CLAIMS

WHAT IS CLAIMED IS:

1. A recombinant gram-negative bacterial host cell for recombinant protein expression, wherein the host cell is:

(a) deficient in a first protease activity, wherein the first protease activity is tail-specific protease activity, wherein the deficient first protease activity results from a mutation in at least one gene encoding a tail-specific protease;

(b) deficient in a second protease activity, wherein the second protease activity is murein DD-endopeptidase activity, wherein the deficient second protease activity results from a mutation in at least one gene encoding a murein DD-endopeptidase.

2. The recombinant gram-negative bacterial host cell of claim 1, wherein the host cell further:

(c) is deficient in at least one additional protease activity, wherein the deficient additional protease activity results from a mutation in at least one gene encoding an additional protease, wherein the additional protease is different from the proteases of (a) and (b);

(d) is deficient in one or more autolytic factor activity, wherein the deficient autolytic factor activity results from a mutation in at least one gene encoding an autolytic factor;
(e) overexpresses one or more inactivated protease;
(f) overexpresses one or more folding modulator; or
(g) any combination of (c), (d), (e) and (f).

3. The recombinant gram-negative bacterial host cell of claim 1 or 2, wherein the deficient tail-specific protease activity results from a mutation in a gene encoding one or more of: (i) a Prc1 tail-specific protease having the amino acid sequence set forth as SEQ ID NO: 33, a homologue of SEQ ID NO: 33, or a Prc1 tail-specific protease related protein having an amino acid sequence at least 30% similar to SEQ ID NO: 33; (ii) a Prc2 tail-specific protease having the amino acid sequence set forth as SEQ ID NO: 35, a homologue of SEQ ID NO: 35, or a Prc2 tail-specific protease related protein having an amino acid sequence at least 30% similar to SEQ ID NO: 35; or (iii) a Tsp tail-specific protease having the amino acid sequence set forth as SEQ ID NO: 71, a homologue of SEQ ID NO: 71, or an Tsp tail-specific protease related protein having an amino acid sequence at least 30% similar to SEQ ID NO: 71.

4. The recombinant gram-negative bacterial host cell of any one of claims 1-3, wherein the deficient murein DD-endopeptidase activity results from a mutation in a gene encoding one or more of:

(i) a MepM1 murein DD-endopeptidase having the amino acid sequence set forth as SEQ ID NO: 1, a homologue of SEQ ID NO: 1, or a murein DD-endopeptidase related protein having an amino acid sequence at least 30% similar to SEQ ID NO: 1;

(ii) a MepM murein DD-endopeptidase having the amino acid sequence set forth as SEQ ID NO: 63, a homologue of SEQ ID NO: 63, or a murein DD-endopeptidase related protein having an amino acid sequence at least 30% similar to SEQ ID NO: 63;

(iii) a MepM1 murein DD-endopeptidase having the amino acid sequence set forth as SEQ ID NO: 65, a homologue of SEQ ID NO: 65, or a murein DD-endopeptidase related protein having an amino acid sequence at least 30% similar to SEQ ID NO: 65; and

(iv) a MepM1 murein DD-endopeptidase having the amino acid sequence set forth as SEQ ID NO: 66, a homologue of SEQ ID NO: 66, or a murein DD-endopeptidase related protein having an amino acid sequence at least 30% similar to SEQ ID NO: 66.

5. The recombinant gram-negative bacterial host cell of any one of claims 2-4, wherein: the host cell of (c) is deficient in 1 to 10 different additional protease activities; the host cell of (d) is deficient in 1-5 different autolytic factor activities; the host cell of (e) overexpresses 1 to 10 different inactivated proteases, wherein each inactivated protease is different; the host cell of (f) overexpresses 1-10 different folding modulators, or any combination thereof.

6. The recombinant gram-negative bacterial host cell of any one of claims 2-5, wherein:

the one or more deficient additional protease activity of (c) results from a mutation of at least one gene encoding an additional protease independently selected from: a serralysin precursor, a membrane-localized protease, a murein L,D transpeptidase, a hemolysin precursor, a D-alanyl-D-alanine carboxypeptidase/endopeptidase AmpH precursor, a

periplasmic serine endoprotease, an AAA+ family proteolytic machine, and a murein DD-endopeptidase different from that of (a);

the one or more deficient autolytic factor activity of (d) results from a mutation of at least one gene encoding an autolytic factor independently selected from: an S-type pyocin, a linear gramicidin synthase subunit D, a hemolysin precursor, a leukotoxin, and a porin;

the one or more inactivated protease of (e) is a mutant periplasmic serine endoprotease; and

the one or more folding modulator of (f) is a disulfide isomerase.

