Elucidating the Structural Dynamics of Carbon Dot Dispersed in Different Solvents and Its Red Fluorescence

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1.1. Reagents and Materials

Alizarin red S, Glucose, p-phenylenediamine, citric acid, ethylenediamine, dimethylformamide (DMF), formamide, dimethyl sulfoxide (DMSO), sodium hydroxide, hydrochloric acid, were purchased from either Central Drug House Pvt. Ltd., India or Sisco Research Laboratories Pvt. Ltd., India. Potassium bromide, deuterated DMSO (DMSO-D6), heavy water (D_2O), dialysis membrane (molecular weight cut off 2 kDa), and silicon wafers were bought from Sigma. Carbon-coated copper TEM grids (300 mesh) were obtained from Ted Pella Inc. Triple distilled water was used as the solvent unless specified.

1.2. Preparation of $NaBH_4$ treated CD_{ARS-G} samples for FTIR study

200 µL V dispersion of CD_{ARS-G} in water was treated with $NaBH_4$ (10µL, 500mM). The reaction mixture was kept for 2 h with occasional stirring. Then excess $NaBH_4$ and its by-products were removed by dialyzing (MWCO 2kDa) the mixture overnight. The dialyzed dispersion was dried using a vacuum concentrator (Eppendorf) for further studies.

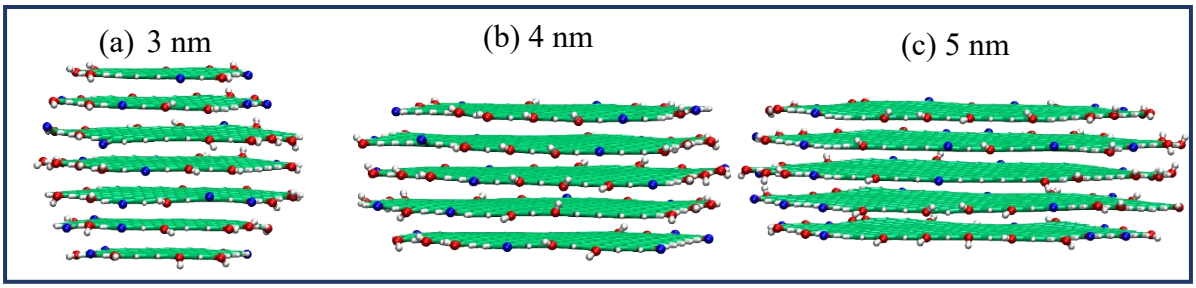


Figure S1. Graphical representation of the three different sized 3:7 CO: OH, CDs modeled in the simulation. Different atoms are color coded as carbon (lime green), hydroxyl oxygen (red), carbonyl oxygen (blue), and hydrogen (white).

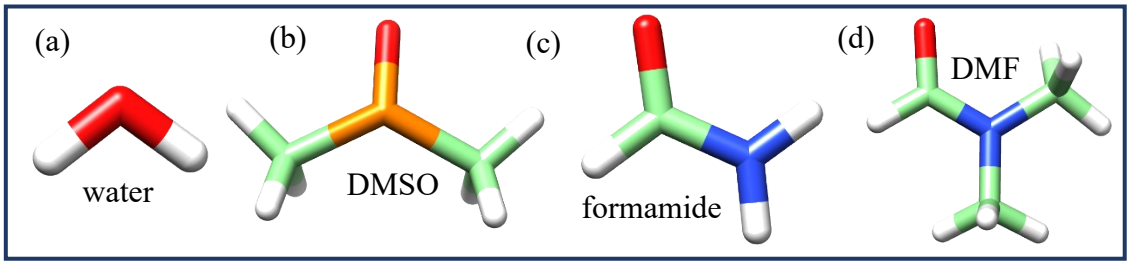


Figure S2. Graphical representation of four different solvent molecules considered in simulations. Different atoms are color coded as carbon (lime green), oxygen (red), hydrogen (white), nitrogen (blue) sulfur (orange).

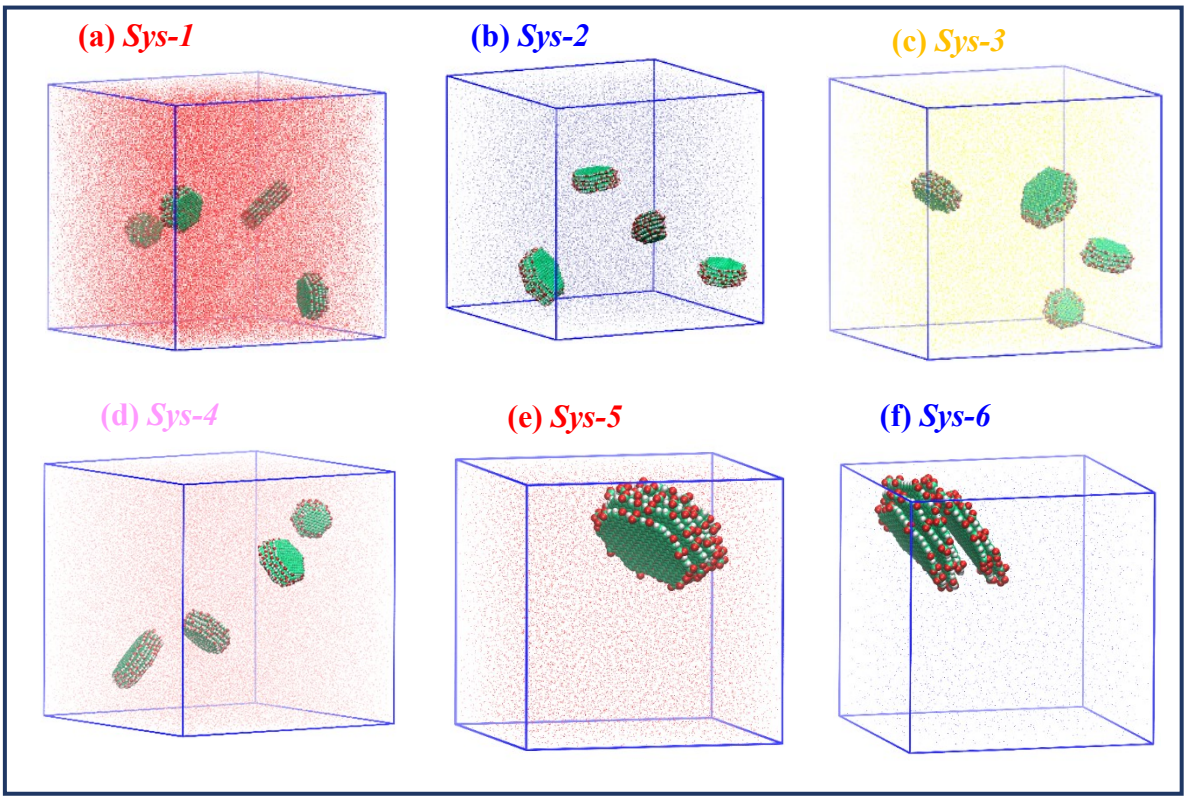


Figure S3. Equilibrated snapshots of the six systems after 100 ns NPT simulation runs. Different solvent systems are represented with characteristic atoms such as oxygen (red) for water, nitrogen (blue) for DMF, sulfur (yellow) for DMSO, and nitrogen (pink) for formamide. CD is shown with carbon atoms color coded with green, hydroxyl oxygen with red, carbonyl oxygen with blue, and hydrogen with white.

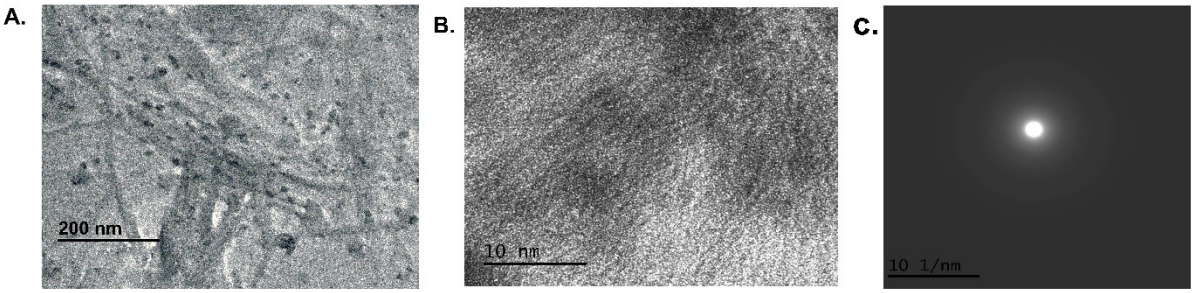


Figure S4. A. TEM, **B.** HR-TEM, and **C.** SAED pattern of CD_{ARS-G}.

