UNRAVELLING SELF-ASSEMBLY OF HEMOGLOBIN IN A LIPID ENVIRONMENT

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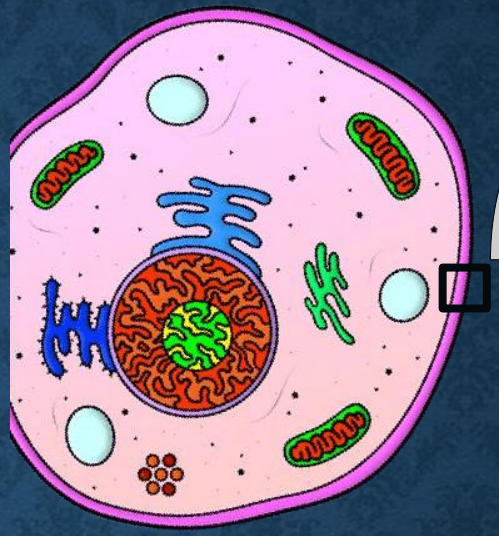
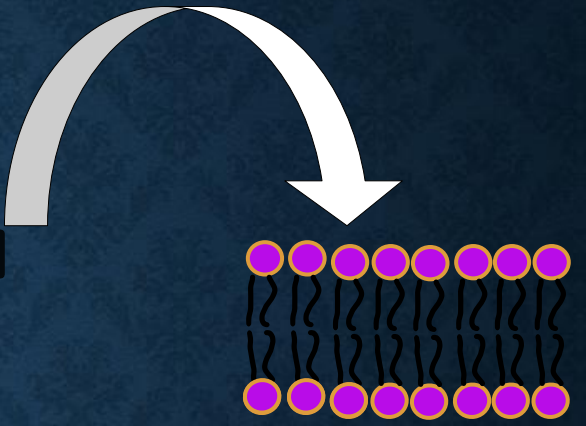
Background

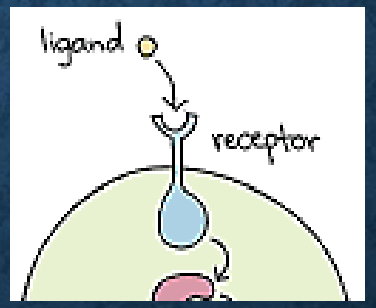
Lipid Bilayer Structures: Vital *in vivo* & Versatile *in vitro*

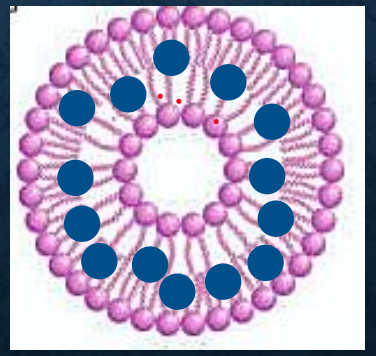
In Vivo:

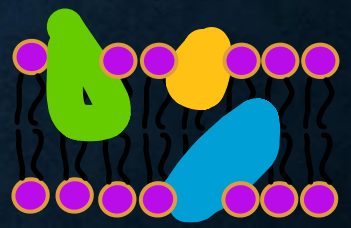
- Cell Membranes
- Compartmentalization
- Transport and Trafficking
- Cell Signalling
- Energy Storage











In Vitro:

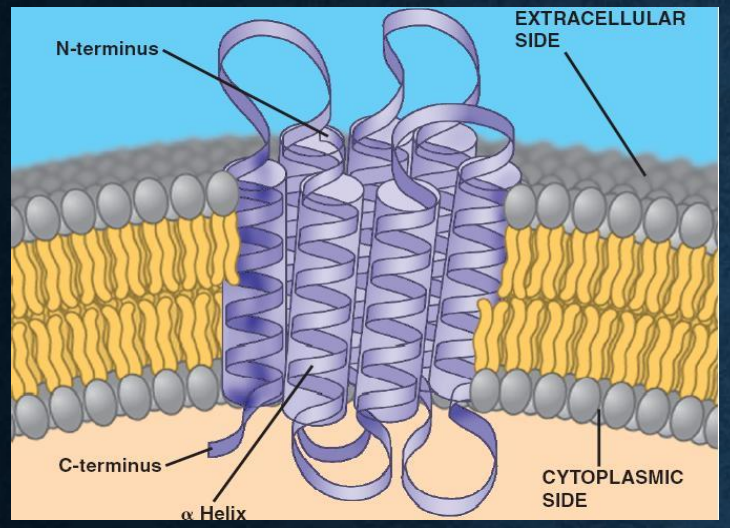
- Model Membranes and Design
- Cell Communication and Signalling
- Drug Targeting and Delivery

Insertion mechanisms

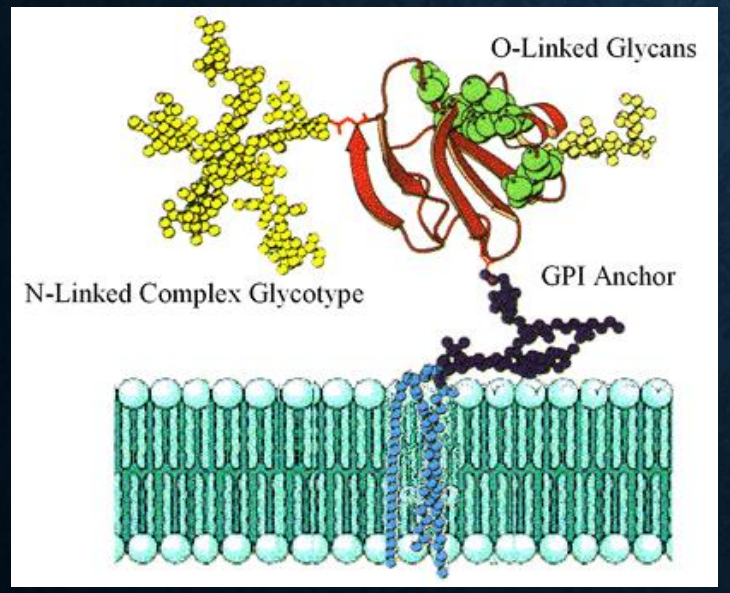
Dynamics and adaptation to the lipid environment

