Electronic Supporting Information

Supramolecular confinement as a tool to control the dynamic molecular assembly of o-nitrosocumene in water

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Experimental details

Materials and Methods: NMR experiments, including titration, characterization, and DOSY measurements were performed on Bruker Avance NMR spectrometers operating at 800 MHz and 500 MHz (both equipped with cryoprobes), as well as a Bruker 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) relative to the residual HDO peak at δ = 4.79 ppm for ^{1}H NMR and δ = 49 ppm for ^{13}C NMR. UV-vis absorption spectra were recorded using a Shimadzu UV 3150 spectrophotometer. Dynamic Light Scattering (DLS) size analysis experiments were done using Malvern Instruments Zetasizer Nano-ZS. Electrospray Ionization (ESI) high resolution mass spectrometry (HRMS) analyses of CB and CD complexes with *o*-NC was performed using a Waters Xevo G2-XS QTOF mass spectrometer. The Electrospray ionization (ESI) high resolution mass spectrometry (HRMS) analysis was performed using a Waters Xevo G2-XS QTOF mass spectrometer The ESI source conditions included: Capillary voltage 0.9-2.2 kV, Sampling cone 40 V, Source offset 80, Source temperature 80 °C, Desolvation temperature 250 °C, Cone gas 0 L/h, Desolvation gas 400 L/h, Lock spray capillary voltage 1.7 kV, Lock mass at 556.2627 in positive resolution mode (leucine enkephalin, peak resolution 30,000). Hosts (OA[1], CB8[2-4]) and guests (*o*-nitrosocumene, and *o*-nitrocumene)[5] were synthesized according to reported procedures. The hosts *α*-CD, *β*-CD, and *γ*-CD were sourced from Wacker Biochem. Corp and used as received.

Sample Preparation for host-guest inclusion studies using NMR spectroscopy: NMR titration experiments were performed to investigate host-guest complexation using a series of supramolecular hosts: octa acid (OA), cucurbit[8]uril (CB8), *α*-cyclodextrin (*α*-CD), *β*-cyclodextrin (*β*-CD), and *γ*-cyclodextrin (*γ*-CD),The general procedure involved the sequential addition of guest molecules to a solution of the host molecule prepared in 0.6 mL D_2O, or vice versa, followed by the acquisition of ^{1}H NMR spectra after each addition to monitor changes in both host and guest NMR signal shifts.

Procedure A (Guest → host): Host solutions prepared as follows: OA (1.0 mM, 10.0 mM sodium borate buffered D_2O), CB8 (0.1 mM). A stock solution of the guest molecule (*o*-NC) was prepared at 60.0 mM DMSO-d_6. For all the titrations, variable equivalents of *o*-NC were added sequentially. After each addition, the NMR tube was vigorously shaken to ensure complete mixing and a ^{1}H NMR spectrum was recorded.

Procedure B (Host → guest): Guest solution of *o*-NC was prepared at 2.0 mM in 0.6 mL D_2O. Variable equivalents of host molecules were added sequentially from a stock solution prepared as follows: OA (30.0 mM, 300.0 mM sodium borate buffered D_2O), α/γ-CD (40 mM), β-CD (20 mM). After each addition, the NMR tube was vigorously shaken to ensure complete complexation and a ^1H NMR spectrum was recorded.

Sample Preparation for host-guest inclusion studies by UV-vis spectroscopy: A solution of *o*-NC was prepared at 0.8 mM in 3.0 mL water. Variable equivalents of a 20.0 mM OA stock solution prepared in 200.0 mM sodium borate were then added sequentially. To minimize dilution effects, the OA stock solution was prepared with a matching concentration of *o*-NC in borate buffer. Following each addition, the solution was vigorously agitated to ensure complete complexation, and a UV-vis spectrum was recorded.

Sample Preparation for dynamic light scattering measurements for guest: A 2 mM stock solution of *o*-NC was prepared by dissolving the compound in 1 mL of filtered water. The water used was filtered through a 0.22 μm pore size filter to remove particulate matter that could interfere with DLS analysis. This procedure ensured consistent sample preparation and minimized potential artifacts arising from suspended particles

Sample Preparation for HRMS analysis: Prior to analysis, solutions were diluted as follows: 0.2 mM *o*-NC + 0.22 mM CB8 was diluted 1:1 with MQ water containing 0.1% formic acid; 1.3 mM α-CD + 1.2 mM *o*-NC and 2 mM *o*-NC + 2.1 mM β-CD, and 1.5 mM *o*-NC + 1.6 mM γ-CD were diluted to a 1:3 ratio with MQ water.

References:

1. C. L. D. Gibb and B. C. Gibb, *J. Am. Chem. Soc.*, 2004, **126**, 11408-11409.
2. R. Behrend, E. Meyer and F. Rusche, *Justus Liebigs Ann. Chem.*, 1905, **339**, 1-37.
3. J. Kim, I.-S. Jung, S.-Y. Kim, E. Lee, J.-K. Kang, S. Sakamoto, K. Yamaguchi and K. Kim, *J. Am. Chem. Soc.*, 2000, **122**, 540-541.
4. W. A. Freeman, W. L. Mock and N.-Y. Shih, *J. Am. Chem. Soc.*, 1981, **103**, 7367-7368.
5. K. R. Reddy, C. U. Maheswari, M. Venkateshwar and M. L. Kantam, *Adv. Synth. Catal.*, 2009, **351**, 93-96.

^{1}H NMR of *o*-NC in DMSO-d_6

Figure S1: ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C) spectrum 60 mM *o*-NC stock solution prepared in a sealed capillary tube with 0.6 mL of DMSO-d_6. The * represents DMSO solvent residual and water in DMSO.

Structures of CB8, CD and OA

Figure S2: Structures of supramolecular host molecules employed in this study, including cavity dimensions - cucurbit[8]uril (CB8), cyclodextrins (α, β, ɣ- CD) and octa acid (OA).

Studies of *o*-NC with CB8

^{1}H NMR titration spectra of *o*-NC with CB8 (host → guest) (host region)

Figure S3: ^{1}H NMR partial titration spectra (500 MHz, D_2O, 25 °C) of (i) 2.0 mM *o*-NC and 2.0 mM guest with (ii) 0.1, (iii) 0.5, (iv) 1.4, (v) 1.8 and (vi) 2.2 mM of CB8. ^{1}H NMR of free CB8 (vii) is also given for reference. Chemical shift of CB8 signals is labelled as H_a-H_c and violet highlights represents aggregate peaks. Guest protons in the starting solution are labelled as a-e in black. Gradual addition of CB8 resulted in the disappearance of aggregate peaks and only bound host signals are observed from the beginning.

