Supporting Information

Binding of silver nanoparticles to bacterial proteins depends on surface modifications and inhibits enzymatic activity

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Summary

SI-1- Cell free extract preparation:

Cells were grown overnight until stationary phase and collected by centrifugation. The cell pellet was then washed 2-3 times with phosphate-buffered saline (PBS) and tris(hydroxymethyl)aminomethane (Tris-HCl) before lysing the cells using ultrasonication (Branson sonifier 150 and 3.9 mm stepped titanium microtip probe). Cell debris was removed by centrifugation at 5,000 *x g* for 10 min yielding a cell-free extract (CFX) containing mostly soluble proteins.

SI-2- Protein identification:

Nanoparticle-protein conjugates were collected by centrifugation at 15,000 *x g* for 25 min and washed in 100 mM Tris, sodium dodecyl sulfate (SDS), or deionized water. Protein bands were excised directly from SDS gels, digested with trypsin, and sequenced at EPFL's Proteomics Core Facilty (http://pcf.epfl.ch/) using liquid chromatography tandem mass spectrometry (LC-MS/MS) (Thermo LTQ linear ion trap or Bruker Daltonics HCT-Ultra).

SI-3- MALDI operation:

We used an ABI (Applied Biosystems) 4800 instrument with a Nd:YAG laser at 355nm and an accelerating potential of 20 kV. Up to 2000 laser shots were typically summed by random sampling of the surface to generate the spectra. The matrix used for all peptide analyses was α-cyano-4-hydroxycinnamic acid. Data were analyzed using mMass 2.4 [20]. Experimental reproducibility was verified by carrying out experiments at least two separate times.

SI-4- TNase activity assay:

TNase (0.2 mg/mL) was incubated for 30 min in 100 ppm AgNPs or diluted in 1M potassium phosphate as a control, then added to a solution containing 200 mM potassium phosphate, 0.041mM pyridoxal 5-phosphate and 5 mM L-tryptophan [20]. The solution was incubated at 37°C for 10 minutes and the reaction was stopped by adding 200 µL 6.1N trichloroacetic acid. Indole was phase extracted in toluene and mixed with p-dimethylaminobenzaldehyde (in 95% ethanol) and an acid-alcohol solution

composed of 859 mM HCl in 95% ethanol. Absorbance was read after 10 minutes at 540nm to monitor for indole release by the active protein for triplicates of each experiment.

SI-5- Summary of nanoparticle characterization methods and results:

Changes in average size (as Z-Average size) and surface charge (as Zeta Potential) of bare silver nanoparticles (bAgNPs) and coated silver nanoparticles (cAgNPs) were measured using dynamic light scattering (DLS) and electrophoretic mobility on a Zetasizer Nano ZS (Malvern Instruments). Three independent measurements were performed on three sample sets: 1) AgNP stock solution (1000 ppm as provided by supplier), 2) stock solution diluted 1:10 with deionized water (mixed thoroughly by repeated pipette plunging), and 3) experimental conditions for peptide binding (10μM $CaCl_2$, 20mM Tris-Cl pH 7.7).

In stock solutions, the average size of cAgNPs (32.2 ± 0.6 nm) and bAgNPs (15.6 ± 0.4 nm) is stable over time (Fig S1). Minor aggregation of bAgNPs after 1:10 dilution is observed. Based on the polydispersity index from DLS measurements, cAgNPs are monodispersed (PdI ≈ 0.3), whereas bAgNPs are more polydisperse (PdI ≈ 0.6), which is consistent with TEM micrographs (Fig. S2), and UV-vis aggregation assays (Fig. S5).

The Zeta potential of cAgNPs (-41.1 ± 0.9 mV) and bAgNPs (-38.2 ± 1.2 nm) stock solution becomes more negative with time, 1:10 dilution, and at experimental conditions (Fig. S3). However, the Zeta potential of cAgNPs and bAgNPs in the same media were not significantly different. This suggests that despite some variability in surface charge, comparisons between AgNP type can still be made for a given experimental condition.

