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Rotaxane-mediated suppression and activation of cucurbit[6]uril for molecular detection by ^{129}Xe hyperCEST NMR

Joel A. Finbloom,[†] Clancy C. Slack,[†,‡] Carson J. Bruns,[†] Keunhong Jeong,[†,‡] David E. Wemmer,[†,#] Alexander Pines,[†,‡] and Matthew B. Francis.*[,†,‡]

[†]*Department of Chemistry, University of California, Berkeley* [‡]*Materials Sciences Division, Lawrence Berkeley National Laboratories, and* [#]*Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720*

**mbfrancis@berkeley.edu*

SUPPORTING INFORMATION

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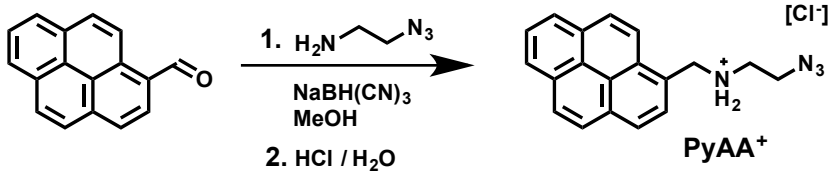
S1. General Methods

All solvents and reagents, including 1-pyrenecarboxaldehyde, cucurbit[6]uril hydrate ($CB6 \cdot XH_2O$), and β-cyclodextrin hydrate ($\beta CD \cdot XH_2O$), were purchased from commercial suppliers and used without further purification. 2-azidoethylamine,[S1] and complex **1**[S2] were prepared according to literature procedures. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} (E. Merck) and visualized under a UV lamp at 254 nm. Column chromatography was carried out on silica gel 60 (E. Merck, 230–400 mesh). A C-18 column was used for analytical and semi-preparative reverse phase high performance liquid chromatography (RP-HPLC) on an Agilent 1100 Series Capillary LC. Runs were eluted with H_2O/MeCN (0.1 % v/v TFA) and monitored using a UV-Vis detector. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance 400 and 600 spectrometers with working frequencies of 400 or 600 MHz for ^{1}H NMR, and 100 or 150 MHz for ^{13}C NMR, respectively. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz), and integration. Data for ^{13}C NMR are reported in terms of chemical shift. Chemical shifts are referenced to the residual non-deuterated solvents for ^{1}H ($CDCl_3$: δ = 7.27 ppm, CD_3CN: δ = 1.94 ppm, $(CD_3)_2SO$: δ = 2.50 ppm) and ^{13}C ($CDCl_3$: δ = 77.0 ppm, CD_3CN: δ = 118.26 ppm, $(CD_3)_2SO$: δ = 39.52 ppm) nuclei. Matrix assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed on a Voyager-DE system (PerSeptive Biosystems, USA) and data were analyzed using Data Explorer software.

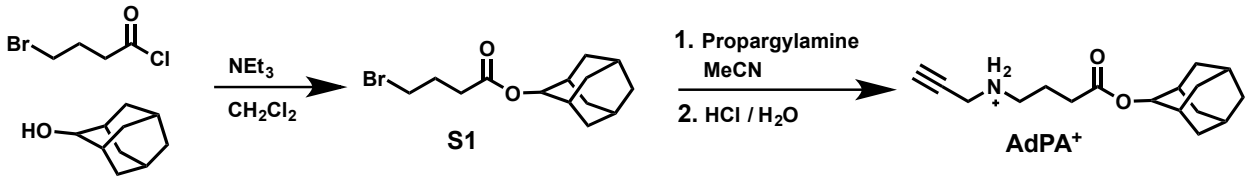
S2. Synthetic Procedures



Scheme S1. Synthesis of 2-azido-*N*-(pyren-1-ylmethyl)ethanaminium chloride (**PyAA**$^+$).

