Insight into Carbon quantum dot-vesicles interaction: Role of functional groups

Jayanta S. Boruah[a,b] , Kamatchi Sankaranarayanan[c] and Devasish Chowdhury*[a]

[a]Material Nanochemistry Laboratory, Physical Sciences Division, Institute of Advanced Study in Science and Technology, Paschim Boragaon, Garchuk, Guwahati-781035, India

[b]Department of Chemistry, Cotton University, Guwahati, Assam, India

[c]Physical Sciences Division, Institute of Advanced Study in Science and Technology, Paschim Boragaon, Garchuk, Guwahati-781035, India

Electronic Supplementary Information

Characterization:

Characterization of citric acid carbon dot (CACD):

The synthesis of citric acid carbon dot (CACD) has been described earlier in the experimental section of the main manuscript. The resultant brown colour CACD solution was characterized by various tools. The particle size was determined by DLS analysis (**Fig S1A**) and TEM image (**Fig S1B**) which indicates that size lies below 10 nm. Zeta potential was calculated and found to have -16 mV as shown in **Fig S1C**. UV-Vis spectrum was also recorded and it possesses a weak absorption peak near 335 nm (**Fig S1D**) arising due to n–π^* transition of C=O bond and another at 220 nm due to π–π^* transition of C=O bond which is not shown here. The spectrum is similar to earlier report.[1] The fluorescent CACD was run to get PL spectrum and the maximum emission at 425 nm (λ_{em}) was observed when excited at 310 nm (λ_{ex}) **(Fig S1E)**. The inset of **Fig S1E** shows light green fluorescence of CACD under UV light (365 nm). The carbon dot was analysed to get the fluorescent decay profile and life time in TRPL instrument and found that it has three lifetimes (τ = 0.9, 3.01, 7.83 ns) as represented in **Fig S1F**. The functional group present on the carbon dot was determined using FTIR spectrometer and results were included in the **Fig S9A** below as comparative spectra and discussed in the results section of the main manuscript.

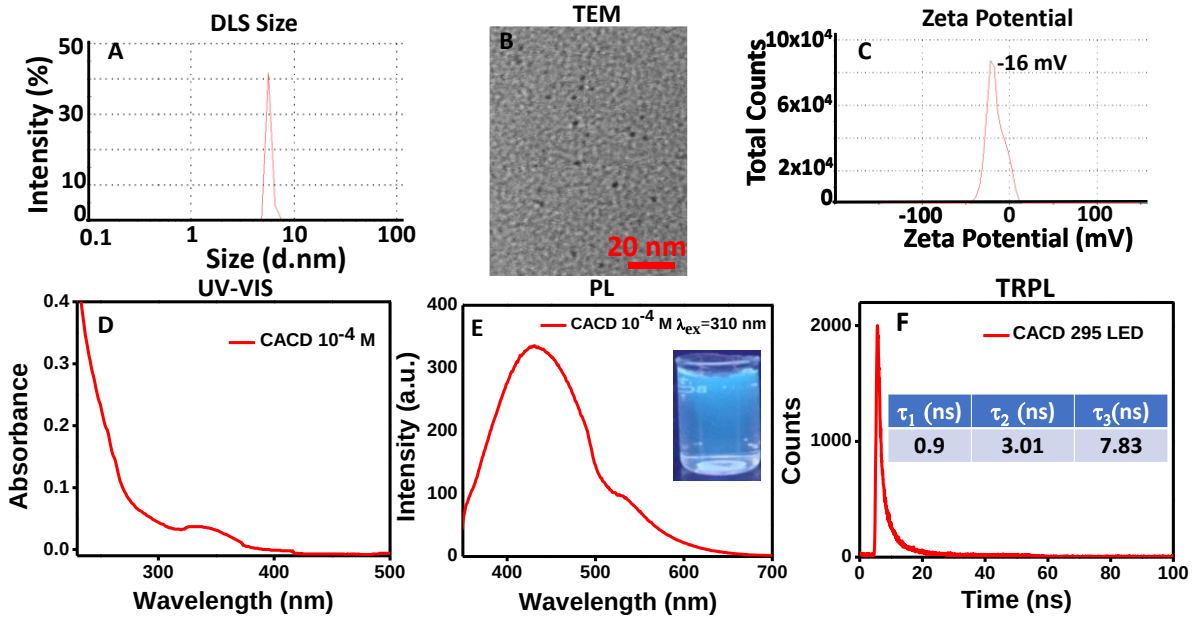

Fig S1: A) Particle size (DLS) B) TEM image C) Zeta potential D) UV-Vis spectrum E) PL spectrum (The light green fluorescence of CACD under UV light is shown in the inset) and F) TRPL decay profile containing lifetime of the CACD. (In all the cases, water is taken as solvent)

Characterization of Polyethyleneimine carbon dot (PEICD):

