Hyper-CEST NMR of metal organic polyhedral cages reveals hidden diastereomers with diverse guest exchange kinetics

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Guest capture and release are important properties of self-assembling nanostructures. Over time, a significant fraction of guests might engage in short-lived states with different symmetry and stereoselectivity and transit frequently between multiple environments, thereby escaping common spectroscopy techniques. Here, we investigate the cavity of an iron-based metal organic polyhedron (Fe-MOP) using spin-hyperpolarized 129Xe Chemical Exchange Saturation Transfer (hyper-CEST) NMR. We report strong signals unknown from previous studies that persist under different perturbations. On-the-fly delivery of hyperpolarized gas yields CEST signatures that reflect different Xe exchange kinetics from multiple environments. Dilute pools with ~10^4-fold lower spin numbers than reported for directly detected hyperpolarized nuclei are readily detected due to efficient guest turnover. The system is further probed by instantaneous and medium timescale perturbations. Computational modeling indicates that these signals originate likely from Xe bound to three Fe-MOP diastereomers (T, C3, S4). The symmetry thus induces steric effects with aperture size changes that tunes selective spin manipulation as it is employed in CEST MRI agents and, potentially, impacts other processes occurring on the millisecond time scale.

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Self-assembled metal organic cages are supramolecular nanostructures that are widely used as hosts for various guests with applications as synthetic ion channels, sensors for organic analytes, in molecular recognition, drug delivery, catalysis, gas adsorption and separation, and magnetic resonance imaging (MRI). Besides the spatial dimensions, time is an important fourth dimension for the overall parameterization of cavity shape and guest exchange kinetics in cages and metal organic frameworks (MOFs). Tailored host cavities have a crucial impact on the capacity and selectivity for diastereomers. However, many initially synthesized M4L6 cages and exhibit dynamic interconversion between the condensed states. NMR is suitable to reveal symmetry differences but requires mandatory spin-labeling of the analyte during synthesis and 1H and 15N NMR in solution state stationary phase in gas chromatography (GC) was recently shown to separate different enantiomers and various types of organic compounds. In general, NMR spectroscopy (1H and 15N NMR) is suitable to reveal symmetry differences but requires relatively high concentrations and is unsuitable for dilute, short-lived species. NMR signals can be enhanced by hyperpolarization (e.g., via dynamic nuclear polarization, DNP) in solution state NMR. However, such DNP at room temperature still requires mandatory spin-labeling of the analyte during synthesis and 10−7 L sample volume. Even this enhancement is insufficient for highly dilute states, particularly when somewhat rapid exchange is involved.

Due to such limitations, certain conformations might have been overlooked in MOFs which can exist as tetrahedral cages constructed from octahedral complex-forming metal ions. These hosts can temporarily bind different hydrophobic guests. Some MOP ligands generate considerable fractions of heterochiral (Cₜₖ) or achiral (Sₜₖ) cages that are guest-responsive and exhibit dynamic interconversion between the diastereomers. However, many initially synthesized M₄L₆ cages (M and L refer to metal and ligand) have been reported to exhibit only homochiral (T) species in solution. An example is the water-soluble tetrahedral [Fe₄L₆]− cage formed by treating 4,4′-diaminobiphenyl-2,2′-disulphonic acid and 2-formylpyridine in water together with Fe(II) and a base. Experimental evidence (e.g., 1H and 15N NMR) is available exclusively for its T species (Fig. 1a). Recent studies on a series of related Co-containing MOPs also found no signs for any other versions.

Conformations with broken symmetry but sparse population could be undetectable amongst such conformers in combination with a certain exchange regime would indeed cause a considerable amount of guests to experience a certain type of host cavity over time. Such inclusion complexes might, however, survive long enough to have an impact on how a guest probes different symmetries and thus also allow for stereoselective recognition of reactants that are processed in tailored cages. Apart from guest recognition, the overall host symmetry is linked to the spatial arrangement of the ligands and enables fine tuning of the portals.

This can have great impact on exchange kinetics. A related aspect is the field of MOFs where the presence of metastable intermediate states in such dynamic structures is in certain cases only observable after loading such structures with gaseous guests or by controlling temperature and pressure. The flexibility limits of most reported self-assembling systems is underexplored and understanding the consequences of fundamental properties like broken symmetry that may impact guest exchange mechanisms in flexible frameworks is key to the successful development of applications. Sensitive tools to monitor all cavity conformations and their guest uptake and release are therefore of high importance for understanding and designing these intriguing systems.

