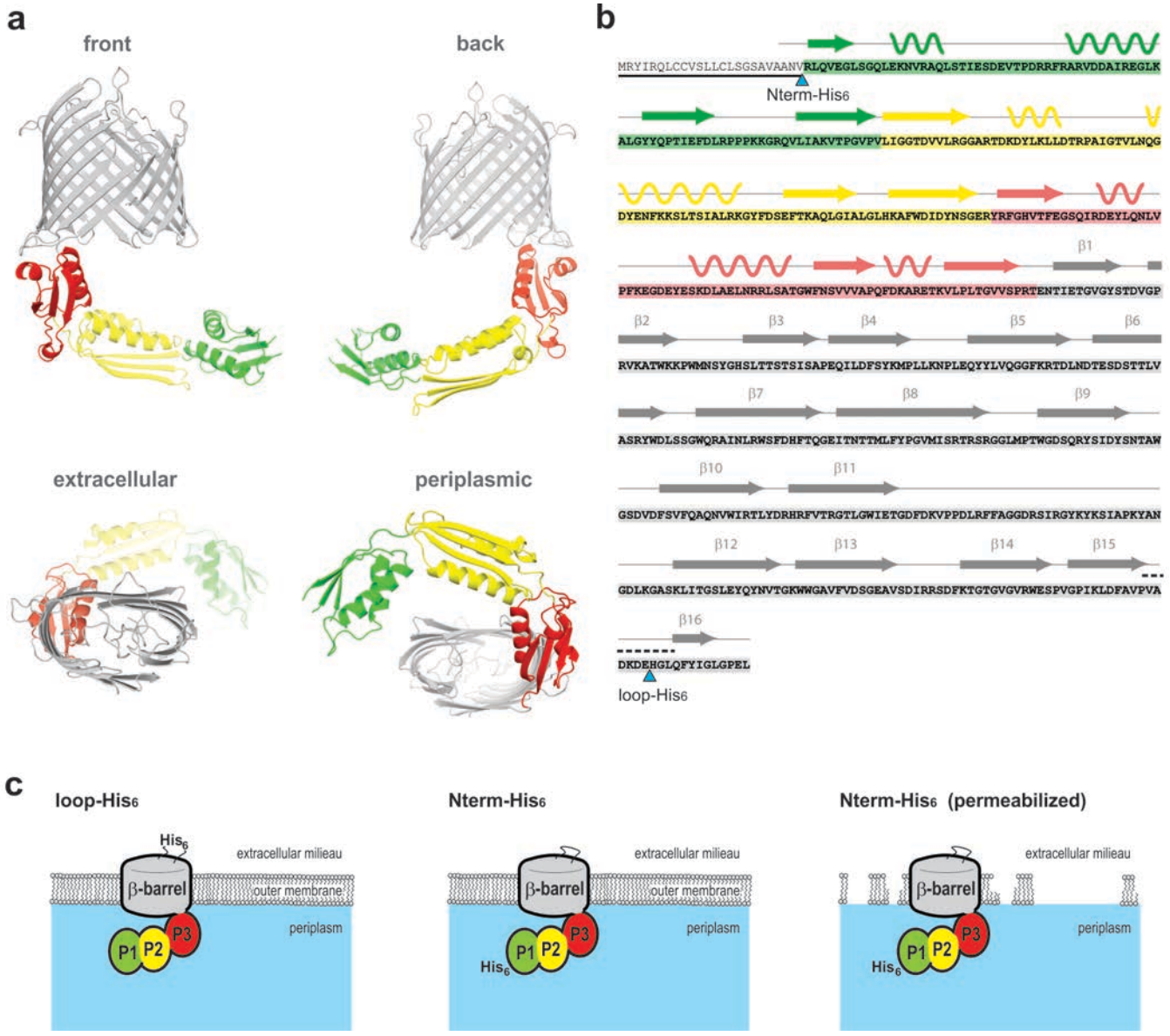
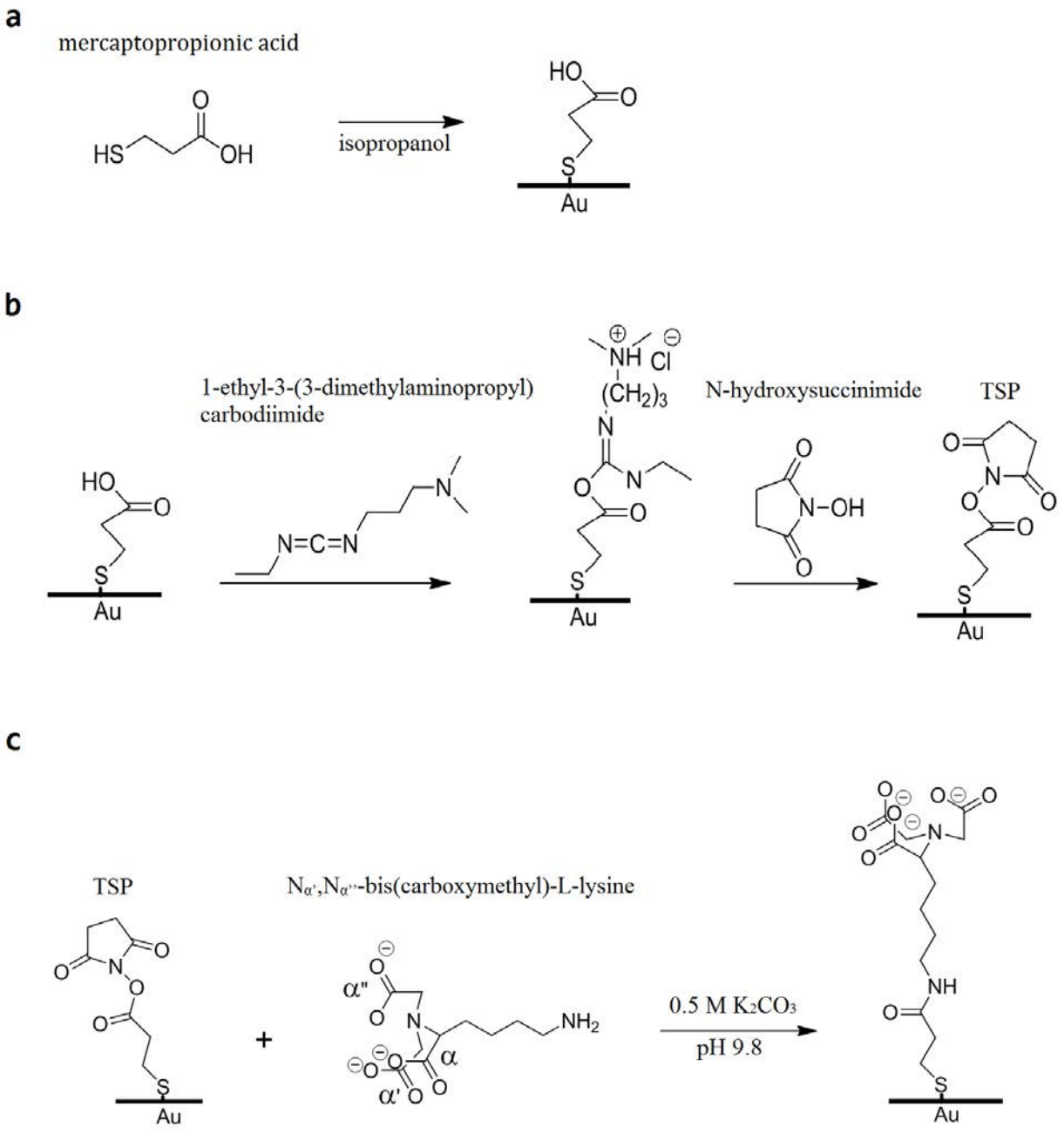


