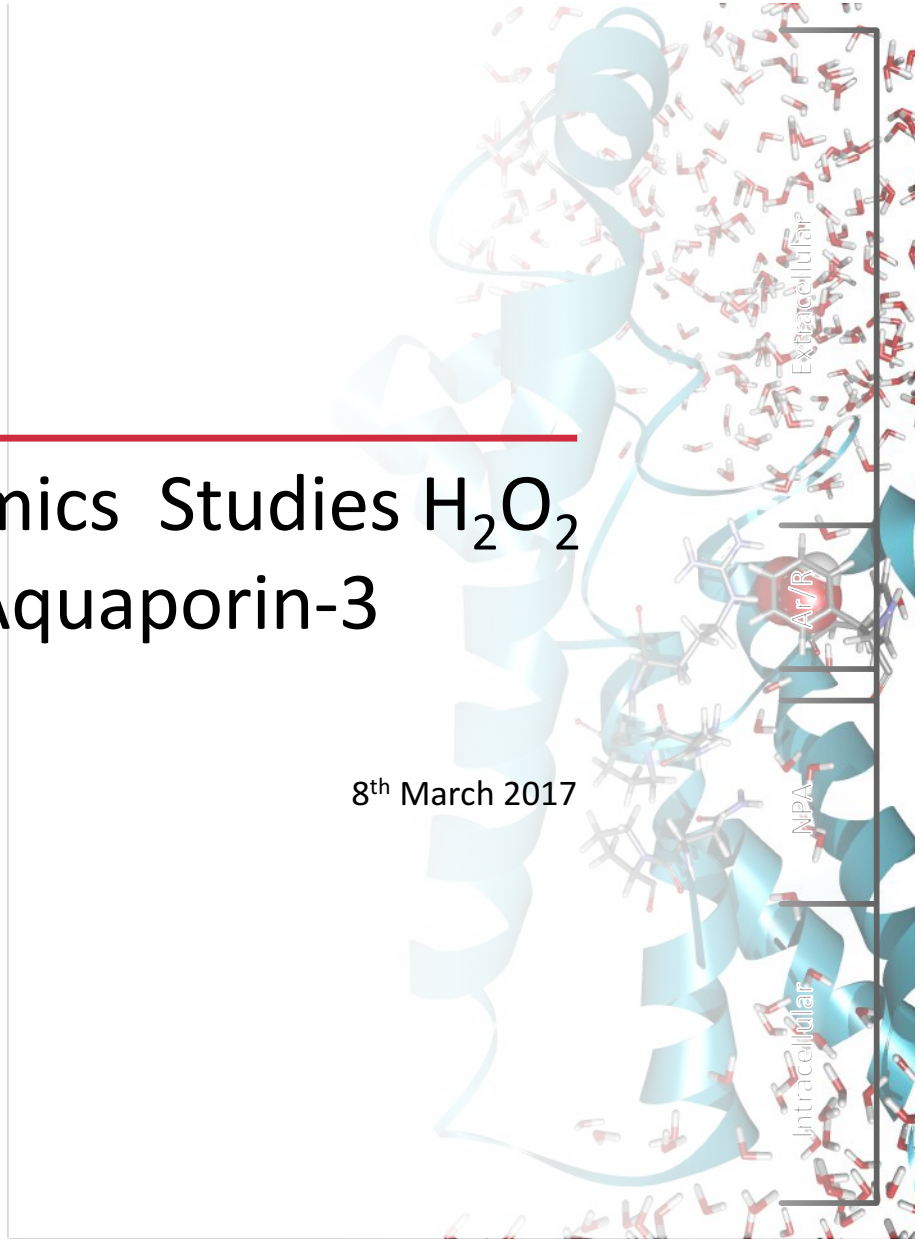
Molecular Dynamics Studies H_2O_2 Permeation via Aquaporin-3

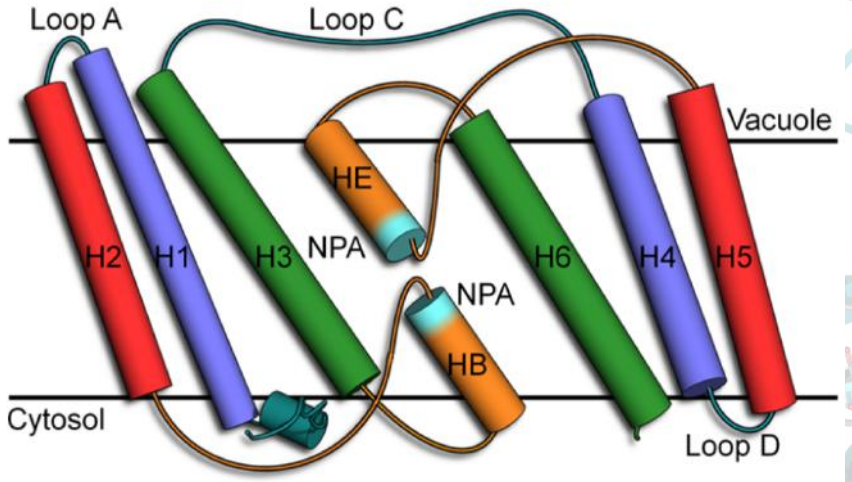
Darren Wragg

8th March 2017



Aquaporins

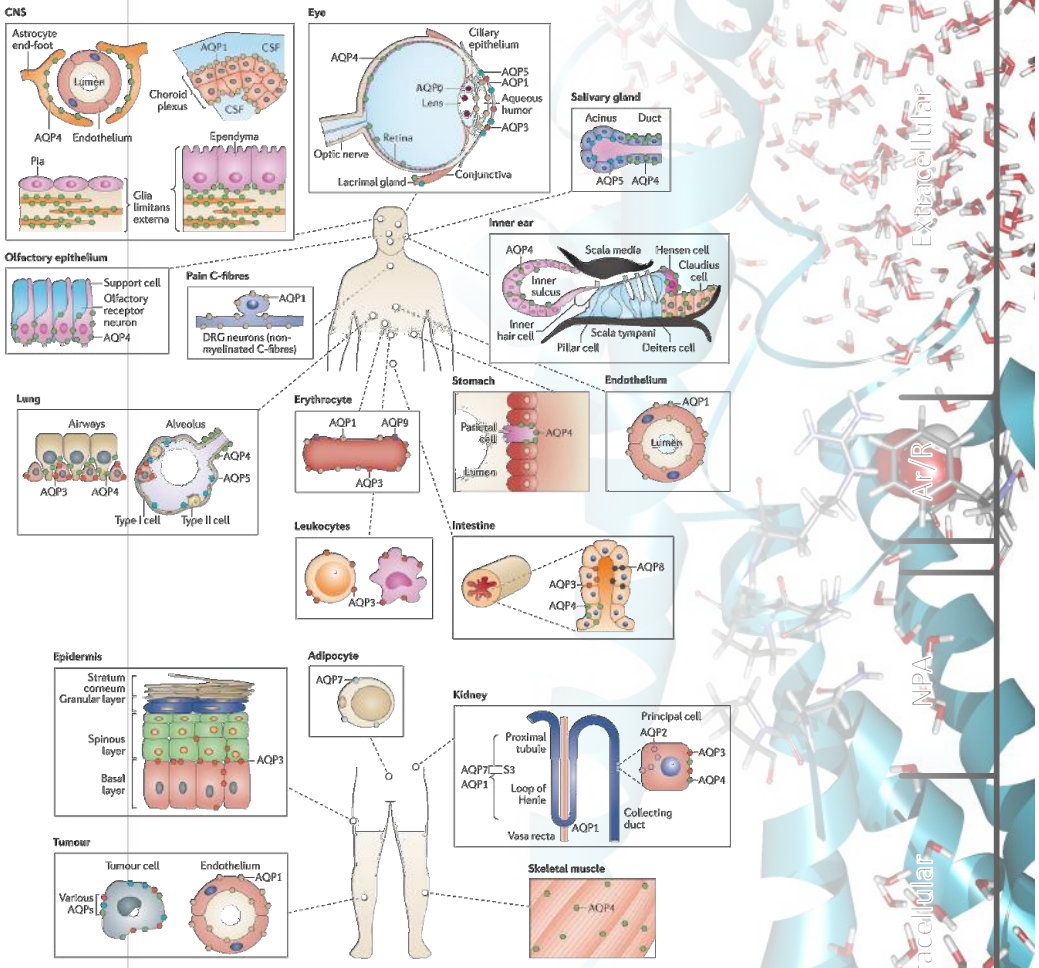
- Water movement is a crucial physiological process in all cells and is controlled by a set of transmembrane proteins called aquaporins (AQPs)
- In humans, the AQP family consists of thirteen isoforms (AQP0 – AQP12), split into two distinct groups:
  - **orthodox aquaporins** (AQP0, AQP1, AQP2, AQP4, AQP5, AQP6 and AQP8)
  - **aquaglyceroporins** (AQP3, AQP7, AQP9, AQP10 and AQP11)
- All have six membrane spanning helices connected by five loops



Aquaporins in health and disease: new molecular targets for drug discovery, G Soveral, S Nielsen and A Casini, eds. CRC Press, Taylor & Francis Group, 2016.
A. S. Verkman, *Nat. Rev. Drug Discov*., 2014, 13, 259–77.
A. Kirscht, *PLoS Biol.*, 2016, **14**, e1002411.

Aquaporins, H_2O_2 and metastasis

- Aquaporins are found in all cell types of the body
- Within the cells:
  - Plasma membrane
  - Mitochondria (AQP8)
  - Cell nucleus
  - Example:
    - Spermatozoa contain AQP3 (tail), AQP7 (head), AQP8 (mitochondria) and AQP11 (intracellular)
- Also overexpressed in a number of cancer cell lines including:
  - Brest cancer
  - Lung cancer
  - Melanoma
  - Leukaemia



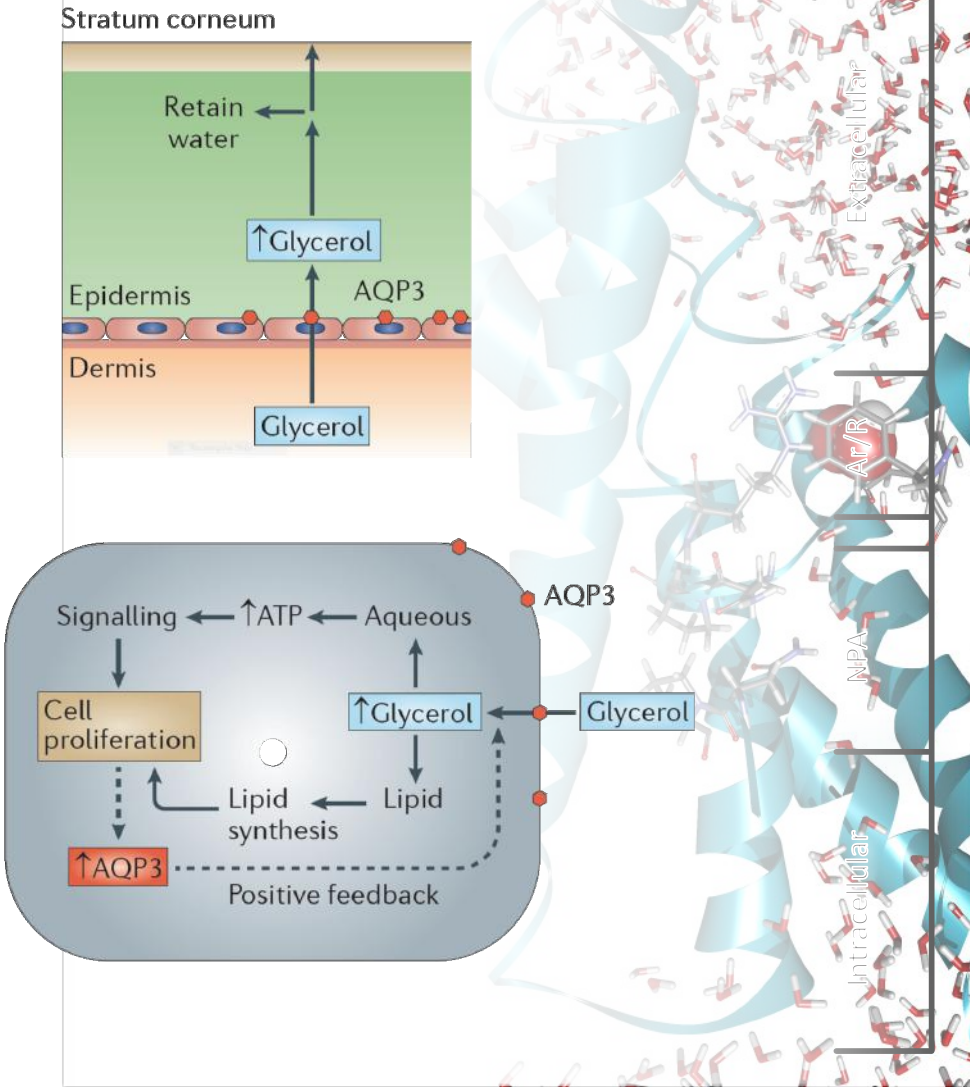
hAQP cell distribution

S. Verkman, *Nat. Rev. Drug Discov.*, 2014, **13**, 259–77.
F. Vieceli Dalla Sega, *Biochim. Biophys. Acta - Mol. Cell Res.*, 2014, **1843**, 806–814.
H. Satooka, *Mol. Cell. Biol.*, 2016, 36, 1206–1218
U. Laforenza, G. Pellavio, A. Marchetti, C. Omes, F. Todaro and G. Gastaldi, *Int. J. Mol. Sci.*, 2016, **18**, 66.

