Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary information

Analytical form of the autocorrelation function for the Fluorescence Correlation Spectroscopy

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S.1 Derivation of the approximate autocorrelation function

Here we present the derivation of Eq. (13) in the main text. First, Eqs. (3) in the main text are expressed in the Fourier representation:

$$\begin{aligned}\frac{\partial \delta \widetilde{C}_A}{\partial t} &= -\left(Dq^2 + k_+[B]^{eq}\right)\delta\widetilde{C}_A - k_+[A]^{eq}\delta\widetilde{C}_B + k_-\delta\widetilde{C}_C,\\ \frac{\partial \delta \widetilde{C}_B}{\partial t} &= -\left(D_Bq^2 + k_+[A]^{eq}\right)\delta\widetilde{C}_B - k_+[B]^{eq}\delta\widetilde{C}_A + k_-\delta\widetilde{C}_C,\\ \frac{\partial \delta \widetilde{C}_C}{\partial t} &= -\left(Dq^2 + k_-\right)\delta\widetilde{C}_C + k_+[B]^{eq}\delta\widetilde{C}_A + k_+[A]^{eq}\delta\widetilde{C}_B,\end{aligned} \tag{1}$$

where $\delta\widetilde{C}_A$, $\delta\widetilde{C}_B$, $\delta\widetilde{C}_C$ are functions of the wave vector $\vec{q}$ and time t, and we have assumed that $D_A = D_C = D$. The normal modes for Eqs. (1) are characterized by three eigenvalues:[1,2]

$$\lambda_0 = -q^2 D, \quad \lambda_\pm = -\frac{1}{2}\left[q^2(D + D_B) + R\right] \pm \frac{1}{2}\sqrt{q^4|\Delta|^2 + 2\epsilon q^2|\Delta|R + R^2}, \tag{2}$$

and by the corresponding eigenvectors:

$$\mathbf{X}_0 = \begin{pmatrix} 1 \\ 0 \\ K[B]^{eq} \end{pmatrix}, \quad \mathbf{X}_\pm = \begin{pmatrix} 1 \\ F_\pm \\ -1 \end{pmatrix}, \tag{3}$$

where $R = k_+([A]^{eq} + [B]^{eq}) + k_-$ is the relaxation rate, $K = k_+/k_-$ is the equilibrium constant, $\Delta = D - D_B < 0$, $\beta = k_+[A]^{eq}/R$, $\epsilon = 2\beta - 1$ and $F_\pm = (q^2D + \lambda_\pm)/(q^2D_B + \lambda_\pm)$. The relevant components of the matrix Z_{jl} (see Eqs. (7)–(10) in the main text) are:

$$\begin{aligned} Z_{BB} &= (F_- e^{t\lambda_-} - F_+ e^{t\lambda_+})/(F_- - F_+),\\ Z_{CC} &= y e^{t\lambda_0} + (1 - y)(F_- e^{t\lambda_+} - F_+ e^{t\lambda_-})/(F_- - F_+),\\ Z_{BC} &= (e^{t\lambda_+} - e^{t\lambda_-})/(F_- - F_+),\end{aligned} \tag{4}$$

where $y = K[B]^{eq}/(1 + K[B]^{eq})$. Next, using the identities:

$$\begin{aligned} F_- - F_+ &= \sqrt{q^4|\Delta|^2 + 2\epsilon q^2|\Delta|R + R^2}/(R\beta),\\ F_+ + F_- &= (q^2|\Delta| + R\epsilon)/(R\beta),\end{aligned} \tag{5}$$

and assuming $Q_B = Q_C = Q$, we express the function $g(q,t)$, given by the general formula: $g = Q_B^2 [B]^{eq} Z_{BB} + 2Q_B Q_C [B]^{eq} Z_{BC} + Q_C^2 [C]^{eq} Z_{CC}$ (see Eq. (10) in the main text), as follows

$$g(q,t) = Q^2 \left\{ y[C]^{eq} e^{t\lambda_0} + \frac{[B]^{eq}}{2(1-\beta)} \left[\chi \left(e^{t\lambda_+} - e^{t\lambda_-} \right) + e^{t\lambda_+} + e^{t\lambda_-} \right] \right\}, \tag{6}$$

where

$$\chi = (R + q^2|\Delta|\epsilon)/\sqrt{q^4|\Delta|^2 + 2\epsilon q^2|\Delta|R + R^2}. \tag{7}$$

