

Protein/Protein and Quantum Dot/Protein Organization in Sequential Monolayer Materials Studied Using Resonance Energy Transfer

Jakub Sławski ¹, Katarzyna Walczewska-Szewc ² and Joanna Grzyb ^{1,*}

¹ Department of Biophysics, Faculty of Biotechnology, University of Wrocław, ul. F. Joliot-Curie 14a,

50-383 Wrocław, Poland; jakub.slawski@uwr.edu.pl

² Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University in Toruń, ul. Grudziądzka 5, 87-100 Toruń, Poland; kszewc@fizyka.umk.pl

* Correspondence: joanna.grzyb@uwr.edu.pl

Table S1. FRET-related characteristics of possible donor-acceptor pairs, used in the study. Overlap integral calculated from experimental spectra, using Fluortool software (www.fluortools.com).

| | Overlap integral | φ^{QDOT} | φ^{FP} | R0 [nm] |
|------------------|---|-------------------------|-----------------------|---------|
| QD510-mBanana | $J = 2.452\text{e}+14 \text{ nm}^4/(\text{M}^*\text{cm})$ | 0.16 | 0.7 | 2.81 |
| QD550-mCherry | $J = 3.858\text{e}+15 \text{ nm}^4/(\text{M}^*\text{cm})$ | 0.61 | 0.22 | 5.56 |
| QD510 homofret | $J = 4.705\text{e}+14 \text{ nm}^4/(\text{M}^*\text{cm})$ | 0.16 | - | 3.13 |
| Qd550 homofret | $J = 2.455\text{e}+15 \text{ nm}^4/(\text{M}^*\text{cm})$ | 0.61 | - | 5.14 |
| mBanana homofret | $J = 1.293\text{e}+14 \text{ nm}^4/(\text{M}^*\text{cm})$ | - | 0.7 | 3.23 |
| mCherry homofret | $J = 1.907\text{e}+15 \text{ nm}^4/(\text{M}^*\text{cm})$ | - | 0.22 | 4.17 |
| QD570- mCherry | $J = 4.868\text{e}+15 \text{ nm}^4/(\text{M}^*\text{cm})$ | 0.414 | | 5.41 |
| QD550- mCherry | $J = 3.858\text{e}+15 \text{ nm}^4/(\text{M}^*\text{cm})$ | 0.61 | | 5.56 |
| QD530- mCherry | $J = 2.261\text{e}+15 \text{ nm}^4/(\text{M}^*\text{cm})$ | 0.59 | | 5.06 |
| QD530- mBanana | $J = 2.801\text{e}+14 \text{ nm}^4/(\text{M}^*\text{cm})$ | 0.59 | | 3.57 |
| QD510- mCherry | $J = 1.537\text{e}+15 \text{ nm}^4/(\text{M}^*\text{cm})$ | 0.16 | | 3.81 |
| QD530 homofret | $J = 1.24\text{E}+15 \text{ nm}^4/(\text{M}^*\text{cm})$ | 0.59 | | 4.58 |
| QD570 homofret | $J = 3.76\text{E}+15 \text{ nm}^4/(\text{M}^*\text{cm})$ | 0.41 | | 5.18 |

Table S2. Components of average fluorescence lifetime values, shown in Figure 3. A1 and A2 represent amplitudes, while t1 and t2 - particular lifetime values [ns]. tAV is an amplitude-weighted average. SD - standard deviation of at least three repetitions. Values are grouped by the scheme of Figure 3, emission wavelength is marked in the headings (respectively - 503 nm, 551 nm and 612 nm).

| no spacer | wtGFP em503 | | | | | mBanana em551 | | | | | mCherry em612 | | | | |
|------------|----------------------------|---------|------|------|------|--------------------------------|---------|------|------|------|--------------------------------|---------|------|------|------|
| | GFP only (first monolayer) | | | | | mBanana only (first monolayer) | | | | | mCherry only (first monolayer) | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 268.66 | 3341.59 | 0.41 | 2.99 | 2.80 | 271.83 | 3344.90 | 0.47 | 3.66 | 3.42 | 239.70 | 3827.00 | 0.53 | 1.62 | 1.56 |
| SD | 25.91 | 43.21 | 0.06 | 0.00 | 0.01 | 14.28 | 34.77 | 0.05 | 0.02 | 0.03 | 20.87 | 138.31 | 0.04 | 0.02 | 0.03 |
| | mBanana + GFP | | | | | GFP + mBanana | | | | | GFP + mCherry | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 437.31 | 3458.90 | 0.47 | 2.92 | 2.65 | 202.79 | 3435.75 | 0.47 | 3.76 | 3.64 | 3473.34 | 514.82 | 2.15 | 3.20 | 2.21 |
| SD | 84.74 | 86.25 | 0.05 | 0.03 | 0.06 | 16.08 | 56.03 | 0.07 | 0.07 | 0.06 | 902.90 | 696.67 | 0.06 | 1.05 | 0.05 |
| | mCherry + GFP | | | | | mCherry + mBanana | | | | | mBanana + mCherry | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 613.58 | 3338.34 | 0.70 | 2.84 | 2.51 | 654.09 | 3240.40 | 0.59 | 3.44 | 2.97 | 906.35 | 3089.83 | 0.65 | 1.50 | 1.27 |
| SD | 80.05 | 30.98 | 0.10 | 0.01 | 0.05 | 81.60 | 38.57 | 0.13 | 0.02 | 0.05 | 370.79 | 225.71 | 0.09 | 0.31 | 0.20 |
| BSA spacer | wtGFP em503 | | | | | mBanana em551 | | | | | mCherry em612 | | | | |
| | GFP only (first monolayer) | | | | | mBanana only (first monolayer) | | | | | mCherry only (first monolayer) | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 188.81 | 3462.63 | 0.46 | 2.97 | 2.84 | 265.69 | 3344.33 | 0.50 | 3.66 | 3.43 | 123.30 | 3618.39 | 0.47 | 1.55 | 1.53 |
| SD | 53.26 | 57.45 | 0.08 | 0.01 | 0.04 | 8.69 | 64.58 | 0.16 | 0.01 | 0.01 | 76.37 | 40.90 | 0.06 | 0.02 | 0.00 |
| | mBanana + GFP | | | | | GFP + mBanana | | | | | GFP + mCherry | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 382.58 | 3365.49 | 0.50 | 2.95 | 2.70 | 190.11 | 3358.59 | 0.40 | 3.86 | 3.68 | 3389.03 | 355.12 | 2.25 | 5.40 | 2.28 |
| SD | 18.13 | 20.71 | 0.09 | 0.00 | 0.01 | 33.24 | 49.92 | 0.05 | 0.03 | 0.05 | 324.44 | 460.15 | 0.11 | 2.50 | 0.11 |
| | mCherry + GFP | | | | | mCherry + mBanana | | | | | mBanana + mCherry | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |

| | | | | | | | | | | | | | | | |
|-------------------|----------------------------|---------|------|------|------|--------------------------------|---------|------|------|------|--------------------------------|---------|------|------|------|
| mean | 678.67 | 3310.15 | 0.63 | 2.82 | 2.46 | 637.10 | 3236.11 | 0.54 | 3.46 | 2.98 | 1143.38 | 2733.27 | 0.57 | 1.39 | 1.20 |
| SD | 219.34 | 74.96 | 0.13 | 0.03 | 0.11 | 74.51 | 58.57 | 0.06 | 0.01 | 0.06 | 1151.45 | 836.71 | 0.17 | 0.15 | 0.05 |
| Jeff amine spacer | wtGFP em503 | | | | | mBanana em551 | | | | | mCherry em612 | | | | |
| | GFP only (first monolayer) | | | | | mBanana only (first monolayer) | | | | | mCherry only (first monolayer) | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 161.30 | 3481.04 | 0.50 | 2.96 | 2.88 | 260.09 | 3324.30 | 0.40 | 3.65 | 3.41 | 2092.38 | 1997.45 | 1.51 | 1.54 | 1.52 |
| SD | 42.42 | 36.76 | 0.02 | 0.05 | 0.02 | 6.71 | 60.30 | 0.05 | 0.01 | 0.01 | 66.46 | 54.65 | 0.06 | 0.01 | 0.03 |
| | mBanana + GFP | | | | | GFP + mBanana | | | | | GFP + mCherry | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 799.21 | 3185.98 | 0.68 | 2.91 | 2.46 | 239.45 | 3390.97 | 0.55 | 3.35 | 3.23 | 162.38 | 3296.86 | 0.23 | 2.55 | 2.42 |
| SD | 107.61 | 55.11 | 0.07 | 0.01 | 0.04 | 0.69 | 62.37 | 0.09 | 0.15 | 0.14 | 15.83 | 66.51 | 0.03 | 0.05 | 0.15 |
| | mCherry + GFP | | | | | mCherry + mBanana | | | | | mBanana + mCherry | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 1019.86 | 3458.48 | 0.76 | 2.83 | 2.37 | ##### | 2641.91 | 0.53 | 3.47 | 2.44 | 1974.42 | 2041.07 | 0.69 | 2.51 | 1.37 |
| SD | 328.18 | 295.60 | 0.19 | 0.03 | 0.08 | 72.81 | 51.99 | 0.04 | 0.03 | 0.07 | 207.80 | 183.01 | 0.44 | 0.74 | 0.31 |

Table S3. Components of average fluorescence lifetime values, shown in Figure 4. A1 and A2 represent amplitudes, while t1 and t2 - particular lifetime values [ns]. tAV is an amplitude-weighted average. SD - standard deviation of at least three repetitions. Values are grouped by the scheme of Figure 4, emission wavelength is marked in the headings (respectively - 503 nm, 551 nm and 612 nm).

| no spacer | wtGFP em503 | | | | | mBanana em551 | | | | |
|----------------------------|-------------|---------|------|------|------|--------------------------------|----------|------|------|------|
| GFP only (first monolayer) | | | | | | mBanana only (first monolayer) | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 37216.56 | 7193.44 | 2.90 | 5.14 | 2.94 | 11836.32 | 30804.64 | 2.27 | 3.68 | 3.29 |
| SD | 20601.89 | 9078.57 | 0.05 | 3.10 | 0.09 | 1873.95 | 1594.57 | 0.13 | 0.05 | 0.04 |
| mBanana + GFP | | | | | | mCherry + mBanana | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 35331.36 | 5071.58 | 2.57 | 4.23 | 2.73 | 11632.34 | 32303.00 | 1.88 | 3.58 | 3.13 |
| SD | 2292.88 | 3661.03 | 0.07 | 0.81 | 0.02 | 817.60 | 1349.77 | 0.07 | 0.03 | 0.02 |
| mCherry + GFP | | | | | | | | | | |
| | A1 | A2 | t1 | t2 | tav | | | | | |
| mean | 55300.49 | 4365.31 | 2.47 | 4.49 | 2.61 | | | | | |
| SD | 1193.77 | 622.06 | 0.02 | 0.14 | 0.00 | | | | | |

| MBP spacer | wtGFP em503 | | | | | mBanana em551 | | | | |
|----------------------------|-------------|----------|------|------|------|--------------------------------|----------|------|------|------|
| GFP only (first monolayer) | | | | | | mBanana only (first monolayer) | | | | |
| | A1 | A2 | t1 | t2 | tav | | A2 | t1 | t2 | tav |
| mean | 29510.61 | 29032.00 | 2.74 | 2.74 | 2.74 | 8820.51 | 32574.17 | 1.94 | 3.58 | 3.24 |
| SD | 15423.73 | 15751.85 | 0.02 | 0.02 | 0.02 | 830.96 | 1162.78 | 0.06 | 0.02 | 0.02 |
| mBanana + GFP | | | | | | mCherry + mBanana | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 39121.66 | 1547.80 | 2.68 | 5.38 | 2.74 | 10945.03 | 33002.95 | 1.54 | 3.45 | 2.98 |
| SD | 2478.54 | 1589.57 | 0.07 | 0.77 | 0.03 | 1619.51 | 4475.65 | 0.05 | 0.03 | 0.03 |
| mCherry + GFP | | | | | | | | | | |
| | A1 | A2 | t1 | t2 | tav | | | | | |
| mean | 5707.88 | 31343.57 | 1.65 | 2.80 | 2.64 | | | | | |

| | | | | | | | | | | |
|-------------|----------------------------|----------|------|------|------|--------------------------------|----------|------|------|------|
| SD | 1045.21 | 2778.59 | 0.17 | 0.05 | 0.07 | | | | | |
| Jeff spacer | wtGFP em503 | | | | | mBanana em551 | | | | |
| | GFP only (first monolayer) | | | | | mBanana only (first monolayer) | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 24210.85 | 21589.36 | 2.78 | 2.78 | 2.76 | 13907.20 | 36109.60 | 2.10 | 3.62 | 3.19 |
| SD | 2019.27 | 2187.33 | 0.00 | 0.00 | 0.00 | 1675.09 | 7679.60 | 0.16 | 0.06 | 0.03 |
| | mBanana + GFP | | | | | mCherry + mBanana | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 37660.34 | 666.02 | 2.71 | 6.15 | 2.76 | 10885.46 | 33289.94 | 1.50 | 3.43 | 2.95 |
| SD | 410.35 | 227.89 | 0.02 | 0.77 | 0.01 | 1196.21 | 3412.05 | 0.05 | 0.02 | 0.03 |
| | mCherry + GFP | | | | | | | | | |
| | A1 | A2 | t1 | t2 | tav | | | | | |
| mean | 40227.98 | 196.71 | 2.67 | 9.11 | 2.69 | | | | | |
| SD | 2247.64 | 47.65 | 0.01 | 1.18 | 0.00 | | | | | |

Table S4. Components of average fluorescence lifetime values, shown in Figure5. A1, A2 and A3 represent amplitudes, while t1, t2 and t3 - particular lifetime values in [ns]. tAV is an amplitude-weighted average. SD - standard deviation of at least three repetitions. Values are grouped by the scheme of Figure 5.