Glycerol – physiological function

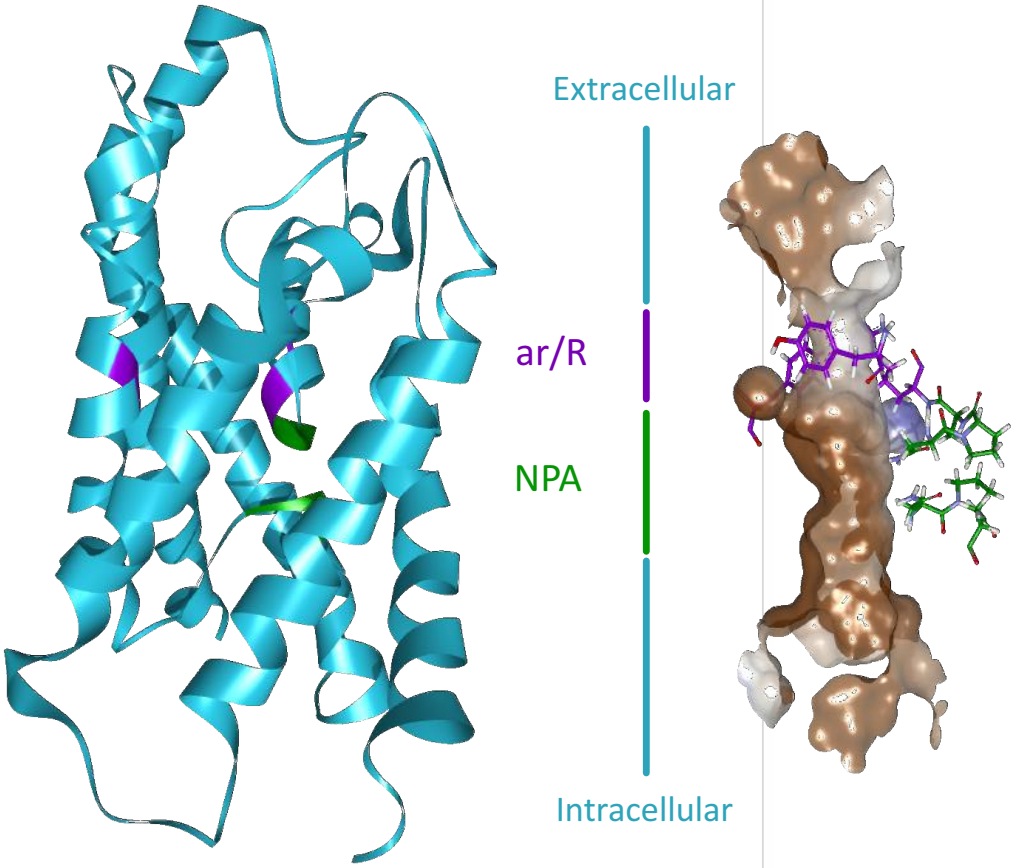
- Glycerol has a roll in a number of physiological functions, including:
  - Skin hydration – helps retain water within the stratum corneum to maintain hydration and elasticity
  - Cell growth (both healthy and tumour cells)
    - ATP generation
    - Lipid synthesis

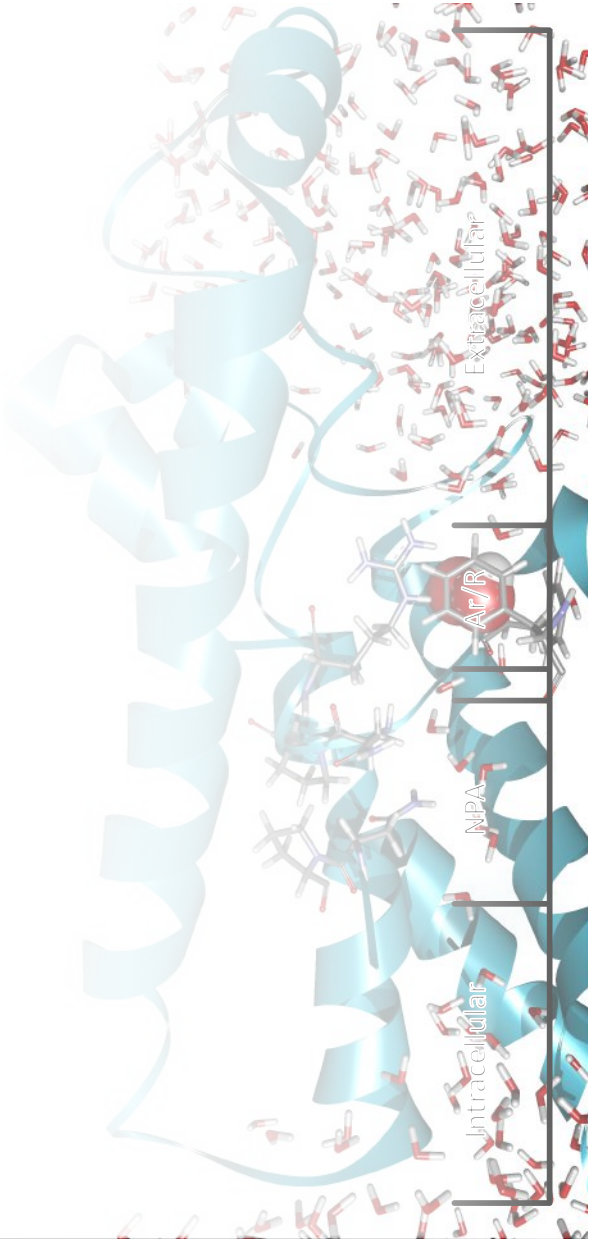
- Tumour cell growth – by reducing uptake of glycerol by tumour cells, cell proliferation can be retarded



A. S. Verkman, Nat. Rev. Drug Discov., 2014, 13, 259–77..

Aquaporin-3



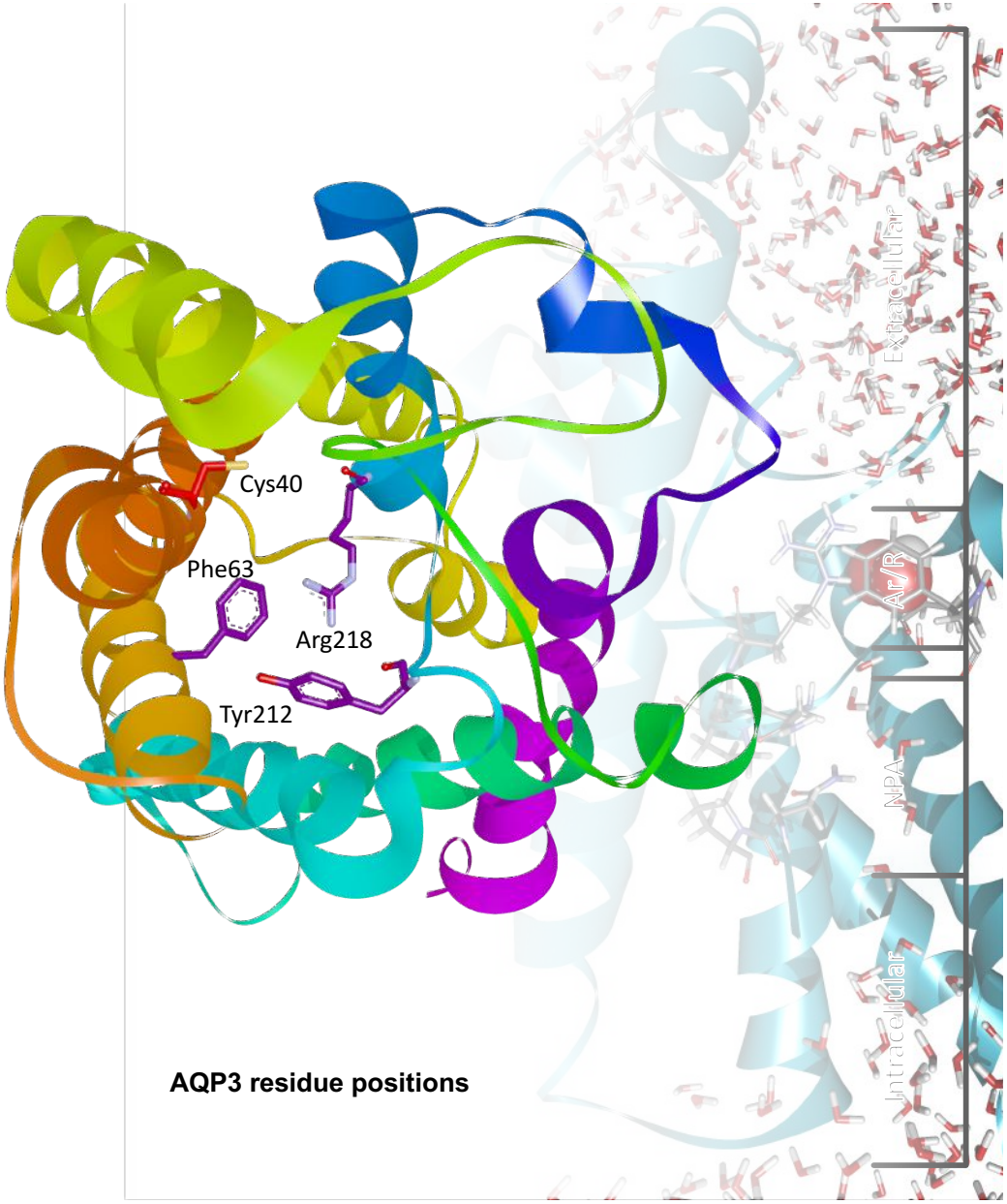


hAQP3 monomer and internal surface, indicating selectivity filters.

Aquaporin Inhibition

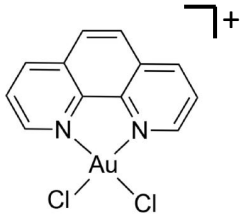
- So far no selective inhibitors have been described, except for the Au(III) complexes in our lab

- The development of selective inhibitors is important for their use as
  - **therapeutic** agents
  - **chemical probes** to study protein function



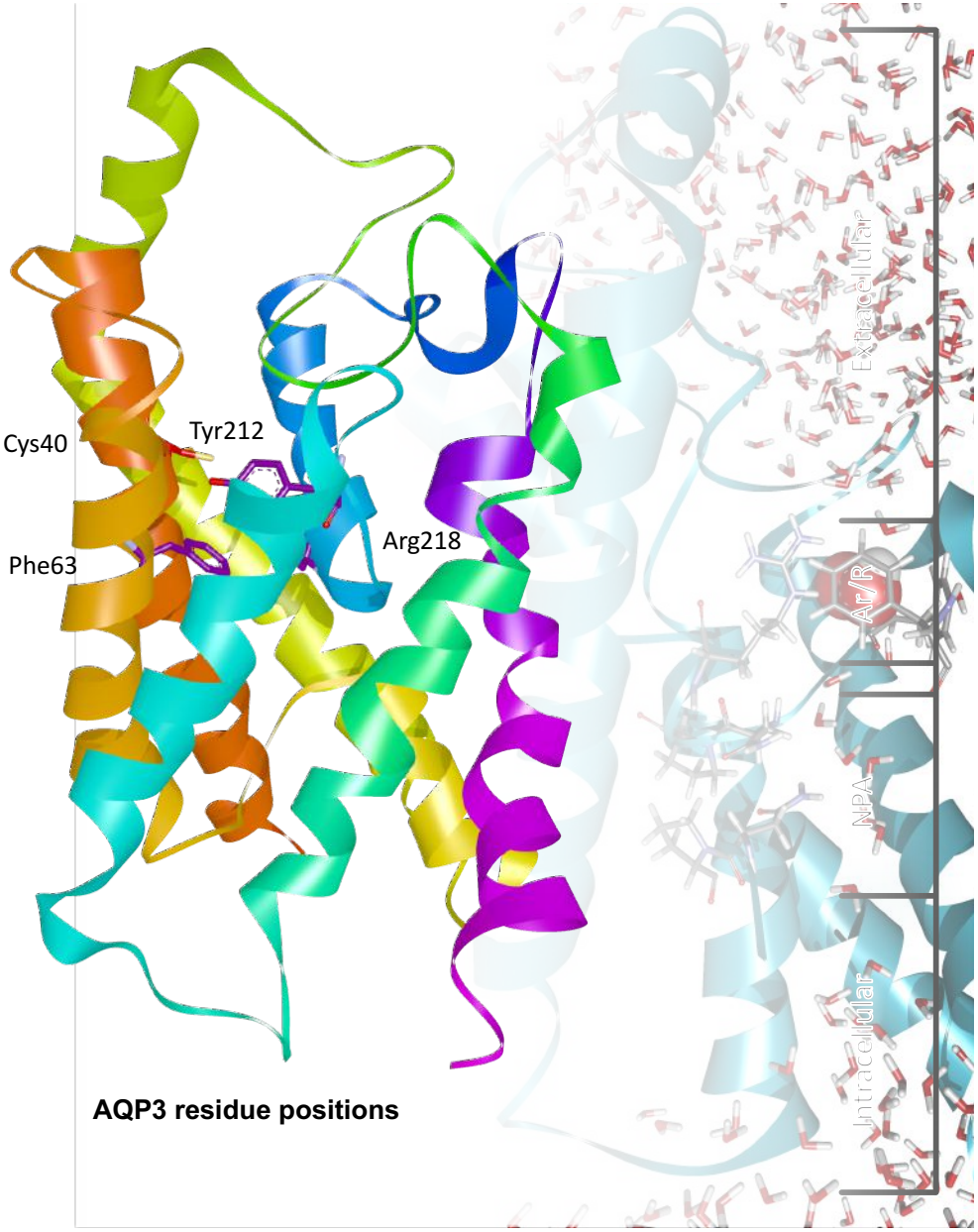
Au(III) and AQP3

- Au(III) complex Auphen
  - Highly selective for AQP3 via Au – S bond (Cys40)
  - Water soluble
  - Inhibits glycerol transport but not water transport (via AQP1)
  - "The Cork Hypothesis"
    - Thought block the channel via steric hindrance by binding to Cys40 located neat the Ar/R selectivity filter



