McConnell-Bloch Modeling of HyperCEST with Xenon Biosensors

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Introduction

The McConnell-Bloch equations describe the MR signal of a single species undergoing kinetic exchange between two or more magnetically distinct sites (1), such as in chemical exchange saturation transfer (CEST). Previously, they have been used to model endogenous CEST (2) and exogeneous PARACEST signal contrast (3) systems. Hyperpolarized xenon-based biosensors that exploit the exchange of ^{129}Xe between bulk solution and cryptophane-A molecular cages (hyperCEST, Figure 1) have demonstrated excellent sensitivity (4), and treatment with the McConnell-Bloch equations would aid in understanding the signal decay kinetics from these agents during hyperCEST detection experiments. Complete knowledge of the parameters that affect hyperCEST contrast would support a more systematic optimization of Xe-based biosensors and pulse sequences for their detection. *The purpose of the present work was to investigate the efficacy of McConnell-Bloch simulations to predict the effects of relevant physical parameters on hyperCEST contrast with xenon biosensors.*

Methods

The biosensor construct consisted of a cryptophane-A molecular cage conjugated to a short peptide chain (EEEE) for increased water solubility (Figure 1), and was dissolved in water containing 5% v/v isopropanol. Pressurized xenon gas (in a mixture of 2% natural-abundance Xe, 10% nitrogen, 88% helium) was hyperpolarized via spin exchange optical pumping with rubidium vapor using a MITI XenoSpin polarizer (Nycomed Amersham) and was solvated by bubbling through a small capillary into a 5mm NMR tube containing 600 µL of the biosensor solution. Xenon was bubbled for 25 s at a flow rate of 0.5 SLM to saturate the solution with hyperpolarized ^{129}Xe, followed by a 2 s wait period to allow the solution to settle and bubbles to clear. All data were acquired on a vertical bore 300 MHz Varian INOVA spectrometer equipped with a 5mm dual tuned (^{1}H, ^{129}Xe), temperature controlled RF probe. Data was collected using a CEST pulse sequence that applied a continuous wave saturation pulse prior to signal excitation (BW = 25 kHz, acquisition time = 0.5 s). The saturation frequency was set on-resonance with the Xe@cryptophane frequency, and control data was generated by applying the saturation off-resonance at an equal distance from the Xe@water frequency but on the opposite side. Fitting was conducted in the time domain using a nonlinear least-squares regression in MATLAB (v.7, The MathWorks, Inc.), and the signal amplitudes were considered for subsequent analyses. Data was collected for each unique combination of the parameters shown in Table 1. For each parameter set, an array of saturation lengths was chosen to produce good dynamic range for the CEST signal decay, and these curves were fit with values predicted by the McConnell-Bloch equations (modified to reflect the use of hyperpolarized signal) using a least-squares nonlinear regression for the purpose of determining the xenon chemical exchange rates (k_{fwd}, k_{rev}, see Figure 1). The equilibrium binding constant, K_a, was determined by comparing the signal intensities of Xe@water and Xe@cryptophane. All other constants were determined from experiment or literature.

Table 1. Parameters tested

Xenon Partial Pressure (kPa)	6.9, 10.3
Temperature (°C)	25, 37
Biosensor (µM)	40, 160
B_1 Intensity (Hz)	217, 431

Results

Simulated saturation curves based on this modified two-site exchange model showed good agreement with experimental data (Figure 2). The rate constant for Xe dissociation from cryptophane, k_{rev}, was shown to be largely dependent upon temperature and cryptophane concentration, though Xe partial pressure also has an effect. The residence time of Xe inside cryptophane was determined from k_{rev} to be in the range of 10 – 40 ms at 25 °C, and 2 – 13 ms at 37 °C depending on the Xe pressure and cryptophane concentration. These values agree with previously reported exchange times (5).

Discussion

The McConnell-Bloch equations have shown to be capable of accurately modeling the hyperCEST contrast of xenon biosensor systems. Further refinement of the data modeling methodology is underway to incorporate fitting constraints between data sets to better represent constants in the physical environment of the investigated system. This work provides the necessary framework to optimize the hyperCEST contrast for a variety of systems. For example, knowledge of the xenon-cryptophane dissociation rates will allow for optimal saturation schemes to maximize CEST efficiency. In particular, *in vivo* applications of the xenon biosensors will benefit from simulations to maximize the hyperCEST contrast while adhering to practical SAR limitations.

References

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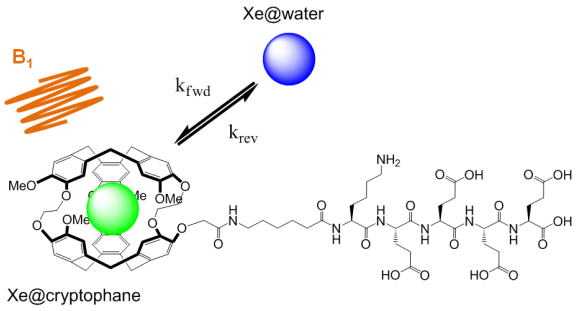

Figure 1 A continuous wave RF pulse selectively saturates Xe nuclei inside cryptophane, which subsequently exchange out into bulk solution decreasing the Xe@water signal intensity.

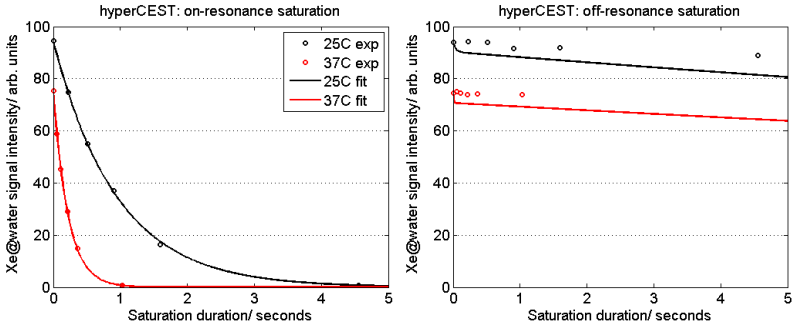

Figure 2 (left) Xe@water signal intensity decays as a function of RF duration for saturation applied on-resonance with Xe@cryptophane. (right) Saturating at an equal distance on opposite side of Xe@water produces neglible effect. Parameters used: T= 25, 37 °C, [cryptophane] = 160 µM, Xe partial pressure = 10.3 kPa, B1 = 217 Hz.