Insertion Mechanism

In vivo

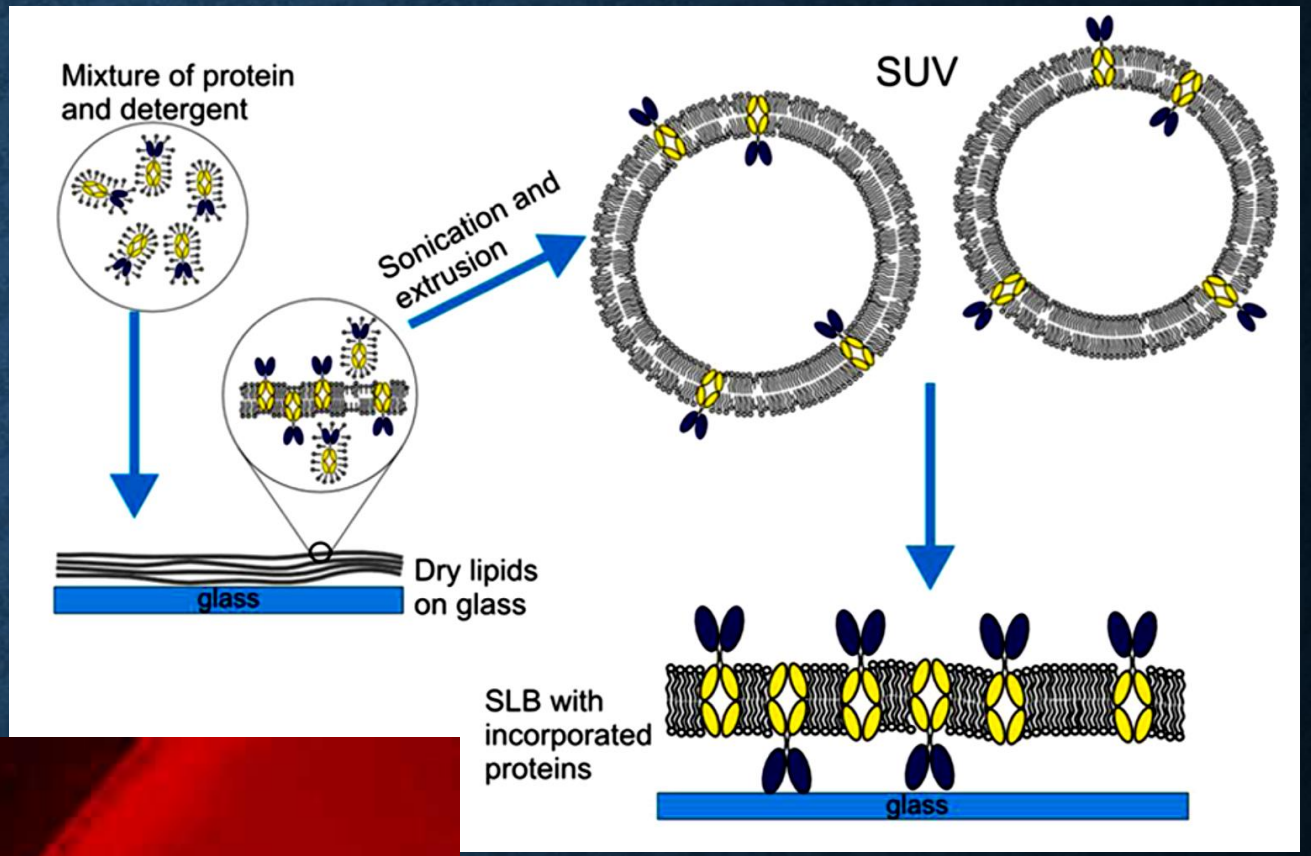


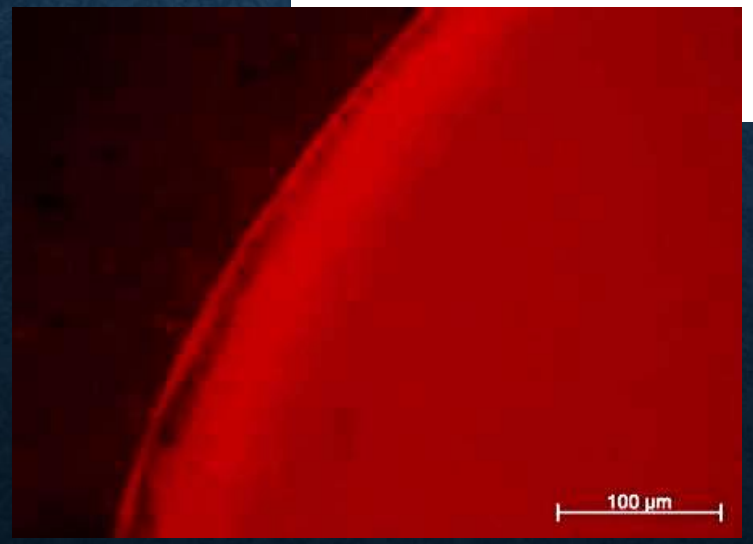
Trans membrane domain



GPI anchor

In vitro

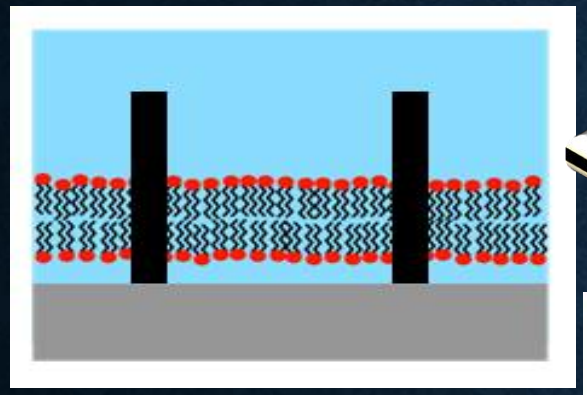


