Electronic supplementary information

for

On the Nature of Ultrabrightness of Nanoporous Fluorescent Particles with Physically Encapsulated Fluorescent Dyes

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Methods of measuring absorbance of encapsulated dye inside of particles which scatter light

To estimate the number of encapsulated dye molecules, to measure its quantum yield, one needs to measure the absorbance of encapsulated dye. One of the major impediments to accurately estimating absorbance of encapsulated dye inside particles is scattering of light by the particles. As we mentioned in the main text, there are four different methods of taking that scattering into account:

1. The absorbance is simply measured with respect to a reference containing the same particles but with no dye inside. Such particles scatter in the same way but do not have fluorescence.
2. The measurements are done in 1% HF acid solution used to dissolve silica matrix of the particles (HF does not change spectra of R6G dye), so the scattering disappears.
3. One can use the media with matching refractive index, which eliminates fluorescence.
4. The scattering can be separated purely mathematically by assuming the additive nature of dye absorbance and particle scattering.

Here we demonstrate examples of using those methods and briefly discuss their limitations.

Method 1. This method is easy to use when the reference particles are the same but with the dye inside bleached. Obviously such approach would be sacrificial to the sample. Therefore we do not use it here.

Method 2. Dissolution of silica matrix with HF. The absorbance of Rhodamine 6G is not affected by HF [1, 2], and therefore, the concentration of dye encapsulated can be determined from the absorbance of the released dye. When there is no aggregation of dye inside the particles this method will give the absorbance of the dye encapsulated inside the particles. But, when the aggregation of dyes inside the particles cannot be ruled out, this method should not be used.

Method 3. Matching the refractive index. It is quite easy to do by titration the refractive index with some ionic salts. In the example below we use calcium chloride. One has to be careful not to disturb the absorbance spectra of the dye by the ions of the refractive index matching salt. This can separately be checked with free dye solution.

Method 4. Subtracting of the baseline. This method is particularly convenient when measuring the absorbance of dyes encapsulated in particles. The measurement of absorbance can be performed with good accuracy by subtracting the shifted baseline [3].

Below we provide examples to demonstrate and compare the above methods. In the provided example, absorbance of nanoparticle solution was measured, Fig. S1A. One can see the absorbance due to the nanoparticles is shifted up due to light scattering by the particles. Then, 40 microliters of 35% HF was added to 1.5 mL to dissolve the silica matrix. The absorbance of

freed dye in solution measured is shown in the same graph. The absorbance obtained from the baseline correction is equal to the absorbance of the freed dye within 5% error.

It needs to be mentioned that the signal to noise ratio (absorbance to scattering) is not same for all particles. The absorbance of the particles is directly related to the amount of dye encapsulated. So for the same amount of suspended particles, the particles with lower amount of encapsulated dye will have a lower signal to noise ratio and a higher error under the same conditions of measurements.

