Characterization of Dynamics of Solvents Encapsulated in Nano-Scale Volumes, by Selective Enhancement of NMR Signal via Dynamic Nuclear Polarization

Audrey Hammack
Chemistry, The University of Texas at Tyler

NNIN REU Site: Nanotech, University of California, Santa Barbara, CA
NNIN REU Principal Investigator(s): Professor Song-I Han, Chemistry and Biochemistry, University of California, Santa Barbara
NNIN REU Mentor(s): John Franck, Department of Chemistry and Biochemistry, University of California, Santa Barbara
Contact: ahammack@patriots.uttyler.edu, songi@chem.ucsb.edu, johnfranck@gmail.com

Abstract:

Lipid vesicles have been spotlighted for their potential applications, but few methods characterize the behavior of encapsulated solvents, either in terms of exchange or in terms of local dynamics inside the vesicle [1]. Our work sought to characterize the dynamics of solvent encapsulated in nano-scale vesicles, which were dispersed in a dilute solution. We achieved dynamic nuclear polarization (DNP), which selectively enhanced the signal from the encapsulated solvent. As the level of enhancement depends on the timescale of solvent dynamics, this gives information on the timescale of intra-vesicular water dynamics.

Introduction:

Since their development, lipid vesicles have been regarded as novel nano-scale containers, and are used as models of cellular compartments [2]. Despite the developments in liposome applications, existing methods have difficulty in characterizing the dynamics of the encapsulated solvent or the rates of exchange across the lipid membrane, since they cannot distinguish the intra-vesicular solvent from the extra-vesicular solvent. Nuclear magnetic resonance (NMR) spectroscopy has high specificity that can elucidate chemical structure and produce images of soft tissues (i.e., magnetic resonance imaging); however, NMR relies on the premise that different chemicals have distinct signatures. In the case of vesicles, the encapsulated solvent and the bulk solvent are the same chemical, and thus, resonate at the same frequency; traditional spectroscopy cannot distinguish these two components.

DNP can selectively enhance an NMR signal, even against a large background signal. DNP arises from cross-relaxation, during which electron spins transfer their polarization to nuclear spins, via dipolar interactions, as a result of excitation by microwave radiation. This polarization transfer results in enhanced signal. Furthermore, it only occurs over short distances, 3-5Å; so when a vesicle encapsulates the spin label, only the NMR signal of protons within the vesicles will be enhanced.

Enhancement of the NMR signal (and polarization) is related to solvent dynamics by the following equation

$$E = 1 - pfs \frac{|\gamma_1|}{\gamma_s} \quad (1)$$

γ_I and γ_S, are the gyromagnetic ratios of the electron and proton, respectively; the leakage factor, *f*, can be calculated from T_1 and $T_{1,0}$, the signal decay time for spin-labeled and non spin-labeled solutions, respectively; and, when extrapolated to infinite power, *s* is 1. This equation thus isolates the coupling factor, ρ, which depends only on the timescales of solvent dynamics, as given by the translational correlation time, τ [3]. With proper modeling and data analysis, a value for τ can be extracted from ρ [4].

Methods:

The vesicles were prepared by depositing 75 mg of a 4:1 mixture of 1,2-dipalmatoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-*sn*-glycero-[phospho-*rac*-(1-glycerol)] (DPPG) in a test tube. To this, a solution of 250 μL of 140 mM CAT_1, dissolved in Hepes-buffered saline, was added. The mixture was warmed above 42°C, the transition temperature of the lipids, vortexed, and extruded through a polycarbonate filter with 400 nm pores. For some samples, 0.35M ascorbic acid was reacted with the unencapsulated radical, in a 5:1 molar ratio.

Results and Conclusions:

The EPR spectra in Figure 1 demonstrate encapsulation of the spin label CAT_1 inside the vesicles. For vesicles containing intra- and extra-vesicular CAT_1, the spectrum has a very broad lineshape, indicating a very high concentration. When the extra-vesicular CAT_1 was partially removed, the spectrum had a broad component, but also included narrow peaks, corresponding to the lowered extra-vesicular CAT_1

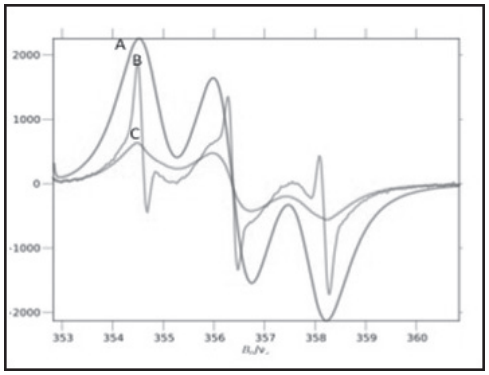

Figure 1: X-band EPR of samples of vesicles, with 140 mM intra- and extra-vesicular CAT_1 (A), partially removed extra-vesicular CAT_1 (B), and no extra-vesicular CAT_1 (C).