Deriving Eq. (6), we have used the identity

$$\beta[B]^{eq} = (1-y)(1-\beta)[C]^{eq}. \tag{8}$$

It follows from the relations: $\beta = K[A]^{eq}\{1+K([A]^{eq}+[B]^{eq})\}^{-1}$ and $K[A]^{eq}[B]^{eq} = [C]^{eq}$, hence $(1-y)(1-\beta) = \{1+K([A]^{eq}+[B]^{eq})\}^{-1} = \beta[B]^{eq}/[C]^{eq}$.

Then the autocorrelation function (see Eq. (6) in the main text) normalized to unity at $t = 0$ assumes the following form

$$G(t) = \frac{y\beta}{1-y+y\beta} h(t/\tau_A) + \frac{1-y}{2(1-y+y\beta)} \left(J_+[\chi,t] - J_-[\chi,t] + J_+[1,t] + J_-[1,t] \right) \tag{9}$$

where

$$J_\pm[\mathcal{A},t] = \Omega \int d^3q\, e^{-\frac{1}{4}(H^2 q_z^2 + L^2 q_\perp^2)} \mathcal{A}(q^2) e^{t\lambda_\pm(q^2)}, \tag{10}$$

$\tau_A = L^2/(4D)$, $\mathcal{A}$ denotes the amplitude and $\Omega = HL^2/(2\sqrt{\pi})^3$ is the normalization factor. In Eq. (9), $\mathcal{A}$ equals either χ or 1.

Although we cannot calculate the integrals $J_\pm$ exactly, we can approximate them by applying the double-tangent construction to the eigenvalues $\lambda_\pm(q^2)$. Thus we assume that $\lambda_\pm(q^2) \approx \lambda_\pm^{\leq}(q^2)$ if $q < q_c$ and $\lambda_\pm(q^2) \approx \lambda_\pm^{\geq}(q^2)$ if $q > q_c$, where $\lambda_\pm^{\leq}(q^2)$ and $\lambda_\pm^{\geq}(q^2)$ are the tangents to $\lambda_\pm(q^2)$ at $q = 0$ and $q = \infty$, respectively, and $q_c^2 = R/|\Delta|$ is their intersect. The tangents are given by the following expressions

$$\begin{aligned} \lambda_+^{\leq} &= -D_+ q^2, \\ \lambda_-^{\leq} &= -D_- q^2 - R, \\ \lambda_+^{\geq} &= -Dq^2 - R(1-\beta), \\ \lambda_-^{\geq} &= -D_B q^2 - R\beta, \end{aligned} \tag{11}$$

where $D_+ = D\beta + D_B(1-\beta)$ and $D_- = D(1-\beta) + D_B\beta$ are the effective diffusion coefficients. The amplitude χ can be expressed as a function of the dimensionless variable $x = (q/q_c)^2$:

$$\chi(x) = \frac{1+\epsilon x}{\sqrt{x^2 + 2\epsilon x + 1}}. \tag{12}$$

The double-tangent construction applied to the denominator leads to the approximation:

$$\sqrt{x^2 + 2\epsilon x + 1} \approx \begin{cases} 1 + \epsilon x & \text{if } x < 1 \\ \epsilon + x & \text{if } x > 1 \end{cases} \tag{13}$$

hence

$$\chi(x) \approx \begin{cases} 1 & \text{if } x < 1 \\ \dfrac{1+\epsilon x}{\epsilon + x} & \text{if } x > 1 \ . \end{cases} \tag{14}$$

