

# Photon induced separation of bio-nano hybrid complex based on carbon nanotubes and optically active bacteriorhodopsin

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**Abstract:** We report optically induced rapid aggregation and subsequent separation of selective single-walled carbon nanotubes (SWNT) functionalized with bacteriorhodopsin. Induced aggregation rate depends on the absorption of bacteriorhodopsin. Optically separated, bio-nano hybrid complexes show stable, preferential binding with SWNTs of specific diameters whereas, unbound SWNTs remains well-dispersed in the solution.

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Bio-nano hybrid complex are materials of active interest due to its immense potential in diverse applications such as bio-mimic devices, sensors, electro-optics, biotechnology, cancer therapy and diagnostics etc [1–4]. Biomolecules, specifically optically active purple membrane (PM) have long been studied for photonic and electro-optic applications. Bacteriorhodopsin (bR) is the optically active center of the PM, which functions as a photo-induced proton pump by converting optical energy into proton gradient across the membrane [5]. bR amounts to 75% of the PM by mass, while the other 25% is due to lipids. Bacteriorhodopsin is a photosensitive seven helix transmembrane protein usually found in the halophilic bacteria, *Halobacterium salinarum*. It is a rare molecule found naturally in crystalline form with size ranging from 400 nm – 800 nm and thickness of 5-10 nm [6]. Crystalline structure provides required chemical and thermal stability to the inner bacteriorhodopsin surrounded by lipids. Due to these intrinsic properties of the PM, including high thermal stability, ability to form thin films with excellent optical and proton transport properties, the PM is widely proposed as an excellent optical material for myriad of applications [7].

Due to unique optoelectronic properties, one dimensional single walled carbon nanotubes (SWNT) are also considered as a promising material for various electro-optical, photonic devices and biosensors [8–10]. Stable donor-acceptor systems, based on optically active bio-nano hybrid complex of PM and SWNT, may play an important role in the development of next generation optical devices. Moreover, solutions of SWNTs provide opportunity to study interesting effects in colloidal solution of one dimensional particle. Stability and controlled assembly are critical challenges for colloidal systems and "bottom-up" assembly of nanoparticles in solution. This exertion describes optically induced aggregation and subsequent separation of pristine SWNTs of selective diameters, preferentially functionalized with purple membrane. Fundamental understanding of these processes should lead to a better stability, directed assembly and controlled charge transfer in donor-acceptor systems based on SWNTs.

SWNT functionalized with optically active molecules, electron-donating polymers and dyes are well- reported [11–13]; however, low solubility of SWNTs in aqueous dispersions limits its applicability, especially functionalization with bio-molecules. This limitation is overcome using bio compatible amine based surfactants. Stable aqueous dispersion of SWNTs has been formed using well established protocol of non-covalent functionalization by PL-PEG [14]. Ultrahigh Purified SWNT were purchased from NanoIntegrals and were used as such without any further purification. Stable dispersions of the SWNTs were made in water using 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine ( $C_{41}H_{82}NO_8P$ ) powder, PL-PEG amine as a surfactant, which was used as purchased from Avanti, Polar Lipids, Inc. Stable SWNT dispersion was prepared by sonication of pristine, hydrophobic nanotubes in aqueous solutions of above mentioned amphiphilic polymer. The hydrophobic lipid chains of PL-PEG

amine strongly anchors to the nanotube surface, whereas the hydrophilic amine chain provides SWNT with water solubility and biocompatibility. Prepared aqueous solutions of SWNT provides for new functionalities to be added to the nanotubes, using bio-molecules such as bacteriorhodopsin. Prepared aqueous solution of SWNTs showed excellent stability for weeks.

