Supporting Information

Selective NMR detection of N-methylated amines using cavitand-decorated silica nanoparticles as receptors†

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† This paper is dedicated to prof. Paolo Scrimin, on the occasion of its 70th birthday

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1. Instruments

All the NMR experiments in this communication were performed at 25 °C on a Bruker AVANCE III spectrometer operating at 500.13 MHz ^{1}H Larmor frequency and equipped with a 5 mm z-gradient broad-band inverse (BBI) non-cryogenic probe. The hydrodynamic particle size and ζ-potential were measured using Dynamic Light Scattering (DLS) with a Malvern Zetasizer Nano-S equipped with a HeNe laser (633 nm) and a Peltier thermostatic system at 25 °C, using a plastic cuvette and a total solution volume of 1 mL. TEM images were recorded on a Jeol 300 PX electron microscope. One drop of sample was placed on the sample grid and the solvent was removed with filter paper. TEM images were analyzed with ImageJ. Chemicals, including the analytes, and solvent were purchased by Merck and used as received. The STD and HPwSTD experiments were performed using previously described pulse sequence (*De Biasi, F.; Rosa-Gastaldo, D.; Sun, X.; Mancin, F.; Rastrelli, F. Nanoparticle-Assisted NMR Spectroscopy: Enhanced Detection of Analytes by Water-Mediated Saturation Transfer. J Am Chem Soc* ***2019****, 141 (12), 4870-4877. DOI: 10.1021/jacs.8b13225.*)

2. Additional experiments

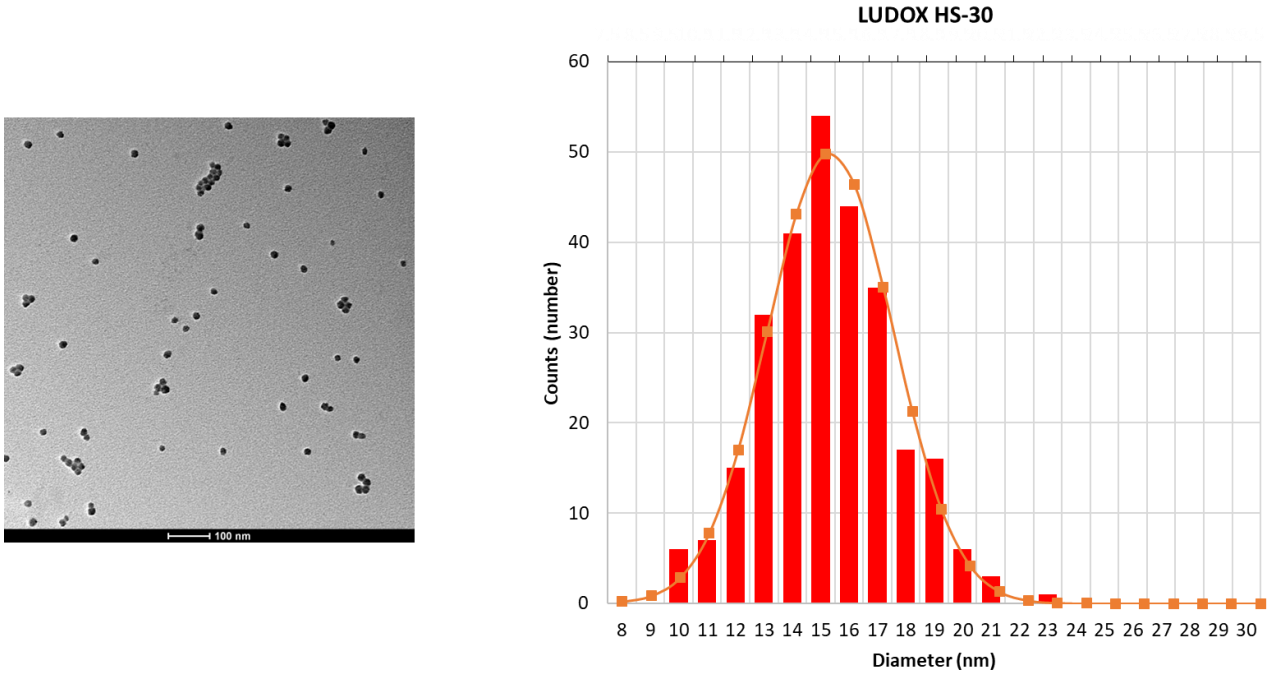

Fig. S1. TEM images of a solution of LUDOX HS-30 (0.03% w/w) and size distribution (d=15.2 nm, σ=2.2 nm).

