Claims

What is claimed:

1. A library comprising a plurality of fibronectin type III module (FN3) domains having a diversified C-CD-D-F-FG-G alternative surface comprising a diversified C beta-strand, a CD loop, a D beta-strand, an F beta-strand, an FG loop and a G beta-strand, wherein an FN3 domain of the plurality of FN3 domains comprises an amino acid sequence of:

MLSPPSNLRVTDVTSTSVTLSWKPPAPITGYXVXYXEXXX
XGEWKXVXVPGSETSYTVTGLKPGTEYXFXVXAVNGAXXG
XPSQXVXVTT (SEQ ID NO: 44)

or an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 44, wherein each X is, independently, any amino acid.

2. The library of claim 1, wherein each X is, independently, any amino acid, except a methionine or a cysteine.

3. The library of claims 1 or 2, wherein the FN3 domain comprises at least one mutated amino acid residue as compared to SEQ ID NO: 24 in one or more of, or each of, the C beta-strand, the CD loop, the D beta-strand, the F beta-strand, the FG loop, or the G beta-strand to form the FN3 domain library having the diversified C-CD-D-F-FG-G alternative surface.

4. The library of any one of claim 1-3, wherein the FN3 domain has one or more mutations (e.g. substitutions, insertions, or deletions) at a position that corresponds to positions 32, 34, 36, 38, 39, 40, 41, 46, 48, 68, 70, 72, 78, 79, 81, 85, and/or 87 of SEQ ID NO: 24.

5. The library of any one of claims 1-4, wherein:

the diversified C beta-strand has an amino acid sequence of TGYXVXYXE (SEQ ID NO: 45), wherein each X is, independently, any amino acid except a methionine or a cysteine;

the diversified CD loop has an amino acid sequence of XXXXGE (SEQ ID NO: 46), wherein each X is, independently, any amino acid except a methionine or a cysteine;

the diversified D beta-strand has an amino acid sequence of WKXVXVP (SEQ ID NO: 47), wherein each X is, independently, any amino acid except a methionine or a cysteine;

the diversified F beta-strand has an amino acid sequence of TEYXFXVXAV (SEQ ID NO: 48), wherein each X is, independently, any amino acid except a methionine or a cysteine;

the diversified FG loop has an amino acid sequence of NGAXXG (SEQ ID NO: 49), wherein each X is, independently, any amino acid except a methionine or a cysteine; or

the diversified G beta-strand has an amino acid sequence XPSQXVXVTT (SEQ ID NO: 50), wherein each X is, independently, any amino acid except a methionine or a cysteine.

6. The library of any one of claims 1-5, wherein the library comprises an amino acid sequence having an amino acid sequence that is at least, or about, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or is identical to a sequence selected from the group consisting of SEQ ID NOs: 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, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, and 43.

7. The library of any one of claims 1-6, wherein:

the diversified C beta-strand has an amino acid sequence of TGYXVXYXE (SEQ ID NO: 45), wherein each X is, independently, any amino acid except a methionine or a cysteine;

the diversified CD loop has an amino acid sequence of XXXXGE (SEQ ID NO: 46), wherein each X is, independently, any amino acid except a methionine or a cysteine;

the diversified D beta-strand has an amino acid sequence of WKXVXVP (SEQ ID NO: 47), wherein each X is, independently, any amino acid except a methionine or a cysteine;

the diversified F beta-strand has an amino acid sequence of TEYXFXVXAV (SEQ ID NO: 48), wherein each X is, independently, any amino acid except a methionine or a cysteine;

the diversified FG loop has an amino acid sequence of NGAXXG (SEQ ID NO: 49), wherein each X is, independently, any amino acid except a methionine or a cysteine; and

wherein the diversified G beta-strand has an amino acid sequence XPSQXVXVTT (SEQ ID NO: 50), wherein each X is, independently, any amino acid except a methionine or a cysteine.

8. A method of producing the library of any one of claims 1-7, the method comprising expressing a polynucleotide encoding the plurality of FN3 domains.

9. A method of making a library of fibronectin module of type III (FN3) domains having a diversified C-CD-F-FG-G alternative surface comprising a diversified one or more, or each of, C beta-strand, a CD loop, an F beta-strand, an FG loop and G-beta strand, comprising

a. providing a reference FN3 domain polypeptide having an amino acid sequence at least 80% identical to that of SEQ ID NO: 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, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44; and

b. introducing diversity into the reference FN3 domain polypeptide by mutating at least one residue in the C beta-strand, CD loop region, F beta-strand region, FG loop region, or G-beta strand region to form the FN3 domain library having the diversified C-CD-F-FG-G alternative surface, wherein:

the diversified C beta-strand has an amino acid sequence of TGYXVXYXE (SEQ ID NO: 45), wherein each X is, independently, any amino acid except a methionine or a cysteine;

the diversified CD loop has an amino acid sequence of XXXXGE (SEQ ID NO: 46), wherein each X is, independently, any amino acid except a methionine or a cysteine;

the diversified D beta-strand has an amino acid sequence of WKXVXVP (SEQ ID NO: 47), wherein each X is, independently, any amino acid except a methionine or a cysteine;

the diversified F beta-strand has an amino acid sequence of TEYXFXVXAV (SEQ ID NO: 48), wherein each X is, independently, is any amino acid except a methionine or a cysteine;

the diversified FG loop has an amino acid sequence of NGAXXG (SEQ ID NO: 49), wherein each X is, independently, any amino acid except a methionine or a cysteine; and

the diversified G beta-strand has an amino acid sequence XPSQXVXVTT (SEQ ID NO: 50), wherein each X is, independently, any amino acid except a methionine or a cysteine.

10. A library produced by the method of claims 8 or 9.

11. A method of obtaining a polypeptide comprising a fibronectin type III module (FN3) domain having a diversified C-CD-D-F-FG-G alternative surface that binds or specifically binds to a target molecule, comprising contacting the library of any one of claims 1-7 with the target molecule and isolating the polypeptide that binds or specifically binds to the target molecule.

12. A polypeptide having an amino acid sequence that is at least, or about, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or is identical to a sequence selected from the group consisting of SEQ ID NOs: 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, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, and 43.

13. A polypeptide comprising an amino acid sequence of :

MLSPPSNLRVTDVTSTSVTLSWKPPAPITGYXVXYXEXXX
XGEWKXVXVPGSETSYTVTGLKPGTEYXFXVXAVNGAXXG
XPSQXVXVTT (SEQ ID NO: 44)

wherein each X is, independently, any amino acid.

14. The polypeptide of claim 13, wherein each X is, independently, any amino acid, except methionine or cysteine.

15. A polypeptide comprising an amino acid sequence of :

LSPPSNLRVTDVTSTSVTLSWKPPAPITGYXVXYXEXXXX
GEWKXVXVPGSETSYTVTGLKPGTEYXFXVXAVNGAXXGX
PSQXVXVTT (SEQ ID NO: 74)

wherein each X is, independently, any amino acid.

16. The polypeptide of claim 15, wherein each X is, independently, any amino acid, except methionine or cysteine.

17. A pharmaceutical composition comprising the polypeptide of claims 12-16.

18. A nucleic acid molecule encoding the polypeptide of claim 17.

19. A plurality of nucleic acid molecules encoding the library of polypeptides of any one of claims 1-7.

20. A host cell comprising the nucleic acid molecule of claim 19.