Covalent Monofunctionalization of Peptide-Coated Quantum Dots for Single-Molecule Assays

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ABSTRACT Fluorescent probes for biological imaging of single molecules (SM) have many stringent design requirements. In the case of quantum dot (QD) probes, it remains a challenge to control their functional properties with high precision. Here, we describe the simple preparation of QDs with reduced size and monovalency. Our approach combines a peptide surface coating, stable covalent conjugation of targeting units and purification by gel electrophoresis. We precisely characterize these probes by ensemble and SM techniques and apply them to tracking individual proteins in living cells.

KEYWORDS Quantum dots, single molecules, particle tracking, cell imaging, fluorescent probes

Single-molecule (SM) fluorescent imaging techniques and tools represent a powerful approach for investigating the behavior of individual biomolecules in vitro and in live cells.1 Yet, the conditions imposed by biological milieu necessitate the design of photostable and high brightness SM probes. Fluorescent quantum dots (QDs) are inorganic semiconductor nanoparticles (∼2–10 nm) that meet these criteria and overcome numerous other optical limitations associated with common organic probes.2,3 As such, they have found numerous applications for imaging SM events.4,5 In vitro, this includes assays for studying the dynamics of enzymes on DNA,6 the function of motor proteins7 or biosensing via Förster resonance energy transfer (FRET),8-10 among others. Long-term, single QD tracking (SQT) of biomolecules in living cells is providing fresh insight into the organization of the plasma membrane,11 the trafficking of receptor ligands,12 the dynamics of neurotransmitter receptors13 and many other open questions in cell biology.5

Thus far, commercial QD probes conjugated to multiple copies (∼8–12) of proteins like streptavidin (SAV) or antibodies have enabled the majority of QD experiments at the SM level. Their wide availability and compatibility with various targeting schemes has favored their use in many applications. These probes are generally constructed from QDs encapsulated in amphiphilic polymers or micelles, which has been a popular route to obtain biocompatibility.14-18 While this design facilitates good conjugate stability and enables specific targeting, it suffers from high multivalency and a large hydrodynamic diameter (HD) in the range ∼25–40 nm,19-22 depending on the particular surface coating and conjugated proteins. Already, several examples have shown how the large size of these QD probes can alter the kinetics and equilibrium of molecular interactions,23 influence the diffusion of receptors in the cell membrane,24,25 prohibit access to size-restricted cellular regions22,24 and limit FRET efficiency toward acceptors in close proximity.9,10 At the same time, the multivalency of QD probes has been shown to trigger undesirable cell signaling events by cross-linking target receptors22 and to complicate the analysis of data from SM assays.8,10,26

In recent years, the challenge of designing improved QD probes for SM imaging has resulted in new surface coatings, conjugation strategies and purification techniques. For instance, compact surface coatings have been developed to reduce the size, but also maintain effective shielding against nonspecific interactions. Poly(ethylene glycol) (PEG) has been shown as a key ingredient to reduce nonspecific interactions27,28 and has been incorporated into dihydrolipoic acid (DHLA) derivatives22,28-30 and compact polymers30-32 to produce QDs with a HD in the range of 8–18 nm. Similarly, surface coatings based on PEGylated peptides containing 6–20 amino acid residues can also yield QDs with a HD of ∼10–12 nm,11,33-35 having the added benefit that they are available from commercial vendors rather than requiring chemical synthesis. A purification technique to prepare QDs with reduced valency has also emerged.22,34,36,37 which was initially applied to prepare gold nanoparticles with defined numbers of DNA.78 It relies on stochastic conjugation of large targeting units to the QD surface and then separation and

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purification of discrete stoichiometries of the complex via gel electrophoresis (GE). This technique has now been applied to prepare monovalent QD probes integrating DHLA or peptide surface coatings and a single copy of SAV, achieving a final probe HD of just 13–15 nm. These designs applied a noncovalent conjugation strategy relying on the self-assembly of polyhistidine-tagged targeting proteins to divalent ions at the nanoparticle surface, which has the advantage that the proteins can be site-specifically attached. However, a disadvantage is that the conjugation is noncovalent, possibly leading to unstable complexes or resulting in the dissociation of the protein from the QD over time (off-rate, $k_{\text{off}} \sim 10^{-4}$ s$^{-1}$).

