Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Information

Title: **Quantum dots clusters as self-assembled antennae for phycocyanin and phycobilisomes acceptor**

Authors: Joanna Grzyb[1], Katarzyna Walczewska-Szewc[2,3], Jakub Sławski[1], Martyna Trojnar[1]

[1] Department of Biophysics, Faculty of Biotechnology, University of Wrocław, F. Joliot-Curie 14a str, 50-383 Wrocław, Poland

[2] Department of Biophysics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University, Grudziądzka 5 str., 87-100 Toruń, Poland

[3] Centre for Modern Interdisciplinary Technologies, Nicolaus Copernicus University, Wileńska 4, 87-100 Toruń, Poland.

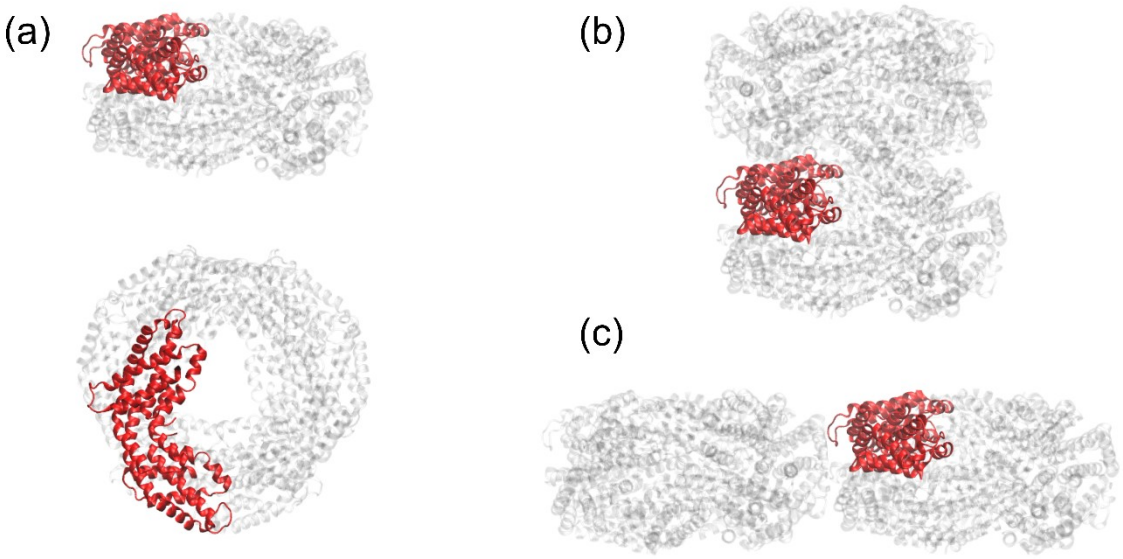


Figure S1. Various configurations of phycobilisome (PBS) models. The basic phycobilisome model consists of twelve phycocyanine subunits forming a torus (a); configurations of tori forming a stack (b), and a line (c). Two phycocyanine subunits forming a dimer (PC) are shown as a red cartoon.

Mechanism of CysHCl induced clustering and assembly with PC/PBS

Previously, it was shown that dithiols may cause covalent coupling of QDs [1]. This is not the case here, as CysHCl is monothiol. The most probable mechanism is partial substitution of β-mercaptopropionic acid molecules from QDs outer surface, by the same mechanism as mentioned dithiol reaction. As a result, however, there is no linkage between two QDs but they obtain positively charged amino groups. With only partial substitution, there are negative and positive charges at the QDs surface, which promotes their aggregation. Neutralization of QD surfaces facilitates interaction with PC/PBS, which are negatively charged.

In order to confirm that partial surface QD substitution occurred, we carried on Fourier-transformed infrared spectroscopy (FTIR) measurement. Measurement was done with a Cary630 FTIR spectrometer, with a diamond ATR module. The samples were deposited on a diamond window by gentle drying from a water solution. For short treatment with CysHCl, QDs were mixed with 2 mM CysHCl, preincubated for 5 min, and spun down (10 min, 10 000g). The resulting pellet was washed with water, resuspended and spun again. The final resuspension was then evaluated with FTIR. For total substitution of a surface, QDs powder was mixed with CysHCl *in substantia*, incubated overnight at 80°C, at resuspended in water. The solution was passed by PD10 column twice to ensure no contamination with free CysHCl molecules. The colored fractions eluting at small elution volumes (corresponding to high molecular weight compounds) were collected and analyzed.

Figure S2 compares two spectral regions with the most intensive vibrations of QDs and QDs treated shortly with CysHCl. The FTIR spectra of CysHCl, as well as QDs with the completely substituted surface, are added for comparison.

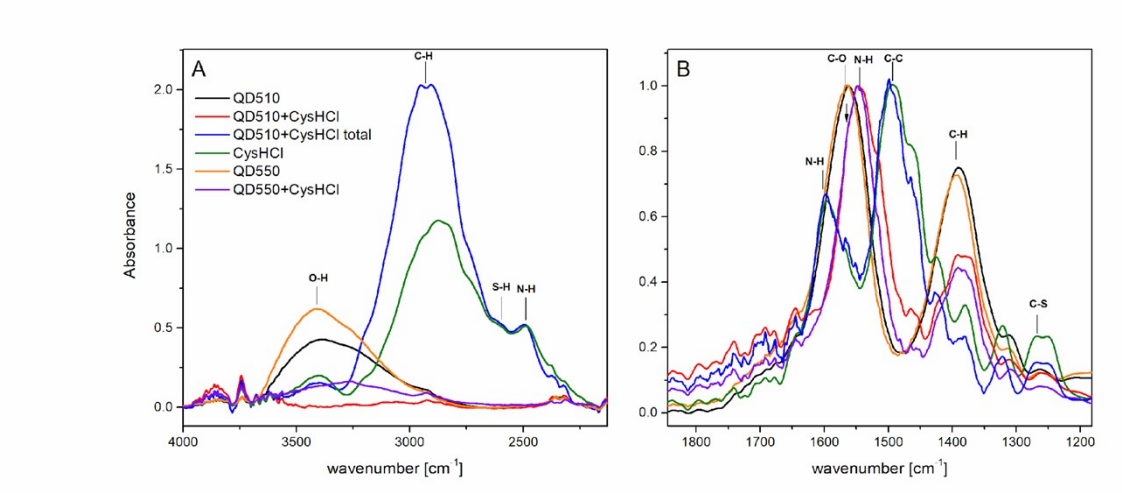


Figure S2. Lower energy (A) and higher energy (B) vibration regions in QDs FTIR spectra. For easier comparison, intensities were normalized to 1 for the band at around 1550 cm^{-1}.