Auphen

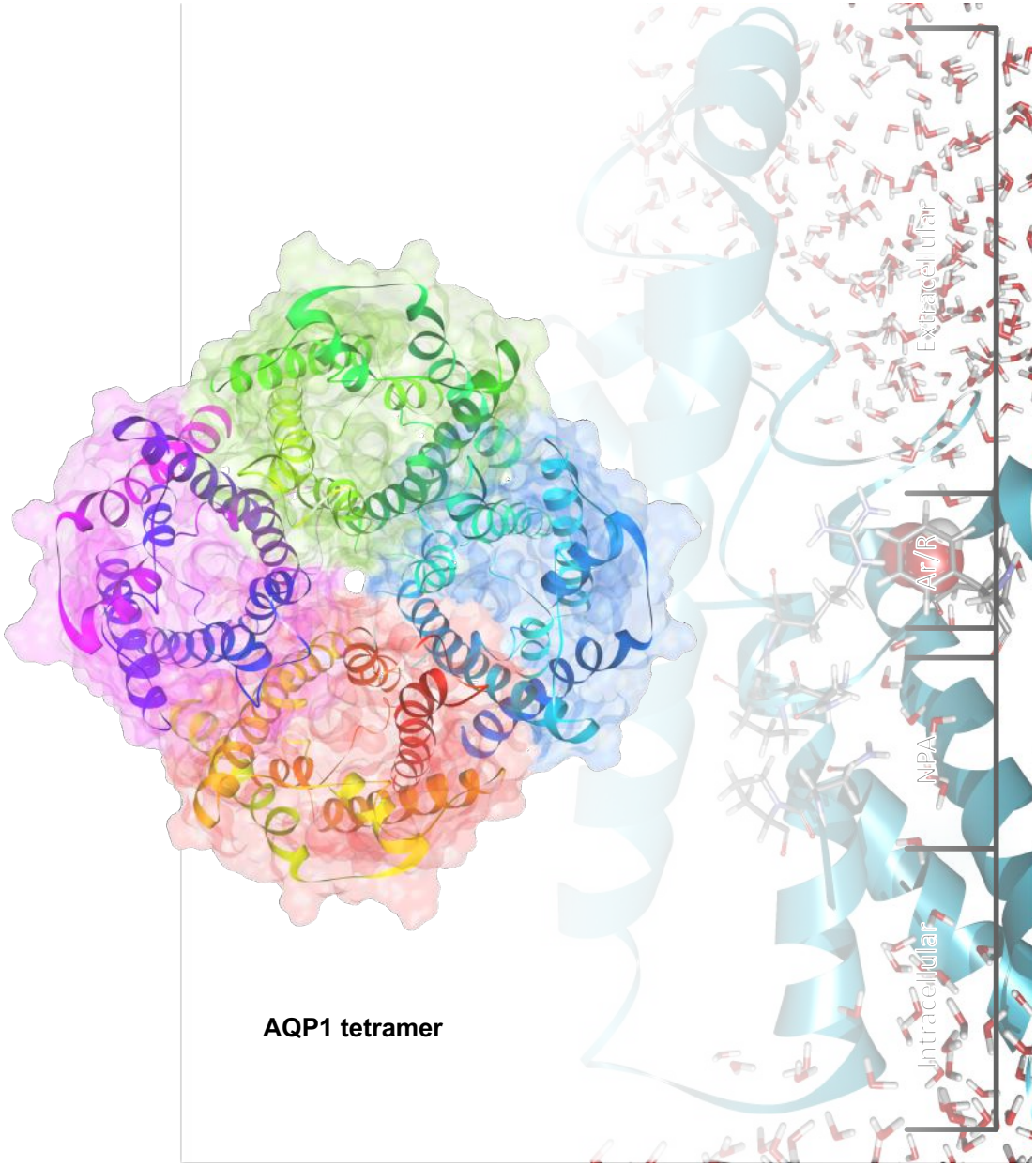
A. P. Martins, *PLoS One*, 2012, **7**, e37435.
A. de Almeida, *Med.Chem.Commun*, 2014, **5**, 1444–1453.



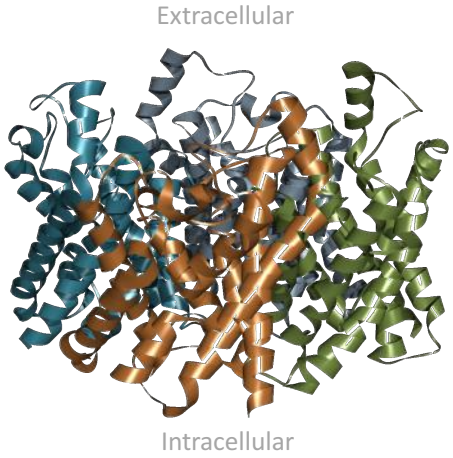
AQP3 residue positions

Project Aims

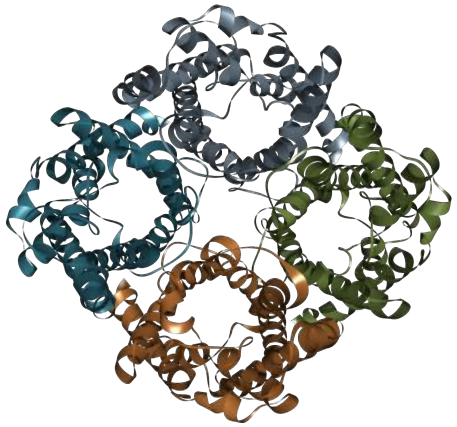
- Elucidation of H_2O_2 and Glycerol transport via AQP's through Molecular Dynamic Simulations
- Increase out understanding of AQP inhibition by Au-coordination complexes through Molecular Dynamic Simulations



Steered Molecular Dynamics(SMD)

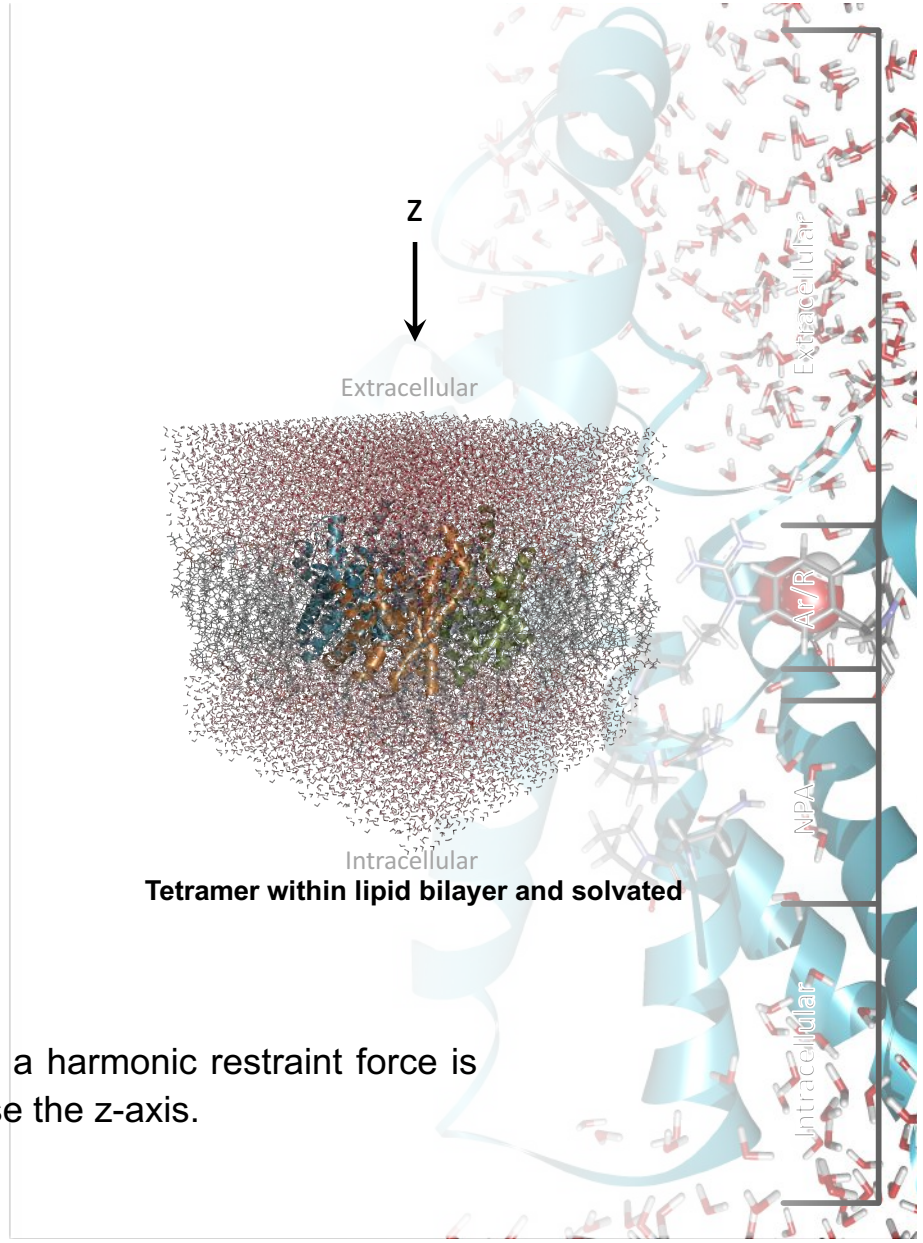


Side view



Extracellular top view

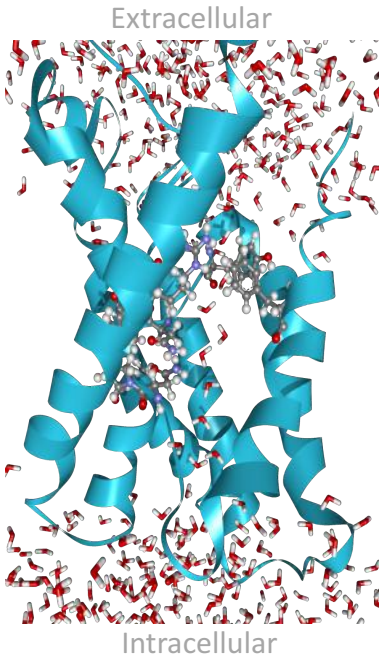
MD model of hAQP3 tetramer



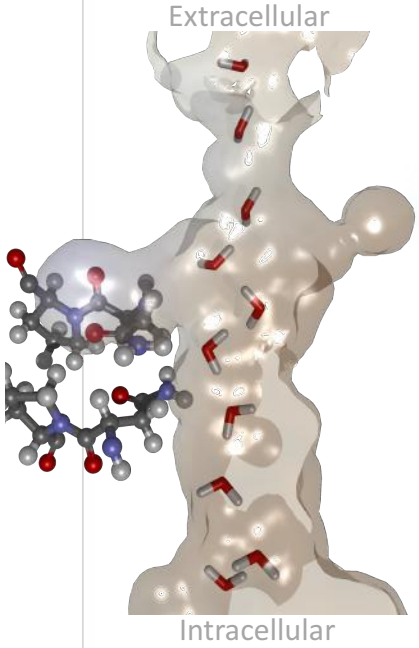
Tetramer within lipid bilayer and solvated

The system is built using a homology model of hAQP3 and a harmonic restraint force is applied to the molecule along the pore coordinate, in this case the z-axis.