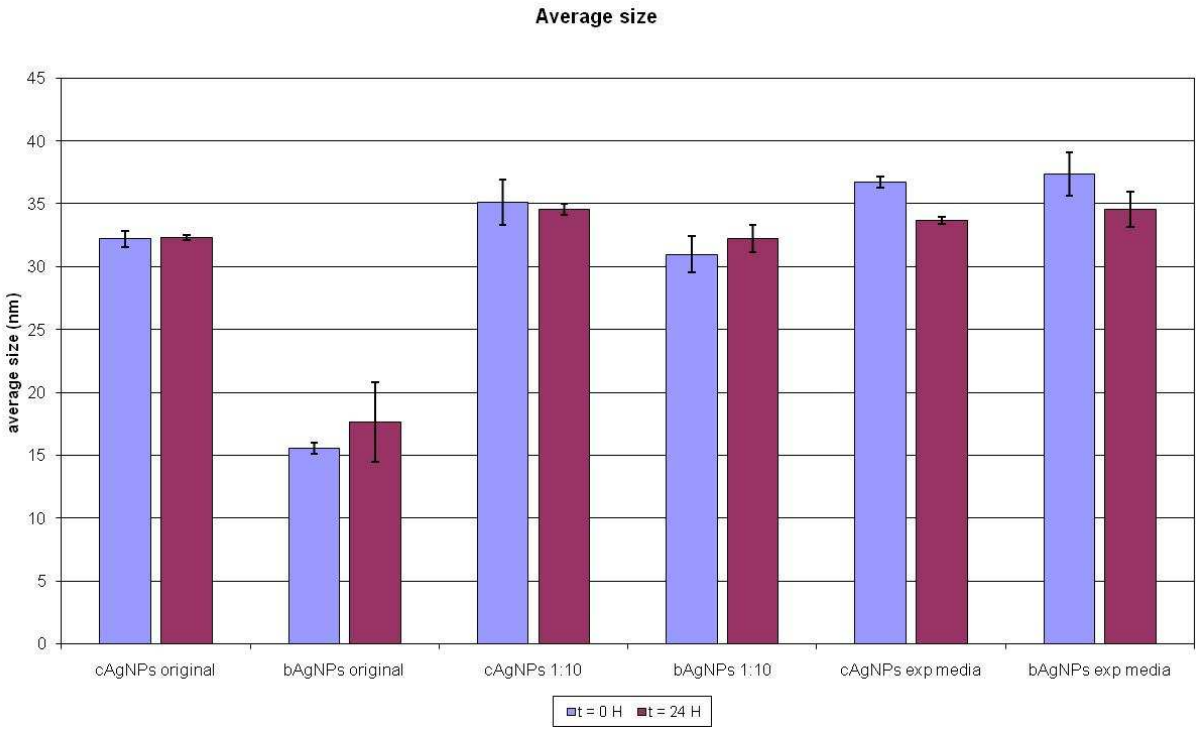

Figure S1 Average size distribution of cAgNPs and bAgNPs in original stock solution, 1:10 dilution (with deionized water), and experimental media (protein digestion solution) after 30 minutes (blue) and 24 hours (purple). Error bars indicate standard deviation after three independent measurements.

