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Supplementary information

Toward the Design of Bio-Solar Cells: High efficiency cascade energy transfer among four donor-acceptor dyes self-assembled in highly ordered protein-DNA matrix

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1. Experimental materials and methods

S1.1 Agarose gel electrophoresis

The chemical modification and net charge on cBSA was evaluated by agarose gel electrophoresis, using a horizontal gel electrophoresis apparatus (Gibco model 200, Life Technologies Inc, MD). Agarose gel (0.5 % w/w) was cast and run in Tris-acetate buffer (40 mM, pH 7.0). Modified and unmodified BSA samples were loaded with 50 % loading buffer (50 % v/v glycerol-dH₂O and 0.01 % w/w Bromophenol Blue) to increase density of samples without further chemical reaction. Samples were spotted into wells in the middle of the gel so that the protein could migrate towards the negative or the positive electrode based on its overall charge. A potential of 100 mV was applied for 30 minutes. The gel was stained overnight with 10 % v/v acetic acid and 0.02 % w/w Coomassie Blue, followed by destaining in 10 % v/v acetic acid overnight. Since a very low percentage of agarose was used (0.5 % w/w) migration distance in the gel was minimal. This relationship was used to calculate the charge on cBSA, and BSA had a net charge of -18 at pH 7.0.

S1.2 Calculation of Binding Constants

The binding constants (K_b) of Fluorescein (F) and Rhodamine B (R) to cBSA in the solution were determined by observing the quenching of the intrinsic fluorescence of cBSA as a function of dye concentration. The fluorescence of BSA ($\lambda_{max, excitation} = 280$ nm, $\lambda_{max, emission} = 340$ nm) is primarily caused by the tryptophan residues, though other aromatic side chains contribute as well. In the presence of extrinsic fluorophores, such as F and R, this fluorescence is often quenched, and thus can be used to determine the binding of the fluorophores.

To observe the quenching of cBSA fluorescence by F and R, 10 μ M cBSA was mixed with increasing amounts of F or R and the emission spectrum obtained (**Fig. S7-8**, $\lambda_{\text{excitation}} = 280 \text{ nm}$). Concentration of each dye ranged from 0 to 200 μ M. The K_b was then determined from the following equation:

$$log_{IO} = \left[\frac{F_0 - F}{F}\right] = log K + nlog [Q]$$

where F_0 is the fluorescence of cBSA in the absence of the quencher, F is fluorescence of cBSA at some quencher concentration, and [Q] is the concentration of the quencher. K is the binding constant and determined from the y-intercept of a plot of log(F_0 -F/F) versus log[Q] (**Fig. S7-8**). Based on this analysis, the K_b of F to cBSA was determined to be 5.1×10^4 M⁻¹, while the K_b of R was 3.12×10^4 M⁻¹. Control studies were completed with unmodified BSA, and the K_b of F and R were found to be 4.6×10^4 M⁻¹ and 1.9×10^4 M⁻¹ respectively, in good agreement with literature reports.

When Coumarin 540A (C) bound to BSA, the emission of the dye increased in intensity while $\lambda_{max, emission}$ was hypsochromically shifted. For a fixed concentration of C, both properties were observed to change as a function of [BSA] and used to determine the K_b. 20 μ M C was titrated with increasing concetrations of cBSA, and the emission spectra collected. All samples were excited at 430 nm, the isosbestic point of free and bound C. The following expression was used to determine the concentration of bound C:

$$[C]_{bound} = \frac{I_{observed} - I_{free}}{\frac{1}{[C]_{total}} (I_{bound} - I_{free})}$$

where $I_{observed}$ is the emission intensity at 544 nm observed in the experiment, I_{free} is the emission intensity of C at 544 nm in the absence of cBSA, and I_{bound} is the emission intensity of C at 544 nm when bound to cBSA. The intensity of completely bound C was determined by plotting emission intensity versus 1/[cBSA] and extrapolating to infinite [cBSA]. The K_b was then determined to be 1.4x10⁵ M⁻¹ using a simple equilibrium model (**Fig. S6**).

S1.3 SEM

Field Emission Scanning Electron Microscopy (FESEM) was performed at various magnifications using a JEOL 6335 operating at 10 kV. The films were coated with palladium before imaging.