[1]H NMR titration spectra of *o*-NC with CB8 (guest → host) (guest region)

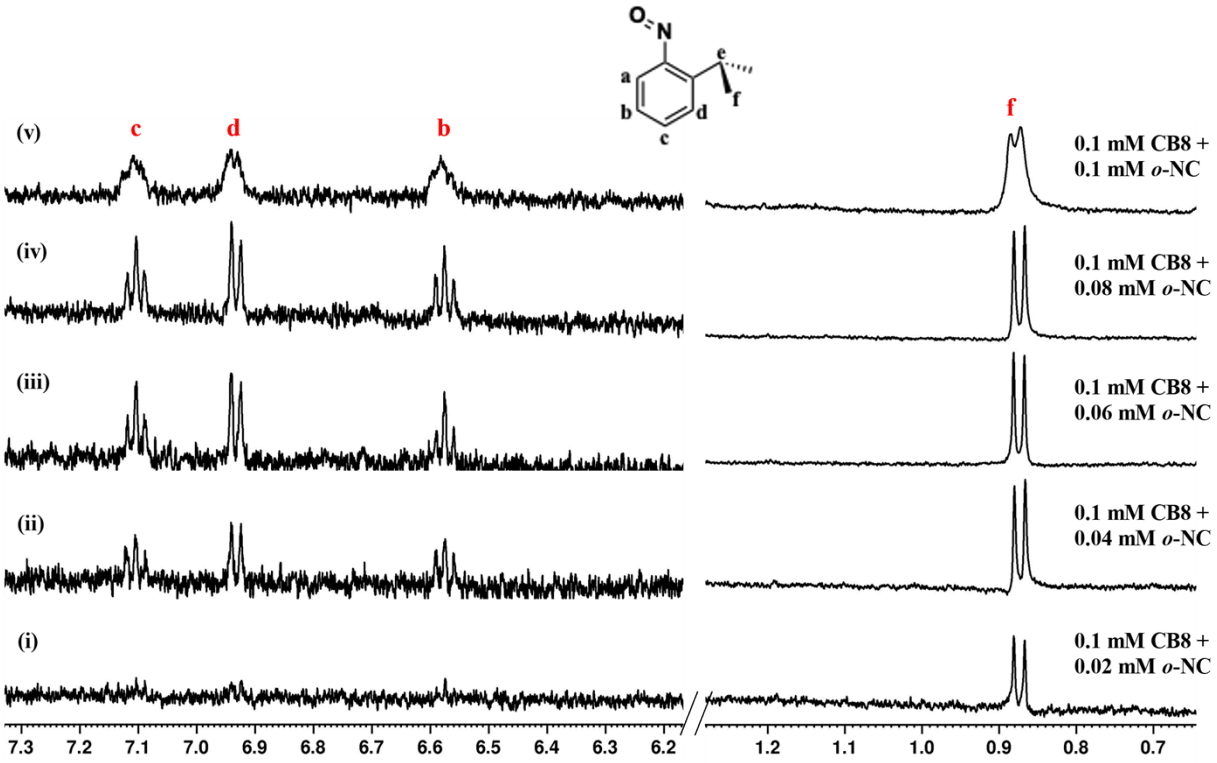


Figure S4: [1]H NMR (500 MHz, D_2O, 25 °C) titration spectra illustrating the binding of *o*-NC to CB8 in water. Titration was performed by adding increasing concentrations of *o*-NC (i) 0.02, (ii) 0.04, (iii) 0.06, (iv) 0.08, and (v) 0.1 mM to a solution of 0.1 mM CB8. Peaks labeled 'b' through 'f' in red corresponds to protons of the *o*-NC guest complexed by CB8. The appearance of only sharp, complexed guest signals in both aromatic and aliphatic regions from the beginning of the titration confirms that all added *o*-NC has been encapsulated by CB8 as monomers. Note that a slight broadening observed in the complexed guest peaks is attributed to a minor excess of free guest molecules competing for binding sites, suggesting that a slightly greater amount of host is required to achieve complete encapsulation of *o*-NC with the CB8 cavity.

Host signals

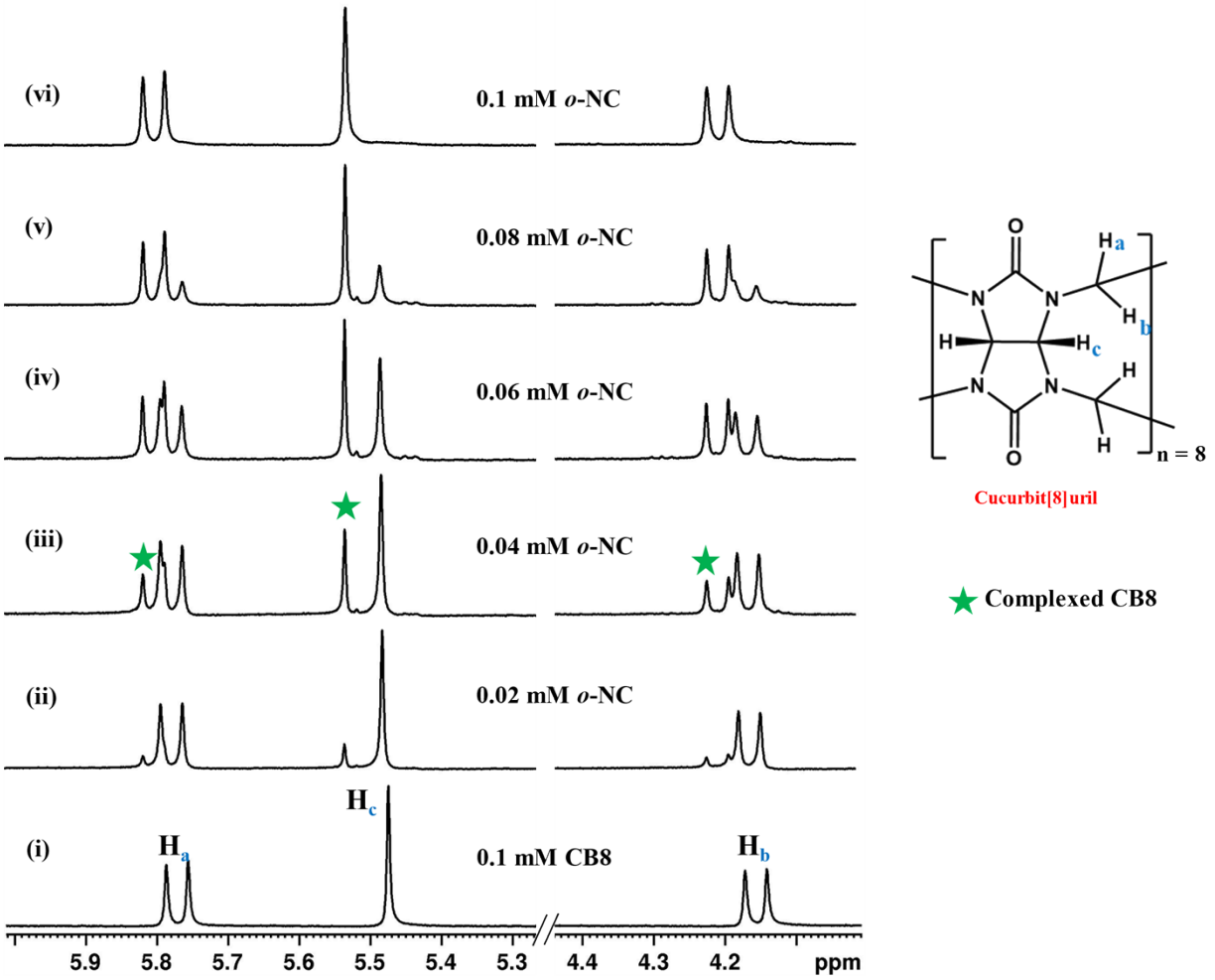


Figure S5: Expanded host regions of *o*-NC@CB8 titration in water. ^{1}H NMR (500 MHz, D_2O, 25 °C) spectra of (i) 0.1mM CB8 and CB8 with (ii) 0.02, (iii) 0.04, (iv) 0.06, (v) 0.8 and (vi) 0.1 mM of guest. Free host protons are labelled from H_a-H_c and the complexed CB8 protons are indicated by green stars.