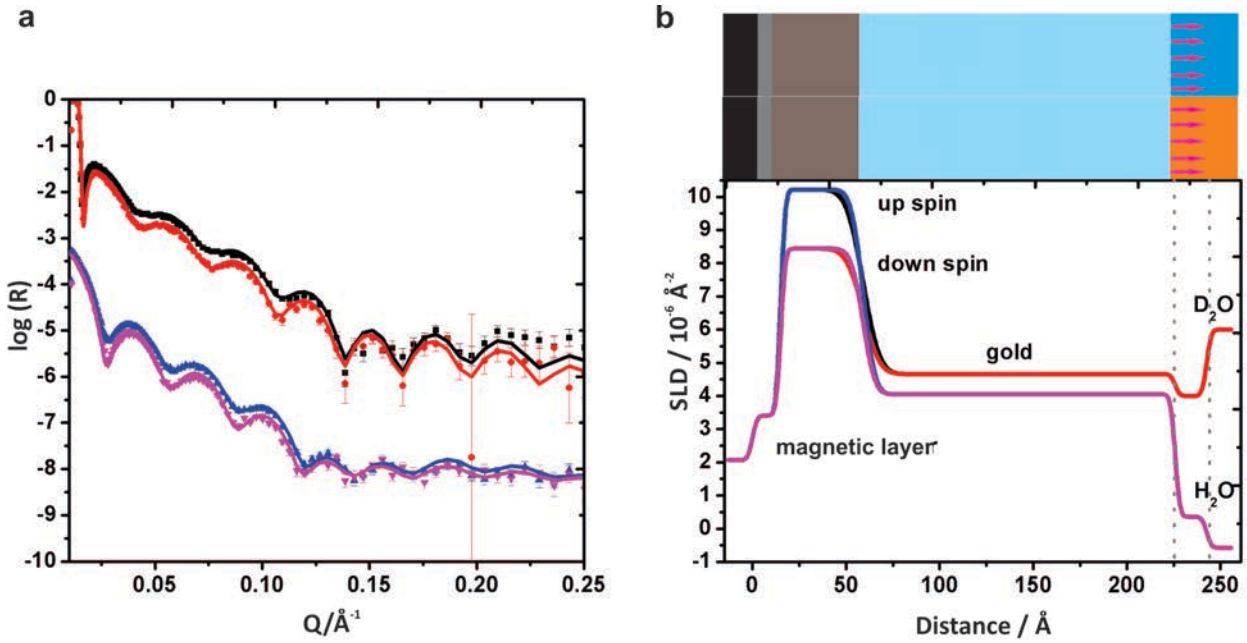
Supplementary Figure 1| Structure-based engineering a hexa-histidine sequence (His_6) into TamA. In order to attach TamA to the Ni-NTA gold surface, a His_6 sequence that can bind Ni-NTA was engineered into an external loop of TamA ("loop-His_6"). As a control for immunofluorescence assessments, a second form of TamA was engineered to have a His_6 sequence in the periplasm, at the N-terminus of the mature protein, by inserting the His_6 sequence immediately after the known signal sequence.

(a) The crystal structure of TamA is shaded according to the domains: the N-terminal POTRA domain P1 is shown in green, POTRA domain P2 shown in yellow, POTRA domain P3 is shown in red and the β-barrel domain is shown in silver. The "extracellular" view corresponds to the aspect of TamA attached to the gold surface for experimental work, while the "periplasmic" view depicts the aspect of TamA available for binding TamB. **(b)** Based on the crystal structure of TamA [pdb P0ADE4] the secondary structure elements of TamA are annotated according to the sequence: α-helices are shown as waved lines and β-strands shown as arrows. The N-terminal signal sequence is underlined, and the sequence corresponding to extracellular loop L8 is indicated with a dashed line. The position at which His_6 sequences were inserted are indicated with a triangle. **(c)** Schematic view of the topology of the engineered His_6 sequences in extracellular loop L8, or the N-terminus, as confirmed by immunofluorescence shown in Figure 1c. The experimentally confirmed topological position of the "loop His_6" sequence and the "N-terminal His_6" sequence are indicated.



