Dynamics of a bacterial multidrug ABC transporter using hydrogen/deuterium exchange

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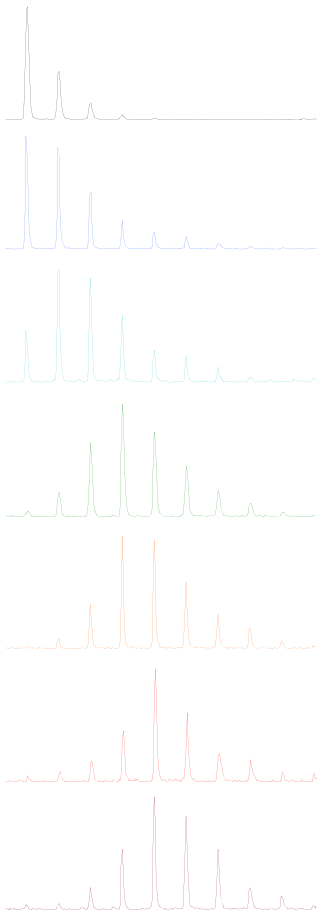
Protein amide hydrogen/deuterium exchange MS

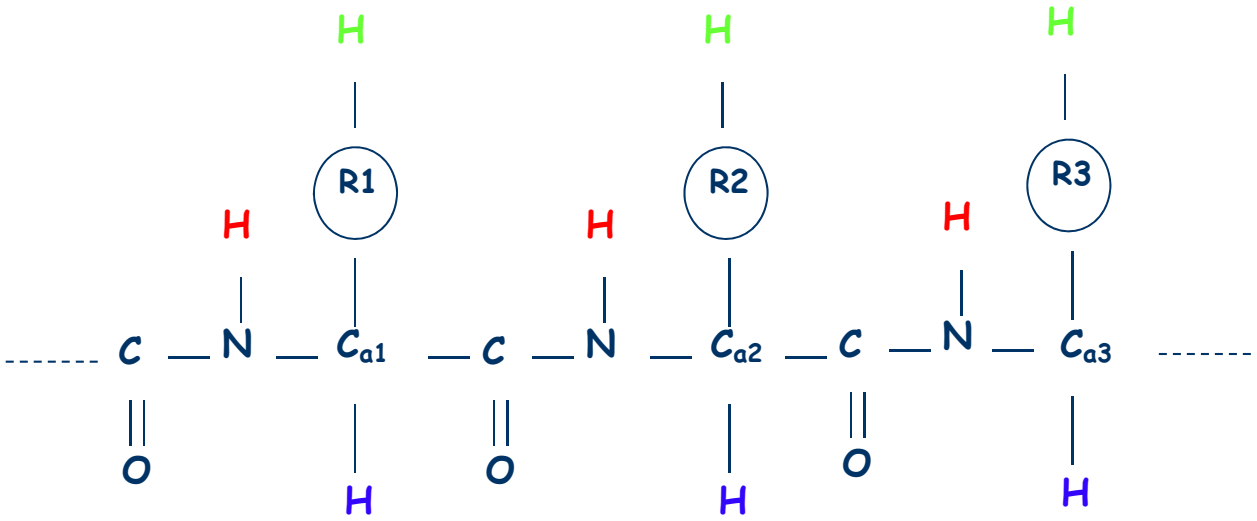
Protein characterization:
interplay of structure, function and dynamics

- conformational changes (ligand, PTM, etc)
- local stability or proper folding
- dynamic picture of activation processes
- interactions (ligand, antigen, membrane, etc)
  e.g. epitopes

Mass spectrometry features:
- sensitivity
- large proteins or complexes
- close to physiological conditions
- resolution
- relatively short time scales



Different H in proteins

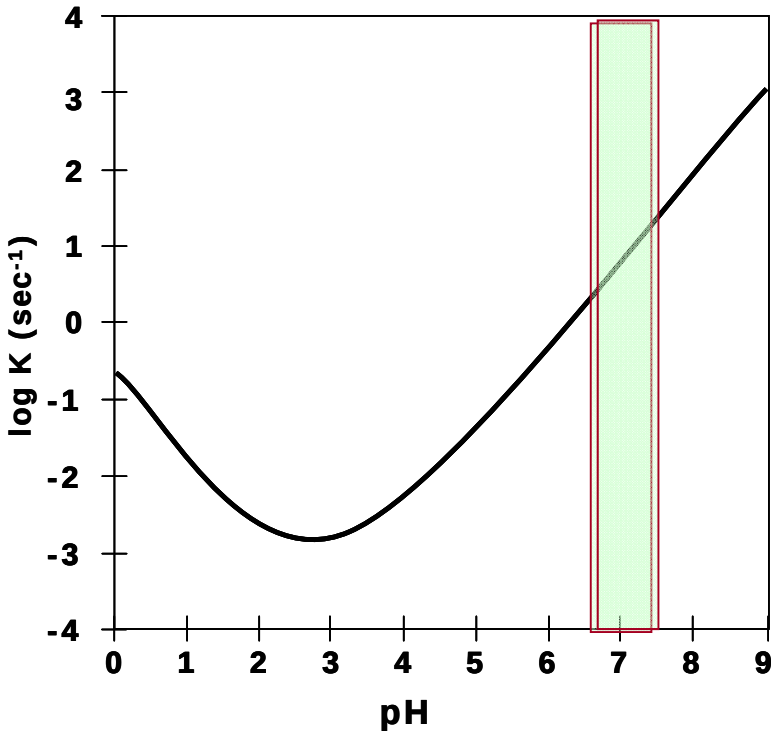


Side chains: very rapid exchange rate

C-H: no exchange

CONH: exchange rate depends on structure and accessibility

Experimental parameters



1 pH unit decrease = 10 times slower exchange

10 °C decrease = 3 times slower exchange

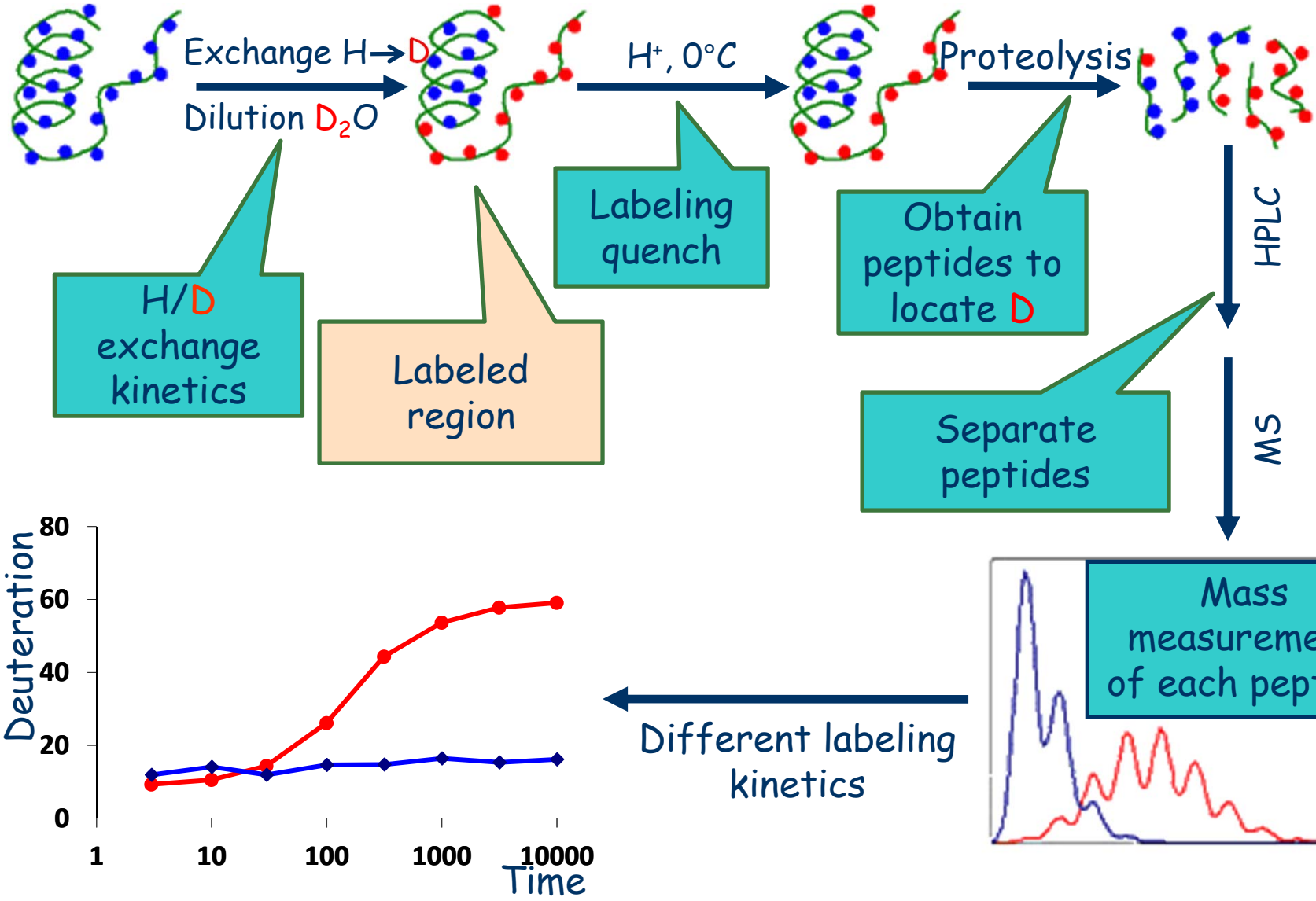
Influence of pH on exchange rate

Exchange conditions

Bai et al 1993 Proteins

Quench conditions

General scheme



Principle

- Conformational changes:

Mass difference for a specific peptide from the protein under different forms:

⟺ difference of deuterium exchange for this peptide

⟹ local conformational change;

- **Interface identification**:

Mass decrease for a peptide from the protein between the free and the complexed form:

⟺ decrease of deuterium exchange for this peptide

⟹ this region is implied in the interface with the partner.

