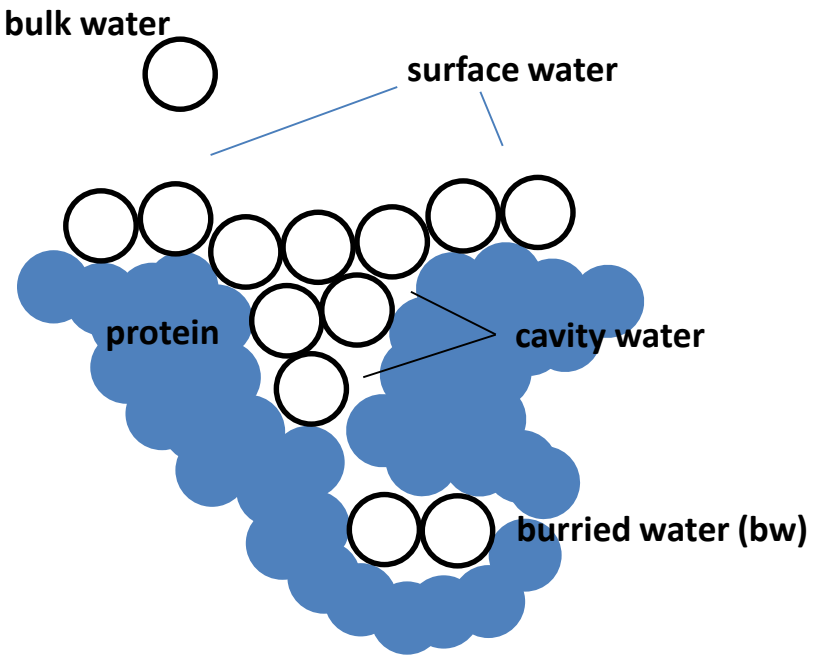
Visualizing Internal Water Interactions in Membrane Proteins by X-ray Footprinting

Sayan Gupta
Sr. Res. Associate., Lawrence Berkeley National Laboratory, CA
Scientific Consultant , Neoproteomics, Inc, OH

Transmembrane Water

75 non-homologus globular protein



- Average number of polar contact by BW is 3.23 (37 % BW makes 4 PCs)
- BW with <2 polar contact forms contact with other BW
- 53 % PC to back bone; 30 % PC to side chain and 17% to other buried water

Williams et. al. Prot. Sc. 1994. 3. 1224-1235

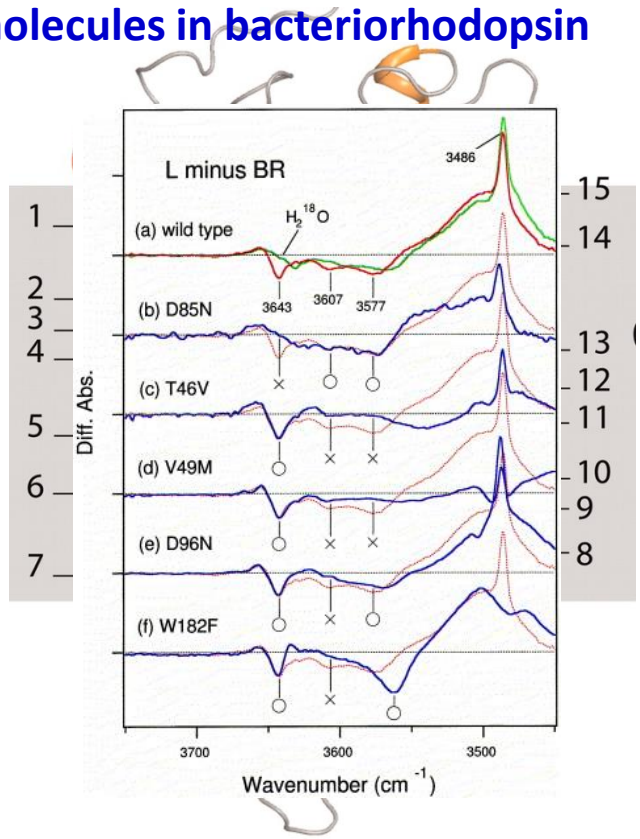
BW in 30 Helical Membrane Protein

- BW in TM protein = globular proteins
- BW in TM helices > BW water in soluble helices. Polar contact by BW in TM > polar contact within helices of globular protein
- Asymmetry of distribution of BW across TM
- Polar contacts are conserved among protein families having different function
- Disease-causing mutations, which are known to result in misfolded TM proteins, occur at buried water contact sites at a higher than random frequency
- BWs play a role in structural stabilization

Renthal R. Prot. Sc. 2007. 17. 294-298

Detecting internal water interaction in TM

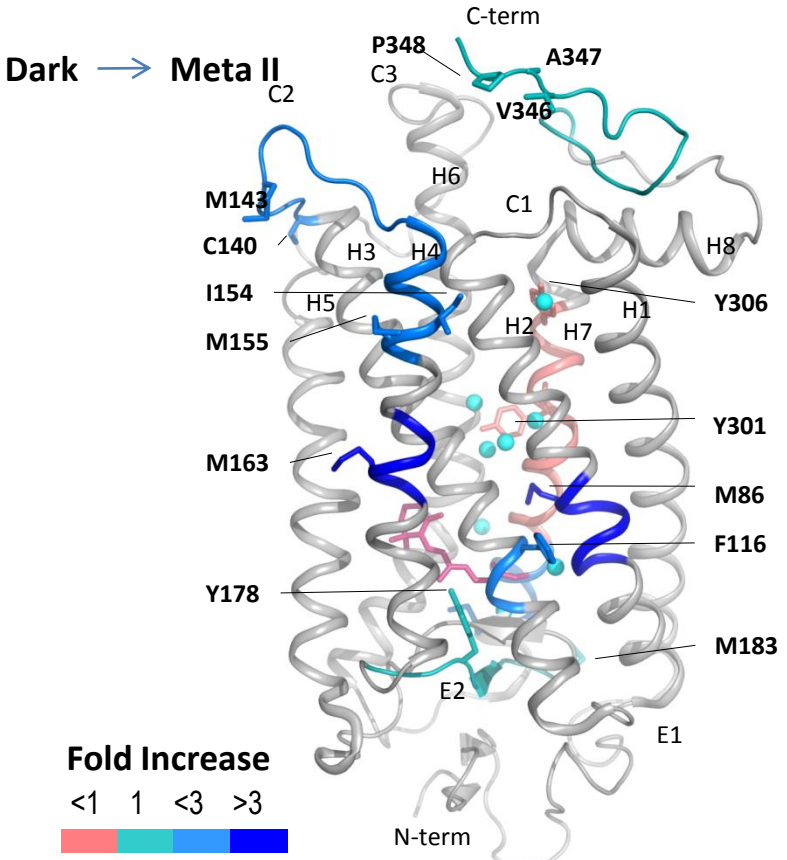
FTIR: Observed water molecules in TM molecules in bacteriorhodopsin



Water molecules observed in the 3750-3450 region. (a) Red and green curves represent spectra upon hydration of H_2O and $H_2{}^{18}O$, respectively (b–f) Blue curves represent the spectra of mutants, while red dotted curves are the spectra of wild type.

Angel TE et al. PNAS 2009. 106(21):8555-60

XF: Photo activation of bovine Rhodopsin



Disruption and reorganization of multiple close packing interactions mediated by both side chains and bound water transmit sufficient information from the chromophore to the cytoplasmic surface for G-protein activation

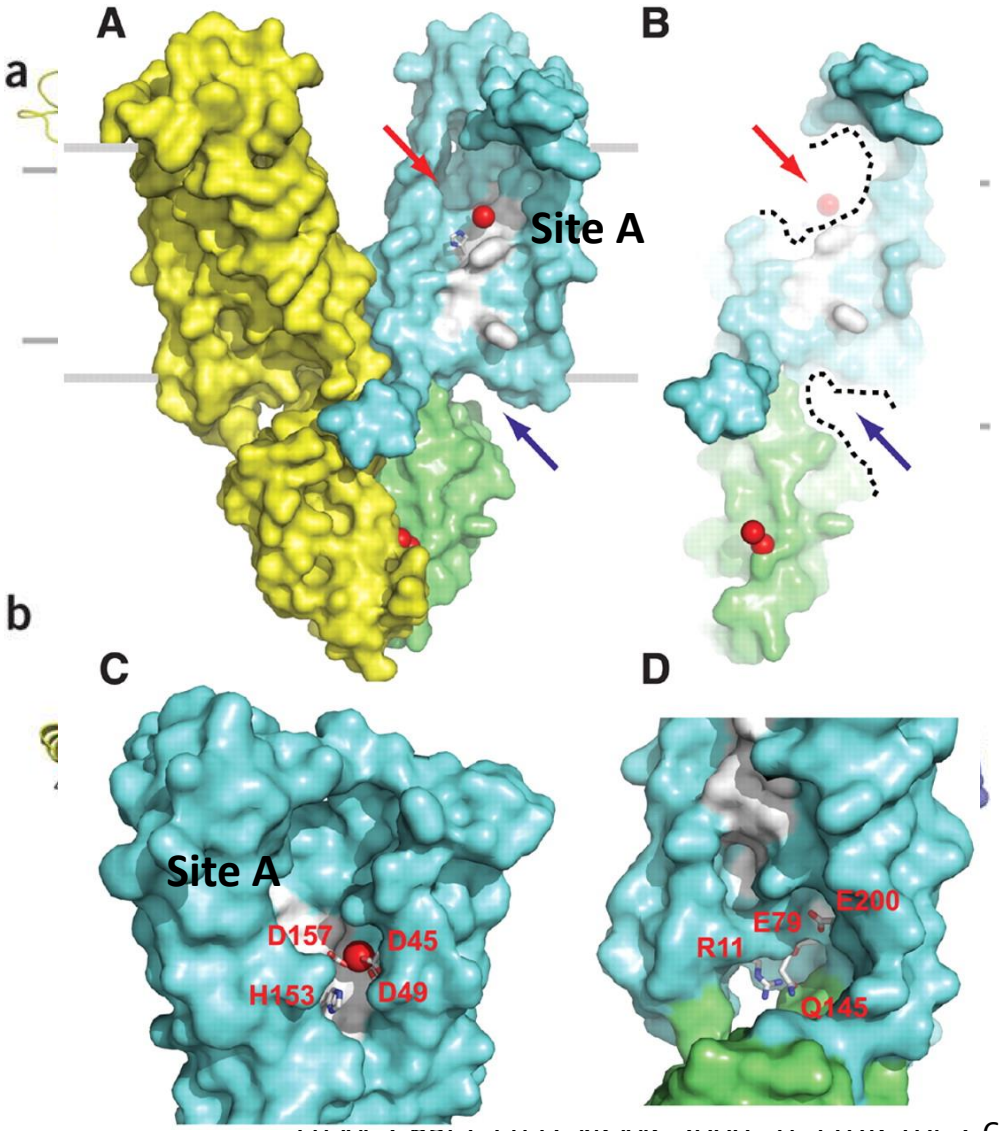
Angel TE et. al. PNAS 2009. 106(34):14367-72

