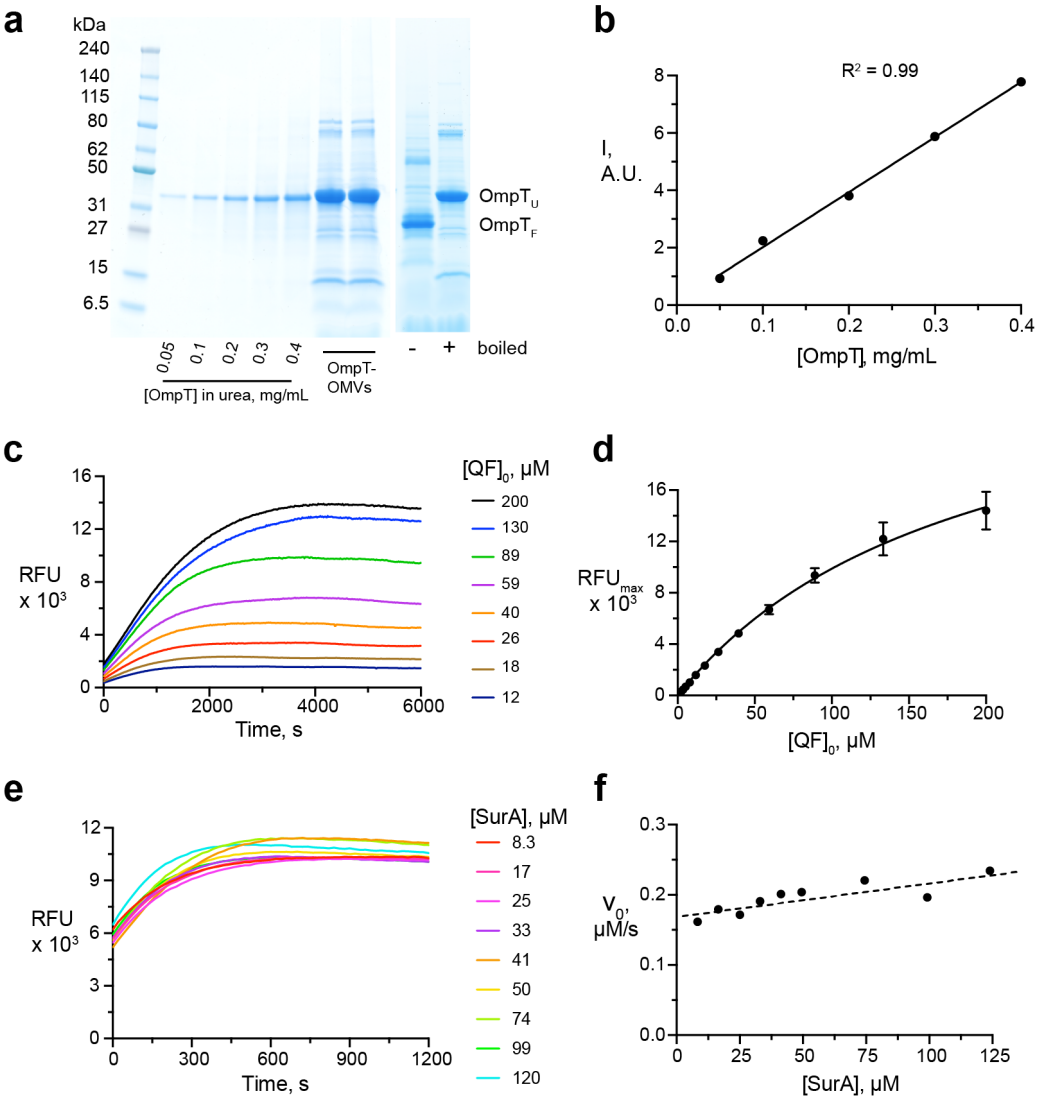
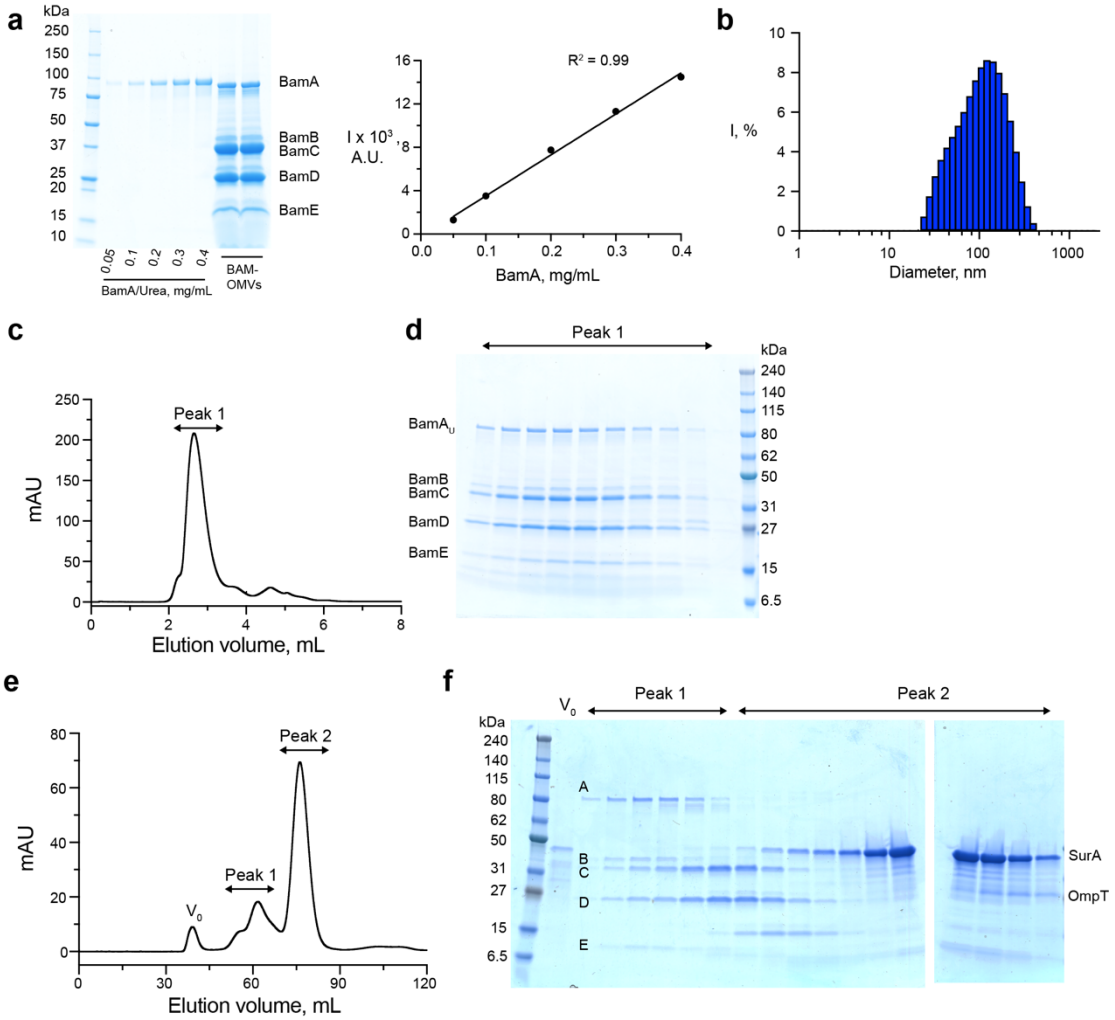
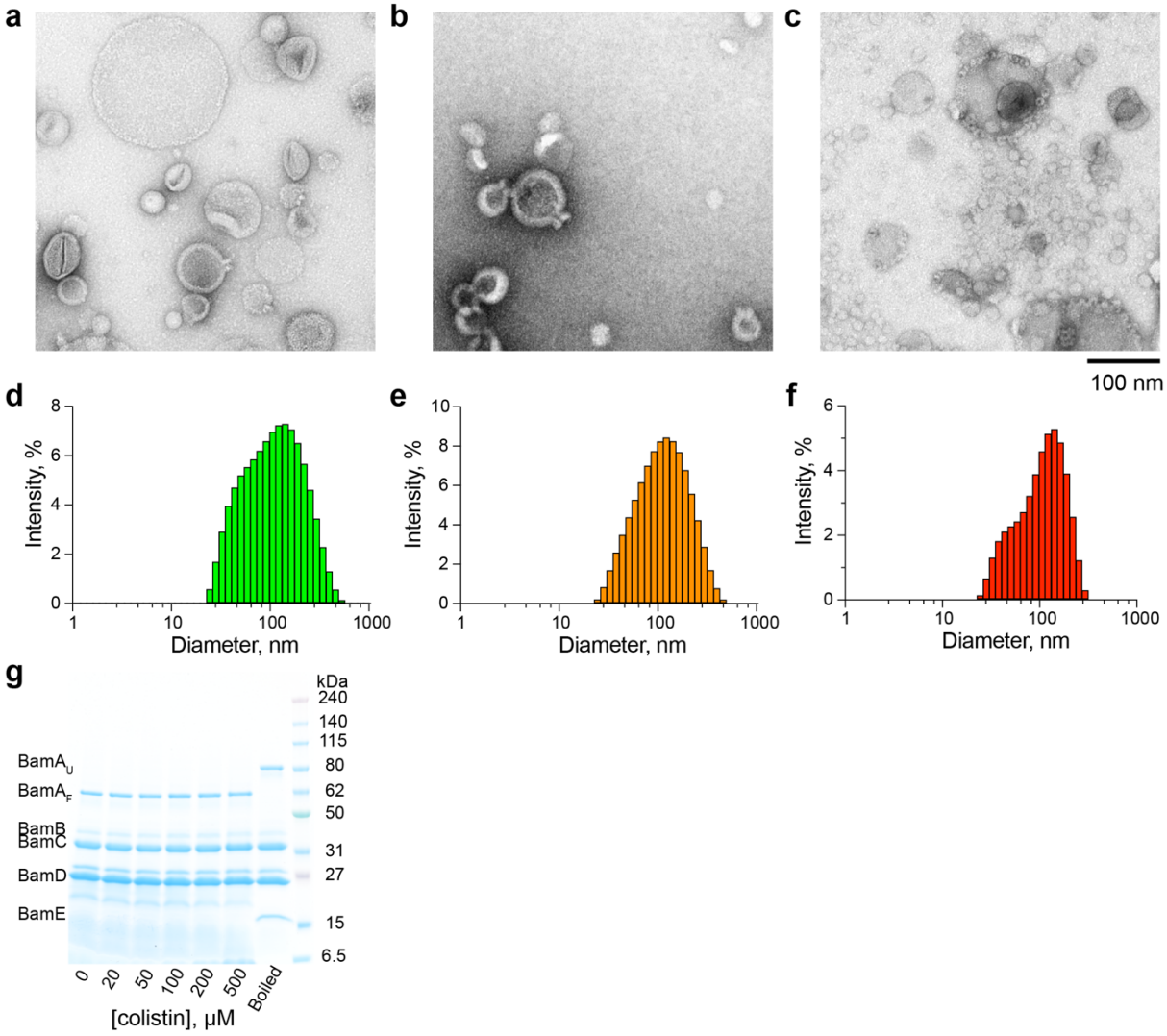
Supplementary Figures