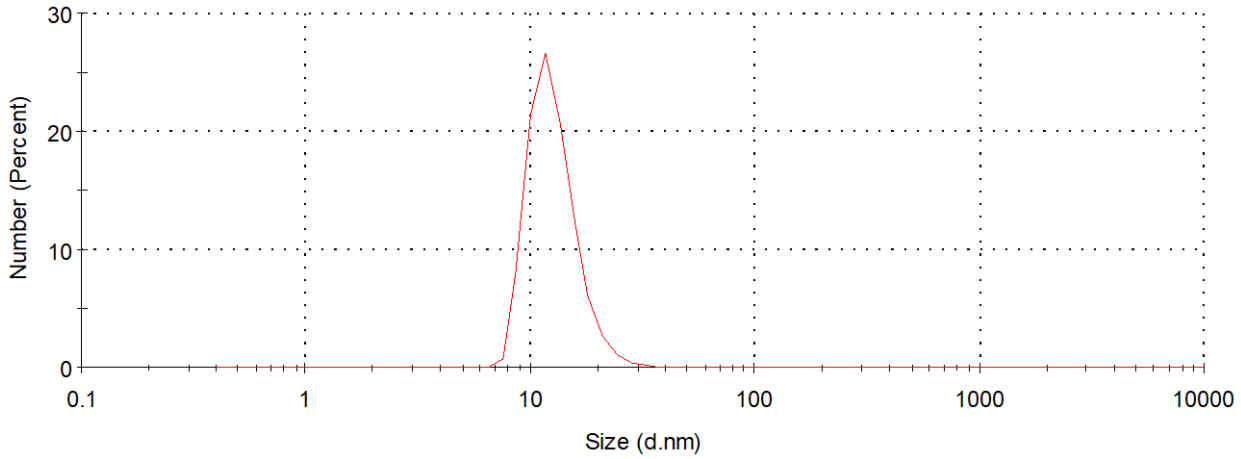

Fig. S2. Size distribution of LUDOX HS-30 (1.5 uM) + buffer phosphate 5 mM (Size=12.8±0.6 nm, PDI=0.25±0.01).

