Structural studies of integral membrane proteins using stealth carrier nanodiscs

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Time-resolved SAXS experiments on MsbA


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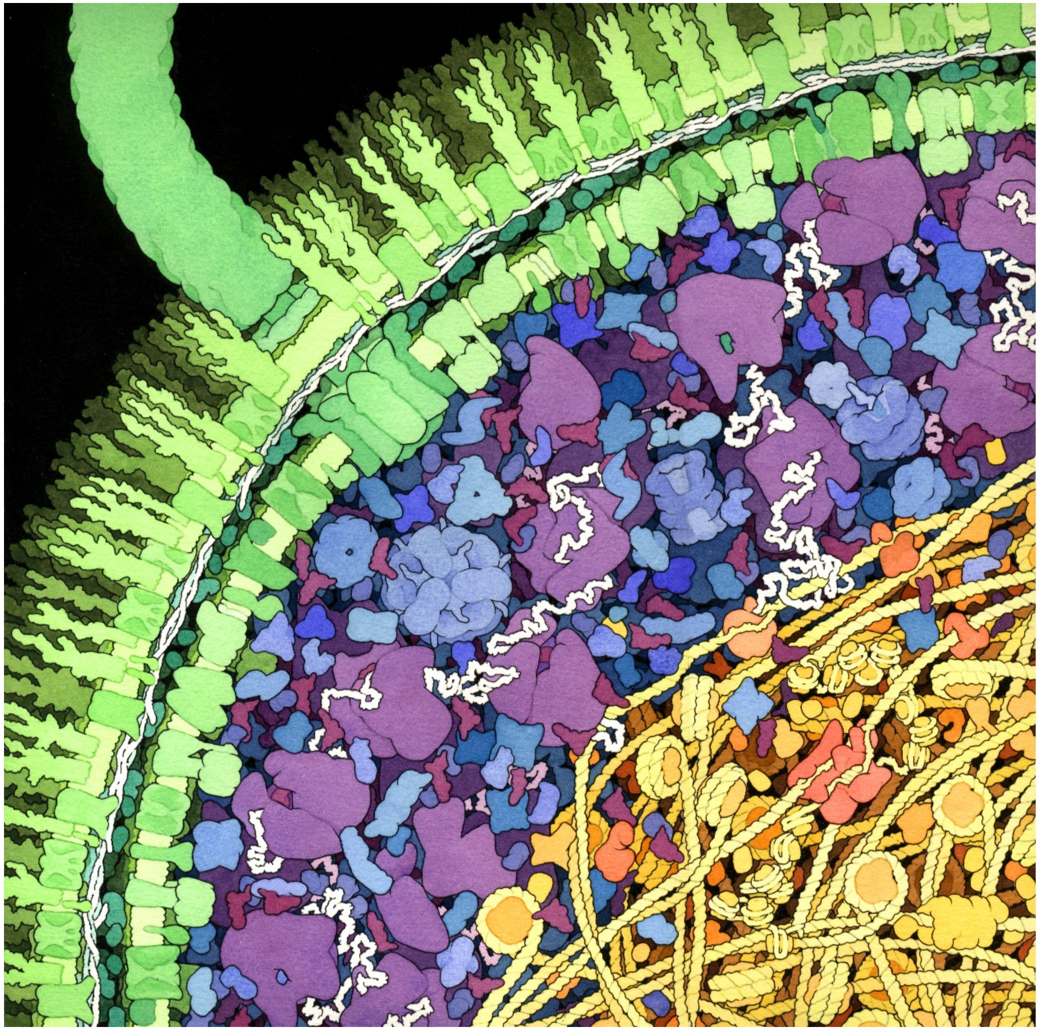
Integral membrane proteins

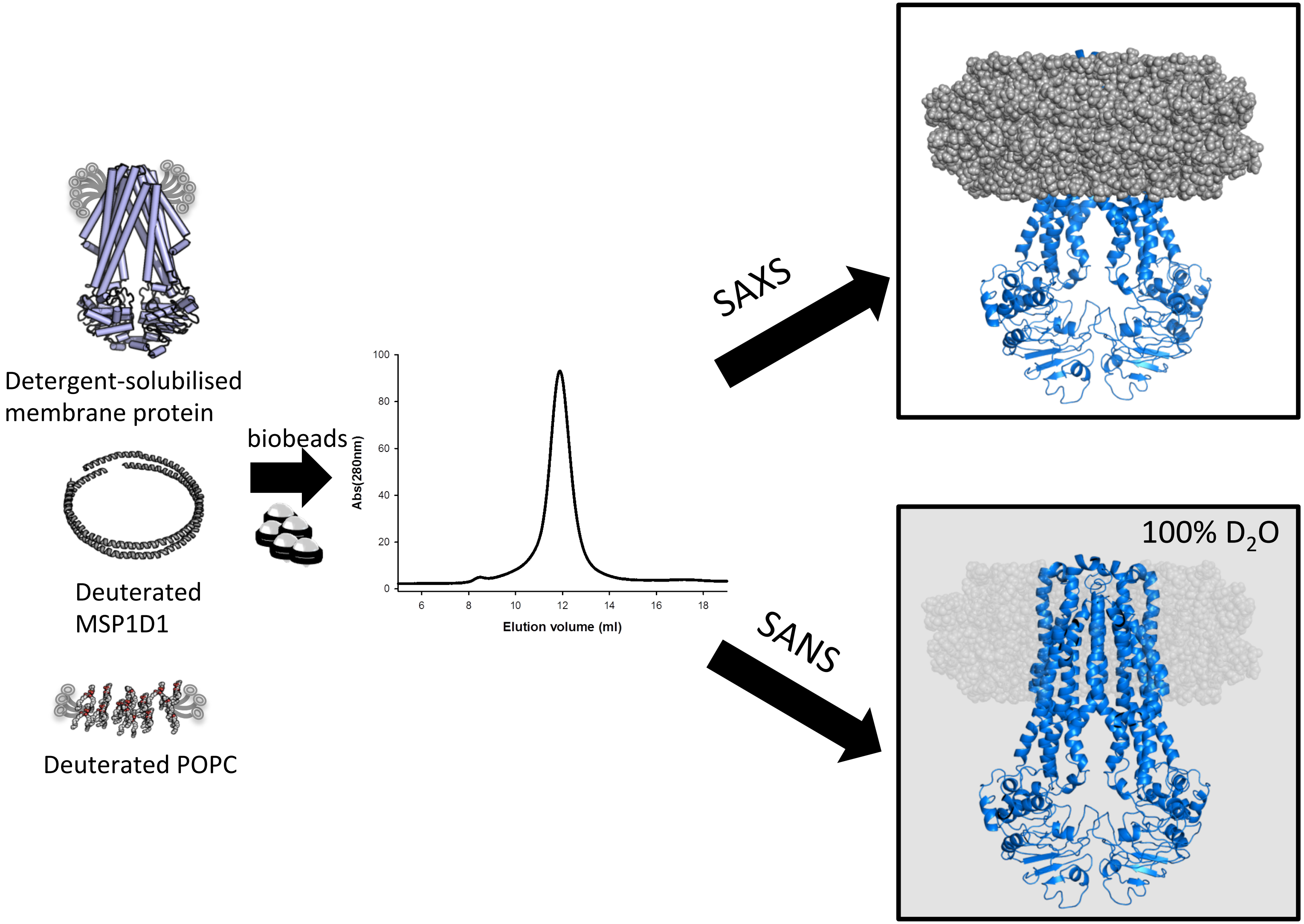


Functions:

Transport
Enzymatic activities
Signal transduction
Intercellular junctions
Cell-cell recognition
Cell shape
Membrane dynamics

David Goodsell, “The Machinery of Life”

Invisible „stealth“ nanodiscs for structural studies of membrane proteins by small-angle scattering



Detergent-solubilised membrane protein

Deuterated MSP1D1

Deuterated POPC

biobeads

SAXS

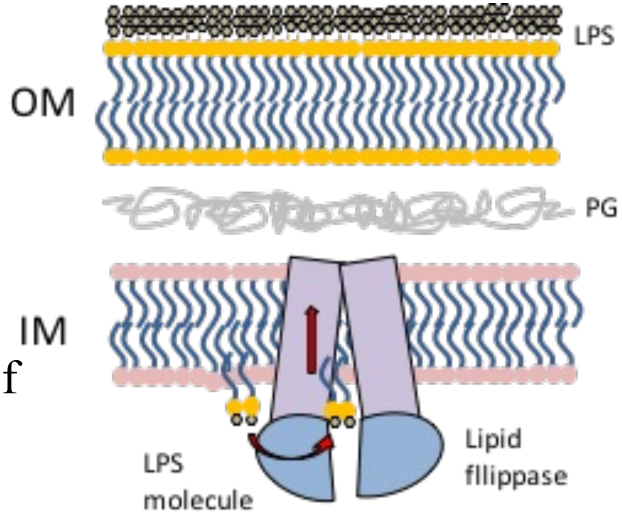
SANS

100% D_2O

Josts, I. *et al*. (2018) Structure

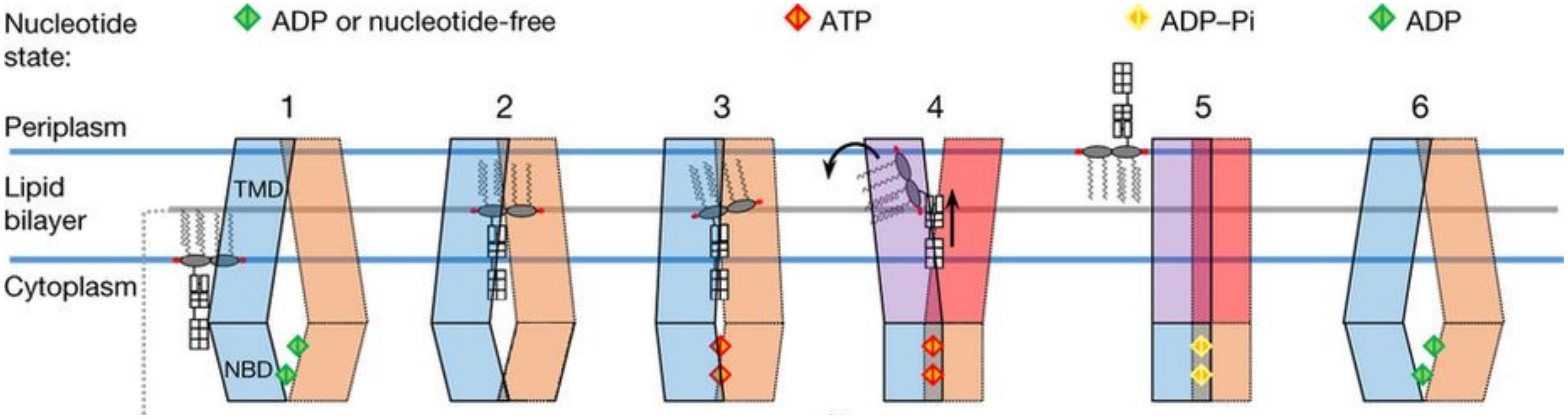
ABC transporter - MsbA

- ABC transporters – ubiquitous integral membrane proteins
- Transport substances across the lipid bilayer - **driven by ATP hydrolysis**
- Transmembrane domain coupled with a soluble ATP-binding cassette domain (1:1)
- Sub-grouped into **importers** and **exporters**
  - ABC exporters – play significant role drug resistance
- **MsbA** is a highly conserved lipid floppase
  - Moves glycolipids from inner leaflet to outer leaflet of the bacterial inner membrane



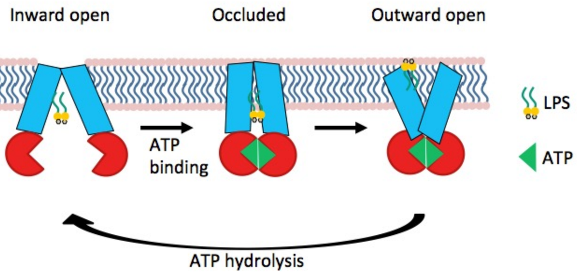
ABC transporter - MsbA

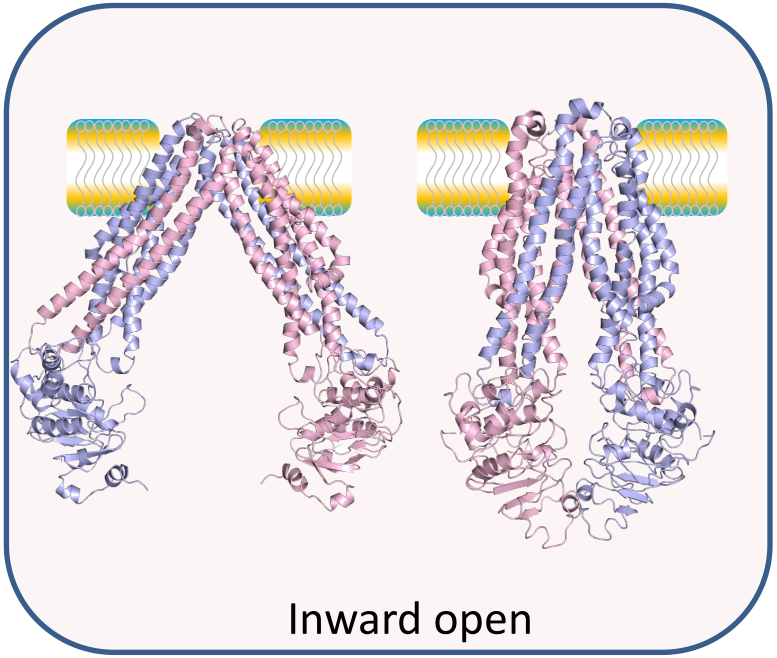
- MsbA is a highly conserved lipid floppase from *E. coli*
- It moves glycolipids from inner leaflet to outer leaflet of the bacterial inner membrane
- MsbA functions via ATP-driven "power stroke" mechanism