The untreated QDs are covered with mercaptoprionic acid. Its binding to a nanocrystal is by thiol, so free S-H vibration should not be expected in the FTIR spectrum. The same occurs for CysHCl attachment to QD. The S-H band at around 2750 cm^{-1} is present in CysHCl, but not in QD or QD+CysHCl. However, the CysHCl treatment causes a significant reduction in the intensity of the broad band of O-H stretching vibrations (3250-3500 cm^{-1}), being most probably a result of the removal of mercaptopropionic acid from a QD surface. The second most significant change is a shift of a maximum of 1562 cm-1, ascribed to the C-O stretch to 1546 cm-1, after a short treatment with CysHCl. The second vibration is

interpreted as N-H bending. An arm of C-O stretch is still present, proving again reminiscence of some mercaptopropionic acid presence, although N-H vibration dominates. It should be noticed that C-H bend vibration at 1400 cm-1 is present for both treated and not treated QDs, although the intensity of this band, in comparison the main band at around 1650 cm-1 lowers. The origin of this change is not clear because cysteamine and mercaptopropionic acid contain the same amount of C-H groups. However, It is possible that the extinction coefficient of N-H vibration is higher than C-O vibration. The C-C stretch vibration, which might be helpful, is present in free CysHCl (and expected in mercaptopropinic acid), although strongly quenched after these compound attachments to the QD surface.

For total substitution with CysHCl, the main vibrations are basically in the same place as for pure CysHCl. There is also a manifestation of free S-H vibration, which may suggest that some free CysHCl stays adsorbed and are hardly removed even by precise desalting.

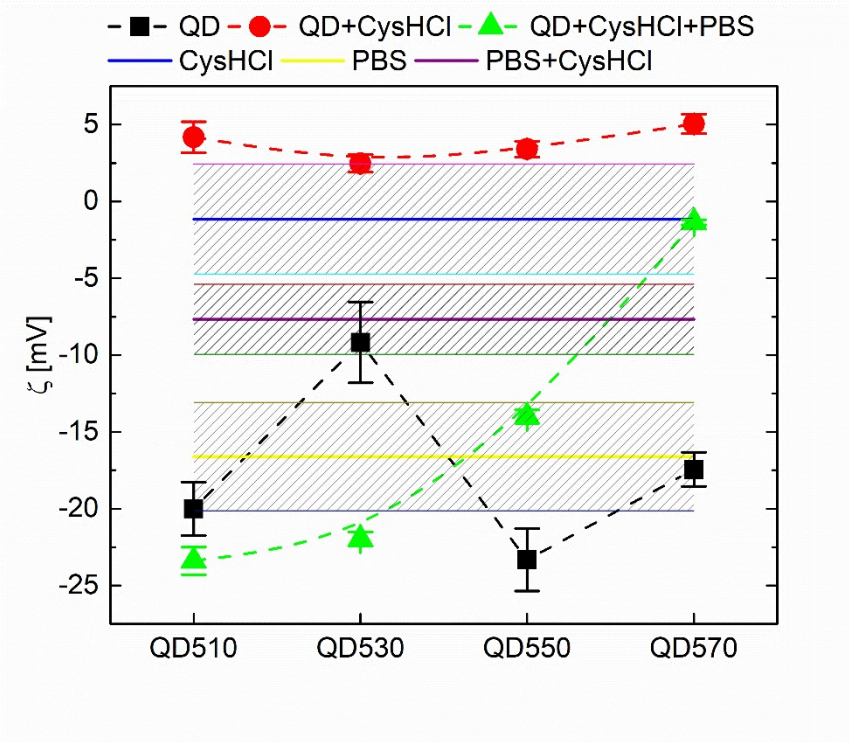


Figure S3. Results of zeta potential evaluation for all tested QD versions (1 µM), CysHCl (2 mM), PBS (1 µM), and their respective mixtures in water. Points are shown for QDs and their mixtures, and bars represent standard deviation of at least three repetitions. Dotted lines connecting points are eyes-guides only. Lines are shown for CysHCl and PBS (with regions corresponding to SD) for easier evaluation regarding particular QDs types. CysHCl results might be treated as background, as the size of these molecules is too small to be actually detected by ZetaSizer.

Zeta potential study (Figure S3) proved that CysHCl presence significantly increases the surface charge of all QDs used. As starting QDs are of negative charge (-10 mV to - 25 mV) Again, QD530 seems to be slightly “out of tendency” for a QD series tested, as also observed for measurements shown in the main manuscript. There, we tested different lots of QDs with fluorescent techniques, getting similar results, and most probably, this is simply a result of some production specificity for this particular size, inducing e.g., higher aggregation tendency or some inhomogeneity of surface.

In the presence of CysHCl, all QDs have ζ at around +2 to+ 5 mV.

It suggests that substitution is with slight domination of NH2 groups, or CysHCl may additionally form some non-covalent outer sphere over QDs surface. It might be the case for PBS alone, as their charge increases in the presence of CysHCl from - 17 mV to - 7 mV. Interestingly, the surface charge of the PBS-QD mixture (here, in 1:1 ratio) is always different from the sum of particular values of components. There is a clear decrease in the charge in a function of QD size. For simplicity, we tested the 1:1 ratio between junction partners; it might be explained by the higher surface of QD occupied by PBS for QD510, and gradually smaller surface for bigger QDs. As PBS are binding to positive charge, and they are overly negative, what is exposed in a PBS:QD complex (especialy for QD510), is a negative surface of a protein complex. It is worth noting that results for QD530 are no longer "out of tendency" so PBS binding may help to destroy unspecific aggregates/increase the homogeneity of a mixture.

