Aquaporin 2

Structural Biology
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Summary

1.Introduction

History

Aquaporines

Family and genetics of AQP2

SCOP classification

2. Function

Renal Function

Ligand

Translocation

Signalling Pathway

Residues

3. Structure

Polarity

Salt bridges

Subunit interactions

Cd/Ca action

ar/R filter

NPA region

4. Family

AQP family

Superimposition

Structural alignment

APQ7-6QZI

Sequence alignment

Phylogenetic tree

5. Evolution

Superimposition

Structural alignment

Bacterial AQP

Plant AQP

Sequence alignment

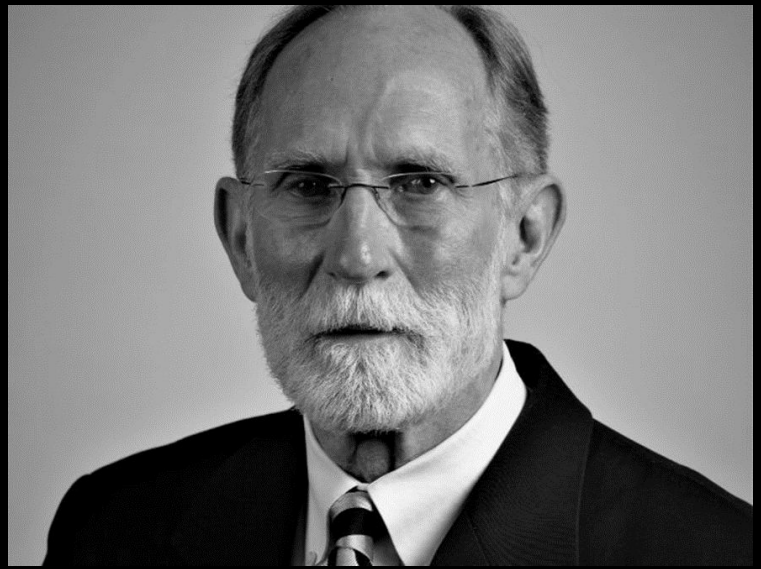
Phylogenetic tree

6. PEM questions

INTRODUCTION

History

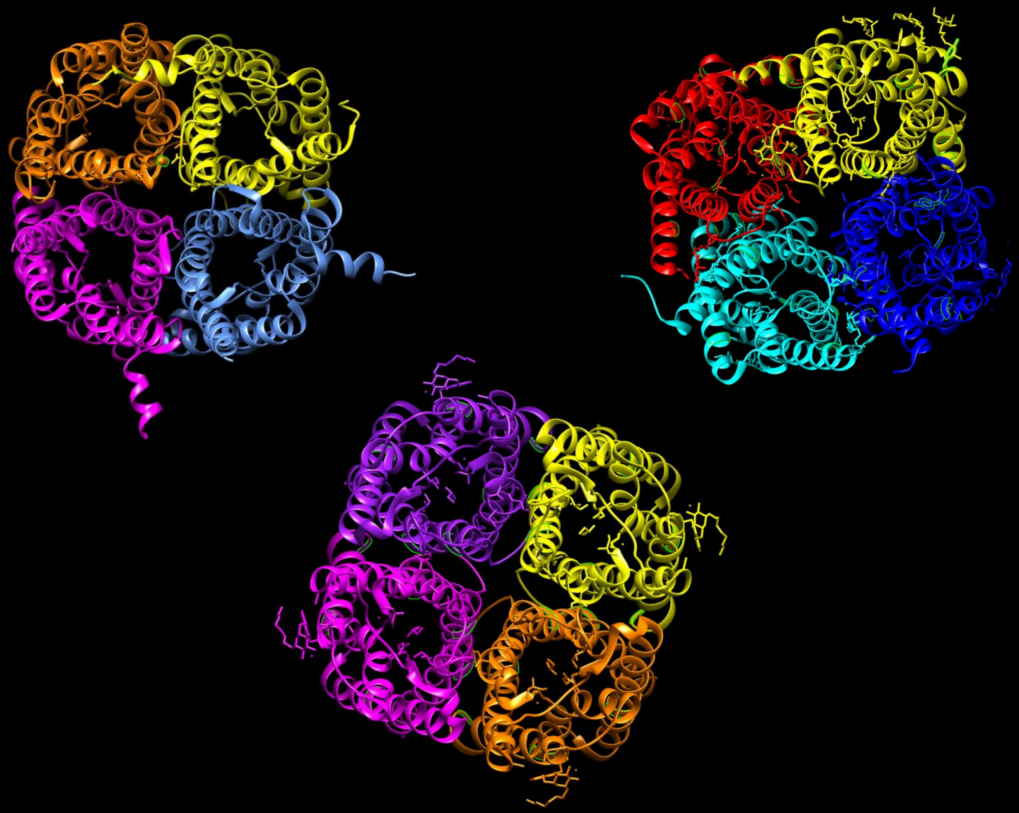
Peter Agre, 2003 Nobel Prize winner in chemistry



Purified aquaporin-1 from human erythrocytes.

They proved AQP1 is a specific water channel by cRNA expression studies in Xenopus oocytes

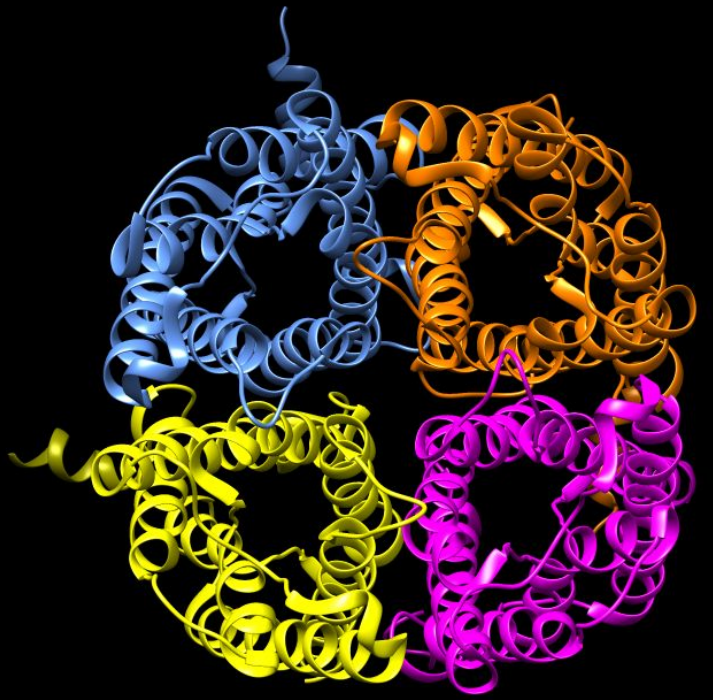
Aquaporines



- Intrinsic membrane proteins found in all organisms.
- Main function: facilitate water movement across membranes.
- Also aquaglyceroporins transport.

AQP2

- Human aquaporin 2 (AQP2) is found in the kidney collecting duct.
- Trafficking between intracellular storage vesicles and the apical membrane
- Water channel responsible for reabsorption across the apical membrane.



Family and genetics of AQP2

- Major intrinsic protein superfamily
- Channels with unique structural features and diverse selectivity filters
- AQP2 protein is encoded by the AQP2 gene, clustered in chromosome 12q13

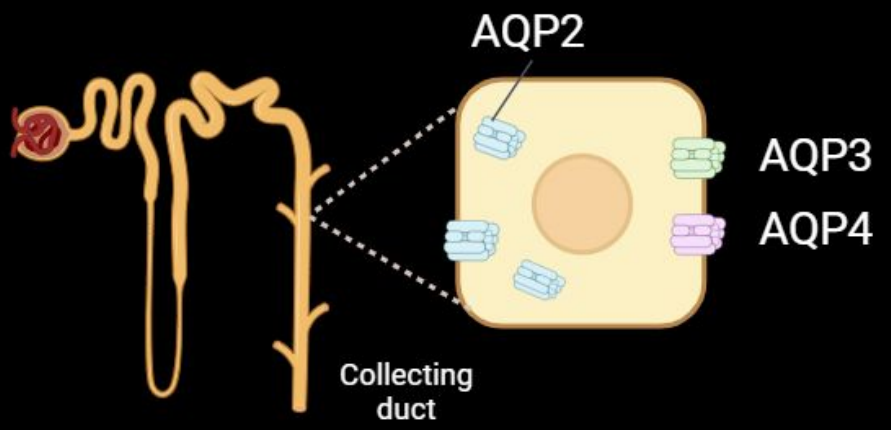
SCOP classification

Class	All alpha proteins
Fold	Aquaporin-like
Superfamily	Aquaporin-like
Family	Aquaporin-like
Domain	8072761

FUNCTION

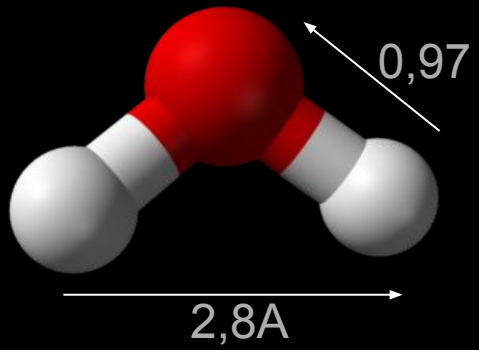
Renal Function

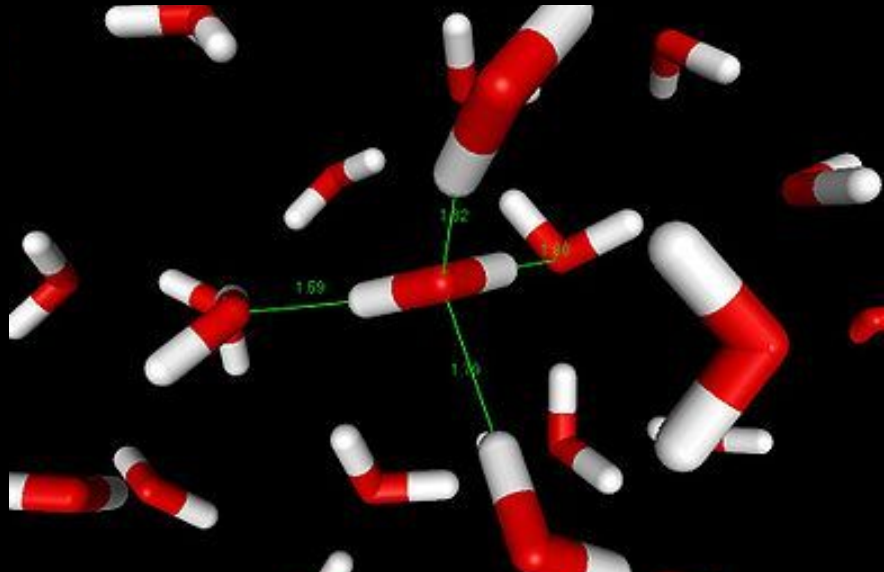
- AQP2 expressed in kidney collecting duct principal cells
- Role urinary concentration
- Importance of vasopressin
- Water reabsorition in colaboration with other AQPs



Ligand: H2O

- It is composed of two hydrogen atoms and one oxygen atom.
- Partial charges create polarity.
- Enable to form hydrogen bonds with electronegative atoms .



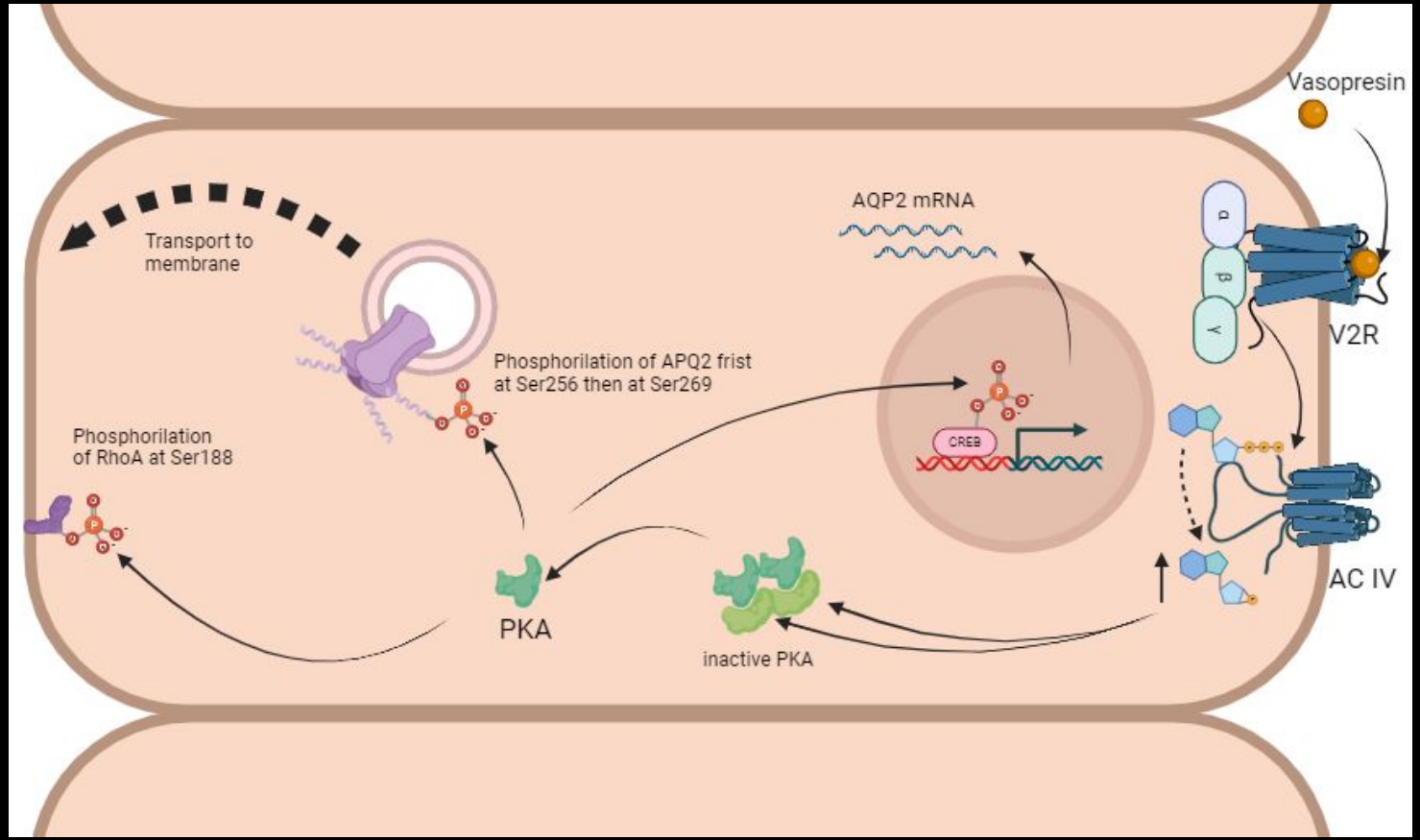


AQP2 translocation

- Vasopressin-stimulated phosphorylation of Ser256
- Additional phosphorylation of Thr269 (Ser269 in mouse) → prolonged residence
- As well as phosphorylation of Ser264

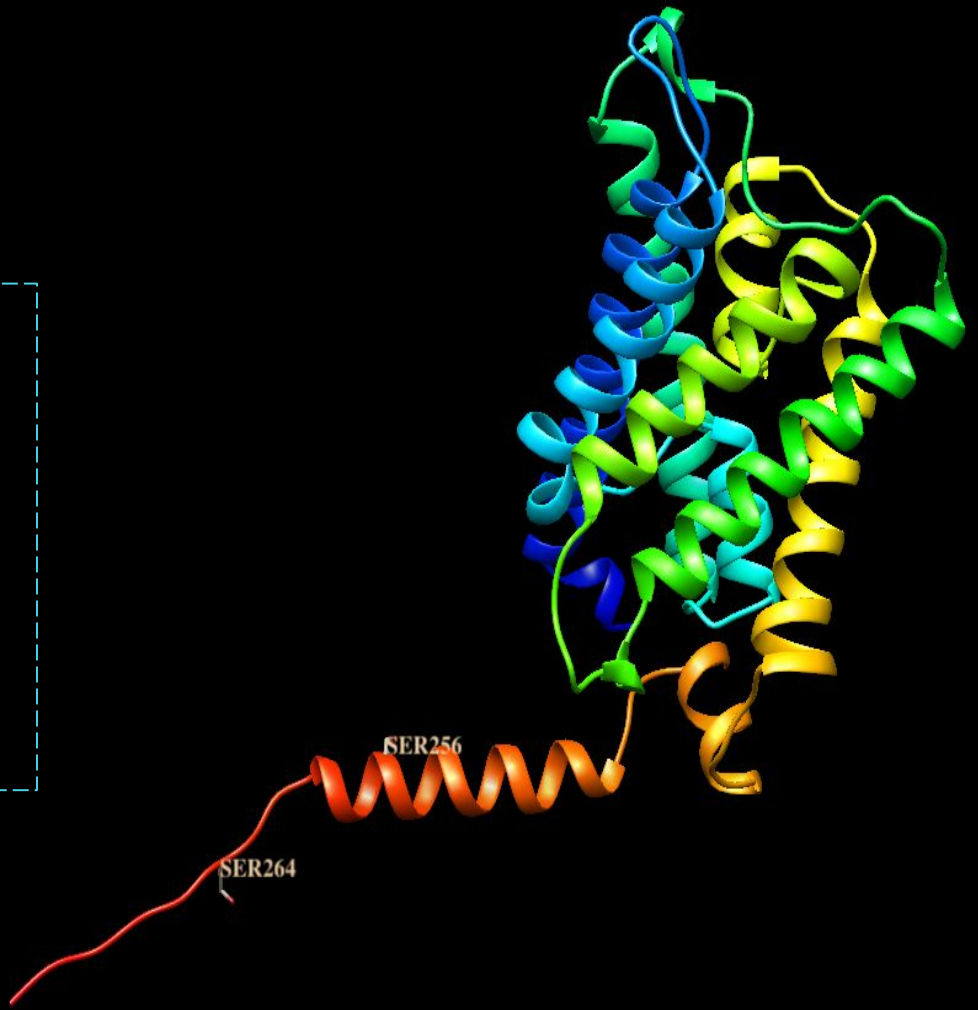
- Phosphorylation of Ser261
- AQP2 removal from apical membrane by ubiquitination of Lys270
- AQP2 stored in vesicles or target multi-vesicular bodies (VMB)

Signalling Pathway



Ser256 and Ser261

- Phosphorylation of Ser256 on the cytoplasmic COOH terminus by PKA
- Ser 264 is also phosphorylated to translocate AQP2
- Dephosphorylation of Ser261 also involved in this process