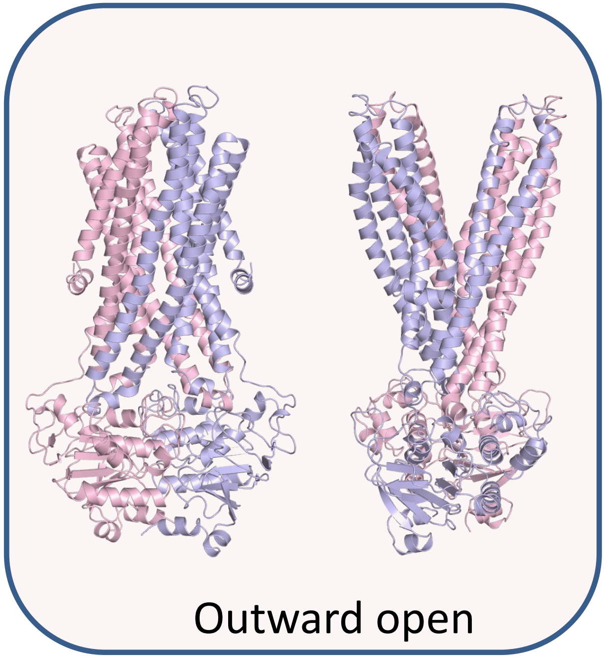


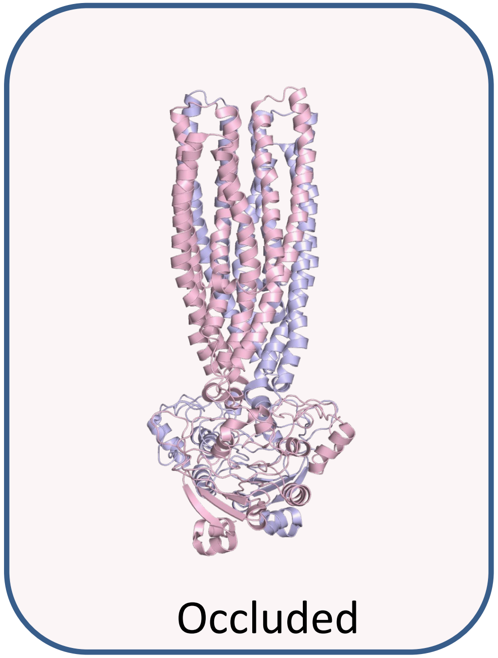
Mi, W. *et al*. (2017) Nature

MsbA functions via ATP-driven "power stroke" mechanism




Inward open


Outward open

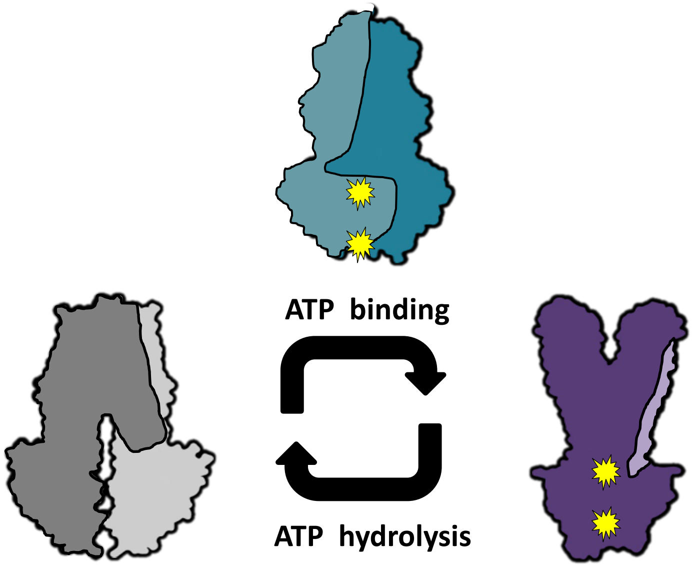

Occluded

Numerous states captured by X-ray crystallography and cryoEM

Ward, A. *et al*. (2007) PNAS

Mi, W. *et al*. (2017) Nature

Understanding the structural kinetics of the ATP-driven "power stroke"



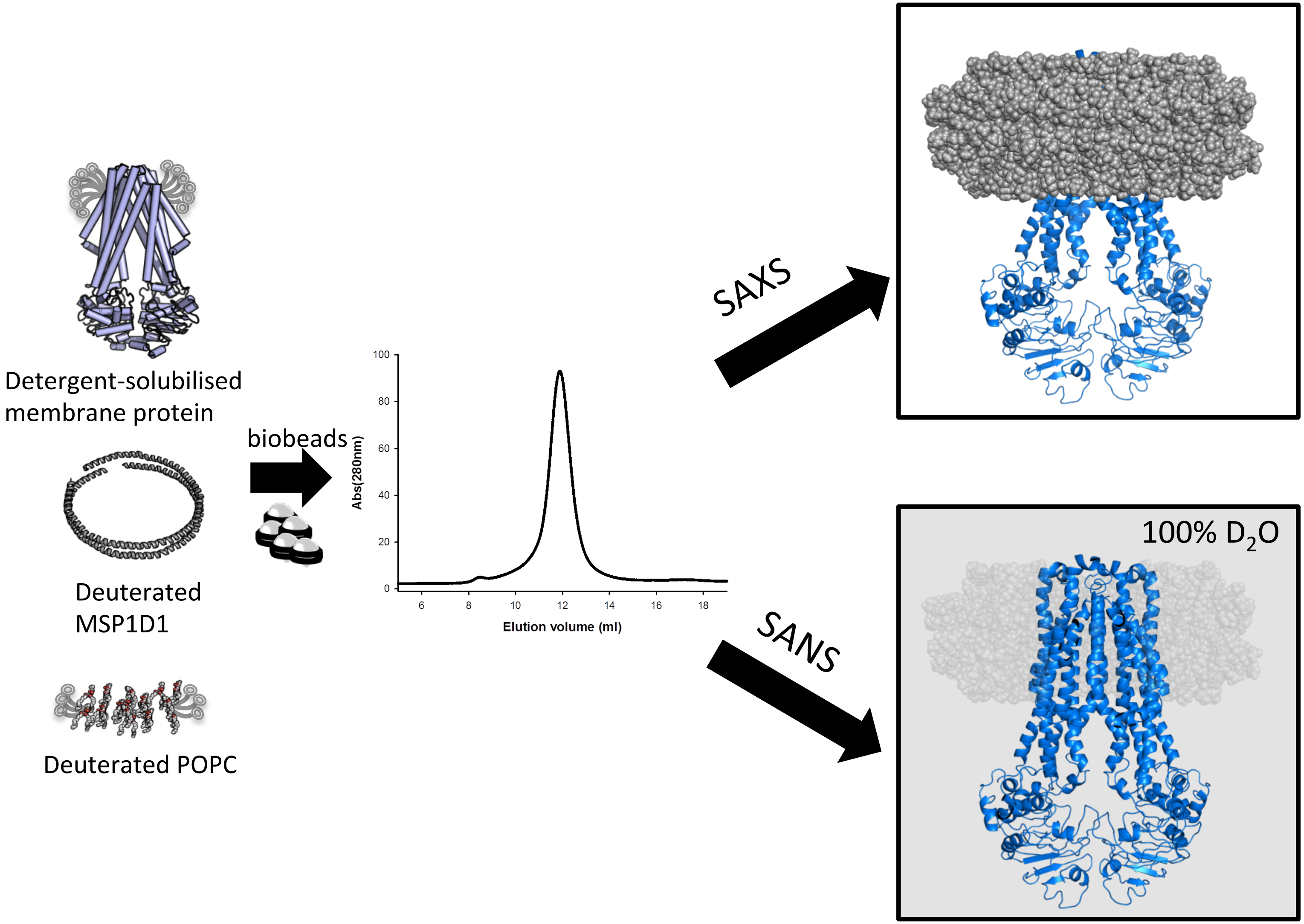
- AIM: study the whole cycle of the "power stroke" of MsbA in **nanodiscs** using time-resolved scattering experiments

- What is the "ground state" conformation?

- What conformational changes can we see?

- Decided to answer these questions using **neutron scattering** and **stealth nanodiscs** followed by **time-resolved SAXS** experiments

Invisible „stealth“ nanodiscs for structural studies of membrane proteins by small-angle scattering



Detergent-solubilised membrane protein

Deuterated MSP1D1

Deuterated POPC

biobeads

SAXS

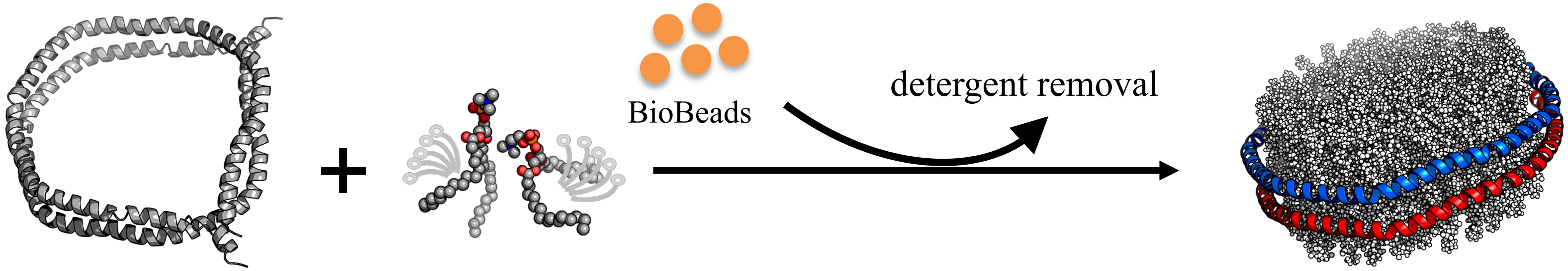
SANS

100% D_2O

Josts, I. *et al*. (2018) Structure

Nanodiscs as soluble sample carriers for solubilised membrane proteins

- Preparation of nanodiscs:



Membrane scaffold protein (MSP)

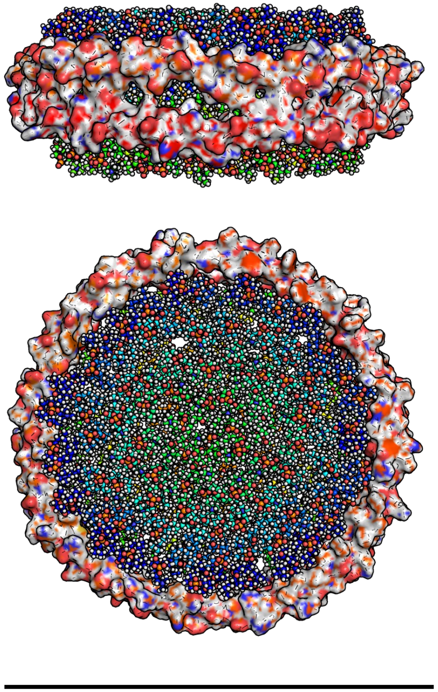
+

Detergent-solubilized lipids

BioBeads

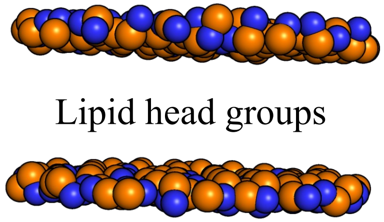
detergent removal

Nanodiscs as soluble sample carriers for solubilised membrane proteins

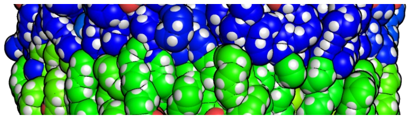


- Eliminate the use of detergents
- Provide a lipid-like environment (important for activity and native conformations)

MSP “belt” protein

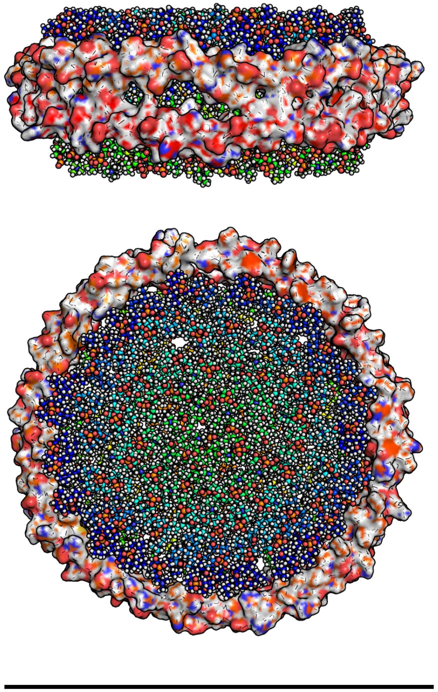


Lipid head groups

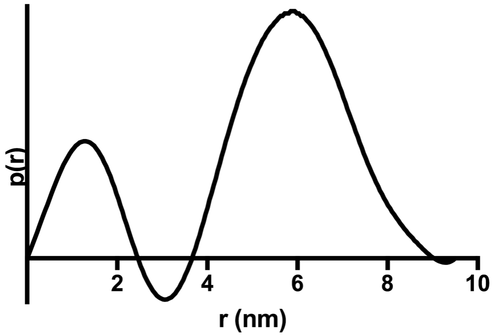


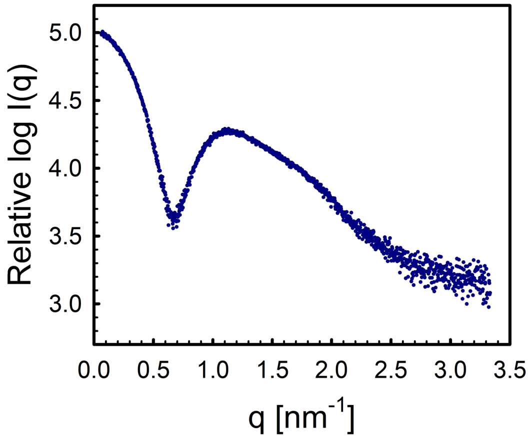
Lipid tails

Nanodiscs as soluble sample carriers for solubilised membrane proteins



- Nanodisc conformations are hard to model against scattering data (multi-contrast system)



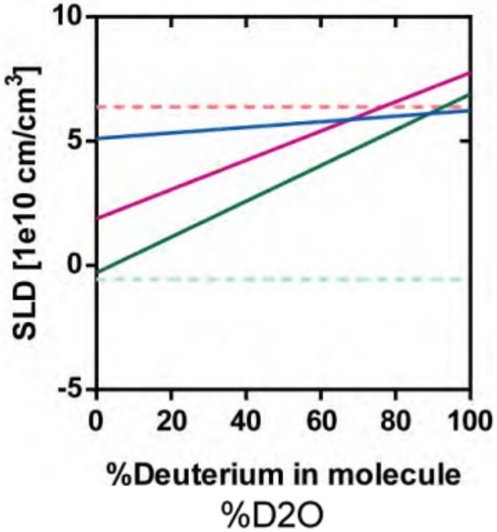


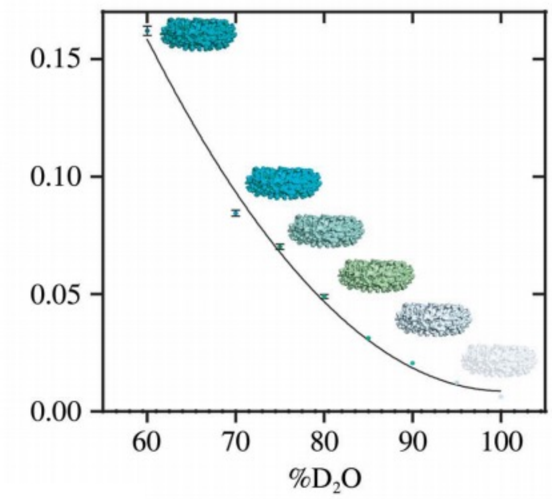
Stealth nanodiscs – practically invisible to neutron scattering

