**Supporting Information**

# A DNA-Directed Light-Harvesting/Reaction Center System

Palash K. Dutta,[1,2] Symon Levenberg,[1,2] Andrey Loskutov,[2] Daniel Jun,[3] Rafael Saer,[3] J. Thomas Beatty,[3] Su Lin,[1,2] Yan Liu,[1,2] Neal W. Woodbury,*[,1,2] Hao Yan*[,1,2]

[1]Department of Chemistry and Biochemistry and [2]The Biodesign Institute
Arizona State University, Tempe, Arizona 85287, United States
[3]Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, V6T 1Z3, Canada

Methods and experimental details

Figures S1-S12

Tables S1-S3

Schemes S1-S4

References cited in the SI

## I. Reaction Center Protein Preparation

**1) Reaction center mutations:** Among a total of eight mutations in the RC, five of them serve to replace the five wild type cysteines with serine and alanine, and the remaining three mutations introduce cysteines on the P side of the RC, by replacing wild type amino acids (glutamic acid or asparagine) with Cys on the surface near P. The mutations are as follows: (H)C156A, (H)C234S, (M)E100C, (L)C92S, (L)C108S, (L)C247S, (L)E72C and (L)N274C. In addition, the engineered RC contains a six-histidine tag at the C-terminus of the H subunit, to facilitate purification with a Ni-sepharose affinity column.[1]

**2) RC isolation and purification:** RCs were isolated from *R. sphaeroides* 2.4.1[2] containing a pRK-based expression plasmid encoding the modified RC puf operon. 2 L of modified LB medium, containing 810 μM $MgCl_2$, 510 μM $CaCl_2$ and 4 mM NaCl, was used to grow cells at 30°C for 3.5 days. The cells were pelleted and resuspended in 50 mM phosphate buffer (pH 8) containing 150 mM NaCl. The cells were then lysed by passing through a French press, followed by addition of small amount of DNase. After removal of any unbroken cells and large cell debris via centrifugation (9000 g for 10 minutes), the remaining supernatant was treated with imidazole (final concentration 5 mM) and the RC protein was solubilized by adding N,N-Dimethyldodecylamine N-oxide (LDAO, final concentration 0.4% by volume). After 20 min incubation at 22°C, the solution was centrifuged at 14000g followed by Ni-sepharose column purification. The eluted RC was dialyzed overnight at 4°C against dialysis buffer (15 mM Tris, 0.025% LDAO, 150 mM NaCl, 1 mM EDTA, pH 8) using 50 kD molecular weight cutoff membrane (Amicon), to remove imidazole and excess LDAO. The concentration of the purified RC was measured using absorbance at 804 nm (ε ~288000 $M^{-1}cm^{-1}$).[3]

## II. RC-DNA Conjugation and Purification

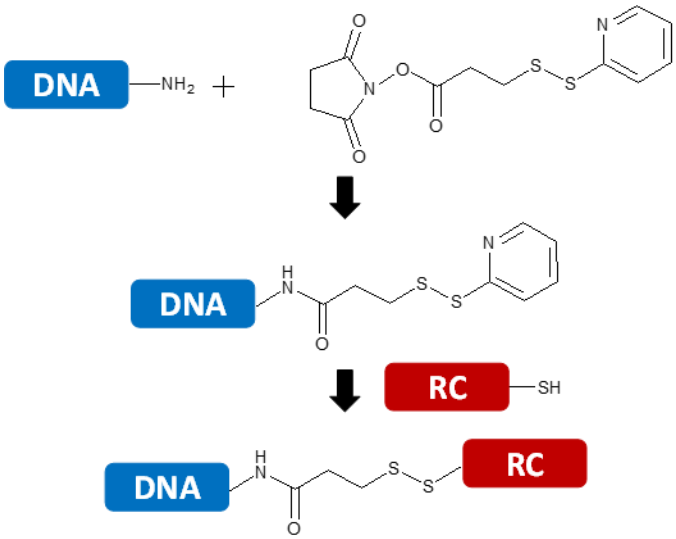
**1) SPDP labeling of DNA:** An amine-modified DNA (Strand 1, 5'-TCGCTAGGAACGG ATTTT-$NH_2$-3') of ~400 μM in 1×PBS, pH 7.6 was treated with 20 fold excess of 50 mM SPDP (N-succinimidyl 3-(2-pyridyldithio) propionate) in dimethyl sulfoxide (DMSO), followed by addition of 1M $NaHCO_3$ (~1/10 of total volume of DNA-SPDP mixture, to adjust pH) and the mixture was shaken gently for 3 hours at room temperature. The DNA-SPDP conjugate was purified with Nap-10 desalting column (GE Healthcare) and then washed 3 times with 1×PBS using 3kD molecular weight cut-off filter (Amicon) to remove the excess SPDP.

**2) Reduction of the disulfide bond in RC:** Before conjugation, the RC was treated with 8 fold excess of 50 mM TCEP-HCl (Tris(2-carboxyethyl)phosphine hydrochloride) for 30 min at 4°C, followed by washing with 1×PBS, 0.025% LDAO, pH 8 using 50kD molecular weight cut-off filter (Amicon) to remove excess TCEP-HCl.

**3) SPDP mediated cross-linking of DNA and RC:** A 10 fold excess of DNA-SPDP conjugate was mixed with TCEP-HCl treated RC and left for ~6 hours at 4°C with gentle mixing (Scheme S1). Then the mixture was treated with 10 mM phosphate buffer with high salt (1.5 M NaCl, 0.025% LDAO, pH 8), followed by washing 3 times with 10 mM phosphate, 0.025% LDAO, pH 8 buffer to remove the NaCl.

**4) Purification of the RC-DNA conjugates:** The sample was then run through an anion exchange column (Mono Q 4.6/100 PE, product code-17-5179-01) using a fast protein liquid chromatography (FPLC) system (AKTA purifier). The desired fractions containing the RC-DNA conjugates with different protein:DNA ratios were washed with dialysis buffer as described previously. The composition of the equilibration buffer used was 10 mM phosphate, 0.025% LDAO, pH 8 and the elution buffer consisted of 10 mM phosphate, 1M NaCl, 0.025% LDAO, pH 8.

**Scheme S1:** RC-DNA conjugation using SPDP as bi-specific cross-linker.




**Scheme S2:** DNA-Alexa Fluor dye conjugation


**III. DNA-dye conjugation and purification:**

Cy3 and Cy5 labeled strands (HPLC purified) (5'-CGCTACATCA/iCy3/TCCTAGCGA-3' and 5'-/5Cy5/ATCCGTTGATGTAGCG-3') were purchased from IDTDNA and used as received. Alexa Fluor dye (AF660 and AF750) labeled DNA strands were prepared as follows.