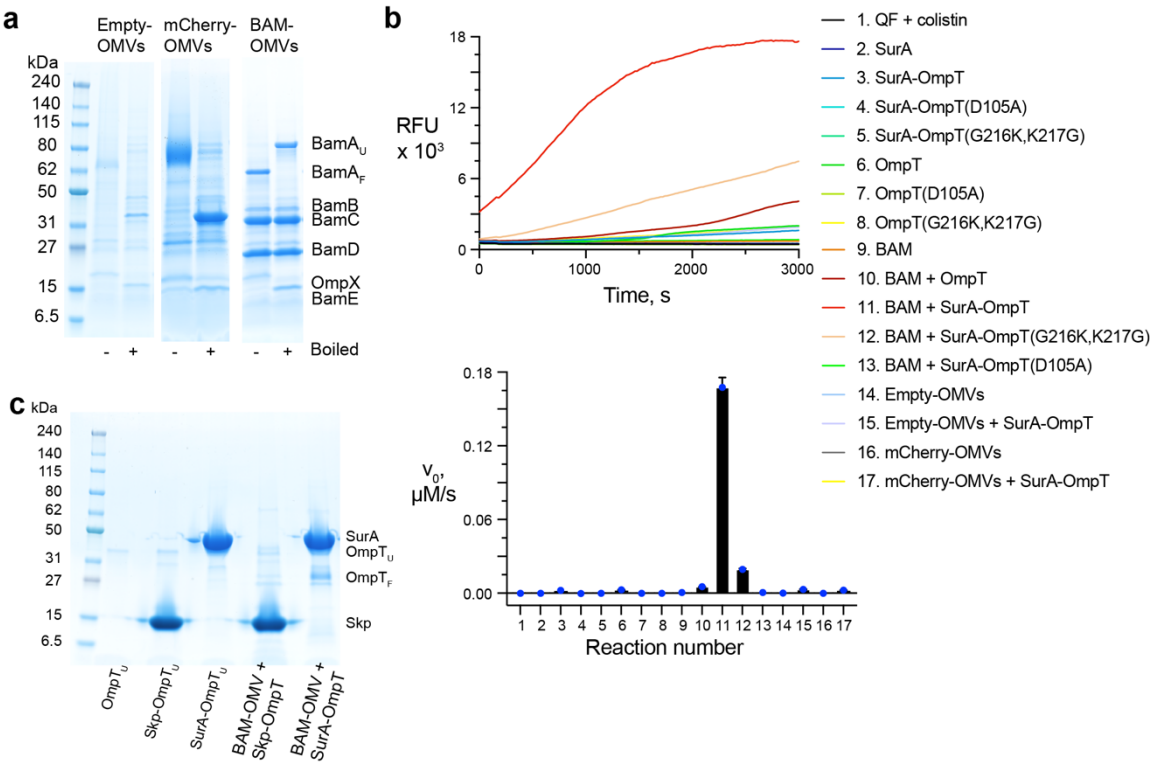
Supplementary Figure 1. Characterization of OmpT-OMVs. (**a**) SDS-PAGE of unboiled and boiled OmpT-OMVs along with pure OmpT in urea solution at known concentrations. The uncropped gel is shown in Supplementary Figure 10. (**b**) Linear fit to calibrate band intensities. (**c**) Fluorescence emitted after the cleavage of QF peptide by OmpT-OMVs using variable $[QF]_0$. (**d**) Scaling curve obtained from fitting the maximum fluorescence values from **c**. n=3 independent experiments. Data are presented as average values ± standard deviation. (**e**) Progress curves of the cleavage of 100 µM QF peptide by 8.4 nM OmpT-OMV at variable SurA concentrations as indicated. (**f**) Initial reaction velocities from **e**, plotted as a function of chaperone concentration. The dashed line is a linear regression to the data. The data underlying panels **b**–**f** are provided as Source Data.