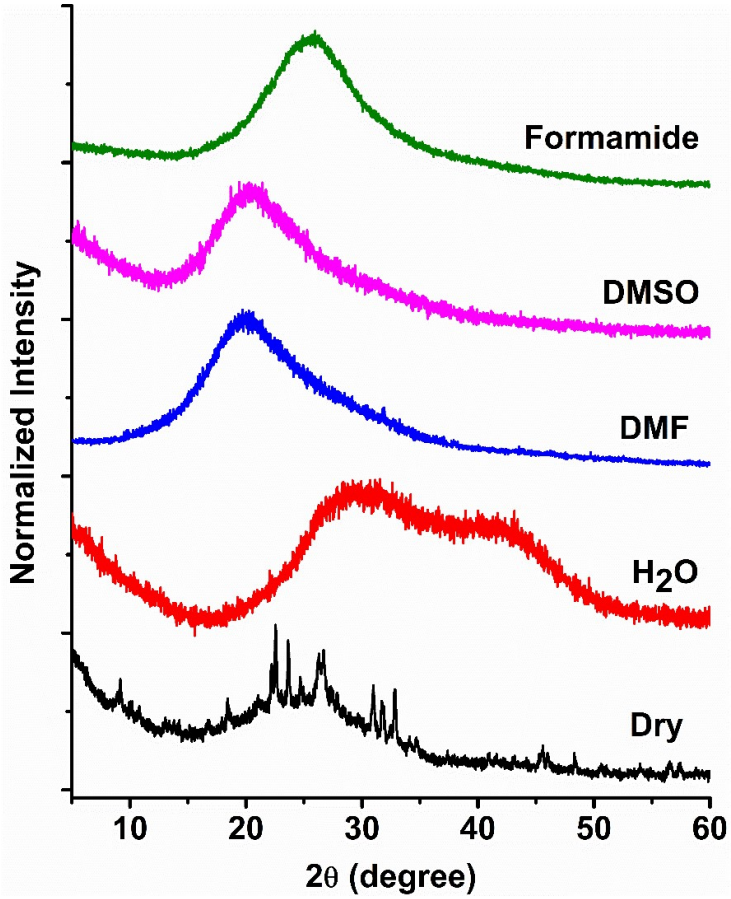


Figure S5. pXRD spectra of CD_{ARS-CA}.

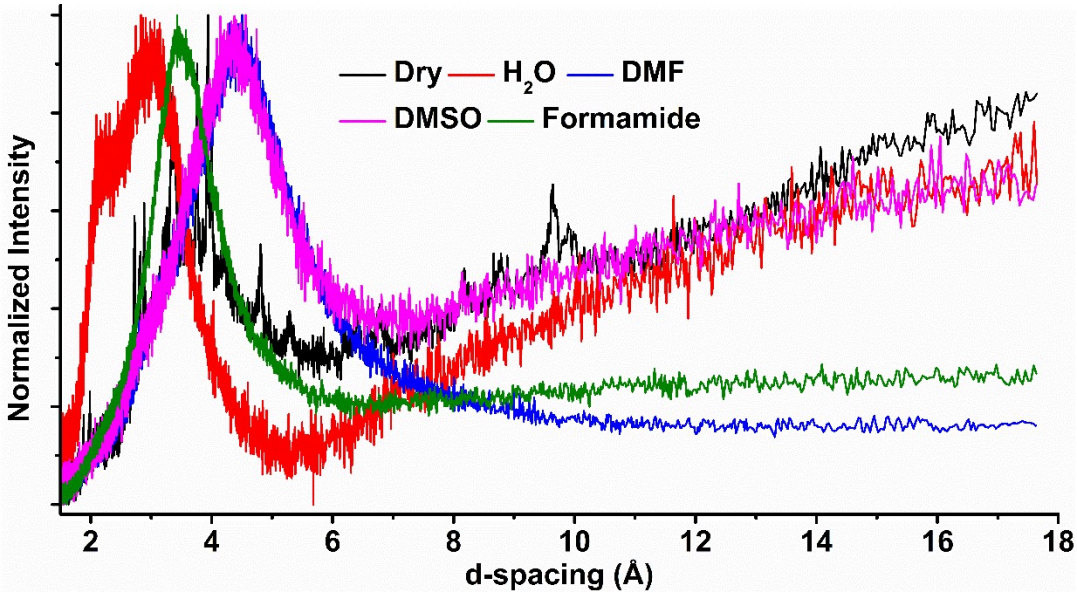


Figure S6. pXRD spectra of CD_{ARS-CA} with respect to d-spacing values.

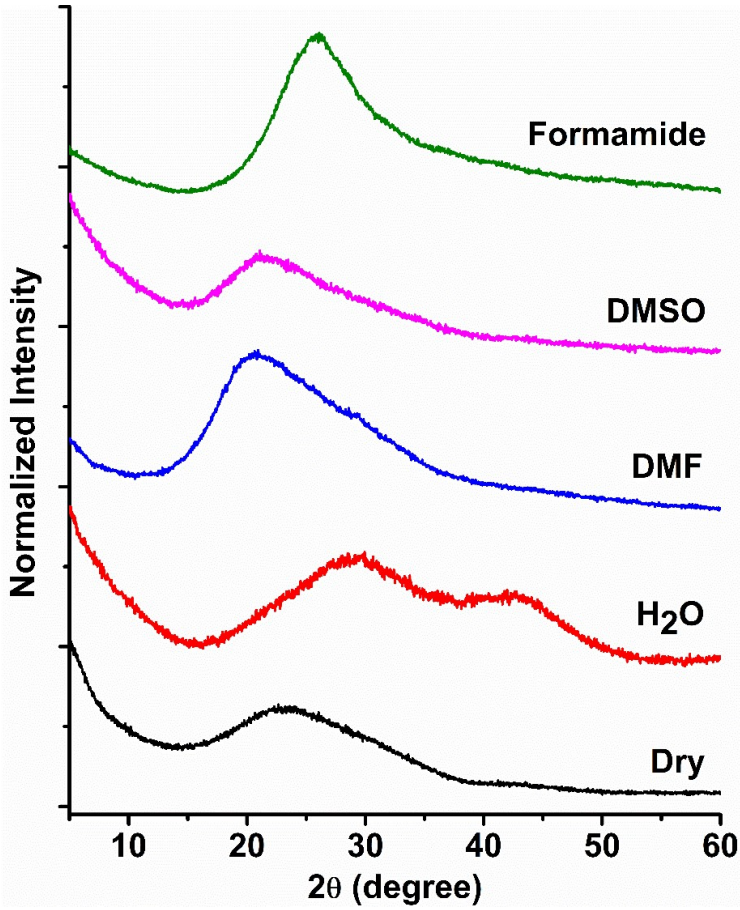


Figure S7. pXRD spectra of CD_{G-EDA}.