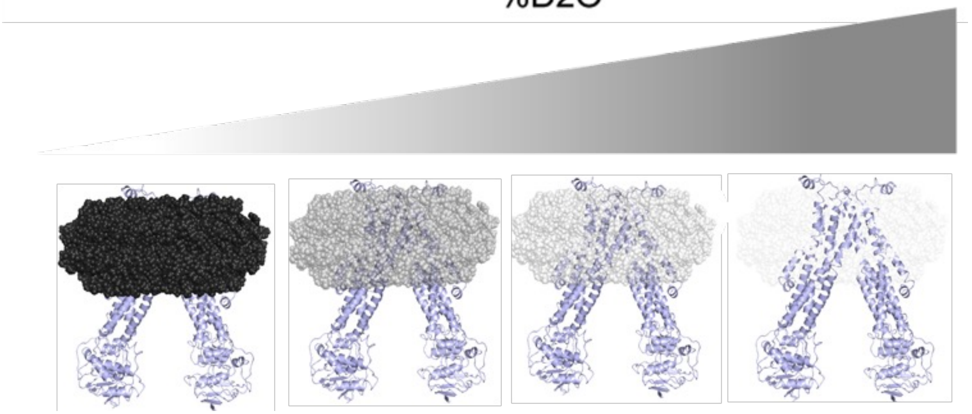
70% d-MSP1D1

Selectively deuterated PC lipids
(78% d-head group, 92% d-tail)

Produced at deuteration lab (ILL)

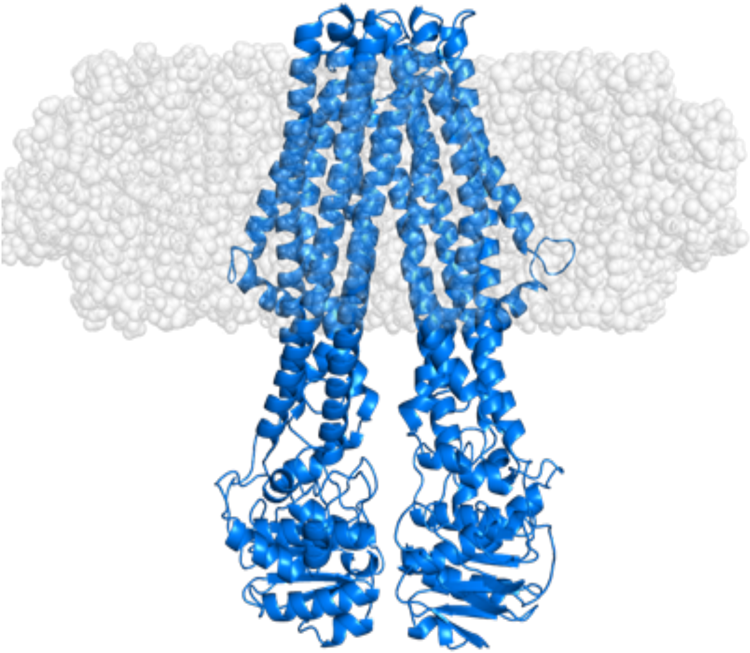


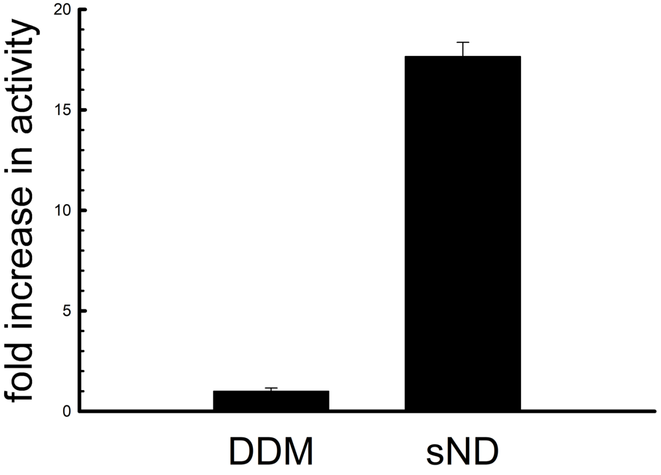


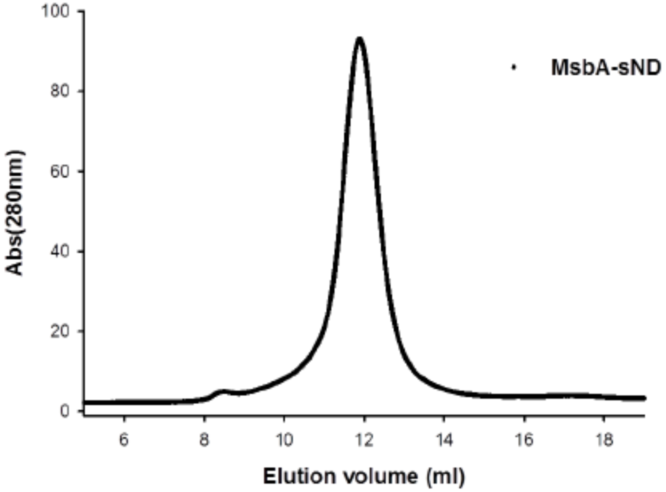


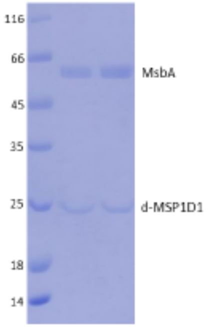
Maric, S. *et al* (2014) Acta Crystallogr D

Incorporation of MsbA in stealth nanodiscs



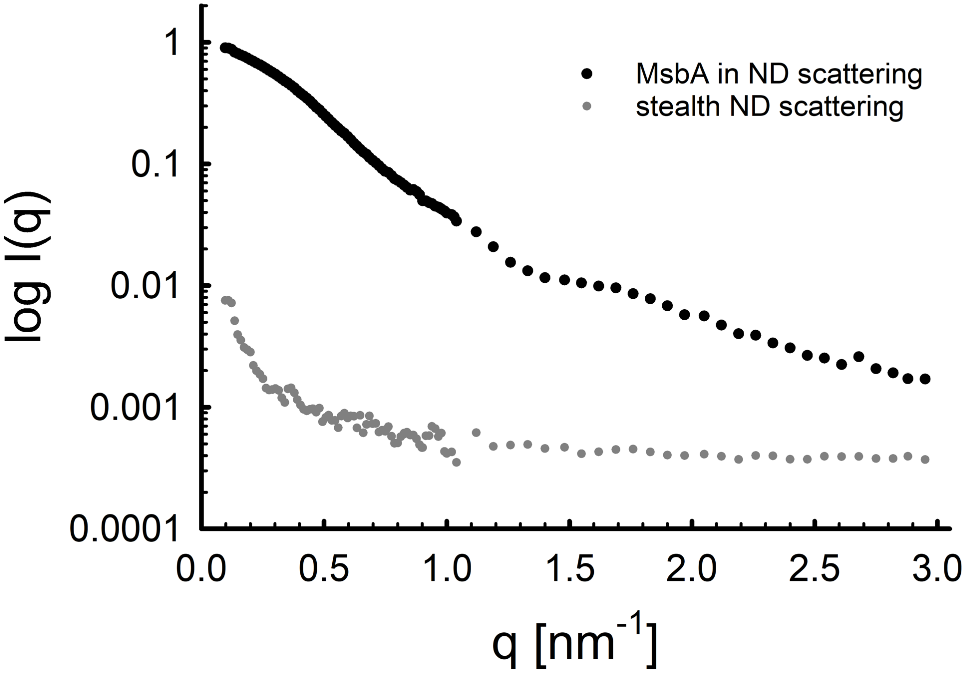


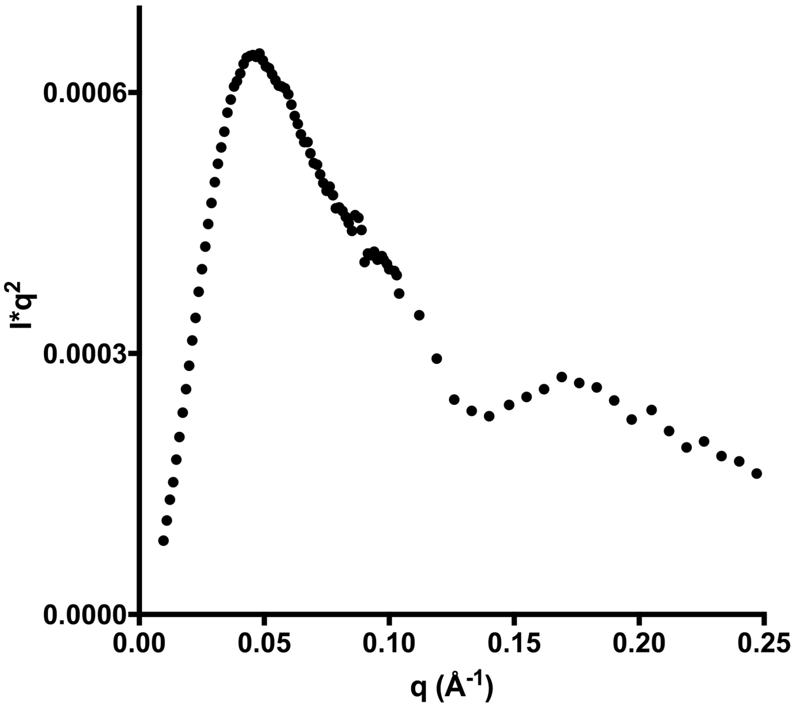




Josts, I. *et al*. (2018) Structure

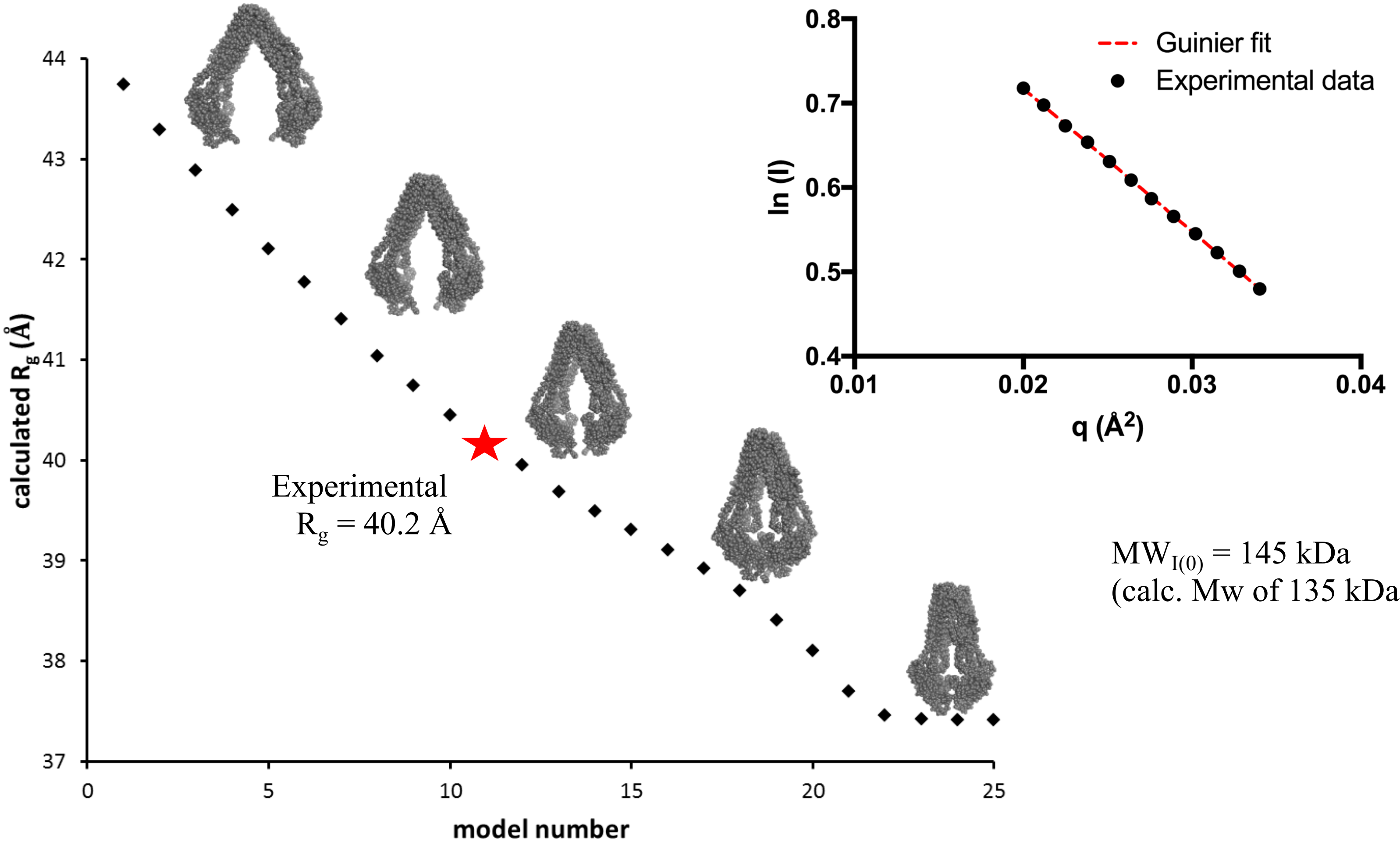
MsbA scatters without the contribution of the lipid nanodisc





Josts, I. *et al*. (2018) Structure

Overall size, shape and conformation of MsbA in solution



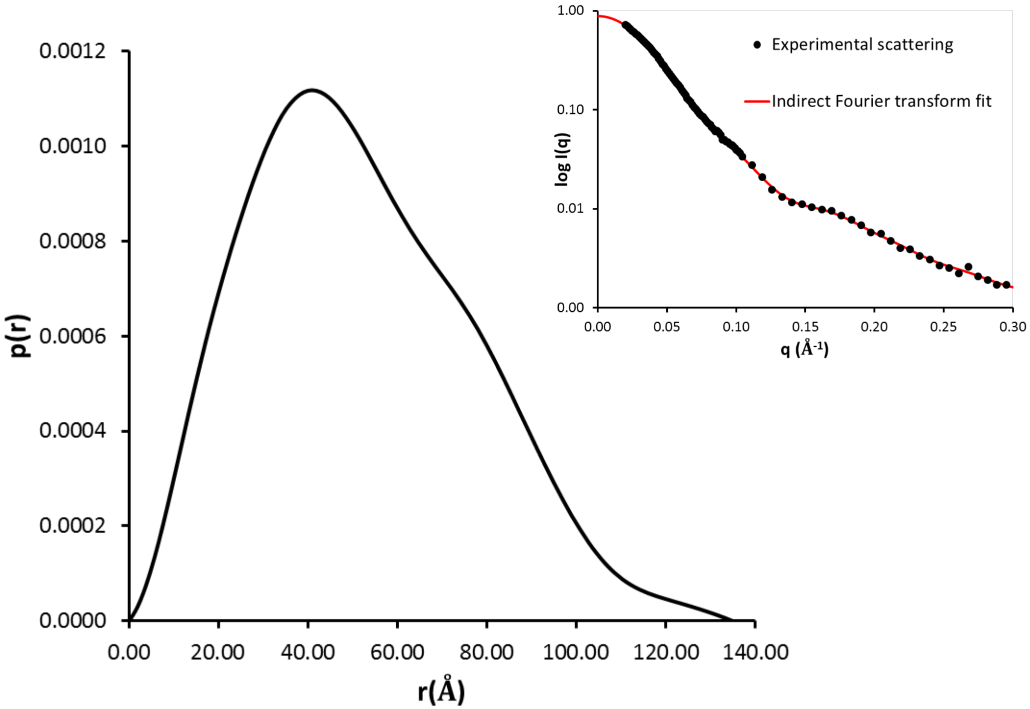
Experimental
R_g = 40.2 Å

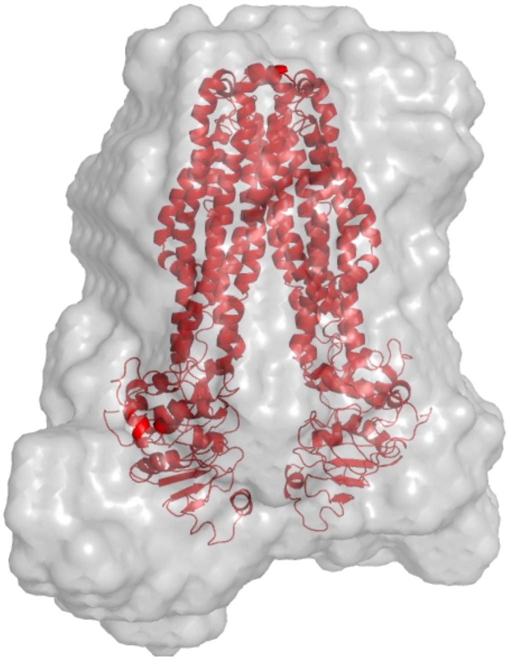
$MW_{I(0)}$ = 145 kDa
(calc. Mw of 135 kDa)