**1) Synthesis of amine-modified DNA and purification:** Amine modified DNAs for dye conjugation were synthesized on a DNA synthesizer (ABI 394 DNA/RNA Synthesizer, Applied Biosystems) via standard protocols by using CPGs (1 μmole scale) with a coupling time of 5 min for amine modified phosphoramidite (amino-modifier C6 dT phosphoramidite for Strand 3 and 5'-amino-modifier C6 phosphoramidite for Strand 2; both purchased from Glen Research). The oligonucleotide was cleaved from the resin by treatment with 1:1 volume mixture of $NH_4OH$ (28% in water) and methylamine (40% in water) for 2 hours at 50°C, and then purified using HPLC (Agilent Technologies 1200 series) with a Phenomenex-C18 column (Solvent A: 100 mM triethylammonium acetate, pH 7; Solvent B: acetonitrile; Flow rate: 4 mL/min). The fractions containing the desired oligonucleotides were collected and lyophilized. After being redissolved in

water, the lyophilized fractions were precipitated in 70% cold ethanol. The pellet of oligonucleotide was washed with 70% ethanol and dried under vacuum, and then dissolved in 0.1 M sodium tetraborate buffer ($Na_2B_4O_7.10H_2O$, pH 8.5) to a final concentration of ~200 μM.

**2) Dye-DNA conjugation and purification:** A 10-fold excess of Alexa Fluor dye (Invitrogen, amine reactive Alexa Fluor 660 and -750) from a ~15 mM stock solution (dissolved in DMSO) was added to the DNA solution described above and incubated overnight with gentle shaking at room temperature (Scheme S2). The DNA was then precipitated using 3 M NaCl and ethanol, and pelleted. The pellet was dissolved in water followed by HPLC purification (as described above for the amine modified DNA). The fraction containing the Dye-DNA conjugate was collected and lyophilized.

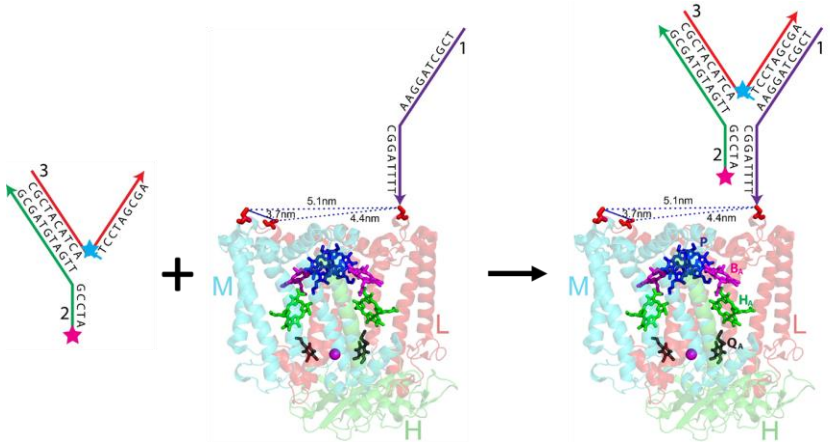
**3) Characterization of the dye-DNA conjugate:** MALDI-mass spectrometry (Applied Biosystem Voyager System 4320 and Bruker Microflex) analysis was carried out before and after the dye conjugation, using 3-hydroxypicolinic acid as the matrix (Figure S1).

**IV. 3arm-RC preparation:**

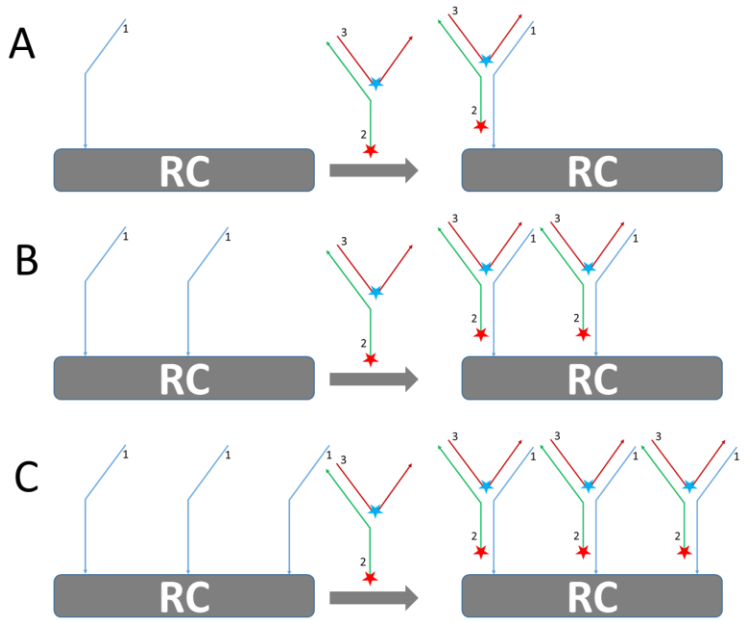
**1) Free 3arm-DNA constructs:** Free 3arm-DNA constructs were prepared by mixing stoichiometric quantities of three DNA strands in TAE/$Mg^{2+}$ buffer (40 mM Tris, 20 mM Acetic acid, 2 mM EDTA, 12.5 mM $Mg^{2+}$, pH 8) and subsequent annealing from 90°C to 10°C. After annealing the structures were purified by 8% native PAGE (polyacrylamide gel electrophoresis) and transferred into Tris buffer (15 mM Tris, 20 mM $Mg^{2+}$, 150 mM NaCl, 1 mM EDTA, pH 8). The stoichiometric formation of the 3arm-DNA constructs were confirmed by native PAGE (Figure S2).

**2) 3arm-RC conjugate:** First, strand-2 and -3 were annealed in the Tris buffer described above from 90°C to 10°C and then mixed with DNA conjugated RC (strand-1 conjugated with RC), with 1.5 fold molar excess followed by annealing from 30°C to 10°C over a 12 hr period (Scheme S3-S4). The mixture was then purified using a 50kD molecular weight cut-off filter (Amicon) using Tris buffer described above plus 0.025% LDAO, to remove the excess DNA strands.

**Scheme S3:** Schematic showing preparation of 3arm DNA-RC conjugate.



**Scheme S4:** Schematic representation of formation of the RC conjugate with different ratios of 3arm DNA. (A) For 1CC or 1-6-7. (B) For 2CC or 2-6-7. (C) For 3CC or 3-6-7.




**V. Spectroscopic Analysis:**

**1) Absorbance and fluorescence spectroscopy:** Absorbance spectra were measured using a quartz cell with 1 cm path length in a Jasco V-670 spectrophotometer. Steady state fluorescence spectra were measured in a Nanolog Fluorometer (Horiba Jobin Yvon), with a quartz cuvette of 1 cm path length. All the steady state emission spectra were corrected for the wavelength dependence of the response of the detection system.