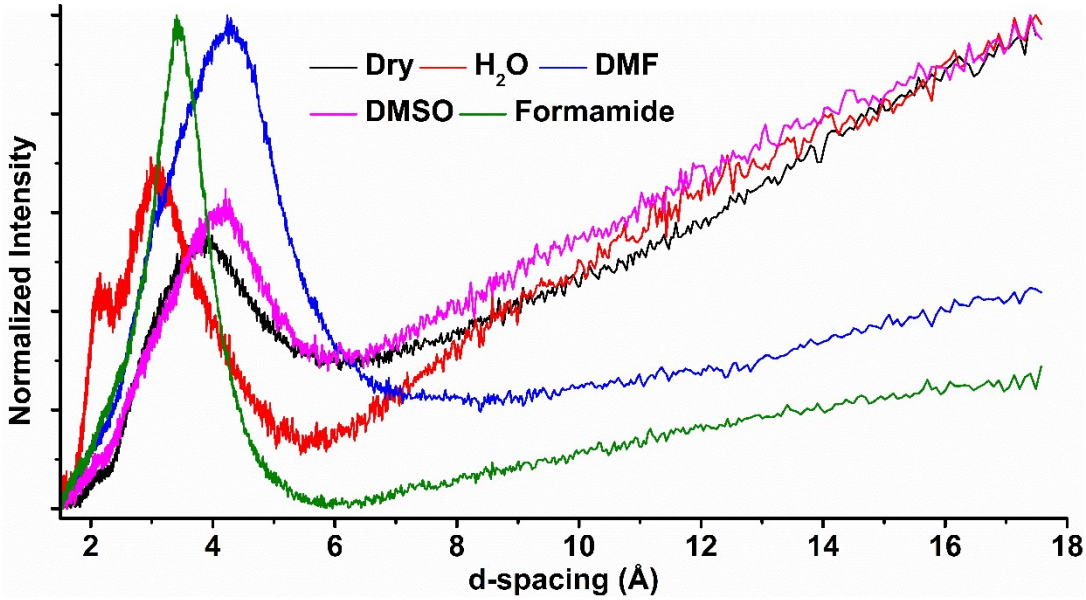


Figure S8. pXRD spectra of $CD_{G\text{-}EDA}$ with respect to d-spacing values.

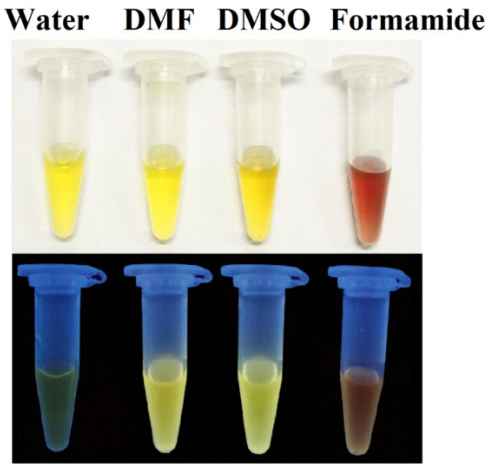


Figure S9. Digital image of $CD_{ARS\text{-}CA}$ in water, DMF, DMSO and formamide.

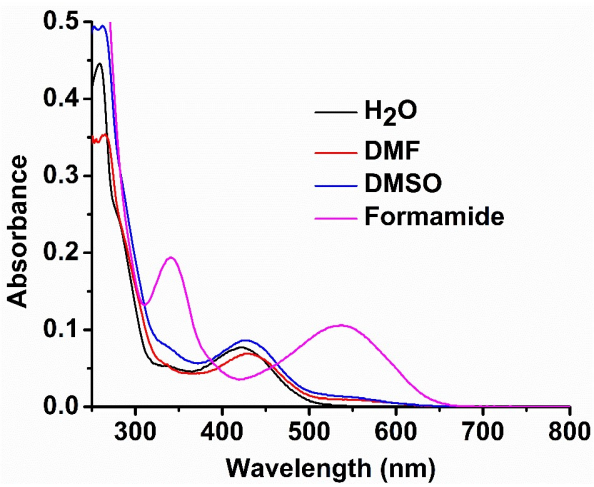


Figure S10. UV-Vis absorption spectra of CD_{ARS-CA} in water, DMF, DMSO and formamide.

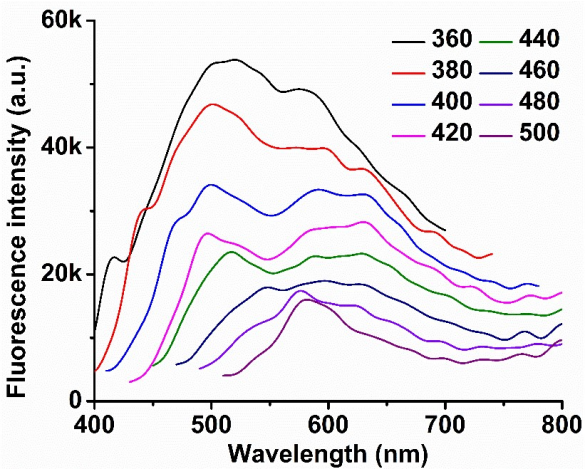


Figure S11. Steady-state fluorescence spectra of CD_{ARS-CA} in water.

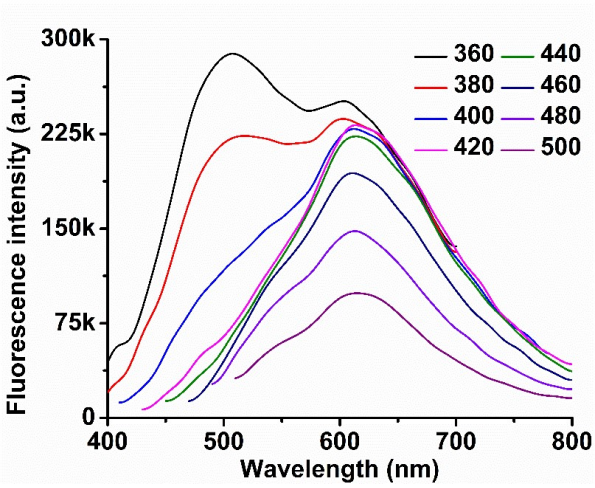


Figure S12. Steady-state fluorescence spectra of CD_{ARS-CA} in DMF.

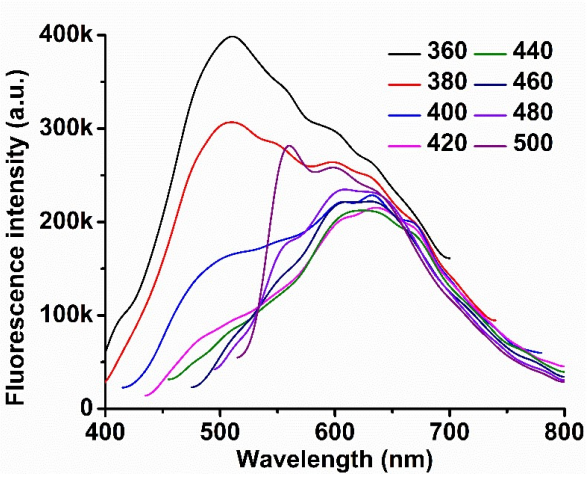


Figure S13. Steady-state fluorescence spectra of CD_{ARS-CA} in DMSO.

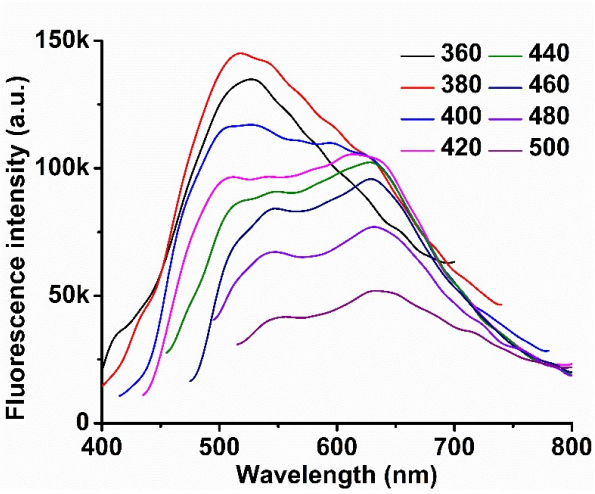


Figure S14. Steady-state fluorescence spectra of CD_{ARS-CA} in formamide.

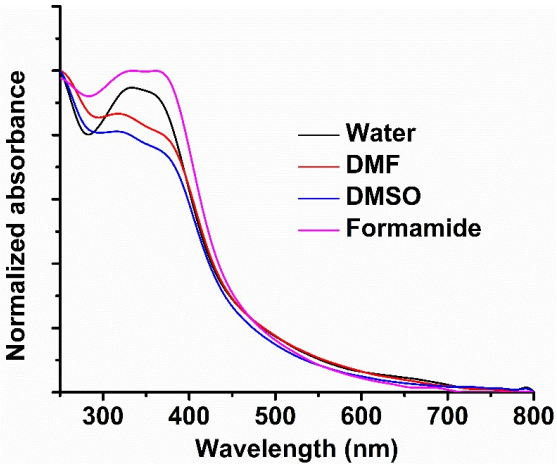


Figure S15. UV-Vis absorption spectra of CD_{G-EDA} in water, DMF, DMSO and formamide.

