

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

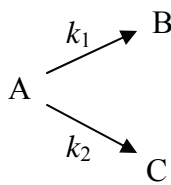
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Derivation of waiting time distribution for branching processes.

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.



The corresponding single-molecule rate equations are:

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

$$\frac{dP_B(t)}{dt} = k_1P_A(t) \quad (2)$$

$$\frac{dP_C(t)}{dt} = k_2P_A(t) \quad (3)$$

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 $P_A(t)$:

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

We can then evaluate the probability density of the time τ 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 $k_1P_A(\tau)\Delta\tau$. Then,

$$f_{A \rightarrow B}(\tau) = \frac{dP_B(\tau)}{d\tau} = k_1P_A(\tau) \quad (5)$$

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

$$f_{A \rightarrow C}(\tau) = \frac{dP_C(\tau)}{d\tau} = k_2P_A(\tau) \quad (6)$$

Using equation (4), we have

$$f_{A \rightarrow B}(\tau) = k_1 e^{-(k_1+k_2)\tau} \quad (7)$$

$$f_{A \rightarrow C}(\tau) = k_2 e^{-(k_1+k_2)\tau} \quad (8)$$

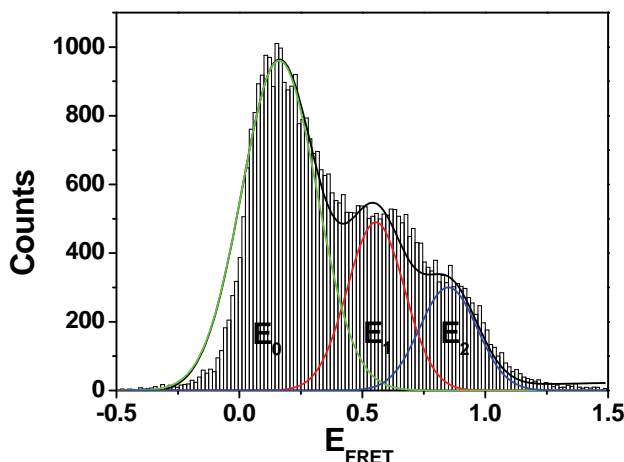
Therefore, both the distribution of $\tau_{A \rightarrow B}$ and that of $\tau_{A \rightarrow C}$ follow exponential distribution with the same decay constant of k_1+k_2 . Note $\int_0^\infty (f_{A \rightarrow B}(\tau) + f_{A \rightarrow C}(\tau))d\tau = 1$, as expected.

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:

$$R_{br} = \frac{N_{A \rightarrow B}}{N_{A \rightarrow C}} = \frac{\int_0^\infty f_{A \rightarrow B}(\tau)d\tau}{\int_0^\infty f_{A \rightarrow C}(\tau)d\tau} = \frac{k_1}{k_2} \quad (9)$$

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 $\sim 0.16 \pm 0.15$, 0.55 ± 0.12 , 0.84 ± 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 $K_1 \sim 5 \pm 1 \mu\text{M}$ and $K_2 = 8 \pm 2 \mu\text{M}$, consistent with those calculated from the kinetic constants.