7. The recombinant gram-negative bacterial host cell of claim 6, wherein:

the serralysin precursor is selected from:
a serralysin precursor having the amino acid sequence set forth as SEQ ID NO: 9; a homologue of the serralysin precursor having the amino acid sequence set forth as SEQ ID NO: 9; a serralysin precursor related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 9;
a serralysin precursor having the amino acid sequence set forth as SEQ ID NO: 47; a homologue of the serralysin precursor having the amino acid sequence set forth as SEQ ID NO: 47; and a serralysin precursor related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 47;

the membrane-localized protease is an HtpX having the amino acid sequence set forth as SEQ ID NO: 39, a homologue of the HtpX having the amino acid sequence set forth as SEQ ID NO: 39, or an HtpX related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 39;

the murein L,D transpeptidase is a murein L,D transpeptidase having the amino acid sequence set forth as SEQ ID NO: 41, a homologue of the murein L,D transpeptidase having the amino acid sequence set forth as SEQ ID NO: 41, or a murein L,D

transpeptidase related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 41;

the hemolysin precursor is a hemolysin precursor having the amino acid sequence set forth as SEQ ID NO: 43, a homologue of the hemolysin precursor having the amino acid sequence set forth as SEQ ID NO: 43, or a hemolysin precursor related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 43;

the D-alanyl-D-alanine carboxypeptidase/endopeptidase AmpH precursor is a D-alanyl-D-alanine carboxypeptidase/endopeptidase AmpH precursor having the amino acid sequence set forth as SEQ ID NO: 45, a homologue of the D-alanyl-D-alanine carboxypeptidase/endopeptidase AmpH precursor having the amino acid sequence set forth as SEQ ID NO: 45, or a D-alanyl-D-alanine carboxypeptidase/endopeptidase AmpH precursor related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 45;

the periplasmic serine endoprotease is selected from:
a DegP2 having the amino acid sequence set forth as SEQ ID NO: 31; a homologue of the DegP2 having the amino acid sequence set forth as SEQ ID NO: 31; a DegP2 related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 31;
a DegP having the amino acid sequence set forth as SEQ ID NO: 69; a homologue of the DegP having the amino acid sequence set forth as SEQ ID NO: 69; a DegP related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 69;
a DegP having the amino acid sequence set forth as SEQ ID NO: 62; a homologue of the DegP having the amino acid sequence set forth as SEQ ID NO: 62; and a DegP related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 62;

the AAA+ family proteolytic machine comprises: an HslU protease having the amino acid sequence set forth as SEQ ID NO: 37, a homologue of the HslU protease having the amino acid sequence set forth as SEQ ID NO: 37, or a HslU related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 37; and an HslV protease having the amino acid sequence set forth as SEQ ID NO: 38, a homologue of the HslV protease having the amino acid sequence set forth as SEQ ID NO: 38, or a HslV related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 38;

the murein DD-endopeptidase is selected from:
a *P. fluorescens* MepM2 protease having the amino acid sequence set forth as SEQ ID NO: 3; a homologue of the MepM2 protease having the amino acid sequence set forth as SEQ ID NO: 3; a MepM2-related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 3;
a MepM2 protease having the amino acid sequence set forth as SEQ ID NO: 64; a homologue of the MepM2 protease having the amino acid sequence set forth as SEQ ID NO: 64; a MepM2-related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 64;
a MepM2 protease having the amino acid sequence set forth as SEQ ID NO: 67; a homologue of the MepM2 protease having the amino acid sequence set forth as SEQ ID NO: 67; a MepM2-related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 67;
a MepM2 protease having the amino acid sequence set forth as SEQ ID NO: 68; a homologue of the MepM2 protease having the amino acid sequence set forth as SEQ ID NO: 68; and
a MepM2-related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 68;

the S-type Pyocin is an S-type Pyocin having the amino acid sequence set forth as SEQ ID NO: 49, a homologue of the S-type Pyocin having the amino acid sequence set forth

as SEQ ID NO: 49, or an S-type Pyocin related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 49;

the linear gramicidin synthase is a linear gramicidin synthase having the amino acid sequence set forth as SEQ ID NO: 51, a homologue of the linear gramicidin synthase having the amino acid sequence set forth as SEQ ID NO: 51, or a linear gramicidin synthase related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 51;

the leukotoxin is a leukotoxin having the amino acid sequence set forth as SEQ ID NO: 53, a homologue of the leukotoxin having the amino acid sequence set forth as SEQ ID NO: 53, or a leukotoxin related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 53;

the ShlB hemolysin transporter is an ShlB hemolysin transporter having the amino acid sequence set forth as SEQ ID NO: 55, a homologue of the an ShlB hemolysin transporter having the amino acid sequence set forth as SEQ ID NO: 55, or an ShlB hemolysin transporter related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 55;

