

ENGINEERED BINDING PROTEINS FOR RECOGNITION OF BACTERIA

RELATED APPLICATION

5 This application claims the benefit under 35 U.S.C. 119(e) of U.S. provisional application number 63/082,686, filed September 24, 2020, which is incorporated by reference herein in its entirety.

REFERENCE TO A SEQUENCE LISTING SUBMITTED AS A TEXT FILE VIA EFS-
WEB

10 The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on September 22, 2021, is named M065670499WO00-SEQ-CRP and is 57,599 bytes in size.

15 FIELD

Described herein are antigen-binding molecules that bind to bacteria (*e.g.*, *Listeria monocytogenes*, *Salmonella typhi*, or *E. coli*) and methods of use thereof. Also described herein are compositions and kits comprising these antigen-binding molecules.

20 BACKGROUND

In the United States, there are an estimated 9.4 million cases of foodborne illnesses, resulting in approximately 56,000 hospitalizations and 1,350 deaths per year (1). *Listeria monocytogenes* is a Gram-positive foodborne pathogenic bacterium that can grow even in harsh environments (0.4 to 45 °C, pH 4.0 to 9.6, and in aerobic or anaerobic conditions) (2).
25 It is found in various food products, including fresh produce and milk. Other pathogenic bacteria such as *E. coli* and *Salmonella* also present health risks to society.

SUMMARY

30 *Listeria monocytogenes* causes a rare but serious disease called listeriosis. Although listeriosis is uncommon, it has a high fatality rate, encompassing an estimated 19% of deaths related to foodborne illnesses (1). Furthermore, *L. monocytogenes* results in a large economic burden—an estimated \$2 billion annually—from health care costs, lost productivity, and

reduced quality of life (3). Pregnant women and those with weak immune systems—including neonates, the elderly, and cancer patients—are particularly vulnerable to listeriosis (2, 4). It can result in abortion and premature birth, as well as meningoencephalitis (in 55-70% of cases) or septicemia (15-50% of cases) in the immunocompromised (4, 5).

- 5 Therefore, timely detection of *L. monocytogenes* is vital to reduce the burden of this detrimental disease. Similarly salmonellosis is an infection caused by *Salmonella* and is a major health risk. Additionally legionella is another pathogenic organism that can cause sickness. Types of *E. coli* can also be pathogenic and cause sickness. Rapid diagnostic tests that can be used directly in the field site, manufacturing plants, and farms would allow for
- 10 early detection of potential contaminants to reduce the current large burden of these foodborne pathogens.

Accordingly, in some aspects, the disclosure relates to antigen-binding molecules comprising a reduced charge Sso7d(rcSso7d) antigen-binding protein that binds to a bacterium. In some embodiments, an rcSso7d antigen-binding protein binds to a gram-

15 positive bacterium. In some embodiments, an rcSso7d antigen-binding protein binds to a gram-negative bacterium. In some embodiments, an rcSso7d antigen-binding protein binds to a pathogenic bacterium. In some embodiments, an rcSso7d antigen-binding protein binds to a bacterium that causes food poisoning. In some embodiments, an rcSso7d antigen-binding protein binds to a bacterium that causes food spoilage. In some embodiments, an

20 rcSso7d antigen-binding protein binds to a bacterium of the genus *Listeria*, *Escherichia*, *Salmonella*, and/or *Legionella*. In some embodiments, the rcSso7d antigen-binding protein binds to a *Listeria monocytogenes*, *Salmonella typhi*, and/or *E. coli*.

In some embodiments, the antigen-binding molecule binds to at least two antigens. In some embodiments, two or more of the at least two antigens are specific to different types of

25 bacteria.

In some embodiments, the antigen-binding molecule binds to a protein antigen, a glycosylated protein antigen, and/or a carbohydrate antigen.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein that binds *Listeria monocytogenes* protein LMOF2365_0639, wherein the

30 antigen-binding protein comprises a variable region having: (i) the amino acid sequence of any one of SEQ ID NOs: 1-8; or (ii) an amino acid sequence having 2 or 1 amino acid differences with any one of SEQ ID NOs: 1-8.

In some embodiments, the antigen-binding molecule comprises a variable region having: (i) the amino acid sequence of any one of SEQ ID NOs: 3, 5 or 7; or (ii) an amino acid sequence having no more than 2 or 1 amino acid differences with any one of SEQ ID NOs: 3, 5 or 7.

5 In some embodiments, the rcSso7d antigen-binding protein comprises: (i) the amino acid sequence of any one of SEQ ID NOs: 13-15; (ii) an amino acid sequence having no more than ten amino acid differences with any one of SEQ ID NOs: 13-15; or (iii) an amino acid sequence having at least 80% identity with any one of any one of SEQ ID NOs: 13-15. In some embodiments, the rcSso7d antigen-binding protein comprises the amino acid sequence
10 of any one of SEQ ID NOs: 13-15.

In some embodiments, the antigen-binding protein comprises a variable region having: (i) the amino acid sequence of any one of SEQ ID NOs: 1, 2, 4, or 6; or (ii) an amino acid sequence no more than 2 or 1 amino acid differences with any one of SEQ ID NOs: 1, 2, 4, or 6.

15 In some embodiments, the rcSso7d antigen-binding protein comprises: (i) the amino acid sequence of any one of SEQ ID NOs: 16-19; (ii) an amino acid sequence having no more than seven amino acid differences with any one of SEQ ID NOs: 16-19; or (iii) an amino acid sequence having at least 80% identity with any one of any one of SEQ ID NOs: 16-19. In some embodiments, the rcSso7d antigen-binding protein comprises the amino acid sequence
20 of any one of SEQ ID NOs: 16-19.

In some embodiments, the antigen-binding molecule further comprises an enzyme, a fluorescent protein, an immunoglobulin, a peptide tag, a small molecule, or a combination thereof.

In some embodiments, the antigen-binding molecule comprises an enzyme selected
25 from the group consisting of alkaline phosphatase, horseradish peroxidase, and β -galactosidase.

In some embodiments, the antigen-binding molecule comprises a fluorescent protein selected from the group consisting of TagBFP, mTagBFP2, Azurite, EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric
30 Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange, mKO κ , mKO2, mOrange, mOrange2, mRaspberry,

mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.

In some embodiments, the antigen-binding molecule comprises a peptide tag selected from the group consisting of a His tag, a biotin acceptor tag, an HA tag, a c-Myc tag, a streptavidin tag, and a FLAG tag.

In some embodiments, the antigen-binding molecule comprises biotin.

In some embodiments, an antigen-binding molecule comprising a reduced charge Sso7d (rcSso7d) antigen-binding protein that binds *Salmonella typhi* Native Omp, said Native Omp comprising the amino acid sequence of *Salmonella typhi* membrane proteins OmpA, OmpW, OmpC, OmpF, OmpD, and phoE, wherein the antigen-binding protein comprises a variable region having: (i) the amino acid sequence of any one of SEQ ID NOs: 58-71; or (ii) an amino acid sequence having 2 or 1 amino acid differences with any one of SEQ ID NOs: 58-71.

In some embodiments, the rcSso7d antigen-binding protein comprises: (i) the amino acid sequence of any one of SEQ ID NOs: 33-46; (ii) an amino acid sequence having no more than ten amino acid differences with any one of SEQ ID NOs: 33-46; or (iii) an amino acid sequence having at least 80% identity with any one of any one of SEQ ID NOs: 33-46.

In some embodiments, the rcSso7d antigen-binding protein comprises the amino acid sequence of any one of SEQ ID NOs: 33-46.

In some embodiments, the antigen-binding molecule further comprises an enzyme, a fluorescent protein, an immunoglobulin, a peptide tag, a small molecule, or a combination thereof.

In some embodiments, the antigen-binding molecule comprises an enzyme selected from the group consisting of alkaline phosphatase, horseradish peroxidase, and β -galactosidase.

In some embodiments, the antigen-binding molecule comprises a fluorescent protein selected from the group consisting of TagBFP, mTagBFP2, Azurite, EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange, mKOκ, mKO2, mOrange, mOrange2, mRaspberry,

mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.

In some embodiments, the antigen-binding molecule comprises a peptide tag selected from the group consisting of a His tag, a biotin acceptor tag, an HA tag, a c-Myc tag, a streptavidin tag, and a FLAG tag.

In some embodiments, the antigen-binding molecule comprises biotin.

In some embodiments, an antigen-binding molecule comprising a reduced charge Sso7d (rcSso7d) antigen-binding protein that binds *E. coli* OmpW, wherein the antigen-binding protein comprises a variable region having: (i) the amino acid sequence of any one of SEQ ID NOs: 73-80; or (ii) an amino acid sequence having 2 or 1 amino acid differences with any one of SEQ ID NOs: 73-80.

In some embodiments, the rcSso7d antigen-binding protein comprises: (i) the amino acid sequence of any one of SEQ ID NOs: 47-54; (ii) an amino acid sequence having no more than ten amino acid differences with any one of SEQ ID NOs: 47-54; or (iii) an amino acid sequence having at least 80% identity with any one of any one of SEQ ID NOs: 47-54.

In some embodiments, the rcSso7d antigen-binding protein comprises the amino acid sequence of any one of SEQ ID NOs: 47-54.

In some embodiments, the antigen-binding molecule further comprises an enzyme, a fluorescent protein, an immunoglobulin, a peptide tag, a small molecule, or a combination thereof.

In some embodiments, the antigen-binding molecule comprises an enzyme selected from the group consisting of alkaline phosphatase, horseradish peroxidase, and β -galactosidase.

In some embodiments, the antigen-binding molecule comprises a fluorescent protein selected from the group consisting of TagBFP, mTagBFP2, Azurite, EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange, mKOκ, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.

In some embodiments, the antigen-binding molecule comprises a peptide tag selected from the group consisting of a His tag, a biotin acceptor tag, an HA tag, a c-Myc tag, a streptavidin tag, and a FLAG tag.

In some embodiments, the antigen-binding molecule comprises biotin.

5 In some embodiments, an antigen-binding molecule comprising a reduced charge Sso7d (rcSso7d) antigen-binding protein that binds *E. coli* OmpA, wherein the antigen-binding protein comprises a variable region having: (i) the amino acid sequence of any one of SEQ ID NOs: 81-83; or (ii) an amino acid sequence having 2 or 1 amino acid differences with any one of SEQ ID NOs: 81-83.

10 In some embodiments, the rcSso7d antigen-binding protein comprises: (i) the amino acid sequence of any one of SEQ ID NOs: 55-57; (ii) an amino acid sequence having no more than ten amino acid differences with any one of SEQ ID NOs: 55-57; or (iii) an amino acid sequence having at least 80% identity with any one of any one of SEQ ID NOs: 55-57. In some embodiments, the rcSso7d antigen-binding protein comprises the amino acid sequence
15 of any one of SEQ ID NOs: 55-57.

In some embodiments, the antigen-binding molecule further comprises an enzyme, a fluorescent protein, an immunoglobulin, a peptide tag, a small molecule, or a combination thereof.

In some embodiments, the antigen-binding molecule comprises an enzyme selected
20 from the group consisting of alkaline phosphatase, horseradish peroxidase, and β -galactosidase.

In some embodiments, the antigen-binding molecule comprises a fluorescent protein selected from the group consisting of TagBFP, mTagBFP2, Azurite, EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric
25 Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange, mKO κ , mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.

30 In some embodiments, the antigen-binding molecule comprises a peptide tag selected from the group consisting of a His tag, a biotin acceptor tag, an HA tag, a c-Myc tag, a streptavidin tag, and a FLAG tag.

In some embodiments, the antigen-binding molecule comprises biotin.

In some aspects, the disclosure relates to compositions comprising an antigen-binding molecule described herein and a buffer, wherein the buffer has a pH $\leq$ 6.0. In some embodiments, the buffer has a pH of 5.0 – 6.0. In some embodiments, the buffer has a pH of about 5.5.

In some embodiments, the buffer comprises sodium acetate and/or phosphate-buffered saline.

In some embodiments, the composition further comprises a LMO2365_0639 protein, an OmpA protein, an OmpW protein, an OmpC protein, an OmpF protein, an OmpD protein, a phoE protein, or a combination thereof.

In some embodiments, the composition further comprises a *Listeria monocytogenes* cell, a *Salmonella typhi* cell, an *E. coli* cell, or a combination thereof.

In some aspects, the disclosure relates to methods of detecting a protein of interest in a sample.

In some embodiments, a method of detecting LMO2365_0639 in a sample comprises: (a) contacting the sample with an antigen-binding molecule described herein that binds to LMO2365_0639; and (b) detecting, if present, LMO2365_0639 protein bound by the antigen-binding molecule.

In some embodiments, a method of detecting OmpA, OmpW, OmpC, OmpF, OmpD, phoE, or a combination thereof in a sample comprises: (a) contacting the sample with an antigen-binding molecule described herein that binds to OmpA, OmpW, OmpC, OmpF, OmpD, phoE, or a combination thereof; and (b) detecting, if present, OmpA, OmpW, OmpC, OmpF, OmpD, phoE, or a combination thereof bound by the antigen-binding molecule.

In some embodiments, a method of detecting OmpW in a sample comprises: (a) contacting the sample with an antigen-binding molecule described herein that binds to OmpW; and (b) detecting, if present, OmpW bound by the antigen-binding molecule.

In some embodiments, a method of detecting OmpA in a sample comprises: (a) contacting the sample with an antigen-binding molecule described herein that binds to OmpA; and (b) detecting, if present, OmpA bound by the antigen-binding molecule.

In some embodiments, the sample is from a food product. In some embodiments, the sample is from a subject. In some embodiments, the subject is a mammal. In some

embodiments, the subject is a human. In some embodiments, the method further comprises treating the subject.

In some embodiments, the sample is contacted with the antigen-binding protein at a pH ≤ 6.0 . In some embodiments, the sample is contacted at a pH of 5.0 – 6.0. In some
5 embodiments, the sample is contacted at a pH of about 5.5.

In some embodiments, the sample comprises sodium acetate and/or phosphate-buffered saline.

In some embodiments, step (a) further comprises contacting the sample with a detection agent, wherein the detection agent binds to or is bound by the antigen-binding
10 molecule.

In some embodiments, the detection agent comprises an enzyme, a fluorescent protein, an immunoglobulin, a peptide tag, a small molecule, and/or a combination thereof.

In some embodiments, the detection enzyme comprises an enzyme selected from the group consisting of alkaline phosphatase, horseradish peroxidase, and β -galactosidase.

In some embodiments, the detection agent comprises a fluorescent protein selected
15 from the group consisting of TagBFP, mTagBFP2, Azurite, EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP,
20 Monomeric Kusabira-Orange, mKO κ , mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.

In some embodiments, the detection agent comprises a peptide tag selected from the group consisting of a His tag, a biotin acceptor tag, an HA tag, a c-Myc tag, a streptavidin
25 tag, and a FLAG tag.

In some embodiments, the detection agent comprises biotin.

In some aspects, the disclosure relates to methods of detecting bacteria in a sample.

In some embodiments, the method comprises: (a) contacting the sample with an antigen-binding molecule described herein that binds to LMOF2365_0639; and (b) detecting,
30 if present, bacteria bound by the antigen-binding molecule. In some embodiments, the method is a method of detecting a *Listeria monocytogenes* cell in a sample comprising: (a) contacting the sample with an antigen-binding molecule described herein that binds to

LMOF2365_0639; and (b) detecting, if present, *Listeria monocytogenes* bound by the antigen-binding molecule.

In some embodiments, the method comprises: (a) contacting the sample with an antigen-binding molecule described herein that binds to OmpA, OmpW, OmpC, OmpF, OmpD, phoE, or a combination thereof; and (b) detecting, if present, bacteria bound by the antigen-binding molecule. In some embodiments, the method is a method of detecting a *Salmonella typhi* cell in a sample comprising: (a) contacting the sample with an antigen-binding molecule described herein OmpA, OmpW, OmpC, OmpF, OmpD, phoE, or a combination thereof; and (b) detecting, if present, *Salmonella typhi* bound by the antigen-binding molecule.

In some embodiments, the method comprises: (a) contacting the sample with an antigen-binding molecule described herein that binds to OmpW; and (b) detecting, if present, bacteria bound by the antigen-binding molecule. In some embodiments, the method is a method of detecting a *E. coli* cell in a sample comprising: (a) contacting the sample with an antigen-binding molecule described herein that binds to OmpW or OmpA; and (b) detecting, if present, *E. coli* bound by the antigen-binding molecule.

In some embodiments, the sample is from a food product. In some embodiments, the sample is from a subject. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human. In some embodiments, the method further comprises treating the subject.

In some embodiments, the sample is contacted with the antigen-binding protein at a $\text{pH} \leq 6.0$. In some embodiments, the sample is contacted at a pH of 5.0 – 6.0. In some embodiments, the sample is contacted at a pH of about 5.5.

In some embodiments, the sample comprises sodium acetate and/or phosphate-buffered saline.

In some embodiments, step (a) further comprises contacting the sample with a detection agent, wherein the detection agent binds to or is bound by the antigen-binding molecule.

In some embodiments, the detection agent comprises an enzyme, a fluorescent protein, an immunoglobulin, a peptide tag, a small molecule, and/or a combination thereof.

In some embodiments, the detection enzyme comprises an enzyme selected from the group consisting of alkaline phosphatase, horseradish peroxidase, and β -galactosidase.

