Pressure Dependent Signal Enhancement in Hyper-CEST

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Introduction

The so called hyper-CEST method promises tremendous potential on molecule-specific MR imaging using hyperpolarized ^{129}Xe caged in functionalized cryptophane cages [1]. It was shown that these biosensors can be utilized as highly sensitive temperature probes [2] and that molecule-specific signals in the lower nM regime can be detected when experimental parameters are optimized to yield maximum saturation of the caged ^{129}Xe. Here an alternative approach for enhancing the saturation efficiency and thus improving the overall sensitivity of hyper-CEST, by variation of the total xenon concentration in the solution is presented.

Methods

For the measurements an NMR tube of 13 mm diameter and fused with a glass valve on top was filled with the biosensor (cage-HA [3]) dissolved in 2 ml PBS buffer. After degassing the sample the MR tube was pressurized with hyperpolarized ^{129}Xe gas (enriched to ~ 90 % ^{129}Xe, P_{Xe}~15 %). For hyper-CEST experiments (performed on a BRUKER 30/100 whole body imager) we repeatedly (n=1 to 10) measured two FID signals (S_{2n-1} and S_{2n}, Fig. 1a) of dissolved ^{129}Xe ($\delta_{bulk} \approx 196$ ppm) within a 1 cm horizontal slice. Between these two excitations the caged ^{129}Xe was saturated with a train of $N_{sat} = 1000$ successive selective pulses with a repetition time of $\Delta t_{sat} = 15$ ms. Since the Xe nuclei transfer between cage and solution, the saturation of cage Xe causes a loss in the bulk signal. The signal reduction $S_{red}=S_{2n}/S_{2n-1}$ is governed by three major effects: T1-relaxation, signal loss from the first RF excitation of the bulk resonance, and damping by saturation transfer. To compensate for the first two effects, saturation is performed once on the cage resonance $\delta_{cage} \approx 64$ ppm and once at the mirror frequency referred to the bulk signal (i.e. at 330 ppm). The normalized hyper-CEST signal $S_{CEST}=S_{red}$(on res.)/S_{red}(off res.) thus reflects the reduction solely due to saturation transfer. It is proportional to the difference of bulk xenon concentration C_{bulk} and N_{sat} times the cage concentration C_{cage}, $S_{CEST} = \left(C_{bulk} - kN_{sat}C_{cage}\right)/C_{bulk}$, where $k=k_{sat}\times k_{129Xe}\times k_{oc}$ accounts for imperfect saturation k_{sat}, ^{129}Xe enrichment k_{129Xe} and fractional xenon occupancy k_{oc} of the cages. For $k_{oc} \approx K_a \times C_{buk} / (1+K_a \times C_{bulk})$ with the xenon cage association constant $K_a \approx 6000$ [2] one obtains $S_{CEST} = 1 - k_{sat}\times k_{129Xe}\times K_a \times N_{sat} \times C_{cage} / (1+K_a \times C_{bulk})$ (Eq.1). By varying the xenon gas pressure p_{Xe} in the NMR tube, the bulk concentration may be set freely, e.g. $C_{bulk} = p_{Xe} \times 3.4$ mM/bar at T=35 °C [5].

Results and Discussion

Hyper-CEST was performed with biosensor concentrations of $C_{cage} = 5$ µM and $C_{cage} = 0.5$ µM. In Fig. 1a) two consecutive FIDs are shown with caged xenon saturation in between. In Fig. 1b) the results of the signal reduction are shown for the 5 µM sample. The average signal reduction for off-resonance saturation is 0.85±0.01 which is slightly below the value of 0.87±0.01 as determined by T1-decay and flip-angle measurements [4]. Such deviations are characteristic to each sample and call for the normalization procedure described above (see also Fig. 1b).

A similar experiment series was performed on the 0.5 µM sample but at even lower xenon gas pressures (Fig. 1c). Here, due to the lower biosensor concentration efficient saturation can only be achieved when the xenon bulk concentration in the solution is reduced appropriately. By fitting Eq. 1 to the data for both samples, a saturation efficiency of $k_{sat} \approx 0.38$ is obtained. For the experimental parameters used one expects $k_{sat} \approx 1 - 2 \times \exp(-15 \text{ ms} / 9 \text{ ms}) \approx 0.6$ ('saturation' was achieved by inversion of the caged xenon magnetization every 15 ms at 9 ms exchange time between bulk and caged xenon).