| | QD510 | | | | | | | QD530 | | | | | | |
|------|-----------------|---------|---------|------|------|-------|-------|-----------------|---------|---------|------|-------|-------|-------|
| | QD510 monolayer | | | | | | | Qd530 monolayer | | | | | | |
| | A1 | A2 | A3 | t1 | t2 | t3 | tav | A1 | A2 | A3 | t1 | t2 | t3 | tav |
| mean | 961.47 | 2155.15 | 1997.06 | 0.94 | 5.12 | 20.73 | 10.43 | 1250.65 | 2656.90 | 1470.52 | 4.48 | 18.13 | 44.27 | 22.08 |
| SD | 70.95 | 45.90 | 76.63 | 0.23 | 0.43 | 0.67 | 0.54 | 242.24 | 63.03 | 243.86 | 0.38 | 1.33 | 2.85 | 1.29 |
| | mBanana + QD510 | | | | | | | mBanana + QD530 | | | | | | |
| | A1 | A2 | A3 | t1 | t2 | t3 | tav | A1 | A2 | A3 | t1 | t2 | t3 | tav |
| mean | 1519.84 | 2499.94 | 1505.92 | 0.87 | 4.45 | 17.59 | 7.05 | 1362.97 | 2434.76 | 1171.83 | 4.23 | 15.28 | 39.99 | 18.07 |
| SD | 150.90 | 85.36 | 74.73 | 0.19 | 0.34 | 0.49 | 0.18 | 50.55 | 47.22 | 43.76 | 0.15 | 0.42 | 1.25 | 0.34 |

| | QD550 | | | | | | | QD570 | | | | | | |
|------|-----------------|---------|---------|------|-------|-------|-------|-----------------|---------|---------|------|-------|--------|-------|
| | QD550 monolayer | | | | | | | Qd570 monolayer | | | | | | |
| | A1 | A2 | A3 | t1 | t2 | t3 | tav | A1 | A2 | A3 | t1 | t2 | t3 | tav |
| mean | 674.65 | 2801.67 | 1231.23 | 6.65 | 25.13 | 58.07 | 30.52 | 680.33 | 3004.00 | 947.23 | 8.27 | 30.08 | 120.32 | 34.52 |
| SD | 140.48 | 367.29 | 566.26 | 1.26 | 3.29 | 9.68 | 0.68 | 152.03 | 311.50 | 483.06 | 1.14 | 4.15 | 95.68 | 0.33 |
| | mCherry + QD550 | | | | | | | mCherry + QD570 | | | | | | |
| | A1 | A2 | A3 | t1 | t2 | t3 | tav | A1 | A2 | A3 | t1 | t2 | t3 | tav |
| mean | 2339.55 | 1641.17 | 2170.00 | 2.28 | 4.97 | 31.85 | 13.37 | 5282.25 | 1454.14 | 2063.55 | 1.21 | 6.97 | 35.23 | 10.78 |
| SD | 1315.20 | 497.87 | 314.83 | 2.21 | 3.86 | 1.54 | 3.72 | 2604.77 | 183.71 | 284.16 | 0.13 | 1.59 | 1.42 | 3.65 |

Table S5. Components of average fluorescence lifetime values, shown in Figure 8. A1, A2 and A3 represent amplitudes, while t1, t2 and t3 - particular lifetime values [ns]. tAV is an amplitude-weighted average. SD - standard deviation of at least three repetitions. Values are grouped by the scheme of Figure8.

mBanana em551

| | mBanana monolayer | | | | | QD510 + mBanana | | | | | mBanana + QD510 | | | | |
|------|-----------------------|---------|------|------|------|-----------------------|---------|------|------|------|-----------------|---------|------|------|------|
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 531.51 | 4156.74 | 0.48 | 3.61 | 3.26 | 3042.31 | 1860.44 | 2.41 | 7.34 | 3.88 | 913.68 | 4302.89 | 0.55 | 3.92 | 3.33 |
| SD | 54.64 | 61.34 | 0.05 | 0.03 | 0.02 | 1956.95 | 2161.58 | 1.67 | 3.01 | 0.36 | 193.52 | 152.02 | 0.05 | 0.06 | 0.13 |
| | QD510 + mBanana + PLL | | | | | mBanana + QD510 + PLL | | | | | | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 425.43 | 4238.95 | 0.47 | 3.67 | 3.38 | 693.19 | 4287.84 | 0.59 | 3.58 | 3.16 | | | | | |
| SD | 98.89 | 176.02 | 0.07 | 0.03 | 0.06 | 149.16 | 27.75 | 0.14 | 0.04 | 0.13 | | | | | |

| | QD530 + mBanana | | | | | mBanana + QD530 | | | | |
|------|-----------------------|---------|------|-------|------|-----------------------|---------|------|-------|------|
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 4178.27 | 753.71 | 3.88 | 18.78 | 6.16 | 3598.63 | 1413.09 | 3.27 | 15.11 | 6.62 |
| SD | 64.93 | 78.18 | 0.11 | 0.52 | 0.38 | 420.06 | 88.12 | 0.14 | 1.19 | 0.49 |
| | QD530 + mBanana + PLL | | | | | mBanana + QD530 + PLL | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 748.51 | 4247.75 | 0.68 | 3.97 | 3.48 | 3348.94 | 1906.48 | 2.29 | 8.95 | 3.83 |
| SD | 48.62 | 25.18 | 0.11 | 0.07 | 0.10 | 1825.86 | 2172.91 | 1.48 | 4.51 | 0.68 |

mCherry em612

| mCherry only (first monolayer) | | | | | | QD510 + mCherry | | | | | mCherry + QD510 | | | | |
|--------------------------------|--------|---------|------|------|------|-----------------------|--------|------|-------|------|-----------------------|---------|------|-------|------|
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 758.79 | 4266.74 | 0.68 | 1.59 | 1.46 | 4277.55 | 560.39 | 1.79 | 5.86 | 2.20 | 4511.42 | 204.73 | 2.05 | 10.59 | 2.42 |
| SD | 203.95 | 177.55 | 0.12 | 0.03 | 0.01 | 217.14 | 231.94 | 0.21 | 2.00 | 0.20 | 38.46 | 17.45 | 0.23 | 3.02 | 0.34 |
| | | | | | | QD510 + mCherry + PLL | | | | | mCherry + QD510 + PLL | | | | |
| | | | | | | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| | | | | | mean | 4073.40 | 990.46 | 1.32 | 2.86 | 1.61 | 3531.85 | 1401.19 | 1.41 | 4.57 | 1.81 |
| | | | | | SD | 125.26 | 291.18 | 0.03 | 0.09 | 0.05 | 1691.40 | 1939.73 | 0.45 | 2.37 | 0.19 |
| | | | | | | QD530 + mCherry | | | | | mCherry + QD530 | | | | |
| | | | | | | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| | | | | | mean | 4432.67 | 178.25 | 2.32 | 16.16 | 2.80 | 4525.63 | 347.17 | 2.67 | 71.42 | 2.58 |
| | | | | | SD | 73.93 | 68.06 | 0.10 | 5.79 | 0.17 | 25.43 | 67.38 | 0.06 | 40.59 | 0.46 |
| | | | | | | QD530 + mCherry + PLL | | | | | mCherry + QD530 + PLL | | | | |
| | | | | | | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| | | | | | mean | 4352.66 | 401.26 | 1.69 | 4.89 | 1.95 | 4475.01 | 215.30 | 1.97 | 9.62 | 2.31 |
| | | | | | SD | 132.38 | 108.85 | 0.14 | 0.86 | 0.16 | 66.48 | 58.73 | 0.09 | 1.37 | 0.13 |

