

Probing Transient Copper Chaperone–Wilson Disease Protein Interactions at the Single-Molecule Level with Nanovesicle Trapping [*J. Am. Chem. Soc.* **2008**, *130*, 2446–2447]. Jaime J. Benítez, Aaron M. Keller, Patrick Ochieng, Liliya A. Yatsunyk, David L. Huffman,* Amy C. Rosenzweig, and Peng Chen*

The distributions of the individual waiting times in Figure 2D were analyzed incorrectly. In the protein interaction scheme (Figure 2C), each of the E_{FRET} states (E_0 , E_1 , and E_2) branches directly to two other states. The decay constant from each of the six waiting time distributions (i.e., $\tau_{0\rightarrow1}$, $\tau_{0\rightarrow2}$, $\tau_{1\rightarrow0}$, $\tau_{1\rightarrow2}$, $\tau_{2\rightarrow0}$, and $\tau_{2\rightarrow1}$) does not directly correspond to a particular kinetic constant in the interaction scheme, but instead is the sum of the rate constants of the two kinetic processes that branch from the same state (i.e., E_0 , E_1 , or E_2). The individual rate constants can subsequently be determined using the ratios of the number of transition events for each kinetic process. The relations between the decay constants of the waiting time distributions and the rate constants are given in the revised Figure 2D below; their derivations are in the additional Supporting Information. The decay constants of the $\tau_{0\rightarrow1}$ and

$\tau_{0\rightarrow2}$ distributions should be the same, as should those of the $\tau_{1\rightarrow0}$ and $\tau_{1\rightarrow2}$ distributions and those of the $\tau_{2\rightarrow0}$ and $\tau_{2\rightarrow1}$ distributions. By omitting the first bin in each waiting time distribution, which is often inaccurate due to limited time resolution, all six waiting time distributions can be fitted consistently with single-exponential decay functions (Figure 2D). The results give $k_1 = (1.6 \pm 0.2) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, $k_{-1} = 0.88 \pm 0.04 \text{ s}^{-1}$, $k_2 = (1.4 \pm 0.2) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, $k_{-2} = 1.3 \pm 0.1 \text{ s}^{-1}$, $k_3 = 0.42 \pm 0.04 \text{ s}^{-1}$, and $k_{-3} = 0.7 \pm 0.1 \text{ s}^{-1}$. From these rate constants, we can also obtain the dissociation constants for the two interaction complexes with $K_1 = 5.6 \pm 0.6 \mu\text{M}$ and $K_2 = 9 \pm 1 \mu\text{M}$ (see also revised Figure S6 in the Supporting Information). Except for the quantitative values of the kinetic parameters listed here, this correction does not affect any other conclusions in our study. We thank Taekjip Ha for alerting us to the error.

Supporting Information Available: Derivation of waiting time distribution for branching processes and revised Figure S6. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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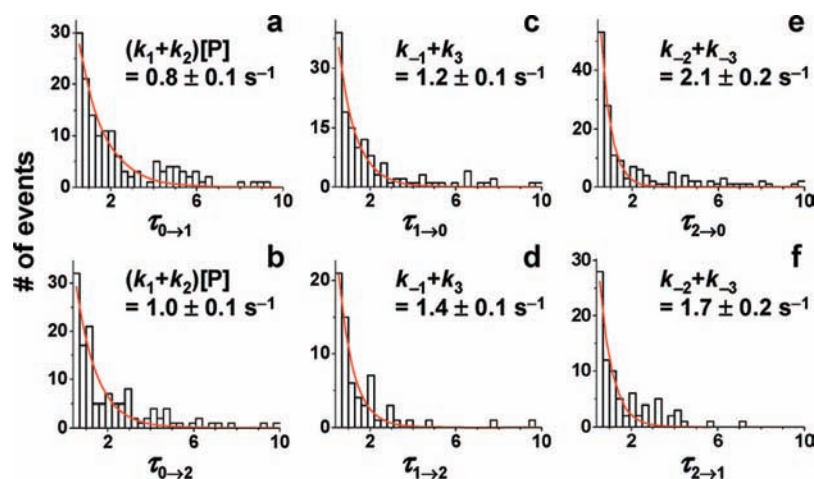


Figure 2. (D) Distributions of the waiting times before each $E_0 \rightarrow E_1$ transition ($\tau_{0\rightarrow1}$, a), $E_0 \rightarrow E_2$ transition ($\tau_{0\rightarrow2}$, b), $E_1 \rightarrow E_0$ transition ($\tau_{1\rightarrow0}$, c), $E_1 \rightarrow E_2$ transition ($\tau_{1\rightarrow2}$, d), $E_2 \rightarrow E_0$ transition ($\tau_{2\rightarrow0}$, e), and $E_2 \rightarrow E_1$ transition ($\tau_{2\rightarrow1}$, f). Solid lines are exponential fits; insets give the decay constants of the exponential fits and the relations to the protein interaction rate constants in Figure 2C (see the original paper). [P] is the effective concentration ($\sim 3 \mu\text{M}$) of a single protein molecule in the nanovesicle.