Characterization of Protein-Detergent Mixed Micelles Using NMR Spectroscopy

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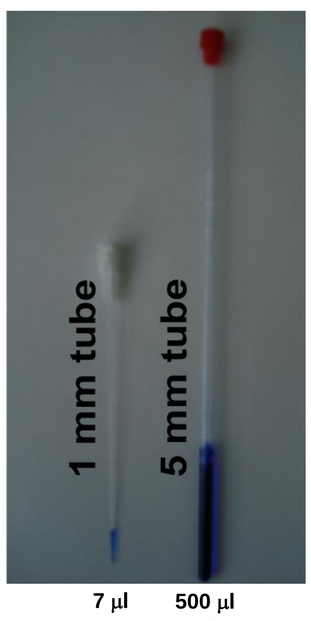
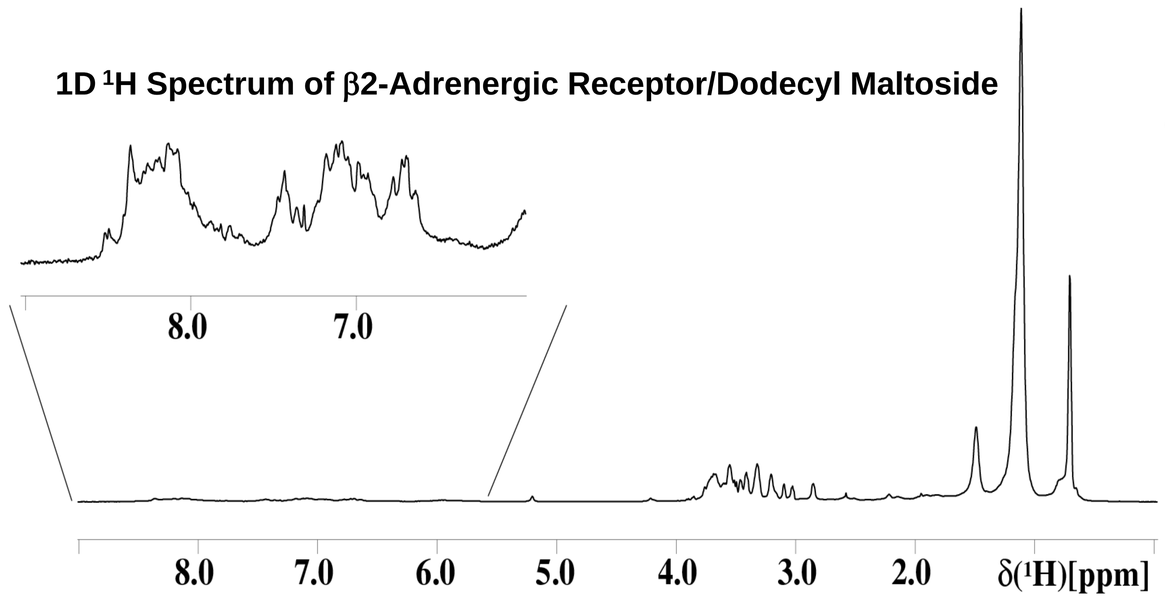
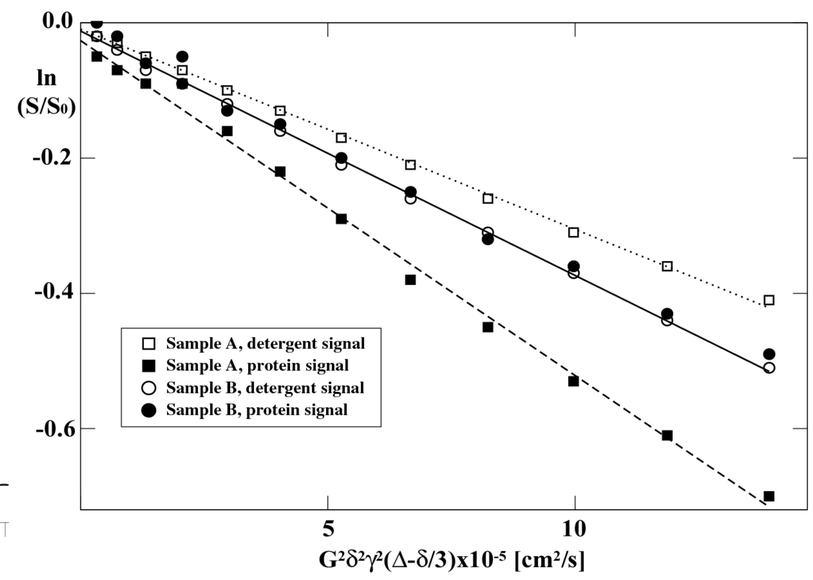
Initial screening of integral membrane protein preparations using 1D ^{1}H NMR spectroscopy, which has been quite extensively used with soluble proteins, is limited by the fact that non-deuterated detergents introduce a set of background signals which partially overlap with the signals from the membrane protein. Furthermore, the detergent adds typically 25–50 kDa to the molecular weight of the mixed micelles. We have therefore established a new protocol to characterize proteins in mixed protein–detergent micelles, using TROSY-based NMR experiments in combination with uniform ^{2}H,^{15}N-labeling of the membrane proteins, and MicroProbe technology which enables to measure multidimensional NMR experiments with μg-amounts of protein.