**2) Time-correlated single-photon counting measurements:** Fluorescence lifetime measurements were analyzed by time-correlated single-photon counting (TCSPC). A fiber supercontinuum laser (Fianium SC450) was used as the excitation source, with a repetition rate of 20 MHz. The laser output was sent through an Acousto-Optical Tunable Filer (Fianium AOTF) to obtain excitation pulses at wavelengths of 510 nm, 600 nm, 620 nm and 740 nm. Fluorescence emission was collected at a 90° geometry setting and detected using a double-grating monochromator (Jobin-Yvon, Gemini-180) and a microchannel plate photomultiplier tube (Hamamatsu R3809U-50). The polarization of the emission was 54.7° relative to that of excitation. Data acquisition was done using a single photon counting card (Becker-Hickl, SPC-830). The typical instrument response function had a full width half maximum of 50 ps, measured using light scattered from the sample at the excitation wavelength. The data were fitted using a locally written software package ASUFIT.

**3) Calculation of FRET efficiency, average lifetime of dye molecules and decay rate constants:** FRET efficiencies (E) were calculated according to the following equation:

$$E = 1 - \frac{I_{DA}/A_{DA}}{I_D/A_D} \qquad (1)$$

Where $I_{DA}$ and $I_D$ are the integrated area of fluorescence from the donor with and without an acceptor. $A_{DA}$ and $A_D$ are the absorbance of the donor at the excitation wavelength with and without an acceptor.

The average lifetime was calculated using the following equation.

$$\tau_{ave} = \frac{\sum_i A_i \tau_i}{\sum_i A_i} \qquad (2)$$

Where $A_i$ is the amplitude of the i$^{th}$ exponential component in the fit and $\tau_i$ is the corresponding lifetime.

The energy transfer efficiency calculated from the lifetime measurements was determined as:.

$$E_{lifetime} = 1 - \frac{\tau_{ave,DA}}{\tau_{ave,D}} \qquad (3)$$

Where $\tau_{ave,DA}$ and $\tau_{ave,D}$ are the average lifetimes of the donor with and without an acceptor obtained from the TCSPC data.

The average lifetime (τ1) determined for Cy3 in the 3arm DNA-Cy3 molecules is 1.79 ns (Table 2).

$$\tau 1 = \frac{1}{k_{r,Cy3} + k_{nr,Cy3}} \qquad (4)$$

Where $k_{r,Cy3}$ and $k_{nr,Cy3}$ are the radiative and nonradiative decay rate constants of Cy3. Thus, $k_{r,Cy3} + k_{nr,Cy3} = 0.55\ ns^{-1}$.

In the case of 3arm DNA-Cy3-Cy5, the measured average lifetime of Cy3 (τ2) is 0.50 ns (Table 2).

$$\tau 2 = \frac{1}{k_{Cy3-Cy5} + k_{r,Cy3} + k_{nr,Cy3}} \qquad (5)$$

Where $k_{Cy3\text{-}Cy5}$ is the rate constant for Cy3 to Cy5 energy transfer. By combining (4) and (5), $k_{Cy3\text{-}Cy5}$ can be determined as 1.45 ns$^{-1}$.

In the case of **1C**, the average lifetime of Cy3 (τ3) is 1.06 ns (Table 2).

$$\tau 3 = \frac{1}{k_{Cy3-RC} + k_{r,Cy3} + k_{nr,Cy3}} \qquad (6)$$

Where $k_{Cy3\text{-}RC}$ is the rate constant for Cy3 to RC energy transfer. Combining (4) and (6) gives 0.39 ns$^{-1}$ for $k_{Cy3\text{-}RC}$.

Similarly for **1CC**, the average lifetime of Cy3 (τ4) is

$$\tau 4 = \frac{1}{k_{Cy3-RC}+k_{Cy3-Cy5}+k_{r,Cy3}+k_{nr,Cy3}} \qquad (7)$$

Based on the values determined for the microscopic rates in the denominator of this expression, one would expect τ4 to be 0.42 ns. The experimentally observed lifetime of Cy3 in **1CC** is 0.28 ns (Table 2).

Values of $k_{AF660\text{-}RC}$, $k_{AF660\text{-}750}$, and ($k_{r,AF660}$+$k_{nr,AF660}$) can be calculated in an analogous manner and are 0.20 $ns^{-1}$, 0.30 $ns^{-1}$, 0.59 $ns^{-1}$. Based on these values, the calculated lifetime of AF660 in **1-6-7** should be 0.92 ns, in close agreement with the experimental value of 0.90 ns.

The fact that the microscopic rate constants estimated and the observed average lifetimes are internally consistent supports the kinetic model used and the resulting energy transfer efficiencies determined.

**4) Cytochrome *c* oxidation experiment:** Before measuring the cytochrome *c* oxidation kinetics, bovine heart cytochrome *c* was reduced by treating with a 10-fold molar excess of sodium ascorbate in 10 mM sodium phosphate buffer (pH 6.9),[4] followed by purification with Nap-25 column (GE Healthcare). The oxidation kinetics of cytochrome *c* in presence of the 3arm DNA-RC were measured by monitoring the change in the absorbance at 550 nm in the presence of a 650 nm or 800 nm excitation beam (Figure S12). The 800 nm excitation beams was generated by passing white light (Dolan-Jenner MH-100 Metal Halide Fiber optic illuminator) through an 800 nm band pass filter (FB800-40, FWHM 40 nm). The 650 nm excitation beam was generated by passing the white light beam through both an RG610 (long pass) and a IF650 (band pass, FWHM 10 nm) filter. The sample contained 0.1 μM RCs (RC-wild type, RC, **1C**, **2C**, **3C**, **1CC**, **2CC** and **3CC**), 100-fold molar excess of decylubiquinone (extinction coefficient at 409 nm in ethanol = 343 $M^{-1}cm^{-1}$) and 10-fold molar excess of reduced cytochrome *c* in the dialysis buffer described in section 2 in part I.

**5) Light-minus-dark measurements:** The light-minus-dark measurements were performed by measuring the absorbance spectra of a sample taken in the dark (dark spectra) and in presence of 550 nm (bandwidth ~10 nm) continuous light (light spectra), and then subtracting the dark spectra from the light spectra. The samples were illuminated with 550 nm light for 6 minutes prior to the measurement. The path of the excitation light was perpendicular to the path of the probe light from the UV-Vis absorbance spectrophotometer. The excitation light at 550 nm was obtained by passing a white light source (Dolan-Jenner MH-100 Metal Halide Fiber optic illuminator) through two filters (BG 38 and IF550, 10 nm band pass). For all measurements, samples contained a 50-fold excess of 1,10-phenanthroline compared to the RC concentration.

