Supplementary Info

Enhanced electric field sensitivity of quantum dot/rod two-photon fluorescence and its relevance for cell transmembrane voltage imaging

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NP morphology

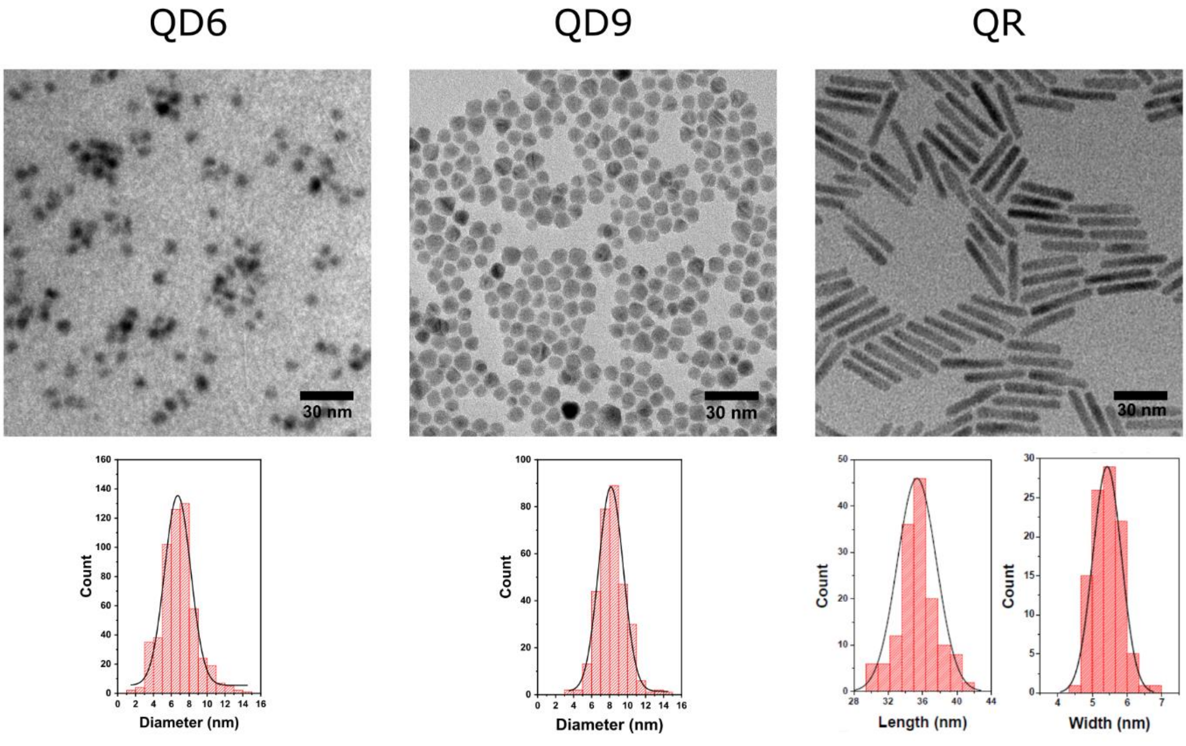


Figure S1: Transmission electron microscopy (TEM) images of the small CdSe/ZnS QDs (QD6) with an average diameter of (6.3 ± 1.5) nm, large CdSe/CdS QDs (QD9) with a diameter of (8.9 ± 1.1) nm and CdSe/CdS dot-in-rods with a core size of 3.7 nm, a length of (35.3 ± 2.3) nm and width of (5.4 ± 0.4) nm.

The core size of the CdSe/CdS QD and QR is 3.7 nm ($\lambda_{1S, QD}$ = 572 nm and $\lambda_{1S, QR}$ = 573 nm) as determined according to the sizing curve by Jasieniak *et al.* [1].

Silica encapsulation of CdSe/CdS QDs.

As an alternative route to PSMA encapsulation for water transfer, CdSe/CdS QDs (QD9) were coated with a 8.5 nm thick silica shell via a reverse microemulsion method. To this end, the QDs (4 nmol) were dispersed in a mixture of heptane (20 ml), Brij L4 (6.4ml), and 5% ammonia solution in water (1 ml). After 1 hour, tetraethyl orthosilicate (25 µl) was added and the reaction was left to react for 3 days. Next, mPEG-silane (9-12 PE units, 16µl) was added and the reaction was left to proceed for another day. The particles were then collected and washed with ethanol and water by centrifugation using spin filters (Vivaspin 20, 100 kDa MWCO). The sample was finally redispersed and stored in water.