Characterization of IMP–detergent mixed micelles using Microprobe 1D ^{1}H NMR spectroscopy:



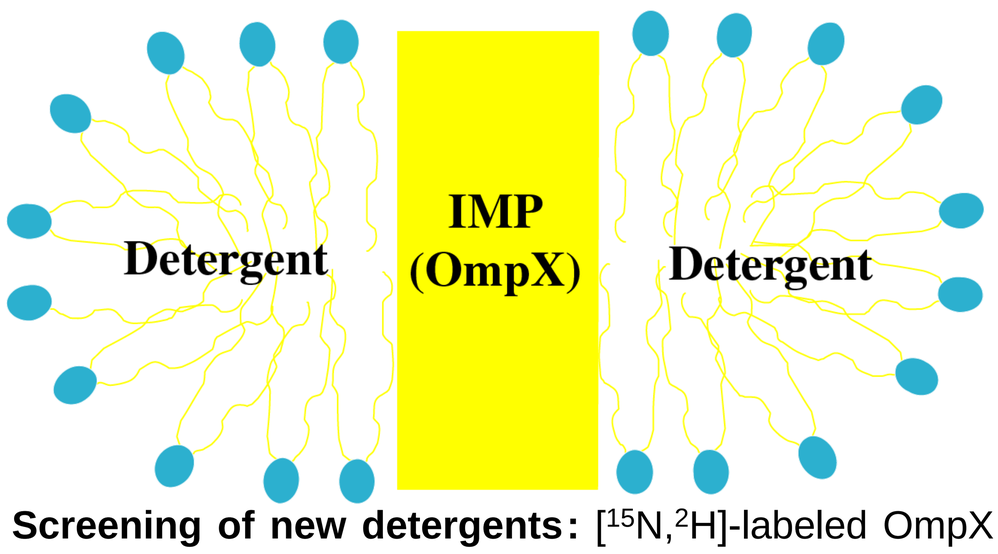


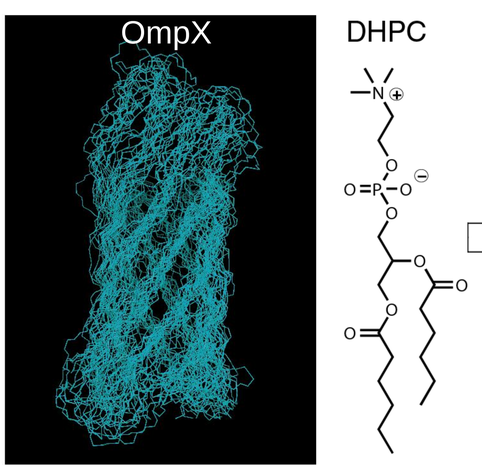


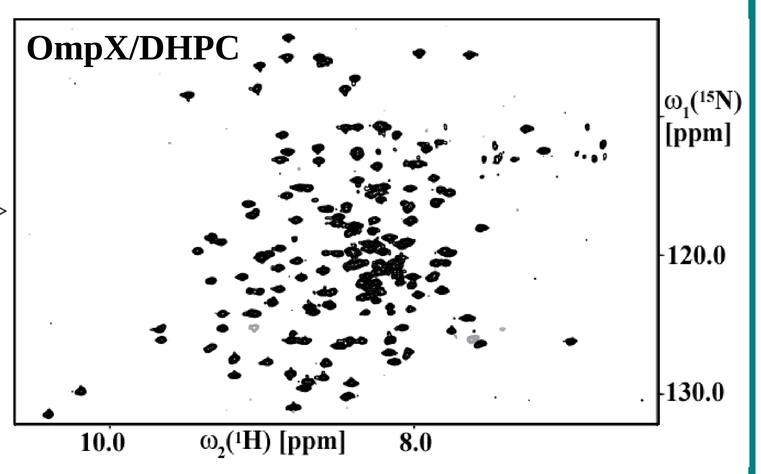
Screening for globular folds: The high mass sensitivity and the excellent solvent suppression properties of the Bruker 1mm Microprobe allowed the assessment of β2-AR reconstituted into DDM using only 7 μl of solution. The measurement time was 20’ (In collaboration with R. Stevens and B. Kobilka).

Characterization of IMP-detergent complexes: Stejskal-Tanner plots from two samples of β2-AR/DDM: The different slopes in A indicate a inhomogeneous micelle-size distribution.

