Supporting Information

Biomimetic Fabrication of Biotinylated Peptide Nanostructures upon Diatom Scaffold; A Plausible Model for Sustainable Energy

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Experimental section

General- Avidin and HABA were purchased from Sigma-Aldrich and used without further purifications. Milli-Q water, Ethanol, and other general chemicals were purchased from Spectrochem, Mumbai, India, and used without further purification.

Peptide synthesis – Biotinylated peptide (1) used in this study was synthesized by using standard protocol mentioned in the research paper published by our group.^{1,2} Purity and identity was also confirmed prior to use.

Atomic Force Microscopy (AFM) – Neat and co-incubated sample (at 37 °C for 0-1 day in ethanol/water) of diatoms and peptide-diatoms solution were imaged with an atomic force microscope. The solution was incubated at ambient temperature for 3-12h followed by centrifuging. The supernatant was decanted which contains the unbounded peptide and diatom solution. The residue (pellet) was washed with methanol followed by milli-Q water and redissolved it into appropriate amount of milli-Q water. The 5 µL aliquots of this sample were fixed onto fresh and neat glass surface by using methanol as a fixing agent. The samples were dried at dust free space under 60W lamp for 4h followed by high vacuum drying and subsequently examined under atomic force microscope (AFM) (INNOVA, ICON Analytical Equipment, Bruker, Sophisticated Instrument Center-Dr. Harisingh Gour Central University, Sagar-M.P.) operating under the Acoustic AC mode (AAC or Tapping mode), with the aid of a cantilever (NSC 12(c) from MikroMasch, Silicon Nitride Tip) by NanoDrive[™] version 8 software. The force constant was 2.0 N/m, while the resonant frequency was ~280 kHz. The images were taken in air at room temperature, with the scan speed of 1.0-1.5 lines/sec. The data analysis was done using of Nanoscope Analysis Software. The sample-coated substrates were dried at dust free space under 60W lamp for 6h followed by high vacuum drying and subsequently examined under AFM.

Scanning Electron Microscopy (SEM) - 20 μ L aliquot of the fresh and aged samples of diatoms (Number of diatoms ~4x10⁶/ μ L) were dried at room temperature on a glass mirror (5x5x1mm³) surface and coated with gold. Scanning electron microscopy

images were made using a FEI QUANTA 200 microscope equipped with a tungsten filament gun operating at WD 10.6 mm and 20 kV.

Fluorescence studies- Fluorescence spectra were recorded on RF-5301PC Spectrofluorophotometer (Shimadzu) with a 10 mm quartz cell at room temperature. Fluorescence lifetime was measured by single photon counting method using a commercial setup from Jobin-Yvon. For lifetime measurement, Samples was excited at 287 nm using a nano-LED.

To investigate the observed quenching in fluorescence intensity is result of FRET or PET, we performed the following experiments and our observations reveal that the observed quenching in fluorescence intensity is solitary result of Photo-induced electron transfer (PET) process, not by Forster resonance energy transfer (FRET) process.

Photoinduced electron transfer (PET) is usually de-excitation pathway for fluorescence chemosensors. PET systems are composed of two components, a fluorophore, and an electron donor. The electron donor component typically possesses a non-bonding electron pair, typically of a nitrogen atom, whose energy lies between the HOMO/LUMO gaps of the fluorophore. Electron transfer from the non-bonding lone pair of the electron donor to the excited state fluorophore, quenches fluorescence.⁴ For PET, the energy of the fluorophore's excited state must be sufficient to oxidize the electron donor and reduce the fluorophore. To prove that, first, we have taken well-studied pure tryptophan molecule and monitor its fluorescence quenching by acrylamide (Fig. S1a & c). It has been well established that the fluorescence quenching of tryptophan by acrylamide is caused only by photo-induced electron transfer.⁴⁻⁶ The obtained Stern-Volmer constant is 21.14 M⁻¹. In a similar fashion, now we took Biotin-Trp-Trp-OH (1) in place of tryptophan, here, we obtained similar observation (Fig. S1b, c) with different Stern-Volmer constant (17.74 M⁻¹) and, a case of PET process. Now, when we replaced quencher molecule (i.e. acrylamide) by diatom, we observed monotonous decrease in fluorescence intensity of 1 by adding diatom solution which is believed to be a PET process. Another reason is not to be believe in FRET process, because FRET is special technique to determine the distance between two chromophores (i.e. fluorophores) and highly dependent on the distances between two chromophores.⁴⁻⁶ An excited state donor fluorophore transfers energy (not an electron) to an acceptor fluorophore through a non-radiative process. In present case, 1 and diatom do not act ideal FRET system. In principle, diatom must act as energy acceptor, however, diatom literally unable to accept the energy released by excited molecules of 1(Fig. S1d). In other words, this is an absolute energy mismatch system for FRET process.



