Investigation of Transmembrane Helix Interactions in Perfluorinated Detergent

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Introduction

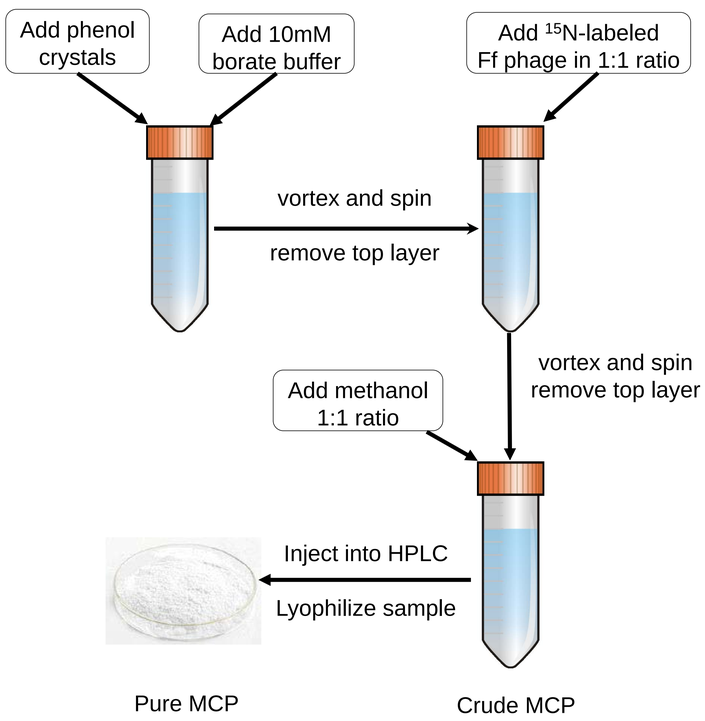
The transmembrane of the major coat protein (MCP) from filamentous bacteriophage is known to dimerize in detergent and lipid bilayer systems. This interaction has been studied in great depth through mutagenesis, but little about the dimer structure is known. This information can potentially be gleaned from solution NMR studies. This project aims to study the effect of solubilizing agents, specifically detergents, on the interactions between the transmembrane helices in membrane proteins.

Good detergents for solution NMR should be able to solubilize a high amount of protein, among other properties. One promising, but relatively obscure detergent, is perfluorooctanoic acid (PFO). Fluorocarbons have very different properties compared to hydrocarbons, and the measure of the difference in stabilization energy provided by PFO could be a useful reference for the development of new solvents for the application of solution NMR to study membrane protein structure. To this end, the dimerization properties of the MCP from the M13 Ff phage, which has previously been characterized in a range of hydrocarbon-based detergents, will be studied in PFO.

Methods

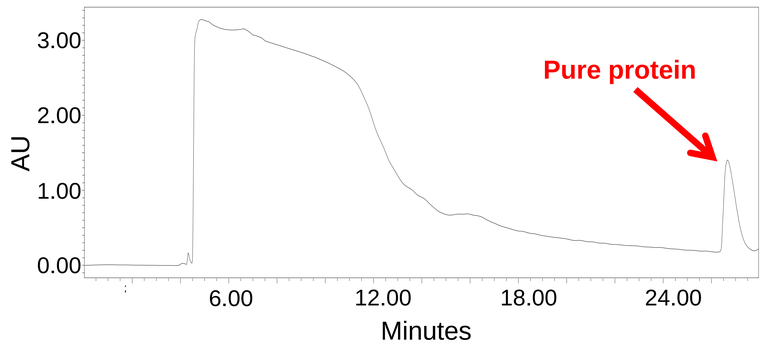
Filamentous bacteriophage has been cultured in ^{15}N-enriched media for ^{1}H-^{15}N HSQC NMR studies. Phage is purified, then treated to a standard phenol extraction protocol to separate the MCP from the virus.

