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Solubilization of Quantum Dots with a Recombinant Peptide from *E.coli*

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The molecular interfacing of biomolecules with advanced materials such as semiconductor nanocrystals, carbon nanotubes and conducting polymers has enormous potential in the field of biomedical applications and optoelectronics. [1-4] Currently, combinatorial biology approaches such as phage display and cell surface display methods permit a better study of these interactions.[5-9] Computational modeling combined with phage display of selected septapeptides with different affinity for metallic platinum revealed the importance of interface-reactive side chains, their special arrangement and backbone stereochemistry for peptide-interface recognition. [10-12]. Genetic engineering of phage display has also been employed to interface peptides with semiconductor nanocrystals also known as quantum dots (QDs). [8] Selective modification of physiological conditions of prokaryotic *E. coli* resulted in capping and controlled growth of CdS nanocrystals [13], although, nanocrystal formation in bacteria has not yet been found in nature.[14, 15] In addition, high affinity metal ligand clusters have been shown to promote the formation of CdS and ZnS nanocrystals in the presence of cysteine and glutathione thiolates *in vitro*. [16-18] A classical organic-inorganic interaction has evolved in eukaryotic yeast, where the presence of heavy metal stress results in increased accumulation of glutathione and glutathione-like peptides termed phytochelatins. [19] Phytochelatins are thiolate peptides which, in the presence of sulfide anions, sequester toxic metal ions by forming QD-peptide complexes in the cell cytoplasm.[20, 21] These intracellular peptide coated nanocrystals appear to be very stable and have photophysical properties similar to QDs synthesized chemically. [21] We previously showed that

rationally designed phytochelatin related synthetic peptides can bind and stabilize CdSe/ZnS core/shell QDs. [22]

A genetic engineering approach to synthesize a rationally designed recombinant peptide GSESGGSESGFCCFCCFCCF (dubbed thereafter rFC3, peptide #1 in Table 1) in *E. coli,* was adopted for solubilization of QDs. The rFC3 sequence contains only natural amino acids as compared to previously tested synthetic peptides that were initially designed with unnatural amino acids.[22] We found that the surface exchange of this natural rFC3 peptide with hydrophobic solvent TOPO (trioctylphosphine oxide) coated CdSe-ZnS QDs was successful as assessed by gel electrophoresis and fluorescence correlation spectroscopy (FCS). Finally, we estimate the number of peptides using a defined ratio of rFC3 and a fluorescent peptide (FITC-CC3, Table 1) – a critical step necessary for future biological applications as one can control the stoichiometry of the reactants with appropriate peptide sequence with functional groups at the N-termini (–NH_2 , –SH groups).

For the recombinant expression of peptide, *E .coli* codon optimized oligo-nucleotides encoding for the peptide rFC3 was synthesized. The pET-31b based DNA vector for expression of the QD binding peptide-rFC3 is depicted in Fig.1a & b. Use of KSI as a carrier protein for rFC3 peptide directs the fusion (KSI-rFC3) protein to inclusion bodies when over-expressed in *E. coli*, protecting the bacteria from the potential toxicity (if any) of these peptides during expression. In theory, a higher number of tandem peptide repeats will give higher yields, but in practice peptide yield decreased when it reached more than 50% of the total encoded protein. The purified His-tagged rFC3 fusion protein was dissolved in 70% formic acid and incubated with a 100-fold

excess of cyanogen bromide (CNBr) [23] over methionine residues. The final KSI-rFC3 was cleaved by CNBr at the methionine residue (ATG codon) to liberate the monomeric rFC3 peptides with a homoserine lactone group at the C-terminal residue [23] from the insoluble KSI fusion protein and His-tag cleaved peptide. CNBr cleaved peptide mixture was centrifuged to remove insoluble material, diluted 10 fold in water, vacuum dried, dissolved in acetic acid and purified via HPLC (Fig.2a) SDS- PAGE analyses of the final dissolved cleaved products (Fig. 2b) revealed greater than 90% cleavage (lane 1, Fig. 2b). In addition, CNBr-cleaved non-specific reaction products were also observed and were purified by RP-HPLC (lane 1, Fig. 2b). The rFC3 peptide elutes at 17–18 min (Fig. 2a) also shown in lane 2 (Fig. 2b).

Having obtained the purified rFC3 peptide, we tested its ability to solubilize 620 nm emitting QDs. The final genetic engineered rFC3 peptide consisted of a bipartite motif – a hydrophobic adhesive motif critical for binding to the core-shell CdSe-ZnS QDs and a hydrophilic motif that confers optimal colloidal properties and solubility to the QDs (Scheme. 1). It has been previously shown that the stability of amphiphilic molecules bound to QDs can be enhanced by the presence of multiple thiol groups [12]. Other non-thiol groups, such as oligomeric phosphine [24], poly(dimethylaminoethyl methacrylate) [25] and 4-substituted pyridine [26] have also been described to exchange hydrophobic surfactant molecules with hydrophilic ligands. Here we chose to incorporate six cysteine residues in the adhesive domain of the rFC3 peptide. The presence of hydrophobic phenylalanine (F) residues around these cysteines was found to be critical in the solubilizing the QDs (Scheme 1). In addition, the hydrophobicity of the phenyl

moieties renders the cysteines in the adhesive domain less prone to react with each other and favorably competes off the surfactant TOPO molecules.