Here, we report several advances in the design of reduced-size and valency QD probes for SM imaging. We show that the combination of a compact peptide surface coating, stable covalent conjugation of biotin or SAV targeting units and GE purification can yield monofunctional QD probes with high conjugate stability (>1 week), and a HD of ~13–15 nm, which represents a reduction in diameter by a factor of 2 compared to commercial QD probes prepared with an amphiphilic polymer coating. Importantly, our design reduces the technical complexity associated with QD probe preparation, which should allow researchers from a wide range of backgrounds to apply them in different applications. Using ensemble methods, we precisely characterize the size, activity, valency, and stability of the QD probes. We also use a SM assay to directly count the number of functional binding sites on individual QDs and we apply these probes for tracking the diffusion of individual proteins in the membrane of living cells.

**Design and Synthesis of pQDs.** To prepare compact and biocompatible QDs, we selected a surface coating based on engineered, two-domain peptides of ~20 amino acid residues in length. These peptides contain a cysteine-rich adhesive domain that binds to the QD surface, cysteine, and lysine terminal amino acids that serve as reactive sites for conjugation and PEG to minimize nonspecific interactions (Supporting Information). Next, we exchanged the native hydrophobic surfactants of green- ($\lambda_{\text{em}} = 520$ nm) or red-emitting ($\lambda_{\text{em}} = 605$ nm) CdSe/ZnS QDs with these peptides. The resulting peptide-coated QDs (pQDs) were highly soluble in various aqueous buffers and they retained the characteristic optical properties of the original hydrophobic QDs (Supporting Information Figure S1), with a modest decrease in quantum yield from 34 to 25% (Supporting Information).

We determined the size of the pQDs by electron microscopy (EM) after negative staining with uranyl acetate (Figure 1a). The inorganic core/shell CdSe/ZnS nanoparticle was revealed by regions of high contrast, while the organic surface coating appeared as a low contrast halo surrounding the core/shell nanoparticle. Scale bar = 25 nm. (b) Conjugation scheme for pQDs. Scheme I: Bifunctional PEG linkers containing a thiol-reactive maleimide and a small targeting unit (e.g., biotin) are covalently conjugated to cysteine residues on the pQDs. Scheme II: Maleimide-activated antibodies or proteins (e.g., streptavidin) are formed with the bifunctional cross-linker SMCC and covalently conjugated to cysteine residues on the pQDs.
consistent with their expected net negative charge. The in a narrow band in the gel toward the positive electrode, 2a). In the absence of the biotin-PEG-mal, the pQDs migrated in the peptide coating. The pQDs were titrated with increasing amounts of the biotin-PEG-mal and analyzed by gel electrophoresis (GE) of pQDs covalently conjugated to the pQDs via thiol groups of cysteine residues on the other end (biotin-PEG-mal) was selected to enable conjugation to the pQDs via a linker. (a) Gel electrophoresis (GE) of pQDs covalently conjugated to the pQDs via a linker. (a) Gel electrophoresis (GE) of pQDs covalently conjugated to different stoichiometries of biotin via a linker. (a) Gel electrophoresis (GE) of pQDs covalently conjugated to increasing amounts of a bifunctional 10 kDa biotin-PEG-maleimide. Discrete bands represent pQDs with N = 0, 1, and 2 copies of biotin, (b) GE of a bulk mixture of biotin-conjugated pQDs before (left) and after (right) the addition of excess SAV. There is a large shift in migration for only bands corresponding to \( N \geq 1 \) copies of biotin, indicating the high level of specific activity retained by the biotin at the end of the linker following conjugation to the pQDs (c) Effect of PEG linker length on the separation of different conjugate stoichiometries during GE. Discrete bands are resolved in the case of the 10 and 20 kDa PEG-maleimide, but not for the 5 kDa PEG-maleimide linker. All samples were loaded at the start position and the direction of applied voltage is indicated.

The diameter of green-emitting pQDs was 6.9 ± 1.4 nm, while that of red-emitting pQDs was 9.4 ± 1.6 nm, owing to the larger nanoparticle size (≈5 versus 2.5 nm). In both samples, the peptide coating was ~2 nm thick, contributing ~4 nm to the overall diameter. For comparison, we also examined commercial, SAV-conjugated QD probes (cQD-SAV, \( \lambda_{\text{em}} = 655 \) nm) containing an amphiphilic polymer-based surface coating. The cQD-SAV were 25.1 ± 3.7 nm in diameter, more than twice the size of the pQDs, because of both a larger nanoparticle size and thicker surface coating (~8 nm in diameter). Finally, we used cryo-EM to determine the diameter of wild-type streptavidin (SAV), which was found to be 3.0 ± 0.6 nm (Figure 1a).