Membranes proteins and ABC transporters

- 1/3 of the human genome encodes membrane proteins

Marketed small-molecule drug targets: 2/3 are membrane proteins

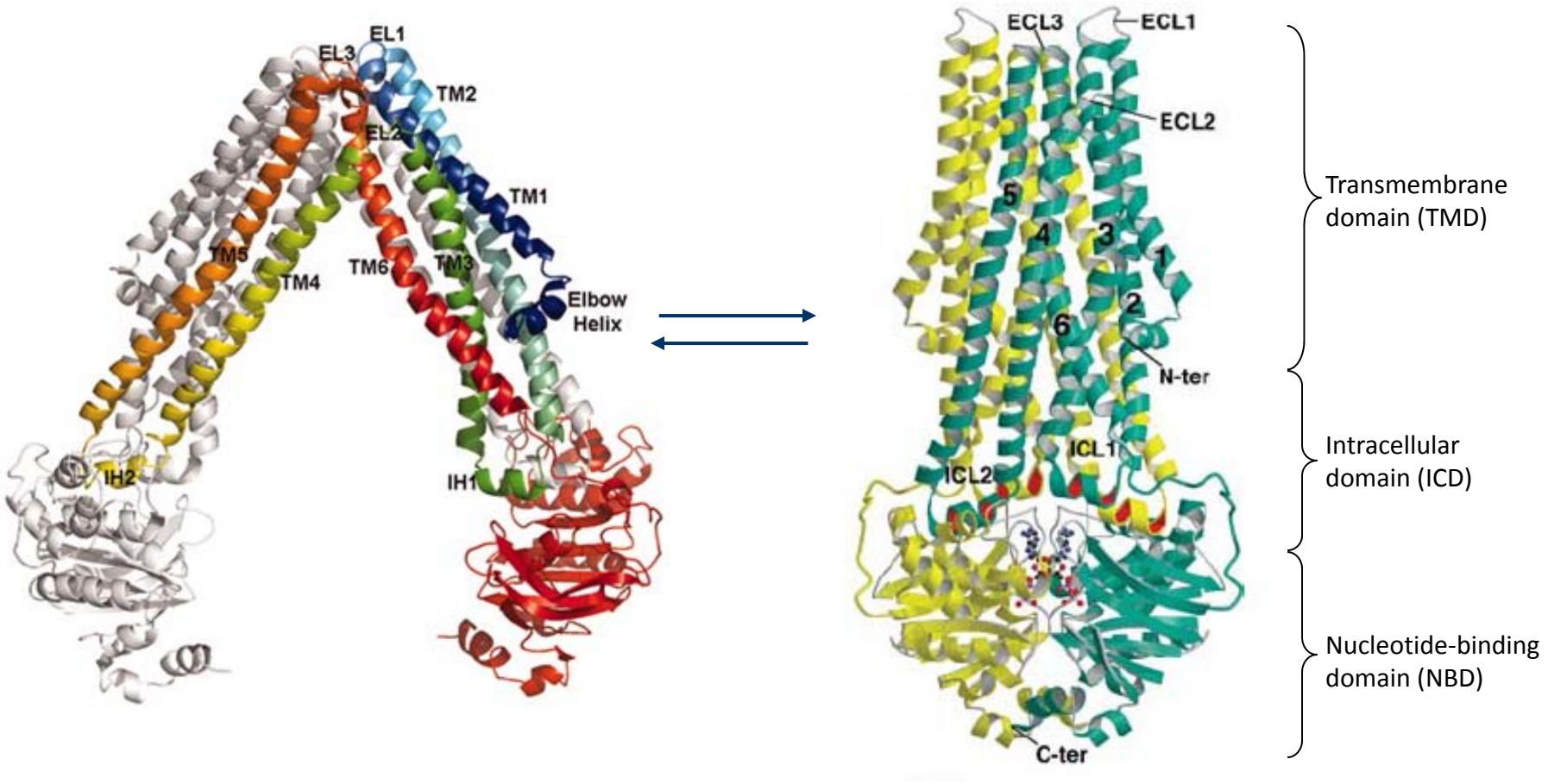
GPCR, transporters, ion channels, receptors, etc

- ABC transporters: importers or exporters of many substrates

  ++ Nutrients import and detoxification

  - - Antibiotics and chemotherapy resistance

Structures of ABC exporters



ABC exporter MsbA ‘open’

Ward et al 2007 PNAS

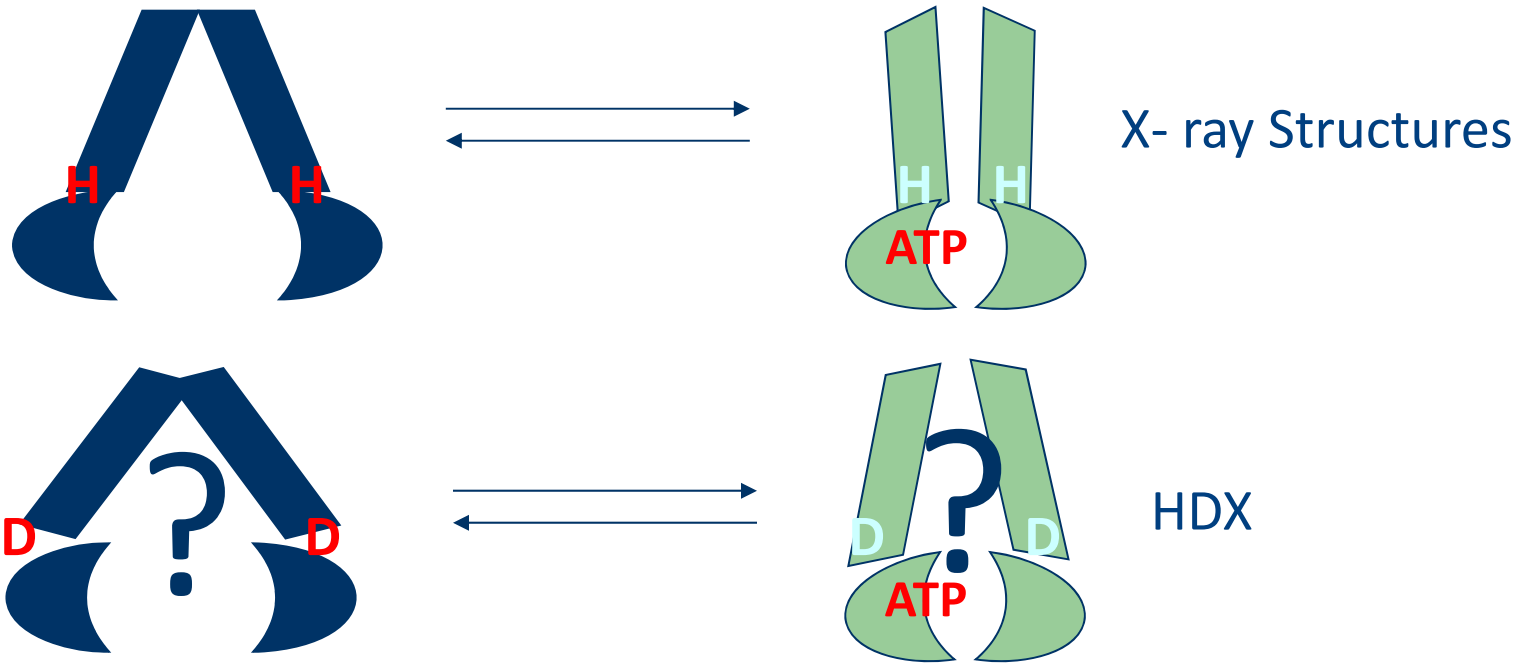
ABC exporter Sav1866 ‘closed’

Dawson and Locher 2006 Nature

Objective of the study

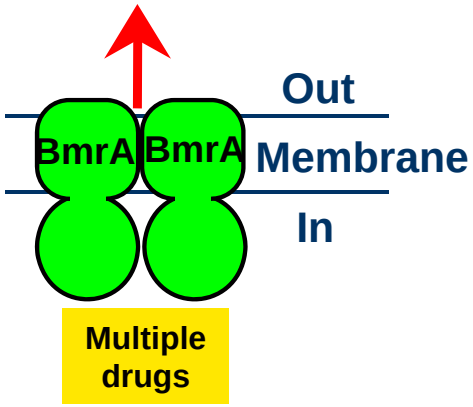
X-ray structures of ABC exporters show a rigid body motion of both subunits to hydrolyze ATP:

interaction between TMD & NBD similar in open or closed state



Dynamics of an ABC transporter in two extreme conformations by HDX?

BmrA

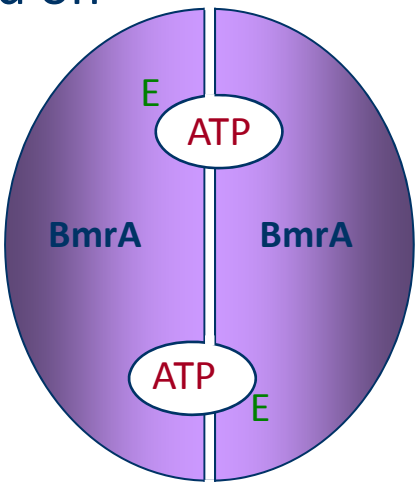


- BmrA is a homodimer ABC exporter from Bacillus subtilis

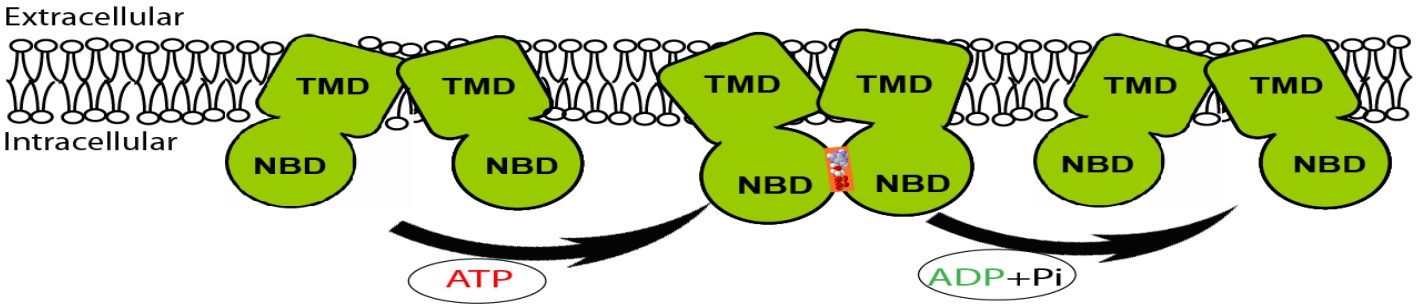
- Like other ABC transporters, BmrA exports multiple drugs out of the cell in vivo, hence providing ***M***ultiple ***D***rug ***R***esistance properties

- Open & closed 3D models of BmrA are available (based on MsbA & Sav1866)

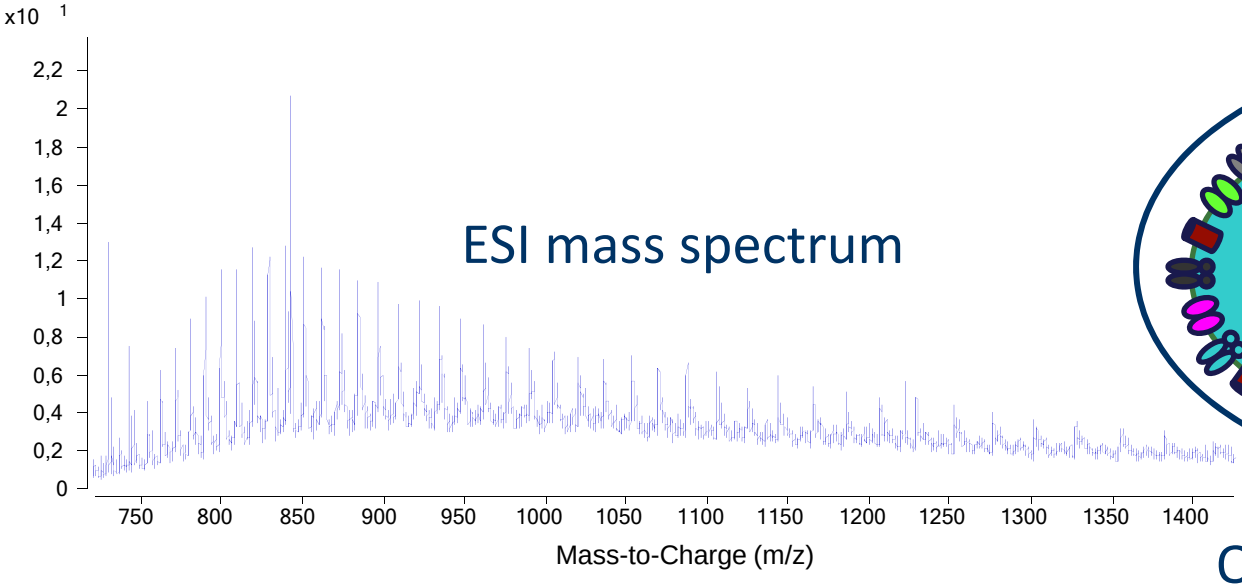
- Conserved E504 directly involved in ATP hydrolysis (presumably as catalytic base) Orelle et al 2003 JBC