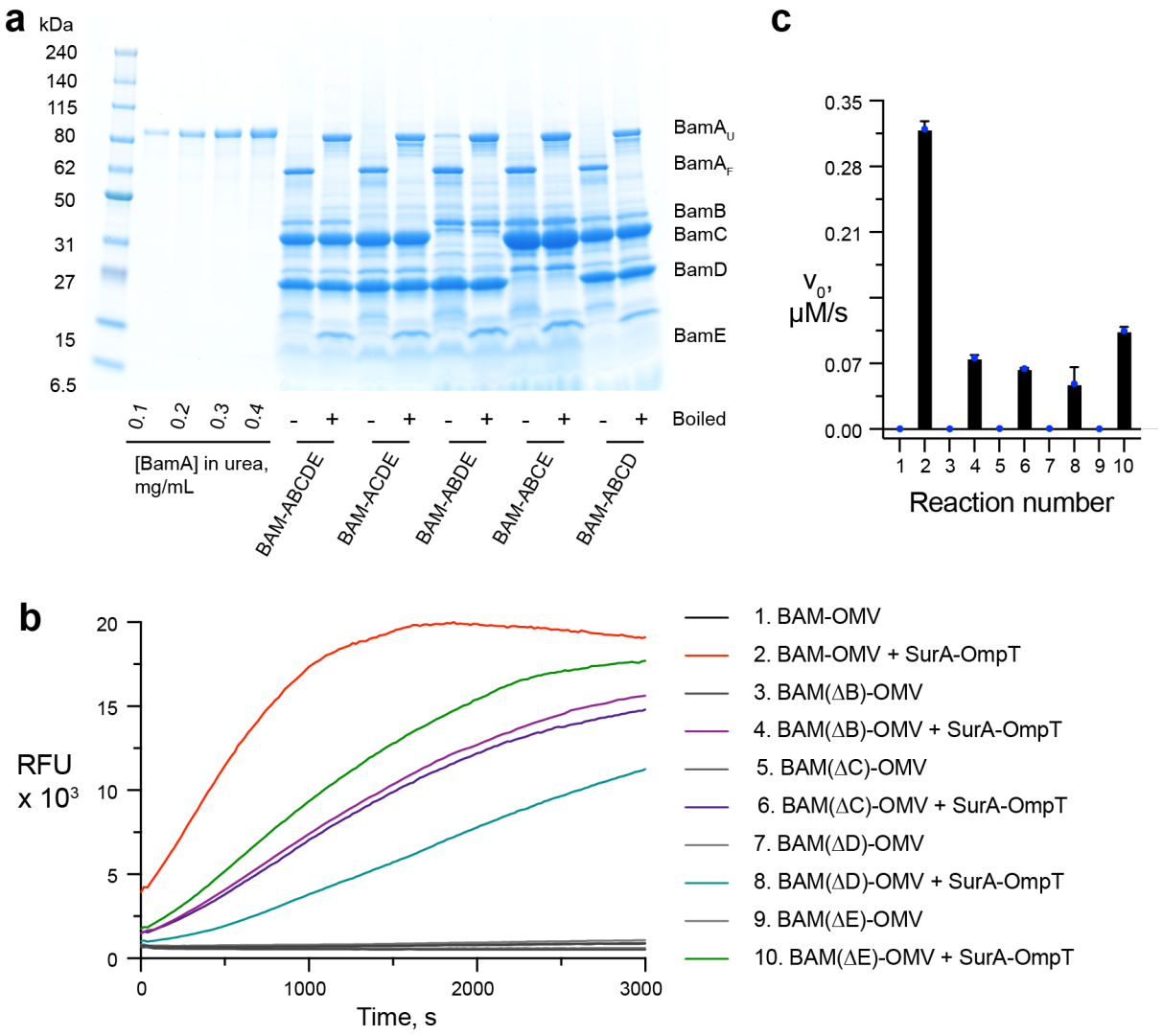
Supplementary Figure 2. Characterization of BAM-OMVs. (**a**) SDS-PAGE of BAM-OMVs along with pure BamA in urea at known concentrations and a linear fit to calibrate band intensities. The uncropped gel is shown in Supplementary Figure 11. (**b**) Size distribution of OMVs as observed by dynamic light scattering (DLS). (**c**) Size exclusion chromatography characterization of BAM complex extracted from BAM-OMVs with 1% DDM on a manually packed 4 mL superdex S200 column equilibrated in 20 mM Tris-Cl, pH 8.0, 150 mM NaCl containing 0.05% DDM. (**d**) SDS-PAGE of elution fractions from peak 1 demonstrating individual component of BAM complex. Note that a majority of the peak volume comprises complete BAM complexes and only a minority corresponds to excess BamC and BamD. The uncropped gel is shown in Supplementary Figure 12. (**e**) SEC elution profile of a DDM-extracted reaction mixture comprising BAM-OMVs, SurA, urea-unfolded OmpT and colistin. Two separate elution peaks were observed besides the void volume peak (V_0). (**f**) SDS-PAGE of elution fractions from peak 1 and 2, demonstrating intact BAM complex and SurA-OmpT. The uncropped gels are shown in Supplementary Figure 13. The data underlying panels **a**–**c** and **e** are provided as Source Data.



