Hybrid gold nanoparticles-quantum dots self-assembled nanostructures driven by complementary artificial proteins

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Electronic Supplementary informations

Morphological and optical characterization of AuNP and QD

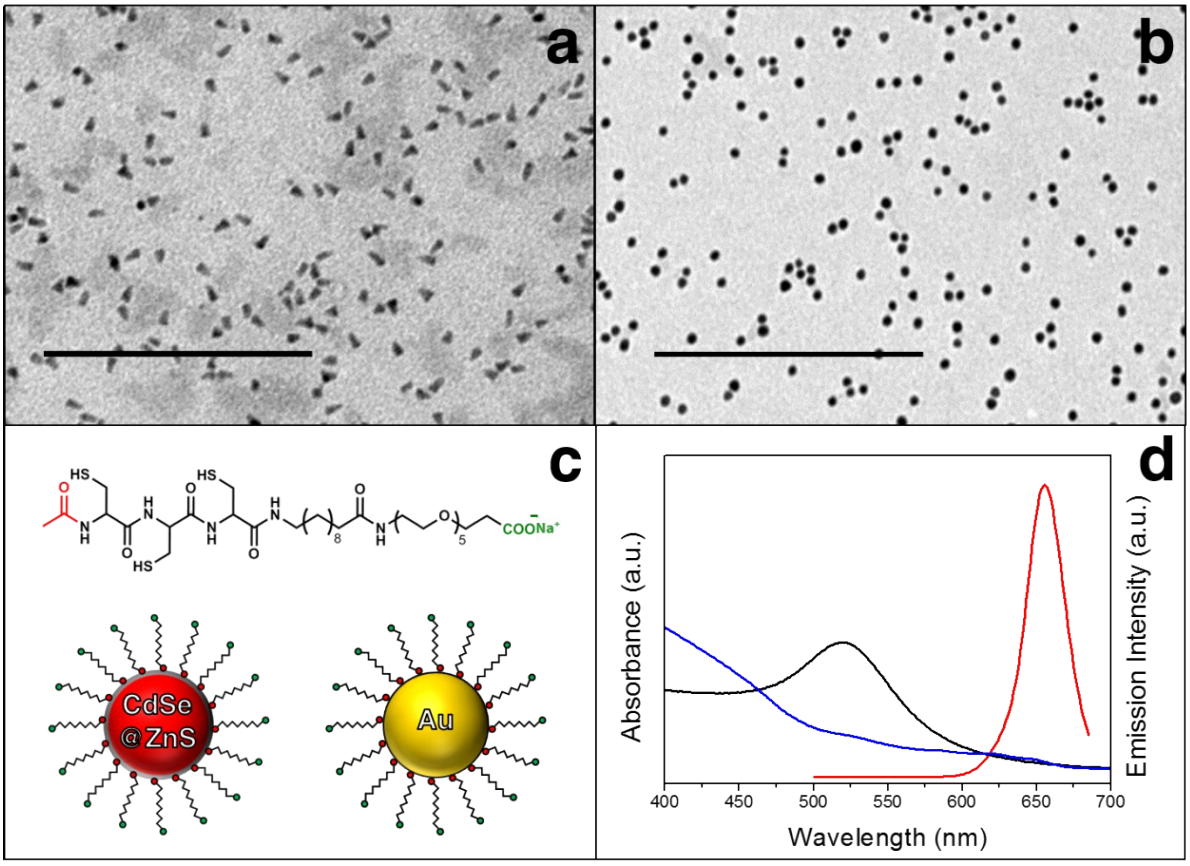


Figure S1. TEM pictures of peptide-grafted **(a)** CdSe@ZnS quantum dots and **(b)** gold nanoparticles AuNP. **(c)** Chemical structure of the tricystein ligand C5PEG4 and schematic representation of water-soluble QD-C5PEG4 and AuNP-C5PEG4. **(d)** Normalized UV-Vis absorption and emission intensity spectra of QD-C5PEG4 (blue and red curves, respectively) and normalized UV-Vis absorption spectrum of gold nanoparticles (black curve). Scale bar: 100 nm.

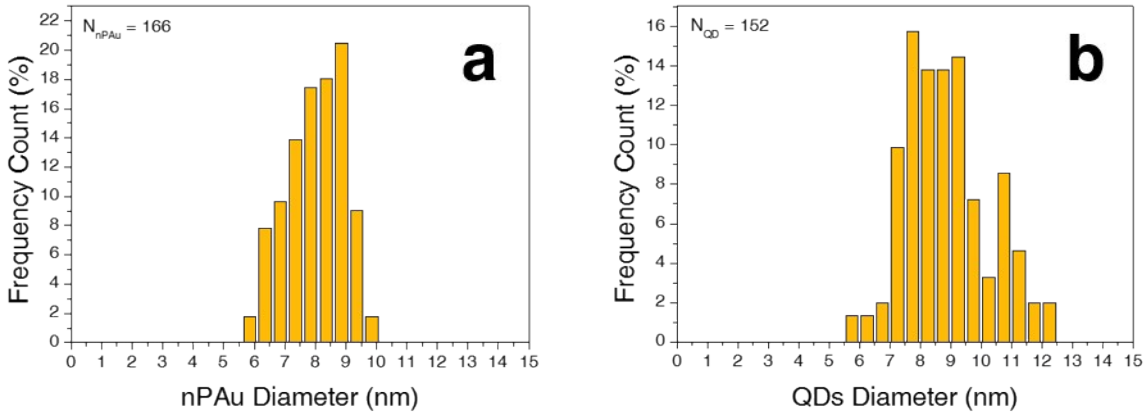


Figure S2. Particle size distribution histograms of peptide-stabilized **(a)** gold nanoparticles AuNP-C5PEG4 and **(b)** quantum dots QD-C5PEG4.