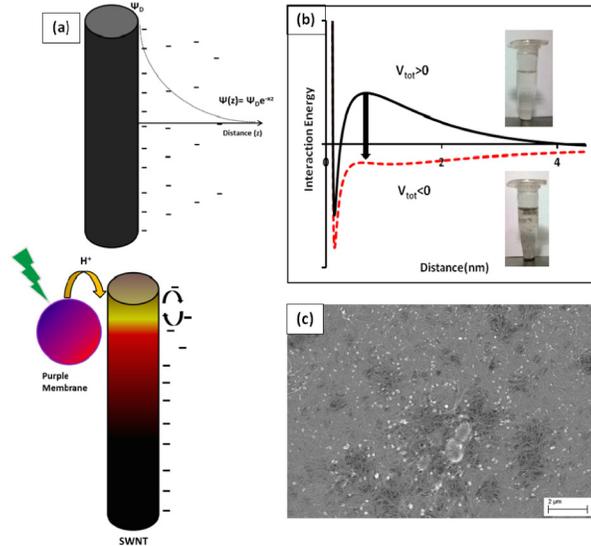


Fig. 1. (a) Conceptual model of light induced aggregation of PM functionalized SWNTs due to charge transfer from optically active PM. (b) Interaction and inhomogeneous surface charges leads to aggregation due to decrease in repulsive potential. (c) SEM of aggregated PM functionalized SWNTs showing random surface binding of PM with SWNTs.

Optoelectronic and thin film applications demand high quantity (several milligrams) of the PM with better optical properties [15]. Hence, Halobacterium cultivation was performed in a 7 liter photo-bioreactor to give high yield of 14.4 mg/l. 79.36 micro molar ( $\mu\text{M}$ ) from the stock protein was used for further functionalization with SWNTs. SWNTs tend to coagulate, steadily with the PM over 24 hours, in dark. However, prepared aqueous solution of PM-SWNT was irradiated horizontally through a slit, by broadband mercury white lamp (New port, oriel instruments, USA) for time periods in ratio of [1:2:3:4] starting with 1hour. Corresponding to each of the samples kept under light exposure, samples of the same concentrations were also kept in dark for similar duration. Figure 1(a) illustrates the experimental set up and conceptual model for our observation of optically induced aggregation of the functionalized SWNT dispersions. Pristine dispersions of SWNT do not show any change in concentration over the course of these experiments. Although, PM functionalized SWNTs coagulate very slowly in dark; optically induced rapid aggregation is observed for the PM functionalized SWNTs, kept under broadband illumination.

DLVO theory named after Derjaguin, Landau, Verwey and Overbeek is traditionally used to understand stability of colloidal solutions. Even with its inherent limitation, due to assumptions on size, charge dielectric properties and dimensions of interacting particles, it is widely being used to understand fundamental interactions in solutions of nanoparticles [16,17]. Bhattacharya [18] recently asked if light has the ability to aggregate particles. This is an interesting question of wider interest. Although optical effects in solutions of gold nanoparticles and functionalized SWNT have limitedly been reported, the phenomenon is not well understood [19,20]. Here, we attempt to provide theoretical frame work for our experimental observations. According to the theory, total interaction potential ( $V_{tot}$ ) of two

interacting spheres with radius  $R_1$ ,  $R_2 \gg D$ , the inter-particle distance is given by

$$V_{tot} = \left( \frac{-A_H}{6D} + Ze^{-\kappa D} \right) \left[ \frac{R_1 R_2}{R_1 + R_2} \right].$$

Here, the first term is due to the attractive Van der Waals

force and depends on the Hamaker constant  $A_H$ . Hamaker constant is related to the dielectric properties of the system. For  $\epsilon_i$  being the dielectric constants and  $n_i$  the refractive index of SWNTs and medium (water) respectively,  $\nu_e$  being the frequency of lowest electron transition:

$$A_H \approx \frac{3}{4} K_B T \left[ \frac{\epsilon_1 - \epsilon_3}{\epsilon_1 + \epsilon_3} \right] \left[ \frac{\epsilon_2 - \epsilon_3}{\epsilon_2 + \epsilon_3} \right] + \frac{3hV_e}{8\sqrt{2}} \times \frac{(n_1^2 - n_3^2)(n_2^2 - n_3^2)}{\sqrt{(n_1^2 + n_3^2)}\sqrt{(n_2^2 + n_3^2)}[\sqrt{n_1^2 + n_3^2} + \sqrt{n_2^2 + n_3^2}]} \quad (1)$$

