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A Structural Basis for ^{129}Xe Hyper-CEST Signal in TEM-1 β -Lactamase

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Abstract

Genetically encoded (GE) contrast agents detectable by magnetic resonance imaging (MRI) enable non-invasive visualization of gene expression and cell proliferation at virtually unlimited penetration depths. Using hyperpolarized ^{129}Xe in combination with chemical exchange saturation transfer, an MR contrast approach known as hyper-CEST, enables ultrasensitive protein detection and biomolecular imaging. GE MRI contrast agents developed to date include nanoscale proteinaceous gas vesicles as well as the monomeric bacterial proteins TEM-1 β -lactamase (bla) and maltose binding protein (MBP). To improve understanding of hyper-CEST NMR with proteins, structural and computational studies were performed to further characterize the Xe-bla interaction. X-ray crystallography validated the location of a high-occupancy Xe binding site predicted by MD simulations, and mutagenesis experiments confirmed this Xe site as the origin of observed CEST contrast. Structural studies and MD simulations with representative bla mutants offered additional insight regarding the relationship between local protein structure and CEST contrast.

Graphical Abstract

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TEM-1 β -lactamase (bla) is a genetically encoded contrast agent detectable at nanomolar concentrations in mammalian cells via ^{129}Xe hyper-CEST NMR. Protein mutagenesis, X-ray crystallography and MD simulations offer insight into the mechanism of Xe-bla exchange and the factors that govern CEST contrast.

Keywords

xenon; hyperpolarized; CEST; contrast; magnetic resonance

Introduction

Genetically encoded (GE) contrast agents (a.k.a. reporter genes) such as fluorescent and bioluminescent proteins have made it possible to label cell populations as well as visualize protein expression, localization, and functionality.^[1] The delivery of reporter genes to target cells and tissues can be accomplished using relatively non-invasive transgenesis methods such as viral infection.^[2] Alternatively, these genes can be expressed recombinantly, tagged with a biorecognition element (e.g., antibody), and administered as exogenous contrast agents.^[3] Quantitative measurement of optical reporter genes, however, is typically limited to depths of a few millimeters due to the strong scattering of light by living tissue.^[4] Monitoring gene expression in larger, opaque organisms requires the use of reporter genes detectable by alternative modalities such as magnetic resonance imaging (MRI). MRI is a non-invasive, non-ionizing imaging technique that offers good spatial and temporal resolution at virtually unlimited penetration depths. However, conventional ^1H MRI is characterized by low sensitivity as MR contrast arises from the net magnetization that results from the small population difference between the nuclear Zeeman energy levels of ^1H nuclei.^[5] Additionally, any MR contrast produced by a reporter gene must be detectable against background signal of water protons present in tissue at $\sim 90\text{ M}$.^[6] Consequently, the sensitivities of reporter genes detectable by ^1H MRI have largely been limited to the micromolar-to-millimolar range.^[7]

^{129}Xe -based MRI reporter genes can overcome this detection limit through the combined use of two techniques: nuclear hyperpolarization and chemical exchange saturation transfer (CEST). Hyperpolarization of ^{129}Xe nuclei through spin-exchange optical pumping (SEOP) increases their detectable magnetization by several orders of magnitude.^[8] Hyper-CEST is an MRI contrast approach in which solute-bound hyperpolarized (hp) ^{129}Xe nuclei that resonate at a frequency different from Xe in solution are selectively spin-flipped using RF

saturation pulses (Scheme 1).^[9] Xe exchange between solute and solvent on the kHz timescale leads to readily observed loss of Xe polarization in water, where Xe has millimolar solubility.^[8a] Hyper-CEST has been shown by our laboratory^[10] and others^[11] to enable ultrasensitive detection of Xe biosensors that localize magnetic resonance (MR) contrast to molecular targets of interest. Pioneering work by Shapiro and coworkers validated the use of nanoscale, multi-gene bacterial gas vesicles as genetically encoded hyper-CEST reporters detectable at picomolar concentrations.^[12] To advance this approach for biomolecular imaging applications, we characterized the monomeric *E. coli* protein TEM-1 β -lactamase (bla) as a hyper-CEST reporter both *in vitro* and in mammalian cells.^[13] Bla produces a CEST response 60 ppm downfield of the $^{129}\text{Xe}_{(\text{aq})}$ frequency, though the origin of such MR contrast and the mechanism of Xe exchange had yet to be elucidated. Gaining additional insight regarding the structural basis for ^{129}Xe hyper-CEST signal in bla can inform rational mutagenesis experiments to enhance MR contrast as well as guide the search for novel GE contrast agents for ^{129}Xe hyper-CEST MRI.

