Expanded View Figures

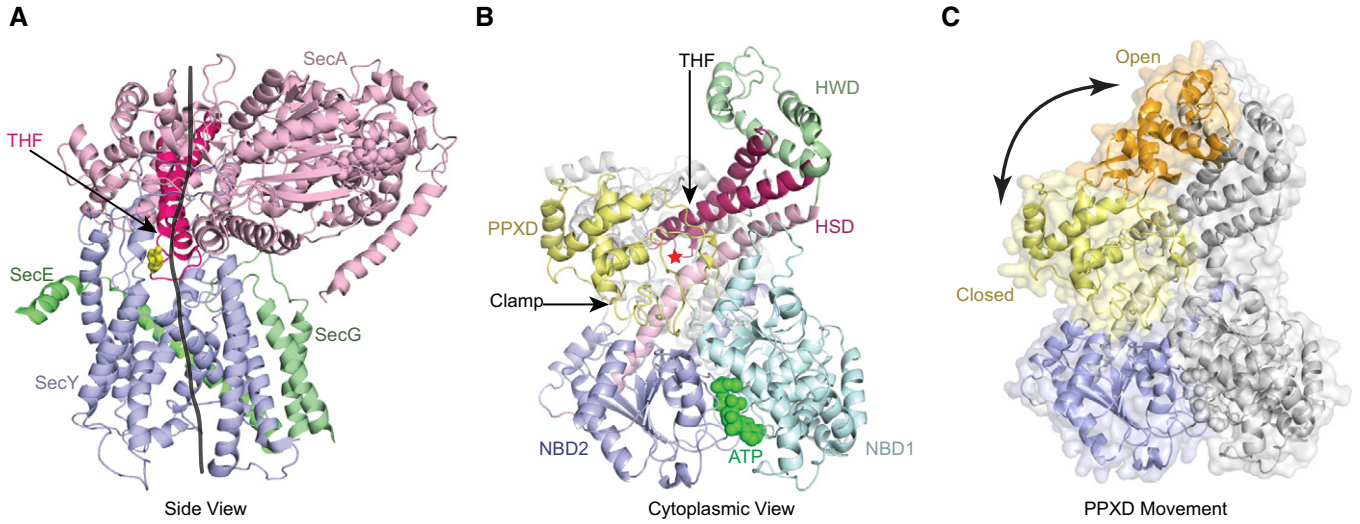

Figure EV1. Structure of the SecA-SecY complex.

A Side view with a translocating polypeptide (black line) modeled into the crystal structure (PDB 3DIN). SecA is in pink, SecY in blue, and SecG and SecE in green. The lateral gate of the SecY channel is in the front. The two-helix finger (THF) of SecA is highlighted in red and a conserved Tyr residue at its tip shown in yellow in stick presentation.

B View from the cytosol. SecA's domains are shown in different colors, and ATP is shown in its binding site. The red star indicates the position of the translocating polypeptide.

C Clamp conformational change. The PPXD from the crystal structure of SecA with an open clamp conformation (PDB 1M74) aligned to SecA in the closed conformation. NBD2 is in blue. The closed PPXD is in yellow and the open conformation is in orange. The arrow indicates the PPXD movement.

