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

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

We report a method for blocking interactions between [129]Xe and cucurbit[6]uril (CB6) until activation by a specific chemical event. We synthesized a CB6–rotaxane that allowed no [129]Xe interaction with the CB6 macrocycle component until a cleavage event released the CB6, which then produced a [129]Xe@CB6 NMR signal. This contrast-upon-activation [129]Xe NMR platform allows for modular synthesis and can be expanded to applications in detection and disease imaging.

Localized molecular detection with high sensitivity and selectivity is of paramount importance in early disease detection. Current techniques such as magnetic resonance imaging (MRI), the clinical version of nuclear magnetic resonance (NMR), and X-ray computed tomography benefit from being noninvasive imaging techniques with high resolution and excellent tissue penetration.[1] However, these techniques lack molecular information that could provide valuable insight for disease diagnosis. While advances in responsive ^{1}H NMR contrast agents that turn on only in select environments have led to the detection of enzymes,[1b] signalling molecules,[2] and redox conditions,[3] none of these advances offer direct molecular detection with high signal contrast. Hyperpolarized xenon MRI provides a sensitive complement to ^{1}H MRI. The use of hyperpolarization increases signal strength by several orders of magnitude, and there is no natural background [129]Xe signal in biological environments.[4] Xenon participates in a range of supramolecular interactions, which produce distinct molecular signals for different xenon environments inside various hosts. This allows xenon NMR to be used for molecular imaging applications.[5] Coupling hyperpolarization with chemical exchange saturation transfer (CEST)

between xenon in free and host encapsulated forms, termed [129]Xe hyperCEST NMR, allows highly sensitive molecular detection at levels that are required for the early detection of disease biomarkers.[6]

Taking advantage of this sensitivity, hyperCEST has been used to detect cancer markers,[7] small molecule analytes,[8] and cell surface glycans.[9] These methods rely on either the targeted delivery of xenon hosts to a region of interest, or small chemical shift differences between bound and unbound xenon sensors. A desirable alternative approach would be to suppress the [129]Xe@host signal completely until the sensor reaches a region of interest, where it is selectively activated. Previous examples of [129]Xe hyperCEST have relied on cryptophane-A (CryA) xenon hosts that are hydrophobic, costly, and difficult to functionalize.

Cucurbit[6]uril (CB6) is an excellent xenon host for activated [129]Xe NMR detection, as it produces a distinctive [129]Xe@CB6 signal, has better exchange parameters for hyperCEST when compared to CryA, is soluble in most buffers and biological environments, and is commercially available.[10] While CB6 has only recently been reported for use with [129]Xe hyperCEST NMR,[10a] it is attracting significant attention over CryA-based sensors due to these advantages. However, a major limitation of CB6 sensors is the difficult chemical functionalization to generalize them for diverse spectroscopic applications.[11]

While the covalent functionalization of CB6 remains a challenge, its ability to participate in a wide range of supramolecular host–guest interactions has been well studied.[12] Competing CB6 guests have been shown to suppress [129]Xe@CB6 signals to varying degrees, although in each case an excess of competing molecules was needed to suppress the [129]Xe@CB6 signal.[10a,13] Recently, Schröder *et al.* took advantage of CB6 host–guest interactions to design an enzyme-sensing platform based on a competition between [129]Xe and an enzyme product for binding of the CB6 cavity.[13] However, it would be beneficial to have a more modular [129]Xe sensor platform that does not rely on a specific molecule's affinity toward cucurbituril. It would likewise be valuable to create a system that will block [129]Xe@CB6 interactions with greater control, as such an approach could

[a] Department of Chemistry, University of California, Berkeley, Berkeley, CA, 94720, USA

[b] Materials Sciences Division, Lawrence Berkeley National Laboratories, Berkeley, CA, 94720, USA