The microwave treatment has been employed to synthesize the Polyethyleneimine carbon dot (PEICD) as mentioned earlier in the experimental section of the main manuscript. The carbon dot is analysed by different spectroscopic methods. The speciality of carbon that is size below 10 nm was appeared in by DLS analysis (**Fig S2A**) and TEM image (**Fig S2B**). The surface charge was found to be +39 mV measured from Zeta potential data (**Fig S2C**). The sample was run to get UV-Vis spectrum and found that it has two absorption peak, one near 225 nm (due to $\pi-\pi^*$ transition) and other one at 360 nm (arising due to $n-\pi^*$ transition) as shown in **Fig S2D**. It is interesting to note that the PEICD prepared in microwave synthesis reactor (Anton Paar, Monowave 200) could form two UV-Vis peaks while the earlier report showed only one peak near 350 nm.[2] The pH adjustment to 7 may have some role on the observed UV-Vis spectrum. The PL spectrum of PEICD was recorded and it gives maximum emission at 465 nm (λ_{em}) was observed when excited at 340 nm (λ_{ex}) **(Fig S2E)**. The inset of **Fig S2E** shows green fluorescence of PEICD under UV light (365 nm). The fluorescent decay profile and life time of PEICD were measured in TRPL instrument and the observed three lifetimes (τ) were (1, 4.03, 9.91 ns) as shown in **Fig S2F**. The functional group present on the carbon dot was determined using FTIR spectrometer and results were included in the **Fig 4B** as comparative spectra in the main manuscript.

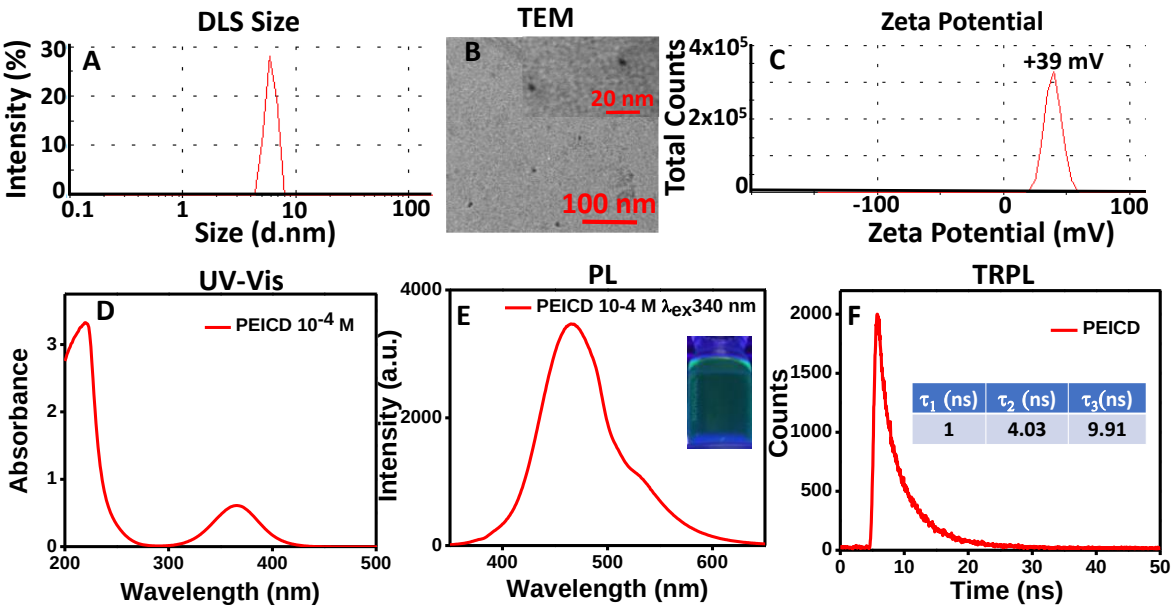

Fig S2: A) Particle size (DLS) B) TEM image C) Zeta potential D) UV-Vis spectrum E) PL spectrum (The green fluorescence of PEICD under UV light is shown in the inset) and F) TRPL decay profile containing life time of the PEICD. (In all the cases, water is taken as solvent)

Characterization of Polyethylene glycol carbon dot (PEGCD):