This approximation of $\chi(x)$ is continuous at $x = 1$. Then the integration over the wave vectors is split into two intervals: $q < q_c$ and $q > q_c$, and $J_\pm[\mathcal{A}, t]$ is approximated by

$$J_\pm[\mathcal{A}, t] \approx J_\pm^<[\mathcal{A}, t] + J_\pm^>[\mathcal{A}, t], \tag{15}$$

where

$$\begin{aligned} J_\pm^<[\mathcal{A}, t] &= 2\pi\Omega \int_0^{q_c} dq_z\, e^{-\frac{1}{4}H^2 q_z^2} \int_{q_z^2}^{q_c^2} dq^2 e^{-\frac{1}{4}L^2 q_\perp^2} \mathcal{A}(q^2) e^{t\lambda_\pm^<(q^2)}, && (16) \\ J_\pm^>[\mathcal{A}, t] &= 2\pi\Omega \left[\int_0^{q_c} dq_z\, e^{-\frac{1}{4}H^2 q_z^2} \int_{q_c^2}^{\infty} dq^2 e^{-\frac{1}{4}L^2 q_\perp^2} \mathcal{A}(q^2) e^{t\lambda_\pm^>(q^2)} \right. \\ &\quad + \left. \int_{q_c}^{\infty} dq_z\, e^{-\frac{1}{4}H^2 q_z^2} \int_{q_z^2}^{\infty} dq^2 e^{-\frac{1}{4}L^2 q_\perp^2} \mathcal{A}(q^2) e^{t\lambda_\pm^>(q^2)} \right] && (17) \end{aligned}$$

and $q_\perp^2 = q^2 - q_z^2$. Moreover, if we assume that $\frac{1}{4}H^2 q_c^2 \gg 1$, which is equivalent to the condition $R\tau_\Delta \gg \omega^{-2}$, where $\tau_\Delta = L^2/(4|\Delta|)$ and $\omega = H/L$, then the main contributions to the integrals come from $q_z \approx 0$. Thus the integration over q_z from 0 to q_c can be extended to infinity and the second term in (17) can be neglected, which gives

$$\begin{aligned} J_\pm^<[\mathcal{A}, t] &\approx 2\pi\Omega \int_0^{\infty} dq_z e^{-(\frac{1}{4}H^2 + tD_\pm)q_z^2} \int_0^{q_c^2} dq_\perp^2 e^{-\frac{1}{4}L^2 q_\perp^2} \mathcal{A}^<(q_\perp^2) e^{t\lambda_\pm^<(q_\perp^2)}, \\ J_+^>[\mathcal{A}, t] &\approx 2\pi\Omega \int_0^{\infty} dq_z e^{-(\frac{1}{4}H^2 + tD)q_z^2} \int_{q_c^2}^{\infty} dq_\perp^2 e^{-\frac{1}{4}L^2 q_\perp^2} \mathcal{A}^>(q_\perp^2) e^{t\lambda_+^>(q_\perp^2)}, \\ J_-^>[\mathcal{A}, t] &\approx 2\pi\Omega \int_0^{\infty} dq_z e^{-(\frac{1}{4}H^2 + tD_B)q_z^2} \int_{q_c^2}^{\infty} dq_\perp^2 e^{-\frac{1}{4}L^2 q_\perp^2} \mathcal{A}^>(q_\perp^2) e^{t\lambda_-^>(q_\perp^2)}, \end{aligned} \tag{18}$$

where $\mathcal{A}^<$ and $\mathcal{A}^>$ correspond to $q_\perp < q_c$ and $q_\perp > q_c$, respectively. Then using (14) and the relation

$$(1 + \epsilon x)/(x + \epsilon) = \epsilon + (1 - \epsilon^2)/(x + \epsilon), \tag{19}$$

where now $x = q_\perp^2 |\Delta|/R$, we obtain

$$\begin{aligned} J_\pm^<[\chi, t] &= J_\pm^<[1, t], \\ J_\pm^>[\chi, t] &= \epsilon J_\pm^>[1, t] + (1 - \epsilon^2) J_\pm^>[(x + \epsilon)^{-1}, t]. \end{aligned} \tag{20}$$