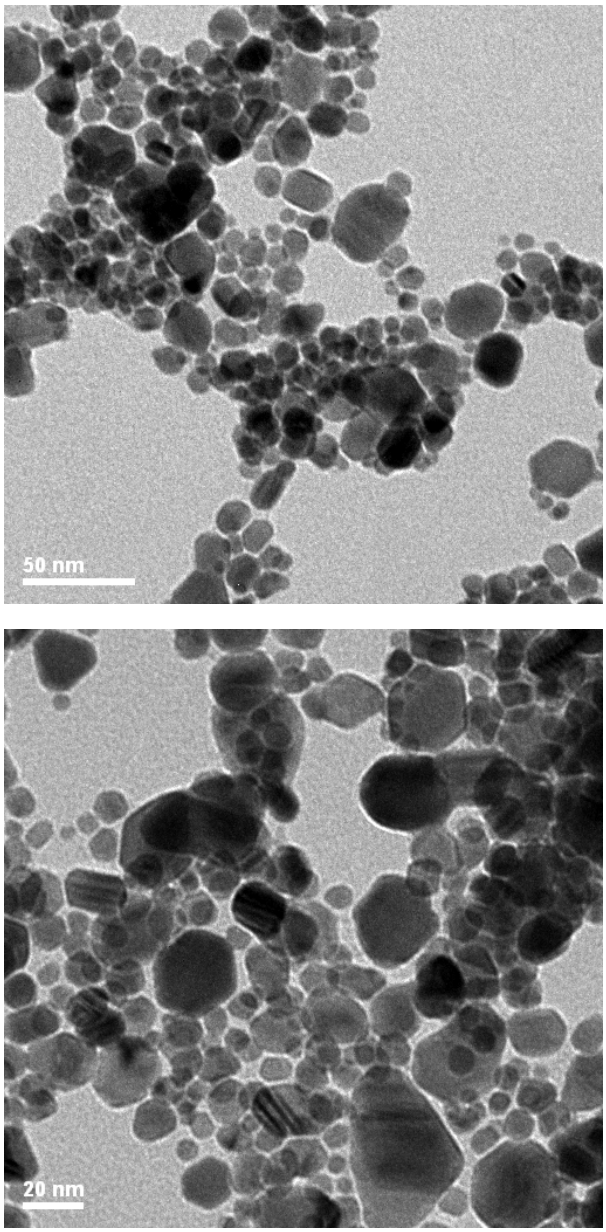

Figure S2: Representative TEM micrographs of cAgNPs (top) and bAgNPs (bottom) used in this study.

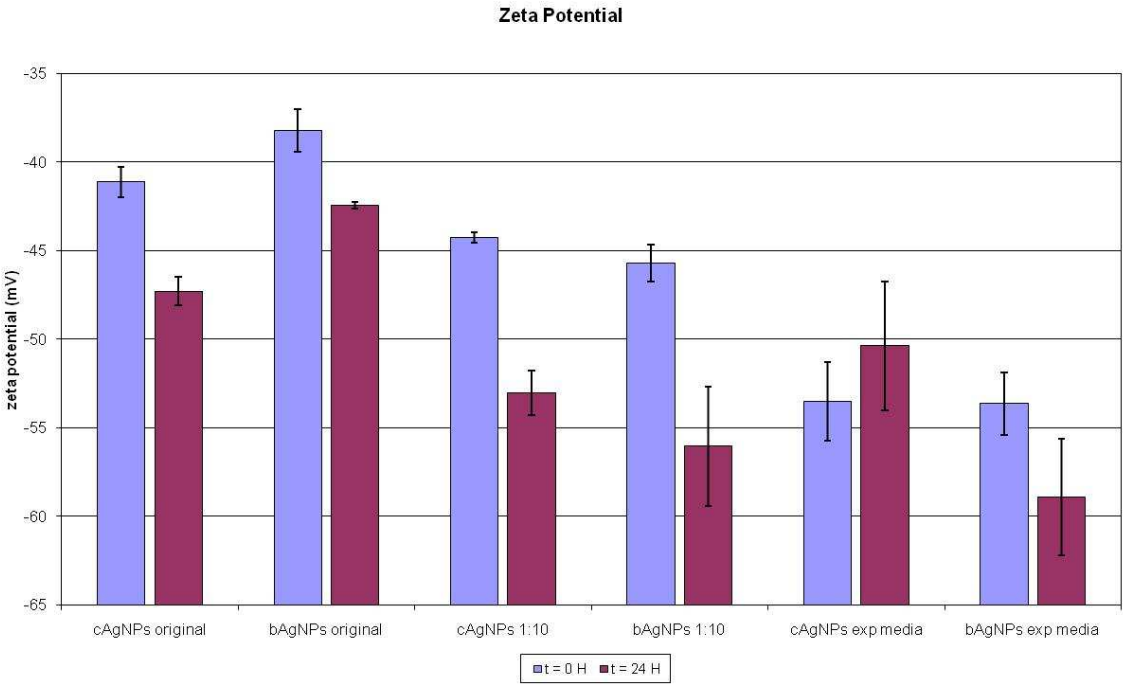

Figure S3 Average Zeta potential of cAgNPs and bAgNPs in original stock solution, 1:10 dilution (with deionized water), and experimental media (protein digestion solution) after 30 minutes (blue) and 24 hours (purple). Error bars indicate standard deviation after three independent measurements.