2-azido-N-(pyren-1-ylmethyl)ethanaminium chloride (PyAA$^+$). A portion of 2-Azidoethylamine (79 mg, 0.92 mmol) was added to a solution of 1-pyrenecarboxaldehyde (214 mg, 0.93 mmol) in a mixture of CH_2Cl_2 (5 mL) and MeOH (7 mL) under an atmosphere of nitrogen. After stirring at

ambient temperature for 30 min, $NaBH_3CN$ (93 mg, 1.5 mmol) was added in one portion and the reaction continued stirring for 3 d. The solvent was then removed under a stream of N_2. The residue was taken up in CH_2Cl_2 (20 mL) and washed with 5 M HCl (10 mL), leading to the formation of a pale yellow precipitate, which was collected by vacuum filtration and dried under vacuum (170 mg, 54%). ^{1}H NMR (400 MHz, $(CD_3)_2SO$, 293 K) δ = 9.82 (s, 2H), 8.58 (d, J = 9.0 Hz, 1H), 8.40–8.33 (m, 5H), 8.26 (d, J = 9.0 Hz, 1H), 8.22 (d, J = 9.0 Hz, 1H), 8.13 (t, J = 7.5 Hz, 1H), 4.96 (s, 2H), 3.86 (t, J = 6.0 Hz, 2H), 3.32 (t, J = 6.0 Hz, 2H). ^{13}C NMR (100 MHz, $(CD_3)_2SO$, 293 K) δ = 131.4, 130.7, 130.2, 129.4, 129.3, 128.2, 128.1, 127.3, 126.6, 125.8, 125.6, 125.5, 124.8, 123.9, 123.6, 123.3, 47.1, 46.8, 45.7. HRMS (ESI-TOF-MS): *m/z* calc'd for $C_{19}H_{17}N_4$ [M – Cl]$^+$ 301.1448, observed 301.1443.

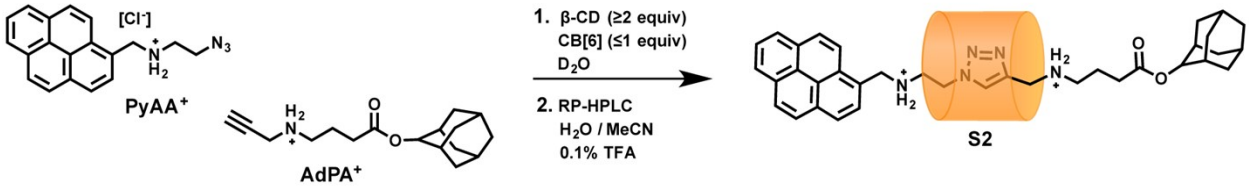


Scheme S2. Synthesis of propargylammonium-functionalized adamantyl ester stopper **AdPA**$^+$.

2-Adamantyl 4-bromobutanoate (S1). A portion of 4-Bromobutyryl chloride (1.6 mL, 14 mmol) was added to a mixture of 2-adamantanol (650 mg, 4.2 mmol) and triethylamine (1.6 mL, 11 mmol) in CH_2Cl_2/THF (1:1 v/v, 30 mL). The reaction stirred at ambient temperature for 3 h, under an atmosphere of N_2. The reaction mixture was poured into 0.1 M HCl (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic extracts were combined, dried ($MgSO_4$), filtered, concentrated, and subjected to flash column chromatography on SiO_2, eluting with CH_2Cl_2 to afford the product as a white solid (530 mg, 41%). ^{1}H NMR (400 MHz, $CDCl_3$, 293 K) δ = 4.95 (t, J = 3.0 Hz, 1H), 3.49, (t, J = 7.0 Hz, 2H), 2.54 (t, J = 7.0 Hz, 2H), 2.20 (q, J = 7.0 Hz, 2H), 2.04–1.97 (m, 4H), 1.88–1.82 (m, 4H), 1.80–1.72 (m, 4H), 1.60–1.54 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, 293 K) δ = 171.9, 77.2, 37.3, 36.3, 33.0, 32.8, 31.8, 31.8, 27.9, 27.2, 26.9. HRMS (ESI-TOF-MS): *m/z* calc'd for $C_{14}H_{22}BrO_2$ [M + H]$^+$ 301.0803, observed 301.0795.

