Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Supporting Information

Photobleaching of Quantum Dots by Non-resonant Light

Xingbo Shi[2], *Yang Tu*[1], *Xiaojun Liu*[1], *Edward S. Yeung*[3], *Hongwei Gai* [1]*

1, School of Chemistry and Chemical Engineering, and Jiangsu Key Laboratory of Green Synthetic Chemistry for Functional Materials, Jiangsu Normal University, Xuzhou, Jiangsu, China, 221116
2, College of Food Science and Technology, Hunan Agricultural University, Changsha, Hunan, China, 410128
3, School of Biology, Hunan University, Changsha, Hunan, China, 410082

Experimental Section

Single QD imaging Three kinds of commercial Core/shell QDs (ZnS/CdSe) (carboxyl QD525, amino (PEG) QD585, carboxyl QD655, Invitrogen) were used in sodium borate buffer (pH 9.4 for QD525 and QD655, and pH 8.5 for QD655). An aliquot (4-8μL, 0.6nM) of QD solution was deposited on a glass slide and was immediately covered by a coverslip. Mono-dispersed single QDs were observed at the surface of the coverslip by an epi-fluorescence microscope (Olympus BX51) with a 100× oil immersion objective (NA ×1.45, UPLSAPO, Olympus, Tokyo, Japan) and an electron multiplied charge-coupled device (EMCCD, DV887, Andor Technology, North Ireland). For the EMCCD, the gain was set at 200, the temperature was maintained at −60 ^{o}C, and the exposure times were kept at 0.1-0.5 seconds. The power of the mercury lamp is 100 W.
Non-resonant light: There are two ways to generate non-resonant light to irradiate the QD solution. Firstly, the mercury lamp was used as the light source and the chosen bandpass filter was placed in the excitation pathway. When recording the photobleaching process, the matched filter set was switched back. Secondly, a 655nm laser with 10-nm width (0.5-200mW, Shanxi Richeng Technology Development Co. Ltd.) was used as an illumination source.
Fluorescence spectra: The QD solution was continuously illuminated by the 650nm laser in a fluorescence cuvette for a given time. Then the fluorescence spectrum of QDs was measured by a fluorescence spectrophotometer (F-7000, Hitachi).
Imaging acquisition and processing. Images were processed using ImageJ software (National Institutes of Health, USA). In brief, counts above the appropriate threshold which was modified in each frame according to the background level were treated as the fluorescence signal, and the number of fluorescence spots at the stacks was added up by "analyze particles tools" of ImageJ.
Heating QDs solution: QD solution was packaged into 6 centrifugal tubes wrapped with the foil. Each tube contains 2 nM 585 QD. They were placed into the oven at 40 ^{o}C for 0, 30, 60, 90, 120, 150 and 180 minutes. Then the heated samples were analyzed with fluorescence spectrometer.

S1: Normalization process for the mercury lamp power at different wavelengths.

The mercury lamp at different wavelengths has different powers. To eliminate the effects of power variation, we have to introduce a power correction factor. Different wavelength light of the mercury lamp was scattered into the EMCCD to record their intensity "I". The quantum efficiency of the EMCCD and the transmission efficiency of the filters were defined by the manufacturers. Since the dichroic mirror is taken away from the light path, the excitation beam cannot directly enter into the detector. Only the scattered light reaches the EMCCD. This follows Rayleigh scattering. So that the scattering intensity is inversely proportional to λ^4; the value of the correction factor δ can be estimated by the following function.

$$\delta = I/(QE \times \lambda^4)$$

Using this function, we obtained the relative correction factor δ at different excitation wavelengths. The following table is the corresponding data.

Excitation filter	*Relative intensity*	*Relative λ^4*	*Relative QE*	*Correction factor δ*
#1 ex387/11	1	1	1	**1**
#2 ex435/40	52.32335	1.596295	6.363636	**5.150828**
#3 ex472/30	14.14703	2.212708	5.795455	**1.103198**
#4 ex482/35	17.93912	2.40627	6.965909	**1.070235**
#5 ex531/40	104.38	3.544331	8.727273	**3.374463**
#6 ex562/40	208.1218	4.447351	8.727273	**5.362133**
#7 ex628/40	26.69475	6.934174	8.590909	**0.448118**
#8 ex692/40	17.41691	10.22305	8.272727	**0.205941**