The integration with the amplitude $\mathcal{A} = 1$ yields

$$\begin{aligned} J_+^<[1, t] &= h(t/\tau_+) \left[1 - e^{-R\tau_\Delta(1 + t/\tau_+)} \right], \\ J_+^>[1, t] &= h(t/\tau_A) e^{-R\tau_\Delta(1 + t/\tau_+)}, \\ J_-^<[1, t] &= e^{-Rt} h(t/\tau_-) \left[1 - e^{-R\tau_\Delta(1 + t/\tau_-)} \right], \\ J_-^>[1, t] &= e^{-Rt} h(t/\tau_B) e^{-R\tau_\Delta(1 + t/\tau_-)}, \end{aligned} \tag{21}$$

where $\tau_\pm = L^2/(4D_\pm)$, $\tau_B = L^2/(4D_B)$, and $h(\bar{t}) = (1+\bar{t})^{-1}(1+\bar{t}/\omega^2)^{-1/2}$ is the autocorrelation function for the single-component diffusion (see the main text). The integrals $J^{\gtrless}_{\pm}[\mathcal{A}, t]$ for $\mathcal{A} = (x+\epsilon)^{-1}$ adopt the following form

$$\begin{aligned} J^{>}_{+}[(x+\epsilon)^{-1}, t] &= R\tau_\Delta h_z(t/\tau_A)\, e^{-R\tau_\Delta(1+t/\tau_+)}\mathcal{E}_1(2\beta R\tau_\Delta(1+t/\tau_A)), \\ J^{>}_{-}[(x+\epsilon)^{-1}, t] &= R\tau_\Delta e^{-Rt}\, h_z(t/\tau_B)\, e^{-R\tau_\Delta(1+t/\tau_-)}\mathcal{E}_1(2\beta R\tau_\Delta(1+t/\tau_B)), \end{aligned} \tag{22}$$

where $h_z(\bar{t}) = (1+\bar{t}/\omega^2)^{-1/2}$, $\mathcal{E}_1(z) = e^z \mathrm{E}_1(z)$ and

$$\mathrm{E}_1(z) = \int_z^\infty \frac{e^{-u}}{u}\, du \tag{23}$$

denotes the exponential integral.[3] We recall that for $z \to \infty$, $\mathrm{E}_1(z) \approx e^{-z}/z$, whereas for $z \to 0$, $\mathrm{E}_1(z) \approx -\gamma - \ln z$, where $\gamma \approx 0.5772$ is the Euler constant.

Finally, combining Eqs. (9), (15), (20), (21) and (22), we arrive at the approximation $G_a(t)$ for the FCS autocorrelation function (see Eq. (13) in the main text):

$$\begin{aligned} G_a(t) = {} & \frac{y\beta}{1-y+y\beta}\, h(t/\tau_A) + \frac{1-y}{1-y+y\beta}\Big\{ h(t/\tau_+)\left[1 - e^{-R\tau_\Delta(1+t/\tau_+)}\right] \\ & + \beta h(t/\tau_A) e^{-R\tau_\Delta(1+t/\tau_+)} + (1-\beta)e^{-Rt} h(t/\tau_B) e^{-R\tau_\Delta(1+t/\tau_-)} \\ & + 2\beta(1-\beta)R\tau_\Delta\Big[h_z(t/\tau_A)\, e^{-R\tau_\Delta(1+t/\tau_+)}\mathcal{E}_1(2\beta R\tau_\Delta(1+t/\tau_A)) \\ & - e^{-Rt}\, h_z(t/\tau_B)\, e^{-R\tau_\Delta(1+t/\tau_-)}\mathcal{E}_1(2\beta R\tau_\Delta(1+t/\tau_B))\Big]\Big\}. \end{aligned} \tag{24}$$