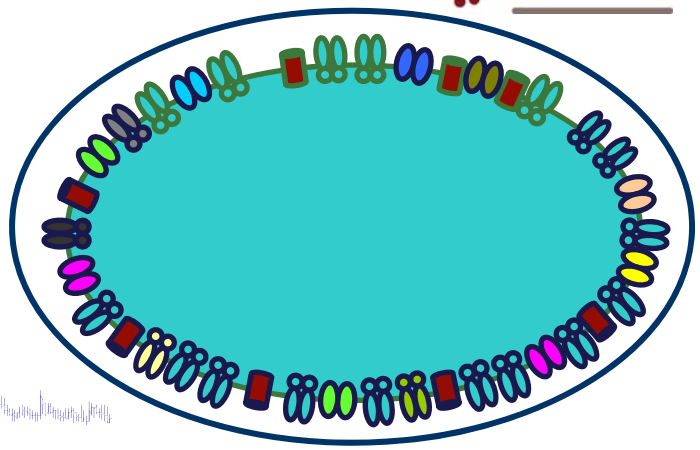
BmrA motions



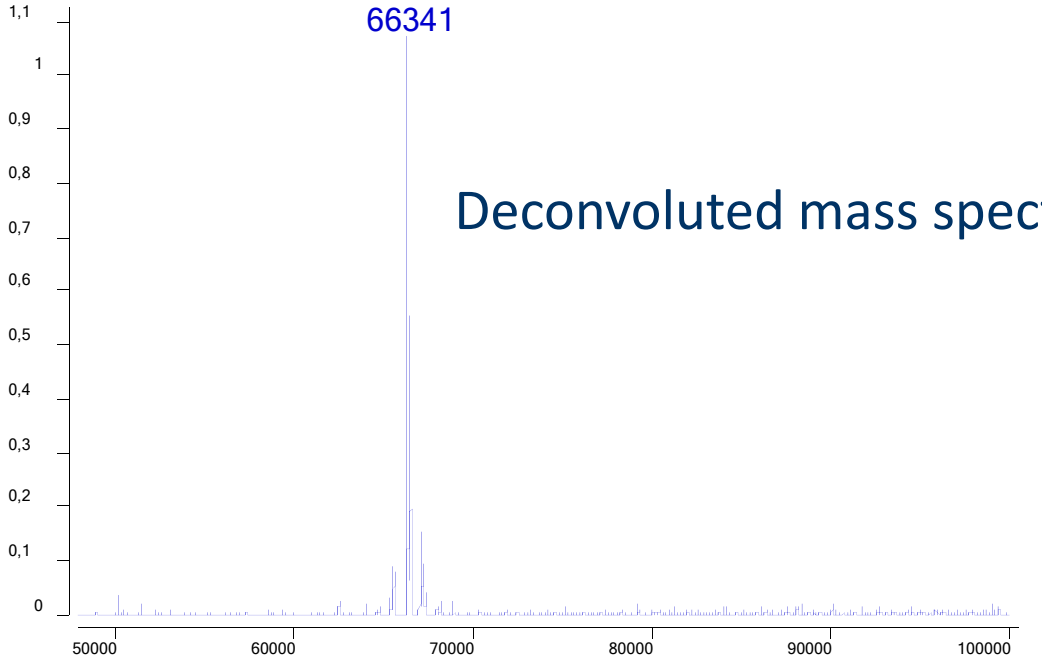
BmrA mass spectrum from membrane



ESI mass spectrum

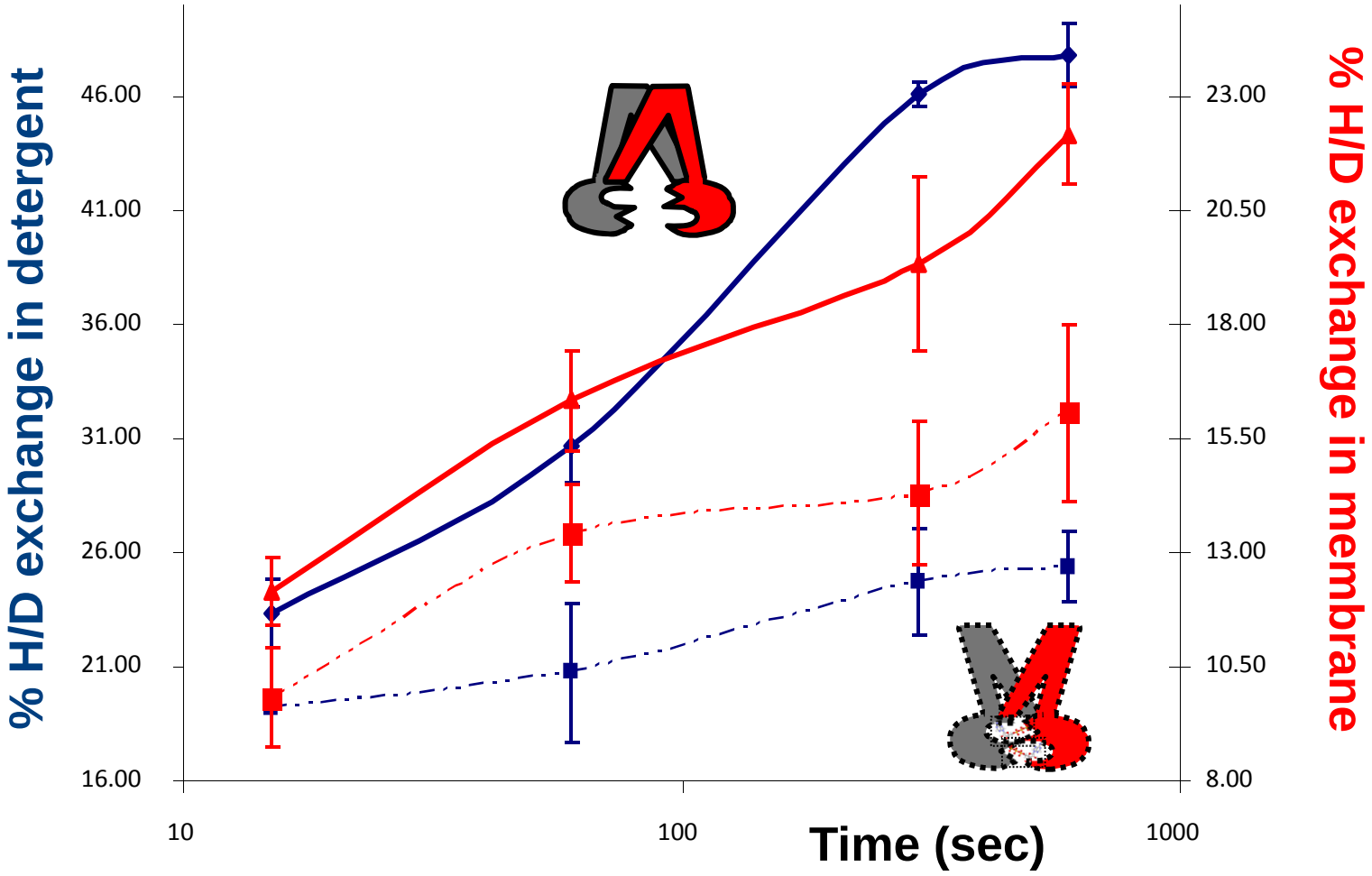


Overexpression in E. coli



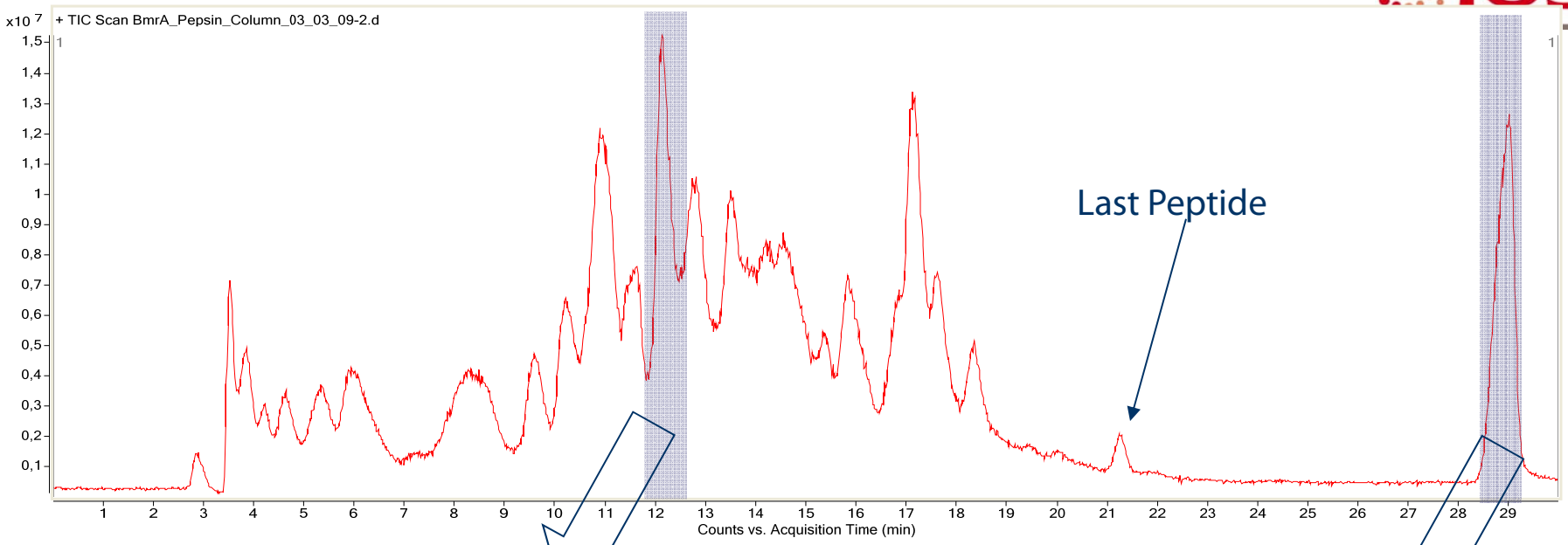
Deconvoluted mass spectrum

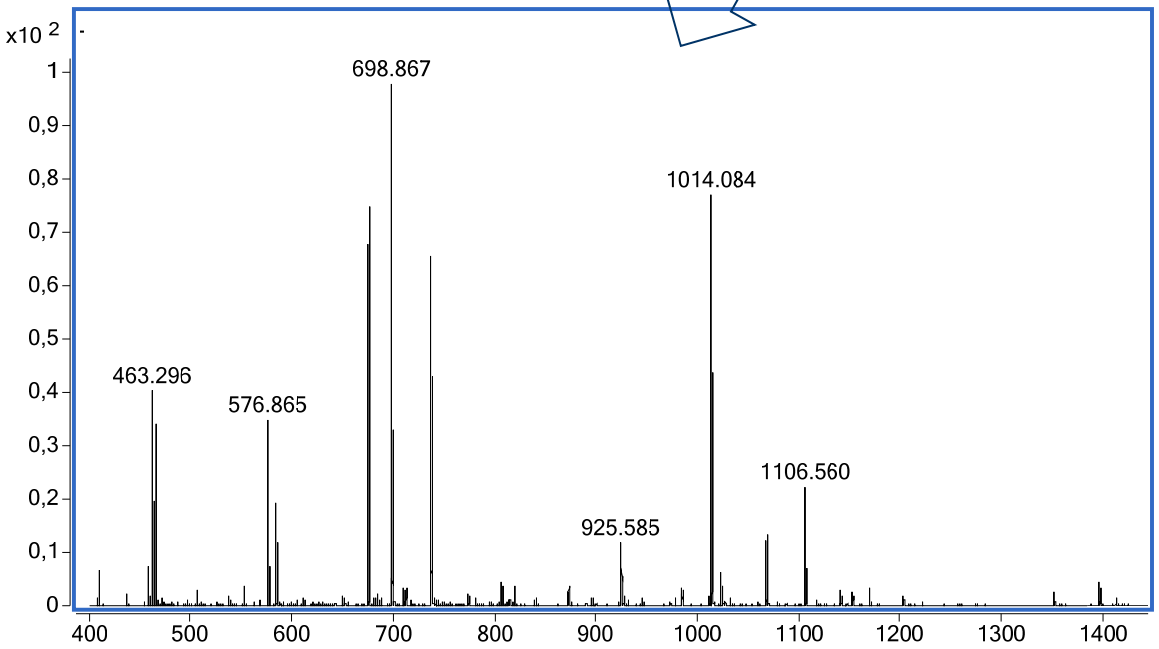