The synthesize the Polyethylene glycol carbon dot (PEGCD) was based on an earlier mentioned in the experimental section of the main manuscript. The carbon dot produced through pyrolysis was undergone different spectroscopic techniques. DLS analysis was performed and size of the PEGCD lies below 10 nm (**Fig S3A**). The TEM image (**Fig S3B**) has also same size with spherical shaped particles. PEGCD was recorded to have surface charge of -16 mV (**Fig S3C**) which can be attributed the surface -OH group on the carbon dot. The UV-Vis spectrum was taken and shown in **Fig S3D**. Here, one absorption peak near 225 nm was observed (due to $\pi-\pi^*$ transition) and another one very weak (a small hump) at 280 nm (arising due to $n-\pi^*$ transition of -OH bond). The PL spectrum of PEGCD was recorded as represented in **Fig S2E.** It shows maximum emission near 460 nm (λ_{em}) when excited at 370 nm (λ_{ex}). There was some shifted peak here contrary to the earlier report which has emission peak near 400 nm.[3] This may arise from the point of pH adjustment to 7. It also has another small peak near 530 nm which may be present due to excess polyethylene glycol in the mixture. The green fluorescence of PEGCD under UV light (365 nm) was included in the inset of **Fig S3E**. The fluorescent decay profile and life time of PEGCD are also relevant and were measured in TRPL instrument. It has three lifetimes (τ) value viz. 1.28, 3.95, 14.76 ns as shown in **Fig S3F**. The functional group present on the carbon dot was determined using FTIR spectrometer and results were included in the **Fig S9B** below as comparative spectra and discussed in the results section of the main manuscript.

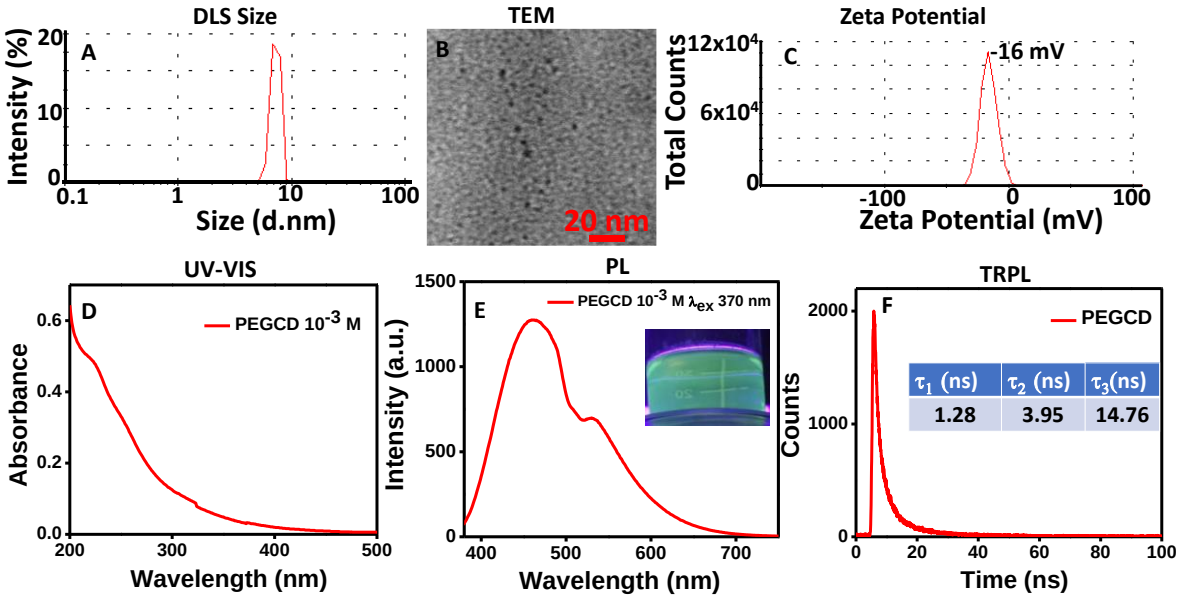

Fig S3: A) Particle size (DLS) B) TEM image C) Zeta potential D) UV-Vis spectrum E) PL spectrum (The bright green fluorescence of PEGCD under UV light is shown in the inset) and F) TRPL decay profile containing lifetime of the PEGCD. (In all the cases, water is taken as solvent)

UV-Vis spectrum of BSA and PL behavior during preparation BSA coated Polyethylene glycol carbon dot (PEGCD BSA):