Supplementary Figure 2| Method developed for derivatization of gold surfaces. Magnetic contrast neutron reflectrometry (MCNR) and quartz crystal microbalance with dissipation (QCM-D) analysis require TamA to be orientated on an engineered gold surface. Controlling protein orientation at interfaces uses histidine tags: an alternative to Ni/NTA.

(a) The clean gold ("Au") was exposed to a solution of 5mM mercaptopropionic acid in isopropanol overnight followed by rinsing with isopropanol three times to remove any unbound mercaptopropionic acid and the derivatized gold dried under a stream of nitrogen gas. **(b)** The dried modified substrate was placed into a mixture of 75mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and 25 mM N-hydroxysuccinimide aqueous solution for 30 minutes to form the TSP monolayer and then washed with milli-Q water. **(c)** The self-assembled TSP monolayer was immersed in 150mM Nα',Nα''-bis(carboxymethyl)-L-lysine in a 0.5 M K_2CO_3 buffer at pH 9.8 for 3 hours which formed a carboxamide linkage.

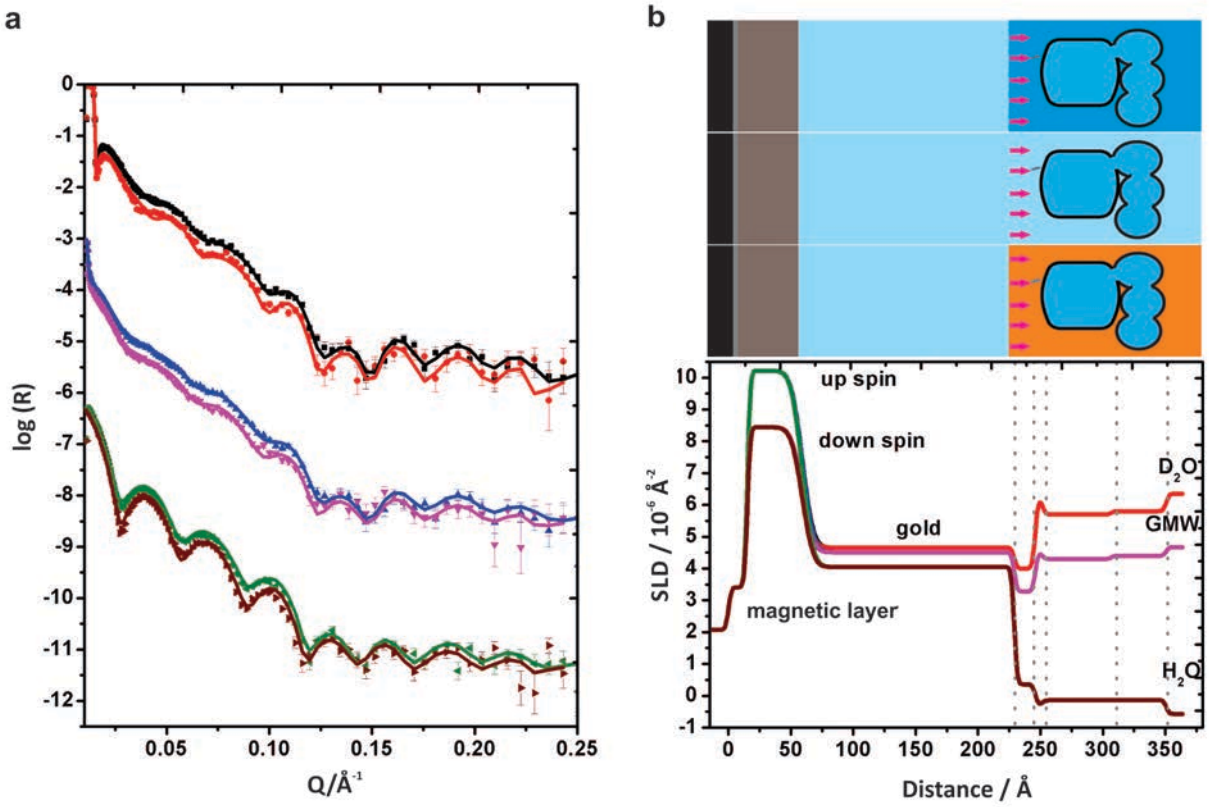


c

Layer	t / Å	nSLD / $\times 10^{-6}$ Å^{-2} ±0.05		Φ / %	ζ / Å
Silicon	---	2.07		---	2
Silicon oxide	15±2	3.41		---	2
Permalloy	44±2	10.22/8.44		---	2
Gold	170±3	4.66	4.05	91±5	7
NTA	16.8±1	4.00	0.36	48±5	2
Bulk solvent	---	6.35	-0.57	---	2

Supplementary Figure 3| Characterization of the Ni-NTA array on the gold surface. The quality control experiment characterizes the functional NTA group on the gold surface (Supplementary Fig. 2) used in all MCNR analyses. The magnetic layer displays two different scattering length densities (SLDs) for spin-up and spin-down neutrons, thereby providing two sets of reflection data in each experiment.

(a) MCNR profiles of NTA in D_2O (black spin-up and red spin-down) and H_2O (blue spin-up and magenta spin-down) based buffer. The symbols with error bars are the collected data and the solid lines are the fits. The H_2O data is offset for clarity. **(b)** The SLD profiles corresponding to the data in Supplementary Fig. 3a and a scaled cartoon of the surface layers is shown. **(c)** The results of the fitted and calculated parameters for the NTA layer using a four-layer model. t: thickness, Φ: volume fraction, ζ: roughness.



