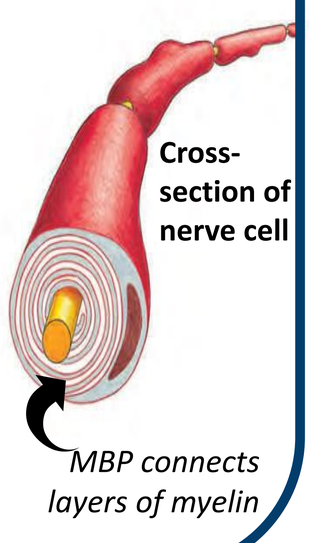
The Myelin Basic Protein (MBP) and its Phase Behaviour

I. Graf von Westarp[1,2], A. Radulescu[3], B. Wu[3], S. Förster[1], A. Stadler[1,2]

[1]Jülich Centre for Neutron Science (JCNS), Forschungszentrum Jülich GmbH, Wilhelm-Johnen-Straße, 52428 Jülich, Germany
[2]Institute of Physical Chemistry (IPC), RWTH Aachen University, Landoltweg 2, 52074 Aachen, Germany
[3]Jülich Centre for Neutron Science (JCNS), Forschungszentrum Jülich GmbH, Outstation at MLZ, Lichtenbergstraße 1, 85747 Garching, Germany

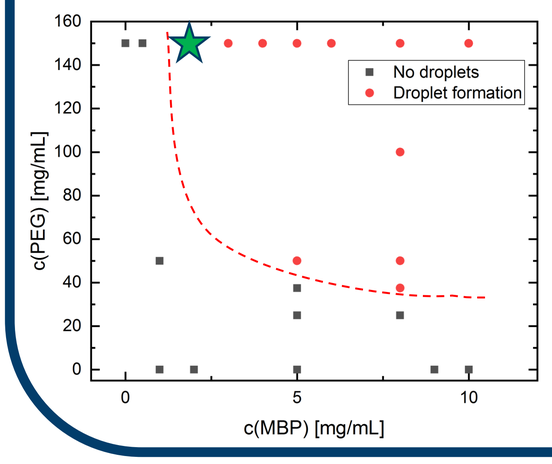
The importance of phase separation

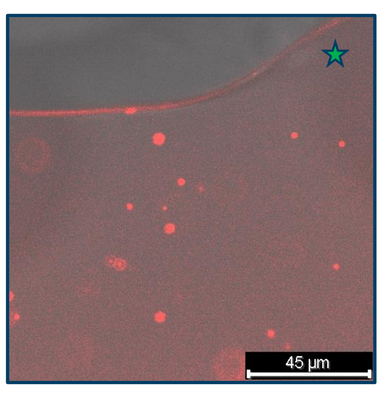
Phase separated MBP is essential for a compact myelin sheath and axonal signal transport.
Damages can result in Multiple Sclerosis, hence research on the phase behaviour is crucial.

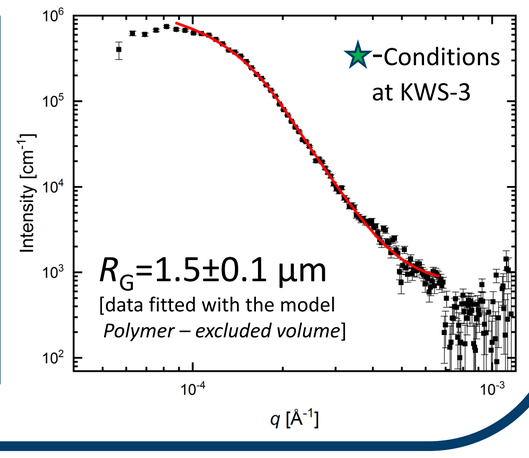


Liquid-Liquid Phase Separation in vitro

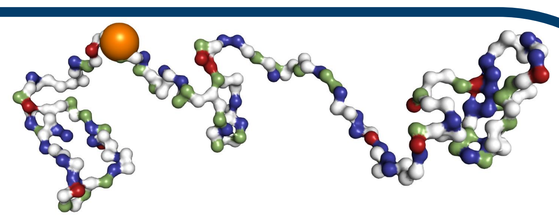
LLPS at pH 7 in presence of 150 mM NaCl and Polyethylene glycol (PEG)
➔ Formation of labeled condensates visible with confocal microscopy
➔ Size distribution in low µm-range confirmed with SANS at KWS-3 [3]







