A protein-dye hybrid system as narrow range tunable intracellular pH sensor

Palapuravan Anees, Karivachery V. Sudheesh, Purushothaman Jayamurthy, Arunkumar R. Chandrika, Ramakrishnapillai V. Omkumar and Ayyappanpillai Ajayaghosh*

P. Anees, K. V. Sudheesh, Prof. Dr. A. Ajayaghosh

Chemical Sciences and Technology Division and Academy of Scientific and Innovative Research (AcSIR), CSIR-National Institute for Interdisciplinary Science and Technology (CSIR-NIIST), Thiruvananthapuram, 695019, India

E-mail: ajayaghosh@niist.res.in

Dr. P. Jayamurthy

Agroprocessing and Natural Products Division and Academy of Scientific and Innovative Research (AcSIR), CSIR-National Institute for Interdisciplinary Science and Technology (CSIR-NIIST), Thiruvananthapuram, 695019, India

A. R. Chandrika, Dr. R. V. Omkumar

Molecular Neurobiology Division, Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram 695 014, India

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1. Experimental section

1.1. Description of experimental techniques

UV-vis absorption and emission spectral measurements

Electronic absorption spectra were recorded on a Shimadzu UV-3101 PC NIR scanning spectrophotometer and emission spectra were recorded on a SPEX-Flourolog F112X spectrofluorimeter with a 1cm quartz cuvette. All experiments were carried out at 298 K.

Circular dichroism spectroscopy (CD)

CD spectra were recorded on JASCO-J-810 spectropolarimeter by appropriately choosing sensitivity, response time and scanning speed. CD spectra were recorded as θ in millidegrees. pH dependent chiroptical studies of BSA protein were carried using 2 μM solutions in water.

Dynamic light scattering (DLS) measurements

DLS analyses were carried out with a Zetasizer Nano S from Malvern Instruments at 25 °C. Average of at least five measurements were taken. The samples were prepared in phosphate buffer (25 mM NaH_2PO_4, 10 mM NaCl). 15 μL of **Sq** dye from a 1.2×10^{-3} M stock solution in acetonitrile was injected to 3 mL phosphate buffer in quartz cuvette with constant stirring, the solution was kept for 10 min at room temperature before measurement. The average hydrodynamic radii were calculated from Stork-Einstein equation ($R_h = k_B T/(6\pi\eta D)$). All solutions used to detect hydrodynamic diameter by DLS were passed through Millipore filters with a pore size of 0.22 μm to remove dust.

2. Supplementary figures

2.1. pH dependent stability of Sq dye

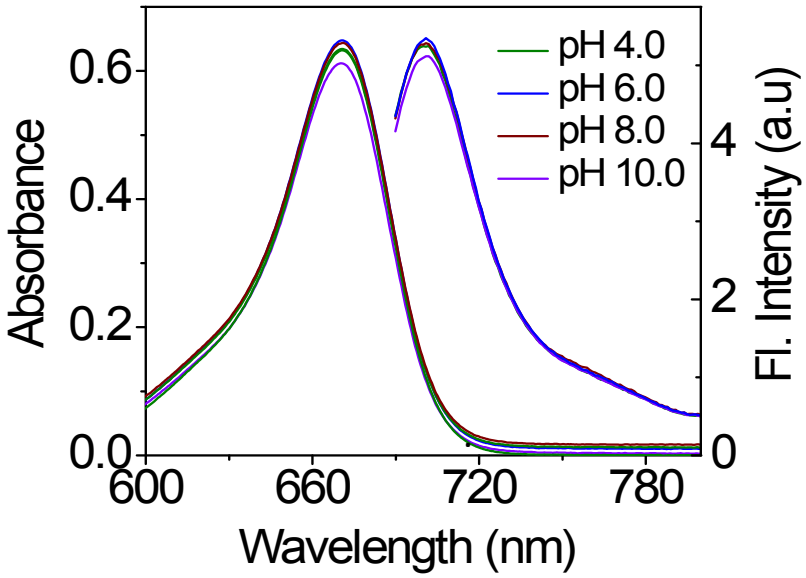

Fig. S1 Absorption and emission (λ_{ex} @ 640 nm) spectral changes of Sq dye (2 μM) in 30% ACN/25 mM phosphate buffer in different pH solutions.