For comparison purposes, solubilization of QDs was also carried out with a previously described unnatural amino acid Cha peptide[22] dubbed CC3 (peptide #3, Table 1) and synthetic phenylalanine peptide dubbed sFC3 (peptide #2, Table 1) peptide obtained commercially. Peptide-coated QDs (pcQDs, emission ~620 nm, FWHM ~ 35 nm) were prepared according to methods reported in the literature. [22] Solubilization of QDs with different peptides from Table 1 confirmed that the interaction of the peptides with the inorganic ZnS shell is dependent on thiol functional groups and hydrophobic amino acid residues - Cha and F. This result is in agreement with our previous study with the synthetic unnatural peptide sequence CC3 where the importance of amino aicds in the hydrophobic and hydrophilic motifs were systematically studied.[22] Upon ligand exchange and solubilization of TOPO-coated QDs, colloidal properties of pcQDs indicated narrow size distribution as evaluated by fluorescence detection after agarose gel electrophoresis (Lanes 1, 2 and 3, Fig. 3). While CC3 and sFC3-coated QDs had similar electrophoretic mobility, the mobility of rFC3-coated QDs (Lane 3) was reduced. Yet, all three peptides have similar charge at the pH of separation. In fact, rFC3-coated QDs appear to have a reduced mobility because of the presence of residual DTT during the purification of rFC3 (see Methods and supplementary info). Indeed DTT can react on the surface of QDs[27] and compete with the binding of rFC3 peptides. This competition may result in a reduction in the number of peptides bound to QDs and affect the total charge of the QDs (one sFC3 peptide provides a -3.0 charge at pH 8.3, during electrophoresis). This translates into a reduced mobility in agarose gels. We have confirmed this effect in an experiment where DTT was intentionally added to compete with the binding of sFC3 peptides on the surface of QDs. We found that the mobility of QDs during agarose electrophoresis is indeed reduced with increasing amounts of DTT (supplementary info).

The absence of smeared bands of the various pcQDs in the agarose gel is a good indicator for monodispersivity and uniform size distribution. Furthermore, pcQDs in aqueous buffer (PBS, pH7.2 or 50 mM Na-borate, 10 mM NaCl, pH7.2) were stable and showed no signs of aggregation over 4-6 months at 4^0C (data not shown).

Having obtained stable and bright peptide coated QDs, it was essential to estimate the number of functional groups provided by the N-termini of the peptides. The surfactant exchange with peptides is a self-limiting reaction. As previously described [22],TMAOH is added in the pyridine:DMSO co-solvent where both the QDs and the peptides are solvated. TMAOH is a strong base and allows the pH of the co-solvent to be raised above

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10.0, pH at which the cysteine (pKa around 8.3) of the peptides form thiolates anion that can coordinate to Zn^{2+} on the ZnS shell of the QDs. We also observed that TMAOH favors the removal of TOPO from the QDs surface and, therefore, allows the interaction of the peptide directly with the surface of the QDs without steric hindrance by TOPO molecules. This was evident upon addition of TMAOH to QDs in the absence of peptides which resulted in precipitation of QDs. The QDs could not be re-dissolved in any polar or apolar solvents, which was is consistent with the removal of surface ligands. Rosenthal et al[28] have also made similar observation of the effect of TMAOH on surface TOPO.

We utilized optical absorption spectroscopy in order to determine the number of bound peptides to the QD at the end of the exchange reaction. In contrast to aromatic amino acids, the phenylalanine residue has a weak extinction coefficient (ϵ = 195 $M^{-1}cm^{-1}$ at 257.5 nm), insufficient for the spectroscopic determination of stoichiometry. We therefore used a N-terminal fluorescein isothiocyanate (FITC) peptide dubbed FITC-CC3 (peptide #4, Table 1). Alternatively mixtures of FITC-CC3 and rFC3 (peptide #1, Table 1) were used to vary and further quantitate peptides on the surface of QDs.

The absorption of light at a wavelength λ by a QD coated with a mixture of peptides #1 and #3 can arise from: (i) absorption by the QD if $\lambda < \lambda_{QD}$ (where λ_{QD} is the excitonic bandgap), (ii) absorption by FITC if $\lambda < \lambda_{FITC}$ (where λ_{FITC} is the longest wavelength of FITC absorption), and (iii) absorption by the peptides themselves. The latter is negligible at visible wavelengths, and therefore it is easy to obtain two separate situations: (1) only the QDs are absorbing ($\lambda_{FITC} < \lambda < \lambda_{QD}$), and (2) both FITC and QDs are absorbing ($\lambda < \lambda_{FITC} < \lambda_{QD}$).

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Let n be the unknown average number of FITC-peptide per QD. The extinction coefficient of FITC at 493 nm, $\varepsilon_{FITC}(493)$, is provided by the manufacturer ($\varepsilon_{FITC}(493)$ = 85,200 cm^{-1} M^{-1}). The first exciton peak of the QD used in this experiment is 610 nm. If we measure the absorption of a peptide-coated QD at 493 nm and 610 nm (Fig. 4), we will obtain data corresponding to the two different situations described above.