We thus explored ultra-sensitive NMR saturation transfer with 129Xe to search for additional conformations of a yet exclusively assumed homochiral host. Xe as a monoatomic, spherical guest serves as a neutral “spy” to explore multiple symmetry environments. Hyperpolarized (HP) 129Xe can be easily dispersed into solution and saturated magnetization is readily replaced by on-the-fly delivery of fresh HP gas. The experiment can be repeated within a few seconds to allow for stepwise acquisition of well-resolved chemical shift information and/or signal averaging. Even though this type of spectroscopy unlike multi-dimensional NMR does not directly reveal structural information on the cavity seen by the guest atom, differences in the Xe exchange timescales become visible in the line shape of the saturation responses. Exchange kinetics are highly relevant in host-guest systems with non-covalent nature where Xe can serve as a model guest to explore cavity access. The noble gas is a powerful hyperpolarized NMR reporter because it can be more or less directly applied to bulk material and sense exchange kinetics in host sites without the need for cumbersome isotope labeling of the cavity-shaping atoms but rather provides a reversibly bound spin label itself to probe the existence of multiple conformations.

We investigated the water-soluble tetrahedral [Fe₄L₆]− cage by performing chemical exchange saturation transfer (CEST) NMR experiments using HP 129Xe, since Xe forms inclusion complexes with this Fe-MOP. Combining the sensitivity enhancement of HP nuclei with CEST, i.e., hyper-CEST, yields a ca. 105 - 106 fold signal enhancement over conventional NMR. Recently, the 129Xe hyper-CEST technique was applied for demonstrating a significant enhancement of the NMR signal of dissolved gas using a water soluble MOF. In another recent study, the capability of 129Xe hyper-CEST was demonstrated for differentiating a complex mixture containing MOFs as nano-cages in which the latter displayed similar chemical compositions. This was possible by tuning the MOF structure and by encoding multiple false colors for observed Xe chemical shifts. Saturation transfer (ST) techniques are a powerful tool that find an increasing number of applications. The magnetization in the bound pool is saturated by utilizing a continuous wave rf pulse applied over a time that is significantly longer than the average residence time of transiently bound spins. During this saturation period, chemical exchange causes a signal loss in bulk pool by affecting hundreds to thousands of spins per exchange site, thereby leading to accumulation of destroyed magnetization in the detection (bulk) pool. ST techniques improve NMR sensitivity, retain the spectral dimension, and can be “tuned” in sensitivity to different time scales of exchange processes. Furthermore, Xe significantly extends the detection range towards exchange rates of 104 Hz due to its large chemical shift range. This enables detecting so far elusive host conformations of stereochemistry interest.

In this work, the Xe@Fe-MOP system is investigated by 129Xe NMR under different perturbation conditions (e.g., temperature cycling, portal blocking, and guest competition). We observe multiple signals of HP 129Xe interacting with Fe-MOP and...
validate normal cage integrity for guest access. The exchange pathways that Xe undergoes between different chemical environments of Fe-MOP and solution are checked further by inversion recovery (IR) and 2D CEST (short saturation) measurements. The multiple responses in the CEST spectra are interpreted using complementary theoretical methods and first principles modeling as computational methods are considered an integral part to understand such systems13.

Results and discussion

Thermal stability of supramolecular Fe-MOP. Tetrahedral Fe-MOPs ([Fe₄L₆]₄⁺−([CH₃]₄N)₄⁺) were self-assembled under basic conditions at 50 °C (Supplementary Fig. 1 and Section 3)42. The product was structurally characterized using ¹H-NMR and ESI-MS (Supplementary Figs. 2 and 3). Fe-MOP (100 μM) encapsulation of Xe was investigated by Xe hyper-CEST NMR at temperatures ranging between T = 25 ... 50 °C (see Supplementary Section 3.2, for additional tests between 4 and 20 °C). Beside the direct saturation signal of free Xe in water at 0 ppm, the CEST spectra displayed three responses at 13, 24, and 30 ppm, respectively, at 25 °C (Fig. 2; it is common practice in CEST experiments that the chemical shifts are referenced with respect to the signal that is observed, i.e., in this case, the signal of free Xe in solution). This is in contrast to only one peak corresponding to the 13 ppm signal of bound Xe observed using direct ¹²⁹Xe NMR detection48. Also, the Co-containing versions [CoₙFe₄(CH₃CO)₄L₆]₄⁺⁻ (n = 1–3) have been reported to display only one signal each for encapsulated Xe44. With increasing temperature, the intensities of the narrow signals at 13 and 24 ppm increased while the broad one at 30 ppm disappeared into the baseline upon reaching 35 °C. When reducing T from 50 °C back to 25 °C, the data matched the initial result (Fig. 2 and Supplementary Fig. 4 together with Supplementary Video 1). We thus conclude that the 30 ppm signal represents already relatively fast exchange at room temperature (RT), which yields inefficient CEST conditions at higher temperatures. The two stronger signals, however, represent intermediate exchange at RT, which yields even better saturation transfer at higher exchange rates. Overall, the temperature cycling revealed the structural robustness of the self-assembled Fe-MOP via reproducible hyper-CEST signatures.