2.2. CD spectra of BSA protein at different pH conditions

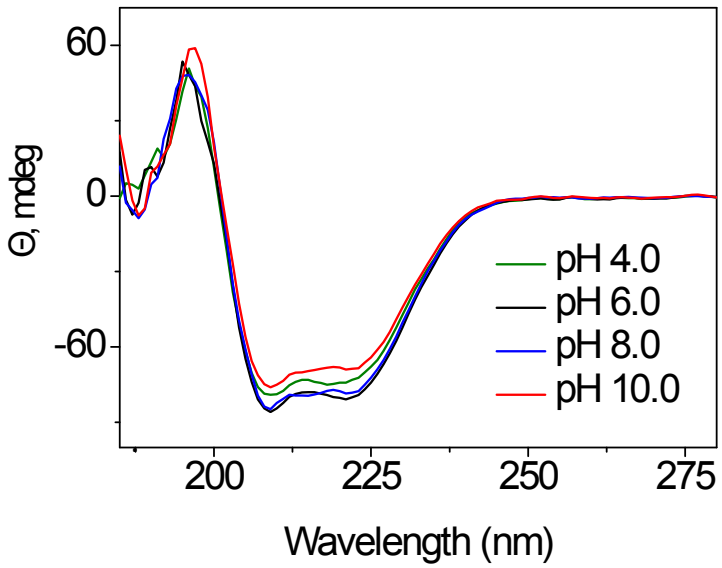

Fig. S2 Circular dichroism spectra of BSA protein (2 μM, water) at different pH conditions.

2.3. Reversible interaction of protein-dye hybrid

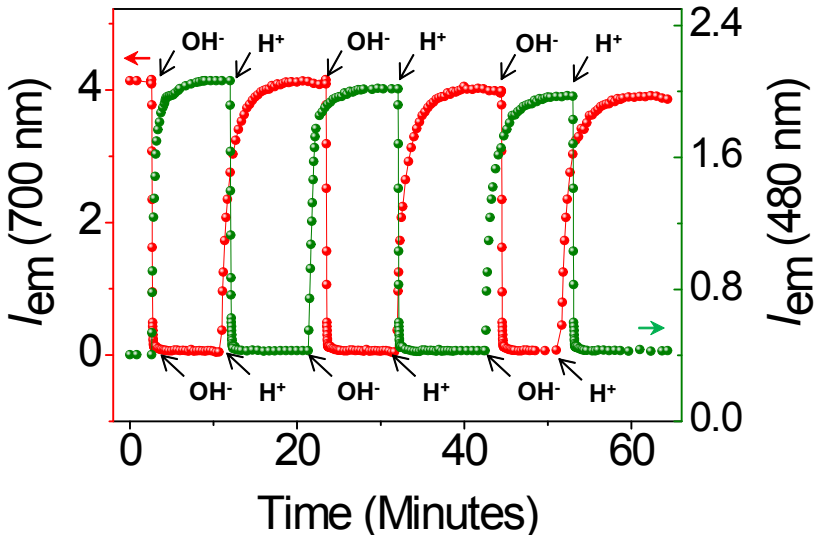

Fig. S3 Time dependent fluorescence responses of 16:1 BSA-SqNPs at 480 (λ_{ex} @ 380 nm) and 700 nm (λ_{ex} @ 640 nm) regions to acid/base cycles.