Determination of A3 protein concentration on AuNP-A3

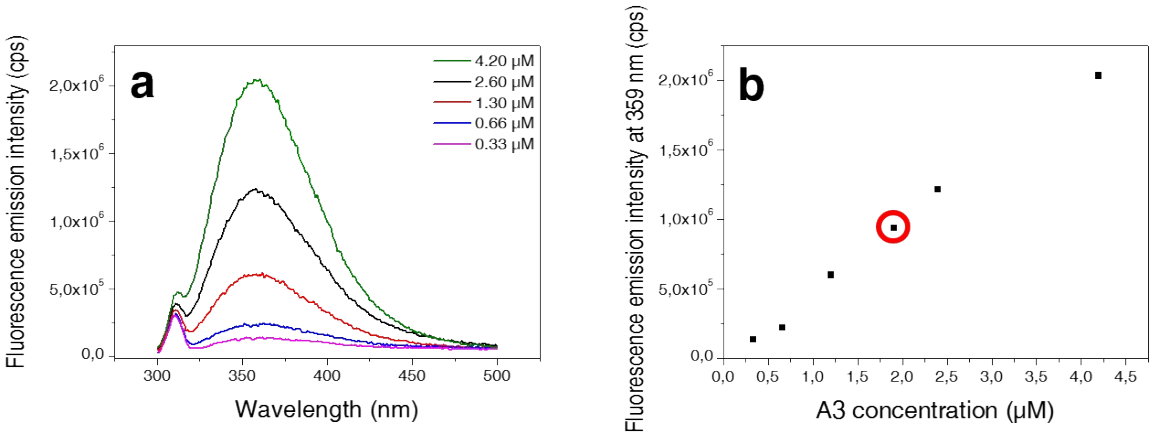


Figure S3. (a) Fluorescence emission spectra of a range of A3 suspensions with concentrations from 0.33 to 4.20 µM. **(b)** Calibration curve of the fluorescence emission intensity at 359 nm according to the A3 concentration. The circled value is the emission intensity obtained with dissolved AuNP-A3 saturated bioconjugates.

Determination of A3 protein concentration on QD-A3

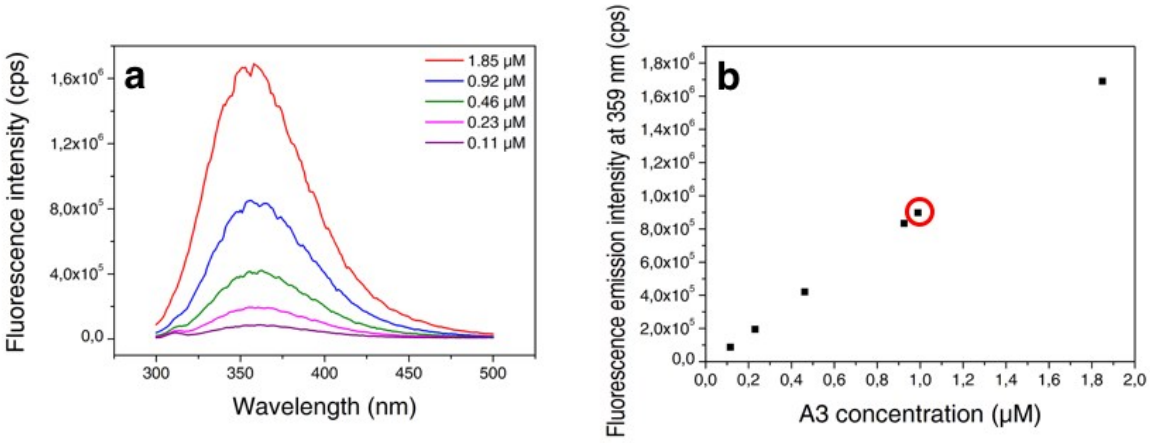


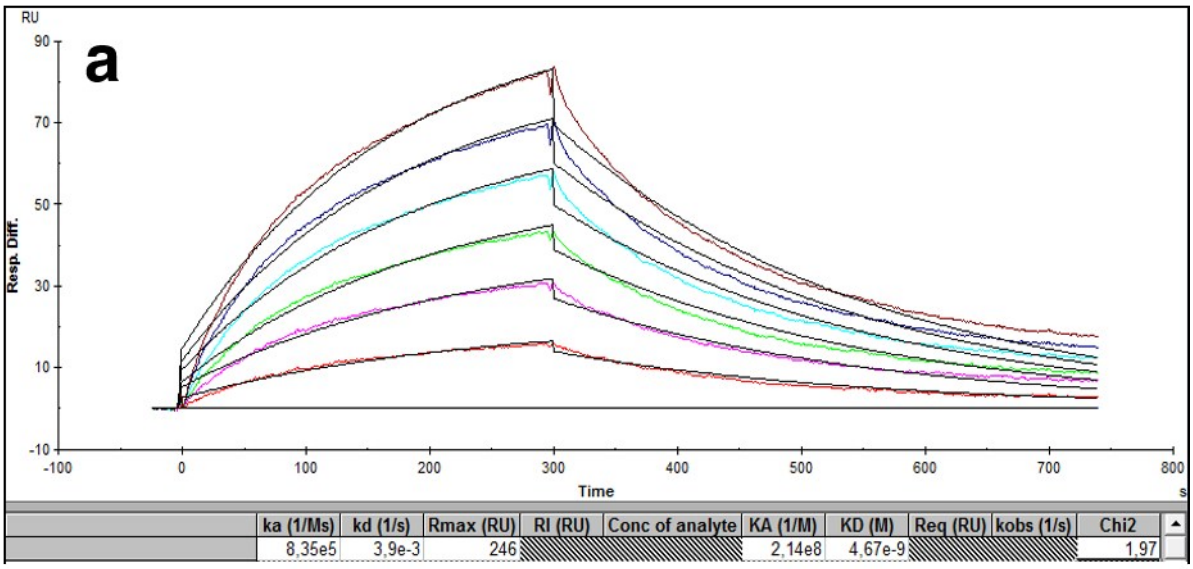
Figure S4. (a) Fluorescence emission spectra of standard A3 solutions of different concentrations from 0.11 to 1.85 µM. **(b)** Corresponding calibration curve of the A3 fluorescence emission intensity at 359 nm v.s. concentration. The circled value is the measured emission intensity of the QD-A3 bioconjugates after dissolution of the QD.