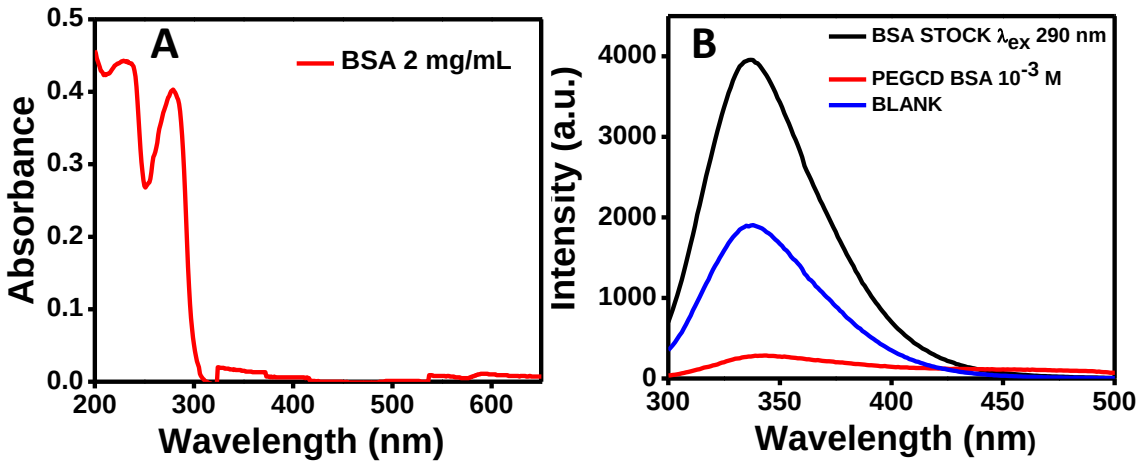

Fig S4: A) UV-Vis spectrum of BSA in water (10^{-5} M or 2 mg/mL) B) PL quenching study to confirm the preparation of BSA coated PEGCD (PEGCD BSA)

Characterization of BSA coated Polyethylene glycol carbon dot (PEGCD BSA):

The BSA coated Polyethylene glycol carbon dot (PEGCD BSA) was prepared as discussed in the experimental section of the main manuscript. Various spectroscopic techniques were employed to PEGCD BSA to characterise well. The size of the nanoparticles was measured in DLS analysis and TEM as represented in **Fig S5(A-B)**. It reveals size of the PEGCD BSA below 10 nm. Zeta potential was also recorded and PEGCD BSA has surface charge -11 mV (**Fig S5C**). Slight change of zeta potential from -16 mV (for PEGCD) to -11 mV (PEGCD BSA) may be due to BSA (bovine serum albumin) coating on the carbon dot. The **Fig S5D** represents UV-Vis spectrum of the carbon dots which contains two absorption peaks, one near 225 nm was observed (due to $p-\pi^*$ transition) and another one very weak (a small hump) at 280 nm (arising due to $n-\pi^*$ transition). Actually, BSA has UV-Vis peaks near 230 nm and 280 nm in water which has been added in **Fig S6** below for references. So, the present peak in case of PEGCD BSA may come from the BSA coating onto the carbon dot. The PL spectrum as shown in **Fig S5E** shows maximum emission near 465 nm (λ_{em}) when excited at 370 nm (λ_{ex}). Here, the PL emission peak shifted slightly compare to PEGCD (460 nm) indicating BSA coating on the surface of the carbon dot. The inset of **Fig S5E** is the representation of the green fluorescence of PEGCD BSA under UV light (365 nm). The fluorescence life time was also determined in a TRPL instrument. It was found to have three lifetimes (τ) value viz. 1.15, 3.87, 14.6 ns as shown in **Fig S5F**. The corresponding decay profile has been included. The functional group present on the carbon dot was determined using FTIR spectrometer and results were included in the **Fig S9C** below as comparative spectra and discussed in the results section of the main manuscript.

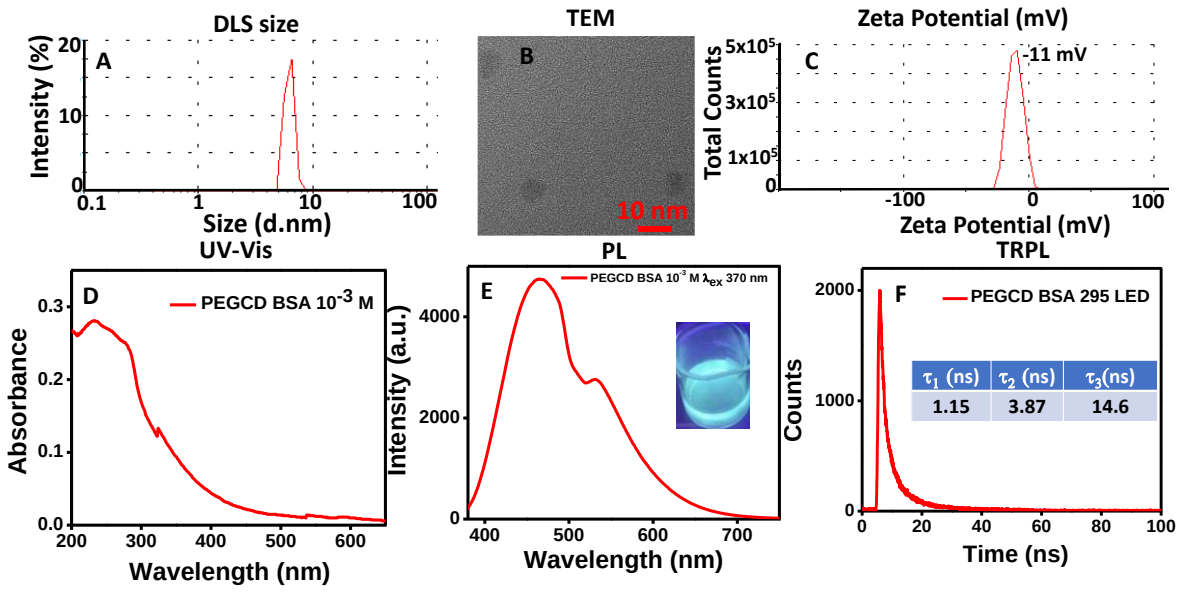

Fig S5: A) Particle size (DLS) B) TEM image C) Zeta potential D) UV-Vis spectrum E) PL spectrum (The bright green fluorescence of PEGCD BSA under UV light is shown in the inset) and F) TRPL decay profile containing lifetime of the PEGCD BSA. (In all the cases, water is taken as solvent)