4-(2-adamantoxy)-4-oxo-N-(prop-2-ynyl)butan-1-aminium (AdPA$^+$). Propargylamine (600 mg, 11 mmol) was added to a solution of compound **S1** (315 mg, 1.04 mmol) in MeCN (6 mL) and the mixture was stirred at 60 °C for 18 h, under an atmosphere of N_2. The solvent was removed under

reduced pressure and the residue was combined with CH_2Cl_2 (4 mL), producing an orange precipitate of predominantly propargylammonium bromide. The precipitate was removed via filtration and the filtrate was diluted with CH_2Cl_2 (8 mL) and washed with 2 M HCl (10 mL). The organic layer was dried under vacuum to afford the product as a pale tan-colored solid (301 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$, 293 K) δ = 9.97 (s, 2H), 4.91 (d, J = 3.0 Hz, 1H), 3.91 (td, J = 5.0, 2.5 Hz, 2H), 3.22 (tt, J = 6.0, 5.0 Hz, 2H), 2.64 (t, J = 2.5 Hz, 1H), 2.53 (t, J = 7.0 Hz, 2H), 2.22 (q, J = 7.0 Hz, 2H), 1.99–1.94 (m, 4H), 1.86–1.79 (m, 4H), 1.78–1.70 (m, 4H), 1.57–1.51 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, 293 K) δ = 171.6, 78.3, 77.6, 72.8, 45.6, 37.2, 36.2, 36.1, 31.8, 31.7, 31.7, 31.6, 27.1, 26.9. HRMS (ESI-TOF-MS): *m/z* calc'd for $C_{17}H_{26}NO_2$ $[M - Cl]^+$ 276.1964, observed 276.1954.



Scheme S3. Synthesis of **S2**

PyAA-CB6-AdPA-rotaxane (S2). Compounds **PyAA**$^+$ (3.5 mg, 10 μmol), **AdPA**$^+$ (3.2 mg, 10 μmol), βCD·XH_2O (40 mg), and CB6·XH_2O (10.8 mg) were mixed in D_2O (2 mL) and stirred at 60 °C for 1 h under an atmosphere of N_2. ^{1}H NMR spectroscopy indicated that **AdPA**$^+$ was converted quantitatively to the βCD-capped CB6-rotaxane. The solution was purified by semi-preparative RP-HPLC, which subsequently removed the βCD caps, eluting in an aqueous gradient of 5% to 95% MeCN/0.1% TFA in H_2O/0.1% TFA over 40 min at a flow rate of 3.0 mL/min. After removal of the solvent, **S2** was obtained as an off-white powder. ^{1}H NMR (600 MHz, CD_3CN, 293 K) δ = 8.85 (d, J = 9.0 Hz, pyrene, 1H), 8.41–8.33 (m, pyrene, 5H), 8.27 (d, J = 9.0 Hz, pyrene, 1H), 8.23 (d, J = 9.0 Hz, pyrene, 1H), 8.14 (t, J = 8.0 Hz, pyrene, 1H), 8.03 (br s, $–NH_2^+–$, 2H), 7.10 (br s, $–NH_2^+–$, 2H), 6.53 (s, triazole, 1H), 5.70 (d, J = 15 Hz, CB6, 6H), 5.69 (d, J = 15 Hz, CB6, 6H), 5.31 (t, J = 5 Hz, 2H), 5.27 (s, CB6, 12H), 4.92 (t, J = 2.5 Hz, 1H), 4.26–4.23 (m, 2H), 4.06–4.02 (m, 2H), 3.87–3.81 (m, 2H), 3.31–3.25 (m, 2H), 2.74 (t, J = 7.5 Hz, 2H), 2.36 (q, J = 7.5 Hz, 2H), 2.11–2.05 (m, 2H), 2.00–1.75 (m, adamantyl, 12H), 1.59–1.54 (m, adamantyl, 2H). ^{13}C NMR (150 MHz, CD_3CN, 293 K) δ = 155.7, 132.3, 129.4, 128.6, 127.3, 126.7, 126.1, 124.9,

123.0, 82.5, 81.1, 80.2, 76.8, 51.3, 51.0, 47.3, 37.0, 35.9, 31.8, 31.5, 27.2, 27.0. HRMS (MALDI-TOF-MS): *m/z* calcd for $C_{72}H_{80}N_{29}O_{14}$ $[M+H]^+$ 1575.59, observed 1573.06.