SPR measurements analysis

The kinetics (shown in Figure 3b) were first evaluated using the BIA evaluation software, version 4.1 (GE Healthcare). The data were processed by fitting the binding profiles to a 1:1 Langmuir interaction model (See figure S4 a). The quality of the fit was assessed by the statistical Chi2 value provided by the software (Chi2 values <10 were considered as acceptable). The extracted value of the dissociation constant is found to be K_D = 4.67 nM.

Secondly the fitting of each dataset yields rates for association (k_{on}) and dissociation (k_{off}), from which the equilibrium dissociation constant K_D was calculated ($K_D = k_{off}/k_{on}$) and found to be K_D = 6.80 nM.

Finally the Scatchard linearization (see Figure S4b) provides the dissociation constant K_D = 5.60 nM.



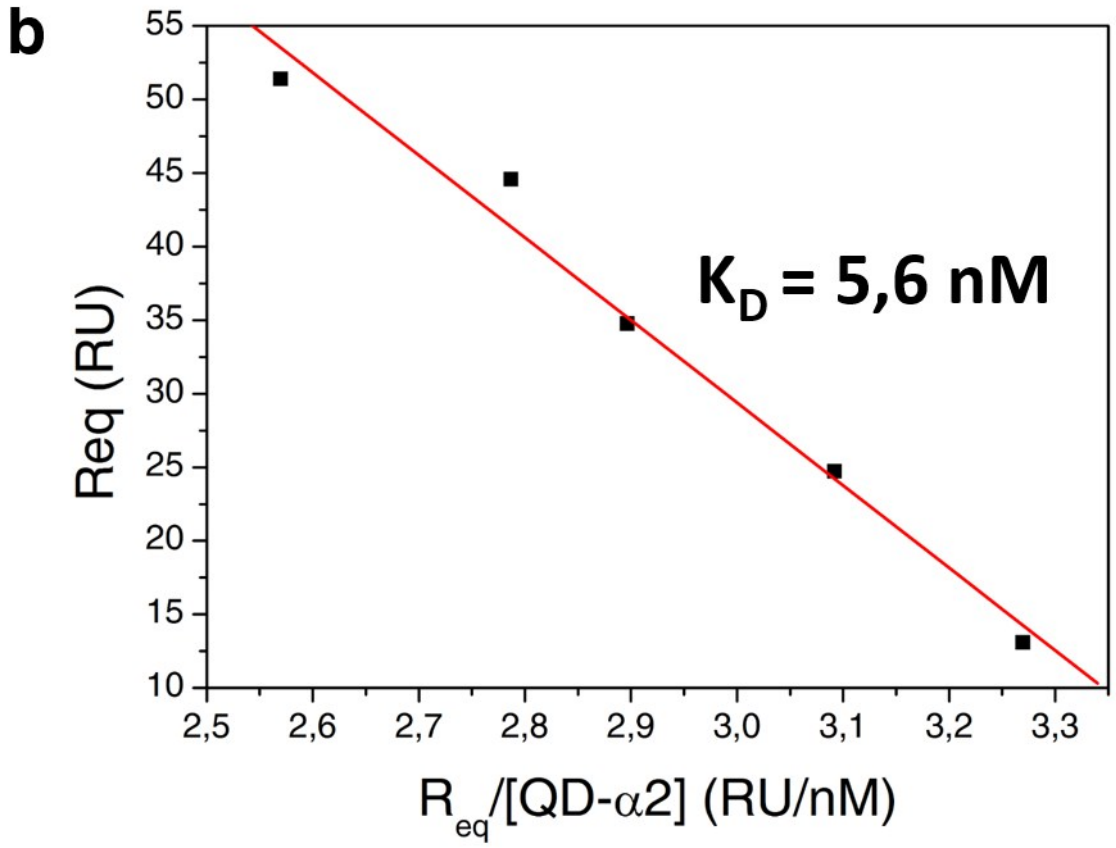


Figure S5. (a) Kinetics measurements performed using six concentrations of QD-α2 applied to immobilized A3. The data were fitted using the Langmuir model and the BIA evaluation software. (b) Scatchard plot for the estimation of the dissociation constant K_D, which is obtained from the slope of the linear fit.

Table S1: Zeta potential measurements of the quantum dots and the gold nanoparticles grafted with the initial ligand and the two proteins obtained in pH 8 borate buffer. The pI of the proteins are respectively around 7.05 for α2 and 5.43 for A3.

Zeta potential	*C5PEG4*	*α2 protein*	*A3 protein*
QD	$-26 \pm 1\ mV$	$-12 \pm 1\ mV$	$-6 \pm 1\ mV$
AuNP	$-27 \pm 1\ mV$	$-14 \pm 1\ mV$	$-7 \pm 1\ mV$

Analysis of QD-A3•α2-QD superstructures from TEM measurements

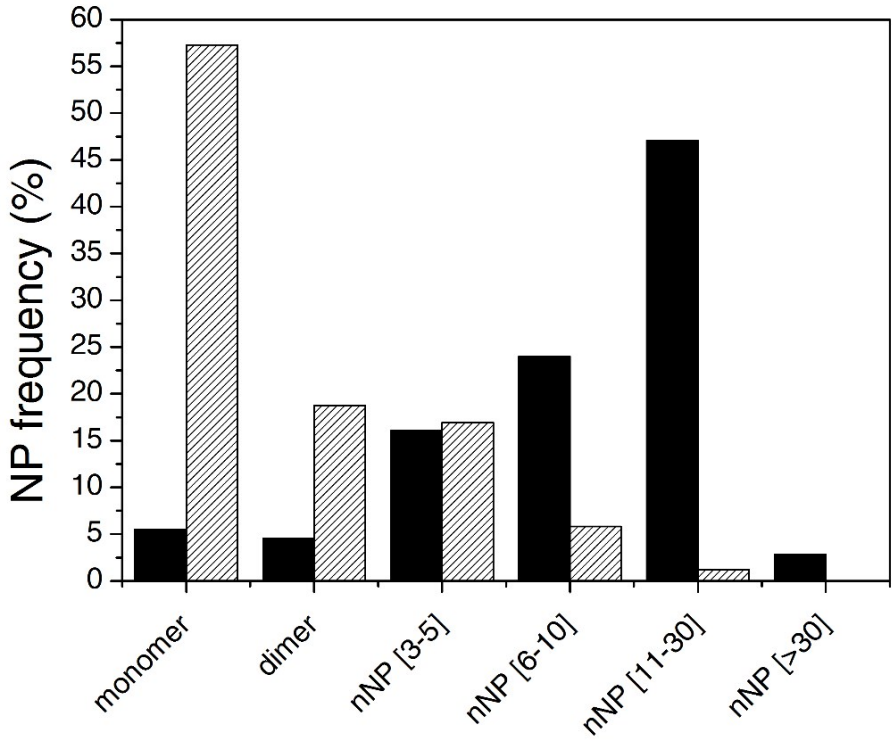


Figure S6. Histogram of the relative parts of QD involved in QD-A3•α2-QD aggregates of different sizes in the case of a complementary 1:1 mixture of QD-A3 and QD-α2 (black bars) or in the case of pure QD-A3 (crosshatched bars).