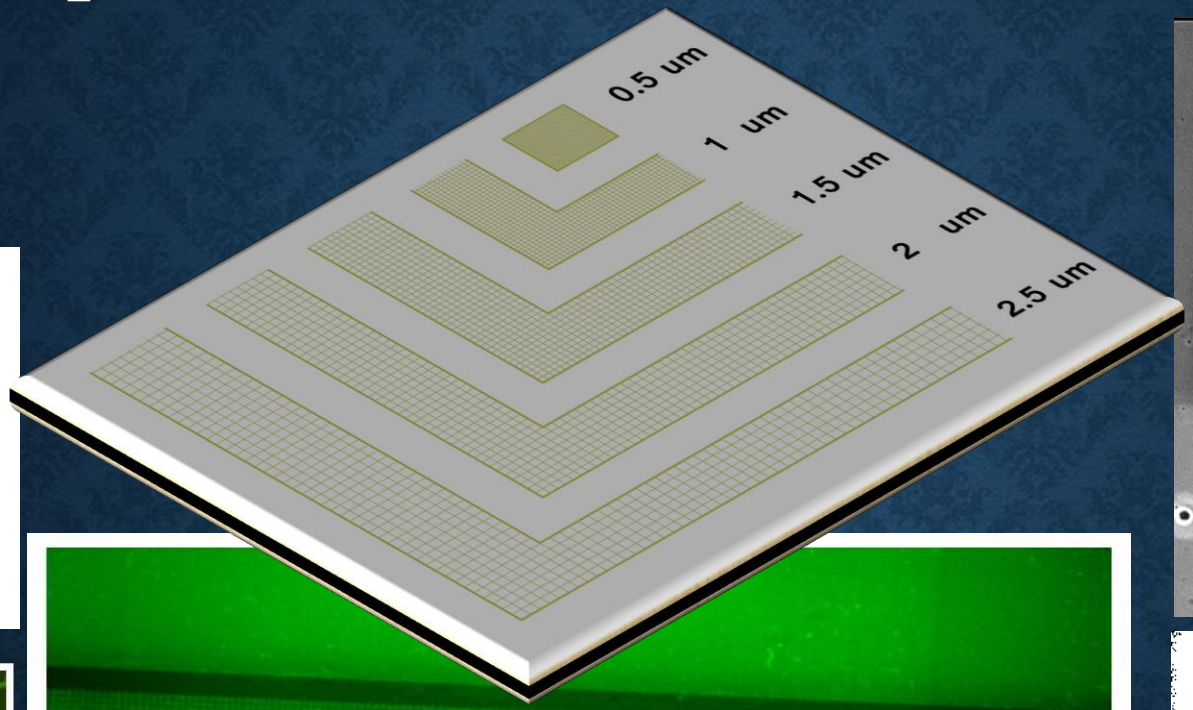


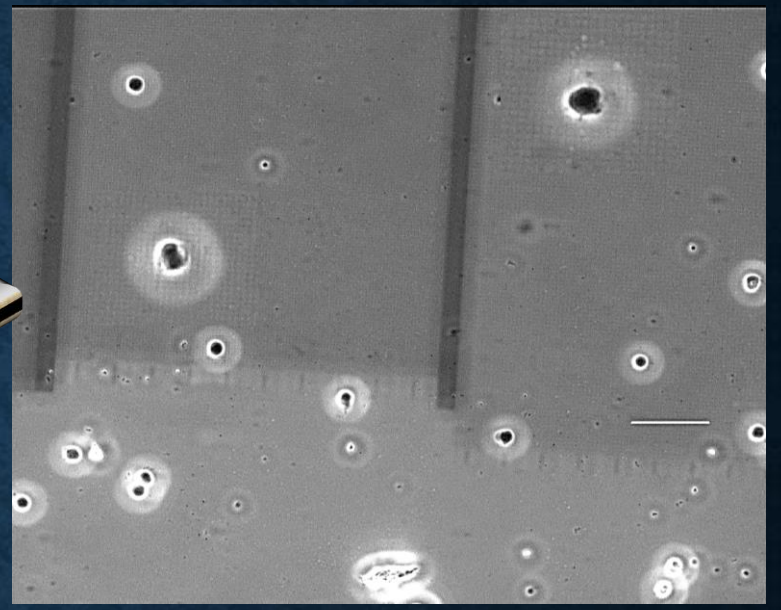
Fluorescence observed from membrane incorporated labelled neural adhesion protein EphrinA5-Fc