| QD550 + mCherry | | | | | | mCherry + QD550 | | | | |
|-----------------------|---------|---------|------|-------|------|-----------------------|--------|------|-------|------|
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 2953.94 | 2215.15 | 2.35 | 2.35 | 2.35 | 4659.71 | 240.32 | 2.28 | 43.83 | 4.74 |
| SD | 34.76 | 31.67 | 0.02 | 0.02 | 0.02 | 62.28 | 108.70 | 0.25 | 34.61 | 2.98 |
| QD550 + mCherry + PLL | | | | | | mCherry + QD550 + PLL | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 3984.09 | 809.76 | 2.02 | 20.77 | 2.25 | 4611.98 | 156.52 | 1.84 | 11.53 | 2.11 |
| SD | 879.50 | 1302.70 | 0.02 | 21.13 | 0.24 | 43.12 | 77.60 | 0.08 | 5.03 | 0.12 |

| QD570 + mCherry | | | | | | mCherry + QD570 | | | | |
|-----------------------|---------|---------|------|------|------|-----------------------|--------|------|-------|------|
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 2879.94 | 2297.95 | 2.57 | 2.57 | 2.57 | 4178.87 | 688.01 | 2.54 | 23.10 | 5.38 |
| SD | 51.07 | 64.75 | 0.06 | 0.06 | 0.06 | 220.72 | 236.23 | 0.01 | 2.04 | 0.73 |
| QD570 + mCherry + PLL | | | | | | mCherry + QD570 + PLL | | | | |
| | A1 | A2 | t1 | t2 | tav | A1 | A2 | t1 | t2 | tav |
| mean | 3576.68 | 1333.89 | 2.32 | 7.77 | 2.43 | 4405.32 | 436.25 | 2.17 | 13.77 | 2.91 |
| SD | 845.31 | 1121.03 | 0.18 | 9.26 | 0.01 | 161.31 | 74.68 | 0.30 | 4.18 | 0.28 |

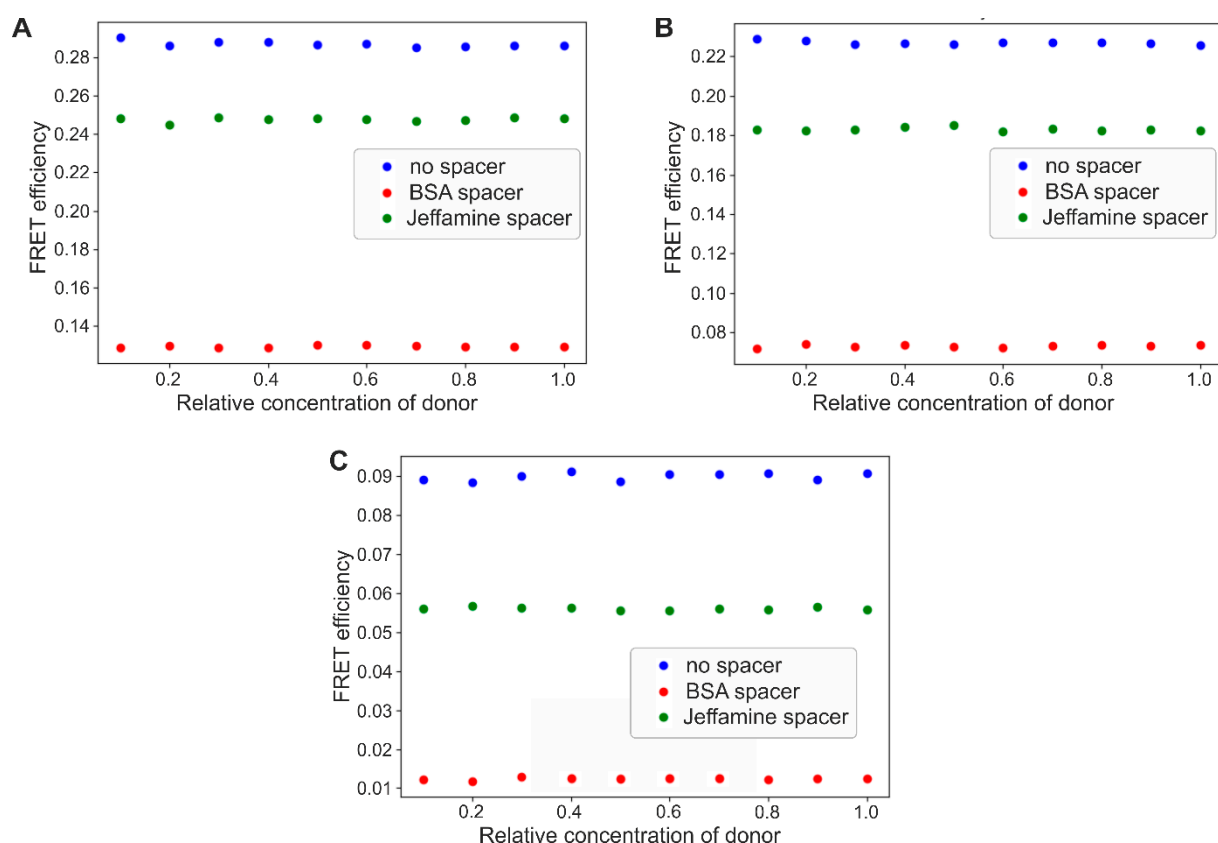


Figure S1. FRET efficiencies calculated for varied relative concentration of donor proteins. (A) a GFP as first layer, an mBanana as a second layer, (B) a GFP as first layer, an mCherry as a second layer, and (C) an mBanana as first layer, an mCherry as a second layer.

