

## Electronic Supplementary Information

### **Recognition-mediated Cucurbit[7]uril-Heptamolybdate Hybrid Material: A Facile Supramolecular Strategy for $^{99m}\text{Tc}$ Separation**

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#### **Experimental**

Ammonium molybdate was obtained from SRL, India. CB7 was synthesized and purified following the reported procedures in the literatures.<sup>1</sup> Purity of the CB7 was checked using  $^1\text{H}$ -NMR spectroscopy. Nanopure water (conductivity less than  $0.06\ \mu\text{S cm}^{-1}$ ), obtained from a Millipore Gradient A10 system, was used to prepare the sample solutions.

**Spectroscopic measurements:** X-ray diffraction (XRD) measurements were carried out using a Philips powder ( $\lambda = 1.54056\ \text{\AA}$ ) X-ray diffractometer (model PW 1071) with Ni filtered  $\text{Cu-K}\alpha$  radiation.

$^{13}\text{C}$ -CPMAS-NMR (cross polarization magic angle spinning nuclear magnetic resonance) experiments were recorded using a Bruker Avance 500 machine having a standard 4 mm bore MAS accessory at a basic frequency of 125 MHz for  $^{13}\text{C}$  nuclei. Samples were spun at a frequency of 10 kHz. All chemical shift values are expressed with respect to tetra-methyl silane as the reference material.

VG PQ ExCell (Thermo Elemental) inductively coupled mass spectrometry (ICP-MS) was used to determine the Mo content in ppm in the supernatant liquid immediately after precipitation of the heptamolybdate. IR spectrum was recorded using a JASCO FTIR 4100 spectrophotometer (Jasco Corporation, Tokyo, Japan).

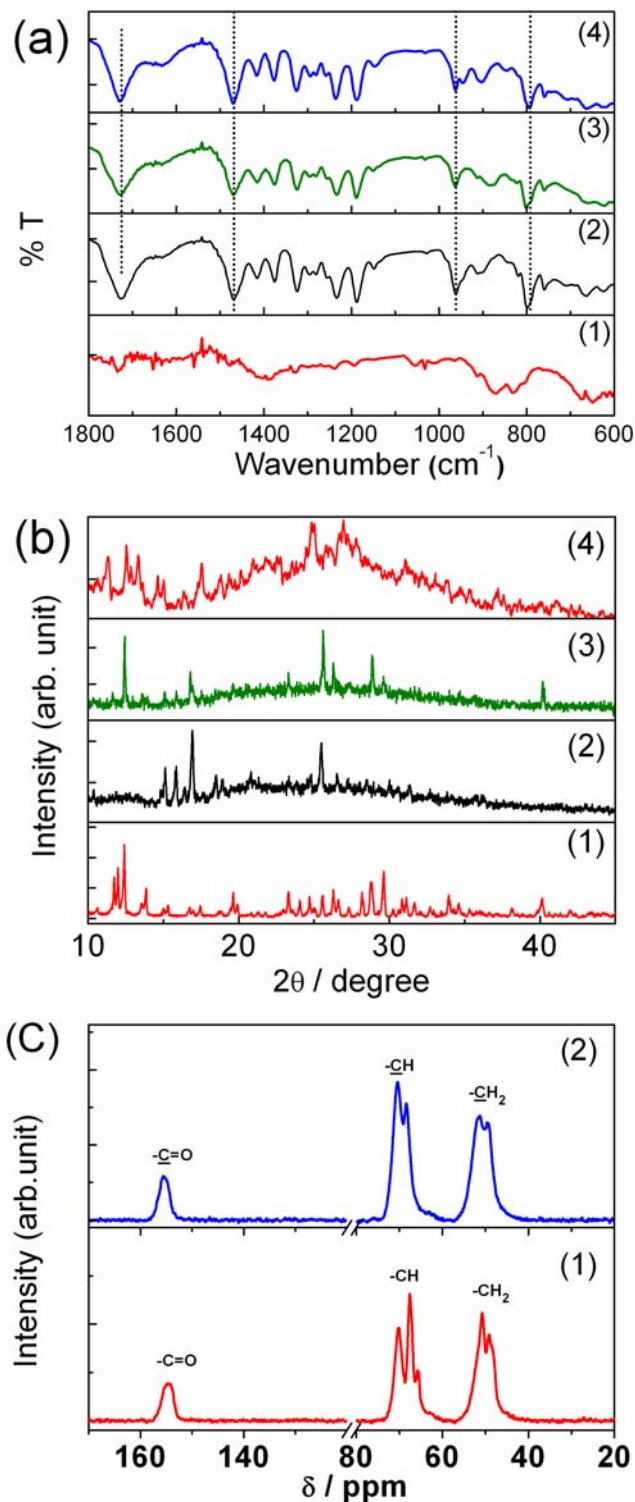
**Elution:** In a lab scale setup, 1 ml solution of 5 mM of CB7 at pH  $\sim 6$  is mixed with  $32.5\ \mu\text{L}$  of  $\text{Mo}_7\text{O}_{24}^{6-}$  having Specific activity of 371.9 mCi/g of molybdenum. The reaction mixture was then heated at  $60^\circ\text{C}$  for 5 min to obtain a curdy white fluffy precipitate. The mixture was cooled to room temperature and centrifuged. The supernatant was separated and analyzed. Elution of the  $\text{CB7-}^{99}\text{Mo}_7\text{O}_{24}^{6-}$  precipitate was carried out after  $\sim 24$  hours for about 3 days using 1 mL of 0.9% saline solution. Supernatant ( $\sim 1$  ml) is carefully collected, filtered through  $0.22\ \mu\text{m}$  filter (to eliminate the possibility of the precipitate) and analyzed for  $^{99m}\text{Tc}$  tracer and  $^{99}\text{Mo}$  impurity by a  $\gamma$ -counter.