NOESY of complexed *o*-NC@CB8 complex at two mixing times (300 & 700 ms)

At 300 ms mixing time

Figure S6: NOESY spectrum (500 MHz, D_2O, 25 °C) of a 0.2 mM *o*-NC guest and 0.22 mM CB8 host complex, acquired with a 300 ms mixing time. Blue dots indicate signals from the complexed *o*-NC guest, while colored dots represent distinct CB8 proton resonances. The absence of cross-peaks between the guest and CB8 protons at this mixing time is because of the host peaks pointing outwards.

At 700 ms mixing time

Figure S7: NOESY spectrum (500 MHz, D_2O, 25 °C) of a 0.2 mM *o*-NC guest and 0.22 mM CB8 host complex, acquired with a 700 ms mixing time. Blue dots indicate signals from the complexed *o*-NC guest, while colored dots represent distinct CB8 proton resonances. The absence of cross-peaks between the guest and CB8 protons at this mixing time is because of the host peaks pointing outwards.

Studies of *o*-NC with CDs

^{1}H NMR titration spectra of *o*-NC with α-CD (host → guest) (Guest region)

Figure S8: ^{1}H NMR (500 MHz, D_2O, 25 °C) partial spectra of (i) *o*-NC (2.0 mM) and (ii) to (vii) 2.0 mM guest with 0.067, 0.13, 0.33, 1.0, 2.0 & 4.0 mM α-CD. Peaks labelled from a-d in black and red represents free and complexed guest protons respectively.

Host signals

Figure S9: ^{1}H NMR (500 MHz, D_2O, 25 °C) partial spectra (i) - (vii) show sequential additions of increasing concentrations of α-CD (0.067 - 4.0 mM) to an initial solution of 2.0 mM *o*-NC. 2.0 mM α-CD spectrum (viii) is given for reference. Cyclodextrin protons are labelled from H_1-H_6 in black. The purple dotted line is to check the changes for internal cavity H_3 and H_5 CD protons with guest addition.

^{1}H NMR titration spectra of *o*-NC with β -CD (Adding host → guest) (guest region)

Figure S10: ^{1}H NMR titration spectra (500 MHz, D_2O, 25 °C) demonstrating the interaction of (i) 2.0 mM *o*-NC with (ii)– (x) various concentrations of β-CD in water. As the concentration of β-CD increases, both dimeric species and aggregates of *o*-NC dissociate, forming a complex with the monomeric guest. The chemical shift change for the complexed protons is minimal, only Δδ 0.08 ppm relative to the free *o*-NC. Notably, protons 'c' and 'd' which appeared as a single resonance in the free *o*-NC spectrum, are resolved into separate peaks when complexed within the β-CD cavity.

Guest signals

Figure S11: ^{1}H NMR spectra (500 MHz, D_2O, 25 °C) showing the interaction of (i) 2.0 mM *o*-NC with increasing concentrations of β-CD in water (ii)– (x). The encapsulation of *o*-NC within the β-CD cavity is further supported by the observation of splitting of isopropyl methyl signals of the guest because of the chiral β-CD. The splitting indicates proximity and binding of *o*-NC to the β-CD cavity.

Host signals

Figure S12: ^{1}H NMR spectra (500 MHz, D_2O, 25 °C) showing the interactions of (i) 2.0 mM *o*-NC with (ii)– (xi) various concentrations of β-CD in water. Shifts observed for inward H_3 and H_5 protons (see the picture below) of β-CD compared to (xii) free β-CD, indicate encapsulation of the guest molecule within the CD cavity.

NOESY of complexed *o*-NC@ β-CD complex at 300 ms mixing time

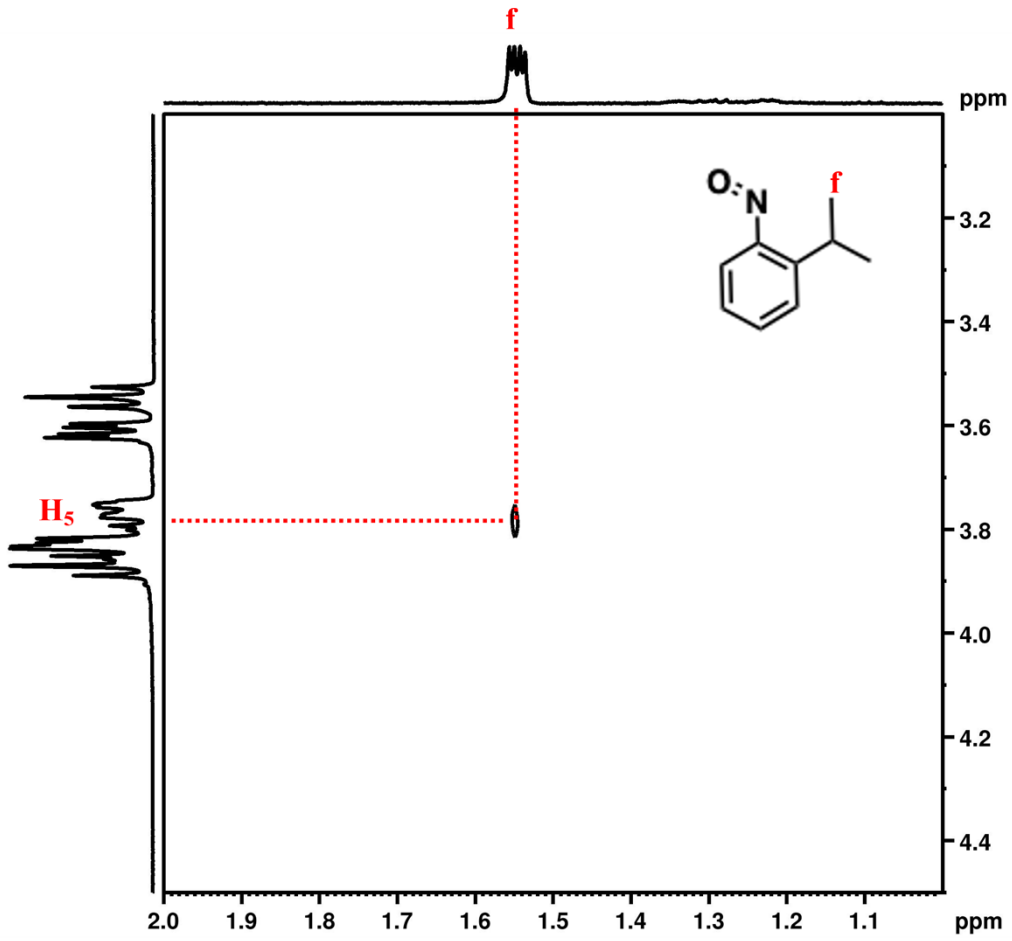


Figure S13: NOESY spectrum (500 MHz, D_2O, 25 °C) of a 2.0 mM *o*-NC guest and 2.1 mM β-CD host complex, acquired with a 300 ms mixing time. A notable NOE correlation is observed between the isopropyl protons of the guest molecule and an inner proton (H_5) of the β-CD, providing evidence for encapsulation of *o*-NC within the β-CD cavity.

^{1}H NMR titration spectra of *o*-NC with γ -CD (host → guest) (guest region)

Figure S14: ^{1}H NMR (500 MHz, D_2O, 25 °C) partial titration spectra showing the interaction of *o*-NC with various concentrations of γ-CD. Spectrum (i) represents free *o*-NC (2.0 mM), while spectra (ii) – (vii) show solutions containing 2.0 mM guest and varying concentrations of γ-CD (0.067, 0.13, 0.33, 1.0, 2.0 and 4.0 mM). As the concentration of γ-CD increases, both dimeric species and aggregates of *o*-NC dissociate, forming a complex with the monomeric guest.