In some embodiments, the detection agent comprises a fluorescent protein selected from the group consisting of TagBFP, mTagBFP2, Azurite, EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SFCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange, mKOκ, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.

In some embodiments, the detection agent comprises a peptide tag selected from the group consisting of a His tag, a biotin acceptor tag, an HA tag, a c-Myc tag, a streptavidin tag, and a FLAG tag.

In some embodiments, the detection agent comprises biotin.

In some aspects, the disclosure relates to methods of killing a bacterial cell.

In some embodiments, the method comprises contacting a *Listeria monocytogenes* cell with an antigen-binding molecule described herein that binds to LMO2365_0639, wherein the antigen-binding molecule further comprises a cytotoxic agent.

In some embodiments, the method comprises contacting a *Salmonella typhi* cell with an antigen-binding molecule described herein that binds to OmpA, OmpW, OmpC, OmpF, OmpD, phoE, or a combination thereof, wherein the antigen-binding molecule further comprises a cytotoxic agent.

In some embodiments, the method comprises contacting an *E. coli* cell with an antigen-binding molecule described herein that binds to OmpW or OmpA, wherein the antigen-binding molecule further comprises a cytotoxic agent.

In some embodiments, the sample is contacted with the antigen-binding protein at a pH ≤ 6.0 . In some embodiments, the sample is contacted at a pH of 5.0 – 6.0. In some embodiments, the sample is contacted at a pH of about 5.5.

In some embodiments, the sample comprises sodium acetate and/or phosphate-buffered saline.

In some aspects, the disclosure relates to methods of monitoring growth of a population of bacteria. In some embodiments, the method comprises: (a) contacting a sample comprising at least a subset of the population with an antigen-binding molecule described herein; and (b) repeating step (a) at least once.

In some embodiments, the method is a method of monitoring growth of *Listeria monocytogenes* comprising: (a) contacting a sample comprising at least a subset of the population with an antigen-binding molecule described herein that binds to LMOF2365_0639; and (b) repeating step (a) at least once.

5 In some embodiments, the method is a method of monitoring growth of *Salmonella typhi* comprising: (a) contacting a sample comprising at least a subset of the population with an antigen-binding molecule described herein that binds to OmpA, OmpW, OmpC, OmpF, OmpD, phoE, or a combination thereof; and (b) repeating step (a) at least once.

10 In some embodiments, the method is a method of monitoring growth of *E. coli* comprising: (a) contacting a sample comprising at least a subset of the population with an antigen-binding molecule described herein that binds to OmpW or OmpA; and (b) repeating step (a) at least once.

15 In some embodiments, step (a) further comprises contacting the sample with a detection agent, wherein the detection agent binds to or is bound by the antigen-binding molecule.

In some embodiments, the detection agent comprises an enzyme, a fluorescent protein, an immunoglobulin, a peptide tag, a small molecule, and/or a combination thereof.

In some embodiments, the detection enzyme comprises an enzyme selected from the group consisting of alkaline phosphatase, horseradish peroxidase, and β -galactosidase.

20 In some embodiments, the detection agent comprises a fluorescent protein selected from the group consisting of TagBFP, mTagBFP2, Azurite, EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, 25 Monomeric Kusabira-Orange, mKO κ , mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.

30 In some embodiments, the detection agent comprises a peptide tag selected from the group consisting of a His tag, a biotin acceptor tag, an HA tag, a c-Myc tag, a streptavidin tag, and a FLAG tag.

In some embodiments, the detection agent comprises biotin.

In some aspects, the disclosure relates to kits comprising an antigen-binding molecule described herein.

In some embodiment, the kit further comprises a buffer. In some embodiments, the buffer has a pH of ≤ 6.0 . In some embodiments, the buffer has a pH of 5.0 – 6.0. In some
5 embodiments, the buffer has a pH of about 5.5. In some embodiments, the buffer comprises sodium acetate and/or phosphate-buffered saline.

In some embodiments, the kit further comprises a substrate. In some embodiments, the substrate comprises paper, nitrocellulose, cellulose powder, and/or a liquid colloid. In some embodiments, the antigen-binding molecule is bound to the substrate.

10 In some embodiments, the kit further comprises a growth media that allows for bacterial growth.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to
15 further demonstrate certain aspects of the present disclosure, which can be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein. It is to be understood that the data illustrated in the drawings in no way limit the scope of the disclosure.

FIGs. 1A-1C. FIG. 1A. Schematic of the combinatorial yeast-surface display library
20 for rcSso7d (diversity: $\sim 1.4 \times 10^9$). FIG. 1B. Schematic of the yeast surface display complex. rcSso7d (PDB: 1SSO) is expressed with HA/c-Myc epitope tags and genetically fused to the Aga2p protein, which is linked to Aga1p in the cell wall via disulfide bonds. The variable binding face of rcSso7d is indicated. FIG. 1C. Schematic of *L. monocytogenes* and the target surface protein, LMOF2365_0639 (“LSP”).

25 FIG. 2. Representative FACS dot plots for the FACS selections (“a”: MBS and FACS against His-LSP, top; “b”: MBS against His-LSP and FACS against LSP-BA, middle; “c”: MBS and FACS against LSP-BA, bottom). Positive sorts are shown for FACS #1, #2, and #4 to show enrichment. Negative sorts were conducted prior to FACS #2a, #4a, #2b, and #4b. Concentration of His-LSP or LSP-BA used are listed above the plots, with a reduction in
30 concentration in subsequent FACS rounds for increased stringency. Numbers indicate the percentage of the population in each quadrant. Gates drawn indicate the gates used for sorting.

FIGs. 3A-3B. Target-specific binding of identified rcSso7d clones. FIG. 3A. Schematic of protein complex for rcSso7d clonal analysis on yeast-surface display. FIG. 3B. FACS histograms of target-specific binding for the eight identified rcSso7d clones against 20 nM of LSP from the three different selection processes. All clones show specific binding, except clone 8, which has off-target binding to the labeling reagents.

FIGs. 4A-4C. Heat stability of identified rcSso7d clones. FIG. 4A. Aligned amino acid sequences of the seven unique rcSso7d clones specific to LSP. Clones 3, 5, and 7 are full-length, and clones 1, 2, 4, and 6 are truncated (early stop codon). Binding face sequences are underlined. Highlighted amino acids were previously found to be important for rcSso7d core stability. Clone 7 has an additional inserted amino acid (bolded). FIG. 4B. Protein ribbon structure of rcSso7d (PDB: 1SSO), depicting the amino acids of the binding face and the hydrophobic core. F5, Y7, F31, and Y33 are particularly important in rcSso7d core stability (24). FIG. 4C. FACS histograms of rcSso7d thermal stability, demonstrating reduction in target binding activity (percentage represents reduction in signal based on the geometric mean fluorescence intensity) after heating the rcSso7d-displayed clonal yeast for 10 minutes at 80 °C.

FIGs. 5A-5D. rcSso7d.LSP.3 binding to live, whole cells. FIG. 5A. Schematic of desired protein complex on the surface of *L. monocytogenes* cells for rcSso7d binding. FIGs. 5A-5D. FACS histograms of rcSso7d.LSP.3 binding to live, whole cell (FIGs. 5B-5C) *L. monocytogenes* or (FIG. 5C) *Bacillus subtilis*. rcSso7d incubations with whole *L. monocytogenes* cells were tested in (FIG. 5B) PBS (pH 7.4) and (FIG. 5C) sodium acetate (pH 5.5). Higher signal was seen in the lower pH buffer. Incubations with whole *B. subtilis* cells were tested in (FIG. 5D) sodium acetate (pH 5.5) for comparison.

FIGs. 6A-6C. SDS-PAGE gel images for (FIG. 6A) His-LSP, (FIG. 6B) LSP-BA, and (FIG. 6C) rcSso7d.LSP.3 after purification on Ni-NTA columns. His-LSP (FIG. 6A) and LSP-BA (FIG. 6B) were collected into one fraction each and both demonstrated high purity. Gel image for rcSso7d.LSP.3 (FIG. 6C) shows lanes with the clarified lysate prior to purification, the flowthrough after loading the Ni-NTA column, and the two fractions collected during purification. Fraction #2 was used based on presence and purity of the product.

FIG. 7. FACS dot plots for the post-MBS sub-libraries (left: post-MBS #1 and right: post-MBS #2; “a” denotes that these are the rounds of MBS conducted using His-LSP.

Samples shown in the top plots were incubated with His-LSP, and samples shown in the bottom plots were incubated without His-LSP. Percentages shown indicate the percentage of the population in the upper quadrants.

FIG. 8. FACS dot plots for the FACS selections against His-LSP, originating from the MBS population screened using His-LSP (FACS “a”). Negative sorts (top row) were conducted prior to positive sorts (middle row) for FACS #2a, #3a, and #4a. Bottom row depicts the negative controls (samples incubated without His-LSP) for the positive FACS sub-populations. Concentration of His-LSP used are listed above the plots, with a reduction in concentration from 1 μ M to 1 nM for increased stringency. Numbers indicate the percentage of the population in each quadrant. Gates drawn indicate the gates used for sorting.

FIG. 9. FACS dot plots for the sub-libraries generated from MBS and FACS selections against His-LSP. The sub-libraries were challenged against LSP-BA to assess whether the orientation of the target label (N-terminus vs. C-terminus) affects the sorting populations. Low levels of positive binding were seen in all sub-libraries.

FIG. 10. FACS dot plots for the FACS selections against LSP-BA, originating from the MBS population screened using His-LSP (FACS “b”). Negative sorts (top row) were conducted prior to positive sorts (middle row) for FACS #2b, #3b, and #4b. Bottom row depicts the negative controls (samples incubated without LSP-BA) for the positive FACS sub-populations. Concentration of LSP-BA used are listed above the plots, with a reduction in concentration from 100 nM to 1 nM for increased stringency. Numbers indicate the percentage of the population in each quadrant. Gates drawn indicate the gates used for sorting.

FIG. 11. FACS dot plots for the FACS selections against LSP-BA, originating from the MBS population screened using LSP-BA (FACS “c”). Negative sorts (top row) were conducted prior to positive sorts (middle row) for FACS #3c. Bottom row depicts the negative controls (samples incubated without LSP-BA) for the positive FACS sub-populations. Concentration of LSP-BA used are listed above the plots, with a reduction in concentration from 100 nM to 5 nM for increased stringency. Numbers indicate the percentage of the population in each quadrant. Gates drawn indicate the gates used for sorting.

FIG. 12. Schematic of desired protein complex on the surface of *E. coli*, *Salmonella*

ssp., *L. monocytogenes*, and *Listeria spp.* cells for rcSso7d binding. Exemplary target proteins include OmpA, OmpW, LSP, OmpC, OmpF, OmpD, and phoE.

FIG. 13. Alignment of OmpA amino acid sequences from *Salmonella* species and *E. coli*. Regions of identity are highlighted.

5 FIG. 14A-14B. Design, production and characterization of additional antigens. FIG. 14A. Crystal structures of *E. coli* OmpA, *E. coli* OmpW and *Salmonella typhi* Native Omp. FIG. 14B. SDS PAGE gels of the antigens used in selection of rcSso7d variants.

FIG. 15. Magnetic bead sorts (MBS) to reduce library diversity from 1.4×10^9 to the upper limit for FACS (1×10^7).

10 FIGs. 16A-16C. FACS dot plots for the libraries generated from MBS and FACS selections against Native Omp (FIG. 16A), OmpW (FIG. 16B) and OmpA (FIG. 16C). The subpopulations of antigen-binders in each round were selected, expanded in culture, and eventually sequenced to determine the identity of the clones that are specific to each antigen.

FIG. 17. Aligned amino acid sequences of unique rcSso7d clones specific to Native
15 Omp. Binding face sequences are underlined. Highlighted amino acids were previously found to be important for rcSso7d core stability.

FIG. 18. Aligned amino acid sequences of unique rcSso7d clones specific to OmpW. Binding face sequences are underlined. Highlighted amino acids were previously found to be important for rcSso7d core stability.

20 FIG. 19. Aligned amino acid sequences of unique rcSso7d clones specific to OmpA. Binding face sequences are underlined. Highlighted amino acids were previously found to be important for rcSso7d core stability.

DETAILED DESCRIPTION

25 The traditional method of detecting bacteria (*e.g.*, *Listeria*, such as *L. monocytogenes*; *Salmonella* and *E. coli*) in food and environmental samples uses a culture-based method, which involves multiple sequential pre-enrichment steps by plating on selective agar before additional biochemical tests for confirmation (6). This process is quite time-consuming (>96 hours due to enrichment steps) and can be quite labor-intensive (2, 4, 7, 8). Additionally, the
30 enrichment process may lead to false negative results if other non-pathogenic bacteria is also present in the sample and allowed to outgrow the *L. monocytogenes* (9). Non-culture-based, rapid detection methods have been investigated to reduce the processing time; these methods

include nucleic acid based tests—such as polymerase chain reaction (PCR), real-time PCR, and loop-mediated isothermal amplification (LAMP), mass spectrometry, and immunoassays and biosensors (2, 4, 7, 8). Unfortunately, many of these methods still require lengthy pre-enrichment steps, require expensive equipment, are not able to distinguish live cells from
5 dead cells, or have inadequate sensitivity and specificity.

Rapid diagnostic tests that can be used directly in the field site, manufacturing plants, and farms would allow for early detection of potential contaminants to reduce the current large burden of these foodborne pathogens. These tests would ideally not require complicated equipment or trained personnel, be inexpensive, and provide results within hours
10 rather than days. Typical rapid diagnostic tests use antibodies to detect presence of the target molecule in the sample. Antibodies have been developed for *L. monocytogenes* detection by targeting surface biomarkers on the bacteria; however, most of these antibodies have not been specific to *L. monocytogenes* (7). In recent years, alternative binding proteins have been investigated for rapid diagnostic tests to replace antibodies as the affinity reagents due to their
15 thermal stability, easy and inexpensive mass-production, and manipulability of the scaffold for additional properties (10-14).

Here, the reduced-charge Sso7d (rcSso7d) alternative binding scaffold was used to engineer affinity reagents against bacteria (*e.g.*, *L. monocytogenes*) via *in vitro* selection processes using yeast-surface display. For example, a *L. monocytogenes* surface protein,
20 LMOF2365_0639, that had previously been identified as a surface marker for *L. monocytogenes* (15) was targeted. rcSso7d clones were identified that bind to live *L. monocytogenes* whole cells with minimal cross-reactivity to a non-pathogenic Gram-positive bacterium, *Bacillus subtilis*. Those skilled in the art will realize that other bacterial will have unique surface proteins that can be used as targets for the selection of engineered binding
25 proteins. The methods described here can be used to develop engineered binding proteins for all types of bacteria. Once identified these binding proteins can be used to create methods to selectively detect different bacteria. Although not limited to pathogenic bacteria, efficient detection methods enabled by these binding proteins are of particular interest to ensure the safety of food and beverages. Additionally, non-pathogenic bacteria that leads to spoilage of
30 food and beverages is of interest, to prevent food waste by allowing early detection of these organisms in the food production process.

I. Antigen-Binding Molecules.

In some aspects, this disclosure relates to antigen-binding molecules that bind to surface proteins associated with bacteria of interest, such as LSP (LMOF2365_0639, a surface protein of *Listeria monocytogenes* (15)), OmpA, OmpW, OmpC, OmpF, OmpD, and/or
5 phoE. In some embodiments, an antigen-binding molecule comprises a reduced charge Sso7d (rcSso7d) antigen-binding protein. In some embodiments, an antigen-binding molecule comprises multiple rcSso7d antigen-binding proteins. In some embodiment, each of the antigen binding molecules are the same. In other embodiments, an antigen-binding molecule comprises two or more distinct rcSso7d antigen-binding proteins (*e.g.*, each of
10 which binds to a unique antigen).

The general structure of rcSso7d antigen-binding proteins have been described previously. *See e.g.*, FIGs. 4A-4B. Of particular note, an rcSso7d antigen-binding protein comprises a scaffold region (*i.e.*, stable core region in FIG. 4B) and a variable region (*i.e.*, binding face in FIG. 4B). The amino acids of the variable region are surface exposed and
15 enable an rcSso7d antigen-binding protein to specifically bind a target (*e.g.*, LMOF2365_0639).

It is understood that the amino acids that form the variable region need not be positioned sequentially within the amino acid sequence of the antigen-binding protein; in other words, the amino acids corresponding to the variable region may be separated by one or
20 more amino acid of the scaffold region. Notwithstanding this point, the amino acids corresponding to the scaffold region and the variable region of an rcSso7d antigen-binding protein are readily identifiable by one having ordinary skill in the art (16-22).

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region having a defined amino acid sequence. In light
25 of the disclosure above (*i.e.*, relating to the structures of rcSso7d antigen-binding proteins), it is understood that the amino acid sequences provided herein for an rcSso7d antigen-binding protein variable region do not strictly reflect an N-terminal to C-terminal sequence of amino acids within the polypeptide chain of the rcSso7d antigen-binding protein. Instead, it is understood that – although the amino acid sequences are provided in the N-terminal to C-
30 terminal orientation – one or more amino acids of the scaffold region may separate the amino acids of the variable domains described herein. *See e.g.*, FIG. 4A.