Interaction studies of Phosphatidylcholine vesicles and citric acid carbon dots: (PHOS VES CACD):

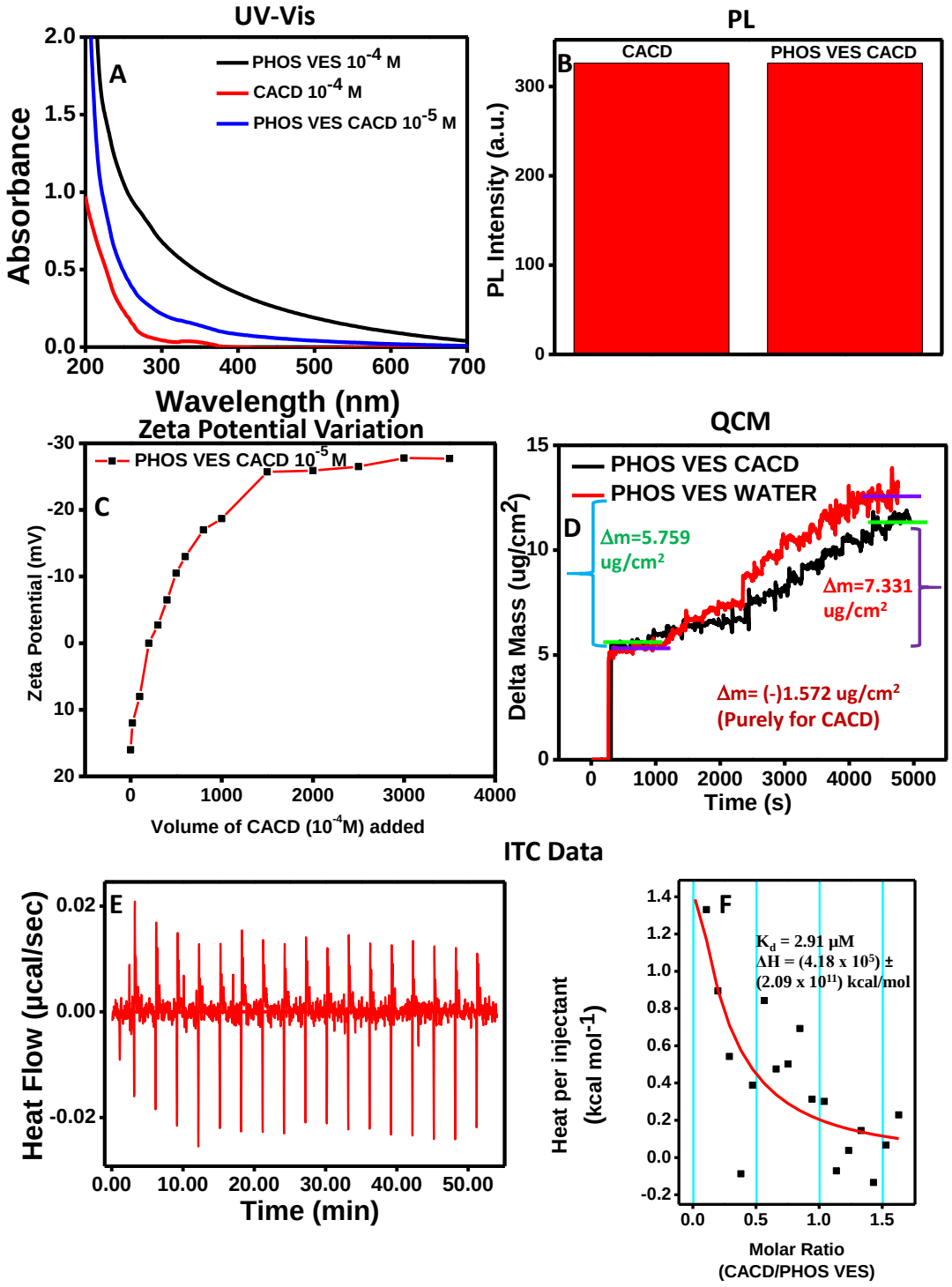

Fig S6: Experiment of **(A)** Change of UV-Vis spectra of PHOS VES (10^{-4} M) with addition of CACD (10^{-4} M) **(B)** Change of PL intensity of CACD (10^{-4} M) with addition of PHOS VES (10^{-4} M) **(C)** Change of Zeta Potential of PHOS VES (10^{-4} M) with addition of CACD (10^{-4} M) **(D)** Change of mass (m)/ frequency (f) of PHOS VES (10^{-4} M) with addition of CACD (10^{-4} M) in QCM **(E)** Heat flow/Change Vs time plot and **(F)** Heat produced per injectant with rise in molar ratio of the two materials in ITC measurements**.** The inset of Fig S6F shows the dissociation constant, K_d and enthalpy change, ΔH for the titration. (Aqueous medium is used as blank)