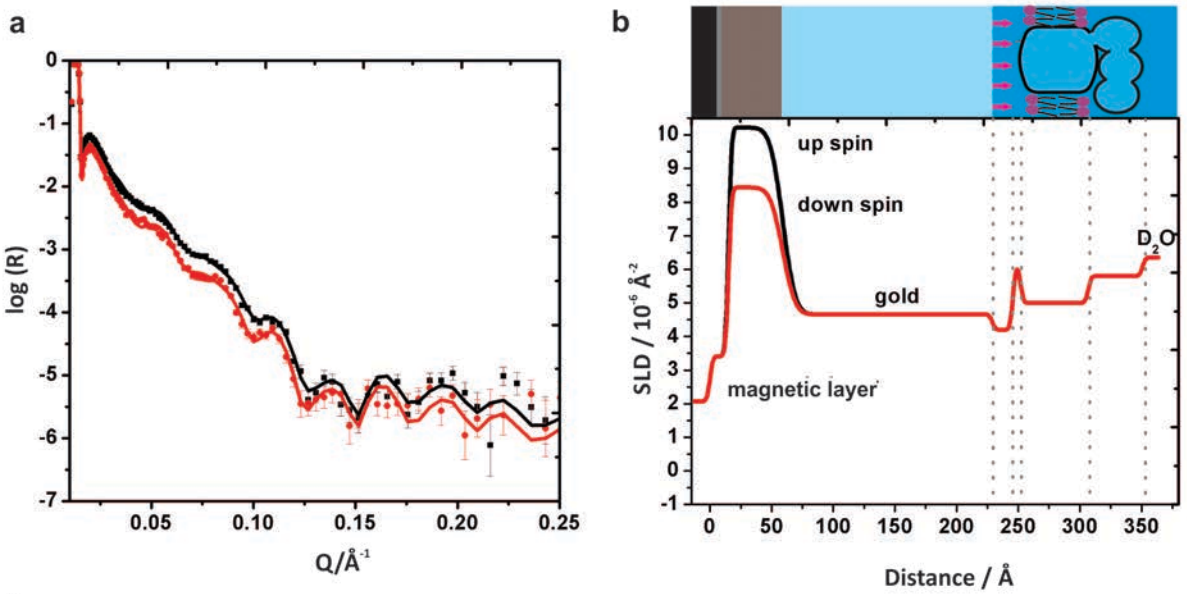
c

Layer	t / Å	nSLD / $\times 10^{-6}$ Å^{-2} ±0.05			Φ / %	ζ / Å
Silicon	---	2.07			--	2
Silicon oxide	15±2	3.41			--	2
Permalloy	44±2	10.22 /8.44			--	2
Gold	170±3	4.66	4.51	4.05	91±5	7
NTA	16.8±1	4.00	3.27	0.36	48±3	2
His 6	6±2	6.20	4.50	-0.28	7±2	2
TamA β-barrel	55±2	5.70	4.30	-0.18	34±2	2
TamA POTRA	44±2	5.80	4.40	-0.21	16±2	2
Bulk solvent	---	6.35	4.67	-0.57	--	--

Supplementary Figure 4| Characterization of TamA molecules on the Ni-NTA-modified surface. Experimental approach for contrast variations in MCNR analysis makes use of the two neutron spin states and isotopic contrast [by rinsing the protein layer using three different buffer compositions: D_2O (SLD=6.35 × 10^{-6}), H_2O (SLD=-0.57 × 10^{-6}) and gold matched water (GMW, SLD=4.67 × 10^{-6})]. Gold matched water (GMW) refers to a mixture of D_2O and H_2O to match to the SLD of the underlying gold, allowing for selective measurement of the reflections from the gold layer.

(a) MCNR profiles recorded after the addition of 0.1 mg/ml TamA in the detergent to Ni-NTA layer, rinsing away the detergent using D_2O (blue spin up; cyan spin down), GMW (blue spin up; cyan spin down) and H_2O (green spin up; brown spin down) based buffers. The symbols with error bars are the collected data and the solid lines are the fits. Data for TamA in D_2O, H_2O and GMW are offset for clarity. **(b)** The corresponding SLD profile and a scaled cartoon of TamA in D2O, GMW and H2O based buffer are shown. The orientation of TamA imposed by the Ni-NTA layer is indicated. **(c)** Results

of the fitted and calculated parameters from three solvent contrasts for the TamA on the NTA layer using a seven-layer model based on the result in Supplementary Fig. 3c where the first four layers were fixed. The volume fraction of TamA corresponds to the percentage of (v/v) of solvent in the layers determined using solvent contrasts. t: thickness, Φ: volume fraction, ζ: roughness.