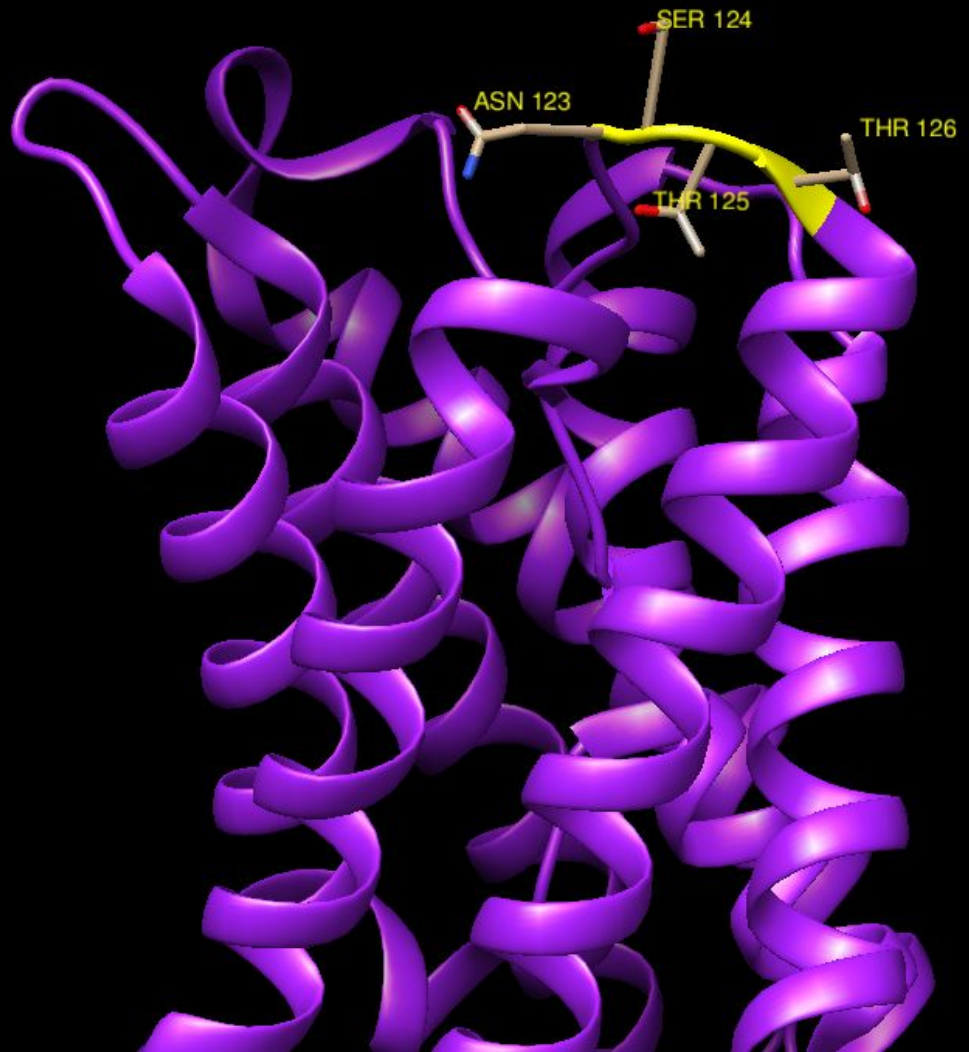
Lys270 and Ser264

- Activation of receptors by hormones, such as PGE2, ATP, and dopamine
- Induce ubiquitination on Lys270 → internalization and lysosomal degradation
- Also Ser261 phosphorylated is reinstalled by PKC

Glycosylation

- Region: Asn-X-Thr/Ser (where X can be every aa except Pro)

- Importance for AQP2 to exit the Golgi Complex and its proper routing the plasma membrane

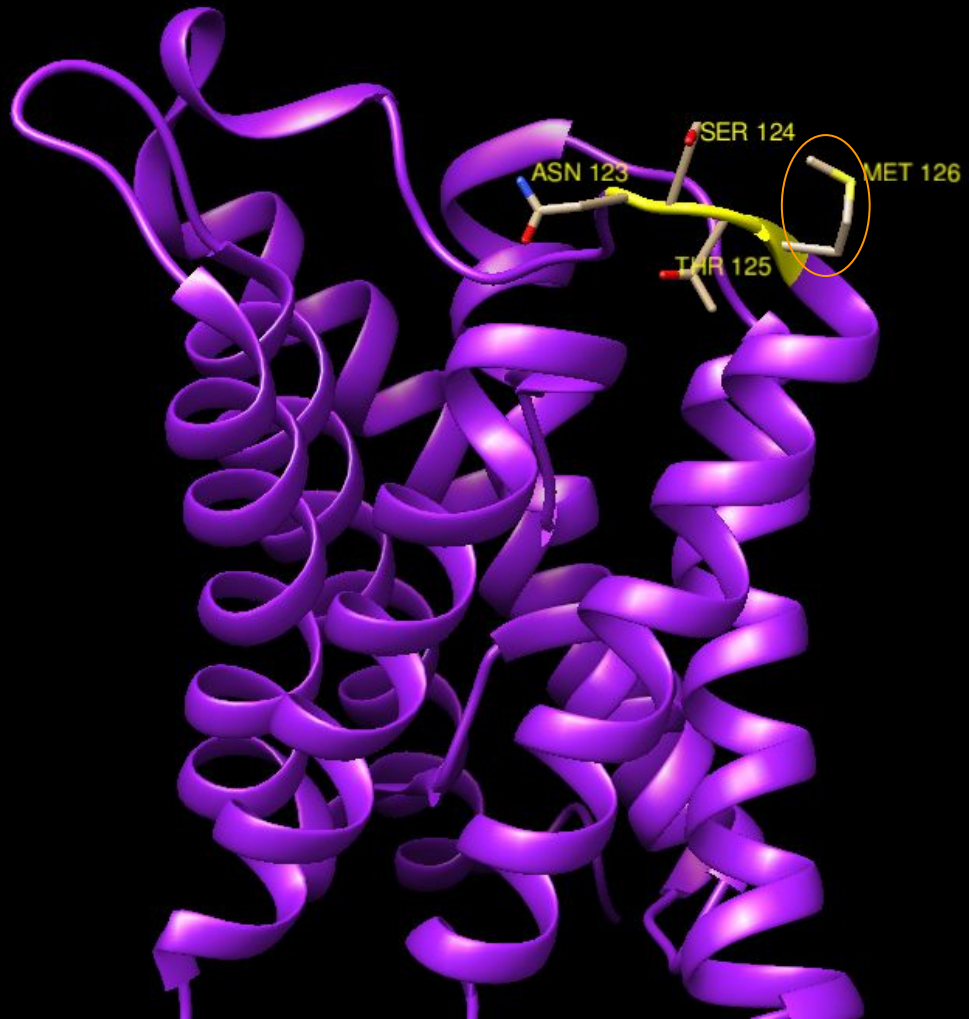


Mutant T125M

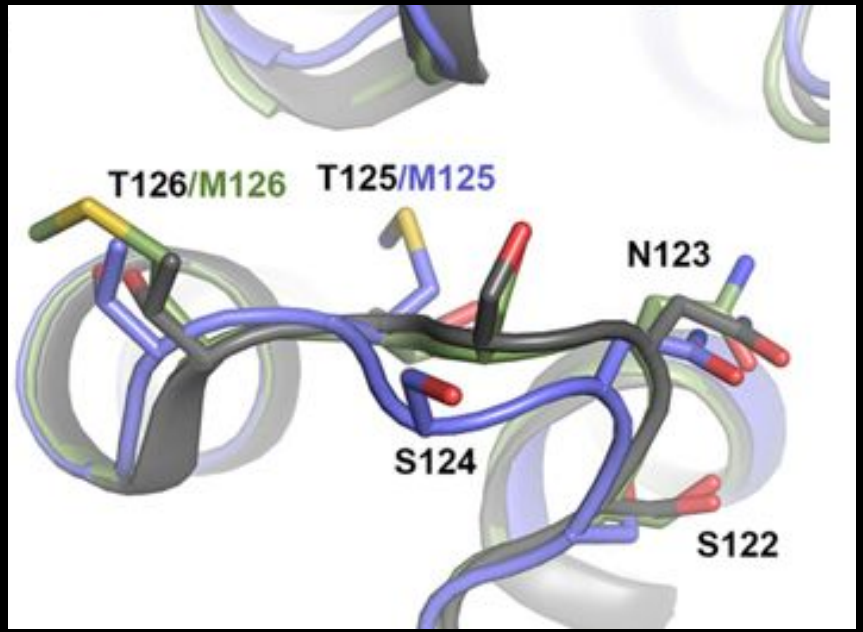
- Mutation: change of Thr125 → Met125
- Located within the glycosylation consensus sequence (Asn-X-Ser/Thr). This changes suppresses glycosylation
- Protein being retained in the ER and Golgi

Mutant T126M

- Mutation: change of Thr126 → Met126
- In this case glycosylation occurs, by UGT. Protein being retained in the ER
- Both related with Nephrogenic Diabetes insipidus



Nephrogenic Diabetes Insipidus



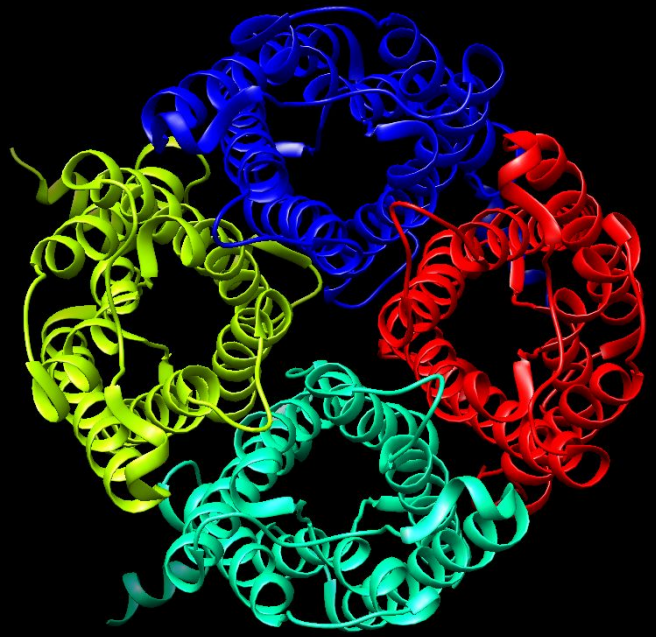
Hagströmer CJ, Steffen JH, Kreida S, Al-Jubair T, Frick A, Gourdon P, et al. Structural and functional analysis of aquaporin-2 mutants involved in nephrogenic diabetes insipidus. Scientific Reports | [Internet]. 123AD [cited 2024 Feb 23];13:14674. Available from: https://doi.org/10.1038/s41598-023-41616-1

Nephrogenic diabetes insipidus (NDI) is an inability to concentrate urine due to impaired renal tubule response to vasopressin.

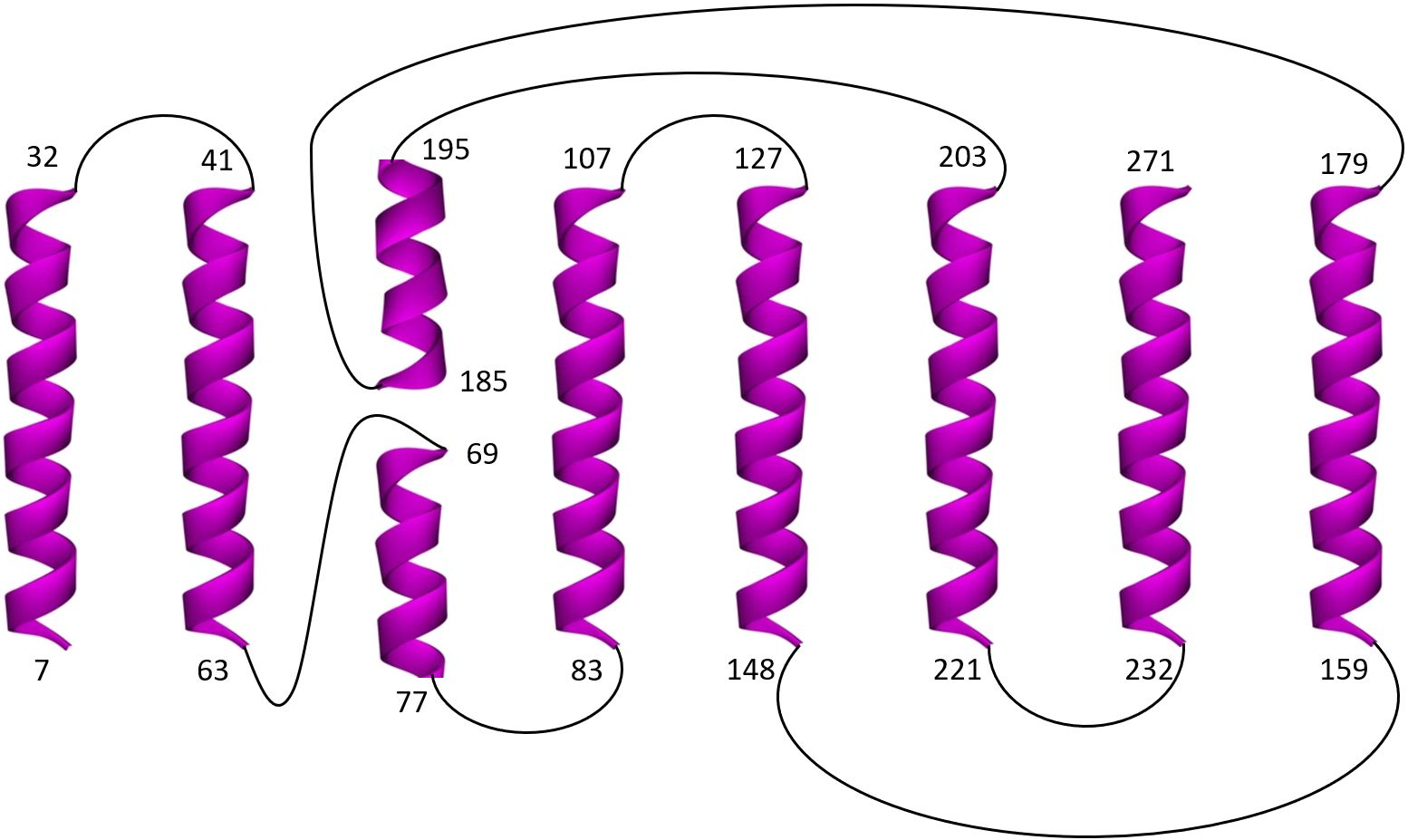
This leads to excretion of large amounts of dilute urine that need to be compensated with fluid intake to avoid severe dehydration and hypernatremia.

STRUCTURE

Structure

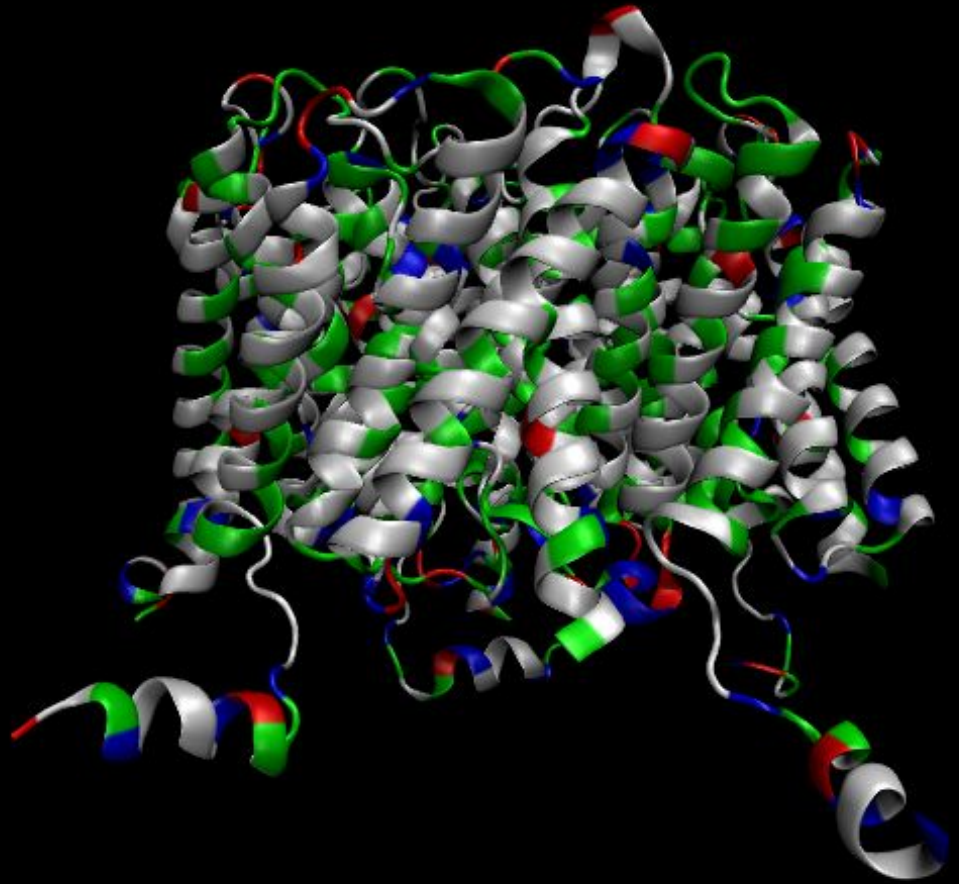


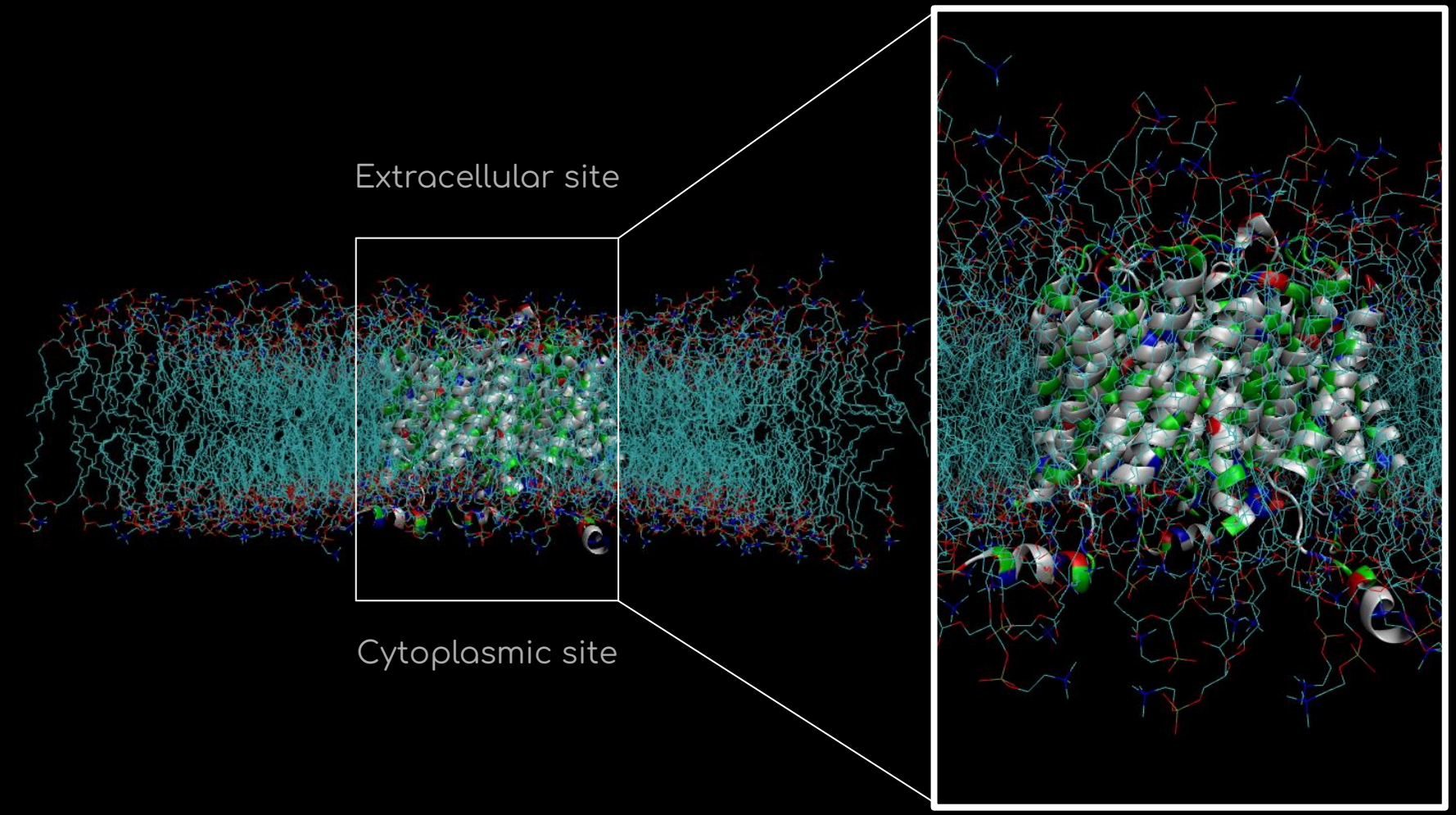
- Six transmembrane α-helices and five connecting loops.
- N and C-termini are cytosolic
- Two loops fold toward the membrane and move toward each other to form the pore.