each of the one or more overexpressed inactivated proteases is independently selected from: *P. fluorescens* DegP2 S219A; an inactivated DegP2 comprising an amino acid substitution or disruption of the amino acid sequence set forth as SEQ ID NO: 31; an inactivated DegP2 comprising an amino acid substitution or disruption of a homologue of the amino acid sequence set forth as SEQ ID NO: 31; an inactivated DegP2-related protein comprising an amino acid substitution or disruption of a DegP2 having at least 60% similarity or at least 35% identity to amino acid sequence set forth as SEQ ID NO: 31; an inactivated DegP comprising an amino acid substitution or disruption of the amino acid sequence set forth as SEQ ID NO: 69; an inactivated DegP comprising an amino acid substitution or disruption of a homologue of the amino acid sequence set forth as SEQ ID NO: 69; an inactivated DegP-related protein comprising an amino acid substitution or disruption of a DegP having at least 60% similarity or at least 35%

identity to amino acid sequence set forth as SEQ ID NO: 69; an inactivated DegP/HtrA comprising an amino acid substitution or disruption of the amino acid sequence set forth as SEQ ID NO: 62; an inactivated DegP/HtrA comprising an amino acid substitution or disruption of a homologue of the amino acid sequence set forth as SEQ ID NO: 62; an inactivated DegP/HtrA-related protein comprising an amino acid substitution or disruption of a DegP having at least 60% similarity or at least 35% identity to amino acid sequence set forth as SEQ ID NO: 62; an inactivated DegP, DegP-related protein, or DegP homologue having a substitution or disruption of an amino acid at a position corresponding to any one of 131 (His), 134 (Asp) and 236 (Ser) (SEQ ID NO: 62, numbering including leader sequence 1-26), or respective positions 105, 108, and 210, when excluding the leader sequence; an inactivated DegP, DegP-related protein, or DegP homologue having an amino acid substitution corresponding to *E. coli* Htr S210A; an inactivated DegP, DegP-related protein, or DegP homologue having an amino acid substitution corresponding to *E. coli* Htr H105R; and an inactivated DegP, DegP-related protein, or DegP homologue having a substitution or disruption of any one or more amino acid at a position corresponding to any one of: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 146, 147, 148, 149, 150, 151, 152, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, and 234 of SEQ ID NO: 31; and

each of the one or more folding modulators is independently selected from: a disulfide bond isomerase having an amino acid sequence set forth as any one of SEQ ID NOS: 60, 76, 77, 78, 80, and 81; a homologue of a disulfide bond isomerase having an amino acid sequence set forth as any one of SEQ ID NOS: 60, 76, 77, 78, 80, and 81; a disulfide bond isomerase-related protein having at least 60% similarity or at least 60% identity to an amino acid sequence set forth as any one of SEQ ID NOS: 60, 76, 77, 78, 80, and 81; a protein disulfide isomerase having an amino acid sequence set forth as any one of SEQ ID NOS: 27 and 82-98; a homologue of a protein disulfide isomerase having an amino acid sequence set forth as any one of SEQ ID NOS: 27 and 82-98; and a protein disulfide isomerase-related protein having at least 60% similarity or at least 60% identity to an amino acid sequence set forth as SEQ ID NOS: 27 and 82-98.

8. The recombinant gram-negative bacterial host cell of any preceding claim, wherein the gram-negative bacterial host cell further comprises a functional protease activity, wherein the functional protease activity is the activity of: a MepS1 having the amino acid sequence set forth as SEQ ID NO: 5; a homologue of the MepS1 having the amino acid sequence set forth as SEQ ID NO: 5; or a MepS1 related protein having at least 50% sequence similarity to the *P. fluorescens* MepS1 protease amino acid sequence set forth as SEQ ID NO: 5.

9. The recombinant gram-negative bacterial host cell of any preceding claim, wherein the gram-negative bacterial host cell further comprises a functional protease activity, wherein the functional protease activity is: a MepS2 having the amino acid sequence set forth as SEQ ID NO: 7; a homologue of the MepS2 having the amino acid sequence set forth as SEQ ID NO: 7; or a MepS2 related protein having at least 50% sequence similarity to the *P. fluorescens* MepS2 protease amino acid sequence set forth as SEQ ID NO: 7.

10. The recombinant gram-negative bacterial host cell of any preceding claim, wherein the gram-negative bacterial host cell is a *Pseudomonad*, and the deficient first protease activity results from a mutation of a coding sequence and/or noncoding sequence of a gene encoding *P. fluorescens* Prc1 and/or a mutation of a coding sequence and/or noncoding sequence of a gene encoding *P. fluorescens* Prc2.

11. The recombinant gram-negative bacterial host cell of any preceding claim, wherein the second protease activity is deficient due to a mutation that results in a conservative or non-conservative substitution in an active site amino acid or an allosteric site amino acid of a protease having the second protease activity.

