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Xenon Biosensor Amplification via Dendrimer−Cage Supramolecular Constructs

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Hyperpolarized xenon magnetic resonance imaging (^{129}Xe MRI) is becoming a useful method for imaging lungs, tissue, blood flow, and tumors in vivo.[1] However, in these applications, polarized ^{129}Xe partitions in a nonspecific manner between blood, lipid, and gas environments, and thus it is desirable to find ways to target ^{129}Xe to specific biological targets in order to maximize image contrast. We have previously introduced a "xenon biosensor" consisting of a water-soluble construct of a cryptophane-A cage and a targeting peptide. This targeted cage construct binds xenon ($K_a > 10^3$), providing a resonance signal for cage-bound xenon that is easily distinguished from the signals from gaseous and solution-dissolved xenon.[2]

High sensitivity is an important requirement for in vivo imaging, and the current xenon biosensor methodology allows for spectroscopy at nanomolar concentrations and in vitro imaging at micromolar concentrations.[3] Unfortunately, the xenon biosensor suffers from limitations inherent to its original design, including the separate ^{129}Xe signals afforded by each of the two diastereomers of the biosensor, which decreases the overall signal-to-noise ratio by a factor of 2, and the significant line-broadening and subsequent signal intensity loss that results from attachment of the Xe cage to a large biomolecule.[4]

Approaches that partly overcome these limitations involve extensive synthetic work and the tedious separation of the cage enantiomers.[4] Here we introduce a simple method for coupling a racemic mixture of cryptophane-A cages (Figure 2b) to a carrier molecule that circumvents the sensitivity limitations and affords signal amplification through the attachment of multiple cages to a single dendrimer-based targeting moiety.

The regular, highly branched architecture of dendrimers[5] provides multiple opportunities for the attachment of Xe-binding cages to peripheral functionalities while also incorporating a targeting moiety. Such an approach has been used with gadolinium-based macromolecular MRI contrast agents.[6] Since cryptophane-A contains one carboxylic acid, attachment to a dendrimer could be easily accomplished; however, attaching multiple cages to one dendrimer would yield multiple diastereomers, thereby diminishing the overall sensitivity of the cage−dendrimer construct.

We hypothesized that supramolecular constructs could be obtained through a combination of acid−base and hydrophobic interactions of the cage with the interior of water-soluble polypropylene imine dendrimers (PPI) or polyamidoamine dendrimers (PAMAM). Such a supramolecular approach would avoid the creation of diastereomers due to the noncovalent and dynamic nature of the linkage between the carboxylic acid moiety of the cage and the multiple interior amine groups of the dendrimer. In addition, with larger dendrimers, it may be possible to insert multiple cages into a single carrier molecule. Indeed, as shown in Figure 1, we have now succeeded in binding multiple copies of cage "functionalized" ^{129}Xe inside these dendrimers, leading to the desired signal amplification, and we have also demonstrated targeting through incorporation of a single targeting moiety onto the carrier dendrimer.

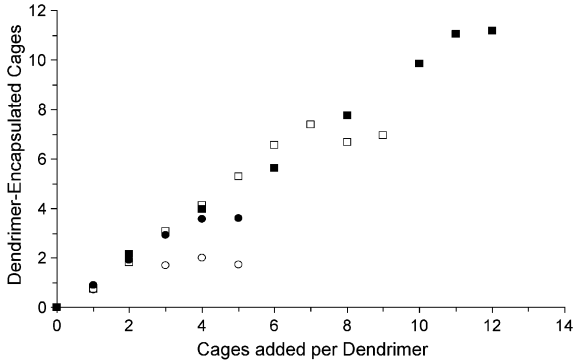

Figure 1. Number of dendrimer-encapsulated cages for G4 PPI (○), G5 PPI (●), G4 PAMAM (□), and G5 PAMAM (■) as a function of the number of cages per dendrimer. The number of dendrimer encapsulated cages reaches a maximum when the cage binding capacity of the dendrimer is saturated.

The synthesis of cryptophane-A has been reported elsewhere.[4a] PPI dendrimers with triethylene glycol peripheral end groups and PAMAM dendrimers with aminoethanol or tris(hydroxymethyl)-aminomethane surface groups were obtained as described in the Supporting Information. Samples were prepared using different cage-to-dendrimer ratios by adding a solution of the cage in acetonitrile in 75 nmol increments to a fixed amount (75 nmol) of PPI in acetonitrile or PAMAM in acetonitrile/water. Following removal of the solvent in vacuo, the cage−dendrimer complexes were resuspended in D_2O, and 1-propanol (∼10%) was added to suppress foaming during the addition of polarized ^{129}Xe. ^{129}Xe polarization and sample introduction were conducted as described previously.[3] The number of cages encapsulated per dendrimer (Figure 1) was determined by monitoring the UV absorbance of the solution at 289 nm and the integral of the cage-encapsulated signal relative to the solution-phase signal with ^{129}Xe NMR. Saturation of the cage-binding capacity of each dendrimer was determined by observation of solution turbidity and of constant UV absorbance and NMR integral values upon addition of excess cage. No UV absorbance or cage-encapsulated ^{129}Xe signal was observed in the absence of dendrimer, indicating that the dendrimer is necessary to solubilize the cage compound.

