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Supplemental information

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Supporting Information

Surface-Tethered Planar Membranes Containing the β-Barrel Assembly Machinery: A platform for Investigating Bacterial Outer Membrane Protein Folding

Stephen C. L. Hall[a], Luke A. Clifton[a] Pooja Sridhar[b], David J. Hardy[b], Jack Wright[d], James Whitehouse[d], Peter Wotherspoon[b], Nadisha Gamage[e], Claire S. Laxton[f], Caitlin Hatton[g], Gareth W. Hughes[b], Mark Jeeves[c], Timothy J. Knowles[b*]

[a]ISIS Pulsed Neutron and Muon Source, Science and Technology Facilities Council, Rutherford Appleton Laboratory, Didcot, Oxfordshire, OX11 OQX. U.K.

[b]School of Biosciences, University of Birmingham, Birmingham, B15 2TT, U.K.

[c]Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, B15 2TT, U.K.

[d]Astbury Centre for Structural Molecular Biology, University of Leeds, LS2 9JT, U.K.

[e]Membrane Protein Laboratory, Diamond Light Source, Harwell Science & Innovation Campus, OX11 0DE, U.K.

[f] School of Life Sciences, University of Nottingham, NG7 2UH, U.K.

[g]School of Life Sciences, University of Warwick, CV4 7AL.

* To whom correspondence should be addressed:

Timothy J. Knowles

School of Biosciences, University of Birmingham, Birmingham, B15 2TT, U.K.

t.j.knowles@bham.ac.uk

Contents

Supplementary Experimental Section

Expression and Purification of hydrogenated BamA$^{(L7H9)}$B.

hBamA$^{(L7H9)}$B was expressed in *E. coli* strain C41(DE3) by growing the strain carrying pSK389H to OD_{600} = 0.4 at 37°C. The temperature was lowered to 18°C, once OD_{600}= 0.6 the cells were induced by addition of IPTG to a concentration of 1 mM. The cells were grown at this temperature for 16 hours and harvested by centrifugation at 6000 x g for 15 minutes at 4 °C. The pellets were re-suspended in 20 mM Tris pH 8, 100 mM NaCl and lysed by 3 passages through a continuous flow cell disruptor (Emulsiflex C3, Avestin). Debris and unlysed cells were removed by centrifugation at 10,000 x g for 10 minutes at 4 °C. The supernatant was then ultracentrifuged at 100,000 x g for 30 minutes at 4 °C to pellet membranes. These were either used immediately or frozen at -80 °C until required. Membranes were re-suspended by solubilisation in 50 mM Tris pH 8, 500 mM NaCl, 2% (w/v) DDM for 2 hours followed by ultra-centrifugation at 100,000 x g for 30 minutes at 4 °C to remove insoluble material. Ni-NTA affinity purification was performed by the addition of Ni-NTA agarose beads to the solution followed by gentle mixing at 4 °C overnight. The beads were loaded on to a disposable column and washed using 5 column volumes of 50 mM Tris pH 8, 500 mM NaCl, 10mM Imidazole, 0.1% (w/v) DDM then eluted in 0.5 mL fractions using 50 mM Tris pH 8, 500 mM NaCl, 250 mM Imidazole, 0.1% (w/v) DDM. The eluted fractions were tested for the presence of protein by SDS-PAGE. Those fractions containing protein were concentrated then further purified using size exclusion chromatography using a Superdex S200 16/60 PG column (GE Healthcare) using 50mM Tris pH 8, 150mM NaCl, 0.03% DDM buffer.

Expression of hydrogenated and deuterated forms of BamCD and assembly and purification of BamA$^{(L7H9)}$BCD.

BamCD was initially expressed in *E. coli* strain BL21(DE3) by growing the strain carrying the pSK46 plasmid in LB (to produce hBamCD) or M9 media in 100% D2O and supplemented with D-glucose (1,2,3,4,5,6,6-D7, 98%) (to produce dBamCD) at 37°C to an OD_{600}=0.4, lowering the temperature to 18°C and inducing with the addition of 1mM IPTG for 16 hours. Cells were harvested by centrifugation at 6000 x g for 15 minutes at 4 °C. The pellets were re-suspended in 20 mM Tris pH 8, 100 mM NaCl and lysed by 3 passages through a continuous flow cell disruptor (Emulsiflex C3, Avestin). Cell debris and unlysed cells were removed by centrifugation (75,000 x g, 30 minutes, 4°C) and the supernatant was filtered (0.2 µm PVDF syringe filter). Purified hBamA$^{(L7H9)}$B in 50 mM Tris pH 8, 150 mM NaCl, 0.03% DDM buffer was loaded on to a pre-equilibrated 5ml His-Trap column (GE Healthcare). Filtered supernatant containing either hBamCD or dBamCD was then loaded on to the column enabling in situ formation of either the hBamA$^{(L7H9)}$BCD or hBamA$^{(L7H9)}$BdCD complex. The column was washed using 5 column volumes of 50 mM Tris pH 8, 500 mM NaCl, 10 mM imidazole, 0.03% (w/v) DDM prior to elution in 0.5 mL fractions using 50 mM Tris pH 8, 500 mM NaCl, 250 mM Imidazole, 0.03% (w/v) DDM. Fractions containing BamA$^{(L7H9)}$BCD, as evidenced by SDS-PAGE, were concentrated then further purified using size exclusion chromatography using a Superdex S200 16/60 PG column (GE Healthcare) using 50 mM Tris pH 8, 150 mM NaCl, 0.03% DDM buffer.

Expression of hydrogenated and deuterated forms of BamE and assembly and purification of BamA$^{(L7H9)}$BCDE.

BamE was initially expressed in *E. coli* strain BL21(DE3) by growing the strain carrying the pET16b-BamE plasmid in LB (to produce hBamE) or M9 media in 100% D2O and supplemented with D-glucose (1,2,3,4,5,6,6-D7 98%) (to produce dBamE) at 37°C to an OD_{600}=0.4 then lowering the temperature to 18°C and inducing with the addition of 1 mM IPTG for 16 hours. Cells were harvested by centrifugation at 6000 x g for 15 minutes at 4 °C. The pellet was re-suspended in 20 mM Tris pH 8, 100 mM NaCl and lysed by 3 passages through a continuous flow cell disruptor (Emulsiflex C3, Avestin). Cell debris and unlysed cells were removed by centrifugation (75,000 x g, 30 minutes, 4°C). The supernatant was filtered using a 0.2 μm PVDF syringe filter and loaded on to a pre-equilibrated 5 mL His-Trap column (GE Healthcare). The column was washed with 5 column volumes of 50 mM Tris pH 8, 500 mM NaCl, 50 mM imidazole prior to elution in 0.5 mL fractions using 50 mM Tris pH 8, 500 mM NaCl, 250 mM Imidazole. Those fractions containing BamE, as evidenced by SDS-PAGE, were pooled and incubated with Factor Xa (GE Healthcare, 1 unit for every 100 μg of protein) and dialyzed into 50 mM Tris pH 8, 150 mM NaCl to remove the N-terminal hexa-histidine tag. The sample was further purified to remove the cleaved tag by repeat passage through a 5ml His-trap (GE Healthcare) column using the procedure listed above. Those fractions containing cleaved protein were pooled, concentrated, and further purified by size exclusion chromatography using a Superdex S75 26/60 PG column (GE Healthcare) using 50 mM Tris pH 8, 150 mM NaCl.