In some embodiments, an antigen-binding molecule comprises a rcSso7d antigen-

binding protein that binds to a bacterium. In some embodiments, an rcSso7d antigen-binding protein binds to a gram-positive bacterium. In some embodiments, an rcSso7d antigen-binding protein binds to a gram-negative bacterium. In some embodiments, an rcSso7d antigen-binding protein binds to a pathogenic bacterium. In some embodiments, an rcSso7d antigen-binding protein binds to a bacterium that causes food poisoning. In some embodiments, an rcSso7d antigen-binding protein binds to a bacterium that causes food spoilage.

In some embodiments, an rcSso7d antigen-binding protein binds to a bacterium of the genus *Listeria*, *Escherichia*, *Salmonella*, or *Legionella*. Bacterial species, sub-species and strains falling within each of these genera are known to those having ordinary skill in the art. For example, exemplary species falling within the genus *Listeria* include *Listeria monocytogenes*, *Listeria seeligeri*, *Listeria ivanovii*, *Listeria welshimeri*, *Listeria marthii*, *Listeria innocua*, *Listeria grayi*, *Listeria fleischmannii*, *Listeria floridensis*, *Listeria aquatica*, *Listeria newyorkensis*, *Listeria cornellensis*, *Listeria rocourtiae*, *Listeria weihenstephanensis*, *Listeria grandensis*, *Listeria riparia*, and *Listeria booriae*.

In some embodiments, an rcSso7d antigen-binding protein binds to an antigen that is shared by bacteria of different genera, species, sub-species, and/or strains. For example, in some embodiments an rcSso7d antigen-binding protein binds to a conserved surface antigen of such genera, species, sub-species, and/or strains.

In other embodiments, an rcSso7d antigen-binding protein binds to an antigen that is unique to a specific bacterial species, sub-species, or strain.

In some embodiments, an antigen-binding molecule binds to a protein antigen, a glycosylated protein antigen, and/or a carbohydrate antigen, which may be common to more than one genera, species, sub-species, and/or strains, or which may be unique to a specific bacterial species, sub-species, or strain.

Antigens that can be used for selective detection of one or more genera, species, sub-species, and/or strains by the antigen-binding proteins described herein can be identified using methods known to those of skill in the art, *e.g.*, by database searching. In some embodiments, an antigen is present on the surface of a cell and can be used, for example, to detect viable/intact cells. In some embodiments, an antigen is present in the interior of a cell. In some embodiments, the antigens are surface proteins that are specific to a particular species of bacteria or alternatively are widely expressed across one or more species or genera.

In some embodiments, the antigens contain carbohydrate residues that are specific to a particular species of bacteria, or alternatively that are widely expressed across one or more species or genera. Thus, the antigen-binding proteins and methods of use thereof disclosed herein can be used to target different groups or individual types of bacteria by selecting the engineered binding proteins that target an antigen of interest. In some embodiments, multiple binding proteins that bind multiple antigens can be identified or engineered, and this collection of multiple binding proteins is used in assays that have selectivity for one or more different types of bacteria.

In some embodiments, an rcSso7d antigen-binding protein binds to LSP (LMOF2365_0639, a surface protein of *Listeria monocytogenes* (15)).

In some embodiments, an rcSso7d antigen-binding protein binds to OmpA.

In some embodiments, an rcSso7d antigen-binding protein binds to OmpW.

In some embodiments, an rcSso7d antigen-binding protein binds to OmpC.

In some embodiments, an rcSso7d antigen-binding protein binds to OmpF.

In some embodiments, an rcSso7d antigen-binding protein binds to OmpD.

In some embodiments, an rcSso7d antigen-binding protein binds to phoE.

In some embodiments, an rcSso7d antigen-binding protein binds to LMOF2365_0639, a surface protein of *Listeria monocytogenes* (15). In some embodiments, an antigen-binding molecule comprises a reduced charge Sso7d (rcSso7d) antigen-binding protein that specifically binds LMOF2365_0639. The general structure of rcSso7d antigen-binding proteins have been described previously, and rcSso7d antigen-binding proteins that specifically bind LMOF2365_0639 are further described herein.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region having:

- (i) the amino acid sequence of DGSNCY (SEQ ID NO: 1);
- (ii) the amino acid sequence of DGHKCL (SEQ ID NO: 2);
- (iii) the amino acid sequence of IKYIDSRWI (SEQ ID NO: 3);
- (iv) the amino acid sequence of DGYRCW (SEQ ID NO: 4);
- (v) the amino acid sequence of WRAWDAKYI (SEQ ID NO: 5);
- (vi) the amino acid sequence of DGHHCW (SEQ ID NO: 6);
- (vii) the amino acid sequence of IAYYYYSSIK (SEQ ID NO: 7);
- (viii) the amino acid sequence of NIYWVNISY (SEQ ID NO: 8).

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region that has an amino acid sequence having 2 or 1 amino acid differences relative to one or more of SEQ ID NOs: 1-8.

An “amino acid difference,” as used herein, may be an amino acid addition, an amino acid deletion, or an amino acid substitution. In some embodiments, an amino acid difference described herein is an amino acid addition. In some embodiments, an amino acid difference described herein is an amino acid deletion. In some embodiments, an amino acid difference described herein is an amino acid substitution. In some embodiments, the amino acid substitution is a conservative amino acid substitution (*i.e.*, a substitution with an amino acid with similar biochemical properties).

For example, in some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region that has an amino acid sequence having 2 or 1 amino acid deletion(s) relative to one or more of SEQ ID NOs: 1-8. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region that has an amino acid sequence having 2 or 1 amino acid addition(s) relative to one or more of SEQ ID NOs: 1-8. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region that has an amino acid sequence having 2 or 1 amino acid substitution(s), optionally a conservative amino acid substitution(s), relative to one or more of SEQ ID NOs: 1-8.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region having:

- (i) the amino acid sequence of
MATVKFTYQGEEKQVDISKIKX_vAX_vRX_vGQX_vIX_vFX_vYDEGGGAX_vGX_vGX_vVSEKDA
PKELLQ (SEQ ID NO: 9);
- (ii) the amino acid sequence of
MATVKFTYQGEEKQVDISKIKX_vVX_vRX_vGQX_vIX_vFX_vYDEGGGAX_vGX_vGX_vVSEKDA
PKELLQ (SEQ ID NO: 10);
- (iii) the amino acid sequence of
MATVKFTYQGEEKQVDISKIKX_vAX_vX_vRX_vGQX_vIX_vFX_vYDEGGGAX_vGX_vGX_vVSEK
DAPKELLQ (SEQ ID NO: 11); or
- (iv) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKX_vVX_vRX_vGQX_vIX_vFX_vL (SEQ ID NO: 12);

wherein X_v corresponds to an amino acid of the variable region.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region that has an amino acid sequence having ten or fewer, nine or fewer, eight or fewer, seven or fewer, six or fewer, five or fewer, four or fewer, three or fewer, two or fewer, or one amino acid difference(s) with one or more of SEQ ID NOs: 9-12. In some embodiments, the amino acids corresponding to amino acids of the variable region are not considered when determining the number of amino acid differences.

For example, in some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region that has an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid deletion(s) relative to one or more of SEQ ID NOs: 9-12, optionally wherein the amino acids of the variable region are not considered. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region that has an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid additions relative to one or more of SEQ ID NOs: 9-12, optionally wherein the amino acids of the variable region are not considered. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region that has an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1, optionally a conservative amino acid substitution(s), relative to one or more of SEQ ID NOs: 9-12, optionally wherein the amino acids of the variable region are not considered.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region that has at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, or at least 98% identity with the amino acid sequence of one or more of SEQ ID NOs: 9-11. In some embodiments, the amino acids corresponding to amino acids of the variable region are not considered when determining the sequence identity. Methods of determining the extent of identity between two sequences (*e.g.*, two amino acid sequences or two polynucleic acids) are known to those having ordinary skill in the art. One exemplary method is the use of Basic Local Alignment Search Tool (BLAST®) software with default parameters (blast.ncbi.nlm.nih.gov/Blast.cgi).

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising:

- (i) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKIAKRYGQIIDFSYDEGGGARGWGIVSEKDAPKELLQ
MLEKQ (SEQ ID NO: 13);

(ii) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKWVRRAGQWIDFAYDEGGGAKGYGIVSEKDAPKELL

5 QCWKS (SEQ ID NO: 14);

(iii) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKIAVYRYGQYIYFSYDEGGGASGIGKVSEKDAPKELL

QMLEKQ (SEQ ID NO: 15);

(iv) the amino acid sequence of

10 MATVKFTYQGEEKQVDISKIKDVGRSGQNICFYL (SEQ ID NO: 16);

(v) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKDVGRHGQKICFLL (SEQ ID NO: 17);

(vi) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKDVGRYGQRICFWL (SEQ ID NO: 18); or

15 (vii) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKDVGRHGQHICFWL (SEQ ID NO: 19).

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein that has an amino acid sequence having ten or fewer, nine or fewer, eight or fewer, seven or fewer, six or fewer, five or fewer, four or fewer, three or fewer, two or fewer,
20 or one amino acid difference(s) with one or more of SEQ ID NOs: 13-19. In some embodiments, the amino acids corresponding to amino acids of the variable region are not considered when determining the number of amino acid differences.

For example, in some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region that has an amino acid
25 sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid deletion(s) relative to one or more of SEQ ID NOs: 13-19, optionally wherein the amino acids of the variable region are not considered. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or
30 1 amino acid additions relative to one or more of SEQ ID NOs: 13-19, optionally wherein the amino acids of the variable region are not considered. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1, optionally a conservative amino acid

substitution(s), relative to one or more of SEQ ID NOs: 13-19, optionally wherein the amino acids of the variable region are not considered.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein that has an amino acid sequence that has at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, or at least 98% identity with the amino acid sequence of one or more of SEQ ID NOs: 13-19. In some embodiments, the amino acids corresponding to amino acids of the variable region are not considered when determining the sequence identity.

In some embodiments, an rcSso7d antigen-binding protein binds to Native Omp, a protein comprising the amino sequences of *Salmonella typhi* membrane proteins, OmpA, OmpW, OmpC, OmpF, OmpD, and phoE.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region having:

- (i) the amino acid sequence of WKDSYWIAH (SEQ ID NO: 58);
- (ii) the amino acid sequence of SSRGASRYH (SEQ ID NO: 59);
- (iii) the amino acid sequence of IAAWWWHRG (SEQ ID NO: 60);
- (iv) the amino acid sequence of GWWWWWARY (SEQ ID NO: 61);
- (v) the amino acid sequence of WIHGYSIGS (SEQ ID NO: 62);
- (vi) the amino acid sequence of WHHWGDIDS (SEQ ID NO: 63);
- (vii) the amino acid sequence of WISRNAGYG (SEQ ID NO: 64);
- (viii) the amino acid sequence of YSGDSRIIY (SEQ ID NO: 65);
- (ix) the amino acid sequence of HNHNDNRNI (SEQ ID NO: 66);
- (x) the amino acid sequence of GYAHKRGHG (SEQ ID NO: 67);
- (xi) the amino acid sequence of AYNKIIYHH (SEQ ID NO: 68);
- (xii) the amino acid sequence of HYAYKGAGH (SEQ ID NO: 69);
- (xiii) the amino acid sequence of NYHYKRSYS (SEQ ID NO: 70); or
- (xix) the amino acid sequence of YIWHWYWSN (SEQ ID NO: 71).

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region that has an amino acid sequence having 2 or 1 amino acid differences (as defined herein) relative to one or more of SEQ ID NOs: 58-71. For example, in some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region that has an amino acid sequence having

2 or 1 amino acid deletion(s) relative to one or more of SEQ ID NOs: 58-71. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region that has an amino acid sequence having 2 or 1 amino acid addition(s) relative to one or more of SEQ ID NOs: 58-71. In some embodiments, an

5 antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region that has an amino acid sequence having 2 or 1 amino acid substitution(s), optionally a conservative amino acid substitution(s), relative to one or more of SEQ ID NOs: 58-71.

In some embodiments, an rcSso7d antigen-binding protein that binds to Native Omp

10 comprises a scaffold region having the amino acid sequence of
 MATVKFTYQGEEKQVDISKIX_vVX_vRX_vGQX_vIX_vFX_vYDEGGGAX_vGX_vGX_vVSEKDA
 PKELLEKQ (SEQ ID NO: 72), wherein X_v corresponds to an amino acid of the variable region. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region that has an amino acid sequence having ten or

15 fewer, nine or fewer, eight or fewer, seven or fewer, six or fewer, five or fewer, four or fewer, three or fewer, two or fewer, or one amino acid difference(s) with SEQ ID NO: 72. In some embodiments, the amino acids corresponding to amino acids of the variable region are not considered when determining the number of amino acid differences.

For example, in some embodiments, an antigen-binding molecule comprises an

20 rcSso7d antigen-binding protein comprising a scaffold region that has an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid deletion(s) relative to SEQ ID NO: 72, optionally wherein the amino acids of the variable region are not considered. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region that has an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2

25 or 1 amino acid additions relative to SEQ ID NO: 72, optionally wherein the amino acids of the variable region are not considered. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region that has an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1, optionally a conservative amino acid substitution(s), relative to SEQ ID NO: 72, optionally wherein the amino acids of the variable

30 region are not considered.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region that has at least 80% identity, at least 85%

identity, at least 90% identity, at least 95% identity, or at least 98% identity with the amino acid sequence of SEQ ID NO: 72. In some embodiments, the amino acids corresponding to amino acids of the variable region are not considered when determining the sequence identity.

5 In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising:

(i) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKXVKRDGQSIYFWYDEGGGAIGAGHVSEKDAPKELLEKQ (SEQ ID NO: 33);

10 (ii) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKSVSRRGQGIAFSYDEGGGARGYGHVSEKDAPKELLEKQ (SEQ ID NO: 34);

(iii) the amino acid sequence of

15 MATVKFTYQGEEKQVDISKIKIVARAGQWIWFWYDEGGGAHGRGGVSEKDAPKELLEKQ (SEQ ID NO: 35);

(iv) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKGVWRWGQWIWFWYDEGGGAAGRGYVSEKDAPKELLEKQ (SEQ ID NO: 36);

(v) the amino acid sequence of

20 MATVKFTYQGEEKQVDISKIKWVIRHGQGIYFSYDEGGGAIGGGSVSEKDAPKELLEKQ (SEQ ID NO: 37);

(vi) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKWVHRHGQWIGFDYDEGGGAIGDGSVSEKDAPKELLEKQ (SEQ ID NO: 38);

25 (vii) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKWVIRSGQRINFAYDEGGGAGGYGGVSEKDAPKELLEKQ (SEQ ID NO: 39);

(viii) the amino acid sequence of

30 MATVKFTYQGEEKQVDISKIKYVSRGGQDISFRYDEGGGAIGIGYVSEKDAPKELLEKQ (SEQ ID NO: 40);

(ix) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKHVNRHGQNIDFRYDEGGGANRGIVSEKDAPKELLEKQ (SEQ ID NO: 41);

EKQ (SEQ ID NO: 41);

(x) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKGVYRAGQHIKFRYDEGGGAGGHGGVSEKDAPKELL

EKQ (SEQ ID NO: 42);

5 (xi) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKAVYRNGQKIIFIYDEGGGAYGHGHVSEKDAPKELLE

KQ (SEQ ID NO: 43);

(xii) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKHVYRAGQYIKFGYDEGGGAAGGGHVSEKDAPKELL

10 EKQ (SEQ ID NO: 44);

(xiii) the amino acid sequence of

MATVKFTYQGEEKQVDISKIKNVYRHGQYIKFRYDEGGGASGYGSVSEKDAPKELL

EKQ (SEQ ID NO: 45); or

(xiv) the amino acid sequence of

15 MATVKFTYQGEEKQVDISKIKYVIRWGQHIWFFYYDEGGGAWGSGNVSEKDAPKEL

LEKQ (SEQ ID NO: 46).

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein that has an amino acid sequence having ten or fewer, nine or fewer, eight or fewer, seven or fewer, six or fewer, five or fewer, four or fewer, three or fewer, two or fewer,
20 or one amino acid difference(s) with one or more of SEQ ID NOs: 33-46. In some embodiments, the amino acids corresponding to amino acids of the variable region are not considered when determining the number of amino acid differences.

For example, in some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising an amino acid sequence having 10, 9, 8, 7, 6, 5,
25 4, 3, 2 or 1 amino acid deletion(s) relative to one or more of SEQ ID NOs: 33-46, optionally wherein the amino acids of the variable region are not considered. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid additions relative to one or more of SEQ ID NOs: 33-46, optionally wherein the amino acids of the variable region are not
30 considered. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1, optionally a conservative amino acid substitution(s), relative to one or more of SEQ ID

NOs: 33-46, optionally wherein the amino acids of the variable region are not considered.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein that has an amino acid sequence that has at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, or at least 98% identity with the amino acid sequence of one or more of SEQ ID NOs: 33-46. In some embodiments, the amino acids corresponding to amino acids of the variable region are not considered when determining the sequence identity.