A comparison of the triethylene glycol-terminated PPI dendrimers with tris(hydroxymethyl)aminomethane-terminated PAMAM dendrimers showed that the former were clearly less effective in their ability to solubilize the cage compound. For example, the generation 4 and 5 PPI dendrimers were capable of encapsulating only 2 and

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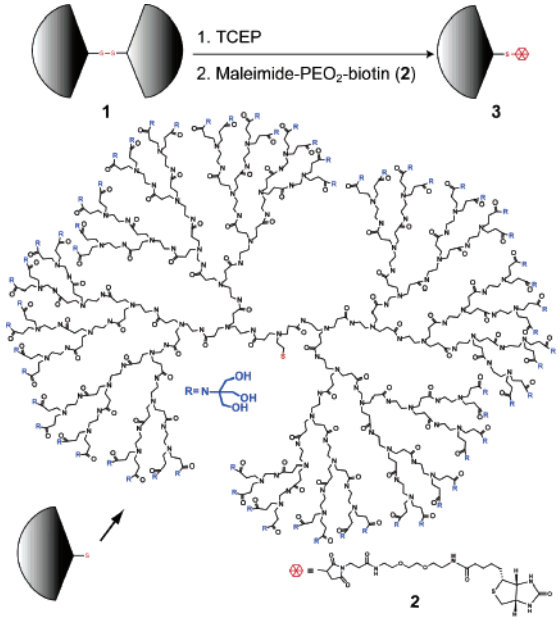
Scheme 1. Synthetic Strategy for a Singly Biotinylated PAMAM Dendrimer



4 cages, respectively, while their G4 and G5 PAMAM counterparts could encapsulate 7 and 11 cages, respectively (Figure 1). This finding correlates well with the structure of the dendrimers, as the PAMAM dendrimers are less compact and have a larger hydrodynamic volume than PPI dendrimers of the same generation. In addition, PAMAM dendrimers contain not only tertiary amine but also amide groups. The ^{129}Xe NMR spectra of the Xe cage−dendrimer complexes showed a single peak at 59 ppm (12 Hz line width) for PAMAM dendrimers, and at 63 ppm (80 Hz line width) for PPI dendrimers. There was no significant variation in chemical shift or line width for different cage-to-dendrimer ratios or different generations of the same type of dendrimer, suggesting that the cages are involved in dynamic equilibria, with fast exchange occurring between different sites within the same dendrimer.

To demonstrate amplification of the ^{129}Xe signal, we synthesized a dendrimer with one biotin moiety attached to its core. A G5 cystamine core PAMAM dendrimer (see Supporting Information) was reduced to the thiol and attached to maleimide−PEO_2−biotin to afford the targeted dendrimer **3** (Scheme 1). This dendrimer was capable of encapsulating up to three cages. Upon binding of dendrimer **3** to avidin, the number of bound cages decreased to two, perhaps as a result of unfavorable electrostatic interactions between the protein surface and the cage−dendrimer complex. There was no significant change in line width or chemical shift of the functionalized ^{129}Xe signal upon avidin binding.

A comparison of the NMR signal for ^{129}Xe held in the avidin-bound PAMAM−cage construct **3** and the signal afforded by our previously optimized biotinylated peptide−cage xenon biosensor[4b] indicates that there is an approximately 8-fold gain in sensitivity (Figure 2). This results from a doubling of the signal-to-noise ratio due to the presence of two bound cages per biotin ligand, another doubling resulting from the absence of diastereomer peaks, and a final doubling resulting from the decrease in line width from ∼25 to ∼12 Hz. A gain in sensitivity could also possibly be obtained through the direct binding of xenon to the PAMAM dendrimer (K_a < 500 M^{-1}), as this would effectively concentrate xenon directly around the dendrimer-bound cages. However, as shown in the Supporting Information, the local increase in xenon concentration directly around the cages was determined to be relatively small.

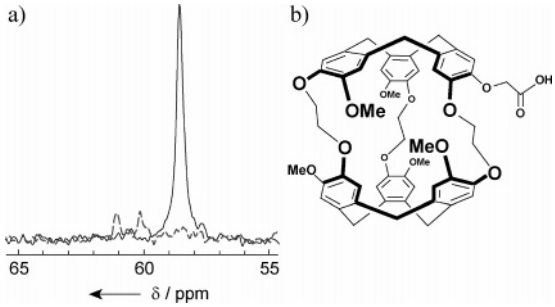


Figure 2. (a) Comparison of an optimal avidin-bound biotinylated xenon biosensor peptide construct (gray dashed line; 30 μM biosensor, 7.5 μM avidin)[4b] with the biotinylated PAMAM construct **3** of this study (black solid line; 30 μM dendrimer, 7.5 μM avidin, two cages per dendrimer). As shown here, the dendrimer−cage construct leads to a sensitivity gain of ∼8×. (b) Structure of the cryptophane-A acid (±).

In summary, we have provided a facile route toward novel supramolecular constructs that amplify the ^{129}Xe NMR signal. By carefully choosing specific dendrimers, we can encapsulate multiple cages, providing water-solubility to the "functionalized" ^{129}Xe and avoiding the interference of diastereomers without additional synthetic steps or covalent attachment of cages to dendrimers. Future work will utilize these supramolecular constructs for imaging.

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Supporting Information Available: Experimental procedures and characterization data provided for dendrimers. This material is available free of charge via the Internet at http://pubs.acs.org.

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