Interaction of Phosphatidylcholine vesicles and polyethylene glycol carbon dots (PHOS VES PEGCD):

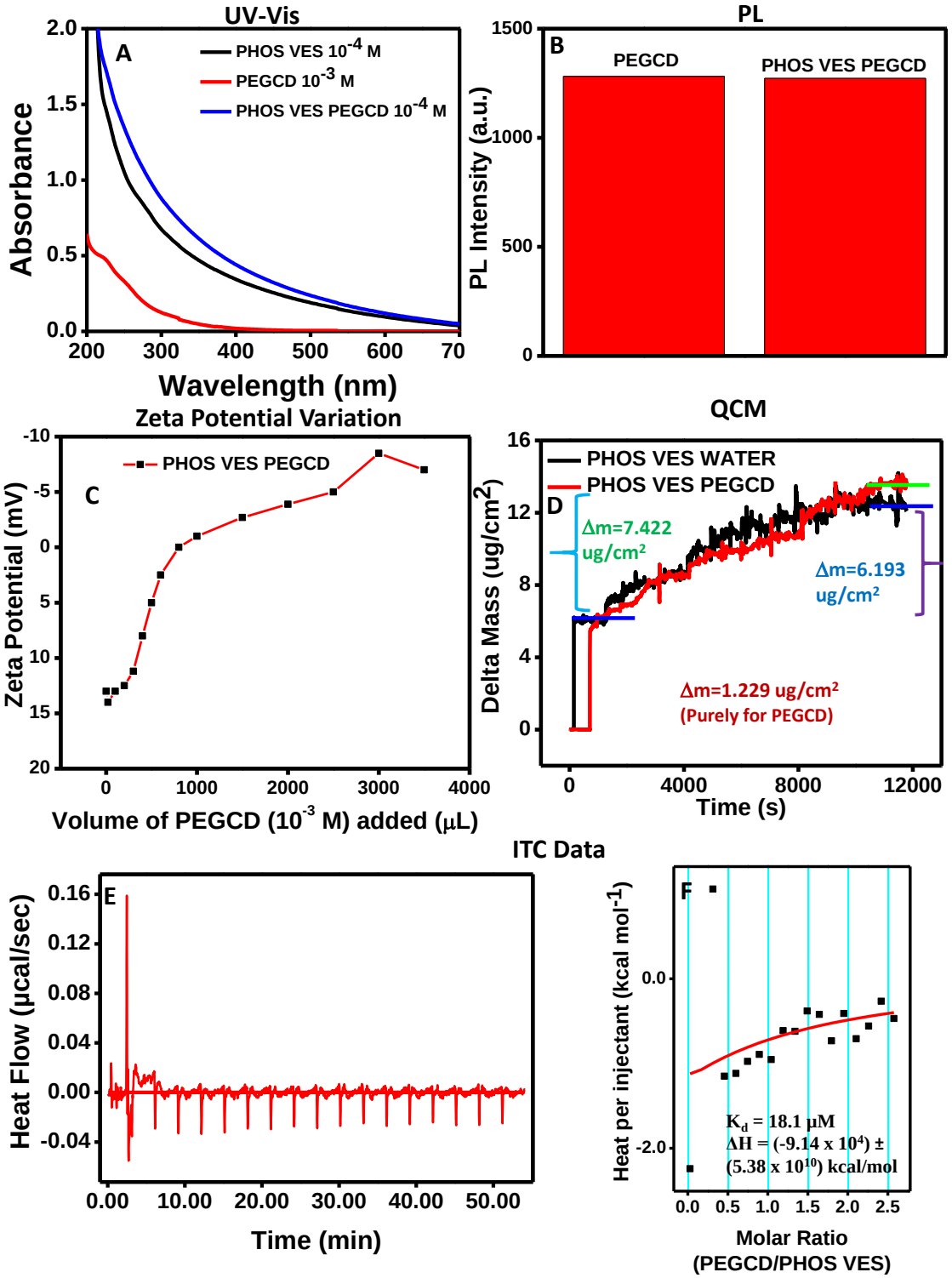

Fig S7: Experiment of **(A)** Change of UV-Vis spectra of PHOS VES (10^{-4} M) with addition of PEGCD (10^{-3} M) **(B)** Change of PL intensity of PEGCD (10^{-3} M) with addition of PHOS VES (10^{-4} M) **(C)** Change of Zeta Potential of PHOS VES (10^{-4} M) with addition of PEGCD (10^{-3} M) **(D)** Change of mass (m)/ frequency (f) of PHOS VES (10^{-4} M) with addition of PEGCD (10^{-3} M) in QCM **(E)** Heat flow/Change Vs time plot and **(F)** Heat produced per injectant with rise in molar ratio of the two materials in ITC measurements**.** The inset of Fig S7F shows the dissociation constant, K_d and enthalpy change, ΔH for the titration. (Aqueous medium is used as blank)