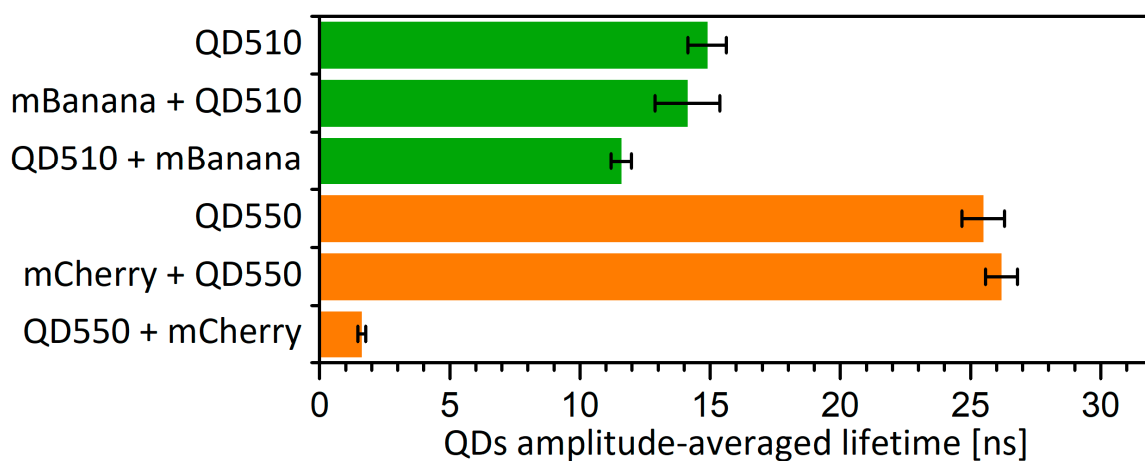


Figure S2. Fluorescence lifetimes of QD510 (green bars) and QD550 (yellow bars) immobilized onto PVDF membrane. The excitation was by 405 nm laser, emission signal was collected at 510 nm (for QD510) or 550 nm (for QD550). Fluorescence lifetime values are amplitude-weighted averages of three-exponential decays. Order in sample names (e.g. mBanana + QD510 or QD510 + mBanana) reflects the sequence of QDs and proteins immobilization onto PVDF.

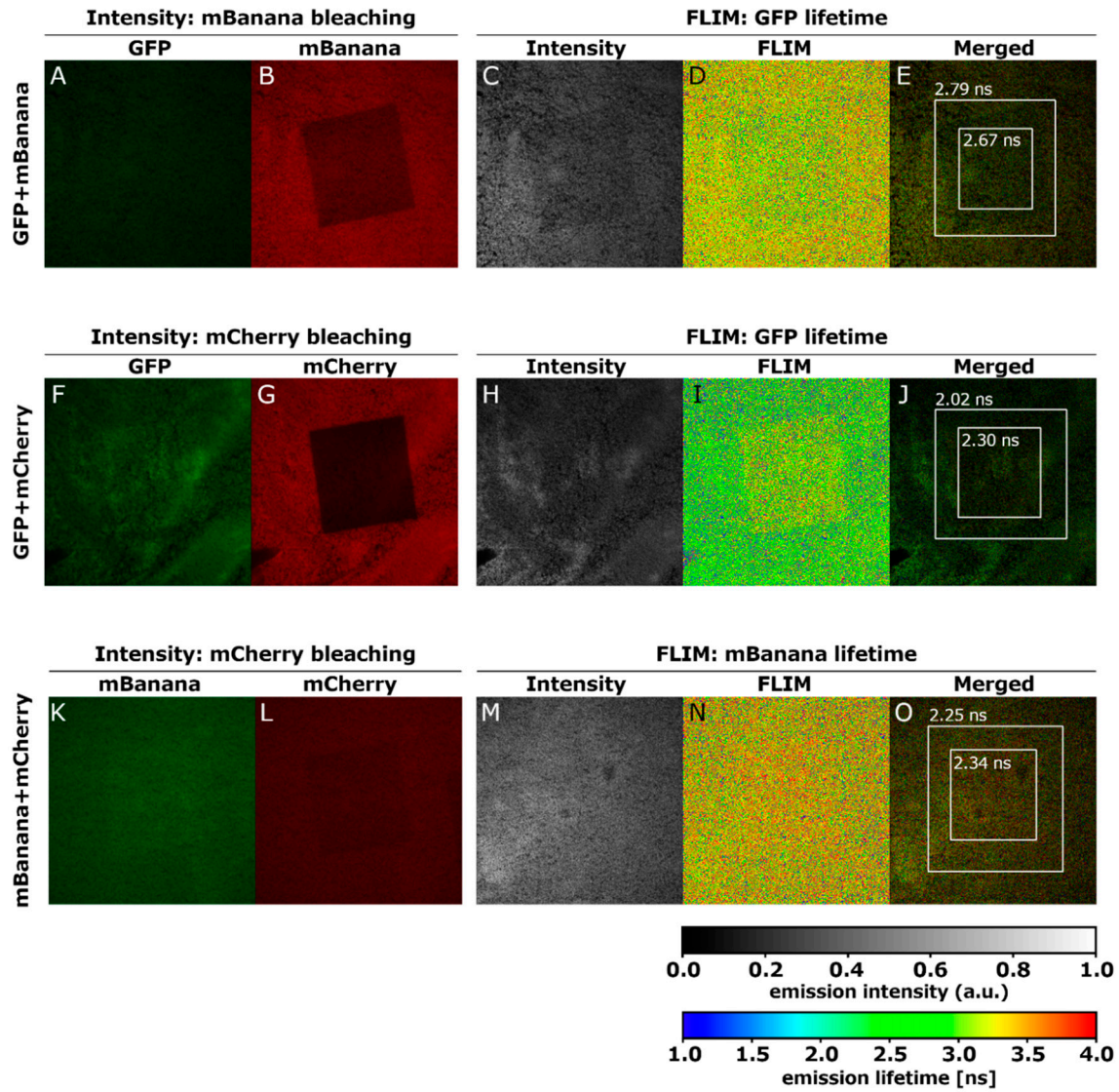


Figure S3. Representative images obtained during bleaching experiment. (A-E) GFP-mBanana samples, (F-J) GFP-mCherry samples, (K-O) mBanana-mCherry samples. For A-E, square fragment was bleached by 514 nm laser (100% intensity) and image was taken with 458 nm (20% laser intensity) and 514 nm (8% laser intensity) excitation wavelength. The emission of GFP (A, green channel, BP 470-500 nm) and mBanana (B, red channel, BP 530-600 nm) are shown. FLIM image was recorded for 470 nm excitation, fluorescence intensity channel (C), mean lifetime channel (D), and merged image (E) are shown. Squares indicated on E show ROIs used for lifetime calculation for bleached (inside smaller square) and unbleached (outside larger square) fragments. For F-J square fragment was bleached by 561 nm and 594 nm lasers (100% intensity) and image was taken with 488 nm (1,5% laser intensity) and 561 nm (1,2% laser intensity) excitation wavelength. The emission of GFP (F, green channel, BP 505-530 nm) and mCherry (G, red channel, LP 575 nm) are shown. FLIM image was recorded for 470 nm excitation, fluorescence intensity channel (H), mean lifetime channel (I), and merged image (J) are shown. For K-O square fragment was bleached by 594 nm laser (100% intensity) and image was taken with 488 nm (2,6% laser intensity) excitation wavelength. The emission of mBanana (K, green channel, BP 505-565 nm) and mCherry (L, red channel, LP 650 nm) are shown. FLIM image was recorded for 470 nm excitation, fluorescence intensity channel (M) mean lifetime channel (N), and merged image (O) are shown.