NP optical properties

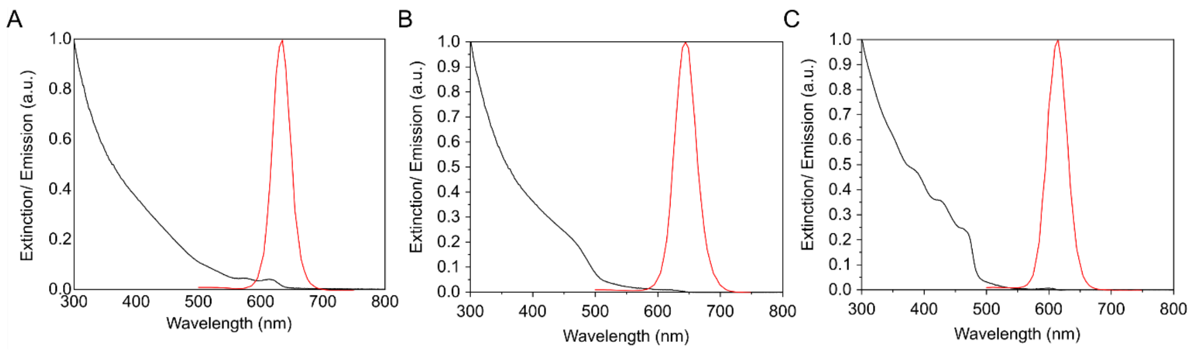


Figure S2: 1P extinction (black) and emission (red, 450 nm excitation) spectra of (A) QD6-PSMA, (B) QD9-PSMA and (C) QR-PSMA in water (pH 7.5 25 °C).

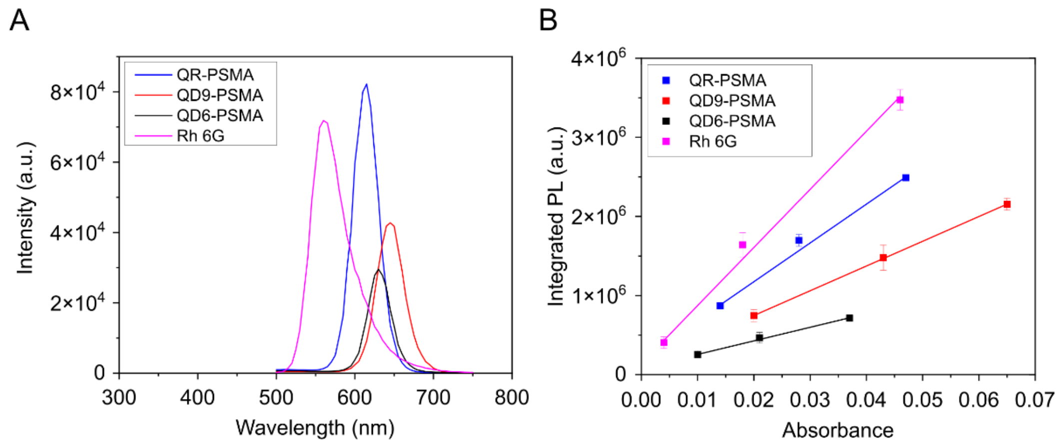
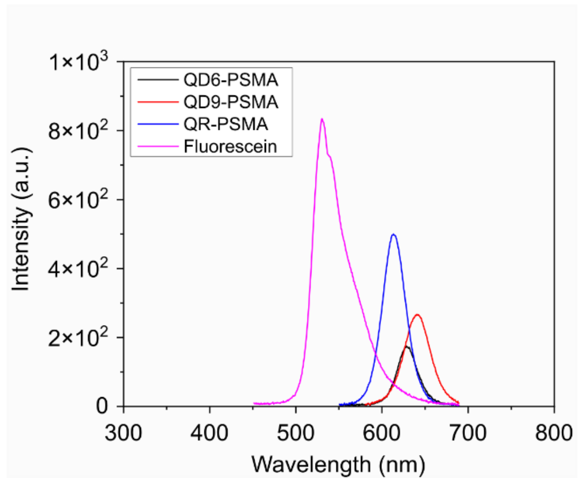


Figure S3: (A) Emission spectra (450 nm excitation) of a QR-PSMA, QD9-PSMA, QD6-PSMA and Rhodamine 6G (Rh 6G) solution with an absorbance of 0.06 at 450 nm. (B) Integrated fluorescence as a function of absorbance. From the ratio of the slopes (with respect to Rh 6G; $QY_{Rh\ 6G}$ = 0.96) the QY of the NPs is estimated in Table S1 [3].