[c] Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720. E-mail: mbfrancis@berkeley.edu
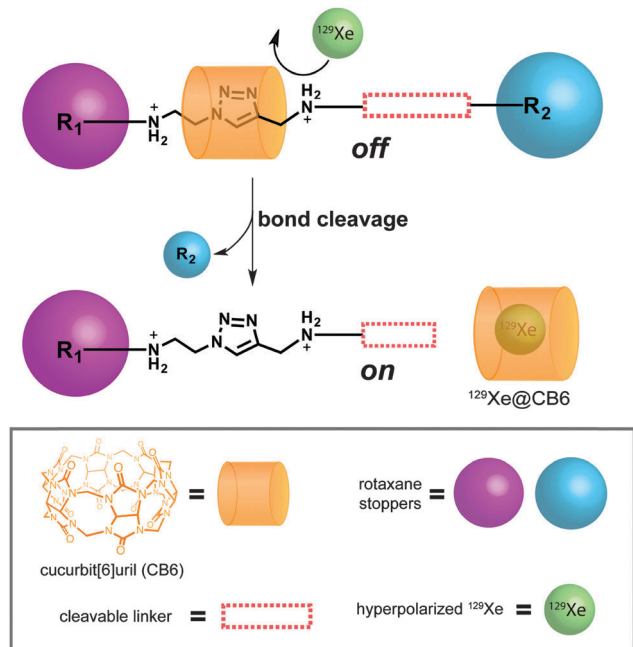
Scheme 1 General strategy for activating CB6–rotaxanes for ^{129}Xe NMR. A rotaxane with a triazole–diammonium–CB6 center and a cleavable linker blocks ^{129}Xe–CB6 interactions and thus suppresses ^{129}Xe@CB6 NMR signals. Once cleaved, the CB6 ring can dissociate and serve as a host for hyperpolarized ^{129}Xe, producing a ^{129}Xe@CB6 NMR signal.

Fig. 1 Complex **1** demonstrates competitive host–guest interactions between **1** and ^{129}Xe@CB6. (A) Scheme outlining the dynamic host–guest interactions of **1** and ^{129}Xe. (B) ^{129}Xe HyperCEST NMR spectrum of 100 μM CB6 complex **1**. A strong ^{129}Xe@CB6 peak was observed.

eliminate background signals until the CB6 reaches a region of interest, where it is then released to produce a ^{129}Xe@CB6 signal.

Herein, we report the design, synthesis, and implementation of a chemically activated CB6–rotaxane platform for ^{129}Xe NMR (Scheme 1). In this system, the ^{129}Xe@CB6 interactions are blocked by a mechanical bond, where the "dumbbell" stopper component of a rotaxane prevents ^{129}Xe from accessing the CB6 cavity. Upon cleavage of the rotaxane stopper, dissociation of the CB6 ring allows it to host ^{129}Xe, producing a ^{129}Xe@CB6 signal. While we focused on CB6 for this report, this system could be diversified using different synthetic strategies to afford rotaxanes with other moieties to optimize xenon exchange rates or competitive thermodynamics.

The rotaxanes used in this work were synthesized by CB6-catalyzed azide–alkyne 1,3-dipolar cycloaddition, which is routinely used for CB6–rotaxane synthesis, allows for increased solubility of CB6 through complexation, and is a facile method for the preparation of rotaxanes with diverse functional groups for various applications.[14] Dmochowski et al. previously reported that CB6 incubated with excess putrescine guests led to a suppression of ^{129}Xe@CB6 NMR signals.[10a] However, in equal concentrations of competitors relative to CB6, little change in the ^{129}Xe@CB6 hyperCEST NMR signal was observed. Schröder et al. similarly observed that excess concentrations of cadaverine were able to suppress ^{129}Xe@CB6 signals. Since both of these competing guests were alkane–diammonium ions, the triazole–diammonium recognition unit of our rotaxane platform might therefore pose a challenge to CB6 detection if it were able to
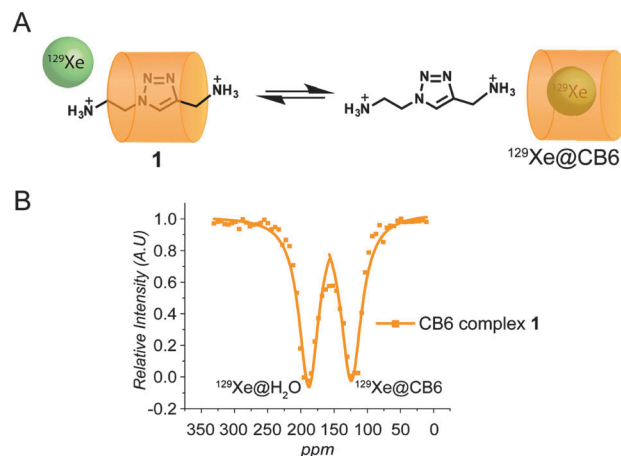
overwhelm ^{129}Xe@CB6 interactions through complex competition. To test if this competition could suppress ^{129}Xe@CB6 signals, we synthesized CB6 complex **1** via a CB6-catalyzed azide–alkyne 1,3 dipolar cycloaddition (Fig. 1). Complex **1** lacks the stoppers of a full rotaxane, allowing the CB6 ring and triazole–diammonium axle components to exchange easily in solution. This construct therefore represents a model for the post-cleavage product of an activated CB6–rotaxane, where the CB6 host and the triazole–diammonium guest are in equal concentrations. A strong ^{129}Xe@CB6 peak was observed for 100 μM CB6 complex **1** by ^{129}Xe hyperCEST NMR, approaching complete saturation of the dissolved xenon signal, similar to 10 μM free CB6 in solution (Fig. S2, ESI†). While there was some signal suppression due to competition between complexes **1** and ^{129}Xe@CB6, xenon was still able to exchange rapidly between the bulk water and the CB6 molecules, giving a strong hyperCEST response. These observations are in agreement with previous competition studies.[10a,12]