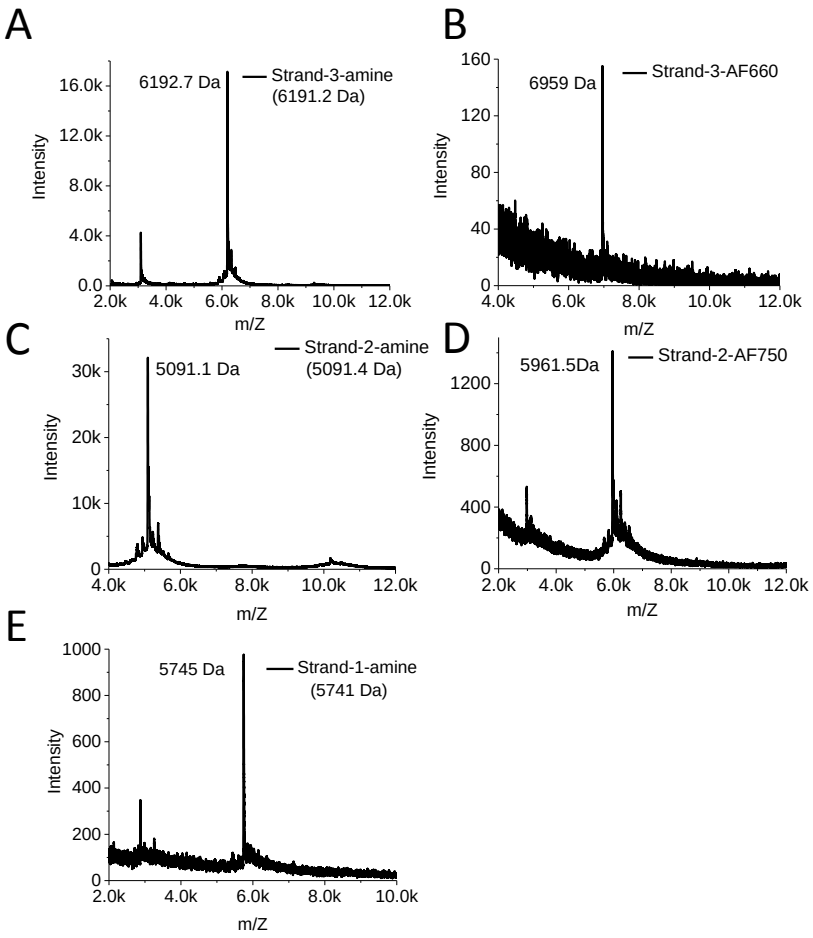



**Figure S1:** MALDI-TOF spectra of (A) amine modified Strand-3, (B) Alexa Fluor 660 conjugated Strand-3, (C) amine modified Strand-2, (D) AF750 conjugated Strand-2, and (E) amine modified Strand-1.

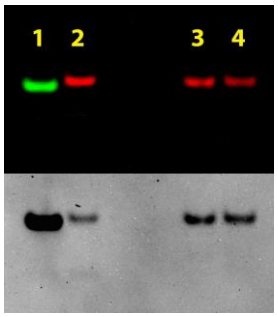



**Figure S2:** Images of an 8% nondenaturing polyacrylamide gel electrophoresis (PAGE). (Top) Gel image measured with a Typhoon™ Trio multifunction imager (Amersham Biosciences) exciting at 532 nm and 633 nm with emission at 580 nm and 670 nm, respectively. (Bottom) Ethidium bromide stained gel image. (1), (2), (3) and (4) represent the purified 3arm labeled with Cy3, Cy3-Cy5, AF660 and AF660-AF750, respectively.

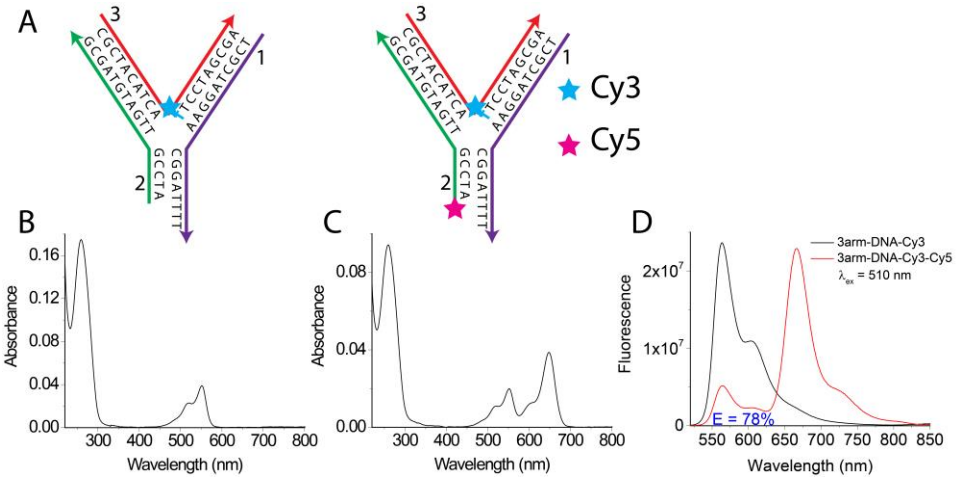



**Figure S3:** (A) Schematic of 3arm-DNA structure with Cy3 only (3arm-DNA-Cy3) and with both Cy3 and Cy5 (3arm-DNA-Cy3-Cy5). (B)-(C) Absorption spectra of 3arm-DNA-Cy3 and 3arm-DNA-Cy3-Cy5. (D) Corresponding fluorescence emission spectra with excitation at 510 nm. The spectra were corrected by adjusting for the independently determined wavelength-dependent detector response and normalized by dye absorbance at 510 nm. A 78% energy transfer efficiency was observed from Cy3 to Cy5 organized within the 3arm-DNA nanostructure.

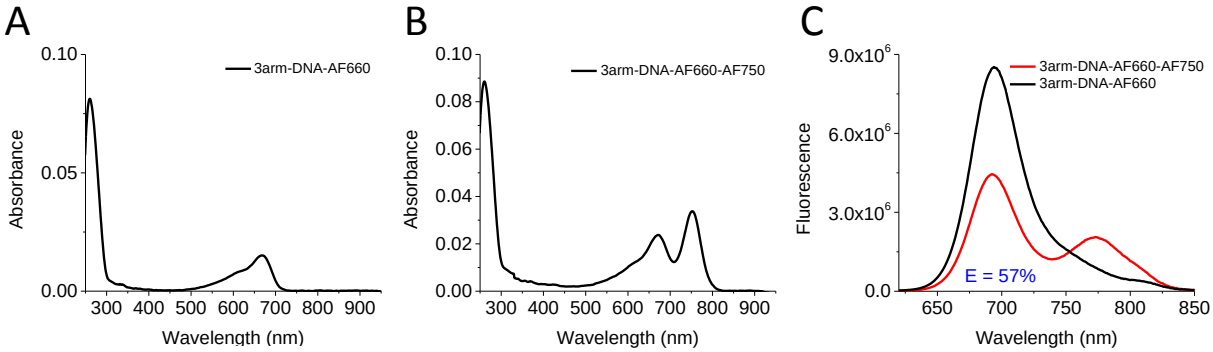



**Figure S4:** (A)-(B) Absorption spectra of 3arm-DNA-AF660 and 3arm-DNA-AF660-AF750. (C) Corresponding fluorescence emission spectra with excitation at 600 nm. The spectra were corrected for the wavelength dependence of the detector sensitivity and normalized by dye absorbance at 600 nm. A 57% energy transfer efficiency was observed from AF660 to AF750 organized within the 3arm DNA nanostructure.