2.4. Noncovalent interaction of Sq dye with BSA protein under acidic pH

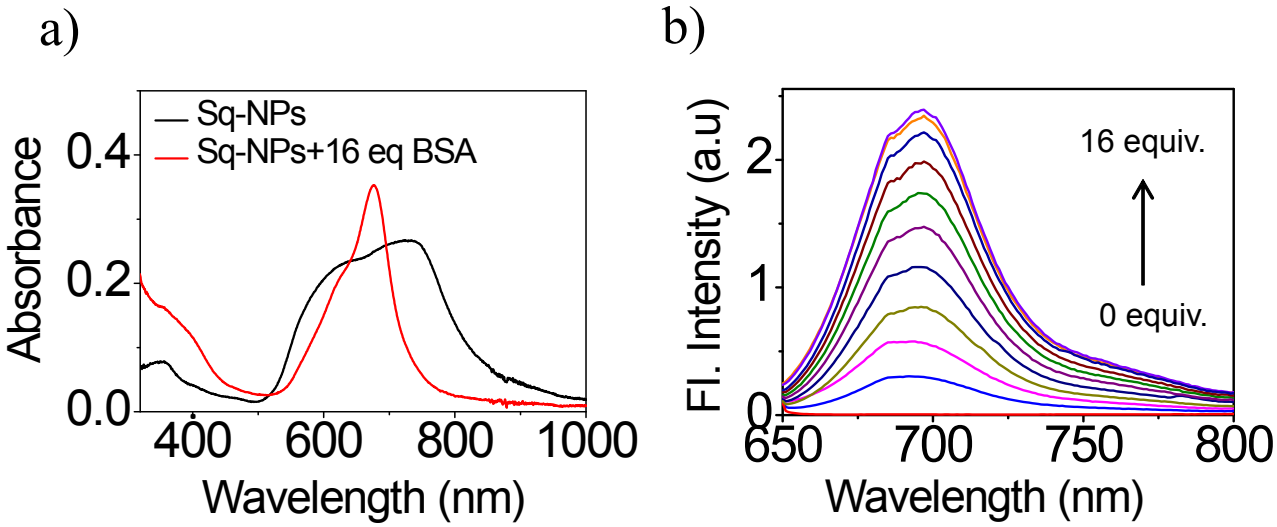

Fig. S4 (a) UV-Vis absorption spectral changes and (b) fluorescence changes of SqNPs upon addition of BSA. All experiments were performed using 6 μM SqNPs solution and 96 μM BSA in 25 mM phosphate buffer at pH 4.0.

2.5. DLS analysis of SqNPs in the presence and absence of BSA protein

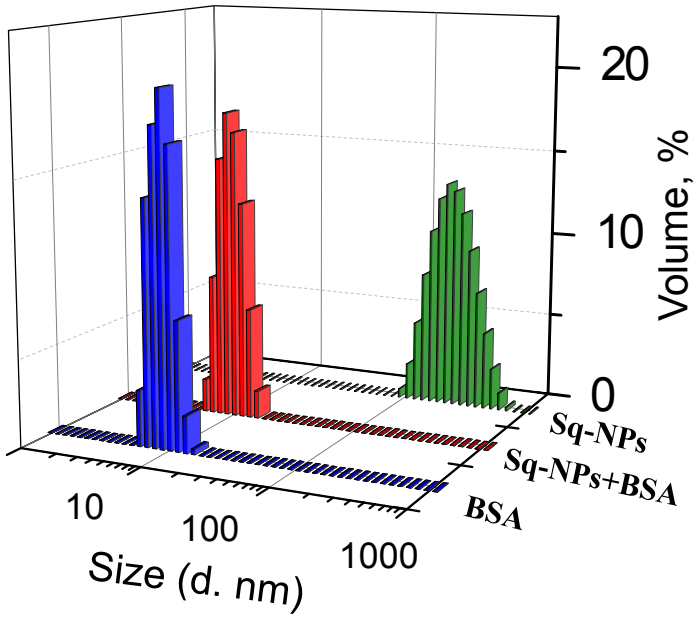

Fig. S5 DLS analysis of SqNPs (6 μM, 25 mM phosphate buffer, pH 4.0) in the presence and absence of BSA protein (96 μM). DLS data of BSA protein alone (6 μM) are also shown.