- **Part 1: Visualization of Zn2+ / H+ Anti-port mechanism by XF in Zinc-Transporter YiiP**

- **Part 2: pH induced conformational change in KcsA: voltage gated potassium ion channel**

YiiP Zn-Transporter

- Zn2+ is a trace nutrient (nM inside cell. Zn – homeostasis is critical
- CDF and ZIP are 2 major classes of TM protein for Zn – homeostasis
- Bacterial YiiP is a CDF: Mammalian homolgues are responsible for Zn-sequestration into secretory vesicle
- Zn^{2+} in efflux is couple to H^{+} influx in 1:1



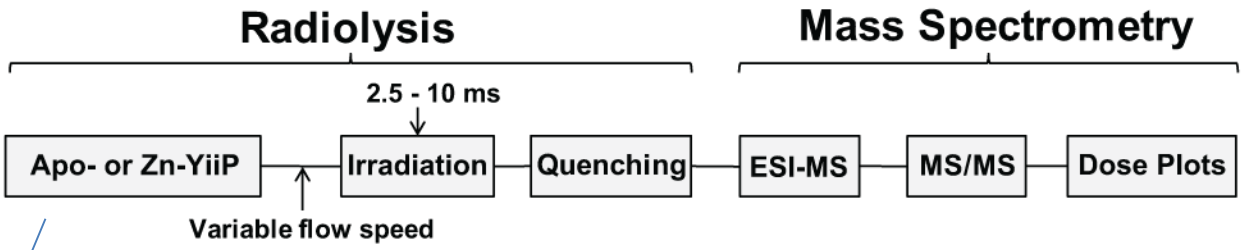
Chao Y, Fu D. JBC 2004. 279: 39251-59

Lu M, Chai J, Fu D. NSMS 2009. 16 (10): 1063-68

Lu M, Chai J, Fu D. NSMS 2007. 317: 1695-96

Steady State XF

Sample irradiation using modified KinTek® at Beamline X28C of NSLS

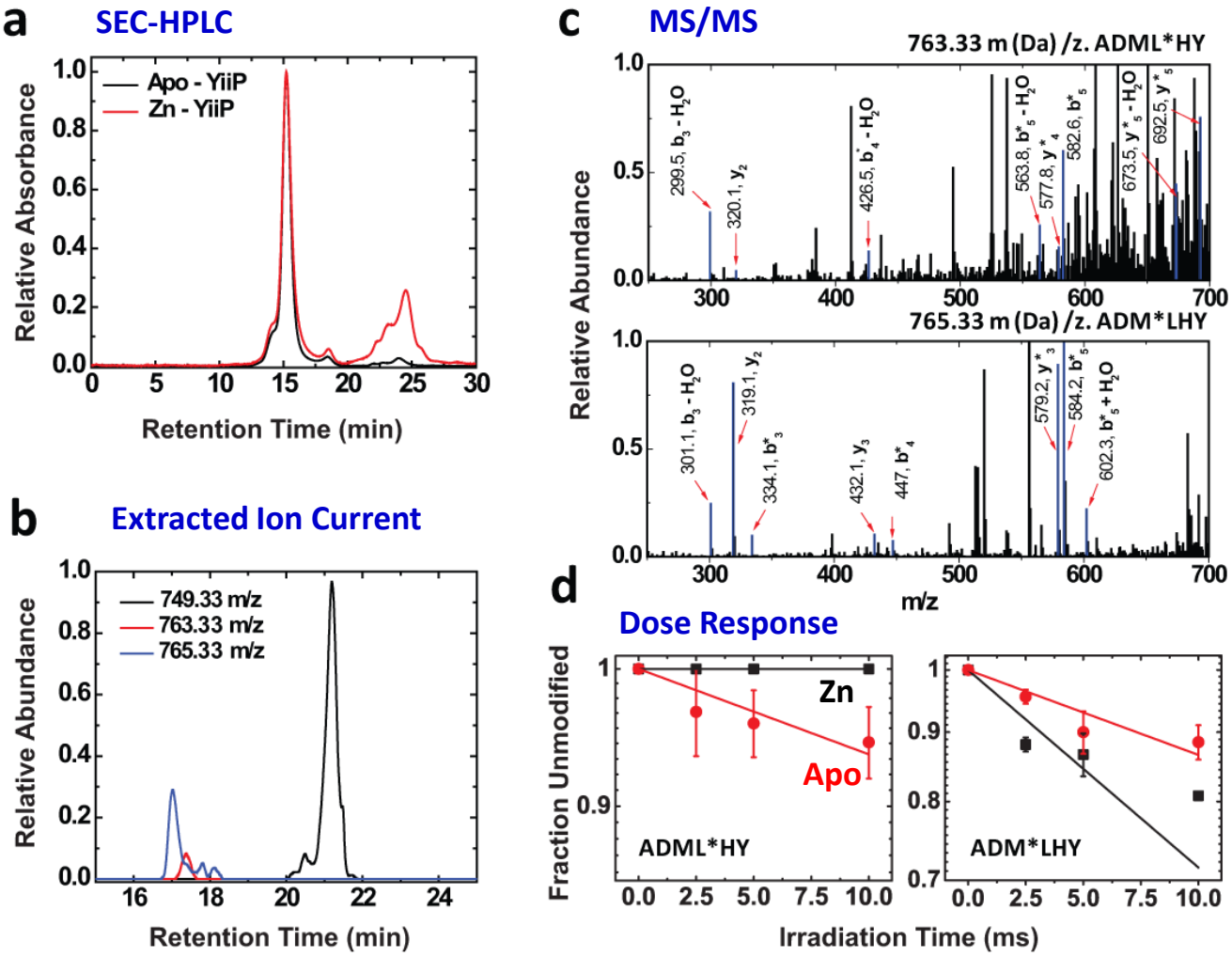


Within 30 min of sample preparation,
Characterized by pre- & post-irradiation SEC-HPLC

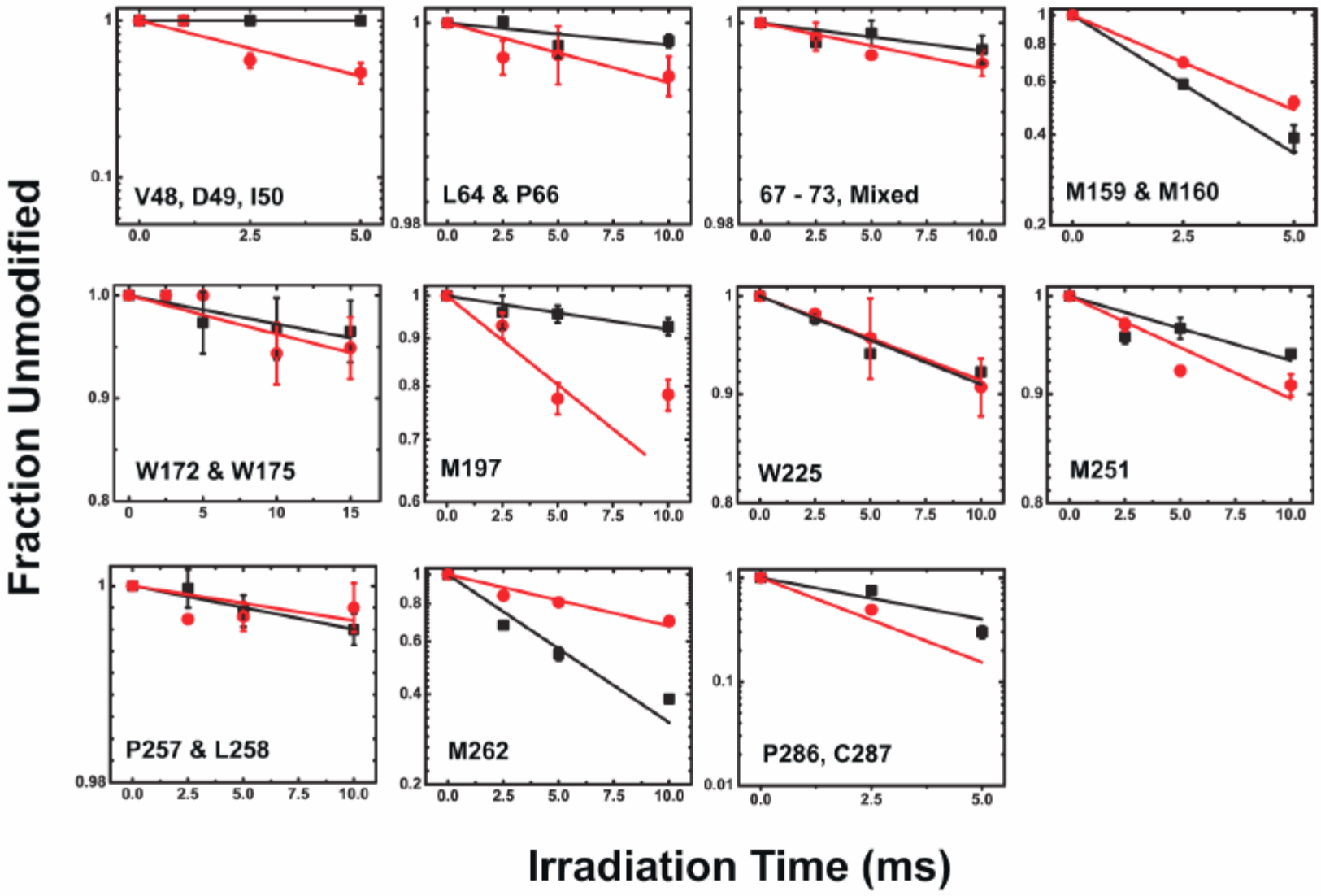
Pepsin. Trypsin and Trypsin-Chymotrypsin yeilded a sequence coverage ~ 80%. Data analyzed using - MASCOT, Protein Propector, ProtMap and manual evaluation