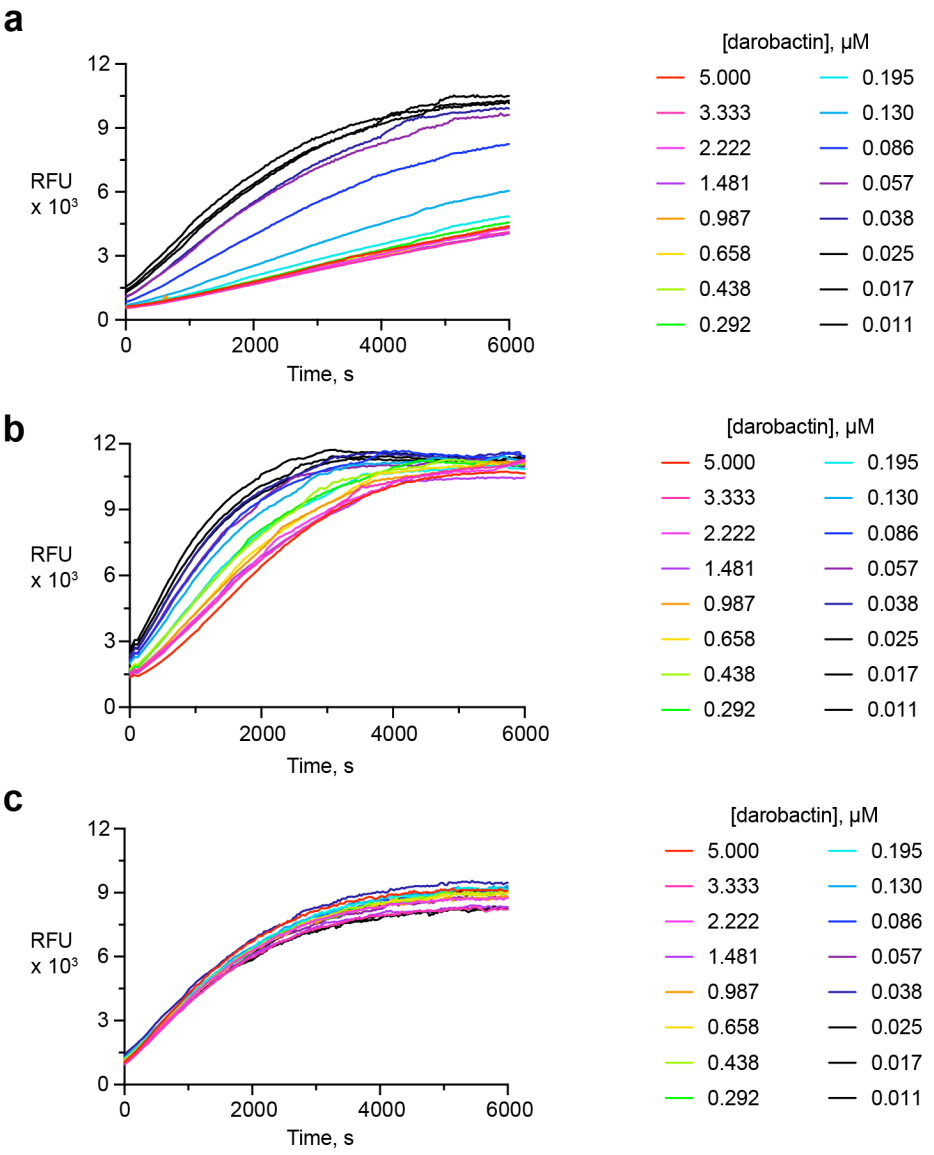
Supplementary Figure 3. Negative staining electron micrographs of BAM-OMVs incubated with variable colistin concentrations. (**a**) 20 μM, (**b**) 50 μM, and (**c**) 100 μM colistin. (**d**–**f**) The average size distribution of OMVs at (**d**) 20 μM, (**e**) 50 μM, and (**f**) 100 μM colistin as observed by DLS measurement. (**g**) SDS-PAGE gel-shift assay of BAM-OMVs treated with colistin concentrations up to 500 μM, demonstrating proper folding of BamA at all concentrations. The uncropped gel is shown in Supplementary Figure 14. The data underlying panels **d**–**f** are provided as Source Data.



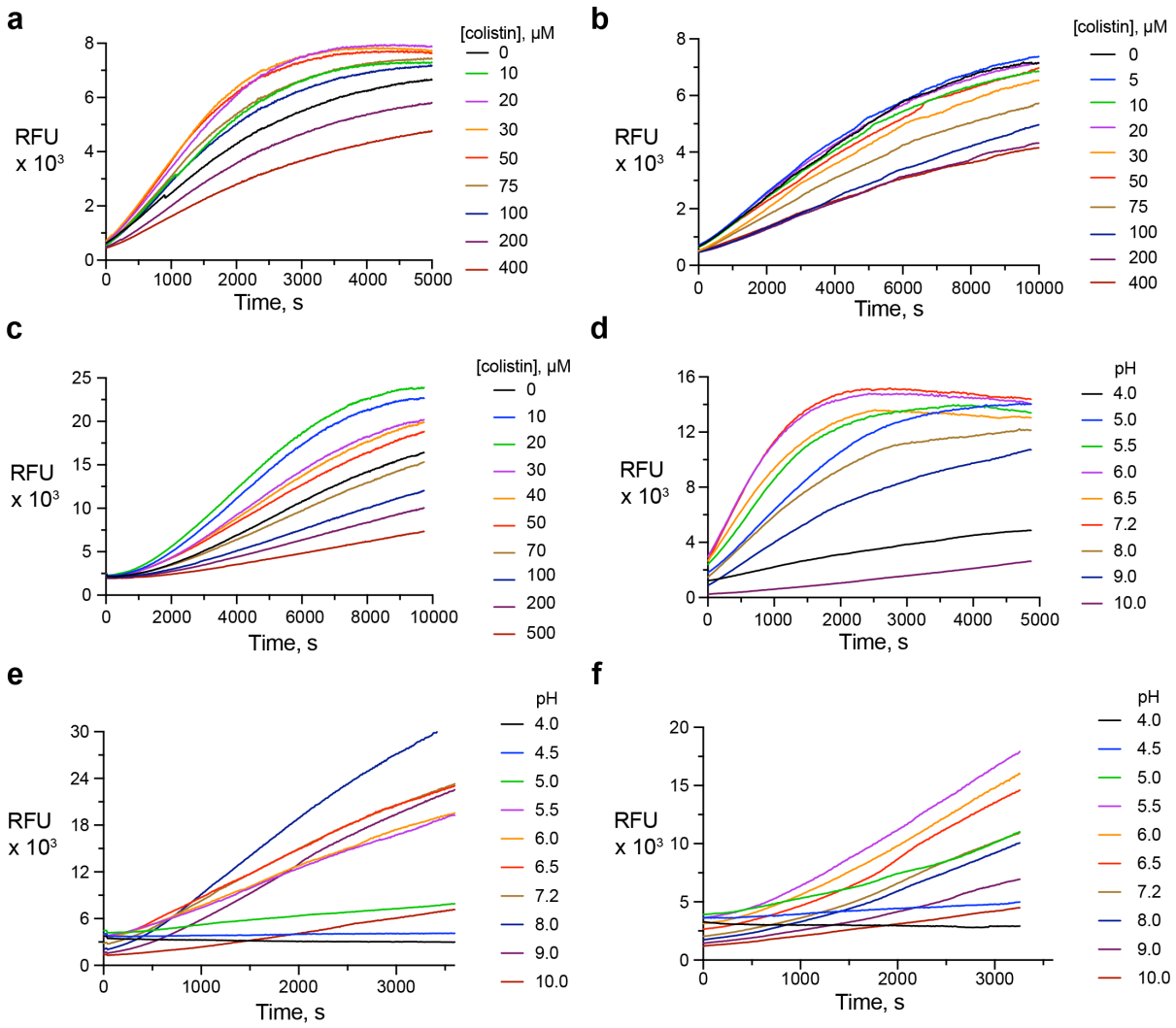
Supplementary Figure 4. BAM-mediated OmpT folding with control reactions. (**a**) SDS-PAGE analysis of empty-OMVs, mCherry-OMVs, and BAM-OMVs. The BAM complex is not detectable in empty OMVs and mCherry OMVs, but is present in BAM-OMVs. The strong band in mCherry-OMVs at 70 kDa/35 kDa for -/+ boiling is the mCherry dimer and monomer, respectively. The uncropped gel is shown in Supplementary Figure 15. (**b**) Fluorescence emission progress curves of cleavage of QF peptide in presence of variable reaction components 1–17, as indicated, and bar graph of the resulting initial reaction velocities v_0. n=2 independent experiments. Data are presented as average values ± standard deviation. (**c**) SDS-PAGE of unboiled reaction mixtures demonstrating the presence of folded OmpT ($OmpT_F$) in BAM-OMVs, starting from unfolded OmpT ($OmpT_U$) bound to the chaperones Skp or SurA. The uncropped gel is shown in Supplementary Figure 16. The data underlying panel **b** are provided as Source Data.