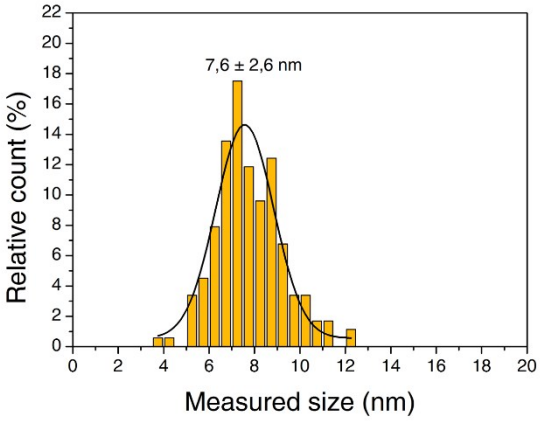


Figure S7. Distribution histogram of surface-to-surface interparticle distances extracted from QD-A3•α2-QD self-assemblies TEM images.

Hybrid AuNP—QD large self-assemblies (protein-saturated NP surface)

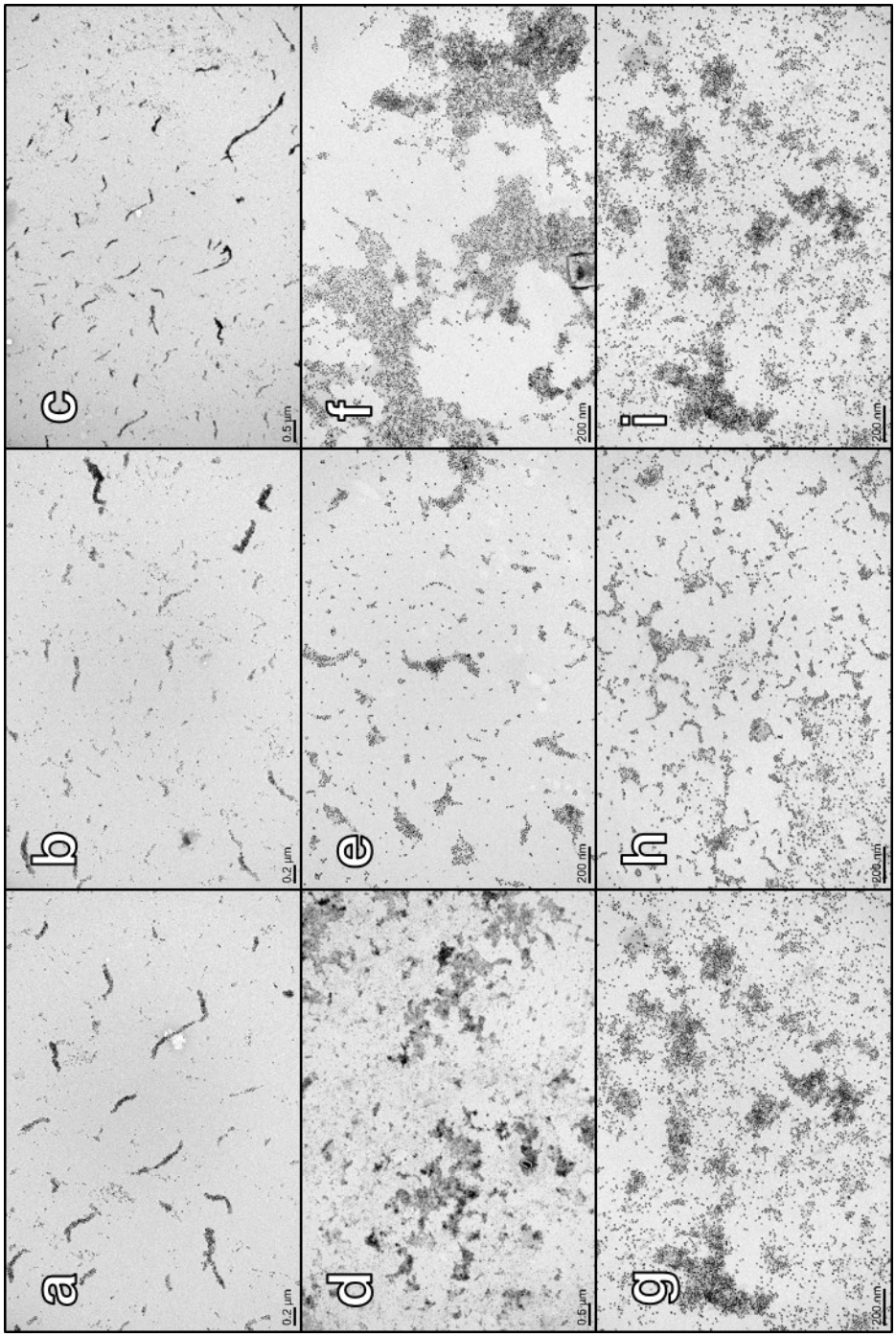


Figure S8. TEM images of large superstructures of QD-A3•α2-AuNP with mixtures molar ratios of (a—c) 1:1, (d—f) 1:2 and (g—i) 1:5. The surface of the NP was saturated with 100 molar eq of proteins.