Table S1: 1PF quantum yield (QY) as measured relative to Rhodamine 6G at 450 nm, 25°C.

Nanoparticle	Solvent	Capping	pH	QY (%)
Type I QD CdSe/ZnS (QD6)	$CHCl_3$	octadecylamine	/	**48 ± 1**
	Water	MSA	10	**40 ± 3**
	Water	PSMA/M600-EA	7.5	**25 ± 2**
Type I QD CdSe/ZnS (QD9)	Toluene	Oleic acid	/	**72 ± 3**
	Water	PSMA/M600-EA	7.5	**42 ± 2**
	Water	SiO_2-PEG (9-12 PE units)	7.5	**69 ± 6**
Quasi-Type II QR CdSe/CdS (QR)	Toluene	Phosphonic acids	/	**30 ± 2**
	Water	PSMA/M600-EA	7.5	**69 ± 5**

A



B

$$\sigma_{NP} = \frac{\sigma_{REF}\,\eta_{REF}\,C_{REF}}{\eta_{NP}\,C_{NP}} \frac{\langle F(t)\rangle_{NP}}{\langle F(t)\rangle_{REF}}$$

	QD6-PSMA	QD9-PSMA	QR-PSMA
σ_{NP} (GM)	(24 ± 4) x 10^2	(7 ± 1) x 10^3	(22 ± 4) x 10^4

Figure S4: (A) 2P (900 nm) emission spectra of fluorescein (aqueous, 0.1 mM pH 12), QD6-PSMA (200 nM, pH 7.5), QD9-PSMA (65 nM, pH 7.5) and QR-PSMA (170 nM, pH 7.5). (B) Calculation of the NP's 2PA cross section (σ_{NP}) using fluorescein (16 GM) as a reference with η the quantum yield, C the sample concentration (M) and $\langle F(t)\rangle$ the fluorescence emission per unit time[4].

IDE topography and spacing

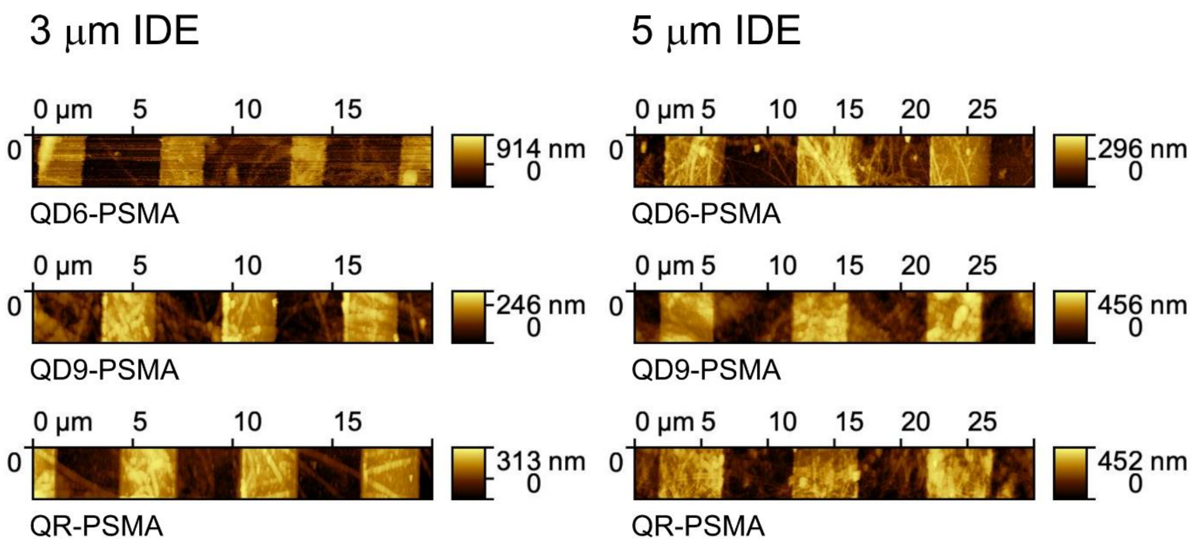


Figure S5: Atomic force microscopy (AFM) topography images of 3 µm and 5 µm spaced IDEs coated with QD-decorated collagen fibers.

