Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

An intracellular Diamine Oxidase Triggered Hyperpolarized ^{129}Xe Magnetic Resonance Biosensor

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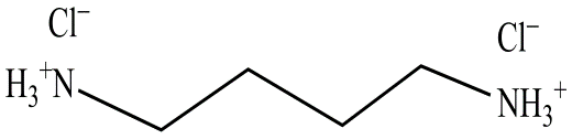
Abbreviations

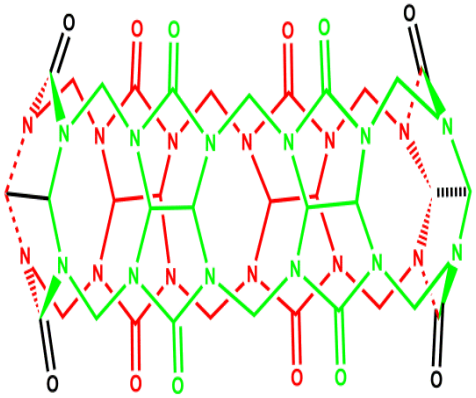
CB6: cucurbit[6]uril; DAO: diamine oxidase; Put: putrescine dihydrochloride; MRI: magnetic resonance imaging; NMR: nuclear magnetic resonance; NH_4OAc: ammonium acetate; CEST: chemical exchange saturation transfer; IVEC: small intestinal villi and epithelial cells; ELISA: fluorescence spectrophotometer and enzyme linked immunosorbent assay; Hyd: hydroxylamine hydrochloride.

Materials

Organic reagents and solvents were used as purchased from the following commercial sources: Sigma-Aldrich: cucurbit[6]uril hydrate (CB6); Putrescine hydrochloride: (Put, 98%); Diamine Oxidase (DAO, 0.05 unit/mg); Pluronic L-81 (Sinopharm Chemical Reagent Co., Ltd:); ammonium acetate (NH_4OAc, AR); Acetic acid ($C_2H_4O_2$, AR); Ammonia solution ($NH_3 \cdot H_2O$, AR); Sai Qi (Shang Hai) biological engineering co., ltd: small intestinal villi and epithelial cells (IVEC).



(Put)



(CB6)

Isothermal Titration Calorimetry (ITC) studies

MicroCal ITC 200 was used for obtaining the binding constant between Put and CB6. The cell (Volume = 1.4 mL) was filled with degassed CB6 (200 μM). Put (22.5 μM) was injected (1 step of 4 μL and 24 more steps of 11 μL each) into Put. The duration of each injection was 22 s. An interval of 180 s was allowed between each injection. The injector stirred the solution at 371 rpm to ensure complete mixing within a few seconds. Calorimetric data analysis was carried out using Origin 7.0 software (MicroCal). The details of the ITC titration are as follows: (1) titration of CB6 aqueous solution with Put solution, (2) titration of buffer with the same Put solution (blank). Appropriate subtractions of blank were performed in order to explore the possible direct binding interaction between the buffer and Put.

Enzyme assays

In a typical experiment, a solution (4 mL total volume) was prepared containing 50 μM CB6, and 100 μM Put in 20 mM NH_4OAc buffer, pH 7.2. After addition of DAO, the sample was placed in the thermostatic shaker (T = 37℃, RPM=300/min). After a period of time, the products of enzymatic reactions are used for NMR and MRI studies. The experiment without enzyme was used as the control group

NMR studies

NMR experiments were performed on a 9.4T NMR spectrometer (Bruker Bio-spin, Ettlingen, Germany) with gradient coils for imaging and a variable temperature unit. A 10 mm inner-diameter double resonant probe (^{129}Xe and ^{1}H), was used for excitation and detection. The gas flow was controlled by flow controllers at the sample gas outlet. Hyperpolarized ^{129}Xe (ca.5% polarization) was generated by spin exchange optical pumping using a 65 W continuous (794.7 nm) in a custom-designed continuous flow set up at 4.51 bar absolute pressure using a gas mixture of 2% Xe (87% enriched abundance of ^{129}Xe), 8% N_2 and 90% He. The gas mixture was directly bubbled into solution after polarization. For CEST spectroscopy and Hyper-CEST MRI, the hyperpolarized ^{129}Xe gas mixture was bubbled for 20 s at a total flow rate of 0.12 SLM followed by a 3 s delay (to allow possible remaining bubbles to collapse) before signal frequency. The saturation parameters (B_1 field strength and saturation time t_{sat}) are given in the figure captions. The saturation frequency is referenced to dissolved ^{129}Xe resonance which is set to 0 ppm.