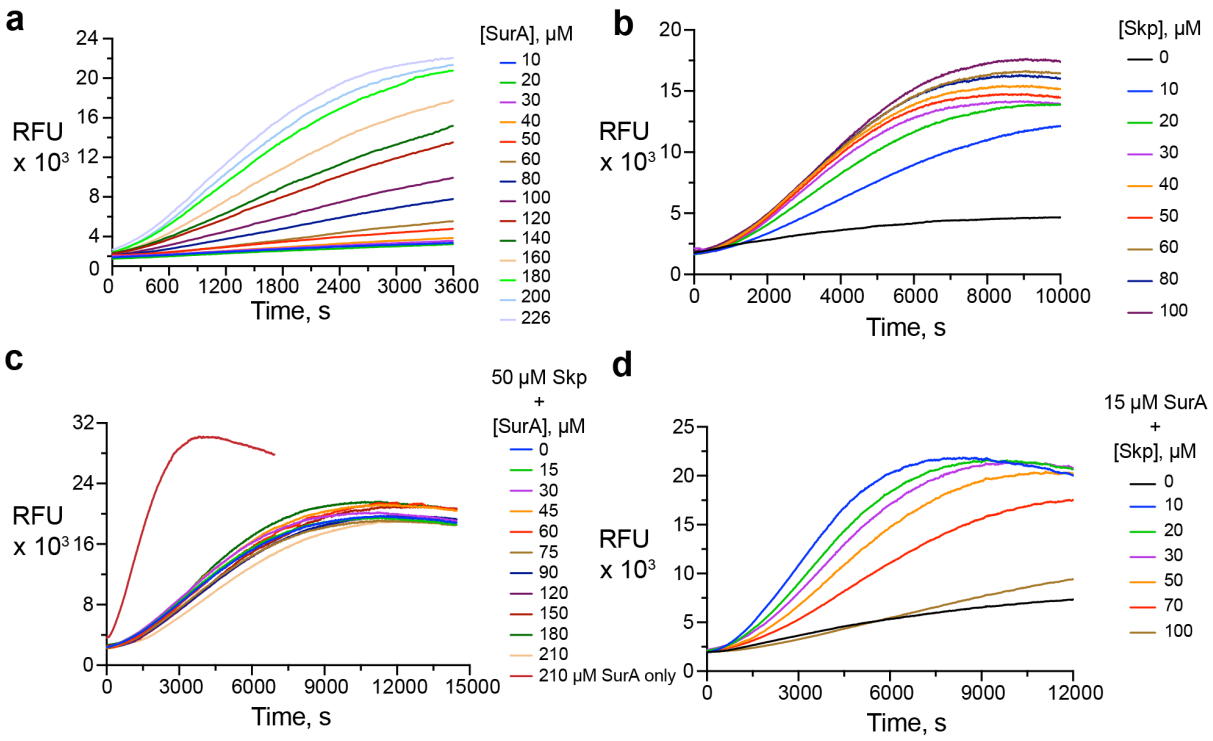
Supplementary Figure 5. Effect of BAM lipoproteins on BAM-mediated OmpT folding in OMVs. (**a**) SDS-PAGE of different preparations of BAM lipoprotein knockouts in OMVs along with pure BamA in urea at known concentrations. The uncropped gel is shown in Supplementary Figure 17. (**b**) Fluorescence emission of BAM-mediated OmpT folding of different reaction mixtures 1–10 as mentioned. (**c**) Initial reaction velocities for different reaction mixes 1–10 as indicated in **b**. n=2 independent experiments. Data are presented as average values ± standard deviation. The data underlying panels **b**–**c** are provided as Source Data.



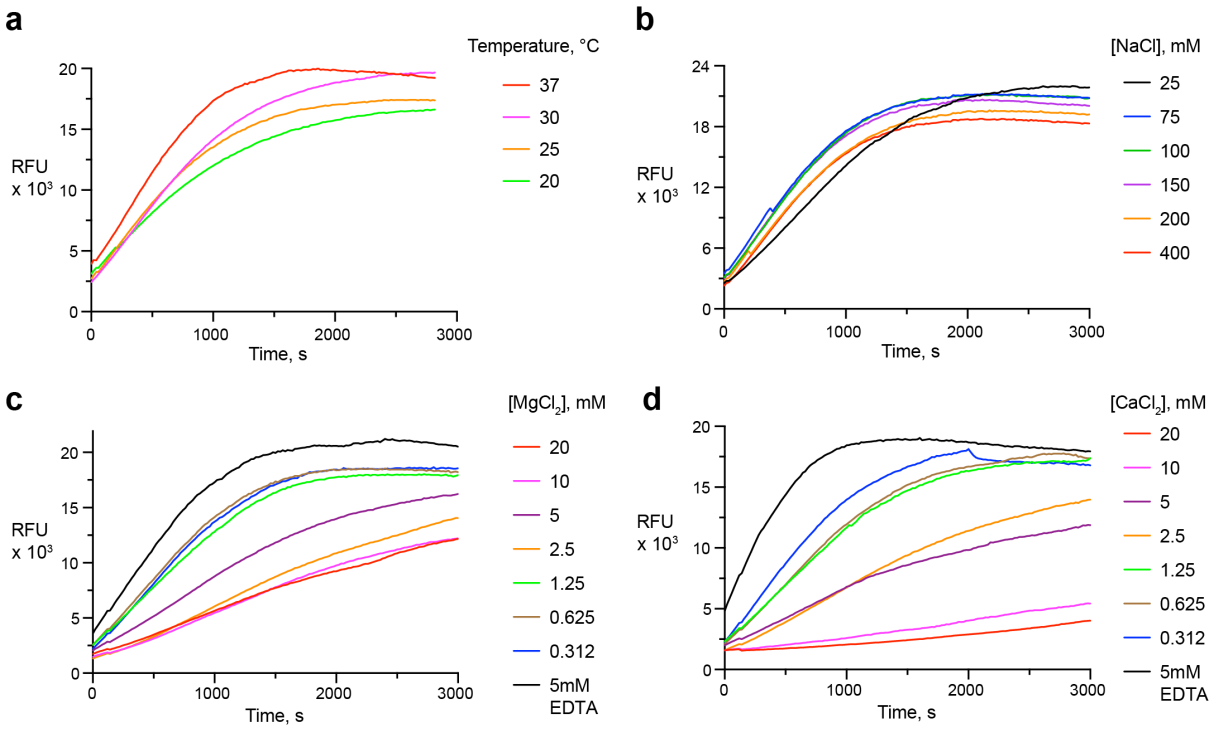
Supplementary Figure 6. Effect of darobactin on BAM-mediated folding of OmpT in OMVs. Fluorescence emission of BAM-mediated OmpT folding at variable darobactin concentration, as indicated, in (**a**) presence of colistin and (**b**) absence of colistin with sonicated BAM-OMVs. (**c**) Peptidase activity of OmpT in OmpT-OMVs at variable darobactin concentration, as indicated. The data underlying panels **a**–**c** are provided as Source Data.