Interaction of Phosphatidylcholine vesicles and BSA coated polyethylene glycol carbon dots (PHOS VES PEGCD BSA):

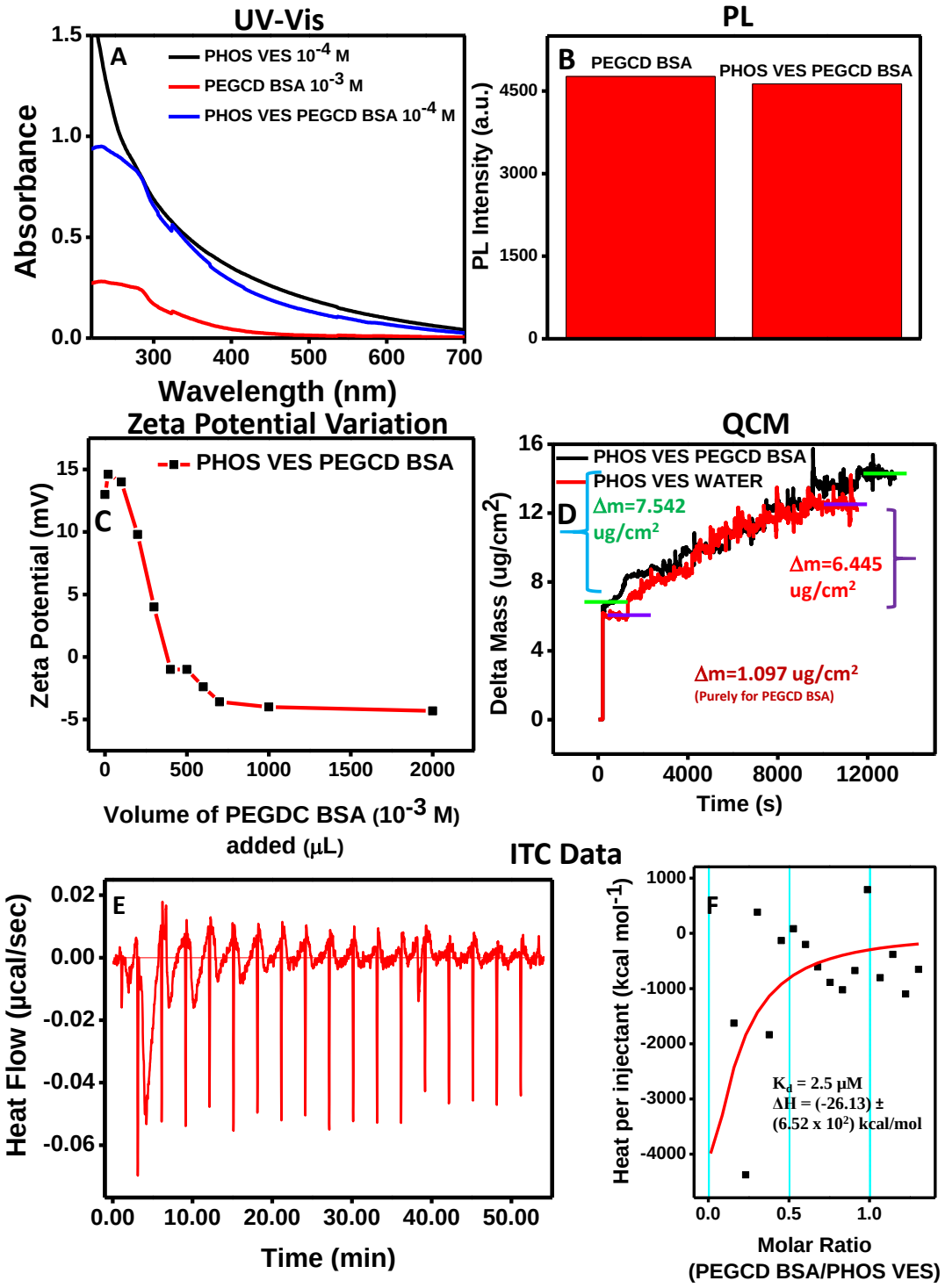

Fig S8: Experiment of **(A)** Change of UV-Vis spectra of PHOS VES (10^{-4} M) with addition of PEGCD BSA (10^{-3} M) (**B)** Change of PL intensity of PEGCD BSA (10^{-3} M) with addition of PHOS VES (10^{-4} M) (**C)** Change of Zeta Potential of PHOS VES (10^{-4} M) with addition of PEGCD BSA (10^{-3} M) (**D)** Change of mass (m) of PHOS VES (10^{-4} M) with addition of PEGCD BSA (10^{-3} M) in QCM. **(E)** Heat flow/Change Vs time plot and **(F)** Heat produced per injectant with rise in molar ratio of the two materials in ITC measurements**.** The inset of Fig S8F shows the dissociation constant, K_d and enthalpy change, ΔH for the titration. (Aqueous medium is used as blank)