Water permeation



Single file water molecules

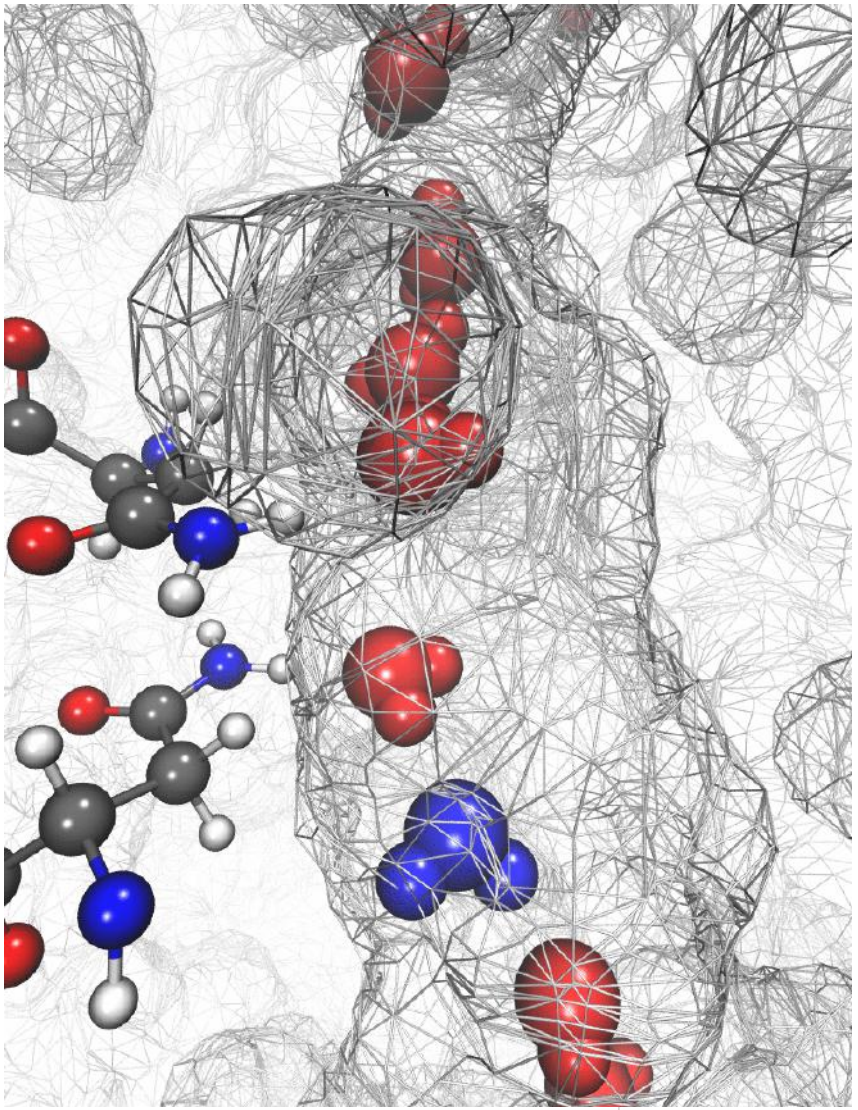


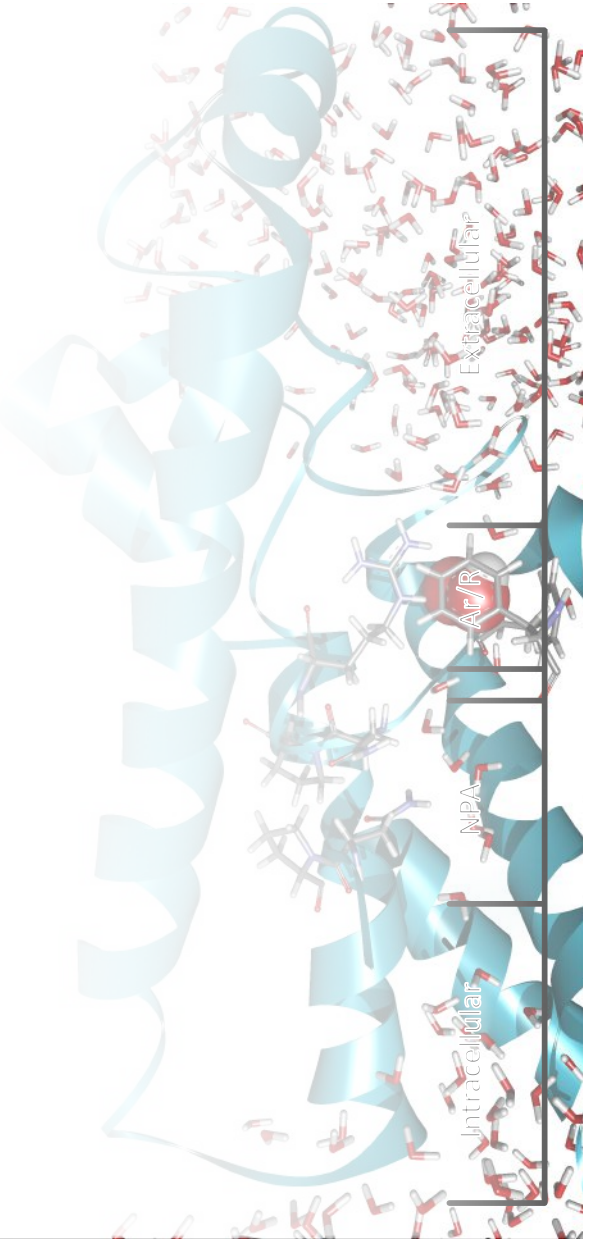
Water molecules passing though NPA SF

- The ar/R selectivity filter (ar/R SF) creates a steric hindrance, blocking larger molecules and creating the single file flow of water molecules.

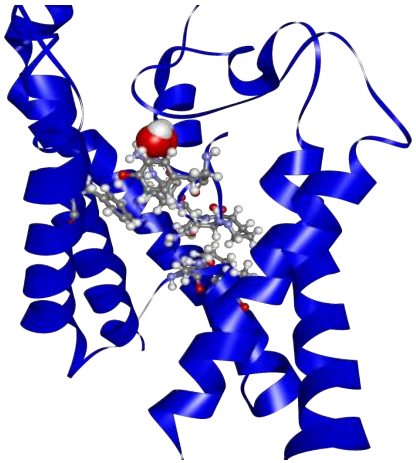
- As the water molecules pass the second SF (NPA), each molecule is flipped due to a combination of electrostatic interactions and a partially hydrophobic internal pore surface, thus preventing backflow and permeation by charged species.

Water permeation

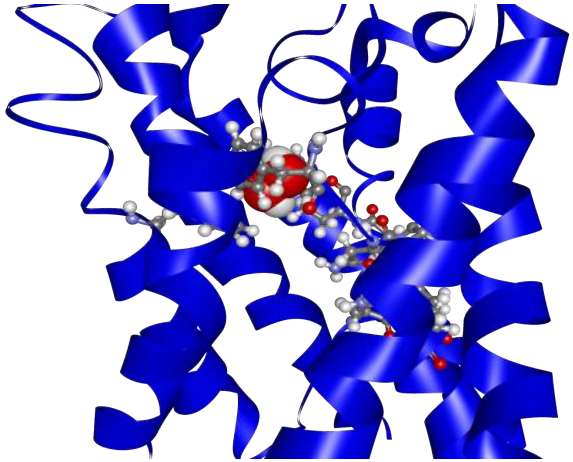




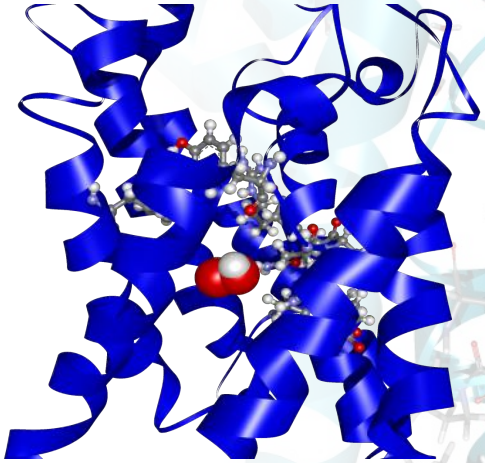
H_2O_2 permeation



Entering ar/R



Within ar/R

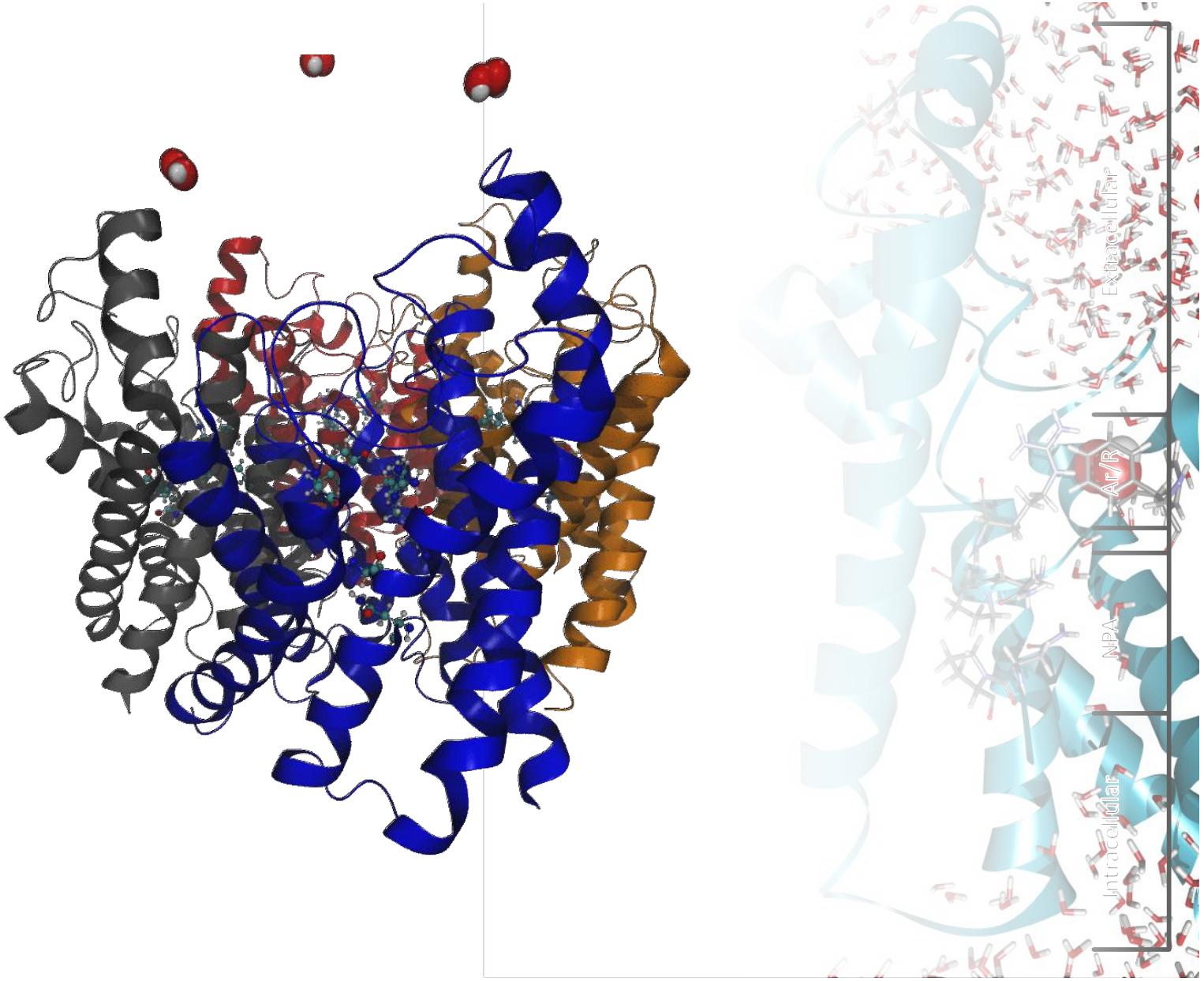


Within NPA

H_2O_2 permeation through the AQP3 pore, from extracellular to intracellular side

- H_2O_2, although being more similar in size to water when compare to glycerol, also adopts a longitudinal orientation when passing through the Ar/R S/F.
- As for glycerol, the flipping motion observed in water permeation is not observed in the case of H_2O_2, while H-bond formation between the substrate and the NPA S/F is observed.

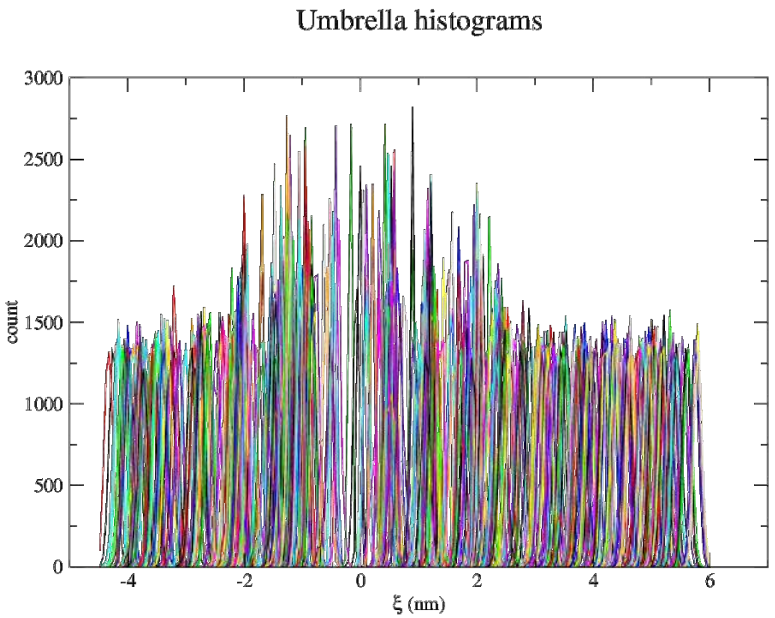
H_2O_2 permeation

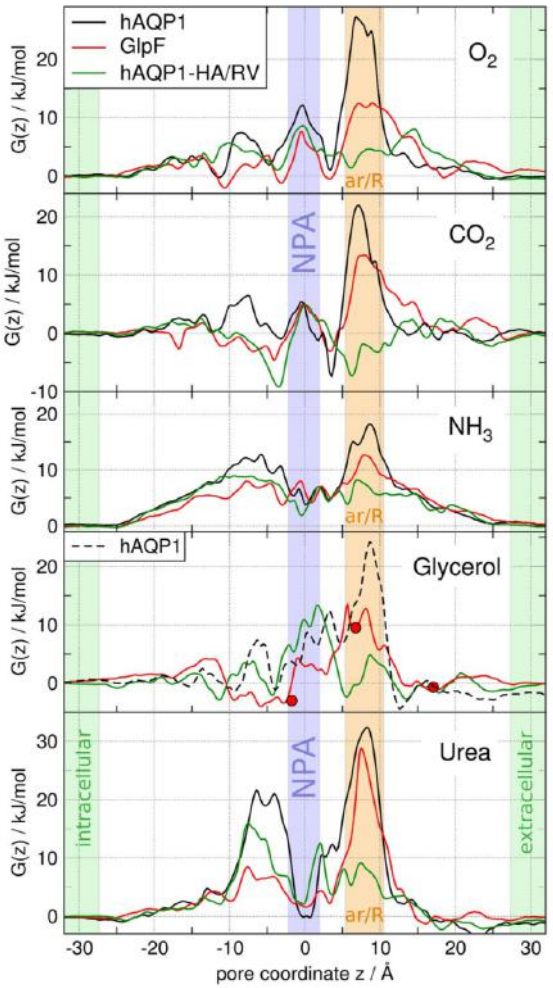


Calculating Potentials of Mean Force (PMF)

- Weighted Histogram Analysis Method (WHAM)