**Determination of Radiochemical Purity (RCP):** Radioactivity distribution on paper chromatography strip (Whatman No. 3) was recorded on MiniGITA  $\gamma$ -radioactivity TLC scanner, procured from Raytest, Germany. Paper chromatography strip (11.5 cm long) was prepared by spotting  $\sim 4 \mu\text{L}$  of the test solution (Pertechnetate obtained from CB7- $^{99}\text{Mo}_7\text{O}_{24}^{6-}$  generator) 1.5 cm from the bottom of the strip. The strip was developed in physiological saline till the solvent front reach 1 cm from the top of the strip. The radioactivity distribution on the developed strip was recorded on a TLC scanner with the energy window of the detector set for  $^{99\text{m}}\text{Tc}$ . From the peak area measurement, RCP of pertechnetate was calculated. A typical paper chromatography pattern of pertechnetate in saline, showing only one component, is shown in Fig. S3, ESI.

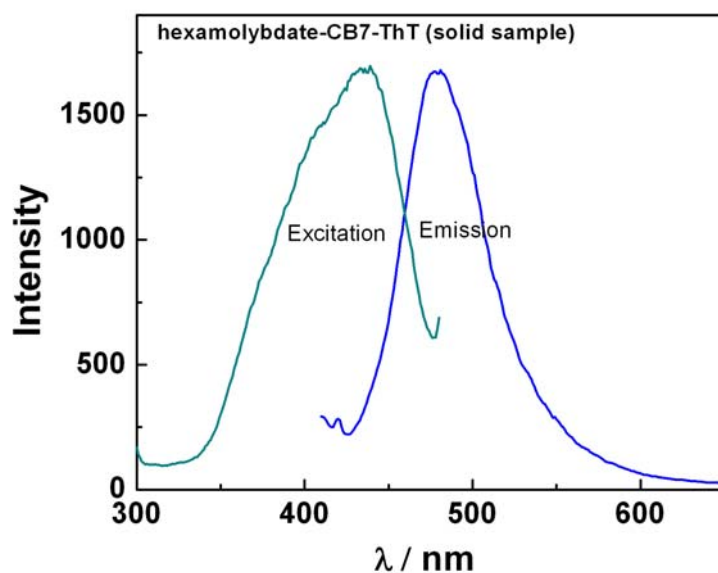
**Determination of Radionuclidic Purity (RNP):** The  $\gamma$  activity was followed for the determination of  $^{99}\text{Mo}$  in the supernatant liquid (containing  $^{99\text{m}}\text{Tc}$ ) collected after washing CB7-heptamolybdate precipitate using a NaI (TI) multichannel analyzer which allows simultaneous determination of both the isotopes.<sup>2</sup> The fraction of  $^{99}\text{Mo}$  activity as well as  $^{99\text{m}}\text{Tc}$  activity present in the supernatant is calculated by comparing their respective activities with solutions of  $^{99}\text{Mo}$  and  $^{99\text{m}}\text{Tc}$  of known activity.

**Synthesis of the CB7-heptamolybdate hybrid material:** On adding CB7 solution to the ammonium molybdate solution at pH  $\sim 5.5$ , turbidity appeared. On slight heating the solution at  $\sim 60^\circ\text{C}$  for about 5 min, a white precipitate settled down giving easy separation using centrifugation. The supernatant solution was removed and the solid was then dried, which was found to be with a yield of  $\sim 70\%$ . The solid thus obtained was characterized using FT-IR spectroscopy, X-ray diffraction, solid state NMR etc.