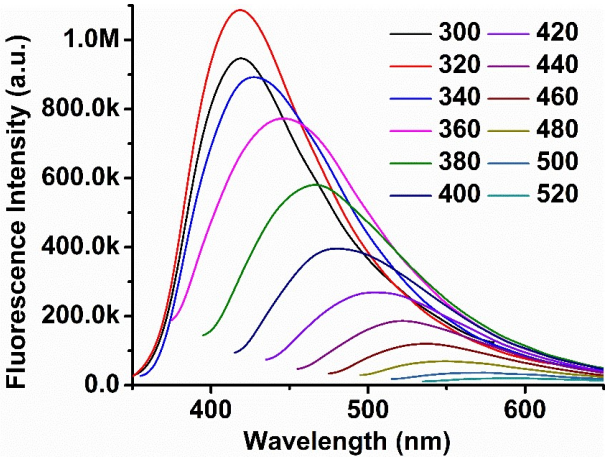


Figure S16. Steady-state fluorescence spectra of $CD_{G\text{-}EDA}$ in water.

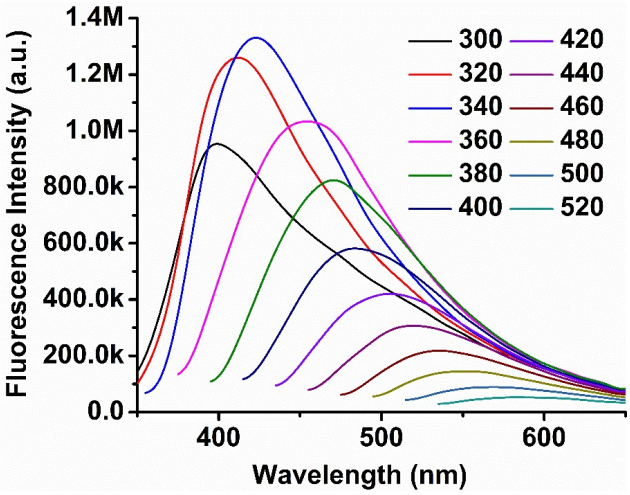


Figure S17. Steady-state fluorescence spectra of $CD_{G\text{-}EDA}$ in DMF.

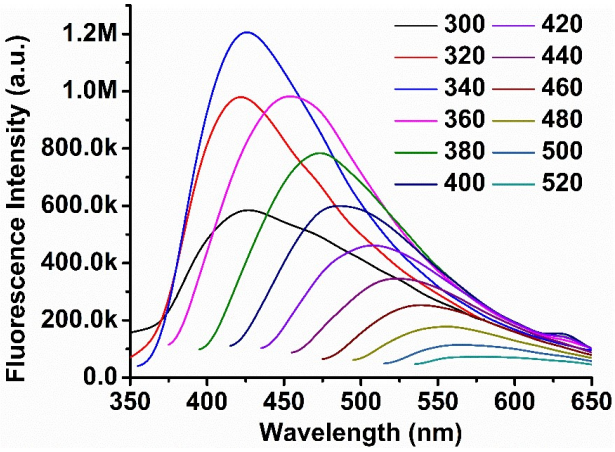


Figure S18. Steady-state fluorescence spectra of $CD_{G\text{-}EDA}$ in DMSO.

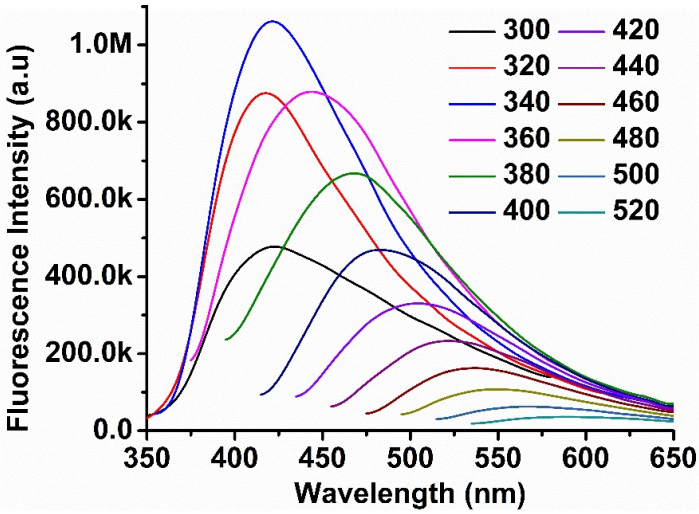


Figure S19. Steady-state fluorescence spectra of $CD_{G\text{-}EDA}$ in formamide.

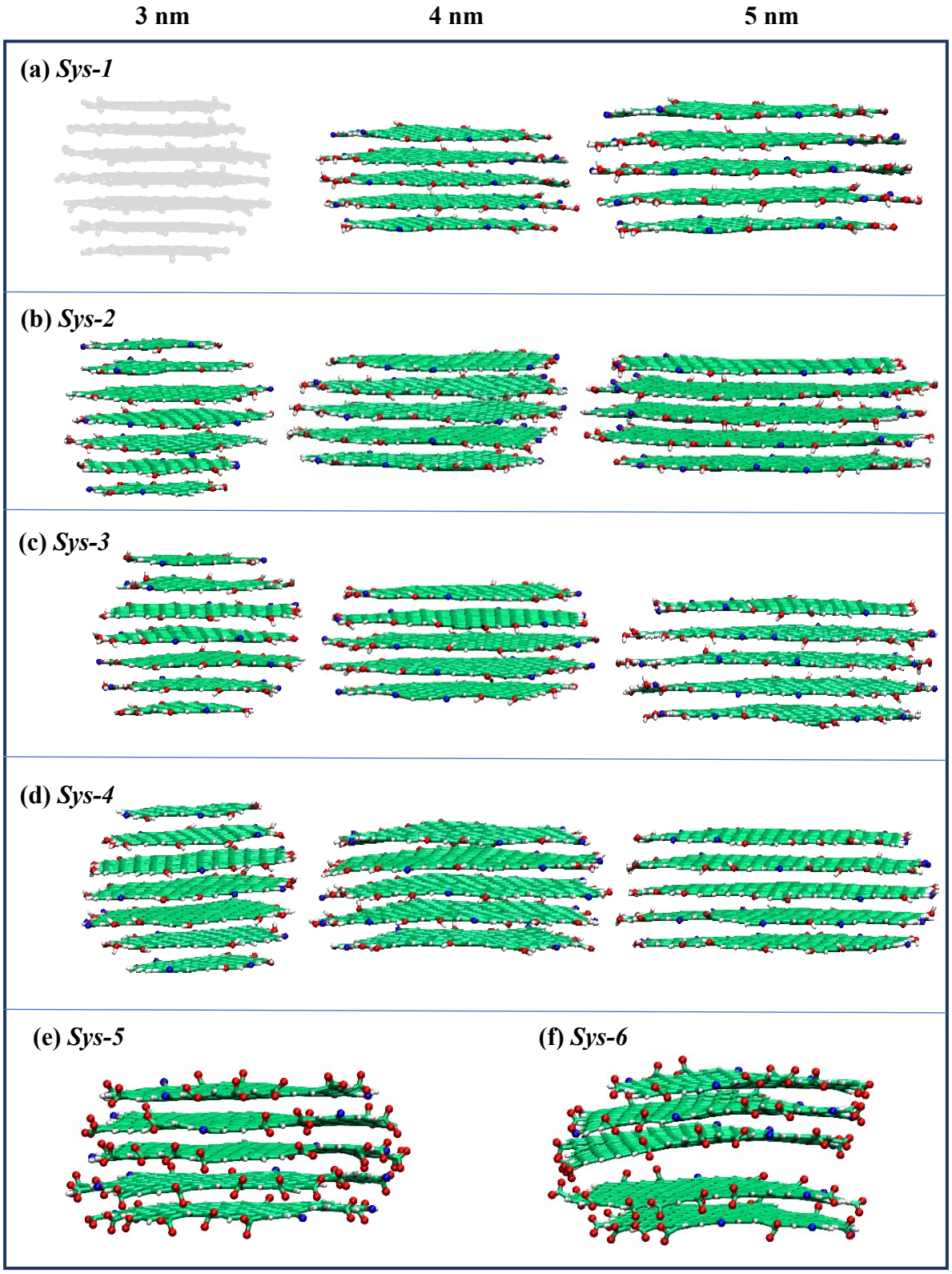


Figure S20. Equilibrated snapshots of the CD in all six simulated systems. Different atoms are color coded as carbon (lime green), hydroxyl and carboxyl oxygen (red), carbonyl oxygen (blue), and hydrogen (white).