2.6. DLS analysis of BSA-SqNPs in the presence and absence of DNSA

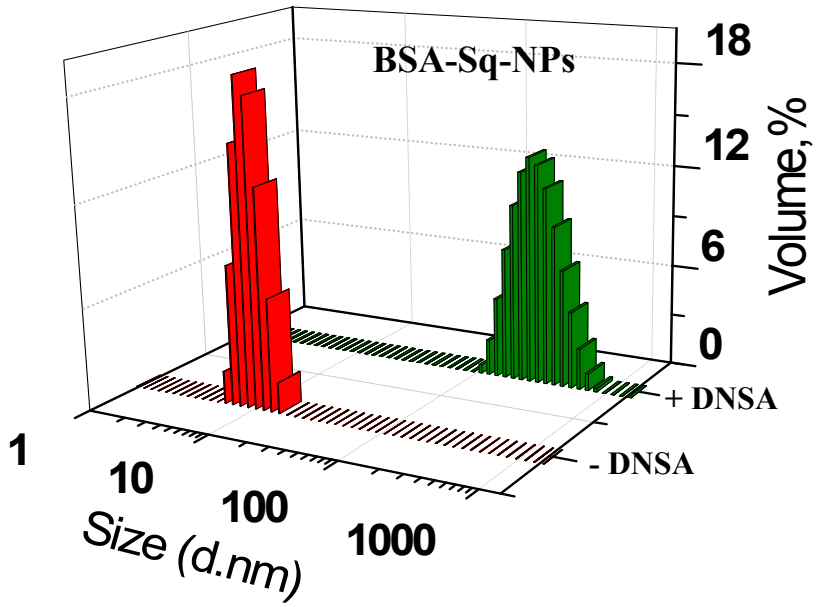

Fig. S6 DLS analysis of 16:1 BSA-SqNPs (25 mM phosphate buffer, pH 4.0) in the presence and absence of DNSA (120 μM).