To test if there is any specific effect of CysHCl on PC/PBS, we used a related protein, phycoerythrin (PE). This is also a constituent of phycobilisomes in some algi (e.g. *Calothirx* sp.), with a spectral range blue-shifted in comparison to PC [2]. Absorption and emission characteristics of PE makes it a good acceptor from QD510, QD530 and QD550. Surprisingly, we found no FRET between QD and PE in presence or absence of CysHCl. This indicates that some specific interaction between CysHCl and PC must occur.

To verify the hypothesis, we tested several chemicals, containing thiol group (dithiotreitol, β-mercaptoetanol), amino group (cystamine, Tris, urea, poly-L-lysine) or both of them (cysteine, homocysteine). We also used mixtures of amino and thiol compounds, to simulate probable action of CysHCl. The structural formulas of chemicals are shown in Fig. S4. To sum up, there was no single chemical or a chemical mixture, which simply simulated CysHCl. For prolonged incubation of QD+PC/PBS with cystamine, signatures of FRET energy transfer were found. This fact was explained by slow hydrolysis of cystamine molecules, realizing cysteamine with both thiol on amine groups.

Interestingly, poly-L-lysine caused significant decrease in QD fluorescent intensity as well as emission maximum red shift. Poly-L-lysine is a positively charged polypeptide, with high molecular weight, and is often used in immobilization matrices. Thus, the QD clustering we observed here was due to its surface charge neutralization. PC/PBS might be also immobilized in this matrix, however this was not enough to promote FRET.

Homocysteine, which itself did not induce FRET, was found to inhibit (to prevent occurrence, and also to revert) the effects caused by CysHCl. Cysteine, shorter by one methylene group, was not enough to repeat such a process. It shades some light on the mode of CysHCl-PC interaction. We may hypothesize that the amino group interacts with protein, anchoring it or rather stabilizing it or just the fluorophore in more preferable conformation. The full explanation would demand more studies, which are beyond the scope of this manuscript.

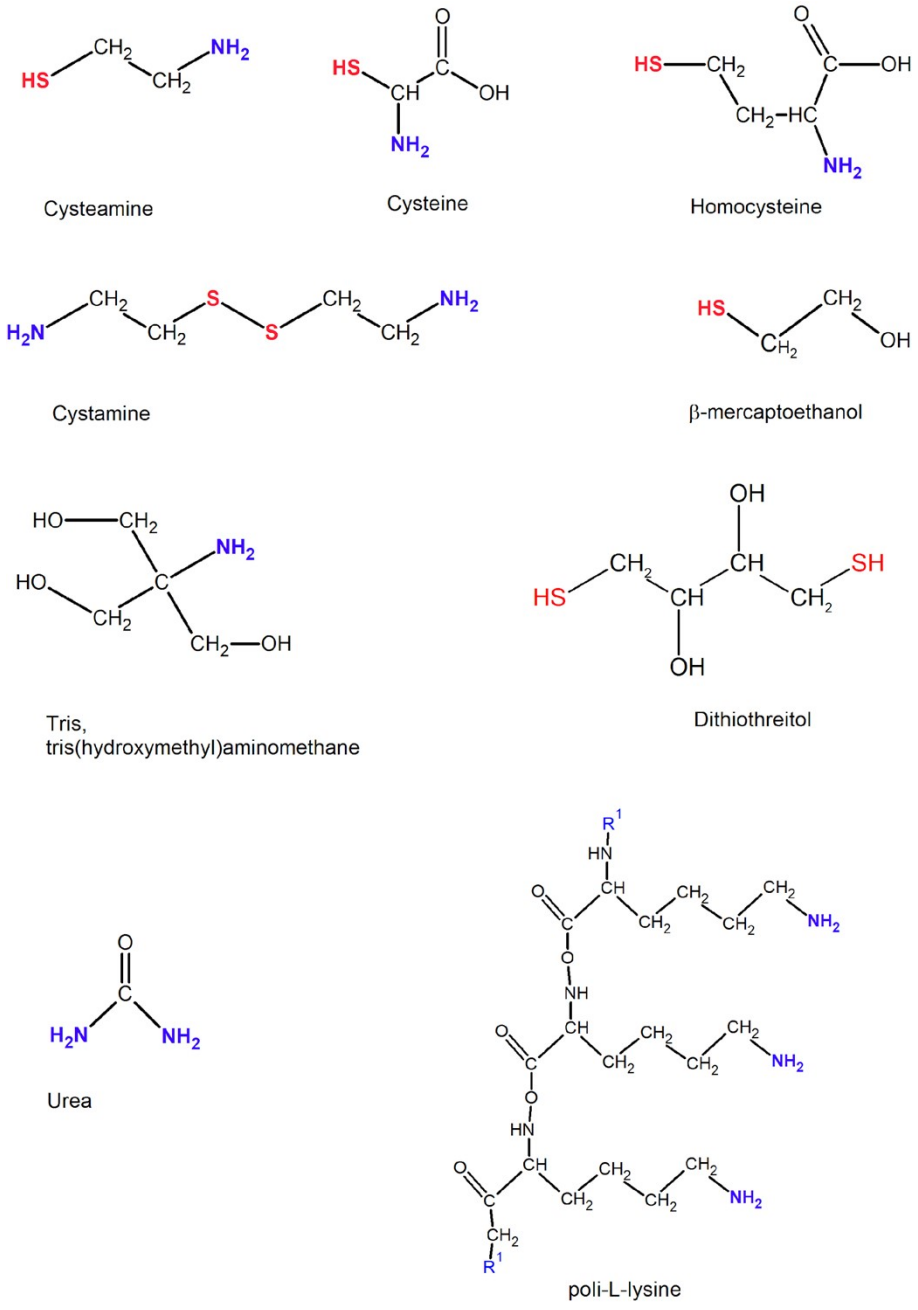


Figure S4. The chemical formula of cysteamine and other chemicals tested in order to simulate cysteamine effects on QD and PC/PBS. Thiol groups are marked red and amino groups blue.