Host signals

Figure S15: ^{1}H NMR (500 MHz, D_2O, 25 °C) partial titration spectra showing the interaction of *o*-NC with various concentrations of γ-CD. Spectrum (i) represents free *o*-NC (2.0 mM), while spectra (ii) – (vii) show solutions containing 2.0 mM guest and varying concentrations of γ-CD (0.067, 0.13, 0.33, 1.0, 2.0 and 4.0 mM). 2.0 mM γ-CD spectrum (viii) is given for reference. Cyclodextrin protons are labelled from H_1-H_6 in black. The purple dotted line is to check the changes for internal cavity H_3 and H_5 CD protons with guest addition.

Studies of *o*-NC with OA

^{1}H NMR titration spectra of *o*-NC with OA (host → guest)

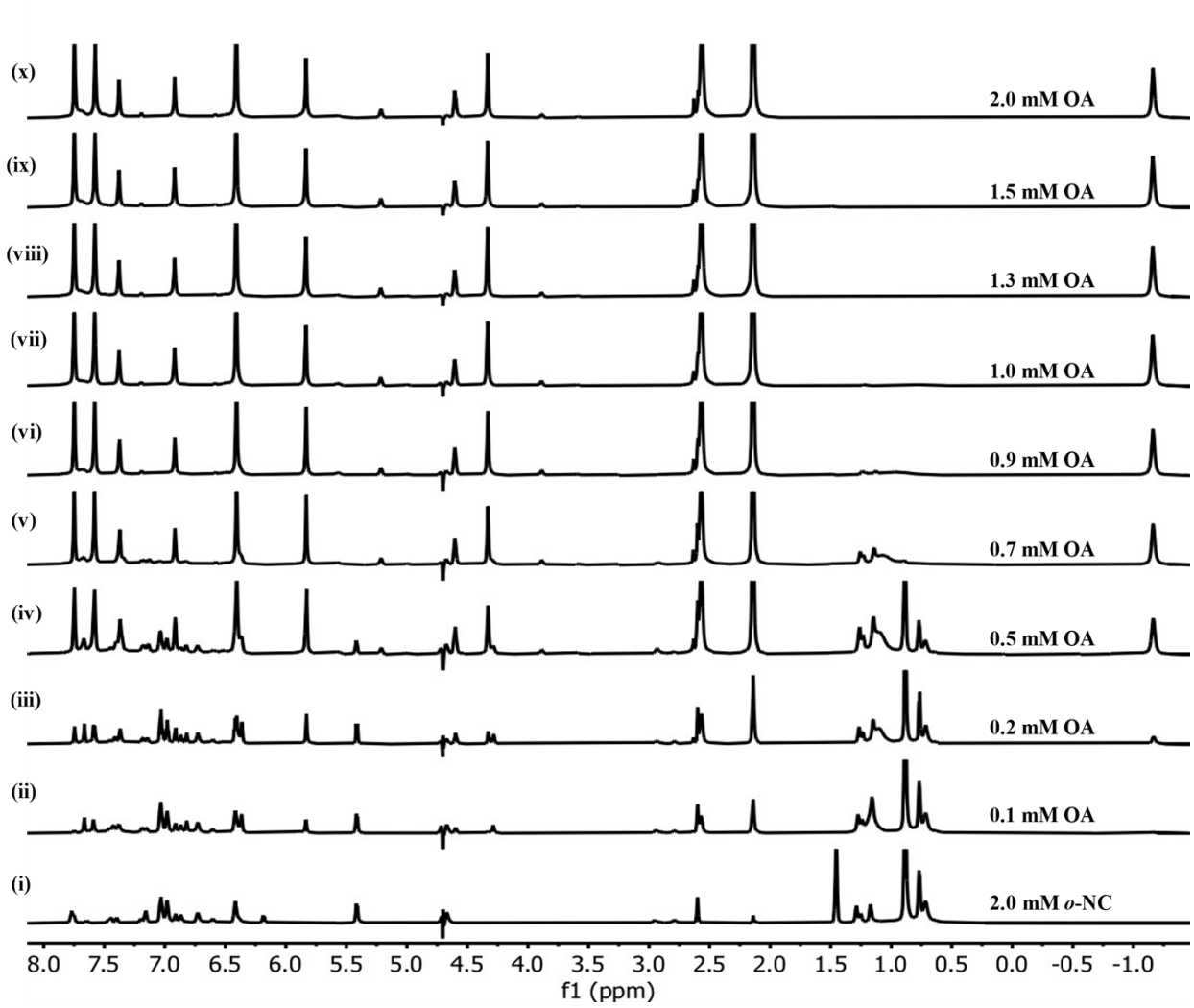


Figure S16: ^{1}H NMR full titration spectra (800 MHz, 20 mM borate buffered D_2O, 25 °C) of (i) 2.0 mM *o*-NC with (ii) 0.1, (iii) 0.2, (iv) 0.5, (v) 0.7, (vi) 0.9, (vii) 1.0, (viii) 1.3, (ix) 1.5 and (x) 2.0 mM concentrations of OA. As the concentrations of OA increases, aggregates and dimers dissociate, forming complexed monomers.