32
41
195
107
127
203
271
179
185
69
7
63
77
83
148
221
232
159

Polarity

Non-polar/ hydrophobic	**white**
Polar/hydrophilic	**green**
Basic residues	**blue**
Acidic residues	**red**

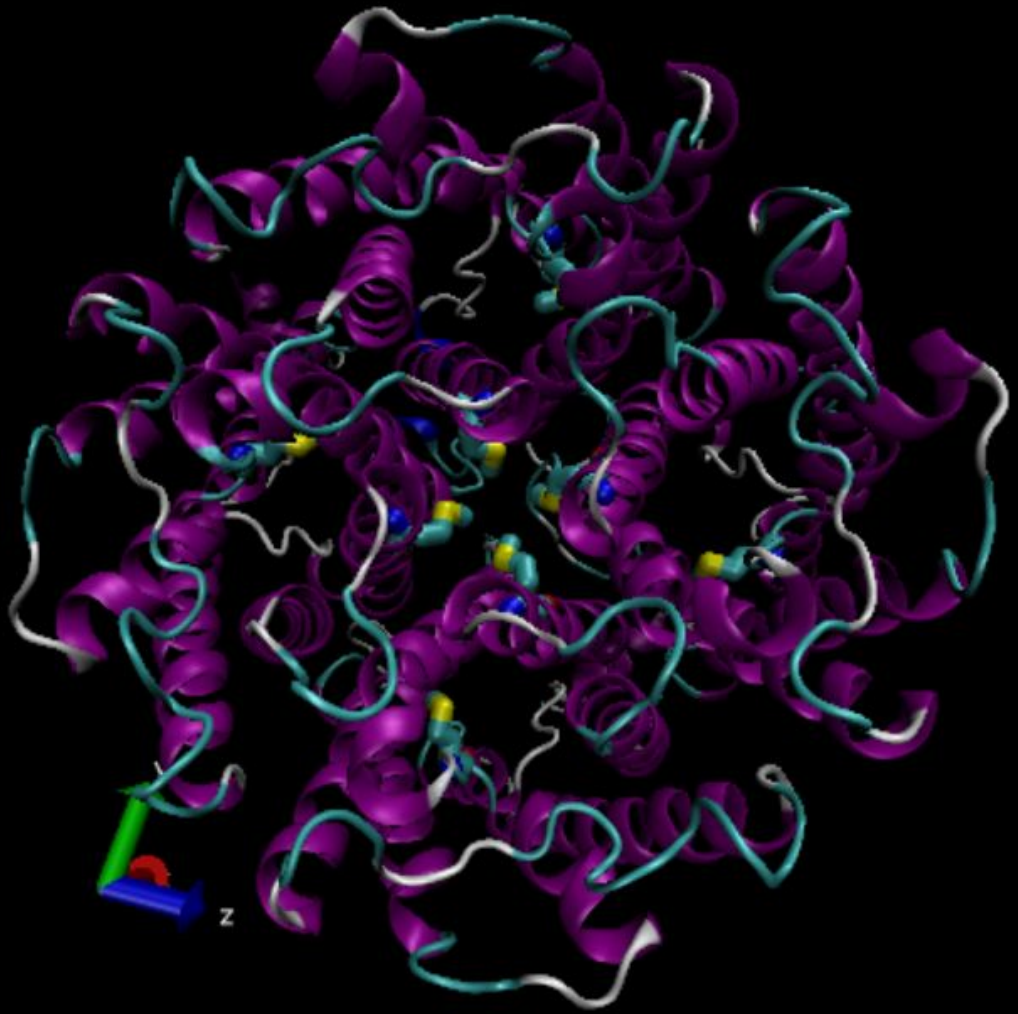



Extracellular site
Cytoplasmic site

Polarity

- Hydrophilic atoms focused towards interior alpha helices
- Also focused on both the top (outer) and bottom (cytoplasm)
- Hydrogen bonds

Polarity

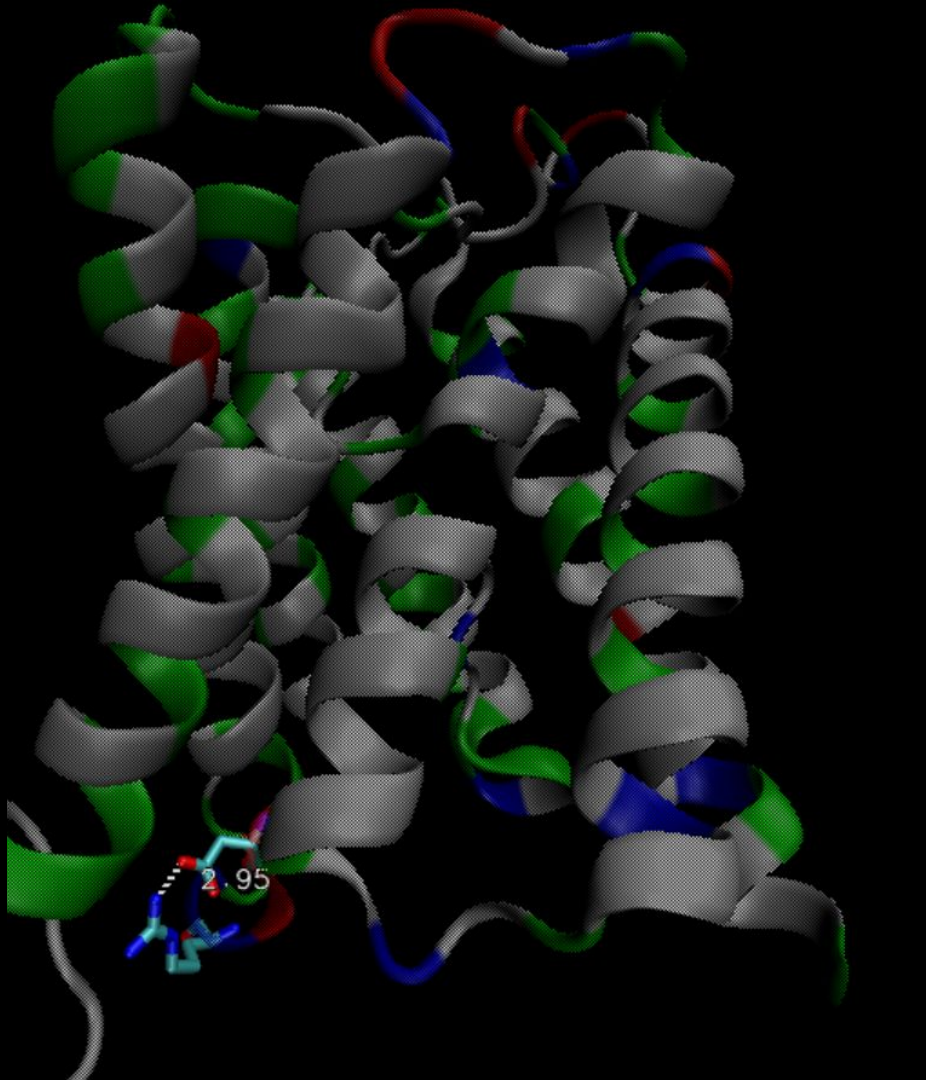


Salt bridges

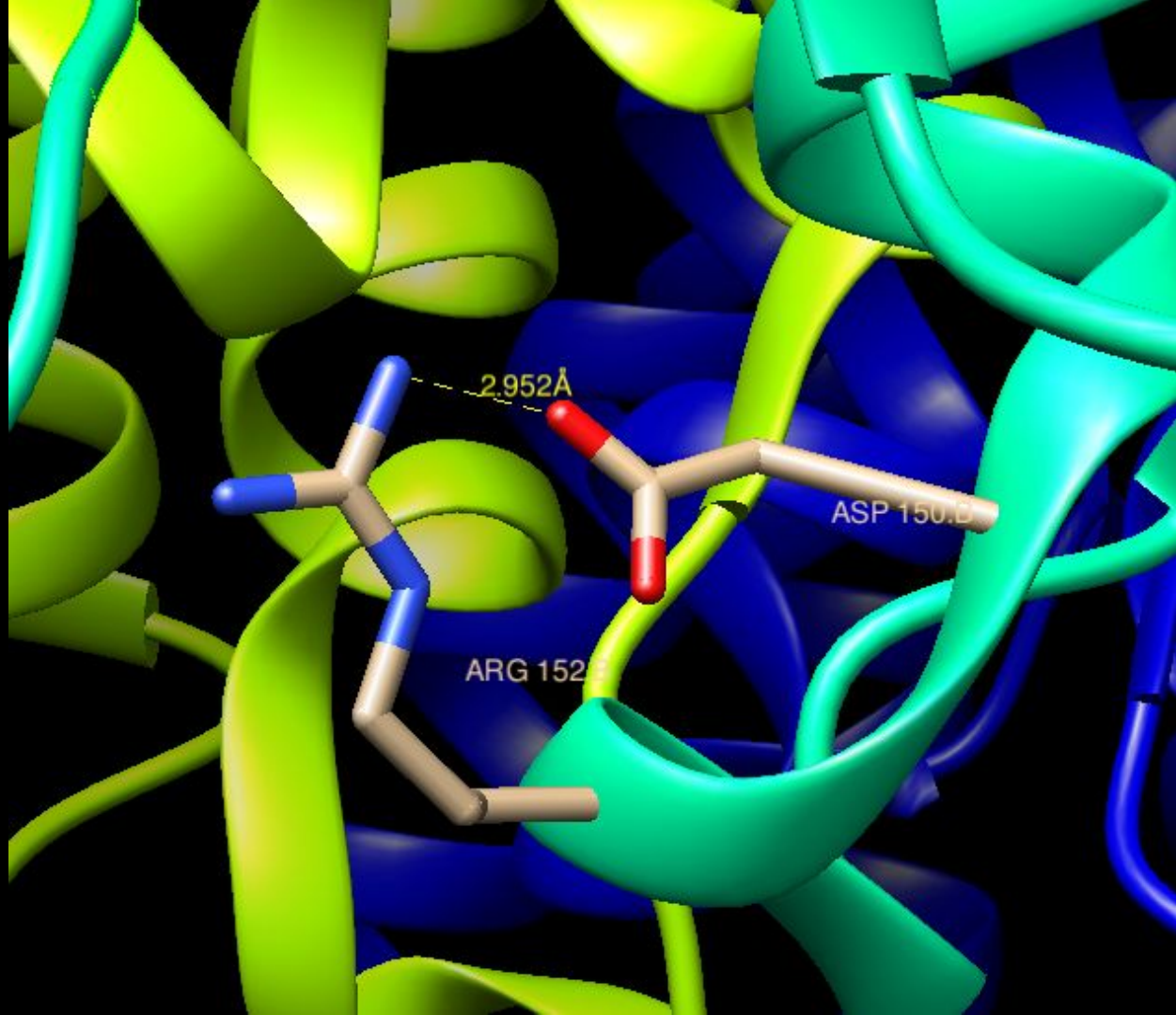
Asp150-Arg152	Glu3-Arg85	Glu232-His80
Chain B Chain C Chain D	Chain A	Chain C

Salt bridges

- In the case of chains B, C and D we find a Salt Bridge between Asp150 and Arg152.
- Distance is 2.95 Å
- Located innermost face (core)

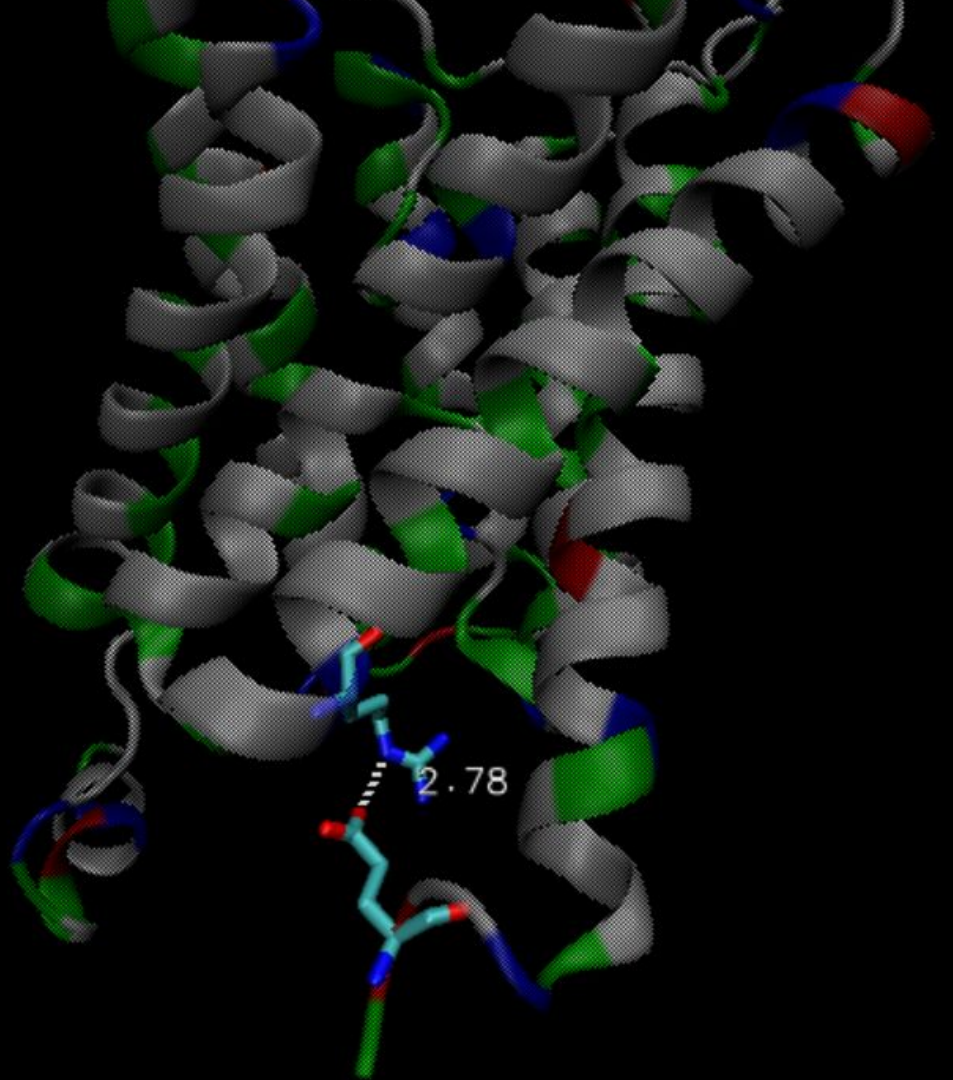


Asp150-Arg152

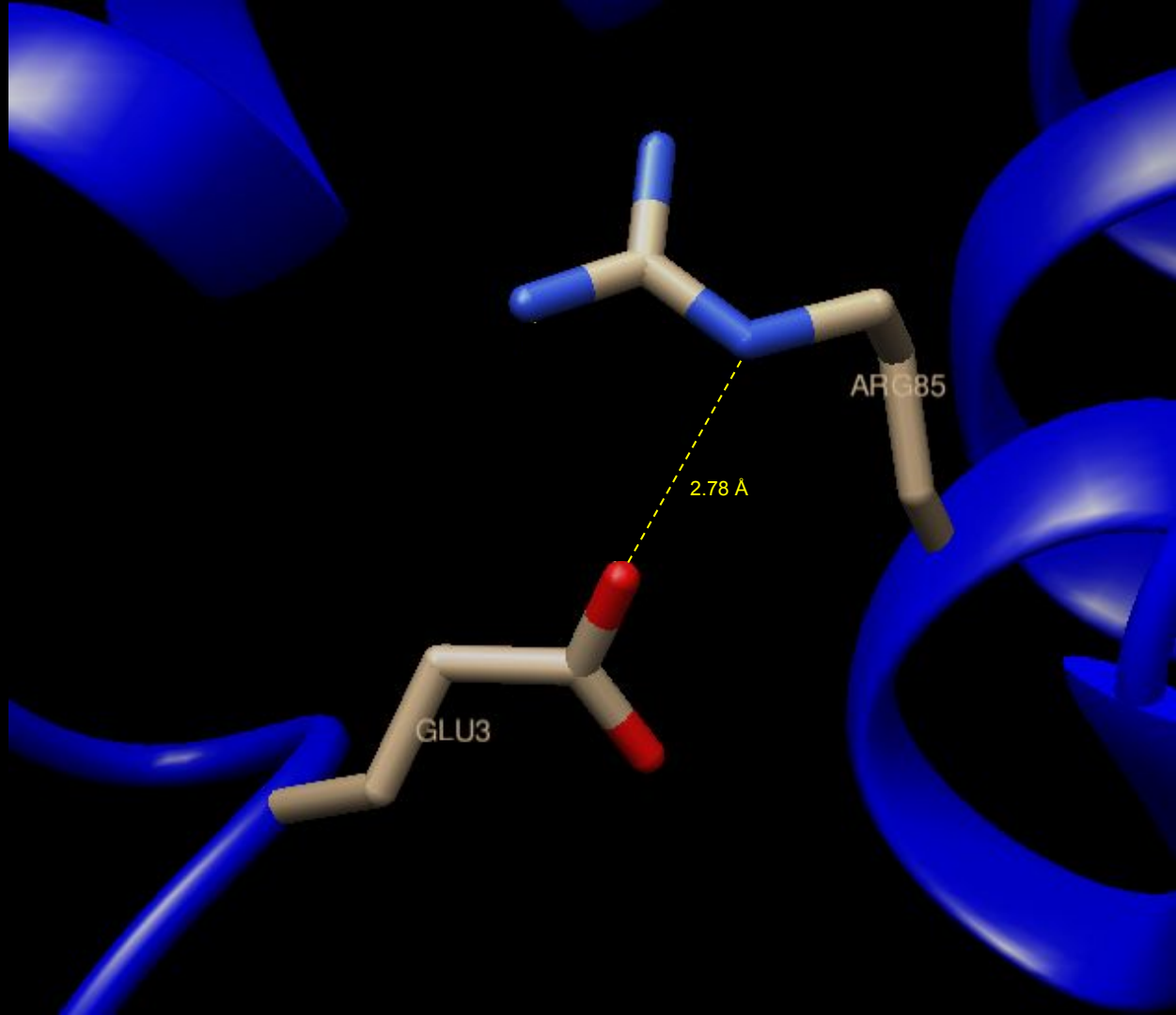


Salt bridges

- Chain A single Salt Bridge between Glu3 and Arg85.
- Distance 2.78 Å
- Located outermost, closer membrane