SS-XF & Mass Spectrometry Analysis

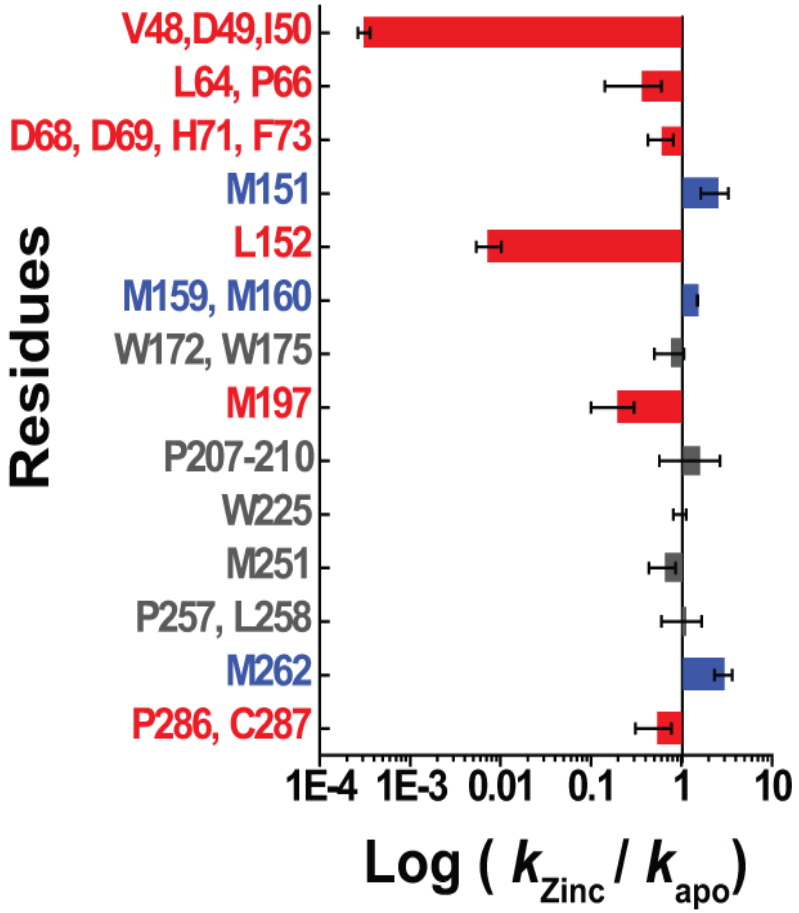
^{149} ADMLHY^{154} of TM5



SS-XF & Mass Spectrometry Analysis



Quantification of Water Accessibility from Steady State-XF

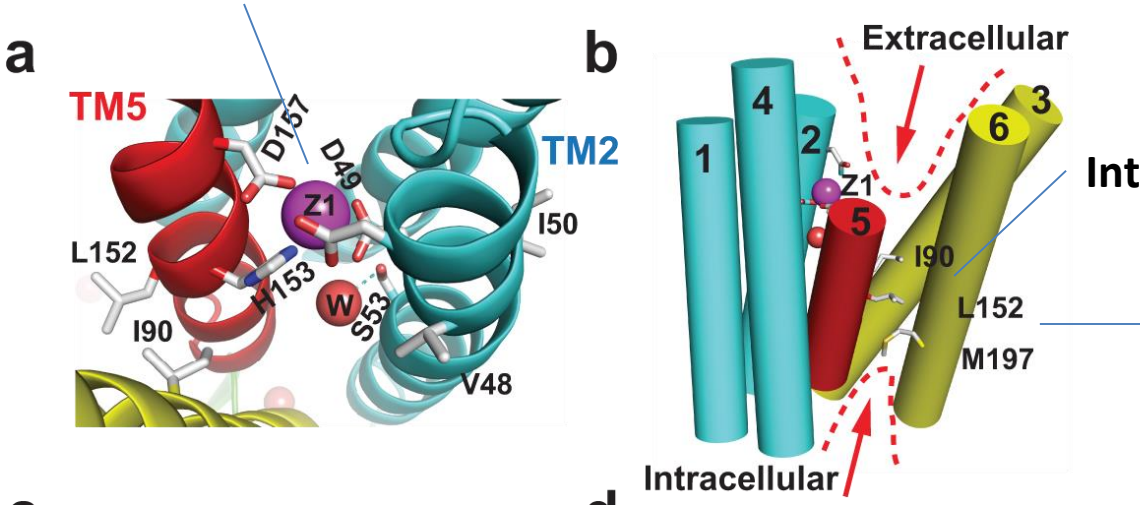


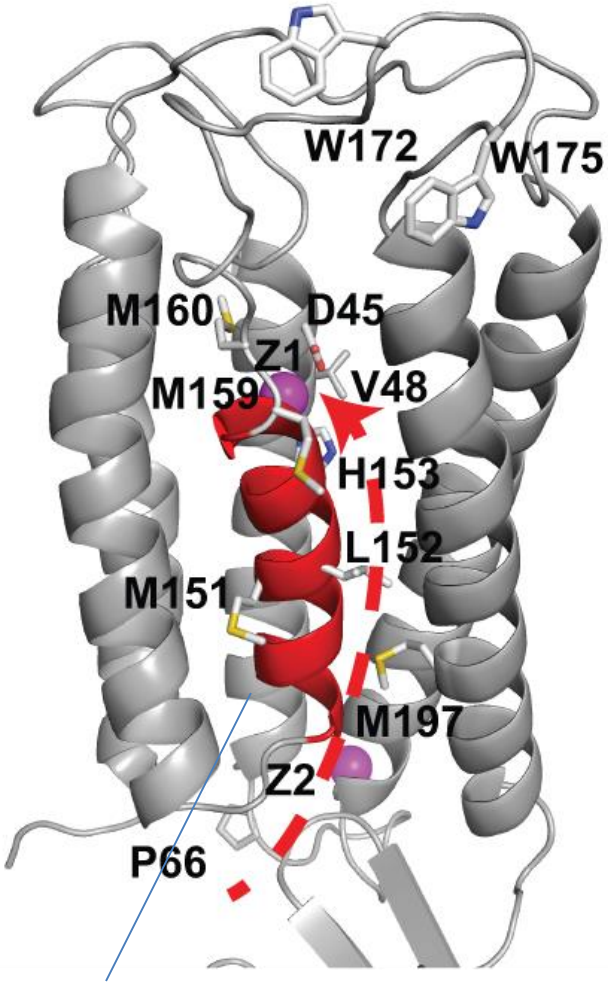
- Ratio from more than 6 independent measurements
- Ratiometric water accesibilty (WA) is independent of intrinsic side chain reactivity
- Two sites undergoes >100 fold decrease in WA – TM2 (D49 ..) & TM5 (L152)
- M197 also show 70% reduction in WA
- M151, M159 & M160 (TM5) show 50-130% increase in WA (reciprocal change)
- Residues showing no changes are away from Zn transport pathway and used as internal control