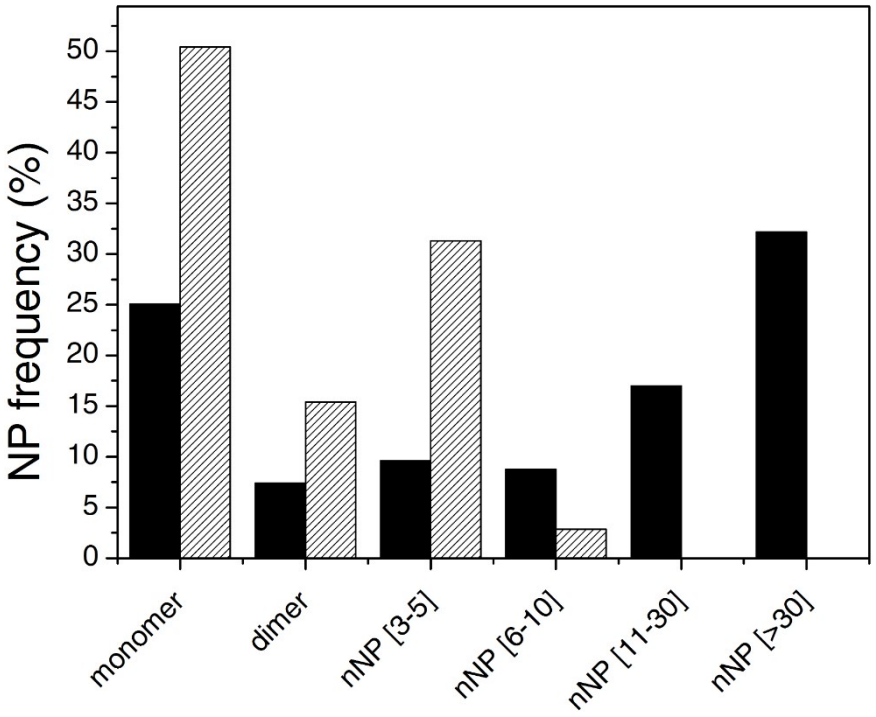


Figure S9. Histogram of the relative parts of QD and AuNP involved in hybrid QD-A3•α2-AuNP aggregates of different sizes in the case of a complementary 1:1 mixture of QD-A3 and AuNP-α2 (black bars) or in the case of a non complementary 1:1 mixture of QD-α2 and AuNP-α2 (crosshatched bars).

Hybrid AuNP—QD spatially limited self-assemblies

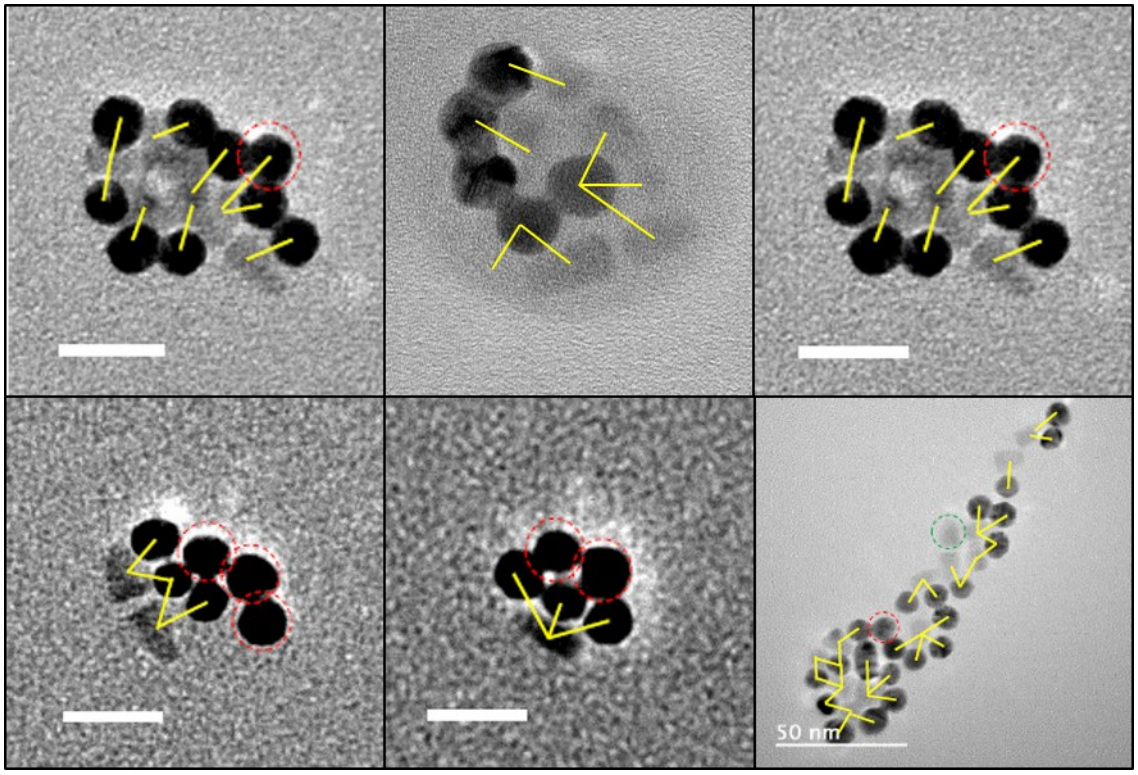


Figure S10. HRTEM images of spatially limited QD-A3 + AuNP-α2 self-assemblies. Yellow bar represent QD-AuNP possible links, red dotted circles represent AuNP with no obvious link to QD and green dotted circles represent QD with no obvious link to AuNP. Scale bar is 20 nm.

Table S2. Statistical analysis of QD-AuNP oligomers.

