Figures and figure supplements

In vivo experiments do not support the charge zipper model for Tat translocase assembly

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Figure 1. The charge zipper model for TatA assembly. (A) Cartoon showing the TatA domain structure, comprising a transmembrane helix (TMH), an amphipathic helix (APH), and a densely-charged region (DCR). Below the cartoon are shown the amino acid sequences for E. coli TatA (top) and B. subtilis TatAd (bottom) starting at the beginning of the APH and with acidic (red) and basic (blue) residues indicated. The APH is assigned in each case from the corresponding NMR structures (PDB 2MN7 and 2L16) and is indicated by a gray cylinder. The E. coli TatA sequence is C-terminally truncated to residue 69. Salt bridge pairs predicted by Walther and co-workers are indicated above each sequence. For E. coli TatA the predicted salt bridge pairs tested in this study are indicated in black and the acidic DDE motif targeted in this study is underlined. For B. subtilis TatAd, the assigned intra- and inter-molecular pairs are distinguished using dotted or solid lines respectively. (B) Structure of E. coli TatA (PDB 2MN7) shown in three orientations with the charged APH side chains indicated. (C) Schematic diagram of the charge zipper model for TatA folding and assembly applied to E. coli TatA. Folding back the DCR against the APH is proposed to allow pairing of amino acids with complementary charges to form either intra-molecular or inter-molecular salt bridges. Three adjacent TatA protomers are shown with the residues of the acidic DDE motif and their potential salt bridge partners outlined in red and blue respectively. The charge zipper model does not predict which residue pairs would form inter- and intra-molecular salt bridges and one of several possible configurations is represented here. (D) The salt bridges shown in (C) are proposed to mediate self-assembly of multiple adjacent TatA molecules (1). The assembled APH/DCR units are then proposed to insert across the membrane to form the passage for substrate protein transport (2).

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Figure 2. Transport activity of TatA charge zipper variants. All TatA variants were expressed from the phage lambda attachment site in the ΔtatAΔtatE strain JARV16. Mutant strains are identified by the amino acid
Figure 2 continued

substitutions present in TatA where EDD is K37E/K40D/K41D, and KKK is D45K/D46K/E47K. WT refers to the ΔtatE strain J1M1, and ΔTatA refers to the parental strain JARV16. (A) Whole cell (W), periplasm (P), and spheroplast (S) fractions of cells overproducing CueO from plasmid pQE80-CueO were subject to immunoblotting with antibodies against CueO or the cytoplasmic marker protein DnaK. m is the transported form of CueO from which the signal peptide has been removed and p the precursor protein. (B) Growth of the strains when cultured in LB/glycerol/TMAO medium under anoxic conditions. Error bars represent the S.E.M of three biological replicates. (C) Serial ten-fold dilutions of log-phase cultures were spotted onto LB-agar containing the indicated amount of SDS. (D) Immunoblot of membranes isolated from the strains used in A-C, probed with TatA antibodies (upper panels) or antibodies against TatB and TatC (lower panels).

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**Figure 3.** TatA oligomerization behavior of charge zipper variants. Representative fluorescence images of TatA-YFP in *E. coli* cells. A tatA-yfp fusion was expressed from the chromosome in three different backgrounds: ELV16 λAry which contains all other tat genes (designated AyBCE), JARV16 λAry which lacks tatE (designated AyBC), or DADE λAry which possesses no other tat genes (designated Ay). The TatA-YFP variant produced is indicated to the left of the panels, where WT is the parental protein and KKK is a D45K/D46K/E47K variant. Where indicated, CueO was overproduced from plasmid pQE80-CueO by adding IPTG to early exponential phase cultures for 30 min prior to imaging (+CueO columns). 50 μM CCCP was subsequently added, as indicated (+CCCP column), and the cells incubated for 30-45 min prior to imaging. Scale bar = 1 μm.

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**Figure 3—figure supplement 1.** TatA oligomerization behavior of additional charge zipper variants. (A) Immunoblot of membranes isolated from the strains used in panel (B) and in Figure 3, probed with TatA antibodies (upper panels) or antibodies against TatB and TatC (lower panels). (B) Representative fluorescence images of strains ELV16 λArY EDD (AyEDDBCE), JARV16 λArY EDD (AyEDDBC), and MΔABC λArY EDD (AyEDDE). The scaling used to display these images differs from that employed in Figure 3 and uses 1000 a.u. as the minimum (black) and 4000 a.u. as the maximum (white). DOI: https://doi.org/10.7554/eLife.30127.005