Optimization of Xenon Biosensors for Increased Sensitivity

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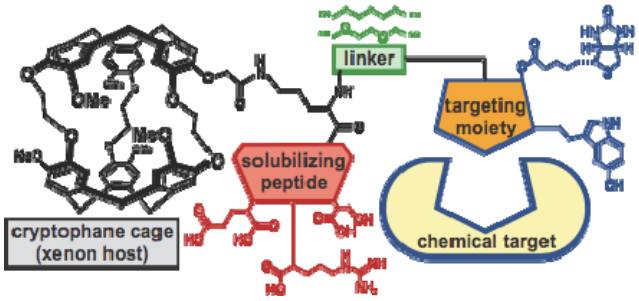

Figure 1. Schematic representation of the modular xenon biosensor.

Purpose: Recently developed biosensors allow investigators to tether the chemical shift-sensitive, zero-background signal from ^{129}Xe to a specific chemical moiety, generating potential to expand the diagnostic range of MRI in both *in vitro* and *in vivo* experiments. In this study, we focus on the design of new biosensors and detection schemes that significantly increase the sensitivity of MRI for molecular imaging. By increasing the sensitivity in a chemically sensitive manner, we seek to help MRI become more competitive with techniques like PET and optical methods.

Methods: Previous work with xenon biosensors in solution [1] was troubled by an inability to correctly quantitate the concentration of biosensor *in vitro*, in part because of undesirable interactions between the biosensor molecules and the glass walls of the phantom. To overcome this depletion of signal due to phantom-sensor attraction, we altered the solubilizing peptide (Fig. 1) to generate a negatively charged biosensor, whose concentration did not vary during the course of an *in vitro* experiment. In this way, all biosensor molecules were available for detection using the HYPER-CEST detection method [2]. In order to detect low concentrations in solution, the temperature of the NMR sample was increased to 37°C with the VT control unit of a 7.05 T Varian Unity Inova spectrometer equipped with a 10 mm double-resonant ^{1}H/^{129}Xe probe. The higher temperature accelerates the exchange kinetics of the xenon atoms associated with the biosensor, increasing the amount of chemical exchange saturation transfer (CEST) onto the bulk peak of xenon in solution, observed at ~193 ppm. Delivery of the hyperpolarized gas (^{129}Xe at natural abundance of 26% in a mixture of 90% He, 9% N_2, 1% Xe) into the NMR phantom was achieved in a stopped-flow mode, as described in [3]. ^{129}Xe NMR spectra were acquired with increasing length (0–20s) of a cw rf pulse selectively saturating the invisible biosensor signal "on-resonant" at 68 ppm, 125 ppm lower in frequency than the solution peak, and observing the intense resonance at 193 ppm. A control dataset was acquired with selective saturation "off-resonant" at 318 ppm (193 ppm + 125 ppm).

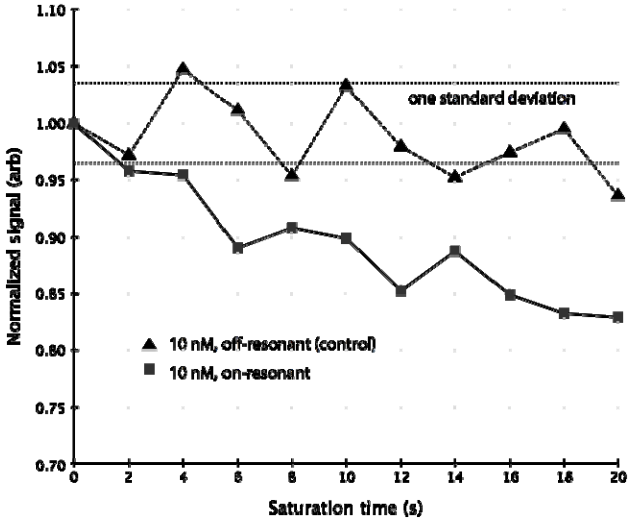

Figure 2. Normalized HYPER-CEST signal of a 10 nM xenon biosensor construct.

Results: Due to increased biosensor concentration in solution and the increased xenon exchange kinetics at higher temperature, concentrations as low as 10 nM of the sensor could be detected with this setup (this corresponds to a concentration of functionalized ^{129}Xe of only 1.4 nM). The CEST signal decrease after 20 s of on-resonant saturation was 16.5% whereas the noise of the control experiment was only 3.5% (Fig. 2). To achieve the same signal-to-noise ratio with direct detection, a measurement time of ~55 years would be required. The experiments as performed took 106 seconds.

Conclusion: By manipulating the chemical properties of the sensor and the exchange properties of the xenon, we have shown a 4000-fold increase in *in vitro* xenon biosensor sensitivity detection factor, demonstrating the great potential the HYPER-CEST technique offers for optimized biosensor constructs. Future experiments with new molecular probes could close the sensitivity gap between PET and NMR molecular imaging.

REFERENCES:

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