Once it was confirmed that a competitive triazole–diammonium guest would not significantly block ^{129}Xe–CB6 interactions, we set out to synthesize a chemically activated CB6–rotaxane that could completely suppress the ^{129}Xe@CB6 NMR signal until undergoing a controlled cleavage and subsequent release of CB6 for xenon binding. Rotaxane **2** was synthesized with pyrene-functionalized 2-azidoethylamine (**PyAA⁺**) and an adamantyl-ester-functionalized propargylamine (**AdPA⁺**) as rotaxane stoppers (Fig. 2). β-Cyclodextrin (βCD) caps were added to improve the solubility of the end groups and to accelerate the rotaxane capture reaction.[14c,e] Compared to previous xenon sensors, which have relied on direct CryA conjugates,[7–9] this synthetic strategy provides a facile and modular approach. To confirm that the βCD caps would not interact with xenon and affect the ^{129}Xe hyperCEST response of **2**, βCD alone in solution was tested and revealed no measurable background ^{129}Xe hyperCEST signals.

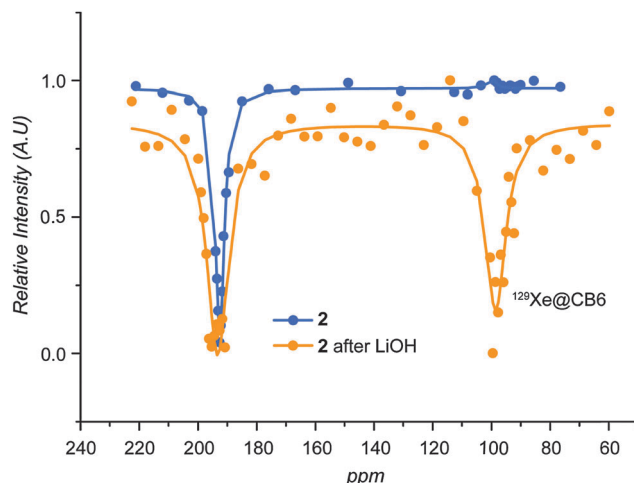The ^{129}Xe hyperCEST response of **2** revealed no detectable ^{129}Xe@CB6 signal even at 100 μM (Fig. 3). Free CB6 in solution
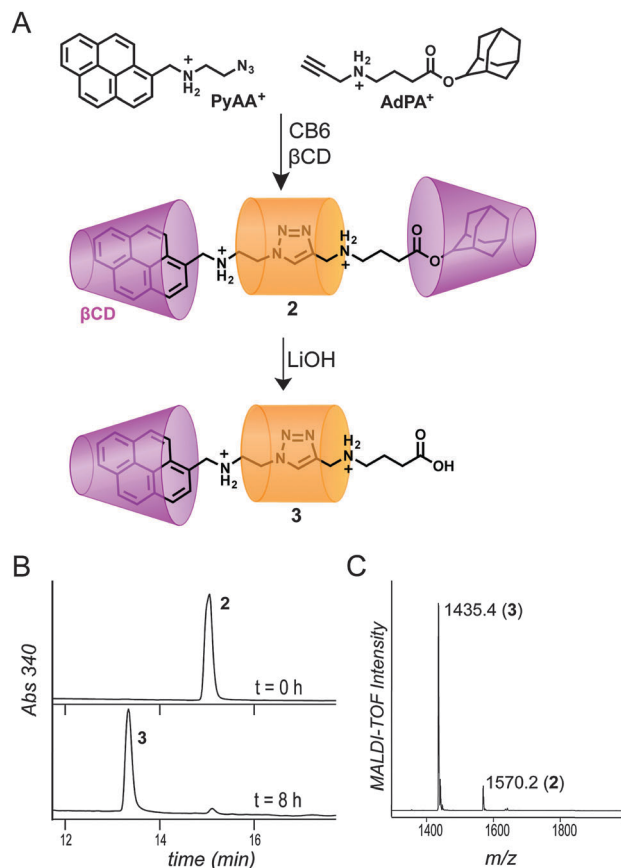
Fig. 2 Rotaxane **2** exhibits responsive cleavage. (A) Rotaxane **2** containing a labile ester bond was synthesized with β-cyclodextrin (βCD) caps to improve water solubility and to accelerate the rotaxane capture reaction. (B) HPLC traces monitoring the cleavage rate of **2** (15 min) when exposed to 10 equiv. of LiOH at 40 °C. Near complete conversion to **3** (13 min) was observed by 8 h. (C) MALDI-TOF MS confirmed the presence of ester-hydrolysis product **3** at 1435 *m/z*.