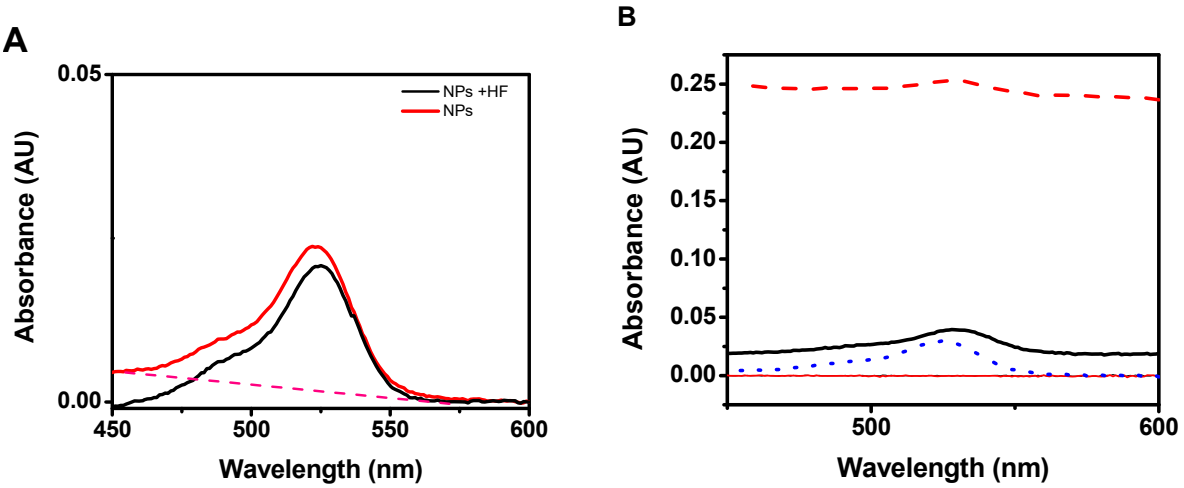


Fig. S1. Examples of measurement of absorbance.

A) Examples for nanoparticles. Absorbance spectra of nanoparticles suspended in water before and after addition of 40ul of 35% HF are shown. The dash line is the baseline due to scattering of light by nanoparticles. It can be subtracted as the baseline.

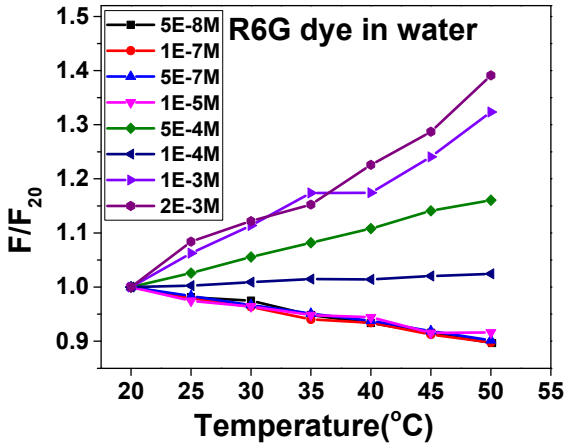
B) Examples for discoids. Eliminating scattering by refractive index matching. Discoids suspended in water before (dash) and after etching with HF (dots), suspended in $CaCl_2$ (RI =1.44) solution (straight).

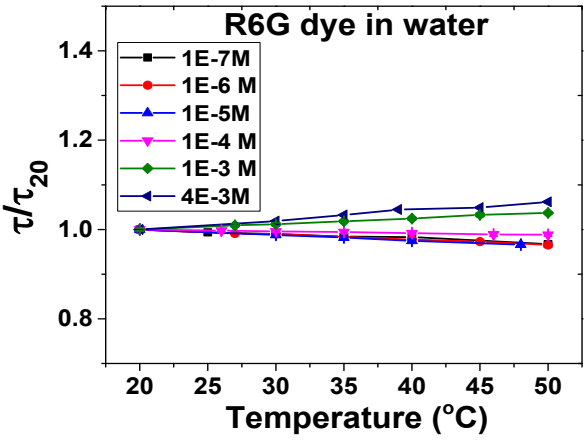
A example for discoids is shown in Fig. S1B. The case of micron sized discoids presents a more difficult challenge to handle the scattering. The scattering from these large particles are high that only high concentration discoids show any good indication of absorbance by the dye. The amount of scattering can be reduced by using a high refractive index solution (n=1.44), for example, ~40% $CaCl_2$ solution as a medium to suspend the particles for absorbance

measurement. Dissolution of the silica matrix can again be used to estimate the amount of dyes encapsulated inside the particle quantity do not observe dye dimerization. As an example, the measured absorbance for the solution is 0.028. It can be seen that the absorbance of particles suspended in water is not comparable to that obtained by releasing the dye (absorbance ~0.005). On the other hand, the particles suspended in $CaCl_2$ solution show a low contribution form scattering. The absorbance measured is 0.020. The scattering due to particles is decreased due to the refractive index of the suspending medium being close to that of the particles. The error in the measured absorbance may also arise from the particle's aggregation. The viscosity of the salt solution is 8.83 cP compared to 1 cP of water at 20°C. The high viscosity causes problems to homogenous dispersion of particles. It is difficult to de-aggregate discoid aggregates when the viscosity of the medium is high. This is also compounded by the fact that neutralization of charge on silica surface occurs due to the excess amount of counter ions present and there is no barrier for aggregation for particles in the solution.