12. The recombinant gram-negative bacterial host cell of any preceding claim, wherein the deficient second protease activity results from at least one mutation of the second protease gene, wherein the mutation results in a disruption of the amino acid sequence at a position corresponding to: (i) any one or more of residues 134 to 145 of SEQ ID NO: 1; (ii) any

one or more of residues 319 to 411 of SEQ ID NO: 1; (iii) one or more of residues any 361 to 378 of SEQ ID NO: 1; (iv) any one or more residue selected from 248, 319, 330, 332, 334, 337, 378, 410, and 411 of SEQ ID NO: 1; or any combination of (i), (ii), (iii), and (iv).

13. The recombinant gram-negative bacterial host cell of any preceding claim, wherein the bacterial host cell is *Pseudomonas fluorescens*, wherein the deficient second protease activity results from a gene mutation that results in an amino acid substitution of SEQ ID NO: 1 selected from: Y248stop, G332S, D334N, A337T, H411Y, P410L, and any conservative or non-conservative amino acid substitution of any one of R319, H330, D334, H378, and H411.

14. The recombinant gram-negative bacterial host cell of any preceding claim, wherein the host cell is capable of high-density cell growth in culture.

15. The recombinant gram-negative bacterial host cell of claim 18, wherein the high-density cell growth in culture comprises growth to OD575 of about 80 to about 300.

16. The recombinant gram-negative bacterial host cell of claim 18 or 19, wherein the high-density cell growth in culture is increased in comparison to a control cell by about 2-fold to about 15-fold.

17. The recombinant gram-negative bacterial host cell of claim 20, wherein the recombinant gram-negative bacterial host cell and the control cell, respectively, are selected from:

> (i) a recombinant gram-negative bacterial host cell deficient in the first protease activity and the second protease activity, and a corresponding gram-negative bacterial host cell deficient in the first protease activity and wherein the second protease is functional;
>
> (ii) a recombinant gram-negative bacterial host cell deficient in the first protease activity, the second protease activity, and an additional protease activity as recited in 2(c), and a corresponding gram-negative bacterial host cell deficient in the first protease activity and the second protease activity, and wherein the additional

protease activity of 2(c) that is deficient in the compared recombinant gram-negative bacterial host cell is functional; and

(iii) a recombinant gram-negative bacterial host cell deficient in the first protease activity and the second protease activity, and comprising a functional protease that is: a MepS1 having the amino acid sequence set forth as SEQ ID NO: 5; a homologue of the MepS1 having the amino acid sequence set forth as SEQ ID NO: 5; or a MepS1 related protein having at least 50% sequence similarity to the *P. fluorescens* MepS1 amino acid sequence set forth as SEQ ID NO: 5, and a corresponding gram-negative bacterial host cell deficient in the activity of the first protease and the second protease, and deficient in the functional protease of the compared recombinant gram-negative bacterial host cell.

18. The recombinant gram-negative bacterial host cell of claim 21, wherein the additional protease activity of 2(c) is an activity of a serralysin precursor having the amino acid sequence set forth as SEQ ID NO: 9; a homologue of the serralysin precursor having the amino acid sequence set forth as SEQ ID NO: 9; or a serralysin precursor related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 9.

19. The recombinant gram-negative bacterial host cell of any preceding claim, further comprising at least one expression construct, each expression construct comprising at least one nucleic acid sequence encoding a recombinant protein of interest.

20. The recombinant gram-negative bacterial host cell of claim 23, wherein the recombinant protein of interest is selected from: an antibody, antibody fragment, or derivative of an antibody or antibody fragment; an antibody-based drug, a non-antibody binding protein (e.g., an antibody mimetic, including, but not limited to, an alphabody, an iBody, an affibody, an affilin, an affitin, or an anticalin), a reagent protein; a vaccine antigen; a therapeutic protein or enzyme; non-natural protein; a pathogen protein or derivative thereof; a microbial toxin, a lipoprotein; an extracellular receptor or ligand; a protease; a kinase; a blood protein; a chemokine; a cytokine; a bone morphogenic protein; an anticoagulant; a blood factor; a bone morphogenetic protein; an engineered protein scaffold; an enzyme, e.g., a biocatalytic enzyme; a growth factor; an interferon; an interleukin; a thrombolytic agent; a hormone; and a TGF-beta family member protein.