Host region

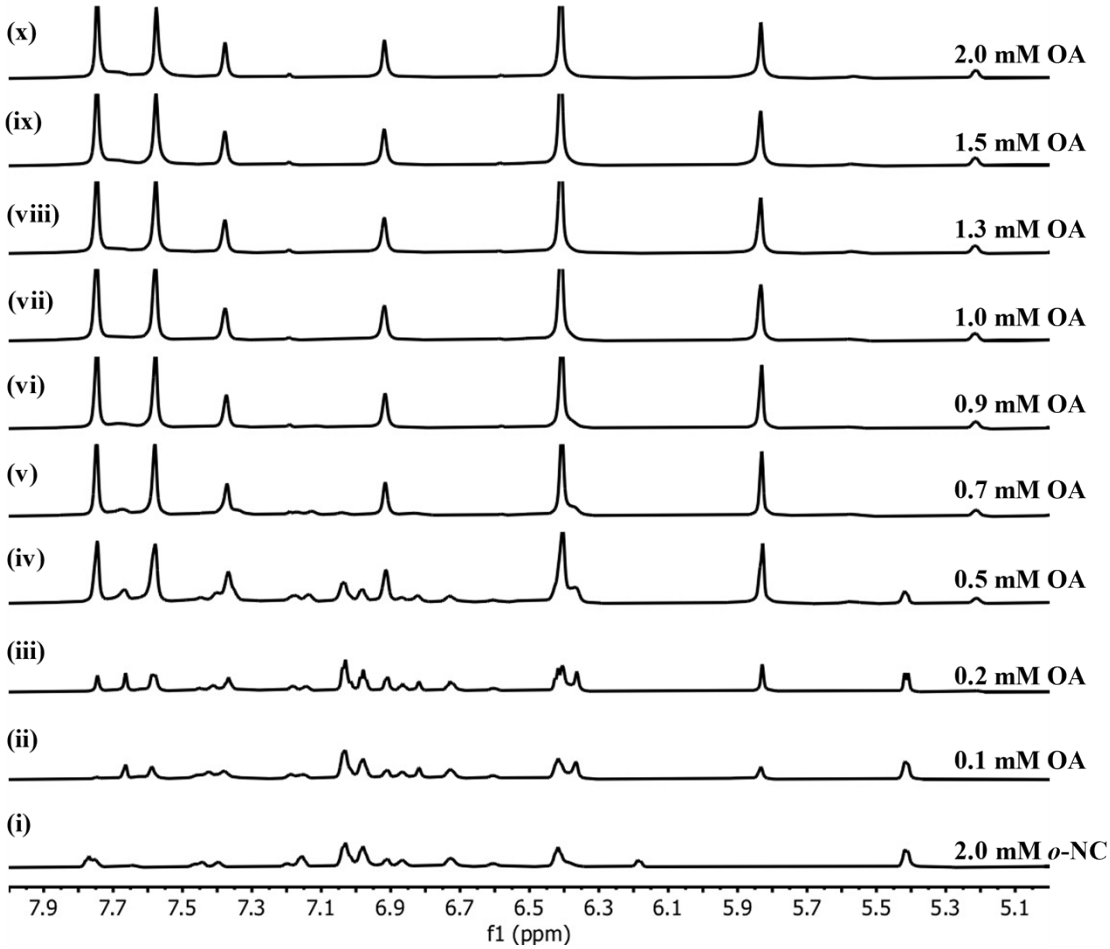


Figure S17: ^{1}H NMR partial titration spectra (800 MHz, 20 mM borate buffered D_2O, 25 °C) of (i) 2.0 mM *o*-NC with (ii) 0.1, (iii) 0.2, (iv) 0.5, (v) 0.7, (vi) 0.9, (vii) 1.0, (viii) 1.3, (ix) 1.5 and (x) 2.0 mM concentrations of OA. As the concentrations of OA increases, aggregates and dimers dissociate, forming complexed monomers.

Guest region

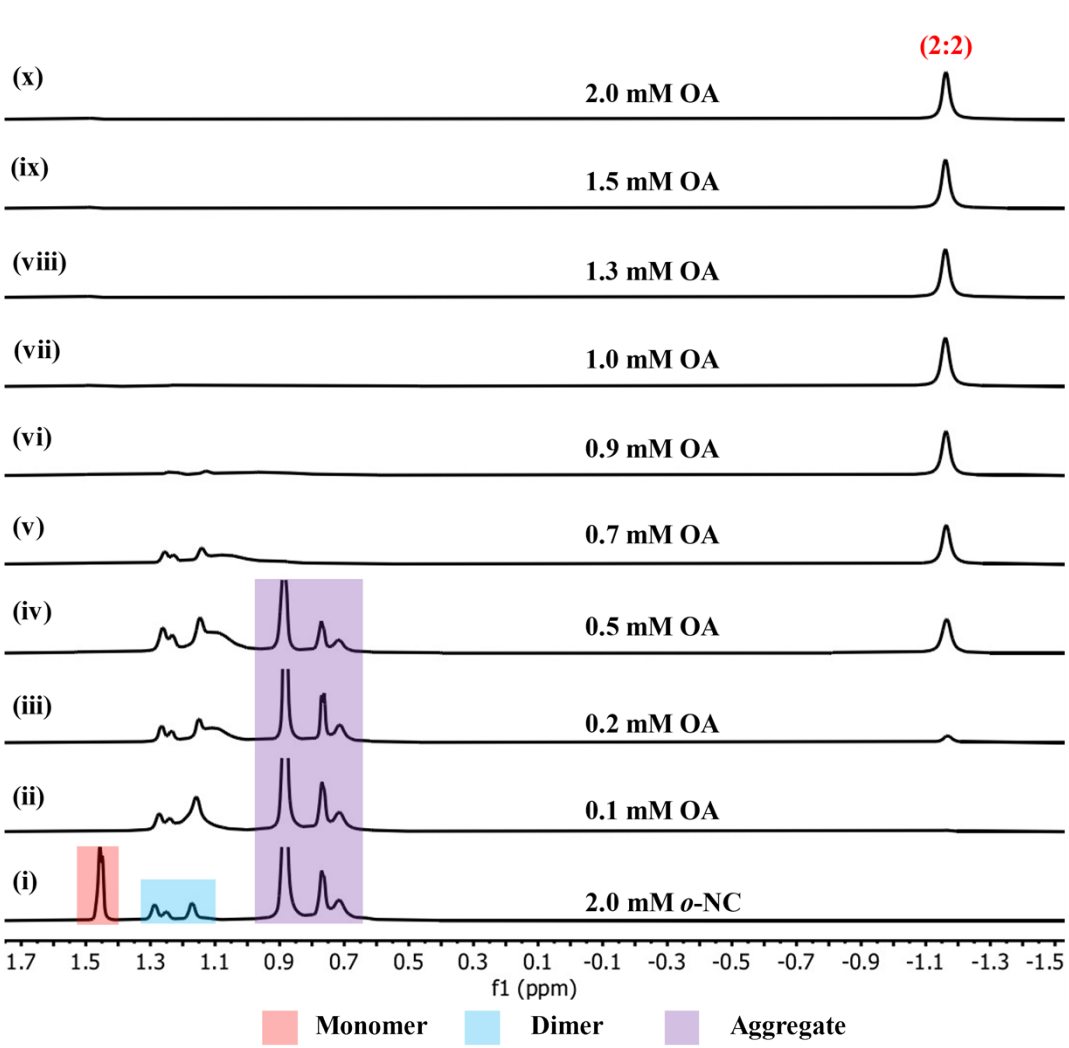
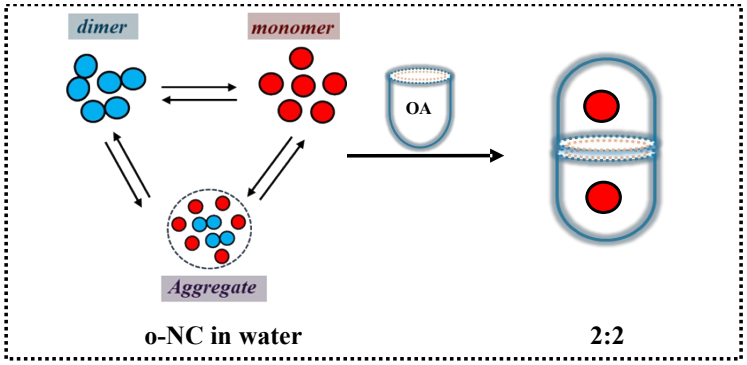


Figure S18: ^{1}H NMR partial titration spectra (800 MHz, 20 mM borate buffered D_2O, 25 °C) of (i) 2.0 mM *o*-NC with (ii) 0.1, (iii) 0.2, (iv) 0.5, (v) 0.7, (vi) 0.9, (vii) 1.0, (viii) 1.3, (ix) 1.5 and (x) 2.0 mM concentrations of OA. As the concentrations of OA increases, aggregates and dimers dissociate, forming complexed monomers.