Xenon is a well-established atomic probe of protein structure and function. Xe preferentially binds void spaces within proteins *via* van der Waals forces without inducing significant structural rearrangement.^[14] Observed distances between bound Xe and protein side chain atoms usually range from 3.0 Å to 4.5 Å.^[15] Xe has been employed in protein crystallography to phase structures,^[16] trace exchange pathways for gaseous substrates (e.g., O₂, N₂, H₂, CO, CH₄),^[17] and characterize structural plasticity.^[18] Protein NMR experiments have also employed Xe, where the sensitivity of ^{129}Xe chemical shift to its local environment has been used to study non-specific^[19] and specific^[20] Xe-protein interactions as well as monitor protein conformational changes.^[21] These studies, however, are limited by the sensitivity of the NMR methods used to directly measure the ^{129}Xe -protein chemical shift. Direct detection ^{129}Xe NMR spectra of proteins such as maltose binding protein (MBP) and T4 lysozyme report a single resonance peak, corresponding to the $^{129}\text{Xe}_{(\text{aq})}$ environment, due to low Xe binding affinity and rapid exchange of Xe between protein and solvent.^[22] As such, Xe-protein interactions typically perturb the $^{129}\text{Xe}_{(\text{aq})}$ peak by less than 3 ppm per mM protein.^[21, 23] The low affinity of Xe for proteins necessitates the use of high protein concentrations for direct detection ^{129}Xe NMR experiments. The aforementioned hyper-CEST approach extends the sensitivity of ^{129}Xe NMR as a probe of biomolecular structure and function.

In this report we characterize the Xe-bla interaction by X-ray crystallography and perform protein mutagenesis combined with additional X-ray crystallography and MD simulations to elucidate the structural basis for hyper-CEST response. The crystal structure of bla reveals a major Xe binding site, with Xe entry and egress pathways identified through MD simulations. Mutations made to the major Xe binding site in bla confirm its role in generating the 60-ppm downfield shift observed in the hyper-CEST z-spectrum. Further analysis of Xe exchange with these mutants by X-ray crystallography and MD simulations reveals additional insights regarding the relationship between Xe-protein interactions and CEST.

Results and Discussion

Xe binding to bla

The Xe derivative of wild-type (WT) bla was isomorphous to native protein, with a root-mean-square deviation (rmsd) of 0.11 Å between the C_α positions of the native and Xe-derivative crystal structures. Bla crystallized as a tetramer, and three Xe atoms (hereafter designated Xe1, Xe2, and Xe3) were observed at the same positions in all four bla molecules in the asymmetric unit (Fig. S1). Density present in the anomalous Fourier map further confirmed the presence of Xe at these three sites (Fig. S2). Because Xe binding was nearly identical among all four bla chains, for simplicity a representative bla molecule (chain A in the crystal structure) is discussed in the remainder of this report.

The protein side chains surrounding Xe1, Xe2, and Xe3 are predominantly hydrophobic (Figs. 1, S3) and are within van der Waals distances of the bound Xe (Tables S3–S5). Notably, none of the three Xe binding sites contains water in the native bla structure, indicating that Xe binding is not driven by hydrophobic stabilization – an observation consistent with Xe binding to myoglobin.^[24] Additionally, computational studies of Xe binding to organic host molecules such as cryptophane concluded that the occupancy of water molecules in the hydrophobic interior of the cryptophane is anti-correlated to the free energy of Xe binding.^[25] The bla-Xe complex shows an additional Xe atom, Xe4, bound at the interface between protein chains A and D (Fig. S1). Xe4, however, is likely an artifact of crystal packing given its proximity to the protein surface and the fact that Xe4 is enclosed by neighboring bla molecules in the crystal structure.