Zheng *et al.* (2007) Proteins

Josts, I. *et al.* (2018) Structure

Overall size, shape and conformation of MsbA in solution

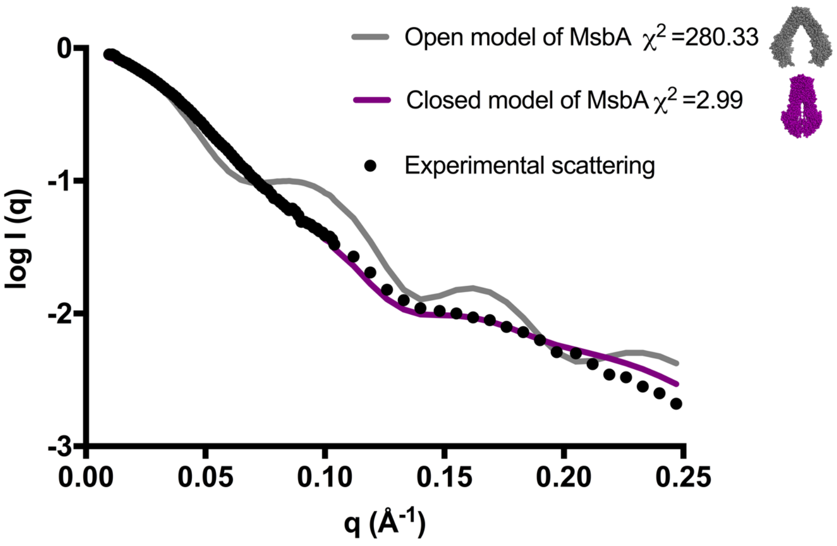


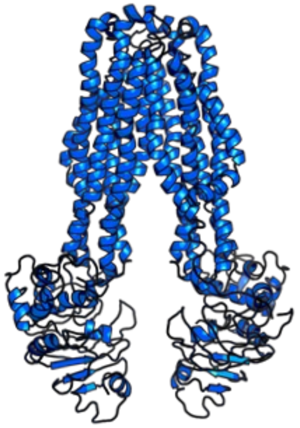


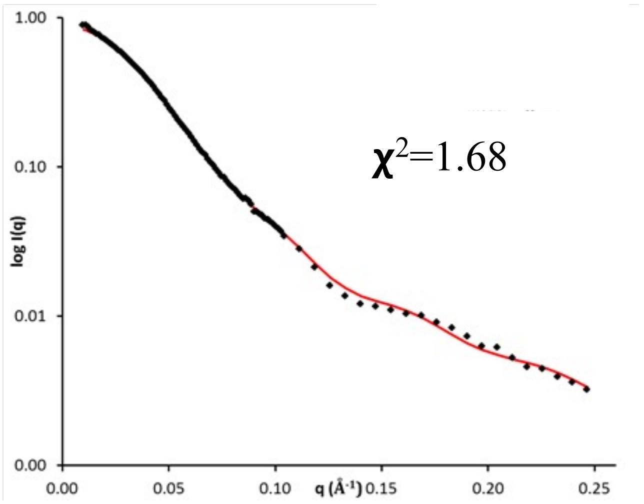
Ab initio model of MsbA

Josts, I. *et al*. (2018) Structure

Rigid body modelling sheds insights into the apo state of MsbA

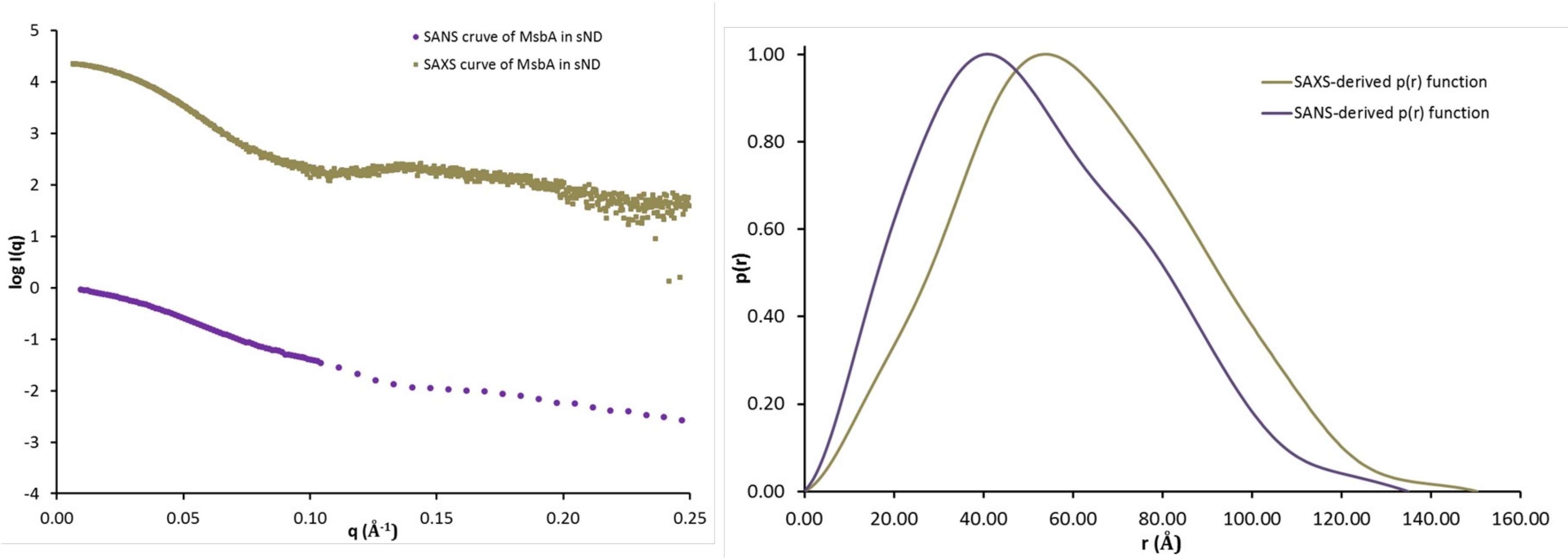






Josts, I. *et al*. (2018) Structure

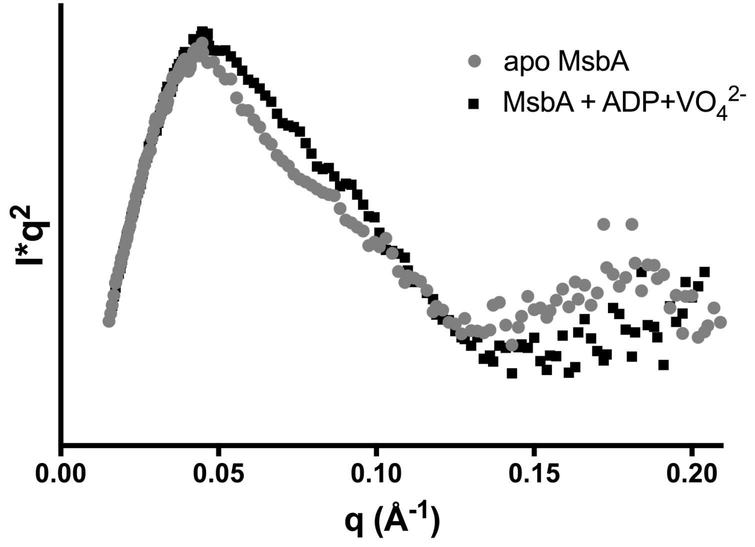
Comparison of neutron and x-ray scattering with IMPs incorporated in stealth carrier nanodiscs



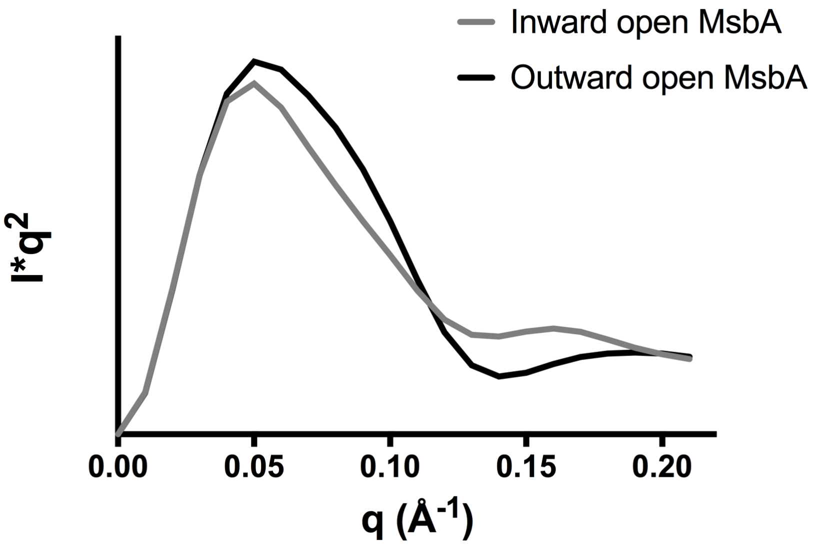
- Comparison of SANS (violet) and SAXS (gold) scattering profiles and distance distribution plots of identical MsbA-sND samples

Josts, I. *et al*. (2018) Structure

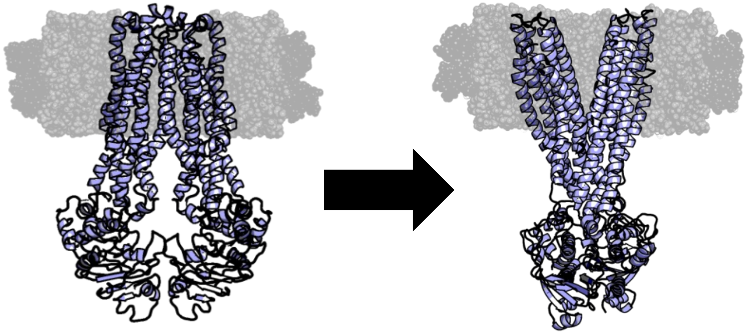
Observing different conformational states of MsbA