**Covalent Conjugation of Discrete Numbers of Targeting Units.** Having established the compact size of the pQDs, we tested the ability to covalently conjugate discrete numbers of targeting units (Figure 1b). First, we selected biotin, to enable specific targeting toward SAV and avidin variants. Because of its small size (240 Da), direct conjugation of biotin to the pQDs did not enable us to separate different stoichiometries of the complex via GE (data not shown). To increase the effective size of biotin, we used a long, flexible linker between the biotin and the pQDs. A 10 kDa PEG linker containing a single biotin on one end and a single maleimide on the other end (biotin-PEG-mal) was selected to enable conjugation to the pQDs via thiol groups of cysteine residues in the peptide coating. The pQDs were titrated with increasing amounts of the biotin-PEG-mal and analyzed by gel electrophoresis (GE) after a short incubation period (Figure 2a). In the absence of the biotin-PEG-mal, the pQDs migrated in a narrow band in the gel toward the positive electrode, consistent with their expected net negative charge. The addition of increasing amounts of biotin-PEG-mal led to the formation of new, discrete bands in the gel with a lower mobility than the unconjugated pQDs. These bands were assigned to pQDs conjugated to different stoichiometries of biotin via the linker (\( N = 0, 1 \) and 2). The biotin on the end of the linker remained highly active after the conjugation, because the addition of a 50-fold molar excess of SAV to the bulk mixture led to a quantitative shift in migration of only the pQD bands containing biotin (\( N \geq 1 \)) (Figure 2b). We further explored the effect of linker length on the ability to resolve discrete bands by GE following conjugation to the pQDs. PEG-maleimide linkers of length 5, 10, and 20 kDa were reacted with the pQDs in a 3-fold molar excess. An obvious trend emerged during GE of these samples; the distance between the discrete bands in the gel increased with the linker length (Figure 2c). Clear separation between bands could not be seen when the linker was 5 kDa in length, imposing a practical size limit.

Given the positive results obtained with a small targeting unit, we extended the GE technique to prepare pQDs with discrete numbers of proteins. We selected the wild-type SAV protein (~56 kDa) to enable specific targeting of the probes toward biotinylated biomolecules. First, we prepared a maleimide-activated SAV by reacting free amines on the protein with a 3-fold molar excess of the bifunctional cross-linker succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) (Figure 1b). As with the biotin-PEG-mal, titration of the SAV-maleimide with the pQDs led to the formation of discrete bands during GE of the samples, confirming their successful conjugation to the peptide coating. We selected a ratio of SAV-maleimide to produce a bulk mixture of pQDs containing 0, 1, and 2 copies of SAV (Figure 3a). To test the activity of the conjugated SAV, we reacted this bulk mixture with a 50-fold molar excess of a large biotinylated target, a 10 kDa biotin-PEG. Analysis by GE revealed a quantitative shift in migration of only the pQD bands containing SAV (\( N \geq 1 \)), indicating a high level of specific activity retained by the SAV. We could purify the different stoichiometries of the complex from the bulk mixture by cutting the desired bands out of the gel and eluting the pQDs from the agarose. Reanalysis of the samples containing 1 (pQD-SAV₁) or 2 (pQD-SAV₂) of the proteins per QD showed that they could be recovered with very high purity (Figure 3b). Importantly, the purified pQD-SAV₁ was stable over storage periods exceeding one week at 4 °C, with no apparent dissociation of the SAV from the nanoparticle surface, even in at nanomolar concentrations (Figure 3c).