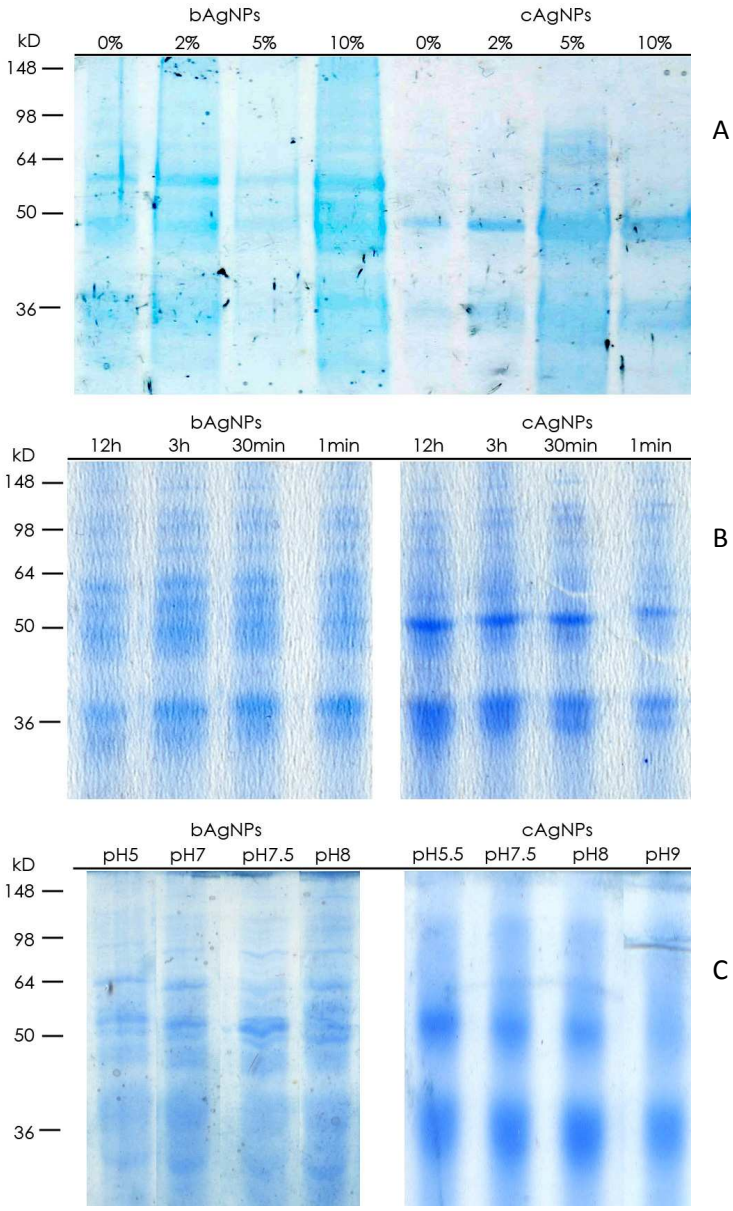

Figure S4: (A) SDS-PAGE gel of *E. coli* soluble CFX portion bound to bAgNPs and portion bound to cAgNPs before and after successive 2%, 5%, and 10% SDS washing steps. (B) Effect of reaction time on proteins associated with bAgNPs and cAgNPs. (C) Effect of reaction pH on proteins associated with bAgNPs and cAgNPs.

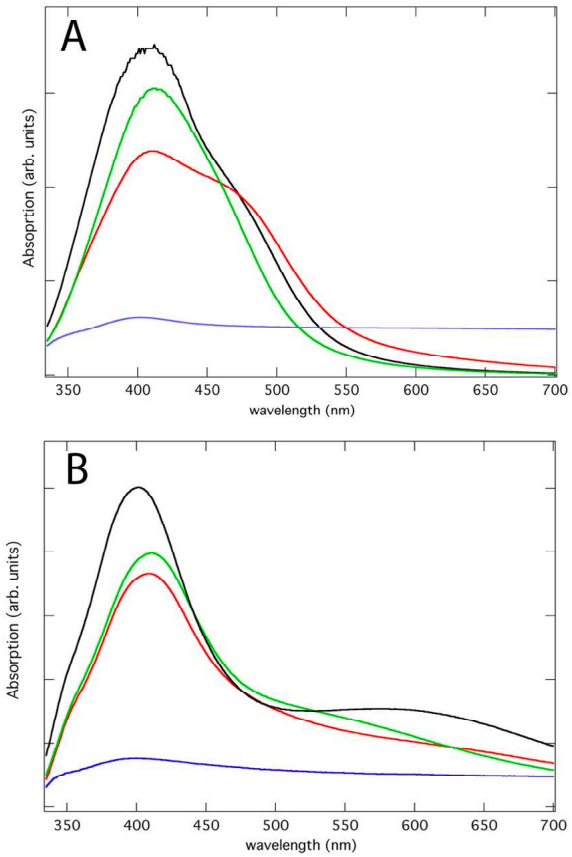

Figure S5. Absorption spectra of TNase protection against aggregation of bare (A) and coated (B) AgNPs. Black curves are AgNP absorption spectra, green curves are AgNP reacted first with 10 ug/mL TNase followed by addition of NaCl, red curves are AgNP reacted first with 1 ug/mL TNase followed by addition of NaCl, and blue curves are AgNP in NaCl.