Structural implication of XF data

Active site

Sub-domain crossover





Extracellular cavity side

Intracellular cavity side

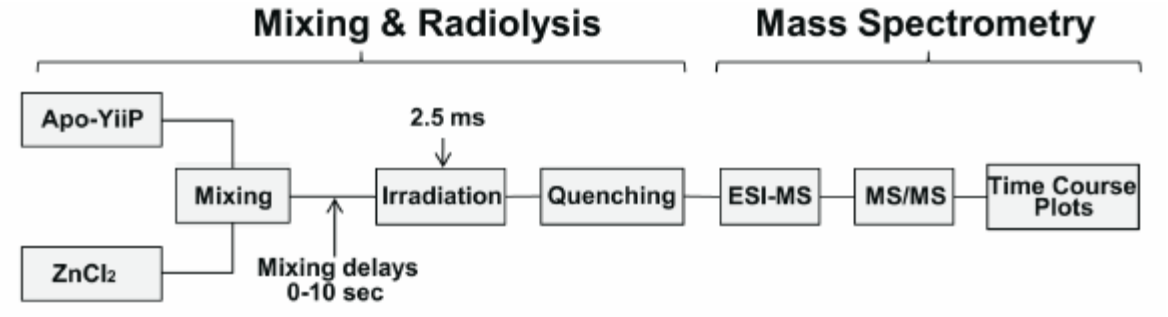
L152 acts as a hydrophobic barrier

TM5 re-orientation

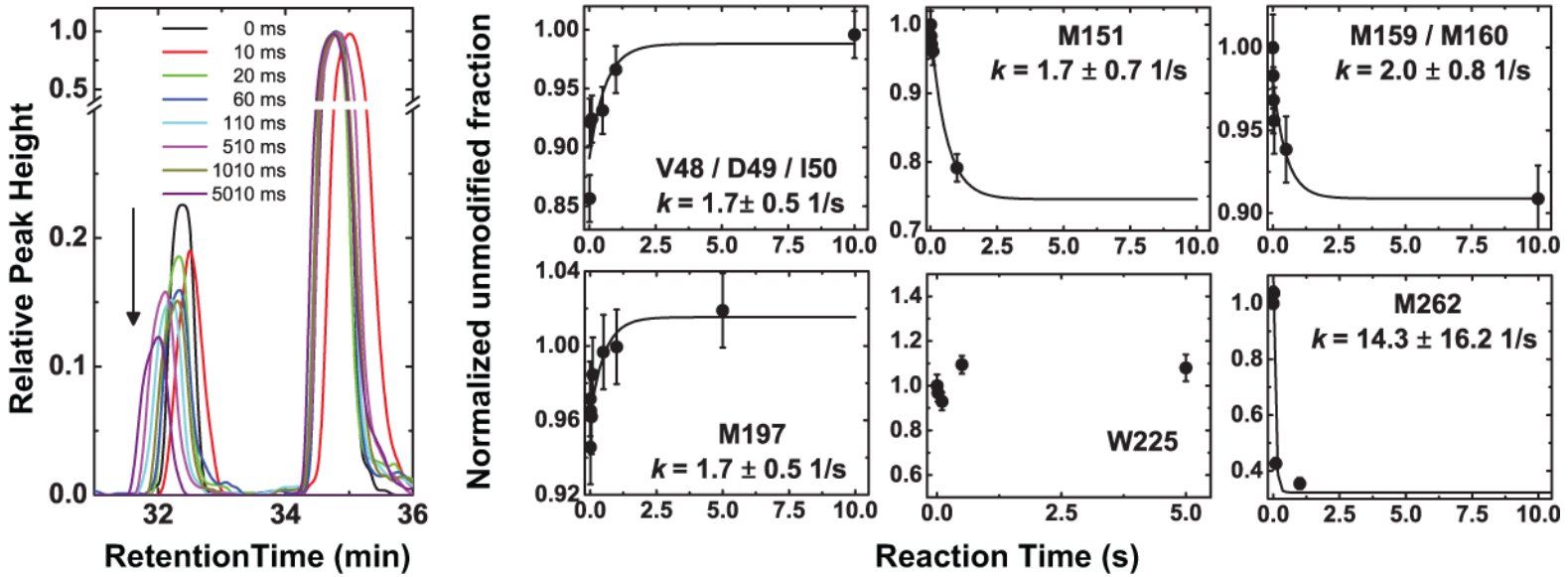
PDB 3H90

Kinetics of Water Accessibility Change

Time resolved XF using modified KinTek® at Beamline X28C of NSLS

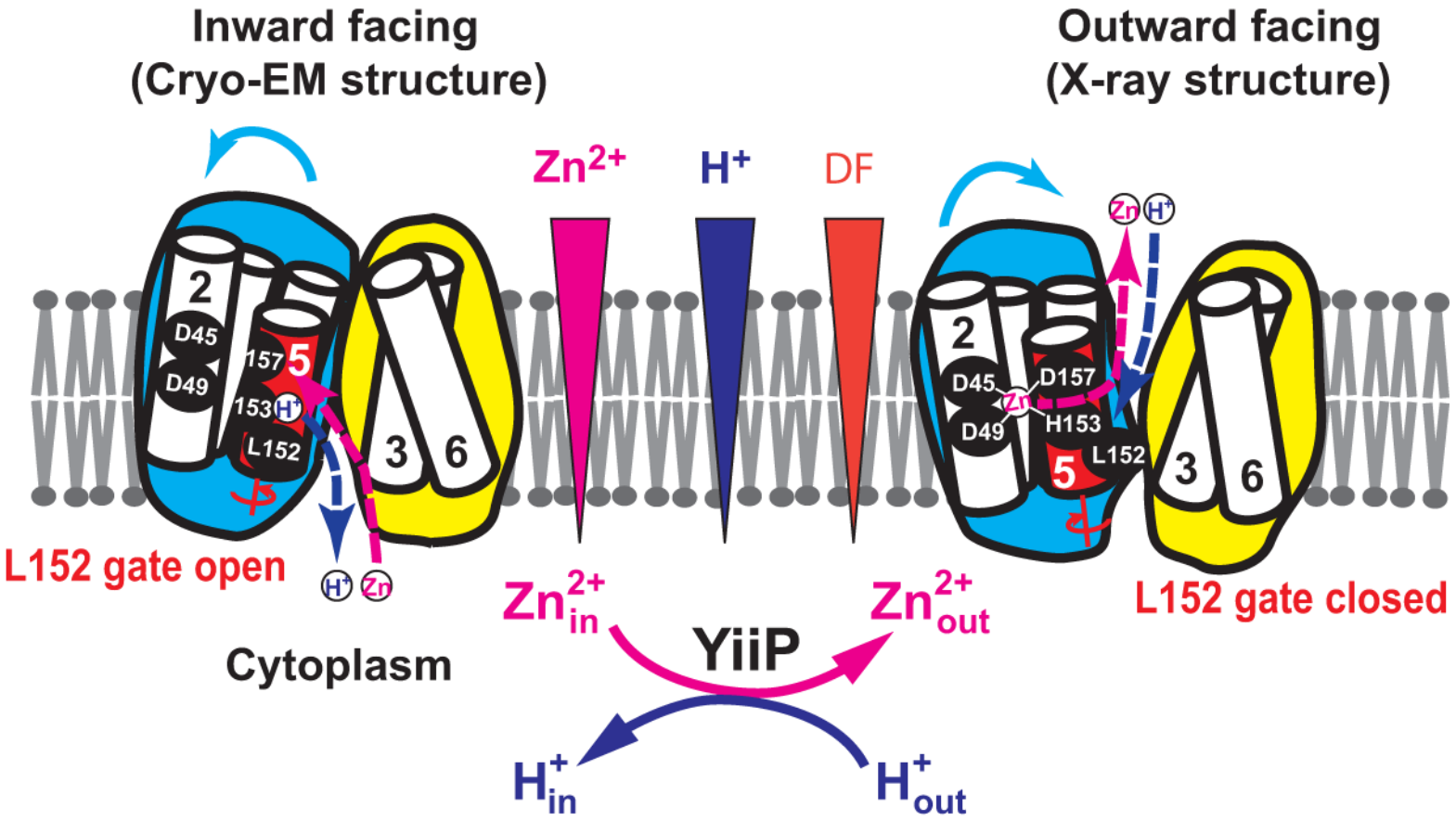


Time course of water accessibility changes of the most reactive residues



Concerted and reciprocal / Overall TM5 rate = 1.8 s^{-1} / Transport rate is 2-5 s^{-1}

