

Supporting Information 1

**Rotational and translational diffusion of biomolecules in complex
liquids and HeLa cells**

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ROTATIONAL DIFFUSION COUPLED TO A FIRST-ORDER REACTION

A. The Model

We consider a spherical nanoparticle with a fluorescent dye attached to its surface. The dye is represented by an electric dipole moment, the orientation of which can be normal or tangential to the particle's surface. We assume that the orientation of the dye can change as a result of a chemical reaction



where the A and B species represent nanoparticles with normal and tangential dye orientation, respectively. The reaction rate constants for the forward and backward reactions are k_+ and k_- . We consider rotational diffusion coupled to reaction (1) via the reaction-diffusion equation:

$$\frac{\partial \delta c_A}{\partial t} = -D \hat{J}^2 \delta c_A - k_+ \delta c_A + k_- \delta c_B, \quad (2a)$$

$$\frac{\partial \delta c_B}{\partial t} = -D \hat{J}^2 \delta c_B + k_+ \delta c_A - k_- \delta c_B, \quad (2b)$$

where D is the rotational diffusion coefficient and \hat{J}^2 is the square of the angular momentum operator. δc_i , for $i = A, B$, depends on time and Euler angles ϕ , θ and ψ , denoted Ω for brevity, as follows: $\delta c_i(\Omega, t) = P(\Omega, t) \delta C_i(t)$. $P(\Omega, t)$ is the probability density of finding the particle's principal axes rotated by Ω with respect to the laboratory frame, whereas $\delta C_i(t) = \int d\Omega \delta c_i(\Omega, t)$ is the deviation of the concentration of component i from its equilibrium value \bar{C}_i . Then Eq. (2) is decomposed into the rotational diffusion equation [1, 2]

$$\frac{\partial P}{\partial t} = -D \hat{J}^2 P \quad (3)$$

and the reaction equation

$$\frac{\partial \delta C_A}{\partial t} = -k_+ \delta C_A + k_- \delta C_B, \quad (4a)$$

$$\frac{\partial \delta C_B}{\partial t} = k_+ \delta C_A - k_- \delta C_B. \quad (4b)$$

The solution of Eq. (3) that satisfies the initial condition $P(\Omega, 0)$ is

$$P(\Omega, t) = \int d\Omega' G(\Omega, \Omega', t) P(\Omega', 0), \quad (5)$$

where

$$G(\Omega, \Omega', t) = \frac{1}{8\pi^2} \sum_{l=0}^{\infty} (2l+1) e^{-Dl(l+1)t} \sum_{k,m=-l}^l D_{km}^l(\Omega) D_{km}^{l*}(\Omega'), \quad (6)$$

is the Green's function, and $D_{km}^l(\Omega)$ are the Wigner rotation matrices [3]. They satisfy orthogonality relations

$$\int_0^\pi \sin \theta d\theta \int_0^{2\pi} d\phi \int_0^{2\pi} d\psi D_{km}^{l*}(\phi, \theta, \psi) D_{k'm'}^l(\phi, \theta, \psi) = \frac{8\pi^2}{2l+1} \delta_{l,l'} \delta_{k,k'} \delta_{m,m'}. \quad (7)$$

When $t = 0$, the Green's function is equal to the Dirac function $\delta(\Omega, \Omega')$.

The solution of Eq. (4) that satisfies the initial conditions $\delta C_A(0)$ and $\delta C_B(0)$ is

$$\delta C_A(t) = \frac{1}{R} [(k_- + k_+ e^{-Rt}) \delta C_A(0) + k_- (1 - e^{-Rt}) \delta C_B(0)], \quad (8a)$$

$$\delta C_B(t) = \frac{1}{R} [k_+ (1 - e^{-Rt}) \delta C_A(0) + (k_+ + k_- e^{-Rt}) \delta C_B(0)], \quad (8b)$$

where $R = k_+ + k_-$ is the chemical relaxation rate. Finally, correlation functions, defined as $\langle \delta c_i(\Omega, t) \delta c_j(\Omega', 0) \rangle$, are expressed as follows