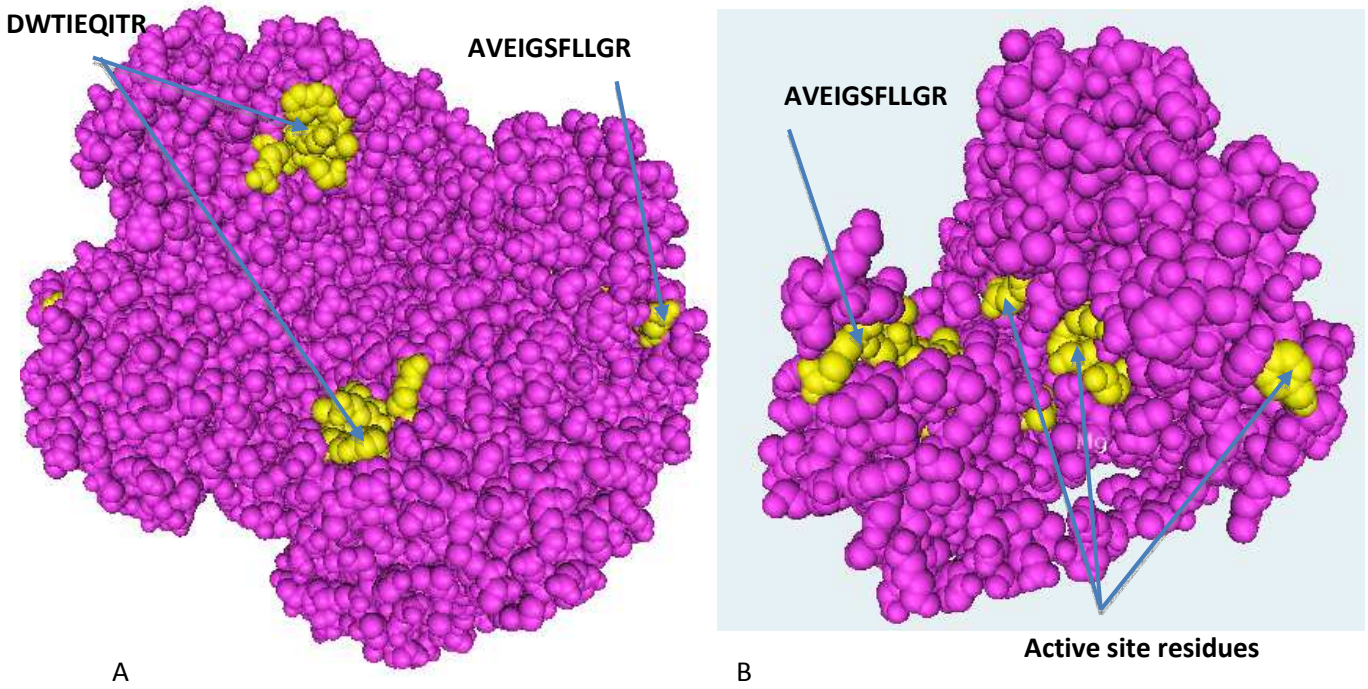

Figure S6: Localization in the protein TNase of two peptides hat bind to bAgNPs but not to cAgNPs: DWTIEQITR and AVEIGSFLLGR. (A) Localization of the two peptides in the full tetrameric protein. (B) Localization of AVEIGSFLLGR in close proximity to the active site of a monomer.

Table S1 (next page): Proteins identified from excised bands numbered in Fig. 1. Only proteins identified with more than 10 unique peptides in one band are listed. The proteins listed in bold correspond to those for which more than 10 unique peptides were found associated with both bAgNPs and cAgNPs. The absolute abundance of the proteins in the cytosol of *E. coli* is taken from [24, 25].