21. The recombinant gram-negative bacterial host cell of claim 25 or 26, wherein the antibody, antibody fragment, or derivative thereof is selected from: a monoclonal antibody; a complementarity determining region (CDR) fragment; a CDR-grafted antibody; a single-chain antibody; a single chain antibody fragment; modified antibody, a bispecific antibody, a chimeric antibody; a diabody; a triabody; a tetrabody; a minibody; a linear antibody; a chelating recombinant antibody; a bibody; a tribody; an intrabody; a nanobody; a small modular immunopharmaceutical (SMIP); an antigen-binding-domain immunoglobulin fusion protein; a camelid antibody; a shark single domain antibody, an avian antibody (e.g., a chicken antibody), a VHH-containing antibody; a F(ab); a F(ab)'; F(ab)'$_2$; scFv; an Fc fragment generated from the heavy chain constant region of an antibody; a reduced IgG fragment (e.g., generated by reducing the hinge region disulfide bonds of IgG); an Fc fusion protein (e.g., comprising the Fc domain of IgG fused together with a protein or peptide of interest); a domain antibody; a VL; a VNAR; a VH; and a VHH.

22. The recombinant gram-negative bacterial host cell of claim 27, wherein the VHH-containing antibody is a VHH concatenated antibody.

23. The recombinant gram-negative bacterial host cell of any one of claims 25 to 28, wherein the antibody, antibody fragment, or derivative thereof, binds to a target selected from: a cytokine; a chemokine; a drug; a cell-surface protein, e.g., a receptor, cell-surface marker, pathogen surface-protein, etc.; a growth factor; a growth factor receptor; immune checkpoint molecule, and a blood factor.

24. The recombinant gram-negative bacterial host cell of any one of claims 25 to 29, wherein the antibody, antibody fragment, or derivative thereof is a Fab'.

25. The recombinant gram-negative bacterial host cell of claim 30, wherein the Fab' binds to a target selected from: Carcinoembryonic antigen (CEA); CD22; fibrin II, beta chain; TNF-alpha; and NCA-90 (granulocyte antigen).

26. The recombinant gram-negative bacterial host cell of any one of claims 25 to 31, wherein the at least one expression construct encoding the antibody, antibody fragment, or derivative thereof comprises at least one nucleic acid sequence encoding a heavy chain, at least one nucleic acid sequence encoding a light chain, or both, wherein the heavy chain is full-length or a heavy chain fragment, and the light chain is full-length or a light chain fragment.

27. The recombinant gram-negative bacterial host cell of claim 32, wherein the at least one expression construct encoding the antibody, antibody fragment, or derivative thereof comprises at least two nucleic acid sequences, each encoding a heavy chain.

28. The recombinant gram-negative bacterial host cell of claim 32 or 33, wherein the at least one expression construct encoding the antibody, antibody fragment, or derivative thereof comprises a nucleic acid sequence encoding a heavy chain and a nucleic acid sequence encoding a light chain, wherein the heavy and light chain are expressed from the same mRNA transcript.

29. The recombinant gram-negative bacterial host cell of claim 32 or 33, wherein the at least one expression construct encoding the antibody, an antibody fragment, or derivative thereof comprises a nucleic acid sequence encoding a heavy chain and a nucleic acid sequence encoding a light chain, wherein the heavy and light chain are expressed from different mRNA transcripts.

30. The recombinant gram-negative bacterial host cell of claim 34 or 35, wherein each heavy chain-encoding nucleic acid sequence and each light chain-encoding nucleic acid sequence is individually operably linked to an independently selected nucleic acid sequence encoding a periplasmic secretion signal.

31. The recombinant gram-negative bacterial host cell of claim 36, wherein the periplasmic secretion signal has the amino acid sequence set forth as SEQ ID NO: 11, 13, 15, or 17.

32. The recombinant gram-negative bacterial host cell of any one of claims 34 to 37, wherein the expression construct comprises: a nucleic acid sequence encoding an antibody heavy

chain, operably linked to a nucleic acid sequence encoding a periplasmic secretion signal, wherein the periplasmic secretion signal has the amino acid sequence set forth as SEQ ID NOS: 11, 13, 15, or 17; a nucleic acid sequence encoding a light chain, operably linked to a nucleic acid sequence encoding a periplasmic secretion signal, wherein the periplasmic secretion signal has the amino acid sequence set forth as SEQ ID NOS: 11, 13, 15, or 17; or both.

33. The recombinant gram-negative bacterial host cell of any one of claims 25 to 38, wherein the antibody, antibody fragment, or derivative thereof is humanized.

34. The recombinant gram-negative bacterial host cell of any one of claims 30 to 39, wherein the Fab' is certolizumab.

35. The recombinant gram-negative bacterial host cell of claim 40, wherein the Fab' heavy chain has the amino acid sequence set forth as SEQ ID NO: 21, and the Fab' light chain has the amino acid sequence set forth as SEQ ID NO: 23.

36. The recombinant gram-negative bacterial host cell of any one of claims 32-41, wherein the nucleic acid sequence encoding the heavy chain is operably linked to a nucleic acid sequence encoding a secretion leader having the amino acid sequence set forth as SEQ ID NO: 11, and the nucleic acid sequence encoding the light chain is operably linked to a nucleic acid sequence encoding a secretion leader having the amino acid sequence set forth as SEQ ID NO: 13.

