

## Supplementary Information

# Bioconjugation of CdSe/ZnS nanoparticles with SNAP tagged proteins

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### Experimental Procedure

Functionalization of QDs with Benzylguanine (BG): QDs with amino functionalized polyethyleneglycol (PEG) stabilizing layer (aQD) were purchased from Invitrogen (Qdot 565 ITK™ „amino (PEG) quantum dots“). BG-GLA-NHS ester was obtained from New England Biolab's. BG-NHS was dissolved in dry DMF to make up the 0.01M stock solution, which was then mixed with aQD in the desired ratios in borate buffer (50 mM, pH 8.3). We typically used concentrations of 0.5 to 1  $\mu$ M aQD. The reaction mixture was mixed at 23 °C for 2 h and resulting bgQDs were purified by ultrafiltration (Vivaspin with PES membrane, cut-off 100 kD,).

#### Optional blocking with Sulfo-NHS-Acetate (SNA):

1 mg of sulfo-NHS-Acetate (thermo scientific) is dissolved in 15  $\mu$ l water and quickly added to the bgQD reaction mixture after 2 hours and was allowed to react for another hour. Washing was done as described above.

#### SNAP-tag Fusion Protein immobilization:

The preparation of SNAP-GFP protein is described in Engin et al., *Langmuir*, 2010, **26**, 6097. bgQD (or SNA blocked bgQD) and SNAP-GFP were mixed in the desired ratio (1:6. 1:12. 1:20) and incubated for at least 5 h (in case of SNAP-mCherry and SNAP-Cadherine the incubation time was increased to at least over night) in HEPES-imidazol buffer (100 mM, pH 7.4 with 100mM imidazol) at r.t. Purification was done by ultrafiltration using Vivaspin or using an automated chromatography system with Superdex size exclusion columns (see below).

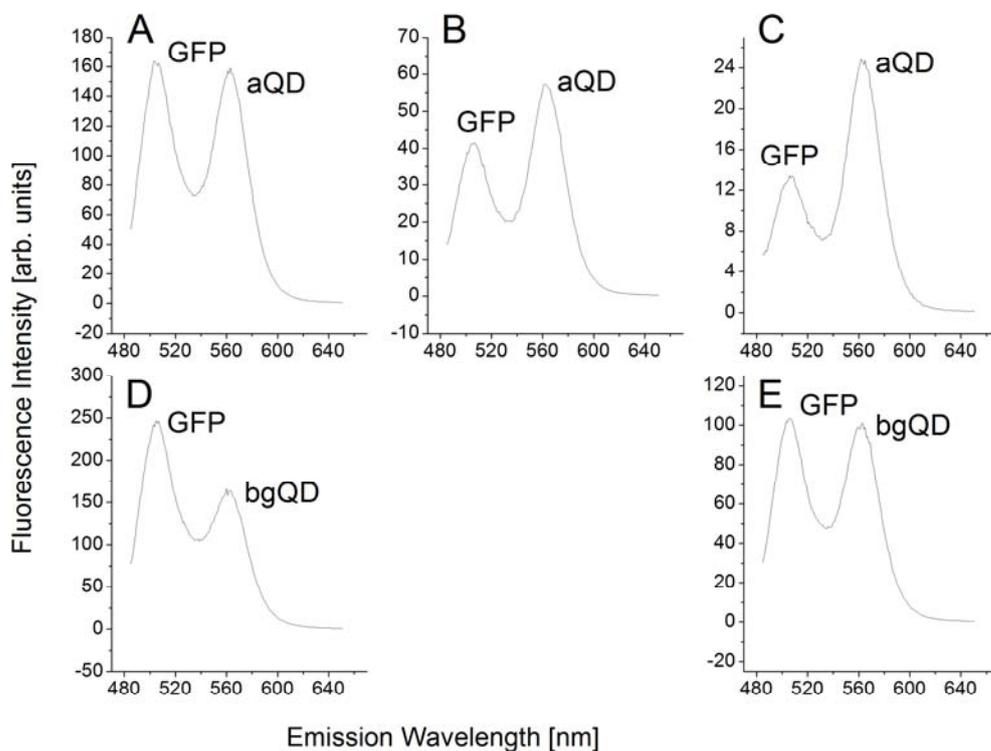
#### Control samples:

To obtain negative control samples, aQD stock solution was directly mixed with the respective protein in the same ratio and under conditions described above.