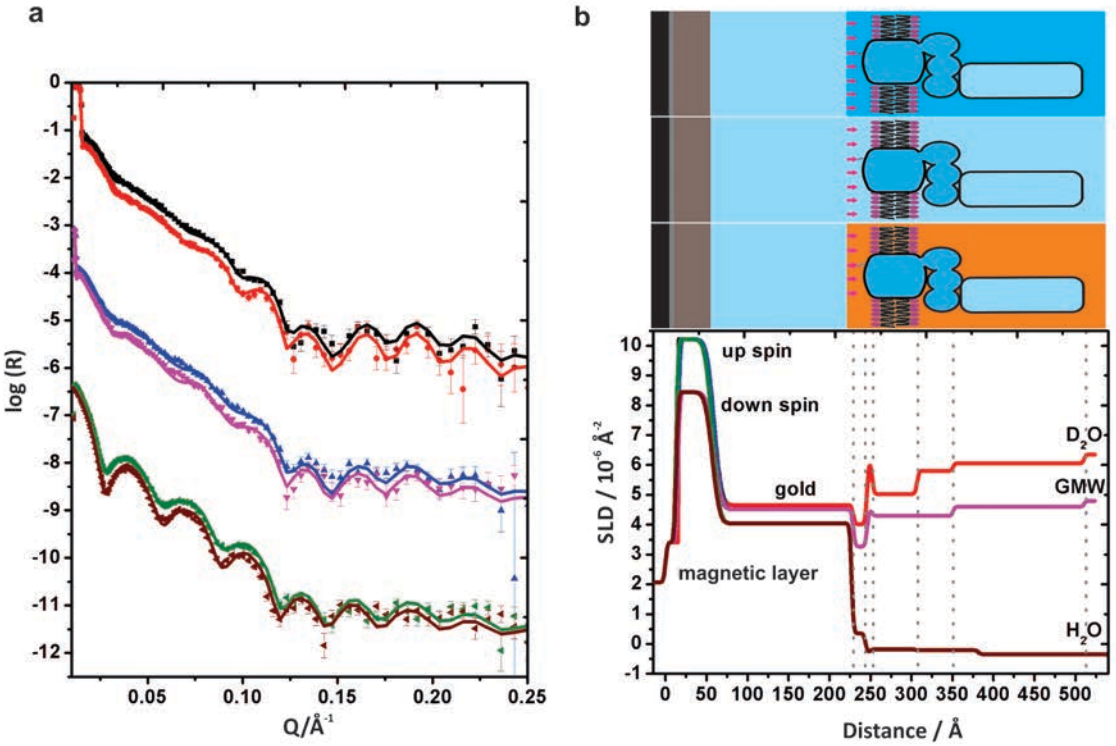


c

Layer	t / Å	nSLD / ×10^{-6} Å^{-2} ±0.05	Φ / %	ζ / Å
Silicon	---	2.07	---	2
Silicon oxide	15±2	3.41	---	2
Permalloy	44±2	10.22 / 8.44	---	2
Gold	170±3	4.66	91±5	7
NTA	16.8±1	4.00	48±3	2
His 6	6±2	6.20	7±2	2
TamA β-barrel	55±2	5.03	34[a]/27[b] ±4	2
TamA POTRA	44±2	5.80	16±2	2
Bulk solvent	---	6.35	---	---

Supplementary Figure 5| Characterization of the membrane layer. A supported membrane was formed by co-adsorption of micelles onto the TamA layer using a molar ratio of 1:6 of 1-Palmitoyl-d31-2-Oleoyl-sn-Glycero-3-Phosphocholine (POPC): β-D-dodecylmaltoside (DDM). This data verifies the quality of the membrane thus formed, and quantifies the protein:lipid composition.

(a) MCNR profiles recorded after the addition of 0.14 mg/ml of POPC:DDM micelles into solution with three time dilution to form a TamA-membrane in D_2O buffer (black spin up and red spin down). The symbols with error bars are the collected data and the solid lines are the fits. **(b)** The corresponding SLD profiles and a scaled cartoon of the TamA-membrane layer are shown. **(c)** The results of the fitted and calculated parameters for the TamA layer in the membrane on the NTA layer using a seven-layer model based on Supplementary Fig. 4c: the TamA volume fraction was fixed, and the decrease of the SLD was counted as membrane filling the gap between TamA molecules. [a] surface coverage of TamA and [b] surface coverage of phospholipid. t: thickness, Φ: volume fraction, ζ: roughness.