The total absorption at a wavelength λ will read:

$$A_{QD+FITC}(\lambda) = \left[\varepsilon_{QD}(\lambda) + n.\varepsilon_{FITC}(\lambda)\right] \cdot c_{QD} L \quad , \quad (1)$$

where c_{QD} and L are the QD concentration and the excitation path length respectively. The extinction coefficient of CdSe QDs (at their first exciton peak wavelength) has been experimentally measured by Peng and collaborators [29] to depend on the first exciton peak wavelength according to:

$$\begin{aligned} &\varepsilon_{QD} = 5{,}857 D^{2.65} \, , \\ &D = 1.6122.10^{-9}\lambda^4 - 2.6575.10^{-6}\lambda^3 + 1.6242.10^{-3}\lambda^2 - 0.4277\lambda + 41.57 \end{aligned} \quad (2)$$

where D is the diameter of the QD core in nm, and ε is expressed in $M^{-1}cm^{-1}$.

From equation. (2), we can calculate $\varepsilon_{QD}(610)$, substitute into equation. (1) and calculate the QDs concentration:

$$c_{QD} = \frac{A_{QD+FITC}(610)}{\varepsilon_{QD}(610)L}. \quad (3)$$

By writing equation. (1) for λ = 493 nm and substituting for the concentration calculated in equation. (3), we derive the equation for *n*:

$$n = \frac{\left[\frac{A_{QD+FITC}(493)}{A_{QD+FITC}(610)}\varepsilon_{QD}(610) - \varepsilon_{QD}(493)\right]}{\varepsilon_{FITC}(493)}. \quad (4)$$

The only unknown in this expression is $\varepsilon_{QD}(493)$, which can be easily obtained from the calculated $\varepsilon_{QD}(610)$ and the measurement of the QD-only absorption spectrum:

$$\varepsilon_{QD}(493) = \varepsilon_{QD}(610).\frac{A_{QD}(493)}{A_{QD}(610)}. \quad (5)$$

The number of FITC-labeled peptide per QD is thus given by the following equation:

$$n = \left[\frac{A_{QD+FITC}(493)}{A_{QD+FITC}(610)} - \frac{A_{QD}(493)}{A_{QD}(610)}\right]\frac{\varepsilon_{QD}(610)}{\varepsilon_{FITC}(493)}. \quad (6)$$

After gel filtration and dialysis, molar concentration of FITC and QDs were evaluated from absorbance values at the first exciton peak, and from extinction coefficients calculated based on the emission wavelength of the CdSe as previously described [29]. The concentration of FITC was determined using absorption values at 493 nm and an extinction coefficient of 85,200 $M^{-1}cm^{-1}$ at this wavelength. [30] The calculated extinction coefficient (at 610 nm) of 620 nm emitting CdSe/ZnS QDs was 353762.8 $M^{-1}cm^{-1}$, and absorption measurements yielded molar concentrations of 821 and 62 nM for FITC and QD respectively. Using equation (6), the calculated number of peptides covering the surface of a single QD was determined to be ~ 9 to 10. A typical uv-vis absorbance spectra of FITC-CC3 coated QDs is shown in Fig. 4. Taking into account that QDs were coated using equimolar concentrations of peptides #1 and #4 we conclude that the surface of 620 nm emitting QDs is covered by approximately 20 peptides. Similar experiments solubilizing QDs with 100% FITC-CC3 peptides also produced identical results.

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Standard characterization methods applied to nanotechnology such as agarose gel electrophoresis and size exclusion high pressure liquid chromatography (SE-HPLC) are limited in resolving size heterogeneity and small aggregates. Indeed, in SE-HPLC, enthalpic interactions of pcQDs with column matrix may preclude accurate quantitative analysis of QD size and its size distribution. Such interactions can be minimized by appropriate mobile phase modifications [31] and recently, aqueous SE-HPLC and STEM (scanning tunneling electron microscopy) was adopted for the separation and characterization of different sizes of CdSe-protein conjugates.[32] To overcome the limitation of gel electrophoresis or SE-HPLC, and study the colloidal properties of natural peptide-coated QDs, we employed fluorescence correlation spectroscopy (FCS).[33] FCS is a powerful tool for detecting conformational fluctuations of biopolymers, for

investigating photophysical properties of organic dyes and fluorescent proteins, and for studying the diffusion kinetics of labeled macromolecules *in vitro* and *in vivo.* [33-35]. The technique was recently extended to studying the aggregation state, the brightness per particle and the hydrodynamic radius of QDs.[34, 36]

We performed FCS measurements on rFC3, sFC3 and CC3 (peptides #1, 2 and 3, Table 1) coated QDs in PBS buffer, pH7.2. FCS revealed hydrodynamic radius of ~11-12 nm for rFC3 and sFC3 and ~13 nm for CC3 pcQDs respectively. Measurments of brightness per particle (BPP) of rFC3, sFC3 and CC3 pcQDs are shown in Fig. 5a and reveal uniform brightness and quantum yields (QYs) ranging between 20-25% in PBS, pH 7.2 (QYs were derived by comparing to a LD690 dye, Exciton). As discussed in Doose et al.[36], the BPP is a measure only for the particles which are in their photoactive "bright" state (it does not take into account dark particles which are not detectable by FCS).