			cAgNPs										bAgNPs									Expected protein abundance	
Identified Proteins	**kDa**	**pI**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**Count/cell[1]**	**Grouping[2]**
Malate dehydrogenase	32	5.61		6	19				14				2	5								3390	high
Cysteine synthase A	34	5.83		11	2																	1450	low
Glyceraldehyde-3-phosphate dehydrogenase A	36	6.58						7	11	1			3	4								170000	high
Protein csiD	37	5.77		13																		-	n/a
Outer membrane protein A	37	5.6		11	16			2	11		1		9	12								-	n/a
Outer membrane protein C	40	4.5		18	12			17	12				15	12								-	n/a
Adenylosuccinate synthetase	47	5.32	13																			7160	high
Isocitrate lyase (ICL)	48	5.2	18	2	6							16									18	-	n/a
Tryptophanase (TNase)	53	5.9	19	8	10	21	15	3	5			23	1	1	2		1			6	21	835	low
Glutamate decarboxylase alpha (GAD-alpha)	53	5.22																	2	13	12	498	low
ATP synthase subunit alpha	55	5.8																	12	2		1990	low
60 kDa chaperonin 1	57	4.85															2	14	7		1	61000	high
Periplasmic dipeptide transport protein (DBP)	60	5.75																5	14	12	1	-	n/a
Periplasmic oligopeptide-binding protein	61	5.85																18	9			-	n/a
Chaperone protein dnaK	69	4.83															13					-	n/a
LPS-assembly protein	90	4.85									7				2	11						-	n/a
Outer membrane protein assembly factor	91	4.9									15				2	20						-	n/a
Aldehyde-alcohol dehydrogenase	96	6.33								13					9							5430	high
Aconitate hydratase 1 (Aconitase 1)	98	5.59								2					12	1						198	low
Pyruvate dehydrogenase E1 component	100	5.5								11	2				10							14500	high
Formate dehydrogenase-O, major subunit	113	6.5								2	17				5	19						672	low
Respiratory nitrate reductase 1 alpha chain	140	6.05																				101	low
Phosphoribosylformyl-glycinamidine synthase	141	5.23																				694	low
Bifunctional protein putA	144	5.69																				329	low

Table S2: List of proteins identified as binding to AgNPs by LC-MS/MS.

Identified Proteins	function/ localization	cofactor
Glyceraldehyde-3-phosphate dehydrogenase A	enzyme	NAD+
Formate dehydrogenase-O, major subunit	**enzyme**	**Fe, W, Se**
Aldehyde-alcohol dehydrogenase	enzyme	Zn, Fe
Respiratory nitrate reductase 1 alpha chain	enzyme	Mo
Tryptophanase (TNase)	**enzyme**	**pyridoxal phosphate**
Cysteine synthase A	enzyme	pyridoxal phosphate
ATP synthase subunit alpha	enzyme/ membrane	
Bifunctional protein putA	enzyme	FAD
Malate dehydrogenase	enzyme	none
Aconitate hydratase 1 (Aconitase 1)	enzyme	4Fe-4S
Pyruvate dehydrogenase E1 component	**enzyme**	**thiamine diphosphate**
Adenylosuccinate synthetase	enzyme	Mg
Phosphoribosylformylglycinamidine synthase	enzyme	Mg
Glutamate decarboxylase alpha (GAD-alpha)	enzyme	pyridoxal phosphate
Isocitrate lyase (ICL)	**enzyme**	**Mg, Mn**
Protein csiD	unknown	
Periplasmic oligopeptide-binding protein	periplasm	
Periplasmic dipeptide transport protein (DBP)	periplasm	
Outer membrane protein A	**membrane**	
Outer membrane protein assembly factor yaeT	**membrane**	
60 kDa chaperonin 1	chaperone/ cytoplasmic	
LPS-assembly protein	membrane	
Chaperone protein dnaK	chaperone/ cytoplasmic	
Outer membrane protein C	**membrane**	

Table S3. Digestion enzymes [trypsin (T); chymotrypsin (C)] used to generate high binding protein fragments shown in Table 1.

Protein	Sequence	Digestion enzyme
TNase	HLPEPFR	T
	KHLPEPF	C
	TIEQITRETY	C
	NIFGYQYTIPTHQGR	T
	GNFDLEGLER	T
	DWTIEQITR	T
	AVEIGSFLLGR	T
	GLTFTYEPK	T
AdhP	HHHHGMASMTGGQQMGR	T
	IRPGQWIAIYGLGGLGNLALQYAK	T
	AAFNSAVDAVR	T
BSA	DTHKSEIAHR	T
	QQCPFDEHVKL	C
	EIARRHPY	C
	YEIAR	T
	RHPEYAVSVLLR	T
	SRRHPEY	C
Cytc	IFVQKCAQCHTVEK	T
	VQKCAQCHTVEKGGKHKTGPNL	C
	TGPNLHGLFGR	T