Model of L152 Gating, TM5 Motion



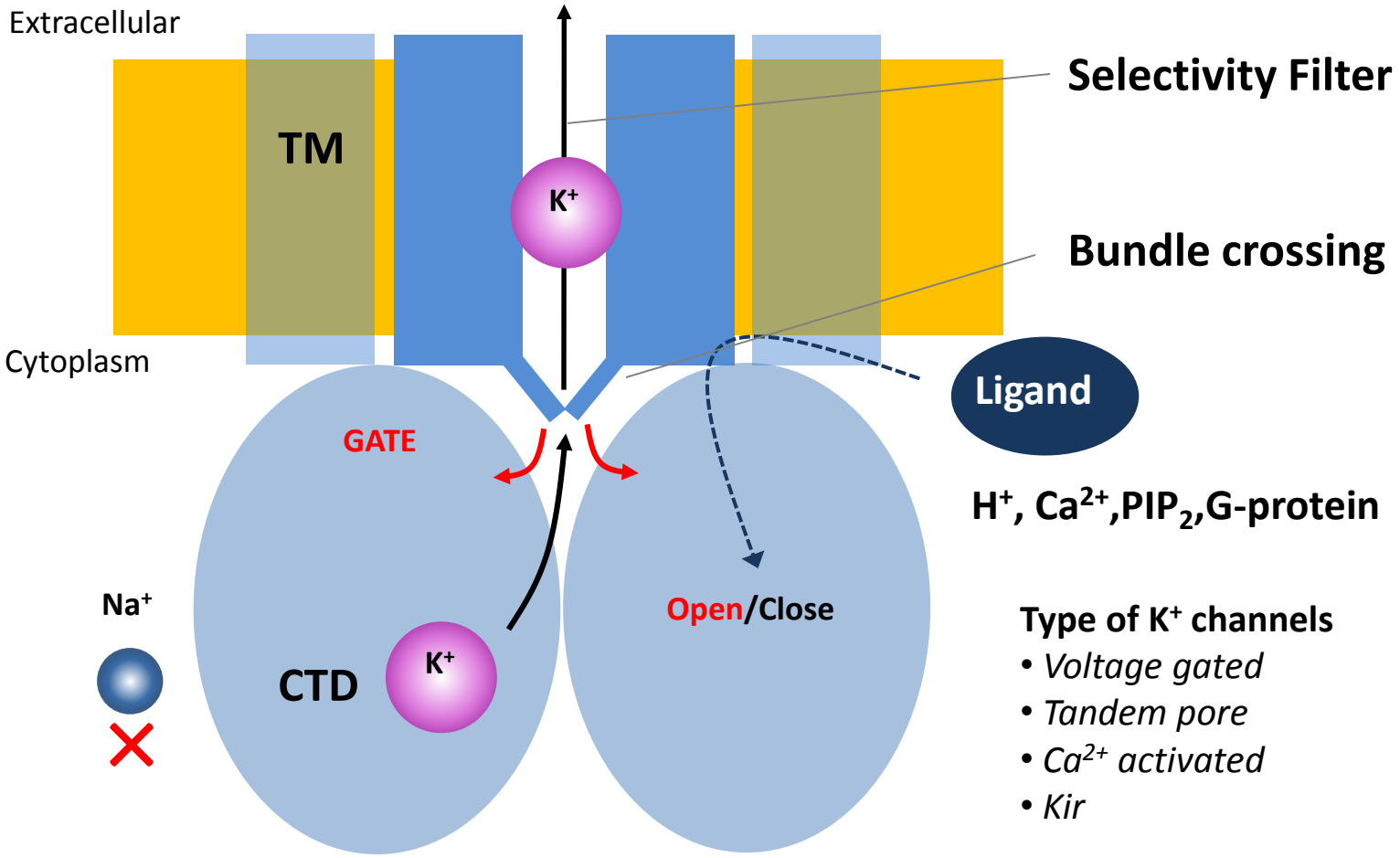
Schematic representation of zinc-for-proton exchange based on two existing structural models with the L152 gate open or closed as indicated. The protein conformational change alternates the membrane facing, on-off mode of zinc coordination and protonation-deprotonation of the transport-site in a coordinated fashion.

Conclusion

The dynamic details revealed in the present study explain how a physiological proton gradient, zinc coordination chemistry and water nanofluidics are orchestrated in a dynamic protein structure to overcome the activation barrier to Zn(II) efflux and promote a vectorial Zn(II) movement through an inter-cavity water portal that is highly conserved in the CDF protein family

Gupta S, Chai J, Cheng J, D'Mello R, Chance MR, Fu D. Nature. 2014 Aug 7;512(7512):101-4.

XF studies on potassium channels

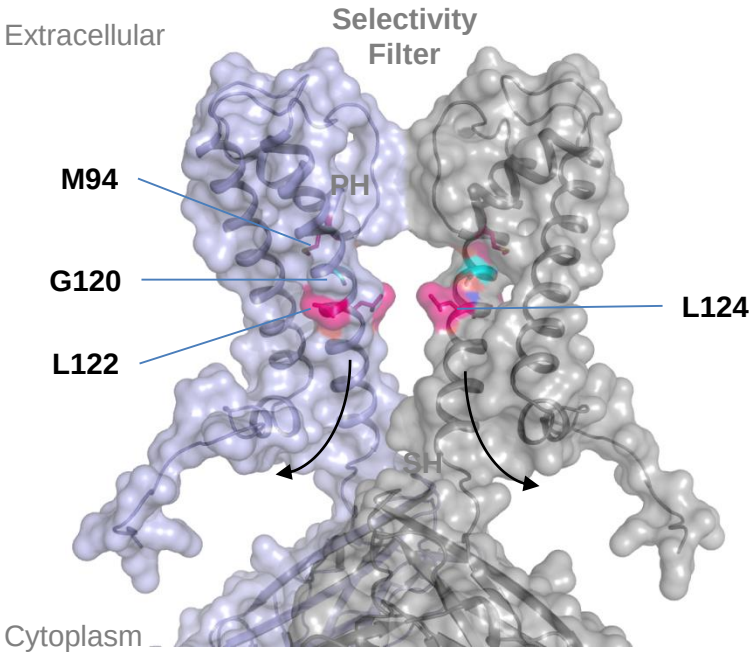


TM – Transmembrane Domain; **CTD** – Cytoplasmic Domain

X-ray Footprinting of KirBac3.1

X-ray Footprinting

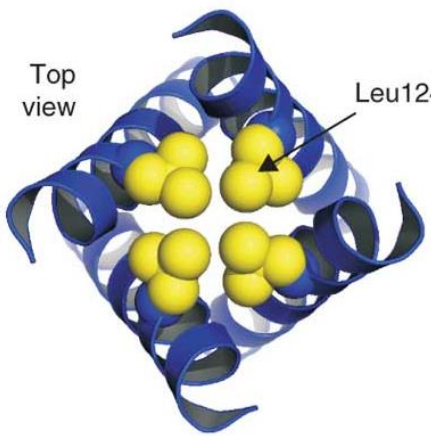
Gupta et al. Structure (2010)



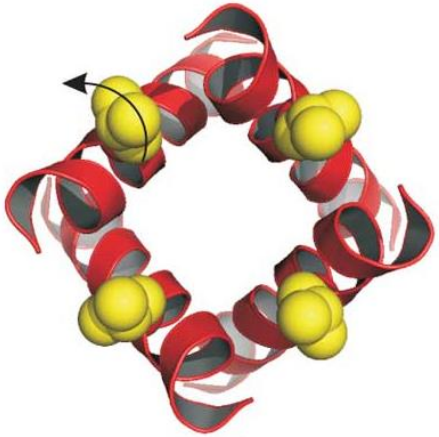
Closed > Open (1XL4)

Channel Opening at 3 Å

Bavro et al. Nat. Struc. Mol. Biol. (2012)



Closed (2WLJ)

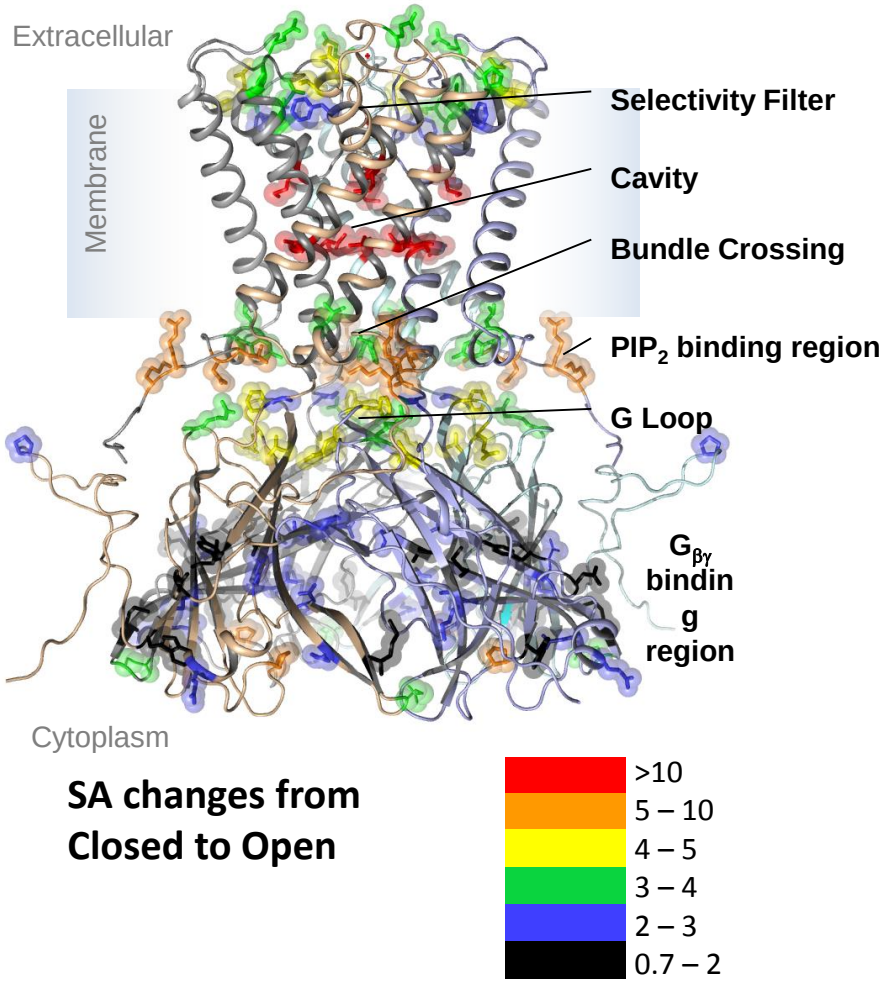


Open (3ZRS)