Figure 1. Reduction in fluorescence intensity of (a) pure L- Tryptophan and (b) Biotin-Trp-Trp-OH (1) with increasing concentration of acrylamide, (c) Stern-Volmer plot for fluorescence quenching of pure tryptophan and 1 using acrylamide and (d) fluorescence spectra of 1 in blue color and absorption spectrum of diatom depicts in red color.

Determination of Florescence quenching Constant:

To know the fluorescence quenching quantitatively, the quenching constant was determined by Stern-Volmer equation as

$$\frac{I_o}{I} = 1 + K_{SV} [\text{no. of diatoms}]$$
$$= 1 + k_a \tau_o [\text{no. of diatoms}]$$

In the above equation, I_0 and I are the fluorescence intensity of 1 in absence and presence of the diatoms, K_{SV} and kq are the Stern-Volmer constant and bimolecular quenching constant. [no. of diatoms] represents the number of diatom species in solution. τ_0 is average life time of molecule 1.

Uv/Vis experiments:- UV-Vis absorption spectra were recorded on LabIndia UV-VIS Spectrophotometer 3000⁺ with 10 mm quartz cell at RT.

Procedure for cell culture of Diatoms and Preparation of Permanent slide:-

Water sample was collected from the Bebas River, Sagar district (PIN: 470003), M.P, India and after centrifugation at 4000r.p.m for 5min and its further cleaning with milli-Q water, the pellet was inoculated in a modified f/2 media.²⁴ Diatom colonies obtained were serial diluted to obtain axenic cultures of *Nitzschia palea*. The number of diatom cells were counted using a Neubauer chamber Bürker, 1.00 mm depth, 1.00 mm² on 10th day of culture.²

Cell Count :

Number of trials	Total no. of counted cell(s)/10 μL (on 10 th day of
	incubation)
1	36026000
2	40400000
3	30347000
4	39236000
5	43400000
Average Cell Count = 37881800/10μL	



Figure S2: Bar graph of diatom samples is showing the cell count/10 μ L on 10th day of inoculation.



Figure S3: Top: (A and B) OM and Bottom: (c) SEM images of N.Palea.



Figure S4: UV-Vis titration spectra of compuond 1 in the presence of increasing concentration of diatoms(N. *palea*) solution depicts the interaction of 1 with diatom cell wall.

HABA assay with avidin and 1

The binding stoichiometry between 1 and avidin was determined by means of the HABAassay. Titration of a solution of saturated HABA with 1 results in a decrease in the UV/Vis absorbance at 500 nm, in which HABA is expelled from the biotin binding pockets of avidin (see Fig.S5). The binding stoichiometry (N) between 1 and avidin is deduced from the inflection point, yielding N = 4.20 (see Fig.S6).



Figure S5: UV/Vis back titration of HABA-saturated avidin with 1 in water.



Figure S6: Area under UV/Vis spectra of HABA-avidin complex versus the ratio between conc. of 1 and avidin.

Determination of binding/association constant of 1- avidin complex

The binding stoichiometry between 1 and avidin has been measured using the Job's plot method through the change in area under the UV/Vis spectra of HABA-avidin complex. This clearly indicates that 1:4.2 stoichiometric complex of 1 and avidin is present in the water (Fig. S6). For the binding constant measurement, we have added 1 in HABA saturated avidin and measured the change in absorbance intensity at 500 nm with an increase in the 1 concentration, where we have observed a monotonous decrease in absorbance intensity of HABA-avidin complex (Fig. S6). For 1:4 complexation between 1 and avidin, the overall equilibrium can be written as

$$A + 4B = AB_4 \tag{1}$$

where A is the Avidin; B is the 1 and AB_4 is the complex. The association constant of complexation is written as

$$K_{a} = \frac{[AB_{4}]}{[A][B]^{4}}$$
(2)