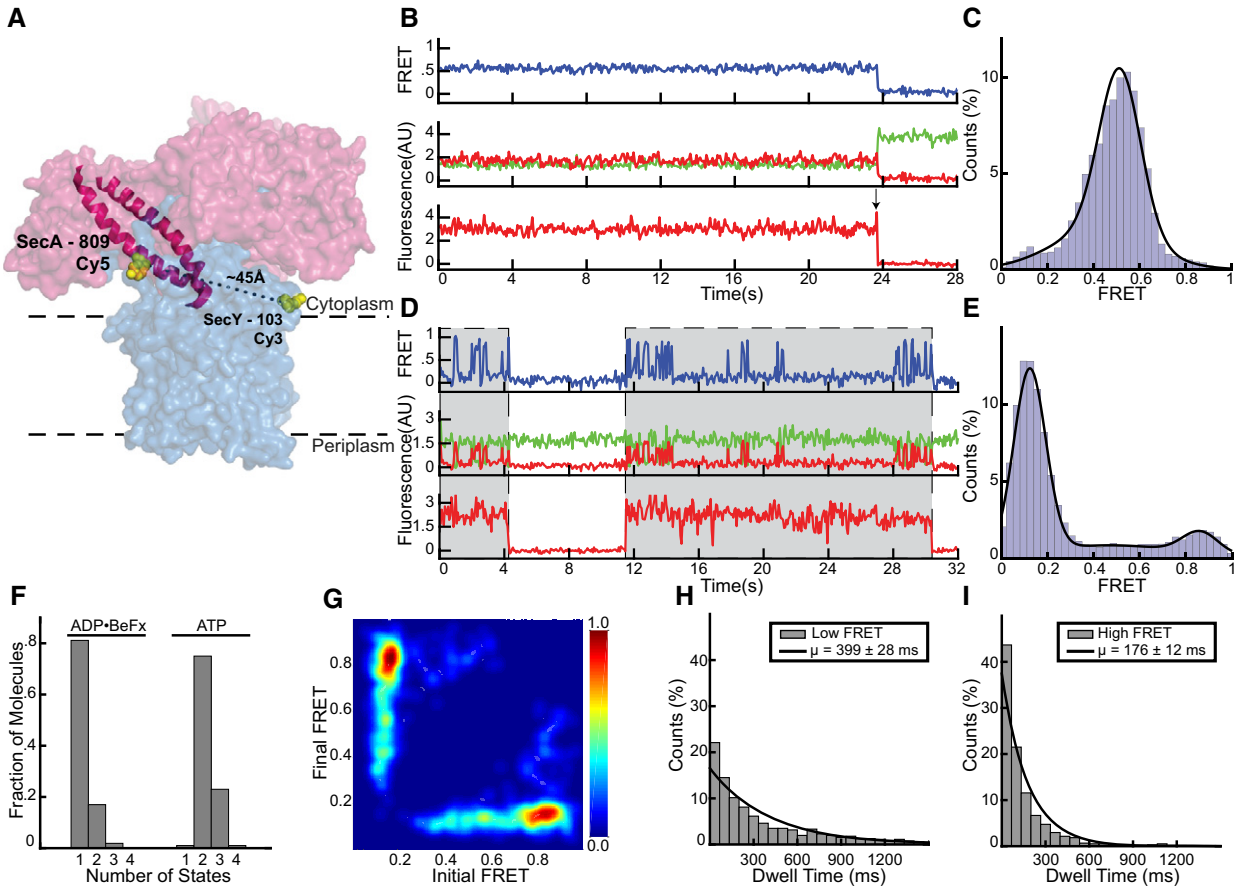

Figure EV2. SecA's two-helix finger movements observed by alternative fluorophore positions.

A Cy5 and Cy3 fluorophores were introduced into the two-helix finger of SecA (PDB 3dIN; red space filling model; helices highlighted) at position 809 and into SecY (blue) at position 103, respectively.

B Representative traces obtained with ADP•BeF_x. The upper FRET trace was calculated from the middle traces obtained by exciting the donor fluorophore and measuring both donor (green) and acceptor (red) fluorescence. The lowest trace was obtained by exciting the acceptor fluorophore directly. The arrow indicates a bleaching event.

C Distribution of FRET values determined from 106 traces as in (B), fit with a Gaussian model (black curve).

D As in (B), but in the presence of ATP. Periods in which a fluorescently labeled SecA molecule is bound are indicated by gray shading.

E As in (C), but with ATP (200 traces).

F Traces obtained in the presence of different nucleotides were used to determine the number of states best fit by the Markov model.

G Transition density plot of idealized ATP FRET states obtained in (F).

H The distribution of dwell times of the low FRET states observed in ATP was fit with exponentials (1,172 low FRET states). The inset shows average dwell time and error, defined as the standard error based on the number of traces.

I As in (H), but with high FRET (1,349 high FRET states).

Source data are available online for this figure.