Examining the origin of multiple CEST peaks. Next, the reason for observing multiple CEST peaks was checked. No indication was found that the oxidation state of the Fe(II) centers changed while the iron was participating in coordination with the ligands (see Supplementary Fig. 6). Likewise, the presence of residual amounts of acetone used during Fe-MOP recrystallization cannot be the origin of additional CEST signals; in fact, large excess of (CH₃)₂CO causes reduced intensity, see Supplementary Fig. 6. We also checked if storage of Fe-MOP in solution might have led to deterioration. However, CEST spectra of freshly mixed Fe-MOP (100 μM) at 25 °C showed 3 responses at 13, 24, and 30 ppm. Stepwise increase in temperature causes two signals (13, 24 ppm) to increase while the third peak (30 ppm) disappeared at T ≥ 35 °C. Cooling the Fe-MOP solution back to 25 °C restored the initial result.

Fig. 1 Schematic illustration of Fe-MOP and its three diastereomers (atoms not to scale). Only one of the ligands oriented along the tetrahedral edge complexing two Fe²⁺ cations is shown for clarity purpose. Depending on how the participating nitrogen atoms (shown in blue) arrange around the metal centers (shiny spheres), the portal size and shape is slightly deformed (illustrated by the pink blurry ring). This impacts Xe exchange kinetics. Schematics of different Fe-MOP diastereomers and their symmetry axes/planes for the T (a), C₃ (b), and S₄ (c) diastereomers. a The T isomer has the ligands coordinated to homochiral metal centers (only green colored Fe²⁺ metal nodes) via the anti-linkages. b, c Conversely the ligands coordinate to the heterochiral metal centers (mix of red and green colored Fe²⁺ metal nodes) in C₃ and S₄ schemes via the syn-linkages.
faces of Fe-MOP was impeded using excess guanidinium hydrochloride (Gu·HCl) which is known to alter the incoming guest kinetics for cyclohexane\textsuperscript{59}. Here, Fe-MOP (100 μM) was gradually blocked by increasing the Gu·HCl concentration. The CEST spectra remained unaffected for [Gu·HCl] < 1 mM. At 1 mM Gu·HCl, a slight left shift of CEST peaks was observed (somewhat anticipated due to higher salt concentration). A 10 mM concentration of Gu·HCl finally resulted in more left shifted peaks while presumably slowing down the Xe exchange for the signal at 30 ppm, which becomes better resolved (Fig. 3). Xe is supposedly small enough to retain sufficient cavity access. Even high concentrations of Gu·HCl can only decelerate the exchange, primarily affecting signals that reflect already enhanced exchange under normal conditions.

Challenging the Fe-MOP cavity with larger competing guests. Assuming sufficient purity and integrity of the hosts, we challenged the interaction of Xe with Fe-MOP directly inside the cavity through competition by adding guests like cyclohexane and SF\textsubscript{6}. Fe-MOP (100 μM) was mixed with cyclohexane (150-fold excess) and a reference CEST spectrum for no cyclohexane encapsulation was acquired at 25 °C (Fig. 4a). The Fe-MOP cavity was then probed by Xe in the presence of cyclohexane at 50 °C over 24 h. The slowly progressing cyclohexane encapsulation was imminent after 8 h as it led to reduced CEST peak intensities. Subsequently, Xe was removed by degassing and the sample mixture containing cyclohexane was maintained at 50 °C for 24 h. Then, re-measuring the sample at 50 °C with Xe displayed 56% and 45% reduction of the 13 and 24 ppm CEST peak intensities compared to Fe-MOP alone (Fig. 4a; the 30 ppm signal is anyway hardly identifiable at 50 °C). Similarly, the Fe-MOP cavity was challenged by encapsulating an even larger and stronger binding guest, SF\textsubscript{6}\textsuperscript{60}. Measuring the SF\textsubscript{6}⊂Fe-MOP (100 μM) at 25 °C after 3 days of introducing the SF\textsubscript{6} atmosphere at 50 °C showed ca. 62 % and 31 % reduction in the intensities of the 13 and 24 ppm CEST peaks compared to untreated Fe-MOP. The fast exchanging CEST peak at 30 ppm disappeared into the baseline after the SF\textsubscript{6} treatment even when measuring the sample at 25 °C (Fig. 4b). Altogether, Xe binding remains possible owing to its smaller size and incomplete competing guest encapsulation (e.g., ~75% for SF\textsubscript{6}\textsuperscript{60}). Here, the CEST technique reveals otherwise missed residual encapsulation. These results illustrate that the newly discovered signals must originate from cavity environments and that not only Xe has access to these.