Hydrodynamic radii were determined from the measured diffusion constants and by comparing the FCS decay curves with that of 26 nm fluorescent beads (used as a standard) according to: $\tau_{QD} / \tau_{bead} = R_{QD} / R_{bead}$. We observed that QDs coated with peptides (rFC3 and sFC3) have small hydrodynamic radii of 11-12 nm similar to those of QDs coated with synthetic Cha peptides which were 12-14 nm (Fig 5b). In addition, aggregation properties were analyzed for the same set of pcQDs. We measured the degree of aggregation of QD samples by taking multiple short FCS measurements (80 times x 30 sec runs) and building histograms of the values of the correlation function at zero correlation time $g^2(0)$ of the different runs. [33] Since $g^2(0) = 1/n$, aggregation would cause the measured average number of particles per run to fluctuate. Histograms of rFC3, sFC3

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and CC3 pcQDs are shown in Fig.6a, b & c respectively, showing slightly narrower distribution for rFC3 coated QDs.

Herein, we reported on the specific QDs surface recognition of a recombinant peptide from *E .coli* forming a hybrid organic-inorganic nanocomposite. We showed that using *E .coli* as a host for the production of cysteine rich peptides is a cost-effective alternate to solid phase peptide synthesis which can be technically challenging and laborious for cysteine rich peptides used in this study. Several surface chemistries replacing the original hydrophobic ligands used during the synthesis of QDs have been described in the literature. Mercapto-based surface exchange chemistry involving mono-thiol ligands were unstable in biocompatible buffers as they detached from the surface of QDs over time. [37] Dithiol ligands with subsequent conjugation to engineered proteins enhanced the stability of QDs in aqueous buffers [12]. Pinaud et al[22]., described the use of unnatural (Cha) amino acids composed of polycysteinyl amino acids which provided stable peptide coating on QD surface. Taking into account the various approaches, we reasoned that peptides could be used as an excellent ligand for coating the surface of QDs. The passivation of QD surface with peptides produced in E. coli is attractive for several reasons. i) peptides mimic the biological environment and are stable in physiological pH, ii) reactive groups such as amines, carboxyl, thiol and peptide tags can be dialed into the hydrophilic domain of the peptide sequence and enable enzymatic or standard conjugation chemistry to biomolecules of interest, iii) molecular evolution strategies to randomize peptides can be adapted to select high affinity binders to QD surface and iv) the possibility to create multifunctional QDs by mixing peptide sequences

in certain molar ratios in a single step for *in vitro* and *in vivo* studies (e.g., a QD with biotin and PEG, conjugating receptor ligand and PEG etc…).

Hence, by laying the ground rules for recombinant peptide sequence that selectively recognize the ZnS shell of QDs, a polymerase chain reaction (PCR) based strategy can be used to randomize the DNA construct to express several different soluble peptides with different functional groups at the N-termini for bio-conjugation using standard chemistry. It is therefore expected that recombinant DNA design in combination with expression in *E. coli* will provide a platform for further refinement of the organic-inorganic interface. In conclusion, we expanded our "peptide toolbox" for QD coating [2] by encompassing recombinant DNA technology strategies, an active *E. coli* expression system, biochemical purification and colloidal and photophysical characterization.

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Figure Legends

Table 1. Peptides used for solubilizing quantum dots.

Scheme 1. Schematic of the peptide coated quantum dots. The adhesive motif consists the hydrophobic aminoacid phenylalanine (F) and thiol containing cysteines (C) bound to the inorganic ZnS shell and the hydrophilic motif conferring solubility to quantum dots.

Figure 1. Schematic diagram of the KSI-rFC3-His expression DNA construct. (a) The peptide sequence of the recombinant peptide rFC3. The DNA sequence indicated in (b) encoding the rFC3 peptide was cloned into the AIWNI site downstream of the KSI fusion protein. The ATG and its complementary codon for methionine was used in the construction of tandem repeats of the rFC3 peptide. Note that the ATG codon is a chemical cleavage site for cyanogen bromide.

Figure 2. Purification of recombinant peptide FC3 expressed in *E.coli*. Cyanogen bromide cleaved fusion KSI-rFC3 protein was injected into 4.6 x 250 mm C-18 column. Pure peptide eluted with a retention time at around 18 min as shown in (a), and analyzed on a 4-12% denaturing SDS-PAGE gel. (b) Lanes 1, cyanogen bromide cleaved fusion recombinant protein; 2, purified peptide after reverse phase HPLC step.

Figure 3. Agarose gel electrophoresis of various peptide coated quantum dots (QDs) emitting at 620 nm. Lanes 1- sFC3 peptide; 2 – CC3 peptide; 3 – rFC3 peptide. rFC3 is slightly delayed because of residual DTT during purification of the peptide.

Figure 4. Comparison of normalized absorbance spectra of peptide coated quantum dots emitting at 620 nm. FITC-CC3 and rFC3 peptide coated (red) quantum dots has excitonic

peaks at 493 and 610 nm respectively while rFC3 peptide coated (black) quantum dots has an exciton peak at 610 nm alone.

Figure 5. Histograms obtained for rFC3, sFC3 and CC3 peptide coated quantum dots using fluorescence correlation spectroscopy (FCS). (a) Brightness per particle (BPP) was approximately uniform for all the peptide coated quantum dots, (b) The hydrodynamic diameter of rFC3, sFC3 and CC3 was measured to be 11 and 13 nm respectively.