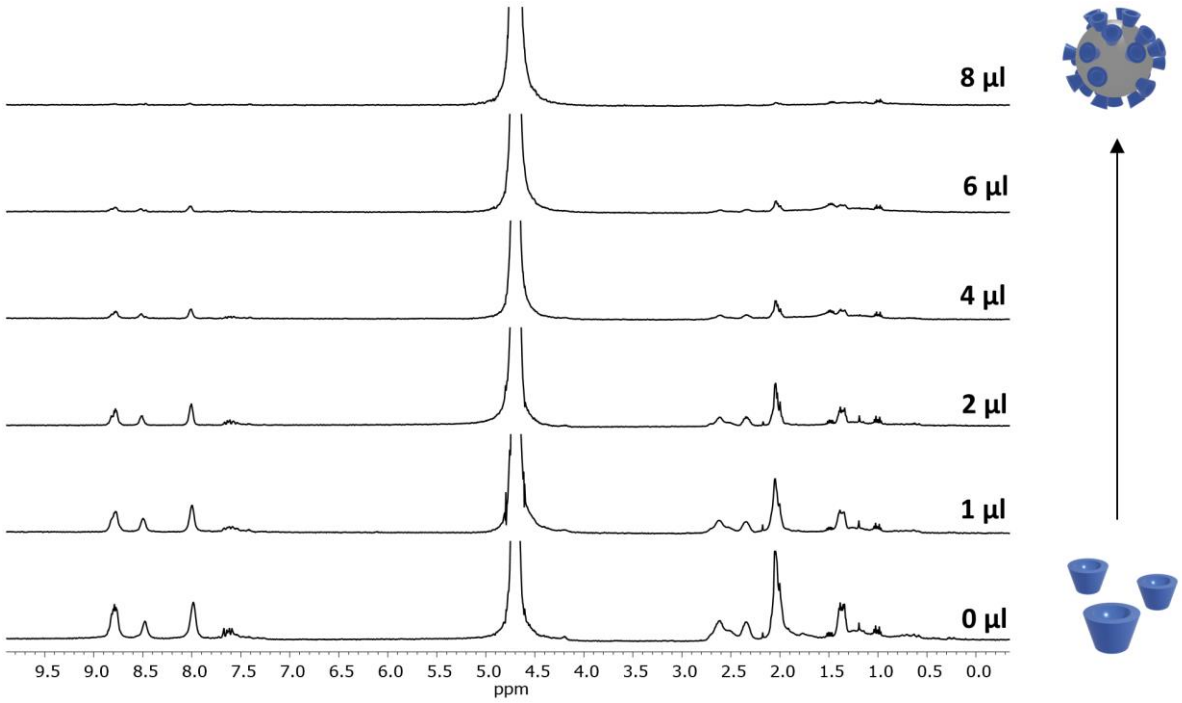

Fig. S3. ^{1}H NMR (500 MHz, 25 °C, phosphate buffer 5 mM, D_2O) spectra of Tiiii-Py 0.5 mM in presence of (from bottom to top) 0, 0.15, 0.3, 0.6, 0.9 and 1.2 µM of LUDOX HS-30.

This experimental observation allows the approximate determination of the Tiiii-Py loading on the silica nanoparticles. As a matter of fact, complete signals disappearing indicates full binding of the cavitand to the particles (due to the reduction of T_2 relaxation times). Since this is observed when the concentration LUDOX is 1.2 µM and that of Tiiii-Py is 0.5 mM, one cand easily calculate that full loading occurs when $4.2 \cdot 10^2$ Tiiii-Py units per particle are present in the sample.

Such value is in agreement with simple geometric calculations. The average size of LUDOX of about 14 nm, corresponding to a surface area of 616 nm^2. The maximum and minimum distances of two adjacent pyridyl groups in Tiiii-Py is 1.4 nm (extended to outside of the square defined by the cavitand top ring) or 0.95 nm (average distance between two adjacent P=O oxygen atoms, corresponding to a more contracted disposition of the pyridyl groups), respectively (see figure S4). With these numbers it is possible to calculate a maximum loading between $(3.1 - 6.8) \cdot 10^2$ cavitands per LUDOX nanoparticle, which well compares with the experimentally determined value.

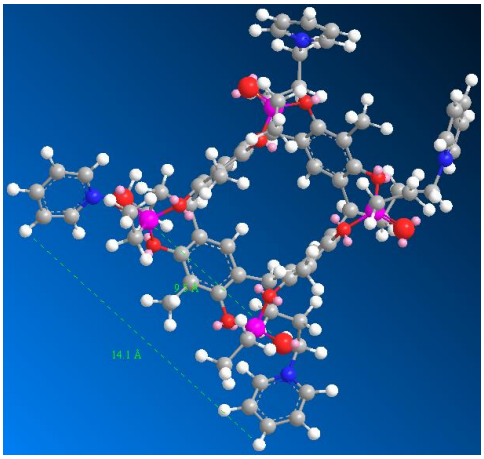

Fig. S4. Crystal structure of cavitand Tiiii-Py. Color code: white: hydrogen; grey: carbon; blue: nitrogen; red: oxygen; pink: phosphorous

Tab. S1. Parameters obtained with DLS measurements (H_2O milliQ, phosphate buffer 5 mM, pH=7.0).

Ludox (μM)	PhosC (μM)	Size (nm)	Z-average	PDI	ζ-POT
1.5	0	12.8±0.6	22.7±0.1	0.25±0.01	-24.1±5.1
1.5	10	14.2+.0.3	27.2±0.8	0.48±0.02	-27.5±0.7
1.5	20	14.7±0.1	33.5±0.7	0.59±0.03	-25.1±0.5
1.5	60	14.7±0.5	72±2	0.92±0.06	-28.0±0.4
1.5	100	17.4±0.7	109±3	0.99±0.01	-23.7±0.3

Binding constant calculation

The binding constant of between NMPEA and the Tiiii-Py@LUDOX system was measured through an NMR shift titration: increasing amounts of a concentrated solution of NMPEA (100 mM) were added to a solution of Tiiii-Py@LUDOX ([Tiiii-Py]=0.125 mM) in phosphate buffer 5 mM (pD=7.0). The variation in δ of the analyte signals was monitored and plotted against the analyte concentration. The data points obtained from the aliphatic signals were fitted with a 1:1 binding model using DynaFit® as reported in previous works. The reported association constant is the average on the constant obtained from each signal.