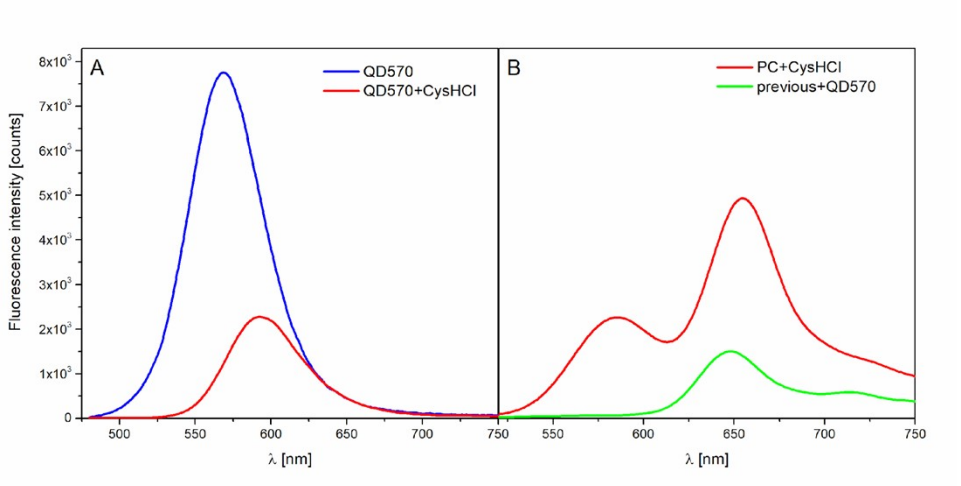
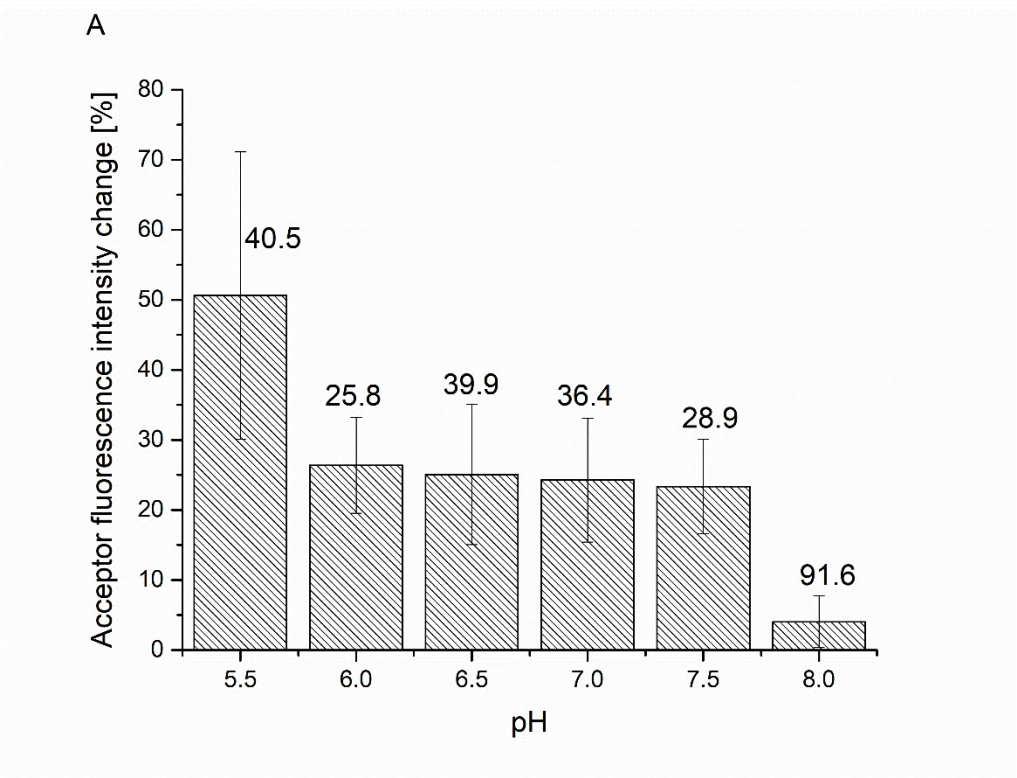


Figure S5. Example of steady-state measurements, showing effect of CysHCl (2 mM) addition to QD570 (0.1 µM) solution (A) or the same amount of QD570 addition to solution containing 0.5 µM PC and CysHCl.