Figure 6. Size distribution histograms of peptide coated quantum dots using fluorescence correlation spectroscopy (FCS). Distribution of rFC3 coated quantum dots (a) indicate a narrower distribution as compared to sFC3 (b) and CC3 (c) peptide coated quantum dots

Table 1. Peptides used for solubilization of quantum dots.

No	Acronym	Peptide sequence
1	rFC3	NH_2-Gly-Ser-Glu-Ser-Gly-Gly-Ser-Glu-Ser-Gly-Phe-Cys-Cys-Phe-Cys-Cys-Phe-Cys-Cys-Phe-HS*
2	sFC3	NH_2-Gly-Ser-Glu-Ser-Gly-Gly-Ser-Glu-Ser-Gly-Phe-Cys-Cys-Phe-Cys-Cys-Phe-Cys-Cys-Phe-$CONH_2$
3	CC3	NH_2-Gly-Ser-Glu-Ser-Gly-Gly-Ser-Glu-Ser-Gly-Cha§-Cys-Cys-Cha-Cys-Cys-Cha-Cys-Cys-Cha-$CONH_2$
4	FITC-CC3	FITC-Gly-Ser-Glu-Ser-Gly-Gly-Ser-Glu-Ser-Gly-Cha-Cys-Cys-Cha-Cys-Cys-Cha-Cys-Cys-Cha-$CONH_2$
5	C3	NH_2-Cha-Cys-Cys-Cha-Cys-Cys-Cha-Cys-Cys-Cha-$CONH_2$

(*) – homoserine residue denoted by HS, obtained after CNBr cleavage of methionine residue. (§) – 3-cyclohexylalanine denoted by Cha, an unnatural aminoacid.

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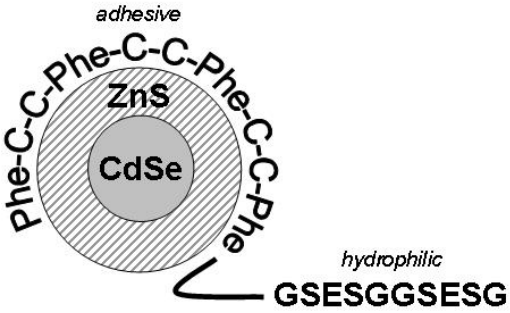


Scheme 1. Iyer et al.

(a) **Target peptide : GSESGGSESGFCCFCCFCCF**

(b)

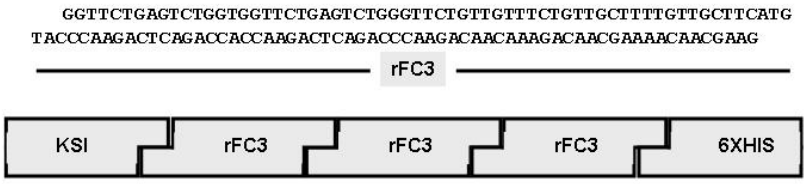


Figure 1. Iyer et al.

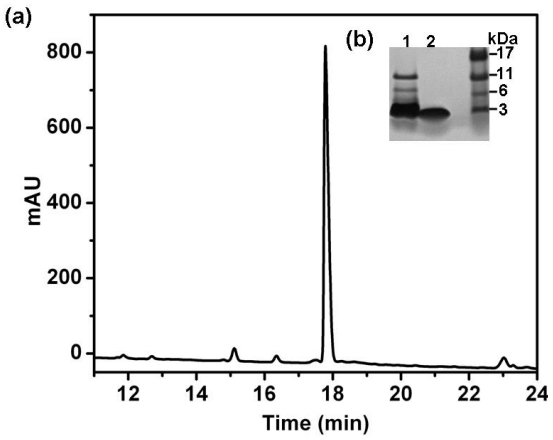


Figure 2. Iyer et al.

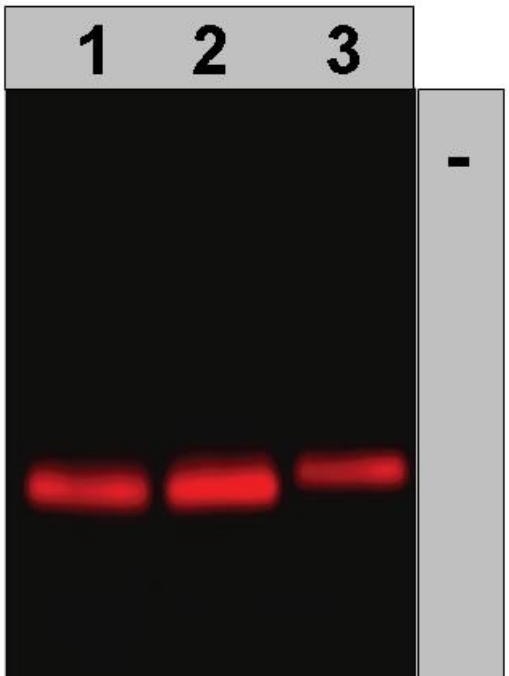


Figure 3. Iyer et al.