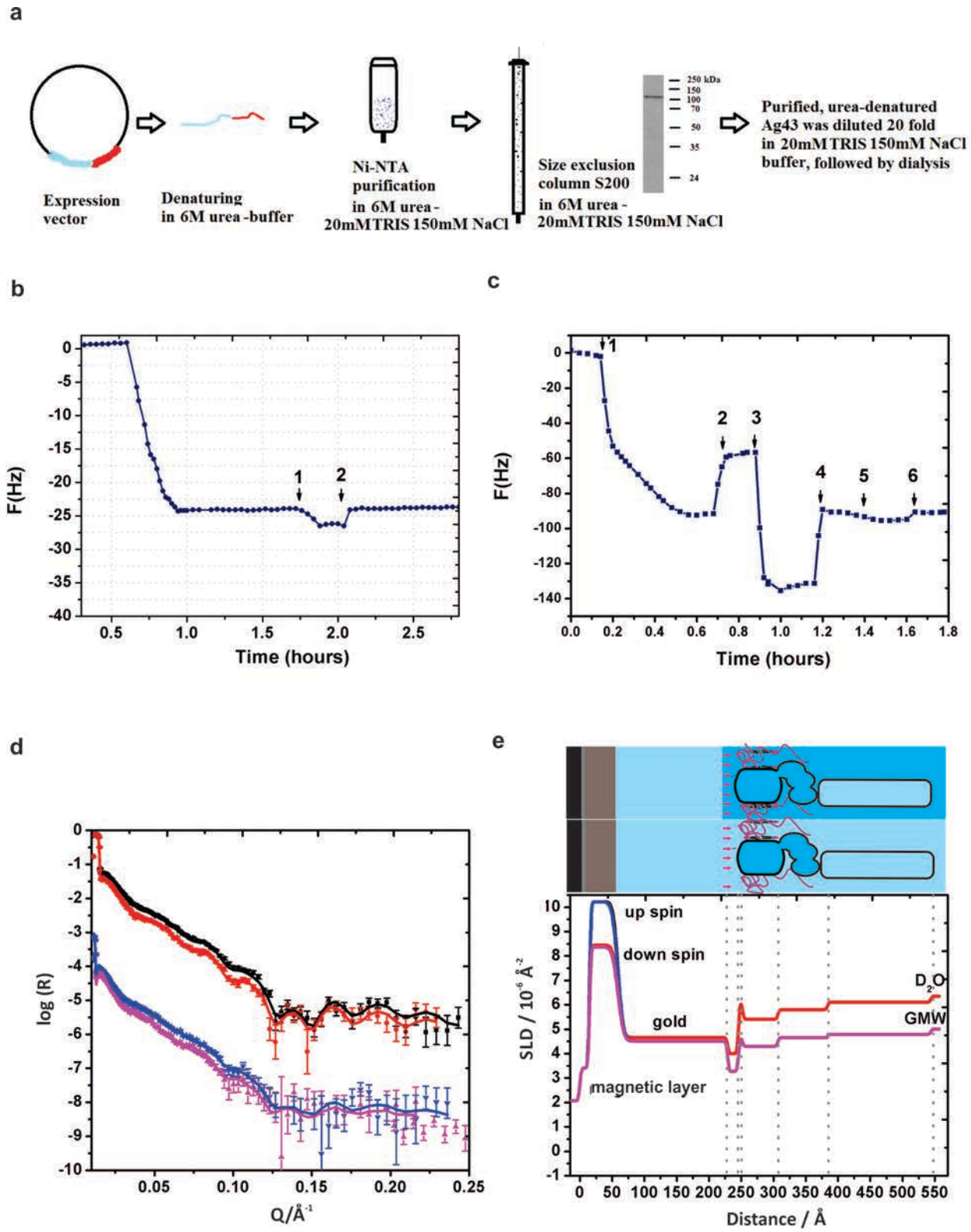


c

Layer	t / Å	nSLD / $\times 10^{-6}$ Å^{-2} ±0.05			Φ / %	ζ / Å
Silicon	---	2.07			---	2
Silicon oxide	15±2	3.41			---	2
Permalloy	44±2	10.22 / 8.44			---	2
Gold	170±5	4.66	4.51	4.05	91±5	7
NTA	16.8±1	4.00	3.27	0.36	48±3	2
His6	6±1	6.20	4.50	-0.28	7±2	2
TamA β-barrel	55±2	5.03	4.30	-0.18	34[a] /27[b] ±4	2
TamA POTRA	44±2	5.80	4.40	-0.21	16±4	2
TamB	160±20	6.02	4.60	-0.34	10±1	2
Bulk solvent	---	6.35	4.80	-0.57	---	2

Supplementary Figure 6| Orientation of TamB relative to the membrane layer. Given no constraints in the aqueous layer, TamB orientation would be dictated by the nature of its interaction with the TamA-membrane surface. This data describes the elongate TamB molecule as sitting perpendicular to the membrane surface. Gold matched water (GMW) refers to a mixture of D_2O and H_2O to match to the SLD of the underlying gold, allowing for selective measurement of the reflections from the gold layer.

(a) MCNR profiles recorded after TamB was added into the layer of TamA on the Ni-NTA gold surface in D_2O (black spin up; red spin down), GMW (blue spin up; cyan spin down) and H_2O (green spin up; brown spin down) based buffers. The symbols with error bars are the collected data and the solid lines are the fits. The TAM in D_2O and H_2O data are offset for clarity. **(b)** The corresponding SLD profile and a scaled cartoon of TAM in D_2O (blue), GMW (gold) and H_2O (grey) are shown. **(c)** The results of the fitted and calculated parameters for the TAM layer on the NTA layer using an eight-layer model based on Supplementary Fig. 5c, where parameters in TamA-membrane were fixed and TamB was added on top of TamA. [a] surface coverage of TamA and [b] surface coverage of phospholipid. t: thickness, Φ: volume fraction, ζ: roughness.


a
Expression vector
Denaturing in 6M urea -buffer
Ni-NTA purification in 6M urea - 20mMTRIS 150mM NaCl
Size exclusion column S200 in 6M urea - 20mMTRIS 150mM NaCl
250 kDa
150
100
70
50
35
24
Purified, urea-denatured Ag43 was diluted 20 fold in 20mMTRIS 150mM NaCl buffer, followed by dialysis
b
F(Hz)
Time (hours)
1
2
c
F(Hz)
Time (hours)
1
2
3
4
5
6
d
log (R)
Q/Å^{-1}
e
up spin
down spin
gold
magnetic layer
D_2O
GMW
SLD / 10^{-6} Å^{-2}
Distance / Å

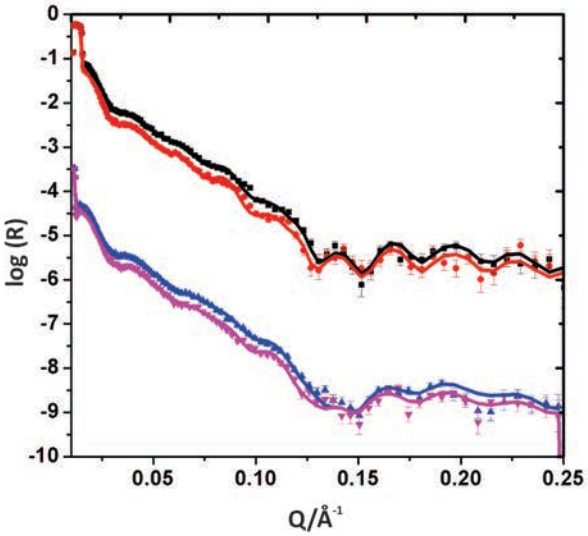
f

Layer	t / Å	nSLD / $\times 10^{-6}$ Å^{-2} ±0.05		Φ / %	ζ / Å
Silicon	--	2.07		---	2
Silicon oxide	15±2	3.41		---	2
Permalloy	44±2	10.22 /8.44		---	2
Gold	170±3	4.66	4.52	91±2	7
NTA	16.8±1	4.00	3.57	48±3	2
His6	6±2	6.20	4.65	7±2	2
TamA β-barrel	55±2	5.40	4.37	34^{a}±4/3^{b}±1/11^{c} ±2	2
TamA POTRA	77±2	5.80	4.49	16±4	2
TamB+Ag43	160±20	6.05	4.91	7.5±1	2
Bulk solvent	--	6.35	5	---	2