2.7. Selectivity studies

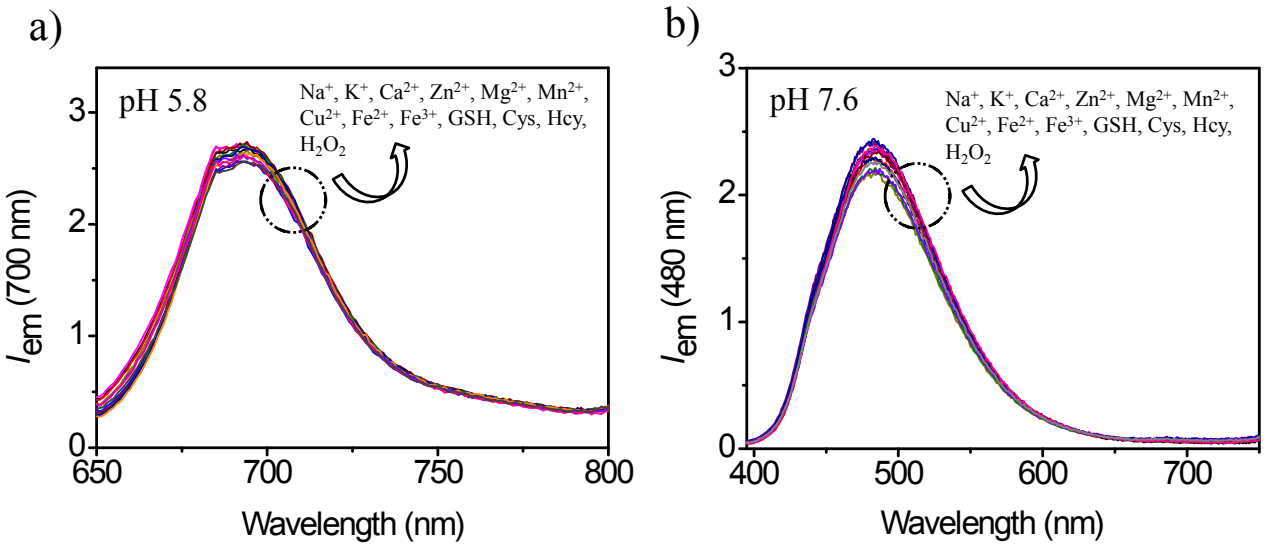

Fig. S7 (a) and (b) Fluorescence spectra of 6:1 BSA-SqNPs in the presence of 20 μM of metal ions (Na^+, K^+, Ca^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}), 1 mM of thiols (GSH, Cys, Hcy), and 100 μM of H_2O_2 in 25 mM phosphate buffer. All data were obtained with 1 μM SqNPs in 25 mM phosphate buffer, λ_{ex} @ 640 nm (a) and λ_{ex} @ 380 nm (b).

2.8. Confocal fluorescence images of HeLa cells incubated with SqNPs alone

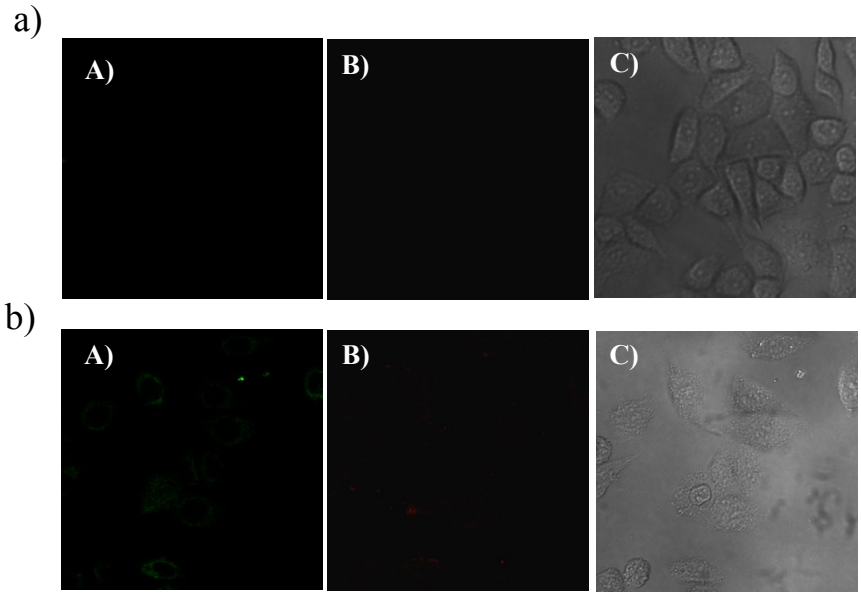

Fig. S8 Confocal fluorescence images of HeLa cells before (a) and after (b) incubation with SqNPs. The three panels represent the green channel (A), red channel (B) and the

bright field (C) images respectively. Green and red images obtained by exciting at 405 and 640 nm, respectively.