During structural refinement of the Xe-bla model, Xe1 converged to the highest average occupancy (68%), whereas Xe2 and Xe3 converged to average occupancies of 21% and 10%, respectively (Table S6). The much higher occupancy of Xe1 indicates that the Xe1 cavity represents the predominant site of Xe exchange in bla. From the occupancy of Xe1, the association constant (K_a) of Xe1 is estimated to be roughly 40 M⁻¹, approximating a solubility of Xe in solution of 43 mM MPa⁻¹.^[22] This K_a for Xe is comparable to the experimentally-determined K_a values for Xe binding to MBP and T4 lysozyme, which are 20 ± 10 M⁻¹ and 60.2 ± 0.2 M⁻¹, respectively.^[22] Xe1 lies at the terminus of a cryptic (i.e., not visible in the native bla structure) channel lined by predominantly hydrophobic side chains. This hydrophobic channel is the same one uncovered in crystal structures of bla complexed with allosteric inhibitors.^[26] The back wall of the Xe1 cavity consists of residues A36, L40, and V44, flanked by T265 and Q278. The methylenes of the R275 side chain cap the binding site, shielding Xe1 from solvent. Three isoleucine side chains – I263, I282, and I279 – separate the Xe1 cavity from the rest of the hydrophobic channel and together comprise the entry portal for Xe1 binding. Notably, the positions and conformations of the side chains surrounding Xe1 are nearly identical in native and Xe-derivatized bla, revealing that the native conformation of bla has a pre-existing cavity capable of accommodating Xe. The refined B-factor of Xe1 is 11.0 Å², and the average B-factor of protein atoms within 6 Å of Xe1 is 7.8 Å². These comparable thermal mobility values indicate that Xe1 is well localized in the pocket, as expected given that the van der Waals volume of Xe is approximately 42 Å³ and the Xe1 cavity volume is 62 Å³.

Allostery with active site

To further probe the structural dynamics of the Xe1 cavity, the ^{129}Xe hyper-CEST z-spectrum of bla was acquired in the presence of the competitive β -lactamase inhibitor tazobactam ($K_i = 20 \text{ nM}$).^[27] We hypothesized that because ligand binding at the Xe1 site affects the hydrolytic activity of bla,^[26, 28] inhibitor binding at the active site should have some measurable effect on Xe1 binding and exchange. Indeed, the z-spectrum of bla complexed with tazobactam shows broadening of the Xe-bla peak (Fig. 2; Fig. S4), indicative of faster Xe exchange between protein and solvent. This result suggests that inhibitor binding at the active site of bla increases the accessibility of the Xe1 cavity through the allosteric mechanism identified by Horn et al.^[26]

Mechanism of Xe1 exchange

The results of our X-ray crystallography experiments were compared to our prior Xe-bla MD simulations.^[13] These MD simulations were performed in the presence of excess Xe (i.e., “flooding”) for a 1 μs trajectory. The atomic coordinates of unliganded bla (PDB ID 1BTL)^[29] were used as the starting point of the simulation. At the outset of the simulation, bla remained in its closed conformation with the position of the C-terminal α -helix (helix 12) blocking Xe access to the protein interior. Over the course of the simulation, helix 12 moved away from helix 11 and the nearby β -sheet wall to allow Xe entry to the hydrophobic channel leading to the Xe1 cavity. Inspection of the Xe density map through the final 400 ns of the trajectory reveals a continuous “Y”-shaped density throughout the channel, revealing pathways of Xe exchange (Fig. 3). There are two distinct Xe entry points into the hydrophobic channel. Xe can pass either above or below the contact point between helices 11 and 12. Both pathways converge into a single route leading to the Xe1 cavity at the terminus of the hydrophobic channel. The contours of the Xe density approximate the hydrophobic moieties of detergent-like ligands complexed with bla.^[26] The Xe density shows discrete lobes along this pathway, suggesting that the outward movement of helix 12 leads to the formation of a series of connected cavities that transiently bind Xe. Our X-ray crystallography experiments affirm the results of the MD simulations, as Xe3 is positioned at the entrance to one of these exchange pathways and Xe1 lies at the terminus of the Xe density. Although our MD simulations did not predict the binding of Xe2, this binding site was recently identified as a cryptic pocket in bla using Markov State Models.^[30]