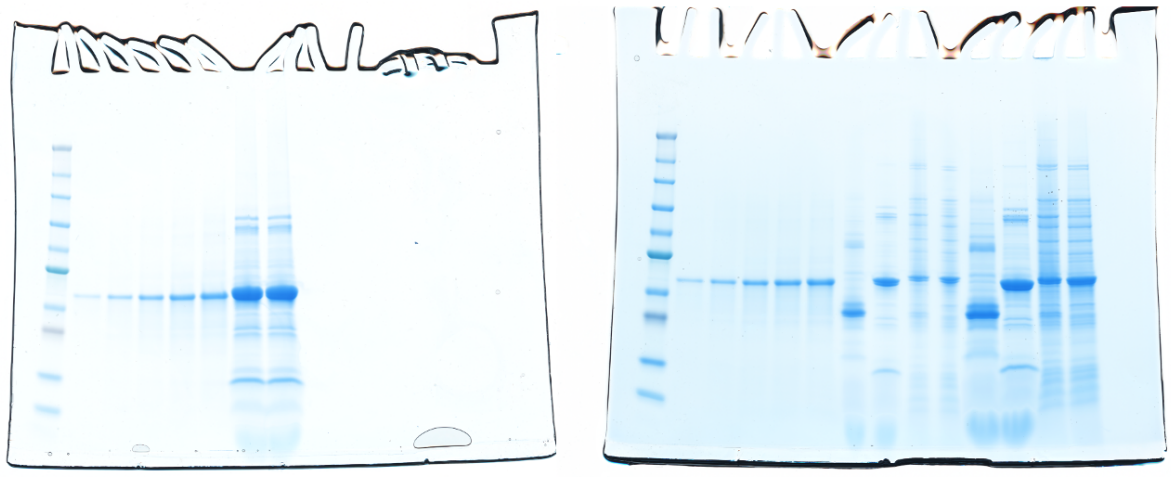
Supplementary Figure 7. Fluorescence emission for optimization of colistin concentration and reaction buffers. (**a**–**c**) Fluorescence emission progress curves of BAM-mediated OmpT activity in presence of (**a**) reaction mixes comprised of 16 nM BAM, 100 µM QF, 100 µm SurA and 2 µm OmpT and variable colistin concentration, as indicated, (**b**) reaction mixes comprised of sonicated BAM OMVs with 16 nM BAM, 100 µM QF, 100 µm SurA and 2 µm OmpT and variable colistin concentration, as indicated. (**c**) Reaction mixes comprised of 16 nM BAM, 500 µM QF, 50 µm Skp and 2 µm OmpT and variable colistin concentration, as indicated. (**d**) Reaction mixes comprised of 0.8 nM OmpT, 100 µM QF, 50 µm colistin at variable pH, as indicated. (**e**) Reaction mixes comprised of 16 nM BAM, 500 µM QF, 50 µm colistin, 50 µm SurA and 2 µm OmpT at variable pH, as indicated. (**f**) Reaction mixes comprised of 16 nM BAM, 500 µM QF, 50 µm colistin, 50 µm Skp and 2 µm OmpT at variable pH, as indicated. The initial reaction velocities from these experiments are shown in Figure 3. The data underlying panels **a**–**f** are provided as Source Data.



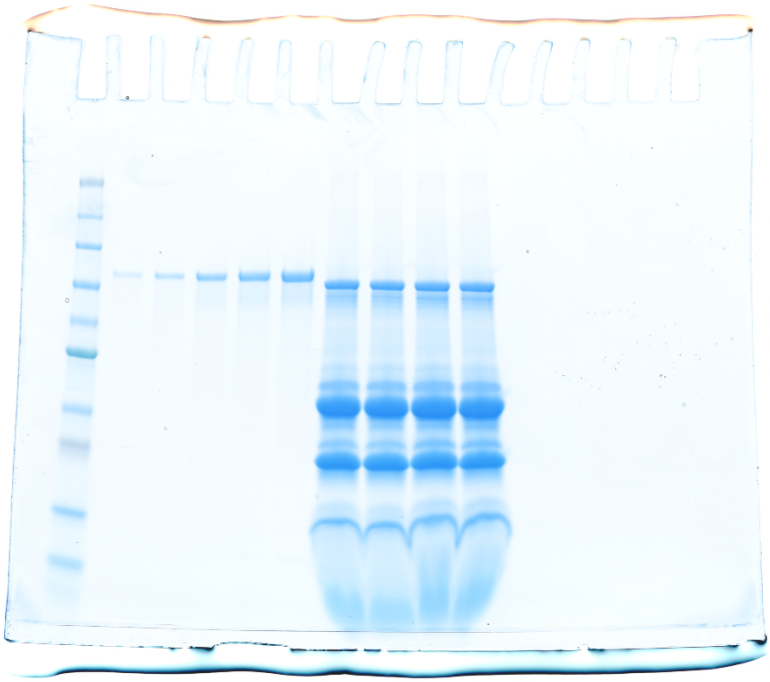
Supplementary Figure 8. Optimization of chaperone concentration and their competitive effect on BAM-mediated OmpT folding. (**a**, **b**) Fluorescence emission of BAM-mediated OmpT folding at variable (**a**) SurA and (**b**) Skp concentrations, as indicated. (**c**) Fluorescence emission of BAM-mediated OmpT activity at 50 μM Skp and variable SurA concentration, as indicated. (**d**) Fluorescence emission of BAM-mediated OmpT activity at 15 μM SurA and variable Skp concentration, as indicated. Each reaction mix comprised of 16 nM BAM in OMVs, 20 μM colistin, 500 μM QF peptide, 2 μM unfolded OmpT in addition to Skp and SurA at the indicated concentrations. The initial reaction velocities from these experiments are shown in Figure 3. The data underlying panels **a**–**d** are provided as Source Data.



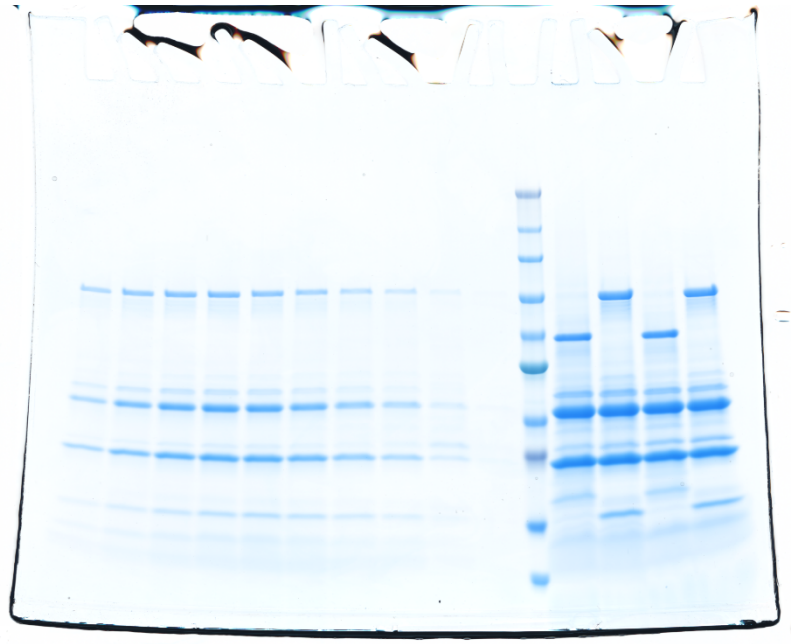
Supplementary Figure 9. Fluorescence emission for optimization of reaction parameters for BAM-mediated OmpT folding. Fluorescence emission of BAM-mediated OmpT folding at variable (**a**) reaction temperatures, (**b**) NaCl, (**c**) $MgCl_2$, and (**d**) $CaCl_2$ concentrations as indicated. The reaction mixture comprised of 100 nM BAM in OMVs, 20 µM colistin, 100 µM QF peptide, 100 µm SurA and 2 µm OmpT. The initial reaction velocities from these experiments are shown in Figure 3. The data underlying panels **a**–**d** are provided as Source Data.