Exchange pathways between different CEST pools. We next revisited direct HP Xe NMR spectroscopy at fairly high Fe-MOP concentration (10.3 mM) and could show a previously unknown second and third Xe@Fe-MOP peak at 24 and 30 ppm, respectively (Supplementary Fig. 11a, b). This enabled us to estimate the populations of the suspected additional species (Supplementary Fig. 11c). The relative populations of the 13, 0 ppm (solution pool), 24 and 30 ppm pools at 10.3 mM cage concentration estimated from the direct spectrum are 1, 0.32, 0.010, and 0.0017,
respectively (see Supplementary Fig. 11c). Despite their low population, these are acting as rather efficient CEST pools and are indicative for relative fast exchange rates. The strongest peak represents ca. 5.6 mM of occupied MOPs (see Supplementary Information), which is ca. 55 % of the overall host concentration.

In addition, we carried out inversion recovery (IR) experiments at conditions where the solution peak and the two most prominent cage signals can be observed, while one of these three signals was selectively inverted (Fig. 5 and Supplementary Fig. 12 together with Supplementary Video 2). The two bound Xe signals at 13 and 24 ppm were arbitrarily assigned as Cage1 and Cage2, respectively. Regarding this initial subset, the data supports a three site exchange model (Cage1 <-> Solution <-> Cage2), in which the two more intense cage signals were directly connected with the solution pool, but not directly connected with each other (Section 10.1, Supplementary information).

The IR measurements at high concentrations sense the immediate flux of magnetization after a selective, instantaneous perturbation of one pool. They showed that the system equilibrates within ca. 350 ms (Supplementary Movie 2). CEST measurements with detecting pools other than the solution pool provides complementary insights at increased sensitivity for intermediate timescale fluxes between the pools. This must be detected before saturated Xe accumulates in the solution pool and then spreads throughout the network. We thus investigated CEST spectra at 25 and 10°C with global readout (Supplementary Fig. 13). A short saturation (\( t_{\text{sat}} = 250 \) ms) was used for measuring the exchange with limited sensitivity enhancement. Saturing the solution peak at 25°C yields a strong signal for Cage2 and a weak response for Cage1. This is because the small Cage2 pool is immediately saturated from the bulk pool whereas Cage1 represents a large enough pool that is not completely exchanged within 250 ms. The solution peak showed a (rather weak) response to Cage2 saturation and a decent response for Cage1 saturation. However, Cage2 saturation yields an overproportional strong CEST response for the solution pool. This again reflects the rather efficient exchange from Cage2 into the solution pool. As Cage1 does practically not respond to Cage2 saturation, we conclude that the flux via the solution pool has not yet reached Cage1 within 250 ms. For the reverse direction, data suggests that despite the indirect flux of saturation from Cage1 via the solution pool into Cage2, this is indeed detectable after 250 ms and comparable to direct flux from the solution pool to Cage2 (Supplementary Fig. 14).

We then cooled the Fe-MOP solution to 10°C for decelerated exchange from Cage3 signal and employed a higher saturation
strength \((B_1 = 5 \mu T)\) for a length \((t_{\text{rad}})\) of 250 ms. Only the solution peak reacted to Cage3 saturation. This suggests that either it is very inefficient pathway or too fast flux is observed from Cage3 into Cage1 or Cage2 (Supplementary Fig. 14b, 15).

Overall, the 2D CEST experiment enabled us to establish the possible CEST network between highly and sparsely populated CEST pools and the respective Fe-MOP states.