S3. Responsive cleavage of CB6-rotaxanes

LiOH-induced cleavage of 2, monitored by HPLC and MS. To a solution of 5 mM **S2** in 500 μL H_2O was added βCD·XH_2O (6.5 mg). The solution was heated to 40 °C and gently sonicated until soluble to afford rotaxane **2**. To that solution was added 10 equiv of LiOH as a 1 M solution. The solution was stirred and heated at 40 °C. Aliquots of 100 μL were taken at timepoints of interest and analyzed by RP-HPLC, eluting in an aqueous gradient 10% to 90% MeCN/0.1% TFA in H_2O/0.1% TFA over 20 min at a flow rate of 0.5 mL/min, monitoring at the Abs_{340} of pyrene. At the last timepoint of 8 h, a 100 μM solution was prepared in ddH_2O for analysis by ^{129}Xe NMR. At 8 h, an additional aliquot was taken for analysis by MALDI-TOF-MS to confirm the cleavage product **3**. HRMS (MALDI-TOF-MS): *m/z* calcd for $C_{62}H_{66}N_{29}O_{14}$ *[M+H]*$^+$ 1441.37, observed 1435.4.

S4. Xenon NMR

Xenon polarization was obtained using a home built spin-exchange optical-pumping setup resulting in a 10% polarization of a xenon gas mixture (2 % Xe, 10% N_2, 88% He).[S3] The hyperpolarized gas was bubbled directly into a 5 mm phantom containing the solution of interest for 20 s then left to settle for 2 s. The sample was held at 3.4 atm and 25 °C throughout. A 9.4 T (400 MHz) Varian VNMRS console was used for all hyperCEST experiments with optimized saturation power and duration for each sample (**1** sample: 15 dB, 5 s, **2**: 0 dB, 8 s). A standard hyperCEST pulse sequence was used sweeping the saturation frequency in 100 Hz or 500 Hz increments over a 200 ppm range.[S4] Data processing was carried out using MATLAB. FIDs were zero-filled to 16384 points, baseline was corrected, apodized with an 11 Hz exponential, and a Fourier transform was performed. Each ^{129}Xe@H_2O areas in the spectra were integrated and the contrast of each spectrum was compared between the maximum and minimum area in each data series. Each profile was fitted with Lorentz profile using ORIGINLAB.[S5]

S5. References

[S1] J. Hannant, J.H. Hedley, J. Pate, A. Walli, S.A. Farha Al-Said, M.A. Galindo, B.A. Connolly, B.R. Horrocks, A. Houlton, and A.R. Pike, *Chem. Commun.* 2010, **46,** 5870–5872.

[S2] W.L. Mock, T.A. Irra, J.P Wepsiec, and M. Adhya. *J. Org. Chem.* 1989, **54** (22), 5302–5308.

[S3] M.S. Albert, G.D. Cates, B. Driehuys, W. Happer, B. Saam, C.S. Spring Jr., and A. Wishnia, *Nature*. 1994, **370,** 199-201.

[S4] L. Schroder, T.J. Lowery, C. Hilty, D.E. Wemmer, and A. Pines, *Science.* 2006, **314** (5798), 446-449.

[S5] K.K. Palaniappan, R.M. Ramirez, V.S. Bajaj, D.E. Wemmer, A. Pines, and M.B. Francis, *Angewandte Chemie*. 2013, **52** (18)**,** 4849-4853.