Number of QD	QD with no obvious link to AuNP	Number of AuNP	Au with no obvious link to QD	Number of QD—AuNP links	Average link per QD	Average link per AuNP
56	1 (1.7 %)	100	8 (8 %)	104	1.85	1.04

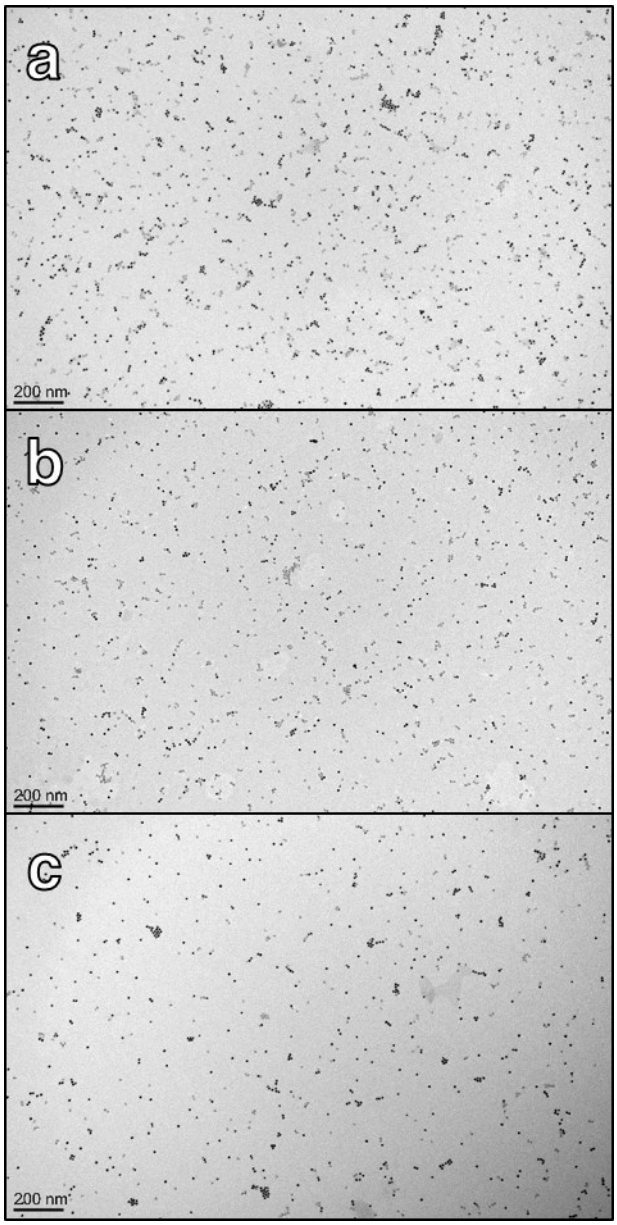


Figure S11. TEM images of QD-A3/AuNP-α2 mixture in the presence of A3 (9.6 μM) at the different QD:AuNP molar ratio of **(a)** 1:1; **(b)** 1:2 and **(c)** 1:5.

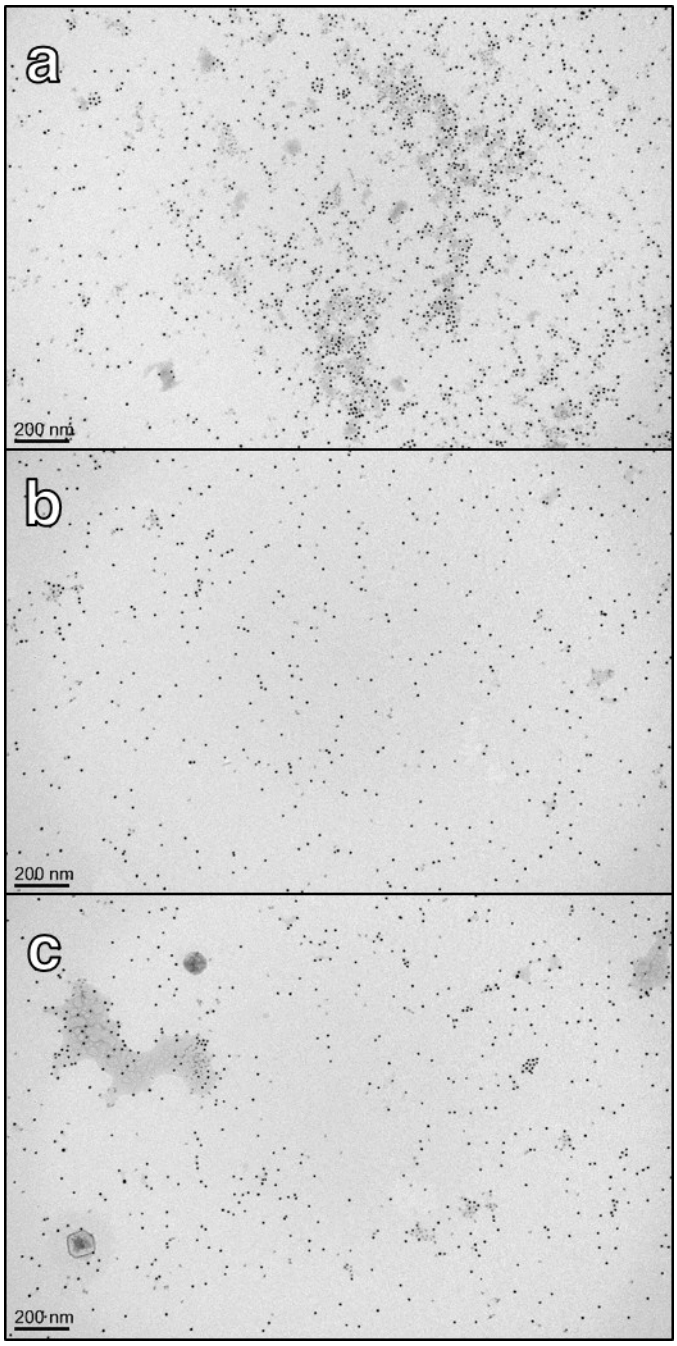


Figure S12. TEM images of mixtures of QD-α2 and AuNP-α2 at a QD-α2/AuNP-α2 molar ratio of 1:1 (a), 1:2 (b) and 1:5 (c).