#### Size Exclusion Chromatography:

Size Exclusion Chromatography was performed using GE Äkta Explorer and GE Superdex 200 10/300 GL column, 100  $\mu$ l sample volume, 10 mM HEPES (pH 7.4) as elution buffer and a flow of 0.4 ml/min.

Fig. S1:



The effect of imidazole on the protein-QD binding: Fluorescence spectra of a negative control solution (aQD mixed with SNAP-GFP) after purification by ultrafiltration with HEPES buffer (A), purification by ultrafiltration with HEPES-imidazole buffer (B) and after incubation in HEPES – imidazole buffer and subsequent wash (C). D and E represent bgQD- SNAP GFP conjugate after ultrafiltration with HEPES buffer only (D) and incubation in HEPES-imidazole and subsequent wash steps (E).

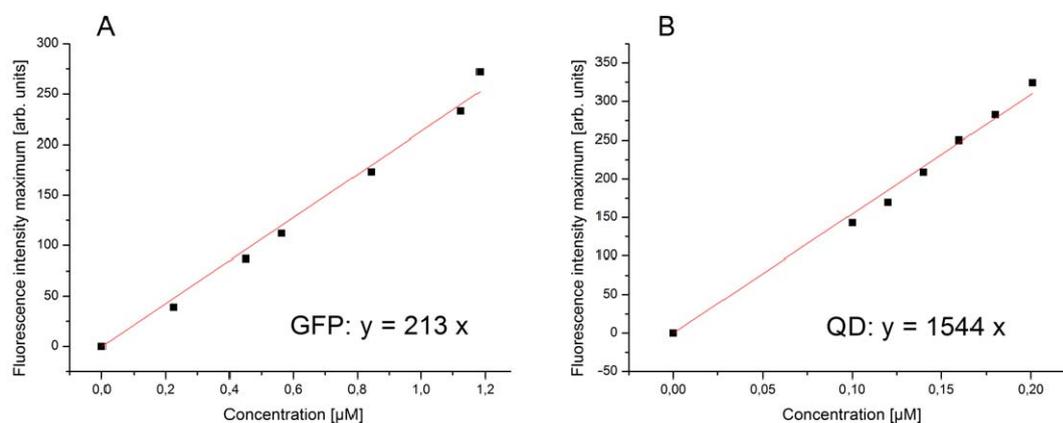
Fig. S2:



Agarose gel electrophoresis (0.6 % agarose, 100 V); 1: aQDs from the stock solution, 2: BG functionalized QDs (bgQD) washed with 0,5 % Tween 20 containing borate buffer, 3: bgQDs washed with borate buffer without Tween 20.

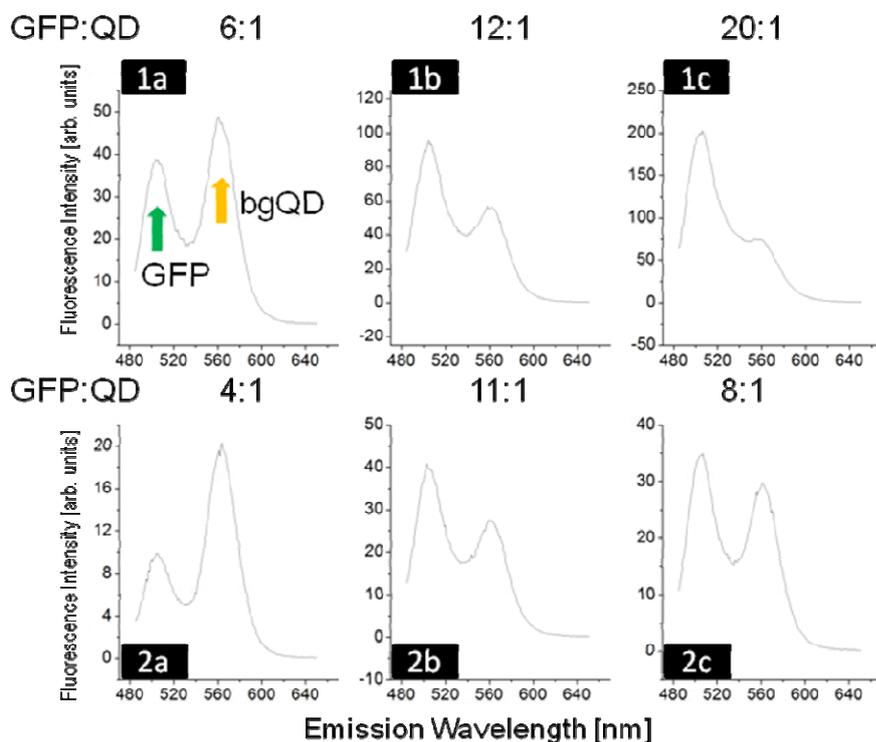
## Determination of the average number of proteins per QDs