Obtaining ^{15}N -labeled MCP



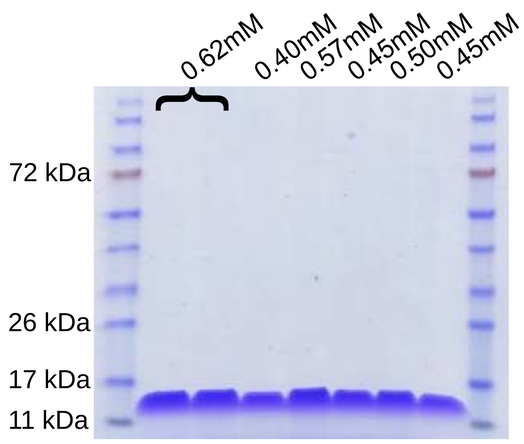
Results

HPLC purification profile of MCP protein

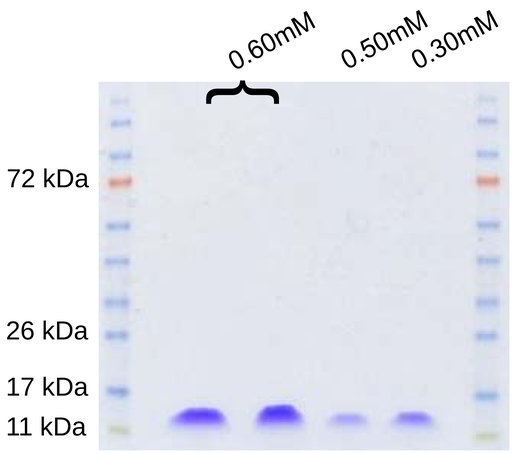


Gels

Gels show band 'stretches', which is characteristic of MCP dimerization. DNA ladder are given on first and last lanes, with molecular weight of selected bands. Protein concentrations are indicated above each lane, as determined by BCA assays.

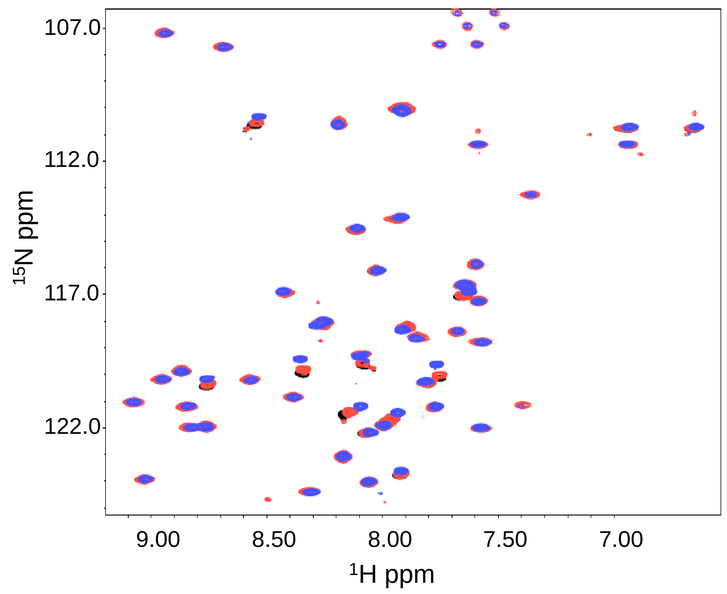


Gel run on aliquots taken from before and after the NMR sample has undergone 3 serial dilutions.



Gel run on aliquots taken from NMR samples having undergone 2 2 serial dilutions, the spectra of which are shown on the right.

Three overlaid ^{1}H-^{15}N HSQC NMR spectra



In **black**: 0.60mM MCP, **red**: 0.50mM, **blue**: 0.30mM MCP
All ^{1}H-^{15}N HSQC NMR experiments were run in 250mM PFO, 10% D_20, pH 5.1.

HSQC is the most common experiment in protein NMR. Protons attached to the heteronucleus (^{15}N) on each amino acid residue of the protein gives rise to a peak on the spectrum with a specific chemical shift.

Conclusions

From the^{1}H-^{15}N HSQC NMR spectra obtained through serial dilution, it is evident that:

- peaks showed a difference in chemical shift;
- there is no clear evidence of protein dimerization;
- the data goes in opposition from what is observed on the gels, from which both monomeric and dimeric species are observed

This necessitates further studies to better understand the behavior of MCP in the perfluorinated detergent.

References

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2. R.A. Menlnyk, A.W. Partridge, C.M. Deber, Transmembrane domain mediated self-assembly of major coat protein subunits from Ff bacteriophage, J. Mol. Biol. (2002) 315, 63-72.
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Acknowledgements

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