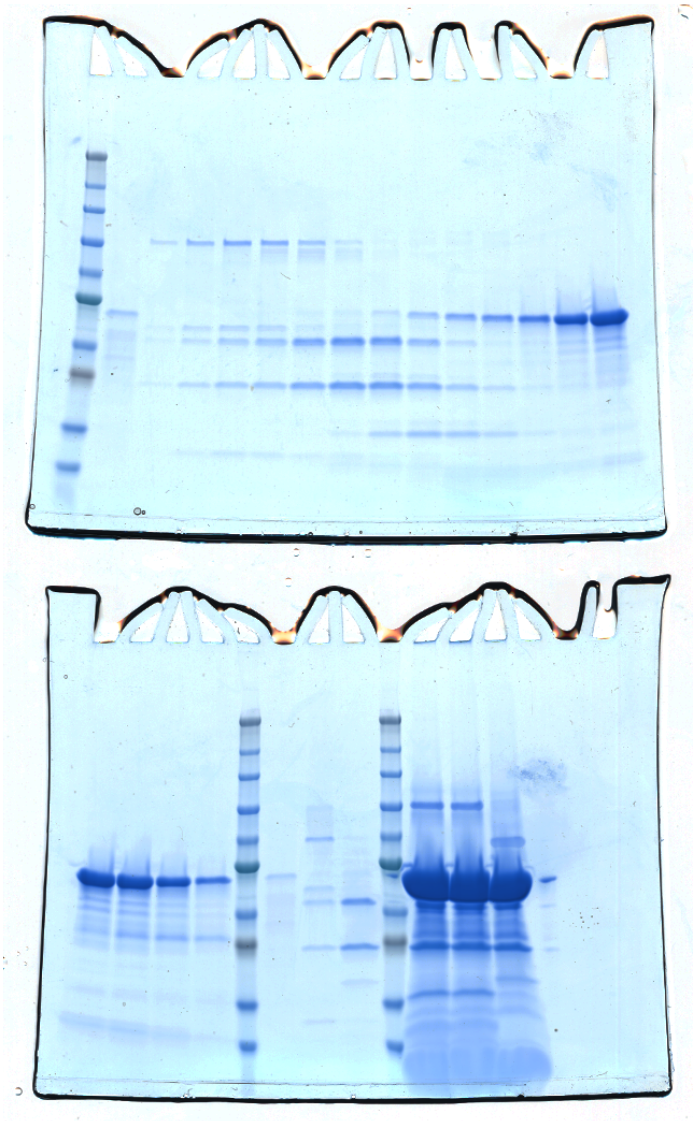
Supplementary Figure 10. Uncropped gels of Supplementary Figure 1a. Coomassie Blue stained 4–20% SDS-PAGE with molecular weight standard in the first lane.



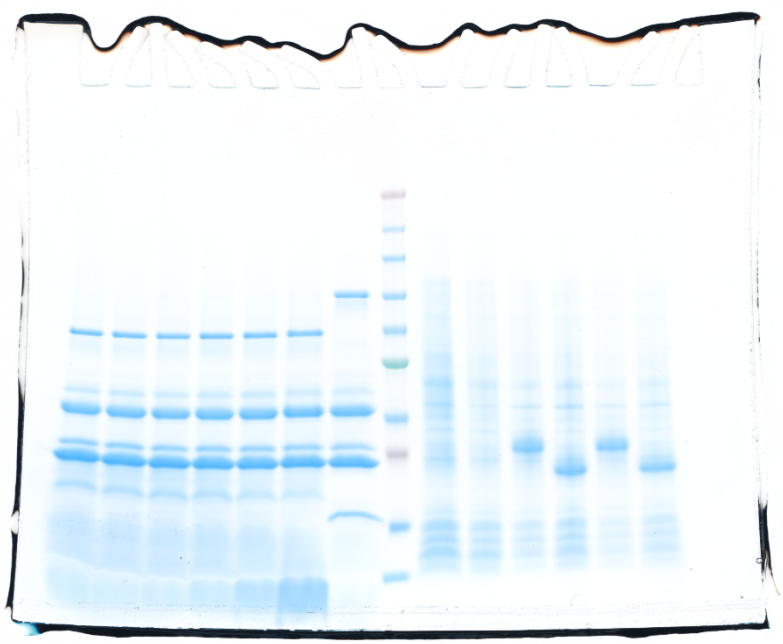
Supplementary Figure 11. Uncropped gel of Supplementary Figure 2a. Coomassie Blue stained 4–20% SDS-PAGE with molecular weight standard in the first lane.



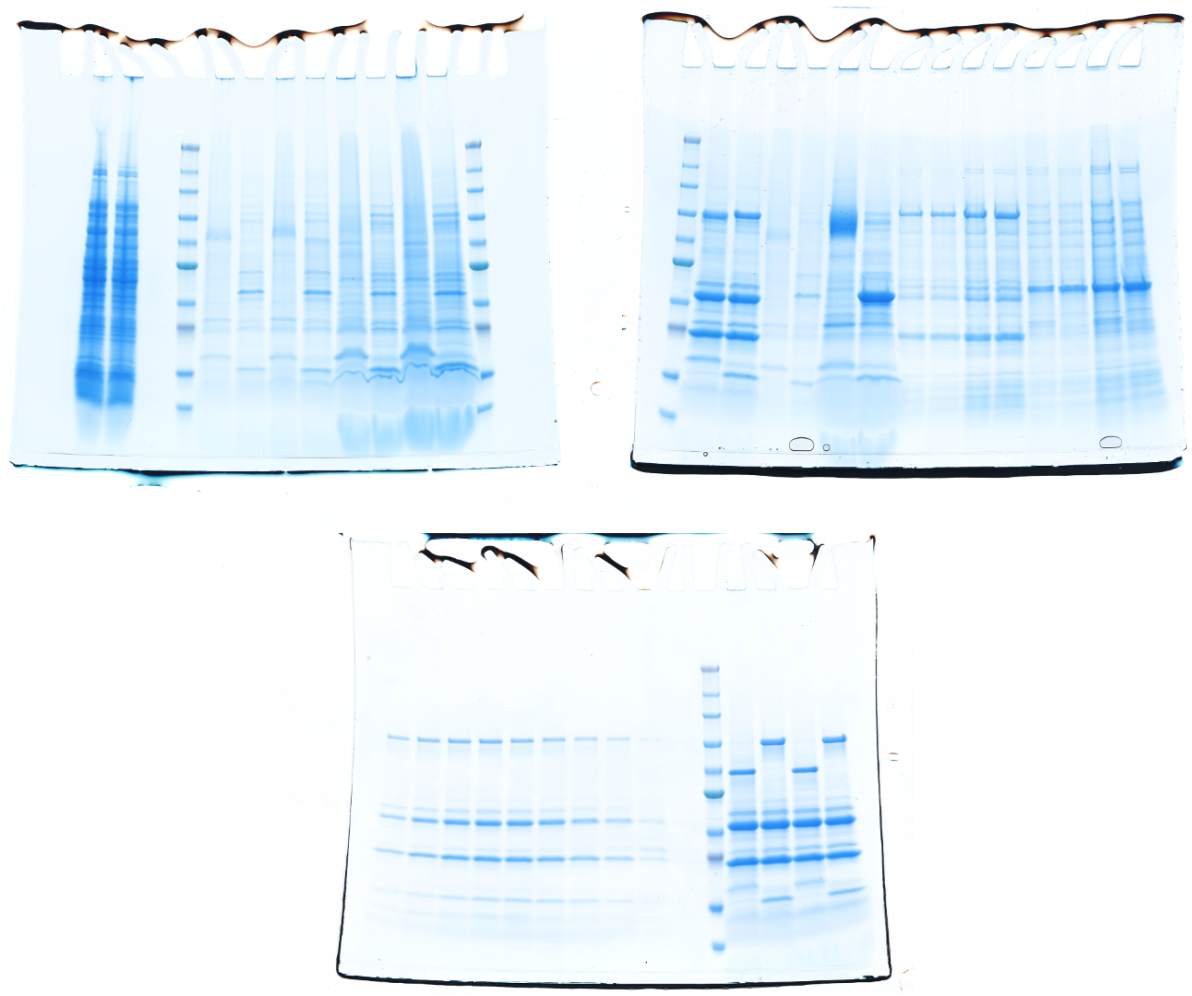
Supplementary Figure 12. Uncropped gel of Supplementary Figure 2d. Coomassie Blue stained 4–20% SDS-PAGE with molecular weight standard in lane 11.