Temperature dependence of fluorescence and lifetime

Details of temperature dependence (relative to 20°C) of fluorescence and lifetime of free dye, nanoparticles, and discoids are shown in figure S2.





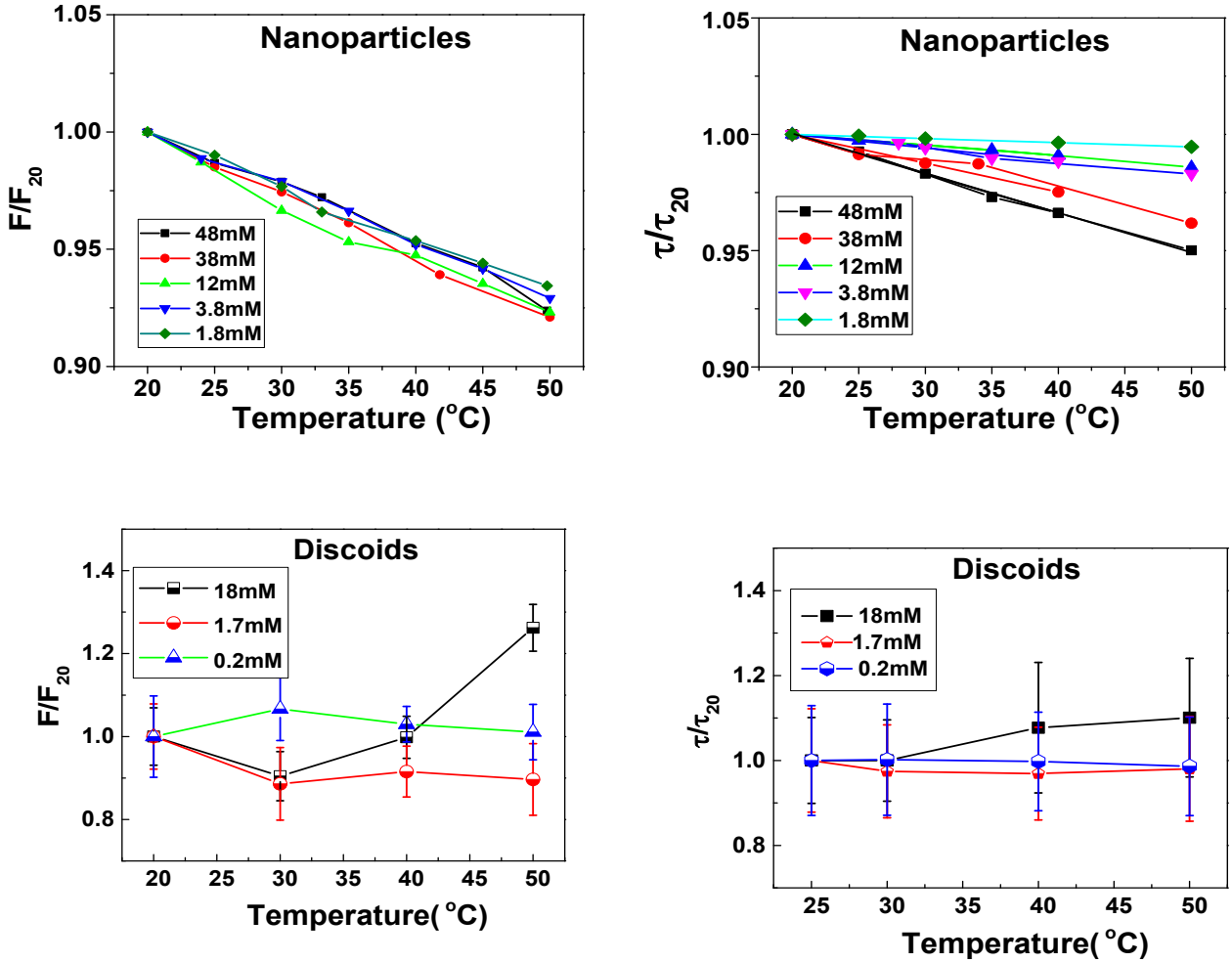


Fig. S2. Temperature dependence of fluorescence and lifetime (relative to 20°C) for R6G dye encapsulated in nanoparticles, discoids, and free dye. Excitation/emission wavelength is 488/550 nm.

Problem of scattering

This is not a typical problem when working with molecular fluorophores. However, when dealing with fluorescent nanoparticles, the scattering can be substantial. It depends upon the ratio of refractive indexes of the media, the particle, and the wavelength of light. As an example, one

can see a strong extinction of ultraviolet light by 30nm silica particles reported in [2] due to the Mie scattering, which could easily be confused with the absorbance.

In the described measurements, the scattering contributes to both absorbance and fluorescence behavior. In both cases light does not always penetrate inside of the particles to be either absorbed or transferred into fluorescence. The scattering effectively decreases anisotropy of fluorescence of the particles, see the below. Most of the effect is seen in absorbance as we described above. When dealing with fluorescent silica particles, the portion of the extinction coefficient corresponding to scattering can be found from the absorbance measurements using one of the four methods described above.