We also notice that despite the assumption $R\tau_\Delta \gg \omega^{-2}$ made to derive the above approximation, $G_a(t)$ behaves correctly in the limit $R \to 0$, i.e., it tends to $G_0(t)$ (see Eq. (12) in the main text). Moreover, if $R\tau_\Delta$ is small the last term in (24) can be neglected and a simplified version of $G_a(t)$ without the exponential integral can be used:

$$\begin{aligned} G_s(t) = {} & \frac{y\beta}{1-y+y\beta}\, h(t/\tau_A) + \frac{1-y}{1-y+y\beta}\Big\{ h(t/\tau_+)\left[1 - e^{-R\tau_\Delta(1+t/\tau_+)}\right] \\ & + \beta h(t/\tau_A) e^{-R\tau_\Delta(1+t/\tau_+)} + (1-\beta)e^{-Rt} h(t/\tau_B) e^{-R\tau_\Delta(1+t/\tau_-)}\Big\}. \end{aligned} \tag{25}$$

S.2 Comparison of $G(t)$ with approximate autocorrelation functions

In Figs. 1–3, we compare two approximations of the autocorrelation function: $G_a(t)$ and $G_s(t)$, with $G_\mathrm{n}(t)$ obtained directly from the theoretical model[2] by numerical integration. We also show the limits of fast reaction, $G_\infty(t)$, and fast diffusion, $G_0(t)$ (see the main text). We fix the value of the parameter $d = D/(D+D_B)$ at 0.01 and change both β and R. For $R = 10$ (the fastest reaction) and $R = 0.01$ (the slowest reaction), $G_a(t)$ and $G_\mathrm{n}(t)$ are very close to each other, whereas the largest discrepancies occur at $R = 1$. $G_s(t)$ also reproduces the limits of fast reaction and fast diffusion correctly but it decays too fast with time for $R = 1$ and $R = 0.1$. In the case $R = 0.01$, $G_s(t)$ approximates $G(t)$ almost as well as $G_a(t)$, which means that it can be used for slow reactions.

S.3 Autocorrelation function for $Q_B \neq Q_C$

Here we generalize the derivation of $G_a(t)$ presented in section S.1 for the case of different fluorescence of components B and C. For $Q_B \neq Q_C$, the function $g(q,t)$ assumes the following form

$$\begin{aligned} g(q,t) &= Q_C^2[C]^{eq} y e^{t\lambda_0} + \frac{[B]^{eq}}{2(1-\beta)}\left[Q_B^2(1-\beta)+Q_C^2\beta\right]\left(e^{t\lambda_+}+e^{t\lambda_-}\right) \\ &+ \frac{[B]^{eq}}{2(1-\beta)}\left\{\chi Q_B Q_C + \phi(Q_B-Q_C)\left[Q_B(1-\beta)+Q_C\beta\right]\right\}\left(e^{t\lambda_+}-e^{t\lambda_-}\right), \end{aligned} \tag{26}$$

where

$$\phi = (q^2|\Delta| + \epsilon R)/\sqrt{q^4|\Delta|^2 + 2\epsilon q^2|\Delta| R + R^2}. \tag{27}$$

Substituting $Q_B = Q_C$ in (26), we recover Eq. (6). The autocorrelation function normalized to unity at $t = 0$ is given by

$$\begin{aligned} G(t) &= y\beta\bar{Q}_C^2 h(t/\tau_C) + \frac{1}{2}(1-y)\left[\bar{Q}_B^2(1-\beta)+\bar{Q}_C^2\beta\right]\left(J_+[1,t]+J_-[1,t]\right) \\ &+ \frac{1}{2}(1-y)\big\{\bar{Q}_B\bar{Q}_C\left(J_+[\chi,t]-J_-[\chi,t]\right) + (\bar{Q}_C-\bar{Q}_B)\left[\bar{Q}_B(1-\beta)+\bar{Q}_C\beta\right] \\ &\times \left(J_+[\phi,t]-J_-[\phi,t]\right)\big\}, \end{aligned} \tag{28}$$