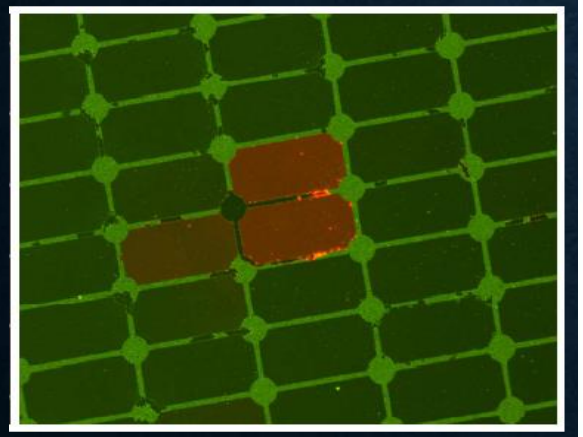
Ghosh Moulick et.al; Langmuir 2016

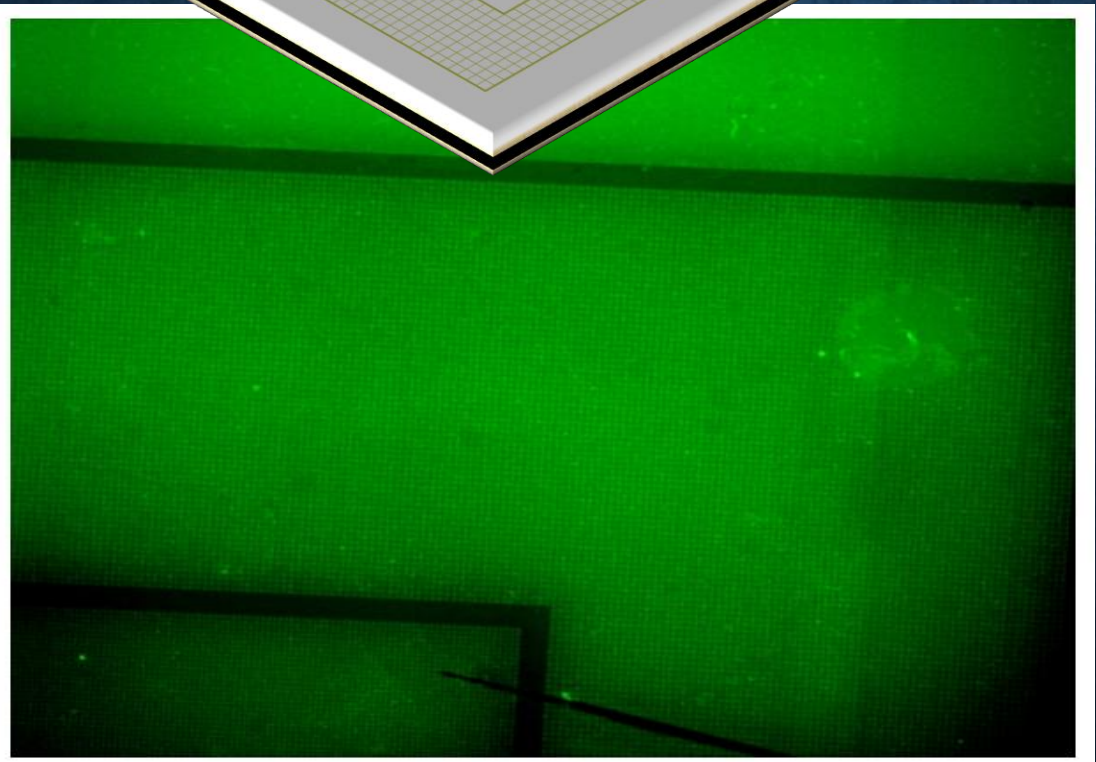
Studying Compartmentalization and adhesion of Neurons

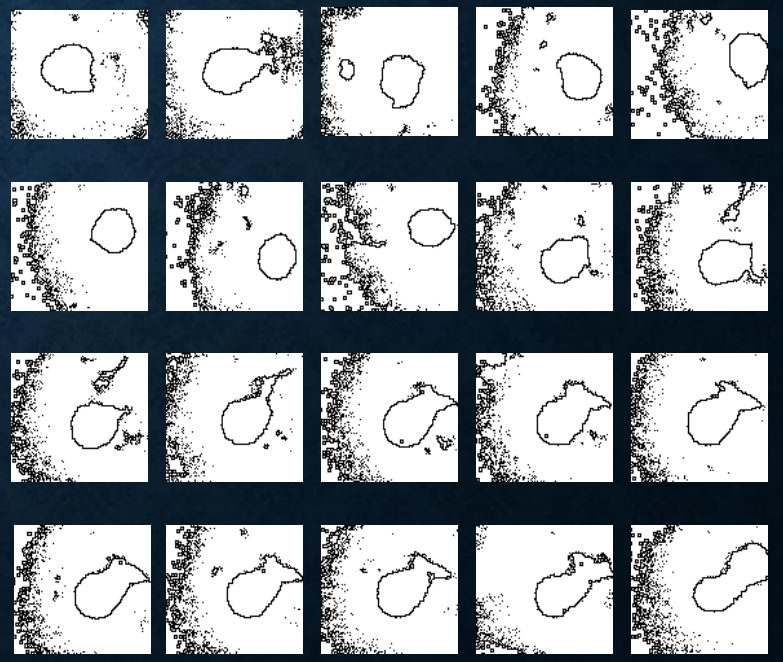








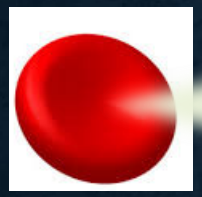




Ghosh Moulick et.al;
Nanoscale 2018

Current Investigation

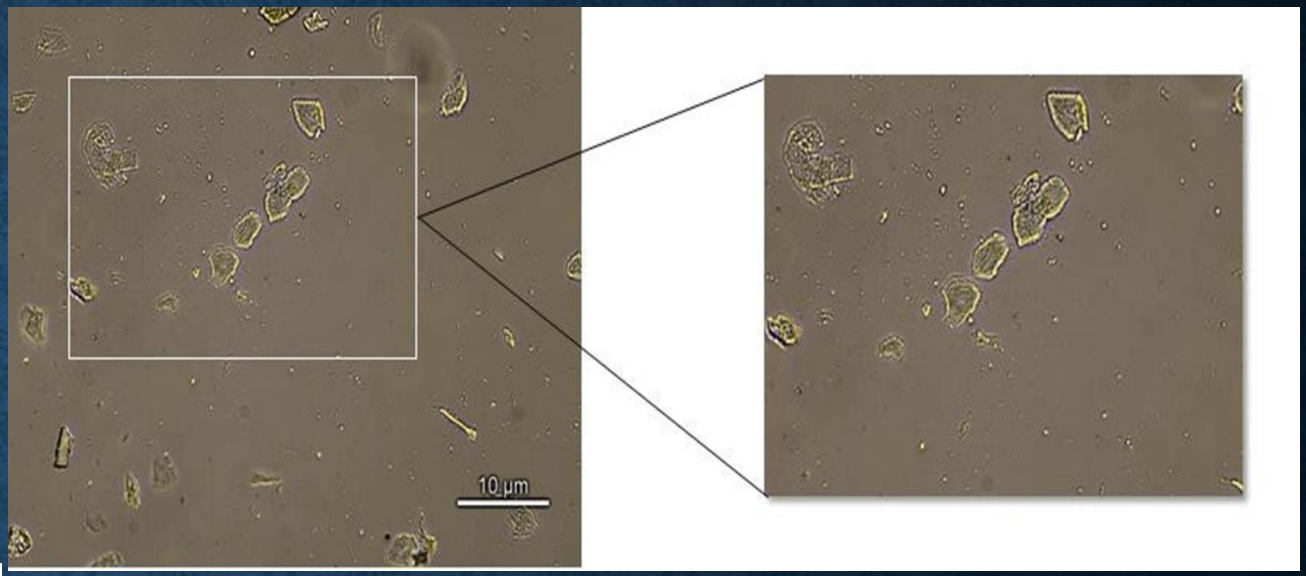
Insertion of a Nonmembranous Blood Protein Hemoglobin