S1.4 Circular Dichroism- Antenna Components

Circular dichroism spectra were obtained on a J-710 Spectropolarimeter from Jasco (Oklahoma City, OK). Using 0.05 cm quartz circular cuvette, each of the components of the antenna complex was tested. Solution concentrations of the dyes were the same as used for the fluorescence studies. Several scans were averaged routinely.

S1.5 Calculation of concentrations in the film and Förster volume occupancy.

The concentration of each component in the film phase was determined assuming that that the volume of all dry components were additive. Using the masses and the densities of DNA, cBSA, H, C, F, R, and phosphate buffer (1.32 mg/mL), the volume of each component and the total volume was calculated. Using the density of water, the volume of water retained was estimated by weighing the film and subtracting the masses of all the remaining components. The volume of the film was thus the sum of the volumes of DNA, cBSA, H, C, F, R, phosphate buffer and residual water. The concentration of each component in the film calculated from the total film volume and the mole numbers of individual components.

The number of donors and acceptors occupying the Förster volume (occupancy number) was then estimated. The average Förster volume was determined to be 6.6×10^5 Å³, based on the the average of the Forster radii of the three pairs (H-C, C-F, and F-R). The total volume of the film was divided by the average Forster volume to obtain the total number of quenching "cells" in the film. Finally, the occupancy numbers were calculated from the mole numbers and the number of Forster cells.

S1.6 Energy transfer efficiency estimates from the absorption and emission data

The gain in emission at 590 nm for the antenna when excited at 350 nm was calculated as the ratio of the emission of the 4 dyes embedded in the DNA/protein film to the emission of R bound to DNA/protein film, at the same R concentration. The excitation and emission wavelengths, and all other parameters were kept the same. The gain in emission at 590 nm for the assembly when compared to the film of R/DNA/protein was found to be 2.4.

To derive an expression to estimate the overall efficiency of the system, we first start with the following relationship (Equation 1):

(1)
$$\frac{I_{F,AD} - I_{F,A}}{I_{abs,AD} - I_{abs,A}} \propto \phi_{ET}$$

where I_F is emission intensity of the acceptor at its $\lambda_{max,emission}$ (590 nm), I_{abs} is the intensity of absorbed light at the $\lambda_{max,absorbance}$ of the donor (350 nm), and the subscripts A and AD indicate systems with the acceptor alone or the acceptor and the donor, respectively. Simply speaking, Equation 1 indicates that the quantum yield of energy transfer is proportional to the change in acceptor emission divided by the change in absorbance at the excitation wavelength upon addition of the donor.

In order to quantitate I_{abs}, we first look to the Beer-Lambert law (Equation 2): (2) $\frac{I}{I_0} = 10^{-\varepsilon bc}$

where I is the intensity of light transmitted by the sample, I_0 is the intensity of light received by the sample, ε is the molar absorptivity of the sample, b is the path length, and c is the concentration of the absorbing species. We can also state that I_{abs} is equal to the intensity of received light minus the intensity of transmitted light (Equation 3).

$$(3) \quad I_{abs} = I_0 - I$$

By solving Equation 2 with respect to I, we can substitute for I in Equation 3 to obtain Equation 4:

(4)
$$I_{abs} = I_0 (1 - 10^{-\varepsilon bc})$$

This allows us to express $I_{abs,A}$ and $I_{abs,AD}$ in terms of I_0 , ε , b, and c (Equations 5 and 6). To obtain $I_{abs,AD}$, we assume that the intensity of absorbed light of a sample containing both the acceptor and the donor is the sum of the intensity of light absorbed by the acceptor and by the donor, respectively (Equation 6).

(5)
$$I_{abs,A} = I_0 (1 - 10^{-\varepsilon_A b c_A})$$

(6)
$$I_{abs,AD} = I_{abs,A} + I_{abs,D} = I_0 (2 - 10^{-\varepsilon_A b c_A} - 10^{-\varepsilon_D b c_D})$$

We can the substitute Equations 5 and 6 into Equation 1. After combining like terms, we obtain Equation 7:

(7)
$$\frac{I_{F,AD} - I_{F,A}}{I_0 \left(1 - 10^{-\varepsilon_D b c_D}\right)} \propto \phi_{ET}$$