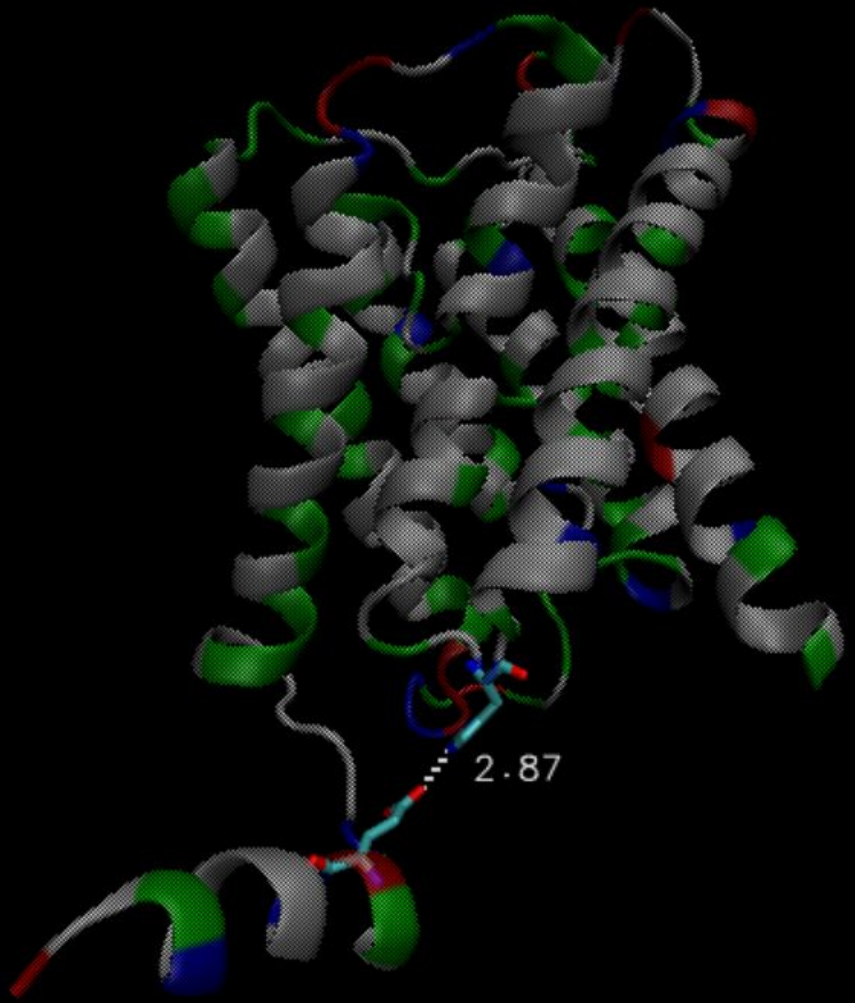


Glu3-Arg85

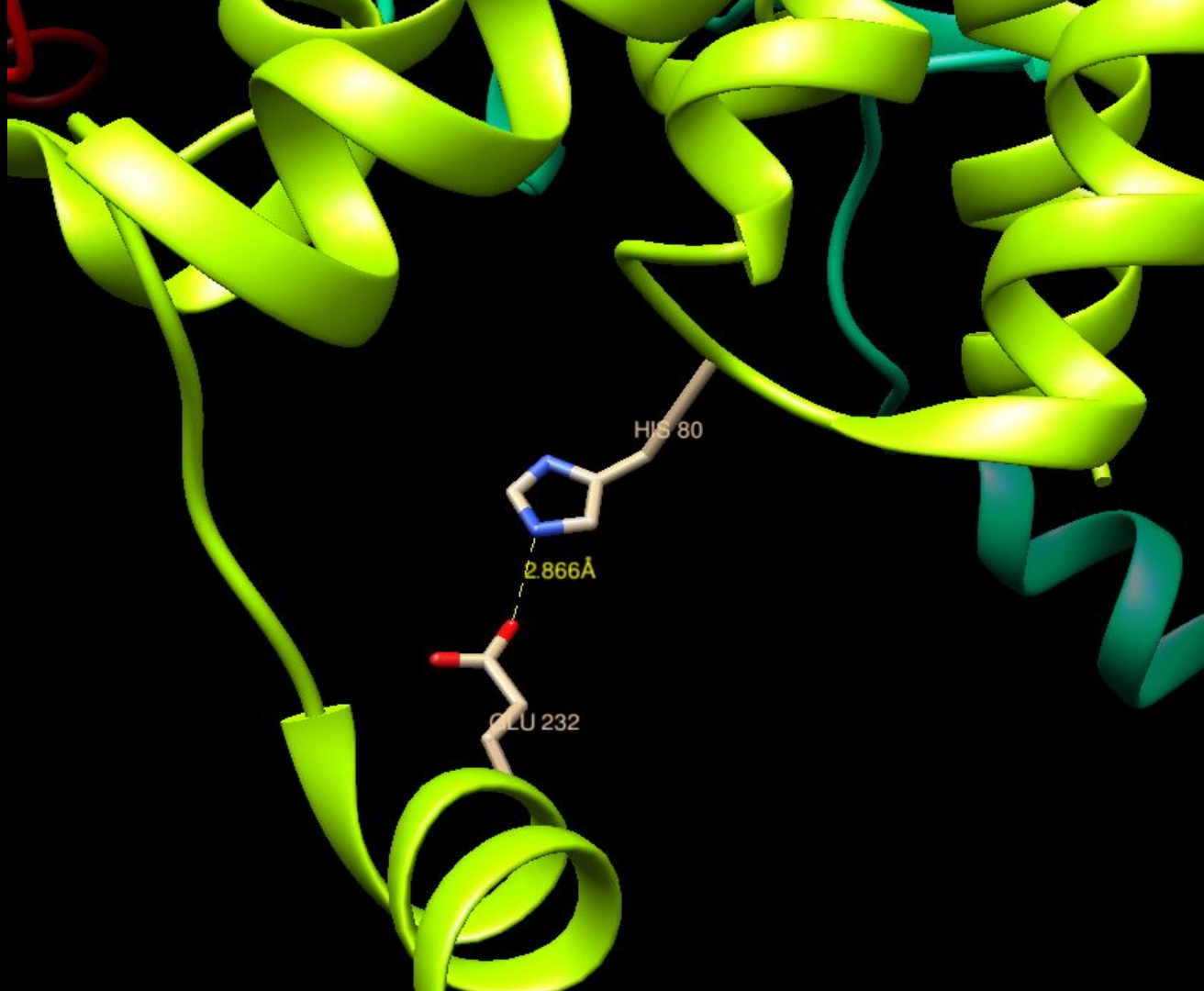


Salt bridges

- Chain C additional Salt Bridge between Glu232 and His80
- Distance 2.87 Å
- Located between one tail and external alpha helices



Glu232-His80



Cadmium action

There is interaction with two Cd2+ ions

Cd2 partial occupancy (65%) located between loop and the C-terminal tail in protomer C

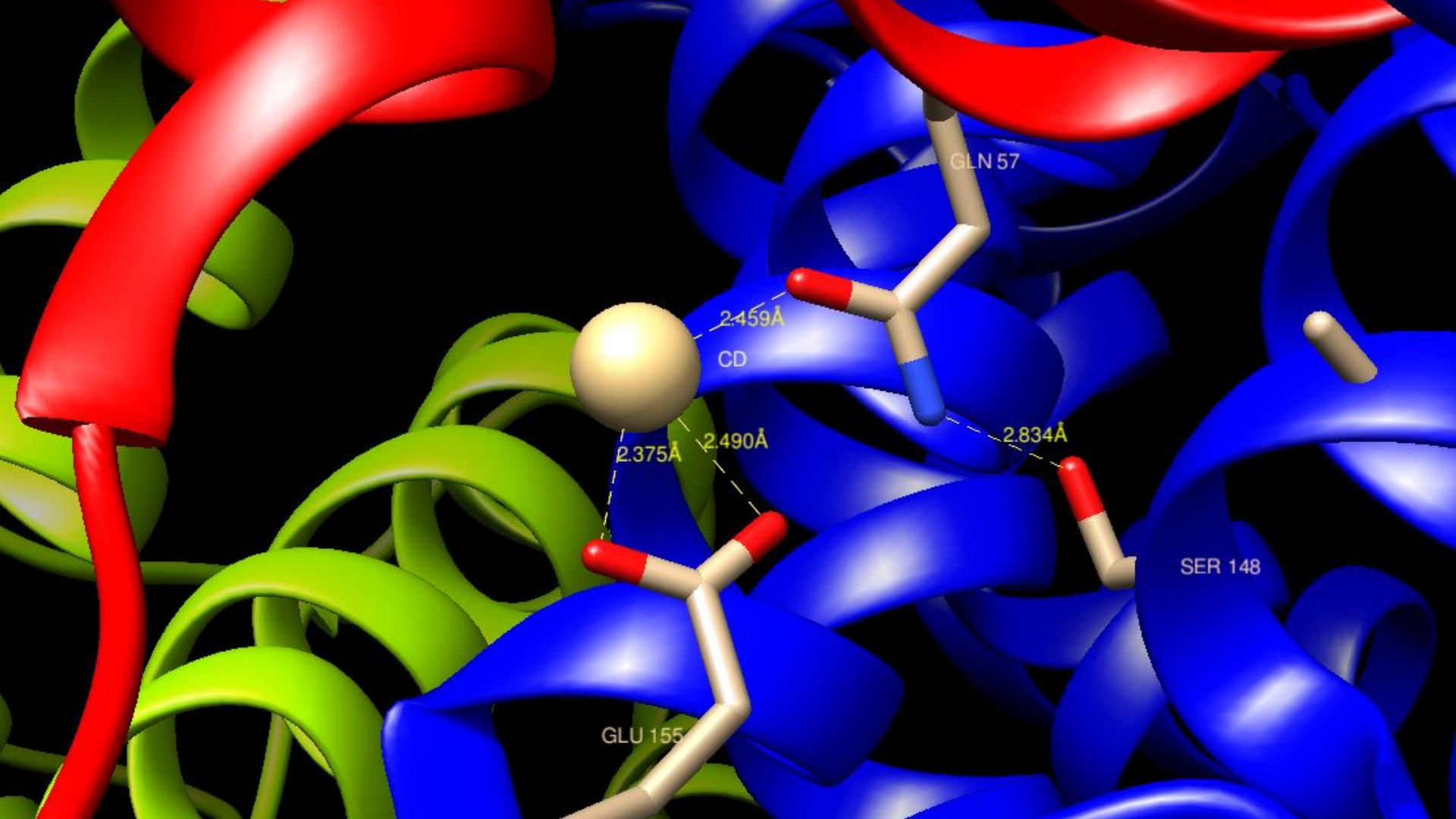
Ligated by HisC80, GluC232, and one water molecule

Helps position the C-terminal helix for this interaction

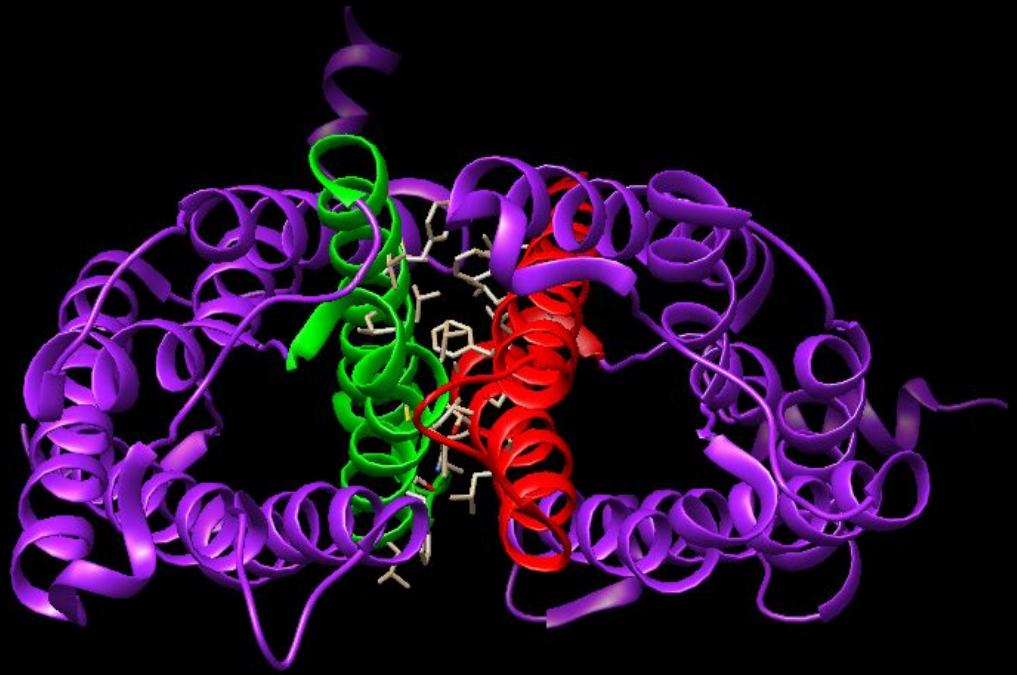
Cd1 has full occupancy and bind with promoter A and D

Between GluA155 of loop D and GlnD57 of TM helix 2 as well as two water molecules

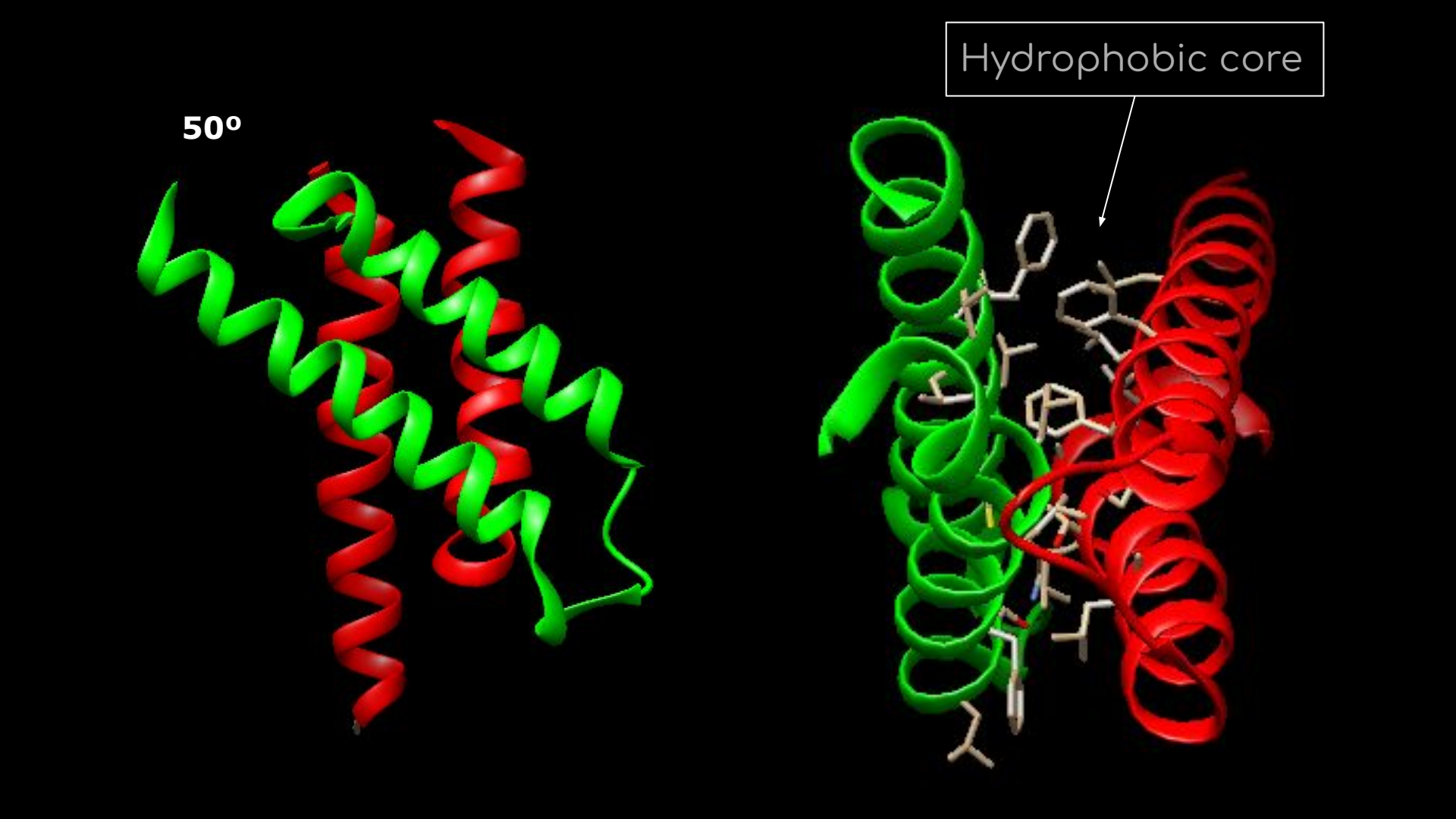
Displaces loop D to the center AQP2, hydrogen bond between AsnA156, AsnD156, and GluB155


GLN 57
2.459Å
CD
2.375Å
2.490Å
2.834Å
SER 148
GLU 155

Subunits interactions

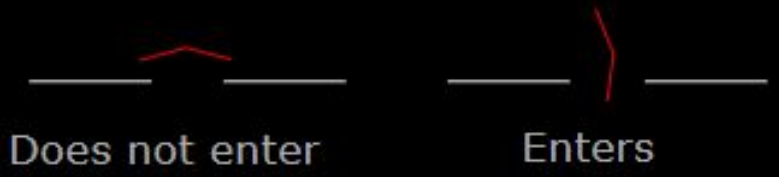


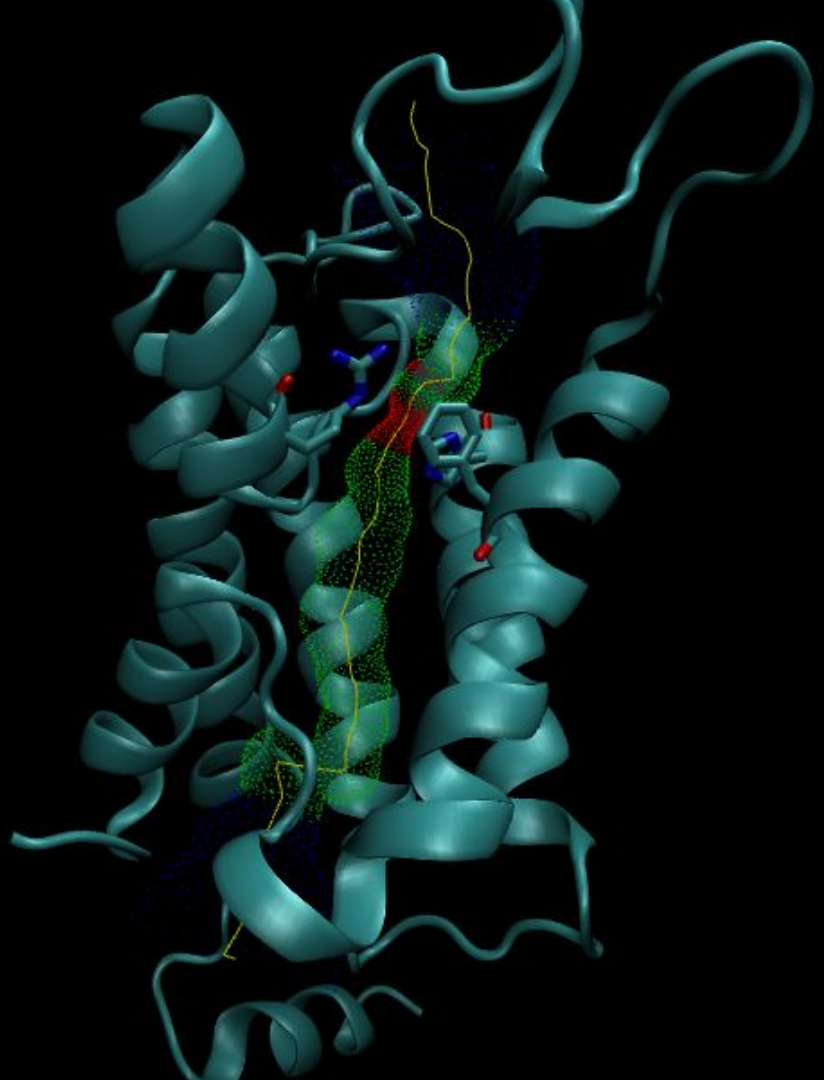
Interaction between lateral residues from helices TM1 and TM2 from one chain with the residues from helices 4 and 5 from the next subunits.