To produce hBamA$^{(L7H9)}$BCDE, hBamA$^{(L7H9)}$BCD was incubated with approximately 2-fold excess hBamE in 50 mM Tris pH 8, 150 mM NaCl, 0.03% DDM for 30 minutes prior to purification by size exclusion chromatography using a Superdex S200 16/60 PG column (GE Healthcare). To produce hBamA$^{(L7H9)}$BdCDE, hBamA$^{(L7H9)}$BdCD was incubated with dBamE in excess in 50mM Tris pH 8, 150mM NaCl, 0.03% DDM for 30 minutes prior to purification by size exclusion chromatography using a Superdex S200 16/60 PG column (GE Healthcare).

Expression and purification of SurA.

SurA was expressed and purified using the method of Iadanza et al. 2016 [1]. Plasmid pET-SurA was transformed in to *E. coli* (DE3) cells and grown in 4L of LB broth containing 50 μg/ml Kanamycin at 37°C with shaking (200rpm). At OD_{600}=0.4 the temperature was reduced to 18°C and left until OD_{600}=0.6. At this point the cells were induced with 1mM IPTG. After 16 hours the cells were harvested by centrifugation (6000 x g, 15 minutes, 4°C). Cells were re-suspended in 50 mM Tris pH 8, 500 mM NaCl, 50 mM imidazole, containing EDTA-free protease inhibitors (Roche) and lysed using a cell disruptor (Emulsiflex C3, Avestin). Cell debris and unlysed cells were removed by centrifugation (75,000 x g, 30 minutes, 4°C), the supernatant was filtered (0.2 μm PVDF syringe filter) and loaded on to a 5 mL HisTrap column (GE Healthcare). Washing was performed by passage of 5cv 50 mM Tris pH 8, 500 mM NaCl, 50 mM imidazole. His-tagged SurA was denatured on column using 50 mM Tris pH 8, 500 mM NaCl, 6 M guanidine-HCl and the protein eluted with a gradient of 50 mM Tris pH 8, 500 mM NaCl, 6 M guanidine and 500 mM imidazole. Protein containing fractions were assessed by SDS-PAGE then dialyzed against 50 mM Tris pH 8. His-tag removal was performed by incubation with thrombin (40-80 units depending on yield)

overnight at 4℃. Removal of un-cleaved His-SurA was performed by repeat passage through a 5 mL His-Trap column. Refolded-SurA was concentrated to 200 µM using Amicon Ultra (10 kDa MWCO) concentrators, aliquoted, then snap frozen in liquid nitrogen and stored at -80℃.

Expression and purification of OmpT.

pET-OmpT was transformed into *E. coli* (DE3) cells (NEB) and grown in 2 L of lysogeny broth (LB) medium containing 50 µg/ml kanamycin at 37℃ with shaking (200rpm). At OD_{600}=0.6 expression was induced by the addition of 1mM IPTG. After 4 hours the cells were harvested by centrifugation (6000 x g, 15 minutes, 4℃). Cells were re-suspended in 50 mM Tris pH 8 and lysed by three passages through a cell disrupter (C3 emulsiflex, Avestin). The insoluble fraction was collected by centrifugation (75,000 x g, 30 minutes, 4℃) and then resuspended in 50 mM Tris pH 8, 2% (v/v) Triton X-100 and incubated for 1 hour at room temperature. The insoluble fraction was pelleted by centrifugation (75,000 x g, 10 minutes, 4℃) and the pelleted material was then washed twice with 50 mM Tris pH 8, with centrifugation (75,000 x g, 10 minutes, 4℃) after each washing. The washed inclusion body was then solubilized in 50 mM Tris pH 8, 6 M guanidine HCl for 1 hour then centrifuged (75,000 x g, 30 minutes, 4℃) to remove insoluble material. The purity was assessed by SDS-PAGE. The supernatant was then filtered (0.2 µm PVDF syringe filter) and concentrated to 120 µM and snap frozen in liquid nitrogen and stored at -80℃.

Expression and purification of pertactin.

Plasmid pET26b expressing Pertactin (Genescript) was transformed into *E. coli* (DE3) cells (NEB) and grown in 2 L of lysogeny broth (LB) medium containing 50 µg/ml kanamycin at 37oC with shaking (200rpm). At OD_{600}=0.6 expression was induced by the addition of 1mM IPTG. After 4 hours the cells were harvested by centrifugation (6000 x g, 15 minutes, 4℃). Cells were re-suspended in 50 mM Tris pH 8, 150 mM NaCl and lysed by three passages through a cell disrupter (C3 emulsiflex, Avestin). The insoluble fraction was collected by centrifugation (75,000 x g, 30 minutes, 4℃) and then resuspended in 50 mM Tris pH 8, 2% (v/v) Triton X-100 and incubated for 1 hour at room temperature. The insoluble fraction was pelleted by centrifugation (35,000 x g, 10 minutes, 4℃) and the pelleted material was then washed twice with 50 mM Tris pH 8, with centrifugation (35,000 x g, 10 minutes, 4℃) after each washing. The washed inclusion body was then solubilized in 50 mM Tris pH 8, 8 M Urea for 1 hour and then centrifuged (75,000 x g, 30 minutes, 4℃) to remove insoluble material. The purity was assessed by SDS-PAGE. The supernatant was then filtered (0.2 µm PVDF syringe filter) and concentrated to 20 µM and snap frozen in liquid nitrogen and stored at -80℃.

Polarized Neutron Reflectometry Data Analysis

In modelling the interfacial structure, all experimental datasets were constrained against data collected for the Si-Py-Au-DTSP-ANTA-Cu^{2+} interface in D_2O, prior to the addition of β-OG solubilized BamABCDE. This interfacial structure was modelled as 5 layers between bulk Si and solvent, representing, SiO_2, permalloy, gold and the DTSP-ANTA-Cu^{2+} SAM. The scattering length density (SLD) of these layers were fixed according to known values (Table S1), with the exception of permalloy, where sputter coating can lead to a variable elemental composition and magnetic properties, resulting in slightly different nuclear and magnetic SLDs. The thickness and roughness was fit for

each of these layers, in addition to the hydration of the SAM, accounting for incomplete coverage and labile hydrogen exchange in different isotopic contrasts. These parameters were constrained across all experimental data sets collected on each substrate. Upon the addition of β-OG solubilized hBamA$^{(L7H9)}$BCDE in D_2O, three additional layers were included, representing a water gap containing extracellular loops of BamA which bind to the Cu^{2+}-SAM, a 'transmembrane' region composed of BamA and β-OG, and a final layer composed of periplasmic domains of each component of the Bam complex. The thickness and roughness of each of these layers was fit. Proteins contain labile hydrogens which exchange with the bulk solvent, which when the isotopic composition of the bulk solvent is changed, causes the SLD of the protein to be modified. The single SLD was calculated for Bam in each solvent (Table S1) and used throughout this analysis. The volume fraction of Bam (χ_{Bam}) was fit independently for each of these protein-containing layers, accounting for the intrinsic hydration of proteins in addition to incomplete coverage as a result of both binding density and the protein structure. This allows the SLD of peripheral membrane protein layers to be calculated as

$$SLD_{Peripheral\ Protein\ Layer} = \chi_{Bam}SLD_{Bam} + (1 - \chi_{Bam})SLD_{Solvent}$$