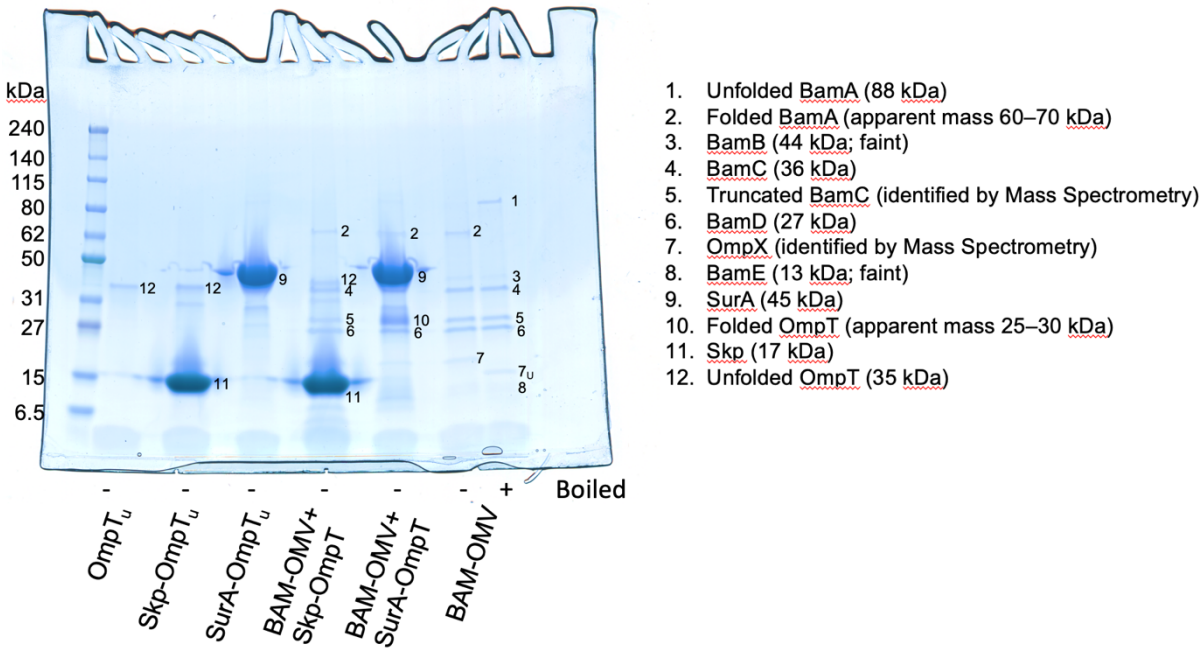
Supplementary Figure 13. Uncropped gels of Supplementary Figure 2f. Coomassie Blue stained 4–20% SDS-PAGE with molecular weight standard in lanes 1 / lanes 5 & 9.



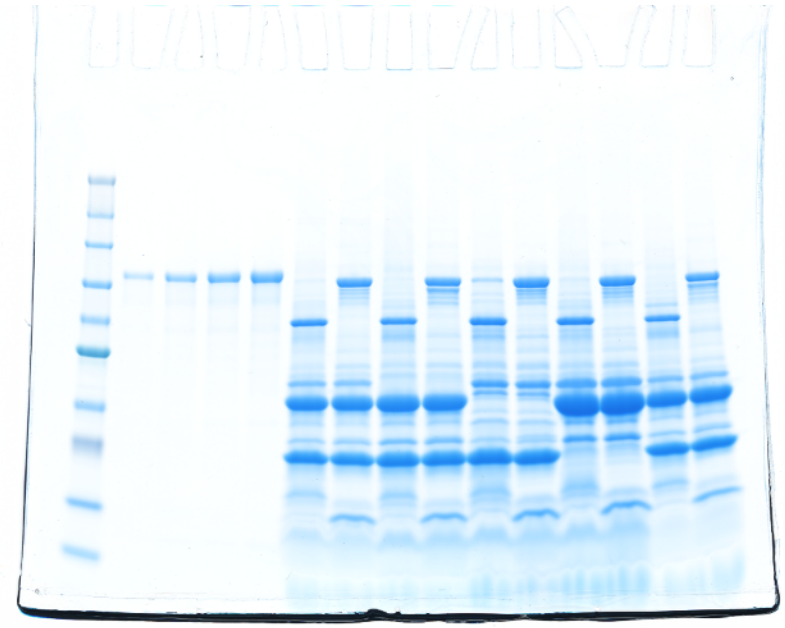
Supplementary Figure 14. Uncropped gel of Supplementary Figure 3g. Coomassie Blue stained 4–20% SDS-PAGE with molecular weight standard in lane 8.



Supplementary Figure 15. Uncropped gels of Supplementary Figure 4a. Coomassie Blue stained 4–20% SDS-PAGEs with molecular weight standards in lanes 5 & 14 / lane 1 / lane 11. Top left: Empty-OMVs, top right: mCherry-OMVs, bottom: BAM-OMVs.



Supplementary Figure 16. Uncropped gel of Supplementary Figure 4c. Coomassie Blue stained 4–20% SDS-PAGE with molecular weight standard in the first lane. Samples were loaded with or without boiling, as indicated below the gel. Annotations of all bands are given on the right.



Supplementary Figure 17. Uncropped gel of Supplementary Figure 5a. Coomassie Blue stained 4–20% SDS-PAGE with molecular weight standard in the first lane.