In some embodiments, an rcSso7d antigen-binding protein binds to *E. coli* OmpW.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region having:

- (i) the amino acid sequence of WSWYYSIGG (SEQ ID NO: 73);
- (ii) the amino acid sequence of IHAWGXWSI (SEQ ID NO: 74), wherein X is any naturally-occurring amino acid, such as wherein X is alanine (A), arginine (R), asparagine (N), aspartic acid (D), cysteine (C), glutamic acid (D), glutamine (Q), glycine, (G), histidine (H), isoleucine (I), leucine (L), lysine (K), methionine (M), phenylalanine (F), proline (P), serine (S), threonine (T), tryptophan (W), tyrosine (Y), or valine (V);
- (iii) the amino acid sequence of HSGWDAHDA (SEQ ID NO: 75);
- (iv) the amino acid sequence of WSWYYSSGS (SEQ ID NO: 76);
- (v) the amino acid sequence of WWWWKYAWI (SEQ ID NO: 77);
- (vi) the amino acid sequence of YKWYWIAYN (SEQ ID NO: 78);
- (vii) the amino acid sequence of WWYRYWWI (SEQ ID NO: 79); or
- (viii) the amino acid sequence of IWWARWGRY (SEQ ID NO: 80).

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region that has an amino acid sequence having 2 or 1 amino acid differences (as defined herein) relative to one or more of SEQ ID NOs: 73-80. For example, in some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region that has an amino acid sequence having 2 or 1 amino acid deletion(s) relative to one or more of SEQ ID NOs: 73-80. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region that has an amino acid sequence having 2 or 1 amino acid addition(s) relative to one or more of SEQ ID NOs: 73-80. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a

variable region that has an amino acid sequence having 2 or 1 amino acid substitution(s), optionally a conservative amino acid substitution(s), relative to one or more of SEQ ID NOs: 73-80.

In some embodiments, an rcSso7d antigen-binding protein that binds to *E. coli*

- 5 OmpW comprises a scaffold region having the amino acid sequence of
 MATVKFTYQGEEKQVDISKIKX_vVX_vRX_vGQX_vIX_vFX_vYDEGGGAX_vGX_vGX_vVSEKDA
 PKELLEKQ (SEQ ID NO: 72), wherein X_v corresponds to an amino acid of the variable
 region. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-
 binding protein comprising a scaffold region that has an amino acid sequence having ten or
 10 fewer, nine or fewer, eight or fewer, seven or fewer, six or fewer, five or fewer, four or fewer,
 three or fewer, two or fewer, or one amino acid difference(s) with SEQ ID NO: 72. In some
 embodiments, the amino acids corresponding to amino acids of the variable region are not
 considered when determining the number of amino acid differences.

- For example, in some embodiments, an antigen-binding molecule comprises an
 15 rcSso7d antigen-binding protein comprising a scaffold region that has an amino acid
 sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid deletion(s) relative to SEQ ID NO:
 72, optionally wherein the amino acids of the variable region are not considered. In some
 embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein
 comprising a scaffold region that has an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2
 20 or 1 amino acid additions relative to SEQ ID NO: 72, optionally wherein the amino acids of
 the variable region are not considered. In some embodiments, an antigen-binding molecule
 comprises an rcSso7d antigen-binding protein comprising a scaffold region that has an amino
 acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1, optionally a conservative amino acid
 substitution(s), relative to SEQ ID NO: 72, optionally wherein the amino acids of the variable
 25 region are not considered.

- In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-
 binding protein comprising a scaffold region that has at least 80% identity, at least 85%
 identity, at least 90% identity, at least 95% identity, or at least 98% identity with the amino
 acid sequence of SEQ ID NO: 72. In some embodiments, the amino acids corresponding to
 30 amino acids of the variable region are not considered when determining the sequence
 identity.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-

binding protein comprising:

- (i) the amino acid sequence of
MATVKFTYQGEEKQVDISKIKWVSRWGQYIYFSYDEGGGAIGGGGVSEKDAPKELL
EKQ (SEQ ID NO: 47);
- 5 (ii) the amino acid sequence of
MATVKFTYQGEEKQVDISKIKIVHRAGQWIGFXSYDEGGGAWGSGIVSEKDAPKELL
EKQ (SEQ ID NO: 48);
- (iii) the amino acid sequence of
MATVKFTYQGEEKQVDISKIKHVSRRGQWIDFAYDEGGGAHGDGAVSEKDAPKELL
10 EKQ (SEQ ID NO: 49);
- (iv) the amino acid sequence of
MATVKFTYQGEEKQVDISKIKWVSRWGQYIYFSYDEGGGASGGGSVSEKDAPKELL
EKQ (SEQ ID NO: 50);
- (v) the amino acid sequence of
15 MATVKFTYQGEEKQVDISKIKWVWRWGQWIKFYIYDEGGGAAGWGIVSEKDAPKE
LLEKQ (SEQ ID NO: 51);
- (vi) the amino acid sequence of
MATVKFTYQGEEKQVDISKIKYVKRWGQYIWFYIYDEGGGAAGYGNVSEKDAPKELL
EKQ (SEQ ID NO: 52);
- 20 (vii) the amino acid sequence of
MATVKFTYQGEEKQVDISKIKWVWRWGQYIRFYIYDEGGGAWGWGIVSEKDAPKEL
LEKQ (SEQ ID NO: 53); or
- (viii) the amino acid sequence of
MATVKFTYQGEEKQVDISKIKIVWRWGQAIRFWYDEGGGAGGRGYVSEKDAPKEL
25 LEKQ (SEQ ID NO: 54).

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein that has an amino acid sequence having ten or fewer, nine or fewer, eight or fewer, seven or fewer, six or fewer, five or fewer, four or fewer, three or fewer, two or fewer, or one amino acid difference(s) with one or more of SEQ ID NOs: 47-54. In some
30 embodiments, the amino acids corresponding to amino acids of the variable region are not considered when determining the number of amino acid differences.

For example, in some embodiments, an antigen-binding molecule comprises an

rcSso7d antigen-binding protein comprising an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid deletion(s) relative to one or more of SEQ ID NOs: 47-54, optionally wherein the amino acids of the variable region are not considered. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid additions relative to one or more of SEQ ID NOs: 47-54, optionally wherein the amino acids of the variable region are not considered. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1, optionally a conservative amino acid substitution(s), relative to one or more of SEQ ID NOs: 47-54, optionally wherein the amino acids of the variable region are not considered.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein that has an amino acid sequence that has at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, or at least 98% identity with the amino acid sequence of one or more of SEQ ID NOs: 47-54. In some embodiments, the amino acids corresponding to amino acids of the variable region are not considered when determining the sequence identity.

In some embodiments, an rcSso7d antigen-binding protein binds to *E. coli* OmpA.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region having:

- (i) the amino acid sequence of RWWGYWDNW (SEQ ID NO: 81);
- (ii) the amino acid sequence of RHYWRRHH (SEQ ID NO: 82); or
- (iii) the amino acid sequence of HHWWWW (SEQ ID NO: 83).

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region that has an amino acid sequence having 2 or 1 amino acid differences (as defined herein) relative to one or more of SEQ ID NOs: 81-83. For example, in some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region that has an amino acid sequence having 2 or 1 amino acid deletion(s) relative to one or more of SEQ ID NOs: 81-83. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a variable region that has an amino acid sequence having 2 or 1 amino acid addition(s) relative to one or more of SEQ ID NOs: 81-83. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a

variable region that has an amino acid sequence having 2 or 1 amino acid substitution(s), optionally a conservative amino acid substitution(s), relative to one or more of SEQ ID NOs: 81-83.

In some embodiments, an rcSso7d antigen-binding protein that binds to *E. coli* OmpA comprises a scaffold region having:

(ii) the amino acid sequence of
MATVKFTYQGEEKQVDISKIKX_VVX_VRX_VGQX_VIX_VFX_VYDEGGGAX_VGX_VGX_VVSEKDA
PKELLEKQ (SEQ ID NO: 72) or

(ii) the amino acid sequence of
10 MATVKFTYQGEEKQVDISKIKX_VVX_VRX_VGQX_VIX_VFX_VL (SEQ ID NO: 12);
wherein X_V corresponds to an amino acid of the variable region.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region that has an amino acid sequence having ten or fewer, nine or fewer, eight or fewer, seven or fewer, six or fewer, five or fewer, four or fewer,
15 three or fewer, two or fewer, or one amino acid difference(s) with one or more of SEQ ID NO: 12 and 72. In some embodiments, the amino acids corresponding to amino acids of the variable region are not considered when determining the number of amino acid differences.

For example, in some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region that has an amino acid
20 sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid deletion(s) relative to SEQ ID NO: 12 or 72, optionally wherein the amino acids of the variable region are not considered. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region that has an amino acid sequence having 10, 9, 8, 7, 6, 5,
25 4, 3, 2 or 1 amino acid additions relative to SEQ ID NO: 12 or 72, optionally wherein the amino acids of the variable region are not considered. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region that has an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1, optionally a conservative amino acid substitution(s), relative to SEQ ID NO: 12 or 72, optionally wherein the amino acids of the variable region are not considered.

30 In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising a scaffold region that has at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, or at least 98% identity with the amino

acid sequence of SEQ ID NO: 12 or 72. In some embodiments, the amino acids corresponding to amino acids of the variable region are not considered when determining the sequence identity.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising:

- (i) the amino acid sequence of
MATVKFTYQGEEKQVDISKIKRVWRWGQGIYFWYDEGGGADGNGWVSEKDAPKE
LLEKQ (SEQ ID NO: 55);
- (ii) the amino acid sequence of
MATVKFTYQGEEKQVDISKIKRVHRYGQWIWFRYDEGGGARGHGHVSEKDAPKEL
LEKQ (SEQ ID NO: 56); or
- (iii) the amino acid sequence of
MATVKFTYQGEEKQVDISKIKHVHRWGQWIWFWL (SEQ ID NO: 57).

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein that has an amino acid sequence having ten or fewer, nine or fewer, eight or fewer, seven or fewer, six or fewer, five or fewer, four or fewer, three or fewer, two or fewer, or one amino acid difference(s) with one or more of SEQ ID NOs: 55-57. In some embodiments, the amino acids corresponding to amino acids of the variable region are not considered when determining the number of amino acid differences.

For example, in some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid deletion(s) relative to one or more of SEQ ID NOs: 55-57, optionally wherein the amino acids of the variable region are not considered. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid additions relative to one or more of SEQ ID NOs: 55-57, optionally wherein the amino acids of the variable region are not considered. In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein comprising an amino acid sequence having 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1, optionally a conservative amino acid substitution(s), relative to one or more of SEQ ID NOs: 55-57, optionally wherein the amino acids of the variable region are not considered.

In some embodiments, an antigen-binding molecule comprises an rcSso7d antigen-binding protein that has an amino acid sequence that has at least 80% identity, at least 85%

identity, at least 90% identity, at least 95% identity, or at least 98% identity with the amino acid sequence of one or more of SEQ ID NOs: 55-57. In some embodiments, the amino acids corresponding to amino acids of the variable region are not considered when determining the sequence identity.

5 In some embodiments, an antigen-binding molecule comprises an enzyme, a fluorescent protein, an immunoglobulin, a peptide tag, a small molecule, or a combination thereof.

 In some embodiments, an antigen-binding molecule comprises an enzyme. In some embodiments, the enzyme is selected from the group consisting of alkaline phosphatase,
10 horseradish peroxidase, and β -galactosidase.

 In some embodiments, an antigen-binding molecule comprises an immunoglobulin. In some embodiments, the immunoglobulin specifically binds to LMO2365_0639. In some embodiments, the immunoglobulin does not bind to LMO2365_0639; for example, in some embodiments, the immunoglobulin specifically binds to a non-LMO2365_0639 target on the
15 surface of a cell (*e.g.*, *L. monocytogenes* cell).

 In some embodiments, an antigen-binding molecule comprises a fluorescent protein. In some embodiments, the fluorescent protein is selected from the group consisting of TagBFP, mTagBFP2, Azurite, EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP,
20 mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange, mKOk, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.

25 In some embodiments, an antigen-binding molecule comprises a peptide tag. In some embodiments, the peptide tag is selected from the group consisting of a His tag, a biotin acceptor tag, an HA tag, a c-Myc tag, a streptavidin tag, and a FLAG tag.

 In some embodiments, an antigen-binding molecule comprises a small molecule. In some embodiments, the small molecule is a fluorescent molecule. Examples of fluorescent
30 small molecules are known to those having ordinary skill in the art. In some embodiments, the small molecule is biotin. In some embodiments, the small molecule is cytotoxic.

In some aspects, the disclosure relates to polynucleic acid molecules comprising a nucleic acid sequence encoding an antigen-binding molecule described herein (or the proteinaceous portion thereof).

5 **II. Compositions Comprising an Antigen-Binding Molecules.**

In some aspects, the disclosure relates to compositions comprising an antigen-binding molecule described herein. In some embodiments, a composition comprises a plurality of antigen-binding molecules, wherein each of the antigen-binding molecules are the same. In some embodiments, a composition comprises a plurality of antigen-binding molecules,
10 wherein two or more of the antigen-binding molecules are chemically distinct, *e.g.*, having different amino acid sequences, such as different variable region amino acid sequences. For example, in some embodiments, a composition comprises two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more chemically distinct antigen-binding molecules. In some embodiments, a composition
15 comprises 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 2-3, 2-4, 2-5, 2-6, 2-7, 2-8, 2-9, 2-10, 3-4, 3-5, 3-6, 3-7, 3-8, 3-9, 3-10, 4-5, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, 5-10, 6-7, 6-8, 6-9, 6-10, 7-8, 7-9, 7-10, 8-9, 8-10, or 9-10 chemically distinct antigen-binding molecules. In some embodiments, a composition comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 chemically distinct antigen-binding molecules.

20 In some embodiments, a composition further comprises a substrate. In some embodiments, the substrate comprises paper, nitrocellulose, cellulose powder, and/or a liquid colloid. In some embodiments, an antigen-binding molecule described herein is localized on the substrate. For example, in some embodiments, an antigen-binding molecule is localized at an interface. The interface can be solid as in a functional cellulose, or at the surface of a
25 particle, or at the surface of a liquid-liquid colloid. See *e.g.*, Miller et al., Beyond Epitope Binning: Directed in Vitro Selection of Complementary Pairs of Binding Proteins, ACS Combinatorial Science, 2020, 22 (1), 49-60; Sung et al., Binding performance of engineered rcSso7d affinity reagents versus commercial antibodies against Zika virus NS1, Analyst, 2020 145, 2515-2519; Zhang et al., Emulsion Agglutination Assay for the Detection of
30 Protein-Protein Interactions: An Optical Sensor for Zika Virus, ACS Sensors, 2019, 4 (1): 180–184, the contents of each of which is incorporated herein. Nanoparticles can be used to produce optical signals or can be magnetic. Functionalization of magnetic particles with the

antigen binding proteins selected by the methods described here, can be used to localize, separate, and/or concentrate bacteria. Attachment to liquid colloids can also provide methods to create optical signals for the detection.

In some embodiments, a composition further comprises a buffer.

5 In some embodiments, the buffer has a pH of less than or equal to 6.0, less than or equal to 5.9, less than or equal to 5.8, less than or equal to 5.7, less than or equal to 5.6, less than or equal to 5.5, less than or equal to 5.4, less than or equal to 5.3, less than or equal to 5.2, less than or equal to 5.1, less than or equal to 5.0, less than or equal to 4.9, less than or equal to 4.8, less than or equal to 4.7, less than or equal to 4.6, less than or equal to 4.5, less than or equal to 4.4, less than or equal to 4.3, less than or equal to 4.2, less than or equal to 4.1, or less than or equal to 4.0. In some embodiments, the buffer has a pH of about 4.0 to about 6.0, about 4.0 to about 5.5, about 4.0 to about 5.0, about 4.0 to about 4.5, about 4.5 to about 6.0, about 5.0 to about 6.0, about 5.5 to about 6.0, or about 4.5 to about 5.0. In some
10 embodiments, the buffer has a pH of about 4.0, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 4.7, about 5.8, about 5.9, or about 6.0.

In some embodiments, the buffer comprises sodium acetate and/or phosphate-buffered saline.

20 In some embodiments, the composition contains growth media that promote the proliferation of bacteria.

In some embodiments, the composition comprises a LMO2365_0639 protein.

In some embodiments, the composition comprises a *Listeria monocytogenes* cell, a *Salmonella typhi* cell, and/or an *E. coli* cell. In some embodiments, the composition comprises a viable *Listeria monocytogenes* cell, a *Salmonella typhi* cell, and/or an *E. coli* cell.
25

III. Methods of Detection.

In some aspects, the disclosure relates to methods of detection that utilize an antigen-binding molecule described herein or a composition comprising an antigen-binding molecule described herein.

30 In some aspects, the disclosure relates to methods of detecting bacteria in a sample. In some embodiments, the method comprises: (a) contacting the sample with an antigen-binding molecule described herein or a composition comprising an antigen-binding molecule

described herein; and (b) detecting, if present, bacteria bound by the antigen-binding molecule.

In some aspects, the disclosure relates to methods of detecting a *Listeria monocytogenes* cell (e.g., a viable cell) in a sample. In some embodiments, the method
5 comprises: (a) contacting the sample with an antigen-binding molecule described herein or a composition comprising an antigen-binding molecule described herein; and (b) detecting, if present, *Listeria monocytogenes* bound by the antigen-binding molecule.

In some aspects, the disclosure relates to methods of detecting a *Salmonella typhi* cell (e.g., a viable cell) in a sample. In some embodiments, the method comprises: (a) contacting
10 the sample with an antigen-binding molecule described herein or a composition comprising an antigen-binding molecule described herein; and (b) detecting, if present, *Salmonella typhi* bound by the antigen-binding molecule.

