

Dynamic Properties of a Type II Cadherin Adhesive

Domain: Implications for the Mechanism of

Strand-Swapping of Classical Cadherins

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Supplemental Experimental Procedures

ZZ-exchange

To improve the precision in determination of kinetic rate constants a quadratic approximation to a ratio of cross-peak and auto-peak amplitudes is used. The major advantage of this approach is that differences in R_1 relaxation rate constants during the mixing period, transfer efficiency differences during the preparation period, and linewidth differences during the detection period, of the ZZ-exchange experiment are suppressed. The ratio does not depend on initial intensities of individual states and hence lends itself to global fitting of multiple residues. Individual forward and reverse rate constants are obtained by supplying relative populations, obtained from a fully relaxed HSQC experiment.

The general description for the evolution for the auto and cross – peaks under R' is decomposed,

$$\hat{M}(t) = e^{-Rt} \hat{M}(0) = e^{-Dt} e^{-(R'-D)t} \hat{M}(0) = e^{-Dt} e^{-R^*t} \hat{M}(0) \quad [\text{S.1}]$$

$$D = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} (\bar{p} + k_{ex}/2) \quad [\text{S.2}]$$

$$\begin{aligned} R^* &= \begin{bmatrix} p_1 + k_1 & -k_{-1} \\ -k_1 & p_2 + k_{-1} \end{bmatrix} - \begin{bmatrix} \bar{p} + k_{ex}/2 & 0 \\ 0 & \bar{p} + k_{ex}/2 \end{bmatrix} \\ &= \begin{bmatrix} -\frac{1}{2}\Delta + \frac{1}{2}(k_1 - k_{-1}) & -k_{-1} \\ -k_1 & \frac{1}{2}\Delta - \frac{1}{2}(k_1 - k_{-1}) \end{bmatrix} \quad [\text{S.3}] \end{aligned}$$

$$\bar{p} = (p_1 + p_2)/2$$

$$\Delta = p_2 - p_1$$

In the above k_1 and k_{-1} are the forward and reverse kinetic rate constants, respectively, and p_x are the R_1 relaxation rate constants for site X. The characteristic rate for the reaction, k_{ex} is the sum of forward and reverse rates. Expansion to second order,

$$e^{-R^*t} = E - R^*t + \frac{t^2}{2}R^*R^* + O(t^3) \quad [S.4]$$

with,

$$R^*R^* = \begin{bmatrix} (\frac{1}{2}\Delta - \frac{1}{2}(k_1 - k_{-1}))^2 + k_1k_{-1} & 0 \\ 0 & (\frac{1}{2}\Delta - \frac{1}{2}(k_1 - k_{-1}))^2 + k_1k_{-1} \end{bmatrix} \quad [S.5]$$

leads to the following evolutions under R^* for the individual resonances,

$$M_{11}(t) = M_1(0) \left[1 + (\frac{1}{2}\Delta - \frac{1}{2}(k_1 - k_{-1}))t + ((\frac{1}{2}\Delta - \frac{1}{2}(k_1 - k_{-1}))^2 + k_1k_{-1})\frac{t^2}{2} \right] \quad [S.6]$$

$$M_{22}(t) = M_2(0) \left[1 - (\frac{1}{2}\Delta - \frac{1}{2}(k_1 - k_{-1}))t + ((\frac{1}{2}\Delta - \frac{1}{2}(k_1 - k_{-1}))^2 + k_1k_{-1})\frac{t^2}{2} \right] \quad [S.7]$$

$$M_{21}(t) = M_1(0)k_1t \quad [S.8]$$

$$M_{12}(t) = M_2(0)k_{-1}t \quad [S.9]$$

The products of auto and cross – peak resonances to second order,

$$M_{11}(t)M_{22}(t) = M_1(0)M_2(0)[1 + k_1k_{-1}t^2] \quad [S.10]$$

$$M_{12}(t)M_{21}(t) = M_1(0)M_2(0)[k_1k_{-1}t^2] \quad [S.11]$$

motivate the ratio $\Xi(t)$, where all the factors of the full expression containing e^{-Dt} cancel,

$$\Xi(t) = \frac{M_{12}(t)M_{21}(t)}{M_{11}(t)M_{22}(t) - M_{12}(t)M_{21}(t)} = k_1k_{-1}t^2 \quad [S.12]$$

Alternatively, the exact ratio is given by,

$$\Xi_{exact}(t) = \frac{4k_1k_{-1} \text{Sinh}^2 \left[\frac{1}{2}t\sqrt{k_{ex}^2 - 2\Delta(k_1 - k_{-1}) + \Delta^2} \right]}{k_{ex}^2 - 2\Delta(k_1 - k_{-1}) + \Delta^2} \quad [S.13]$$

Series expansion shows that $\Xi(t)$ does not depend on state specific R_1 relaxation rates up to third order in time. Transfer efficiency differences between individual states are cancelled by the ratio, thus removing a source of error in systems which have significant state specific differences in R_2 .

The specific case of the monomer dimer equilibrium obeys the following differential equations, where the factors of two account for transfer of 2 spins / molecule between each state (given 100 % isotopic labeling). The amplitude of the resonance representing transfer from state X to state Y is $a_{YX}(t)$, the kinetic on-rate and off-rate are k_{on} and k_{off} respectively, and $[M]$ is the equilibrium monomer concentration.

$$\begin{aligned}
 \frac{da_{DD}(t)}{dt} &= -2k_{off}a_{DD}(t) + 2k_{on}[M]a_{MD}(t) \\
 \frac{da_{MD}(t)}{dt} &= 2k_{off}a_{DD}(t) - 2k_{on}[M]a_{MD}(t) \\
 \frac{da_{MM}(t)}{dt} &= -2k_{off}a_{MM}(t) + 2k_{on}[M]a_{DM}(t) \\
 \frac{da_{DM}(t)}{dt} &= 2k_{off}a_{MM}(t) - 2k_{on}[M]a_{DM}(t) \\
 a_{DM}(0) &= a_{MD}(0) = 0
 \end{aligned}
 \tag{S.14}$$

The quadratic ratio expression is,

$$\Xi(t) = \zeta t^2 = 4k_{off}k_{on}[M]t^2
 \tag{S.15}$$