**Ensemble Characterization of pQD-SAV.** We then determined the contribution of discrete numbers of SAV to the overall hydrodynamic diameter (HD) of pQDs by both size-exclusion chromatography (SEC) and fluorescence correlation spectroscopy (FCS). In the case of SEC, we detected the fluorescent signal from the pQDs as they eluted from the chromatography column (Figure 3d) and determined their size with a set of protein standards of known HD (Supporting Information Figure S2). For the unconjugated pQDs, the HD was determined to be 14.0 nm. This measurement is ~4 nm larger than the diameter obtained by EM (9.6 nm) and
accounts for hydrodynamic and hydration effects in solution. In the case of pQD-SAV 1, conjugation of a single SAV increased the HD by \( \sim 1.6 \) to 15.6 nm. Additional HD measurements on these samples were also performed using FCS, where the mean HD was calculated from autocorrelation functions (Figure 3e). These results were consistent with the SEC measurements with a HD of 12.8 nm for the unconjugated pQDs and 16.0 nm for pQD-SAV 1, representing an increase of 3.2 nm due to the SAV. In addition, we used SEC and FCS to measure the HD of a broader sample set, including pQDs with 2 copies of the SAV and cQD-SAV (Supporting Information Figure S2). These results indicate a progressive increase in HD of the pQDs following conjugation of discrete copies of SAV, and also highlight the fact that pQD-SAV 1 is less than half the HD of the cQD-SAV probes (\( \sim 15 \) nm versus 35 nm).

Single-Molecule Characterization of pQD-SAV. For the purpose of measuring the precise number of biotin-binding sites on pQD-SAV 1, we designed a SM counting assay based on the stepwise photobleaching of a biotinylated organic fluorophore.\(^{43,44}\) We synthesized a biotin derivative containing a single Alexa647 dye (biotin-A647, \( \lambda_{em} = 673 \) nm) and mixed it in 100-fold molar excess with pQD-SAV 1 before analyzing and purifying the complex by GE (Figure 4a). In gels, binding of biotin-A647 to pQD-SAV 1 was confirmed by colocalization of their fluorescent emission, while the excess unbound biotin-A647 migrated at a faster rate than the QD-dye complex. We confirmed that the binding of the dye was specific to the SAV and not the QD surface or coating, because it was eliminated by coincubation of pQD-SAV 1 and biotin-A647 in the presence of a 1000-fold molar excess of free biotin (Figure 4a). We then purified the QD-dye complex by extracting the colocalized region from the gel and deposited it on glass coverslips at nanomolar concentrations for further characterization.

To analyze this hybrid QD-dye complex, we used SM imaging by dual color total internal reflection fluorescence (TIRF). Individual QD-dye complexes, attached to the coverslip, were excited at 488 or 633 nm and the respective fluorescent emission of pQD-SAV 1 and biotin-A647 were separated and detected on different halves of the same EMCCD camera (Figure 4b, Supporting Information Figure S2).
FIGURE 4. Counting binding sites on SAV-conjugated pQDs via an organic dye reporter. (a) GE of a mixture of pQD-SAV1 and biotin-A647 (left lane) and a control when a 1000-fold molar excess of free biotin was added to the mixture (right lane). The yellow band indicates colocalization of pQD-SAV1 (green) and biotin-A647 (red). The QD-dye complex was purified from unbound dye by extracting the yellow region of the gel. Samples were loaded at the start position and the direction of applied voltage is indicated. (b) Total internal reflection fluorescence (TIRF) excitation scheme for the QD-dye complex. The pQDs are directly excited at 488 nm and biotin-A647 is independently and directly excited at 633 nm. The emission of the QDs and dye are separated and detected on two halves of the same camera. (c) TIRF microscopy of single QD-dye complexes nonspecifically attached to a glass coverslip. Images are a maximum intensity projection of two image sequences acquired sequentially for pQD-SAV1 with 488 nm excitation (left, QD channel) and then for pQD-SAV1 with 633 nm excitation (right, dye channel). A high level of colocalization (∼90%) was observed between the fluorescent spots in the dye and QD channels (white circles). Scale bar = 2 µm. (d) Typical intensity time traces of spots in the dye channel. Single and multistep photobleaching is observed up to 4 unique steps (arrows). (e) Distribution of biotin-binding sites for pQD-SAV1, and pQD-mSAV1, prepared with wild-type (top, N = 256) or monovalent (bottom, N = 57) SAV protein, respectively. These distributions were determined from the number of photobleaching steps observed for spots in the dye channel.

S1). Excitation of the pQD-SAV1 at 488 nm produced diffraction-limited fluorescent spots in the QD channel with the characteristic intermittency (blinking) of individual QDs. Under these conditions, many correlated spots also appeared in the dye channel, resulting from QD FRET to biotin-A647 (Supporting Information Figure S3). However, to accurately count the number of biotin-A647 per pQD-SAV1, we applied direct excitation at 633 nm and collected time-lapse images in the dye channel until all dye molecules had photobleached (∼50 s, Figure 4c). Individual QD-SAV1 were highly colocalized (∼90%) with the dye molecules as verified by excitation at 488 nm (Figure 4c). In the dye channel, intensity time traces from individual spots showed consecutive photobleaching steps as expected from single or few molecules of biotin-A647 bound to pQD-SAV1 (Figure 4d).