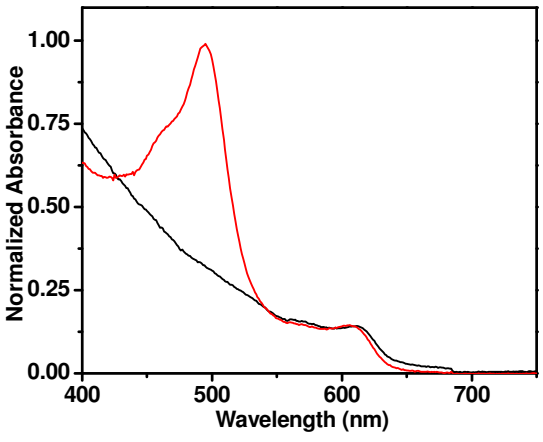
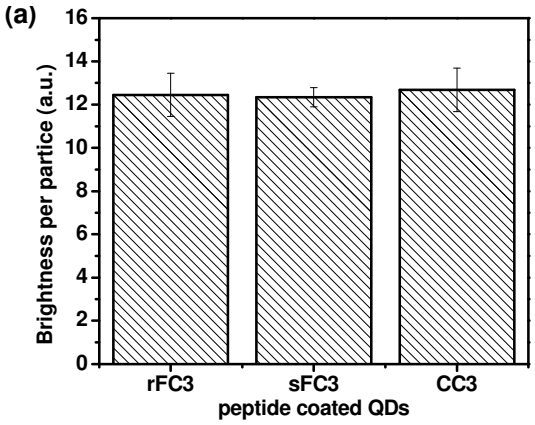


Figure 4. Iyer et al.



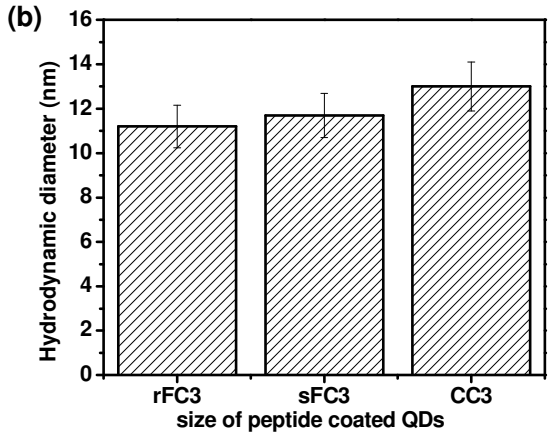
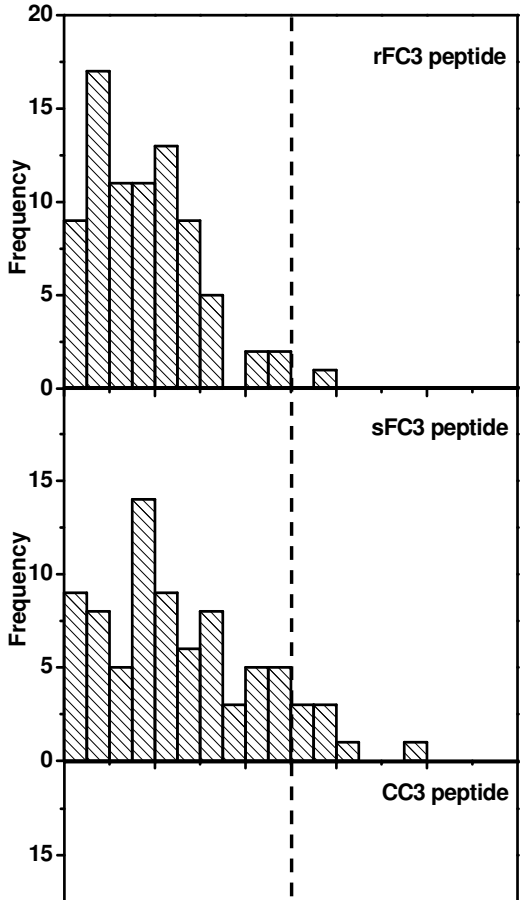


Figure 5. Iyer et al.

(a)



(b)

(c)

Figure 6. Iyer et al.

Supporting Information:

Methods

Bacterial strains. *E.coli* strain **DH5α** F$^-$ ϕ80d*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17(r_k^-, m_k^+) *pho*A *sup*E44 λ$^-$ *thi*-1 *gyr*A96 *rel*A1 was used as a host for subcloning screening for positive clones by colony PCR and plasmid amplification. Gene expression and peptide expression studies were performed in *E.coli* strain **BL 21 (DE3)pLysS**. F$^-$*ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* Δ(*srl-recA*)*306*::Tn*10* (Tc^R) (DE3) pLysS

(Cm^R) (Novagen, Madison, WI) Plasmid maintenance and bacterial stocks were maintained at 50 μg/ml ampicillin.