$$\langle \delta c_i(\Omega, t) \delta c_j(\Omega', 0) \rangle = G(\Omega, \Omega', t) \langle \delta C_i(t) \delta C_j(0) \rangle, \quad (9)$$

where $\langle \rangle$ is the ensemble average. Concentration fluctuations depend on time and the position vector and are subject to Poisson statistics. Since the initial positions of different molecules are not correlated, zero-time correlations are

$$\langle \delta C_i(\mathbf{r}, 0) \delta C_j(\mathbf{r}', 0) \rangle = \bar{C}_i \delta_{ij} \delta(\mathbf{r} - \mathbf{r}'), \quad (10)$$

where \bar{C}_i is the average concentration [4]. The $\langle \delta C_i(\mathbf{r}, t) \delta C_j(\mathbf{r}', 0) \rangle$ correlations contribute to the autocorrelation function for translational diffusion, which is much slower than rotational diffusion. Therefore, for the rotational diffusion time scale, we can replace the local concentration fluctuations with their average over the observation volume V , i.e. $\delta C_i(t) = V^{-1} \int_V d^3r \delta C(\mathbf{r}, t)$. Consequently, Eq. (10) should be replaced with the initial condition $\langle \delta C_i(0) \delta C_j(0) \rangle = \bar{C}_i \delta_{ij} / V$. Using the last relation in Eq. (8) gives the autocorrelation and cross-correlation functions

$$\langle \delta C_A(t) \delta C_A(0) \rangle = \frac{\bar{C}_A}{VR} (k_- + k_+ e^{-Rt}) = \frac{\bar{C}_A}{V(1+K)} (1 + K e^{-Rt}), \quad (11a)$$

$$\langle \delta C_B(t) \delta C_B(0) \rangle = \frac{\bar{C}_B}{VR} (k_+ + k_- e^{-Rt}) = \frac{\bar{C}_B}{V(1+K)} (K + e^{-Rt}), \quad (11b)$$

$$\langle \delta C_A(t) \delta C_B(0) \rangle = \frac{\bar{C}_B}{VR} k_- (1 - e^{-Rt}) = \frac{\bar{C}_B}{V(1+K)} (1 - e^{-Rt}), \quad (11c)$$

$$\langle \delta C_B(t) \delta C_A(0) \rangle = \frac{\bar{C}_A}{VR} k_+ (1 - e^{-Rt}) = \frac{\bar{C}_A}{V(1+K)} (1 - e^{-Rt}), \quad (11d)$$

where $K = k_+/k_-$ is the reaction (1) equilibrium constant.

B. FCS correlation function

We need to calculate the correlation functions resulting from rotational diffusion of species A and B to apply the model described in Sec. A for measurements using fluorescence correlation spectroscopy (FCS). It is convenient to define A and B in terms of two different dipole orientation distributions, $V_A(\chi, \zeta)$ and $V_B(\chi, \zeta)$, where the polar angle χ and azimuthal angle ζ are determined in the system of the particle's principal axes. In general, $V_i(\chi, \zeta)$, $i = A, B$, can be expanded in spherical harmonics $Y_{lm}(\chi, \zeta)$:

$$V_i(\chi, \zeta) = \sum_{l=0}^{\infty} \sum_{m=-l}^l v_{lm}^i Y_{lm}(\chi, \zeta), \quad (12)$$

where

$$Y_{lm}(\chi, \zeta) = (-1)^m \sqrt{\frac{(2l+1)(l-m)!}{4\pi(l+m)!}} P_l^m(\cos \chi) e^{im\zeta}, \quad (13)$$

if $m \geq 0$ and $Y_{lm}(\chi, \zeta) = (-1)^m Y_l^{-m*}(\chi, \zeta)$ if $m < 0$. Hence, the expansion coefficients are

$$v_{lm}^i = \int_0^\pi \sin \chi d\chi \int_0^{2\pi} d\zeta V_i(\chi, \zeta) Y_{lm}^*(\chi, \zeta). \quad (14)$$

In order to express V_i in the lab frame, spherical harmonics must be rotated by Ω , which gives

$$V_i(\chi, \zeta | \Omega) = \sum_{l=0}^{\infty} \sum_{m=-l}^l v_{lm}^i \sum_{k=-l}^l D_{km}^l(\Omega) Y_{lk}(\chi, \zeta). \quad (15)$$