Fig. S3:



Calibration curves for determination of protein coverage on QDs. The fluorescence intensity maximum dependence on concentration of GFP ( $\lambda_{\text{ex}} = 475 \text{ nm}$ ,  $\lambda_{\text{em}} = 504 \text{ nm}$ ) in A) and QD ( $\lambda_{\text{ex}} = 475 \text{ nm}$ ,  $\lambda_{\text{em}} = 564 \text{ nm}$ ) in B).

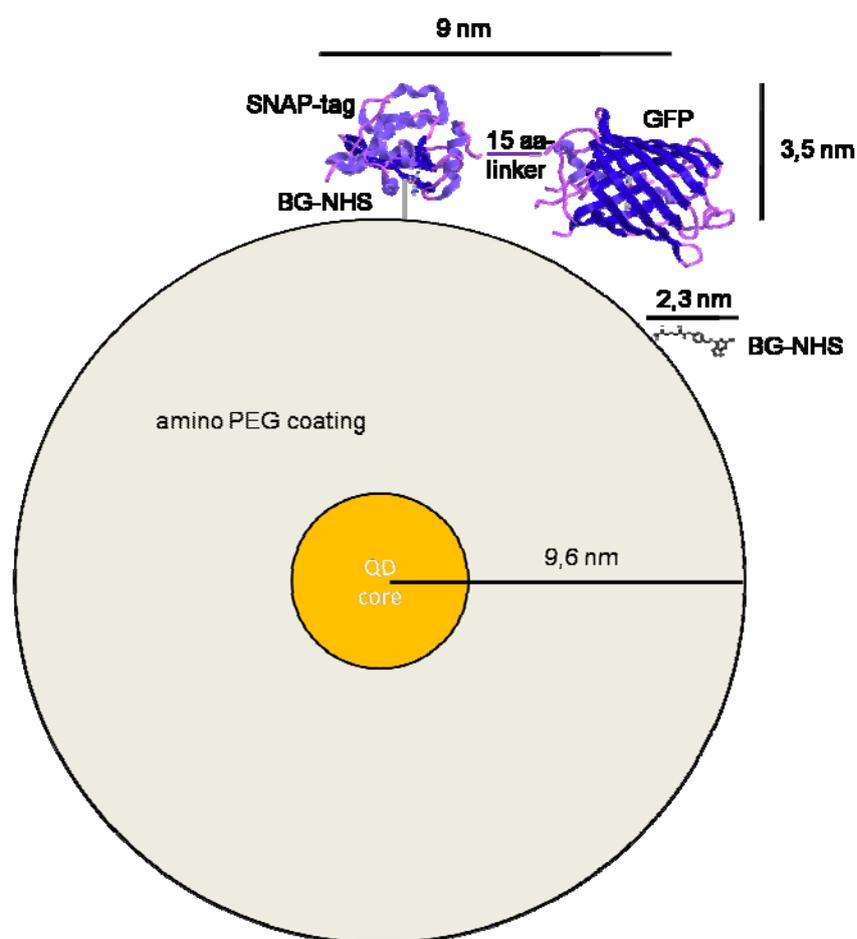
Fig. S4:



Fluorescence spectra of gfpQD conjugates prepared using different ratios of bgQD and SNAP-GFP ( $\lambda_{\text{ex}} = 475 \text{ nm}$ ,  $\lambda_{\text{em}} \text{ QD} = 564 \text{ nm}$ ,  $\lambda_{\text{em}} \text{ GFP} = 504$ ) before (1a, b, c) and after (2a, b, c) purification with ultrafiltration. The ratio of bgQD to GFP after washing has been determined with the help of calibration curves (Fig. S3).