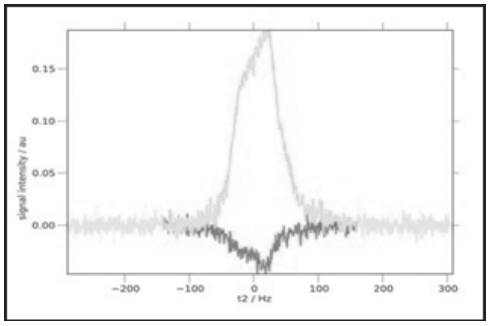

Figure 2: NMR spectra of 400 nm vesicles, containing 140 mM CAT_1, before and after, DNP enhancement.

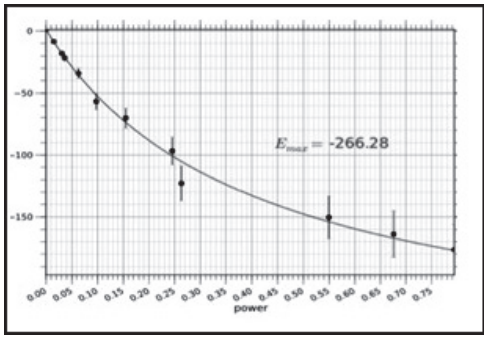

Figure 3: Measurement of DNP enhancement of signal from 140 mM CAT_1.

concentration. Finally, when reaction with ascorbic acid removed all extra-vesicular CAT_1, only the broad components remained, indicating that CAT_1 remained encapsulated.

The T_1 time of the NMR signal can determine the residence time of water in the two environments. Therefore, we measured the T_1 time for a 140 mM spin label solution, before and after treatment with ascorbic acid, as 23.7 ms and 2.3 s, respectively. For a sample of vesicles containing 140 mM CAT_1 and treated with ascorbic acid, T_1 was found to be 1.13 s, a weighted average between the two cases which indicates rapid exchange of water across the membrane.

Figure 2 shows an unenhanced spectrum, and a spectrum that had been substantially enhanced by DNP. Since it had been demonstrated that CAT_1 was encapsulated, it can be concluded that DNP exclusively enhanced the NMR signal of water inside the vesicle.

Finally, signal enhancement, from a solution of 140 mM CAT_1, was acquired as a function of microwave power, as shown in Figure 3. Extrapolating to infinite power yields, E_{max} = -266.28, and by means of Equation 1, $\rho = 0.40$, yielded a τ for this sample of 12.6 ps.

Future Work:

Future work includes measurement of ρ for a sample of vesicles that encapsulate CAT_1, thus selectively determining τ for water diffusion dynamics inside the vesicles. Additionally, it has been shown that it is possible to slow water exchange across the membrane and resolve the two simultaneous T_1 time constants for intra-vesicular CAT_1 solution and extra-vesicular pure water [5]. This would allow determination of rates of exchange and permeation across the membrane.

Acknowledgements:

I would like to thank my mentor John Franck, my principal investigator, Song-I Han, the Han lab, the National Nanotechnology Infrastructure Network Research Experience for Undergraduates (NNIN REU) Program and the National Science Foundation support and funding.

References:

[1] P. Walde, S. Ichikawa Biomolecular Engineering. 18,4 (2001) 143-77.

[2] K. L. Chan, P. R. C. Gascoyne, F. F. Becker and R. Pethig. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism,1349, 2 (1997) 182-196.

[3] K.H. Hausser and D. Stehlik, D. Adv. Magn. Reson., 3, (1968), 71-139.

[4] Armstrong, B and Han, S. J. Am. Chem. Soc. 131, 2009, 4641-4647.

[5] G. Bacic, M.R. Niesman, H.F. Bennett, R.L. Magin, H.M. Swartz, Mag. Reson. Med., 6 (1988), 445-458.