# EFFECT OF DETERGENT ON HSQC QUALITY FOR *M.TUBERCULOSIS* MEMBRANE PROTEIN Rv3004

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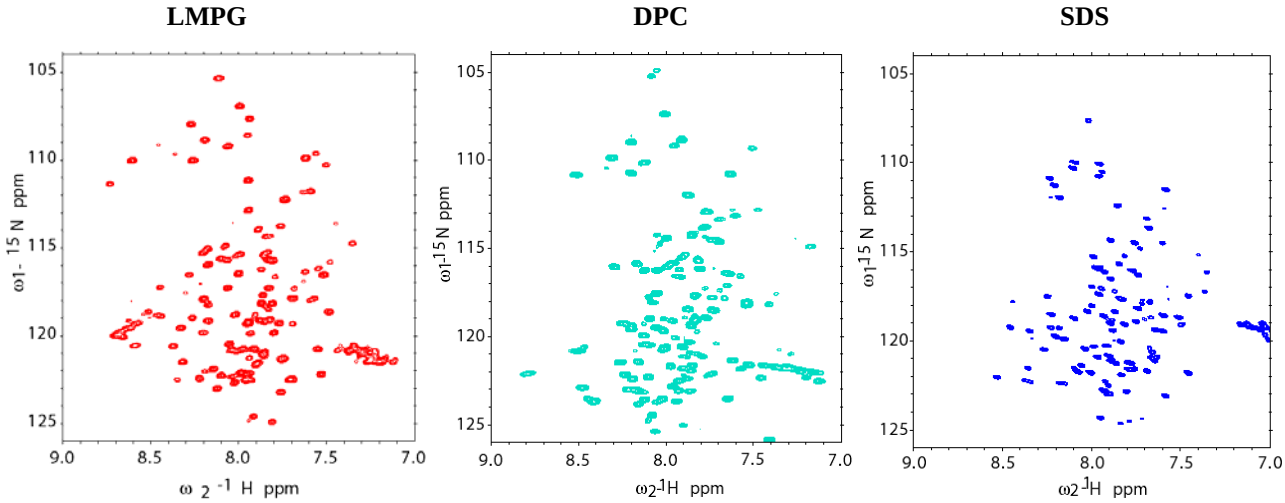
Our protein of interest is Rv3004, from the membrane of *M.tuberculosis*. Rv3004 is a 122 amino acid fusion protein with one transmembrane helix. It has no proven function, but based on the sequence, it may be a signal peptide. We have successfully expressed Rv3004 in *E.coli* at a yield of ~12mg/liter of culture. The next step is to find the appropriate environment to characterize the structure using NMR. We sampled four different detergents. Sodium-n-dodecylsulfate (SDS) is a 12 carbon chain, anionic detergent that can be denaturing depending on the conditions and the protein. Dodecylphosphocholine (DPC) is a 12 carbon chain zwitterionic detergent. The last two are anionic lipid derivatives of 14 and 16 carbon chain lengths, respectively, 1-myristoyl-2-hydroxy-sn-glycero-3-[phospho-RAC-(1-glycerol)] (LMPG) and 1-palmitoyl-2-hydroxy-sn-glycero-3-[phospho-RAC-(1-glycerol)] (LPPG).

Uniformly $^{15}$N labeled protein was expressed in *E.coli* according to methods introduced by Marley et al.[1] The protein was purified by His-tag affinity chromatography then allowed to precipitate. After precipitation, it was subjected to successive 50% acetone washes to remove any residual detergent introduced during purification. The protein was then lyophilized.

Samples were prepared to a final concentration of 1mM Rv3004 in 25mM sodium acetate, 5% detergent, and 10% $D_2O$, at pH4, with all experiments run at 50°C. $^{15}$N-$^{1}$H Heteronuclear Single Quantum Coherence (HSQC) spectra were acquired on the Varian 600MHz spectrometer at the NHMFL.




In SDS there are 106 peaks, DPC 109 peaks, LMPG 119 peaks, and in LPPG 133 peaks, with an average signal to noise ratio of 92, 125, 144, and 133 respectively. The best of these spectra, LMPG, is labeled Fig. 1. The resonances are sharp and of relatively uniform intensity.

Given the data acquired during these experiments, detergents are a significant factor in the quality of a spectrum, indicating that detergent screens are a useful primary step. LMPG yields more resonances than both SDS and DPC. LMPG, despite showing fewer peaks than LPPG, has a higher signal to noise ratio, excellent dispersion for an α-helical membrane protein and uniform signal intensity.

## References

[1] Marley, J., *et al*., Journal of Biomolecular NMR, **20**, 71-75 (2001).