We then consider the probabilities of excitation and detection of a photon with polarization parallel (\parallel) or orthogonal (\perp) to the excitation polarization, where the latter is oriented along the x -axis of the lab frame. These probabilities are proportional to the functions $U_i^\parallel(\Omega)$ and $U_i^\perp(\Omega)$, respectively, where [1, 2]

$$U_i^\parallel(\Omega) = \int_0^\pi \sin \chi d\chi \int_0^{2\pi} d\zeta \sin^4 \chi \cos^4 \zeta V_i(\chi, \zeta | \Omega), \quad (16a)$$

$$U_i^\perp(\Omega) = \int_0^\pi \sin \chi d\chi \int_0^{2\pi} d\zeta \sin^4 \chi \cos^2 \zeta \sin^2 \zeta V_i(\chi, \zeta | \Omega). \quad (16b)$$

In the case of non-polarised detection studied in this work, U_i^\parallel and U_i^\perp are replaced by their sum

$$U_i(\Omega) = U_i^\parallel(\Omega) + U_i^\perp(\Omega) = \int_0^\pi \sin \chi d\chi \int_0^{2\pi} d\zeta \sin^4 \chi \cos^2 \zeta V_i(\chi, \zeta | \Omega). \quad (17)$$

Inserting Eq. (15) into Eq. (17) gives

$$U_i(\Omega) = \sum_{l=0}^{\infty} \sum_{m=-l}^l v_{lm}^i \sum_{k=-l}^l u_{lk} D_{km}^l(\Omega), \quad (18)$$

where

$$u_{lk} = \int_0^\pi \sin^5 \chi d\chi \int_0^{2\pi} d\zeta \cos^2 \zeta Y_{lk}(\chi, \zeta). \quad (19)$$

The only non-zero coefficients are

$$u_{0,0} = \frac{8}{15}\sqrt{\pi}, \quad u_{2,0} = -\frac{16}{21}\sqrt{\frac{\pi}{5}}, \quad u_{2,\pm 2} = \frac{2}{7}\sqrt{\frac{6\pi}{5}}, \quad (20a)$$

$$u_{4,0} = \frac{8}{105}\sqrt{\pi}, \quad u_{4,\pm 2} = -\frac{2}{21}\sqrt{\frac{2\pi}{5}}. \quad (20b)$$

Then we define the correlation functions

$$\begin{aligned} g_{ij}(t) &= \int d\Omega \int d\Omega' U_i(\Omega) G(\Omega, \Omega', t) U_j(\Omega') = \frac{1}{8\pi^2} \sum_{l=0}^{\infty} (2l+1) e^{-Dl(l+1)t} \\ &\times \sum_{k,m=-l}^l \int d\Omega D_{km}^l(\Omega) U_i(\Omega) \int d\Omega' D_{km}^{l*}(\Omega') U_j(\Omega'). \end{aligned} \quad (21)$$

Since

$$\int d\Omega' D_{km}^{l*}(\Omega') U_j(\Omega') = \frac{8\pi^2}{2l+1} v_{lm}^j u_{lk}, \quad (22)$$

we get

$$g_{ij}(t) = \sum_{l=0}^4 \frac{8\pi^2}{2l+1} e^{-Dl(l+1)t} \sum_{k=-l}^l |u_{lk}|^2 \sum_{m=-l}^l v_{lm}^{i*} v_{lm}^j, \quad (23)$$

where

$$|u_{0,0}|^2 = \pi \left(\frac{8}{15} \right)^2, \quad (24a)$$

$$\sum_{k=-2}^2 |u_{2,k}|^2 = \frac{\pi}{5} \left(\frac{16}{21} \right)^2 + \frac{12\pi}{5} \left(\frac{2}{7} \right)^2 = \pi \left(\frac{8}{15} \right)^2 \frac{215}{196}, \quad (24b)$$

$$\sum_{k=-2}^2 |u_{4,k}|^2 = \pi \left(\frac{8}{105} \right)^2 + \frac{4\pi}{5} \left(\frac{2}{21} \right)^2 = \pi \left(\frac{8}{15} \right)^2 \frac{9}{196}. \quad (24c)$$