Inspection of the Xe-bla crystal shows no open pathway from the protein surface to the Xe1 cavity. Xe1 binding therefore involves dynamic fluctuations of the protein structure to allow Xe access to this buried cavity, an observation made previously to rationalize Xe binding to collagenase.^[31] Comparison of an “open” bla structure complexed with an allosteric inhibitor (PDB ID 1PZO)^[26] to the structure of unliganded bla complexed with Xe (PDB ID 5HW1) shows that the side chains of I263 and I279 shift roughly 2 $\text{\AA}$ apart, thereby allowing access to the Xe1 cavity (Fig. 4a). This expansion allows ~ 3 Xe atoms to simultaneously occupy the cavity (Fig. 4b, c). Inspection of the dynamic distance between I263 and I279 through the MD trajectory reveals that approximately halfway through the 1 μs trajectory, the distance between I263 and I279 sharply increases – a conformation change that coincides with Xe access to the Xe1 cavity. The transient widening of this “isoleucine

portal” controls the rate of Xe1 entry and likely plays an integral role in the hyper-CEST mechanism.

CEST contrast originates from Xe1

At this point the following conclusions were drawn from our X-ray crystallography experiments and MD simulations: (1) bla possesses a high-affinity Xe binding site at the Xe1 cavity; (2) up to three Xe atoms can assemble inside this cavity; (3) Xe exchange is coupled to local protein conformation and is relatively slow. These observations support our initial hypothesis that Xe exchange at the Xe1 cavity gives rise to the 60 ppm shift observed in the ^{129}Xe hyper-CEST z-spectrum. To further test this hypothesis, a series of single point mutations were introduced to bla to perturb Xe1 affinity and/or exchange rates and pathways (Fig. 5). Residues I263 and I279 were chosen because they regulate Xe access to the Xe1 cavity, and likely affect binding affinity as well. I263 was mutated to leucine, asparagine, aspartate, and alanine, and I279 was mutated to asparagine. Residue I282, which also makes van der Waals contacts with Xe1, was mutated to alanine. All bla mutants, with exception of I263D (which expressed exclusively as insoluble inclusion bodies), expressed at levels comparable to WT and were purified following the same procedure. Bla mutants were characterized by circular dichroism (CD, Fig. S5) to verify proper folding, and the CD thermal melts were performed to assess the effects of the point mutations on protein stability (Table S7).

The ^{129}Xe hyper-CEST z-spectra of the bla mutants were acquired following the same protocol used for WT (Fig. 6). The I263A and I279N mutants showed complete loss of downfield CEST contrast; CD and activity assays performed pre- and post-CEST verified that loss of contrast was not the result of protein denaturation during hyper-CEST (Fig. S6; Table S8). The z-spectra of I263A and I279N still showed broadening of the $^{129}\text{Xe}_{(\text{aq})}$ peak compared to PBS alone, characteristic of a chemical exchange mechanism contributing to a magnetic susceptibility difference between bla-bound and solvent-bound Xe.^[12] These spectral data indicate that Xe binding to the I263A and I279N mutants is preserved, but at a more rapid exchange rate compared to WT bla.