50º
Hydrophobic core

ar/R filter

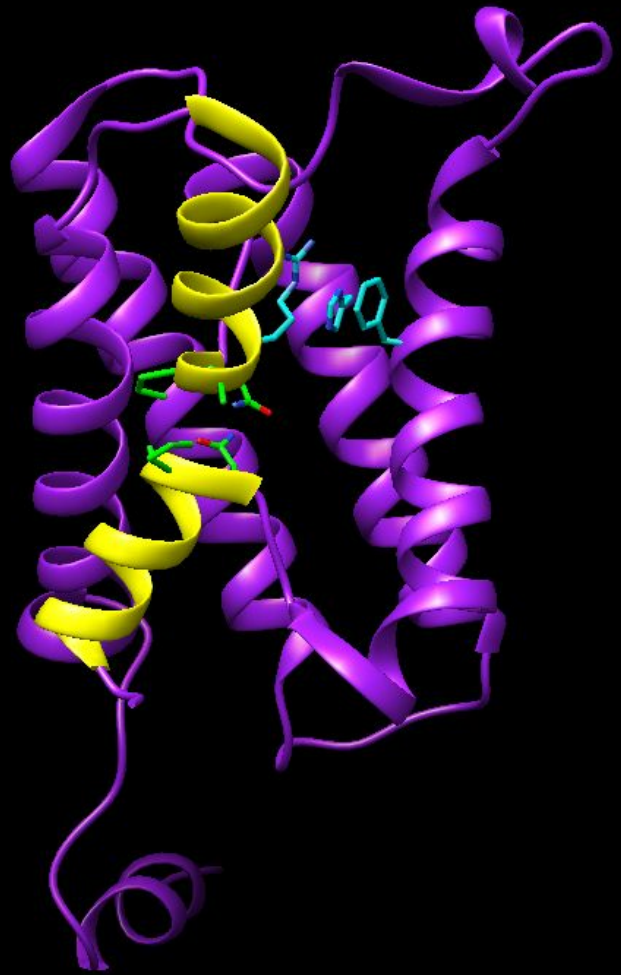
- Red area corresponds to pore entrance (extracelular part) selectivity for Aromatic Arginine (ar/R)
- Creating pore 1.8 Å
- ar/R → Arg187, His172 and Phe48

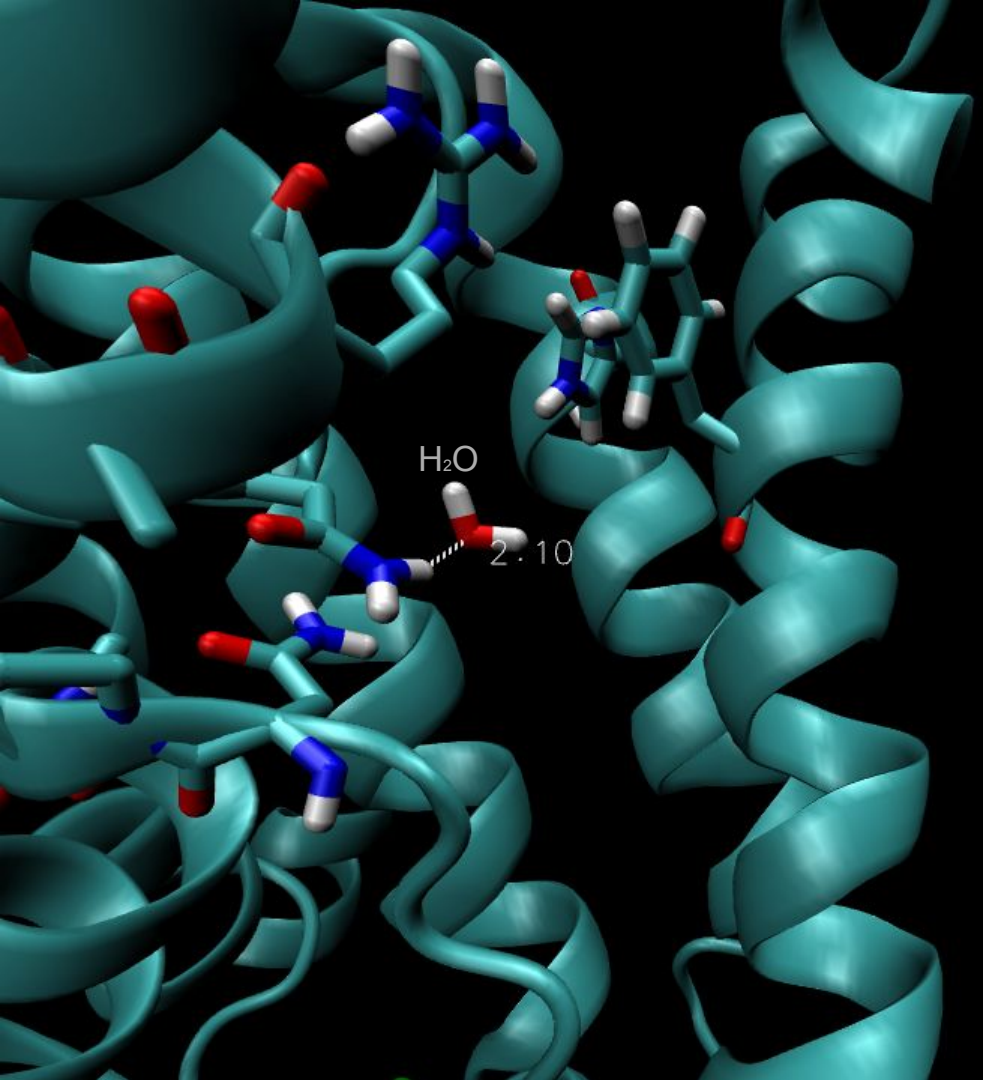




NPA region

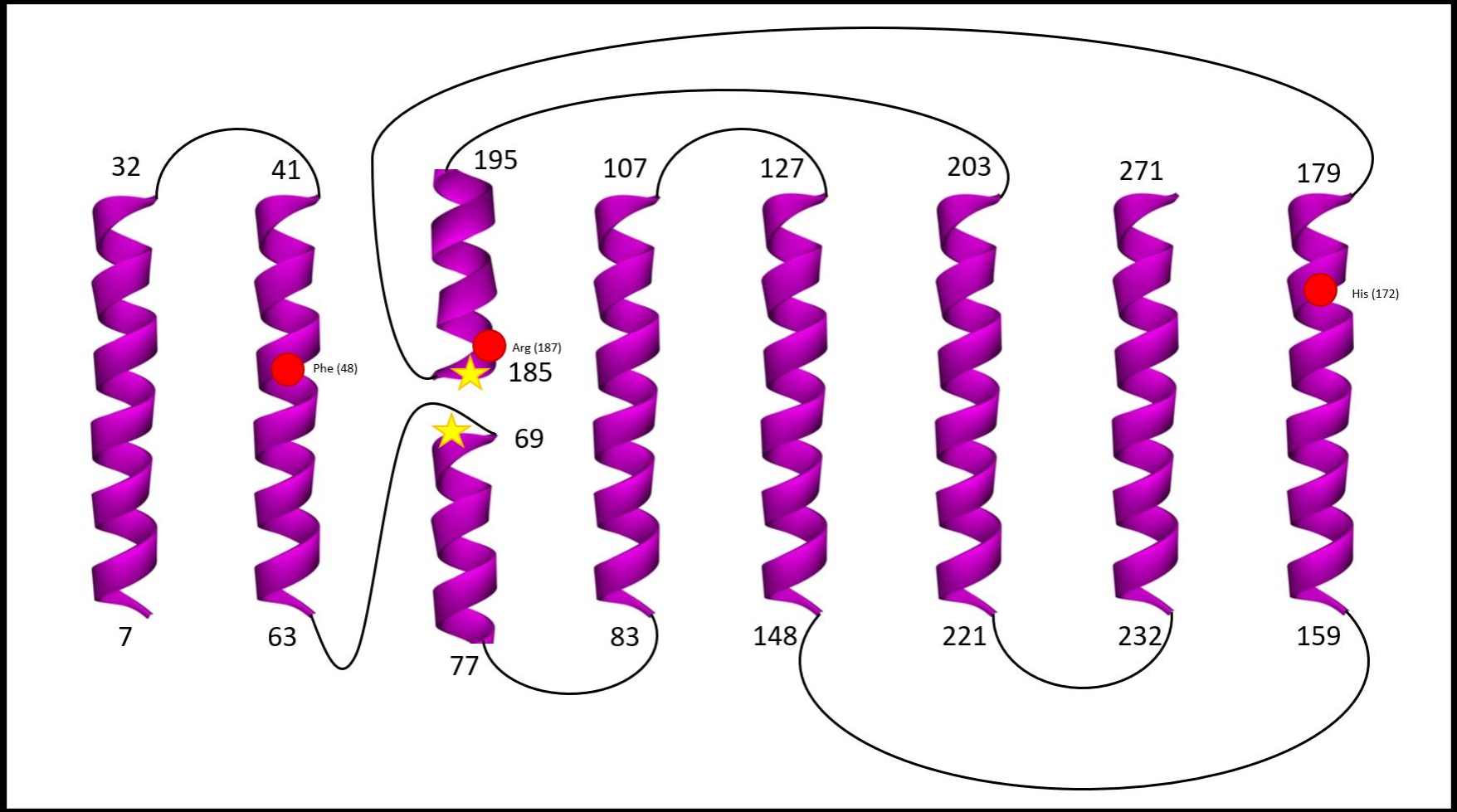
- Key residues for the pore are NPA repetitions
- NPA → Asn68, Pro 69 and Ala70
- Asn extremely conserved
- Only change tolerable in mutagenesis experiments is Asn by Ser (not seen nature)





H2O molecule goes through the pore interacting first with ar/R which only lets water pass.

Then interacts with the different residues of the NPA region which help the water to pass through.


32
41
195
107
127
203
271
179
Phe (48)
Arg (187)
185
69
His (172)
7
63
77
83
148
221
232
159

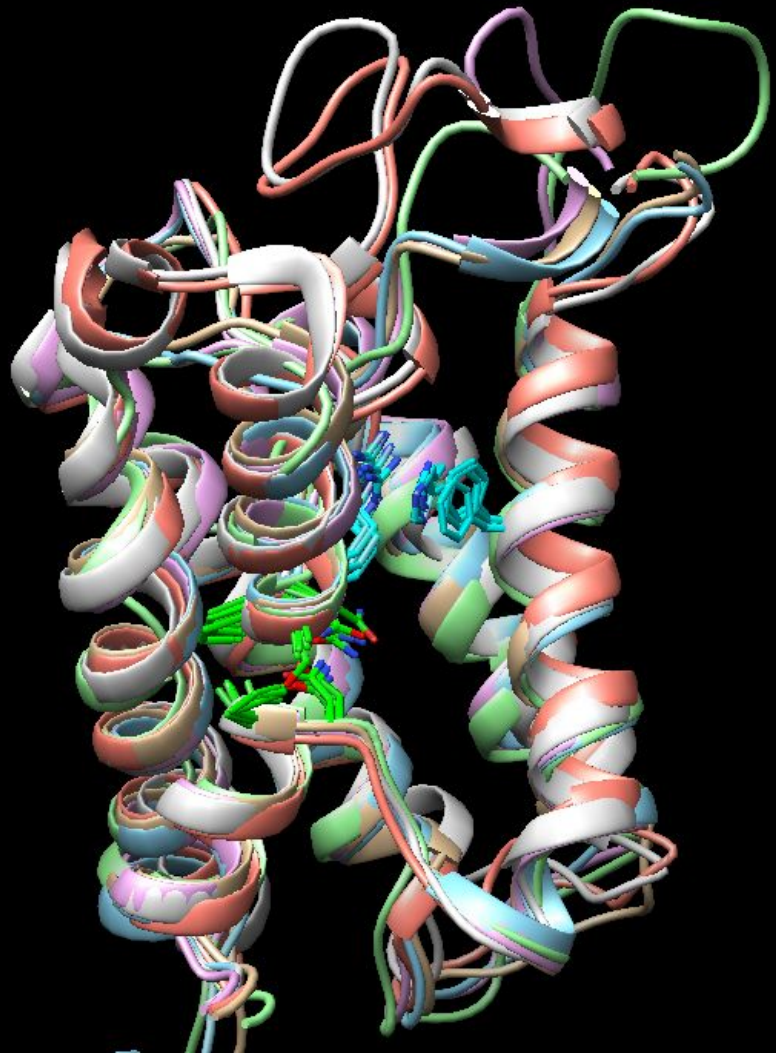
FAMILY

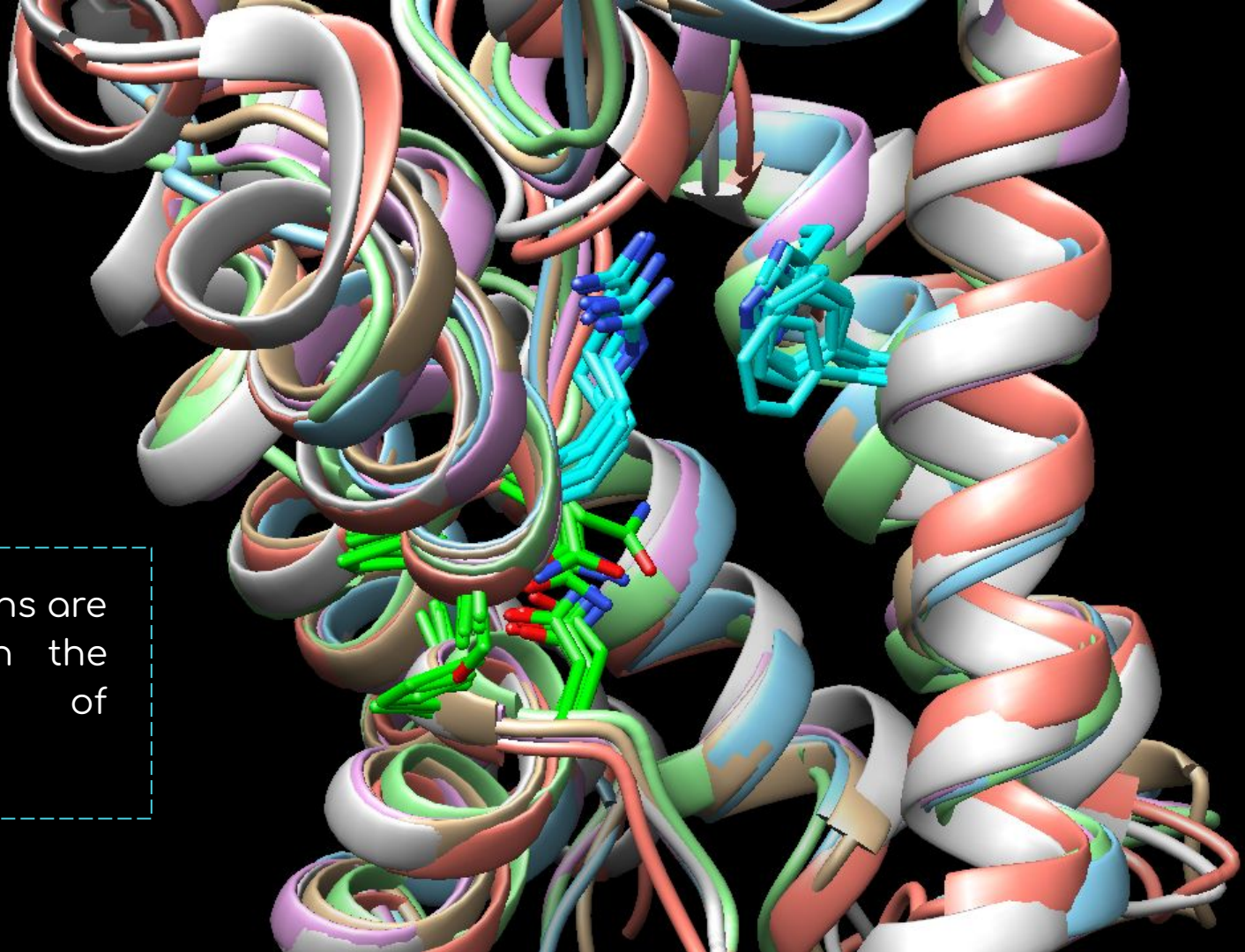
Aquaporine Family

Orthodox AQP	Aquaglyceroporin AQP	Unorthodox AQP
AQP0, AQP1, AQP2, AQP4, AQP5, AQP6 and AQP8	AQP3, AQP7, AQP9 and AQP10	AQP11 and AQP12