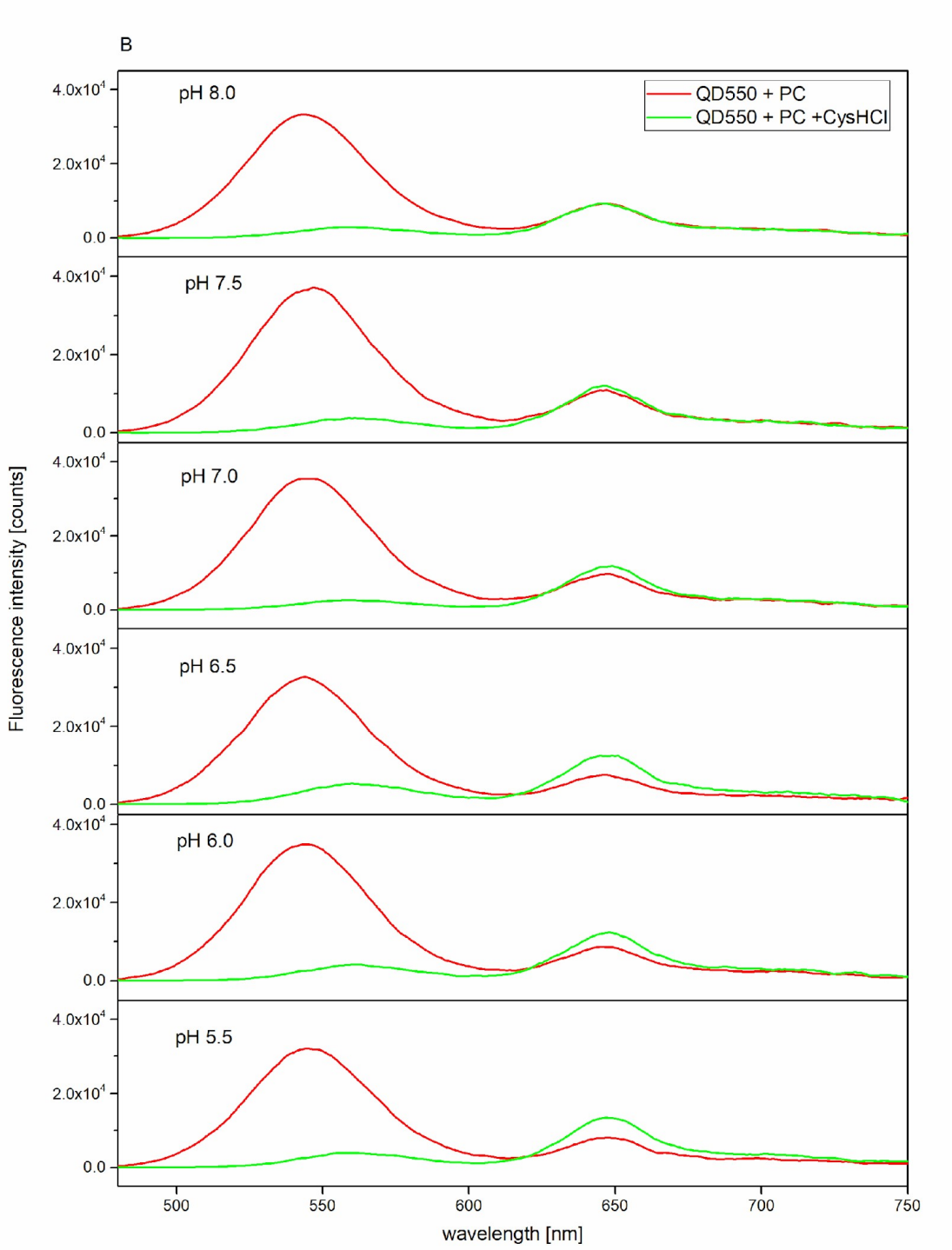


Figure S6. The effect of pH on increase of acceptor PC (0.5 µM) fluorescence (650 nm), in presence of QD550 donor (0.05 µM) and 2 mM CysHCl. First, the emission spectrum (λ_{exc} = 471 nm) of the solution of PC and QD, in 25 mM Hepes/NaOH (pH indicated in the figure) were measured as starting point. Upon addition of CysHCl, second spectrum was registered, and the change in the acceptor intensity was calculated. A) an acceptor intensity change calculated from three independent repetitions; Error bars - SD of three independent repetitions, labels indicate error percentage. B) representative spectra recorded for given pH points.

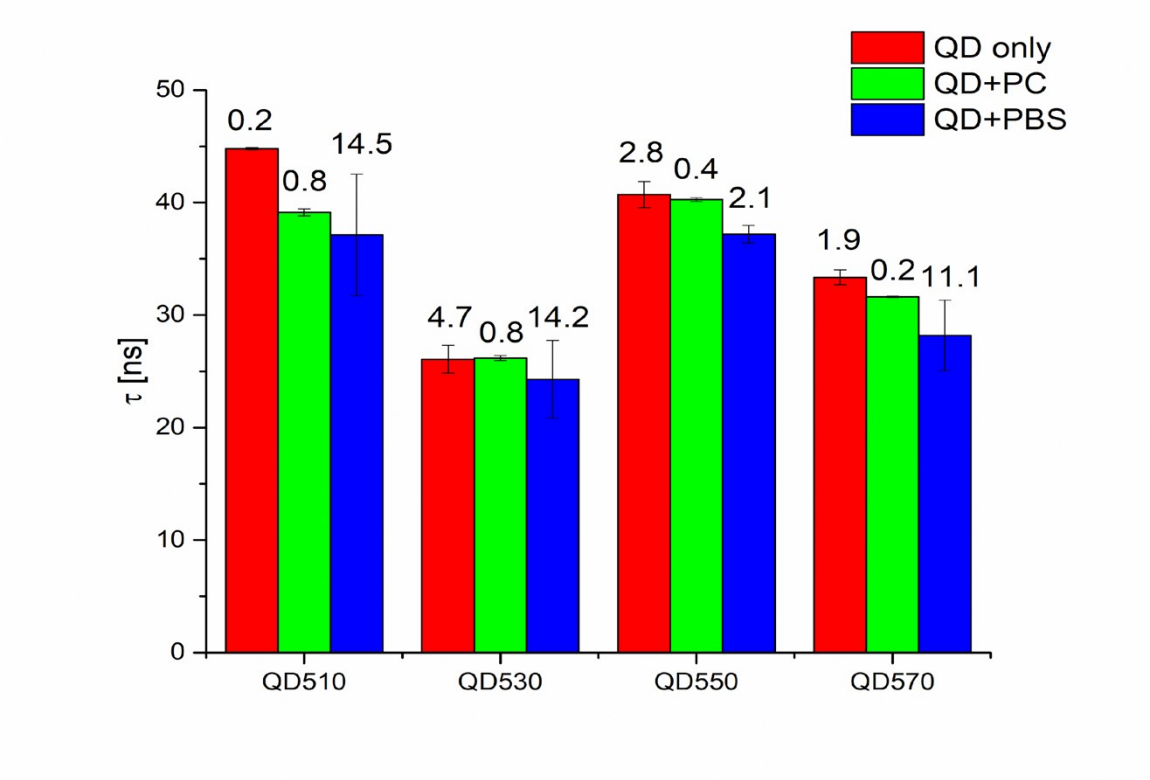


Figure S7. Comparison of average lifetime (amplitude averaged) of QDs in presence of PC/PBS, without CysHCl added. PC/PBS concentration was 0.5 µM, QD concentration was corresponding to plateau at Fig. 2. Error bars represent SD of three independent repetitions. Labels are the error percentage.

