Electronic Supplementary Information

Scattering and Absorption Differ Drastically in Their Inner Filter Effects on Fluorescence, Resonance Synchronous, and Polarized Resonance Synchronous Spectroscopic Measurements

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Table of Contents

Section 1: Comparison of the experimental and mathematic summation spectra of UV-vis spectra of both QD/DNPH and QD/PSNP mixtures

Section 2: Determination of PRS2 VV linear dynamic range in terms of scattering extinction (E^{Sca}) at the 400 nm

Section 3: Iteration PRS2 decomposition of *f*PSNP UV-vis extinction spectrum into absorption and scattering extinction component spectra.

Section 1. Comparison of the experimental and mathematic summation spectra of UV-vis spectra of both QD/DNPH and QD/PSNP mixtures



Fig.S1 (A) UV-vis extinction spectra of pure QD, DNPH and PSNP aqueous solutions. (B) Comparison of (solid line) the experimental and (dash line) mathematic summation spectra of UV-vis spectra of QD/DNPH (C) Comparison of (solid line) the experimental and (dash line) mathematic summation spectra of UV-vis spectra of QD/PSNP

The dash line spectra in Fig. S1 (B) and (C) are the mathematical products of direct addition of QD with DNPH and PSNP UV-vis spectra acquired in Fig.S1 (A). The spectral contribution of each component is derived mathematically according to their concentrations before summation. In both cases of QD/DNPH and QD/PSNP mixtures, the mathematic summation spectra nearly overlap with the experimental ones, with no modification on the any peak position compared to the individual spectra in Fig.S1 (A). It well indicates there is no interactions between QD and DNPH or PSNP.

S2. Determination of PRS2 VV linear dynamic range in terms of scattering extinction (E^{Sca}) at the 400 nm

As shown in Figure 2, PSNP (c=2.14 pM) gives a PRS2 VV intensity of 1.94×10^6 counts at 400 nm when its extinction is merely 1.8×10^{-3} at this wavelength. Since the saturation intensity is 2.0×10^6 counts according to the instrument specification, one can then determine the upper limit of scattering extinction for samples that contain only light scatterers by the instrument without inducing PRS2 signal saturation using Eq. S1.

$$E_{U}^{Sca} = \frac{I_{U}^{PRS2}}{I_{o}^{PRS2}} \times E_{o}^{Sca}$$
 Eq. S1

 E_{o}^{Sca} is 1.8×10⁻³, and I_{o}^{PRS2} is 1.94×10⁶. these are obtained with the data shown in Figure 2 in the main text. I_{U}^{PRS2} is the instrument saturation intensity which is 2.0×10⁶ for the Fluoromax-4 used in this work. Substituting these values into Eq.S1 gives a upper scattering extinction limit of 0.002 at 400 nm.

$$E_U^{Sca} = \frac{2 \times 10^6}{1.94 \times 10^6} \times 1.8 \times 10^{-3} = 0.00185 \approx 0.002$$

Since the water and cuvette produce a PRS2 VV background around 4,700 counts with a standard deviation of 70 counts, the lower limited PRS2 VV signal corresponding to the lower detection limit for light-scattering samples should be $I_{L}^{PRS2} = 4,700 + 3 \times 70$. As a result, one can calculate the lower limit of the light scattering extinction that gives detectable PRS2 signal using Eq. S2. Substituting the values of I_{L}^{PRS2} , E_{o}^{Sca} , and I_{o}^{PRS2} into Eq. S2 yields a lower light scattering extinction of $\sim 5 \times 10^{-6}$

$$E_{L}^{Sca} = \frac{I_{L}^{PRS2}}{I_{o}^{PRS2}} \times E_{o}^{Sca}$$
 Eq. S2

 $E_{L}^{Sca} = \frac{4907}{1.94 \times 10^{6}} \times 1.8 \times 10^{-3} \approx 5 \times 10^{-6}$

S3. Iteration PRS2 decomposition of *f*PSNP UV-vis extinction spectrum into absorption and scattering extinction component spectra.

The fPSNPs are fluorescent nanoparticles. It is therefore possible for fPSNP to be simultaneously photon absorbers, scatterers, and emitters at the wavelength regions the molecule both absorbs and emits. Using the ratiometric bandwidth-varied polarized resonance synchronous spectroscopic (BVPRS2) method,¹ we found that *f*PSNPs are predominantly simultaneous photon absorbers and scatterers under the resonance excitation and detection conditions. This is because the ratiometric BVPRS2 R_{VV} and R_{VH} intensity totally independent of the excitation and detection bandwidth used in the PRS2 measurements (Figure S1(B) and S1(E)). Earlier theoretical derivation and experimental data showed that the fluorescence contribution to the ratiometric BVPRS2 intensity is linearly dependent to the wavelength bandwidth, but the light scattering is independent of the bandwidth.¹



Fig. S2 1st column: (A) *f*PSNP UV-vis extinction spectrum and (D) the Stokes-shifted fluorescence spectrum. 2nd column: (B) and (E) the *f*PSNP ratiometric BVPRS2 spectra obtained with excitation and detection polarization combination of VV and VH, respectively. 3rd column: (E) and (F) are the ratiometric BVPRS2 VV and VH spectral intensity at 550 nm as a function of the wavelength bandwidth.

Having established the *f*PSNP PRS2 spectra are dominated by the light scattering, we then used the iteration PRS2 method described in the main text for estimation of the *f*PSNP light scattering cross-section. Figure S2 shows the calculated S^{OE} and S^{UE} when the PRS2 method was iterated the first (Figure S2(A)), second (Figure S2(B)), and third (Figure S2(C)) times for decomposing the UV-vis extinction spectrum in Figure S1(A) into its absorption and scattering component spectra (Figure S2(E) and S2(F)). The estimation error of the light scattering extinction spectrum is plotted using the logarithm

scale. The maximum relative estimation error is 8.4×10^{-4} , 8.6×10^{-7} , to 1.0×10^{-9} , respectively after the first, second, and third iteration. The final light scattering extinction spectra (Figure S2(E)) is calculated by averaging the S^{OE} and S^{UE} from the third iteration. The absorption extinction spectrum is obtained by subtracting the experimental fPSNP extinction spectrum with the estimated light scattering extinction spectrum (Figure S2(F)).



Fig. S3 (A), (B), and (C) shows the calculated S^{OE} and S^{UE} using iterated PRS2 for the first, second, and third time, respectively. (D) Wavelength dependence of calculated Err in first, second, and third iteration. (E) The final light scattering extinction component spectrum calculated by averaging the S^{OE} and S^{UE} from the third iteration in Figure S2(C). (F) (red) the experimental *f*PSNP extinction spectrum and (black) the estimated absorption component spectrum obtained by subtracting the estimated absorption extinction spectrum from the experimental *f*PSNP extinction spectrum.

Reference

 Xu, J.; Hu, J.; Zhang, D. Quantification of Material Fluorescence and Light Scattering Crosssections using Ratiometric Bandwidth-Varied Polarized Resonance Synchronous Spectroscopy. *revision Submitted*