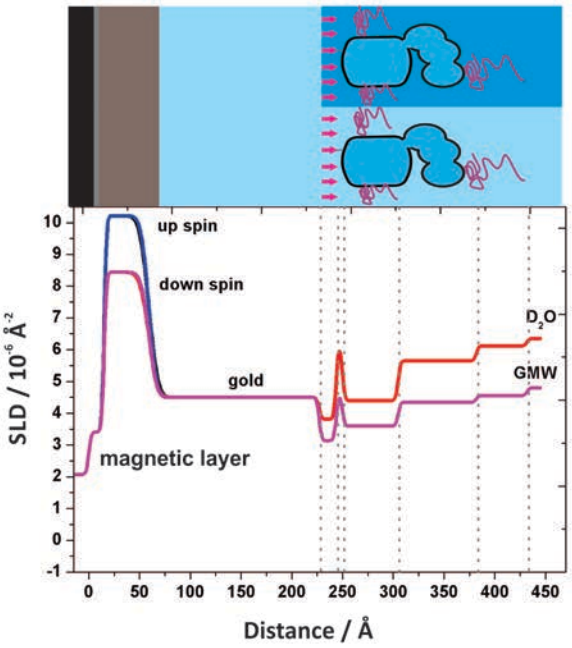
Supplementary Figure 7| Interaction of Ag43 with TAM. Ag43 is an autotransporter: an outer membrane protein that requires the TAM for its assembly *in vivo*. The QCM-D and MCNR data show that Ag43 insertion can be measured *in vitro* in a TAM-dependent reaction. Ag43 only can bind to the membrane with the existence of TAM.

(a) Preparation work flow for Ag43 substrate from *E. coli*. Experimental details are provided in the Methods section. Prior to use, the protein sample in 6M urea was diluted 20-fold into a buffer of 20 mM TRIS, 150 mM NaCl. **(b)** QCM-D measurements show the subsequent frequency responses to (1) adding Ag43 to bilayer and (2) rinsing with buffer to remove the non-specific binding. **(c)** Using membranes reconstituted with TamA (ΔP123), QCM-D measurements show the frequency responses (1) the mass uptake on the sensor surface caused by detergent-solubilized TamA(ΔP123), (2) washing the nickel-NTA:TamA(ΔP123):detergent surface, (3) forming a supported membrane, (4) TamB to TamA(ΔP123), (5) adding Ag43 and (6) rinsing with buffer to remove the non-specific binding. **(d)** MCNR profiles of TAM-Ag43 complex in D_2O (black spin up and red spin down) and GMW (blue spin up and cyan spin down) based buffer. The symbols with error bars are the collected data and the solid lines are the fits. The GMW data are offset for clarity. **(e)** The corresponding SLD profile and a scaled cartoon of TAM-Ag43 mixtures are shown. **(f)** The results of the fitted and calculated parameters for the TAM-Ag43 layer on NTA layer using a seven-layer adsorption model based on the result in Supplementary Fig. 6c. [a] surface coverage of TamA, [b] surface coverage of phospholipid and [c] surface coverage of Ag43. t: thickness, Φ: volume fraction, ζ: roughness

a



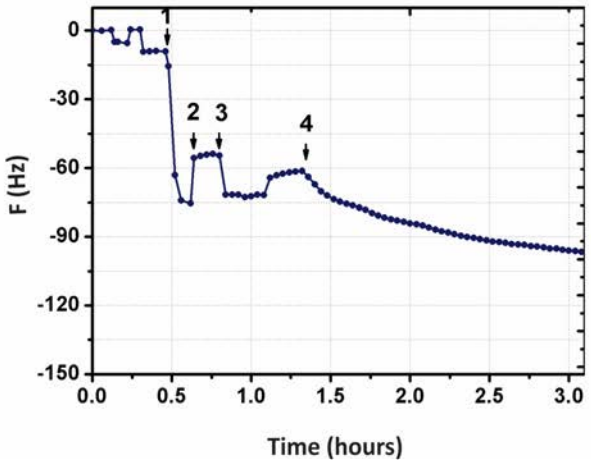
b



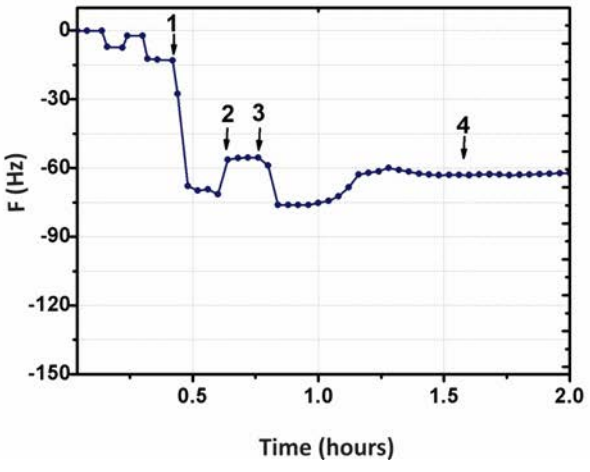
c

Layer	t / Å	nSLD / $\times 10^{-6}$ Å^{-2} ±0.05		Φ / %	ζ / Å
Silicon	---	2.07		---	2
Silicon oxide	15±2	3.41		---	2
Permalloy	44±2	10.22/8.44		---	2
Gold	168±3	4.50	4.50	100±3	7
NTA	16.8±1	3.80	3.33	52±3	2
His6	6±2	6.20	4.65	7±2	2
TamA β-barrel	55±2	4.40	3.60	39[a]/ 0[b]/45[c]±5	2
TamA POTRA	77±5	5.65	4.35	21±4	2
Ag43	50±10	6.11	4.56	8±2	2
Bulk solvent	---	6.35	4.8	---	---