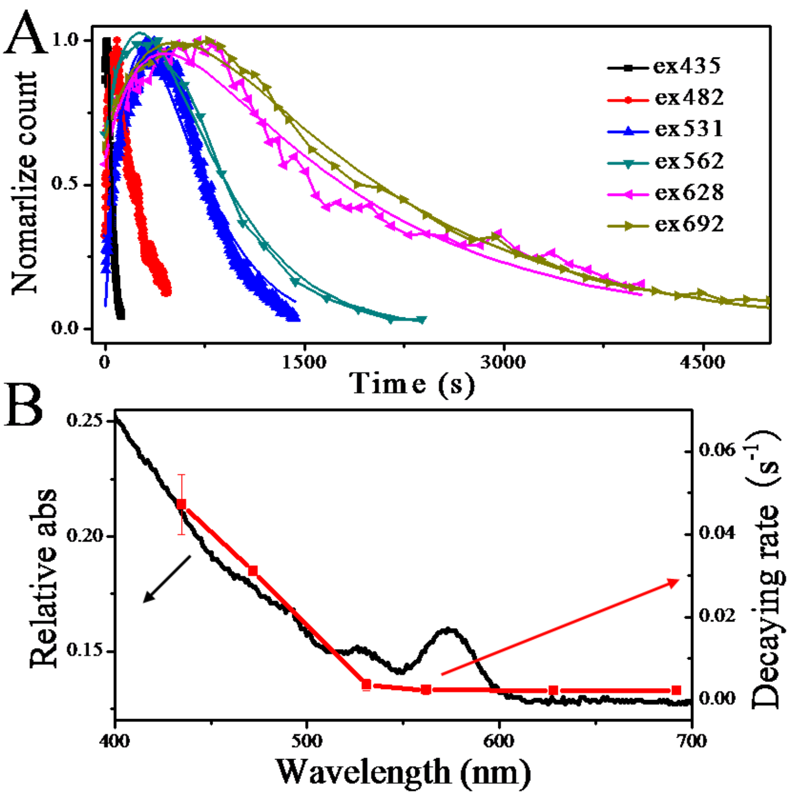
S2: QD525 photobleaching rate constant k_d, photoactivation rate constant k_a and absorption in different wavelength regions

Excitation filter	*Photoactivation rate constant k_a*	*Photobleaching rate constant k_d*	*Absorption*
1# ex387/11	0.00830±0.000350	0.01925± 0.00166	4.406953
2# ex435/40	0.00654±0.000180	0.02350± 0.00286	2.320256
3# ex472/30	0.00124±0.000010	0.01114± 0.00054	1.092405
4# ex482/35	0.00089±0.000008	0.00951±0.00044	1.118960
5# ex531/40	0.00016±0.000005	0.00156±0.00024	0.652015
6# ex562/40	0.00011±0.000002	0.00298± 0.00033	0.134267
7# ex628/40	0.00017±0.000020	0.00118± 0.00025	0.084850

S3-1: QD585 photobleaching rate constant k_d , photoactivation rate constant k_a and absorption in different wavelength regions

Excitation filter	*Photoactivation rate constant k_a*	*Photobleaching rate constant k_d*	*Absorption*
1# ex387/11	-------	-------	0.264
2# ex435/40	0.04195 ±0.00568	0.04712 ±0.00718	0.210
3# ex482/35	0.00524 ±0.00007	0.03106 ±0.00106	0.171
4# ex531/40	0.00327 ±0.00129	0.00355 ±0.00146	0.149
5# ex562/40	0.00158 ±0.0006	0.00241±0.00106	0.152
6# ex628/40	0.00078 ±0.00011	0.00214 ±0.00053	0.129
7# ex692/40	0.00066 ±0.00003	0.00219 ±0.00021	0.127

S3-2: Relationship between the rate of photobleaching and excitation wavelength in QD585. (A) Evolution of the fluorescent QD population under continuous illumination in different wavelength regions. (B) Comparison of the absorption spectrum and photobleaching rate.



S4-1: QD655 photobleaching rate constant k_d , photoactivation rate constant k_a and absorption in different wavelength regions

Excitation filter	*Photoactivation rate constant k_a*	*Photobleaching rate constant k_d*	*Absorption*
1# ex387/11	-------	-------	0.422
2# ex435/40	0.00048± 7.5e-6	0.00161± 0.00005	0.389
3# ex472/30	0.00072± 0.00009	0.00081± 0.00010	0.352
4# ex482/35	0.00058±0.00009	0.00064±0.00009	0.338
5# ex531/40	0.00042±0.00010	0.00047±0.00011	0.296
6# ex562/40	0.00023±0.00007	0.00026± 0.00008	0.256
7# ex628/40	0.000019±0.00015	0.00022± 0.00016	0.217

S4-2: Relationship between the rate of photobleaching and excitation wavelength in QD655. (A) Evolution of the fluorescent QD population under continuous illumination in different wavelength regiosn. (B) Comparison of the absorption spectrum and photobleaching rate.