

# PROTEIN CAGES FILLED WITH GADOLINIUM CONTAINING BRANCHED POLYMERS

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## Introduction

Utilizing high resolution structural information and an appropriate genetic system a protein cage becomes a versatile molecular scaffold upon which multiple chemical and biological functionalities can be added in a spatially defined manner. Biomedical applications of protein cages include targeted delivery of agents for imaging and treatment of tumors and infections. The potential for large magnetic material payloads and the ideal rotational properties of protein cages make them extremely well suited for MRI contrast enhancement. In recent years several groups have reported several variations of this protein cage – Gd chelate theme. While all these publication report high per particle relaxivities, there is a need to develop strategies to fully utilize the Gd payload potential of these cages. Furthermore, it is becoming apparent that the local mobility in the attachment site of the Gd chelates greatly reduces the mobility gains that result from use of these large structures. By creating a polymer network, derived from Gd containing monomers, in the interior cavity of protein cages both the Gd payload and local mobility problems are addressed.

## Strategy

We designed a strategy for filling a cage, Heat Shock Protein (Hsp) from *Methanococcus jannaschii*, with a synthetic polymer. Polymer growth was initiated from cysteine residues located on the interior surface of a genetic Hsp construct (G41C) (Figure 1A), by reacting with a bromo alkyne molecule (generation 0). The exposed alkyne was subsequently reacted with a diazido amine molecule via a Cu(I) catalyzed ‘click’ reaction to yield generation 0.5. Exposed azide functional groups on G0.5 were then ‘clicked’ with a trialkyne molecule to generate a branched structure (generation 1.0). Iterative stepwise reactions with diazido amine and trialkyne were then undertaken to afford generation G1.5, G2.0, G2.5 (Figure 1B). Addition reactions produced no further polymerization. Next the introduced amines were reacted with an amine reactive form of DTPA-Gd (SCN-DTPA-Gd). The complete synthesis scheme is shown in Figure 2.

Figure 1.

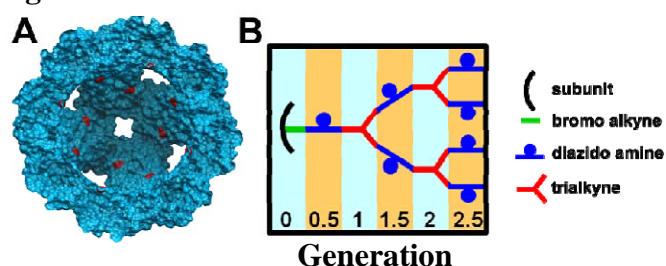
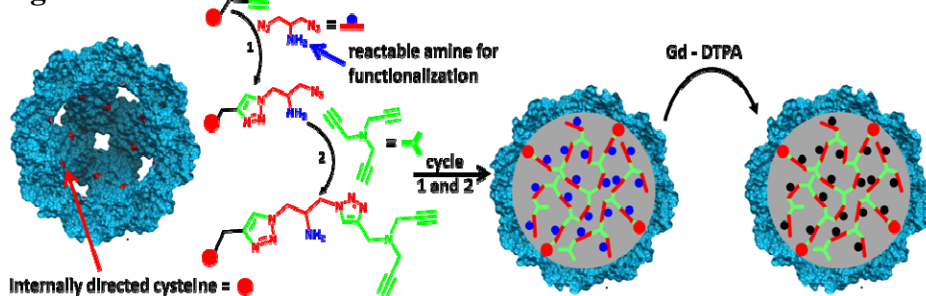


Figure 2.



## Results

The polymerization resulted in eight addressable amine groups per subunit, which corresponds to 192 Gd<sup>3+</sup> ions per cage. Relaxometry experiments produce a field dispersion curve typical of particles with slow tumbling properties with a peak at 31 MHz and an ionic relaxivity value 19.9 sec<sup>-1</sup> mM<sup>-1</sup> at this field strength. This corresponds to a particle relaxivity of 3,821 sec<sup>-1</sup> mM<sup>-1</sup>. The ionic and per particle relaxivity values are similar to other reported protein cage-Gd constructs which is impressive since Hsp is twelve times smaller, by volume, than other reported protein cage Gd<sup>3+</sup> constructs.

Figure 3.