[1]H NMR titration spectra of *o*-NC with OA (guest → host)

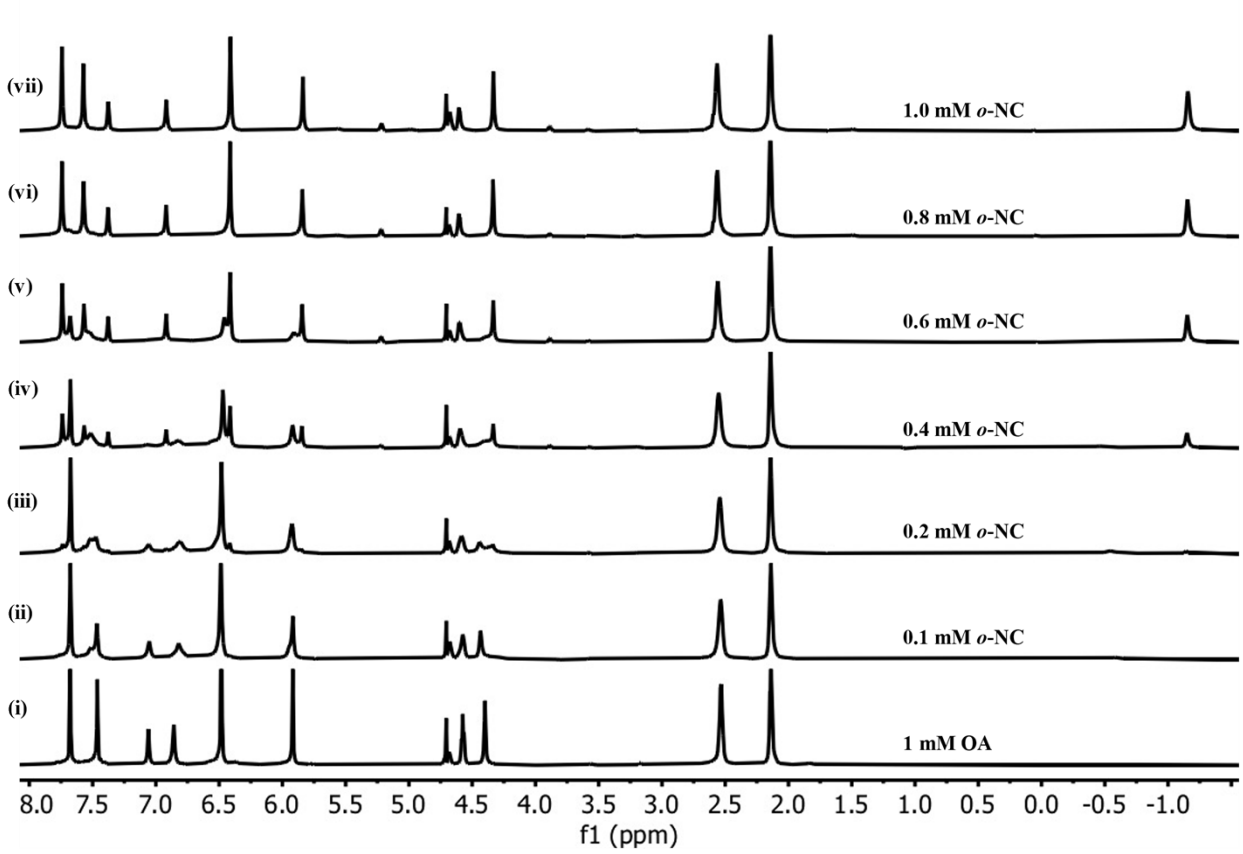


Figure S19: Titration study of OA and *o*-NC complexation. [1]H NMR full titration spectra (800 MHz, 25 ℃) of (i) 1.0 mM OA with (ii) 0.1, (iii) 0.2, (iv) 0.4, (v) 0.6, (vi) 0.8, and (vii) 1.0 mM *o*-NC in 10 mM borate buffered D_2O.

Host region

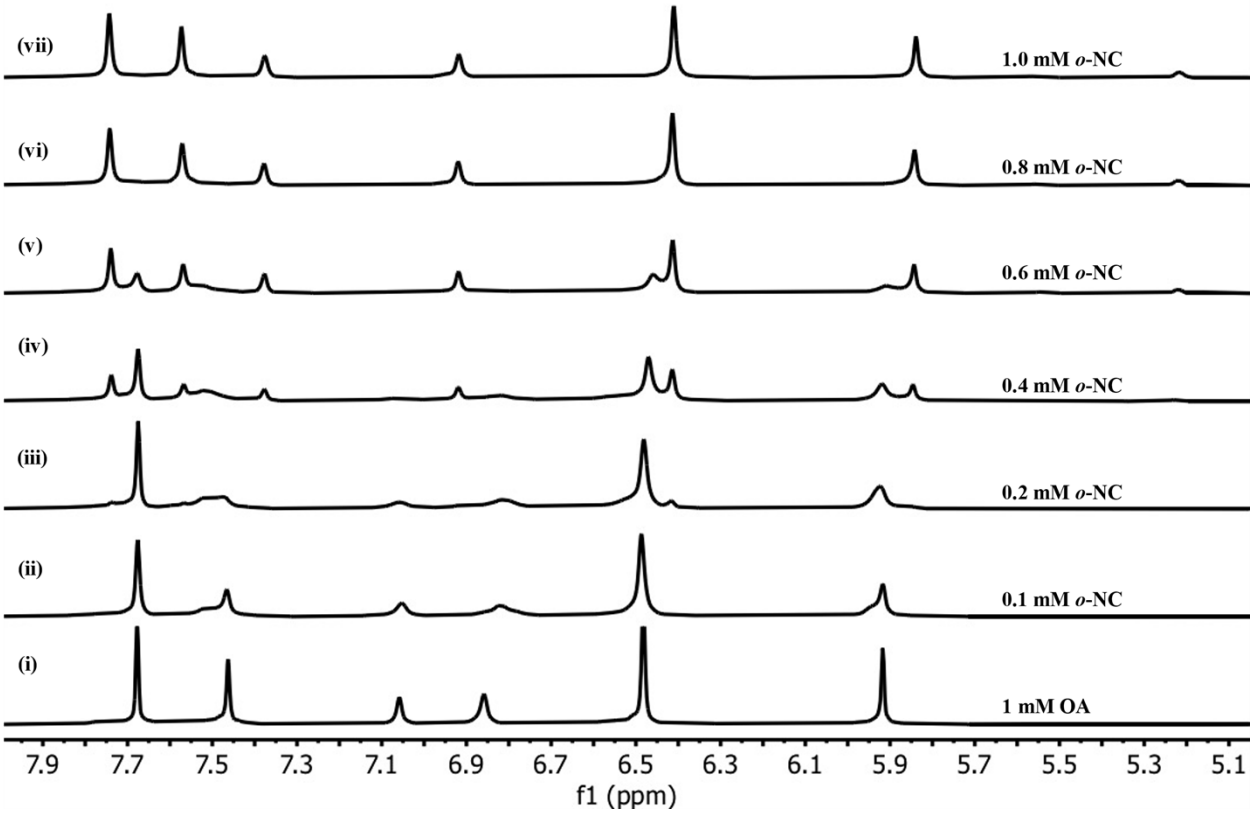


Figure S20: ^{1}H NMR partial titration spectra (800 MHz, 25 ℃) of (i) 1.0 mM OA with (ii) 0.1, (iii) 0.2, (iv) 0.4, (v) 0.6, (vi) 0.8, and (vii) 1.0 mM *o*-NC in 10 mM borate buffered D_2O.