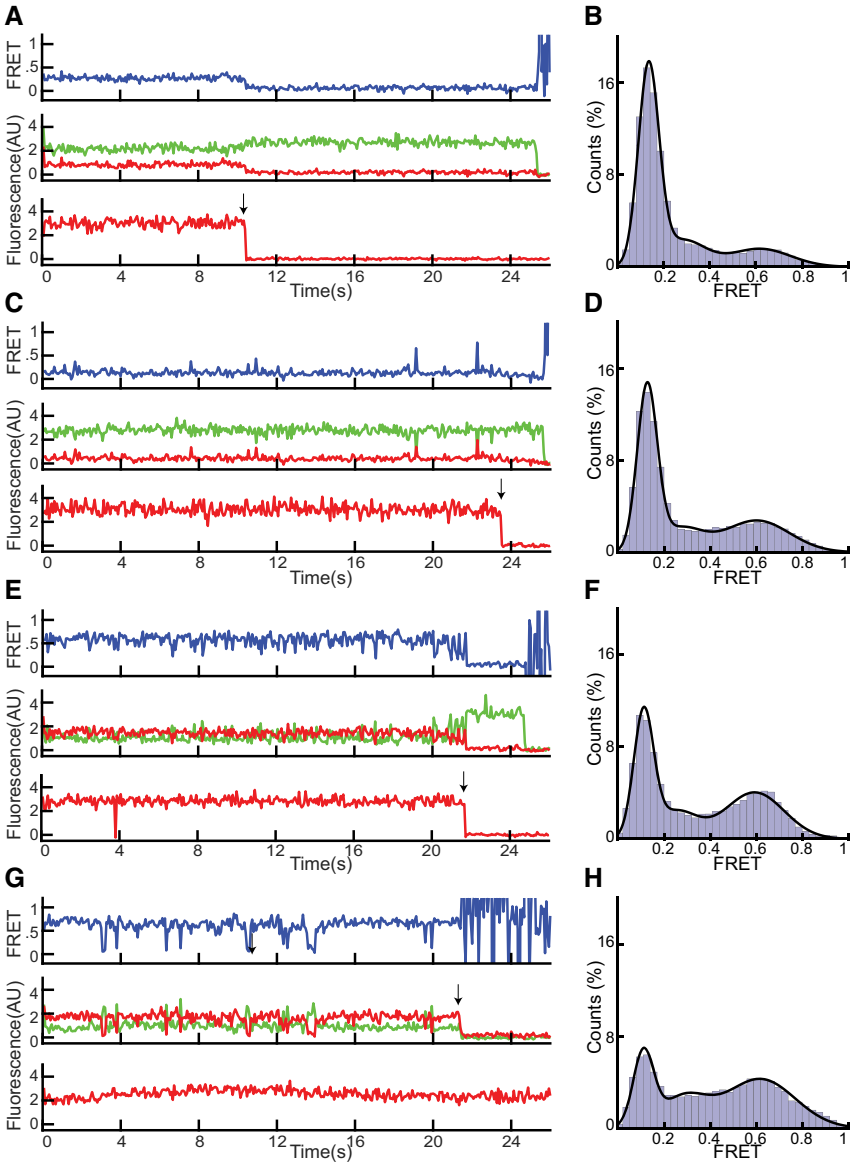

Figure EV3. Representative FRET data for SecA conformations before P_i release.

A Representative traces for the two-helix finger (positions 809 in SecA and 394 in SecY) obtained with ADP•P_i. The upper FRET trace was calculated from the middle traces obtained by exciting the donor fluorophore and measuring both donor (green) and acceptor (red) fluorescence. The lowest trace was obtained by exciting the acceptor fluorophore directly. The arrow indicates a bleaching event.

B Distribution of FRET values determined from 227 traces as in (A) fit with a Gaussian model (black curve).

C As in (A), but with ADP•V_i.

D As in (B), but with ADP•V_i (202 traces).

E As in (A), but for the clamp (positions 233 in SecA and 103 in SecY).

F As in (B), but for the clamp (274 traces).

G As in (A), but for the clamp with ADP•V_i.

H As in (B), but for the clamp with ADP•V_i (157 traces).

Source data are available online for this figure.