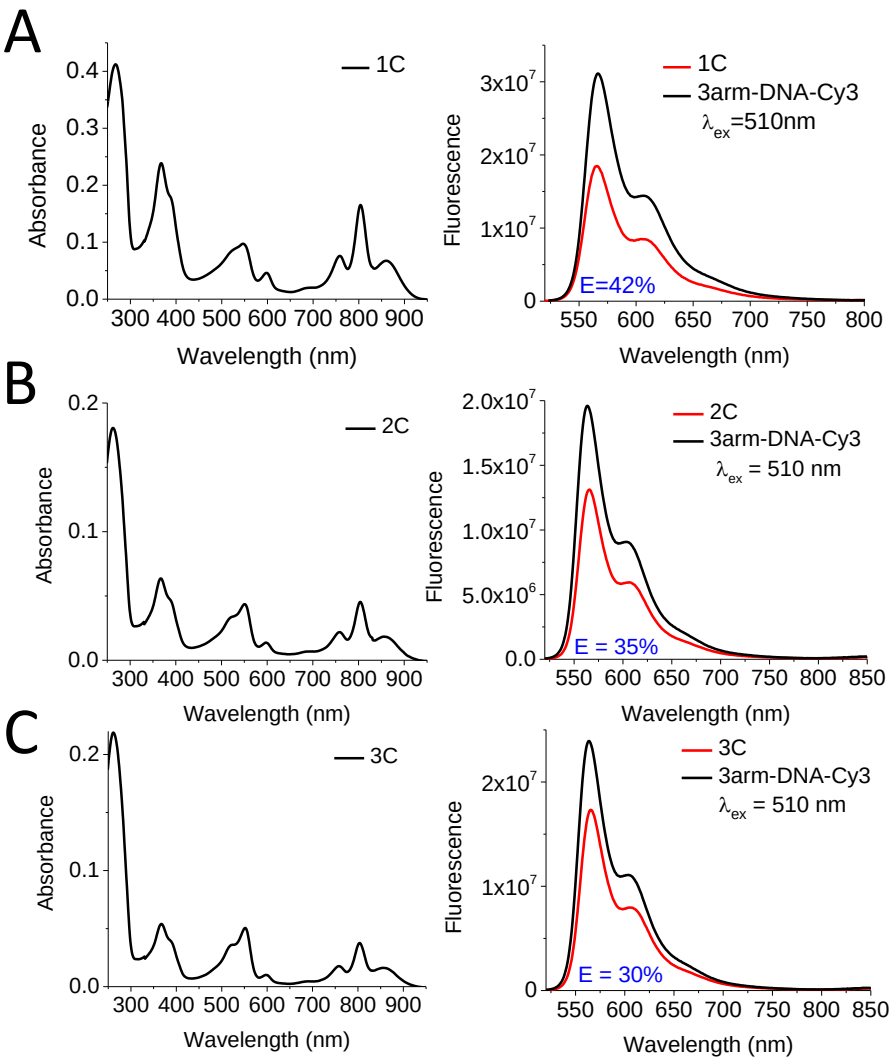



**Figure S5:** Absorbance spectra (left) and fluorescence spectra (right) of RCs with different numbers (1-3) of the 3arm-DNA-Cy3 complexes attached per RC. The energy transfer efficiency (E) values between the Cy3 and the RC are shown in the fluorescence spectra in blue.

