High-resolution studies of protein-lipid interactions using fluorinated lipids and biomolecular 19F NMR

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High resolution NMR studies on the structure, dynamics and molecular interactions of lipids are notoriously hampered by poor spectral ^{1}H dispersion. *Uniform isotopic labeling with ^{13}C* would allow to enhance resolution by $^{1}H,^{13}C$ correlation spectroscopy, but their ^{13}C spectra still show incomplete dispersion and signal splitting from $^{1}J_{CC}$ coupling. Also, ^{13}C would be no unique marker for lipids in interaction studies with ^{13}C labeled proteins. Thus we propose *sparse chemical labeling with fluorine* (100% ^{19}F) in the lipid chain to greatly enhance spectral resolution indirectly, via fluorine induced deshielding of nearby ^{1}H, and directly, via editing in a ^{19}F dimension with surpassing spectral dispersion, simplicity, and intensity. Both deshielding and $J_{H,F}$ coupling reach up to 4 bonds, suggesting an optimal *sparse* fluorination scheme to minimise the biophysical impact of this chemical modification, suppress signal splitting from $^{1}J_{FF}$ coupling, and preserve a high ^{1}H density for intermolecular ^{1}H(lipid),^{1}H(protein) NOE contacts. As a first example for this novel class of membrane mimics we obtained di-(4-fluoro)heptanoylphosphocholine, 4F-DHPC7, that forms stable micelles of similar size as DHPC7. Both 4F-DHPC7 and DHPC7 micelles solubilize and stabilise two representative membrane proteins: (i) photosensory rhodopsin II with 7 transmembrane helices, and (ii) outer membrane protein X with a β-barrel fold. Differences in the $^{1}H,^{15}N$ NMR fingerprint spectra recorded in 4F-DHPC7 and DHPC7 are small, confirming similar protein structures, and correlate with residues near the fluorine position in modelled micelles, suggesting a new method to gauge protein immersion depth. A first ^{19}F filtered NOESY experiment indeed revealed unambiguous NOE contacts between 4-F-DHPC7 and a bihelical integrin fragment. Tests on more fluorinated lipids and membrane proteins with optimised biomolecular ^{19}F NMR experiments are now required to further develop the promising approach.