Fig. 3 Rotaxane **2** can be activated for [129]Xe hyperCEST NMR. [129]Xe-hyperCEST NMR spectra of 100 μM **2** displayed no significant [129]Xe@CB6 signal. After exposure to 10 equiv. of LiOH at 40 °C a substantial increase in the [129]Xe@CB6 peak was observed.

is easily detected at low nanomolar concentrations;[10a,b] thus it is revealing that even at relatively high concentrations of **2**, no CB6 was detected. These results demonstrate that [129]Xe is completely prevented from exchanging with the CB6 cavity and producing a hyperCEST response when CB6 is locked in the rotaxane complex. In contrast to previous work that explored supramolecular CB6 competition between xenon and competing guests,[10a,12] rotaxane **2** demonstrated complete suppression of [129]Xe@CB6 signals without using excess concentrations of the triazole–diammonium guest relative to CB6.

To create an activated CB6–rotaxane for [129]Xe NMR, **2** was synthesized with a labile ester group that can be hydrolysed to **3** and lead to the release of CB6. Treatment with 10 equiv. of LiOH led to the complete ester hydrolysis of **2** by 8 h, as confirmed by high performance liquid chromatography (HPLC) and mass spectrometry (MS) (Fig. 2). After treatment with LiOH, rotaxane **2** was activated, and a significant [129]Xe@CB6 signal was observed at levels nearing complete saturation, similar to CB6 complex **1** (Fig. 3). Thus, even in complexes containing a single bulky rotaxane stopper and a terminal carboxylate on the

axle portion of the rotaxane, it is possible to maintain the association and exchange kinetics between xenon and CB6 that are necessary to produce a hyperCEST response.

As CB6 continues to gain attention for its improved hyperCEST response over previously used CryA constructs, it is increasingly important to be able to manipulate CB6 for diverse applications in NMR detection and MRI. The results described here demonstrate that [129]Xe@CB6 NMR signals can be completely suppressed by locking CB6 into a rotaxane mechanical bond until a specific cleavage event occurs and releases CB6 to produce a [129]Xe@CB6 signal. This activated [129]Xe NMR sensor can be easily synthesized and modulated with different cleavable linkers for tunable activation, and diverse rotaxane stoppers with varying functionalities. This design therefore presents the opportunity for CB6 to be used for applications in activated xenon NMR detection coupled with drug delivery, biomarker targeting, and multimodal imaging. Based on this study, future work will focus on expanding CB6–rotaxanes for different applications and stimuli, and exploring new rotaxane systems with higher sensitivities for *in vivo* detection.

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