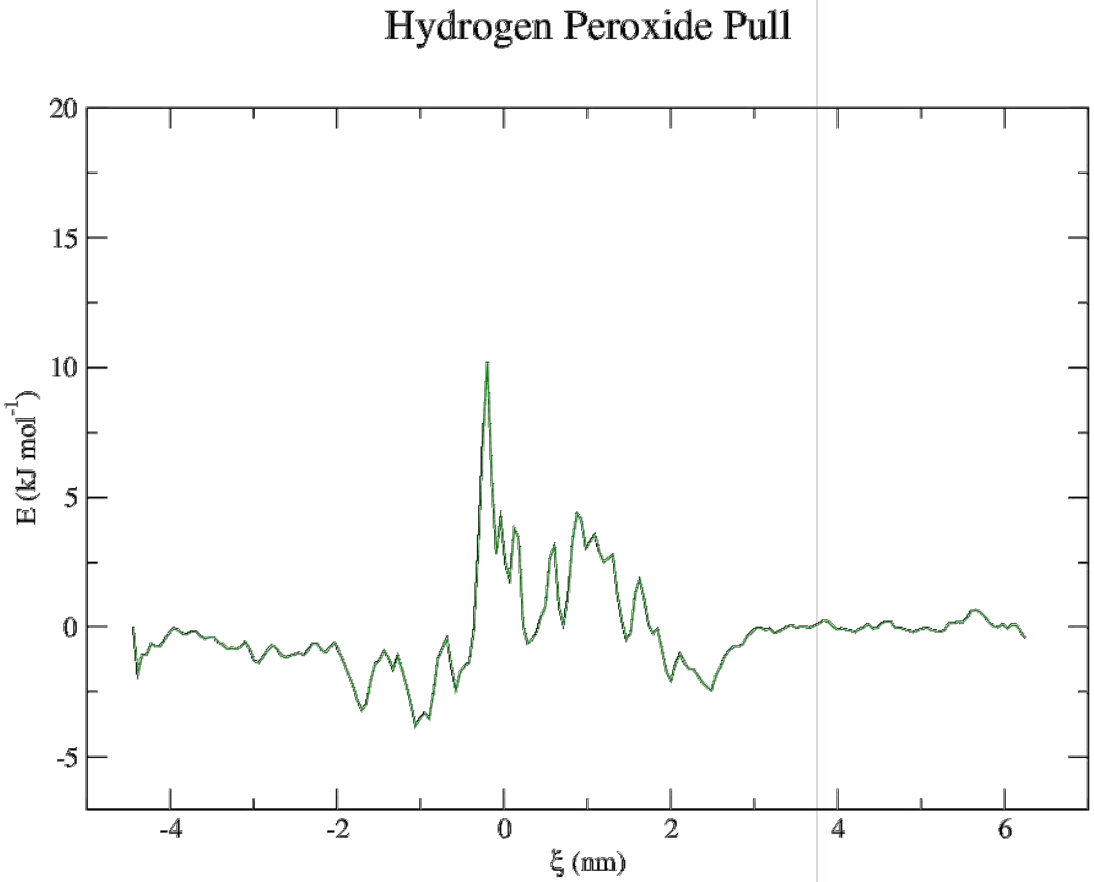
- Histograms for each window are combined, ensuring overlap, to produce an energy profile of the system

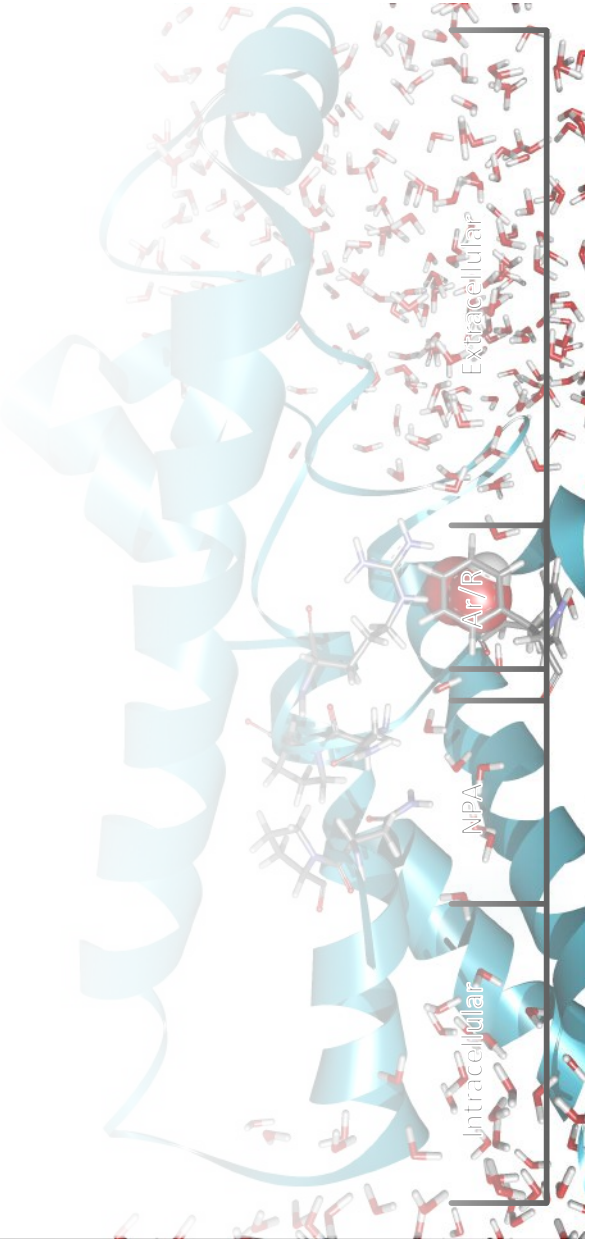




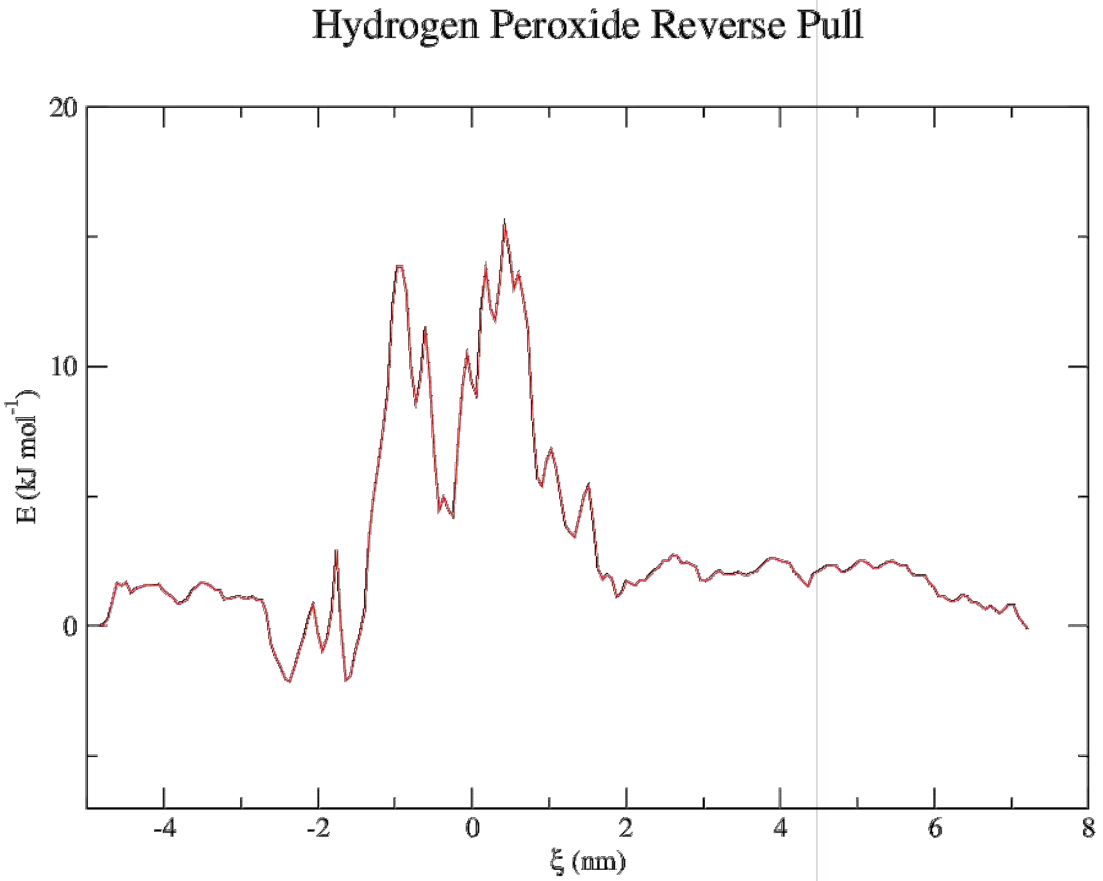
J. S. Hub and B. L. de Groot, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 1198–203.
J. Kästner, *Wiley Interdiscip. Rev. Comput*. Mol. Sci., 2011, 1, 932–942.