BmrA global HDX kinetics

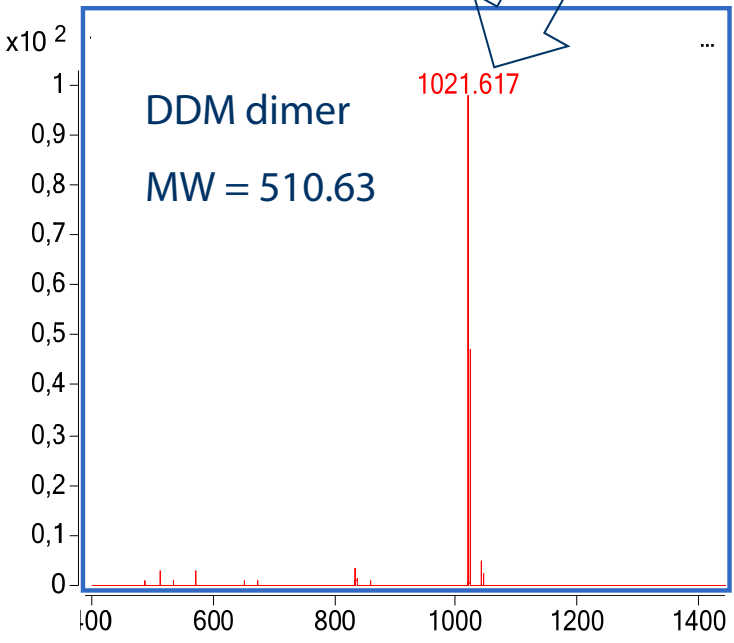


Similar behavior of BmrA in membrane and purified in DDM

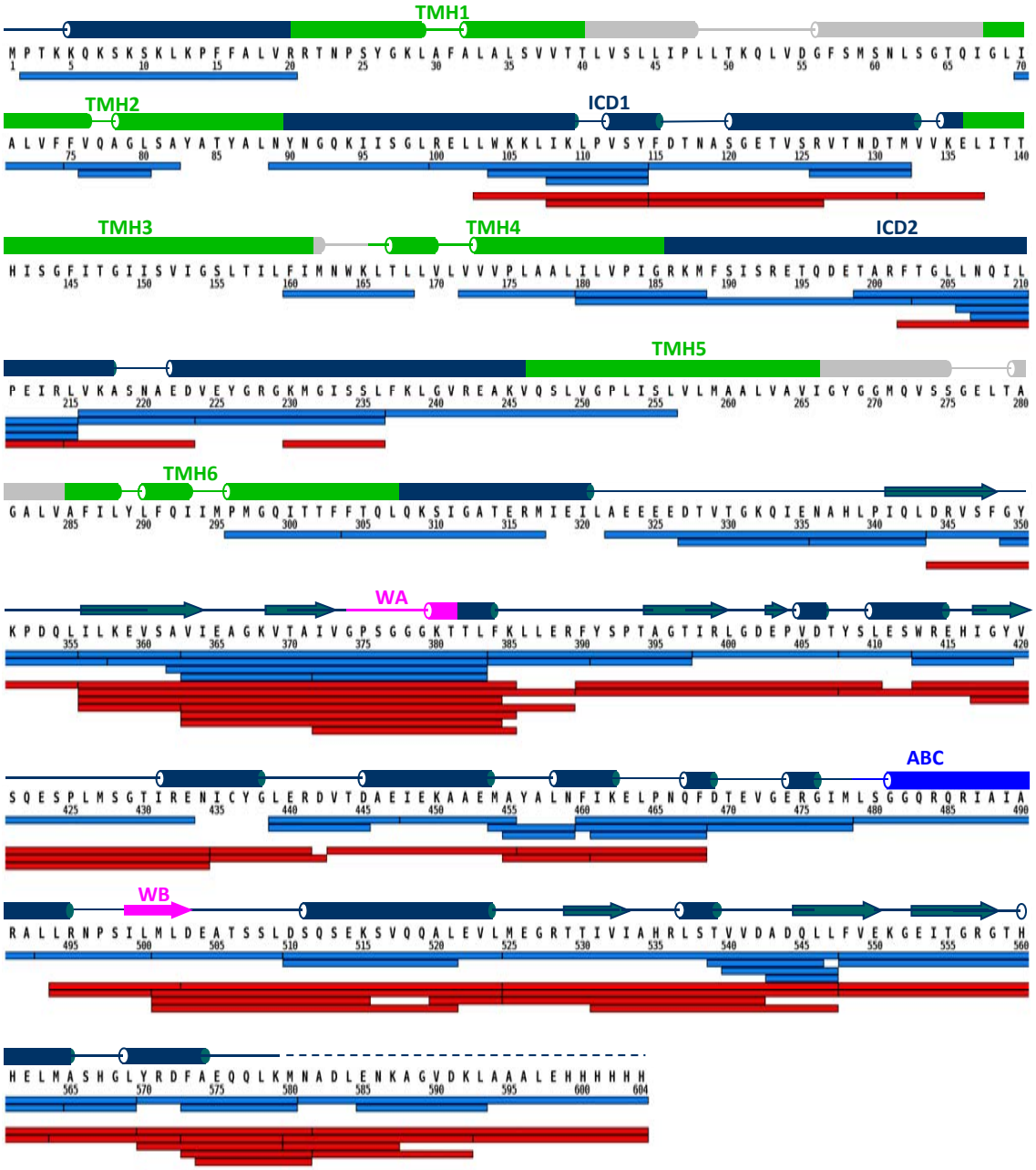
Peptides/detergent separation

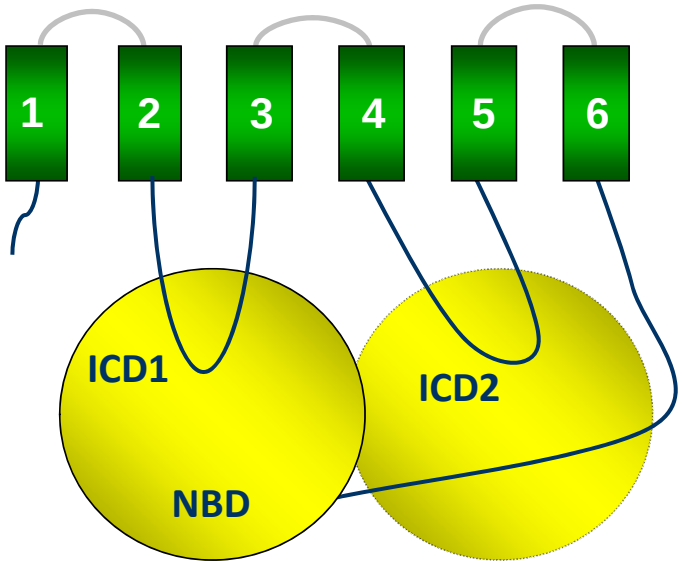






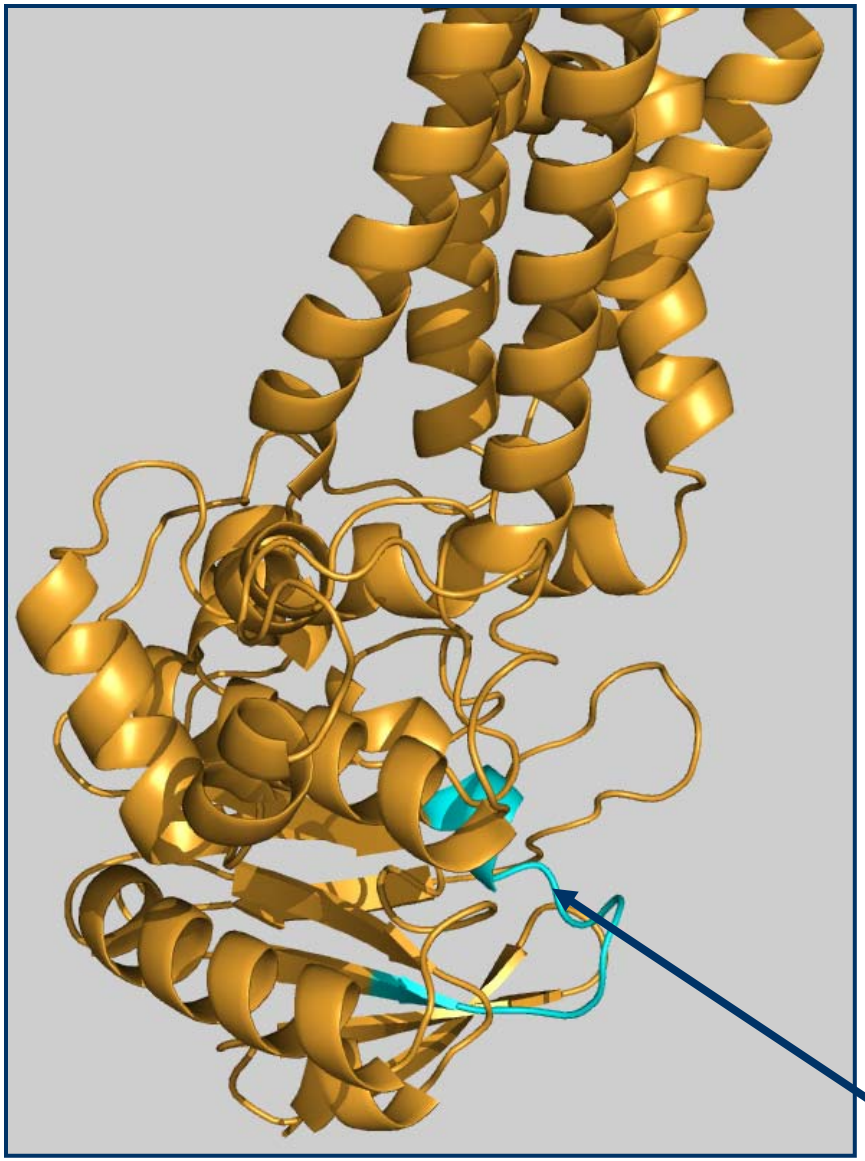
Peptide mapping

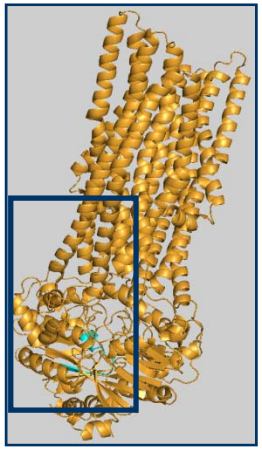