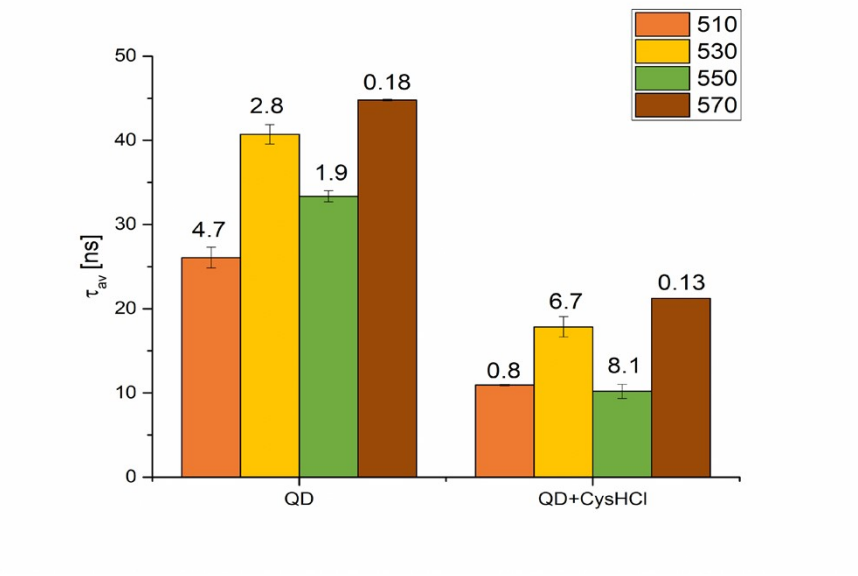


Figure S8. Effect of CysHCl addition (2 mM) on QDs average lifetime. Measurement done for 0.5 µM QD in 25 mM Hepes/NaOH pH 6.5. Excitation was 471 nm and emission was QD specific. Error bars represent SD of three independent repetitions. Labels are the error percentage.

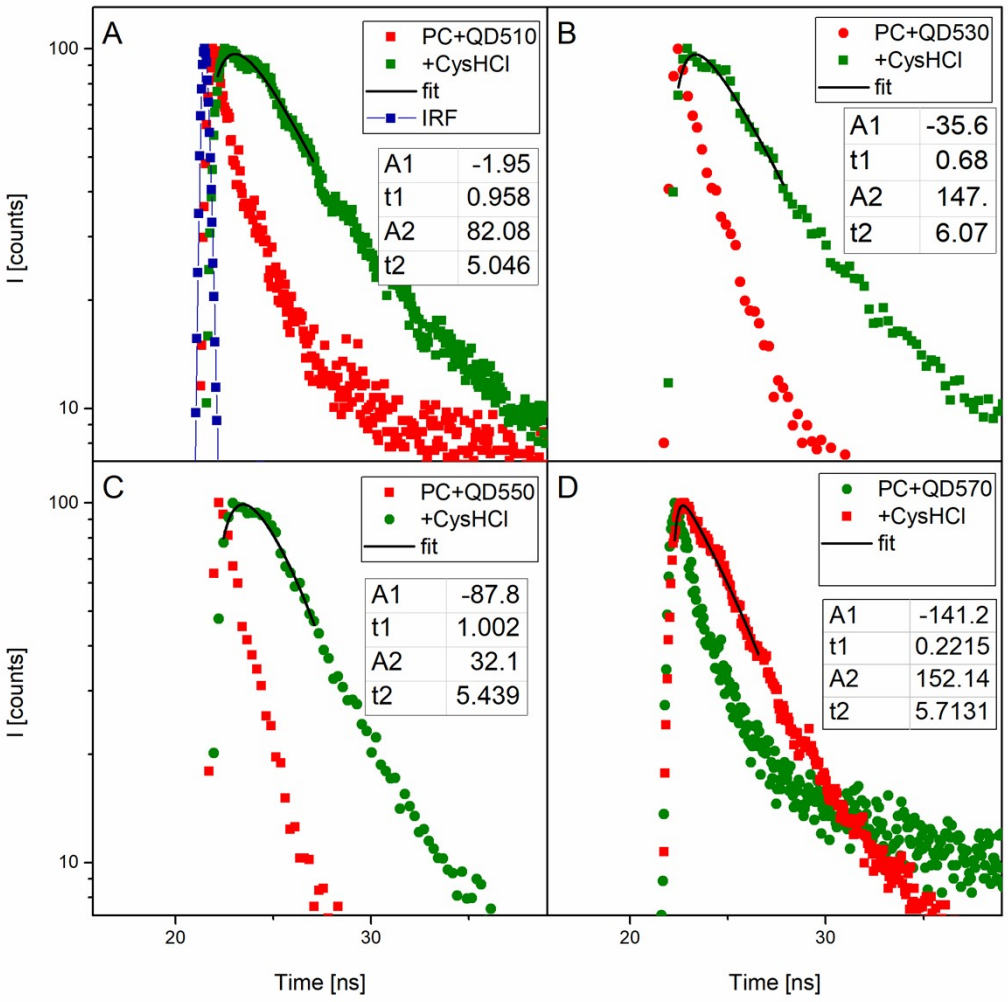


Figure S9. Fluorescence decay traces, recorded at 661 nm (excitation at 471 nm) for a mixture of QD-PC only and after addition of CysHCl. IRF, shown in panel (a), is also characteristics for other cases. Experimental details as for Fig. 3. Insets represent parameters of fit (black solid lines) with negative amplitude allowed. A1, A2 - amplitudes, t1, t2 - their corresponding lifetime constants in nanoseconds.