where $\tau_C = \tau_A = L^2/(4D)$, $\bar{Q}_B = Q_B/\bar{Q}$, $\bar{Q}_C = Q_C/\bar{Q}$ and $\bar{Q}^2 = Q_B^2(1-y)(1-\beta) + Q_C^2\beta$. To perform integrals $J_\pm[\phi,t]$ we need an approximation for $\phi(q)$. As a function of x, ϕ is given by the expression:

$$\phi(x) = \frac{x+\epsilon}{\sqrt{x^2+2\epsilon x+1}} = \frac{d}{dx}\sqrt{x^2+2\epsilon x+1}, \tag{29}$$

thus, using (29) and approximation (13), we obtain

$$\phi(x) \approx \begin{cases} \epsilon & \text{if } x < 1 \\ 1 & \text{if } x > 1\,, \end{cases} \tag{30}$$

hence

$$\begin{aligned} J_\pm^<[\phi,t] &= \epsilon J_\pm^<[1,t], \\ J_\pm^>[\phi,t] &= J_\pm^>[1,t]. \end{aligned} \tag{31}$$

Finally, using Eqs. (28), (15), (20), (21), (22) and (31), we obtain the generalized form of $G_a(t)$ (cf. Eq. (24)):

$$\begin{aligned} G_a(t) &= y\beta\bar{Q}_C^2 h(t/\tau_C) + (1-y)\Big\{\left[\bar{Q}_B(1-\beta)+\bar{Q}_C\beta\right]^2 h(t/\tau_+)\left[1-e^{-R\tau_\Delta(1+t/\tau_+)}\right] \\ &+ \beta(1-\beta)(\bar{Q}_C-\bar{Q}_B)^2 e^{-Rt} h(t/\tau_-)\left[1-e^{-R\tau_\Delta(1+t/\tau_-)}\right] \\ &+ \beta\bar{Q}_C^2 h(t/\tau_C) e^{-R\tau_\Delta(1+t/\tau_+)} + (1-\beta)\bar{Q}_B^2 e^{-Rt} h(t/\tau_B) e^{-R\tau_\Delta(1+t/\tau_-)} \\ &+ 2\beta(1-\beta)\bar{Q}_B\bar{Q}_C R\tau_\Delta\Big[h_z(t/\tau_C)\, e^{-R\tau_\Delta(1+t/\tau_+)}\mathcal{E}_1(2\beta R\tau_\Delta(1+t/\tau_C)) \\ &- e^{-Rt}\, h_z(t/\tau_B)\, e^{-R\tau_\Delta(1+t/\tau_-)}\mathcal{E}_1(2\beta R\tau_\Delta(1+t/\tau_B))\Big]\Big\}, \end{aligned} \tag{32}$$

which reduces to (24) for $Q_B = Q_C$.

References

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[3] G. B. Arfken, H. J. Weber and F. E. Harris, *Mathematical Methods for Physicists*, 7th ed., Academic Press, 2012.