Finally, the functions $g_{ij}(t)$ are expressed in terms of the rotational diffusion coefficient D and the coefficients v_{lm}^A and v_{lm}^B as follows

$$g_{ij}(t) = (2\pi)^3 \left(\frac{8}{15} \right)^2 \left(v_{00}^{i*} v_{00}^j + \frac{43}{196} e^{-6Dt} \sum_{m=-2}^2 v_{2m}^{i*} v_{2m}^j + \frac{1}{196} e^{-20Dt} \sum_{m=-4}^4 v_{4m}^{i*} v_{4m}^j \right). \quad (25)$$

According to our model, the dye orientation is normal to the nanoparticle surface for species A and tangent to it for species B . Therefore, the dipole orientation distributions for $i = A, B$ are

$$V_i(\chi, \zeta) = \frac{\delta(\chi - \chi_i)}{2\pi \sin \chi}, \quad (26)$$

where $\delta(\chi - \chi_i)$ is the Dirac δ -function, $\chi_A = 0$ and $\chi_B = \pi/2$. In the case of A , the dipole is parallel the z -axis of the particle, and in the case of B , any orientation in the xy plane is equally probable. Using Eqs. (14) and (26) gives

$$v_{lm}^A = \sqrt{\frac{2l+1}{4\pi}} P_l(1) \delta_{m,0}, \quad v_{lm}^B = \sqrt{\frac{2l+1}{4\pi}} P_l(0) \delta_{m,0}, \quad (27)$$

where $P_l(\cos \chi)$ are the Legendre polynomials, hence

$$v_{00}^A = \frac{1}{\sqrt{4\pi}}, \quad v_{20}^A = \sqrt{\frac{5}{4\pi}}, \quad v_{40}^A = \sqrt{\frac{9}{4\pi}} \quad (28)$$

$$v_{00}^B = \frac{1}{\sqrt{4\pi}}, \quad v_{20}^B = -\frac{1}{2} \sqrt{\frac{5}{4\pi}}, \quad v_{40}^B = \frac{3}{8} \sqrt{\frac{9}{4\pi}}. \quad (29)$$

Then, we insert these values into Eq. (25) to get

$$g_{AA}(t) = 2\pi^2 \left(\frac{8}{15}\right)^2 \left(1 + \frac{215}{196} e^{-6Dt} + \frac{9}{196} e^{-20Dt}\right), \quad (30a)$$

$$g_{BB}(t) = 2\pi^2 \left(\frac{8}{15}\right)^2 \left[1 + \left(\frac{1}{2}\right)^2 \frac{215}{196} e^{-6Dt} + \left(\frac{3}{8}\right)^2 \frac{9}{196} e^{-20Dt}\right], \quad (30b)$$

$$g_{AB}(t) = g_{BA}(t) = 2\pi^2 \left(\frac{8}{15}\right)^2 \left[1 - \left(\frac{1}{2}\right) \frac{215}{196} e^{-6Dt} + \left(\frac{3}{8}\right) \frac{9}{196} e^{-20Dt}\right]. \quad (30c)$$

Another possibility for species B , not discussed here, is that the dipole could be oriented in the direction $+x$ or $-x$ with the same probability. Finally, the FCS autocorrelation function, $G^{FCS}(t)$, consists of products of $\langle \delta C_i(t) \delta C_j(0) \rangle$ given by Eq. (11) and $g_{ij}(t)$ given by Eq. (30), i.e.

$$\begin{aligned} G^{FCS}(t) &\propto Q_A^2 \langle \delta C_A(t) \delta C_A(0) \rangle g_{AA}(t) + 2Q_A Q_B \langle \delta C_A(t) \delta C_B(0) \rangle g_{AB}(t) \\ &\quad + Q_B^2 \langle \delta C_B(t) \delta C_B(0) \rangle g_{BB}(t). \end{aligned} \quad (31)$$

Q_A and Q_B characterize the fluorescence of the dye in the states A and B , respectively, and the proportionality constant has been omitted in Eq. (31).

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