**CEST spectra simulation and quantification.** After excluding Fe-MOP structural degradation and hyper-CEST artifacts (CEST responses were observed without significant changes even after several months and were eventually also observed with thermally polarized Xe; see Supplementary Fig. 16) or structural imbalance several months and were eventually also observed with thermally induced by Xe bubbling (Supplementary Fig. 10) as explanation, polarized Xe; see Supplementary Fig. 16) or structural imbalance several months and were eventually also observed with thermally induced by Xe bubbling (Supplementary Fig. 10) as explanation, polarized Xe; see Supplementary Fig. 16) or structural imbalance several months and were eventually also observed with thermally induced by Xe bubbling (Supplementary Fig. 10) as explanation, polarized Xe; see Supplementary Fig. 16) or structural imbalance several months and were eventually also observed with thermally induced by Xe bubbling (Supplementary Fig. 10) as explanation, polarized Xe; see Supplementary Fig. 16) or structural imbalance.

The simulation gave the following populations of sites for a Fe-

4. In fact, the pool size of \(114.5 \text{ ppm, follow the above-mentioned chemical shift trend. Obviously, chemical shift differences are much larger than those obtained from the CEST spectra. However, currently neglected finite temperature effects due to motion of the cage atoms as well as solvent molecules may well decrease the chemical shift differences between the three diastereomers. Therefore, the computational modeling suggests that the lowest CEST signal (13 ppm with respect to Xe in water) arises from the well-studied \(T\) diastereomer, while the 24 and 30 ppm signals may originate from \(C_3\) and \(S_4\) diastereomers, respectively.

For \(S_4\) diastereomer, there are nine conformations with large variation in the volume while under similar energy criteria, our sampling approach exhibited only two and three conformations for \(T\) and \(C_3\) cavities, respectively. This indicates that the structure of \(S_4\) diastereomer is more flexible thus allowing larger openings for accommodation of different guests with varied sizes by its cavity. This finding potentially indicates easier and faster in-out exchange of Xe and may explain the specific features of the 30 ppm CEST signal: Firstly, the broader signal (see Fig. 1) is a demonstration of the faster exchange process. Secondly, the signal disappeared above 35 °C, as a consequence of the transition from intermediate to fast exchange regime. Thirdly, the chemical shift of the signal responds more to the temperature cycling than other signals (see Fig. 1 and Supplementary Fig. 4), and the CEST signal

Computational approach and findings. Quantum chemical calculations of \(\text{Xe}^{129}\) NMR chemical shifts were performed (see *Computational methods* section) for Xe atoms placed inside of the three individual diastereomers. The otherwise unknown molecular structures of \(C_3\) and \(S_4\) diastereomers were derived from the X-ray structure\(^{42}\) of the \(T\) diastereomer. The chirality of one or two metal centers was manually changed to create \(C_3\) or \(S_4\) diastereomer, respectively. The conformational space of each isomer was sampled by generating high number of conformers (see Methods section below and Supplementary Section 13). The lowest free energy \((G)\) values for conformations of the \(C_3\), and \(S_4\) are \(\Delta G = 0.6\) and 43.0 kJ/mol, respectively, with respect to the lowest energy conformation of \(T\) diastereomer. The thorough scrutiny of the full conformational space indicate that the lowest \(G\) conformations are the plausible structure candidates. This is supported further by large free energy differences to the next lowest conformations of each diastereomer (\(\Delta G = +4 \ldots +9\) kJ/mol) as well as high energy barriers between them.

Despite the density functional theory (DFT) computed \(\text{Xe}^{129}\) chemical shifts for a few low energy structures of the conformational search are overestimated (see Section 13.3 in Supplementary material), there is an obvious trend of \(\delta(T) < \delta(C_3) < \delta(S_4)\). The energetically most plausible lowest \(G\) structures also follow this trend and, therefore, their structures were studied further.