X-ray Footprinting of KirBac3.1

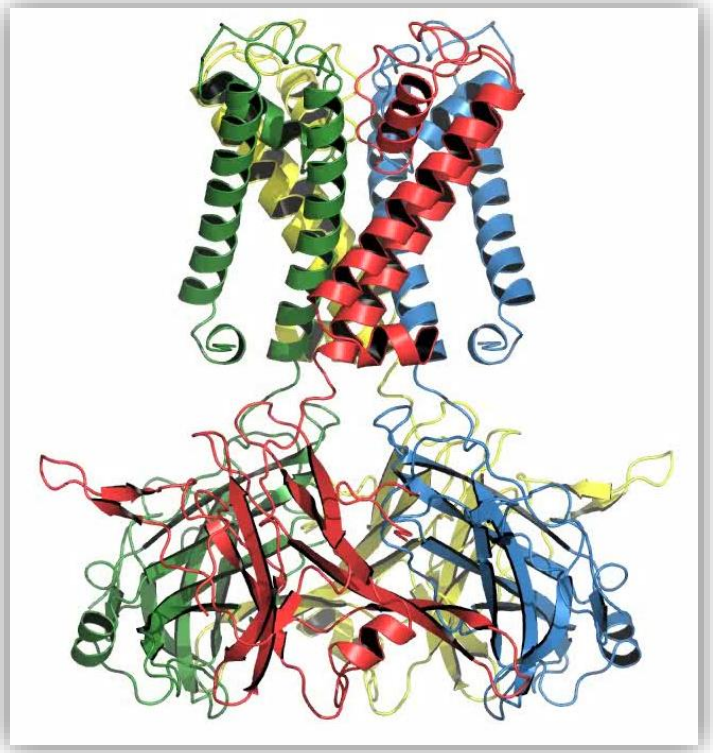
X-ray Footprinting

Gupta S et al. Structure (2010)



Channel Opening at 3 Å

Bavro et al. Nat. Struc. Mol. Biol. (2012)



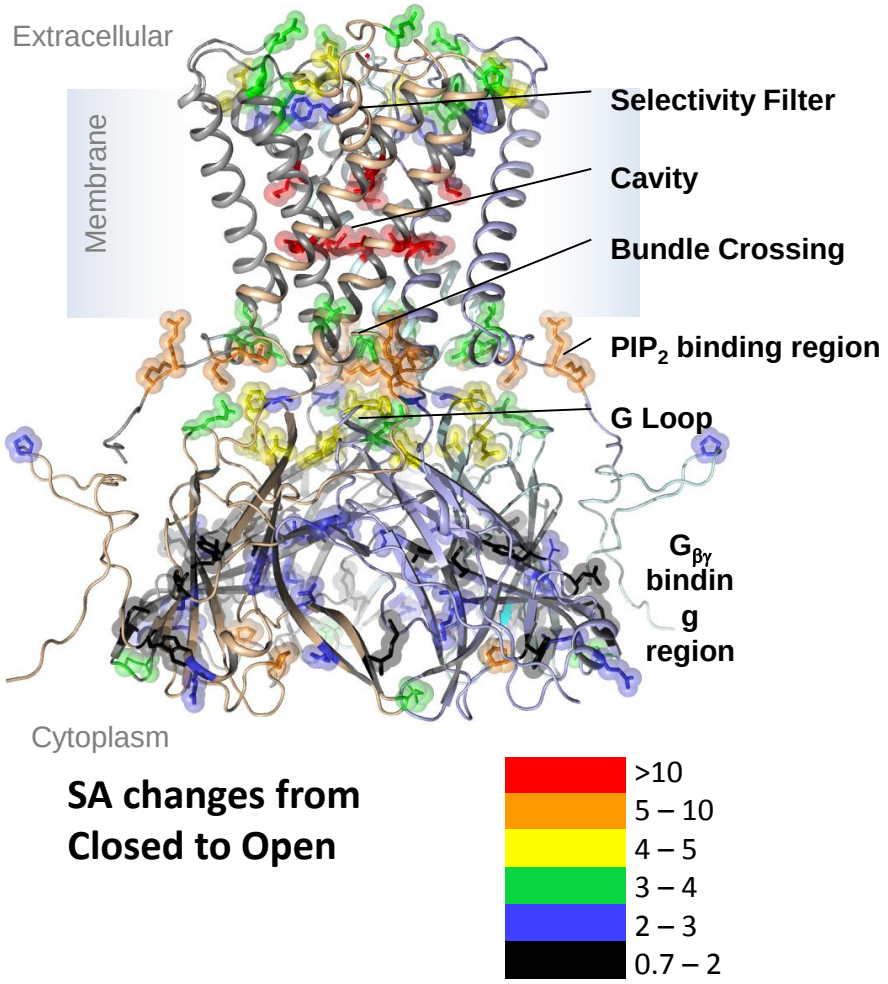
• **Activatory mutants clusters in groups**

Paynter et al. J. Biol. Chem. 2010

X-ray Footprinting of KirBac3.1

X-ray Footprinting

Gupta S et al. Structure (2010)



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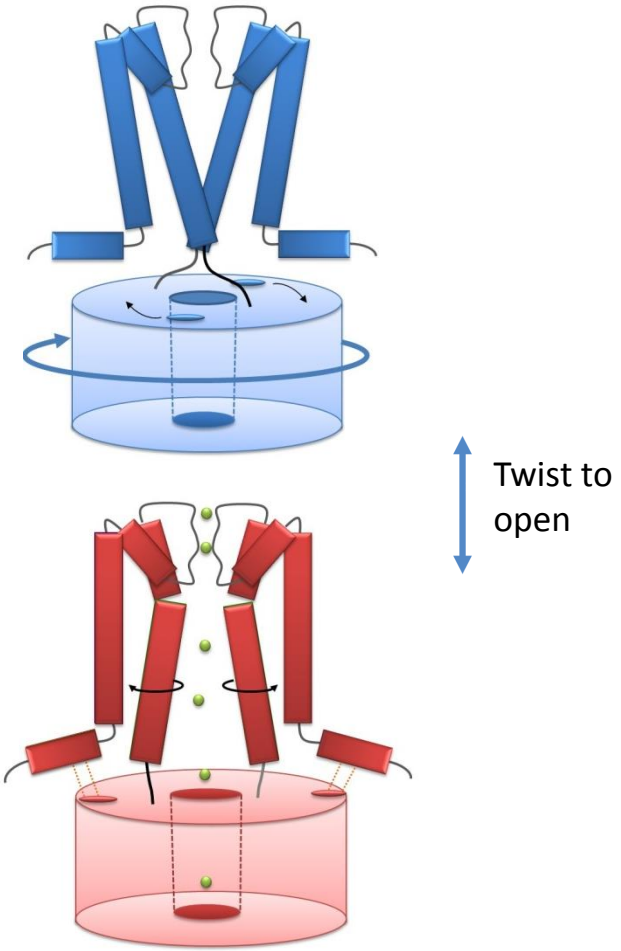
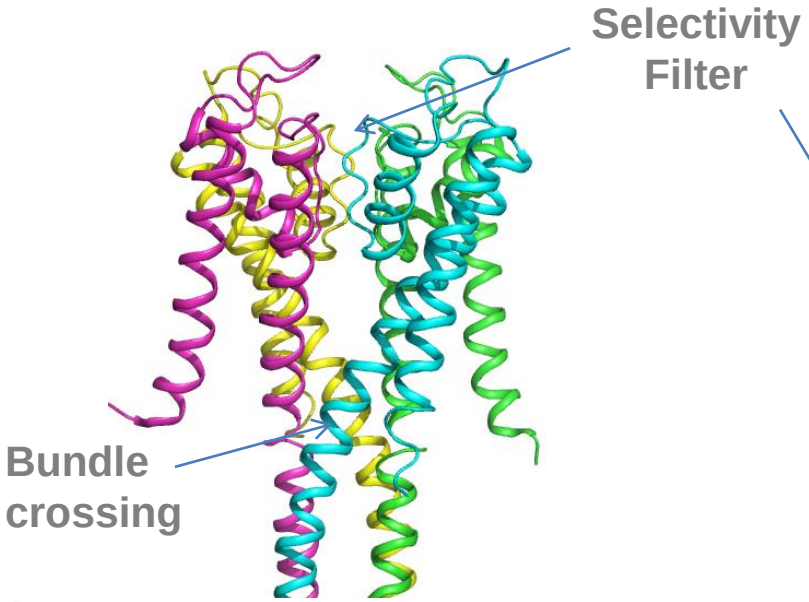
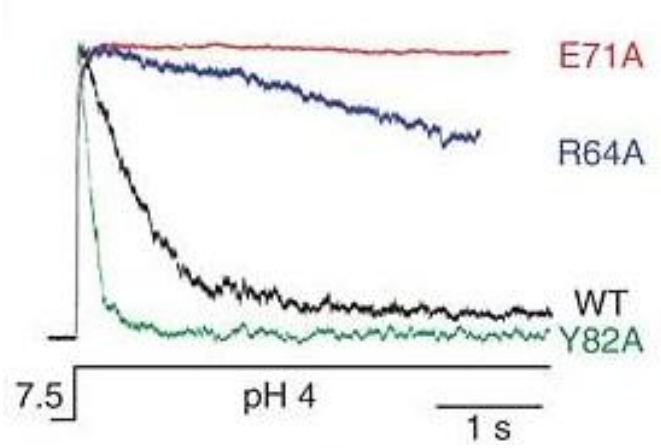
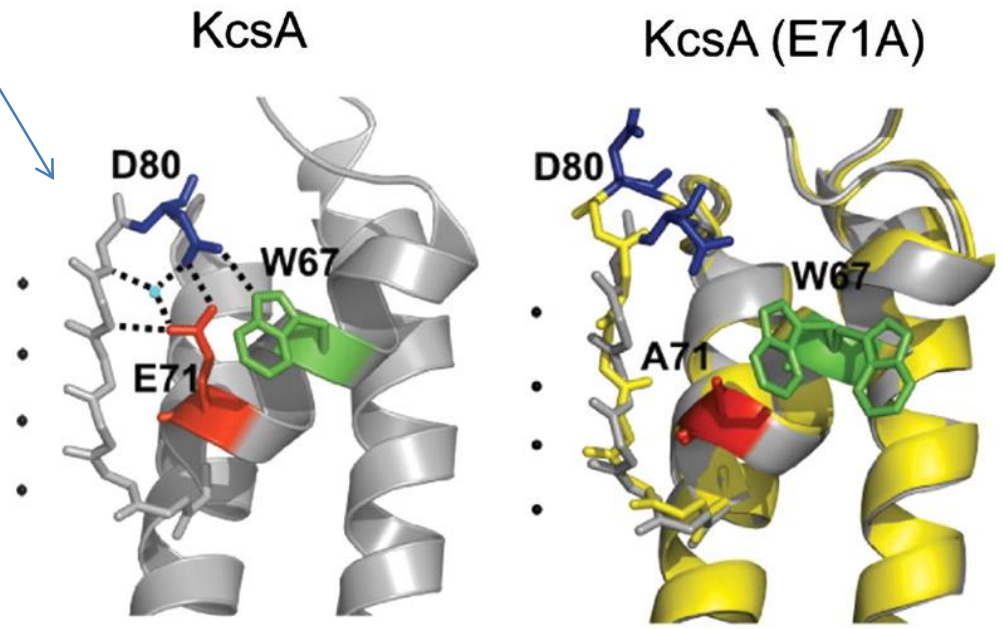