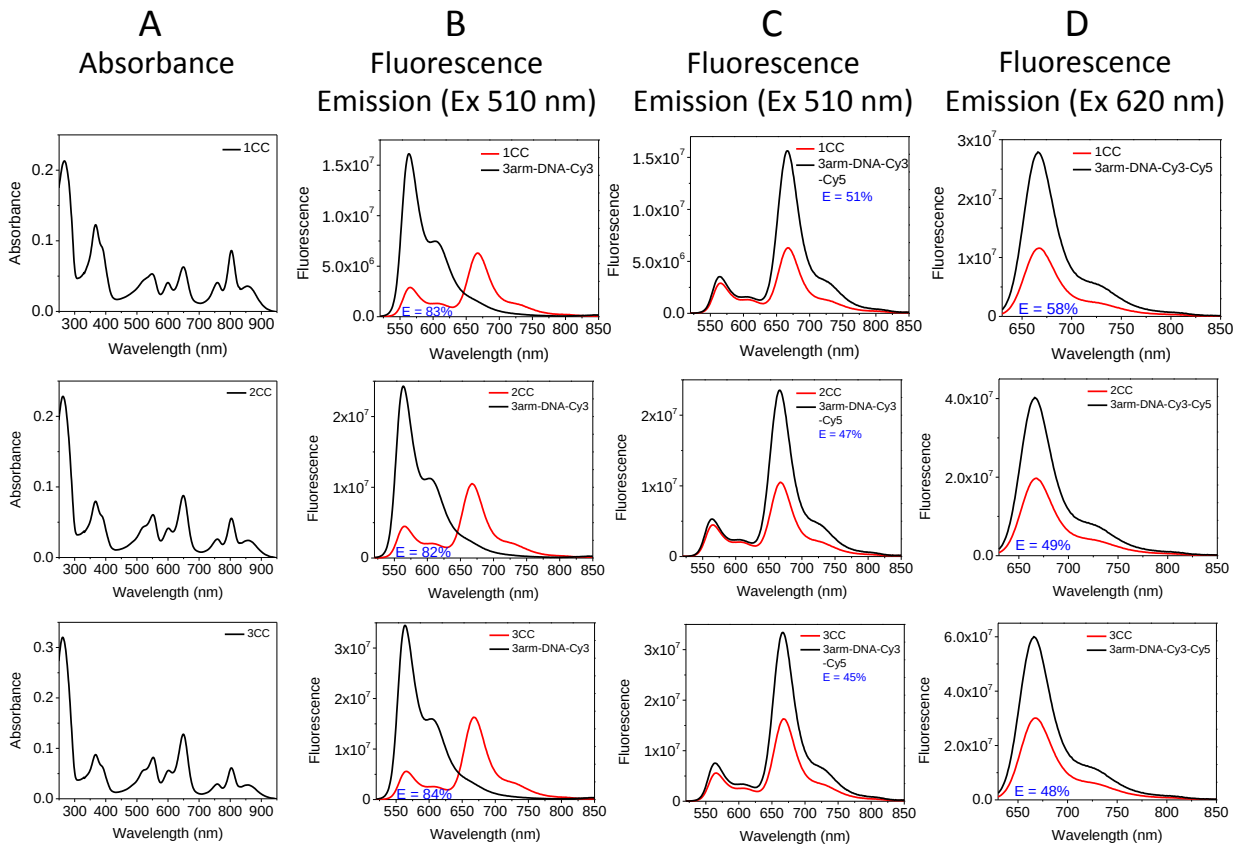



**Figure S6:** Absorbance spectra (panel A) and fluorescence spectra (panel B, C and D) of RCs with different numbers of 3arm-DNA-Cy3-Cy5 complexes per RC. The energy transfer efficiency (E) values are shown on the fluorescence spectra in blue.

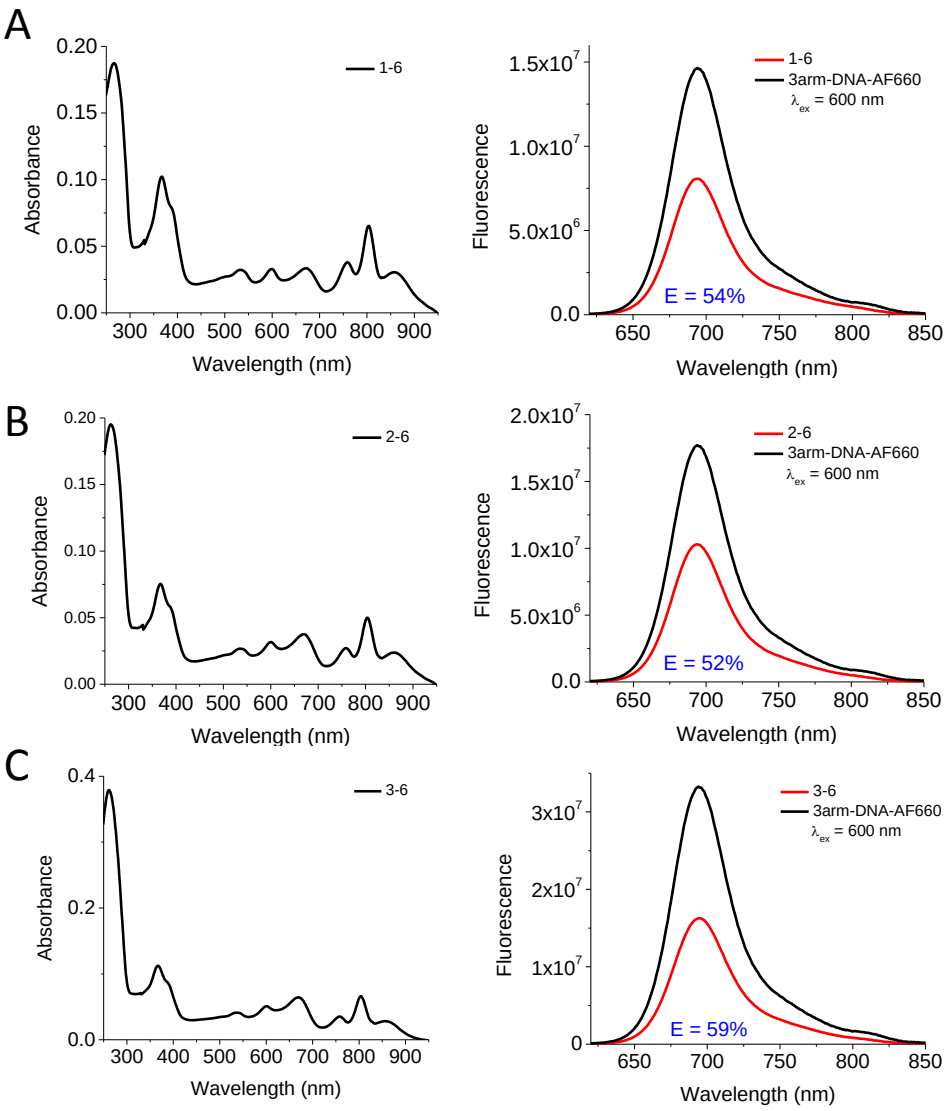



**Figure S7:** Absorbance spectra (left) and fluorescence spectra (right) of RCs with different numbers of 3arm-DNA-AF660 complexes per RC. The energy transfer efficiency (E) values are shown on the fluorescence spectra in blue.