S6. ^{1}H NMR of complex 1

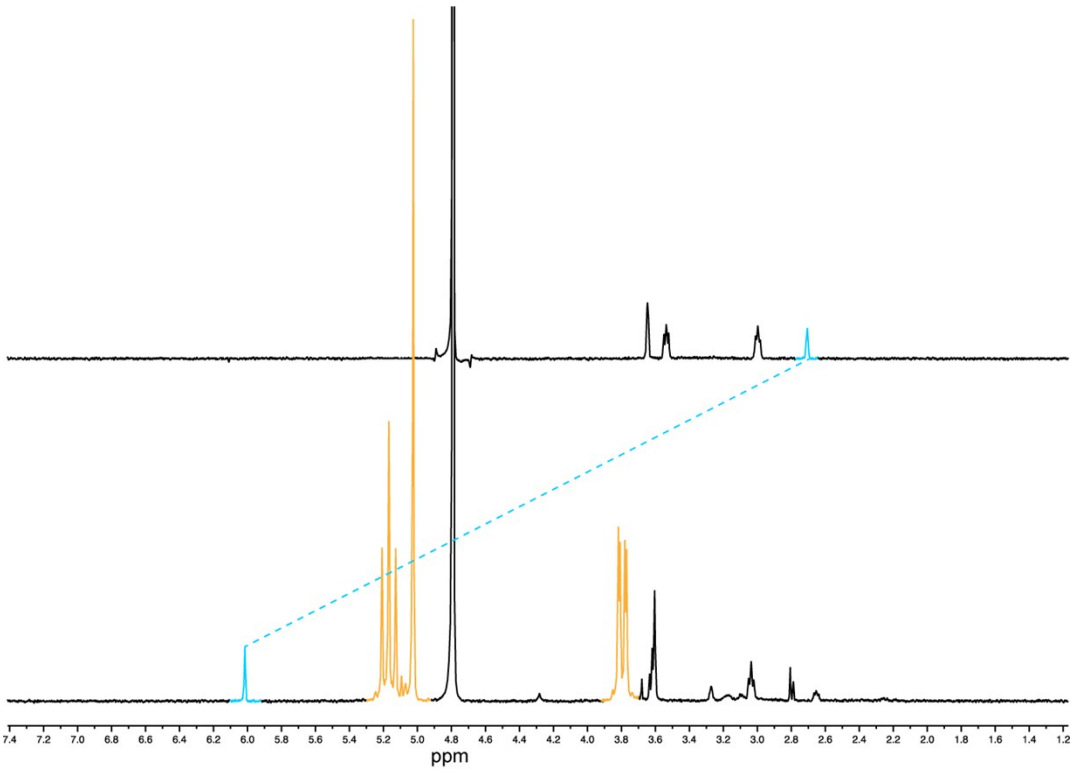


Figure S1. ^{1}H NMR of co-incubated alkyne and azide (top) and complex **1** product (bottom). Blue indicates alkyne (top) and triazole (bottom) chemical shifts, and orange indicates CB6 chemical shifts.

S7. ^{129}Xe HyperCEST NMR of CB6

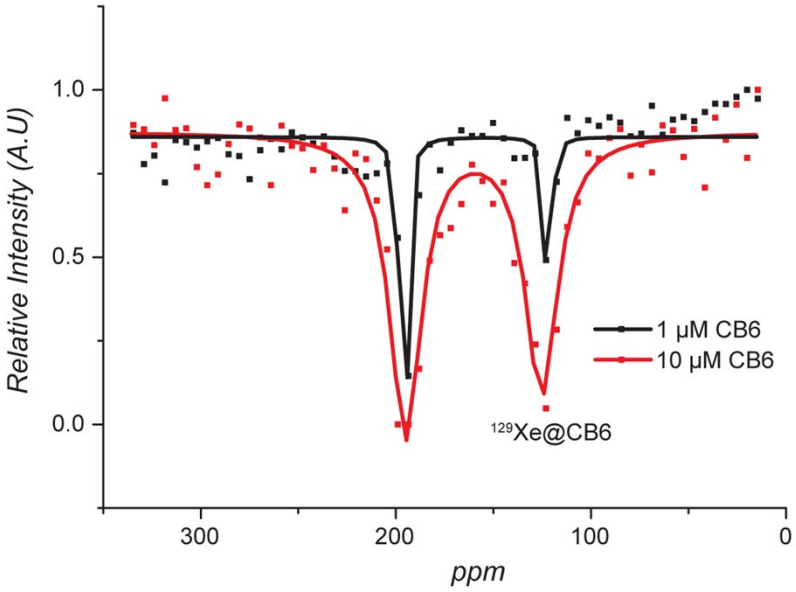


Figure S2. ^{129}Xe hyperCEST NMR spectra of 1 μM (black) and 10 μM (red) CB6 in water.