X-ray Footprinting of KcsA







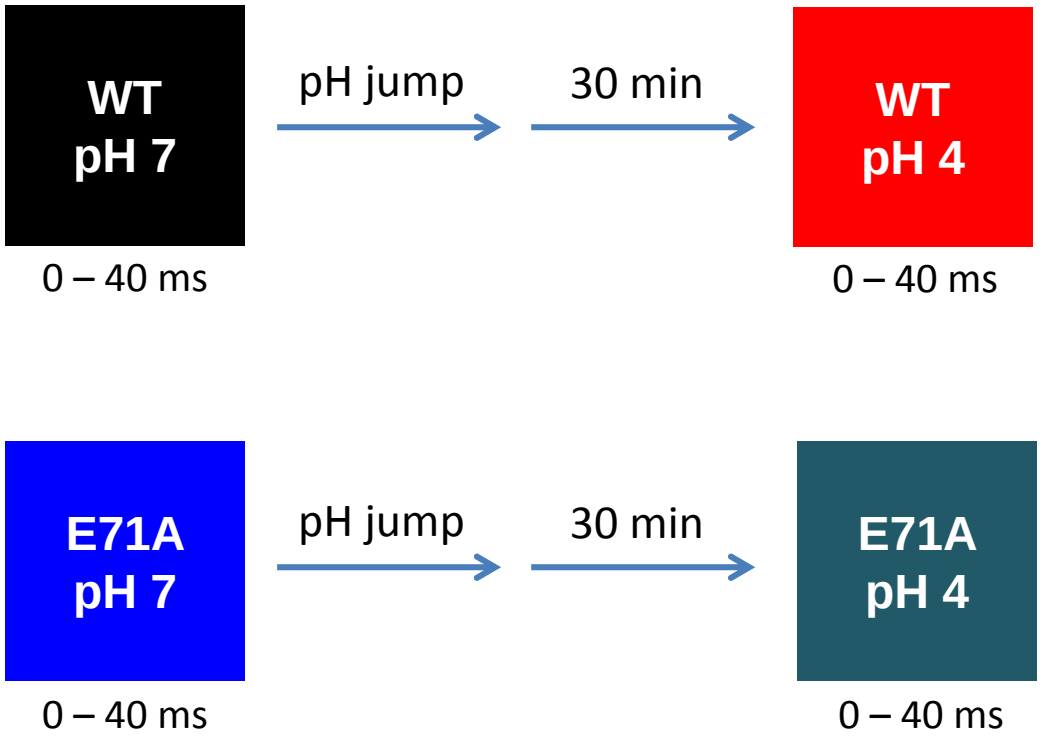
Cheng et al. PNAS (2011)

- **WT shows C- type inactivation (slow process)**
- **E71A abolishes pH dependent inactivation**

Objectives

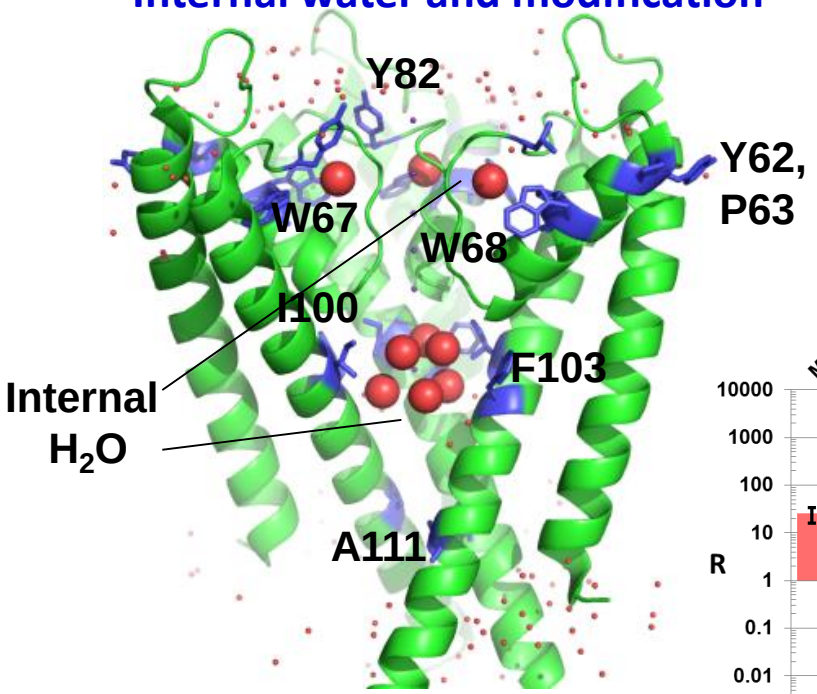
- **To test if footprinting can distinguish conformational changes between active and inactivated KscA and conducting E71A mutant**
- **To identify amino acid network behind modulation of ion selectivity and inactivation mechanism in full length ion channel**

Radiolytic Labeling Experiments



KcsA – Internal Water and XF Results

Internal water and modification

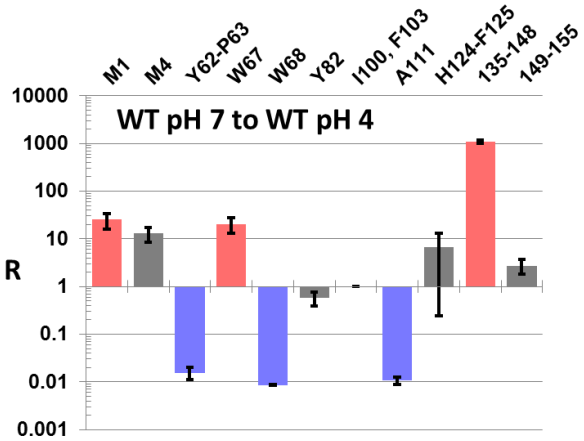


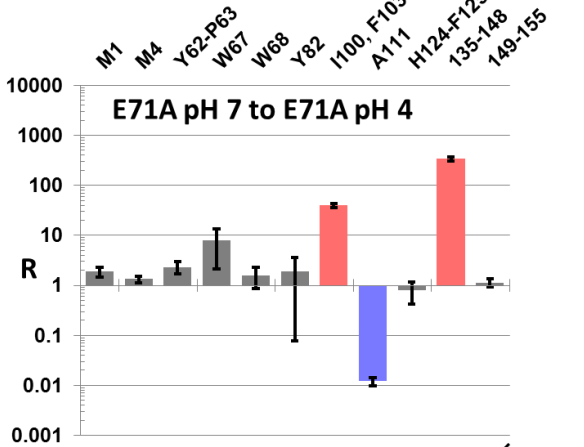
Several polar interactions mediated by structural water molecules (red spheres) near KcsA selectivity filter, 1K4C.pdb). Residues with a 10 fold change in solvent accessibility are shown in blue sticks.

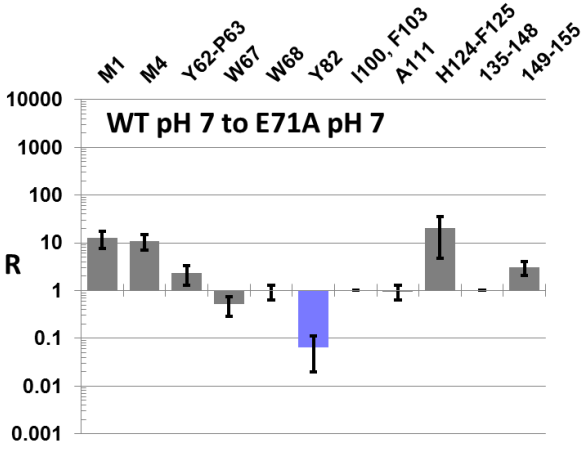
Ostmeyer, J., et al., *Recovery from slow inactivation in K+ channels is controlled by water molecules.* Nature, 2013. **501(7465): p. 121-4.**