In the case of the central 'transmembrane' layer, the presence of β-OG would be expected to modify the SLD of this layer. The SLD of the transmembrane region was calculated from the fitted volume fraction of β-OG ($\chi_{\beta-OG}$) and the fitted volume fraction of Bam (χ_{Bam}), taking into account hydration as discussed above, as

$$SLD_{\beta-OG:Bam\ Layer} = \chi_{Bam}SLD_{Bam} + \chi_{\beta-OG}SLD_{\beta-OG} + (1 - (\chi_{Bam} + \chi_{\beta-OG}))SLD_{Solvent}$$

In modelling the interfacial structure after reconstitution of hBamA$^{(L7H9)}$BCDE within a POPC bilayer, we made the assumption that the structure of the complex remained unchanged, as did the volume fractions of protein in each layer. Therefore, the thickness and roughness of the three protein-containing layers was fit, constrained across datasets collected for adsorbed hBamABCDE in β-OG micelles. Similarly, as the volume fractions of protein in each layer was constrained across datasets collected for hBamABCDE:β-OG, the SLD of the peripheral protein layers remains unchanged by the addition of POPC. In the case of the transmembrane region, the formation of a hydrogenated POPC bilayer around the adsorbed Bam complex would be expected to cause a substantial difference in the SLD of this layer. A single SLD was calculated for POPC (Table S1), and the overall SLD of the transmembrane layer was calculated based on the fitted volume fraction of POPC (χ_{POPC}) and the constrained, fitted volume fraction of Bam (χ_{Bam}) as

$$SLD_{POPC:Bam\ Layer} = \chi_{Bam}SLD_{Bam} + \chi_{POPC}SLD_{POPC} + (1 - (\chi_{Bam} + \chi_{POPC}))SLD_{Solvent}$$

In modelling the interfacial structure of hBamA$^{(L7H9)}$BdCDE, the same approach was taken as with hBamA$^{(L7H9)}$BCDE, except the distribution of deuterated components throughout the protein-containing region was also taken into account. We did initially not make any *a priori* assumptions as to the location of the deuterated components, independently fitting for a volume fraction of dBamCDE in each of the three layers, so the average SLD of the Bam complex within each layer can be calculated as

$$SLD_{Bam\ (Average)} = \chi_{hBamAB}SLD_{hBamAB} + (1 - \chi_{hBamAB})SLD_{dBamCDE}$$

This strategy, however, was unable to provide a statistically significant distribution of dBamCDE throughout the interfacial assembly. We therefore constrained the model to allow dBamCDE presence only in the peripheral protein layer adjacent to the bulk solvent, consistent with both the known structures of the complete BamABCDE complex, and the orientational constraints imparted on the complex by the DTSP-ANTA-Cu^{2+} SAM.

Component volume fraction plots were generated from the MCMC chains of the fitted parameters using a custom MATLAB script, available upon request from the authors. Briefly, absolute volume fraction of all components throughout the interface were calculated (as described above) as a function of distance for each individual MCMC iteration on a layer-by-layer basis, using the 'asymconvstep' function included in RasCAL:

```
function asymconvstep (x,xw,xcen,s1,s2,h)
  %{
    x = vector of x values
    xw = Width of step function
    xcen = Centre point of step function
    s1 = Roughness parameter of left side
    s2 = Roughness parameter of right side
    h = Height of step function.
  %}
  r = xcen + (xw/2);
  l = xcen - (xw/2);

  a = (x-l)./((2^0.5)*s1);
  b = (x-r)./((2^0.5)*s2);

  f = (h/2)*(erf(a)-erf(b));
end
```

This function produces an error function with a width equal to the layer thickness, convoluted with different error functions on each side, with error function widths corresponding to interfacial roughnesses obtained for each layer. The height of the step function corresponds to the volume fraction of each component. Identical components in different layers were then summed together to create continuous distributions spanning multiple layers. Finally, the mean and 95% confidence intervals for the volume fraction of each component was calculated from all MCMC iterations as a function of distance from the Si interface.

Supplementary Data

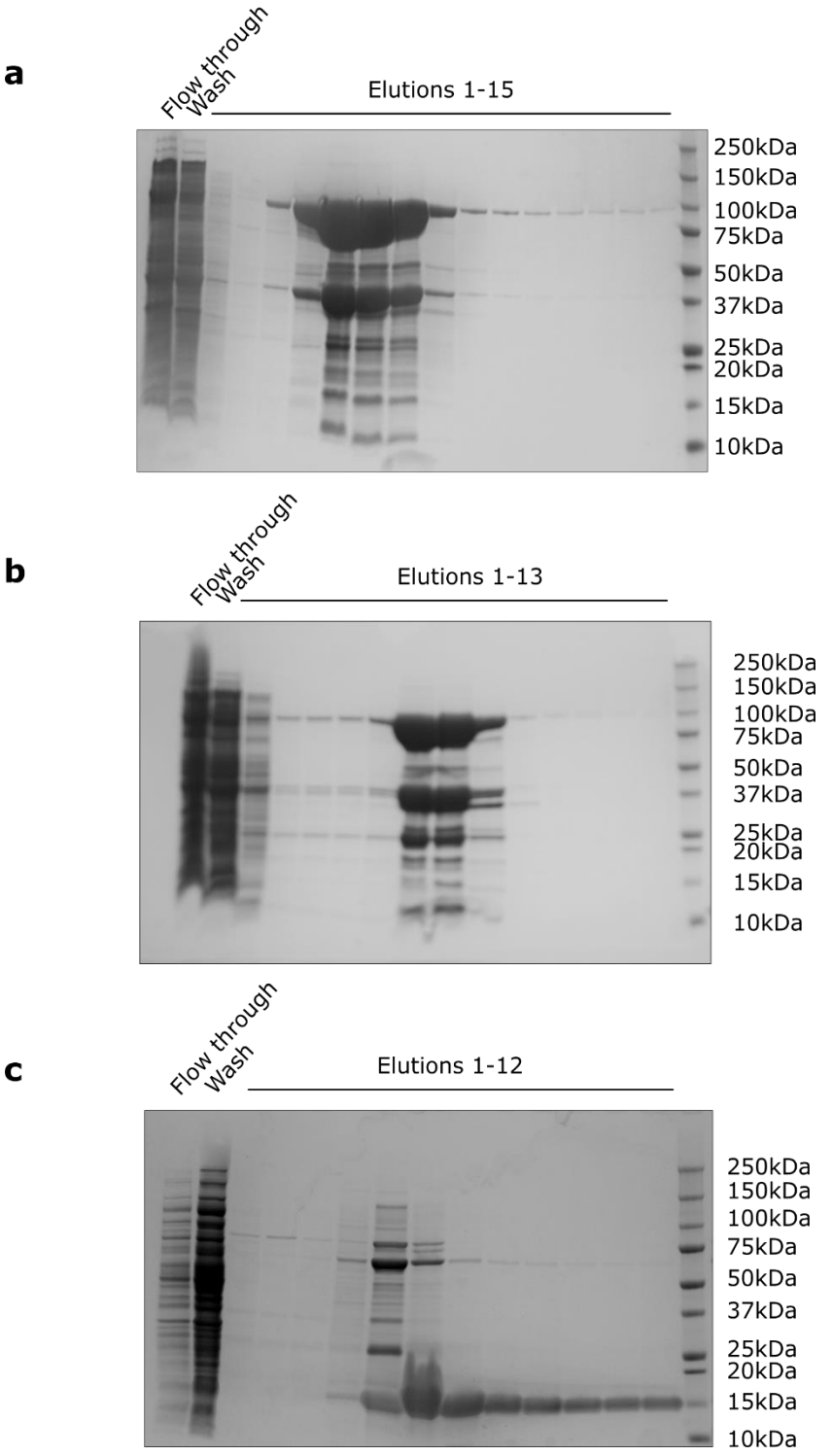

Figure S1 – hBamA$^{(L7H9)}$BCDE Ni-NTA purification. A) Ni-NTA purification of hBamA(L7H9)B over-expressed from *E. coli* strain C41(DE3). B) Ni-NTA purification of BamA(L7H9)B following incubation with detergent solubilized membranes derived from BL21(DE3) overexpressing hBamCD. C) Ni-NTA purification of hBamE over-expressed from *E. coli* BL21(DE3).

