Published in final edited form as:

Small. 2007 May ; 3(5): 793–798. doi:10.1002/sml.200600516.

Solubilization of Quantum Dots with a Recombinant Peptide from *Escherichia coli***

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Keywords

amino acids; biomolecules; fluorescence spectroscopy; peptides; quantum dots

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 better study of these interactions.^[5–9] Computational modeling combined with phage display of selected septapeptides with different affinities for metallic platinum revealed the importance of interface-reactive side chains, and their special arrangement and backbone stereochemistry for peptide-interface recognition.^[10–12] Genetic engineering of phage display has been employed to interface peptides with semiconductor nanocrystals, also known as quantum dots (QDs).^[8] Selective modification of the physiological conditions of prokaryotic *Escherichia 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

**The authors thank Dr. Xavier Michalet for help with data analysis. We thank Restek Corporation for allowing us to test various reverse-phase columns. FCS and fluorescence microscopy were performed at the CNSI Advanced Light Microscopy/Spectroscopy Shared Facility at UCLA. The work was supported by NIH grant 5-R01-EB000312, Keck Foundation grant 04074070, and Defense Advanced Research Projects Agency—Air Force Office of Scientific Research grant FA955004-10048 (to S.W.).

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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 those of 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 synthesizing a rationally designed recombinant peptide GSESGGSESGFCCFCCFCCF (termed hereafter 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 CdSe–ZnS QDs coated with the hydrophobic solvent trioctylphosphine oxide (TOPO) was successful, as assessed by gel electrophoresis and fluorescence correlation spectroscopy (FCS). Finally, we estimated the number of peptides using a defined ratio of rFC3 and a fluorescent peptide (FITC–CC3; FITC: fluorescein isothiocyanate; Table 1)—a critical step necessary for future biological applications, as one can control the stoichiometry of the reactants with an appropriate peptide sequence with functional groups at the N termini (–NH₂, –SH groups).

For the recombinant expression of peptide, an *E. coli* codon-optimized oligonucleotide encoding for the peptide rFC3 was synthesized. The pET-31b-based DNA vector for expression of the QD binding peptide rFC3 is depicted in Figure 1a and b. Use of KSI as a carrier protein for rFC3 peptide directs the fusion (KSI–rFC3) protein to inclusion bodies when overexpressed in *E. coli*, thus 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. The CNBr-cleaved peptide mixture was centrifuged to remove insoluble material, diluted tenfold in water, vacuum-dried, dissolved in acetic acid, and purified by high-performance liquid chromatography (HPLC; Figure 2a). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analyses of the final dissolved cleaved products (Figure 2b) revealed greater than 90% cleavage (lane 1, Figure 2b). In addition, CNBr-cleaved nonspecific reaction products were also observed and were purified by reversed-phase HPLC (RP–HPLC; Figure 2b). The rFC3 peptide elutes at 17–18 min (Figure 2a), also shown in lane 2 (Figure 2b).

Having obtained the purified rFC3 peptide, we tested its ability to solubilize 620-nm-emitting QDs. The final genetically 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 (Figure 3). 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 as exchanging 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 critical in solubilizing the QDs (Figure 3). 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 the previously described unnatural amino acid 3-cyclohexylalanine (Cha) peptide^[22] termed CC3 (peptide 3, Table 1) and a synthetic phenylalanine peptide termed sFC3 (peptide 2, Table 1) obtained commercially. Peptide-coated QDs (pcQDs, emission $\approx$ 620 nm, full width at half-maximum $\approx$ 35nm) 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 on the synthetic unnatural peptide sequence CC3, in which the importance of amino acids in the hydrophobic and hydrophilic motifs was systematically examined.^[22] Upon ligand exchange and solubilization of TOPO-coated QDs, the colloidal properties of pcQDs indicated a narrow size distribution, as evaluated by fluorescence detection after agarose gel electrophoresis (lanes 1–3, Figure 4). While CC3- and sFC3-coated QDs had similar electrophoretic mobilities, the mobility of rFC3-coated QDs (lane 3) was reduced. Yet, all three peptides have similar charges at the pH of separation. In fact, rFC3-coated QDs appear to have a reduced mobility because of the presence of residual dithiothreitol (DTT) during the purification of rFC3 (see Supporting Information). 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 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 (Supporting Information).