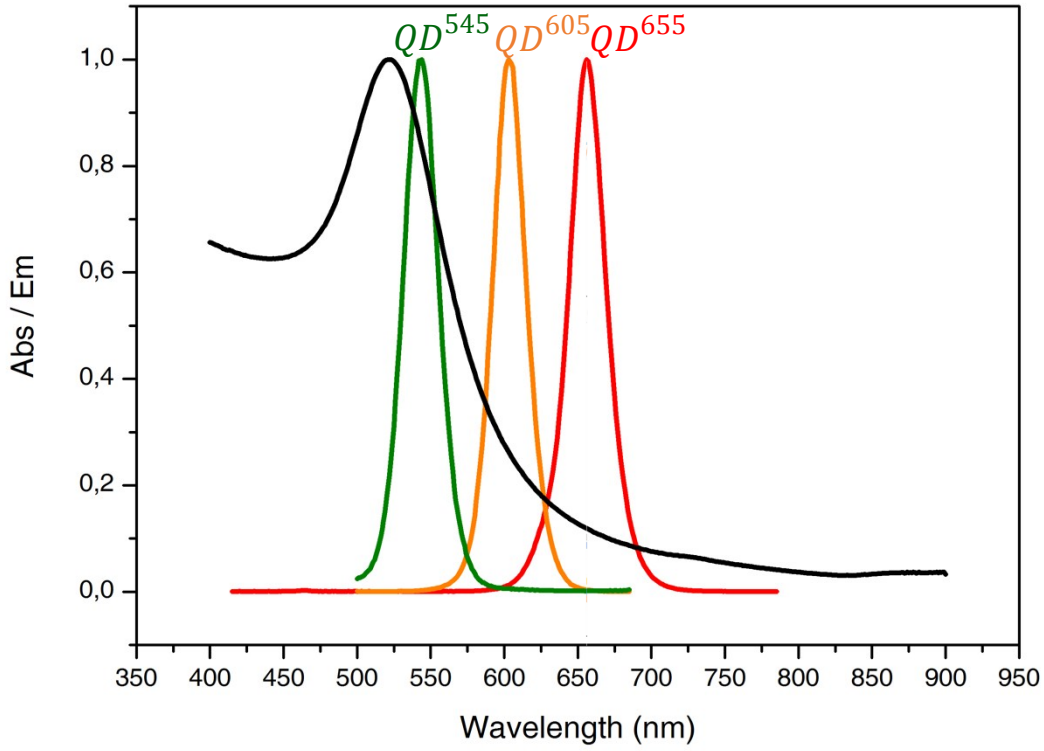


Figure S13. Absorption Spectum of the AuNP (black curve) and fluorescence emission spectra of the red, orange and green QD used (with an excitation wavelength 350 nm). The mean diameter extracted from HRTEM measurements is 6.8 $\pm$ 1 nm for the orange QD^{605} and 3.7 $\pm$ 0.6 nm for the green QD^{545}.

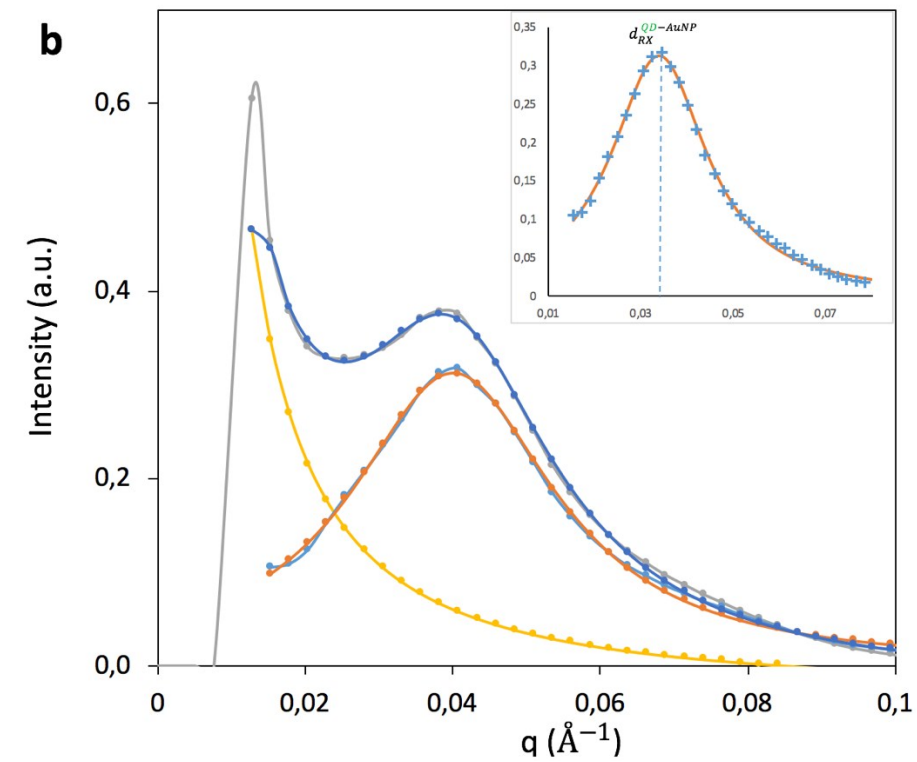


Figure S14. SAXS data of equimolar mixture of AuNP-α2 and QD-A3 with different emission wavelengths: **a)** QD^{605} with orange emission at 605 nm, **b)** QD^{545} with green emission at 545 nm. Characteristic self-assembly peak is shown before (grey curve) and after (orange curve) the blank substraction (yellow curve). The fits are shown as blue curves. **INSET:** peaks of QD-

A3/AuNP-α2 mixture and Lorentzian fit (see text). The center-to-center distances are found to be respectively $16.4\,nm$ for the orange QD^{605} and $14.6\,nm$ nm for the green QD^{545}.