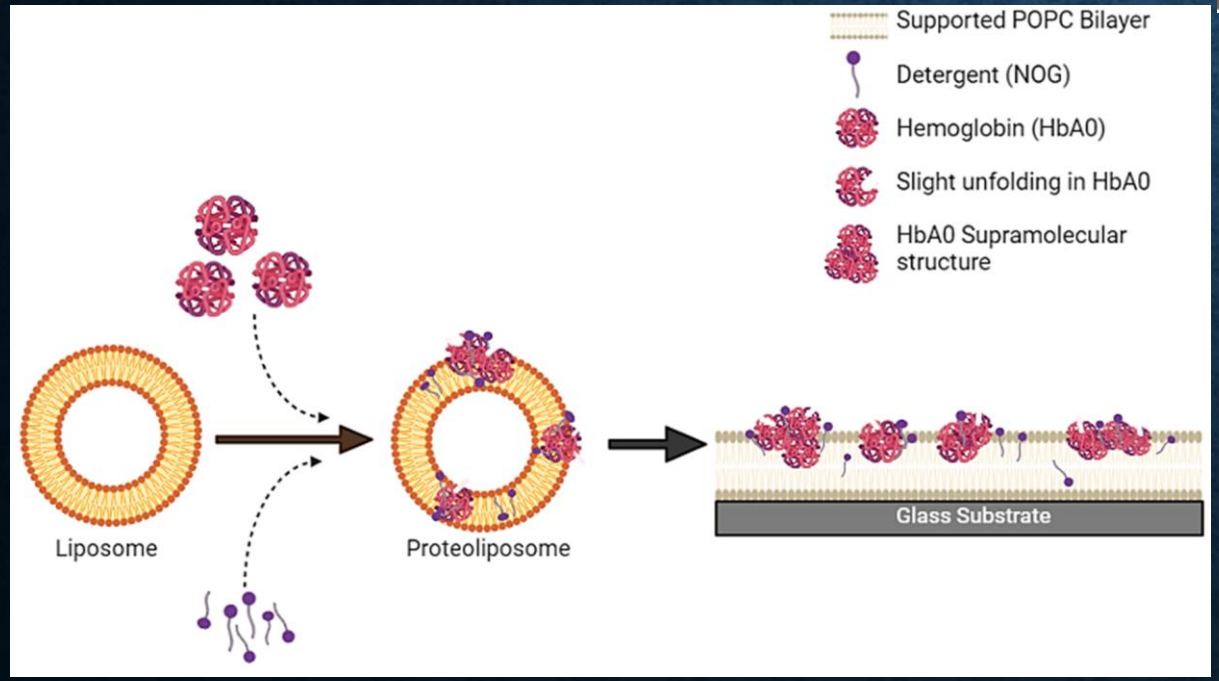
RBC

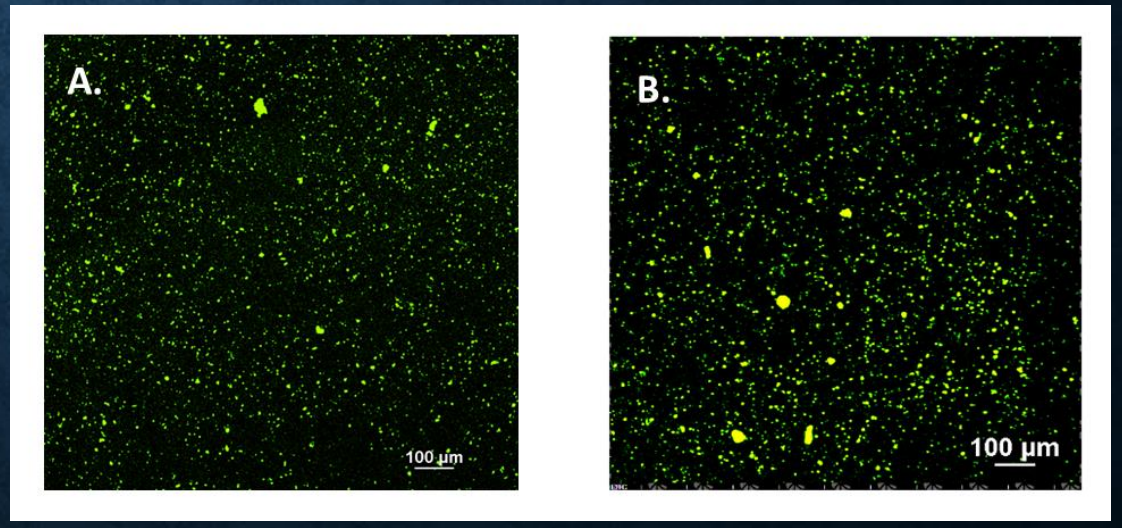
Hemoglobin

Non-membrane proteins (Hemoglobin) lacks distinct transmembrane domain or hydrophobic region



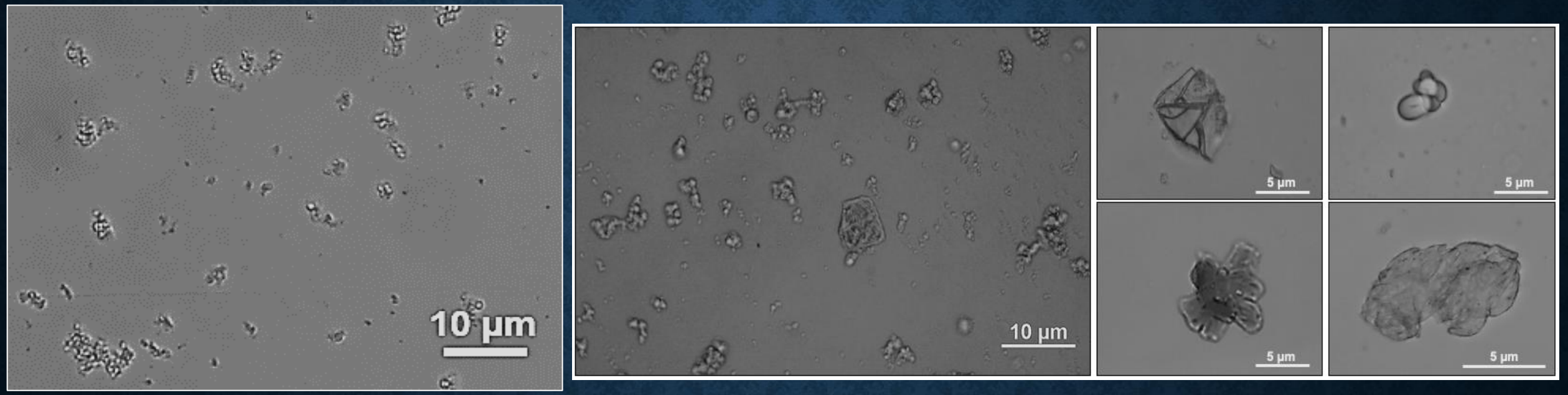
Bright field microscopic image of Hb incorporated in the bilayer





