca 3660

ccgcctggcc ctgagagagt tgcagcaagc ggtccacgct ggtttgcccc agcaggcgaa 3720

aatcctgttt gatggtggtt aacggcggga tataacatga gctgtcttcg gtatcgtcgt 3780

atcccactac cgagatatcc gcaccaacgc gcagcccgga ctcggtaatg gcgcgcattg 3840

cgcccagcgc catctgatcg ttggcaacca gcatcgcagt gggaacgatg ccctcattca 3900

gcatttgcat ggtttgttga aaaccggaca tggcactcca gtcgccttcc cgttccgcta 3960

tcggctgaat ttgattgcga gtgagatatt tatgccagcc agccagacgc agacgcgccg 4020

agacagaact taatgggccc gctaacagcg cgatttgctg gtgacccaat gcgaccagat 4080

gctccacgcc cagtcgcgta ccgtcttcat gggagaaaat aatactgttg atgggtgtct 4140

ggtcagagac atcaagaaat aacgccggaa cattagtgca ggcagcttcc acagcaatgg 4200

catcctggtc atccagcgga tagttaatga tcagcccact gacgcgttgc gcgagaagat 4260

tgtgcaccgc cgctttacag gcttcgacgc cgcttcgttc taccatcgac accaccacgc 4320

tggcacccag ttgatcggcg cgagatttaa tcgccgcgac aatttgcgac ggcgcgtgca 4380

gggccagact ggaggtggca acgccaatca gcaacgactg tttgcccgcc agttgttgtg 4440

ccacgcggtt gggaatgtaa ttcagctccg ccatcgccgc ttccactttt tcccgcgttt 4500

tcgcagaaac gtggctggcc tggttcacca cgcgggaaac ggtctgataa gagacaccgg 4560

catactctgc gacatcgtat aacgttactg gtttcacatt caccaccctg aattgactct 4620

cttccgggcg ctatcatgcc ataccgcgaa aggttttgcg ccattcgatg gtgtccggga 4680

tctcgacgct ctcccttatg cgactcctgc attaggaagc agcccagtag taggttgagg 4740

ccgttgagca ccgccgccgc aaggaatggt gcatgcaagg agatggcgcc caacagtccc 4800

ccggccacgg ggcctgccac catacccacg ccgaaacaag cgctcatgag cccgaagtgg 4860

cgagcccgat cttccccatc ggtgatgtcg gcgatatagg cgccagcaac cgcacctgtg 4920

gcgccggtga tgccggccac gatgcgtccg gcgtagagga tcgagatctc gatcccgcga 4980

aattaatacg actcactata ggggaattgt gagcggataa caattcccct ctagaaataa 5040

ttttgtttaa ctttaagaag gaggagtaca tatgggctga tgataatgat cttcaggatc 5100

cgaattcgag ctccgtcgac aagcttgcgg ccgcactcga gcaccaccac caccaccact 5160

-continued

gagatccggc tgctaacaaa gcccgaaagg aagctgagtt ggctgctgcc accgctgagc 5220

aataactagc ataacccctt ggggcctcta aacgggtctt gaggggtttt ttgctgaaag 5280

gaggaactat atccggat 5298

<210> SEQ ID NO 13
<211> LENGTH: 353
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 13

taagaaggag gagtacatat gggcgctatc gaagttaaag acgttaccga caccaccgct 60

ctgatcacct gggctaaacc gtgggttgac ccgccgccgc tgtggggttg cgaactgacc 120

tacggtatca aagacgttcc gggtgaccgt accaccatcg acctgcagca gaaacacacc 180

gcttactcta tcggtaacct gaaaccggac accgaatacg aagtttctct gatctgcttc 240

gacccgtacg gtatgcgttc taaaccggct aaagaaacct tcaccaccgg tggtggtggt 300

tctggtggtg gtggttctgg tggtggtggt tctggcatat gtactcctcc tta 353

<210> SEQ ID NO 14
<211> LENGTH: 262
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 14