Since, Hamaker constant is related to dielectric properties of the system, the dielectric response of SWNTs functionalized with optically excited PM is expected to be different from that of pristine SWNTs, especially under conditions of significant resonant, absorption. Hence, changes in the dielectric response of functionalized SWNT should lead to consequent changes in the constant  $A_H$ . Moreover, the second term in the total interaction energy is due to repulsive electrostatic double layer depending on the Debye length ( $\kappa$ ) and interaction constant  $Z$  [16–21]. Since both of these factors significantly depend on the surface charges, changes in surface charge are expected to considerably affect the repulsive potential. DLVO theory considers either constant surface potential or charge density of the interacting particles, such as synthetic latex particles of uniform sizes and properties. However, interactions between non-uniformly charged surfaces are complex and correspondingly complicated. In a bio-nano hybrid complex, both the surface potential and charge density may change due to functionalization, especially under conditions of optical excitation and charge transfer. Theoretical studies show colloidal instability and aggregation of particles as a consequence of charge non uniformity on colloidal interactions [6]. Moreover, AFM, TEM images of SWNTs functionalized with PM [22], show random side-wall binding on SWNTs and conformational changes in the adsorbed PM. Hence, non uniformity in surface charges in a one-dimensional SWNT due to random surface functionalization by an asymmetrically charged PM, is expected to play a critical role in the stability of the solution. PM is formed of optically active bR, along with the asymmetrically located charged lipids. Upon optical excitation (500nm–600nm), photoisomerization has been reported causing conformational changes in the bR protein and initiating proton pumping along the membrane [23,24]. Conformational changes and random surface binding of PM on SWNTs, predictably results in a complex system with asymmetric charge distribution. Optical excitation in such a system should affect the repulsive double layer because of enhanced charge non uniformity of surface charges at local binding sites. Hence, observed colloidal instability under optical illumination may be due to cumulative effect of changes in the dielectric and local charge regulation and charge transfer interaction between the SWNTs and optically excited “proton pump” PM.

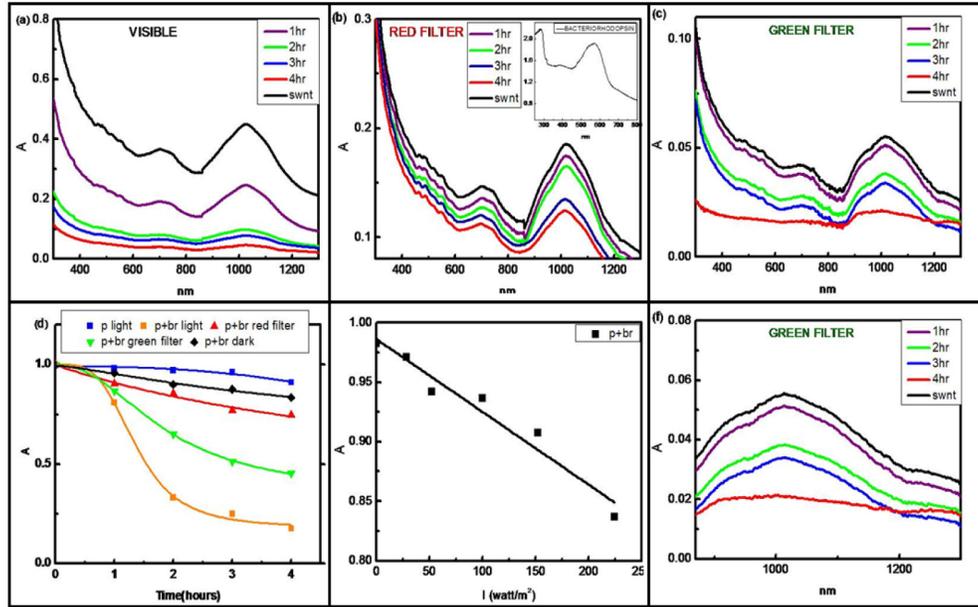


Fig. 2. (a) Absorption spectra of SWNTs functionalized with PM (bacteriorhodopsin) kept in broadband light for varying time intervals from 1 to 4 hours (top to bottom). Inset shows absorption spectra of bacteriorhodopsin with its maximum peak at 570 nm. (b) Showing optically induced rate of aggregation in PM functionalized SWNTs as compared to pristine SWNTs and PM functionalized SWNTs kept in the dark. Enhanced aggregation is seen for green light as compared to red illumination.