Micro-scale screening of new detergents using 2D [^{15}N,^{1}H]-TROSY NMR spectroscopy:

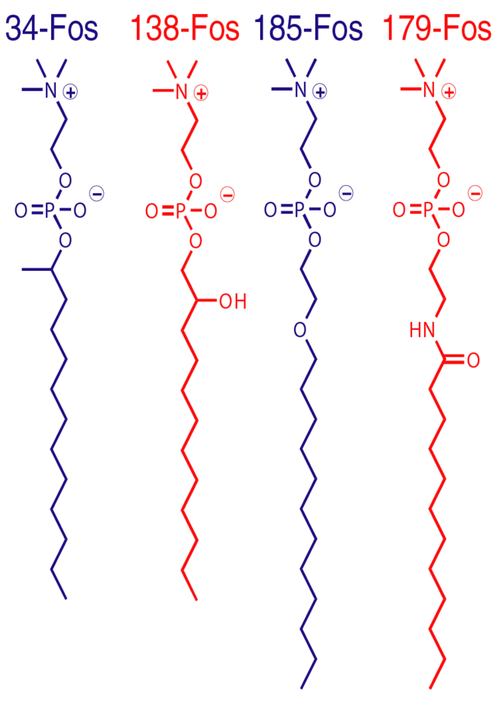


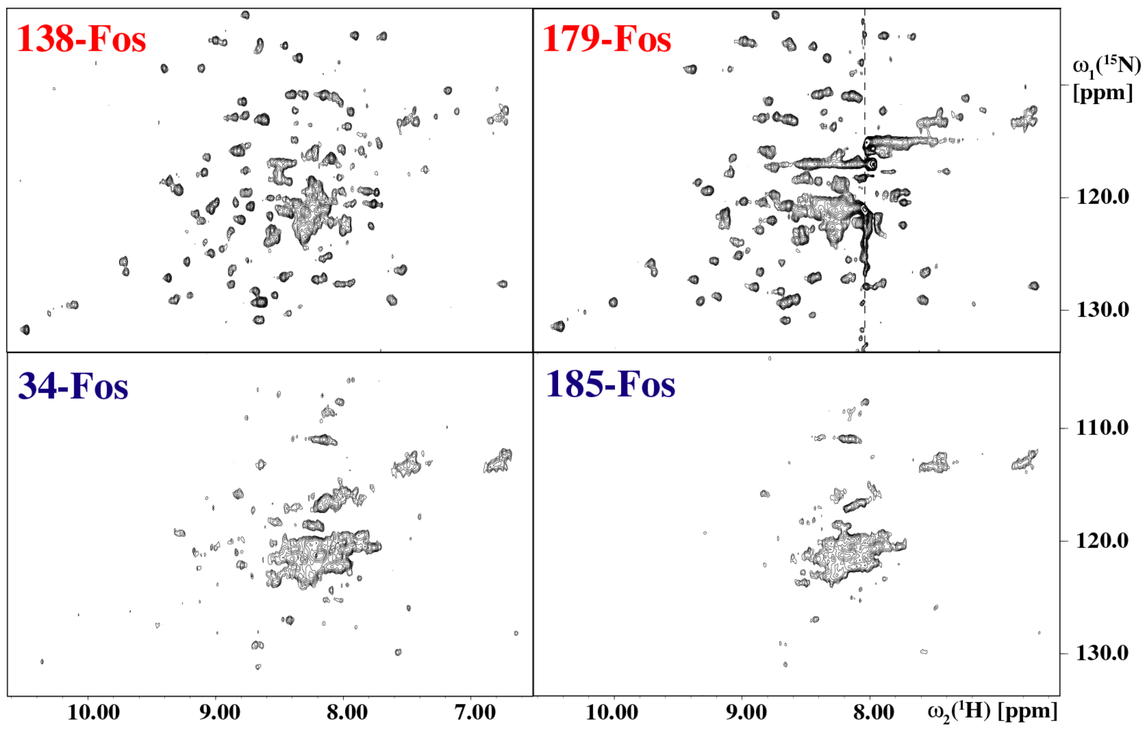




Screening of new detergents: [^{15}N,^{2}H]-labeled OmpX from *E.coli* was reconstituted into unlabeled detergent micelles. The fold of OmpX was then assessed by 2D [^{15}N,^{1}H]-TROSY spectroscopy.

2D [^{15}N,^{1}H]-correlation map as a diagnostic fingerprint: 2D [^{15}N,^{1}H]-TROSY spectrum of OmpX/DHPC measured on a MicroProbe. The sample volume was 7 μl and the total measurement time was 8 h.





Examples of new detergents examined by NMR: Those detergents showing high-quality TROSY spectra are highlighted in red, and those with poor quality spectra are shown in blue. The sample volume was 7 μl and the total measurement time was 8 h.

Conclusions and Outlook:

- A miniaturized pipeline for the assessment of new detergents for structural studies of membrane proteins was established.
- The reconstitution of OmpX in mixed micelles with 138-Fos and 179-Fos seems to be more efficient than the reconstitution with DHPC. These detergents will be retained for further studies, including investigations of protein–lipid interactions with different integral membrane proteins.