Experimental

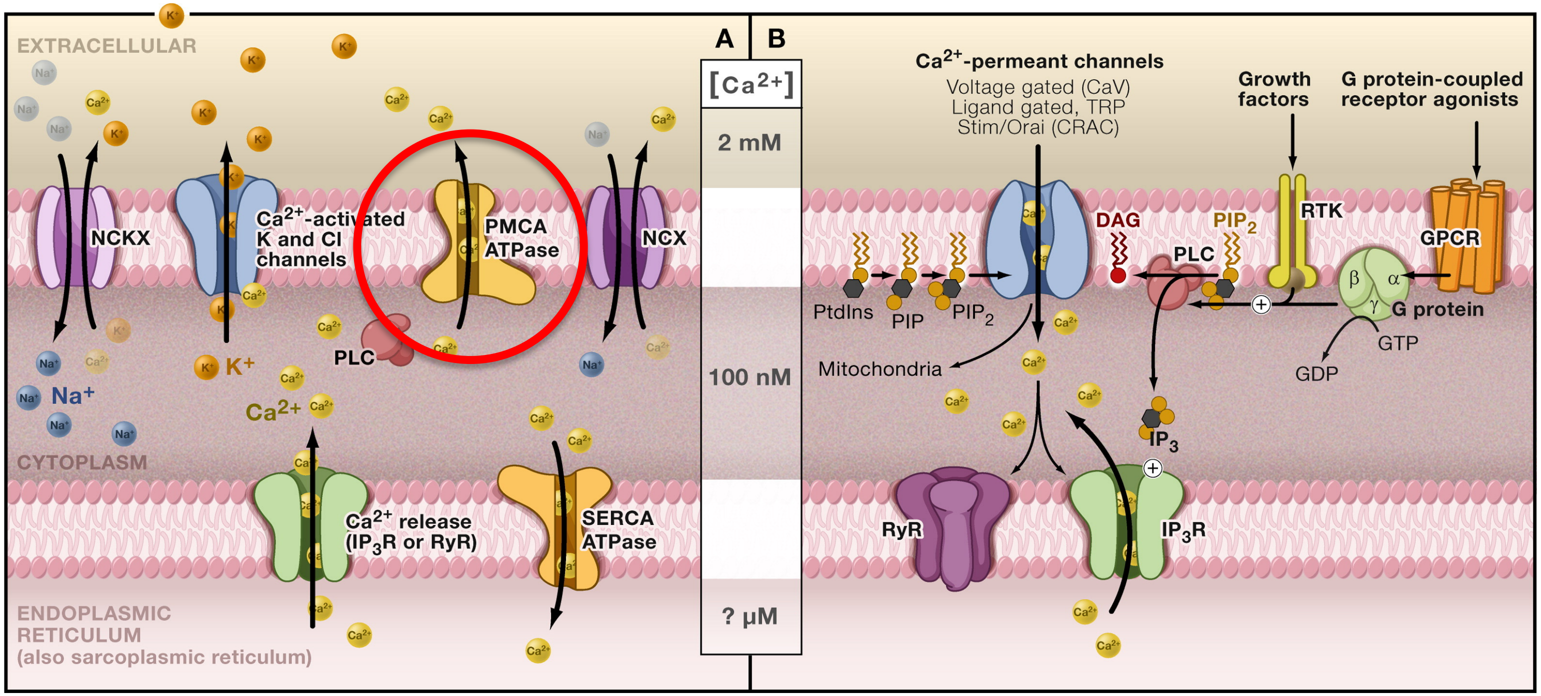


Calculated



Josts, I. *et al*. (2018) Structure

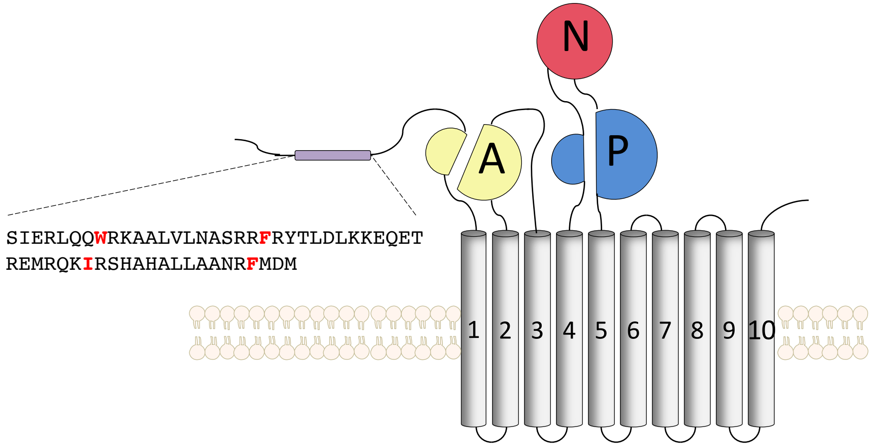
Plasma-membrane Ca^{2+}-ATPase



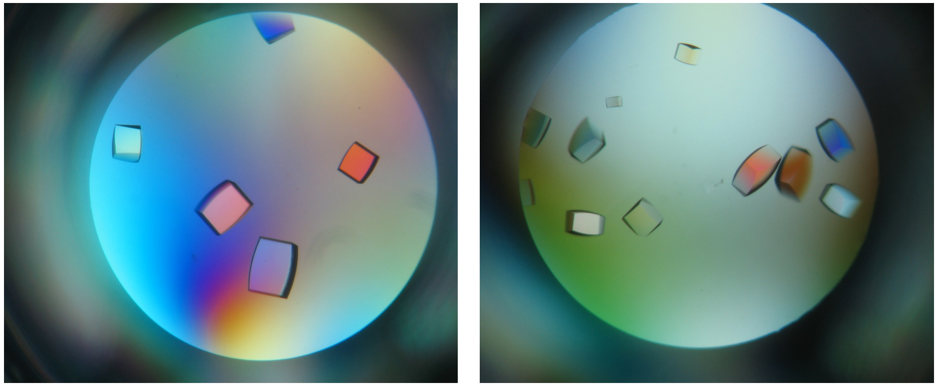
- cytoplasmic Ca^{2+} level is low in resting cells
- excitatory Ca^{2+} signaling network

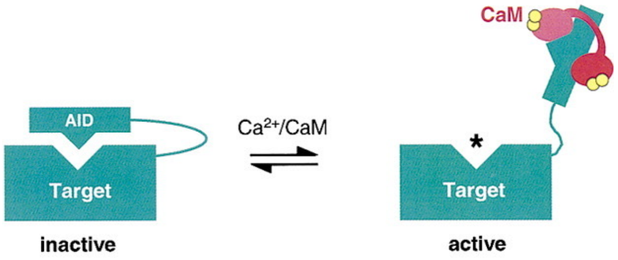
Clapham, D. (2007), Cell

Discovery of a bimodular regulation mechanism in PMCA

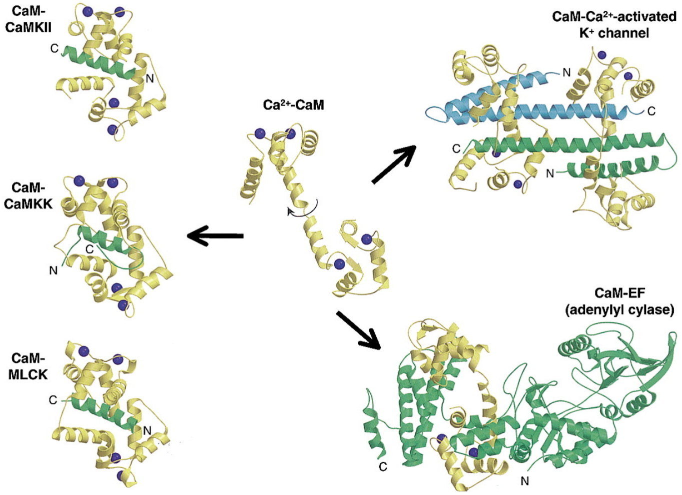


SIERLQQ**W**RKAALVLNASRR**F**RYTLDLKKEQET
REMRQK**I**RSHAHALLAANR**F**MDM





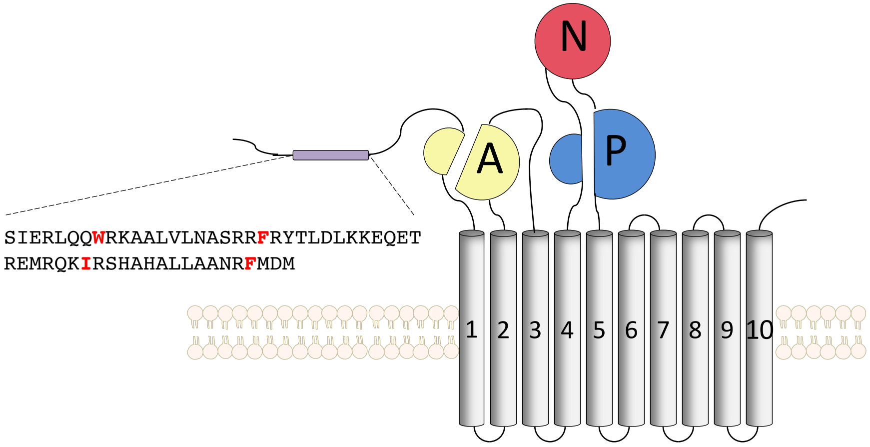
- Relieving autoinhibition



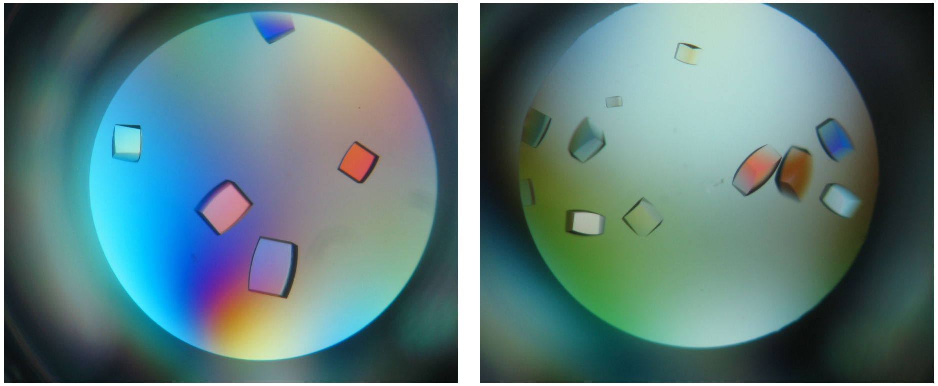
- Structural diversity of calmodulin

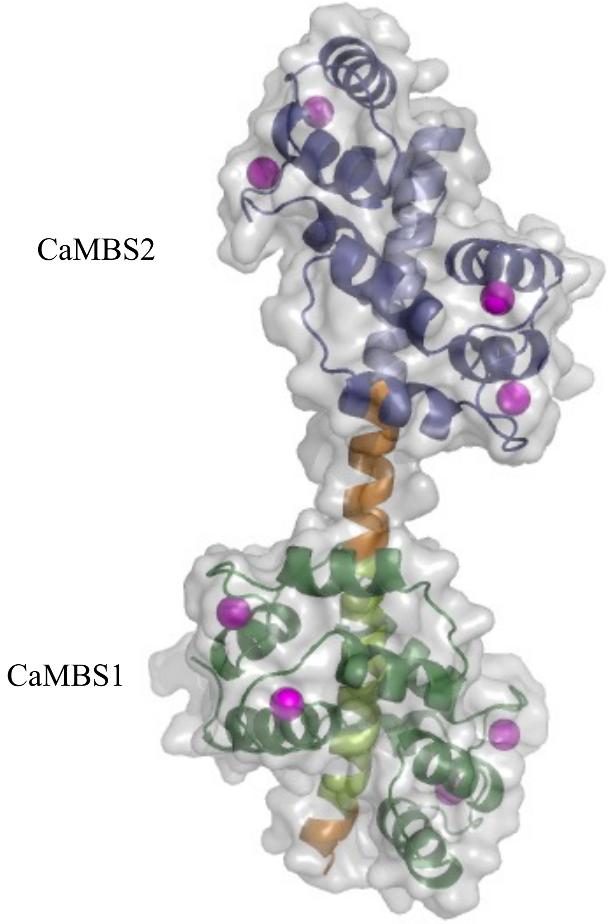
Hoeflich and Ikura (2002), Cell

Discovery of a bimodular regulation mechanism in PMCA



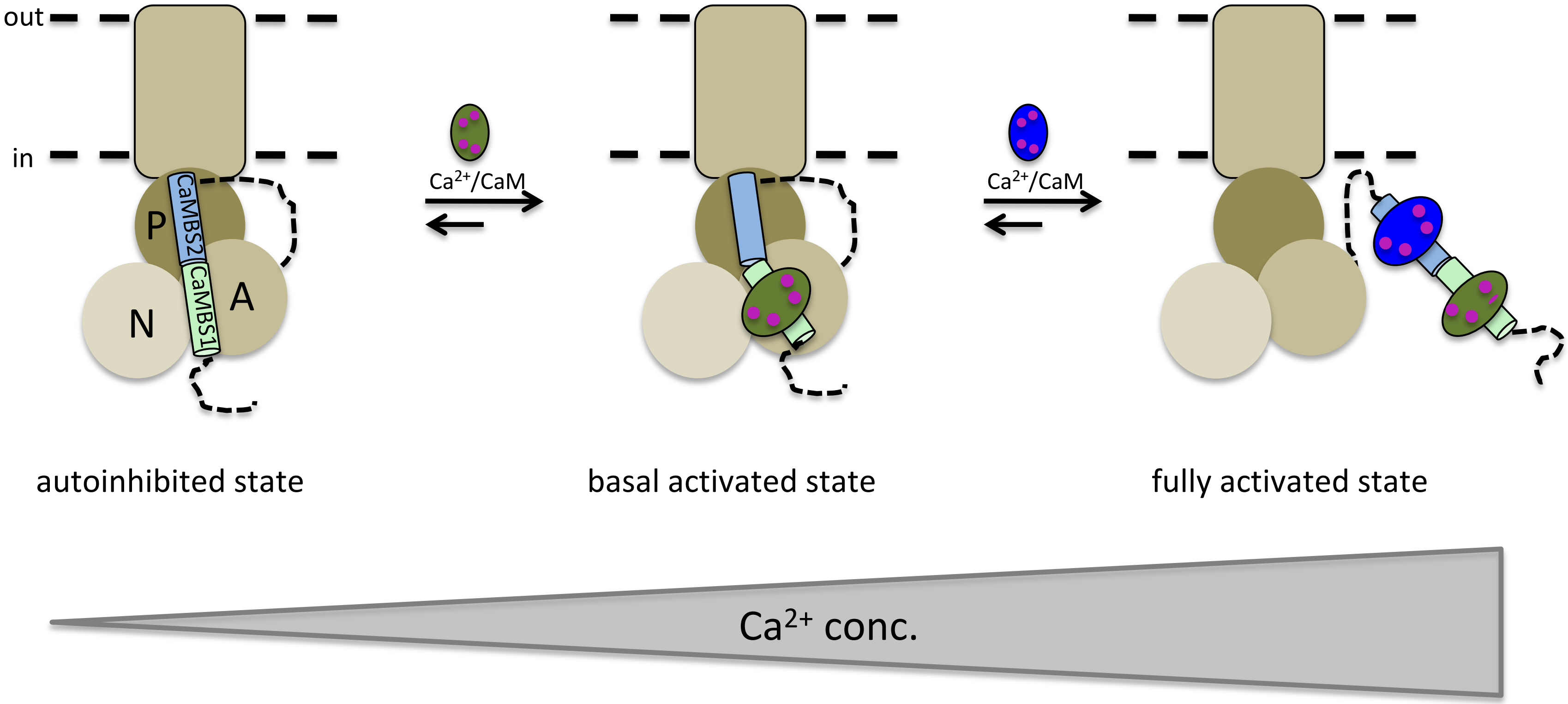
SIERLQQWRKAALVLNASRRFRYTLDLKKEQET
REMRQKIRSHAHALLAANRFMDM





Tidow, H. *et al*. (2012) Nature

PMCA: A bimodular Ca^{2+} sensor for regulation of intracellular Ca^{2+}



autoinhibited state

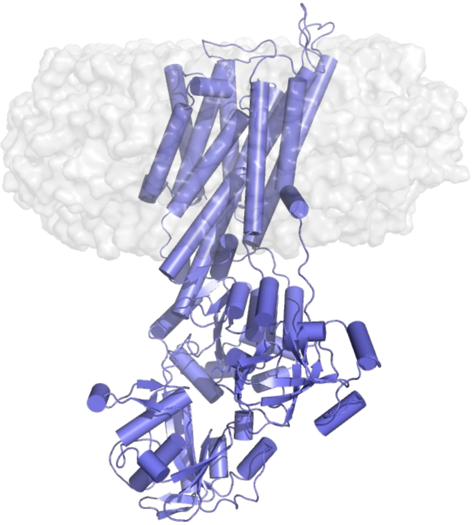
basal activated state

fully activated state

Ca^{2+} conc.