The I282A and I263N mutants showed appreciable CEST contrast at 255 ppm (60 ppm saturation offset), although at roughly half the intensity of WT bla, suggesting that while Xe1 binding is preserved, these alterations to the Xe1 cavity adversely affect binding affinity and/or Xe exchange rates. The signal from I263L at 255 ppm, on the other hand, was only slightly lower than WT, as might be expected from this minor structural perturbation. An unusual feature of the z-spectra of I282A, I263N, and I263L is the broadened signal between 255 and 195 ppm. The spectrum of WT can be deconvolved into two peaks: 255 ppm for Xe-bla, and 195 ppm for Xe-solvent (the minor peak at 235 ppm was interpreted as an artifact). The bla mutants, however, show only a peak at 195 ppm with significant asymmetric broadening towards 255 ppm. The z-spectra of these mutants remeasured at 10 μM protein concentration verified the lack of downfield saturation response (Fig. S7). The signal arising from this “intermediate” range can be interpreted as resulting from Xe bound to bla but not fully shielded from solvent. Collectively, these bla mutant z-spectra confirm that Xe1 is the origin of bla’s unique hyper-CEST signal at 255 ppm.

Xe binding to bla mutants

To further investigate the structural basis of ^{129}Xe hyper-CEST signal, X-ray crystallography experiments were performed on three representative mutants – I263A (total loss of contrast); I263N (major loss of contrast), and I263L (minor loss of contrast). Native protein crystals were derivatized with Xe following the same procedure used for WT. Xe1 binding was preserved for all three mutants (Fig. 7). The occupancy of Xe1 bound to I263L is on par with WT (83% vs. 68%), whereas the occupancies of Xe1 bound to I263N and I263A are 33% and 34%, respectively (Tables S9–11). In the I263N mutant, the carboxamide nitrogen of N263 donates a hydrogen bond to the carbonyl oxygen of G45, orienting the carboxamide directly towards Xe1 and shifting its location slightly compared to Xe1 bound to WT. It is worth noting that despite the presence of the asparagine side chain inside the Xe1 pocket, no water was observed in the native I263N structure, highlighting the hydrophobicity of this buried cavity.

Remarkably, the crystal structures of all three mutants revealed a new cavity capable of binding Xe (hereafter designated Xe5). Similar to the binding sites of Xe1, Xe2, and Xe3, Xe5 is predominantly lined by hydrophobic residues. Inspection of the WT structure shows that Xe5 binding is effectively blocked by the γ 1 and δ carbons of the I263 side chain. The occupancies of Xe5 are 22%, 28%, and 35% in the I263L, I263N, and I263A mutants, respectively (Tables S9–11). Notably, the B-factors of Xe5 are on par with the B-factors of nearby protein atoms, indicating that Xe5 is well-localized inside the cavity. Xe5 is positioned between Xe1, which is completely shielded from solvent, and Xe3, which lies at the entrance of the hydrophobic channel. Xe5 is therefore expected to produce a chemical shift somewhere between 255 and 195 ppm. The presence of Xe5 bound to I263L and I263N can explain the broadened CEST response of these mutants. The Xe interaction with WT bla can be approximated as a two-state system, where Xe is either aqueous or bound in the Xe1 cavity. However, for I263L and I263N, Xe can transiently occupy an intermediate binding site in the hydrophobic channel connecting the protein core to solvent.

Finally, it is revealing that the I263A mutant reports no CEST contrast even though Xe binding at the Xe1 and Xe5 cavities is preserved. This result highlights a fundamental feature of protein hyper-CEST: Xe binding does not guarantee useful MR contrast. Rather, the kinetics of Xe exchange play a more critical role in determining CEST contrast. The importance of Xe exchange kinetics has also been observed in other systems, notably MBP, where our group demonstrated that the protein must adopt a maltose-bound, closed conformation to slow Xe exchange and report CEST contrast.^[32] Additionally, hyper-CEST experiments with sperm-whale myoglobin, the protein with the highest known affinity for Xe ($K_a = 190 \text{ M}^{-1}$),^[33] have shown that no downfield saturation response is observed (unpublished data), likely due to fast exchange ($k_{\text{off}} = 1.5 \times 10^5 \text{ s}^{-1}$).^[33]

Computational studies of bla mutants

To further investigate the effect of the I263 mutations on the structural dynamics of the Xe-binding cavities, and to understand the molecular origins of the perturbed hyper-CEST z-spectra, we performed long time scale MD simulations on the I263L, I263N, and I263A mutants. Simulations were run starting with either a single Xe atom residing in the Xe1 site

or with apo-form bla in the presence of a large excess of Xe atoms in solution (i.e., “flooding”). These two complementary approaches were adopted to avoid artifacts resulting from insufficient statistical sampling (single Xe simulations) and structural perturbation due to the large excess of Xe (“flooding” simulations). The patterns of occupancies calculated from these trajectories were consistent with the experimental structures (Fig. S8a) and between the two computational approaches (Fig. S8b).