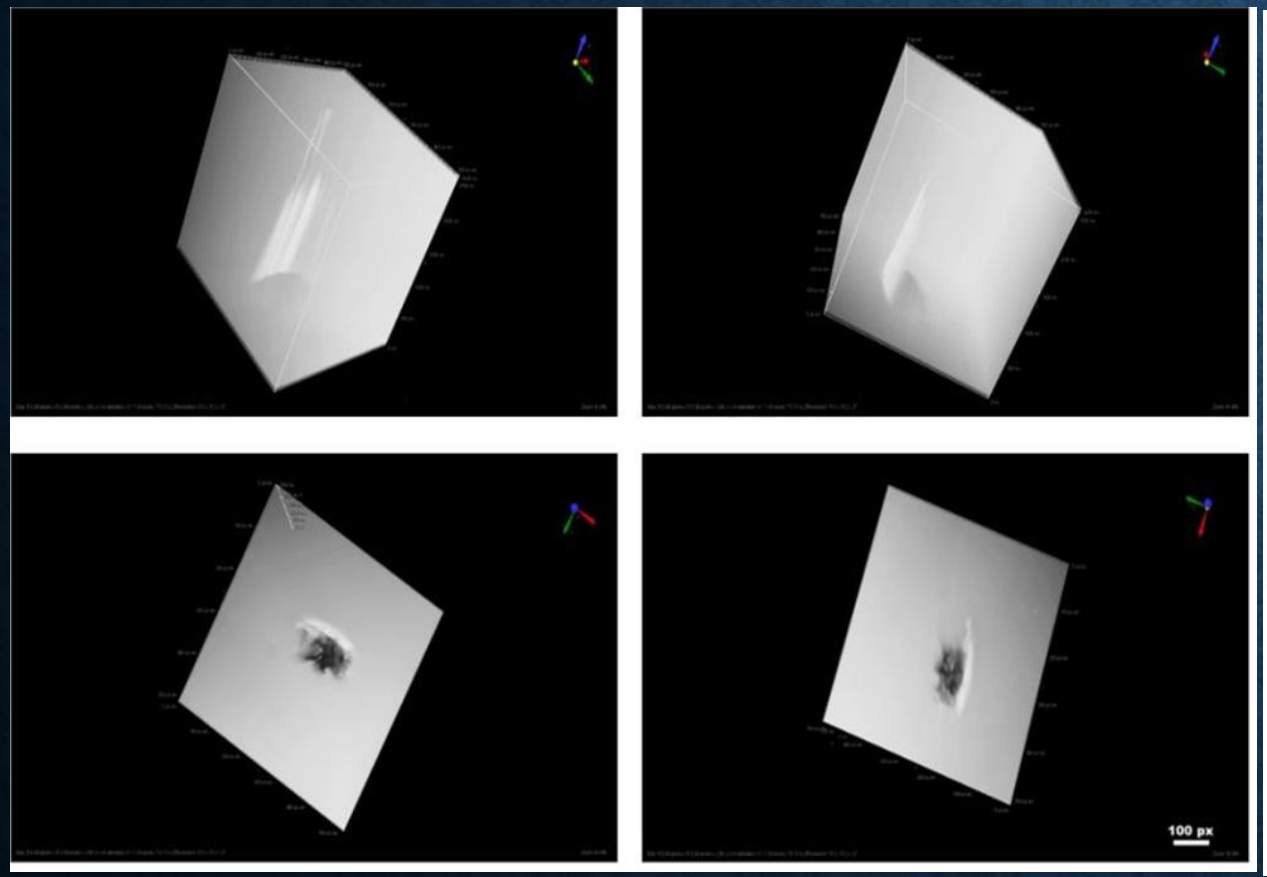
Fluorescence microscopic image of Hb in the bilayer after labelling