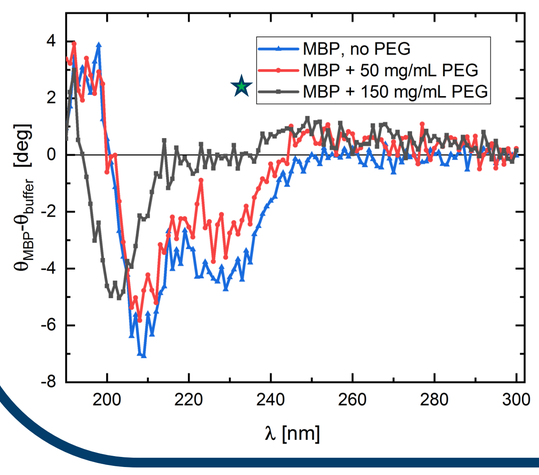
MBP Structure

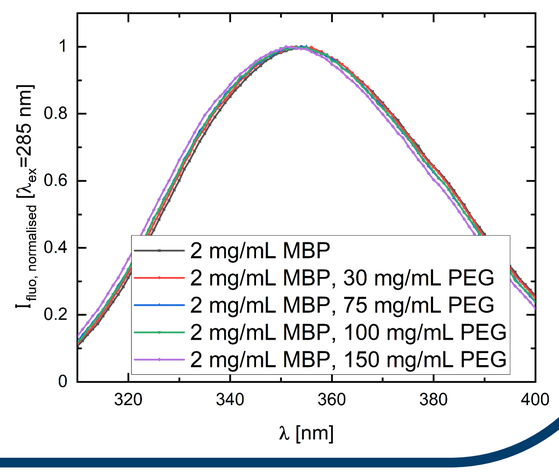


R_h/R_G≈1.1 ➔ Partially unfolded structure in native

CD spectroscopy: Unfolding of α-helices upon PEG addition

Fluorescence Emission Spectroscopy of Tryptophan AA: Environmental polarity remains constant when PEG is added
➔ Trp (orange) is located in an expanded domain in native



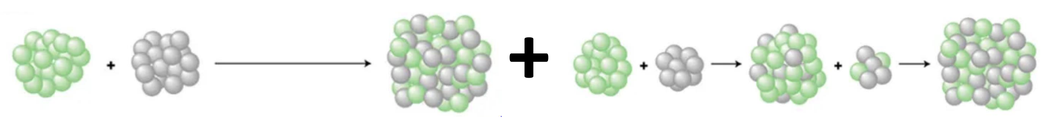


Droplet Growth Kinetics

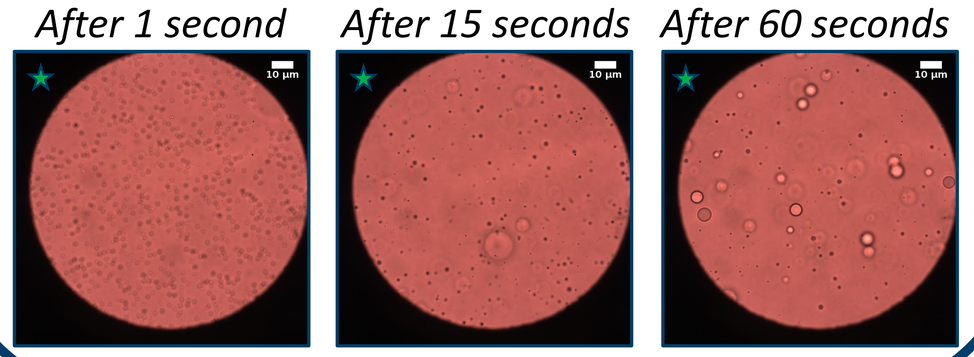
Immediate nucleation when LLPS conditions are set

Droplet size distribution constant after 1 minute

Assumption: **Coalescence + Ostwald-Ripening**



After 1 second *After 15 seconds* *After 60 seconds*



Conclusions

- ✓ MBP undergoes phase separation *in vitro*
  ➔ Formation of µm-sized droplets/condensates
- ✓ α-helical domains unfold upon addition of PEG
- ✓ Tryptophan AA located in natively unfolded region
- ✓ Droplet formation and growth within minutes
  ➔ Mechanisms: Coalescence + Ostwald Ripening

Outlook

- ? Microfluidics of MBP under LLPS conditions
  ➔ Confocal microscopy to follow size evolution
- ? Stopped-Flow tests for nucleation kinetics
  ➔ Light scattering + SANS with deuterated PEG
- ? LLPS in contact with biomimetic membranes
  ➔ Interactions with more physiological systems