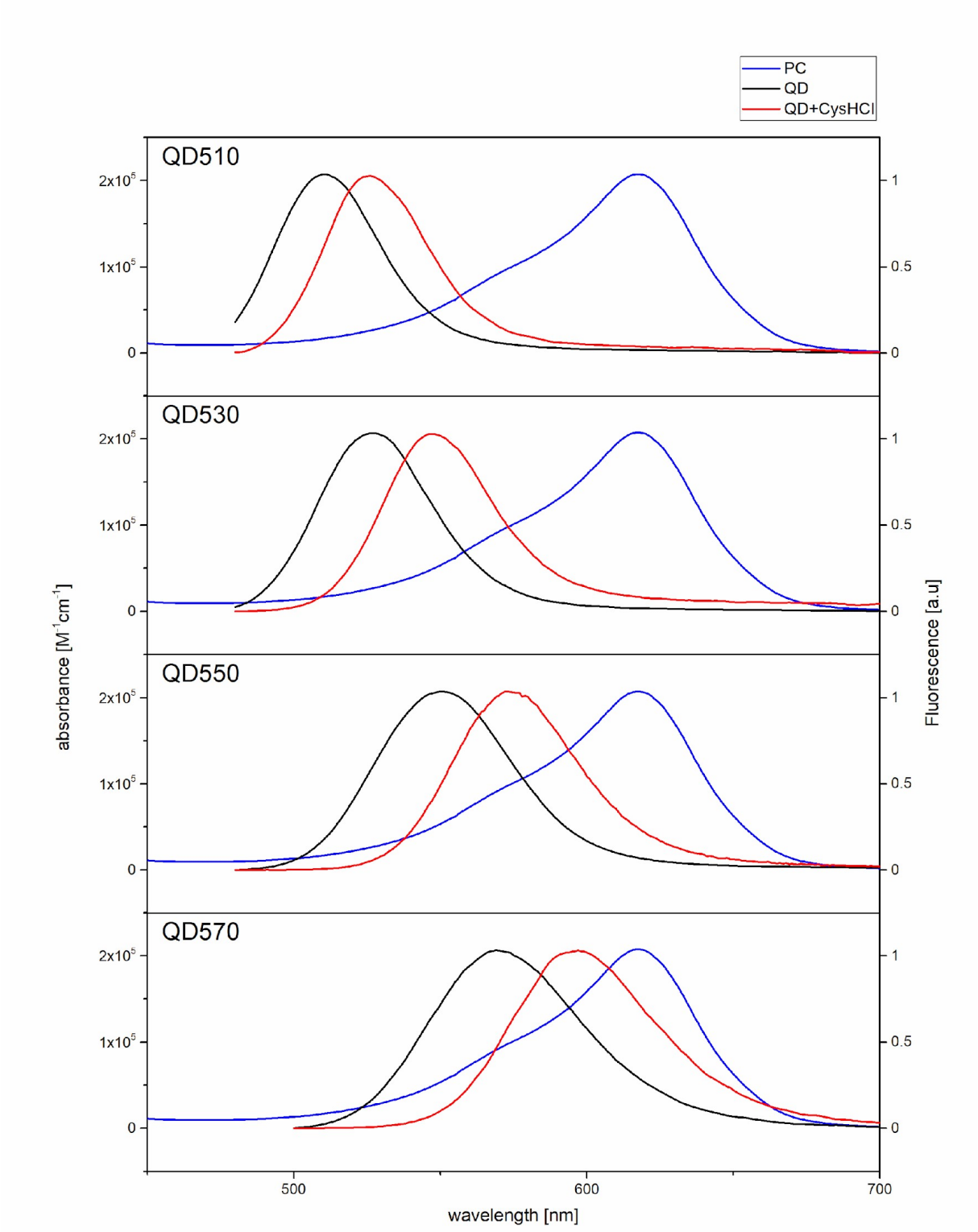


Figure S10. The set of spectra used for calculation of overlap integrals: absorption spectrum of PC (PBS is identical) in molar extinction units and emission spectra (normalized) of QDs or QDs+CysHCl. Type of QD indicated in the figure.

Table S1. Overlap integrals (J)[1] and Forster radius (R_0) for all FRET pairs analyzed in experiments

FRET donor	PC/PBS[2], no CysHCl		PC/PBS+CysHCl		QD HomoFRET (CysHCl present)	
	J [nm^4 M^{-1} cm^{-1}]	R_0 [nm]	J [nm^4 M^{-1} cm^{-1}]	R0 [nm]	J [nm^4 M^{-1} cm^{-1}]	R0 [nm]
QD510	3.73e+15	4.4	1.68e+15	3.9	1.26E+14	2.0
QD530	5.94e+15	5.9	1.40e+15	4.7	3.91E+14	2.7
QD550	1.19e+16	6.7	8.35e+15	6.3	7.68E+14	3.7
QD570	1.62e+16	6.6	7.65E+15	5.8	1.56E+15	3.7

[1]calculated with a|e software (fluortools.com) using experimentally obtained absorption and emission spectra

[2]absorption spectra of PC and PBS are identical

Table S2. Comparison of theoretical estimation (T) and experimental value (I_{max}) of maximum emission intensity for PC or PBS in presence of various QDs.

	ε_{471}[1] [M^{-1} cm^{-1}]	ϕ[2]	n[3] (PC)	T[4] ($\varepsilon_{471}\phi$Jn)	T_{st}[5]	I_{max}[6]	I_{st}[7]
PC							
QD510	23319.9	0.04	20	3.13E+19	1.00	1.75E+05	1.00
QD530	44967.3	0.08	4	2.01E+19	0.64	2.50E+05	1.43
QD550	53470.1	0.25	1.5	1.67E+20	5.34	2.50E+05	1.43
QD570	77618.1	0.14	1.5	1.25E+20	3.98	4.50E+05	2.57
PBS							
QD510	23319.9	0.04	15	2.35E+19	1.00	4.50E+04	1.00
QD530	44967.3	0.08	3	1.51E+19	0.64	6.00E+04	1.33
QD550	53470.1	0.25	1.25	1.40E+20	5.94	6.00E+04	1.33
QD570	77618.1	0.14	1	8.31E+19	3.54	1.00E+05	2.22

[1] Value calculated from experimental spectra of QDs.

[2] QD quantum yield in presence of CysHCl (determined experimentally, this manuscript).

[3]Amount of donors in plateaus (maximum acceptor emission), experimental value for 1 µM PC (compare Fig. 2).

[4] Arbitrary chosen ratio, representing intuition of factors, influencing final fluorescence intensity. T is defined as multiplication of ε_{471}, ϕ, J and n. J was taken from table S1.

[5] The T value normalized by T for QD510. Normalization was done for easier comparison with I_{max}.

[6] Maximum acceptor emission intensity, taken from experimental data for 1 µM PC (compare Fig. 2).