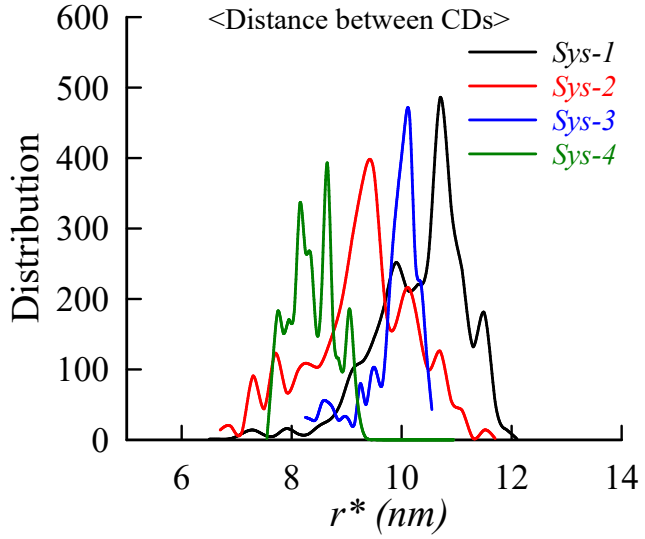


Figure S21. Distribution profile for the average distance between the four CDs in four simulated systems. The CDs are placed sufficiently far enough, and no interactions are observed between them throughout the simulation run. This is also visible in the snapshots presented in Figure S3.

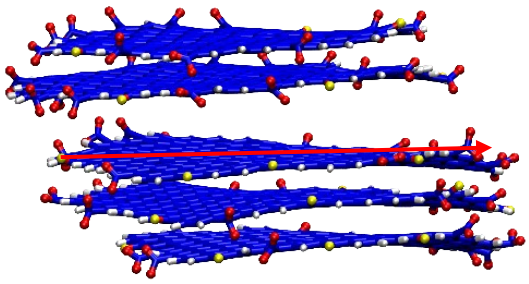


Figure S22. The defined vectors connecting the end carbon atoms of the two innermost layers of 3:7 CO: COO^- CD used for calculating the intramolecular kinetics of CD.

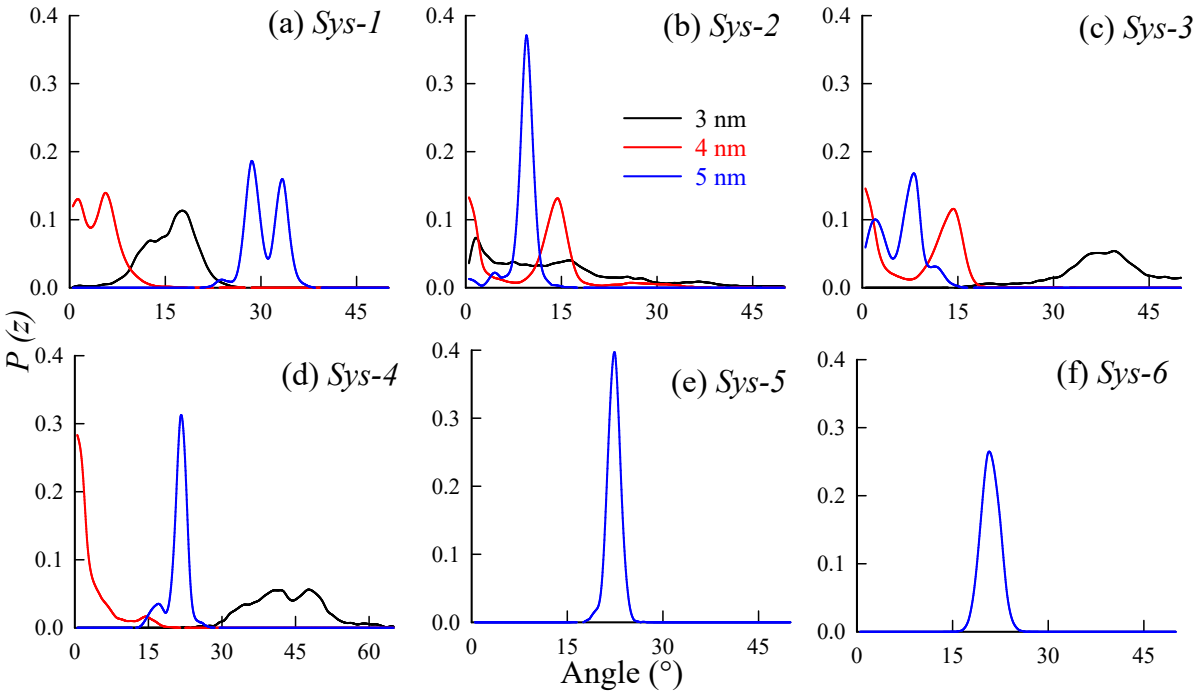


Figure S23. The probability distribution of angular orientation profiles calculated between the vector defined for the two innermost layers of the CD in all six simulated systems.

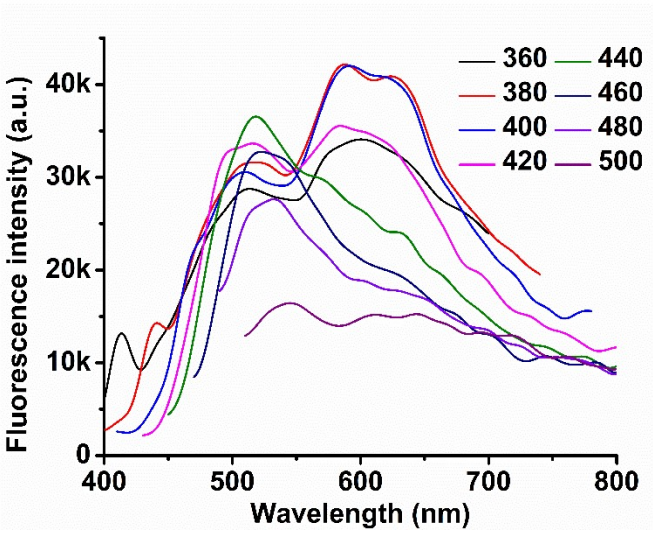


Figure S24. Steady-state fluorescence spectra of the P dispersion of $CD_{ARS\text{-}G}$.

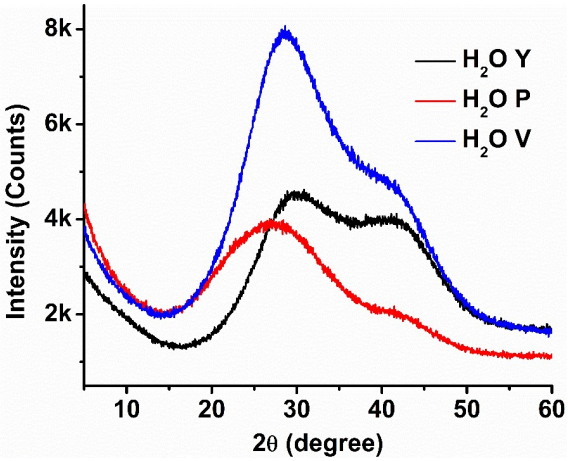


Figure S25. pXRD spectra of the Y, P and V dispersions of CD_{ARS-G} in water.