All I263 mutants showed increased Xe density in the region surrounding the Xe5 cavity, whereas in WT there was a density node at this site. This agreed with our crystallography results, where Xe5 was observed bound to I263L, I263N, and I263A but not to WT. Moreover, I263N showed a smaller, more localized density within the Xe1 cavity, consistent with the reduced occupancy of Xe1 observed in the X-ray crystal structure and decreased hyper-CEST contrast at 255 ppm. Quantitative analysis of the number of Xe atoms occupying the binding sites during the MD simulations confirmed that this was indeed the case (Fig. S9). The occupancy map of I263A, which showed the most perturbed hyper-CEST profile (i.e., complete absence of 255 ppm peak), showed some features distinct from the other three bla variants. Notably, there was continuous Xe density within the Xe1 cavity of I263A, while WT and I263L presented two spheres of density and I263N presented only one. Additionally, in I263A the Xe density along the length of the hydrophobic channel was nearly continuous – only a small gap was observed between Xe densities corresponding to the inner and outer segments of the channel. This is a marked difference from WT, where Xe density was observed as discrete spheres along the channel. These features, collectively, indicate that I263A is generally more accessible to Xe than the other bla variants.

To further ascertain whether mutations to the I263 side chain can affect Xe accessibility as well as occupancy, we analyzed all the Xe entry and egress events that occurred in MD simulations for WT bla and the I263 mutants. As observed for WT bla, Xe enters and exits the bla mutants predominantly via pathway 1, proceeding by way of the Xe3 and Xe5 positions to the Xe1 cavity (Fig. 8; Table 1). The number of Xe atoms following pathways 2 and 3 are minor and fairly conserved for all bla proteins.

Closer inspection of the side chain packing of residues 263 and 279 offers insights relating to the relative accessibilities of Xe. Whereas WT showed a sharp step-wise increase in Xe accessibility upon opening of the I263-I279 “gate”, mutants I263N and I263L showed no such gating phenomenon. The inter-side chain distance of residues 263 and 279 remained relatively unchanged through the MD trajectory, and as a result Xe occupancy in the Xe1 cavity was constant as well. Likewise, the I263A mutant showed almost-immediate Xe binding due to the lack of steric restriction imposed by the 263 and 279 side chain interaction. These MD analyses indicate that well-resolved CEST contrast is achieved when Xe exchange is moderated by side chains acting as gates along the access pathway to the major binding site in bla.

Conclusions

In this study we have investigated the origin of the 60 ppm downfield-shifted peak reported for bla in the ^{129}Xe hyper-CEST NMR experiment. We hypothesized that the 60 ppm shift

was the result of Xe binding to the same hydrophobic pocket initially discovered by Horn et al.^[26] and later characterized *in silico* by Bowman et al.^[26, 28] MD simulations of Xe binding to bla affirmed our original hypothesis and motivated crystallization experiments to further study the interactions between Xe and bla. Crystal structures of WT bla derivatized with Xe revealed a high-occupancy Xe atom (Xe1) bound in the hydrophobic allosteric site as well as low-occupancy Xe bound at two additional sites predicted *in silico* using MD simulations or Markov State Models.^[30] Mutagenesis studies confirmed Xe1 binding as the origin of CEST contrast and offered additional insights as to the relationship between local protein structure and observed ¹²⁹Xe hyper-CEST signal. Two bla mutants, I263A and I279N, showed no hyper-CEST signal whereas three other mutants – I282A, I263N, and I263L – retained contrast at saturation offset 60 ppm but presented hyper-CEST profiles that differed from WT. Xe derivatives of crystallized bla mutants I263L, I263N, and I263A revealed that these mutations impact Xe occupancy at the primary site and also establish a new Xe binding site. Additionally, MD simulations assessing Xe interactions with these mutants revealed that even single point mutations can greatly impact Xe access to the protein which, in turn, affects the measured CEST contrast. The results presented here provide key insights into the mechanism of hyper-CEST with bla, which with further study should provide avenues for engineering the protein to enhance hyper-CEST contrast and modulate the chemical shift for multiplexing applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Chain A of the bla-Xe complex (PDB ID 5HW1), with Xe shown as red spheres (van der Waals radii enlarged for clarity). (Inset) Xe1 binding site, with surrounding protein side chains shown as sticks (Xe-derivative in gray, native bla in cyan). Gray dashes indicate protein-Xe contacts within 4.5 Å. Isomorphous difference Fourier map for Xe1 shown as blue mesh and contoured at 10 σ .