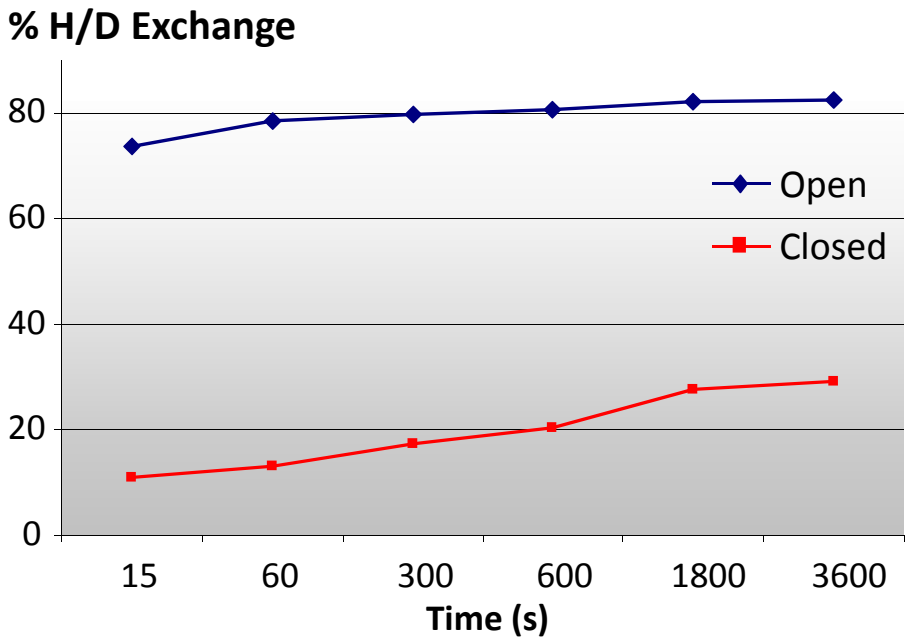


Pepsin	470 of 604 – 78 %
XVIII	300 of 604 – 50 %

BmrA local HDX kinetics



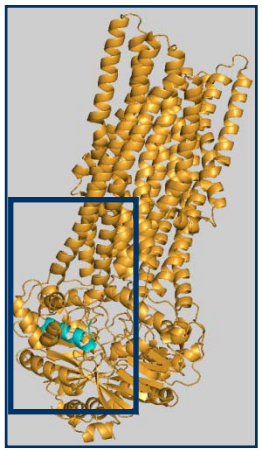


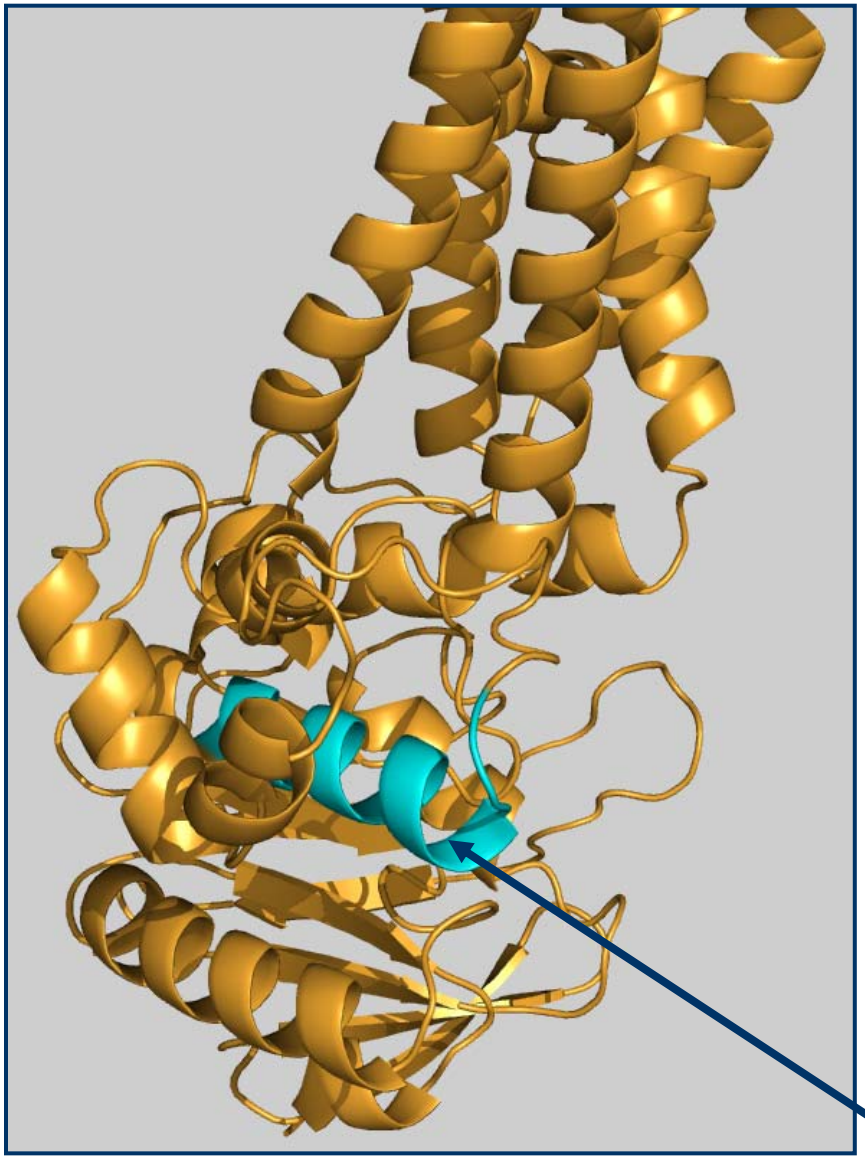


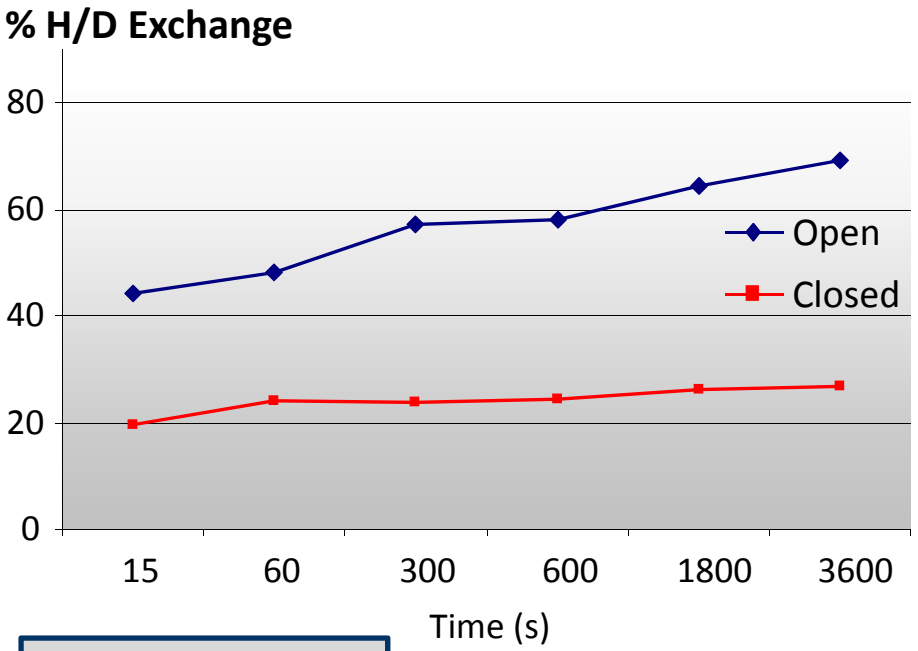
372-383
Walker ‘A’

1 of the 3 distinctive motifs in the ATP Binding Cassette (ABC) which bind and hydrolyze ATP

