Addition to the Supporting Information to

Probing Transient Copper Chaperone—Wilson Disease Protein Interactions at the Single-Molecule Level with Nanovesicle Trapping

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Derivation of waiting time distribution for branching processes.

Consider the following generic branching process in which a species, A, is converted to two different species, B and C, with rate constants $k_1$ and $k_2$, respectively.

![Diagram of branching process]

The corresponding single-molecule rate equations are:

$$\frac{dP_A(t)}{dt} = -(k_1 + k_2)P_A(t)$$ (1)

$$\frac{dP_B(t)}{dt} = k_1 P_A(t)$$ (2)

$$\frac{dP_C(t)}{dt} = k_2 P_A(t)$$ (3)

where $P_i(t)$ represents the probability of finding a particular species, $i$, at time $t$. The initial conditions at $t = 0$ are $P_A(0) = 1$, $P_B(0) = 0$, $P_C(0) = 0$, and at anytime $t$, $P_A(t) + P_B(t) + P_C(t) = 1$.

Using the initial conditions, we can solve for $P_A(t)$:

$$P_A(t) = e^{-(k_1+k_2)t}$$ (4)

We can then evaluate the probability density of the time $\tau$ required to complete the A $\rightarrow$ B transition, $f_{A \rightarrow B}(\tau)$, i.e., the probability density of $\tau_{A \rightarrow B}$. The probability for finding a particular $\tau_{A \rightarrow B}$ is $f_{A \rightarrow B}(\tau)\Delta \tau$, which equals the probability of switching from A to B between $t = \tau$ and $\tau + \Delta \tau$, $\Delta P_B(\tau)$. From equation (2), $\Delta P_B(\tau)$ equals $k_1 P_A(\tau) \Delta \tau$. Then,

$$f_{A \rightarrow B}(\tau) = \frac{dP_B(\tau)}{d\tau} = k_1 P_A(\tau)$$ (5)

Similarly, the probability density of $\tau_{A \rightarrow C}$, $f_{A \rightarrow C}(\tau)$, is,

$$f_{A \rightarrow C}(\tau) = \frac{dP_C(\tau)}{d\tau} = k_2 P_A(\tau)$$ (6)
Using equation (4), we have
\[ f_{A\rightarrow B}(\tau) = k_1 e^{-(k_1 + k_2) \tau} \] (7)
\[ f_{A\rightarrow C}(\tau) = k_2 e^{-(k_1 + k_2) \tau} \] (8)

Therefore, both the distribution of \( \tau_{A\rightarrow B} \) and that of \( \tau_{A\rightarrow C} \) follow exponential distribution with the same decay constant of \( k_1 + k_2 \). Note \( \int_0^\infty (f_{A\rightarrow B}(\tau) + f_{A\rightarrow C}(\tau))d\tau = 1 \), as expected.

The individual rate constants, \( k_1 \) and \( k_2 \), are related to the branching ratio, \( R_{br} \), which is defined as the ratio of the number of \( A\rightarrow B \) and \( A\rightarrow C \) transition events:
\[ R_{br} = \frac{N_{A\rightarrow B}}{N_{A\rightarrow C}} = \frac{\int_0^\infty f_{A\rightarrow B}(\tau)d\tau}{\int_0^\infty f_{A\rightarrow C}(\tau)d\tau} = \frac{k_1}{k_2} \] (9)

where \( N_{A\rightarrow B} \) and \( N_{A\rightarrow C} \) are the numbers of observed \( A\rightarrow B \) and \( A\rightarrow C \) transitions, respectively. Using the experimentally determined branching ratios and the decay constants from the waiting time distributions, we can determine the values of both \( k_1 \) and \( k_2 \).

Finally, if \( A \) represents a bimolecular process, such as single-pair protein association in a nanovesicle as in our experiments, both \( k_1 \) and \( k_2 \) in above equations need to be multiplied by the effective concentration of a single molecule in the nanovesicle, as shown in the original Supporting Information.

Revised Figure S6. Compiled histogram of \( E_{FRET} \) trajectories of Hah1-MBD4 interacting pairs (163 trajectories), showing three peaks corresponding to \( E_0 \), \( E_1 \), and \( E_2 \) states. The solid lines are fits with three Gaussian functions centered at \(-0.16 \pm 0.15, 0.55 \pm 0.12, 0.84 \pm 0.12 \). The relative areas of these three peaks represent the relative stabilities of the dissociated state, complex 1, and complex 2; the calculated dissociation constants are \( K_1 \sim 5 \pm 1 \mu M \) and \( K_2 = 8 \pm 2 \mu M \), consistent with those calculated from the kinetic constants.