**Elemental Analysis:** The elemental analysis of the precipitated complex prepared with CB7 solution (having 100mM NaCl) closely match the 1:1 complex stoichiometry with molecular formula of  $\text{C}_{42}\text{H}_{42}\text{N}_{28}\text{O}_{14}\cdot\text{Na}_6\text{Mo}_7\text{O}_{24}$  (experimental: C 21.23%, H 1.42%, N 14.55%; theoretical: 21.43%, H 1.80%, N 16.64%). The slight discrepancy may be due to the possible presence of oligomeric/polymeric species in the precipitate.



**Figure S1.** Spectroscopic characterization of the hybrid material. (a) FT-IR spectra of (1) Mo<sub>7</sub>O<sub>24</sub><sup>6-</sup>; (2) CB7; (3) Physical mixture of Mo<sub>7</sub>O<sub>24</sub><sup>6-</sup> and CB7; (4) CB7-Mo<sub>7</sub>O<sub>24</sub><sup>6-</sup> precipitated complex. (b) XRD patterns of (1) pure ammonium heptamolybdate; (2) CB7; (3) physical mixture of CB7 and heptamolybdate; (4) CB7-Mo<sub>7</sub>O<sub>24</sub><sup>6-</sup> complex. (c) Solid state <sup>13</sup>C NMR spectra of (1) CB7; (2) CB7-Mo<sub>7</sub>O<sub>24</sub><sup>6-</sup> precipitated complex.



**Figure S2. Spectral characteristics.** Emission and excitation spectra of the precipitate (after wash), confirming the presence of ThT in the precipitate.

**Table S1.** ICP-MS data for the Mo content in the supernatant liquid: immediately after precipitation of the heptamolybdate at pH 5.5-6, using two different concentrations of CB7 (a) and on first wash using saline (b).

	Sample	[CB7] / mM	[Inactive Mo]	
			(ppm)	(%)
(a)	Supernatant	1.0	17.8	2.6
		1.5	18.6	2.8
(b)	1 <sup>st</sup> Washing (0.9% saline solution)	1.0	3.8	0.56
		1.5	2.7	0.4

**Note S1**

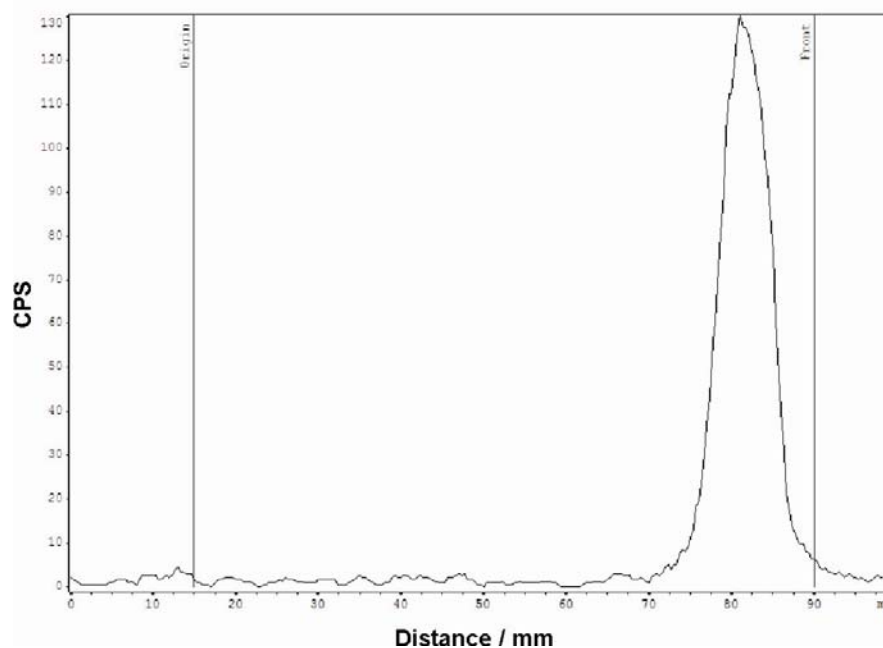
Dissociation of the complex: The concern of CB7 leaching out from the precipitate (even after complete removal of the uncomplexed CB7 in the initial washings itself) to the eluted sample is considered. Any chance of CB7 becoming free on dissociation of the precipitated complex would as well leach out the molybdate in to the solution. Since in isolation, both the CB7 and the heptamolybdate are fairly soluble in the eluent, the dissociation of the complex can be estimated from the  $\gamma$  activity of Mo in the eluate. As evident from the Table S1, the elution

measurements confirm that the presence of, otherwise biocompatible, CB7 is not very significant.