Formation of Supramolecular Structures of Hemoglobin

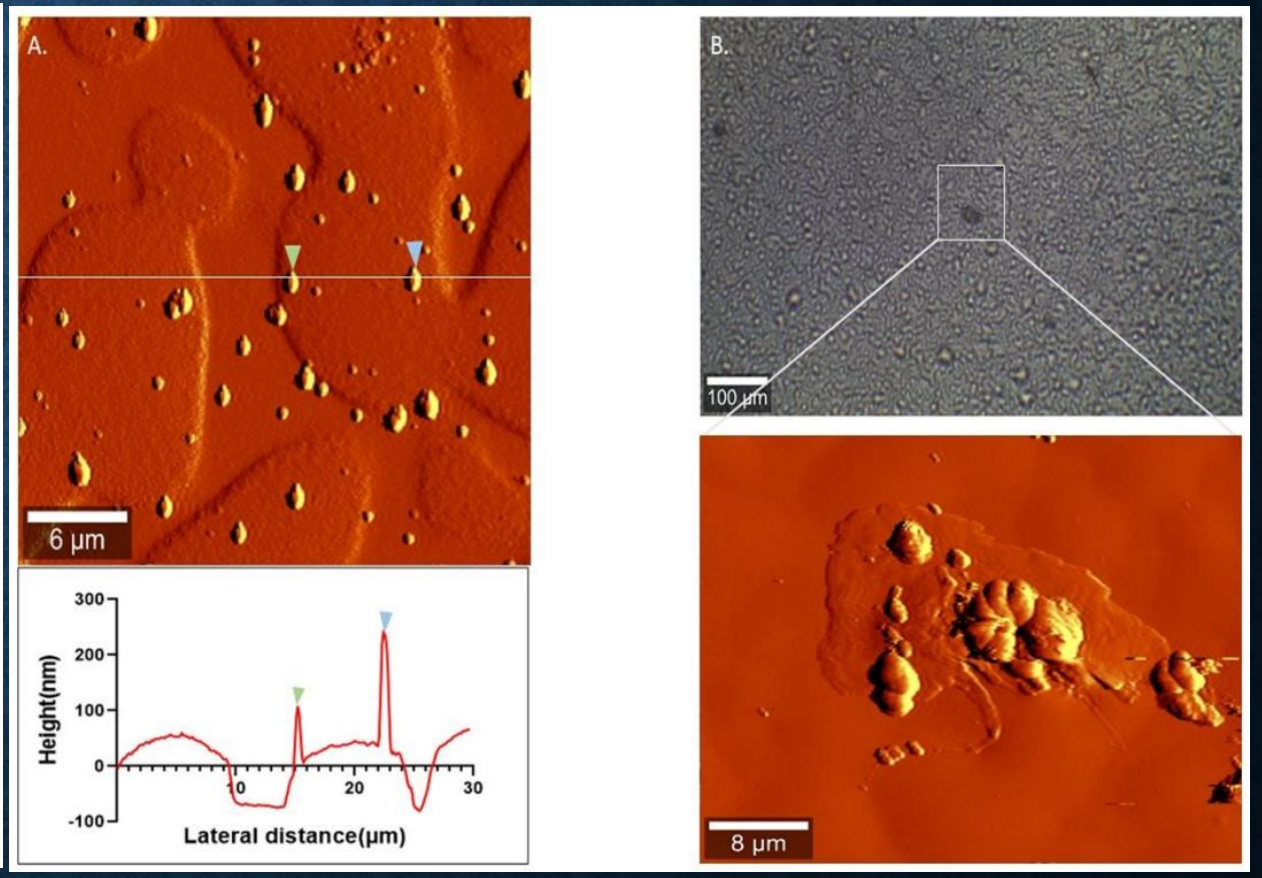


Supramolecular structures of different morphology of Hb in lipid environment encountered during the bright field microscopic study

A close view of the supramolecular Structures – AFM and Confocal Microscopy

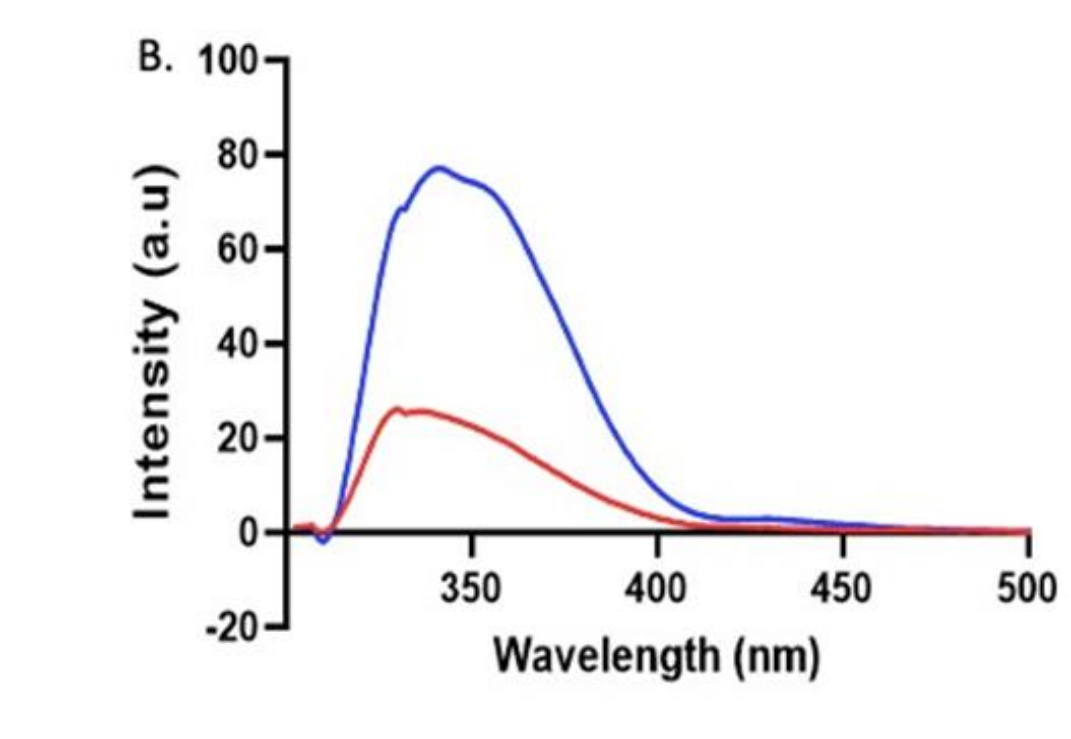


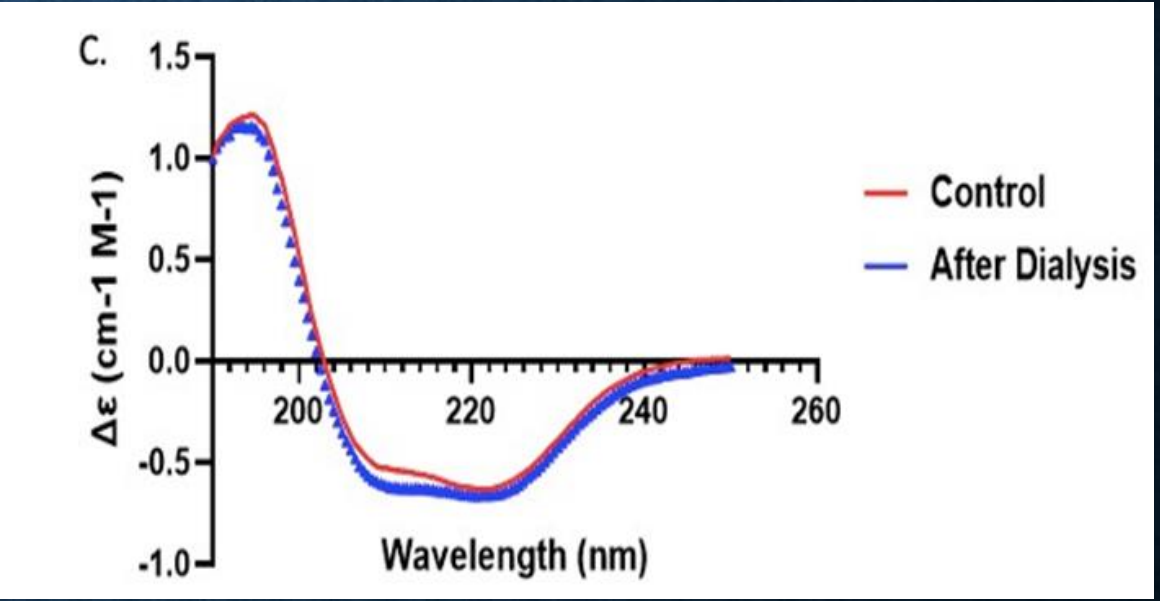
Confocal Z-stack 3D reconstruct of the protein incorporated region in the lipid bilayer