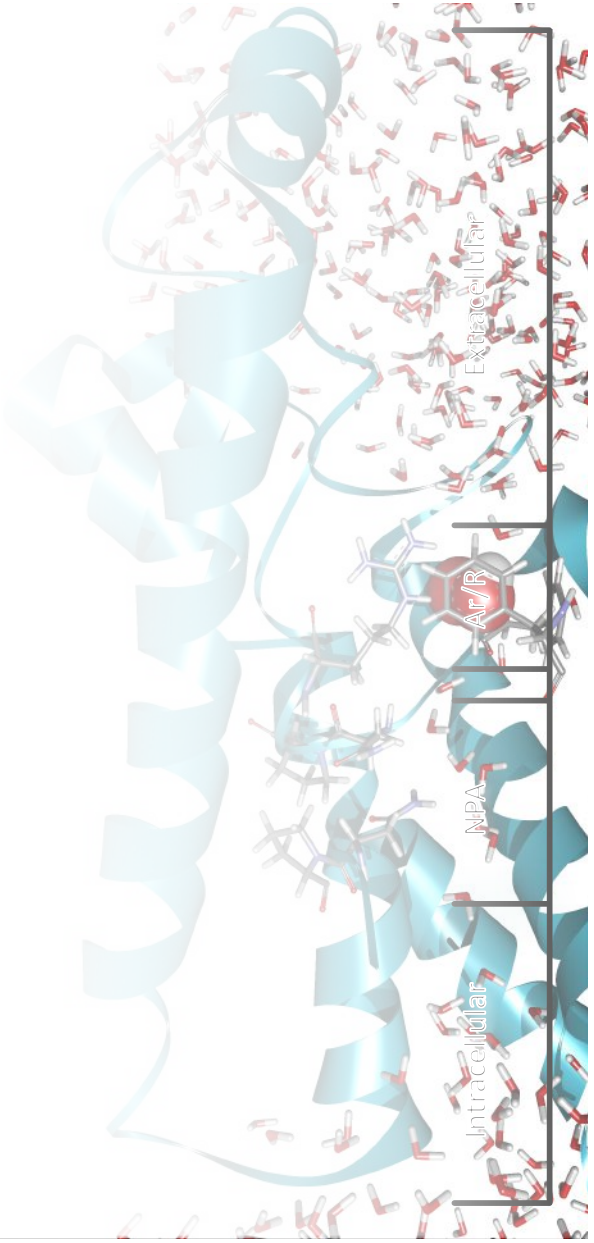
Umbrella sampling – H_2O_2





Umbrella sampling – H_2O_2

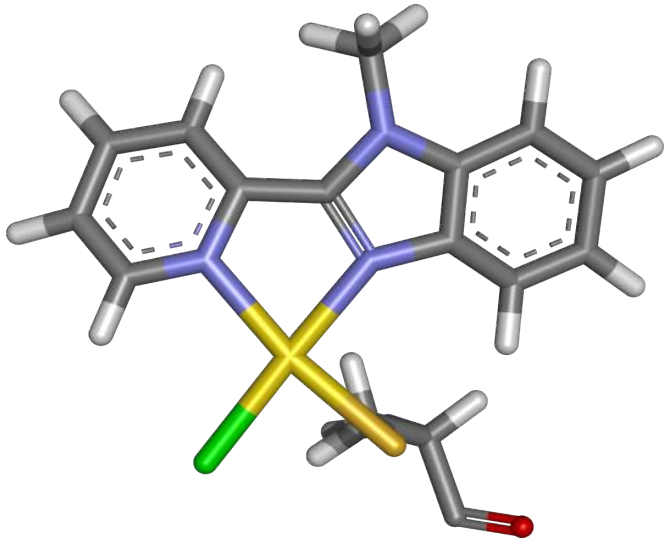


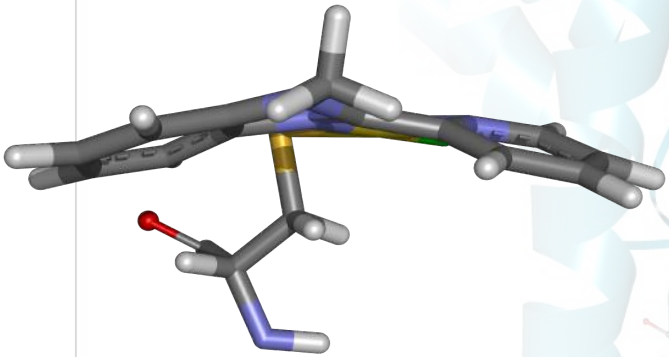


AuPblmME parameterisation

Automated topology builder - QM/MM, DFT

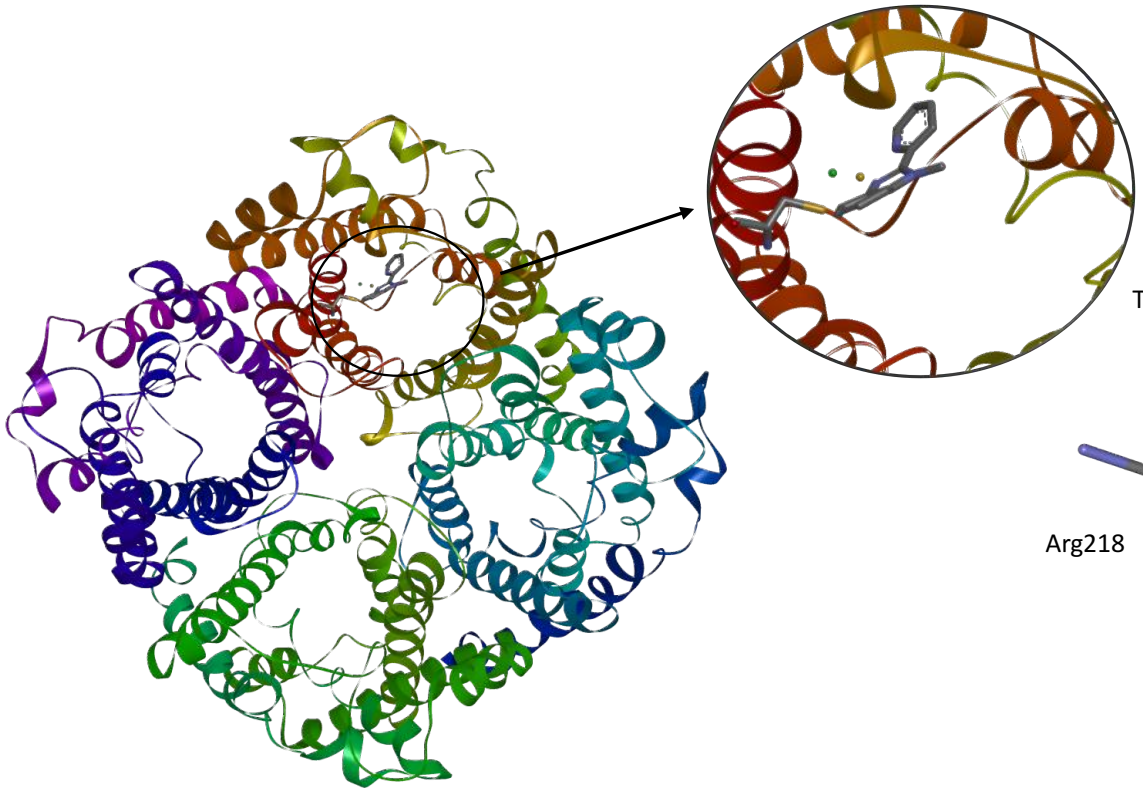
QM/MM



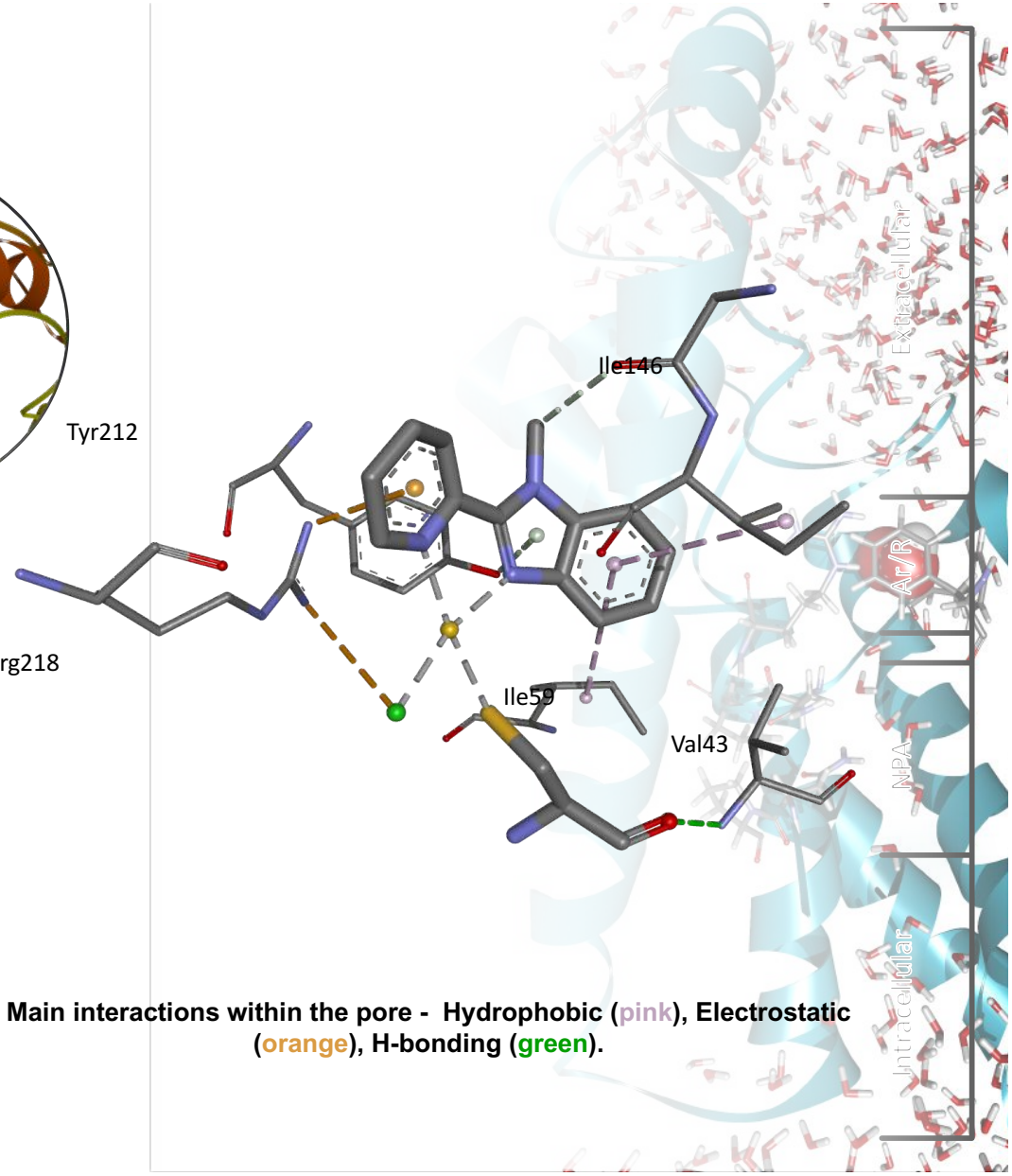


AuPblmME following energy minimisation

AuPblmME parameterisation

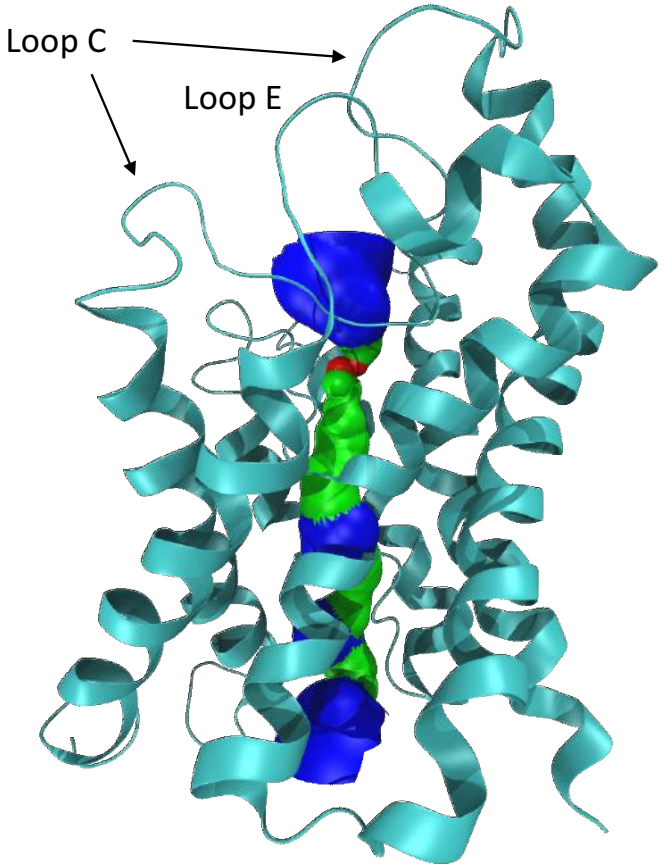


AuPblmME position within the pore

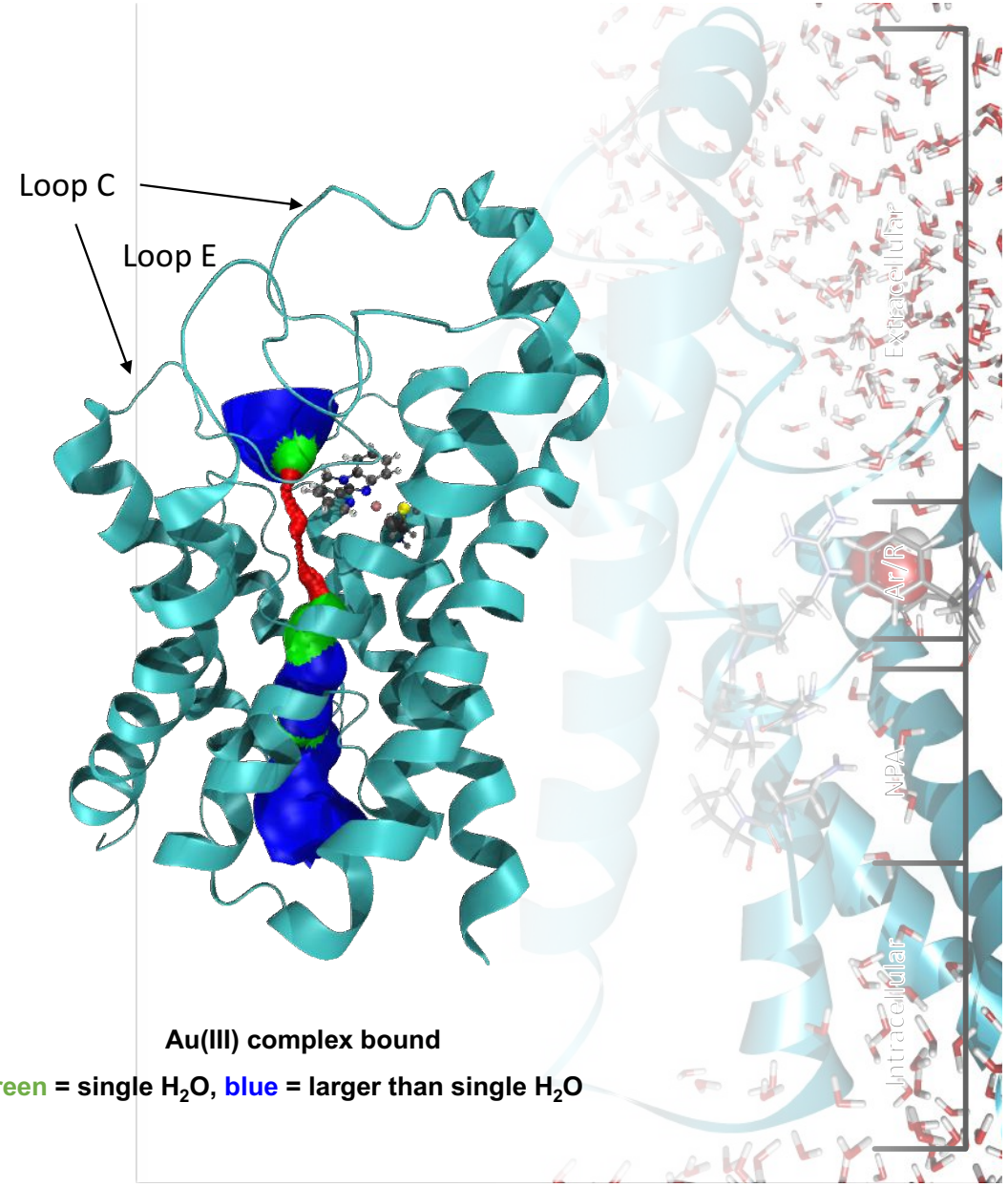


Main interactions within the pore - Hydrophobic (pink), Electrostatic (orange), H-bonding (green).