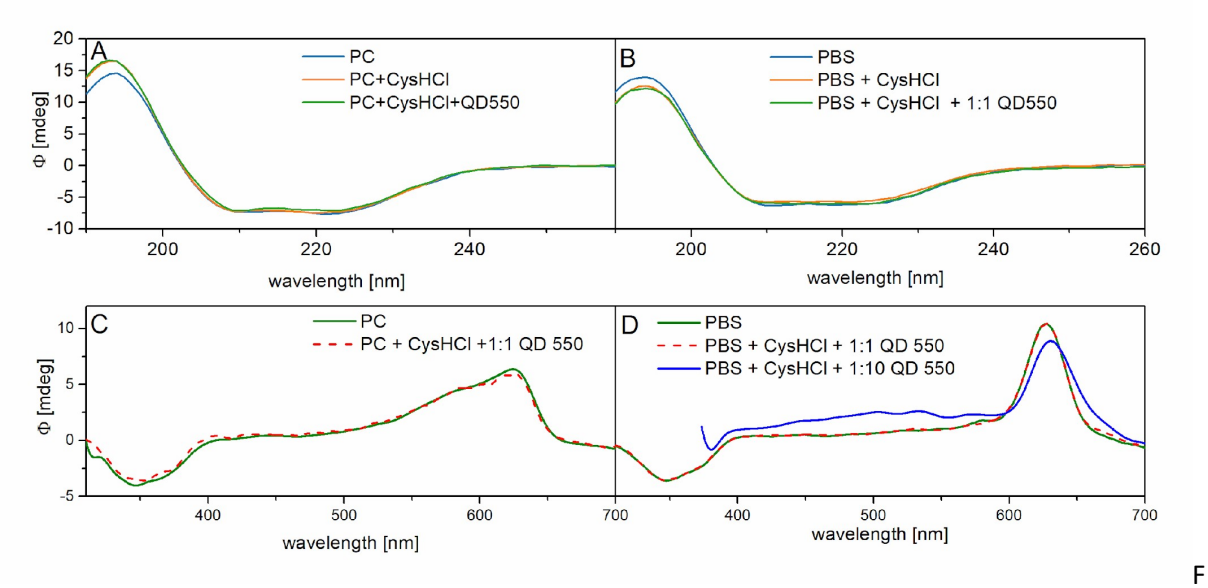
[7] I_{max} standardized to values for QD510

No structural change is induced in PC/PBS by assembly with QDs

In order to investigate possible changes in the secondary or tertiary structure of PC/PBS, we carried on circular dichroism (CD) measurement in the UV range (protein secondary structure) as well as in the visible range (signal originating from phycocyanobilin cofactor). Spectra were recorded using Jasco J700 apparatus, using 0.1 mm cuvette for UV and 10 mm cuvette for Vis range. It should be noted that QDs are not chiral and do not have a CD spectrum other than blank. Analysis of the composition of the secondary structure was performed with the CDPro, the CDSTRR algorithm and the SP56 protein database [3,4]. Proteins were examined as water solution without buffer added. Intensities were corrected for eventual dilution. Results are shown in Figure S10. UV spectra clearly show that both PC

and PBS secondary structure is basically the same and consist mostly (77%) of helices. It is consistent with the known crystal structures of these proteins. The addition of CysHCl slightly modifies the intensity of a band at 193 nm, increasing it for PC or decreasing it for PBS. It results in increasing the helix percentage to 82% for PC, or decreasing to 71% for PBS. This might be simply the effect of pH change. The addition of QD causes only a slight variation in spectra. However, calculations indicate helix percentage increase for both PC and PBS, to 85% and 81%, respectively. It should be noted that normalized root means square deviation (NRSMD) for those fits were in the range 3-9%, which makes calculated differences not significant statistically. To sum up, even if a slight variation in the structure might be caused by QD addition, it is actually a stabilization of the secondary structure, rather than the destruction.

The near Uv-Vis range CD spectra of PC/PBS consist of two main bands: negative at around 350 nm and a positive one, at about 620 nm. These bands correspond to respective absorption regions of PC/PBS. The positive band of PC also contains an additional arm at about 590 nm, which is lacking at PBS spectrum. This might be a signature of weaker coupling between phycocyanobilin cofactors. The addition of CysHCl and QDs did not cause any alteration in mentioned regions, again showing that CysHCl and QD presence is not affecting an orientation of cofactors. We also tested higher than 1:1 molar ratio between PC and QDs. However, due to increasing absorption of added QDs (manifesting as high voltage, HT, exceeding recommended levels), the resulting spectra become less reliable. The example is shown as the blue line in Fig. 10S D - there is a slight red shift and broadening of the 620 nm band, as well as the appearance of broad positive band (or bands) from 450 nm to 600 nm. It corresponds somehow to QDs absorption, and may suggest some coupling, leading to QDs polarization. However, due to the mentioned high HT, this may be treated only as indication, not a final proof.



F

igure S11. Circular dichroism spectra recorded for 5 µM water solution of PC (A, C) or PBS (B, D), alone or in presence of CysHCl (2 mM) and QD550 (5µM), in two spectral ranges: UV (A, B), using 0.1 mm optical path length and near Uv-visible (C, D), with 10 mm optical path length. For the high PC:QD ratio (D, blue line) measurement below 400 nm was impossible due to high voltage, resulting from high absorbance. All spectra corrected for a blank measurement.

Supplementary literature

1. Cohen, E.; Gdor, I.; Romero, E.; Yochelis, S.; van Grondelle, R.; Paltiel, Y. Achieving exciton delocalization in quantum dot aggregates using organic linker molecules. *The journal of physical chemistry letters* **2017**, *8*, 1014-1018.
2. Kosourov, S.; Murukesan, G.; Jokela, J.; Allahverdiyeva, Y. Carotenoid biosynthesis in Calothrix sp. 336/3: composition of carotenoids on full medium, during diazotrophic growth and after long-term H2 photoproduction. *Plant and Cell Physiology* **2016**, *57*, 2269-2282.
3. Sreerama, N.; Woody, R.W. On the analysis of membrane protein circular dichroism spectra. *Protein Sci.* **2004**, *13*, 100-112.
4. Sreerama, N.; Woody, R.W. Estimation of protein secondary structure from circular dichroism spectra: comparison of CONTIN, SELCON, and CDSSTR methods with an expanded reference set. *Anal. Biochem.* **2000**, *287*, 252-260.