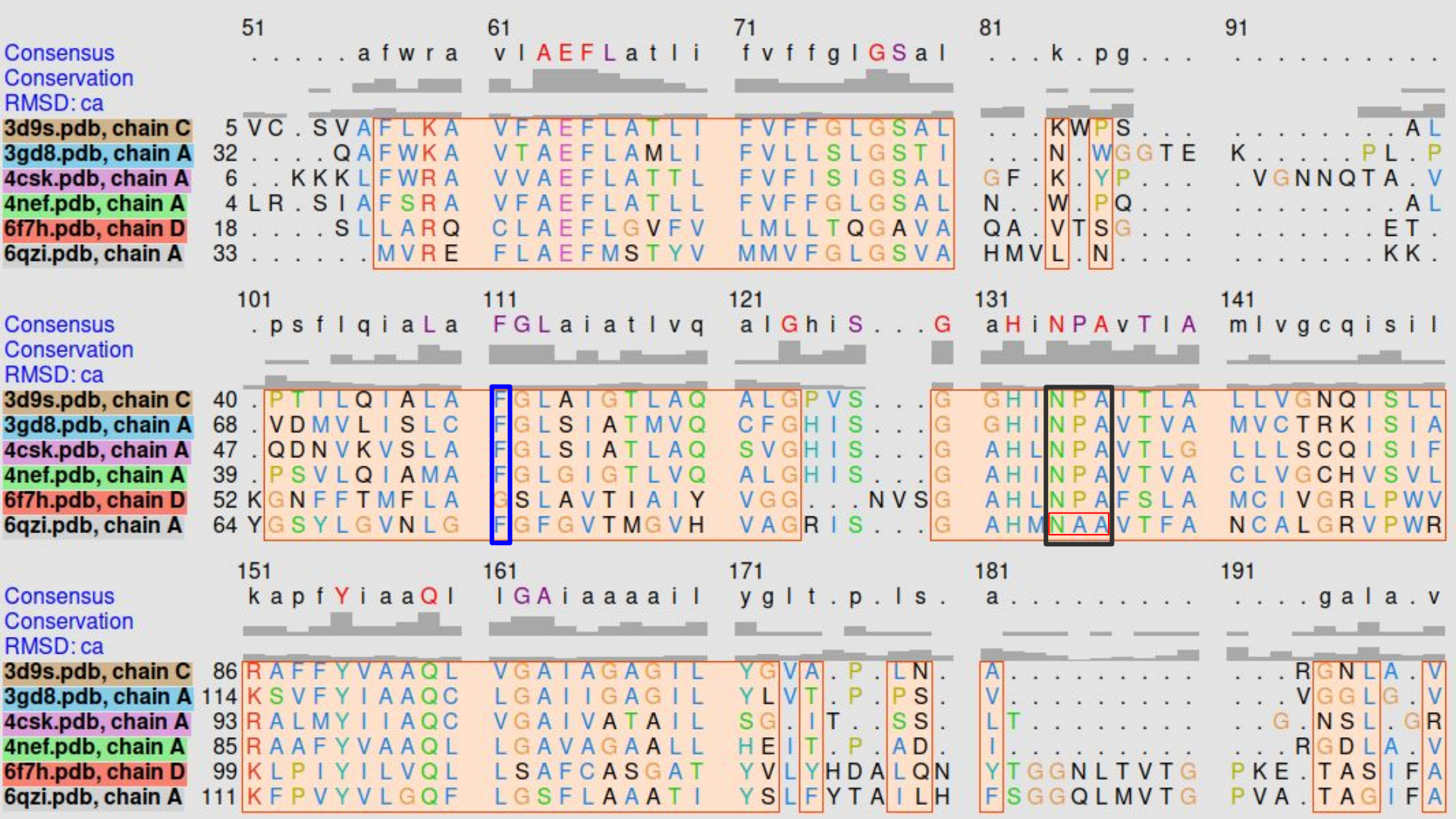
Superimposition

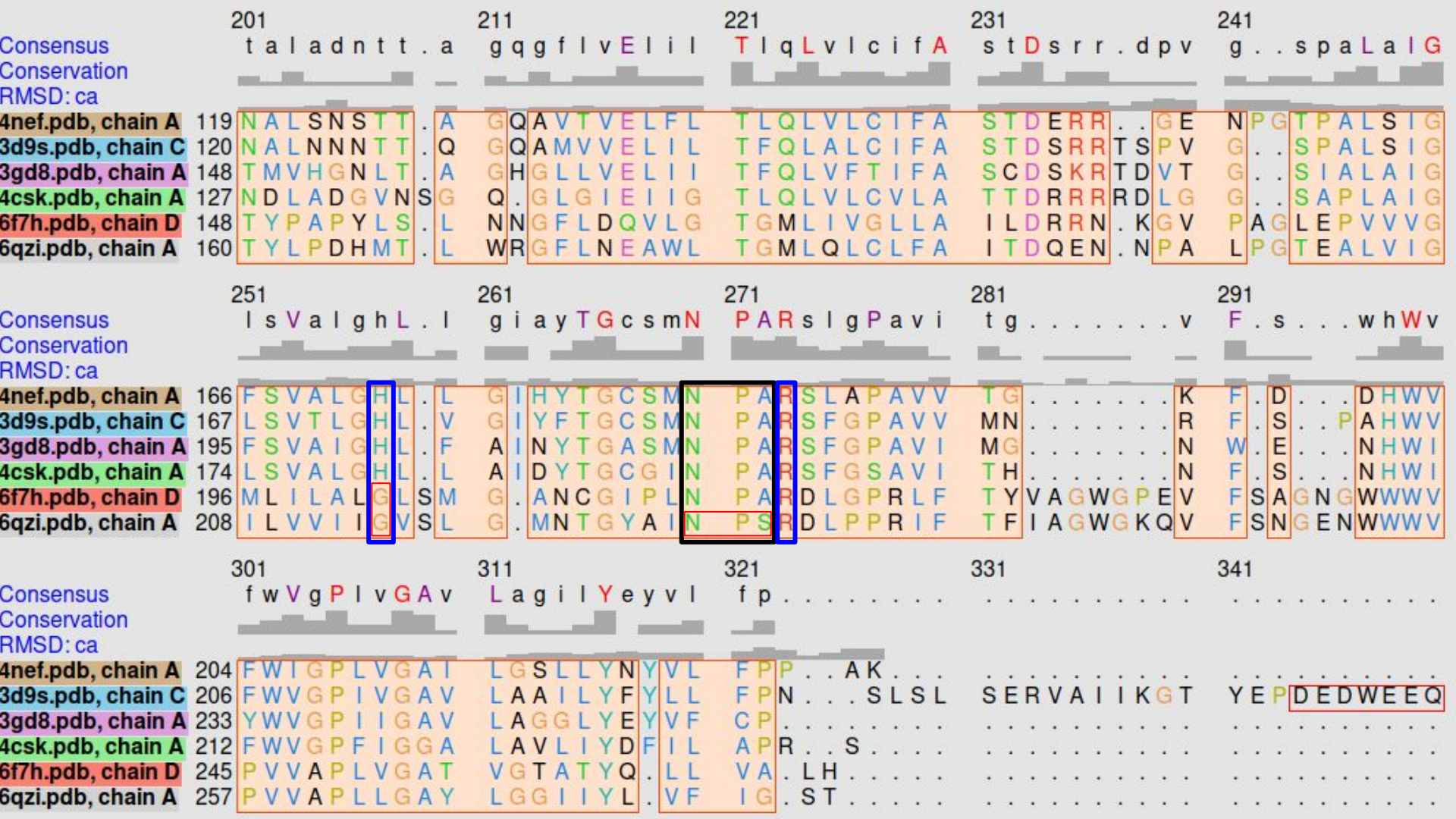
AQP2 (4NEF)	**Green**
AQP5 (3D9S)	**Orange**
AQP4 (3GD8)	**Blue**
AQP1 (4CSK)	**Purple**
AQP10 (6F7H)	**Red**
AQP7 (6QZI)	**Grey**





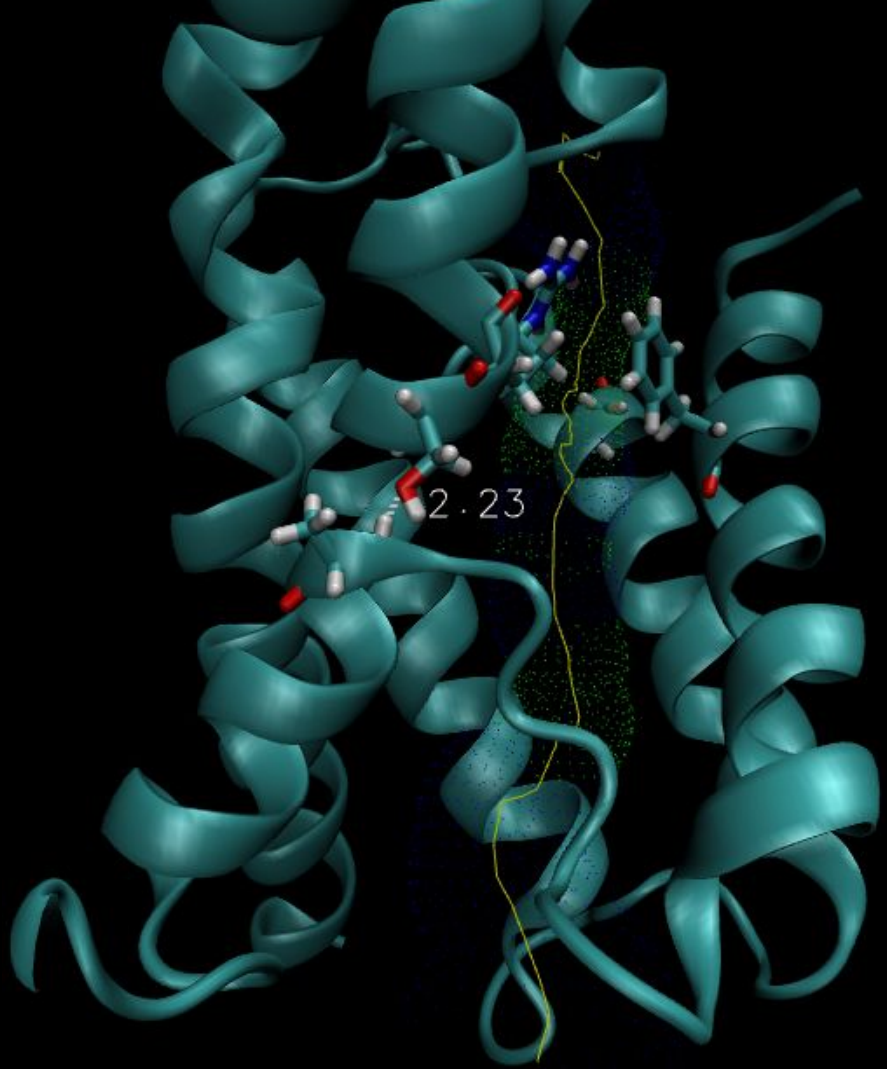
ar/R and NPA regions are highly conserved in the different types of aquaporins


Consensus
Conservation
RMSD: ca
3d9s.pdb, chain C
3gd8.pdb, chain A
4csk.pdb, chain A
4nef.pdb, chain A
6f7h.pdb, chain D
6qzi.pdb, chain A
51
61
71
81
91
101
111
121
131
141
151
161
171
181
191


201 211 221 231 241
Consensus
Conservation
RMSD: ca
4nef.pdb, chain A 119
3d9s.pdb, chain C 120
3gd8.pdb, chain A 148
4csk.pdb, chain A 127
6f7h.pdb, chain D 148
6qzi.pdb, chain A 160
251 261 271 281 291
Consensus
Conservation
RMSD: ca
4nef.pdb, chain A 166
3d9s.pdb, chain C 167
3gd8.pdb, chain A 195
4csk.pdb, chain A 174
6f7h.pdb, chain D 196
6qzi.pdb, chain A 208
301 311 321 331 341
Consensus
Conservation
RMSD: ca
4nef.pdb, chain A 204
3d9s.pdb, chain C 206
3gd8.pdb, chain A 233
4csk.pdb, chain A 212
6f7h.pdb, chain D 245
6qzi.pdb, chain A 257

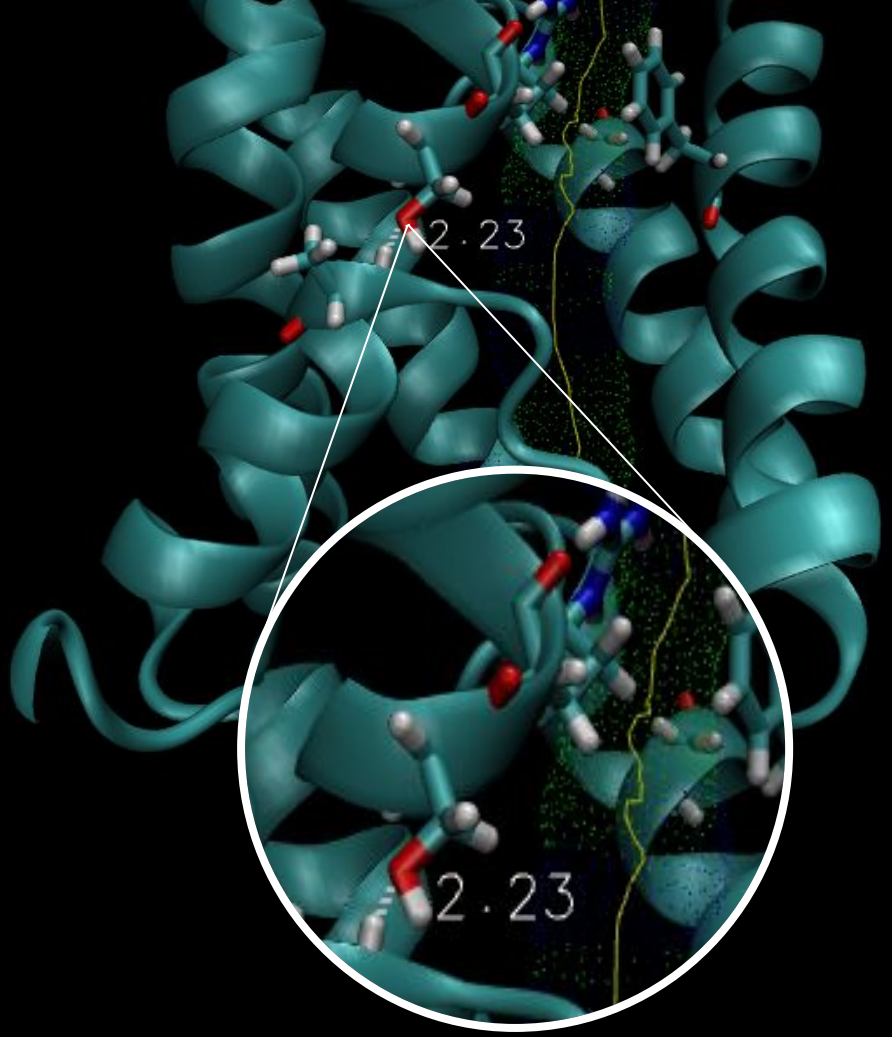
6QZI- AQP7

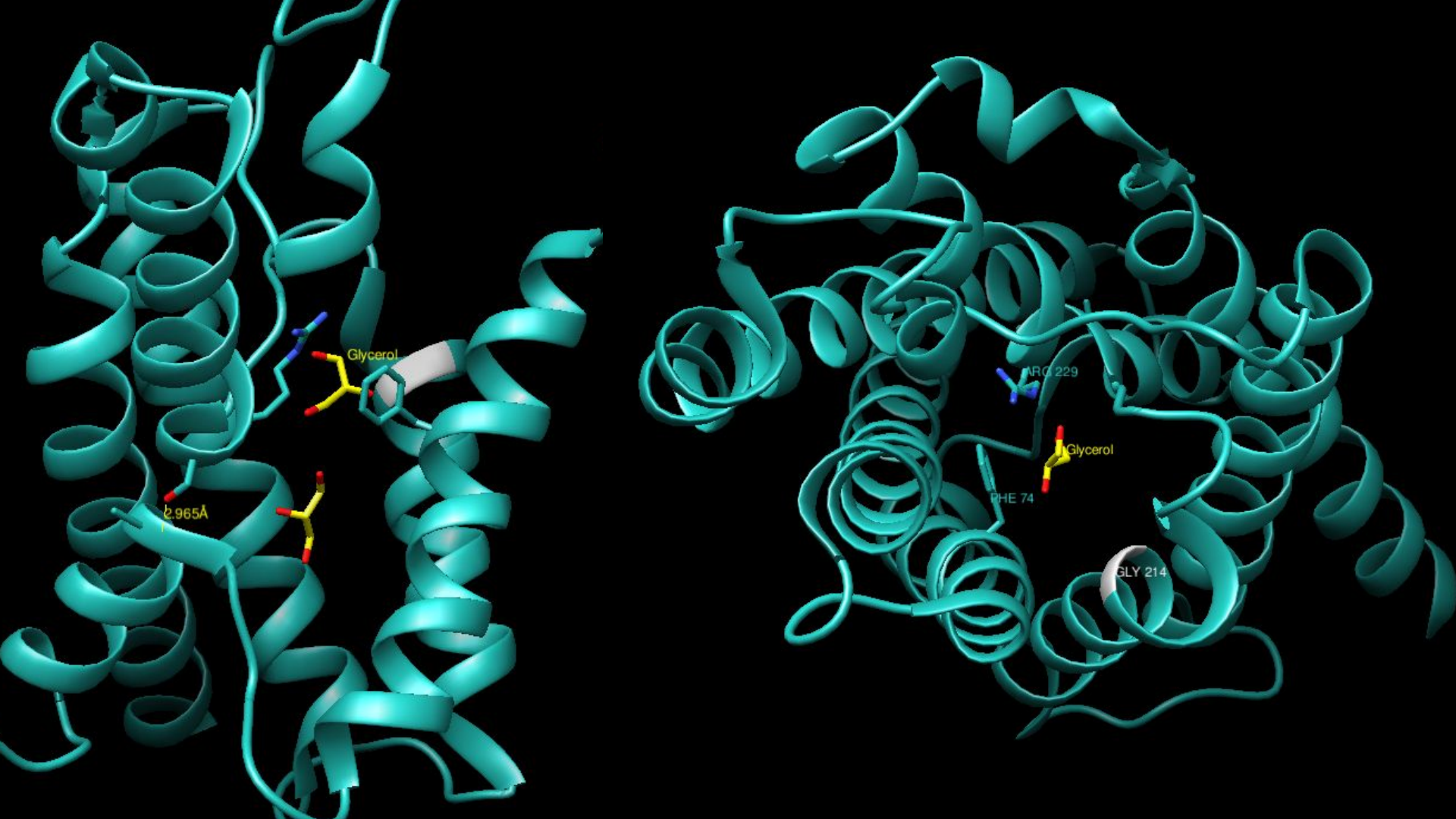
- First NPA (94, 95 and 96), there is a change in Pro95 → Ala95
- Second NPA (226, 227 and 228), change in Ala228 → Ser228
- ar/R filter (In 4NEF: His172, Phe48 and Arg187) change in His214 → Gly214



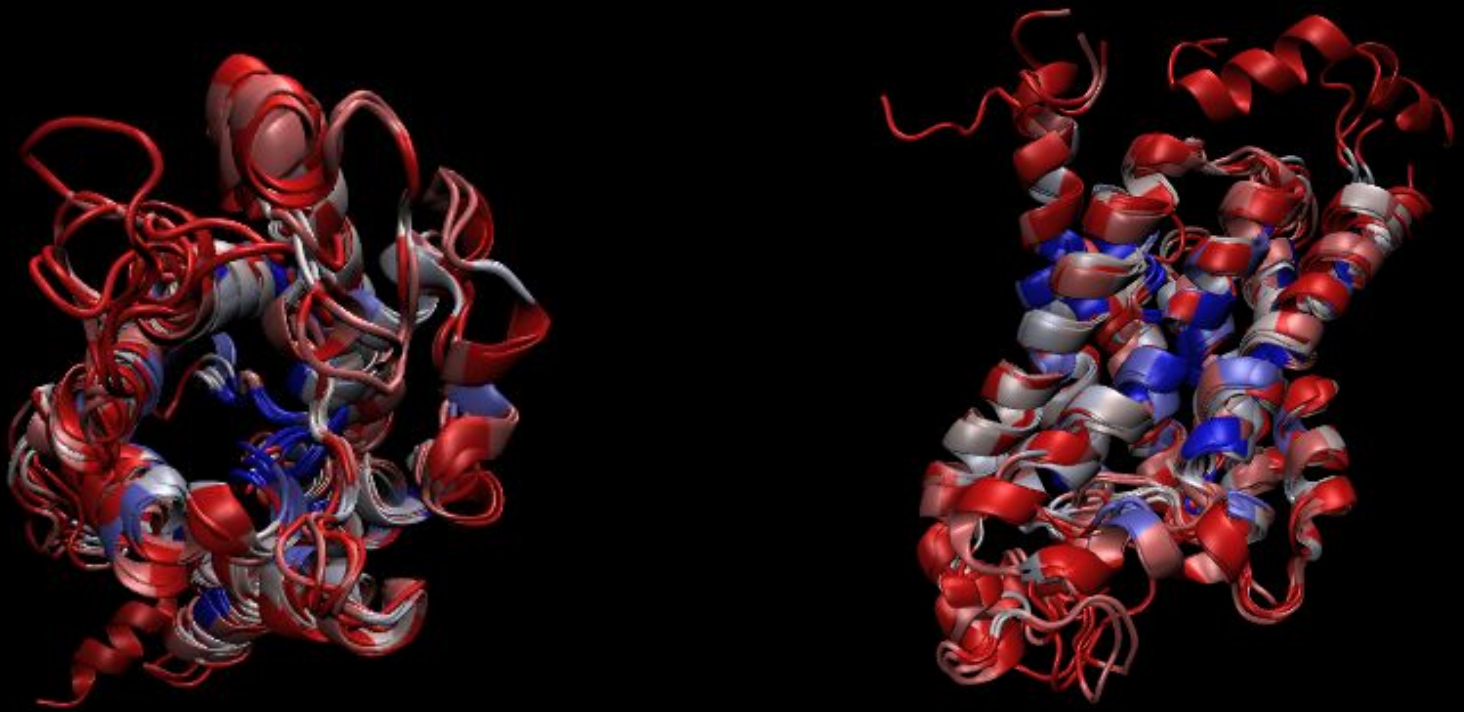
6QZI- AQP7

- Due to the changes in residues of NPA and ar/R the size of the pore increases
- Hydrogen bond between the nitrogen Ala95 and Ser228
- Water transport less efficient than in AQP




Glycerol
2.965Å
ARG 229
Glycerol
PHE 74
GLY 214

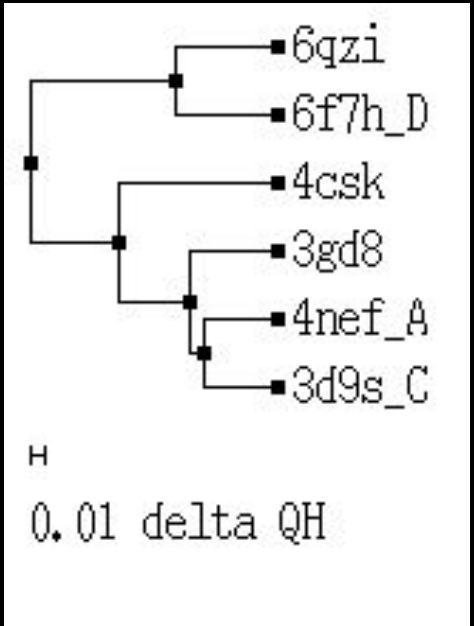
Superimposition of STAMP Structural Alignment



Superimposition Results

Human AQPs	APQ1 (4CSK)	AQP4 (3GD8)	AQP5 (3D9S)	AQP7 (6QZI)	AQP10 (6F7H)
AQP2 (4NEF)	Sc: 7.50 QH = 0.73 RMSD: 1.17 % Id: 42.56%	Sc: 8.04 QH = 0.86 RMSD: 0.78 % Id: 48.88%	Sc: 8.02 QH = 0.82 RMSD: 0.81 % Id: 62.40%	Sc: 6.37 QH = 0.63 RMSD: 1.45 % Id: 31.40%	Sc: 6.31 QH = 0.59 RMSD: 1.49 % Id: 20.66%