Finally, to determine the overall efficiency of energy transfer and emission from the acceptor in the system, Equation 7 must be modified to meet two boundary conditions. First, when there is no energy transfer, the efficiency must be zero. In this case, $I_{F,AD}$ would be equal to $I_{F,A}$, and Equation 7 therefore already satisfies this condition. Second, when the energy transfer is 100% efficient – in other words, all photons absorbed by the donor are transfer to the acceptor – then the efficiency must be equal to 1. This boundary condition can be satisfied by dividing Equation 7 by $(I_{F,A}/I_{abs,A})$, which will also cancel out all proportionality constants that convert I_F and I_{abs} from arbitrary units to number of photons. In doing so, Equation 8 is obtained:

(8)
$$E = \left(\frac{I_{F,AD} - I_{F,A}}{I_{abs,AD} - I_{abs,A}}\right) \left(\frac{I_{abs,A}}{I_{F,A}}\right) = \left(\frac{I_{F,AD} - I_{F,A}}{I_0(1 - 10^{-\varepsilon_D bc_D})}\right) \left(\frac{I_0(1 - 10^{-\varepsilon_A bc_A})}{I_{F,A}}\right)$$

By recognizing that $\varepsilon_A bc_A$ is equal to the absorbance of the acceptor, A_A , and $\varepsilon_D bc_D$ is equal to the absorbance of the donor, A_D , Equation 8 can therefore be expressed solely in terms of quantities that can be readily measured, emission intensity and absorbance (Equation 9).

(9)
$$E = \frac{I_{F,AD} - I_{F,A}}{I_{F,A}} \left(\frac{1 - 10^{-A_A}}{1 - 10^{-A_D}} \right)$$

Because the films were not optically dilute, the absorbance of the donor (H) and acceptor (R) were obtained in solution phase, assuming that the molar absorptivity did not change upon deposition to film phase (**Fig. S10**). By use the above analysis, an overall efficiency of 0.23 was obtained for the current system.

Data files





Figure S2. Agarose gel of BSA (lane 1) and BSA modified with triethylenetetramine (TETA) (cBSA) (lanes 2-5). Lane 5 clearly indicates the positive charge of cBSA in this lane, as opposed to an overall negative charge on the native BSA (-18). Lane 5, calculated to have a +15 charge by using ImageJ software and the proportional relationship between protein charge and distance migrated, was used for all further manipulations in this publication.







Fluorescence emission of R as a function of cBSA concentration. (C) Binding isotherms of R + BSA (blue) and R + cBSA (red). Little difference was seen between the binding constants of R to BSA and R to cBSA. Binding constant of R + cBSA was be 3.1×10^4 M⁻¹.



Table 1. Emission and excitation maxima, binding affinities and % binding of the dyes to

 the protein-DNA film in the solid state.

Dye	Ex/Em (nm)	Binding Constant	% Bound in Film
Hoechst 33258	352/461	$100.0 \text{ x} \ 10^4$	99.99
Coumarin C540A	423/530	14.0 x 10 ⁴	99.93
Fluorescein	490/514	5.1 x 10 ⁴	99.84
Rhodamine B	554/627	3.1 x 10 ⁴	99.80





pH 7.2 with a single peak at 280 nm.



dyes/DNA/protein, and those of the films with one of the four dyes omitted each time. Omitted dye: H (blue) C (green), F (yellow) and R (red).



Figure S10. Solution phase absorbance of 40 μ M Rhodamine B (red curve) and a mixture of 50 μ M H and 40 μ M R. Spectra were baseline corrected at 650 nm normalized to 1 at 553 nm, the wavelength of maximum absorbance of Rhodamine B in solution. The absorbance of Hoescht 33258 in the presence of Rhodamine B was obtained by subtracting the absorbance of Rhodamine B (red curve) from the absorbance of the mixture of Rhodamine B and Hoescht 33258 (blue curve).



Figure S11. Emission spectrum of DNA + H + C + F + R without cBSA. The film was cast from a solution containing 800 μ M DNA, 50 μ M H, 125 μ M C, 100 μ M F, and 40 μ M R. The sample was excited at 350 nm and emission monitored from 460 to 680 nm. No energy transfer is observed in these films without cBSA.





were: [cBSA] = 18.5 mM, [H] = 3.1 mM, [C] = 7.7 mM, [F] = 5.2 mM, and [R] = 2.5 mM.



