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Supporting material

Experimental details

In the case of the **FRAP measurements**, the translational diffusion coefficient D can be found from (2)

$$D = \frac{0.88\omega^2}{4t_{1/2}} \tag{2}$$

where ω is the radius of the bleached spot and D is related to the viscosity by the Stokes Debye Einstein relationship.

$$D = \frac{kT}{6\pi\eta R} \tag{3}$$

where k is the Boltzmann constant, T the absolute temperature, η the viscosity and R the radius of the molecule.

A measure of the number of molecules which are free to diffuse as a percent can be obtained by calculating the mobile fraction R [6].

$$R = (F_\infty - F_0)/(F_i - F_0) \tag{4}$$

where F_∞ is the final fluorescence intensity after full recovery, F_i is the fluorescence intensity before bleaching and F_0 is the fluorescence intensity just after bleaching. The number of hindered molecules (immobile fraction) is therefore equal to 1-R. To correct for bleaching during the recovery of fluorescence an area away from the bleach region was monitored to provide a control curve, which was applied during the analysis. Tests were also performed using different bleach times to ascertain the influence of any recovery during the bleach period.

In relation to the **fluorescence anisotropy measurements**, the sample was excited with linearly polarized light, and the fluorescence decay is measured at polarizations parallel and perpendicular to that of the exciting light. The time-resolved fluorescence anisotropy $r(t)$ is defined as the difference of the parallel and

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perpendicular fluorescence decays, divided by the total fluorescence intensity $I_{\|}(t)+2I_{\perp}(t)$

$$r(t)=\frac{I_{\|}(t)-I_{\perp}(t)}{I_{\|}(t)+2I_{\perp}(t)} \tag{5}$$

This has to be corrected for any polarisation bias of the equipment by measurement of the g-factor, which was found using tail-matching[19]. This accounts for different sensitivities in the detection and is found by dividing the intensity decay curve of the parallel component by that of the perpendicular. When both intensities have decayed into the noise the value of the parallel to perpendicular ratio (the g-factor) can be obtained. This was found to be 1 for our system. It should be noted that care must be taken when using tail-matching for systems where the anisotropy is not expected to decay back to zero. In this case a non-hindered rotor, fluorescein in solution, was used to find the g-factor of the set-up. For a spherically symmetric rotor, *r(t)* decays as a single exponential and is related to the rotational correlation time θ according to

$$r(t)=(r_0-r_\infty)\exp\left(-\frac{t}{\theta}\right)+r_\infty \tag{6}$$

r_0 is the initial anisotropy, and is given by the angle between absorption and emission dipole moment (r_0=0.4 for parallel absorption and emission dipole moments), r_∞ is the limiting anisotropy which accounts for a hindered rotation of the probe and θ is the rotational correlation time. For a spherical rotor in an isotropic medium, θ is directly proportional to the viscosity η of the solvent and the volume V of the rotating molecule:

$$\theta=\frac{\eta V}{kT} \tag{7}$$

The recovered rotational time was obtained by fitting the anisotropy curve (using Microcal Origin software) to an exponential decay to obtain an average rotational time.

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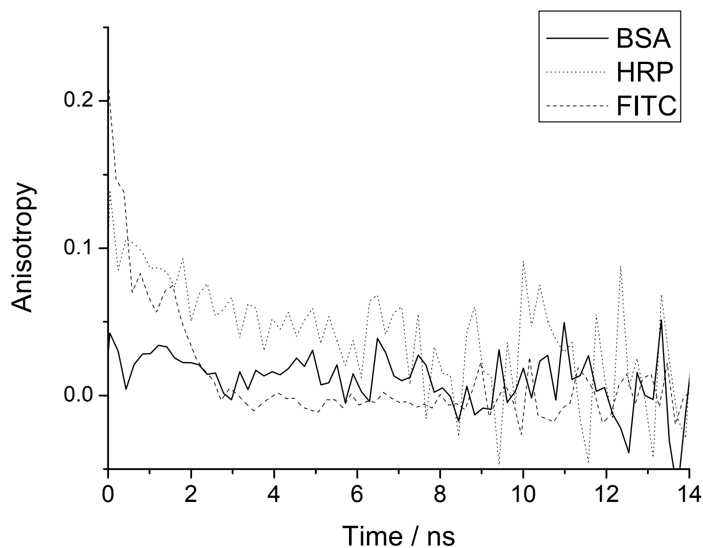
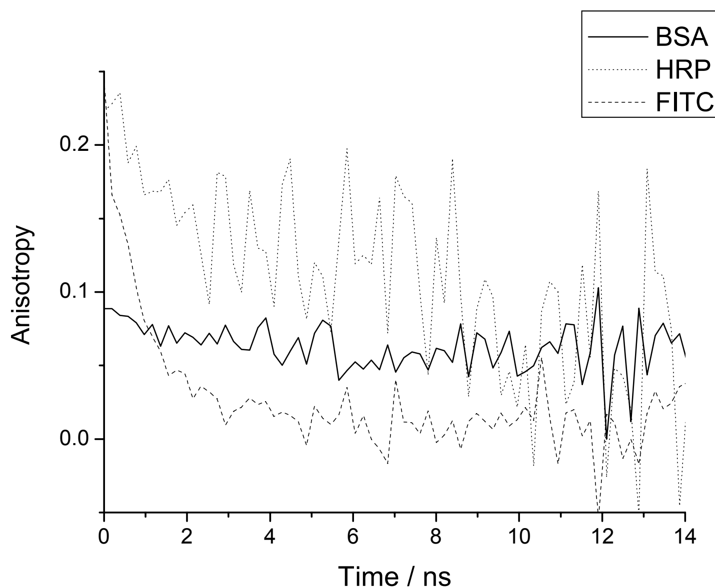
Fluorescence anisotropy data

Anisotropy curves for FITC alone and FITC labelled HRP and BSA incorporated into sol-gel derived media with aging time



Anisotropy decays for day 2 after making sol-gel for all 3 samples.



Anisotropy decays for day 9 after making sol-gel for all 3 samples.

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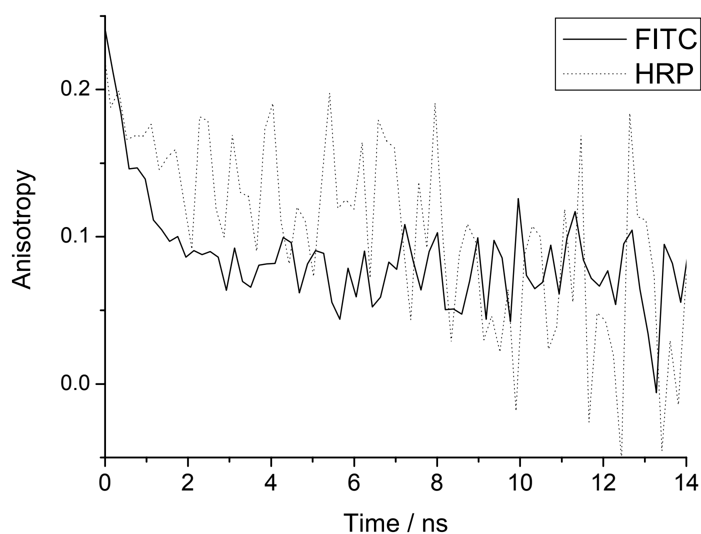

Anisotropy decays for day 12 after making sol-gel for FITC and HRP-FITC

Reference

19. D.V. O'Connor and D. Phillips. *Time-correlated Single Photon Counting*. Academic Press Inc., London, 1984.