Guest region

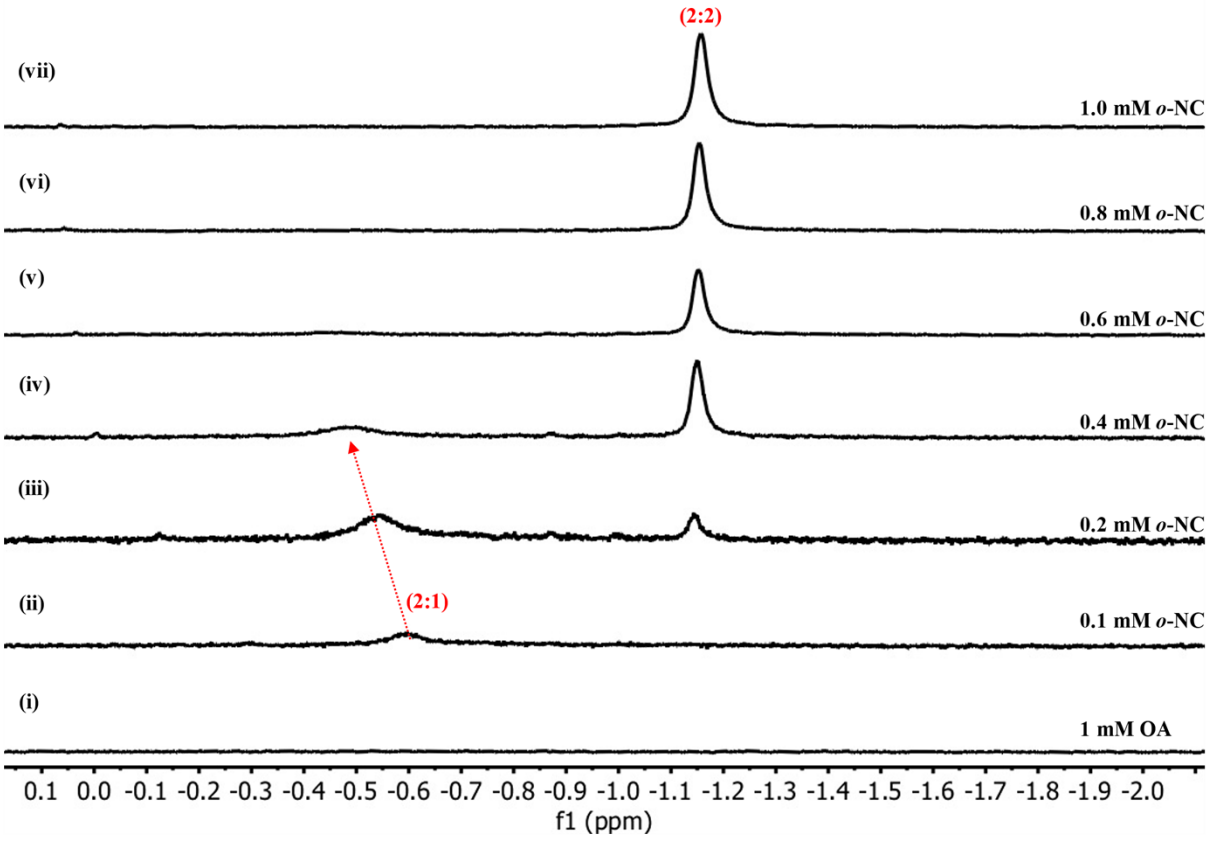
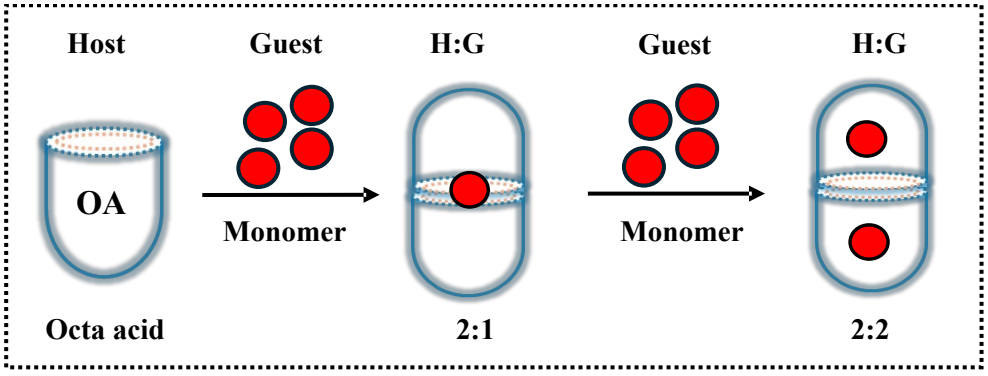


Figure S21: ^{1}H NMR partial titration spectra (800 MHz, 25 °C) of (i) 1.0 mM OA with (ii) 0.1, (iii) 0.2, (iv) 0.4, (v) 0.6, (vi) 0.8, and (vii) 1.0 mM *o*-NC in 10 mM borate buffered D_2O. The complete titration shows that only 2:2 (H: G) complexes are formed, indicating the initial 2:1(H: G) complex has fully converted to 2:2 (H: G).



2D DOSY spectrum of OA, *o*-NC@OA_2 and *o*-NC_2@OA_2

1. **Free OA**

Figure S22: 2D DOSY NMR spectrum (800 MHz, 20 mM borate buffered D_2O, 25 °C) of free 2.0 mM OA. Diffusion measurements yield a diffusion constant (D) of $1.81 \pm 0.02 \times 10^{-10}$ m^2/s, providing information about the molecular size and mobility of OA in 10 mM buffered solution.

2. *o*-NC_2@OA_2

Figure S23: 2D DOSY NMR spectrum (800 MHz, 20 mM borate buffered D_2O, 25 ℃) of the 2.0 mM *o*-NC@ 2mM OA complex. The observed diffusion coefficient of $1.41 \pm 0.02 \times 10^{-10}$ m^2/s indicates the formation of capsuleplex.

3. *o*-NC@OA_2

Figure S24: 2D DOSY NMR spectrum (400 MHz, 20.0 mM borate buffered D_2O, 25 °C) of the 0.5 mM *o*-NC@ 2.0 mM OA complex. The observed diffusion coefficient of $1.38 \pm 0.03 \times 10^{-10}$ m^2/s for the intermediate complex indicates a closed capsuleplex structure, excluding the possibility of an open cavitandplex.

NOESY of complexed *o*-NC_2@ OA_2 complex at 300 ms mixing time

Figure S25: NOESY spectrum (500 MHz, D_2O, 25 °C) of a 2.0 mM *o*-NC guest and 2.1 mM OA host complex, acquired with a 300 ms mixing time. NOE correlations were observed between isopropyl protons of the encapsulated *o*-NC guest and OA protons d, e, f, and g – all located in the rim-to-mid region of the OA cavity. This spatial relationship indicates that the isopropyl group is not deeply buried within the OA cavity, consistent with molecular dynamics (MD) simulations.

DLS and images of o-NC solution before and after OA host addition

Figure S26: Dynamic Light Scattering (DLS) measurements and visual observations illustrate the transition from aggregated to dispersed *o*-NC in aqueous solution. (a) The initial *o*-NC solution exhibits turbidity and a large average Z-size of 856 nm, with a polydispersity index (PDI) of 0.116. The relatively large particle size suggests the presence of larger aggregates in solution which was not observed in NMR experiments. (b) Addition of the OA host results in clearing of the solution. Subsequent DLS analysis reveals that the resulting complexed *o*-NC_2@OA_2 species are below the instrument's detection limit, suggesting their size (<10 Å), is significantly reduced (de-aggregation) and well-dispersed.

