Contribution ID: **73** Type: **Poster**

A 13C NMR MAS method to sort between mobile and rigid molecules in biomembranes

Understanding the membrane dynamics of complex systems is essential to follow their function. As molecules in membranes can be in a rigid or mobile state depending on external (temperature, pressure) or internal (pH, domains, etc.) conditions, we have developed NMR methods to filter highly mobile molecular parts from others that are in more restricted environments. Cross Polarization (CP), Insensitive Nuclei Enhanced by Polarization Transfer (INEPT) with reference to DP (Direct Polarization) ssNMR techniques used in combination with natural abundance MAS 13C-NMR on rigid and fluid model membranes afforded demonstrating that INEPT will detect only very mobile lipid head groups in gel (solid-ordered) phases, the remaining rigid parts are only detected with CP. Interestingly, the 13C-NMR chemical shift of lipid hydrocarbon chains can be used to track order-disorder phase transitions, calculate the fraction of defects and the part of the transition enthalpy due to bond rotamers. Cholesterol-containing membranes (liquid-ordered phases) can be dynamically contrasted as the rigid-body sterol is mainly detected by CP techniques and the phospholipid by INEPT (Fig. 1). A direct correlation is established between the normalized line intensity as obtained by CP and the C-H (C-D) order parameters that are measured from ssNMR or molecular dynamics: when the order is greater than 0.3 (maximum value S=0.5 for chain CH2) only rigid parts can be filtered and detected using CP-MAS. This opens up a new, very simple and robust route for the determination of membrane dynamics from high resolution NMR.

![Fig. 1. Application of magic angle sample spinning and polarization transfer NMR techniques to biomembranes (top) allows filtering of the 13C-NMR spectrum (middle) of the rigid cholesterol body from the more mobile phospholipid resonances (bottom), within the same membrane.

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Session Classification: Clip Session