Construction of Peptide-Encoding DNA Constructs

Synthetic oligonucleotides (Integrated DNA Technologies), encoding the peptide sequence rFC3: 5'-ggttctgagtctggtggttctgagtctgggttctgttgtttctgttgcttttgttgcttcatg-3'and5' gaagcaacaaaagcaacagaaacaacagaacccagactcagaaccaccagactcagaacccat-3' flanked with AlwNI recognition site at its 5' and 3' ends were incubated with T4 polynucleotide kinase (New England Biolabs) at 37 °C for 60 min. 5′-Phosphorylated oligonucleotides were recovered by phenol/chloroform extraction. Annealing was accomplished by heating to 99°C for 10 min with subsequent slow cooling to 30 C over 30 min and room temperature for 3 h. The double-stranded DNAs containing complementary ATG overhangs were then ligated with T4 DNA ligase (New England Biolabs) to form tandem repeats of random lengths. Reaction products were separated by electrophoresis on a 2% Nusieve GTG agarose gel and distinct bands containing oligonucleotides with 1-4 repeats of the FC3 DNA insert were isolated. Purity was confirmed by electrophoresis. These inserts were then ligated into AlwN1 digested pET31b vector (Novagen). The resulting pET31b $(FCQ)_n$ plasmids were transformed into *E. coli* **DH5α** (Invitrogen) for screening via colony PCR. Subsequent electrophoresis permitted identification of clones containing insert DNA. Fusion protein expression levels in IPTG-induced cultures of BL21(DE3)pLysS were tested for constructs containing different repeat numbers of the rFC3 gene, with the pET31b rFC3 plasmid giving the highest yield of fusion protein. The identity of the final construct used for expression was confirmed by DNA sequencing, as shown in Fig. 1.

Protein Expression

KSI-rFC3-6XHis was expressed in *E. coli* strain BL21(DE3)pLysS. Luria broth (LB) culture, supplemented with 50 μg/ml carbenicillin and 34 μg/ml chloramphenicol was inoculated from a glycerol stock and incubated overnight at 37°C. A 1/40 starter culture was inoculated in 1L of LB culture The expression culture was grown to an OD of 0.3–0.4 and induced with 0.7 mM isopropyl-β-d-thiogalactopyranoside (IPTG) for 5 h at 37 C. Cells were then harvested at an O.D. of 1.8, pelleted stored at −70 C, until further use.

Purification of the KSI-rFC3-His fusion protein

The thawed cell pellet was resuspended in 20–30 ml BugBuster buffer (Novagen) containing complete protease inhibitor (Roche, 1 tablet/50 ml), 1.4 mM 2-mercaptoethanol and 0.25 mg/ml lysozyme (Sigma). Cells were lysed by sonication for 2 min at 4 C, and centrifuged at 15,000*g* for 30 min at 4 C. The crude inclusion body pellet was resuspended by sonication in BugBuster and centrifuged. This washing step was repeated with BugBuster solution. Inclusion bodies were subsequently solubilized by incubation in 6 M guanidine HCl (GuHCl) buffer (20 mM Tris-HCl, pH7.9, 1.4 mM 2-mercaptoethanol, 0.5 mM NaCl, 5 mM imidazole.) overnight at 4°C. After brief centrifugation to remove insoluble material, 10–15 ml Ni–NTA resin (Novagen), equilibrated with the same buffer, was poured into a gravity column. Total protein from inclusion bodies mixture was washed with 16 mM imidazole twice with and four times without guanidine hydrochloride and eluted with four volumes 300 mM imidazole, 50 mM sodium acetate, pH 4.0. The final eluate was pH adjusted to pH7.0 and incubated

with 50 mM DTT for 3 h and subsequently dialyzed extensively against 50 mM sodium acetate pH 4.0. The resultant white precipitate was centrifuged and vacuum dried.

CNBr cleavage and reverse phase HPLC purification

The dry protein was dissolved at 10 mg/ml in 70% formic acid and a 100-fold excess of CNBr over Met residues was added. The reaction was purged with N_2 gas, incubated in the dark for 18-20 h at room temperature and quenched by addition of four volumes of water containing 10 mM TCEP, and vacuum was used to dry the sample. CNBr cleavage products were dissolved in 60% Acetonitrile/1%TFA/40 % H_2O, filtered through 0.22 μm, and dried again under vacuum. The final dried pellet was resuspended in 100% acetic acid or 80 % formic acid and peptide was purified by reverse-phase HPLC, using a C18 column (Restek) at 1 ml/min, with a gradient of 5–30% solvent B (99.9% acetonitrile, and 0.1% TFA) in solvent A (99.9% H_2O, and 0.1% TFA) over 20 min. Peptide identity was confirmed by MALDI mass spectrometry. The purity of HPLC purified FC3 peptide was greater than 95%, as determined by MALDI. Typical yields were 40-60 mg per 1.5 L culture.

DTNB Assay

The DTNB assay was performed as described [38]. A solution of 5-5′-dithiobis-2-nitrobenzoate (DTNB) (4 mg/ml) was prepared in ethanol. Purified samples post his-affinity column and reverse phase HPLC chromatography (0.1 ml) and DTNB solution (2.5 μl) were mixed together and allowed to react at room temperature for 20 min. The absorbance of the solution at 412 nm was measured, and the thiol concentration was calculated.

SDS-PAGE Electrophoresis

CNBr cleaved samples were diluted 100 fold with 10 mM Tris, pH 8.0, 5 mM DTT and concentrated under vacuum. Purified peptide HPLC fraction containing acetonitrile/TFA was evaporated and dried by speed vac and redissolved in 10 mM Tris, pH 8.0, 5 mM DTT. The CNBr post cleavage and RP-HPLC samples were treated as per manufacturer's instruction (Invitrogen) and electrophoresed on a 4-12% Bis -Tris at 150 V for 1 hr. The gel was stained with simply blue safe stain (Invitrogen) and imaged under white light.