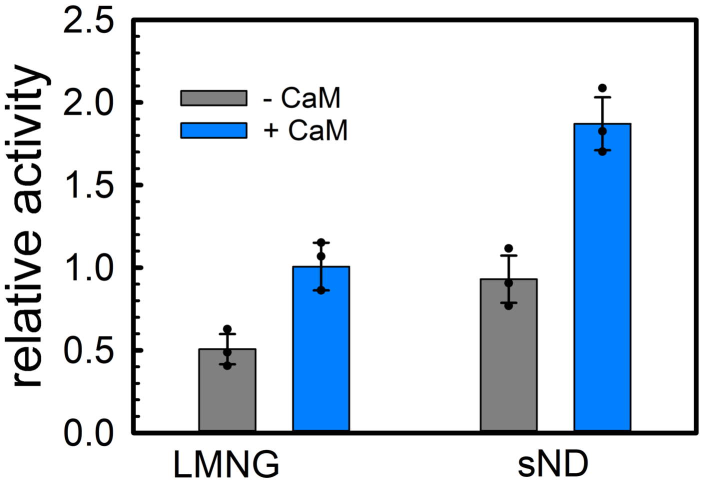
Tidow, H. *et al*. (2012) Nature

Probing conformational changes of ACA8 during activation with CaM

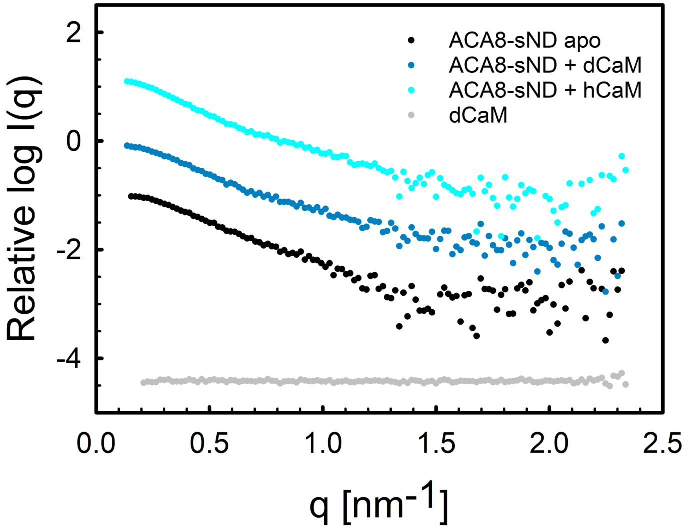


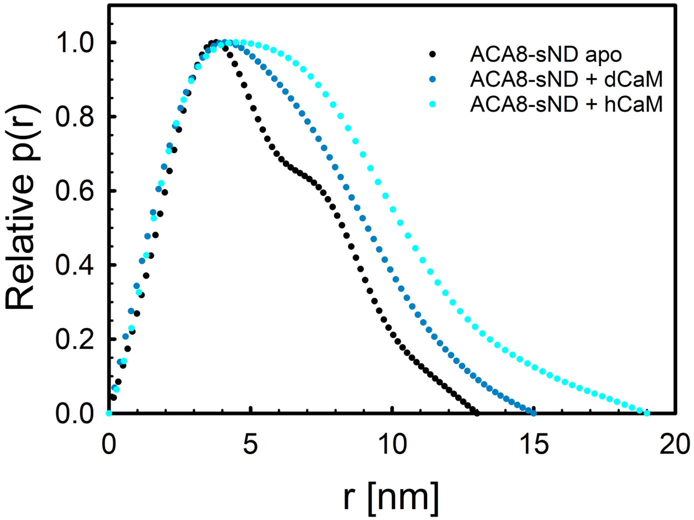
ACA8 in stealth carrier nanodiscs:

- Apo conformation; without CaM
- Fully activated state; with deuterated CaM
  -> only ACA8 is visible
- Fully activated state; with hydrogenated CaM
  -> ACA8 and CaM are visible



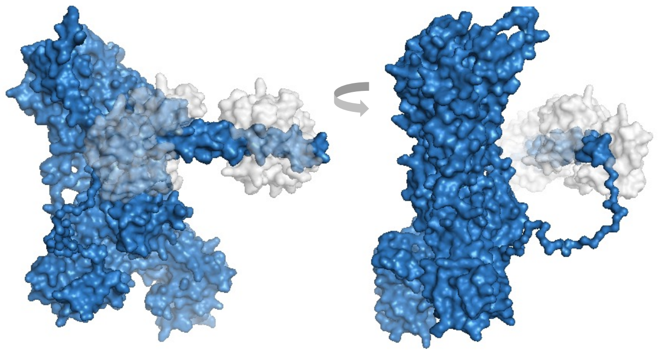
„Invisible“ stealth nanodiscs – PMCA SANS data shows expansion of ACA8 during activation





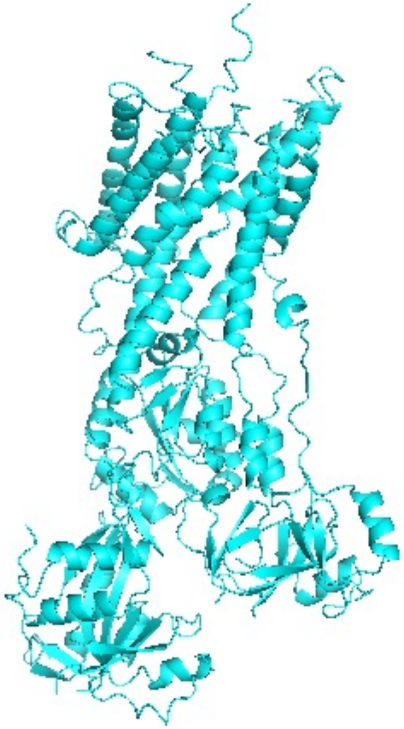
R_g:
ACA8 (apo) = 3.9 nm
ACA8 + dCaM7 = 4.3 nm
ACA8 + hCaM7 = 4.7 nm

D_{max}:
ACA8 (apo) = 13 nm
ACA8 + dCaM7 = 15 nm
ACA8 + hCaM7 = 19 nm

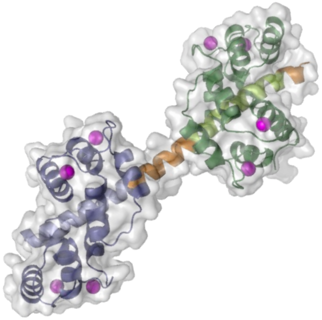


Nitsche, J. *et al*. (2018) Commun Biol

Modelling of ACA8 in its activated state



Homology-model of ACA8 core



Structure of regulatory domain / CaM complex

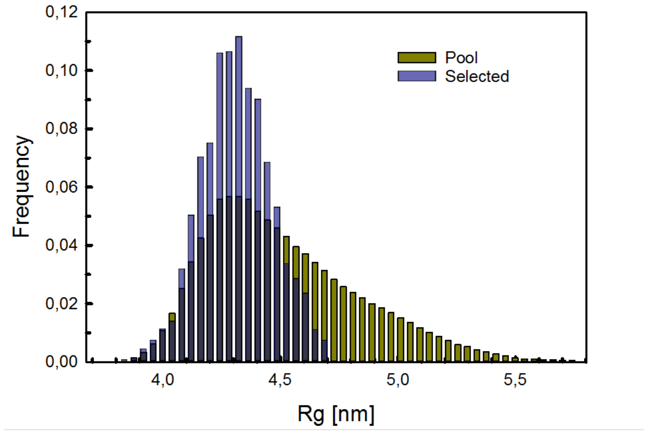
Modelling of ACA8 in its activated state:

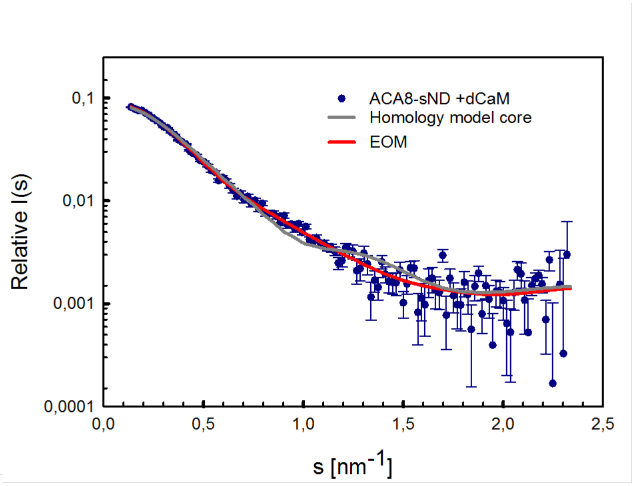
1. Generated pool of 10000 models with the regulatory domain in different conformations

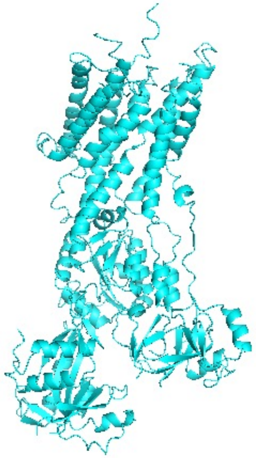
2. Sub-pools of models were used to find ensemble which fits the SANS data the best