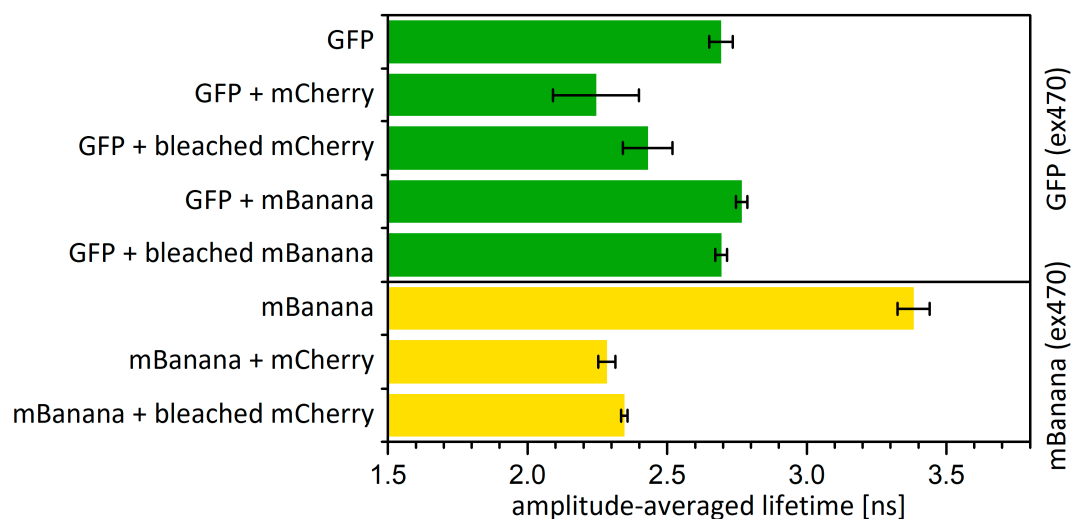


Figure S4. Mean lifetime values of donor proteins in bleaching experiment calculated from FLIM images. Samples on PVDF membrane were excited with 470 nm.

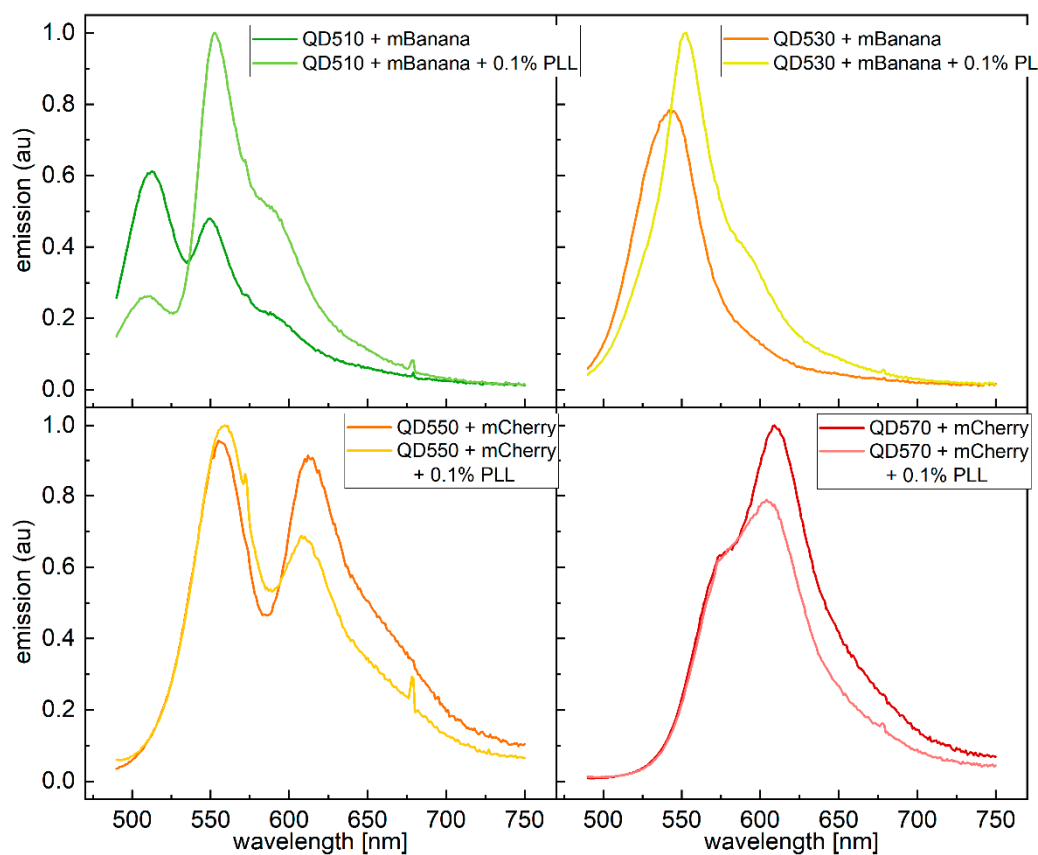


Figure S5. Emission spectra of QD+mBanana/mCherry samples before and after incubation with 0.1% PLL (excitation at 471 nm). Note two emission maxima, originating from emission of QD (550 nm or 570 nm, respectively) and an emission of a fluorescent protein (~555 nm for mBanana and ~610 nm for mCherry).

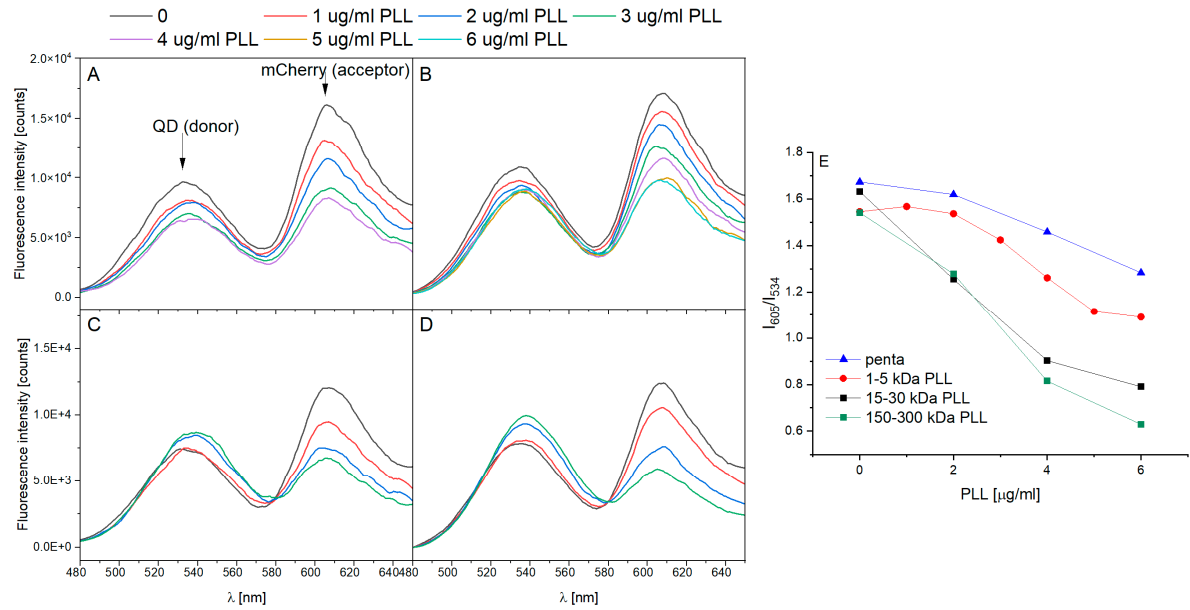


Figure S6. PLL effects on a QD530-mCherry FRET pair in solution changes with a PLL size. The fluorescence spectra of QD530 (100 nM) mixed with mCherry (300 nM), titrated with increasing concentration of (A) penta-Lys, (B) 1-5 kDa PLL, (C) 15-30 kDa PLL and (D) 150-300 kDa PLL. A titrant concentration given in the legend. (E) The actual change in the FRET yield was deciphered as the change in the ratio of mCherry emission intensity (an acceptor, 605 nm) and QD emission intensity (a donor, 534 nm).