It is worth noting that scattering does not produce serious artifacts when measuring fluorescent brightness (unless the Stokes shift is extremely small, which is not the case for the dyes used). This is exampled in **Fig.2** which shows a typical case of fluorescent measurements of ultrabright silica particles when excited by light of different wavelengths. One can clearly see the Mie scattering line in Fig.2 (where the emission and excitation wavelength are the same). To avoid the scattering artifact, fluorescence value in formula 1 was calculated by integrating the fluorescent intensities in the range shown in figure 2, excluding the scattering region.

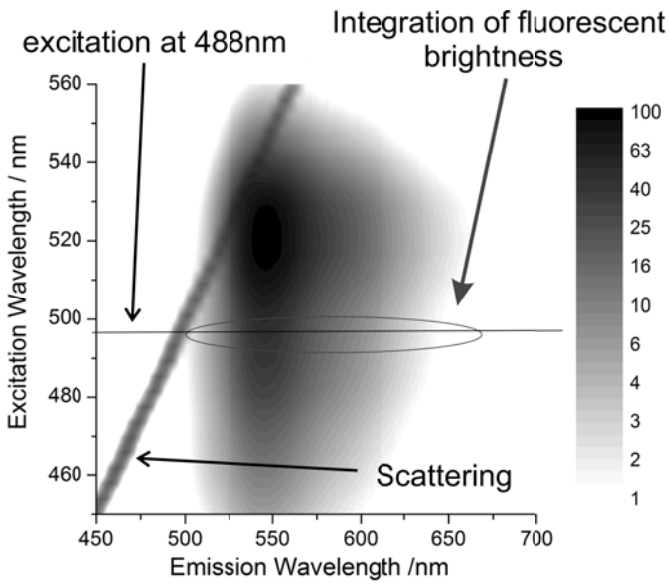


Fig.S3. Fluorescence (arbitrary units) measured for ultrabright fluorescent nanoparticles [1] excited with light of different wavelengths. One can clearly distinguish the scattering and fluorescence.

Cited literature

1. Cho, E.B., D.O. Volkov, and I. Sokolov, *Ultrabright Fluorescent Mesoporous Silica Nanoparticles.* Small, 2010. **6**(20): p. 2314-2319.
2. Sokolov, I. and S. Naik, *Novel fluorescent silica nanoparticles: towards ultrabright silica nanoparticles.* Small, 2008. **4**(7): p. 934-9.
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