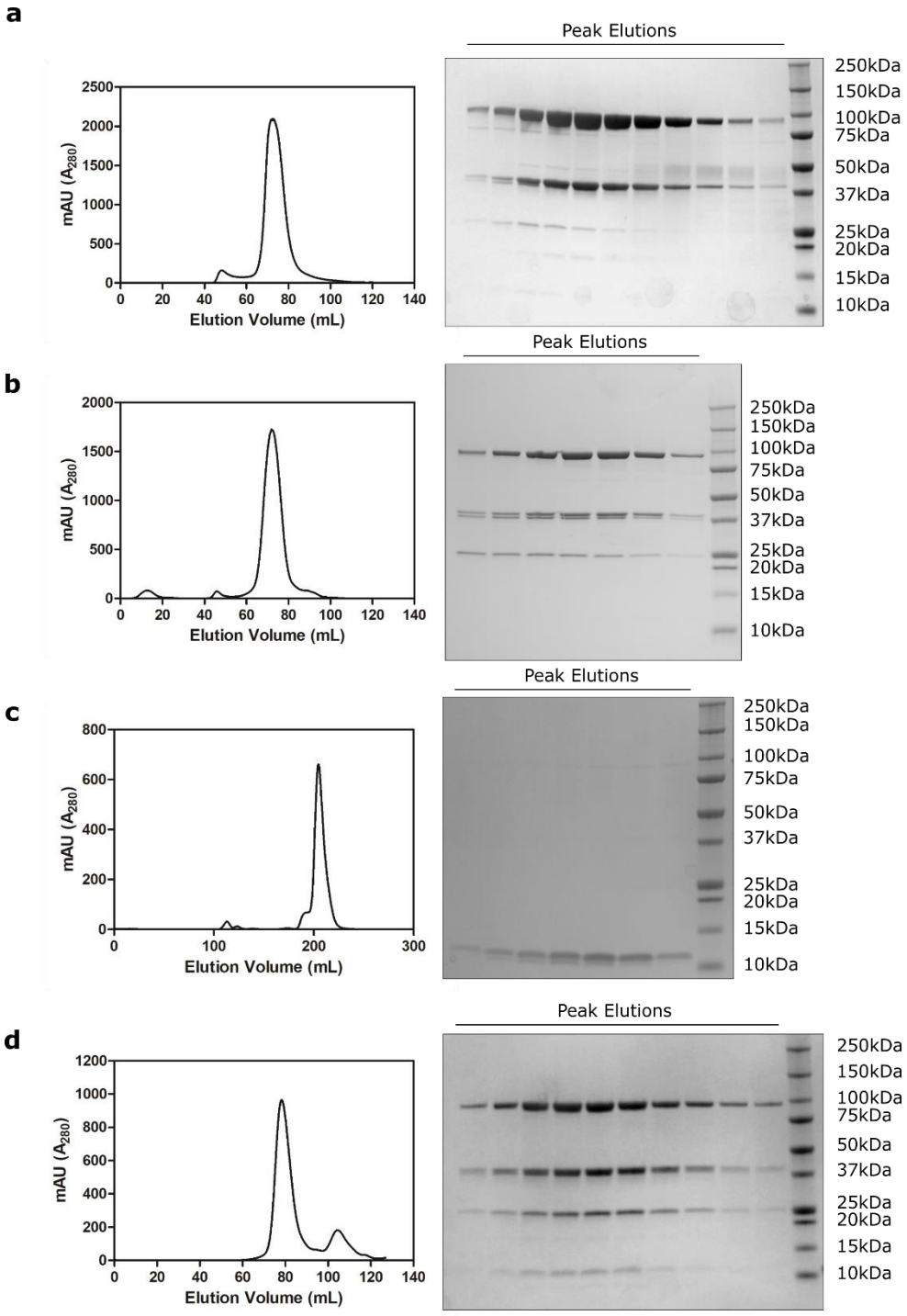

Figure S2 – hBamA$^{(L7H9)}$BCDE size exclusion chromatography purification. A) S200 16/60 Superdex (GE Lifesciences) size exclusion chromatogram of hBamA(L7H9)B (left) and subsequent SDS-PAGE of peak fractions (right). B) S200 16/60 Superdex (GE Lifesciences) size exclusion chromatogram of hBamA(L7H9)BCD (left) and subsequent SDS-PAGE of peak fractions (right). C) S75 26/60 Superdex (GE Lifesciences) size exclusion chromatogram of hBamE (left) and subsequent SDS-PAGE of peak fractions (right). D) S200 16/60 Superdex (GE Lifesciences) size exclusion chromatogram of hBamA(L7H9)BCDE (left) and subsequent SDS-PAGE of peak fractions (right).