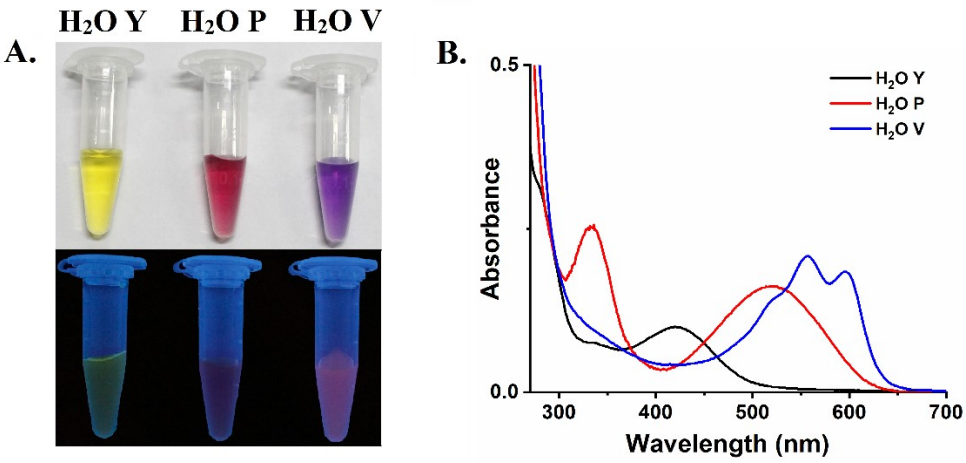


Figure S26. Digital image and UV-Vis absorption spectra of the Y, P, and V dispersions CD_{ARS-CA}.

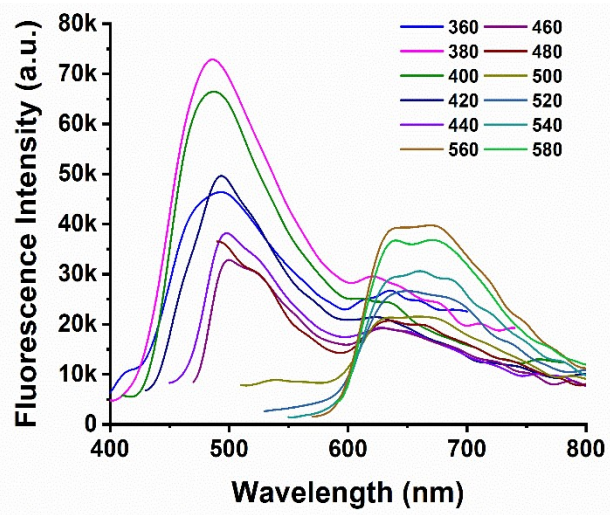


Figure S27. Steady-state fluorescence spectra of the P dispersion of CD_{ARS-CA}.

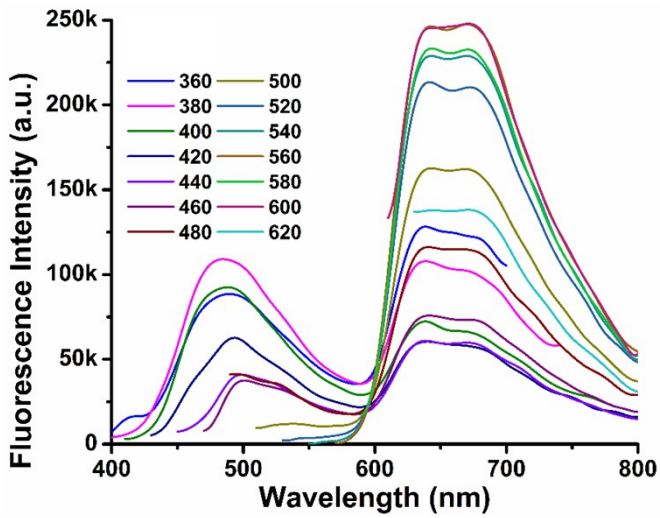


Figure S28. Steady-state fluorescence spectra of the V dispersion of CD_{ARS-CA}.

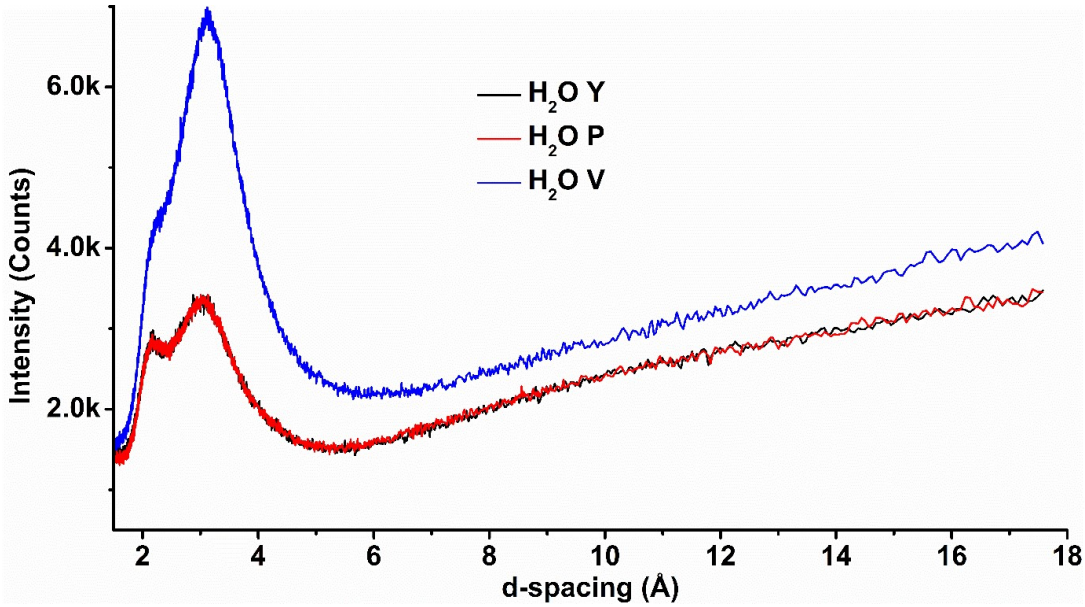


Figure S29. pXRD spectra of the Y, P and V dispersion of CD_{ARS-CA} with respect to d-spacing values.

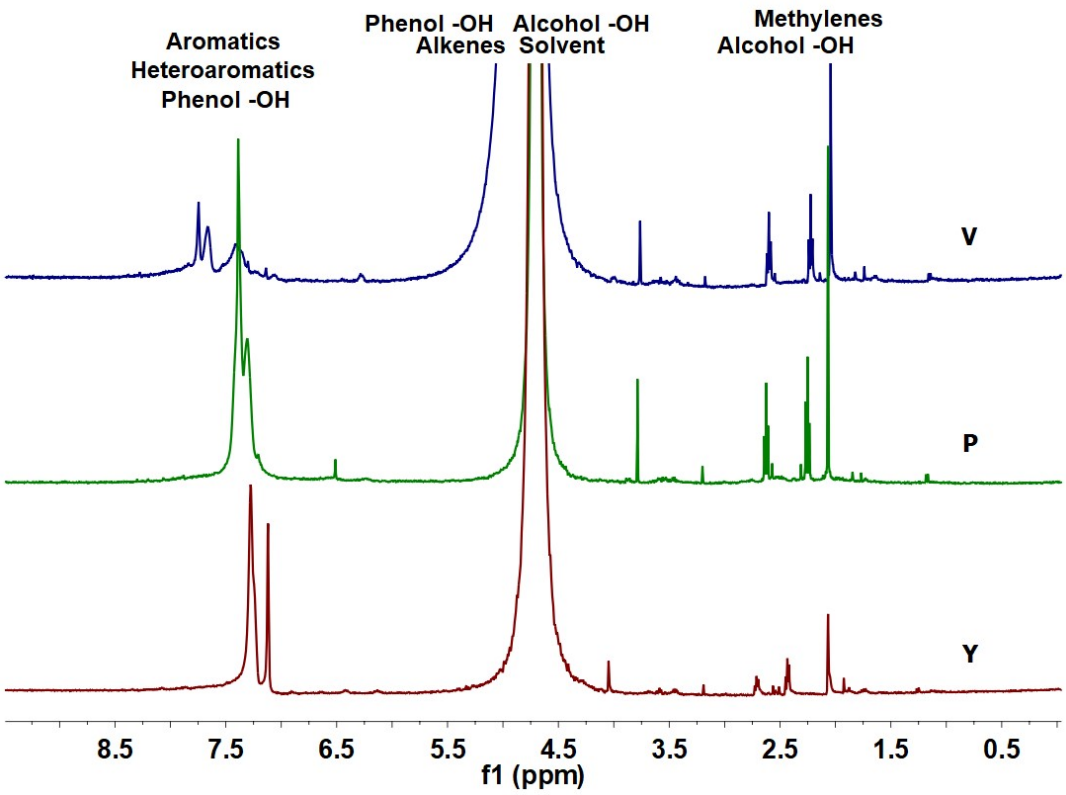


Figure S30. ^{1}H NMR spectra of the Y, P and V dispersion of CD_{ARS-G} in D_2O.