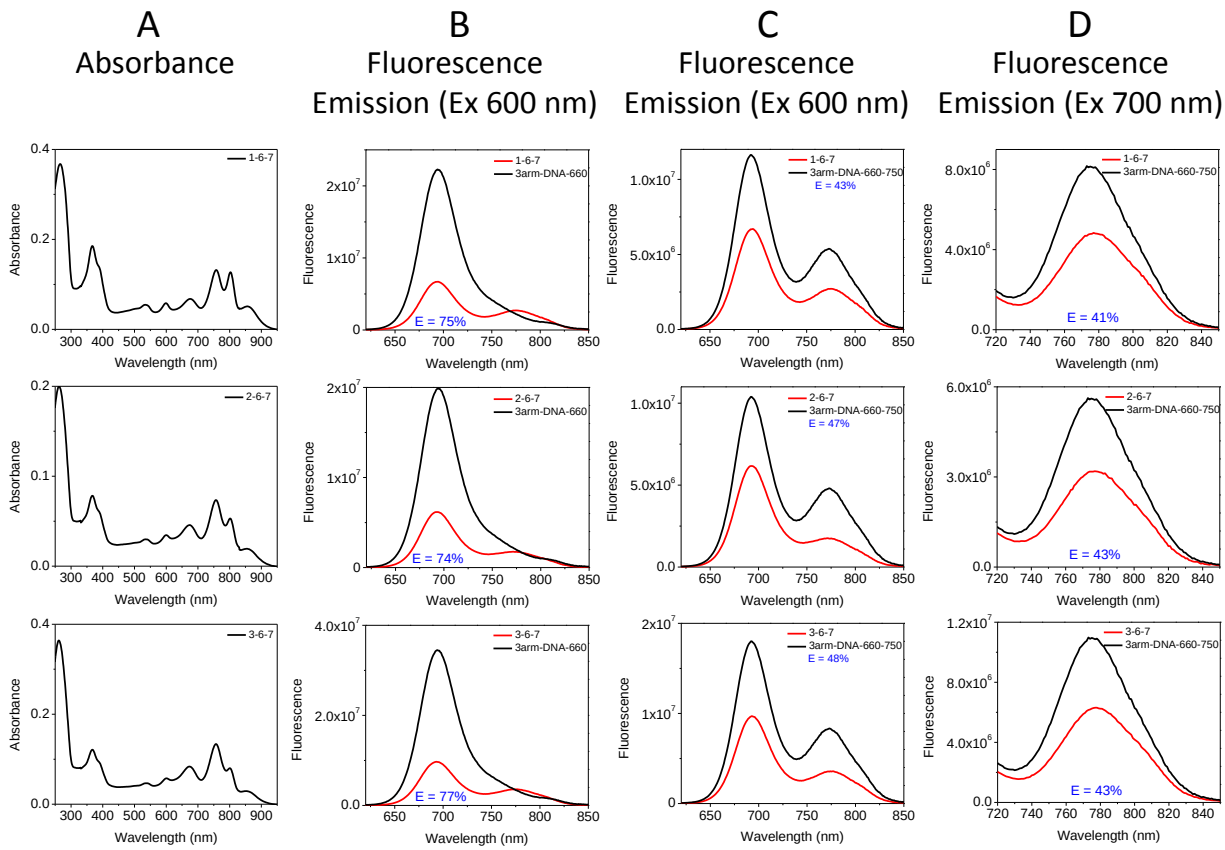



**Figure S8:** Absorbance spectra (panel A) and fluorescence spectra (panel B, C and D) of RCs with different numbers of 3arm-DNA-AF660-AF750 per RC. The energy transfer efficiency (E) values are shown on the fluorescence spectra in blue.

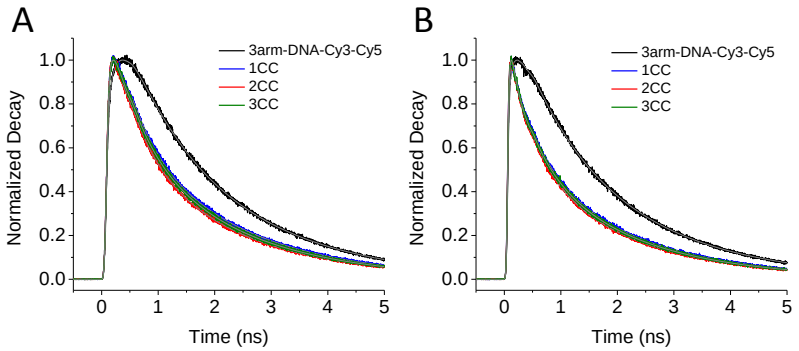



**Figure S9:** Time resolved emission of 3arm-DNA-Cy3-Cy5 with and without the RC. (A) Cy5 emission was monitored at 668 nm after exciting Cy3 at 510 nm. (B) Cy5 emission monitored at 668 nm after exciting Cy5 at 620 nm.

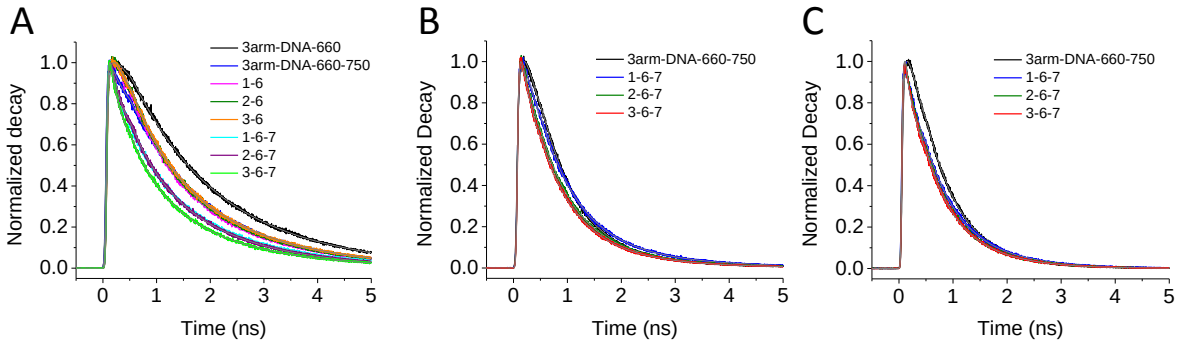



**Figure S10:** Time resolved emission of 3arm-DNA-AF660 and 3arm-DNA-AF660-AF750 samples with and without RCs. (A) AF660 emission was monitored at 698 nm after exciting AF660 at 600 nm. (B) AF750 emission was monitored at 780 nm after exciting AF660 at 600 nm. (C) AF750 emission was monitored at 780 nm after exciting AF750 at 740 nm.

**Table S1:** Fitting parameters for Cy5 lifetime data, monitored at 668 nm ($\lambda_{ex}$ = 620 nm). The results from two replicates of each sample are shown.

| sample | τ1 ns (amplitude %) | τ2 ns (amplitude %) | τ3 ns (amplitude %) | $\kappa^2$ | average lifetime (ns) |
|---|---|---|---|---|---|
| 3arm-DNA-Cy3-Cy5 | 0.77(22.8)<br>0.84(26.2) | 1.92(77.2)<br>1.92(73.8) | | 1.13<br>1.16 | 1.658<br>1.637 |
| **1CC** | 0.10(41.9)<br>0.10(36.8) | 0.48(26.7)<br>0.51(24.8) | 1.89(31.4)<br>1.92(38.4) | 1.06<br>1.07 | 0.763<br>0.900 |
| **2CC** | 0.11(40.3)<br>0.10(37.8) | 0.50(25.7)<br>0.48(26.0) | 1.85(34.0)<br>1.86(36.2) | 1.02<br>1.03 | 0.802<br>0.836 |
| **3CC** | 0.11(45.0)<br>0.10(37.5) | 0.45(27.9)<br>0.51(24.5) | 1.84(27.1)<br>1.87(38.0) | 1.03<br>1.02 | 0.674<br>0.873 |

**Table S2**: Fitting parameters for AF660 lifetime data, monitored at 698 nm ($\lambda_{ex}$ = 600 nm). **1-6** to **3-6** represent AF660 labeled 3arm-DNA conjugated to RCs that have 3arm-DNA to RC ratios between 1 and 3. **1-6-7** to **3-6-7** represent both AF660- and AF750- labeled 3arm-DNA conjugated to RCs that have 3arm-DNA to RC ratios between 1 and 3. The results from two replicates of each sample are shown.