In some aspects, the disclosure relates to methods of detecting a *E. coli* cell (e.g., a viable cell) in a sample. In some embodiments, the method comprises: (a) contacting the
15 sample with an antigen-binding molecule described herein or a composition comprising an antigen-binding molecule described herein; and (b) detecting, if present, *E. coli* bound by the antigen-binding molecule.

In some aspects, the disclosure relates to methods of detecting a protein, a glycosylated protein, or a carbohydrate-based antigen associated with a bacteria of interest in
20 a sample. In some embodiments, the antigen is associated with an intact organism and in others the organism is not intact (e.g., its cell membrane and/or cell wall has been disrupted).

In some aspects, the disclosure relates to methods of detecting protein LSP (such as LMOF2365_0639), OmpA, OmpW, OmpC, OmpF, OmpD, phoE, or a combination thereof in a sample. In some embodiments, the method comprises: (a) contacting the sample with an
25 antigen-binding molecule described herein or a composition comprising an antigen-binding molecule described herein; and (b) detecting, if present, LSP (such as LMOF2365_0639), OmpA, OmpW, OmpC, OmpF, OmpD and/or phoE bound by the antigen-binding molecule.

In some embodiments of the methods described herein, the sample is from a food product. In some embodiments, the food product is produced from a food source that is
30 commonly contaminated with *Listeria monocytogenes*. Exemplary food sources commonly contaminated with *Listeria monocytogenes* are known to those having ordinary skill in the art and include soft cheeses made with unpasteurized milk (e.g., queso fresco, queso blanco,

panela (queso panela), brie, Camembert, blue-veined, feta); raw sprouts (including alfalfa, clover, radish, and mung bean sprouts); melons; certain meat products (hot dogs, pâté, meat spreads, lunch meats, cold cuts, other deli meats (such as bologna), fermented or dry sausages); cold smoked fish, such as salmon, trout, whitefish, cod, tuna, and mackerel; raw
5 (unpasteurized) milk and products made from it (soft cheese, ice cream, yogurt).

In some embodiments of the methods described herein, the sample is from a subject. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human. In some embodiments, the method further comprises treating the subject if LMOF2365_0639 protein is present in the sample. For example, in some embodiments, the
10 subject is treated for listeriosis. Methods of treating listeriosis are described herein (*i.e.*, methods of killing a *Listeria monocytogenes* cell). Additional methods of treating listeriosis are known to those having ordinary skill in the art.

In some embodiments, the sample is from a surface used to prepare a food or beverage. In some embodiments, the sample is of soil or water.

15 In some embodiments of the methods described herein, the sample is contacted with the antigen-binding protein in a buffer have a specific pH. For example, in some embodiments, the buffer has a pH of less than or equal to 6.0, less than or equal to 5.9, less than or equal to 5.8, less than or equal to 5.7, less than or equal to 5.6, less than or equal to 5.5, less than or equal to 5.4, less than or equal to 5.3, less than or equal to 5.2, less than or
20 equal to 5.1, less than or equal to 5.0, less than or equal to 4.9, less than or equal to 4.8, less than or equal to 4.7, less than or equal to 4.6, less than or equal to 4.5, less than or equal to 4.4, less than or equal to 4.3, less than or equal to 4.2, less than or equal to 4.1, or less than or equal to 4.0. In some embodiments, the buffer has a pH of about 4.0 to about 6.0, about 4.0 to about 5.5, about 4.0 to about 5.0, about 4.0 to about 4.5, about 4.5 to about 6.0, about 5.0
25 to about 6.0, about 5.5 to about 6.0, or about 4.5 to about 5.0. In some embodiments, the buffer has a pH of about 4.0, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 4.7, about 5.8, about 5.9, or about 6.0.

In some embodiments, the buffer comprises sodium acetate and/or phosphate-buffered
30 saline.

In some embodiments of the methods described herein, the method step (a) further comprises contacting the sample with a detection agent, wherein the detection agent binds to

or is bound by the antigen-binding molecule. In some embodiments, a detection agent comprises an enzyme, a fluorescent protein, an immunoglobulin, a peptide tag, a small molecule, and/or a combination thereof.

In some embodiments, an antigen-binding molecule comprises an enzyme. In some
5 embodiments, the enzyme is selected from the group consisting of alkaline phosphatase, horseradish peroxidase, and β -galactosidase.

In some embodiments, a detection agent comprises an immunoglobulin that binds to an antigen-binding molecule described herein.

In some embodiments, a detection agent comprises a fluorescent protein. In some
10 embodiments, the fluorescent protein is selected from the group consisting of TagBFP, mTagBFP2, Azurite, EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange,
15 mKOk, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.

In some embodiments, a detection agent comprises a peptide tag. In some
embodiments, the peptide tag is selected from the group consisting of a His tag, a biotin
20 acceptor tag, an HA tag, a c-Myc tag, a streptavidin tag, and a FLAG tag.

In some embodiments, a detection agent comprises a small molecule. In some
embodiments, the small molecule is a fluorescent molecule. Examples of fluorescent small
molecules are known to those having ordinary skill in the art. In some embodiments, the
small molecule is biotin.
25

IV. Methods of Killing a Bacterial Cell.

In some aspects, the disclosure relates to methods of killing a bacterial cell comprising contacting the bacterial cell with an antigen-binding molecule described herein.

In some embodiments, the bacterial cell is a *Listeria monocytogenes* cell, and the
30 method comprises contacting the *Listeria monocytogenes* cell with an antigen-binding molecule described herein that binds to LMOF2365_0639 (or a composition comprising an antigen-binding molecule described herein that binds to LMOF2365_0639), wherein the

antigen-binding molecule comprises a cytotoxic agent.

In some embodiments, the bacterial cell is a *Salmonella typhi* cell, and the method comprises contacting the *Listeria monocytogenes* cell with an antigen-binding molecule described herein that binds to Native Omp, said Native Omp comprising the amino acid
5 sequence of *Salmonella typhi* membrane proteins OmpA, OmpW, OmpC, OmpF, OmpD, and phoE (or a composition comprising an antigen-binding molecule described herein that binds to Native Omp), wherein the antigen-binding molecule comprises a cytotoxic agent.

In some embodiments, the bacterial cell is an *E. coli* cell, and the method comprises contacting the *E. coli* cell with an antigen-binding molecule described herein that binds to
10 OmpW (or a composition comprising an antigen-binding molecule described herein that binds to OmpW), wherein the antigen-binding molecule comprises a cytotoxic agent.

In some embodiments of the methods described herein, the sample is contacted with the antigen-binding protein in a buffer have a specific pH. For example, in some
embodiments, the buffer has a pH of less than or equal to 6.0, less than or equal to 5.9, less
15 than or equal to 5.8, less than or equal to 5.7, less than or equal to 5.6, less than or equal to 5.5, less than or equal to 5.4, less than or equal to 5.3, less than or equal to 5.2, less than or equal to 5.1, less than or equal to 5.0, less than or equal to 4.9, less than or equal to 4.8, less than or equal to 4.7, less than or equal to 4.6, less than or equal to 4.5, less than or equal to 4.4, less than or equal to 4.3, less than or equal to 4.2, less than or equal to 4.1, or less than or
20 equal to 4.0. In some embodiments, the buffer has a pH of about 4.0 to about 6.0, about 4.0 to about 5.5, about 4.0 to about 5.0, about 4.0 to about 4.5, about 4.5 to about 6.0, about 5.0 to about 6.0, about 5.5 to about 6.0, or about 4.5 to about 5.0. In some embodiments, the buffer has a pH of about 4.0, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about
25 5.5, about 5.6, about 4.7, about 5.8, about 5.9, or about 6.0.

In some embodiments, the buffer comprises sodium acetate and/or phosphate-buffered saline.

V. Methods of Monitoring the Growth of a Population of Bacteria.

30 In some aspects, the disclosure relates to methods of monitoring growth of a population of bacteria. In some embodiments, the method comprises: (a) contacting a sample comprising at least a subset of the population with an antigen-binding molecule described

herein or a composition comprising an antigen-binding molecule described herein; and (b) repeating step (a) at least once.

In some embodiments, step (a) further comprises contacting these sample with a detection agent described in “Methods of Detection” above.

5

VI. Kits.

In some aspects, the disclosure relates to kits comprising an antigen-binding molecule described herein (or a composition comprising an antigen-binding molecule described herein).

10 In some embodiments, a kit further comprises a substrate. In some embodiments, the substrate is selected from the group consisting of paper, nitrocellulose, cellulose powder, and liquid colloid. In some embodiments, an antigen-binding molecule is bound to the substrate.

EXAMPLES

15 Example 1: Identification of rcSso7d variants that bind *L. monocytogenes* surface protein.

In this study, the rcSso7d binding scaffold was used as the affinity reagent for development, due to its previous demonstration as an alternative scaffold that is robust, easy to produce, straightforward for engineering new variants, and has similar functional performance to antibodies (16-22). In order to identify an rcSso7d variant that detects *L.*
20 *monocytogenes*, a combinatorial *Saccharomyces cerevisiae* library was used consisting of 1.4×10^9 different rcSso7d variants in a yeast-surface display platform (FIG. 1A) (16, 23). The rcSso7d combinatorial library has a variable binding face (FIG. 1B, red) and is expressed on the surface of yeast via the native *a*-agglutinin proteins Aga1p/Aga2p with c-Myc and HA (hemagglutinin) epitope tags for labeling (FIG. 1B). Therefore, by selecting cells with
25 specific physical characteristics (*i.e.* binding to the target), one can isolate the genetic sequence encoding for that specific rcSso7d variant.

To develop affinity reagents against *L. monocytogenes* whole cells, a *L. monocytogenes* surface protein (“LSP”) identified in Zhang, et al, called LMOF2365_0639 (15) was targeted (FIG. 1C). The LMOF2365_0639 protein was found to have epitopes that
30 were conserved in various *L. monocytogenes* strains but variable among other *Listeria* species, making it a promising surface-exposed biomarker for *L. monocytogenes* (15). LSP was recombinantly produced in *E. coli* as two variants: one variant was constructed with just

an N-terminal 6x-hexahistidine (His) tag for purification and labeling (“His-LSP”) while another variant was cloned with both an N-terminal His tag for purification and a C-terminal biotin acceptor tag (BA) for labeling (“LSP-BA”). Having two different versions of the target protein allows for oriented selection since using a tag on one side of the target protein for labeling may prevent development of affinity reagents against epitopes around that tag due to steric hindrance with the labeling reagents. His-LSP and LSP-BA were expressed and purified on Ni-NTA immobilized metal affinity chromatography (IMAC) following similar protocols as previously described (17) (FIGs. 6A-6C).

Using the rcSso7d library, directed evolution techniques were used to enrich the library for affinity reagents against LSP. In general, magnetic bead sorting (MBS) was conducted to first enrich the library for any rcSso7d clones with binding affinity towards the target protein. After sufficient enrichment, fluorescence activated cell sorting (FACS) was conducted to further enrich for high affinity clones in a controlled fashion. To increase the possibility of developing affinity clones that target a surface exposed epitope of LSP, three different selection schemes using the two different label-oriented LSP variants were used.

In the first selection scheme, MBS was conducted using Ni-NTA magnetic beads coated with N-terminal His-LSP. Moderate enrichment was observed after two rounds of MBS (FIG. 7) with sufficient reduction in library diversity to proceed to FACS. Using this post-MBS sub-library, four rounds of FACS were conducted with His-LSP, using the N-terminal His tag on LSP to label target binding and the N-terminal HA tag on the displayed yeast to label for surface expression of rcSso7d (FACS “a”; FIG. 2 and FIG. 8). To reduce non-specific clones, negative FACS selections were conducted against the labeling reagents (mouse anti-His antibody and goat anti-mouse AF647) for FACS #2a, #3a, and #4a by incubating the populations with labeling reagents in the absence of target LSP and collecting the clones without binding signal (21) (FIG. 8). The collected cells were then immediately relabeled with His-LSP for positive FACS.

To analyze the resulting library diversity after FACS #4a, a subset of the population were sequenced; 30 clones were submitted for sequencing, and five unique clones were identified (TABLE 1; clones 1, 2, 3, 4, and 5). Over 80% of the sequences resulted in the same sequence—clone 1—which indicates a fairly monoclonal population. Clones 1, 2, and 4 had very similar sequences and contained an early stop codon mutation, leading to truncated sequences at approximately 50% of the full length sequence. There was uncertainty

about whether the truncated sequences would be folded properly and the possibility that they may be nonspecific binding proteins. However, after testing the clonal yeast in yeast-surface display, it was found that all five of the identified sequences—full length and truncated—demonstrated target-specific binding to LSP (FIGs. 3A-3B).

5

TABLE 1. Amino acid sequences for the binding face of each unique rcSso7d clone identified.

Clone	Binding face sequence	SEQ ID NO:
rcSso7d.LSP.1	DGSNCY	1
rcSso7d.LSP.2	DGHKCL	2
rcSso7d.LSP.3	IKYIDSRWI	3
rcSso7d.LSP.4	DGYRCW	4
rcSso7d.LSP.5	WRAWDACYI	5
rcSso7d.LSP.6	DGHHCW	6
rcSso7d.LSP.7	IAYYYYSIK	7
rcSso7d.LSP.8	NIYWWNISY	8

To assess whether the His-LSP-enriched sub-libraries also demonstrate binding to LSP-BA—with the tag used for labeling on the C-terminus instead of the N-terminus of the LSP—these sub-libraries were challenged against LSP-BA. Relatively low levels of binding was found to LSP-BA across all the sub-libraries (FIG. 9), suggesting that the clones enriched during selections using N-terminal-tagged LSP bind to an epitope that is inaccessible when using a C-terminal tag to label the LSP.

In order to identify additional rcSso7d clones that may target alternative epitopes of LSP, a second selection scheme was produced using the His-LSP-enriched post-MBS sub-library. Four rounds of FACS was conducted with LSP-BA, using the C-terminal biotin tag to label target binding (FACS “b”; FIG. 2 and FIG. 10). Negative FACS rounds were conducted against the labeling reagent streptavidin (SA) AF647 in FACS #2b, #3b, and #4b to down-select for off-target binding clones. After analyzing the sequences from post-FACS #4b, it was found that over 90% of the sequences resulted in the same sequence as clone 1, indicating that these selections using LSP-BA for FACS identified the same rcSso7d variant as selections using His-LSP for FACS. One unique sequence was also identified (clone 6; TABLE 1), which was similar to the truncated sequences previously identified (clones 1, 2, and 4). These results suggest a lack of diversity in the enriched clones, even after using

different orientation tags on the LSP. Conducting MBS using His-LSP may have applied early selective pressure for affinity clones targeting similar epitopes, even when FACS was conducted using LSP-BA.

In efforts to expand the diversity of identified rcSso7d variants against LSP, another round of selections was conducted, starting from the naïve rcSso7d library and using LSP-BA for both MBS and FACS (FACS “c”; FIG. 2 and FIG. 11). A round of negative selection was conducted prior to FACS #3c to remove nonspecific binding variants against SA AF647. Additionally, in efforts to identify full length variants of LSP, the C-terminal c-Myc tag was used on the yeast-displayed rcSso7d variants to label for expression in FACS #3c and #4c, instead of the N-terminal HA tag. After FACS #4c, the sub-library was sorted into two sub-populations based on the appearance of two potentially monoclonal populations. After sequencing a subset of the two sub-populations, two additional unique rcSso7d sequences were identified (clones 7 and 8; TABLE 1), confirming that the sub-populations were monoclonal. These clones were full length, as expected based on the use of the C-terminal c-Myc tag for labeling expression.

From the three selection schemes, a total of eight unique rcSso7d clones were identified. These clones were analyzed for specific binding against LSP in a yeast-surface display format (FIGs. 3A-3B). All clones except clone 8 demonstrated target-specific binding to LSP. rcSso7d.LSP.8 showed off-target binding to the labeling reagents (mouse anti-His antibody and goat anti-mouse AF647); therefore, this variant was no longer pursued in subsequent studies.

Example 2: Analyses of rcSso7d variant stability.

As previously mentioned, four of the identified clones resulted in truncated sequences. Studies have demonstrated that four amino acid residues in the rcSso7d hydrophobic core are vital to the stability of the protein, forming a “herring bone” structure: F5, Y7, F31, and Y33 (24) (FIGs. 4A-4B). The four truncated rcSso7d variants (clones 1, 2, 4, and 6) contained an early stop codon mutation that led to the absence of the fourth herring bone amino acid (Y33L).

To investigate whether this early truncation affects the thermal stability of the rcSso7d clones, heat stability studies were conducted (25, 26). The rcSso7d-displaying clonal yeast cells were heat treated for 10 minutes at 80 °C and then assessed for target-binding activity

loss. Two of the three full length rcSso7d variants (clones 3 and 5) maintained activity after heat treatment (FIG. 4C). Clone 7 had moderate activity loss, which may be due to the additional amino acid (FIG. 4A) disrupting the hydrophobic core packing or the herring bone structure of the four key amino acids. On the other hand, truncating the rcSso7d sequence
5 appeared to have significant detrimental effects on the thermal stability of the scaffold, with nearly all activity loss for almost all truncated clones (FIG. 4C). These results demonstrate the importance of full length sequences in maintaining the hyperthermostability of the rcSso7d scaffold.

Based on the thermal stability studies and on preliminary data of these clones against
10 whole *Listeria* cells (data not shown), rcSso7d.LSP.3 was chosen for future tests.