37. The recombinant gram-negative bacterial host cell of any one of claims 1-42, wherein the host cell is deficient in:

(i) the first protease activity;

(ii) the second protease activity;

(iii) the activity of a serralysin precursor having the amino acid sequence set forth as SEQ ID NO: 9, a homologue of the serralysin precursor having the amino acid sequence set forth as SEQ ID NO: 9, or a serralysin precursor related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 9;

(iv) an HslU protease having the amino acid sequence set forth as SEQ ID NO: 37, a homologue of the HslU protease having the amino acid sequence set forth as SEQ ID NO: 37, or a HslU related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 37; and

(v) an HslV protease having the amino acid sequence set forth as SEQ ID NO: 38, a homologue of the HslV protease having the amino acid sequence set forth as SEQ ID NO: 38, or a HslV related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 38.

38. The recombinant gram-negative bacterial host cell of claim 43, wherein the host cell further overexpresses an exogenous inactivated DegP, wherein the inactivated DegP is selected from: *P. fluorescens* DegP2 S219A; an inactivated DegP2 derived from the amino acid sequence set forth as SEQ ID NO: 31; an inactivated DegP2 derived from the amino acid sequence set forth as SEQ ID NO: 62; an inactivated DegP2 derived from a homologue of the amino acid sequence set forth as SEQ ID NO: 31; an inactivated DegP2 derived from a DegP2 having at least 60% similarity or at least 60% identity to amino acid sequence set forth as SEQ ID NO: 31; an inactivated DegP2 derived from a DegP2 having at least 60% similarity or at least 60% identity to amino acid sequence set forth as SEQ ID NO: 62; and each of the proteases having the amino acid sequence set forth as SEQ ID NO: 31 comprising a conservative or nonconservative amino acid substitution or disruption of any one or more of positions: 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 146, 147, 148, 149, 150, 151, 152, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, and 234.

39. The recombinant gram-negative bacterial host cell of claim 43 or 44, wherein the host cell overexpresses an exogenous disulfide isomerase selected from any one of: a disulfide isomerase having at least 60% similarity or at least 60% identity to an amino acid sequence set forth as SEQ ID NO: 27, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, or 73, and a homologue of a disulfide isomerase having the amino acid sequence set forth as SEQ ID NO: 27, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, and 73.

40. The recombinant gram-negative bacterial host cell of any one of claims 1-45, wherein the recombinant gram-negative bacterial host cell is selected from: a *Pseudomonad* host cell; an *E. coli* host cell; and a *Vibrio* host cell.

41. The recombinant gram-negative bacterial host cell of claim 46, wherein the *Pseudomonad* host cell is a *Pseudomonas* host cell.

42. The recombinant gram-negative bacterial host cell of claim 47, wherein the *Pseudomonas* host cell is *P. fluorescens*, *P. putida*, *or P. aeruginosa*.

43. The recombinant gram-negative bacterial host cell of claim 47 or 48, wherein the cell is:

(i) lsc::lacIQ1;

(ii) Prc1-

(ii) Prc2-

(iii) HslU-

(iv) HslV-

(v) MepM1-

(vi) PyrF-

and (vii) deficient in a serralysin precursor that is: a serralysin precursor having the amino acid sequence set forth as SEQ ID NO: 9; a homologue of the serralysin precursor having the amino acid sequence set forth as SEQ ID NO: 9; or a serralysin precursor related protein having at least 60% similarity or at least 60% identity to the amino acid sequence set forth as SEQ ID NO: 9; wherein the serralysin precursor deficiency results from a mutation in a gene encoding the serralysin precursor.

44. The recombinant gram-negative bacterial host cell of claim 49, wherein the cell is *P. fluorescens*, the Prc1 has the amino acid sequence set forth in SEQ ID NO: 33, the Prc2 has the amino acid sequence set forth in (SEQ ID NO: 35), the HslU has the amino acid sequence set forth in (SEQ ID NO: 37), the HslV has the amino acid sequence set forth in (SEQ ID NO: 38), the MepM1 has the amino acid sequence set forth in (SEQ ID NO: 1), and the serralysin precursor has the amino acid sequence set forth as SEQ ID NO: 9.

45. The recombinant gram-negative bacterial host cell of claim 50, further comprising an expression vector comprising a nucleic acid sequence encoding DegP2 S219A (SEQ ID NO: 29).

46. The recombinant gram-negative bacterial host cell of claim 50 or 51, further comprising an expression vector comprising a nucleic acid sequence encoding disulfide isomerase PDIA6 (SEQ ID NO: 27).

47. The recombinant gram-negative bacterial host cell of any one of claims 1-52, further comprising an expression vector encoding a recombinant protein.