Figure S14. Quenching of F as a function of [R] (0 – 10.4 mM) in films. Film concentrations of DNA (49.2 mM), cBSA (18.5 mM), H (3.1 mM), C (7.7 mM) and F (6.2 mM) were kept constant. (A) Emission and absorbance spectra were recorded and emission spectra collected from 370 to 650 nm by exciting at 350 nm. (B) The ratio of quantum yield of F emission in the absence of R to that in the presence of R was plotted (Perrin plot) quenching radius obtained from the slope.



constant. Emission and absorbance spectra were recorded and emission spectra collected from 370 to 650 nm by exciting at 350 nm. The ratio of quantum yield of C emission in the absence of F and in the presence of F at each F concentration was plotted and the Forster radius calculated.



Concentrations of DNA (49.2 mM) cBSA (18.5 mM), H (3.1 mM), F (6.2 mM) and R (2.5 mM) were kept constant. Emission and absorbance spectra were recorded and the ratio of quantum yield of H emission in the absence and presence of C concentration was plotted against [C] and the quenching radius estimated.

Table 2. Energy transfer efficiency and thermal stability of biological artificia	al
antenna complexes	

Matrix/Scaffold	No. Dyes	Efficiency Reported	Antenna Effect*	Thermal Stability	Reference
DNA/Protein	4	0.23	~2.4	169+ days at 80 °C	Current work
DNA/Protein	2	~0.95	5.4	N/A	C. V. Kumar and M. R. Duff, J. Am. Chem. Soc., 2009, 131 , 16024-16026.
Tobacco mosaic virus coat protein monomer	3	~0.90ª	4.6	N/A	R. A. Miller, A. D. Presley, and M. B. Francis, <i>J. Am.</i> <i>Chem. Soc.</i> 2007, 129 , 3104–3109.

DNA	3	~0.90 ^b	85-90%	N/A	P. K. Dutta, R. Varghese, J. Nangreave, S. Lin, H. Yan, and Y. Liu, <i>J. Am. Chem. Soc.</i> 2011, 133 , 11985– 11993.
DNA	2	0.55°	12	N/A	J. G. Woller, J. K. Hannestad, and B. Albinsson, <i>J. Am.</i> <i>Chem. Soc.</i> , 2013, 135 , 2759-2768.
DNA origami	4	0.36(±0.17) ^d	N/A	N/A	I. H. Stein, C. Steinhauer, and P. Tinnefeld, J. Am. Chem. Soc. 2011, 133 , 4193–4195.
DNA photonic wire	4 + quantum dot	0.01-0.08°	N/A	N/A	20C.M.Spillmann,M.G.Ancona,S.Buckhout-White,W.R.Algar,M.H.Stewart,K. Susumu,A.L.Huston,EllenR.Goldman,R.Goldman,andI.L.Medintz,ACSNano,2013,7(8),7101-7118.
DNA photonic wire	4 dyes + terbium complex	0.22(±0.06) ^b	N/A	N/A	M. Massey, M. G. Ancona, I. L. Medintz, and W. R. Algar, ACS Photonics 2015, 2 , 639 - 652

N/A = not available

*Where reported, antenna effect was calculated as follows:

Antenna effect =
$$\frac{I_{donor\ excitation}}{I_{direct\ acceptor\ excitation}}$$

where $I_{\text{donor excitation}}$ is the emission intensity of the acceptor when the donor is excited and

 $I_{direct acceptor excitation}$ is the emission intensity of the acceptor when it is excited directly.

^a Efficiency was calculated from the ratio of donor absorbance to donor excitation (monitoring acceptor emission wavelength).

^b Efficiency of donor quenching was reported, calculated from $E = 1 - I_{DA}/I_D$, where I_{DA} and I_D are the emission intensity of the donor in the presence and absence of the acceptor, respectively.

$$E = \frac{\frac{3}{2}\kappa^2 (R_0^{DA})^6}{\frac{3}{2}\kappa^2 (R_0^{DA})^6 + R^2}$$

^c Energy transfer efficiency was calculated from Förster theory, using the equations

and $R_0^{DA} = 0.197 (n^{-4}QY_D * J(\lambda))^{1/6}$, where κ is the orientation factor between donor and acceptor transition dipole moments, R_0^{DA} is the Förster radius, n is the refractive index, QY_D is the donor quantum yield, and $J(\lambda)$ is the integrated spectral overlap of normalized donor fluorescence and acceptor absorption coefficient.

^d Efficiency was calculated using the following equation: F_{E} photon counts of output channel (acceptor λ)

E =

sum of input and output counts

^e Efficiency was determined from the ratio of excited terminal dyes to excited quantum dots (initial donor).