Counting the photobleaching steps permitted an evaluation of the number of biotin-binding sites on the pQD-SAV1. We classified ∼250 of the QD-dye complexes based on the number of observed steps and determined the distribution of the biotin-binding sites on individual pQD-SAV1 to be 1 (41%), 2 (40%), 3 (15%), and 4 (4%) (Figure 4e). This result supports the notion that each pQD is conjugated to a single copy of the SAV protein, and that some the 4 biotin-binding sites of the tetrameric, wild-type SAV are not accessible. As a control, we found the distribution of steps associated with the free biotin-A647 dye to be 1 (94%) and 2 (6%), as expected (Supporting Information Figure S4). Alternatively, we determined the distribution of biotin-binding sites using a histogram analysis of the maximum intensity of spots in the dye channel, which yielded results similar to the step-counting analysis (Supporting Information Figure S4).

We also tested the effect of substituting the wild-type SAV with a monovalent variant containing a single biotin-binding site (mSAV) and prepared pQD-mSAV1, a probe containing a single mSAV. When reacted with biotin-A647, we found that in contrast to the wild-type pQD-SAV1, these pQD-mSAV1 were colocalized with the dye only ∼20% of the time, suggesting that the binding site on mSAV was not accessible in most cases. Nevertheless, for the pQD-mSAV1 that were colocalized with the dye, the distribution of biotin-binding sites was very similar to the free dye control, 1 (89%) and 2 (11%), confirming our ability to achieve QD monovalency (Figure 4e). Overall, data from pQD-SAV1 and pQD-mSAV1 demonstrate that we can finely tune the valency of pQD probes.

SQT of Single Membrane Proteins in Living Cells. Finally, we evaluated the performance of pQD-SAV1 for specific targeting and tracking of a membrane protein in living HeLa cells using wide-field epifluorescent microscopy. As a model system we used HeLa cells expressing cyan fluorescent protein (CFP) anchored to the plasma membrane through the transmembrane domain of platelet-derived growth factor receptor (AP-CFP-TM) (Figure 5a).17,22,28,46 This CFP fusion has a N-terminal 15 amino acid acceptor peptide (AP) tag that can be site-specifically biotinylated with biotin ligase (BirA). The cells were cotransfected with plasmids for AP-CFP-TM and BirA and CFP expression was detected in 50–60% of the cells 24–48 h post-transfection (Figure 5b). When these cells were further incubated for 5 min with nanomolar concentrations of pQD-SAV1, specific QD labeling at the plasma membrane of only AP-CFP-TM expressing cells was observed (Figure 5b). As a further control, no QD labeling was observed when BirA was omit-
ted during the transfection (data not shown). For labeled cells, the density of QDs at the cell surface was low, and allowed for the identification of single QDs diffusing in the membrane (Figure 5c, Supporting Information Movie S1). From the motion of individual pQD-SAV1, we reconstructed the trajectories of the diffusing proteins by Gaussian fitting (localization accuracy $\sim 50$ nm) in each frame of the image sequence (Figure 5c). Using mean square displacement (MSD) analysis of individual trajectories we determined the mean diffusion coefficient ($D$) for AP-CFP-TM to be 0.037 $\mu$m$^2$/s (standard error: 0.032–0.042) at 25 °C (Figure 5d).

As an alternative analysis, we pooled together the individual trajectories and determined $D$ using the probability distribution of square displacements (PDSD). Using mean square displacement (MSD) analysis of individual trajectories we determined the mean diffusion coefficient ($D$) for AP-CFP-TM to be 0.037 $\mu$m$^2$/s (standard error: 0.032–0.042) at 25 °C (Figure 5d). As an alternative analysis, we pooled together the individual trajectories and determined $D$ using the probability distribution of square displacements (PDSD). This analysis identified fast, $D = 0.053 \mu$m$^2$/s (68%), and slow, $D = 0.007 \mu$m$^2$/s (32%), populations of diffusing proteins (Supporting Information Figure S5). As a comparison, the cQD-SAV probe was targeted to AP-CFP-TM and tracked in the same manner, producing nearly identical diffusion coefficients (Supporting Information Figure S5), an indication that both QD designs perform equally well in this application.

