

PASADENA: A Novel Tool to Image Atherosclerotic Plaque

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Introduction: The PASADENA (Parahydrogen And Synthesis Allows Dramatically Enhanced Signal Alignment) method offers a promise of increasing the sensitivity of magnetic resonance (MR) over 10,000 times through hyperpolarization of target ¹³C nucleus during molecular hydrogenation [1-3]. Here, we present a study of a new class of agents, which target binding to atherosclerotic plaque by means of fluoro-carbon moiety and utilize a moiety for PASADENA signal enhancement. ¹³C magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) with signal enhancement over 10,000 fold offer multiple advantages including sub-second experimental time, which is especially attractive for cardiac applications. This class of agents potentially enables the sub-second non-invasive MRI study of cardiac plaque formation with increased spatial resolution and high chemical specificity.

Purpose: We identify a lipid targeted atherosclerotic plaque binding molecule(s), that will bind to vessels of small caliber like coronary artery, employing binding assays to dimyristoylphosphatidylcholine (DMPC) by ¹⁹F solid-state nuclear magnetic resonance (ssNMR) spectroscopy to satisfy the following requirements: PASADENA moiety with C=C double bond for parahydrogen molecular addition, solubility in aqueous buffers, and high affinity for atherosclerotic plaque.

Methods: We utilized acrylate moiety for PASADENA, which has been shown very successful in *in vitro* and *in vivo* application [1]. Hydrofluorocarbon moiety was inserted as corresponding acrylate ester. ¹⁹F NMR spectroscopy was employed for binding assays utilizing ssNMR. ¹⁹F spectroscopy provides high sensitivity similar to ¹H. In addition, fluorinated methyl group has a distinct ¹⁹F chemical shift. When fluorinated molecule binds lipid membrane mimicking atherosclerotic plaque, ¹⁹F resonance of its fluorinated methyl group shifts by up to few ppm and becomes broader due to non-zero chemical shift anisotropy typical for solids, Figure 1. Binding assays were performed in Bruker Avance data acquisition system at 4.7T.

Results: Out of many commercially available candidates two molecules were selected for lipid binding studies to DMPC membranes: 4,4,5,5,6,6,7,7,8,8,9,9,9-tridecafluoro-2-hydroxynonyl acrylate (TDHA) and 2,2,3,3-tetrafluoropropyl acrylate (TFPA) after preliminary screening for solubility in water. While both candidates demonstrated significant binding to hydrated DMPC membranes, TDHA has very low solubility of < 0.2 mM and thus unattractive. TFPA, on the other hand, has solubility of ~20 mM in phosphate buffer at physiological pH ~ 7. The kinetics experiment demonstrated that this class of fluorocarbon acrylates binds to the lipid membrane within the first minute of mixing. TFPA binds to DMPC bilayers in 5:1 molar distribution ratio corresponding to ~5% enrichment of DMPC membranes by TFPA by weight at 45°C, Figure 1. 33% of all TFPA molecules are partitioned in lipid membranes.

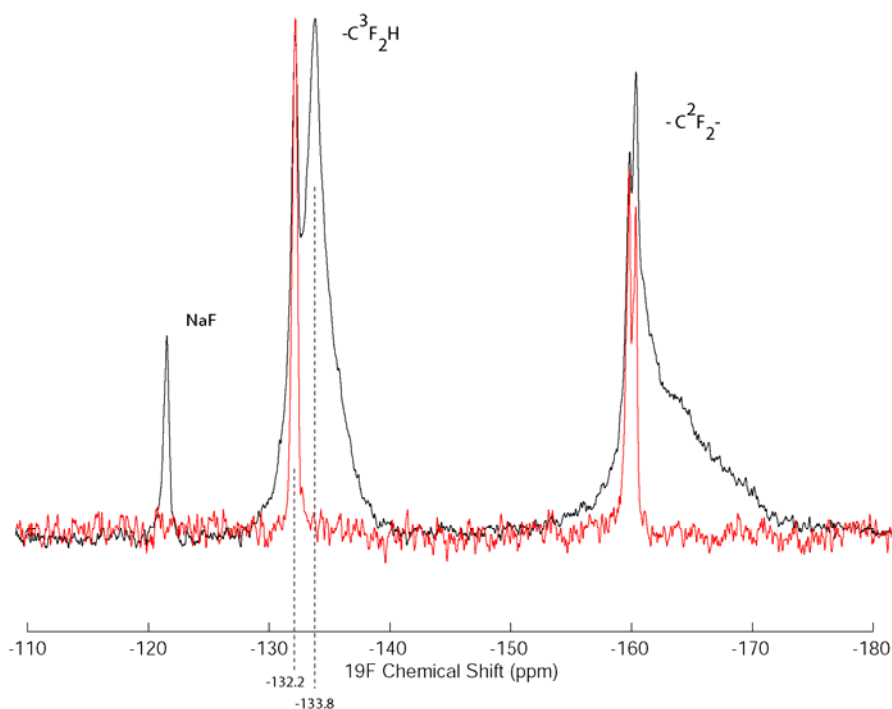


Fig. 1. Black trace corresponds to the binding experiment of TFPA to DMPC lipid membrane while red trace corresponds to the spectrum recorded from 20 mM TFPA solution in water.

Conclusion: Our results demonstrated efficient and rapid binding of TFPA to the lipid membranes mimicking atherosclerotic plaque accompanied by its higher solubility in aqueous buffer, which shows an excellent prospect of employing this class of molecules for *in vivo* hyperpolarized studies on atherosclerotic plaque formation. Experiments with the hyperpolarized ^{13}C spectroscopy and imaging are underway.

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