NP E-field sensitivity

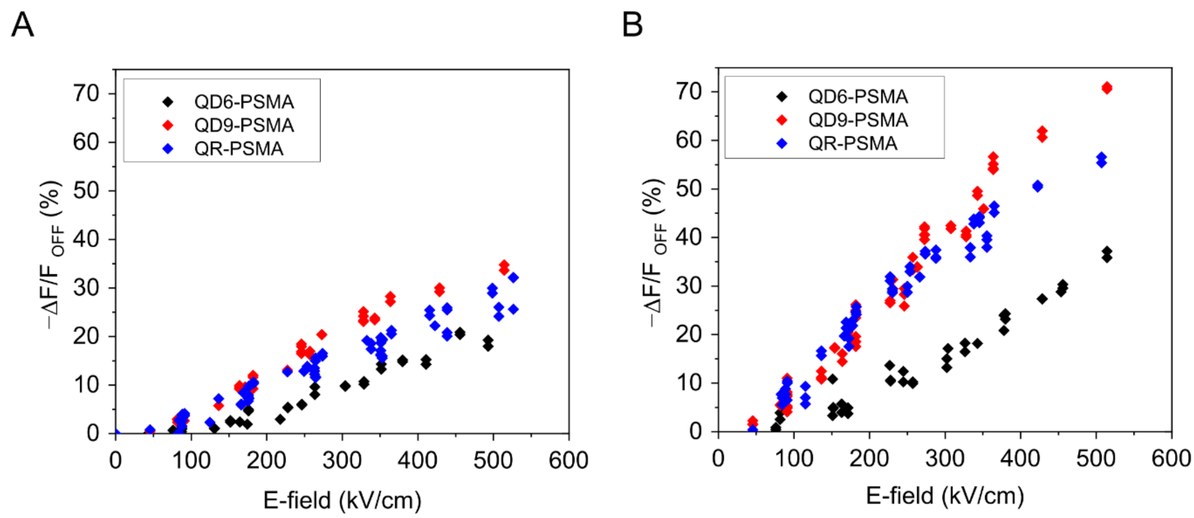


Figure S6: Electric field induced decrease of the fluorescence intensity $\Delta F/F_{OFF}$ under (A) 1PE (488 nm) and (B) 2PE (900nm) for QD6-PSMA (black), QD9-PSMA (red), QR-PSMA (blue).

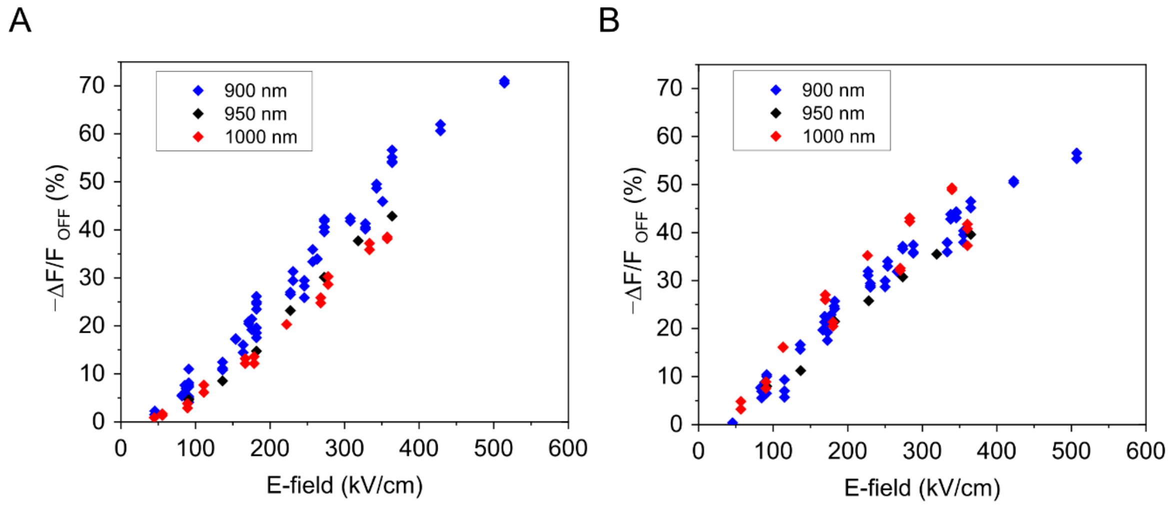


Figure S7: Effect of a small variation in the 2PE wavelength on the electric field induced decrease of the 2PF intensity $\Delta F/F_{OFF}$ of (A) QD9-PSMA and (B) QR-PSMA.

