Supporting Information

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SI Equations

One-Site Ligand Binding. SecYEG binding to SecA promotes an activation of ATPase activity, and was used as a measure of binding to the steady-state complex (SecA:ADP). The data obtained were fitted using a 1-site ligand binding equation with background, defined as:

$$[B] = \frac{B\ \max{\cdot}[L]}{K_d + [L]} + \text{Background} \qquad [1]$$

where $[B]$ is equal to the amount of ligand bound, Bmax is the total capacity of SecA-ligand, $[L]$ is the ligand concentration and K_d is the dissociation constant for SecA-ligand.

Analysis of ATP Binding. The steady-state kinetics of ATP turnover were fitted according to the Michaelis–Menten equation, defined as:

$$v = \frac{V\ \max{\cdot}[S]}{K_M + [S]} \qquad [2]$$

where v is equal to the measured velocity; K_M is the Michaelis constant; V_{max} is the maximum velocity; $[S]$ is the substrate concentration.

One-site quadratic tight ligand binding. Data for proOmpA binding to SecA:ADP from the measurement of ATPase activity were fitted to a 1-site quadratic tight ligand binding equation (as the affinity for translocation sites was close to the concentration of SecA) with background, defined as:

$$v = V\ \max \frac{[L] + [Eo] + K_d - \sqrt{([L] + [Eo] + K_d)^2 - 4[Eo][L]}}{2[Eo]} + \text{Background} \qquad [3]$$

where v is equal to enzyme velocity, V_{max} is the maximum enzyme velocity, $[L]$ is the total ligand concentration, $[Eo]$ is total SecA concentration, and K_d is the dissociation constant for SecA-ligand.

Single Exponential Plus Steady-State. Data from all transient kinetic ATPase experiments were fitted to a single exponential equation, plus the linear component of the steady-state activity, defined as:

$$A = A_0{\cdot}(1 - e^{-k{\cdot}t}) + m{\cdot}t + \text{offset} \qquad [4]$$

where A is the calculated amplitude, A_0 is the amplitude of the exponential phase (stoichiometry of the first hydrolytic turnover), t is the time, k is the rate constant, m is the slope of the reaction (steady-state rate) and the *offset* is the initial value of the data (0 in some cases).

Single Exponential Equation. Data from the ADP release experiment were fitted to a single exponential equation according to Eq. **4** where $m = 0$, because there is no linear component to this transient.