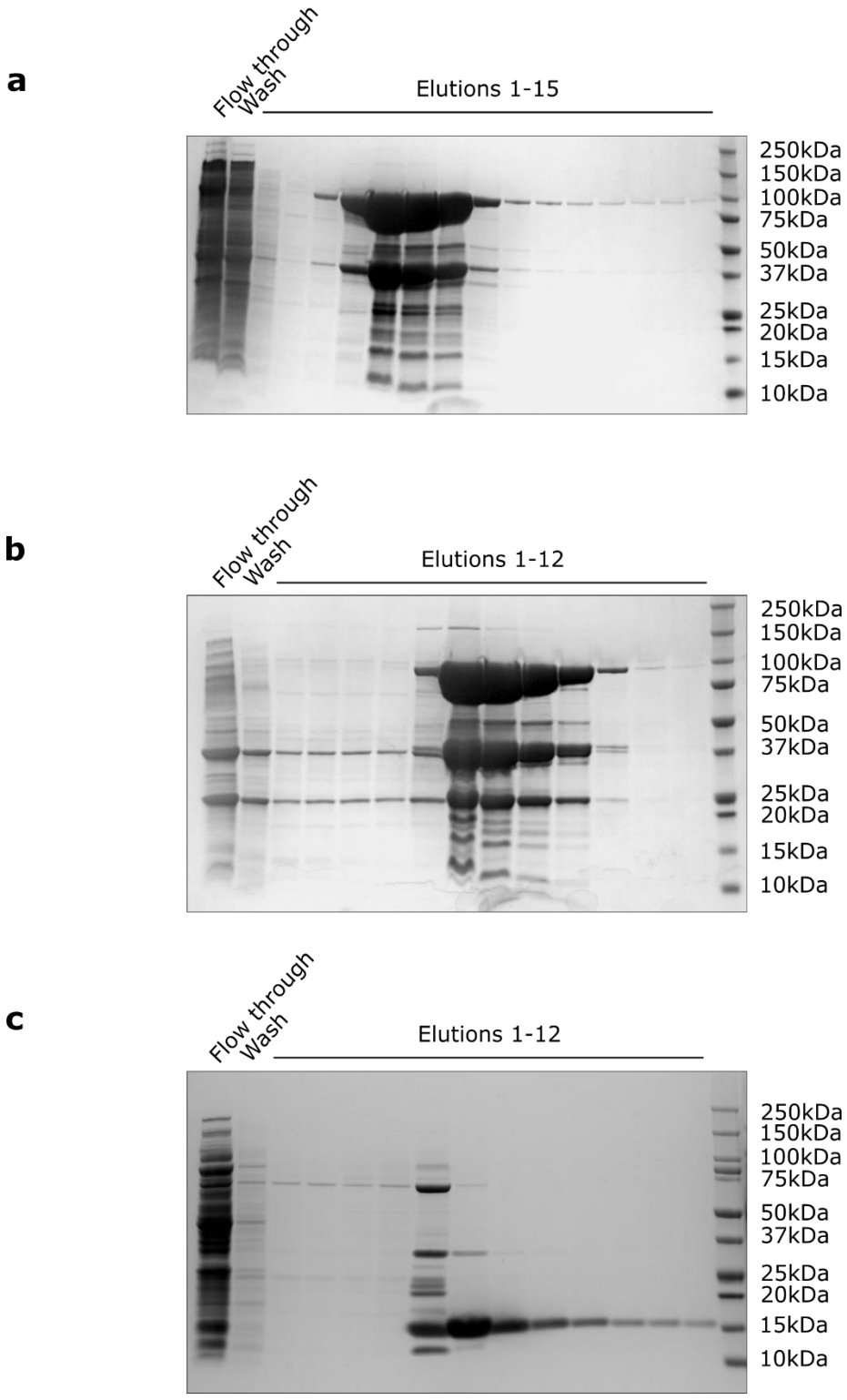

Figure S3 – hBamA$^{(L7H9)}$BdCDE Ni-NTA purification. A) Ni-NTA purification of hBamA(L7H9)B over-expressed from *E. coli* strain C41(DE3). B) Ni-NTA purification of BamA(L7H9)B following incubation with detergent solubilized membranes derived from BL21(DE3) overexpressing dBamCD in deuterated media. C) Ni-NTA purification of dBamE over-expressed from *E. coli* BL21(DE3) in deuterated media.

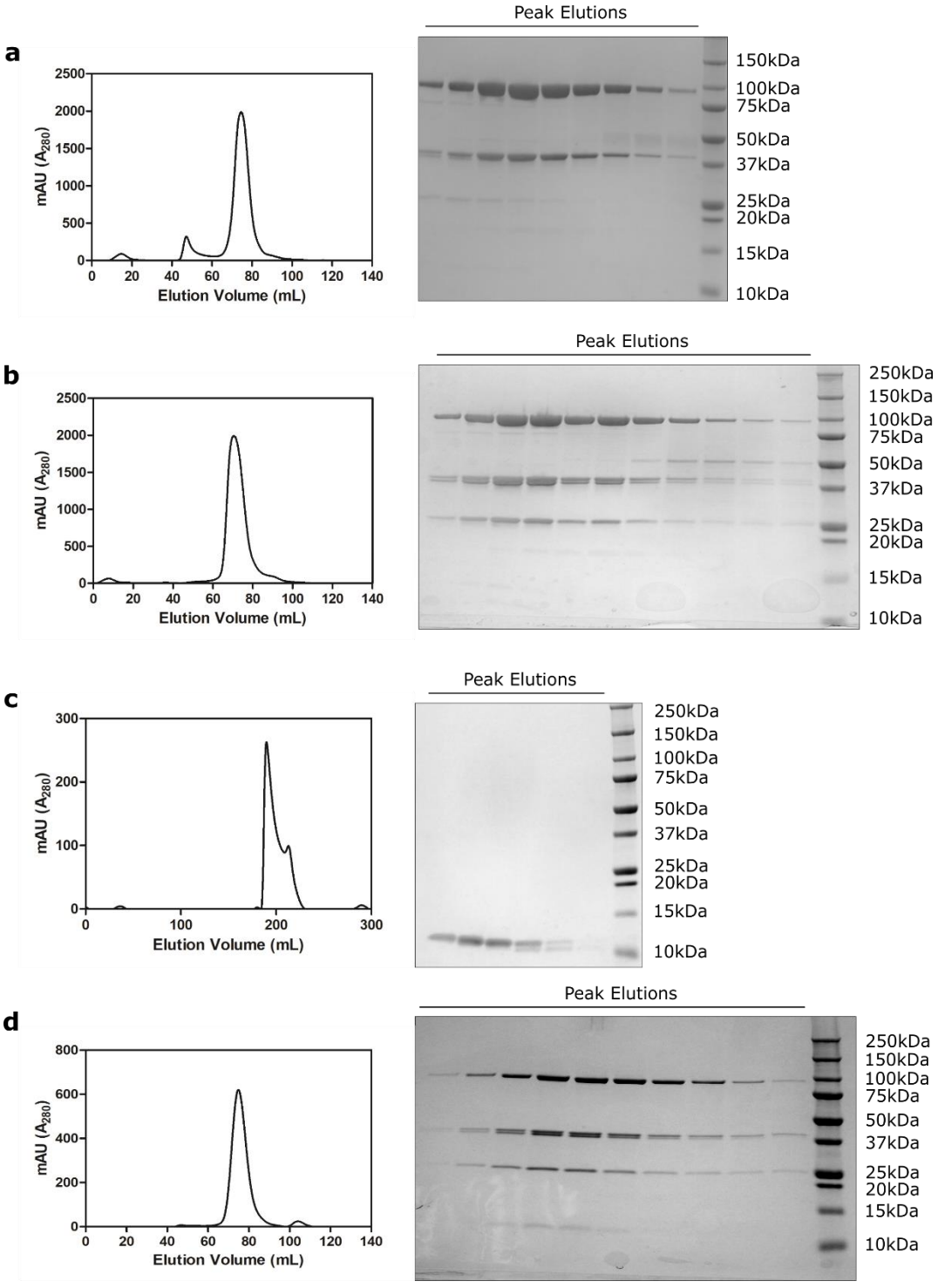

Figure S4 – hBamA$^{(L7H9)}$BdCDE size exclusion chromatography purification. A) S200 16/60 Superdex (GE Lifesciences) size exclusion chromatogram of hBamA(L7H9)B (left) and subsequent SDS-PAGE of peak fractions (right). B) S200 16/60 Superdex (GE Lifesciences) size exclusion chromatogram of hBamA(L7H9)BdCD (left) and subsequent SDS-PAGE of peak fractions (right). C) S75 26/60 Superdex (GE Lifesciences) size exclusion chromatogram of dBamE (left) and subsequent SDS-PAGE of peak fractions (right). D) S200 16/60 Superdex (GE Lifesciences) size exclusion chromatogram of hBamA(L7H9)BdCDE (left) and subsequent SDS-PAGE of peak fractions (right).