Figure 2 illustrates absorption spectra for supernatant of optically aggregated PM functionalized SWNT dispersions. Pristine dispersions of SWNT do not show any change in concentration over the course of these experiments and very slow rate of aggregation is observed for PM functionalized SWNTs kept in dark; however, non linear, rapid aggregation is observed for the PM functionalized SWNT under a broadband illumination. Absorption spectra of SWNTs in aqueous solution show characteristic Van Hove singularities with peaks in VIS/NIR region due to semi-conductor SWNTs, and background absorption in UV and visible frequency is essentially because of plasmonic resonance of metallic SWNTs. Absorption spectra show a consistent decrease in concentration of SWNTs with illumination for different time intervals from one to four hours. To understand the dependence on frequency of illumination on peak absorption of the PM, the phenomenon was further studied using red and green band pass filters. Significantly enhanced aggregation is observed for green filter compared to red for normalized optical intensity. Rate of aggregation under red and green illumination is found to be linear, as compared to logarithmic decrease in concentration, in case of broadband illumination. Absorption spectra of the pure PM (inset Fig. 2) with molar absorption coefficient  $63,000^{-1} \text{ cm}^{-1}$  show broad band absorption with  $\lambda_{\text{max}}$  at 570 nm. In the range 350-470 nm, PM absorption does not exceed 20% of  $\lambda_{\text{max}}$ , whereas absorption significantly decreases beyond 600 nm into visible NIR frequency. Significantly, higher rate of aggregation for green illumination as compared to red emphasizes the dependence of phenomenon on the absorption of PM.

Consistent, linear increase in rate of aggregation is observed with corresponding increase in the intensity of broadband illumination, as shown in Fig. 2(e). NIR absorption band of SWNTs are related to the band gap energy of the semiconducting nanotubes [25]. Hence, discernible changes in the NIR absorption of the separated supernatant with increase in duration of light exposure (Green) indicate preferential binding of the PM with selective diameters of SWNTs as shown in Fig. 2(f).

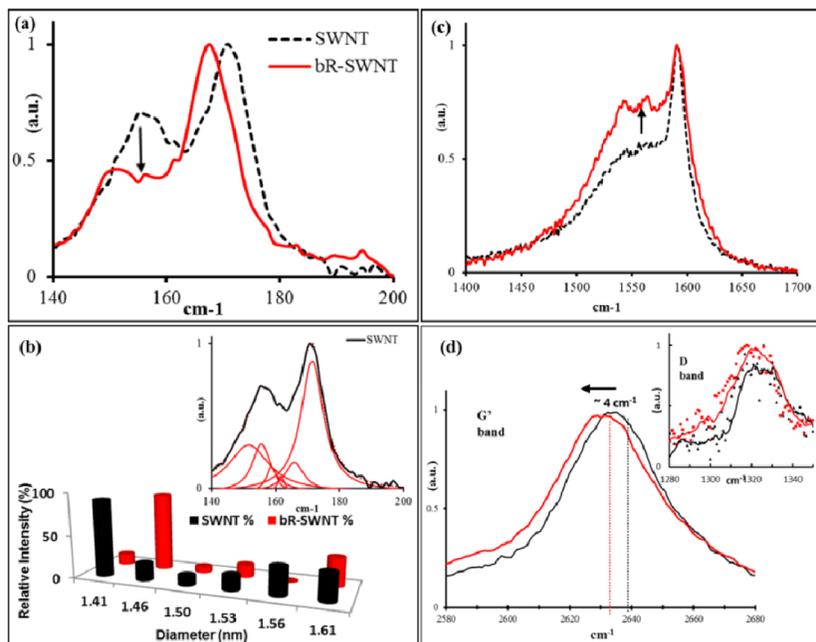


Fig. 3. (a) RBM showing preferential binding of PM with SWNTs of specific diameters (red line) as compared to pristine SWNTs (black line). (b) Histogram as plotted using multiple peak fit in RBM showing relative enrichment in SWNTs of specific diameters. (c) *G* band Raman spectra showing increase in *G* band ~1540-1560  $\text{cm}^{-1}$  in optically aggregated SWNTs functionalized with PM (red solid line) as compared to control pristine SWNTs (black dotted line). (d) *G'* band shows slight red shift indicating strong binding in PM functionalized SWNTs. D band (inset)