^{129}Xe Hyper-CEST MR images in buffer

^{129}Xe Hyper-CEST MR images in buffer were acquired using averages of a FLASH sequence with a slice-selective 90° Gaussian shaped excitation, 2.0×2.0 cm^2 field of view, 20 mm slice thickness, 32×32 matrix size, RARE factor 32, echo time: 2.090 ms. The saturation time and saturation length used varies and are given in the figures.

Cellular Hyper-CEST Spectra Studies

Cellular Hyper-CEST Spectra Studies Intestinal villi and epithelial cells (IVEC) was cultured in Dulbecco modified Eagle medium (DMEM) medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin at 37℃ in 5% CO_2 and 95% humidified environment. The cells were incubated with CB6 [CB6 (2.5mM) and dissolved in culture medium] for 20 h in the first control group. The cells were washed three times with PBS at room temperature and followed by trypsinization and resuspension in culture medium. The cell concentration was kept at 3×10^6 cells/mL. Finally, the cells were transferred to an NMR tube and MRI tube, while the ^{129}Xe NMR spectra and MRI image were acquired by Hyper-CEST. The cells of the second control group were incubated with hydroxylamine hydrochloride (Hyd) [Hyd (2.5 mM) dissolved in culture medium], which could inhibit the activity of DAO, for 2 h. Residual quantities of Hyd not taken up by the cells were removed by washing the cells three times with PBS. The cells were then incubated with CB6 and putrescine dihydrochloride (Put) [CB6 (2.5mM) and Put (5mM) dissolved in culture medium] for another 20 h. Other procedures were carried out similarly to the case of the first control group. In the experiment group the cells were incubated with CB6 [CB6 (2.5mM) and Put (5mM) dissolved in culture medium] for 20 h in the first control group. Other procedures were carried out similarly to the case of the first control group.

^{129}Xe Hyper-CEST MR images in Intestinal villi and epithelial cells

^{129}Xe Hyper-CEST MR images in Intestinal villi and epithelial cells were acquired using averages of a FLASH sequence with a slice-selective 90° Gaussian shaped excitation, 2.0×2.0 cm^2 field of view, 30 mm slice thickness, 32×32 matrix size, RARE factor 16, echo time: 7.0 ms. The saturation time and saturation length used varies and are given in the figures.

^{129}Xe Hyper-CEST MR images in buffer

^{129}Xe Hyper-CEST MR images in buffer were acquired using averages of a flash sequence with a continuous wave saturation, 2.0×2.0 cm field of view, 20 mm slice thickness, 32×32 matrix size, RARE factor 32, echo time: 2.090 ms. The saturation time and saturation length used varies and are given in the figures. No MRI post-processing, such as smoothing, was applied.

Cellular Hyper-CEST Spectra Studies

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^{129}Xe Hyper-CEST MR images in Intestinal villi and epithelial cells

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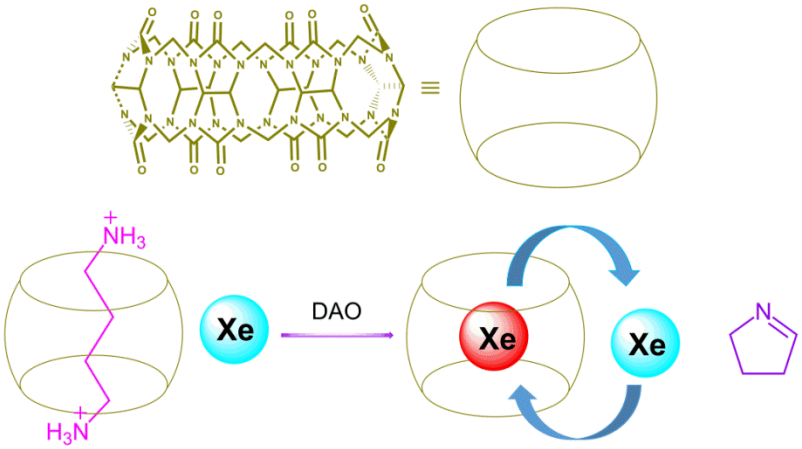
Figures:



Scheme S1 Proposed recognition mechanism of biosensor reaction with DAO

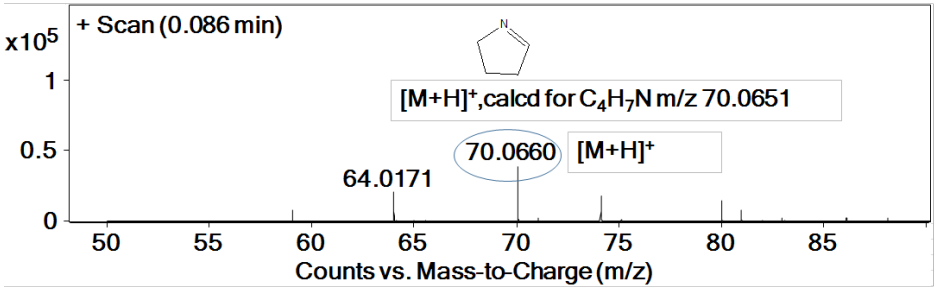


Figure S2. After the reaction with diamine oxidase (DAO)， mass spectrometry (MS) analysis of the biosensor.

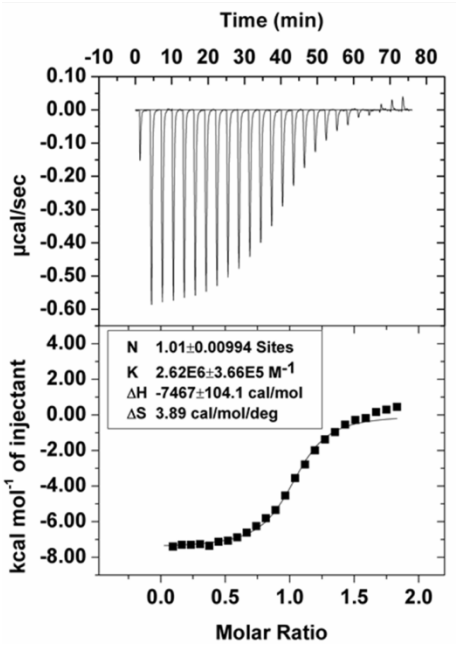
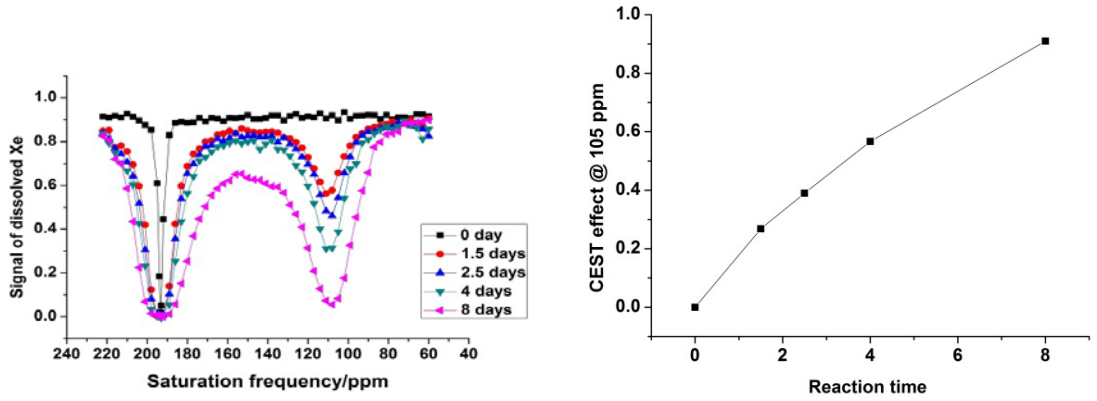


Figure S3. ITC measurements performed with CB6 at 298 K. 200 µM putrescine was titrated into 22.5 µM CB6



(a) (b)

Figure S4. ^{129}Xe@CB6 Hyper-CEST signal appeared upon addition of 2mg/mL DAO. All ^{129}Xe Hyper-CEST NMR spectra were obtain in the same sample at different times.