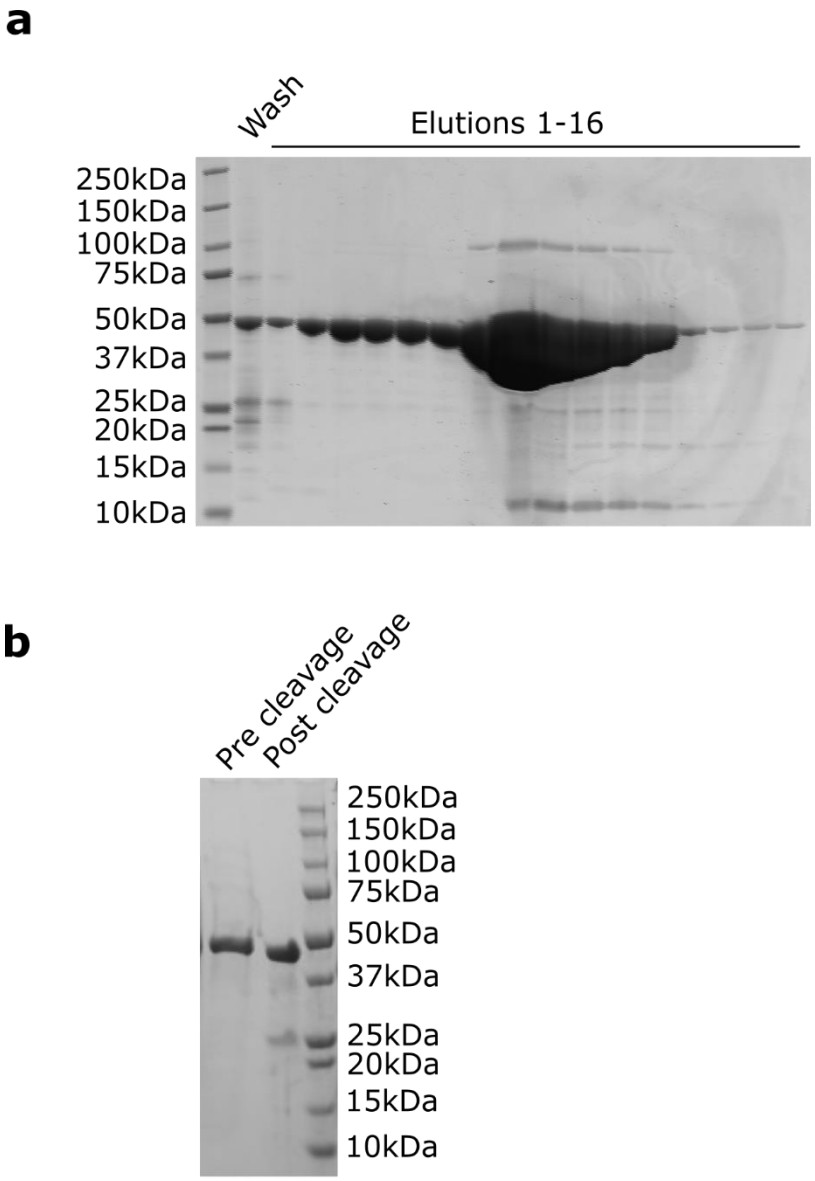

Figure S5 – Purification of SurA. A) SDS-PAGE of fractions following Ni-NTA purification of SurA under denaturing conditions in 6M guanidine hydrochloride. Fractions containing SurA were pooled, refolded by dialysis then subject to thrombin cleavage. B) SDS-PAGE following thrombin cleavage and cleanup using Ni-NTA purification showing pre and post cleavage purified samples.

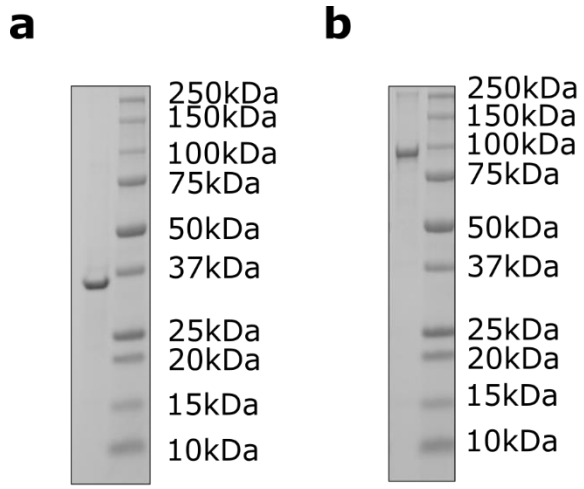

Figure S6 – OmpT & Pertactin inclusion body preparations. SDS-PAGE of A) OmpT and B) Pertactin inclusion body preparations following washing and resuspension in 6M guanidine HCl.