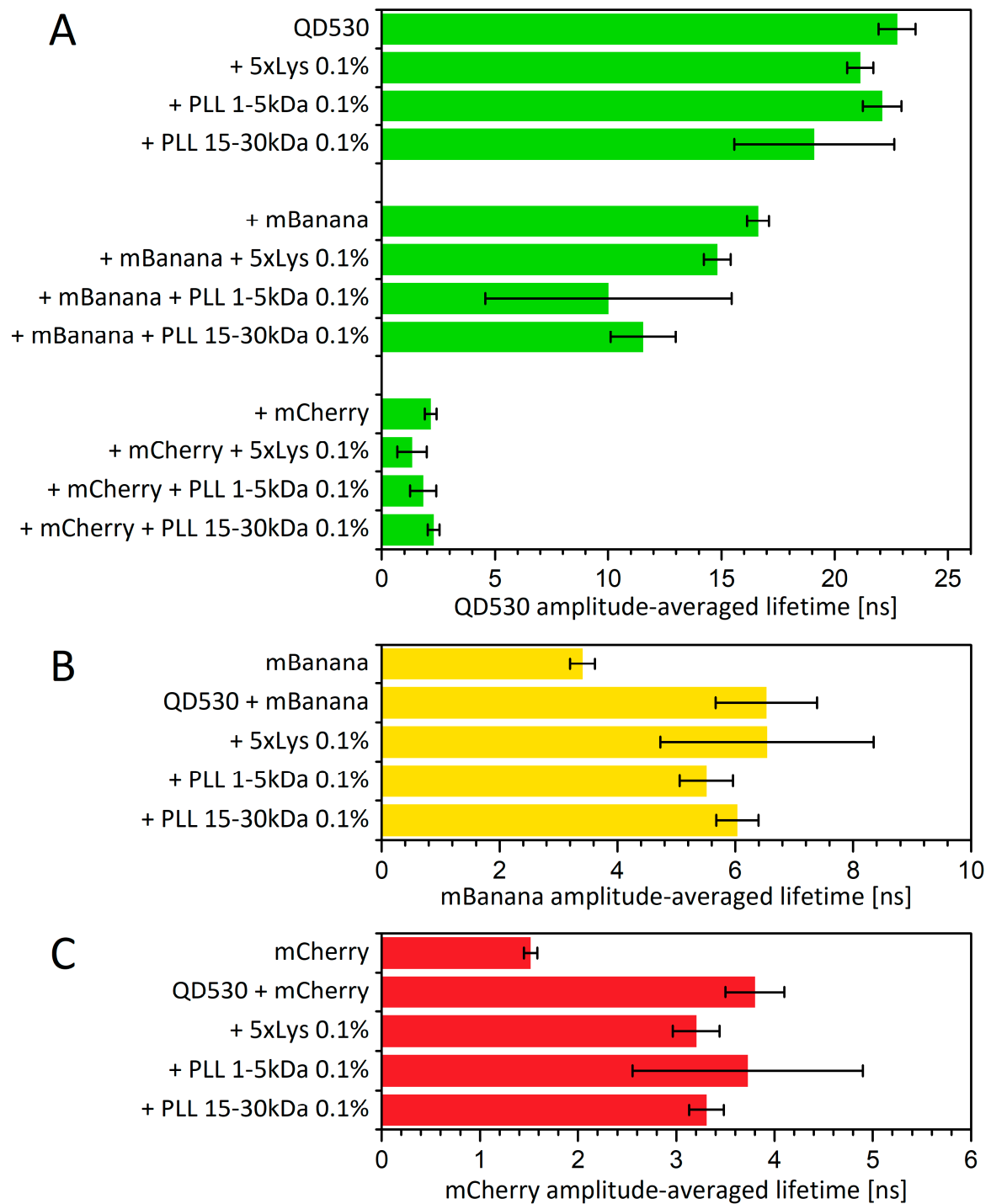


Figure S7. PLL affects FRET efficiency in QD530-FP monolayers, as evidenced by the change of fluorescence lifetime. (A) Fluorescent lifetime of QD530 (λ_{ex} 405 nm, λ_{em} 530 nm), with or without following with an mBanana or an mCherry monolayer, before and after application of smaller PLLs. (B) change in an mBanana fluorescence lifetime or (C) an mCherry fluorescence lifetime, recorded in parallel during the same treatment.

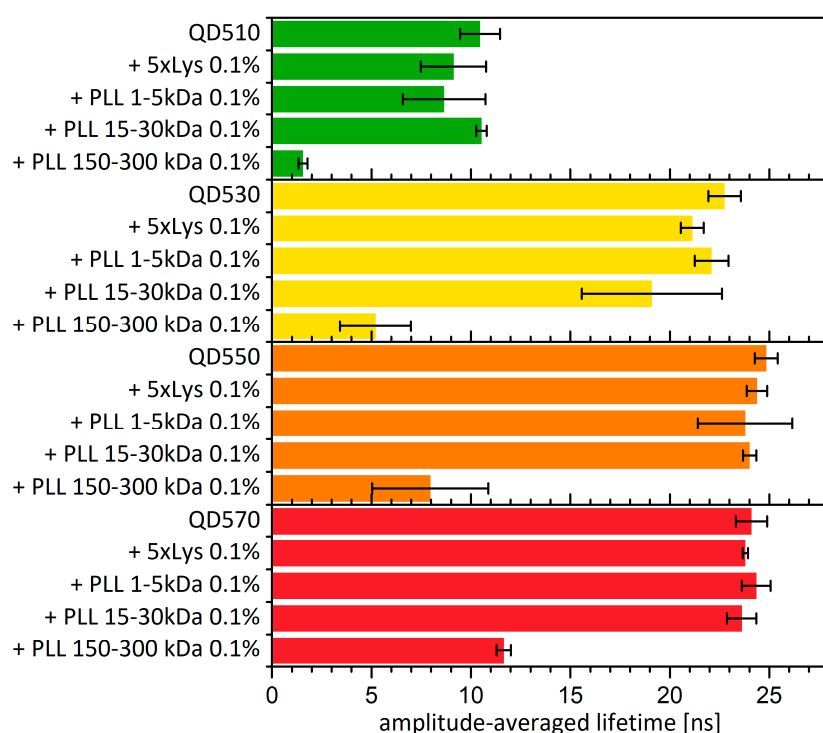


Figure S8. Fluorescence lifetime of QDs forming monolayer is shortened by externally applied PLLs. QDs, respectively QD510, QD530, QD550 and QD570, were measured in the form of monolayer without sequential FP monolayer. PLLs were applied as a drop of 0.1% solution. A type of PLL is given in the figure. Fluorescence was excited with 405 nm laser, emission collected at QDs specific wavelength.