DFT geometry optimization increases their volumes (118.4, 105.7, and 96.0 Å\(^3\) for \(T\), \(C_3\), and \(S_4\), respectively; Xe van der waals volume \(62.4 \text{ Å}^3\)). The cages seem to relax around the Xe atom during the optimization in liquid environment, thereby leading to a decrease in cavity volume compared to X-ray data.\(^{42}\) Larger cavities by DFT optimization results in \(\text{Xe}^{129}\) chemical shifts within the experimental chemical shift range (see Table 5 in Supplementary material). The best estimates of \(\delta(\text{Xe}^{129})\) were obtained by statistical thermal averaging over Xe motion at room temperature. For the lowest \(G\) conformation of \(T\) diastereomer, the obtained \(\delta(T) = 14.1\) ppm (solvant shift w.r.t gas shift is 188 ppm) matches well with the experimental value of 201 ppm. Values for other diastereomers, \(\delta(C_3) = 66.7\) ppm and \(\delta(S_4) = 114.5\) ppm, follow the above-mentioned chemical shift trend. Obviously, chemical shift differences are much larger than those obtained from the CEST spectra. However, currently neglected finite temperature effects due to motion of the cage atoms as well as solvent molecules may well decrease the chemical shift differences between the three diastereomers.
becomes significantly narrow at low temperatures, denoting the transition between exchange regimes.

Theoretically, the additional CEST signals might arise also from cages loaded with several Xe atoms (see Computational methods section). However, loading the much larger solid-state X-ray cavity of the T diastereomer12 with two Xe atoms increased the calculated chemical shift of about 400 ppm compared to loading single Xe atom (accommodating three and four Xe atoms resulted in even larger chemical shifts). Therefore, we can exclude this explanation. Similarly, we were able to exclude the possibility that the additional signals could arise from simultaneous encapsulation of Xe with N2 or He, which were present in the hyperpolarized gas mixture; computational estimates resulted in much larger chemical shift changes than the experimentally observed values and signatures of additional signals were observed even in the thermally polarized experiments without N2 and He. Finally, we expect that, due to its hydrophobicity, the cage does not encapsulate water molecules together with Xe; loading of the T diastereomer with Xe and H2O resulted also counted chemical shifts, which are much higher than the experimentally observed shifts.

In conclusion, we obtained experimental evidence through Xe NMR for two previously unknown bound Xe signals (24 and 30 ppm) for a self-assembling Fe-MOP. Xe even retained accessibility to the cavity upon impeding the portals by excess Gu·HCl, albeit with slower exchange. Competition with larger and stronger binders such as cyclohexane and SF6 considerably reduces (~30–60 %) the transient binding of Xe but also demonstrates that other guests have access to the additionally identified cavity environments. The Xe exchange pathways between different CEST pools were established by IR and 2D CEST experiments and the exchange was found to occur presumably all via the solution pool. This indicates that Xe interaction with different structural forms (diastereomers) of Fe-MOP might be the reason for observing multiple, consistent and reproducible CEST peaks. The hypothesis was supported through extensive first principles modeling showing that structural differences of diastereomers explain both the order of observed Xe chemical shifts and the variety in exchange kinetics. This study demonstrates that the molecular symmetry is linked to different guest exchange regimes with release of bound Xe ranging between 10^3 s^-1 and 10^0 s^-1. The insights thus address two of the central questions in this field12,13: (a) what are the guest exchange time scales occurring in supramolecular nanostructures and (b) what tools are available to observe them? These results illustrate the value of hyperpolarized guests that can be delivered repeatedly within a few seconds to reveal hidden host conformations in detailed solution spectra of bulk material without sophisticated sample preparation. MOP systems thus provide intriguing encapsulation, spin capture, and exchange properties that can be generated by performing selective chemistry either at the metal center (e.g., by metal substitution/exchange) or via the ligand structure (e.g., by post-synthetic ligand exchange, tethering different linker types), respectively. Future studies shall investigate the CEST imaging capabilities of different MOP symmetries and if the time scale that was sufficient for selective RF manipulation inside the formerly hidden conformations would also allow chemical reactions to happen with stereoselective features.

Methods

Materials used. 4,4′-diaminobiphenyl-2,2′-disulfonic acid hydrate (70 wt.% in H2O) was purchased from Santa Cruz Biotechnology, Inc. Heidelberg, Germany. Iron(II) sulfate heptahydrate (~99%), 2-formylypyridine (99%), tetra- methylammonium hydroxide pentahydrate (~97%), cyclohexane (~99.8%), (S)-BINOL i.e., S-(-)-1,1′-Bi(2-naphthol), 99% and Pluronic®L-81 were procured from Sigma Aldrich Chemie GmbH, Steinheim, Germany. Guanidinium hydrochloride (Gu·HCl, ≥ 99.5%), was obtained from Carl Roth GmbH & Co.KG, Karlsruhe, Germany. Sulphur hexafluoride (SF6) gas was kindly provided by Mess Prof. Thomas Braun, Humboldt-Universität zu Berlin, Germany. MilliQ water (18.2 MΩcm) was used for all sample preparations. All chemicals and solvents were used as received without any further purification.