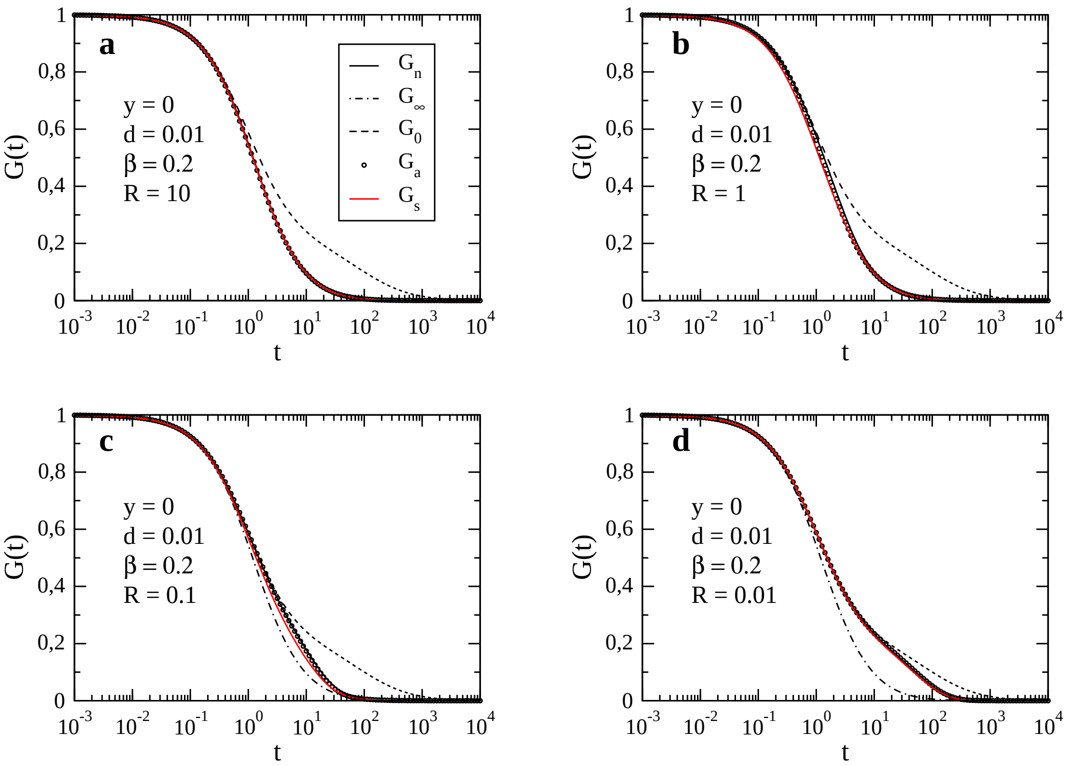

Figure 1: FCS autocorrelation function for the set of parameters y, d, β and R specified in the figure. The time t and inverse of the reaction rate, R^{-1}, are expressed in the units of τ_Δ. The legend in **a** concerns all figures: G_n results from the numerical integration, G_∞ and G_0 correspond to the limits of fast reaction and fast diffusion, respectively, G_a is the approximation given by Eq. (24) and G_s is a simplified version of $G_a(t)$ given by Eq. (25)