2.9. Cell viability test

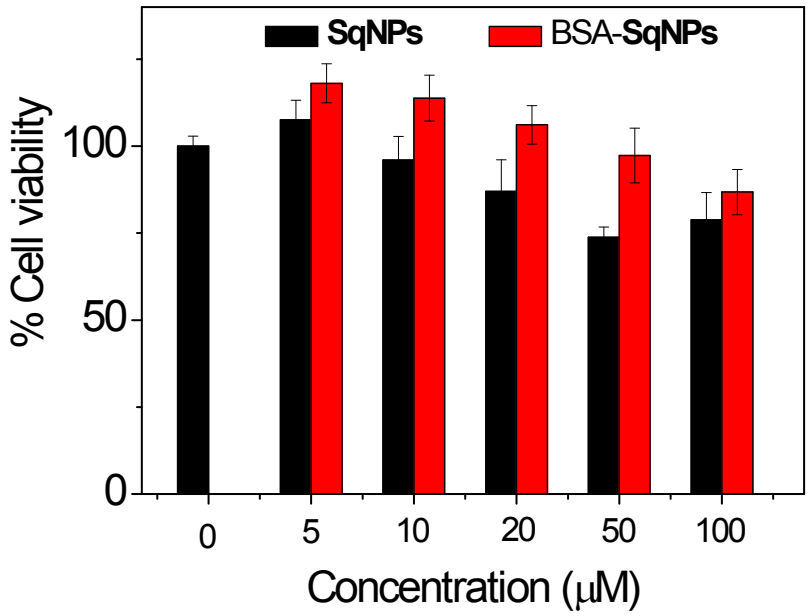

Fig. S9 Cytotoxicity of SqNPs and BSA-SqNPs complex (6:1) in HeLa cells evaluated by MTT assay. The cells were incubated with different concentrations of SqNPs and SqNPs-BSA complex (0-100 μM) for 24 h.

2.10. Confocal fluorescence images of HeLa cells incubated with BSA-SqNPs

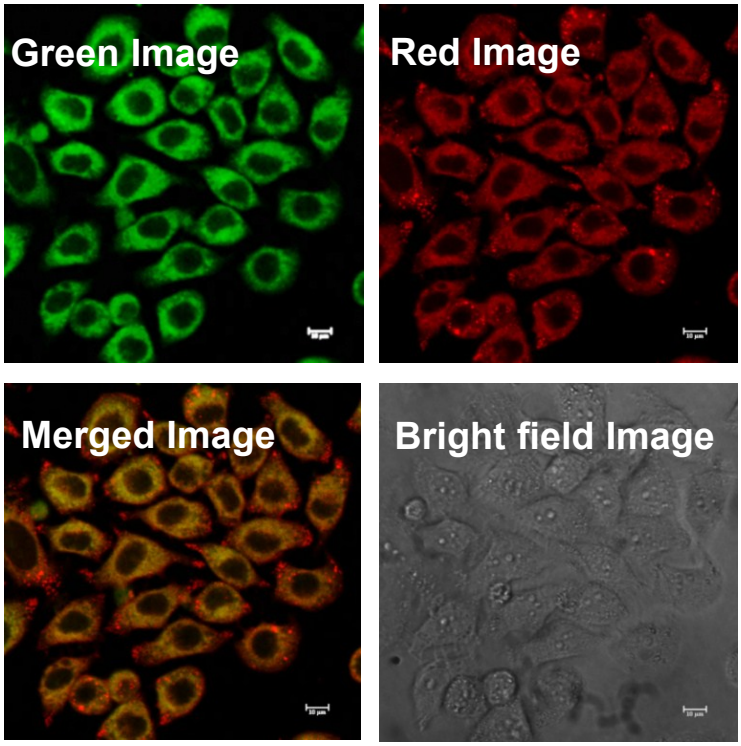

Fig. S10 Confocal fluorescence images of HeLa cells maintained at pH 7.0 incubated with 6:1 BSA-SqNPs for 30 min at 37 ºC.