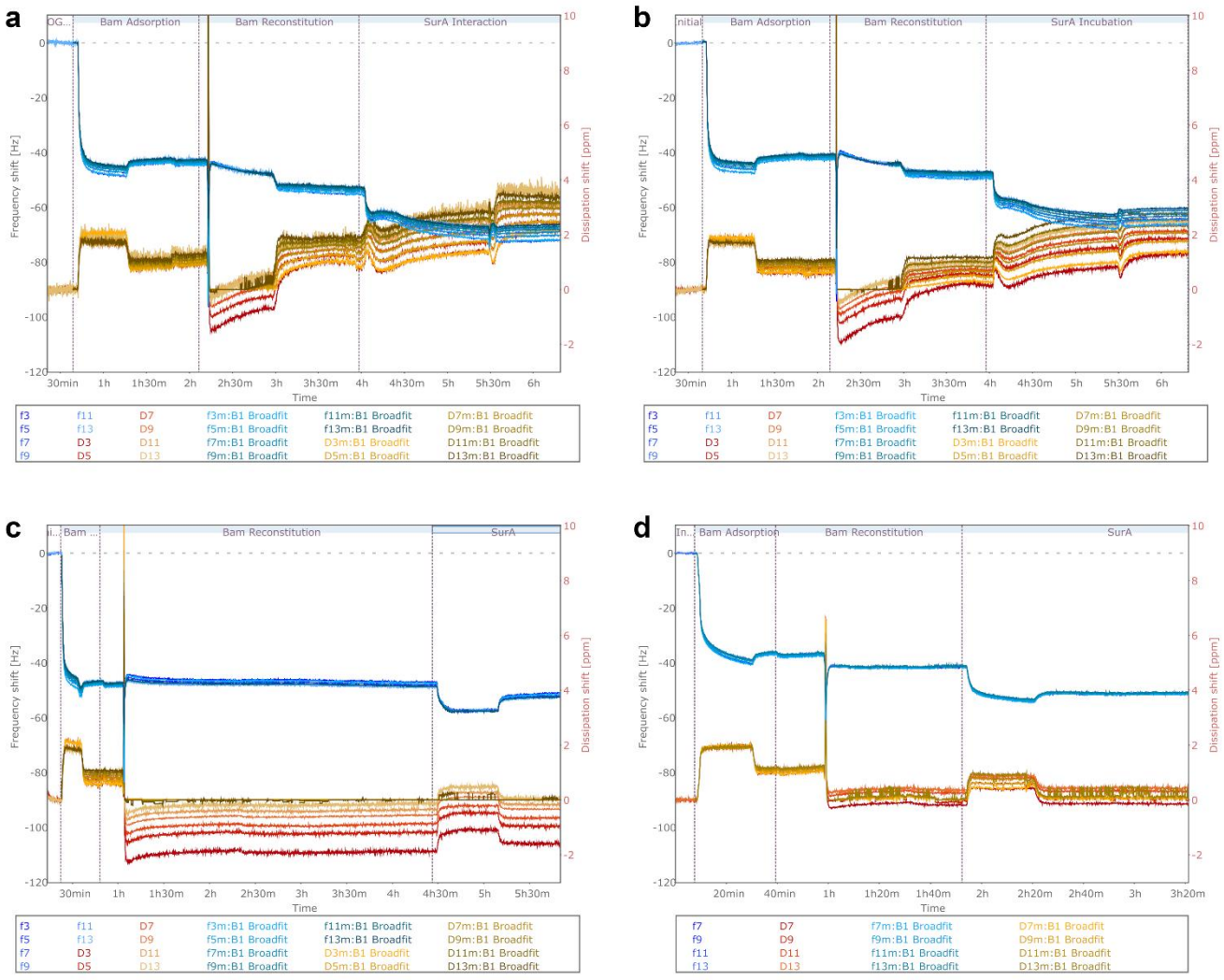

Figure S7. Raw QCMD Fits. Raw data and corresponding fits of 4 repeated QCM-D measurements (a-d.) measuring the interaction of SurA without any OMP with BamABCDE:POPC membranes adsorbed to gold substrates functionalized with DTSP-ANTA-Cu^{2+}

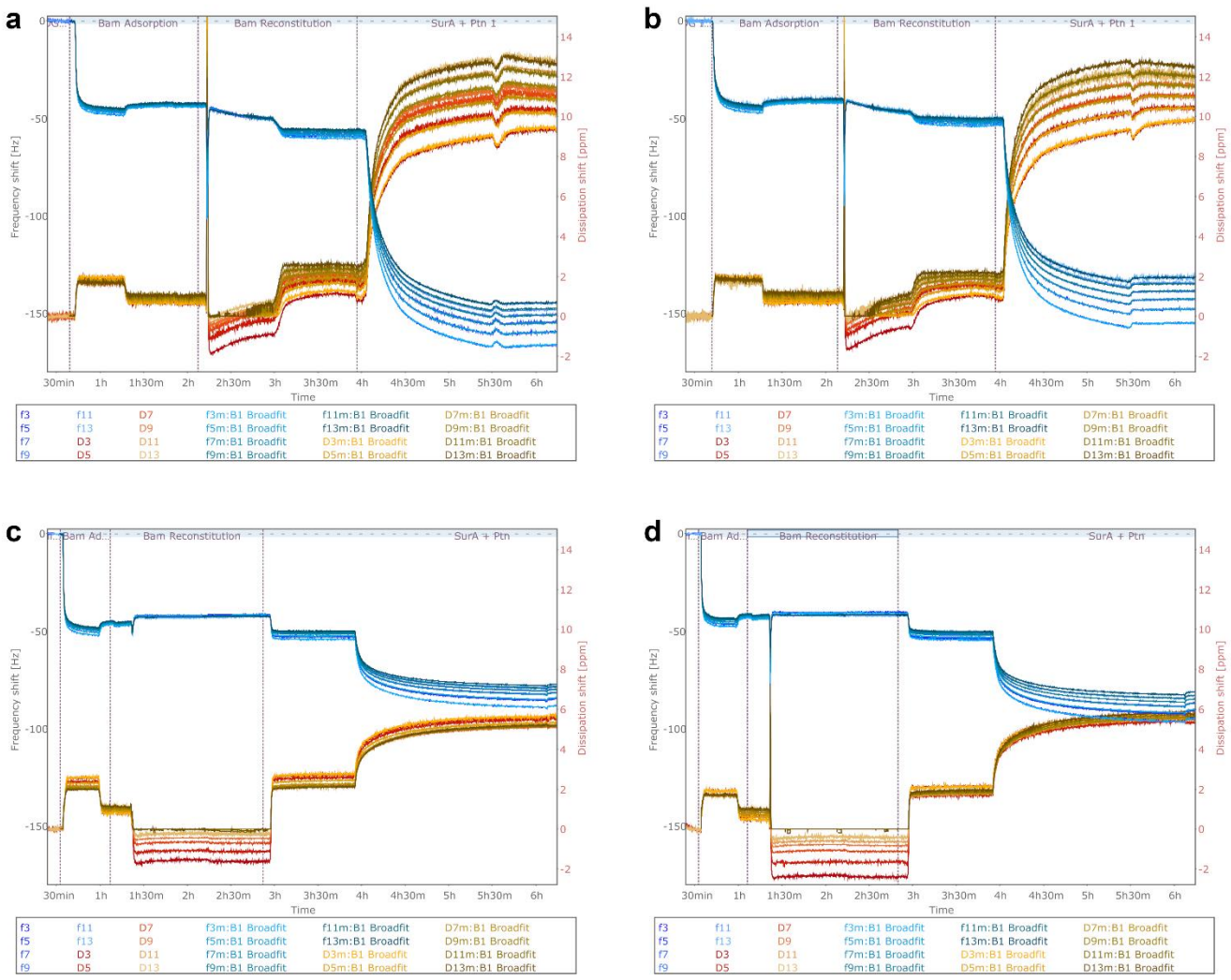

Figure S8. Raw QCMD Fits. Raw data and corresponding fits of 4 repeated QCM-D measurements (a-d.) measuring the interaction of SurA in the presence of pertactin with BamABCDE:POPC membranes adsorbed to gold substrates functionalized with DTSP-ANTA-Cu^{2+}