d

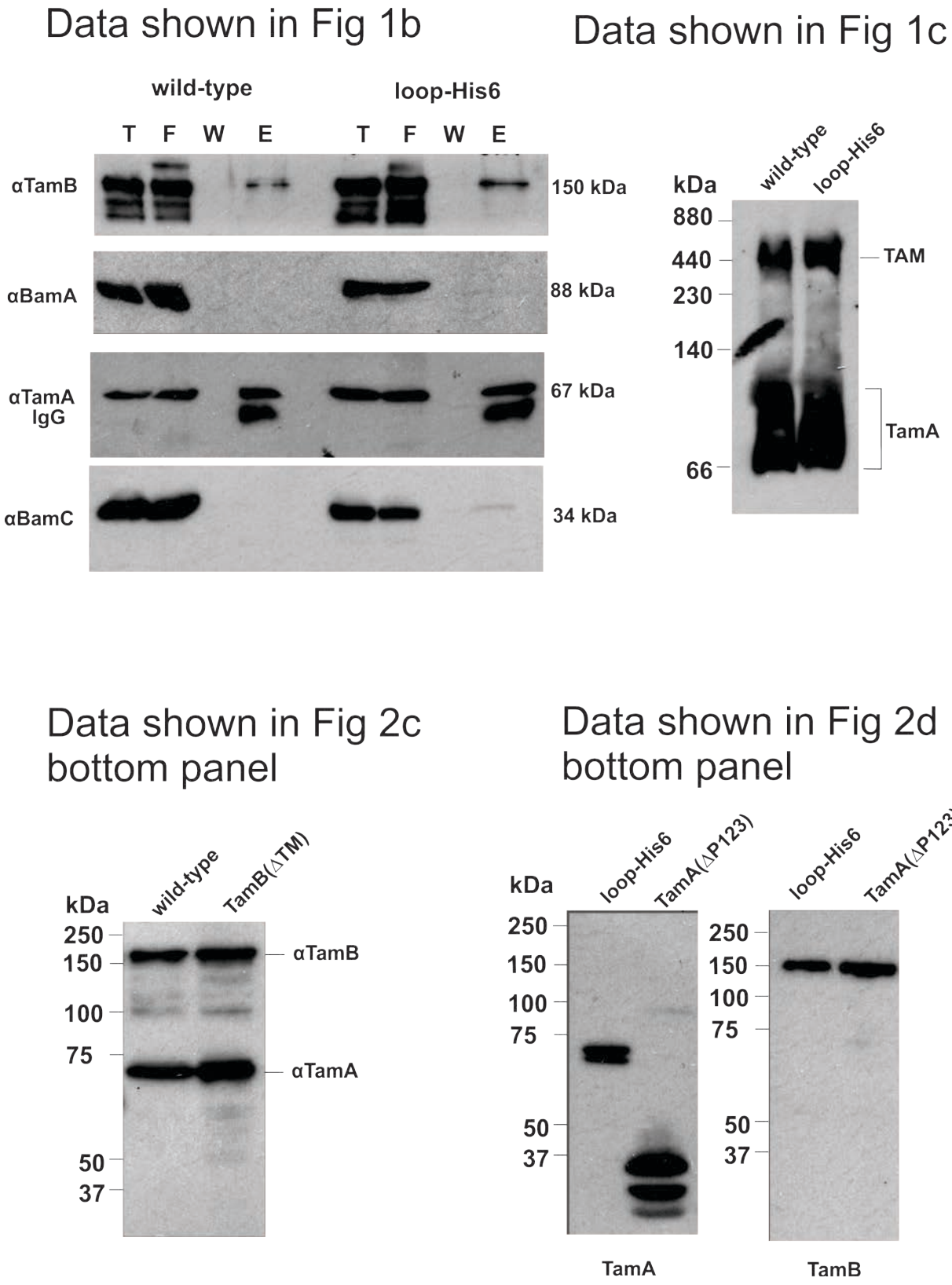


e



Supplementary Figure 8| Characterization of TamA function in substrate binding by the Ag43. The QCM-D and MCNR data show that Ag43 insertion can be measured *in vitro* in a TamA-dependent reaction: a 50 kDa fragment of Ag43 containing the β-barrel domain binds to the membrane, while a 60 kDa fragment of Ag43 containing the passenger domain does not bind to the membrane.

(a) MCNR profiles of TamA-Ag43 complex in D_2O (black spin up and red spin down), and GMW (blue spin up and cyan spin down) based buffer. The symbols with error bars are the collected data and the solid lines are the fits. The GMW data are offset for clarity. **(b)** The corresponding SLD profile and a scaled cartoon of TamA-Ag43 mixtures are shown. **(c)** The results of the fitted and calculated parameters for the TamA-Ag43 layer on NTA layer using a seven-layer model. **(d) & (e)** QCM-D data in which detergent solubilised TamA was added to the gold surface (1) the attachment of the TamA to the gold-surface, and QCM-D measurements show the subsequent frequency responses to (2) washing of detergent and (3) reconstitution of the membrane layer after addition of POPC, and (4) the addition of the 50 kDa barrel-domain of Ag43 **(d)** or the addition of the 60 kDa passenger-domain of Ag43 **(e)** [a] surface coverage of TamA, [b] surface coverage of phospholipid and [c] surface coverage of Ag43. t: thickness, Φ: volume fraction, ζ: roughness.



Supplementary Figure 9| Uncropped gels and immunoblots