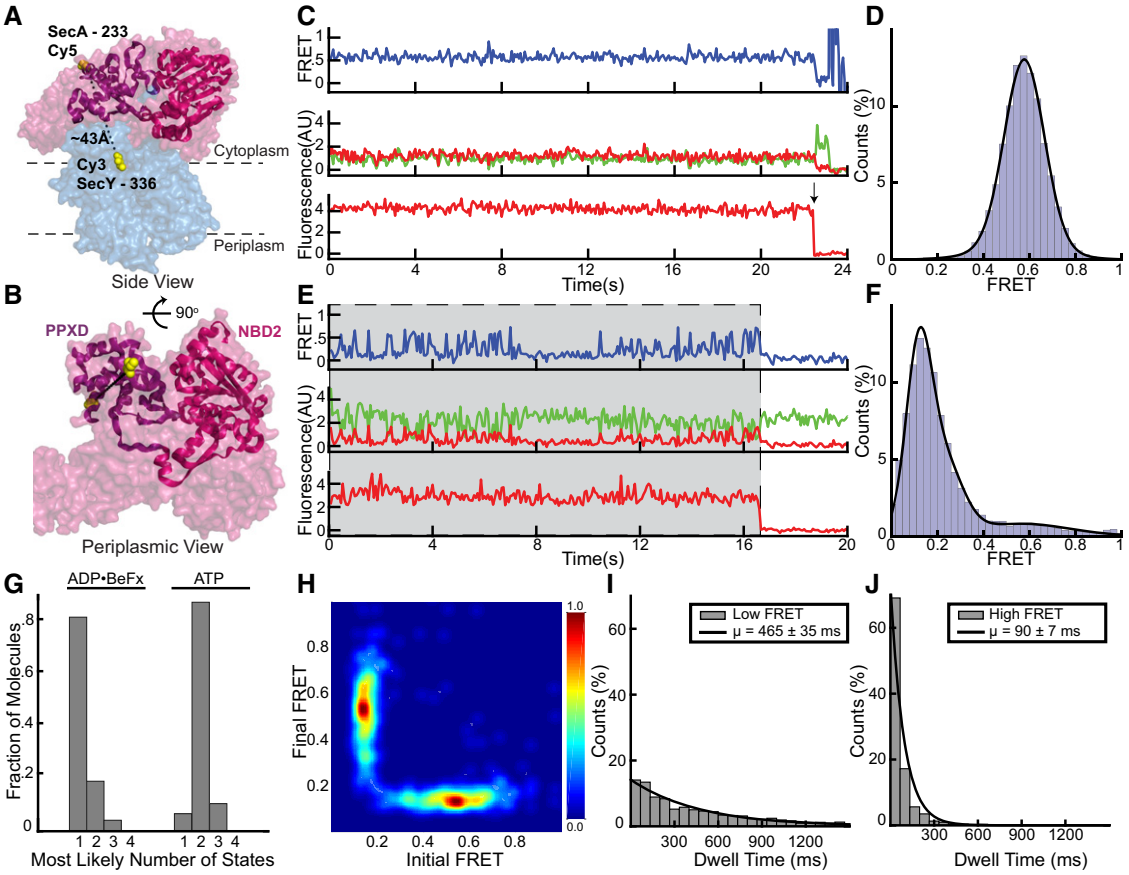

Figure EV4. Clamp movements observed by alternative fluorophore positions.

A Cy5 and Cy3 fluorophores were introduced into the clamp of SecA (PDB 3DIN; red space filling model) at position 233 and into the C-terminal half of SecY (blue) at position 336, respectively. The PPXD and NBD2 making up the clamp are shown as a violet and magenta ribbon models, respectively.

B Rotated view of (A) with SecY masked except for the labeled residue 336.

C Representative traces obtained with ADP•BeF_x. The upper FRET trace was calculated from the middle traces obtained by exciting the donor fluorophore and measuring both donor (green) and acceptor (red) fluorescence. The lowest trace was obtained by exciting the acceptor fluorophore directly. The arrow indicates a bleaching event.

D Distribution of FRET values determined from 163 traces as in (B), fit with a Gaussian model (black curve).

E As in (C), but in the presence of ATP. Periods in which a fluorescently labeled SecA molecule is bound are indicated by gray shading.

F As in (D), but with ATP (178 traces).

G Traces obtained in the presence of different nucleotides were used to determine the number of states best fit by the Markov model.

H Transition density plot of idealized ATP FRET states obtained in (F).

I The distribution of dwell times of the low FRET states observed in ATP was fit with an exponential (1,550 low FRET states). The inset shows average dwell time and error, defined as the standard error based on the number of traces.

J As in (I), but with high FRET (1,778 high FRET states).

Source data are available online for this figure.