Comparative FTIR spectra analysis:

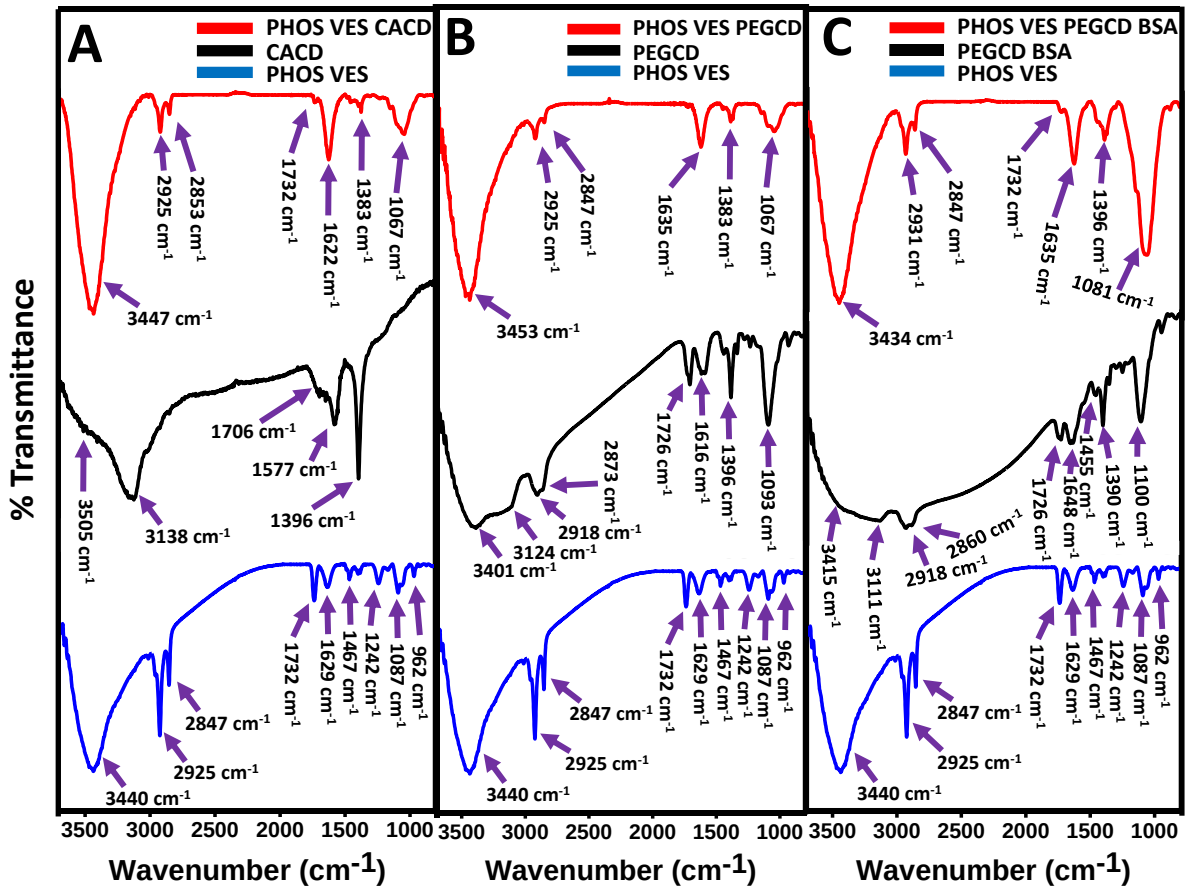

Fig S9: Comparative FTIR spectra of PHOS VES with **A)** CACD and PHOS VES CACD **B)** PEGCD and PHOS PEGCD and **C)** PEGCD BSA and PHOS VES PEGCD BSA

Reference

1. R. Ludmerczki, S. Mura, C. M. Carbonaro, I. M. Mandity, M. Carraro, N. Senes, S. Garroni, G. Granozzi, L. Calvillo, S. Marras, L. Malfatti, and P. Innocenzi, *Chem. Eur.J.,* 2019, **25**, 11963 –11974.
2. B. Han, Y. Li, T. Peng, M. Yu, X. Hu and G. He, *Anal. Methods***,** 2018, **10**, 2989-2993.
3. J. Gogoi and D. Chowdhury, *Journal of Materials Science*, 2020, **55**, 11597–11608.