Structural dendogram



Aquaporin 7

Aquaporin 10

Aquaporin 1

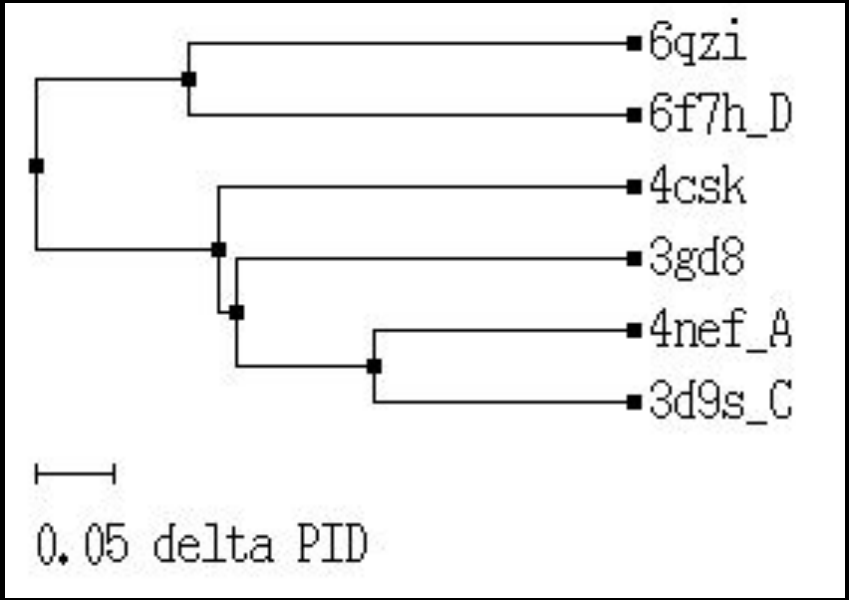
Aquaporin 4

Aquaporin 2

Aquaporin 5

- Two main nodes; one containing aquaglyceroporins (AQP7 and AQP10) and the other orthodox AQP
- AQP5 being the most similar to AQP2

Dendogram using Percent Identity



Aquaporin 7

Aquaporin 10

Aquaporin 1

Aquaporin 4

Aquaporin 2

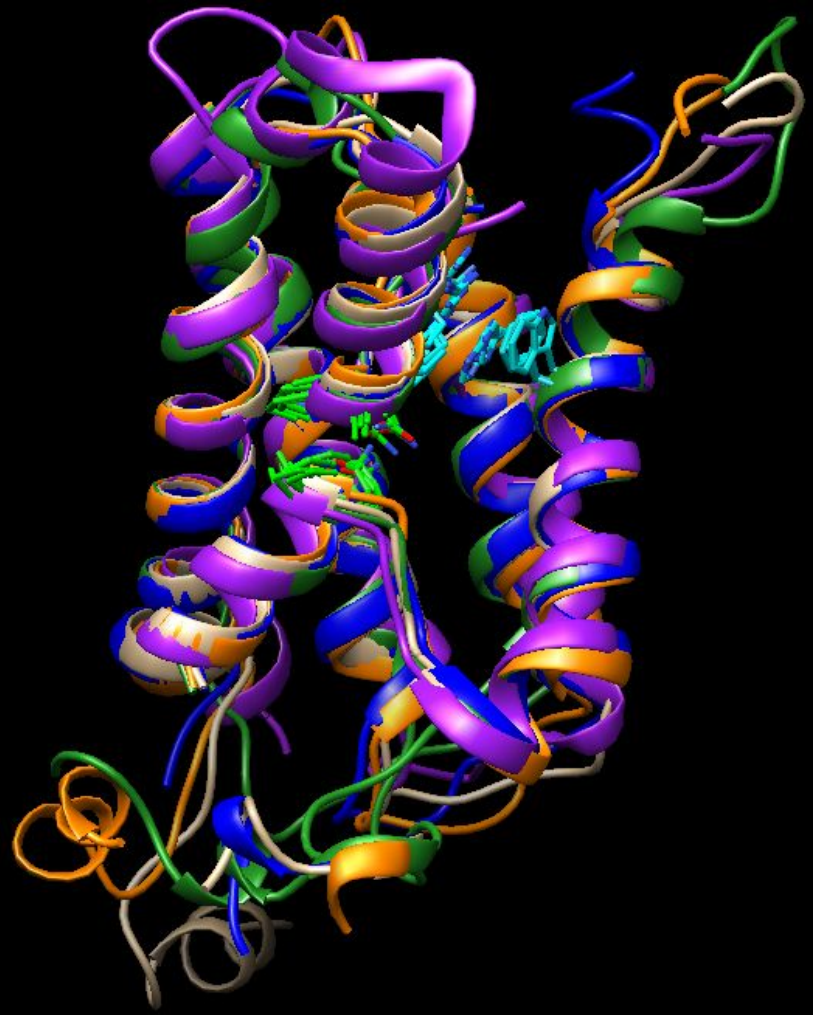
Aquaporin 5

The results obtained with the sequence phylogenetic tree are the sames obtained in the structural analysis

EVOLUTION

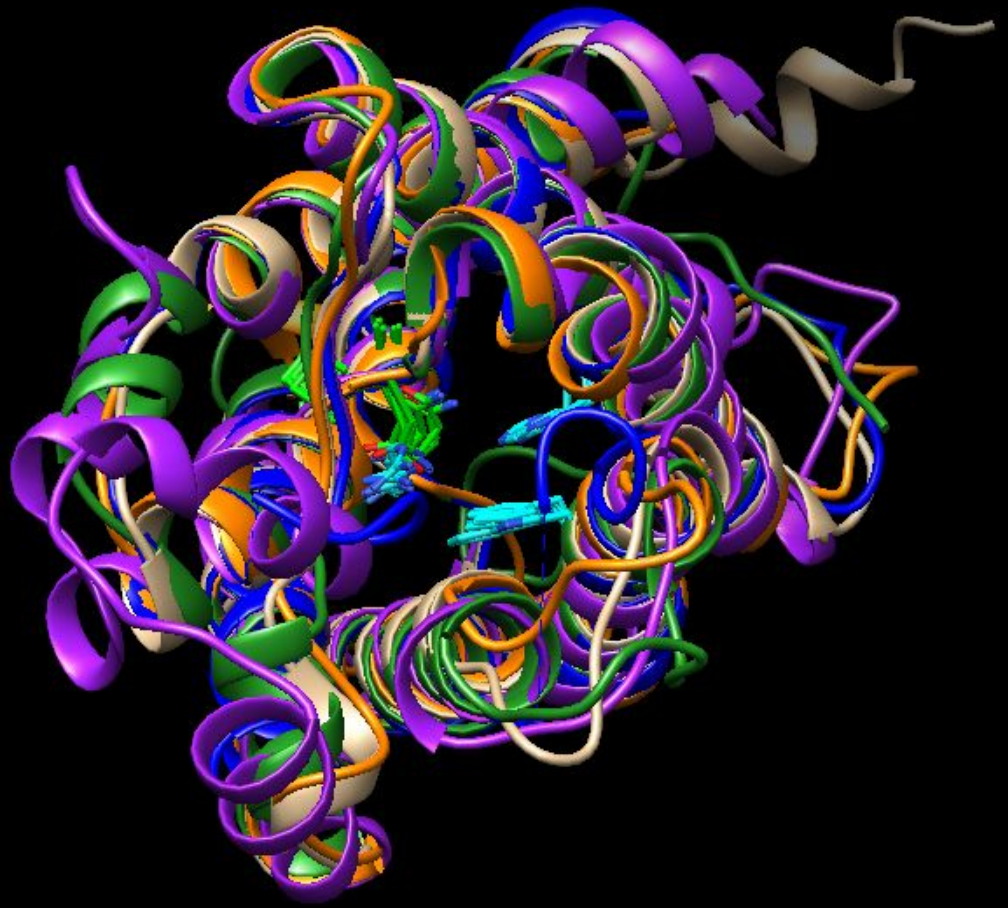
Superimposition

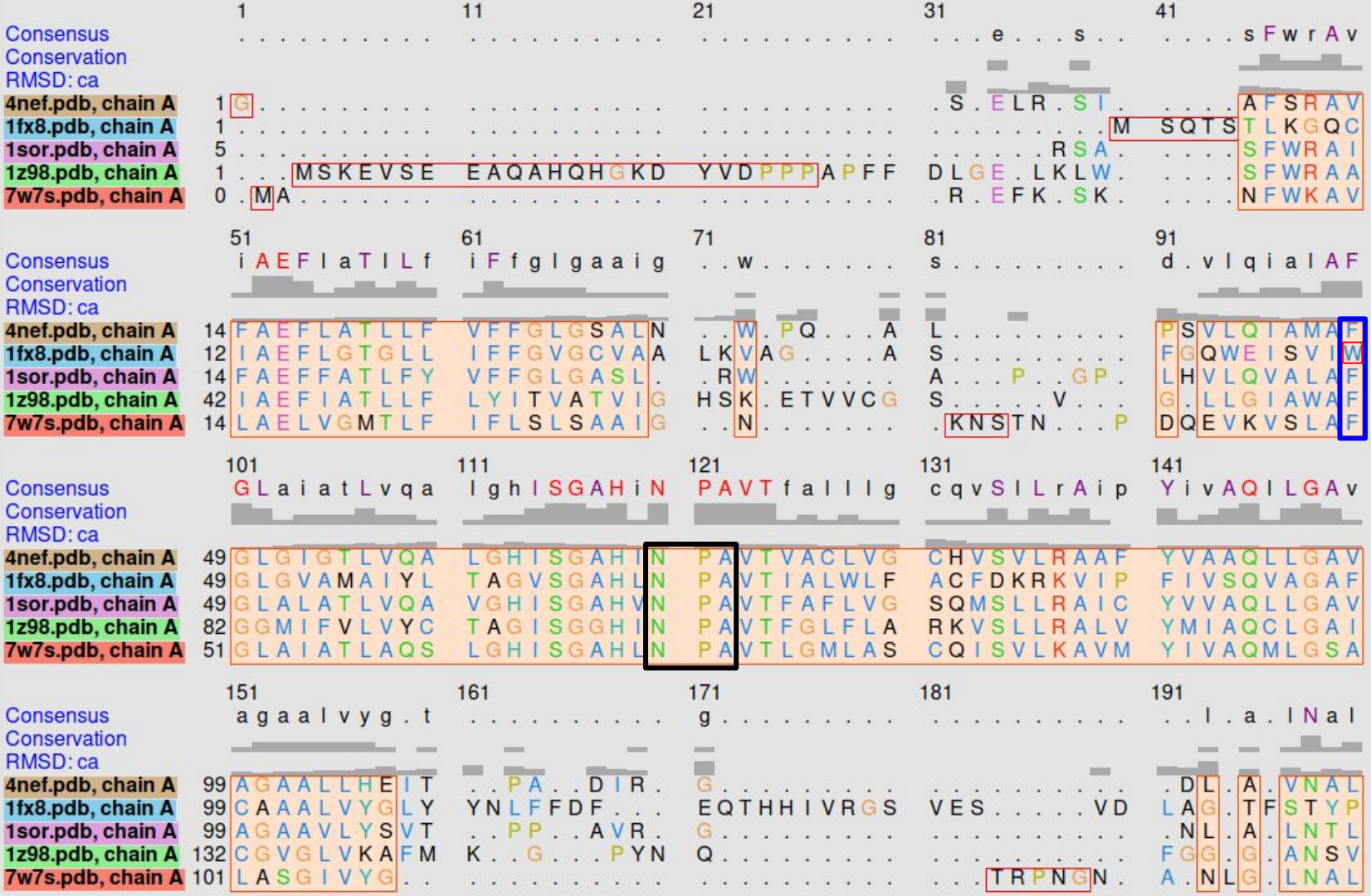
Fish (7W7S)	**Blue**
Spinach (1Z98)	**Green**
Sheep (1SOR)	**Orange**
Bacteria (1FX8)	**Purple**
Human (4NEF)	**Beige**

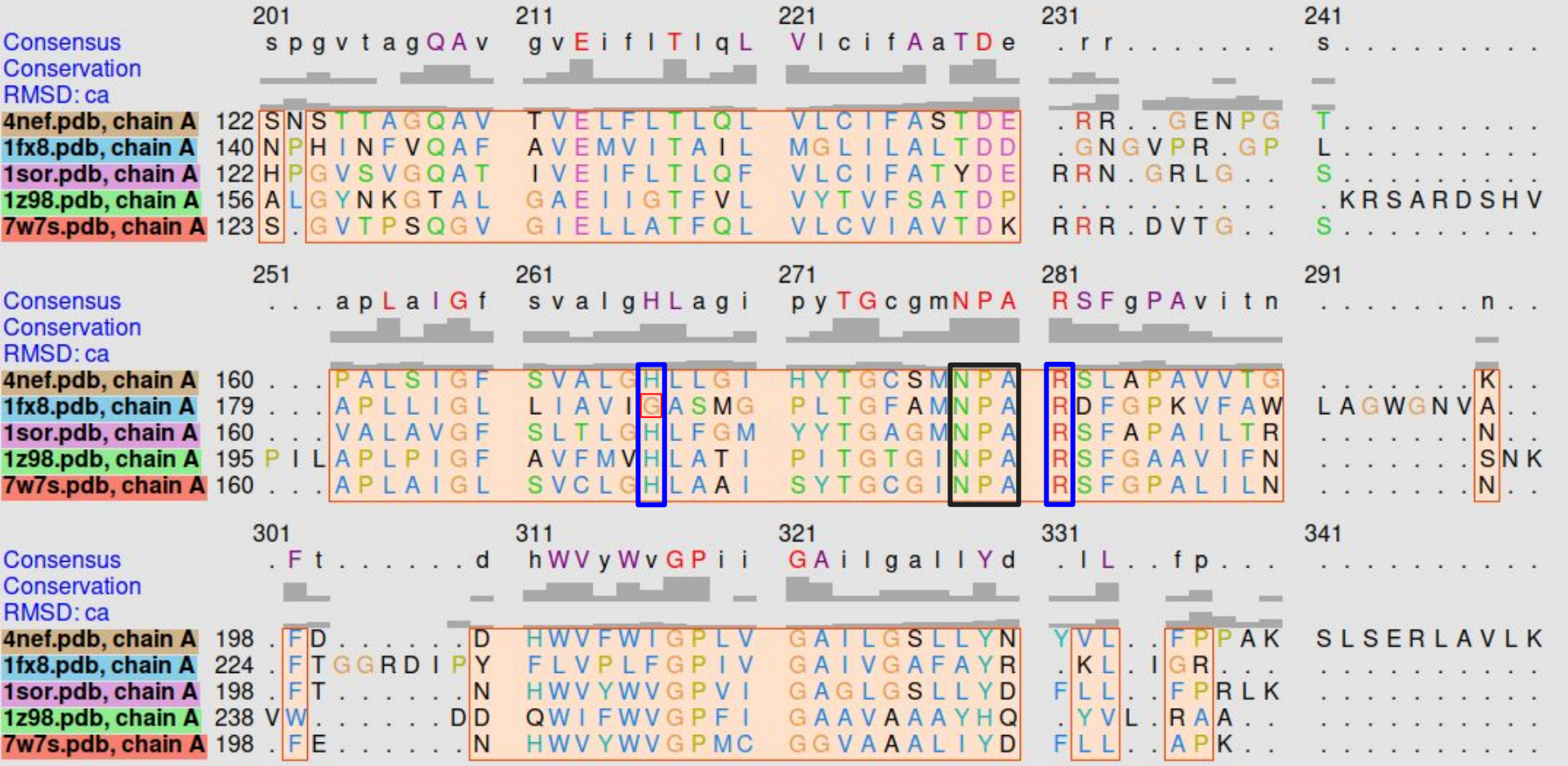


Superimposition

Although animal and plant evolutionary lines separated about 1.6 billion years ago, these highly conserved proteins have an identical structural core

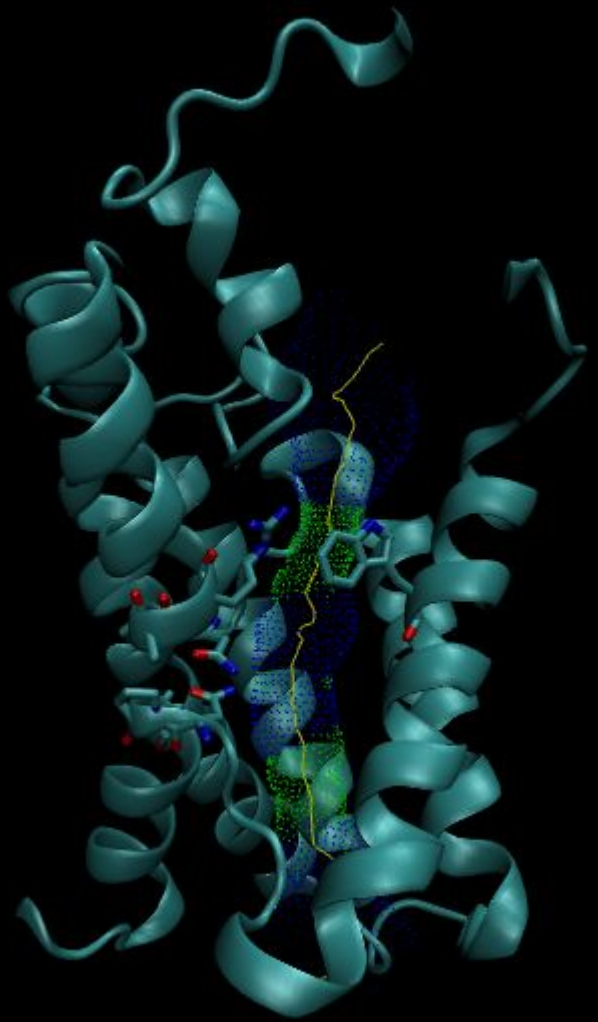



Consensus
Conservation
RMSD: ca
4nef.pdb, chain A
1fx8.pdb, chain A
1sor.pdb, chain A
1z98.pdb, chain A
7w7s.pdb, chain A


201
211
221
231
241
Consensus
Conservation
RMSD: ca
4nef.pdb, chain A 122 SNSTTAGQAV TVELFLTLQL VLCIFASTDE .RR..GENPG T.........
1fx8.pdb, chain A 140 NPHINFVQAF AVEMVITAIL MGLILALTDD .GNGVPR.GP L.........
1sor.pdb, chain A 122 HPGVSVGQAT IVEIFLTLQF VLCIFATYDE RRN.GRLG.. S.........
1z98.pdb, chain A 156 ALGYNKGTAL GAEIIGTFVL VYTVFSATDP .... .KRSARDSHV
7w7s.pdb, chain A 123 S.GVTPSQGV GIELLATFQL VLCVIAVTDK RRR.DVTG.. S.........
251
261
271
281
291
Consensus
Conservation
RMSD: ca
4nef.pdb, chain A 160 ...PALSIGF SVALGHLLGI HYTGCSMNPA RSLAPAVVTG ....K..
1fx8.pdb, chain A 179 ...APLLIGL LIAVIGASMG PLTGFAMNPA RDFGPKVFAW LAGWGNVA..
1sor.pdb, chain A 160 ...VALAVGF SLTLGHLFGM YYTGAGMNPA RSFAPAILTR ....N..
1z98.pdb, chain A 195 PILAPLPIGF AVFMVHLATI PITGTGINPA RSFGAAVIFN ....SNK
7w7s.pdb, chain A 160 ...APLAIGL SVCLGHLAAI SYTGCGINPA RSFGPALILN ....N..
301
311
321
331
341
Consensus
Conservation
RMSD: ca
4nef.pdb, chain A 198 .FD.....D HWVFWIGPLV GAILGSLLYN YVL..FPPAK SLSERLAVLK
1fx8.pdb, chain A 224 .FTGGRDIPY FLVPLFGPIV GAIVGAFAYR .KL.IGR... ....
1sor.pdb, chain A 198 .FT.....N HWVYWVGPVI GAGLGSLLYD FLL..FPRLK ....
1z98.pdb, chain A 238 VW.....DD QWIFWVGPFI GAAVAAAYHQ .YVL.RAA.. ....
7w7s.pdb, chain A 198 .FE.....N HWVYWVGPMC GGVAAALIYD FLL..APK.. ....