Surface Passivation of Quantum Dots using Peptides

TOPO-coated CdSe/ZnS QDs were synthesized by rapid injection of dimethyl-cadmium and TOP-selenide precursors in hot TOPO as described previously [39-42] Two sizes of CdSe cores: 2.4 and 4.7 nm in diameter[39] were over coated with 4-5 monolayers of ZnS and preserved in butanol/TOPO. Green (530 nm) and Red (620 nm) emitting QDs were used for recombinant FCQ peptide exchange. 50 µl of TOPO-coated QDs was pelleted with ~ 100 µl of methanol and resuspended in 450 µl of pyridine to a 1µM concentration. To this, 2 mg of recombinant rFC3, synthetic phenylalanine sFC3 , unnatural amino acid 3-cyclohexylalanine CC3 or N-terminal labeled fluorescein isothiocyanate FITC-CC3 peptides was dissolved in 50 µl of DMSO and mixed with concomitant addition of ~12 µl of tetramethylammonium hydroxide 25%(w/v) in methanol. The resulting mixture was spun at 12,000 rpm for 3 minutes. The supernatant was decanted and corresponding peptide coated QDs were resuspended in 300 µl of DMSO [22].

Deleted: u

Purification and electrophoresis of peptide coated quantum dots

Sephadex G-25 (Amersham) column was equilibrated with milli-Q water. 300 µl of peptide coated QDs was eluted from the column by monitoring the fluorescence using a hand held ultraviolet lamp at 254 nm. Excess peptides from the peptide coated containing fractions was dialyzed against a first change of 50 mM sodium phosphate, 10 mM Na-phosphate overnight and second change of 10 mM sodium phosphate, 10 mM NaCl for 4-6 h using a 500,000 MWCO PVDF dialysis membrane (Spectrapor). The purified peptide coated QDs were electrophoresed on a 1% TBE agarose gel (Tris-Borate-EDTA) at constant 120 V and visualized using a fluorescence gel scanner (Biorad, Hercules, CA) equipped with a laser excitation source at 488 nm and appropriate emission filters.

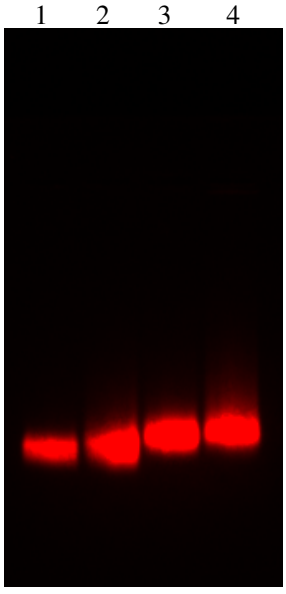
Fluorescence Correlation Spectroscopy of Peptide Coated Quantum Dots

Fluorescence spectra of various peptide coated QDs were acquired on L-201M fluorescence spectrofluorometer (PTI Inc, Lawrenceville, NJ) with excitation at 400 nm. UV-vis absorption spectra were acquired on a Perkin-Elmer lambda 25 spectrophotometer. FCS measurements were performed using a custom-built confocal microscope (Olympus IX71). The emission line of 488 nm of a continuous wave argon Ion laser (Ion Laser Technology, Frankfort, IL) was used as the excitation source. Low excitation power (9 µW) (measured at the back focal plane of the water-immersion objective - 1.2NA, 60x, Olympus) was used in order to reduce blinking and exclude saturation effects (measured saturation intensities were in the range of 100s of µW). The

resulting excitation volume is on the order of 1 fL. Fluorescence was collected by the same objective and separated from excitation light by a dichroic mirror. A 50 µm pinhole was used to reject out-of-focus light. The fluorescence signal was evenly split by a beamsplitter cube and detected by two avalanche photodiodes (APDs, AQR-14, Perkin Elmer Inc.). Detected photon pulses were sent to a hardware correlator card (ALV-6010, ALV GmbH, Langen, Germany), which computed the cross-correlation signal of the two channels with a temporal resolution of 6.5 ns.

Figure S-1. Agarose gel electrophoreses of sFC3 quantum dots in the presence of DTT. Lane 1, - sFC3, 2- sFC3 + 10 µM DTT, 3- sFC3 + 25 µM DTT, 4- sFC3 + 90 µM DTT. The concentrations of DTT tested were based on calculations of the residual amount of DTT present with the KSI-rFC3-His fusion protein even after dialyzing against 4 liters of 50 mM sodium acetate buffer, pH4.0. For 2 mg of rFC3 peptides used during the coating of QDs the final concentration of DTT in pyridine was 25 µM. It was clear that increasing concentrations of DTT influences the mobility of the sFC3 QDs as compared to untreated sFC3 in lane 1.

Deleted: Titration of DTT with sFC3-coated QDs was based on the calculations that 25 uM of DTT was present in the KSI-rFC3-His fusion protein even after dialyzing against 4 liters of 50 mM sodium acetate buffer, pH4.0.



Acknowledgment

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