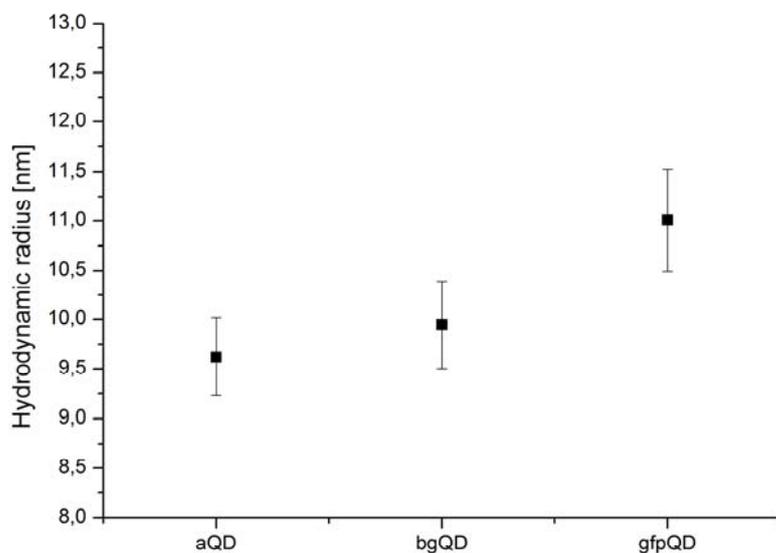
There are 10 BG groups per QDs assuming that all added BG groups are covalently bound. When bgQD and SNAP-GFP were mixed in 1:10 ratio and allowed to react, we have found that the ratio estimated using calibration curves is approximately 1:11. Additionally when 1:20 reaction mixture was used, obtained ratio corresponded to 1:8 which is in a good agreement with estimated 10BG groups available for binding (Fig S4).

Fig.S5:



Schematic representation of gfpQD conjugate, roughly to scale. GFP and SNAP protein structures were obtained from protein data bank (1EMA for GFP and 3KZZ for SNAP-tag).

Fig. S6:

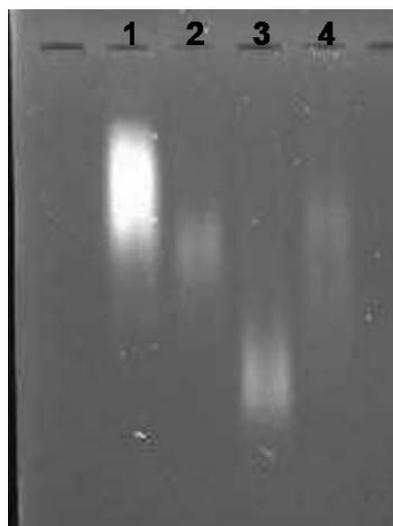


Dynamic light scattering (DLS) data obtained at 20 °C with Malvern Zetasizer Nano. Unmodified aQD were measured in a freshly diluted 0.2  $\mu\text{M}$  solution. bgQD were measured in a concentration of 0.8  $\mu\text{M}$  right before their reaction with SNAP-GFP while gfpQD were measured immediately after the gel filtration chromatography. Reported values are according to size distribution by intensity. The BG to aQD ratio is 20: 1 so there is a maximum of 20 SNAP-GFP proteins bound to the surface.

We have also tried to use Fluorescence Correlation Spectroscopy (FCS) to determine the size of the starting QDs and the final conjugate, but we were not able to obtain consistent results and it seems that the method is not suitable for this particular construct where two fluorescent species are present. We also thought of the use of FCCS. Unfortunately our system with GFP and yellow QDs does not allow the separate excitation of GFP without the excitation of the QD at the same time. Additionally, both emissions overlap as well, so their signals cannot be properly separated as it is required for FCCS.