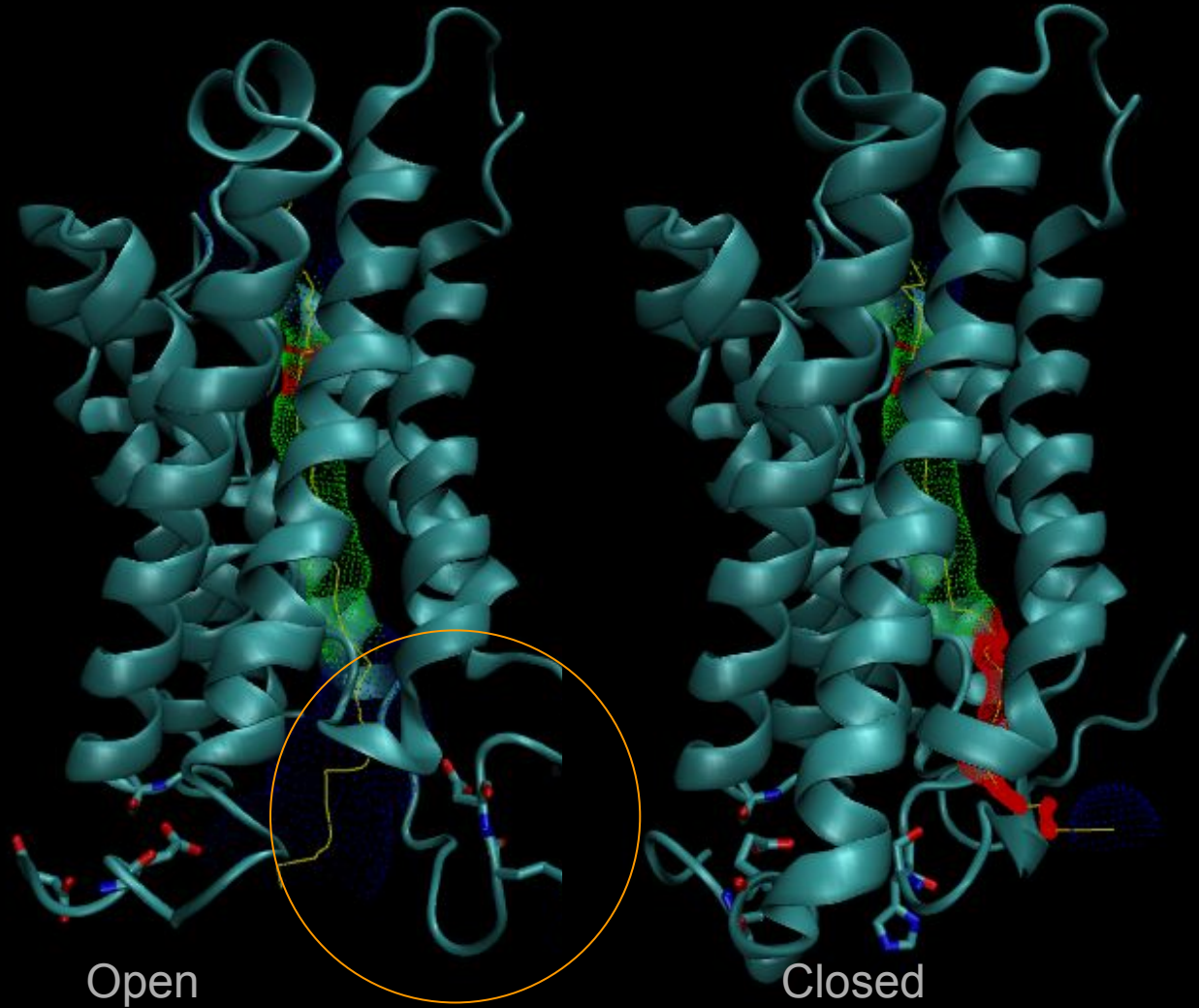
Bacteria

In the case of the Bacteria we observe changes in the selectivity filter. Change of His → Gly and Phe → Trp
These leads to an increase of the pore as we saw in AQP7, this is why bacteria aquaporin acts as aquaglyceroporin

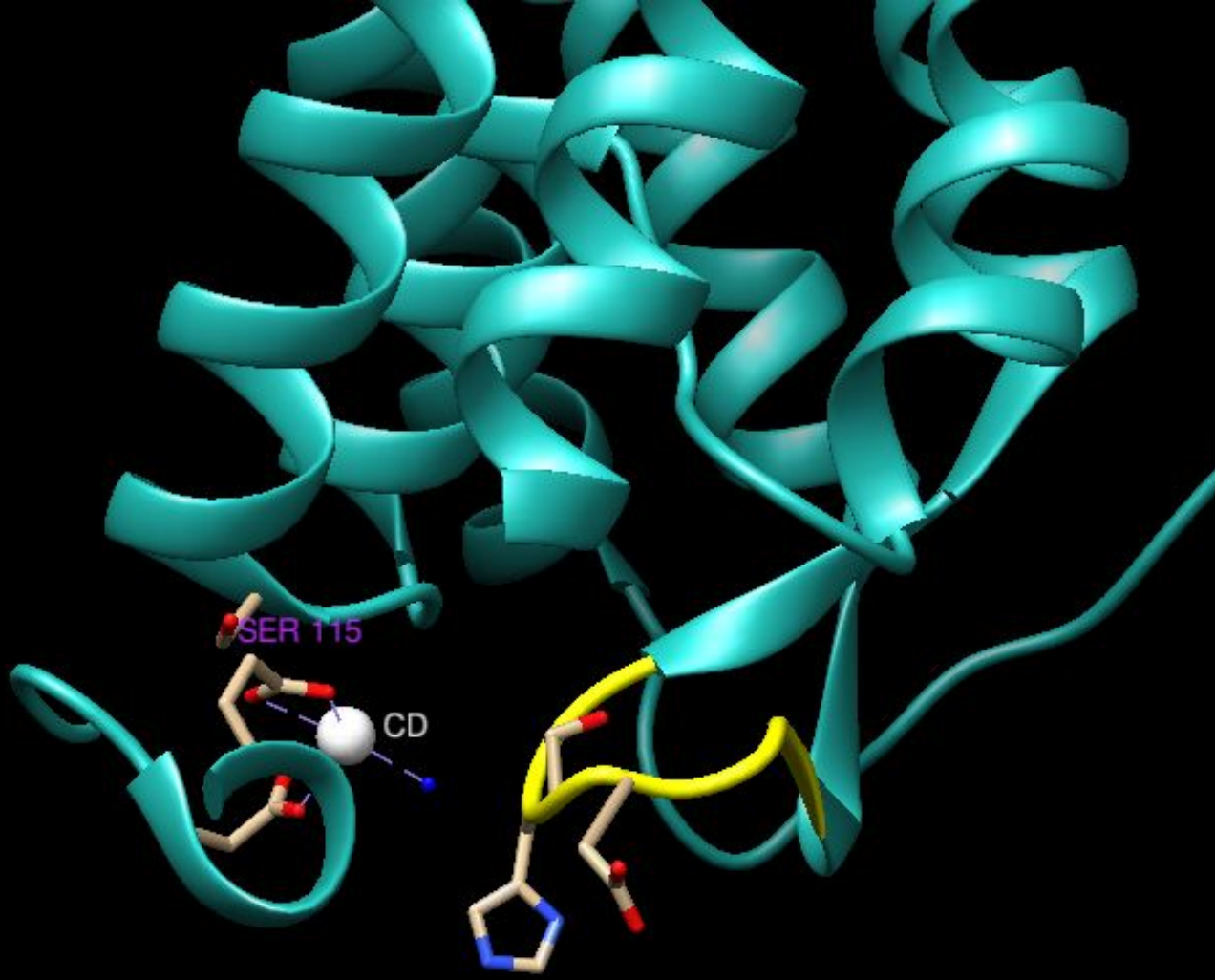


Spinach

Spinach mechanism of open and close conformation. In this case the loop D position plays an important role.
When Ser115 is phosphorylated there's a loss of interaction with Cd → so loop D less proximal to pore

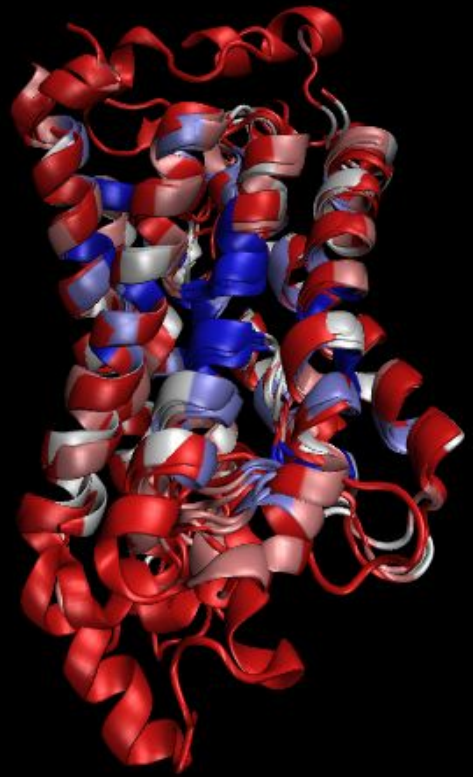


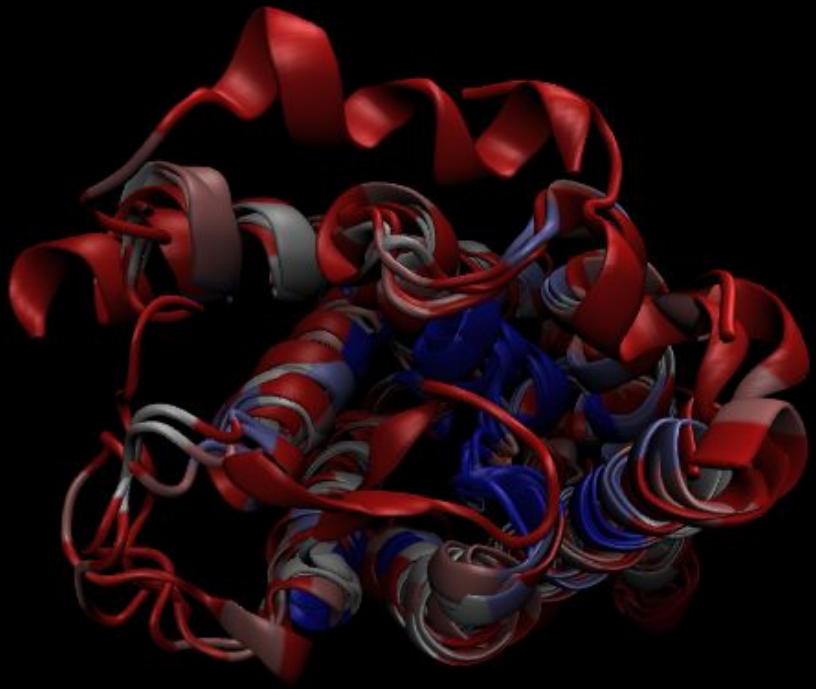
Closer observation of the relevant region related with the closure of the pore

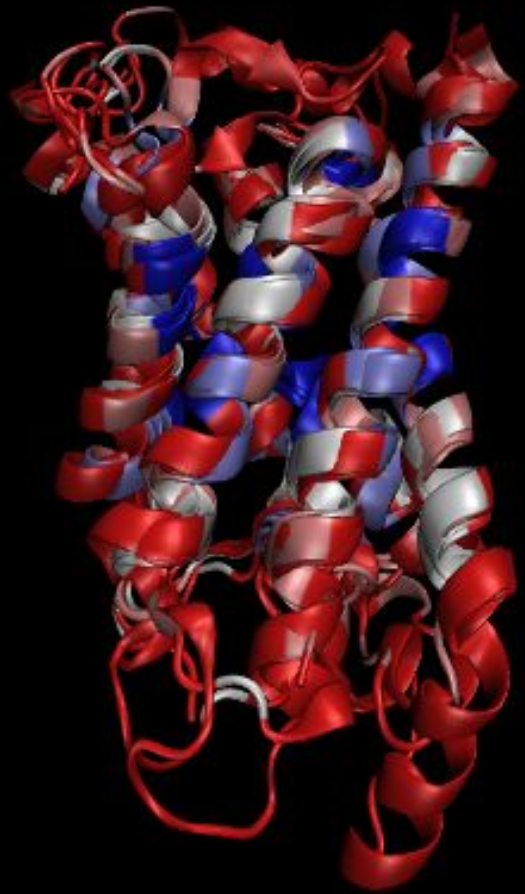


Superimposition of STAMP Structural Alignment

The aligned molecules are colored by their sequence similarity. Each aa is colored according to the degree of conservation with the alignment: blue means highly conserved, whereas red means very low or no conservation



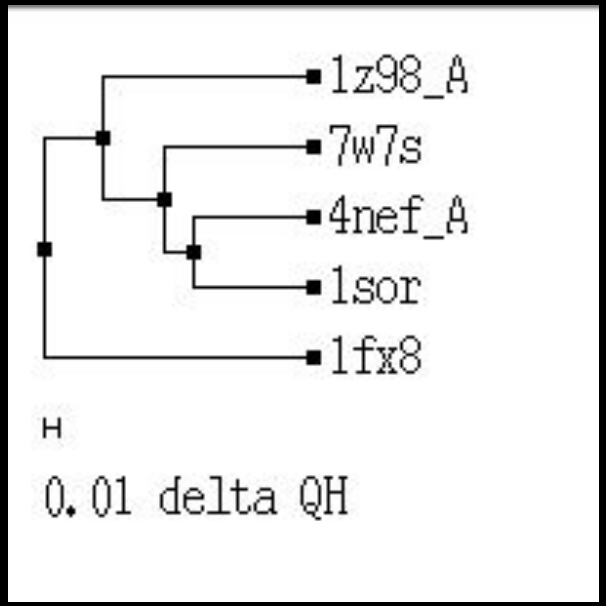




Superimposition Results

Human APQ2s	AQP1 SHEEP (1SOR)	AQP FISH (7W7S)	AQP SPINACH (1Z98)	Bacteria (1FX8)
AQP2 (4NEF)	QH: 0.84 RMSD: 1.02 % Id: 57.45%	QH: 0.83 RMSD: 0.83 % Id: 40.08%	QH:0.74 RMSD: 1.13 % Id: 32.64%	QH: 0.65 RSMD: 1.43 % Id: 26.86%

Structural dendogram



Spinacia oleracea

Anabas testudineus

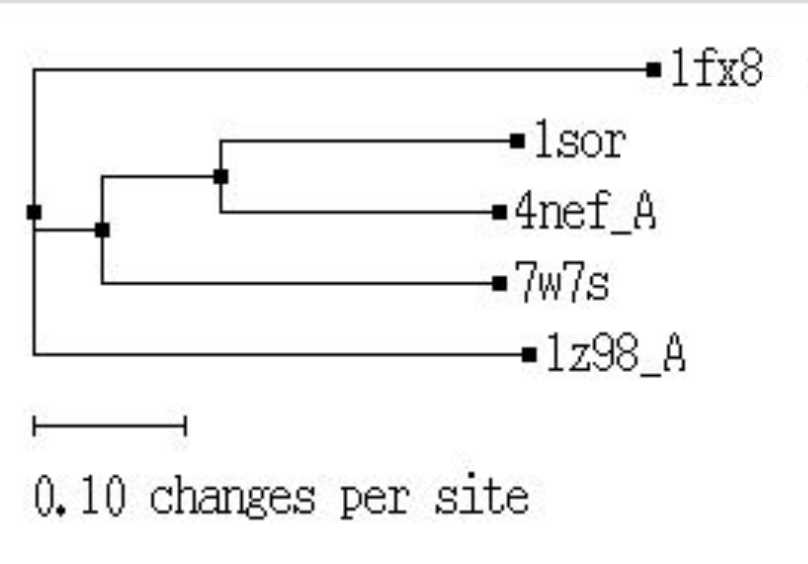
Homo Sapiens

Ovis aries

Escherichia coli K12

Main differences when it comes to structural analysis are in bacteria and plants respect human aquaporin

Dendogram using CLUSTALW



GLPF *Escherichia coli K12*
AQP0 Ovis aries
AQP2 Homo Sapiens
AQP1 Anabas testudineus
SoPIP2;1 Spinacia oleracea

Thanks for your attention!

Diego Moreno Marc

Rodríguez Maria

Figueroa Beatriz

Pou

PEM QUESTIONS

PEM questions

1. Which of the following statements is FALSE:

A) AQP2 has H-Bonds inside the alpha-helix of its structure.
B) AQP2 does not have any kind of salt bridges to stabilize its structure.
C) AQP2 forms a tetramer where each monomer has a functional pore.
D) AQP2 monomers are all alpha-proteins.
E) The AQP2 "tails" are not responsible for the function itself but the correct. transport of the protein.

2. About the NPA region, choose the TRUE statements:

A) It stands for Not Polar Access.
B) The Asparagine in this region is not conserved and tends to variate.
C) It has no relevance for the water conductance.
D) It is completely rigid.
E) It establishes H-Bonds with the passing water molecules.

3. Which of the following statements accurately describes the structural organisation of aquaporins (AQPs)?

A) AQPs consist of seven α-helices arranged perpendicular to the membrane.
B) AQPs are composed of three α-helices that form a transmembrane channel.
C) AQPs are organized into six segments of an α-helix structure that cross the membrane from side to side.
D) AQPs have a single loop that folds toward the membrane and forms the pore.
E) AQPs do not contain any α-helices; instead, they are composed of β-sheets.

4.In the context of the T125M AQP2 mutation, what is the consequence of the amino acid change (Thr125 to Met) within the glycosylation consensus sequence (Asn-X-Ser/Thr)?

A) Enhances glycosylation
B) Suppresses glycosylation
C) Improves protein stability
D) Promotes protein degradation
E) Facilitates protein folding

5. What is the function of the detected salt bridge between Glu3 and Arg85 in Chain A?

A) Stabilisation of the C-terminal intracytoplasmic tail.
B) Interaction with metals, such as cadmium.
C) Enhancing the mobility of the membrane.
D) Facilitating the crystallisation process of proteins.
E) Regulating the movement of sodium molecules within the membrane.

6. What is the function of the area mainly composed of Arg187, His172, and Phe48 in aquaporins?

A) Facilitating the entry of sodium and potassium ions into the pore.
B) Neutralising the pH of acidic molecules.
C) Allowing solutes with positive charge to pass through the pore.
D) Increasing the diameter of the pore to accommodate larger molecules.
E) Forming the selectivity filter to regulate the passage of solutes through the pore.

7. What conserved structural feature remarks similarity between the evolutionary lines of animals and plants despite their separation about 1.6 billion years ago?

A) Tryptophan appearance
B) NPA regions and selectivity filter conservation
C) Pore enlargement
D) Loop A removal
E) Amino acid substitution

8. What role do the residues of the NPA region play in the passage of water through the pore of aquaporins?

A) Blocking the passage of water molecules.
B) Facilitating the entry of other solutes.
C) Providing structural support to the pore.
D) Forming hydrogen bonds with the water molecules.
E) Creating a hydrophobic barrier within the pore.

9. Which molecule or protein is directly phosphorylated by protein kinase A (PKA) in response to vasopressin stimulation, leading to the internalization of AQP2 into vesicles again?

A) Vasopressin receptors.
B) Adenylate cyclases.
C) F-actin polymers and dyneins.
D) RhoA and Aquaporin-2 (AQP2).
E) Aquaporin-7(AQP7).

10. What is the role of Cd1 and Cd2 in the structure and function of aquaporin-2 (AQP2) in vivo?

A) Cd1 binds primarily with HisC80 and GluC232 to stabilize loop D.
B) Cd2 is ligated by GluA155 and GlnD57 to displace loop D.
C) Cd1 helps position the C-terminal helix by forming a hydrogen bond with GluB155.
D) Cd2 displaces loop D to the center of AQP2 by forming an ionic bond with HisC80.
E) Cd does not play a role itself in vivo, it is probably Ca^{2+}.

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