Initial concentration of Avidin and 1can be expressed as

$$[A_{o}] = [A] + [AB_{4}]$$
(3)

$$[B_o] = [B] + 4[AB_4] \tag{4}$$

By using equation (3) and (4), we reformulate the equation (2)

$$\frac{[AB_4]}{([A_o] - [AB_4])} = K_a ([B_o] - 4[AB_4])^4$$
(5)

According to Beer Lambert law, $OD = \varepsilon cl$ or $OD = \varepsilon c$ where OD is the measured absorbance, ε is a wavelength-dependent absorptivity coefficient, *l* is the path length (1 cm), and *c* is the concentration. We modified equation 5 as

$$\frac{OD_{AB_4} / \varepsilon_{AB_4}}{\left(\!\left[A_o\right] - OD_{AB_4} / \varepsilon_{AB_4}\!\right]\!\!} = K_a \left(\!\left[B_o\right] - 4 OD_{AB_4} / \varepsilon_{AB_4}\!\right)\!\!\! \right)$$
(6)

Assuming overall concentration of avidin is being used to form either in HABA-avidin (AH) and Biotin-Trp-Trp-OH-avidin (AB₄) complex or both.

$$[A_o] = [AB_4] + [AH] \tag{7}$$

Rearranging equation 7 with the help of Beer Lambert's law

$$\frac{OD_{AB_4}}{\varepsilon_{AB_4}} = [A_o] - \frac{OD_{HA}}{\varepsilon_{HA}}$$
(8)

Now, the equation 6 is again reformulated as,

$$Y = K_a X^4 \tag{9}$$

where, $Y = \frac{OD_{AB_4} / \varepsilon_{AB_4}}{(A_o] - OD_{AB_4} / \varepsilon_{AB_4}}, X = (B_o] - 4OD_{AB_4} / \varepsilon_{AB_4}$) The binding/association constant

 K_a was determined from the plot of X vs. Y as per equation 9. The estimated value of binding constant by fitting is found to be 6. 13 x10¹⁸ M⁻⁴ (Fig. S7).



Figure S7. Determination of binding/association constant between between 1 and avidin in water using plot of $Y = \frac{OD_{AB_4}}{\varepsilon_{AB_4}} / \left([A_o] - \frac{OD_{AB_4}}{\varepsilon_{AB_4}} \right)$ vs. $X = [B_o] - 4 \frac{OD_{AB_4}}{\varepsilon_{AB_4}}$.

Interaction of Diatom (N. palea) alone with HABA saturated Avidin:

The HABA saturated sample of avidin was further treated with diatoms in water. The UV/Vis spectra of treatment is shown in Fig. S8, which indicates a weaker interaction of diatom with complex.



Figure S8. UV/Vis titration of HABA-saturated avidin with diatoms in water.

Similarly, we have treated HABA-saturated avidin sample with overnight incubated peptide bounded diatoms. These observations are also well corresponded with the freshly coincubated sample of peptide and diatoms.



Figure S9. UV/Vis back titration of HABA-saturated avidin sample with coincubated sample of diatom with **1** in water.



Figure S10. Area under UV/Vis spectra of HABA-avidin complex versus the ratio between concentration of coincubated sample of 1 and Diatoms/avidin.



Figure S11 *Left*: figure depicts that fluorescence quenching of **1** upon increasing number of diatoms, *Right*: Stern-Volmer plot for steady-state fluorescence quenching of tryptophan containing peptide using diatoms.



Figure S12. UV/Vis titration of HABA with avidin which results appearance of HABAavidin complex at 500 nm.



Figure S13. The AFM micrograph of prolonged treated diatom with peptide 1 showing the stability of peptide nanostructures over the diatom surface. (A) 2D and (B) corresponding 3D images of diatom fractions depicted that over deposition of nanostructures 1(marked by arrow), (C) high resolution 2D image clearly shows the over deposition which further confirmed by the (3D) image of different region. This experiment demonstrated the stability of peptide nanostructures in the presence of diatoms.



Figure S14. Few addition 2D and their corresponding 3D high resolutions AFM micrograph of prolonged treated diatom with peptide 1, clearly showing the beautiful hierarchical nanoarray formations over the surface of diatoms without affecting and structure.

Supporting References:

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