Figure 2. ^{129}Xe hyper-CEST z-spectra of 80 μM bla in pH 7.2 PBS at 300 K. Unliganded bla (red) compared to bla in the presence of 1 mM tazobactam (green). Z-spectrum of PBS only (gray) shown for reference. Pulse length, $\tau_{\text{pulse}} = 3.80$ ms; field strength, $B_{1,\text{max}} = 77$ μT .

Figure 3.

Xe occupancy map calculated from a 1 μ s trajectory (only the last 400 ns included in the map). Blue shading is the density at an arbitrary isovalue. Red spheres are Xe atoms from a representative frame of the MD trajectory. Yellow spheres are Xe atoms found in the Xe-derivative crystal structure of WT bla.

Figure 4.

(a) Xe1 shown as a red sphere, with nearby side chains shown as sticks (Xe-derivative of WT bla in gray, "open" bla bound to allosteric inhibitor in magenta). (b) Number of Xe atoms in the Xe1 binding site correlated to the minimum distance between I263 and I279 side chains (minimum among all possible pairwise atom-atom distances). (c) Number of Xe atoms in the major binding site anti-correlated to the number of atom-atom contacts between I263 and I279 side chains.

Figure 5.
Xe1 cavity of the Xe-derivative of WT bla, with mutated side chains highlighted in green.
Closest side chain contacts to Xe1 measured in Å.

Figure 6. ^{129}Xe hyper-CEST z-spectra of 80 μM bla in pH 7.2 PBS at 300 K. Bla mutants (various colors) compared to WT bla (red). Z-spectrum of PBS only (gray) shown for reference. Pulse length, $\tau_{\text{pulse}} = 3.80$ ms; field strength, $B_{1,\text{max}} = 77$ μT .

Figure 7.

Xe1 and Xe5 (red spheres) bound to bla mutants I263L (a), I263N (b), and I263A (c), with distances shown in units Å. Isomorphous difference maps corresponding to Xe shown as blue mesh and contoured at 5 σ . Simulated annealing (SA) omit maps corresponding to mutated I263L and I263N side chains shown as green mesh and contoured at 3 σ . The I263 side chain of WT bla (yellow sticks) and Xe bound to WT bla (yellow sphere) shown for reference.

Figure 8.
 Combined Xe1 entry and exit pathways from 1 μ s MD simulations for WT bla (top-left).
 The thickness of each trace correlates to the number of Xe atoms following that trajectory.
 Blue blobs indicate average Xe density. Plots show the correlation between Xe binding and
 inter-side chain distances of residues 263 and 279 from MD Xe flooding simulations of bla
 mutants following the same parameters used for WT bla (Figs. 4b, 4c).

Scheme 1.

During hyper-CEST, exchangeable solute-bound Xe atoms that resonate at a frequency different from solution-phase hyperpolarized Xe (green) are selectively spin-flipped using RF irradiation. Rapid exchange leads to the depolarization of bulk solution-phase Xe (gray), thereby generating MR contrast.

Table 1.

Comparison of Xe counts for each trajectory

	Xe counts		
	Pathway 1	Pathway 2	Pathway 3
WT	78	11	7
I263L	65	12	9
I263N	33	10	6
I263A	108	7	12