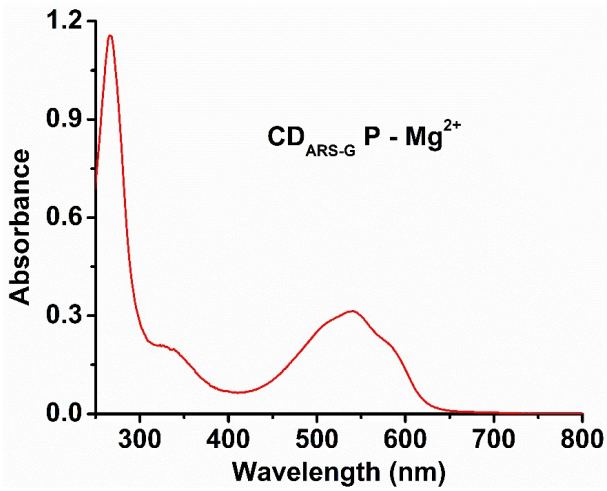


Figure S31. UV-Vis absorption spectra of the CD_{ARS-G} water dispersion in presence of Mg^{2+} ion at physiological pH.

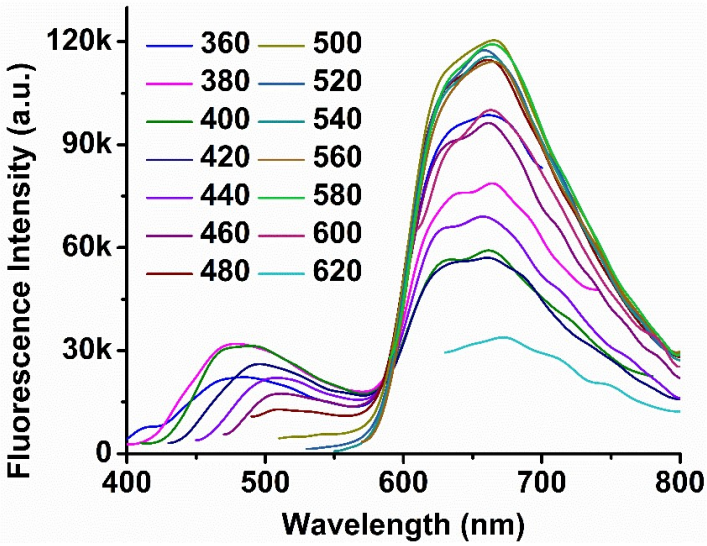


Figure S32. Steady-state fluorescence spectra of the CD_{ARS-G} water dispersion in presence of Mg^{2+} ion at physiological pH.

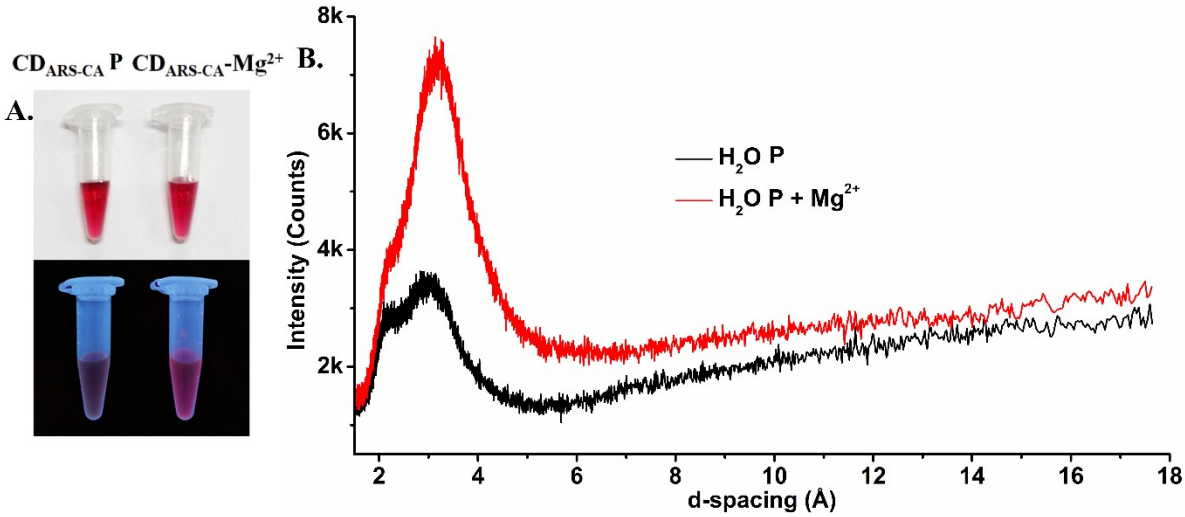


Figure S33. A. Digital image, and **B.** d-spacing distribution of the CD_{ARS-CA} water dispersion in presence of Mg^{2+} ion at physiological pH.

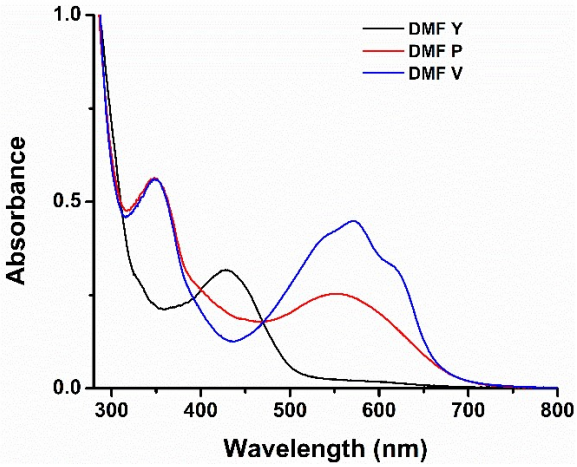


Figure S34. UV-Vis absorption spectra of the CD_{ARS-G} Y, P, and V dispersions in DMF.

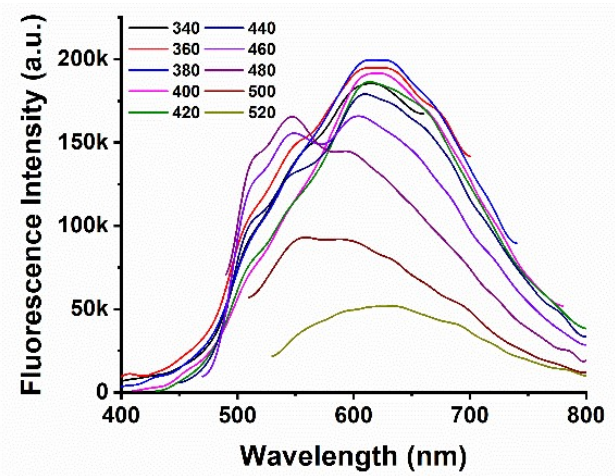


Figure S35. Steady-state fluorescence spectra of the CD_{ARS-G} Y dispersion in DMF.

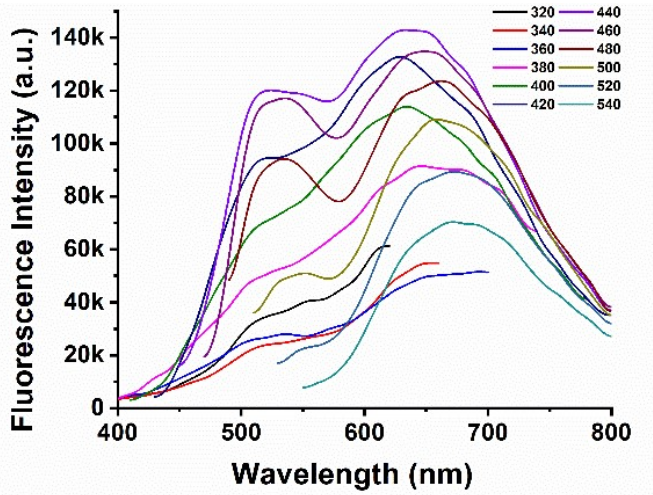


Figure S36. Steady-state fluorescence spectra of the CD_{ARS-G} P dispersion in DMF.