The absence of smeared bands of the various pcQDs in the agarose gel is a good indicator of monodispersity and uniform size distribution. Furthermore, pcQDs in aqueous buffer (phosphate-buffered saline (PBS), pH 7.2, or 50 mM Na borate–10 mM NaCl, pH 7.2) were stable and showed no signs of aggregation over 4–6 months at 4 °C (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. Surfactant exchange with peptides is a self-limiting reaction. As previously described,^[22] tetramethylammonium hydroxide (TMAOH) is added to the pyridine/dimethyl sulfoxide (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 10.0, a pH at which the cysteine residues (pK_a around 8.3) of the peptides form thiolate anions 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 surface of the QDs 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 redissolved in any polar or apolar solvents, which is consistent with the removal of surface ligands. Rosenthal et al.^[28] have also made a similar observation of the effect of TMAOH on surface TOPO.

We used optical absorption spectroscopy to determine the number of peptides bound to the QD at the end of the exchange reaction. In contrast to aromatic amino acids, the phenylalanine residue has a weak extinction coefficient ($\epsilon = 195 \text{ M}^{-1} \text{ cm}^{-1}$ at 257.5 nm),

which is insufficient for the spectroscopic determination of stoichiometry. We therefore used an N-terminal FITC peptide termed 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 4 can arise from: 1) absorption by the QD if $\lambda < \lambda_{\text{QD}}$ (where λ_{QD} is the excitonic bandgap), 2) absorption by FITC if $\lambda < \lambda_{\text{FITC}}$ (where λ_{FITC} is the longest wavelength of FITC absorption), and 3) absorption by the peptides themselves. The latter is negligible at visible wavelengths, and therefore it is easy to obtain two separate situations: first, only the QDs are absorbing ($\lambda_{\text{FITC}} < \lambda < \lambda_{\text{QD}}$) and second, both FITC and the QDs are absorbing ($\lambda < \lambda_{\text{FITC}} < \lambda_{\text{QD}}$).

Let n be the unknown average number of FITC-peptide per QD. The extinction coefficient of FITC at 493 nm is provided by the manufacturer ($\epsilon_{\text{FITC}}(493) = 85\,200\text{ M}^{-1}\text{ cm}^{-1}$). 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 and 610 nm (Figure 5), we will obtain data corresponding to the two different situations described above.

The total absorption at a wavelength λ will read [Eq. (1)]:

$$A_{\text{QD+FITC}}(\lambda) = [\epsilon_{\text{QD}}(\lambda) + n\epsilon_{\text{FITC}}(\lambda)]c_{\text{QD}}L \quad (1)$$

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 Equation (2):

$$\epsilon_{\text{QD}} = 5.857 D^{2.65} \\ D = 1.6122 \times 10^{-9} \lambda^4 - 2.6575 \times 10^{-6} \lambda^3 + 1.6242 \times 10^{-3} \lambda^2 - 0.4277 \lambda + 41.57 \quad (2)$$

where D is the diameter of the QD core in nanometers and ϵ is expressed in $\text{M}^{-1}\text{ cm}^{-1}$. From Equation (2), we can calculate $\epsilon_{\text{QD}}(610)$, substitute into Equation (1), and calculate the QD concentration [Eq. (3)]:

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

By writing Equation (1) for $\lambda = 493$ nm and substituting for the concentration calculated in Equation (3), we derive the equation for n [Eq. (4)]:

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

The only unknown in this expression is $\epsilon_{\text{QD}}(493)$, which can be readily obtained from the calculated $\epsilon_{\text{QD}}(610)$ and the measurement of the QD-only absorption spectrum [Eq. (5)]:

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

The number of FITC-labeled peptides per QD is thus given by the following equation [Eq. (6)]:

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

After gel filtration and dialysis, the molar concentrations of FITC and QDs were evaluated from the 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 $85\,200\text{ M}^{-1}\text{ cm}^{-1}$ at this wavelength.^[30] The calculated extinction coefficient (at 610 nm) of 620-nm-emitting CdSe–ZnS QDs was $353\,762.8\text{ M}^{-1}\text{ cm}^{-1}$, and absorption measurements yielded molar concentrations of 821 and 62 nM for FITC and QDs, 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 absorption spectrum of FITC-CC3-coated QDs is shown in Figure 5. 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 that solubilized QDs with 100% FITC-CC3 peptides also produced identical results (data not shown).