Pore restriction



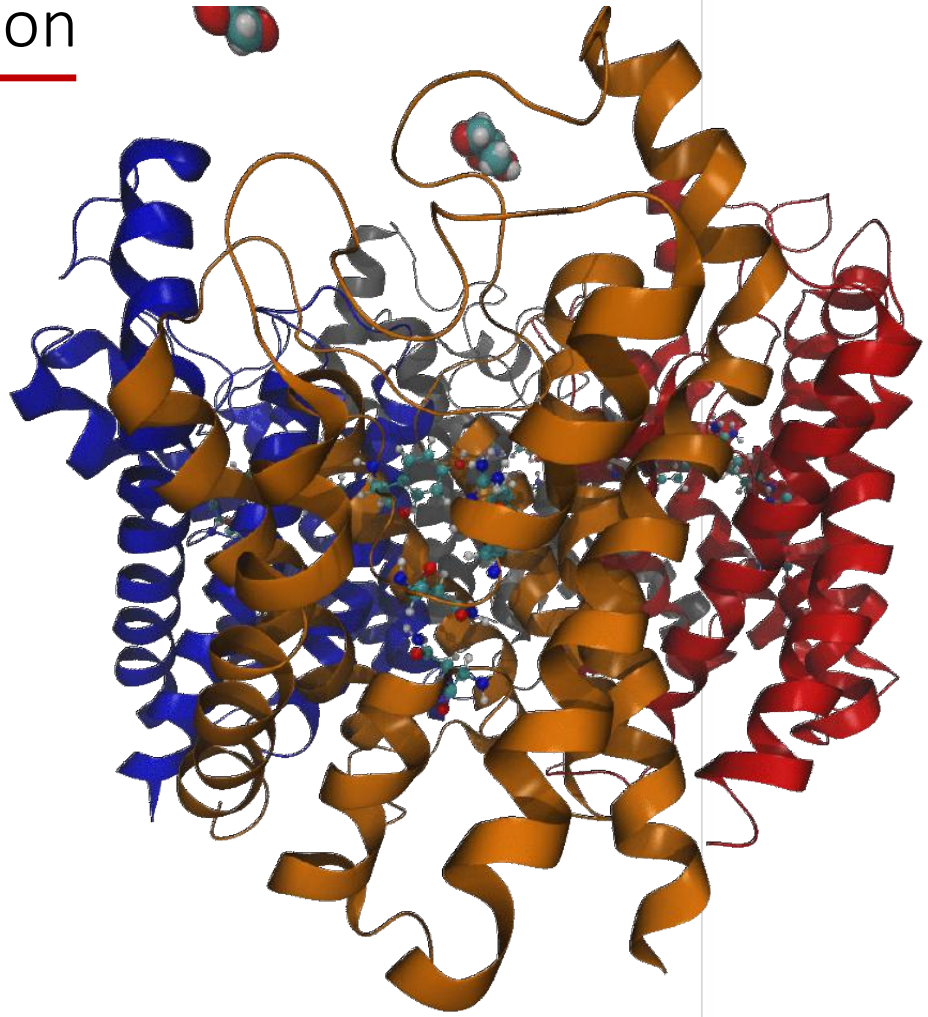
Unbound

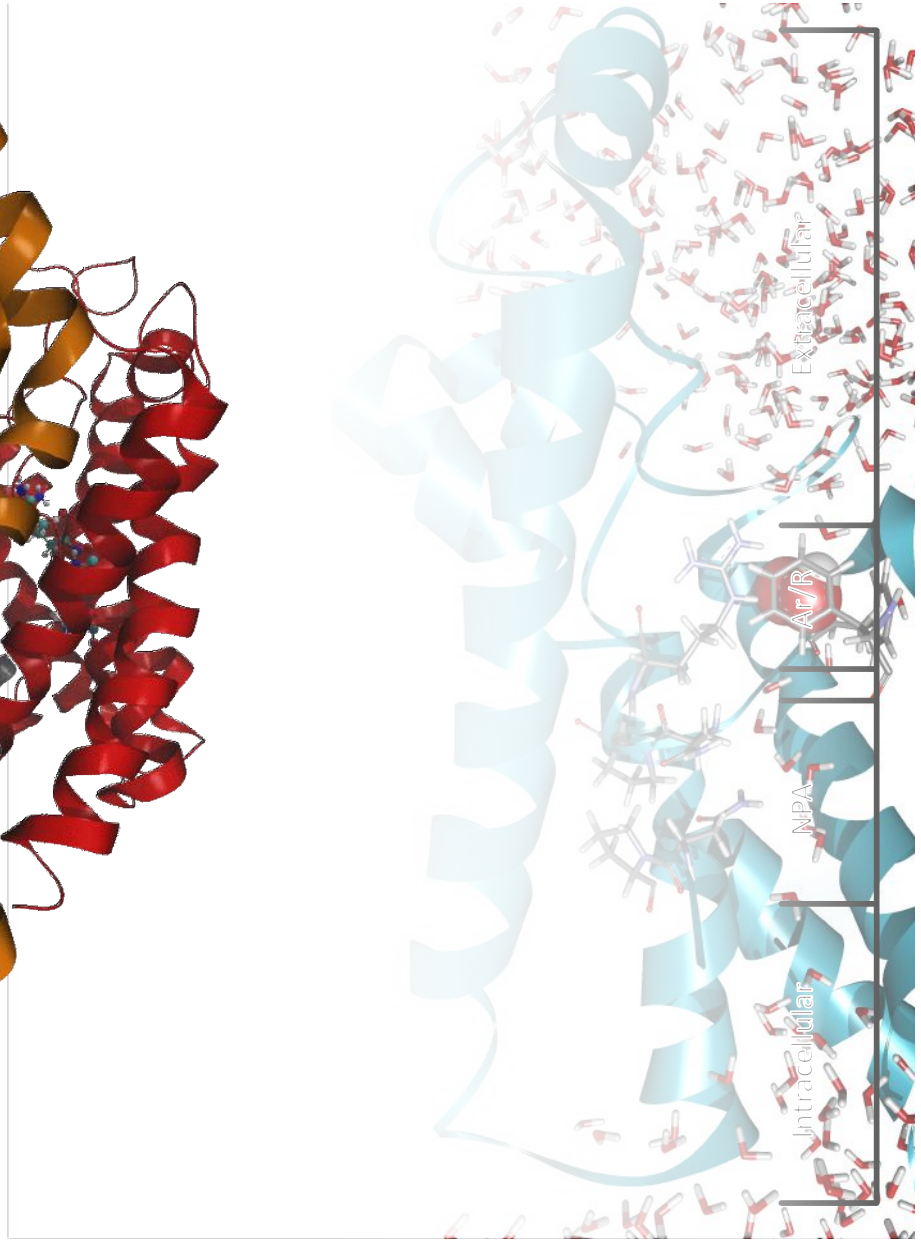


Au(III) complex bound

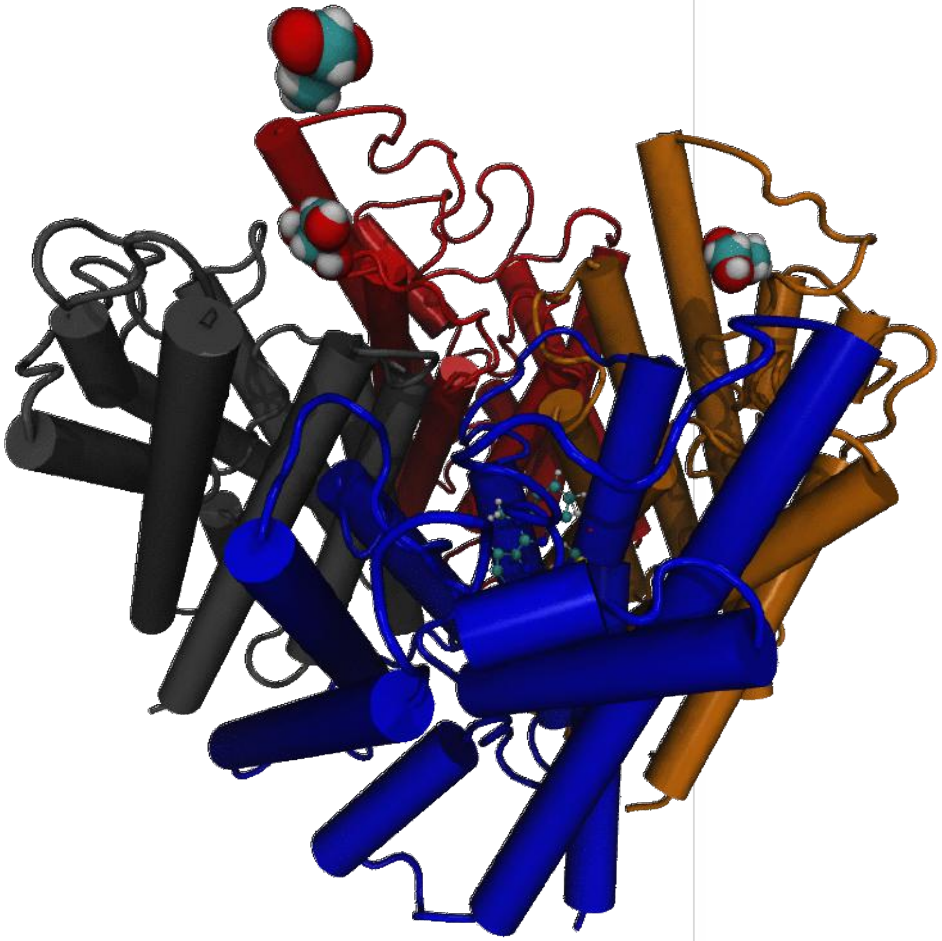
AQP3 pore size based on VDW radii: red = smaller than single H_2O, green = single H_2O, blue = larger than single H_2O