General methods. 1H NMR data of Fe-MOP were recorded in D2O. A potential acetone impurity encapsulated by Fe-MOP was removed by repeating the hyperpolarization and complete removal of acetone was checked by 1H NMR. The electron spray ionization mass spectrometry (ESI-MS) of Fe-MOP was recorded in 1:1 H2O:MeOH mixture using Orbitrap Fusion (Thermo Scientific) with Xcalibur set up. The ESI-MS was performed by a direct injection (4 μL/min) of the sample and measuring in the negative mode using a Standard-Source Spray (3 kV) without using any gas. The circular difference (CD) measurements of Fe-MOP and its enantiomers in H2O were carried out using Jasco J-715 spectrophotometer (Jasco, Maryland, U.S.A.).

Synthesis of supramolecular FeL₄ Fe-MOP). The supramolecular iron-based metal organic polyhedra (FeL₄) was synthesized (Supplementary Fig. 1) as reported in an earlier publication63. Briefly, 4,4′-diaminobiphenyl-2,2′-disulfonic acid (2.05 mmol), 2-formylypyridine (4.06 mmol), tetrakis(dimethylamino)porphyrin (4.06 mmol) and iron (II) sulfate heptahydrate (1.35 mmol) were dissolved in 25 ml de-gassed water. After mixing, the reaction mixture appeared as a dark purple solution. Reaction was carried out for 20h at 50°C under argon atmosphere. The final product was isolated as dark purple crystals by slow vapor diffusion of acetone into the aqueous solution. The structure was validated by 1H NMR (Supplementary Fig. 2) and ESI-MS (Supplementary Fig. 3) in agreement to earlier reports45.

Xenon hyperpolarization and delivery. The NMR experiments were performed with a 9.4 T Bruker AV400 NMR spectrometer (Bruker Biospin, Ettlingen, Germany) equipped with gradient coils for MR imaging. The excitation and detection of the NMR signal was achieved using a 10 mm inner diameter double resonance (129Xe and 1H) probe. A custom-designed continuous-flow polarizer was utilized to produce hyperpolarized (HP) Xe by spin-exchange optical pumping65. A gas mixture of 2% Xe (26.4 % 129Xe natural abundance), 10% N2 and 88% He was used unless otherwise noted. The applied total pressure was 4.5 bar abs., thus the dissolved Xe concentration is ca. 369 μM under these conditions. The achieved 129Xe spin polarization was approximately 25%. The gas mix was directly bubbled into 1.5 ml of the sample placed inside a 10 mm thick-walled NMR tube by using a spectrometer-triggered bubble dispenser (3.5 bar overpressure) via five fused-silica capillaries (Polymicro Technologies, Molex Incorporated, Caudebec les Elbeuf, France). The bubbling was controlled by using spectrometer-triggered gas-flow regulators (mass flow controllers, Omega Newport, Deckenpfronn, Germany).

Hypen-CEST spectroscopy For each data point in the Chemical Exchange Saturation Transfer (CEST) spectrum, the gas mixture was directly bubbled for 12 s into the sample solution (flow rate 0.1 standard liter per minute (SLM)) followed by a 3 s delay to allow the bubbles to collapse. Hyper-CEST spectra were acquired using a continuous wave (CW) saturation pulse (length τsat = 15 s, amplitude B1 = 3.14 μT). The (CW) saturation pulses were applied with 1 ppm increments within 45 to ~26 ppm spectral frequency offsets. The spectral acquisition was implemented by referencing the observed chemical shifts to the free Xe in solution peak at 0 ppm. Z-spectra were fitted to the exponential of a sum of Lorentzians using OriginPro 2018 (OriginLab, Northampton, MA). Unless otherwise indicated, the spectra of all samples were acquired at 25°C.