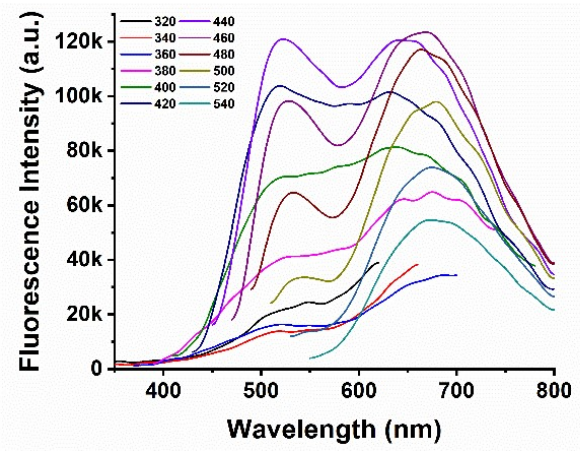


Figure S37. Steady-state fluorescence spectra of the CD_{ARS-G} V dispersion in DMF.

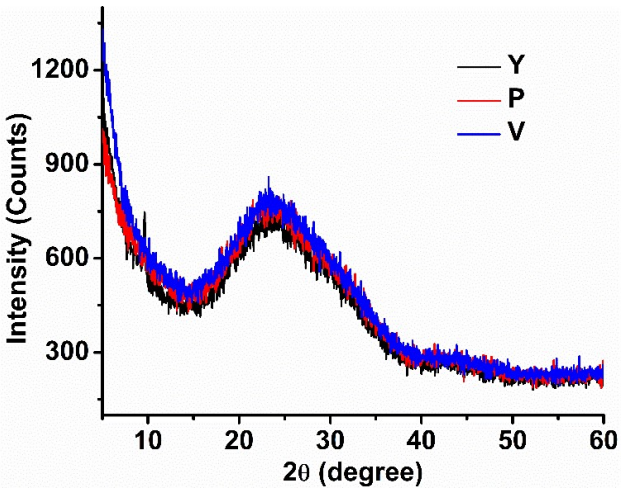


Figure S38. pXRD spectra of the Y, P, and V form of CD_{ARS-G} in dry state.

Table S1. Geometrical details of the four types of CDs modeled in the simulation.

Edge size	# Layers		Diameter of the central layer (nm)	Dot height (nm)	# Functional groups			# Atoms
	above middle layer	total			-OH	-CO	-COO$^-$	
6	3	7	3	2	57	22	–	1435
8	2	5	4	1.5	58	25	–	2051
10	2	5	5	1.5	71	33	–	3164
8	2	5	4	1.5	–	24	56	2080

Table S2. Radius of gyration (R_g) of CDs solvated in different solvents.

Solvent	R_g (nm)		
	3 nm	4 nm	5 nm
Sys-1	1.08	1.33	1.62
Sys-2	1.08	1.33	1.62
Sys-3	1.08	1.33	1.62
Sys-4	1.08	1.33	1.62
Sys-5	-	1.50	-
Sys-6	-	1.51	-

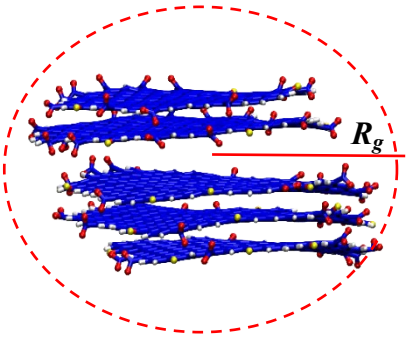
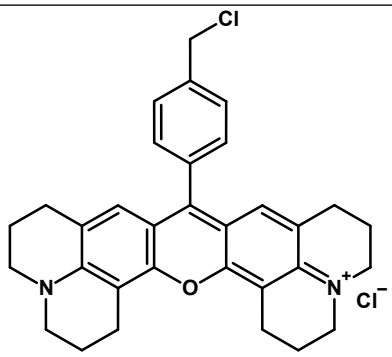
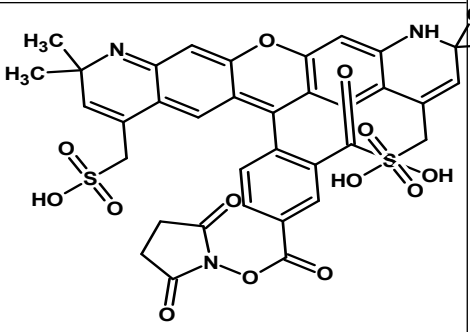
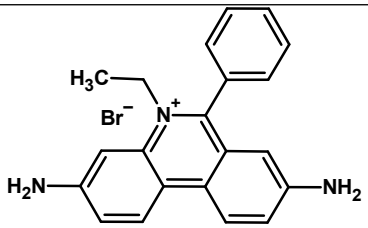
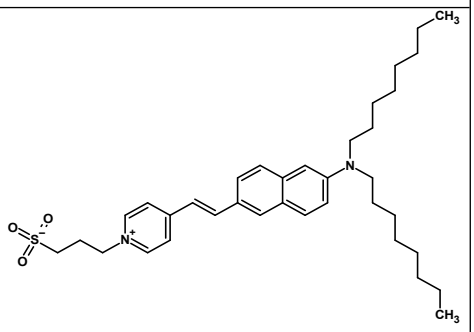
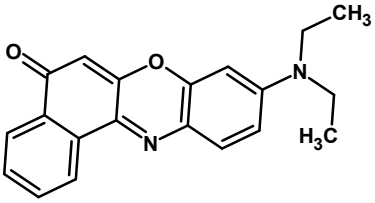
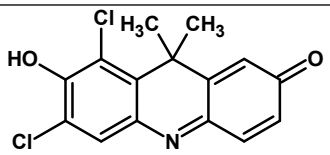
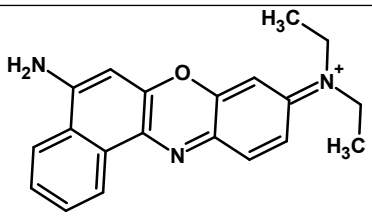
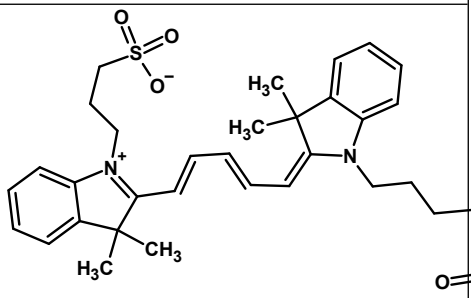
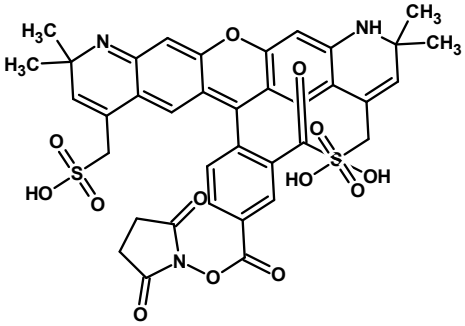
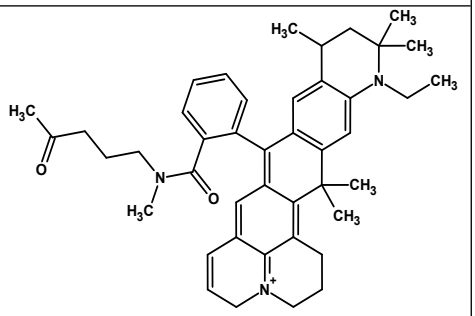


Table S3. Red fluorescent dyes.

Dye	Excitation, nm	Emission, nm	Stokes shift, cm^{-1}	Structure
MitoTracker Red	578	599	606.55	
Alexa 568	576	603	777.36	

Ethidium Bromide	524	605	865.31	
Di-8 ANEPPS	469	630	2555.04	
Nile Red	559	637	646.65	
DDAO pH 9.0	648	657	658.13	
Nile Blue, EtOH	631	660	5448.95	
Cy 5	646	664	2190.50	
BODIPY 650/665-X, MeOH	646	664	211.40	

Atto 647	644	670	209.47	
Carboxynaphthofluorescein, pH 10.0	600	674	696.35