| sample | τ1 ns (amplitude %) | τ2 ns (amplitude %) | τ3 ns (amplitude %) | $\kappa^2$ | average lifetime (ns) |
|---|---|---|---|---|---|
| 3arm-DNA-660 | 1.10(27.4) | 1.90(72.6) | | 1.09 | 1.681 |
| 3arm-DNA-660-750 | 0.08(25.5) | 0.90(19.1) | 1.68(55.4) | 1.08 | 1.123 |
| **1-6** | 0.61(39.5)<br>0.62(32.4) | 1.7(60.5)<br>1.69(67.6) | | 1.06<br>1.15 | 1.267<br>1.343 |
| **2-6** | 0.65(38.5)<br>0.65(35.6) | 1.73(61.5)<br>1.65(64.4) | | 1.11<br>1.10 | 1.314<br>1.294 |
| **3-6** | 0.63(37.6)<br>0.64(37.2) | 1.76(62.4)<br>1.64(62.8) | | 1.06<br>1.14 | 1.335<br>1.268 |
| **1-6-7** | 0.08(36.3)<br>0.09(28.0) | 0.58(25.8)<br>0.68(24.2) | 1.7(37.9)<br>1.65(47.8) | 1.07<br>1.04 | 0.823<br>0.978 |
| **2-6-7** | 0.08(33.6)<br>0.09(29.1) | 0.57(25.8)<br>0.65(22.4) | 1.6(40.6)<br>1.56(48.5) | 1.00<br>1.07 | 0.823<br>0.928 |
| **3-6-7** | 0.07(39.3)<br>0.08(36.7) | 0.52(27.1)<br>0.59(23.2) | 1.57(33.6)<br>1.54(40.1) | 1.12<br>1.13 | 0.696<br>0.784 |

**Table S3:** Fitting parameters for AF750 lifetime data, monitored at 780 nm ($\lambda_{ex}$ = 740 nm).

| sample | τ1 ns (amplitude %) | τ2 ns (amplitude %) | τ3 ns (amplitude %) | $\kappa^2$ | average lifetime (ns) |
|---|---|---|---|---|---|
| 3arm-DNA-660-750 | 0.48(31.1) | 0.72(68.9) | | 1.08 | 0.645 |
| **1-6-7** | 0.08(25.4) | 0.56(59.6) | 1.04(15.0) | 1.07 | 0.510 |
| **2-6-7** | 0.07(31.6) | 0.46(37.0) | 0.81(31.4) | 1.15 | 0.446 |
| **3-6-7** | 0.08(39.0) | 0.47(28.8) | 0.78(32.2) | 1.19 | 0.418 |

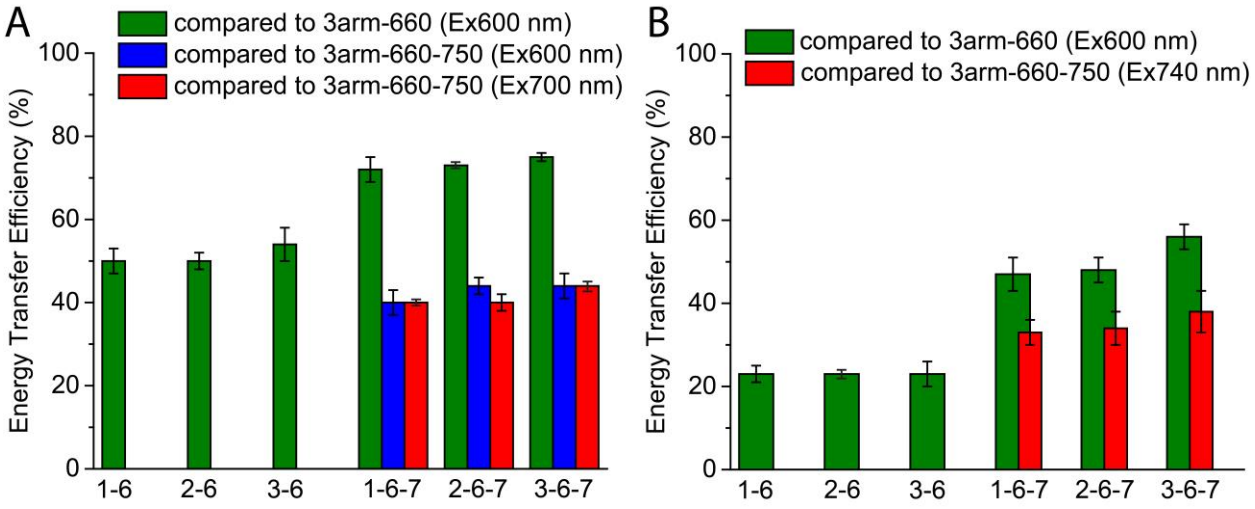



**Figure S11:** Energy transfer efficiencies calculated from (A) steady-state data and (B) lifetime data for **1-6**, **2-6**, **3-6**, **1-6-7**, **2-6-7** and **3-6-7**.

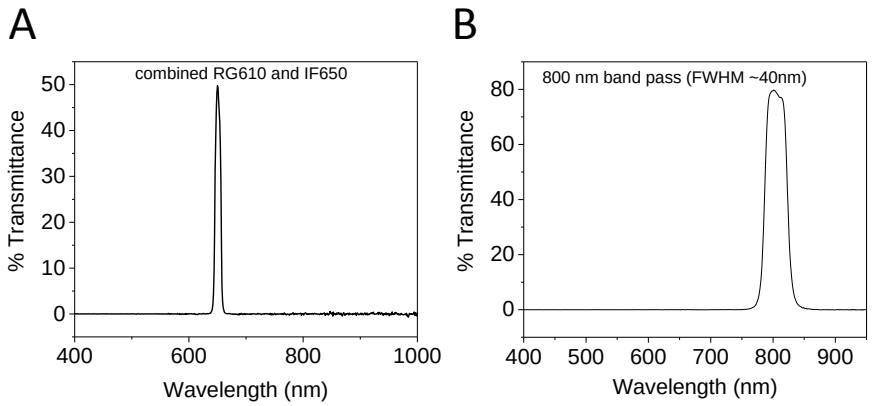



**Figure S12:** Transmittance spectra of filters used in the cytochrome *c* oxidation experiments, (A) for excitation at 650 nm and (B) for excitation at 800 nm.

**References:**


(1) Goldsmith, J. O.; Boxer, S. G. *Biochim. Biophys. Acta* **1996**, *1276*, 171.

(2) Mahmoudzadeh, A.; Saer, R.; Jun, D.; Mirvakili, S. M.; Takshi, A.; Iranpour, B.; Ouellet, E.; Lagally, E. T.; Madden, J. D. W.; Beatty, J. T. *Smart Mater. Struct.* **2011**, *20 (9)*, 094019.

(3) Salafsky, J.; Groves, J. T.; Boxer, S. G. *Biochemistry* **1996**, *35*, 14773.

(4) Spinazzi1, M.; Casarin, A.; Pertegato, V.; Salviati, L.; Angelini, C. *Nature Protocols* **2012**, *7*, 1235.