BmrA local HDX kinetics

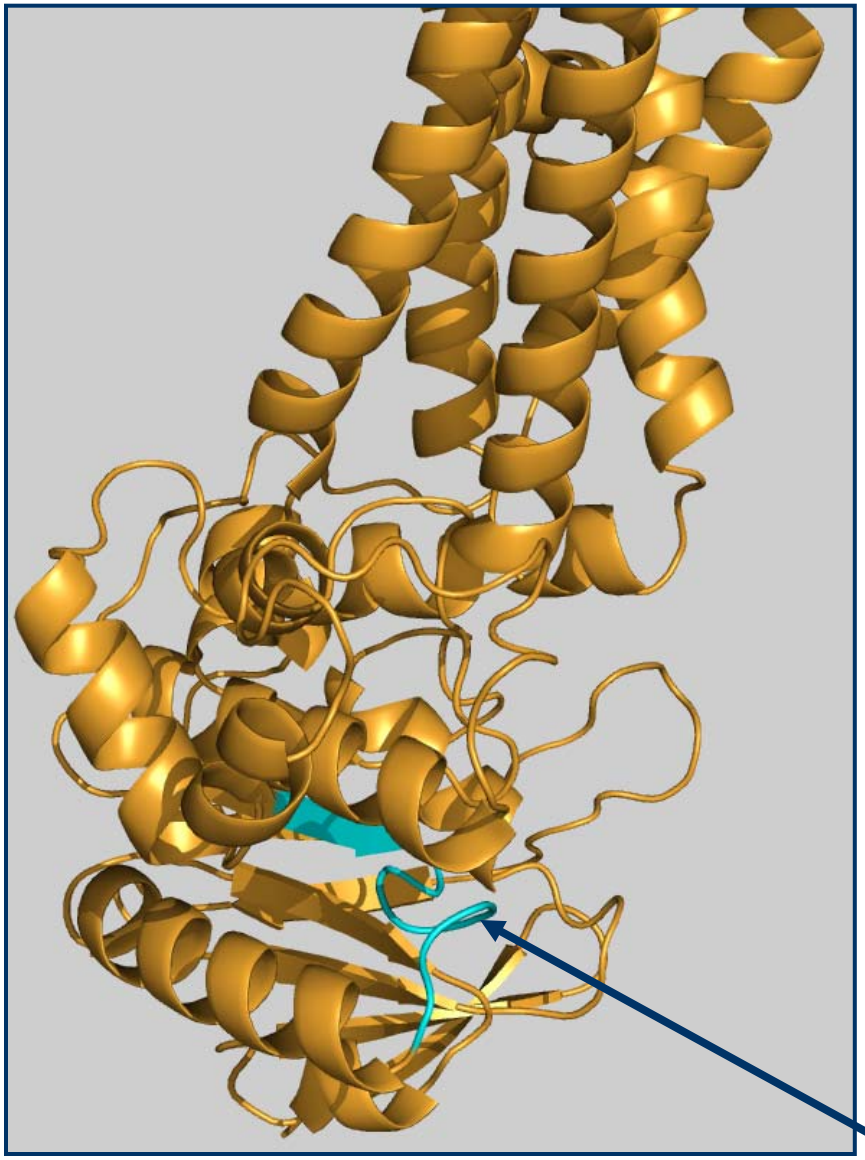


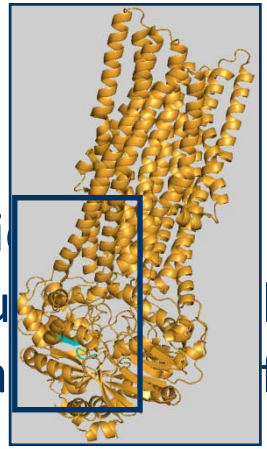




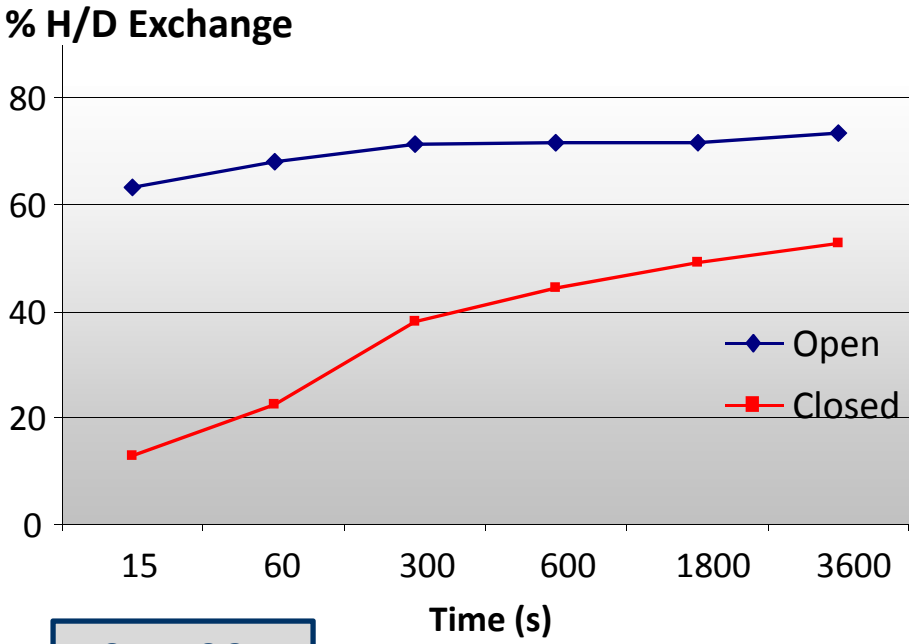
479-492
Signature motif

BmrA local HDX kinetics

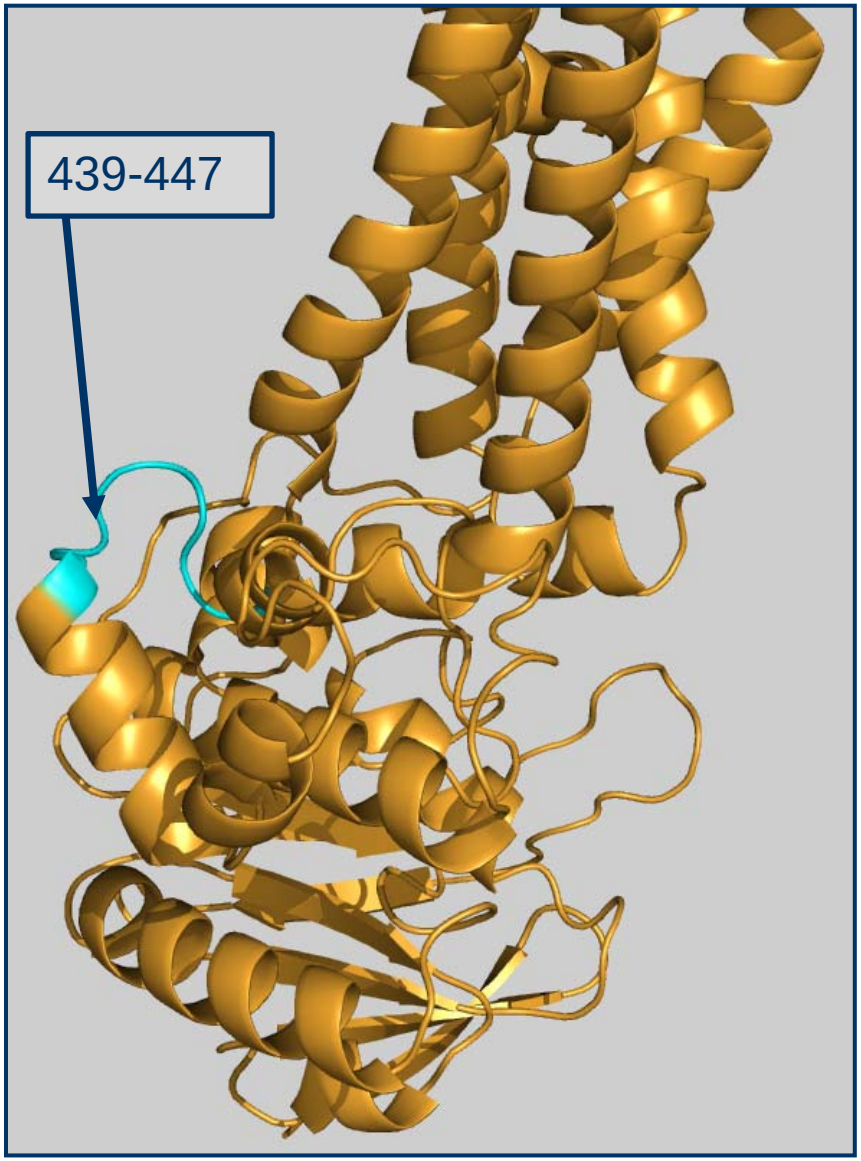


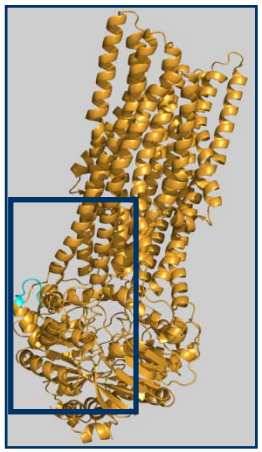


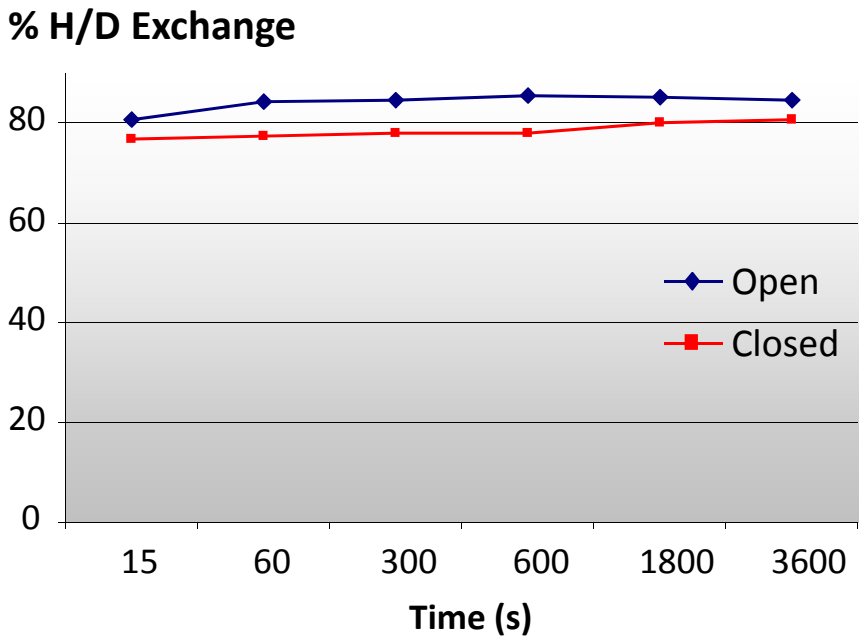
ATP binding regi NBDs showed low deu hange in closed form as a from X-ray structures