agaaataatt ttgtttaact ttaagaagga ggagtacata tgggcgttga taacaaattc 60

aataaagaaa tgtgggcagc ctgggaagaa attcgtaacc tgccgaacct gaatggttgg 120

caaatgaccg ccttcattgc gagcctggtg gatgatccga gccaaagcgc taatctgctg 180

gcggaagcga aaaaactgaa cgacgcccaa gccccgaaag gctgataata atgatcttca 240

ggatccgaat tcgagctccg tc 262

<210> SEQ ID NO 15
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 15

taagaaggag gagtacatat gggcgc 26

<210> SEQ ID NO 16
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 16

taaggaggag tacatatgcc agaaccac 28

<210> SEQ ID NO 17
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 17

Arg Gly Asp Ser
1

<210> SEQ ID NO 18
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: Xaa
<222> LOCATION: (2)..(16)
<223> OTHER INFORMATION: any amino acid wherein none, any one or all of amino acids at positions 2-16 can either be present or absent

<400> SEQUENCE: 18

Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Gly

<210> SEQ ID NO 19
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: Xaa
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: any amino acid except proline

<400> SEQUENCE: 19

Val Pro Gly Xaa Gly
1 5

<210> SEQ ID NO 20
<211> LENGTH: 47
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: Xaa
<222> LOCATION: (1)..(15)
<223> OTHER INFORMATION: any one or all of amino acid at positions 1-15 may either be present or absent
<220> FEATURE:
<221> NAME/KEY: Xaa
<222> LOCATION: (17)..(31)
<223> OTHER INFORMATION: any one or all of amino acid at positions 17-31 may either be present or absent
<220> FEATURE:
<221> NAME/KEY: Xaa
<222> LOCATION: (33)..(47)
<223> OTHER INFORMATION: any one or all of amino acid at positions 33-47 may either be present or absent

<400> SEQUENCE: 20

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Pro
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
35 40 45

We claim:

1. A multivalent fusion protein comprising at least one Fibronectin type III (FnIII) domain that binds TNF-related apoptosis-inducing ligand receptor 2 (TRAILR-2) and comprises SEQ ID NO: 1, and at least one elastin-like polypeptide (ELP) comprising $(VPGXG)_n$ (SEQ ID NO: 19), wherein X is any amino acid except proline and n is an integer greater than or equal to 1, and wherein the ELP has a transition temperature (T_t) of about 10° C. to about 50° C.

2. The multivalent fusion protein of claim 1, wherein n is 60, 120, or 180.

3. The multivalent fusion protein of claim1, wherein X is valine.

4. The multivalent fusion protein of claim 1, wherein the multivalent fusion protein comprises a plurality of the FnIII domain.

5. The multivalent fusion protein of claim 4, wherein the multivalent fusion protein comprises 2, 4, or 6 FnIII domains.

6. The multivalent fusion protein of claim 4, wherein the multivalent fusion protein further comprises a linker positioned between at least two adjacent FnIII domains.

7. The multivalent fusion protein of claim 6, wherein the linker comprises at least one glycine and at least one serine.

8. The multivalent fusion protein of claim 7, wherein the linker comprises an amino acid sequence consisting of SEQ ID NO: 3 $((Gly_4Ser)_3)$.

9. The multivalent fusion protein of claim 6, wherein the linker comprises an amino acid sequence consisting of SEQ ID NO: 4.

10. A method for treating cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of the multivalent fusion protein of claim 1.

11. The method of claim 10, wherein the cancer comprises colorectal adenocarcinoma.

12. The method of claim 10, wherein the multivalent fusion protein is administered intravenously, intraarterially, or intraperitoneally to the subject.

13. The method of claim 10, wherein the multivalent fusion protein is administered intratumorally.

14. The method of claim 10, wherein the multivalent fusion protein forms a depot upon administration to the subject.

15. The method of claim 10, wherein the multivalent fusion protein is administered in a controlled release formulation.

* * * * *