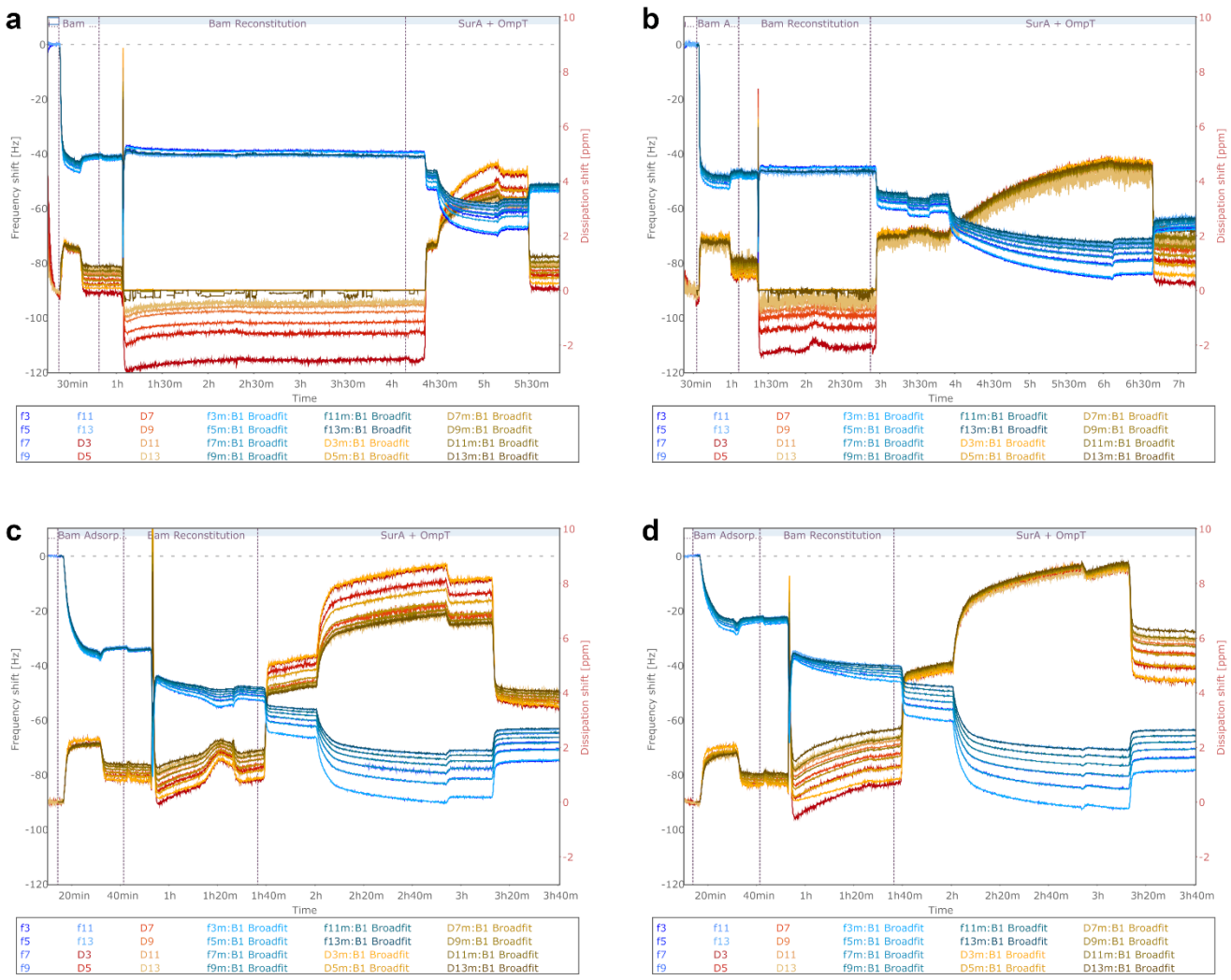

Figure S9. Raw QCMD Ftis. Raw data and corresponding fits of 4 repeated QCM-D measurements (a-d.) measuring the interaction of SurA in the presence of OmpTwith BamABCDE:POPC membranes adsorbed to gold substrates functionalized with DTSP-ANTA-Cu^{2+}

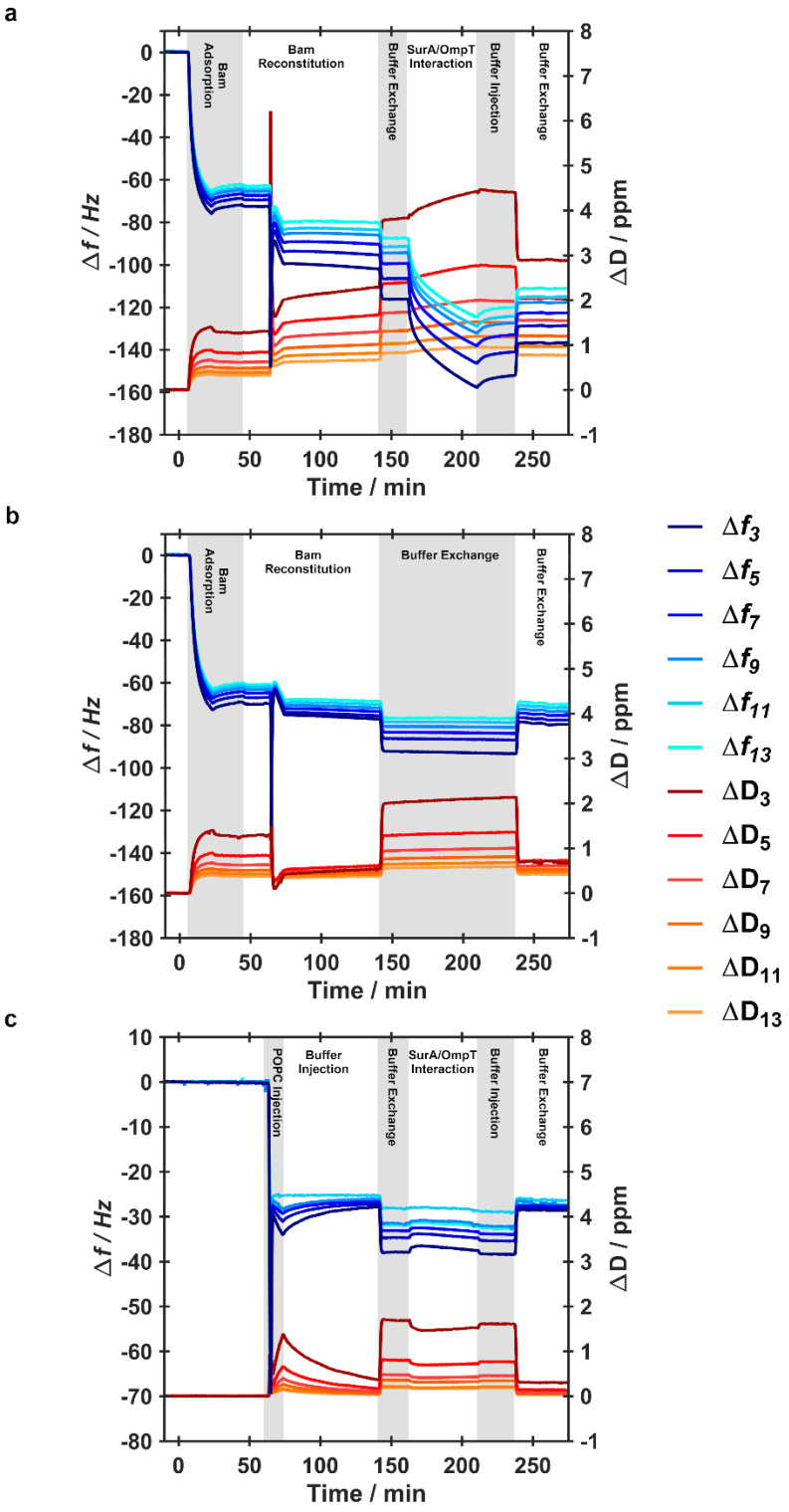

Figure S10. Representative QCM-D data obtained during surface assembly prior to *in-situ* OmpT Activity Assays. **a.** Assembly of BamA$^{(L7H9)}$BCDE:POPC membrane and subsequent interaction with SurA/OmpT. **b.** Assembly of a BamA$^{(L7H9)}$BCDE:POPC membrane without subsequent SurA/OmpT interaction. **c.** Assembly of a POPC membrane in the absence of BamA$^{(L7H9)}$BCDE, and subsequent interaction with SurA/OmpT.

Table S1. Known and calculated scattering length density values for components of the interfacial samples used in NR studies.

Sample Component	**SLD / $\times 10^{-6}$ Å^{-2}**
Silicon[1]	2.07
Silicon Oxide[1]	3.41
Gold	4.6
DTSP-ANTA	1.8
hBamABCDE (H_2O)	1.9
hBamABCDE (D_2O)	3.4
β-OG	1.5
POPC (total)	0.28
80% per d-Protein in H_2O	5.2
80% per d-Protein in D_2O	6.9
D_2O[1]	6.39
H_2O[1]	-0.56

Known SLD values taken from Stidder *et al* [2], Clifton *et al* [3] and Foglia *et al* [4]. Calculated SLD values for proteins were conducted using the protein SLD calculator (http://psldc.isis.rl.ac.uk/Psldc/) using the deuteration value obtained for the proteins by mass spectrometry analysis.

Supplementary References

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