Conclusion

We have introduced a model for the hyper-CEST method. For a given xenon bulk concentration the efficiency of the experimentally applied saturation, and thus the lowest cage concentration to be detectable, can be determined. Assuming perfect saturation and a higher xenon cage association constant of $K_a \approx 30000$, as is due to a modified cage construct [6], one would obtain with 50 nM biosensor concentration at bulk xenon concentration $C_{bulk} = 0.14$ mM ($p_{Xe} = 40$ mbar) a hyper-CEST signal S_{CEST} as large as shown here for the 0.5 µM sample. Applying our model (Eq. 1) to the hyper-CEST measurements presented in [2] ($C_{cage} = 10$ nM, $S_{CEST} = 0.85$ for 20 s CW satruration, $K_a = 6000$ M^{-1}, $k_{129Xe} = 0.26$, $C_{bulk} = 190$ µM) one obtains $N_{sat} \approx 2\times10^4$ assuming perfect saturation. Thus the exchange time between bulk and caged xenon would have to be less than 1 ms. The very smallness of that estimate, as well as the deviation of the model-derived and expected saturation efficiency k_{sat} in our case, call for further investigations to improve the model which finally should allow for an optimization of the sensitivity of hyper-CEST in biosensor applications.

References

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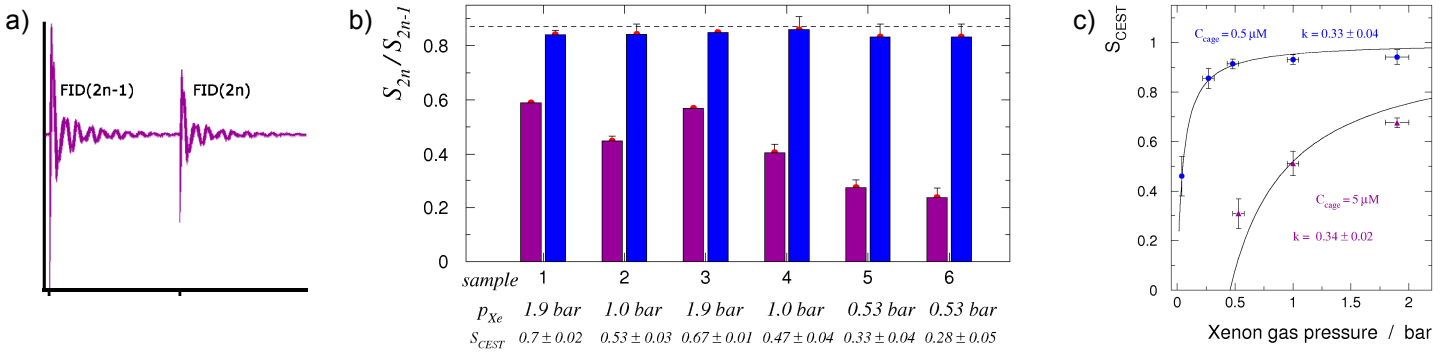

Figure 1: Hyper-CEST results: a) Two FIDs from bulk ^{129}Xe where saturation was applied to caged xenon in between. In b) the ratio of ^{129}Xe bulk-signal $S_{red}=S_{2n}/S_{2n-1}$ is shown for 5 µM biosensor concentration at three different Xenon gas pressures inside the NMR-tube. Applying the saturation pulses off resonance (blue) the signal reduction is due to RF excitation and T1 relaxation as indicated by the dashed horizontal line determined separately. Applying saturation to the caged xenon on-resonance, the ratio of the corresponding ^{129}Xe bulk-signals (purple) is significantly decreased (error bars arise from 10 measurements). c) The normalized hyper-CEST signal $S_{CEST}=S_{red}$(on-res.)/S_{red}(off-res.) (signal loss solely due to hyper-CEST) decreases with lower Xenon gas pressure, as shown by the data for two different biosensor concentrations. By fitting Eq. (1) the efficiency parameter is determined to be $k_{sat} = k / k_{129Xe} \approx 0.38$.