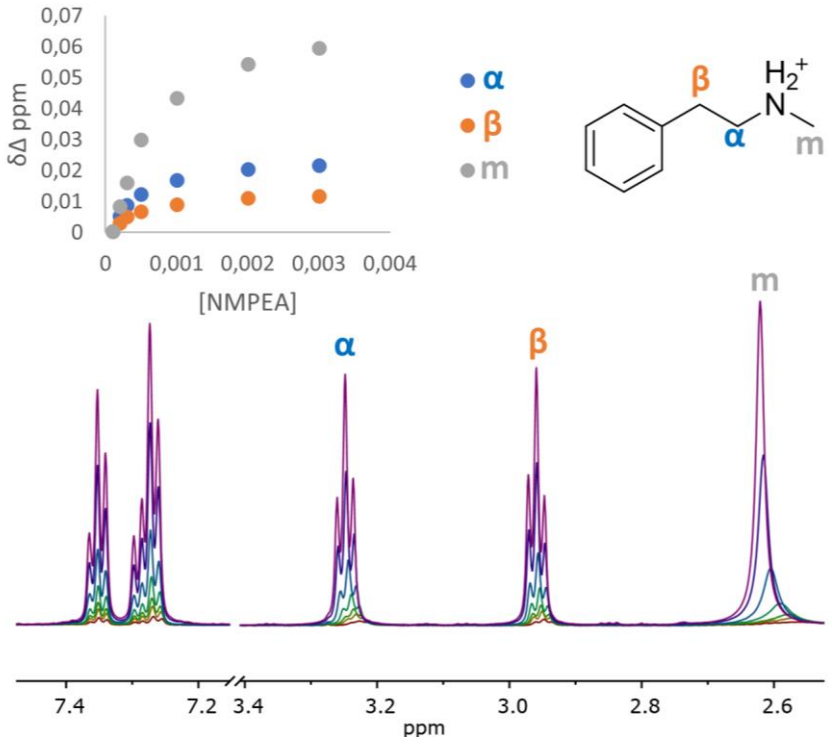

Fig. S5. ^{1}H NMR shift titration (500 MHz, 25 °C, phosphate buffer 5 mM, pD = 7.0, D_2O) of Tiiii-Py@LUDOX with NMPEA (0.2 to 3 mM). Corresponding analytes concentrations and fitting are reported in Figure S5.

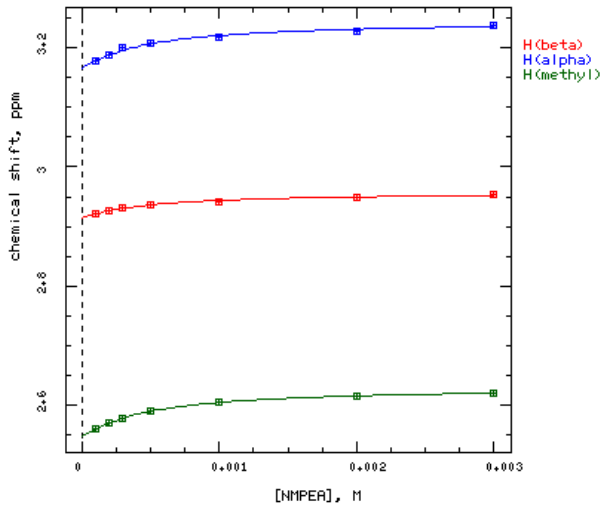

Fig. S6. ^{1}H NMR shift titration of Tiiii-Py@LUDOX with NMPEA: best fit of the data (0.2 to 3 mM). K_a= 3080 ± 580 M^{-1}.