Example 3: rcSso7d.LSP demonstrates specific binding to live, whole cell *L. monocytogenes*.

The rcSso7d.LSP.3 variant was cloned from the yeast-surface display vector into a bacterial expression vector for soluble protein expression. Soluble rcSso7d.LSP.3 was
15 expressed in *E. coli* and purified for testing (FIGs. 6A-6C).

Although the identified rcSso7d.LSP clones demonstrated specific binding to LSP, further studies were performed to develop affinity reagents that can specifically bind to live, whole cell *L. monocytogenes*. Therefore, the soluble rcSso7d.LSP.3 variant was used to label whole *Listeria* cells (FIG. 5A). The buffer used for incubating the rcSso7d with the *Listeria*
20 cells was first optimized. Stronger binding signal was detected in a lower pH buffer (40 mM sodium acetate, pH 5.5) than in a neutral buffer (PBS, pH 7.4) (FIGs. 5B-5C). It was hypothesized that the difference in binding signal is attributed to charge effects. The theoretical isoelectric point of the rcSso7d.LSP.3 protein is 6.2, therefore, in the sodium acetate buffer (pH 5.5), the rcSso7d protein has a net positive charge, while in PBS (pH 7.4),
25 the rcSso7d has a net negative charge. Thus, the net positive charge of rcSso7d in sodium acetate may facilitate interactions with the negatively charged *Listeria* cells.

To further demonstrate that this binding signal to whole cell *Listeria* is target specific, the rcSso7d.LSP.3 clone was challenged in sodium acetate against live, whole cell *Bacillus subtilis* as a model non-pathogenic, Gram-positive bacterium (FIG. 5D). The rcSso7d.LSP.3
30 variant depicted no binding to *B. subtilis*, validating that the binding signal shown against *L. monocytogenes* is not from non-specific interactions against generic bacterial surface epitopes.

In summary, multiple rcSso7d-based affinity reagents have been identified against a *Listeria monocytogenes* surface protein, LMOF2365_0639, using a yeast-surface display library. A few rcSso7d variants with early truncation sequences were identified that led to reduced thermal stability, possibly due to a disruption of the highly stable core. Nonetheless, seven out of the eight identified rcSso7d variants—including the truncated variants—demonstrated target-specific binding to the *Listeria* surface protein. rcSso7d.LSP.3 was selected for testing against live, whole cell *L. monocytogenes* and depicted strong binding to the whole cells when they were incubated in a buffer at pH 5.5—below the isoelectric point of the rcSso7d protein. Furthermore, the rcSso7d did not depict binding to a non-target bacterium, *Bacillus subtilis*. These results demonstrate that this rcSso7d clone can be used as an affinity reagent for use in detecting live, whole cell *L. monocytogenes*.

Example 4: Materials and methods for examples 1-3.

Commercial reagents: Primary labeling reagents and dilutions (bold) used for selection were: chicken anti-HA (AHA; **1:1000**) and chicken anti-cMyc (CMYC; **1:1000**) from Exalpa Biologicals, and mouse anti-6x-His (clone MA1-21315, HIS.H8; **1:1000**) from Thermo Fisher Scientific. Secondary detection reagents were goat anti-mouse AlexaFluor (AF) 647 (A-21235; **1:1000**), goat anti-chicken AF488 (A-11039; **1:1000**), and streptavidin AF647 (S-21374; **1:1000**) from Thermo Fisher Scientific. Magnetic bead selections were conducted using HisPur NiNTA Magnetic Beads (88831) and Dynabeads Biotin Binder (11047) from Thermo Fisher Scientific. Brain Heart Infusion (BHI) broth component Bacto Brain Heart Infusion (BD 237500) and the Enriched Nutrient broth components Bacto Heart Infusion Broth (BD 238400), Difco Nutrient Broth (BD 234000), and Bacto Yeast Extract (BD 212750)) were purchased from VWR.

A pET28b(+) plasmid containing the LMOF2365_0639 (*Listeria monocytogenes* surface protein, LSP) sequence (strain 3D7; VG40303-G) with an N-terminal 6x-histidine tag was codon-optimized and synthesized from GeneWiz.

Listeria monocytogenes (Murray et al.) Pirie (ATCC[®] 43256[™]) CDC F2380 strain (isolated from Mexican-style cheese) and *Bacillus subtilis* (Ehrenberg) Cohn (ATCC[®] 27370[™]) 168 M strain were obtained from ATCC.

Production of recombinant biomarkers: The plasmid construct for the N-terminal 6x-histidine tag variant of LSP (His-LSP) was synthesized in pET28b(+) from GeneWiz. The plasmid construct for the C-terminal biotin acceptor (BA) variant of LSP (LSP-BA)—with N-terminal His tag for purification—was developed following protocols as described previously (17-19, 21). Briefly, polymerase chain reaction (PCR) was conducted on LSP using the pET28b(+)-His-LSP backbone with the primers listed in TABLE 2 and an annealing temperature of 62 °C. The plasmid backbone pET28b(+)-Sso.TB-Link-BA from Sung, et al (19) was used to obtain a pET28b(+) vector with a C-terminal BA sequence. A double digest on the PCR product and this backbone was conducted using *NdeI* and *BamHI* restriction enzymes before running a ligation reaction. The ligation products were purified using the DNA Clean and Concentrator Kit (Zymo Research) before transformation into DH5α *E. coli* via electroporation.

TABLE 2. Oligonucleotide sequences of primers used to clone LSP-BA.

SEQ ID NOs	Oligo Name	DNA Sequence (<i>NdeI</i> and <i>BamHI</i> , restriction sites)
20	LSP-BA-NdeI-for	5'-CTGGC <u>CATATG</u> GTTAATATCCCGGACCCGGTTCTGAAGAGC-3'
21	LSP-BA-BamHI-rev	5'-TATTAGGATCCCGTGTGTTGGGAGGGCGGCGTTA-3'

15

His-LSP and LSP-BA were expressed and purified as described previously (17-19, 21). All biomarker constructs were expressed in BL21(DE3) *E. coli* and induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). LSP-BA was supplemented with free biotin during expression by adding 0.1 mM D-biotin in 10 mM bicine buffer. After overnight expression at 20 °C, the cells were pelleted, lysed via sonication, and purified using immobilized metal affinity chromatography (IMAC) with HisTrap FF crude columns (GE Healthcare). After purification, the proteins were then buffer exchanged into 1x PBS using Amicon Ultra Centrifugal Filters.

All purified proteins were quantified using a bicinchoninic acid (BCA) assay (Thermo Fisher Scientific) and run on a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), as previously described (19).

25

Selections against LSP: Selections were conducted using yeast-surface display as previously described (16, 21, 23, 27). Briefly, *Saccharomyces cerevisiae* EBY100 containing the pCTCON2-rcSso7d combinatorial library (naïve diversity: 1.4×10^9 clones) (16) were cultured in SDCAA media (citrate form) at 30 °C overnight. After passaging the cells, the yeast culture was induced once the OD₆₀₀ approached 4 by passaging the cells into SGCAA (supplemented with 2 g/L dextrose) at an OD₆₀₀ of 1. Yeast cells were induced for 24 hours at 20 °C. At least 20-fold of the library diversity was used in each sorting step. Induced cells were prepared by taking the appropriate number of cells and washing them in PBSF (1x PBS with 0.1% bovine serum albumin, sterile filtered) twice. Yeast populations were centrifuged at 2,000 *xg* for three minutes to pellet the cells gently.

Magnetic bead sorting (MBS) was conducted as previously described (17, 21). For selections against His-LSP, HisPur NiNTA magnetic beads were used to immobilize His-LSP, using at least 100 pmoles of His-LSP per 1 µL of beads, incubated for at least 2 hours at 4 °C. At least 2 µL of coated NiNTA beads were used for at most 1.4×10^9 yeast cells. For selections against LSP-BA, Dynabeads biotin binder magnetic beads were used to immobilize LSP-BA, using 500 pmoles of LSP-BA per 10 µL of beads, incubated for at least 2 hours at 4 °C on a rotary mixer. 10 µL of biotin binder beads were used for at most 1.4×10^9 yeast cells. For each LSP variant, selections were conducted with two rounds of positive sorts against the coated beads by incubating the cells with the coated beads for at least 2 hours at 4 °C on a rotary mixer. Cells that were bound to the beads were washed at least twice. One round of negative sort was conducted immediately prior to the second positive sort using uncoated beads by incubating the cells with the uncoated beads for at least 2 hours at 4 °C on a rotary mixer. Cells that were unbound were collected and used for a positive sort. Yeast cells were outgrown in SDCAA media between each round of selection.

Fluorescence-activated cell sorting (FACS) was conducted as previously described (17, 21). Three different sets of FACS selections were conducted: **1)** FACS “a” using the post-MBS library enriched against His-LSP and further enriching against His-LSP, **2)** FACS “b” using the post-MBS library enriched against His-LSP and further enriching against LSP-BA, and **3)** FACS “c” using the post-MBS library enriched against LSP-BA and further enriching against LSP-BA. Four rounds of FACS were conducted for each sort, decreasing the concentration of LSP used for subsequent sorts for increased selective pressure against higher affinity clones. To label LSP binding, mouse anti-His/goat-anti-mouse AF647 was

used for His-LSP, and SA AF647 was used for LSP-BA. Surface expression of the rcSso7d was labeled using chicken anti-HA/goat anti-chicken AF488 for all sorts, except for FACS #3c and #4c, which used chicken anti-cMyc/goat anti-chicken AF488 to label for full length rcSso7d expression since the HA tag is on the N-terminus of surface-displayed rcSso7d and the c-Myc tag is on the C-terminus of the surface-displayed rcSso7d. Negative selections in FACS (21) were conducted in FACS #2a, #3a, #4a, #2b, #3b, #4b, and #3c by labeling the yeast sub-libraries with the labeling reagents in the absence of LSP and collecting the population that did not display positive binding signal. These collected cells were immediately relabeled for positive sorts.

For all flow cytometry preparations, primary incubation steps were conducted at room temperature for 20-30 minutes, except when the biomarker concentration was low enough to require longer incubation times to reach equilibrium. Secondary incubation steps were conducted at 4 °C for 15-20 minutes. After each labeling step, cells were washed with 1 mL of PBSF. Sorting was conducted on a BD FACS Aria using the FACS Diva software, detecting the fluorescence of AF647 (excitation 640 nm, emission 670/30 nm) and AF488 (excitation 488 nm, emission 582/15 nm). Data was analyzed using FlowJo software.

rcSso7d clonal analysis: After FACS, the enriched yeast sub-libraries were sequenced to determine the diversity, as described previously (17, 21). Briefly, the sub-libraries were minipreped using the ZymoPrep Yeast Miniprep II kit, and the plasmid products were transformed into DH5α *E. coli*. Ten to twenty bacterial colonies were picked and sent off for sequencing via GeneWiz (TABLE 3). The pCTCON2 plasmids for unique clones were transformed back into *S. cerevisiae* EBY100 using the Frozen-EZ Yeast Transformation II Kit (Zymo Research).

TABLE 3. Number of occurrences of each identified unique clone after sequencing the post-FACS #4a, post-FACS #4b, and the two post-FACS #4c (i and ii) sub-library populations.

	<i>Number of occurrences after sequencing</i>			
	FACS #4a	FACS #4b	FACS #4c,i	FACS #4c,ii
Clone 1	25	14	0	0
Clone 2	1	0	0	0
Clone 3	1	0	0	0
Clone 4	2	0	0	0
Clone 5	1	0	0	0

Clone 6	0	1	0	0
Clone 7	0	0	10	0
Clone 8	0	0	0	10

Unique clones were analyzed using flow cytometry, following a similar labeling procedure as outlined above for sorting. Each clone was challenged with 20 nM of His-LSP, followed by mouse anti-His antibody and goat anti-mouse AF647. Surface expression levels were labeled with chicken anti-HA and goat anti-chicken AF488. Results were analyzed on FlowJo software.

Heat stability studies: Thermal stability studies were conducted on the identified rcSso7d clones in the yeast-surface display format, following a procedure described in Traxlmayr, et al (26). In this process, each clonal yeast was cultured and induced as described previously. 5×10^6 cells from each rcSso7d-displaying yeast culture were washed with PBSF and resuspended in 170 μ L of PBSF (for an OD₆₀₀ of 3). The samples were heated in a thermocycler for 10 minutes at 80 °C before placing the samples on ice for 5 minutes. The heat-treated cells and non-heat-treated cells (for a positive control) were labeled with His-LSP, mouse anti-His antibody, and goat anti-mouse AF647 for binding and chicken anti-HA and goat anti-chicken AF488 for expression, following the same protocol as outlined above. The labeled samples were analyzed on flow cytometry. The geometric mean fluorescence signal of AF647 for each sample was calculated using FlowJo software, analyzing only the cells that showed positive expression. The percentage reduction in target-binding activity was calculated by dividing the geometric mean signal after heat-treatment by the signal without heat-treatment.

Production of rcSso7d clones: rcSso7d.LSP.3 was cloned as previously described (17, 20) and detailed above, using the primers listed in TABLE 4, an annealing temperature of 59 °C, *NdeI* and *XhoI* restriction enzymes, and any pET28b(+) backbone. The protein was produced following the same protocol as detailed in the above section.

TABLE 4. Oligonucleotide sequences of primers used to clone rcSso7d.LSP.3.

SEQ ID NO:	Oligo Name	DNA Sequence (<u>NdeI</u> and <u>XhoI</u> , restriction sites)
22	rcSso7d-for	5'-AGGCAGTCT <u>CATATG</u> TGTGCAACCGTGAAATTCAC-3'
23	rcSso7d-rev	5'-ACCCCT <u>CTCGAG</u> TATTGCTTTTCCAGCA-3'

Testing against whole bacterial cells: Brain Heart Infusion (BHI) broth was prepared using 37 g/L of Bacto Brain Heart Infusion in deionized water. Enriched Nutrient (EN) broth was prepared using 12.5 g/L of Bacto Heart Infusion Broth, 5.4 g/L of Difco Nutrient Broth, and 2.5 g/L of Bacto Yeast Extract in deionized water. Broths were autoclaved prior to use. *Listeria monocytogenes* was cultured overnight in BHI broth and *Bacillus subtilis* was cultured overnight in EN broth, both at 30 °C in a shaking incubator.

Approximately 3×10^8 *L. monocytogenes* cells and 1×10^8 *B. subtilis* cells were used for each sample. Cells were pelleted at 4,000 xg for 5 minutes and washed twice with 1 mL of PBSF. Primary incubations occurred with rcSso7d.LSP.3 diluted in either PBSF or 40 mM sodium acetate (pH 5.5) buffer, on a rotary mixer for at least 3 hours at room temperature. After washing the cells in PBSF, they were resuspended in the secondary incubation solution containing mouse anti-His antibody in PBSF (to target the His tag on the rcSso7d.LSP.3 protein) and incubated for 20-30 minutes at room temperature on a rotary mixer. After another set of washes in PBSF, the cells were resuspended in the tertiary incubation solution containing goat anti-mouse AF647 in PBSF and incubated for 15-20 minutes on ice. Samples were washed in PBSF one last time before being processed using flow cytometry. Negative controls followed the same protocol, except with incubations in just PBSF for the secondary incubation step, without mouse anti-His antibody. Results were analyzed using FlowJo software.