48. The recombinant gram-negative bacterial host cell of claim 53, wherein the expression vector encodes a Fab'.

49. The recombinant gram-negative bacterial host cell of claim 54, wherein the expression vector comprising the nucleic acid sequence encoding DegP2 S219A or disulfide isomerase PDIA6 further comprises a nucleic acid sequence encoding the Fab'.

50. The recombinant gram-negative bacterial host cell of claim 54 or 55, wherein the Fab' heavy chain is encoded by SEQ ID NO: 21, and the Fab' light chain is encoded by SEQ ID NO: 23.

51. The recombinant gram-negative bacterial host cell of claim 1, wherein the recombinant gram-negative bacterial host cell is a *Pseudomonad* having the genotype of strain STR94975, STR94976, or STR94977.

52. The recombinant gram-negative bacterial host cell of claim 57, further comprising the expression construct or constructs of STR94975, STR94976, or STR94977, for use in producing a recombinant anti-TNF-alpha Fab'.

53. A method for producing a recombinant protein of interest comprising: (a) recovering the recombinant protein of interest from a recombinant gram-negative bacterial host

cell of any one of claims 1-57 cultured under suitable fermentation conditions, wherein the recombinant gram-negative host cell is transformed with a plasmid comprising a nucleic acid encoding the recombinant protein of interest.

54. The method of any one of claims 59-61, wherein the recombinant gram-negative bacterial host cell can grow to high cell density.

55. The method of claim 62, wherein the high cell density comprises an OD575 of about 80 to about 300.

56. The method of any one of claims 58-63, wherein the suitable fermentation conditions comprise induction of the inducible promoter at: an OD575 of about 80 to about 160, a culture pH of about 5.8 to about 7.0, a temperature of about 28-33 deg C, fed-batch, and a titer range of about 0.2 to about 5 g/L.

57. The method of any one of claims 59-69, wherein the recombinant gram-negative bacterial host cell grows to a cell density that is increased in comparison to a control cell grown under the same fermentation conditions.

58. The method of claim 70, wherein the increase in cell density is about 2-fold to about 15-fold.

59. The method of any one of claims 59-71, further comprising: (b) measuring the yield of intact, soluble, and/or active, recombinant protein of interest recovered from the recombinant gram-negative bacterial host cell.

60. The method of claim 72, wherein the measured yield of intact, soluble, and/or active, recombinant protein is about 0.1 to about 10 g/L.

61. The method of claim 72 or 73, further comprising: (c) measuring the yield of recombinant protein of interest recovered from a control cell that is intact, soluble, active, or a combination thereof.

62. The method of claim 74, further comprising (d) comparing the yield measured in step (b) to the yield measured in step (c).

63. The method of claim 75, wherein the yield measured in step (b) is about 2-fold to about 100-fold higher than that measured in step (c).

64. The method of any one of claims 70-76, wherein the recombinant gram-negative bacterial host cell and the control cell, respectively, are selected from:

> (i) a recombinant gram-negative bacterial host cell deficient in the first protease activity and the second protease activity, and a corresponding gram-negative bacterial host cell deficient in the first protease activity and wherein the second protease is functional;
>
> (ii) a recombinant gram-negative bacterial host cell deficient in the first protease activity, the second protease activity, and an additional protease activity as recited in 2(a), and a corresponding gram-negative bacterial host cell deficient in the first protease activity and the second protease activity, and wherein the additional protease activity of 2(a) that is deficient in the compared recombinant gram-negative bacterial host cell is functional; and
>
> (iii) a recombinant gram-negative bacterial host cell deficient in the first protease activity and the second protease activity, and comprising a functional protease that is: a MepS1 having the amino acid sequence set forth as SEQ ID NO: 5; a homologue of the MepS1 having the amino acid sequence set forth as SEQ ID NO: 5; or a MepS1 related protein having at least 50% sequence similarity to the *P. fluorescens* MepS1 amino acid sequence set forth as SEQ ID NO: 5, and a corresponding gram-negative bacterial host cell deficient in the activity of the first protease and the second protease, and deficient in the functional protease of the compared recombinant gram-negative bacterial host cell.

65. The recombinant gram-negative bacterial host cell of any one of claims 1-45, or any one of claims 47-77, wherein the gram-negative bacterial host cell is not *E. coli*.

66. A recombinant polypeptide comprising: a secretion signal peptide operably linked to a heterologous protein or polypeptide of interest, wherein the secretion signal peptide has the amino acid sequence set forth in SEQ ID NO: 11.

67. The polypeptide of claim 79, wherein the protein or polypeptide of interest is selected from: an antibody, antibody fragment, or a derivative of an antibody or an antibody fragment; an enzyme; a cytokine; a chemokine; a growth factor; a fusion protein; and a vaccine antigen.