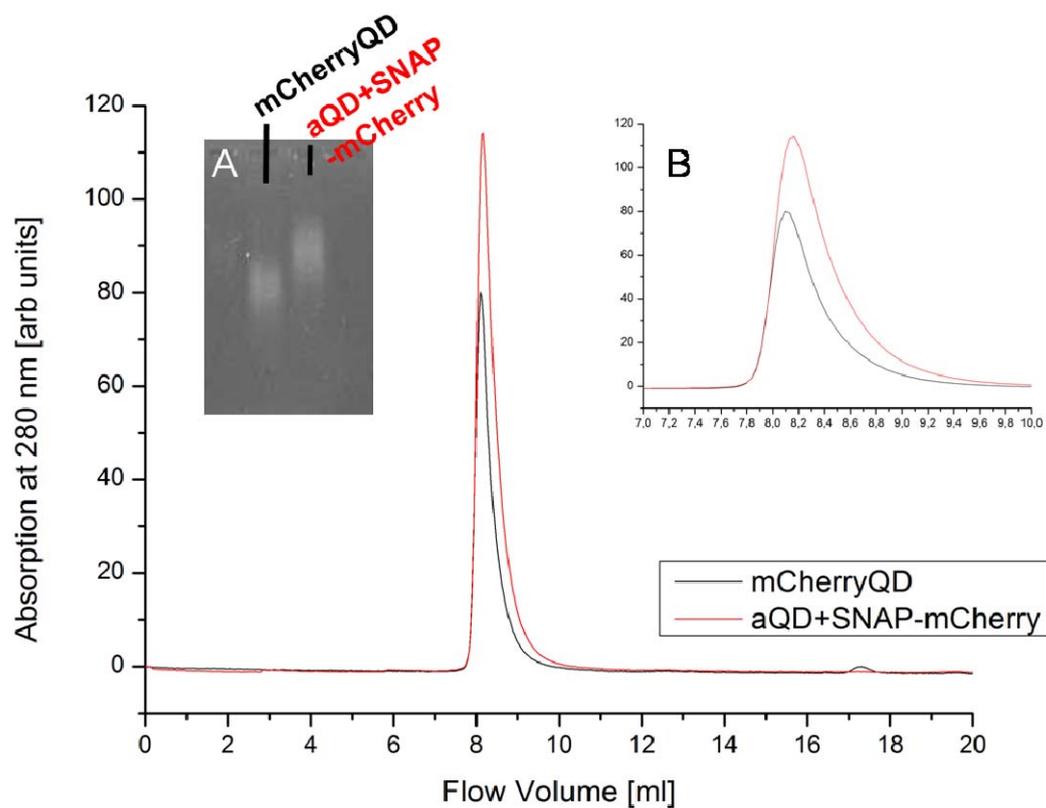
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Fig. S7:



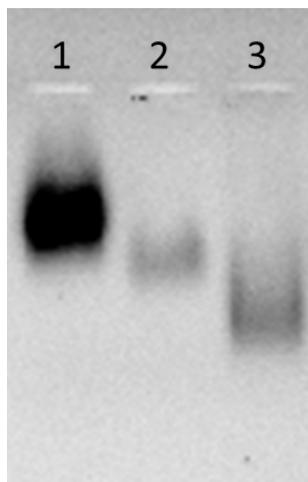
Agarose gel electrophoresis (0,6 % agarose, 100 V); 1: unmodified aQD from the stock, 2: bgQD, 3: mCherryQDs, and 4: control containing unmodified aQD and SNAP-mCherry 50 BG per aQD and 20 SNAP-mCherry per bgQD were used.

Fig.S8



Size Exclusion Chromatography (GE Superdex 200 10/300 GL column) of mCherryQD biocunjugates (black). Negative control with aQD from the stock instead of bgQD (red). Enlarged in inset B. Inset A is showing the agarose gel (0,6 %) of the corresponding conjugation mixtures.

Fig. S9



Agarose gel electrophoresis (0,6 % agarose, 100 V, 1 h); E-CadherinQD conjugate (3) and corresponding controls: aQD (1), BG functionalized QD blocked with SNA (bg-acQD, 2).