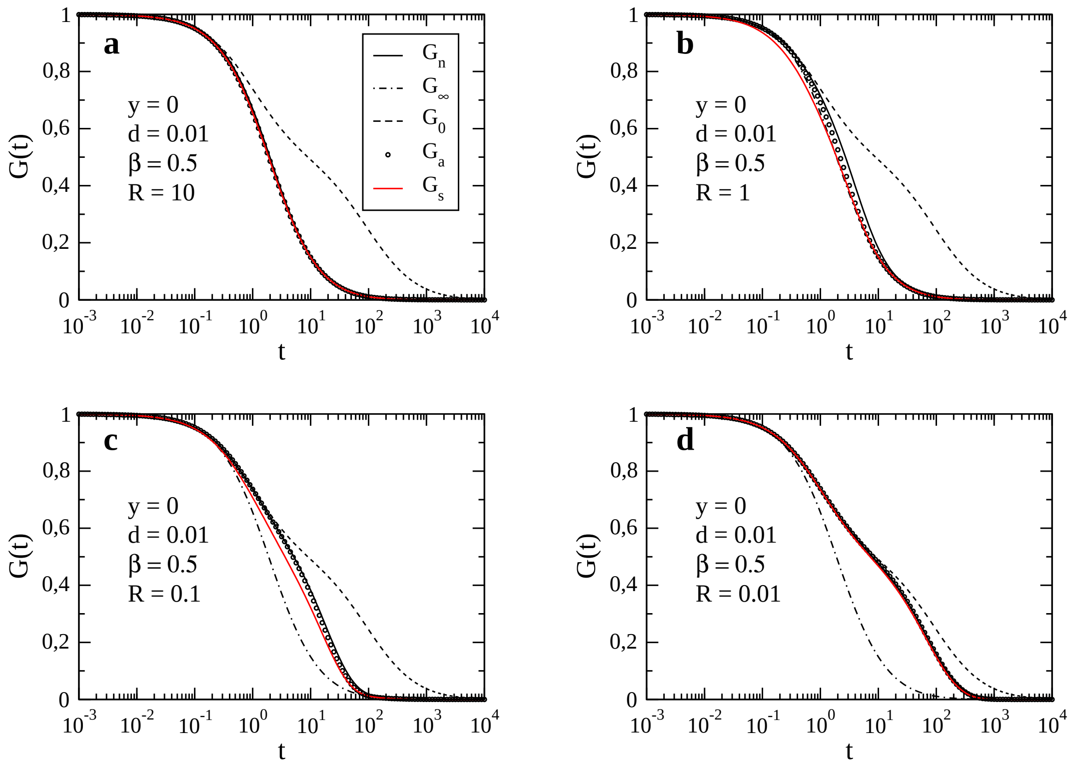

Figure 2: FCS autocorrelation function for the set of parameters y, d, β and R specified in the figure. The meaning of symbols is the same as in Fig. 1

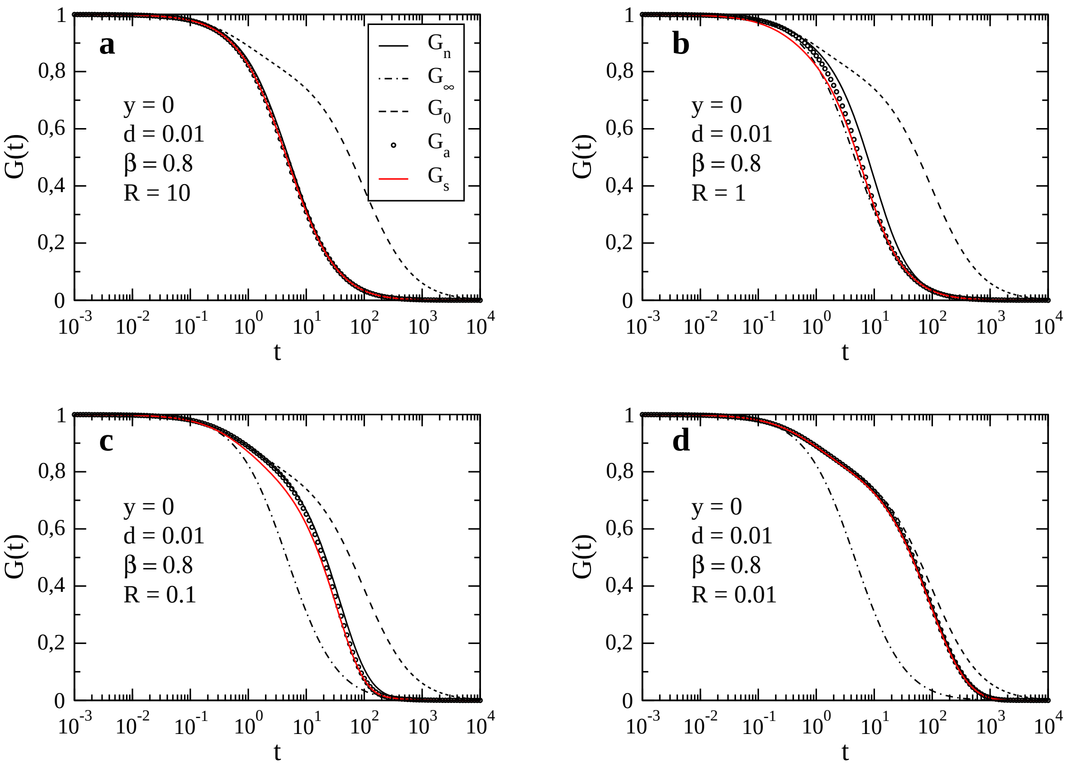

Figure 3: FCS autocorrelation function for the set of parameters y, d, β and R specified in the figure. The meaning of symbols is the same as in Fig. 1