Computational methods. Conformational searches for each diastereomers (T, C groups were carried out for all three conformations (see Supplementary material). First, high number of structures inaccessible from a local minimum by low energy motion were generated by rotating the benzylsulphonate units (5 angles for every unit resulting in 0.24 x 10⁴ rotamers). Then structures with van der Waals (vdW) clashes and other unrealistic conformers were filtered out and obtained plausible structures were minimally solvated with 36 water molecules - one for each oxygen atom in twelve SO₄ groups. The overall charge on each conformation was compensated with four NH₄ cations on each face of the [FeL₄]²⁻ complex and the Xe atom was placed inside its cavity. The structures were then optimized using the semiempirical extended tight binding GF2=xTB method66 via the XTB code (see Code availability statement). In addition to the explicit counterions and water solvent molecules, an implicit GB/SA water solvent model was used in the geometry optimization. The aforementioned optimized structures were selected for further calculations only if Xe remained inside the cavity and SO₄ groups were well exposed to the solvent. To obtain free energy estimates of maximum of twenty lowest energy conformers of each diastereomer, they were subjected to vibrational and conformational calculation with the XTB code.

Density functional theory (DFT) calculations for the lowest free energy (G) conformers of each diastereomer (see more details in Supplementary material and...
Supplementary Table 5) were carried out with the Turbomole code. Geometries of the three complexes including four NH₃ counterions were computed at the complete basis set/cc-pVTZ method with implicit COSMO solvation model of water. At the obtained equilibrium geometries, 122Xe NMR nuclear shielding calculations were carried out at scalar-relativistic local exact two-component (X2C) level. We used BHandHLYP79,80 DFT functional with D4 dispersion correction72 and x2c-TZV1P-S(d)/x2c-SV1P(also) basis sets together with finite nucleus model73. BHandHLYP has been shown to provide best estimates for Xe chemical shifts in several studies of molecular cavities87–84 and molecules78–80. It was observed in those studies that, while the inclusion of SR effects advances the non-relativistic (NR) calculations for Xe shifts, quite small and invariant spin-orbit (SO) relativistic contribution can be neglected. Therefore, SR method like X2C provides high quality results with moderate computational requirements.

While full molecular dynamics treatment of current extensive systems with counterions and large enough explicit solvation is currently not feasible, we focus on treatment of the most important effect, i.e., Xe thermal motion inside the rigid cage. We use the same approach as in previous studies72,85, in which canonical Monte Carlo (MC-NVT) simulations with in house code were carried out on numerical potential energy surface (PES). PES and Xe NMR shift hyper-surface used for averaging were computed at the same level as the equilibrium geometry calculation above (see simulation details and thermal averages in Supplementary Table 5).

The loading effect on Xe chemical shift was studied by placing one to four Xe atoms inside the Xe-cage potential energy surface of T-Fe₄CO₄. The thermal averaging over the dynamics of Xe atoms at $T \approx 300$ K was obtained using the modified version of the in house MC-NVT code, where both T-Fe₄L₄ cage contribution to potential energy and NMR chemical shift of Xe were piecewise-linearly interpolated from the non-uniformly sampled 3D surfaces. For Xe-Xe interactions, the relativistic potential energy and the non-relativistic binary chemical shift functions by Hanni et al.81 were used. Therefore, the Xe chemical shift for each Xe atom is a sum of the thermal averages of the Xe-Xe shift from the binary chemical shift and Xe-$T$-Fe₄L₄ shift (with respect to free Xe atom).

Data availability
All data generated and analysed during this study are included in this article and its Supplementary Information, and are also available from the authors upon request. The details about synthesis, molecular characterization, hyper-CEST spectra under different perturbation conditions, inversion recovery measurements, 2D CEST (short saturation) details about synthesis, structural characterization, hyper-CEST spectra under different perturbation conditions, inversion recovery measurements, 2D CEST (short saturation) experiment and computational methods are available as supplementary information.

Code availability
The code used for computational methods, including in-house MC-NVT python code, is also available from the authors upon request. The semiempirical extended tight binding program package xt ish can be accessed using the following link https://github.com/grimm-lab/xtb. The xTB standalone code, Version 6.2. from the above link was utilized program package xtb can be assessed using the following link https://github.com/grimm-lab/xtb. The xTB standalone code, Version 6.2. from the above link was utilized.

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Author contributions
J.J., L.S., and V.-V.T. designed and J.J. and L.S. executed different experiments presented in the manuscript. C.G., K.R., and I.J. synthesized the MOP cages. S.K., L.M., and P.I. carried out conformational search and first principles modeling of Xe NMR shielding. J.J. and L.S. analyzed and fitted the hyper-CEST data. V.V.Z. performed the four site exchange CEST spectrum simulation and the IR data fitting using the three site exchange model. J.J. wrote the first version of the manuscript. All authors were involved in results discussion and editing of the manuscript.

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Competing interests
The authors declare no competing interests.

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