Additional Sequences

His-LSP

25 Nucleic acid sequence (SEQ ID NO: 24):

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGC
AGCCATATGGAATTCGTTAATATCCCGGACCCGGTTCTGAAGAGCTACCTCAATG
GTCTGCTGGGCCAAAGCAGCACGAGCGATATCACCGAAGCGCAGATGGATACCA
TCACGAATGTGACCATCAGCAACAGCAGCCTCACCGATCTGACCGGCCTCGACTA

CGCCCACAATCTGACGATTCTCCACCTCAGTAACACGGGTGTGACCGACTACGCG
 CTCGTGGCCAAGATTCCGAGTCTGACGAATCTGAGCATTGCGGGTGATAACCTCA
 CCAATGACAGTCTGCCGGATCTGAACAACCTCAGCAACATCACGAACCTCAATCT
 GAGCCCGGGCAAGCTGGATAACAACGCGCTGACGAAGTTCAATAAAATGAGCAA
 5 GCTGAGCTATCTGAATCTGGACAGCAACCCGAGCATCACGAACATCATGCCGCTC
 AAAAGCATCCCGAATCTGGCGACGCTGTTTCGTGCAGTTCTGCGGCATTAACGACT
 TCCGCGGCATCGATACGTTCCCGAAGCTGGTGAGTCTGAGTGCGTATGGTCAGAA
 CGTGGGGCCGCACGGTGCTGATCAACAGCAGCATTAAAGAGCAGTGCGCTGAACCTT
 CGATGAGGCCAACAGACGATCTTCGTGCCATTTACCCTCATGACCGAACGCGGC
 10 GTGAACCTTCGACGGTTACCTCTTCCCATTACACCACCAATACCAGCAGCGCCAGTA
 CGTACTTCACCCTCAACGAGACCAAGATTGACGGTAGTCGTCTCAGATCGATGA
 CAAGGGTATCACGGTGAGCGGTATCACCAAAAGCTACTTCGACACGATTACGAA
 GATGGAGTATAACGCCCTCTACAACAACCCGCGCGGTAGTTATCAGACGCCGCC
 GAACTTCAACAACACTACAGCGTTAGTGGCGGCAGCTACGATCACTACTTCGACATC
 15 GACCACAGCCTCACCATTACGAACGACAGCGCCATCAGCTACGGTGAGCAGACG
 ACCGTGACGGAGGAGCAGTTTCTGAAGGATGTGCACGCGGAAACCGACGATGGC
 ACCCCGGTTACCAGCGACTTCAACACGGTGGTGGATTTCAGCAAGCCGGGCGTGT
 ACACCGTTACGCTGAATGCCGAAAATGCGGCCGGTCTCAAAGCGACGCCAACCC
 AAGTTACGGTTACCATCCACGCCAAGCCGGTGATTACCGCGGACAAAAGCATCA
 20 GCTACACCAAGGACAGTACGAAAACCGATCAGCAGTTTCTGCAAGATATTAGCG
 CGAAGACCAGTGACGGCAGCAAGGTTACGAGTGACTTTGACAGCGTGGTTGACC
 TCGCGAAGGTGGGCACGTATAAGGTGACGCTGAATGCGGTTAGCGCGGATGGTC
 TGAACGCCGACCCAGTGATCGTGCTCGTGAATGTGGTGGAAGGCAATGAACCAC
 CAACCCCAACAGCCCCGGGCCAGATCCGACGCCAGATCCAACCCCGAACCCGA
 25 ACAACCCGAACATCAATCCGAATCCGACAACGGTCAGAGTGCGAACAGCGAGA
 ACGCGAGCAATCCAAGCAACAGCGAAGTTAACGCCGCCCTCCCAAACACGTAA

Amino acid sequence (SEQ ID NO: 25):

MGSSHHHHHHSSGLVPRGSHMEFVNIPDPVLKSYLNGLLGQSSTSDITEAQMDTITN
 30 VTISNSSLTDLTGLDYAHNLTILHLSNTGVTDYALVAKIPSLTNLSIAGDNLNDSLPD
LNNLSNITNLNLSPGKLDNNALTKFNKMSKLSYLNLDNPSITNIMPLKSIPNLATLFV
QFCGINDFRGIDTFPKLVLSAYGQNVGRVTLINSSIKSSALNFDEANQITIFVPFTLMT
ERGVNFDGYLFPFTNTSSASTYFTLNETKIDGSRLTIDDKGITVSGITKSYFDTITKME
YNALYNNPAGSYQTPPNFNYSVSGGSYDHYFDIDHSLTITNDSAISYGEQTTVTEEQ
 35 FLKDVHAETDDGTPVTSDFNTVVDFS KPGVYTVTLNAENAAGLKATPTQVTVTIHA
KPVITADKSISYTKDSTKTDQQFLQDISAKTSDGSKVTSDFDSVVDLAKVGTYKVTL
NAVSADGLNADPVIVLVNVVEGNEPPTPPAPGPDPTDPTPNPNPNINPNPDNGQSA
NSENASNPSNSEVNAAALPNT

40 LSP-BA

Nucleic acid sequence (SEQ ID NO: 26):

ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGC
 AGCCATATGGTTAATATCCCGGACCCGGTTCTGAAGAGCTACCTCAATGGTCTGC
 TGGGCCAAAGCAGCACGAGCGATATACCGAAGCGCAGATGGATACCATCACGA
 45 ATGTGACCATCAGCAACAGCAGCCTACCGATCTGACCGGCCTCGACTACGCCCA
 CAATCTGACGATTCTCCACCTCAGTAACACGGGTGTGACCGACTACGCGCTCGTG
 GCCAAGATTCCGAGTCTGACGAATCTGAGCATTGCGGGTGATAACCTCACCAATG

ACAGTCTGCCGGATCTGAACAACCTCAGCAACATCACGAACCTCAATCTGAGCCC
 GGGCAAGCTGGATAACAACGCGCTGACGAAGTTCAATAAAATGAGCAAGCTGAG
 CTATCTGAATCTGGACAGCAACCCGAGCATCACGAACATCATGCCGCTCAAAAG
 CATCCCGAATCTGGCGACGCTGTTCGTGCAGTTCTGCGGCATTAACGACTTCCGC
 5 GGCATCGATACGTTCCCGAAGCTGGTGAGTCTGAGTGCGTATGGTCAGAACGTG
 GGCCGCACGGTGCTGATCAACAGCAGCATTAAAGAGCAGTGCGCTGAACTTCGAT
 GAGGCCAACCAGACGATCTTCGTGCCATTTACCTCATGACCGAACGCGGCGTGA
 ACTTCGACGGTTACCTCTTCCCATTACCAACCAATACCAGCAGCGCCAGTACGTA
 CTTACCCCTCAACGAGACCAAGATTGACGGTAGTCGTCTCACGATCGATGACAAG
 10 GGTATCACGGTGAGCGGTATCACCAAAAGCTACTTCGACACGATTACGAAGATG
 GAGTATAACGCCCTCTACAACAACCCGGCCGGTAGTTATCAGACGCCGCCGAAC
 TTCAACAACCTACAGCGTTAGTGGCGGCAGCTACGATCACTACTTCGACATCGACC
 ACAGCCTCACCATTACGAACGACAGCGCCATCAGCTACGGTGAGCAGACGACCG
 TGACGGAGGAGCAGTTTCTGAAGGATGTGCACGCGGAAACCGACGATGGCACCC
 15 CGGTTACCAGCGACTTCAACACGGTGGTGGATTTCAGCAAGCCGGGCGTGTACA
 CCGTTACGCTGAATGCCGAAAATGCGGCCGGTCTCAAAGCGACGCCAACCCAAG
 TTACGGTTACCATCCACGCCAAGCCGGTGATTACCGCGGACAAAAGCATCAGCT
 ACACCAAGGACAGTACGAAAACCGATCAGCAGTTTCTGCAAGATATTAGCGCGA
 AGACCAGTGACGGCAGCAAGGTTACGAGTGACTTTGACAGCGTGGTTGACCTCG
 20 CGAAGGTGGGCACGTATAAGGTGACGCTGAATGCGGTTAGCGCGGATGGTCTGA
 ACGCCGACCCAGTGATCGTGCTCGTGAATGTGGTGGAAGGCAATGAACCACAA
 CCCCACCAGCCCCGGGCCAGATCCGACGCCAGATCCAACCCCGAACCCGAACA
 ACCCGAACATCAATCCGAATCCGGACAACGGTCAGAGTGCGAACAGCGAGAACG
 CGAGCAATCCAAGCAACAGCGAAGTTAACGCCGCCCTCCCAAACACGGGATCCA
 25 TGGCGGGCGGCCTGAACGATATTTTTGAAGCGCAGAAAATTGAATGGCATGAAT
 AA

Amino acid sequence (SEQ ID NO: 27):

MGSSHHHHHHSSGLVPRGSHMVNIPDPVLKSYLNGLLGQSSTSDITEAQMDTITNVT
 30 ISNSSLTDLTGLDYAHNLTILHLSNTGVTDYALVAKIPSLTNLSIAGDNLTNDSLPDLN
 NLSNITNLNLSPGKLDNNALTKFNKMSKLSYLNLDNPSITNIMPLKSIPNLATLFVQF
 CGINDFRGIDTFPKLVLSAYGQNVGRVTLINSSIKSSALNFDEANQTIFVPFTLMTER
 GVNFDGYLFPFTTNTSSASTYFTLNETKIDGSRITIDDKGITVSGITKSYFDTITKMEY
 NALYNNPAGSYQTPPNFNYSVSGSYDHYFDIDHSLTITNDSAISYGEQTTVTEEQF
 35 LKDVHAETDDGTPVTSDFNTVVDVFSKPGVYTVTLNAENAAGLKATPTQVTVTIHAK
 PVITADKSISYTKDSTKTDQQFLQDISAKTSDGSKVTSDFDSVVDLAKVGTYKVTLN
 AVSADGLNADPVIVLVNVVEGNEPPTPPAPGPDPTPDPTPNPNPNINPNPDNGQSAN
 SENASNPSNSEVNAALPNTGSMAGGLNDIFEAQKIEWHE

40 rcSso7d.LSP.3

Nucleic acid sequence (SEQ ID NO: 28):

ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGC
 AGCCATATGTGTGCAACCGTGAAATTACATACCAAGGCGAAGAAAAACAGGTG
 GATATTAGCAAAATCAAGAACGTGCATCGTCATGGCCAGAAAATTTACTTTATCT
 45 ATGATGAAGGTGGTGGTGCCAAAGGTCATGGTAAAGTGAGCGAAAAAGATGCAC
 CGAAAGAACTGCTGCAGATGCTGGAAGCAATAA

Amino acid sequence (SEQ ID NO: 29):

MGSSHHHHHHSSGLVPRGSHMCATVKFTYQGEEKQVDISKIKIAKRYGQIIDFSYDE
GGGARGWGIVSEKDAPKELLQMLEKQ

5

Example 5: Additional binding proteins.

In Examples 1-4, binding proteins were engineered that are highly specific to *Listeria monocytogenes*. These binding proteins are directed at a target protein termed LSP, for
 10 *Listeria* Surface Protein. Several rcSso7d sequences that specifically bind the extracellular portion of LSP (e.g. rcSso7d.LSP1 - rcSso7d.LSP8) are disclosed.

This example demonstrates that the strategy described in Examples 1-4 is generalizable to additional bacterial species (FIG. 12). Using the same procedures described in Examples 1-4, additional binding proteins were engineered that target other bacterial
 15 proteins that are constituents of the extracellular portions of bacterial cell walls. These bacterial proteins include the outer membrane protein A, OmpA, and outer membrane protein W, OmpW, of *E. coli*, and outer membrane proteins of *Salmonella typhi* (termed herein Native Omp). The antigen called Native Omp is a combination of parts of the above mentioned outer membrane proteins as well as OmpC, OmpF, OmpD, and phoE.

20 The sequence of OmpA is conserved across many bacteria. For example, these proteins in *Salmonella* species and *E. coli* share a sequence identity of 93% (FIG. 13) (28). OmpA is an abundant protein; each cell expresses approximately 100,000 copies in its cell wall (29). OmpA and some other outer membrane proteins (30) consist of multiple domains, including domains that contain extracellular loops, those that span the cell membranes, and
 25 domains that are soluble (rather than membrane associated) in the periplasm (FIG. 14A) (29).

To target bacteria generally, we produced a recombinant version of the N-terminal domain of OmpA, because the N-terminal domain contains the extracellular loops that are accessible for binding (29). To target *E. coli* more specifically, we produced a recombinant version of OmpW, as the sequence of this membrane protein is much less conserved across
 30 different bacteria (31). To show that the approach applies to *Salmonella*, we used a recombinant chimeric antigen that contains epitopes from many outer membrane proteins of *Salmonella typhi* (32). This antigen is appealing as it has been validated as producing protective immune responses (33-34). FIG. 14B shows SDS PAGE gels of the antigens that were used in selection of rcSso7d variants that bind these bacterial outer membrane proteins.

Magnetic beads that bind proteins containing a hexahistidine tag (OmpW, Native Omp) or a biotin tag (OmpW) were coated with an excess of the antigen sufficient to saturate all binding sites on the magnetic bead, according to binding capacities reported by the manufacturers of the magnetic beads. The naive library of 1.4×10^9 rcSso7d variants, each
 5 displayed on the surface of a different yeast cell, was sorted using the antigen-coated magnetic beads until the library diversity was reduced to 1.4×10^7 , the upper limit that can reasonably be sorted according to quantitative binding activity using FACS (FIG. 15). Multiple rounds of FACS were performed for each antigen (*Salmonella typhi* Native Omp, *E. coli* OmpW, *E. coli* OmpA). FIGs. 16A-16C shows negative control FACS plots (yeast
 10 display library of rcSso7d variants and all staining reagents, but no antigen) and corresponding plots for the same libraries and staining reagents, plus antigen. The shapes indicated with P7 and P8 indicate the populations of cells that were retained in each round. In the negative control plots (-), no or few cells exhibited signals in these areas. When antigen was added (+), a number of rare clones exhibited signals in the indicated regions of
 15 the double positive quadrant of the FACS plot. The subpopulations of antigen-binders in each round were selected, expanded in culture, and eventually sequenced to determine the identity of the clones that are specific to each antigen (FIGs. 17-19).

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OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative
30 feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present disclosure, and without departing from the spirit and scope thereof, can make various changes and modifications of the disclosure to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

5

EQUIVALENTS

While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, *i.e.*, elements that are
5 conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, *i.e.*, “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when
10 used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, “or” should be understood to
15 have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, *i.e.*, the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of
20 exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (*i.e.* “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

25 As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also
30 allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least

one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and
5 optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts
10 of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, *i.e.*, to mean including
15 but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03. It should be appreciated that embodiments described in this document using an open-ended transitional phrase (*e.g.*, “comprising”) are also contemplated, in alternative embodiments, as “consisting
20 of” and “consisting essentially of” the feature described by the open-ended transitional phrase. For example, if the disclosure describes “a composition comprising A and B”, the disclosure also contemplates the alternative embodiments “a composition consisting of A and B” and “a composition consisting essentially of A and B”.

What is claimed is:

CLAIMS

- 5 1. An antigen-binding molecule comprising a reduced charge Sso7d(rcSso7d) antigen-binding protein that binds to a bacterium.
2. The antigen-binding molecule of claim 1, wherein the rcSso7d antigen-binding protein binds to a gram-positive bacterium.
- 10 3. The antigen-binding molecule of claim 1, wherein the rcSso7d antigen-binding protein binds to a gram-negative bacterium.
4. The antigen-binding molecule of any one of claims 1-3, wherein the rcSso7d antigen-binding protein binds to a pathogenic bacterium.
- 15 5. The antigen-binding molecule of any one of claims 1-4, wherein the rcSso7d antigen-binding protein binds to a bacterium that causes food poisoning.
- 20 6. The antigen-binding molecule of any one of claims 1-4, wherein the rcSso7d antigen-binding protein binds to a bacterium that causes food spoilage.
7. The antigen-binding molecule of any one of claims 1-6, wherein the rcSso7d antigen-binding protein binds to a bacterium of the genus *Listeria*, *Escherichia*, *Salmonella*, and/or
- 25 Legionella.
8. The antigen-binding molecule of claim 7, wherein the rcSso7d antigen-binding protein binds to a *Listeria monocytogenes*, *Salmonella typhi* and/or *E. coli*.
- 30 9. The antigen-binding molecule of claim 1-8, wherein the antigen-binding molecule binds to at least two antigens.

10. The antigen-binding molecule of claim 9, wherein two or more of the at least two antigens are specific to different types of bacteria.
11. The antigen-binding molecule of any one of claims 1-10, wherein the antigen-binding molecule binds to a protein antigen, a glycosylated protein antigen, and/or a carbohydrate antigen.
12. An antigen-binding molecule comprising a reduced charge Sso7d (rcSso7d) antigen-binding protein that binds *Listeria monocytogenes* protein LMOF2365_0639, wherein the antigen-binding protein comprises a variable region having:
- (i) the amino acid sequence of any one of SEQ ID NOs: 1-8; or
 - (ii) an amino acid sequence having 2 or 1 amino acid differences with any one of SEQ ID NOs: 1-8.
13. The antigen-binding molecule of claim 12, wherein the antigen-binding molecule comprises a variable region having:
- (i) the amino acid sequence of any one of SEQ ID NOs: 3, 5 or 7; or
 - (ii) an amino acid sequence having no more than 2 or 1 amino acid differences with any one of SEQ ID NOs: 3, 5 or 7.
14. The antigen-binding molecule of claim 13, wherein the rcSso7d antigen-binding protein comprises:
- (i) the amino acid sequence of any one of SEQ ID NOs: 13-15;
 - (ii) an amino acid sequence having no more than ten amino acid differences with any one of SEQ ID NOs: 13-15; or
 - (iii) an amino acid sequence having at least 80% identity with any one of any one of SEQ ID NOs: 13-15.
15. The antigen-binding molecule of claim 13 or claim 14, wherein the rcSso7d antigen-binding protein comprises the amino acid sequence of any one of SEQ ID NOs: 13-15.

16. The antigen-binding molecule of claim 12, wherein the antigen-binding protein comprises a variable region having:

- (i) the amino acid sequence of any one of SEQ ID NOs: 1, 2, 4, or 6; or
- (ii) an amino acid sequence no more than 2 or 1 amino acid differences with any one of SEQ ID NOs: 1, 2, 4, or 6.

17. The antigen-binding molecule of claim 16, wherein the rcSso7d antigen-binding protein comprises:

- (i) the amino acid sequence of any one of SEQ ID NOs: 16-19;
- (ii) an amino acid sequence having no more than seven amino acid differences with any one of SEQ ID NOs: 16-19; or
- (iii) an amino acid sequence having at least 80% identity with any one of any one of SEQ ID NOs: 16-19.

18. The antigen-binding molecule of claim 16 or claim 17, wherein the rcSso7d antigen-binding protein comprises the amino acid sequence of any one of SEQ ID NOs: 16-19.

19. The antigen-binding molecule of any one of claims 1-18, further comprising an enzyme, a fluorescent protein, an immunoglobulin, a peptide tag, a small molecule, or a combination thereof.

20. The antigen-binding molecule of claim 19, wherein the antigen-binding molecule comprises an enzyme selected from the group consisting of alkaline phosphatase, horseradish peroxidase, and β -galactosidase.