**Discussion.** For the design of our QD probes, we selected a peptide surface coating because it generates highly compact and biocompatible QDs, but also because it does not require the complicated chemical synthesis demanded by other compact surface coatings. In fact, our probes are quickly and easily prepared from commercially available components, which is an important advantage to this design.

Using gel electrophoresis, we observed heterogeneity in the number of attached targeting units following their conjugation to the QDs, which is due to the presence of multiple reactive cysteine residues on the QD surface. At least, for a low average ratio of targeting units per QD, the distribution in the stoichiometry following conjugation was consistent with Poisson statistics (Supporting Information Table S1). Deviation from Poisson statistics at higher ratios is possibly a result of the limited number of reactive sites for conjugation and the fact that multiple binding cannot be considered as independent events. Importantly, we could easily separate and purify the QDs containing exactly one targeting unit by GE, which is the desired stoichiometry for many applications. Our choice of biotin and SAV targeting units comes from their widespread use in biology, their compatibility with a variety of targeting schemes and the extremely high affinity and long half-life of the binding interaction. We have also applied the GE technique to prepare QDs conjugated to a single copy of IgG antibodies or Fab fragments (data not shown) and in principle, it should be applicable to a wide range of other large biomolecules (DNA, enzymes, proteins, etc.), as long as...
as their conjugation alters the electrophoretic mobility of the pQDs. On the basis of our experiments with PEG linkers, we determined a size threshold of ~10 kDa, below which it is not possible to separate discrete stoichiometries using GE. Nevertheless, smaller molecules (biotin, receptor ligands, trisNTA, etc.) can be attached to the end of a linker and their stoichiometry controlled in a similar fashion.37

The choice of a covalent conjugation strategy allowed us to prepare QD probes with very good conjugate stability, which is an improvement over other noncovalent conjugation approaches such as those using polyhistidine-tagged proteins,39–41 which may dissociate from the QD surface.31 During characterization of pQD-SAV1, we measured the HD to be ~15 nm, which is less than half that of the commercial cQD-SAV probe. This difference becomes significant in terms of volume, because pQD-SAV1 occupies ~8 times less space than cQD-SAV. A further decrease in pQD-SAV1 size (diameter reduced by ~4 nm) could be achieved by using the smaller green-emitting pQDs. In addition to their reduced size and monovalent QD probes, especially when studying in living cells, an application that can benefit from increased accessibility.

Indeed, we found that near the surface of QDs, only 1 or 2 of the 4 biotin-binding sites in the wild-type SAV are easily accessible after the conjugation. Because a large excess (~100-fold) of biotinylated dye is used and given the strong affinity of the biotin-streptavidin pair, the heterogeneity in number of bound dyes most likely reflects the number of accessible binding sites on the surface of a single QD. This suggests that steric hindrance from the peptide coating or the nanoparticle surface limits access to some of the SAV binding sites. Substituting wild-type SAV with mSAV allowed us to further reduce the number of biotin-binding sites to just one, effectively achieving a monovalent pQD probe. Since many of the pQD-mSAV1 (~80%) did not bind to any biotin molecules, a future improvement could be the site-specific conjugation of mSAV, to ensure that its single biotin-binding site is oriented away from the QD surface, with increased accessibility.

We demonstrated the suitability of pQD-SAV1 for SQT studies in living cells, an application that can benefit from reduced size and monovalent QD probes, especially when applying them in crowded cellular environments, where larger QD probes have limited access.22,24 While diffusion of proteins on the 2D plasma membrane is largely independent of probe size, this is not the case in the cell cytosol, or the nucleus where diffusion is presumably in 3D.48 Thus, our small probes could facilitate targeting and tracking of intracellular targets as techniques for delivering QDs inside cells are further optimized.49 Surface or solution-based in vitro assays should also benefit from the reduced-size and valency. For example, SM FRET measurements employing commercial QD probes as donors have previously suffered from poor energy transfer efficiency, partially because the thick surface coating increases the distance of the acceptors from the nanoparticle surface.9,10 In these applications, pQDs could potentially increase FRET efficiency while ensuring a 1:1 stoichiometry between donor QD and acceptor dye. Aside from biological assays, monovalent peptide-coated nanoparticles could, in principle, enable the controlled bottom-up assembly and spatial organization of more complex nanostructures.50

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Supporting Information Available. Supporting Methods, Supporting Figures S1–S5, Supporting Movie S1, and Supporting Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES AND NOTES