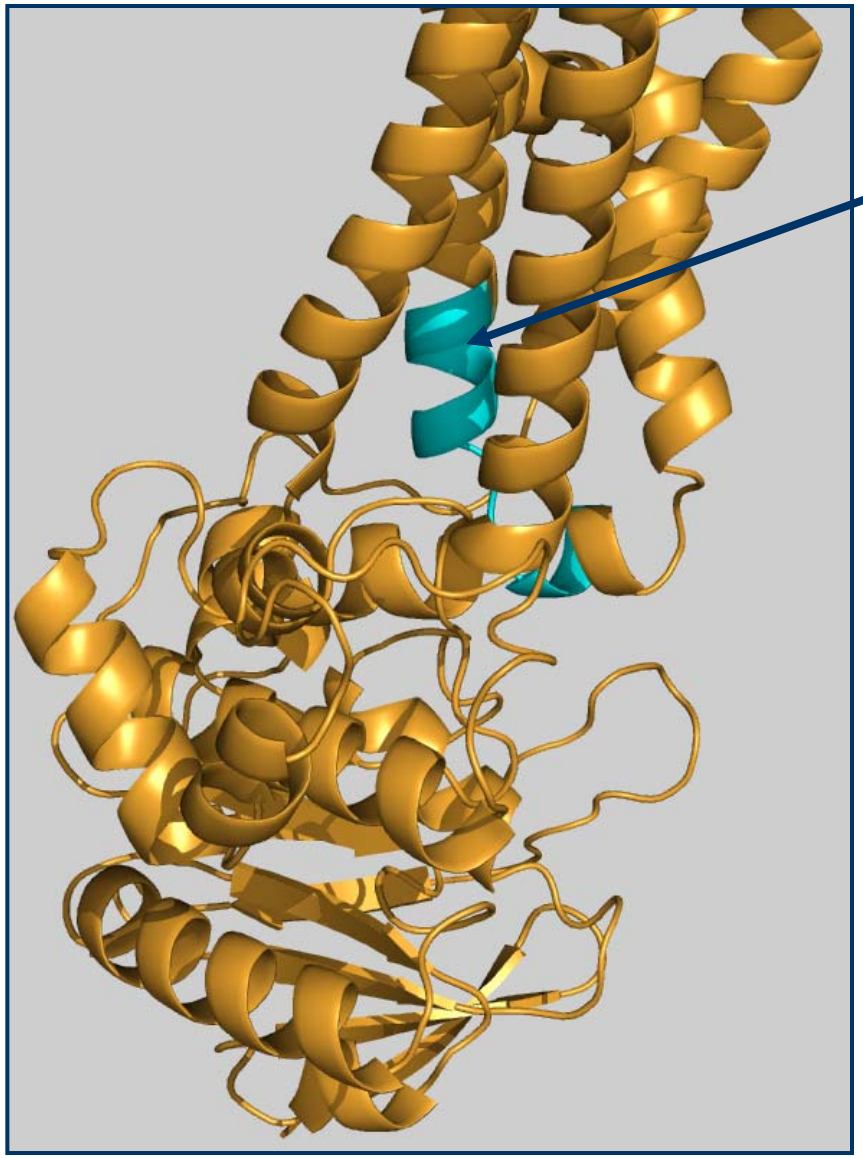
BmrA local HDX kinetics

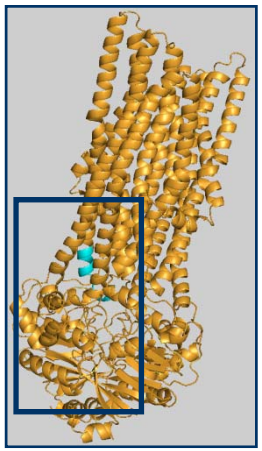


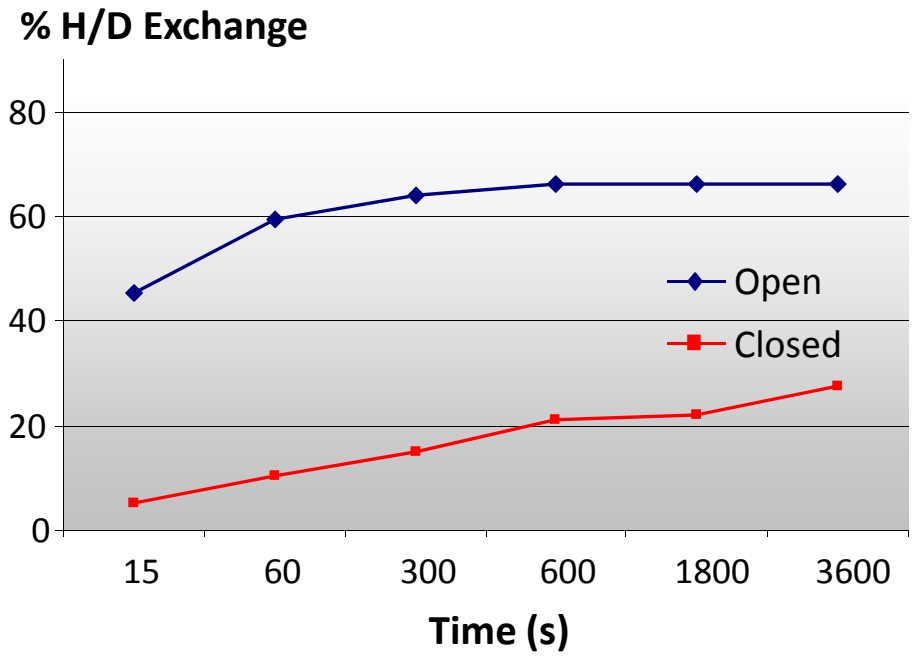




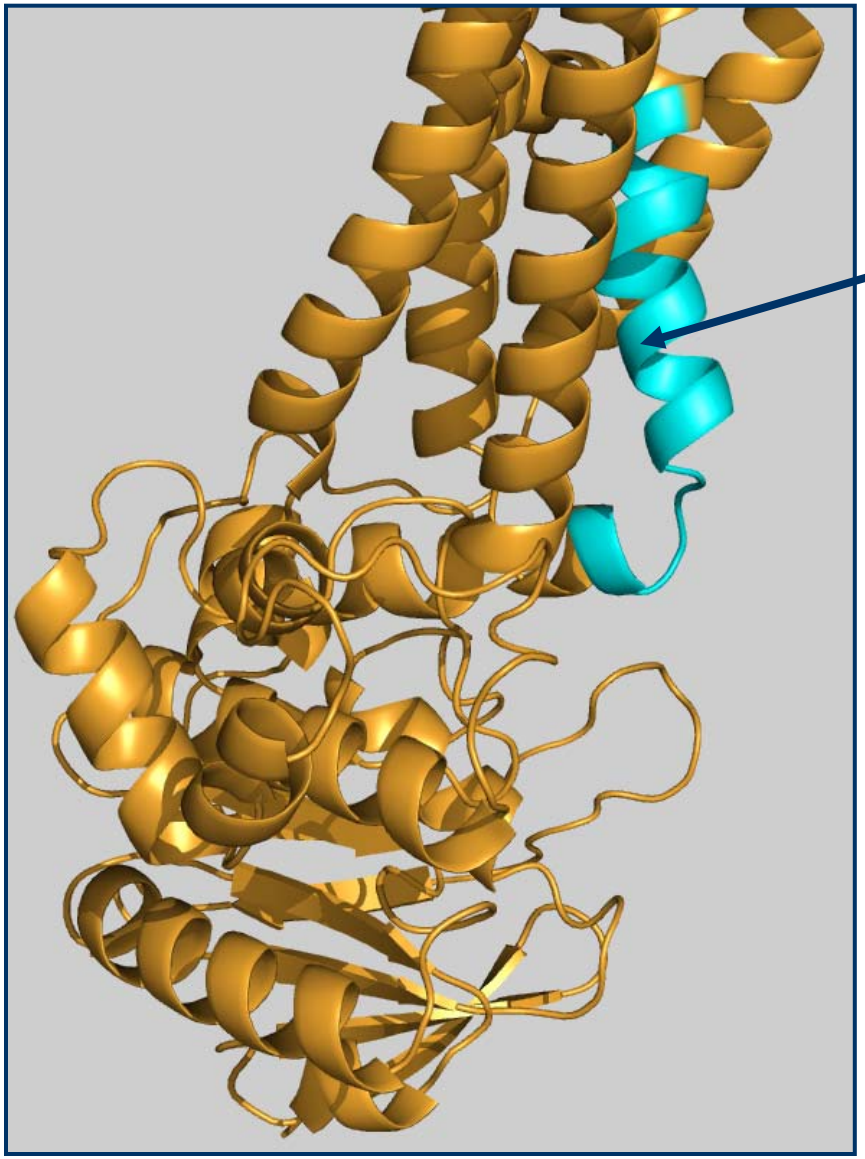
BmrA local HDX kinetics



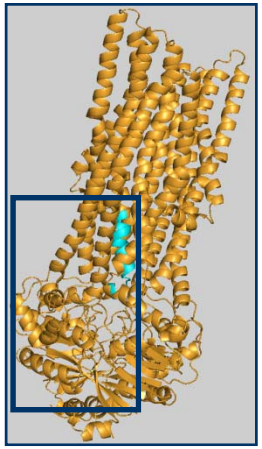


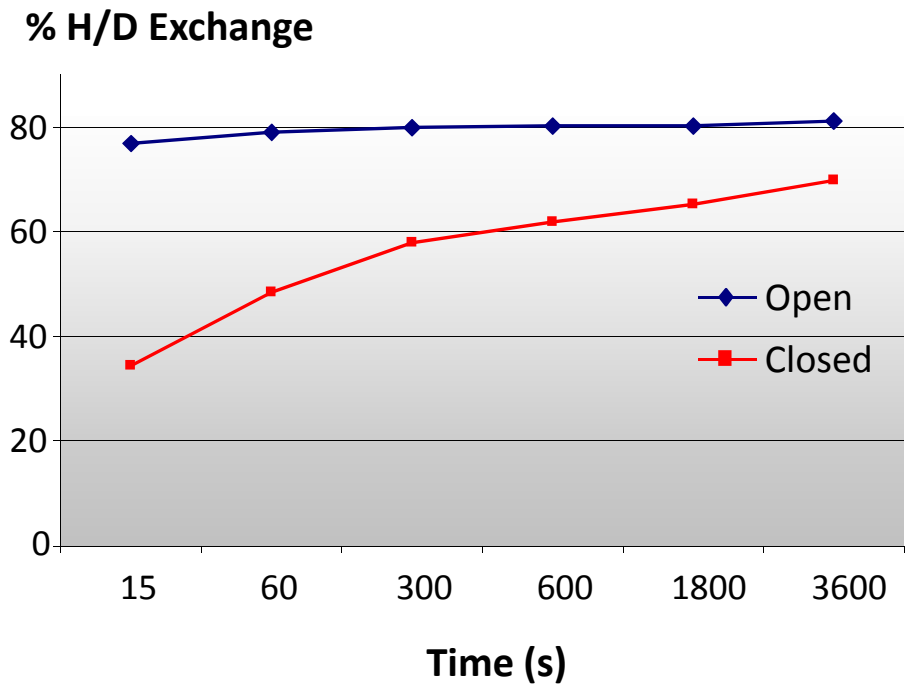


BmrA local HDX kinetics

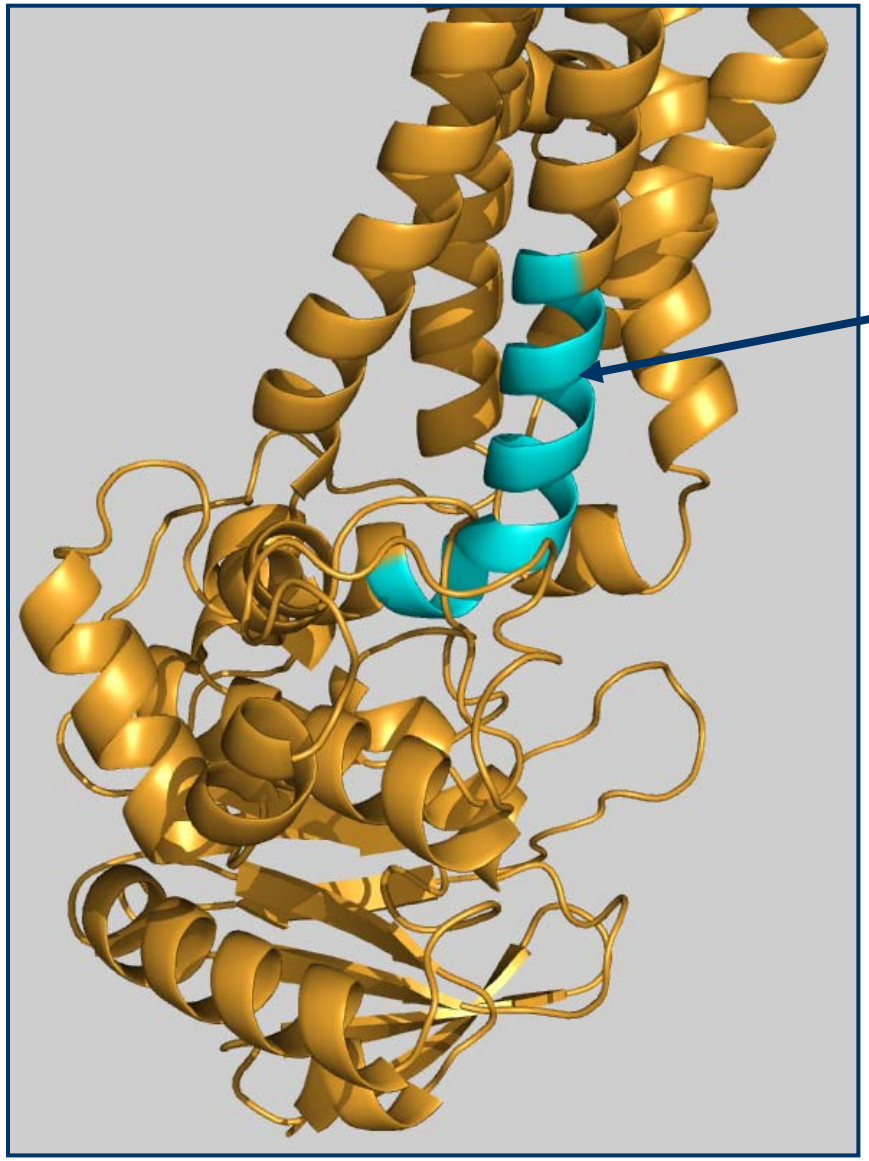


115-132
'ICD 1'