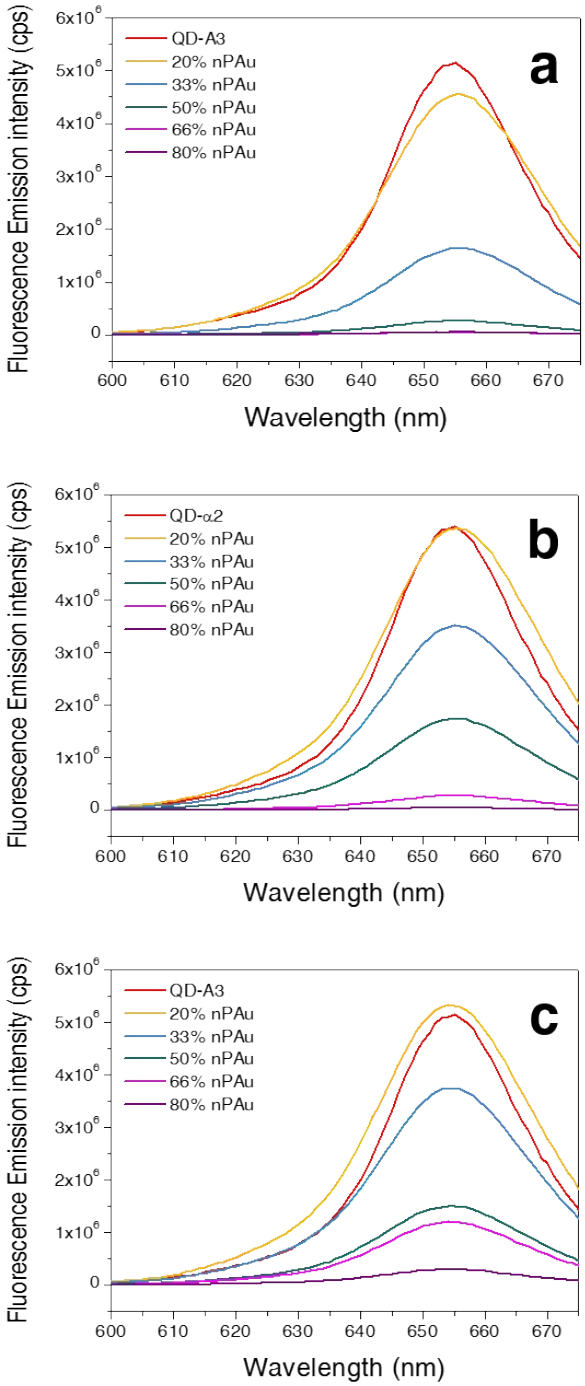


Figure S15. Fluorescence emission spectra of mixtures of (a) QD-A3 and AuNP-α2, (b) QD-A3 and AuNP-A3 and (c) QD-A3 and AuNP-α2 in presence of α2 with different QD/AuNP molar ratio

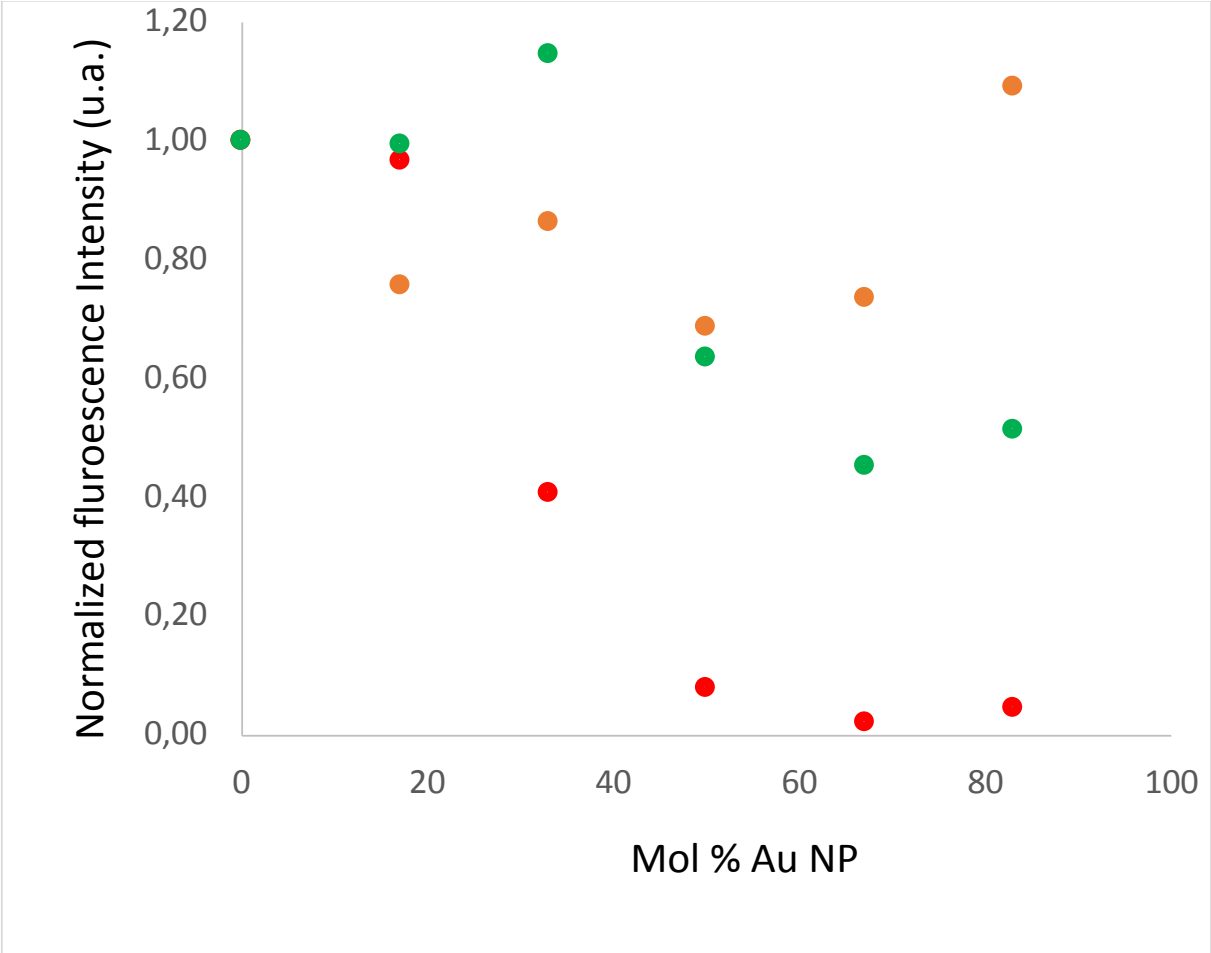


Figure S16. Evolution of the QD-A3 fluorescence in presence of an increasing amounts of the same AuNP-α2: red QD^{655} (red points), orange QD^{605} (orange points) and QD^{545} (green points). Fluorescence intensities are normalized to the value of pure QD-A3 at the same dilution.