Most representative structures of *o*-NC@OA_2, *o*-NC_2@OA_2 and D_E@OA_2 from MD simulation

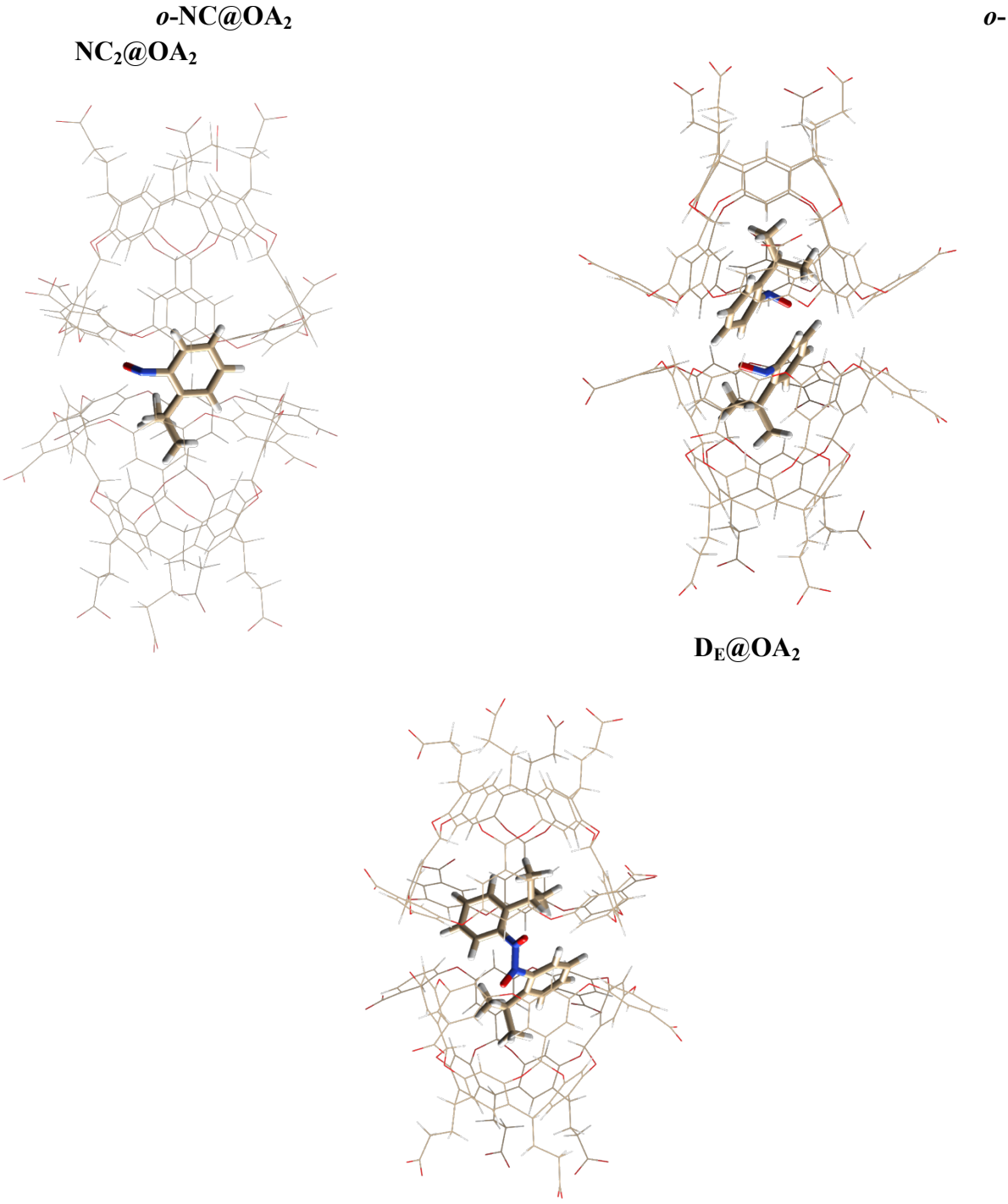


Figure S27: Most representative structures illustrating the encapsulation of *o*-NC, 2 *o*-NC monomers and E-dimer (D_E) within OA, as obtained from MD simulation. These structures demonstrate the ability of OA capsule to encapsulate both monomeric and E-dimeric forms of *o*-NC. The orientation of encapsulated monomers appears to hinder the dimerization process.

Adding *o*-NC to 4,4'-dimethylstilbene(DMS)@OA_2 complexed solution

Figure S28: ^{1}H NMR spectra (500 MHz, D_2O, 25 °C) showing the titration of *o*-NC to complexed DMS@OA_2 solution. The formation of an equally strong complex between *o*-NC and OA resulted in the mixture of both complex. Resulting mixture exhibited only free monomer and dimer peaks, confirming that the initially complexed species originated from aggregates.

	Integral values of OA 'f' protons (normalized to 100) (%)		Integral values of *o*-NC & DMS methyls (normalized to 100) (%)	
	OA (*o*-NC)	**OA (DMS)**	**Me (*o*-NC)**	**Me (DMS)**
1.6 mM DMS@OA_2 + 2 mM *o*-NC	**68**	**33**	**74**	**26**
1.6 mM DMS@OA_2 + 4 mM *o*-NC	**67**	**33**	**77**	**23**

HRMS of *o*-NC complexes with CB8 and CDs

1. ***o*-NC@CB8**

Figure S29: HRMS (ESI^+) spectrum of the *o*-NC@CB8 complex. The peak at m/z = 762.2337 corresponds to the $[CB8 + 2Na + H + o\text{-}NC]^{2+}$ species (calculated m/z = 762.2320). Error: (762.2337 -762.2320)/762.2320 = 2.2 ppm. Above mass spectrum corresponds to 1:1 *o*-NC:CB8 complex.

2. *o*-NC@β-CD

Figure S30: HRMS (ESI^+) spectrum of the *o*-NC@β-CD complex. The peak at m/z = 1306.4480 corresponds to the $[\beta\text{-CD} + Na + o\text{-NC}]^+$ species (calculated m/z = 1306.4436). Error: (1306.4480 -1306.4436)/1306.4436 * 1000000 = 3.4 ppm. Above mass spectrum corresponds to 1:1 *o*-NC:β-CD complex.

Figure S31: HRMS (ESI^+) spectrum of the *o*-NC@β-CD complex. The peak at m/z = 1805.5660 corresponds to the $[3\beta\text{-CD} + Na + K - 3H + o\text{-NC}]^{2+}$ species (calculated m/z = 1805.5616). Error: (1805.5660 – 1805.5616)/1805.5616 = 2.4 ppm. In addition to 1:1 *o*-NC:β-CD complex, 1:3 *o*-NC:3β-CD complexes are also found.

3. *o*-NC@α-CD

Figure S32: HRMS (ESI^+) spectrum of the *o*-NC@α-CD complex. The peak at m/z = 1562.4824 corresponds to the $[3\alpha\text{-CD} + Na + K - 3H + o\text{-NC}]^{2+}$ species (calculated m/z = 1562.4889). Error: (1562.4889 – 1562.4824)/1562.4824 = 4.2 ppm. Above mass spectrum corresponds to 1:1 *o*-NC:3α-CD complex.

4. *o*-NC@ɣ-CD

Figure S33: HRMS (ESI^+) spectrum of the *o*-NC@ɣ-CD complex. The peak at m/z = 2048.6372 corresponds to the $[3ɣ\text{-CD} + Na + K - 3H + o\text{-NC}]^{2+}$ species (calculated m/z = 2048.6409). Error: (2048.6372– 2048.6409)/2048.6409 = -1.8 ppm. Above mass spectrum corresponds to 1:1 *o*-NC:3ɣ-CD complex.