Modelling of ACA8 in its activated state

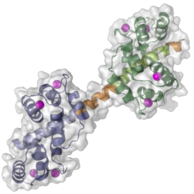
EOM



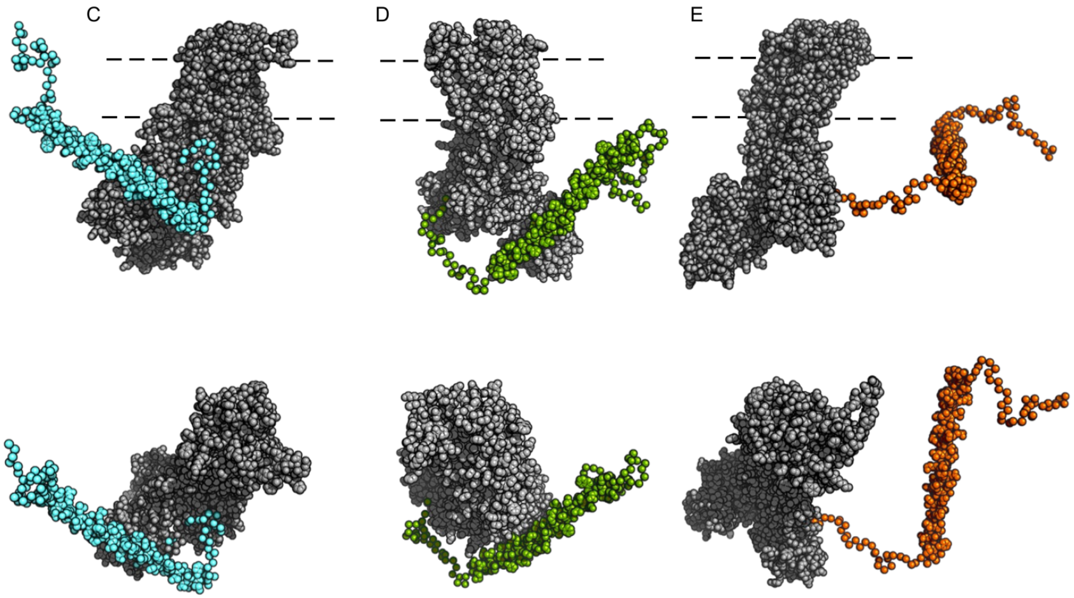




ACA8 core
homology model



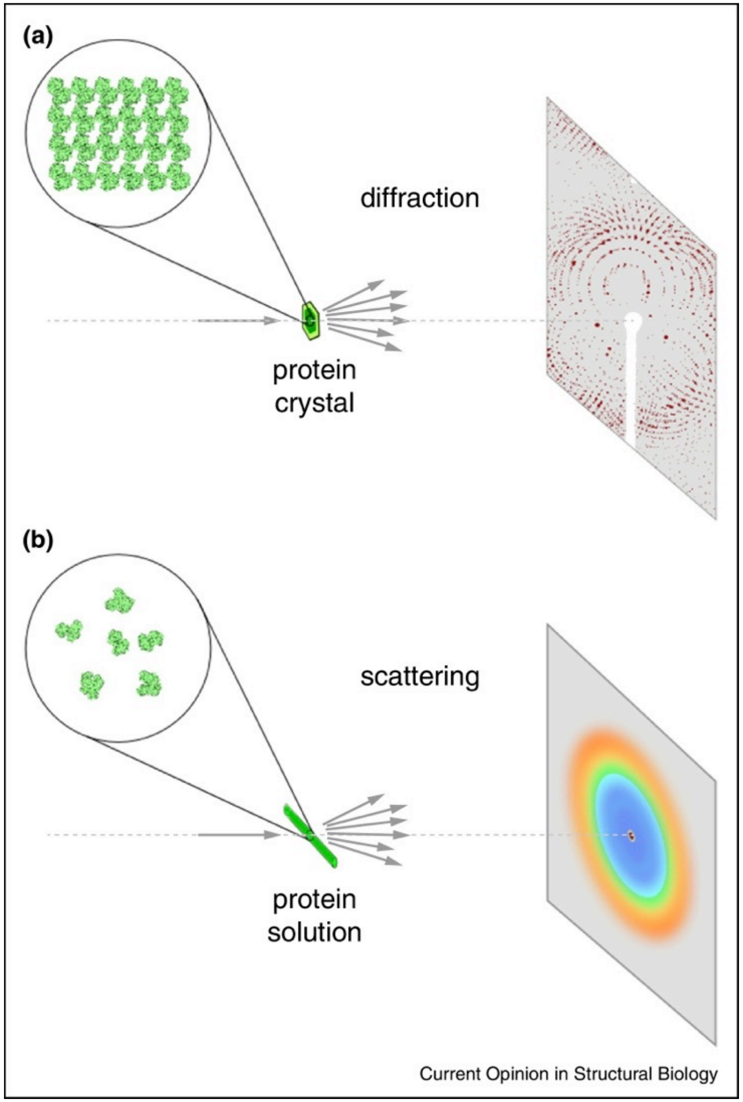
Structure of
ACA8RD/CaM complex

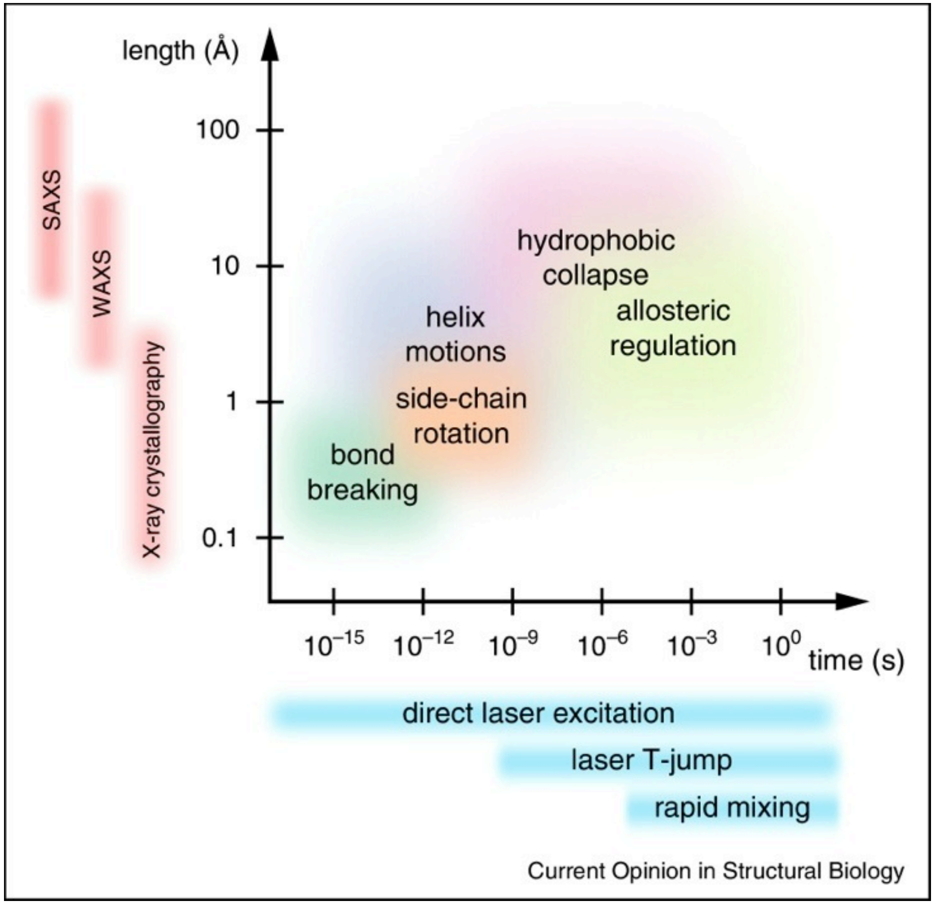


Nitsche, J. *et al*. (2018) Commun Biol

SASBDB: SASDET4

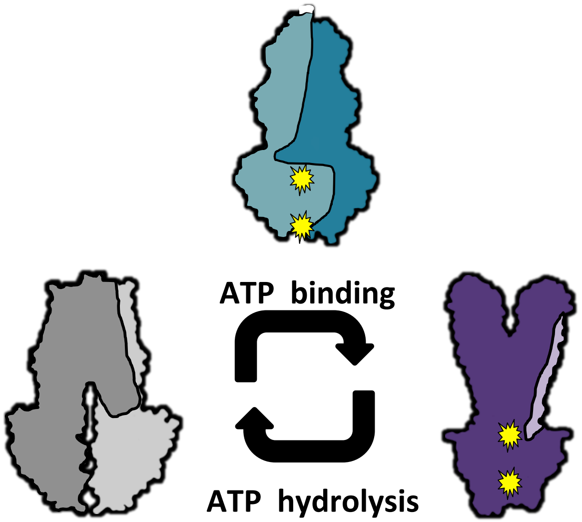
Time-resolved structural biology

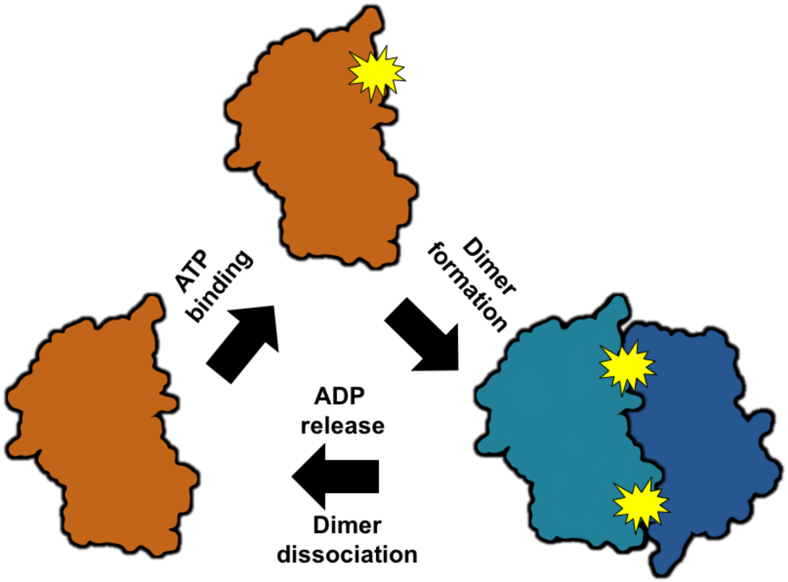




Levantino, M. *et al*. (2015) Curr Opin Struct Biol

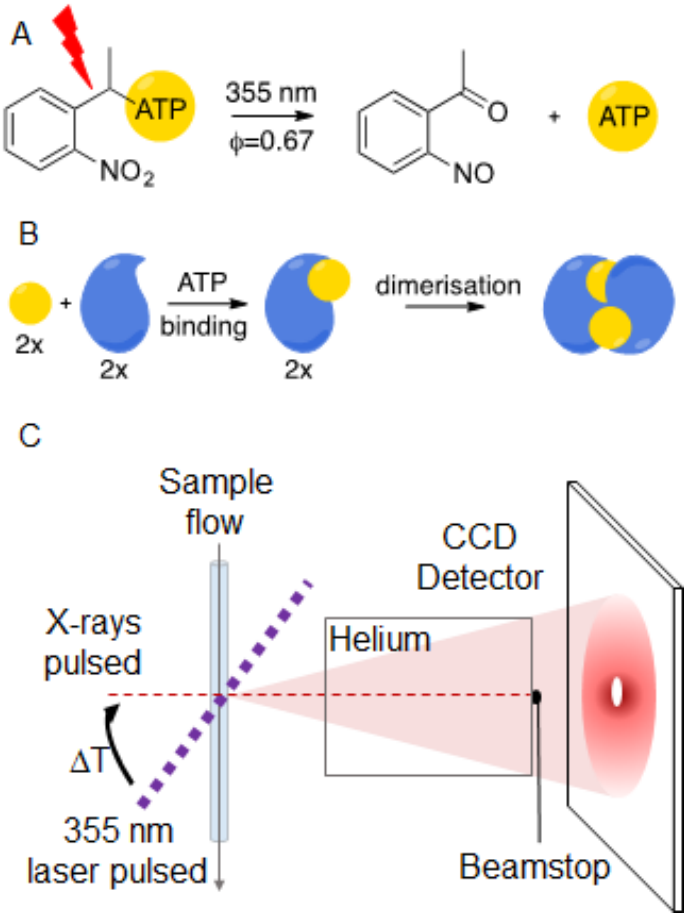
Time-resolved SAXS experiments with MsbA – rapid mixing / stopped-flow

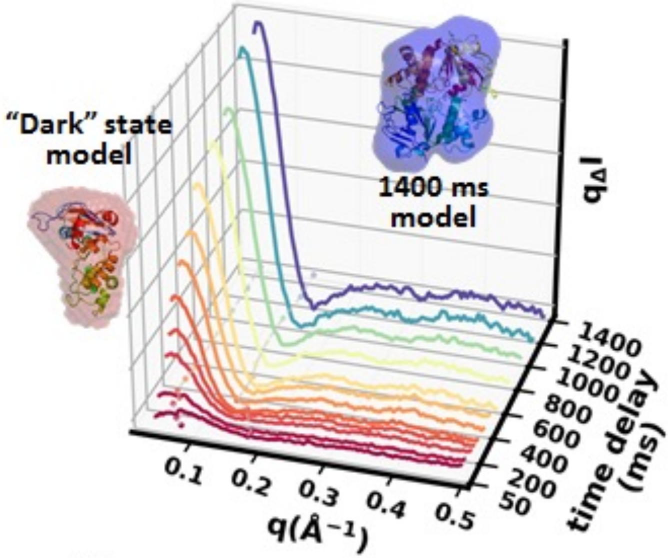


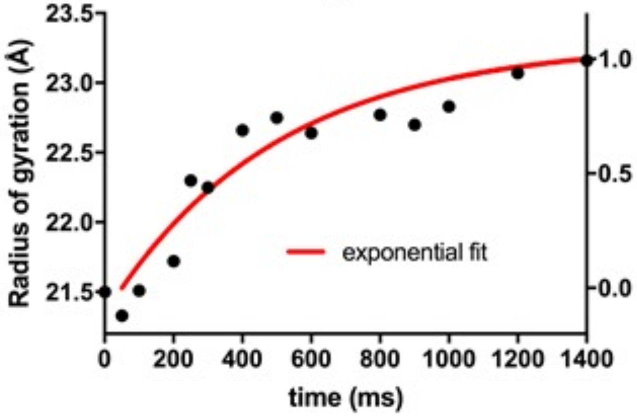


- Full-length MsbA in nanodiscs
- Soluble nucleotide-binding domains

Time-resolved SAXS experiments with MsbA NBD – light-activation

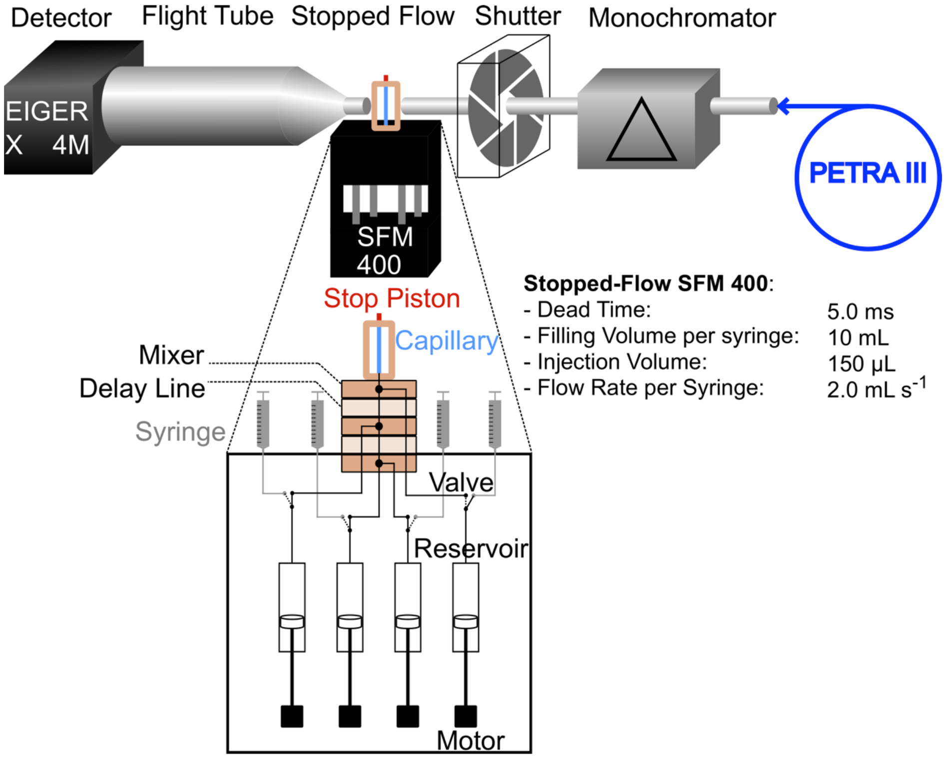






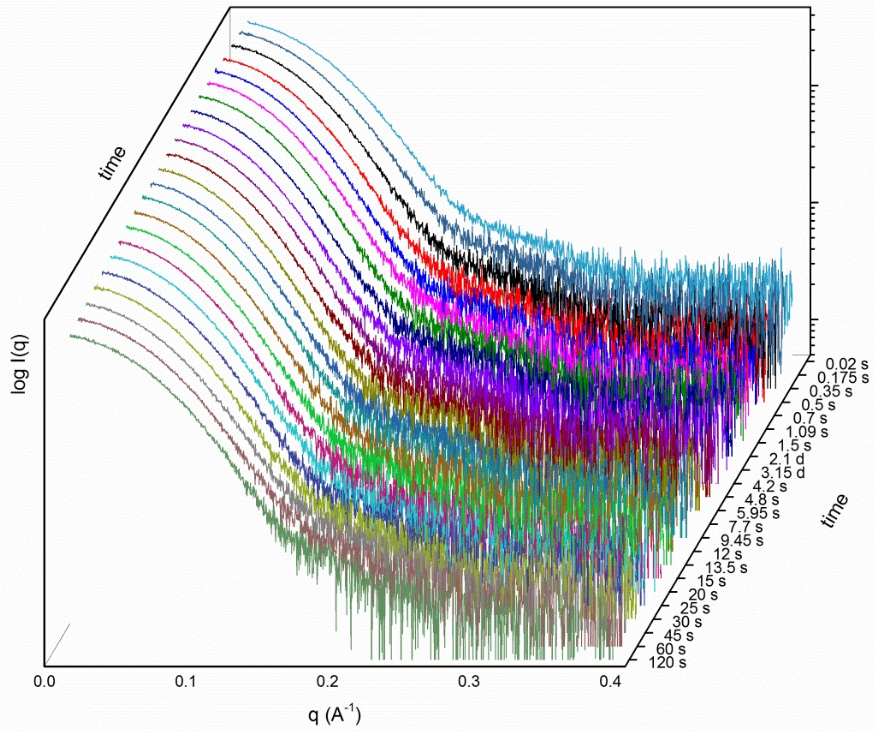
Josts, I. *et al*. (2018) IUCrJ

Time-resolved SAXS experiments with MsbA – rapid mixing / stopped-flow



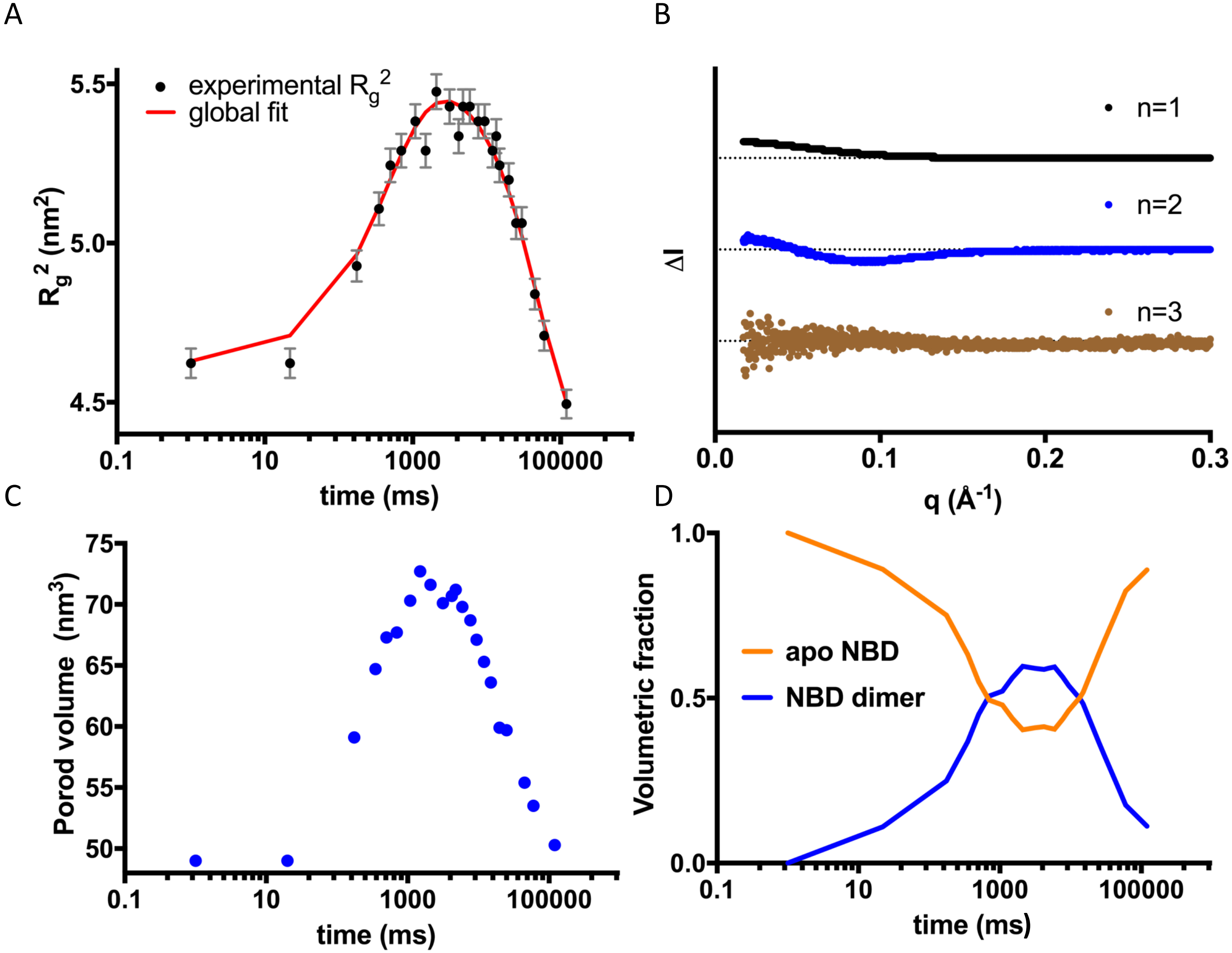
Stopped-Flow SFM 400:

- Dead Time:	5.0 ms
- Filling Volume per syringe:	10 mL
- Injection Volume:	150 µL
- Flow Rate per Syringe:	2.0 mL s^{-1}



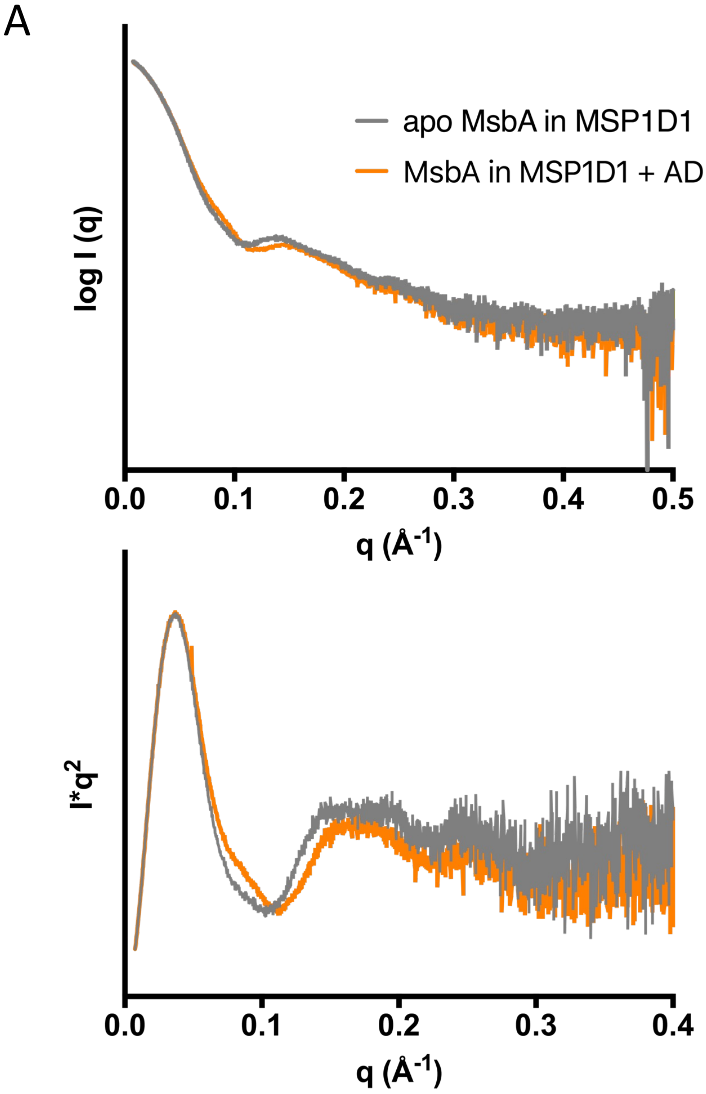
Josts, I. *et al*. (2019) Structure

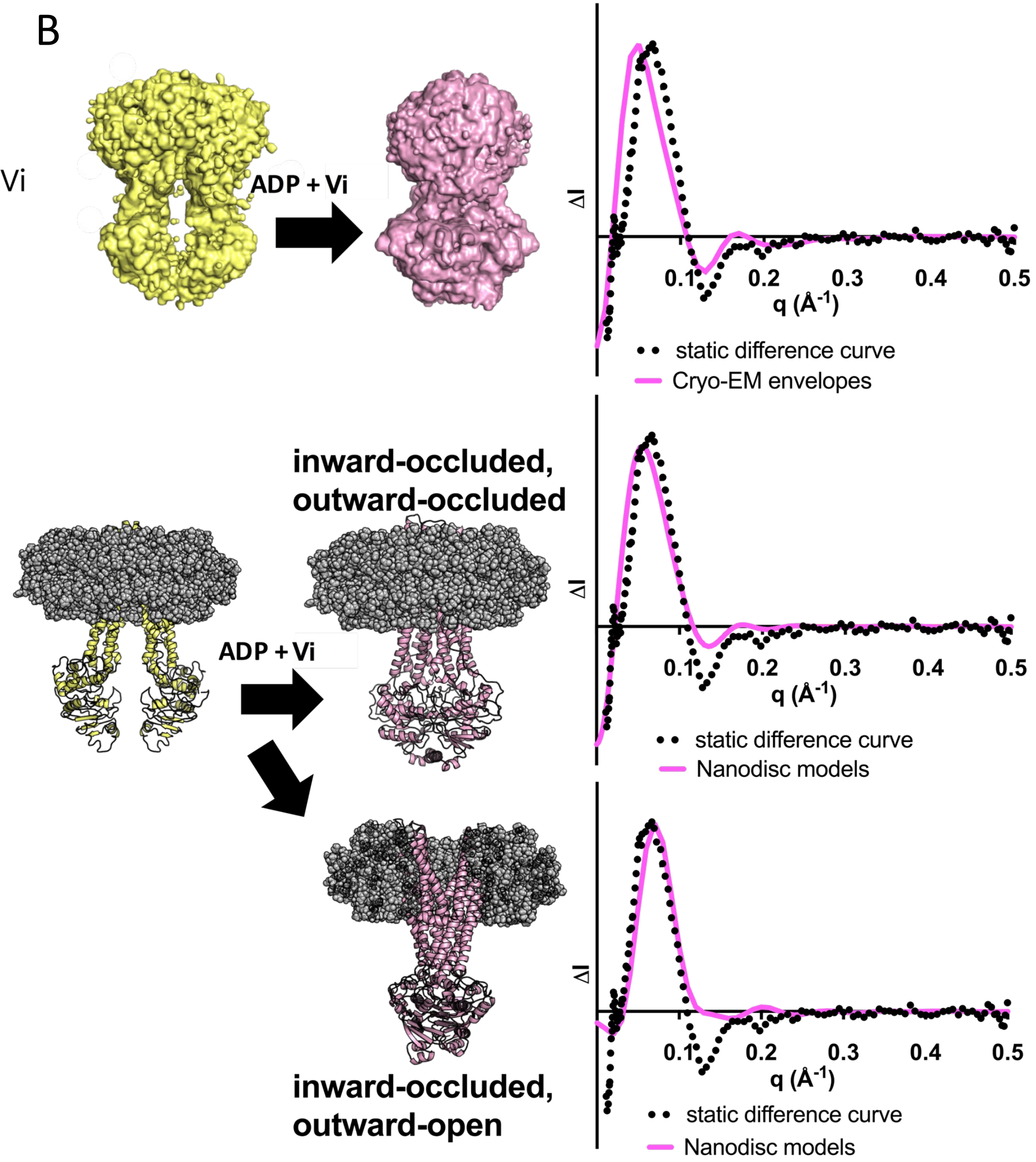
TR-SAXS reveals the structural kinetics of ATP-driven NBD dimerization and subsequent dissociation of isolated NBD domains



Josts, I. *et al*. (2019) Structure

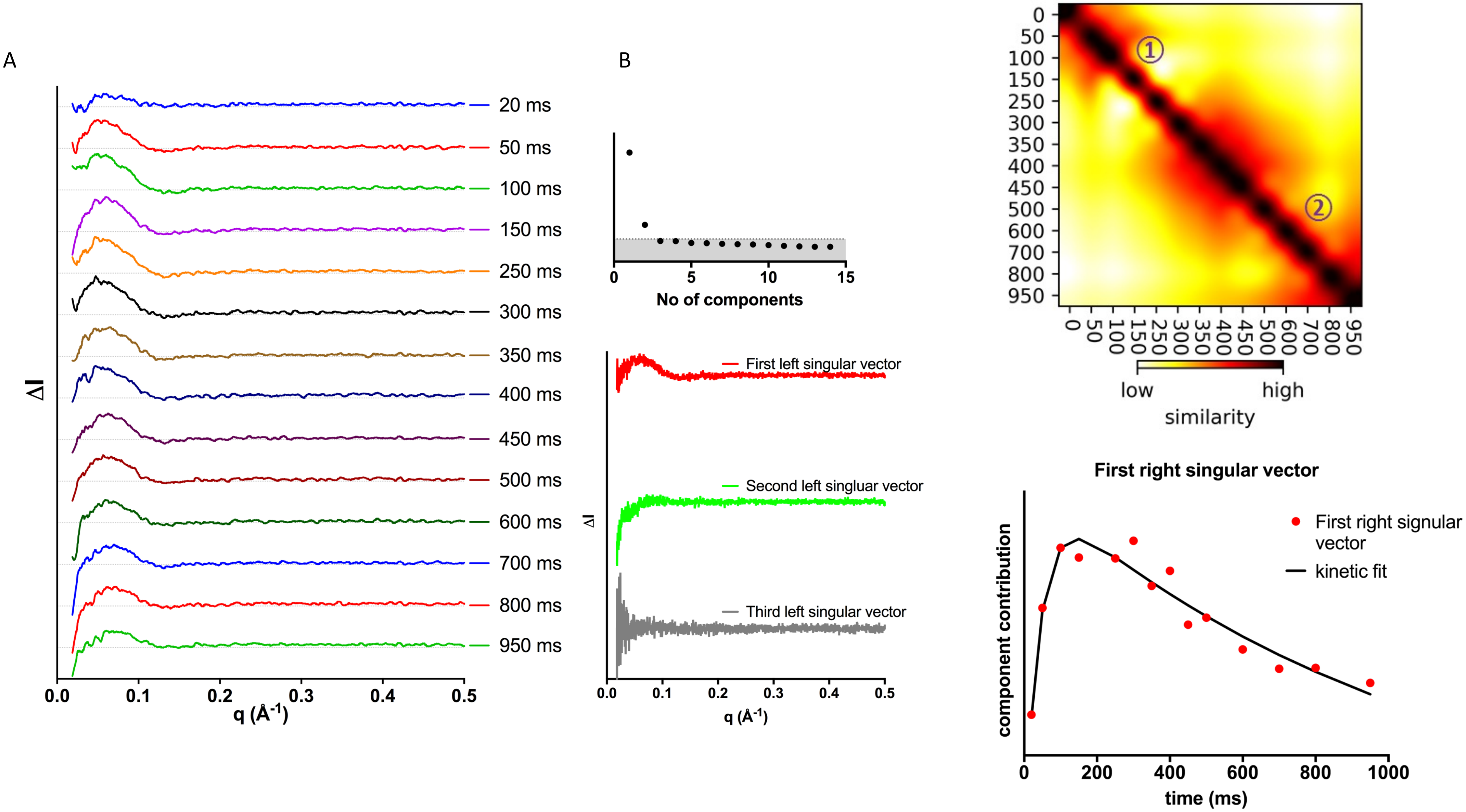
Static SAXS curves reveal conformational changes reflecting the formation of an inward-occluded state of MsbA (ADP-vanadate)





Josts, I. *et al*. (2019) Structure

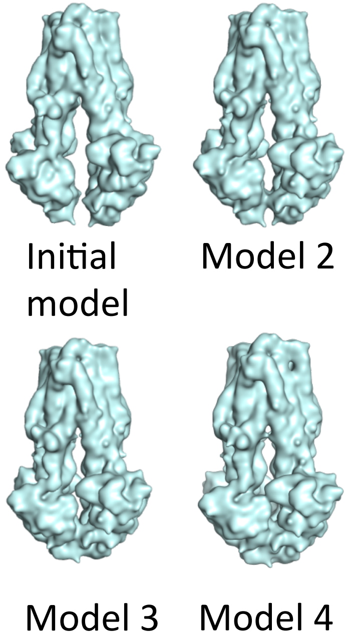
Time-resolved difference scattering curves suggest fast conformational changes in full-length MsbA (in nanodiscs) in response to Mg^{2+}-ATP binding

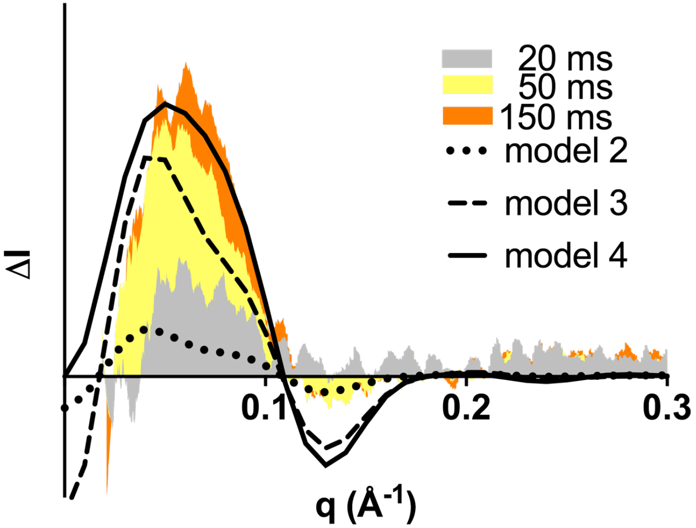


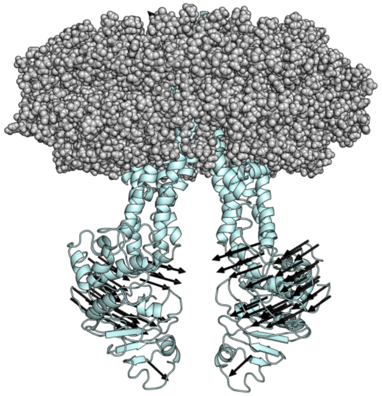
Josts, I. *et al*. (2019) Structure

Normal mode analysis of MsbA conformations suggests the closing of the cytoplasmic chamber through the formation of a closed NBD dimer

Surface representation of models derived from NMA







Sample	Dimerization	Dissociation
MsbA NBD + ATP	**500 ± 100 ms**	**45 ± 10 s**
full-length MsbA in nanodiscs + ATP	**50 ± 30 ms**	**1 ± 0.4 s**

Josts, I. *et al*. (2019) Structure

Conclusions

- Stealth nanodiscs are a useful tool for studying membrane proteins in a native-like lipid environment using SANS in solution
- SANS data using stealth nanodiscs can gain valuable structural information about conformational changes and flexible systems
- Future work on stealth nanodisc and other carrier systems could help to investigate effects of different lipids to the overall conformation
- Time-resolved SAXS experiments with low-ms resolution are possible on IMPs in lipidic environment

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- D11, D22 @ ILL
- ID30B @ ESRF
- ID09 @ ESRF
- MX14.1 @ BESSY

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