Samples were then centrifuged for 10 minutes at 5,000 rpm (Eppendorf® Mini Spin Centrifuge) to carefully separate the supernatant and aggregated floc from the solution. Raman spectrometer, labRAM, with 632 nm laser is used to further investigate the specific binding of the PM functionalized SWNT complexes. Figure 3(a) compares Radial breathing modes (RBM) of pristine SWNTs and that of aggregated floc, separated after 240 minutes of broadband visible illumination. RBM are due to a radial motion of atoms perpendicular to the axis and the resonant frequency is inversely related to the diameter of the tube by  $\omega_{RBM} = (\alpha_{RBM} / d) + \alpha_{bundle}$ . Where,  $d$  is the diameters of nanotubes and  $\alpha_{RBM}$  ( $223.5 \text{ cm}^{-1}$ ),  $\alpha_{bundle}$  ( $12.5 \text{ cm}^{-1}$ ) are constants, respectively [25]. Histogram, as shown in Fig. 3(b) is plotted using RBM spectra, by fitting multiple Lorentzian peaks, representing individual diameters of SWNT in the pristine, control solution, and ones in the aggregated floc. As compared to pristine SWNTs, aggregated floc of SWNTs functionalized with bR shows preferential enrichment of SWNTs with the specific diameters especially of 1.45 nm, where as significant loss of intensity in RBM peaks corresponding to the larger diameter of SWNTs is observed. *G* and *G'* band in Figs. 3(c)-(d), show specific binding and strong interaction between the bR and SWNTs to form stable functional complexes. *G* band in pristine SWNT shows narrow *G*<sup>+</sup> Lorentzian peak at  $1590 \text{ cm}^{-1}$  and broad *G* peak around  $1540 \text{ cm}^{-1}$  due to TO (circumferential) and LO (axial) modes [26]. As reported, functionalized SWNT show changes in *G* band due to interaction between electron donors/acceptor molecules [27]. Significant increase in the characteristic *G* band is observed for functionalized SWNTs as compared to pristine SWNTs. Hence, this discernible changes in the *G* feature of the functionalized SWNT is most likely due to charge transfer and interaction between optically active bacteriorhodopsin and SWNTs. Moreover, the observed red shift of ~4-5  $\text{cm}^{-1}$  in *G'*

band in Fig. 3(d) indicates strong binding and surface interaction between the PM and SWNTs. Moreover, as expected no discernable changes are observed in the D band (Fig. 3(d) inset), confirming that this non-covalent functionalization does not affect the  $Sp^2$  carbon hybridization; thus, retaining pristine electro-optical and charge transport properties of SWNTs.

Understanding fundamental interactions in solutions and using controlled external forces is important for bottom-up, directed assembly of bio-nano hybrid devices. Surely, more research is needed to better understand complex interactions in SWNT functionalized with an optically active PM. However, our results confirm optically induced aggregation and consequent separation of the PM functionalized SWNT of selective diameters from solution. Enhanced aggregation is observed for optical frequency close to peak absorption frequency of bR, indicating strong interaction between the optically active PM and SWNT. The aggregated floc shows enrichment of SWNTs of specific diameters, indicating selective binding of PM with specific SWNTs. These stable bio-nano hybrid complexes based on SWNTs functionalized with optically active PM indicate promising potential for electro-optical applications. Moreover, the PM functionalized SWNT provides opportunity to better understand interactions in colloidal solutions of optically active, donor-acceptor systems. Moreover, this functionalized complex also provides stable system for fundamental studies on charge transfer and interaction between bio-nano hybrid interfaces in solution. Optically induced, selective aggregation of SWNTs functionalized with PM certainly promises potential application in optically induced separation of other functionalized nanoparticles from solution, too. In conclusion, we report optical induced aggregation and separation of stable bio nano hybrid complex of selective diameter of SWNTs, preferentially functionalized with PM.

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