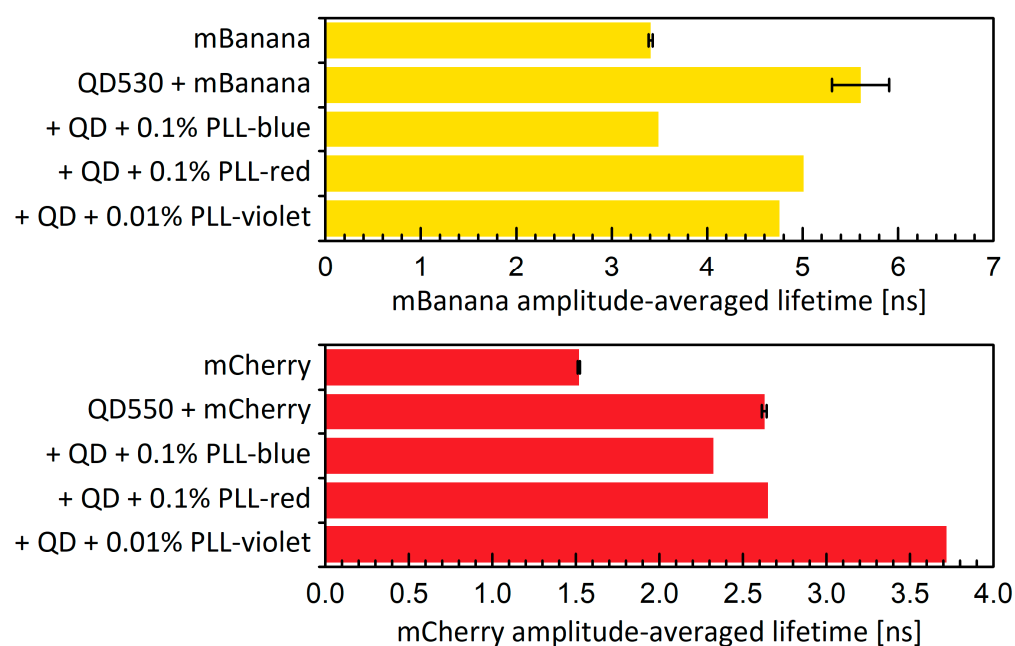


Figure S9. Fluorescence lifetime of FP in QD-FP sequential monolayers is restored by externally applied PLLs. Fluorescence lifetimes of proteins immobilized onto PVDF. Samples are an mBanana, an mCherry (without QDs) and QD530+mBanana, QD530+mCherry (alone and incubated with different PLLs). Excitation was by 471 nm laser, emission signal was collected at 551 nm (mBanana) or 612 nm (mCherry). Lifetime values are amplitude-weighted averages of two-exponential decays. Results are triplicates where error bars indicates that, samples incubated with PLL were measured only once.

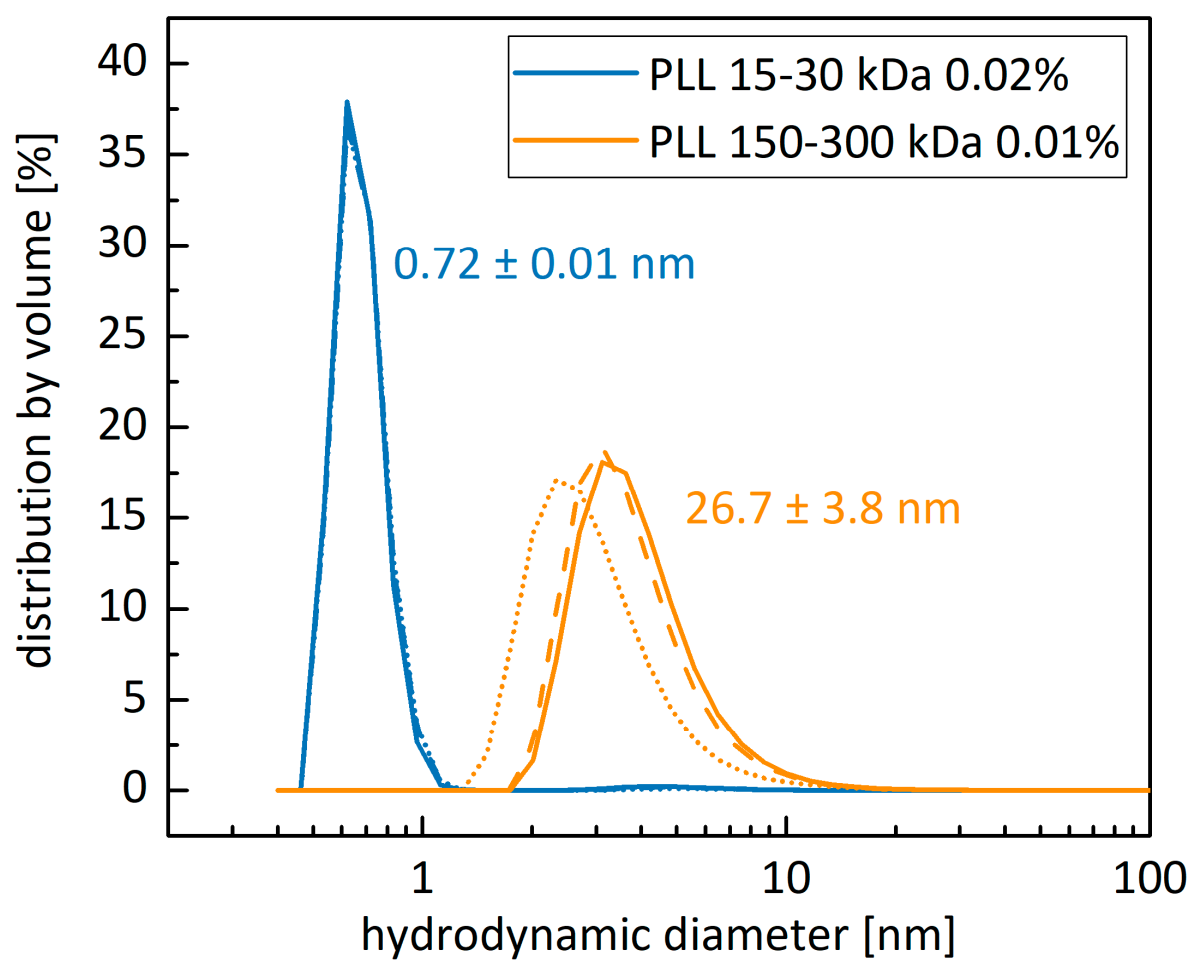


Figure S10. Volume-weighted distribution of hydrodynamic diameters of PLLs (each in two different % concentrations) used in this experiment obtained by DLS measurements. Different line styles (solid, dotted and dashed) represent replicated measurements of the same sample.