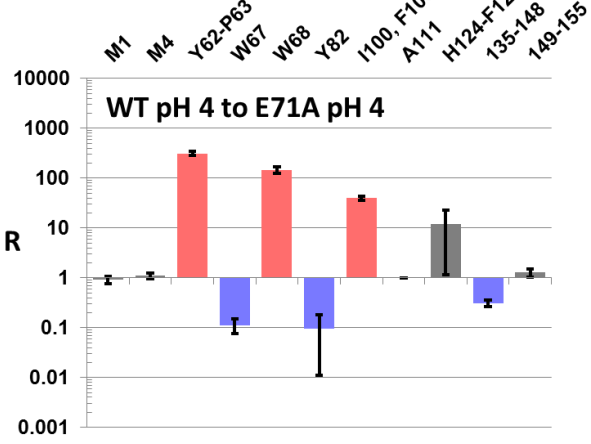
Comparison of pH dependent WA KcsA

WA change of > 10 fold are only considered



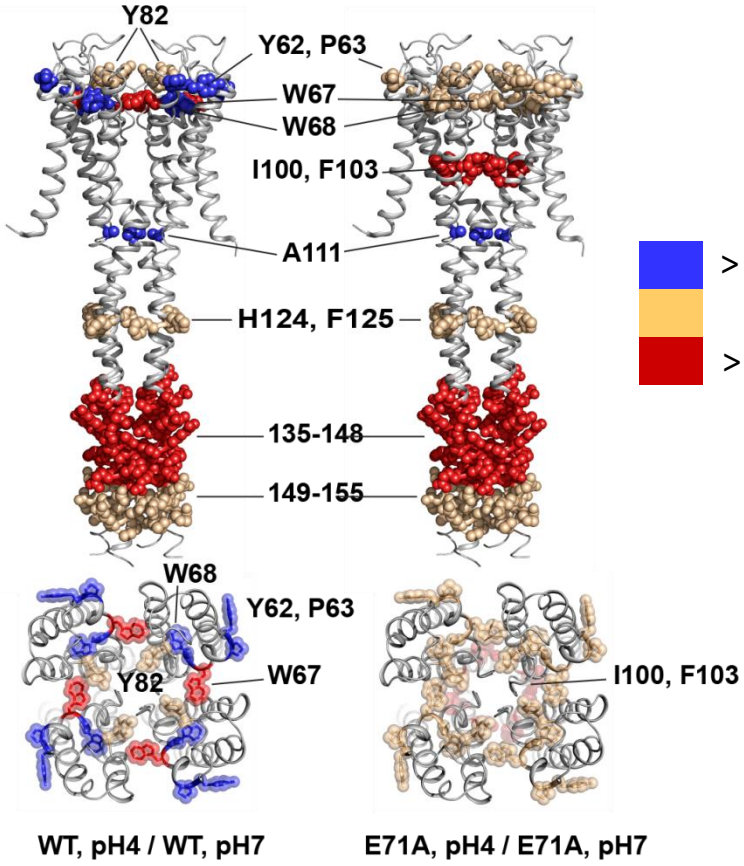






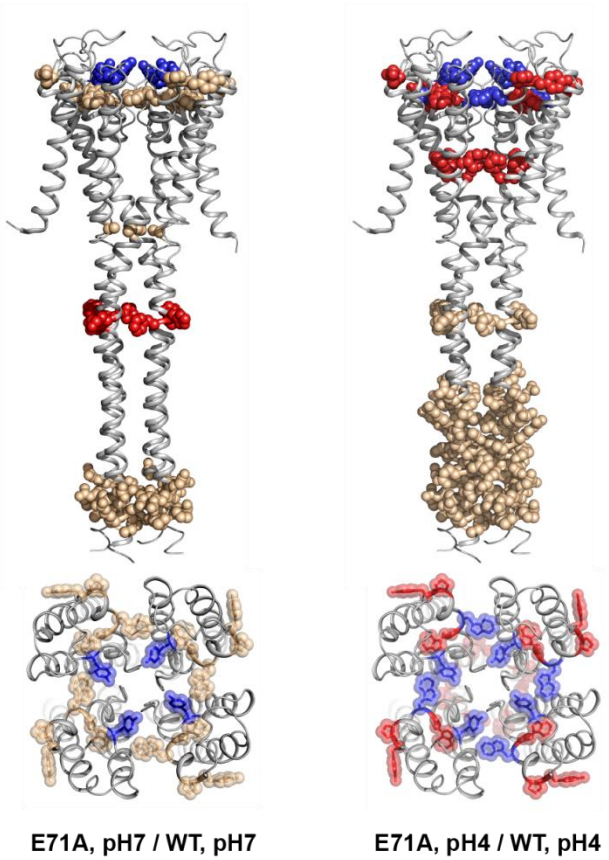
Difference WA map using X-ray crystal structure of full-length KcsA

Functional comparison



> 10 fold decrease
no change
> 10 fold increase

Structural comparison

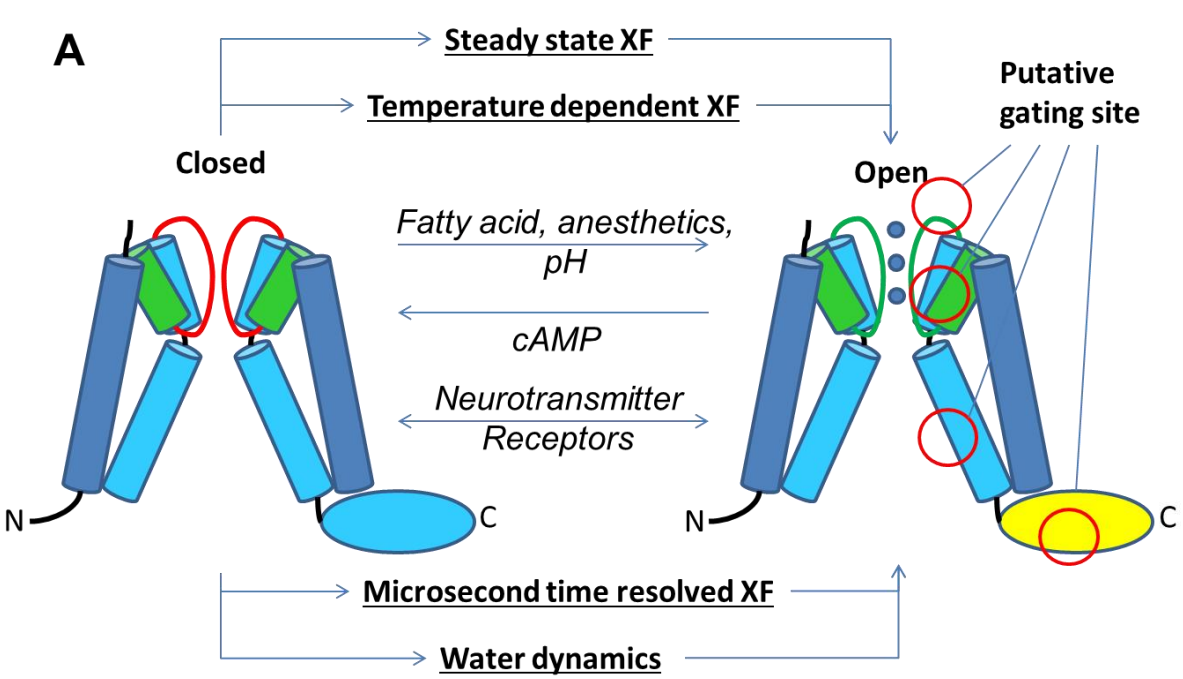


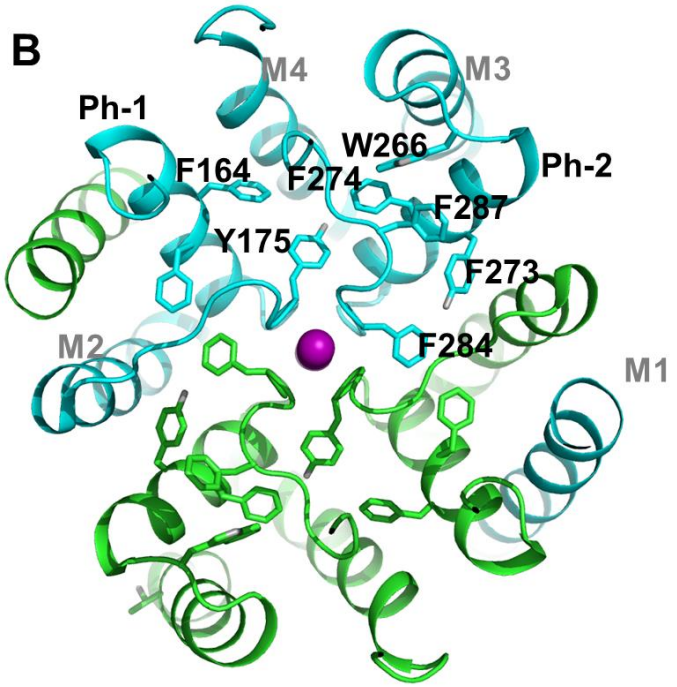
Conclusion – XF of KcsA

- Structural changes of the full-length KcsA (WT and E71A) during channel opening transition in-solution
- Detected a highly differential labeling for key residues involved in the maintenance of the structural stability of the selectivity filter, cavity and bundle crossing
- Structural rearrangement observed in CTD
- Our methodology lands independent support to the recently proposed elegant mechanism of gating and C-type inactivation in KcsA derived from X-ray crystallography
- This study extends the applicability of the XF to study of ion channels in general.

Future Directions

The high flux XF beamline will enable a level of modification sufficient to determine WA changes in μs time scale during channel gating under diverse sample preparation conditions such as liposome and membrane fractions, and will enable the study of dynamics of water-protein interactions inside cavities and selectivity filters





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- **Zn^{2+} Transport**

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BBSRC
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- **Mass Spec.**

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DOE (DE-AC02-98CH10886)