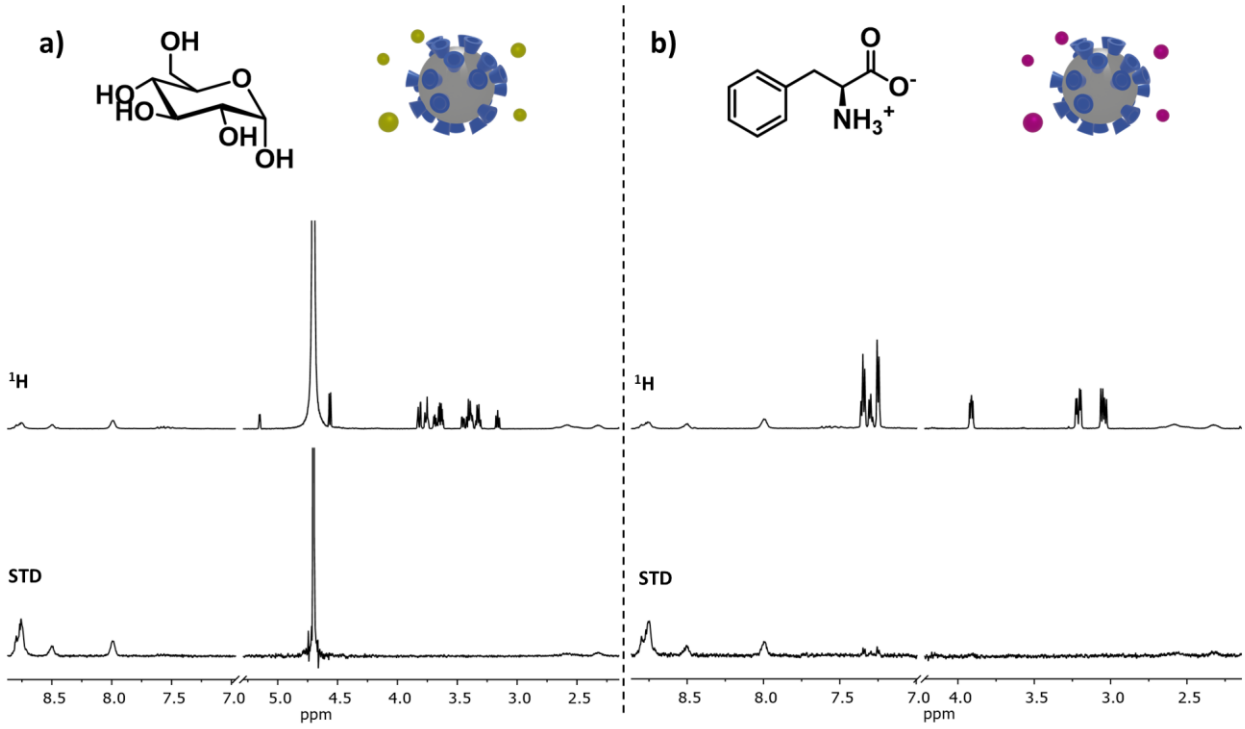

Fig. S7. ^{1}H and STD spectra (500 MHz, 25° C, phosphate buffer 5 mM, pD=7.0) of: a) glucose 0.5 mM and Tiiii-Py @LUDOX (Tiiii-Py 0.25 mM); b) phenylalanine 0.5 mM and Tiiii-Py@LUDOX (Tiiii-Py 0.25 mM).