2.11. Fluorescence intensity plots from wells incubated with BSA-SqNPs hybrids at different pH

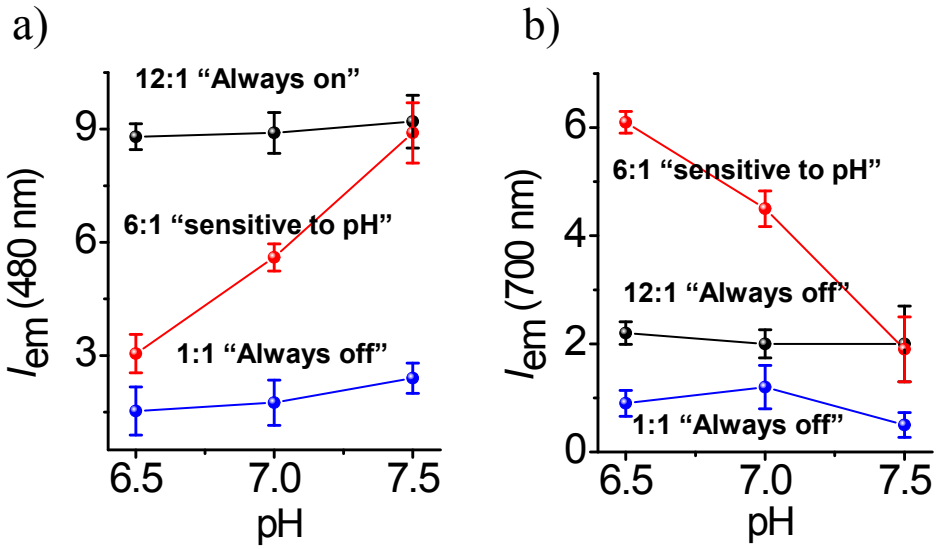

Fig. S11 (a) and (b) Fluorescence intensity plots obtained from different wells treated with BSA-SqNPs conjugates of different ratios at pH 6.5, 7.0 and 7.5.

2.12. Disassembly of the SqNPs by the protein at different pH values

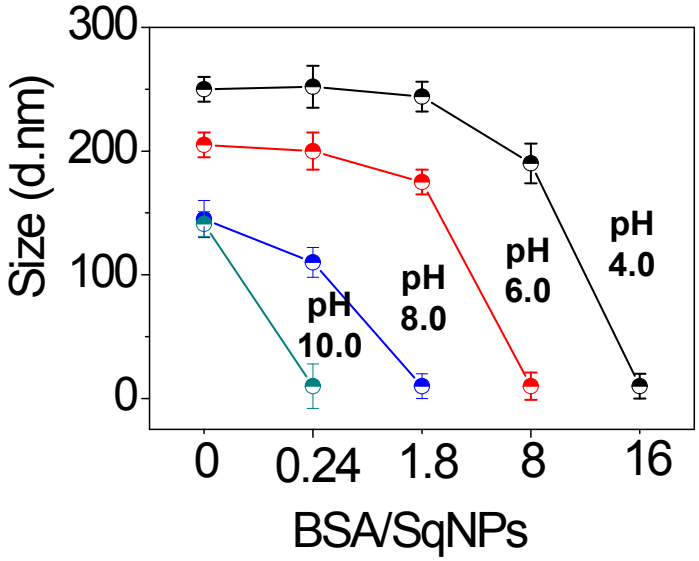

Fig. S12 Volume-weighted hydrodynamic radii of SqNPs (6 μM, in 25 mM phosphate buffer) vs BSA protein of different equiv. at different pH.

2.13. Interaction of various amino acids with Sq dye in the monomeric state at different pH

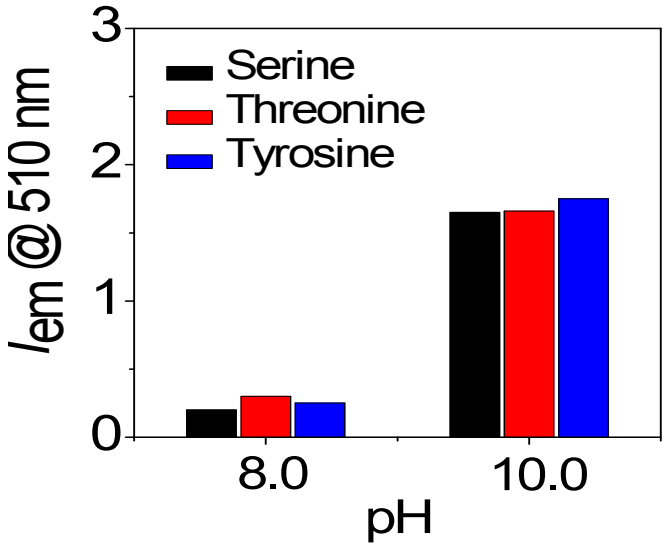

Fig. S13 Fluorescence response of Sq dye (6 μM, in 30% ACN/25 mM phosphate buffer) at 510 nm (λ_{ex} @ 380 nm) monitored against 10 equiv. of different amino acids.

3. References

1. P. Anees, S. Sreejith and A. Ajayaghosh. *J. Am. Chem. Soc.* 2014*,* **136**, 13233-13239.