68. A method of producing a protein or polypeptide of interest in a prokaryotic host cell, the method comprising: producing the protein or polypeptide of interest in the periplasm of a prokaryotic host cell cultured in a cell culture growth medium, wherein the prokaryotic host cell comprises an expression construct comprising a nucleic acid encoding a recombinant polypeptide comprising the protein or polypeptide of interest operably linked to a secretion signal peptide that directs expression of the protein or polypeptide of interest to the periplasm of the prokaryotic host cell, wherein the secretion signal peptide comprises the amino acid sequence of SEQ ID NO: 11, and wherein the secretion signal peptide is not native to the protein or polypeptide of interest.

69. The method of claim 93, further comprising isolating the produced protein or polypeptide of interest.

70. The method of claim 94, wherein the protein or polypeptide of interest is selected from: an antibody, antibody fragment, or a derivative of an antibody or an antibody fragment; an enzyme; a cytokine; a chemokine; a growth factor; a fusion protein; and a vaccine antigen.

71. The method of claim 95, wherein the antibody, antibody fragment, or a derivative of an antibody or an antibody fragment is selected from: a monoclonal antibody; a full chain antibody; a complementarity determining region (CDR) fragment; a CDR-grafted antibody; a single-chain antibody; a single chain antibody fragment; a modified antibody; a variable-region only antibody fragment; a bispecific antibody, a chimeric antibody; a triabody; a tetrabody; a minibody; a linear antibody; a chelating recombinant antibody; a bibody; a dibody; an intrabody; a nanobody; a small modular immunopharmaceutical; an antigen-binding-domain

immunoglobulin fusion protein; a camelid antibody; a shark single domain antibody (VNAR); an avian antibody; a VHH; a VHH-containing antibody; a VHH concatemer; a F(ab); a F(ab)'; F(ab)'$_2$; an scFv; an Fc fragment generated from the heavy chain constant region of an antibody; a reduced IgG fragment; an Fc fusion protein; a domain antibody; a VL; and a VH.

72. The method of claim 95 or 96, wherein the antibody, antibody fragment, or derivative of the antibody or antibody fragment is humanized.

73. The method of claim 95, wherein the enzyme is a therapeutic enzyme.

74. The method of claim 98, wherein the therapeutic enzyme is selected from: a peptidase; a lactase; an amylase; a PEP; a digestive enzyme; a uricase; a rhodanase; a urokinase; a streptokinase; a staphylokinase; a phenylase; a sacrosidase; a lysozyme; a chitinase; a ribonuclease; a glutaminase; an arginase; a vibrilase; a chondroitinase; a hyaluronidase; a galactosidase; a glucuronidase; a glucocerebrosidase; a thymidine phosphorylase; a carbonic anhydrase; a uricase thiosulfate-cyanide; a sulfurtransferase; a phosphothioesterase; an alcohol oxidase; an alcohol dehydrogenase; an asparaginase; a glutamine synthase; an adenosine deaminase; bovine pegademase; alglucerase; dornase alpha; imiglucerase; sacrosidase; rasburicase; agalsidase beta; and nattokinase.

75. The method of claim 95, wherein the fusion protein is selected from: an enzyme fusion protein; a protein A fusion protein; an albumin fusion protein; a thioredoxin fusion protein; a ubiquitin fusion protein; a streptavidin fusion protein; a maltose binding protein fusion protein; a chitin being protein fusion protein; a SUMO fusion protein; and a glutathione-S-transferase fusion protein.

76. The method of any one of claims 93-100, wherein the nucleic acid encodes a linker.

77. The method of claim 101, wherein the linker comprises a cleavage domain.

78. The method of any one of claims 93-102, wherein the prokaryotic host cell is a gram-negative bacterium.

79. The method of any one of claims 93-102, wherein the prokaryotic host cell is a gram-positive bacterium.

80. The method of claim 93, wherein the gram-negative bacterium is a *Pseudomonad, V. natriegens*, or *E. coli*.

81. The method of claim 94, wherein the gram-positive bacterium is a *Corynebacterium* or a *Bacillus*.

82. An expression vector comprising a nucleic acid sequence encoding a recombinant polypeptide of any one of claims 79-92.

83. A prokaryotic host cell comprising an expression vector of claim 107.

84. The prokaryotic host cell of claim 108, wherein the prokaryotic host cell is a gram-negative bacterium.

85. The prokaryotic host cell of claim 108, wherein the prokaryotic host cell is a gram-positive bacterium.

86. The prokaryotic host cell of claim 109, wherein the gram-negative bacterium is a *Pseudomonad, V. natriegens*, or *E. coli*.

87. The prokaryotic host cell of claim 110, wherein the gram-positive bacterium is a *Corynebacterium* or a *Bacillus*.

88. The prokaryotic host cell of any one of claims 108-112, wherein the nucleic acid sequence encoding the recombinant polypeptide is optimized for expression in the prokaryotic host cell.