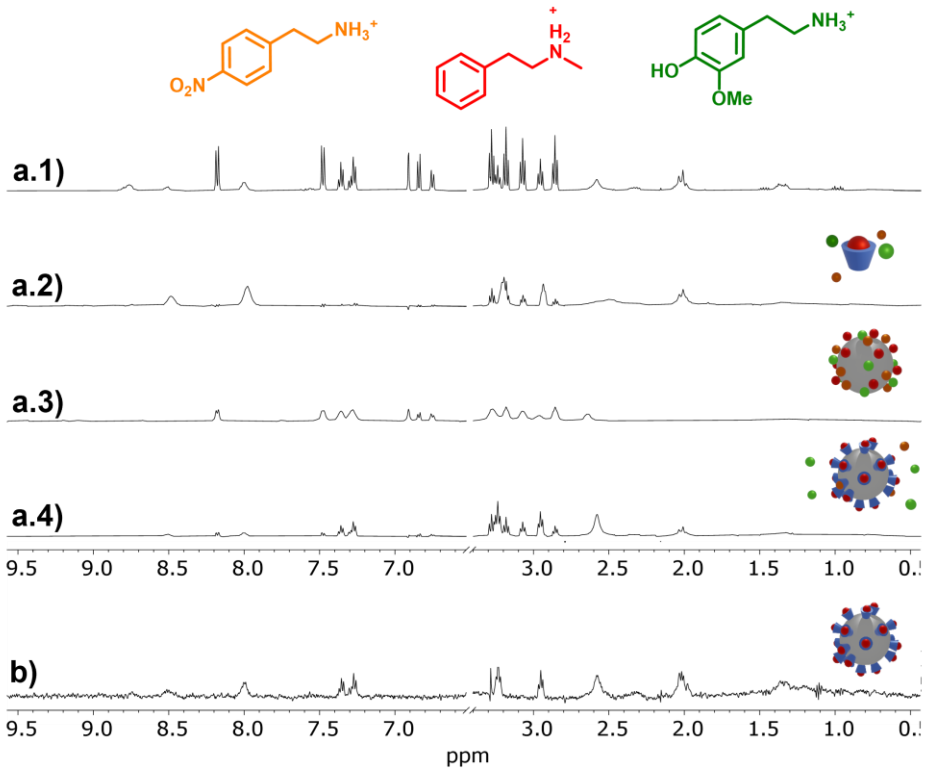

Fig. S8. a.1) ^{1}H-NMR spectrum of a solution of NMPEA (red sphere), 4-NO_2-PEA (orange sphere), 3-MT 0.25 (green sphere) mM each and Tiiii-Py (blue basket) 0.1 mM; a.2) HPwSTD spectrum of the same sample; a.3) HPwSTD spectrum of a solution of NMPEA, 4-NO_2-PEA, 3-MT 0.25 mM each and LUDOX (grey sphere) 0.15 µM; a.4) HPwSTD spectrum of a solution of NMPEA, 4-NO_2-PEA, 3-MT 0.25 mM each, Tiiii-Py 0.1 mM and LUDOX 0.15 µM; b) HPwSTD spectrum of a solution of NMPEA 50 uM, PhosC 0.1 mM and LUDOX 0.15 µM (Conditions: 500 MHz, 25 °C, phosphate buffer 5 mM, pH=7.0, H_2O/D_2O 90:10). Analytes signals in the aliphatic regions are false positives generated by the close position of these resonances to the high-power saturating rf offset.

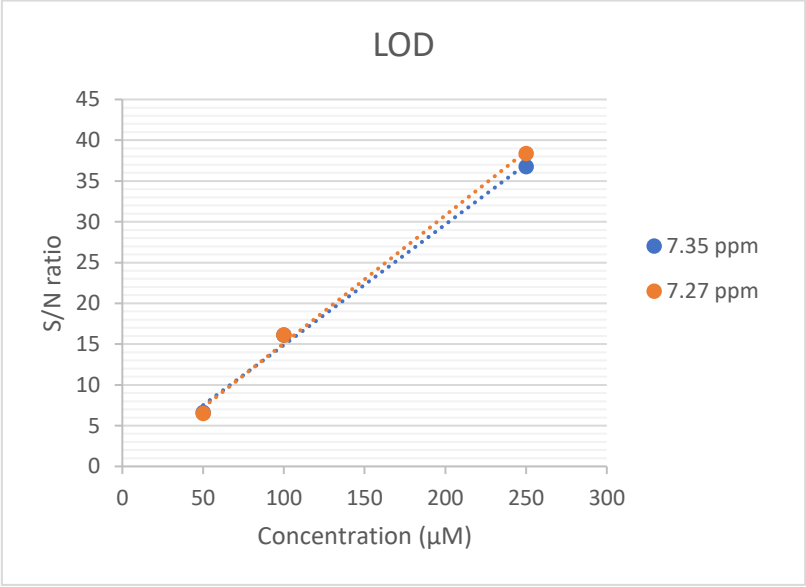

Fig. S9. Signal-to-noise (S/N) ratio measured in HPwSTD spectra (considering NMPEA signals centered at 7.35 ppm and 7.27 ppm) *vs* NMPEA concentration (H_2O/D_2O 90:10, 25° C, phosphate buffer 5 mM, pH=7.0). Average LOD=31 µM, calculated as LOD=3.3*σ_{curve}/slope. R^2=0.9949 (blue curve); R^2=0.9969 (orange curve).

Tab. S2. Comparison of LOD for different sensing methods based on the Tiiii-Py cavitands.

Reference in the manuscript	**Method**	**Analytes**	**LOD (μM)**
(12)	Current change	Sarcosine	20
(13)	Electrochemiluminescence	Sarcosine	30
(14)	Fluorescence dye displacement	Sarcosine	>100
(15)	Fluorescence	Illicit drugs	>5
(16)	Deflection curve	Illicit drug	>100
-	NMR chemosensing (HPwSTD)	NMPEA	31