AFM image of the inserted proteins, Hemoglobin

Exposer of Hydrophobic Regions After Insertion

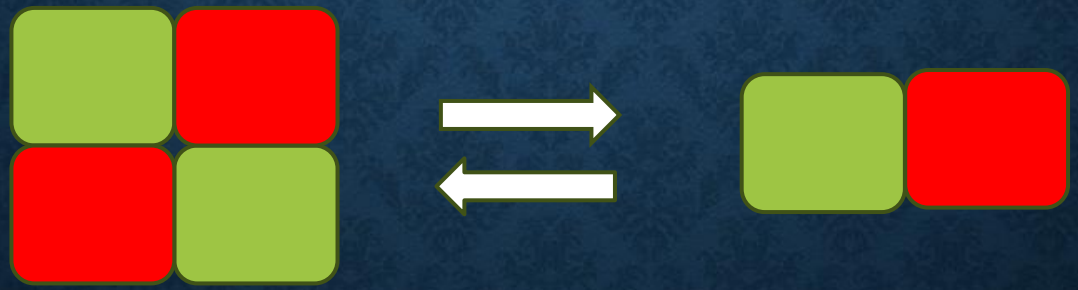




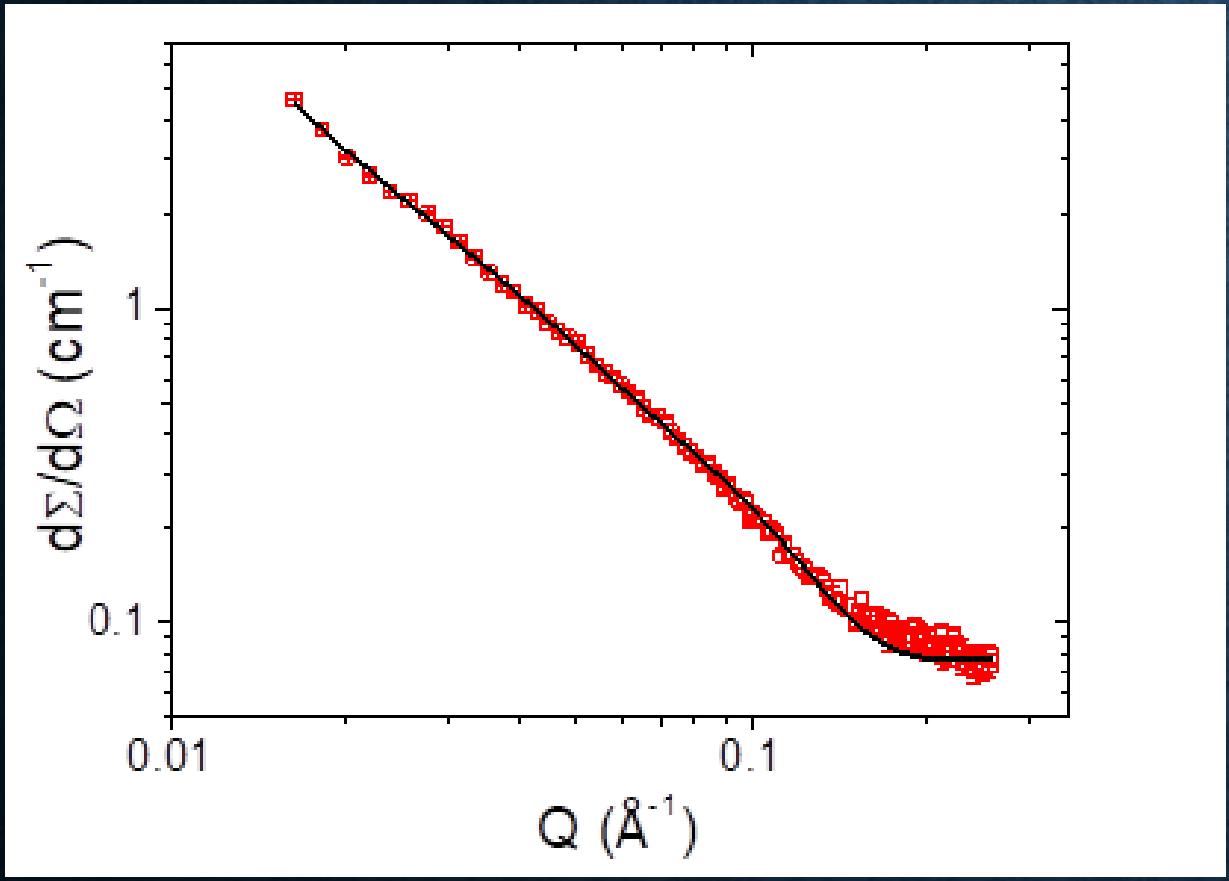
Fluorescence spectra and Circular Dichroism (CD) spectra of only Hemoglobin solution (red) and Hemoglobin-embedded liposome solution (blue)

The Key Question

Is the Tetramer – Dimer Equilibrium hampered and leads to the aggregation



SANS revealed that Hemoglobin retains its tetrameric form



Sample	Structure	Bilayer structure		Hemoglobin (Prolate Ellipsoid)		
		Vesicle Size > $2\pi/Q_{min}$(Å)	Vesicle thickness (Å)	Semiminor Axis (Å)	Semimajor Axis (Å)	Effective Size (Å)
Hb protein in lipid	Mixing of Bilayer with folded Hemoglobin	>400	75.5±5.2	19.4±0.4	67.6±1.5	~32

Measurement of Hemoglobin-embedded liposome solution

Thank you