21. The antigen-binding molecule of claim 19 or claim 20, wherein the antigen-binding molecule comprises a fluorescent protein selected from the group consisting of TagBFP, mTagBFP2, Azurite, EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange, mKOk, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine,

tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.

22. The antigen-binding molecule of any one of claims 19-21, wherein the antigen-
5 binding molecule comprises a peptide tag selected from the group consisting of a His tag, a biotin acceptor tag, an HA tag, a c-Myc tag, a streptavidin tag, and a FLAG tag.

23. The antigen-binding molecule of any one of claims 19-22, wherein the antigen-
10 binding molecule comprises biotin.

24. An antigen-binding molecule comprising a reduced charge Sso7d (rcSso7d) antigen-
binding protein that binds *Salmonella typhi* Native Omp, said Native Omp comprising the
amino acid sequence of *Salmonella typhi* membrane proteins OmpA, OmpW, OmpC, OmpF,
OmpD, and phoE, wherein the antigen-binding protein comprises a variable region having:
15 (i) the amino acid sequence of any one of SEQ ID NOs: 58-71; or
(ii) an amino acid sequence having 2 or 1 amino acid differences with any one of
SEQ ID NOs: 58-71.

25. The antigen-binding molecule of claim 24, wherein the rcSso7d antigen-binding
20 protein comprises:
(i) the amino acid sequence of any one of SEQ ID NOs: 33-46;
(ii) an amino acid sequence having no more than ten amino acid differences with
any one of SEQ ID NOs: 33-46; or
(iii) an amino acid sequence having at least 80% identity with any one of any one
25 of SEQ ID NOs: 33-46.

26. The antigen-binding molecule of claim 24 or claim 25, wherein the rcSso7d antigen-
binding protein comprises the amino acid sequence of any one of SEQ ID NOs: 33-46.

30 27. The antigen-binding molecule of any one of claims 24-26, further comprising an enzyme, a fluorescent protein, an immunoglobulin, a peptide tag, a small molecule, or a combination thereof.

28. The antigen-binding molecule of claim 27, wherein the antigen-binding molecule comprises an enzyme selected from the group consisting of alkaline phosphatase, horseradish peroxidase, and β -galactosidase.

5

29. The antigen-binding molecule of claim 27 or claim 28, wherein the antigen-binding molecule comprises a fluorescent protein selected from the group consisting of TagBFP, mTagBFP2, Azurite, EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange, mKO_κ, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.

15

30. The antigen-binding molecule of any one of claims 27-29, wherein the antigen-binding molecule comprises a peptide tag selected from the group consisting of a His tag, a biotin acceptor tag, an HA tag, a c-Myc tag, a streptavidin tag, and a FLAG tag.

20 31. The antigen-binding molecule of any one of claims 27-30, wherein the antigen-binding molecule comprises biotin.

32. An antigen-binding molecule comprising a reduced charge Sso7d (rcSso7d) antigen-binding protein that binds *E. coli* OmpW, wherein the antigen-binding protein comprises a variable region having:

25

- (i) the amino acid sequence of any one of SEQ ID NOs: 73-80; or
- (ii) an amino acid sequence having 2 or 1 amino acid differences with any one of SEQ ID NOs: 73-80.

30 33. The antigen-binding molecule of claim 32, wherein the rcSso7d antigen-binding protein comprises:

- (i) the amino acid sequence of any one of SEQ ID NOs: 47-54;

(ii) an amino acid sequence having no more than ten amino acid differences with any one of SEQ ID NOs: 47-54; or

(iii) an amino acid sequence having at least 80% identity with any one of any one of SEQ ID NOs: 47-54.

5

34. The antigen-binding molecule of claim 33 or claim 34, wherein the rcSso7d antigen-binding protein comprises the amino acid sequence of any one of SEQ ID NOs: 47-54.

35. The antigen-binding molecule of any one of claims 32-34, further comprising an enzyme, a fluorescent protein, an immunoglobulin, a peptide tag, a small molecule, or a combination thereof.

36. The antigen-binding molecule of claim 35, wherein the antigen-binding molecule comprises an enzyme selected from the group consisting of alkaline phosphatase, horseradish peroxidase, and β -galactosidase.

37. The antigen-binding molecule of claim 35 or claim 36, wherein the antigen-binding molecule comprises a fluorescent protein selected from the group consisting of TagBFP, mTagBFP2, Azurite, EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange, mKO κ , mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.

38. The antigen-binding molecule of any one of claims 35-37, wherein the antigen-binding molecule comprises a peptide tag selected from the group consisting of a His tag, a biotin acceptor tag, an HA tag, a c-Myc tag, a streptavidin tag, and a FLAG tag.

30

39. The antigen-binding molecule of any one of claims 35-38, wherein the antigen-binding molecule comprises biotin.

40. An antigen-binding molecule comprising a reduced charge Sso7d (rcSso7d) antigen-binding protein that binds *E. coli* OmpA, wherein the antigen-binding protein comprises a variable region having:
- 5 (i) the amino acid sequence of any one of SEQ ID NOs: 81-83; or
- (ii) an amino acid sequence having 2 or 1 amino acid differences with any one of SEQ ID NOs: 81-83.
41. The antigen-binding molecule of claim 40, wherein the rcSso7d antigen-binding
- 10 protein comprises:
- (i) the amino acid sequence of any one of SEQ ID NOs: 55-57;
- (ii) an amino acid sequence having no more than ten amino acid differences with any one of SEQ ID NOs: 55-57; or
- (iii) an amino acid sequence having at least 80% identity with any one of any one
- 15 of SEQ ID NOs: 55-57.
42. The antigen-binding molecule of claim 40 or claim 41, wherein the rcSso7d antigen-binding protein comprises the amino acid sequence of any one of SEQ ID NOs: 55-57.
- 20 43. The antigen-binding molecule of any one of claims 40-42, further comprising an enzyme, a fluorescent protein, an immunoglobulin, a peptide tag, a small molecule, or a combination thereof.
44. The antigen-binding molecule of claim 43, wherein the antigen-binding molecule
- 25 comprises an enzyme selected from the group consisting of alkaline phosphatase, horseradish peroxidase, and β -galactosidase.
45. The antigen-binding molecule of claim 43 or claim 44, wherein the antigen-binding molecule comprises a fluorescent protein selected from the group consisting of TagBFP, mTagBFP2, Azurite, EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover,
- 30

mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange, mKOok, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.

5

46. The antigen-binding molecule of any one of claims 43-45, wherein the antigen-binding molecule comprises a peptide tag selected from the group consisting of a His tag, a biotin acceptor tag, an HA tag, a c-Myc tag, a streptavidin tag, and a FLAG tag.

10

47. The antigen-binding molecule of any one of claims 43-46, wherein the antigen-binding molecule comprises biotin.

48. A composition comprising an antigen-binding molecule according to any one of claims 1-47 and a buffer, wherein the buffer has a pH $\leq$ 6.0.

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49. The composition of claim 48, wherein the buffer has a pH of 5.0 – 6.0.

50. The composition of claim 48 or claim 49, wherein the buffer has a pH of about 5.5.

20

51. The composition of any one of claims 48-50, wherein the buffer comprises sodium acetate and/or phosphate-buffered saline.

52. The composition of any one of claims 48-51, wherein the composition further comprises a LMOF2365_0639 protein, an OmpA protein, an OmpW protein, an OmpC
25 protein, an OmpF protein, an OmpD protein, a phoE protein, or a combination thereof.

53. The composition of any one of claims 48-52, wherein the composition further comprises a *Listeria monocytogenes* cell, a *Salmonella typhi* cell, an *E. coli* cell, or a combination thereof.

30

54. A method of detecting protein LMOF2365_0639 in a sample, the method comprising:

(a) contacting the sample with an antigen-binding molecule of any one of claims 12-23; and

(b) detecting, if present, LMOF2365_0639 protein bound by the antigen-binding molecule.

5

55. A method of detecting OmpA, OmpW, OmpC, OmpF, OmpD, phoE, or a combination thereof in a sample, the method comprising:

(a) contacting the sample with an antigen-binding molecule of any one of claims 24-31; and

10 (b) detecting, if present, OmpA, OmpW, OmpC, OmpF, OmpD, phoE, or a combination thereof bound by the antigen-binding molecule.

56. A method of detecting OmpW in a sample, the method comprising:

15 (a) contacting the sample with an antigen-binding molecule of any one of claims 32-39; and

(b) detecting, if present, OmpW bound by the antigen-binding molecule.

57. A method of detecting OmpA in a sample, the method comprising:

20 (a) contacting the sample with an antigen-binding molecule of any one of claims 40-47; and

(b) detecting, if present, OmpA bound by the antigen-binding molecule.

58. The method of any one of claims 54-57, wherein the sample is from a food product.

25 59. The method of any one of claims 54-57, wherein the sample is from a subject.

60. The method of claim 59, wherein the subject is a mammal.

61. The method of claim 59, wherein the subject is a human.

30

62. The method of claim 60 or claim 61, further comprising treating the subject.

63. The method of any one of claims 54-62, wherein the sample is contacted with the antigen-binding protein at a pH $\leq$ 6.0.
64. The method of claim 63, wherein the sample is contacted with the antigen-binding
5 protein at a pH of 5.0 – 6.0.
65. The method of claim 63 or claim 64, wherein the sample is contacted with the antigen-binding protein at a pH of about 5.5.
- 10 66. The method of any one of claims 54-65, wherein the sample comprises sodium acetate and/or phosphate-buffered saline.
67. The method of any one of claims 54-66, wherein step (a) further comprises contacting the sample with a detection agent, wherein the detection agent binds to or is bound by the
15 antigen-binding molecule.
68. The method of claim 67, wherein the detection agent comprises an enzyme, a fluorescent protein, an immunoglobulin, a peptide tag, a small molecule, and/or a combination thereof.
20
69. The method of claim 68, wherein the detection enzyme comprises an enzyme selected from the group consisting of alkaline phosphatase, horseradish peroxidase, and β -galactosidase.
- 25 70. The method of claim 68 or claim 69, wherein the detection agent comprises a fluorescent protein selected from the group consisting of TagBFP, mTagBFP2, Azurite, EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP,
30 Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange, mKO κ , mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.

71. The method of any one of claims 68-70, wherein the detection agent comprises a peptide tag selected from the group consisting of a His tag, a biotin acceptor tag, an HA tag, a c-Myc tag, a streptavidin tag, and a FLAG tag.
- 5
72. The method of any one of claims 68-70, wherein the detection agent comprises biotin.
73. A method of detecting bacteria in a sample comprising:
- (a) contacting the sample with an antigen-binding molecule of any one of claims
- 10 1-47; and
- (b) detecting, if present, bacteria bound by the antigen-binding molecule.
74. A method of detecting a *Listeria monocytogenes* cell in a sample comprising:
- (a) contacting the sample with an antigen-binding molecule of any one of claims
- 15 12-23; and
- (b) detecting, if present, *Listeria monocytogenes* bound by the antigen-binding molecule.
75. A method of detecting a *Salmonella typhi* cell in a sample comprising:
- 20 (a) contacting the sample with an antigen-binding molecule of any one of claims 24-31; and
- (b) detecting, if present, *Salmonella typhi* bound by the antigen-binding molecule.
76. A method of detecting an *E. coli* cell in a sample comprising:
- 25 (a) contacting the sample with an antigen-binding molecule of any one of claims 32-47; and
- (b) detecting, if present, *E. coli* bound by the antigen-binding molecule.
77. The method of any one of claims 73-76, wherein the sample is from a food product.
- 30
78. The method of claim any one of claims 73-76, wherein the sample is from a subject.

79. The method of claim 78, wherein the subject is a mammal.
80. The method of claim 78, wherein the subject is a human.
- 5 81. The method of claim 79 or claim 80, further comprising treating the subject.
82. The method of any one of claims 73-81, wherein the sample is contacted with the antigen-binding protein at a pH $\leq$ 6.0.
- 10 83. The method of claim 82, wherein the sample is contacted with the antigen-binding protein at a pH of 5.0 – 6.0.
84. The method of claim 82 or claim 83, wherein the sample is contacted with the antigen-binding protein at a pH of about 5.5.
- 15 85. The method of any one of claims 73-84, wherein the sample comprises sodium acetate and/or phosphate-buffered saline.
86. The method of any one of claims 73-85, wherein step (a) further comprises contacting
- 20 the sample with a detection agent, wherein the detection agent binds to or is bound by the antigen-binding molecule.
87. The method of claim 86, wherein the detection agent comprises an enzyme, an immunoglobulin, a fluorescent protein, a peptide tag, a small molecule, or a combination
- 25 thereof.
88. The method of claim 87, wherein the detection enzyme comprises an enzyme selected from the group consisting of alkaline phosphatase, horseradish peroxidase, and β -galactosidase.
- 30 89. The method of claim 87 or claim 88, wherein the detection agent comprises a fluorescent protein selected from the group consisting of TagBFP, mTagBFP2, Azurite,

EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange, mKOκ, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.

90. The method of any one of claims 87-89, wherein the detection agent comprises a peptide tag selected from the group consisting of a His tag, a biotin acceptor tag, an HA tag, a c-Myc tag, a streptavidin tag, and a FLAG tag.

91. The method of any one of claims 87-90, wherein the detection agent comprises biotin.

92. A method of killing a bacterial cell comprising contacting the cell with the antigen-binding molecule of any one of claims 1-47, wherein the antigen-binding molecule further comprises a cytotoxic agent.

93. A method of killing a *Listeria monocytogenes* cell comprising contacting the cell with the antigen-binding molecule of any one of claims 12-23, wherein the antigen-binding molecule further comprises a cytotoxic agent.

94. A method of killing a *Salmonella typhi* cell comprising contacting the cell with the antigen-binding molecule of any one of claims 24-31, wherein the antigen-binding molecule further comprises a cytotoxic agent.

95. A method of killing an *E. coli* cell comprising contacting the cell with the antigen-binding molecule of any one of claims 32-47, wherein the antigen-binding molecule further comprises a cytotoxic agent.

96. The method of any one of claims 92-95, wherein the sample is contacted with the antigen-binding protein in a buffer at a pH $\leq$ 6.0.

97. The method of claim 96, wherein the sample is contacted with the antigen-binding protein at a pH of 5.0 – 6.0.
98. The method of claim 96 or claim 97, wherein the sample is contacted with the antigen-binding protein at a pH of about 5.5.
99. The method of any one of claims 96-98, wherein the sample comprises sodium acetate and/or phosphate-buffered saline.
100. A method of monitoring growth of a population of bacteria comprising:
- (a) contacting a sample comprising at least a subset of the population with the antigen-binding molecule of any one of claims 1-47; and
 - (b) repeating step (a) at least once.
101. A method of monitoring growth of *Listeria monocytogenes* comprising:
- (a) contacting a sample comprising at least a subset of the population with the antigen-binding molecule of any one of claims 12-23; and
 - (b) repeating step (a) at least once.
102. A method of monitoring growth of *Salmonella typhi* comprising:
- (a) contacting a sample comprising at least a subset of the population with the antigen-binding molecule of any one of claims 24-31; and
 - (b) repeating step (a) at least once.
103. A method of monitoring growth of *E. coli* comprising:
- (a) contacting a sample comprising at least a subset of the population with the antigen-binding molecule of any one of claims 32-47; and
 - (b) repeating step (a) at least once.
104. The method of any one of claims 100-103, wherein step (a) further comprises contacting the sample with a detection agent, wherein the detection agent binds to or is bound by the antigen-binding molecule.

105. The method of claim 104, wherein the detection agent comprises an enzyme, an immunoglobulin, a fluorescent protein, a peptide tag, a small molecule, or a combination thereof.
- 5
106. The method of claim 105, wherein the detection enzyme comprises an enzyme selected from the group consisting of alkaline phosphatase, horseradish peroxidase, and β -galactosidase.
- 10
107. The method of claim 104 or claim 105, wherein the detection agent comprises a fluorescent protein selected from the group consisting of TagBFP, mTagBFP2, Azurite, EBFP2, EBFP2, Sirius, Sapphire, T-Sapphire, ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midoriishi-Cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP,
- 15
- Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange, mKO κ , mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, mPlum, HcRed-Tandem, mKate2, mNeptune, and NirFP.
108. The method of any one of claims 104-107, wherein the detection agent comprises a
- 20
- peptide tag selected from the group consisting of a His tag, a biotin acceptor tag, an HA tag, a c-Myc tag, a streptavidin tag, and a FLAG tag.
109. The method of any one of claims 104-108, wherein the detection agent comprises biotin.
- 25
110. A kit comprising the antigen-binding molecule of any one of claims 1-47.
111. The kit of claim 110, further comprising a buffer.
- 30
112. The kit of claim 111, wherein the buffer has a pH of ≤ 6.0 .
113. The kit of claim 112, wherein the buffer has a pH of 5.0 – 6.0.

114. The kit of claim 112 or claim 113, wherein the buffer has a pH of about 5.5.
115. The kit of any one of claims 111-114, wherein the buffer comprises sodium acetate
5 and/or phosphate-buffered saline.
116. The kit of any one of claims 110-115, further comprising a substrate.
117. The kit of claim 116, wherein the substrate comprises paper, nitrocellulose, cellulose
10 powder, and/or a liquid colloid.
118. The kit of claim 116 or claim 117, wherein the antigen-binding molecule is bound to the substrate.
- 15 119. The kit of any one of claims 110-118, further comprises a growth media that allows for bacterial growth.