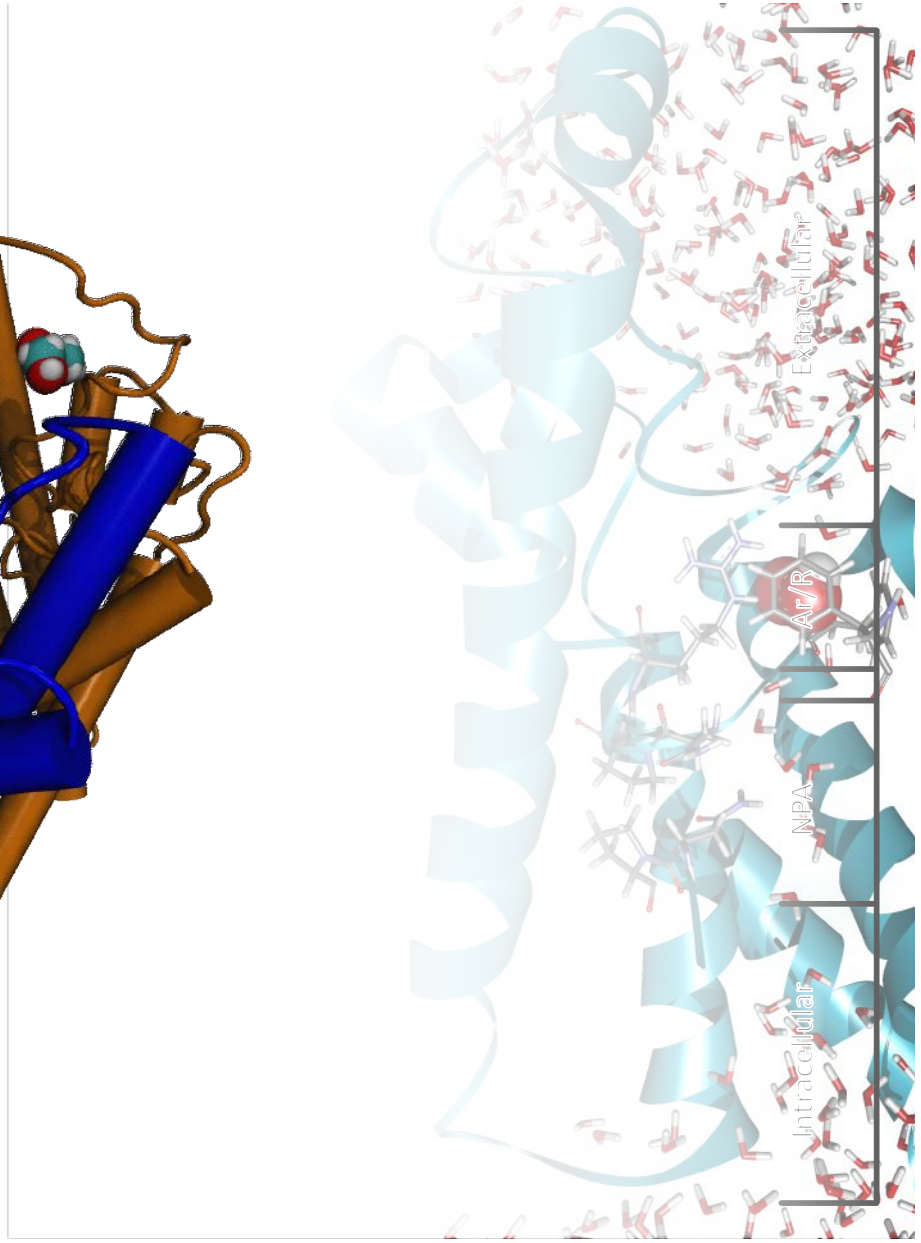
Glycerol permeation



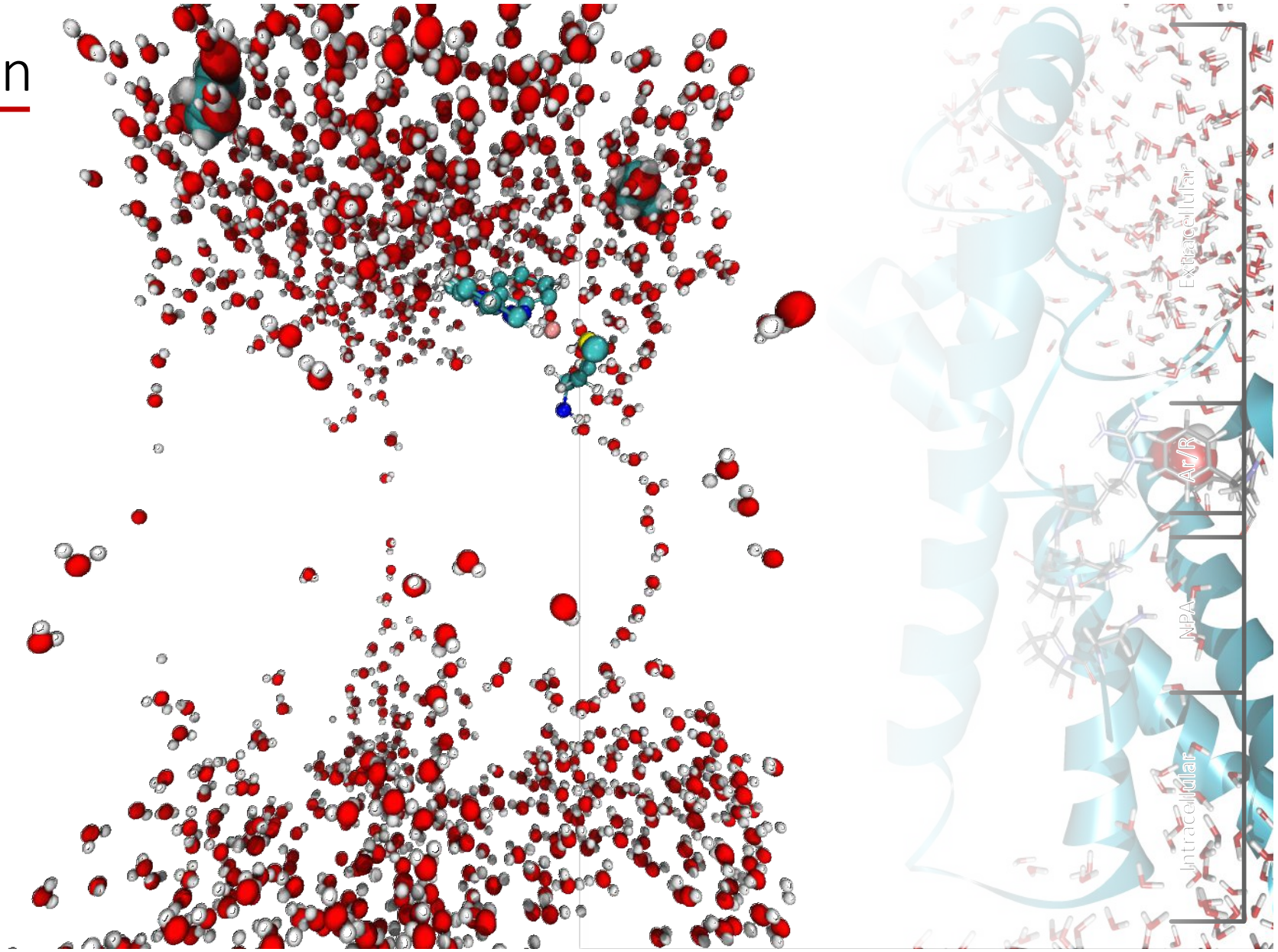


Pore restriction





Pore restriction



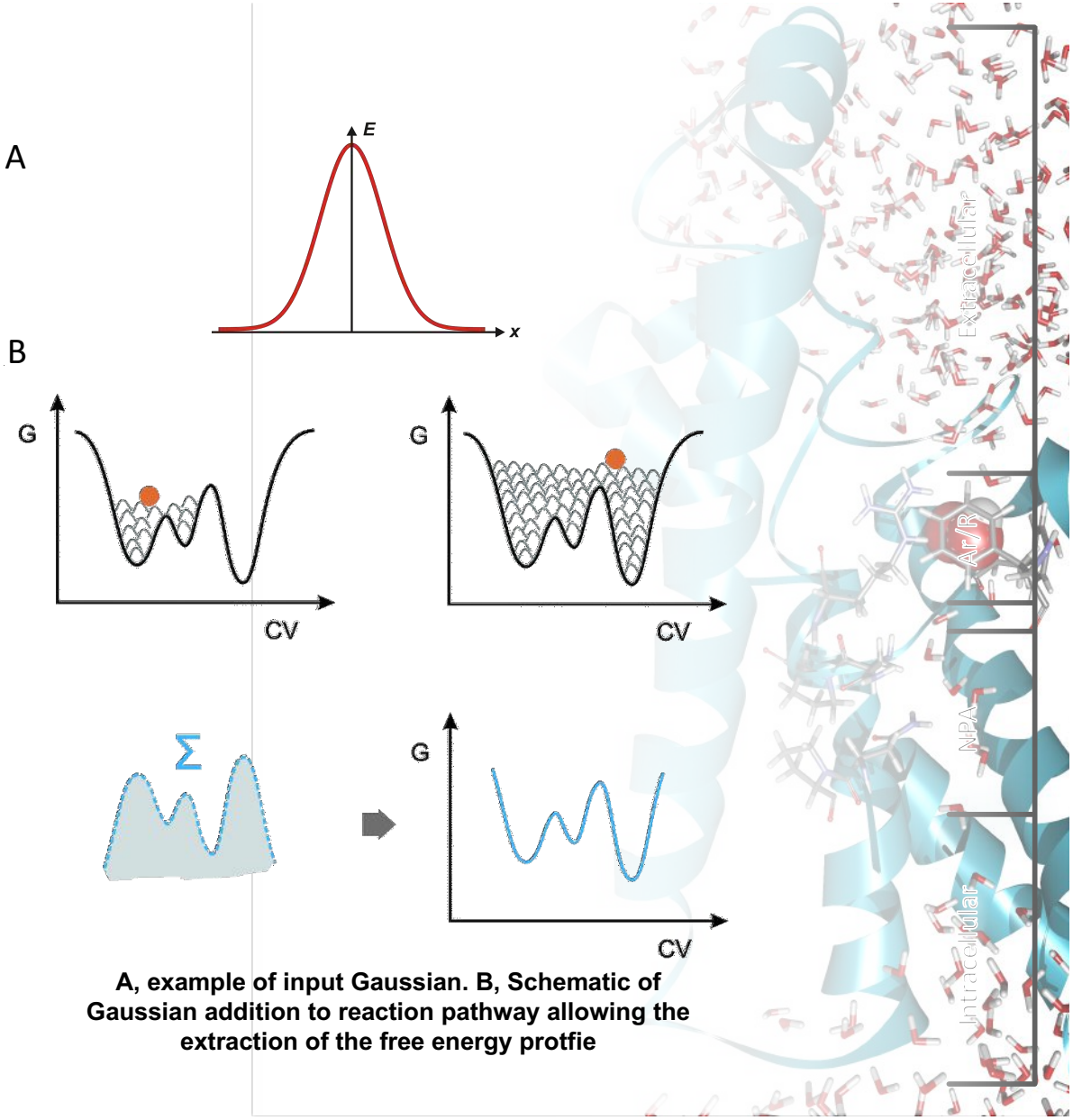
Metadynamic simulations

- Example of input file

```
COM ATOMS=37317-37330 LABEL=com1
COM ATOMS=37331-37344 LABEL=com2
COM ATOMS=37345-37358 LABEL=com3
COM ATOMS=37359-37372 LABEL=com4
COM ATOMS=1-3768 LABEL=comA
COM ATOMS=3769-7536 LABEL=comB
COM ATOMS=7537-11304 LABEL=comCC
OM ATOMS=11305-15072 LABEL=comD

DISTANCE ATOMS=com1,comA LABEL=pos1 SCALED_COMPONENTS
DISTANCE ATOMS=com2,comB LABEL=pos2 SCALED_COMPONENTS
DISTANCE ATOMS=com3,comC LABEL=pos3 SCALED_COMPONENTS
DISTANCE ATOMS=com4,comD LABEL=pos4 SCALED_COMPONENTS
COMBINE LABEL=pos ARG=pos1.c,pos2.c,pos3.c,pos4.c PERIODIC=-10,10

METAD ...
LABEL=metad
ARG=pos
PACE=200
HEIGHT=2 (energy – kJ mol-1)
SIGMA=1 (width – nm)
FILE=HILLS... METAD
PRINT STRIDE=10 ARG=pos,metad.bias FILE=COLVARENDPLUMED
```

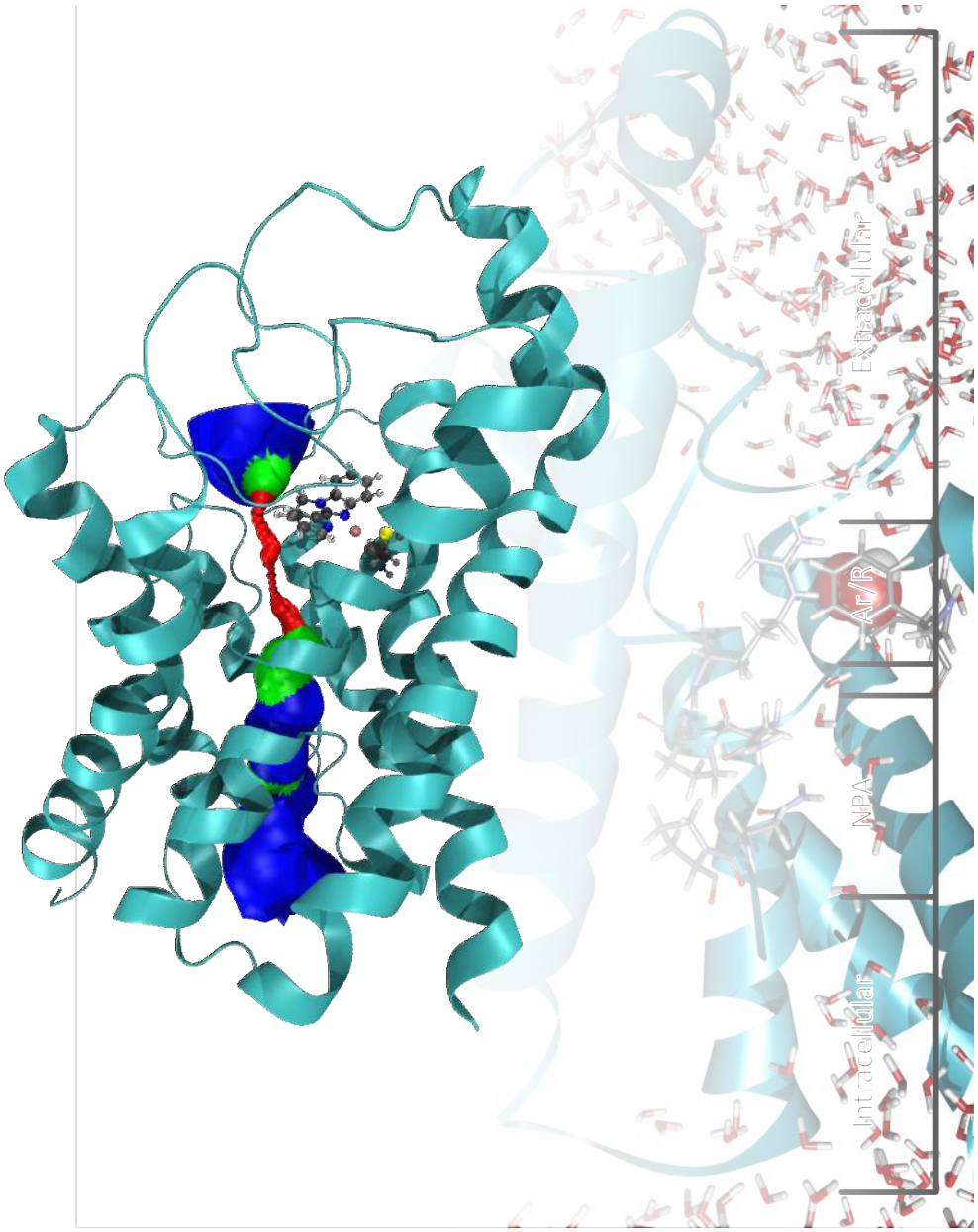


A, example of input Gaussian. B, Schematic of Gaussian addition to reaction pathway allowing the extraction of the free energy protfie

V. Van Speybroeck, *Chem. Soc. Rev.*, 2014, **43**, 7326–7357. (Fig. 11)

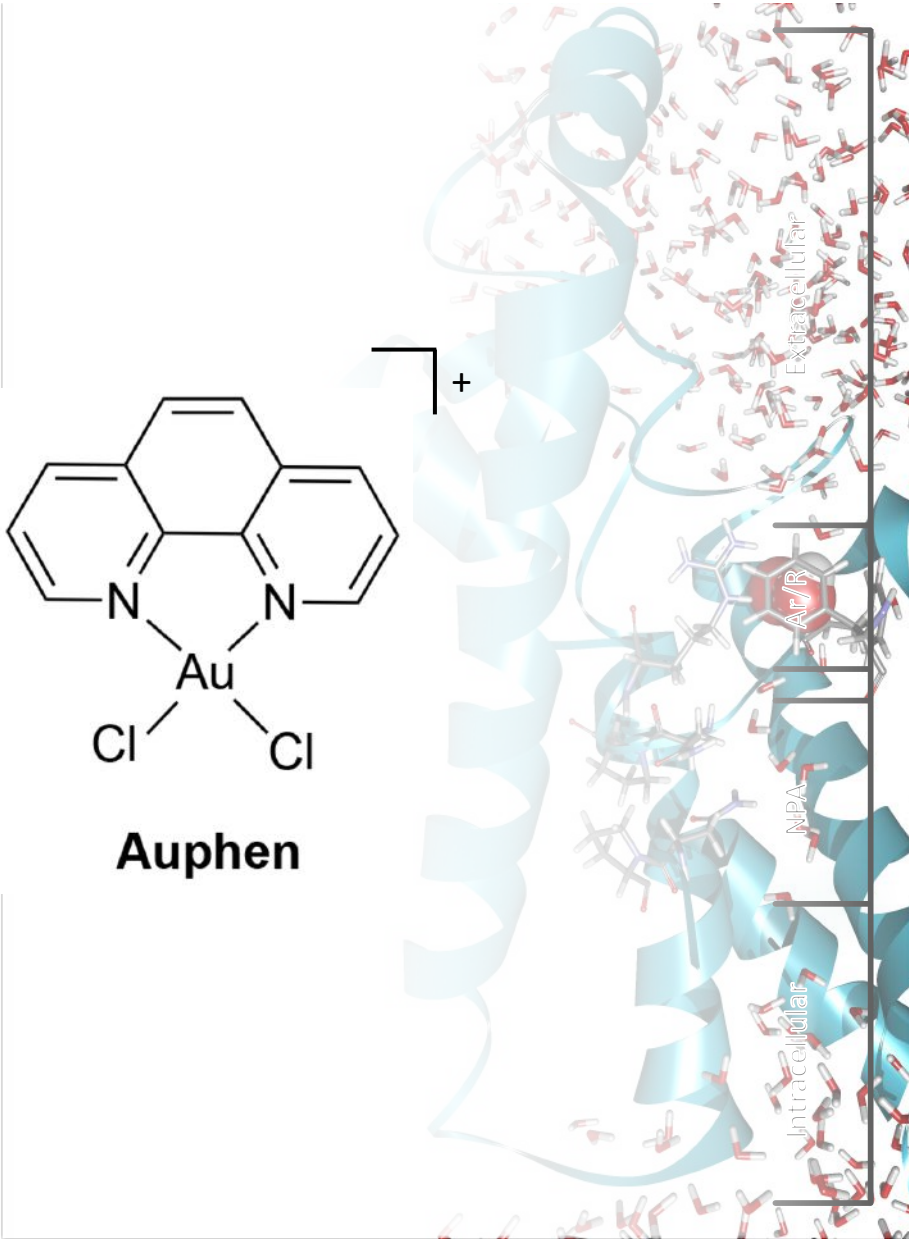
Conclusion

- Bound AuPblmME prevents both glycerol and water transport through the pore
- Complex causes a conformational change of the protein via electrostatic and hydrophobic interactions
- Small slowing effect on second pore diagonal to pore containing complex.
- Remaining monomers are unaffected in regards to both glycerol and water
- Metadynamics
  - Powerful and highly adaptable simulation tool
  - Provides high resolution free energy profiles



Future Studies

- Continue to investigate the effects of potential inhibitor molecules on glycerol and hydrogen peroxide transport
  - Inserting a selection of Au(III) coordination complexes into the system
- Multiple isoform tetramers
  - AQP3 and AQP7
- Metadynamic simulations of aquaporins, including a selection of Au(III) coordination complexes



A. de Almeida, *Med.Chem.Commun*, 2014, **5**, 1444–1453.

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Dr Andreia de Almeida
Brech Aikman
Sam Jobbins

Thank you for your time