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 the column matrix may preclude accurate quantitative analysis of QD size and its distribution. Such interactions can be minimized by appropriate mobile phase modifications^[31] and recently, aqueous SE-HPLC and scanning tunneling electron microscopy (STEM) were adopted for the separation and characterization of different sizes of CdSe–protein conjugates.^[32] We employed FCS to overcome the limitations of gel electrophoresis or SE-HPLC and study the colloidal properties of natural-peptide-coated QDs.^[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 (BPP), and the hydrodynamic radius of QDs.^[34, 36]

We performed FCS measurements on rFC3, sFC3, and CC3 (peptides 1–3, Table 1) coated QDs in PBS buffer of pH 7.2. FCS revealed hydrodynamic radii of ≈ 11 –12 nm for rFC3 and sFC3 and ≈ 13 nm for CC3 pcQDs. Measurements of the BPP of rFC3, sFC3, and CC3 pcQDs are shown in Figure 6a, and reveal uniform brightness and quantum yields (QYs) ranging from 20 to 25% in PBS of pH 7.2 (QYs were derived by comparison with an LD690 dye, Exciton). As discussed by Dooze et al.,^[36] the BPP is a measure only for particles that are in their photoactive “bright” state (it does not take into account dark particles, which are not detectable by FCS).

Hydrodynamic radii (R) 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: $\tau_{\text{QD}}/\tau_{\text{bead}} = R_{\text{QD}}/R_{\text{bead}}$. 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 (Figure 6b). 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 (80×30 s 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, and CC3 pcQDs are shown in Figure 7 a–c, respectively, and indicate a slightly narrower distribution for rFC3-coated QDs.

In conclusion, we have reported the specific QD surface recognition of a recombinant peptide from *E. coli* that forms a hybrid organic–inorganic nanocomposite. We showed that the use of *E. coli* as a host for the production of cysteine-rich peptides is a cost-effective alternative to solid-phase peptide synthesis, which can be technically challenging and laborious for the cysteine-rich peptides used in this study. Several surface chemistries that replace the original hydrophobic ligands used during the synthesis of QDs have been described in the literature. Mercapto-based surface-exchange chemistry involving monothiol ligands was unstable in biocompatible buffers, as the ligands detached from the surface of the 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 a stable peptide coating on the 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 the QD surface with peptides produced in *E. coli* is attractive for several reasons: 1) peptides mimic the biological environment and are stable at physiological pH; 2) 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 obtain biomolecules of interest; 3) molecular evolution strategies to randomize peptides can be adapted to select high-affinity binders to the QD surface; and 4) there is the possibility of creating 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 polyethylene glycol (PEG), conjugating receptor ligand and PEG, etc.).

Hence, by laying the ground rules for recombinant peptide sequences 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 bioconjugation 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. Finally, 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Schematic diagram of the KSI-rFC3-His expression DNA construct. a) Peptide sequence of the recombinant peptide rFC3. b) DNA sequence encoding the rFC3 peptide that was cloned into the A1wN I site downstream of the KSI fusion protein. The ATG and its complementary codon for methionine were 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*. CNBr-cleaved fusion KSI-rFC3 protein was injected into a C18 column (4.6 × 250 mm). a) Pure peptide eluted with a retention time of around 18 min, and analyzed on a 4–12% denaturing SDS-PAGE gel. b) Lane 1: CNBr-cleaved fusion recombinant protein; lane 2: purified peptide after RP-HPLC.

Figure 3. Schematic of the peptide-coated QDs. The adhesive motif consists of the hydrophobic amino acid phenylalanine (F) and thiol-containing cysteines (C) bound to the inorganic ZnS shell, and the hydrophilic motif confers solubility to the QDs.

Figure 4. Agarose gel electrophoresis of various peptide-coated QDs emitting at 620 nm. Lane 1: sFC3 peptide; lane 2: CC3 peptide; lane 3: rFC3 peptide. rFC3 is slightly delayed in migration because of residual DTT during purification of the peptide.

Figure 5. Comparison of normalized absorbance spectra of peptide-coated QDs emitting at 620 nm. FITC-CC3 peptide-coated QDs have excitonic peaks at 493 and 610 nm, while rFC3 peptide-coated QDs have an exciton peak at 610 nm alone.

Figure 6.

Histograms for rFC3, sFC3, and CC3 peptide-coated QDs obtained by FCS. a) The BPP was approximately uniform for all the peptide-coated QDs. b) The hydrodynamic diameter was measured as 11 nm for rFC3 and sFC3, and 13 nm for CC3.

Figure 7. Size-distribution histograms of peptide-coated QDs obtained by FCS. a) Distribution of rFC3-coated QDs, which indicates a narrower distribution compared to b) sFC3 and c) CC3 peptide-coated QDs.

Table 1

Peptides used for solubilization of QDs.

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 ^[a]
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 ^[b] -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

^[a] Homoserine residue denoted HS, obtained after CNBr cleavage of methionine residue.

^[b] 3-Cyclohexylalanine denoted Cha, an unnatural amino acid.