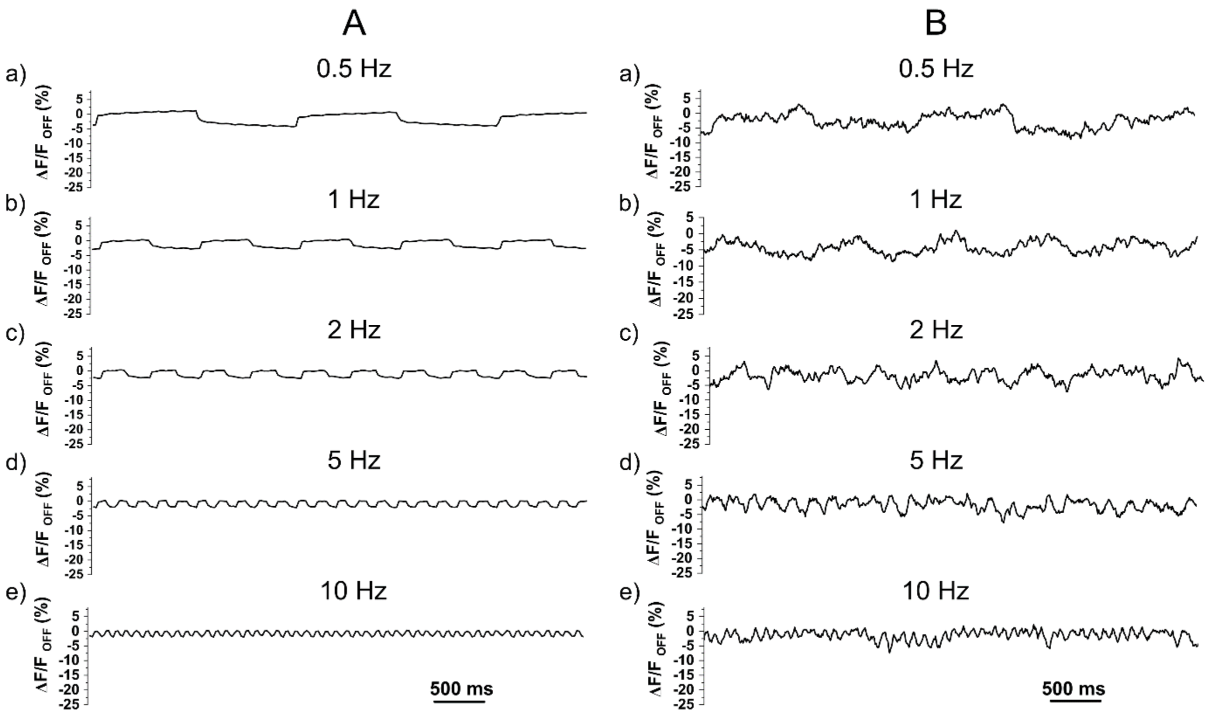


Figure S8: Effect of a frequency modulated E-field with an amplitude of 150 kV/cm on the fluorescence changes of QD6-PSMA under (A) 1PE and (B) 2PE for different frequencies.

Literature

(1) Jasieniak, J.; Smith, L.; van Embden, J.; Mulvaney, P.; Califano, M. Re-Examination of the Size-Dependent Absorption Properties of CdSe Quantum Dots. *J. Phys. Chem. C* **2009**, *113* (45), 19468–19474. https://doi.org/10.1021/jp906827m.

(2) Magde, D.; Wong, R.; Seybold, P. G. Fluorescence Quantum Yields and Their Relation to Lifetimes of Rhodamine 6G and Fluorescein in Nine Solvents: Improved Absolute Standards for Quantum Yields. *Photochem. Photobiol.* **2007**, *75* (4), 327–334. https://doi.org/10.1562/0031-8655(2002)0750327FQYATR2.0.CO2.

(3) Würth, C.; Grabolle, M.; Pauli, J.; Spieles, M.; Resch-Genger, U. Relative and Absolute Determination of Fluorescence Quantum Yields of Transparent Samples. *Nat. Protoc.* **2013**, *8* (8), 1535–1550. https://doi.org/10.1038/nprot.2013.087.

(4) Albota, M. A.; Xu, C.; Webb, W. W. Two-Photon Fluorescence Excitation Cross Sections of Biomolecular Probes from 690 to 960 Nm. *Appl. Opt.* **1998**. https://doi.org/10.1364/ao.37.007352.