**Table S2.** Technical parameters of the proposed supramolecular  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  generator

Day <sup>#</sup>	Reference Counts (cps)		Mo-CB7 complex (740keV) (cps)	% Mo with CB7	Activity in the Eluant	Elution efficiency with 1 ml saline (%)	Activity (mCi/ml)	RCP of $^{99\text{m}}\text{Tc}$ (%)	RNP of $^{99\text{m}}\text{Tc}$ (%)
	$^{99}\text{Mo}$	$^{99\text{m}}\text{Tc}$			$^{99\text{m}}\text{Tc}$	$^{99\text{m}}\text{Tc}$	$^{99\text{m}}\text{Tc}$		
1	274 ± 5	7476 ± 51	253 ± 3	92.5	3865 ± 36	51.7	0.48	>99	94.35
2	201 ± 4	6420 ± 44	175 ± 4	87.4	2803 ± 29	43.7	0.32	>99	97.55
3	153 ± 2	5501 ± 31	135.5 ± 2.5	88.79	2275 ± 22	41.4	0.24	>99	97.12

<sup>#</sup>Generator/generator bed was prepared on day 0.

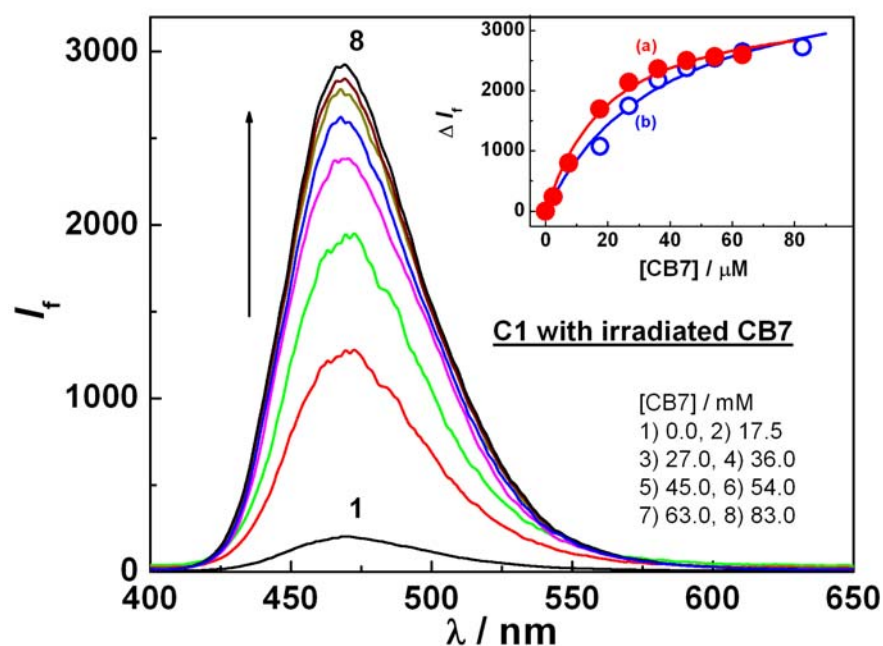


**Figure S3. Radioactivity distribution.** The radioactivity distribution on the developed chromatographic strip recorded on a TLC scanner with the energy window of the detector set for  $^{99\text{m}}\text{Tc}$ .

**Note S2**

**Radiation stability measurements:** Since the added macrocyclic CB7 host, encounters the *in situ* radiation from the active  $^{99}\text{Mo}$  and  $^{99\text{m}}\text{Tc}$  nuclides, the radiation stability of CB7 host was

examined by evaluating the binding interaction of CB7 towards a well known fluorescent dye, namely, coumarin 1 (C1),<sup>3</sup> before and after exposure in a standard  $\gamma$ -chamber. Even with strong radiation field of the  $\gamma$ -source employed (23Gy/min) for about 1 hour, the recognition behaviour of irradiated and un-irradiated CB7 sample differed only slightly, reiterating the excellent stability of CB7 in the low level of activity exposed in the precipitate. From the fluorescence titration of coumarin1 (C1) insets of Fig.S4, it is seen that the binding constant value of CB7-C1 complex decreases from  $\sim 5 \times 10^5 \text{ M}^{-1}$  to  $4 \times 10^5 \text{ M}^{-1}$  with irradiated CB7 sample (exposed dose of 1.38 KGy, which is equivalent to  $41 \times 10^3 \text{ Ci}$ ), which is negligible considering the radiation exposure CB7 from the radioactive  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  mixture during the experiments.



**Figure S4.** Fluorescence spectra and binding curves. Steady-state fluorescence spectra of coumarin 1 (C1) dye of ( $4 \times 10^{-6}$  M) in aqueous solution at different concentrations of irradiated CB7. **Inset:** Fluorescence titration curves for C1 dye with un-irradiated (a) and irradiated (b) CB7 for a comparison.

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