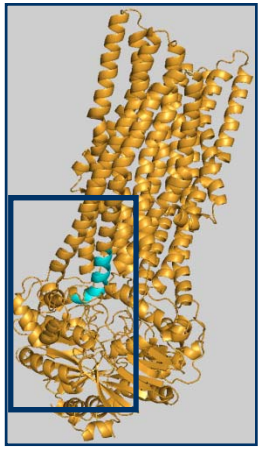


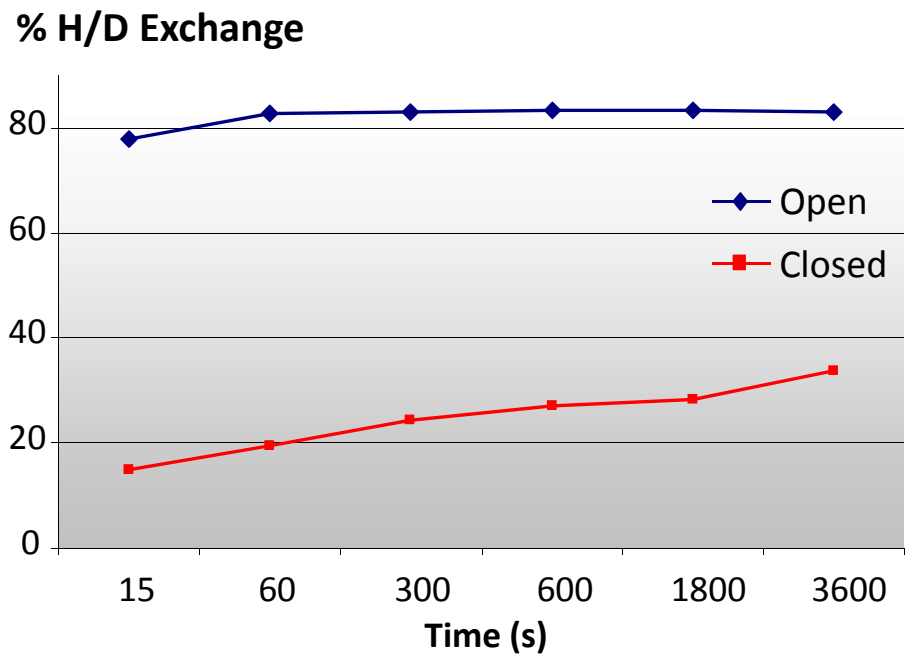


BmrA local HDX kinetics

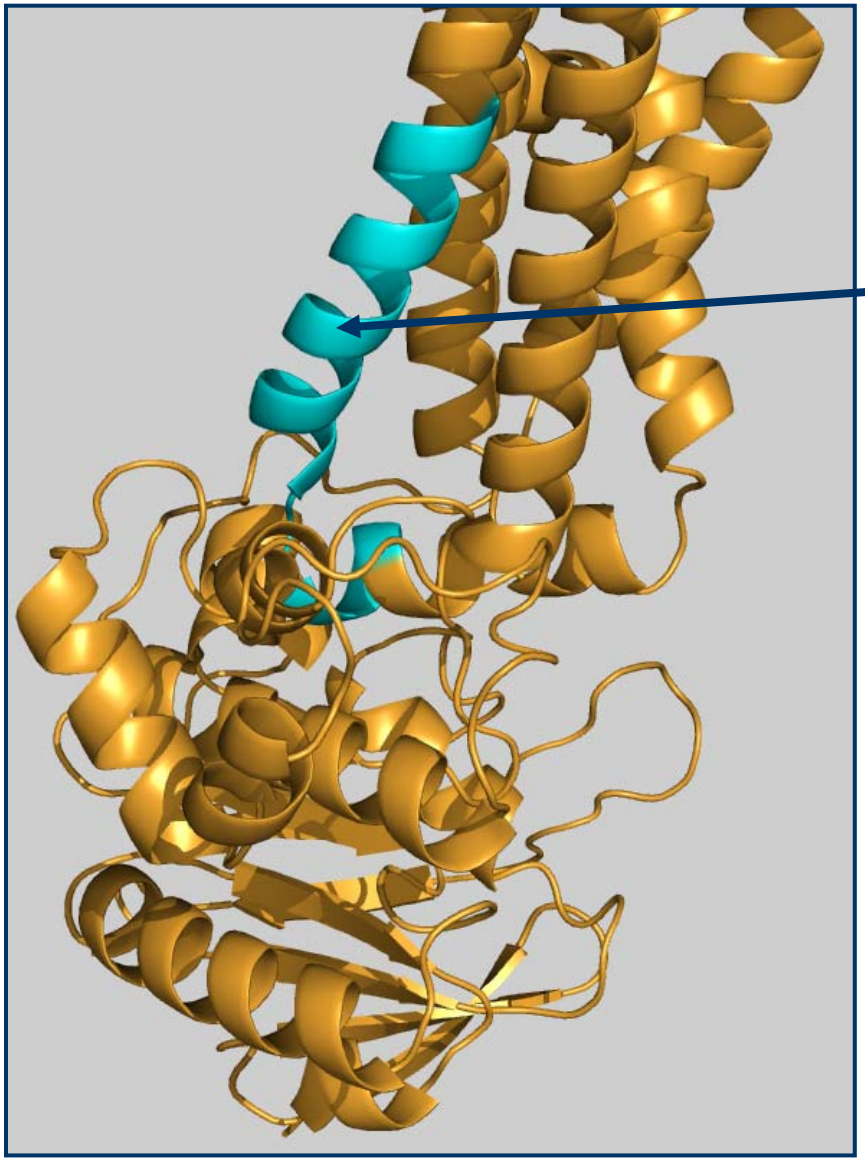


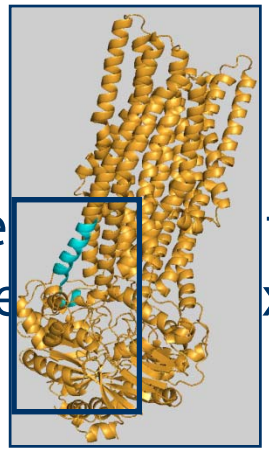
203-215
'ICD 2'





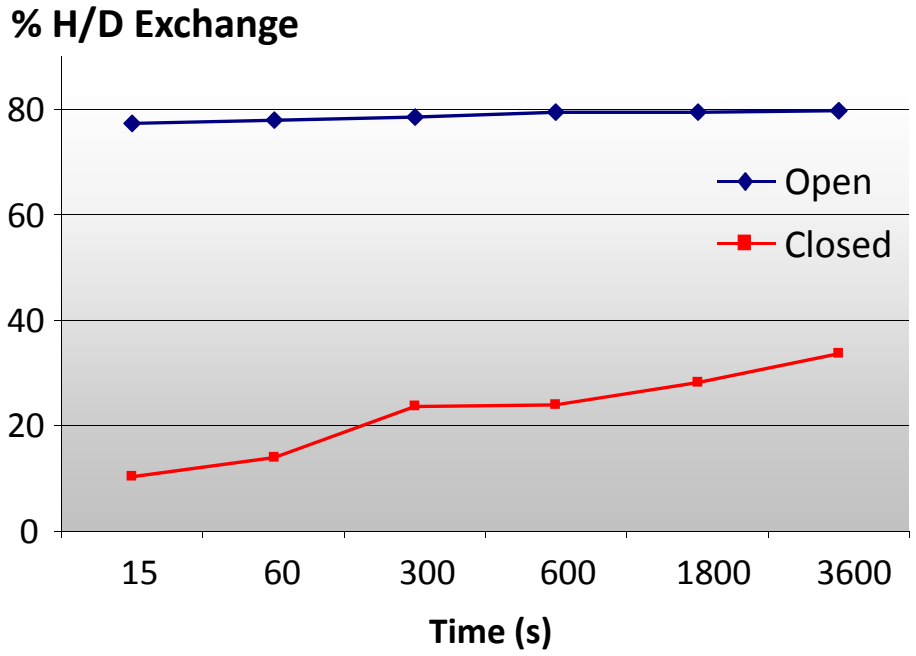
BmrA local HDX kinetics



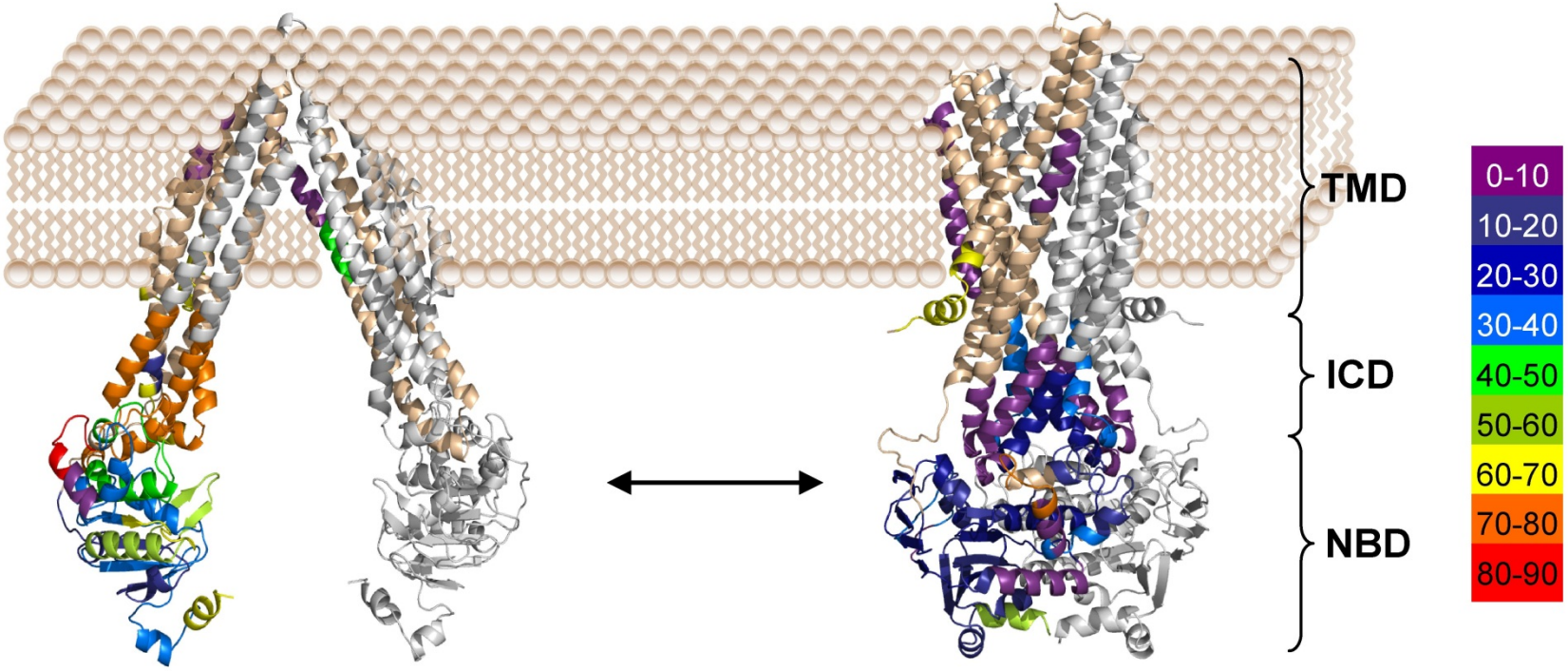


Surprisingly the for ICDs was also change xtreme conf s

216-236
'ICD 2'

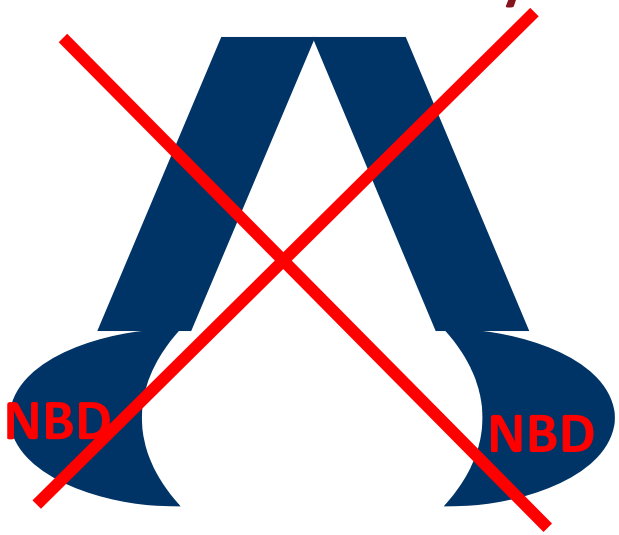


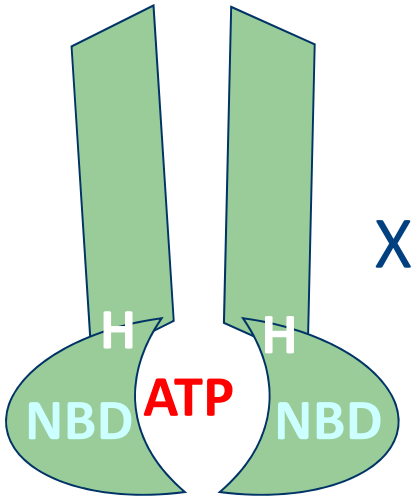
Results summary



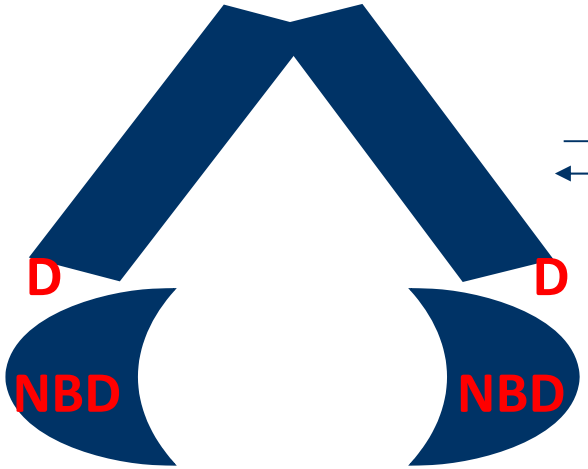
Dynamics of a bacterial multidrug ABC transporter in the inward and outward facing conformations; Mehmood et al 2012 PNAS

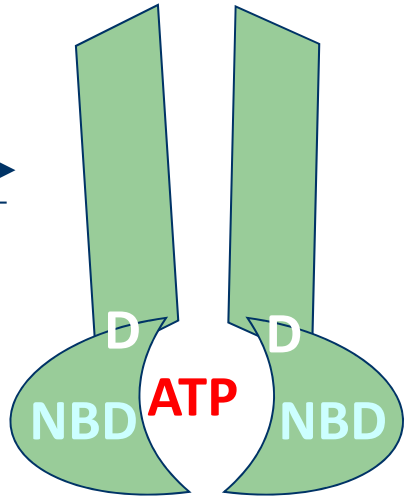
Results summary





X- ray structures





HDX

ICDs disengage from NBDs in open state

(high flexibility of BmrA ICDs)

HDX showed good correlation with BmrA model based on the closed form

Summary and outlook

- HDX MS of the largest membrane protein to date
- Fine dynamics information to characterize a catalytical cycle
- BmrA flexibility enables it to recognize a large panel of molecules
- Complement to X-ray crystallography
- Automated platform at the IBS, open to collaborations

Automation at the IBS

H2-D2

Time saving and reproducibility improvement

Acknowledgements

- Shahid Mehmood
- Jean-Michel Jault
- Carmen Domène (University of Oxford)

- CEA, CNRS, UJF, ANR, HEC of Pakistan