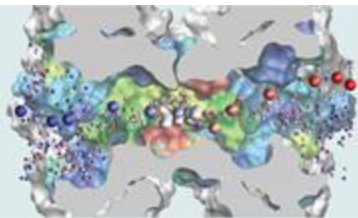# Molecular Dynamics of Paracellular Ion Channels

Frontera User Meeting August 4, 2023

presented by Sarah McGuinness
sfmvisuals.com

The Khalili Group:
COMPUTATIONAL MODELING
OF PROTEINS AND MATERIALS

FRONTERA

NSF

Table of Contents

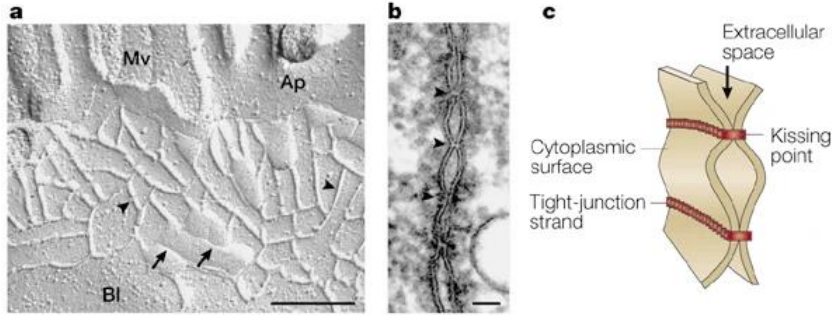Introduction: *Defining the role of claudins in tight junctions*

Methods: *Generating the reduced model*

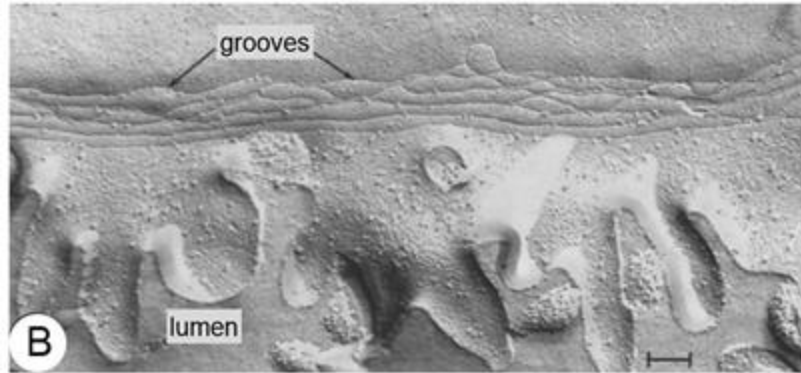Results and Discussion: *Characterizing the claudin-15 WT and mutant selectivity profiles*

Conclusion

Tight junctions are essential for maintaining biological compartments in an organism


(Tsukita et al., 2001)


(Claude and Goodenough, 1973)

Tight junctions are the gatekeepers of the paracellular space

Tight junctions form a **selective barrier** regulating paracellular transport of water and solute across epithelial and endothelial cells.

(Chalcroft and Bullivant, 1970; Tang and Goodenough, 2003; Anderson and Van Itallie, 2009; Furuse, 2010; Lingaraju et al., 2015; Zihni et al., 2016; Odenwald and Turner, 2017)

THE PARACELLULAR SPACE

APICAL

Tight Junction

BASOLATERAL
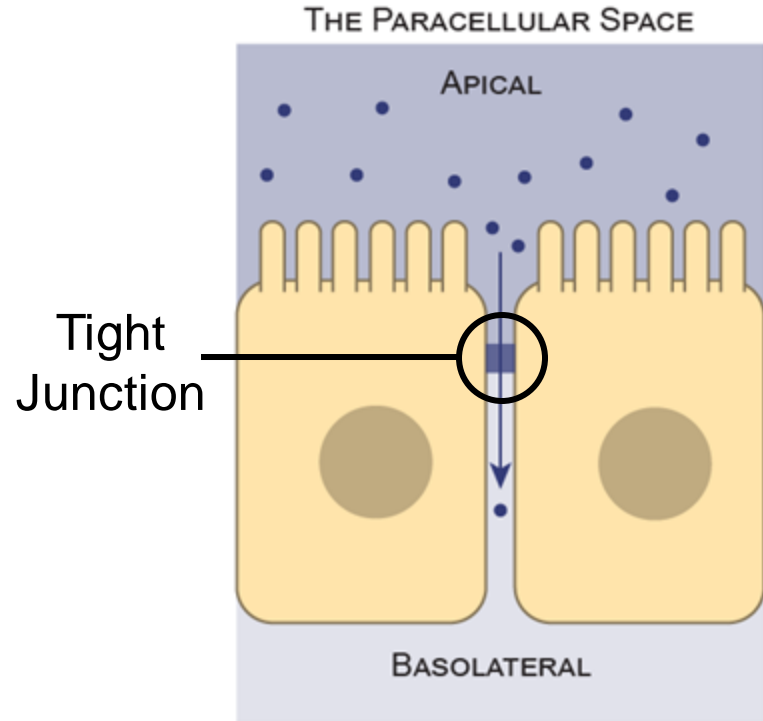
Claudins are the backbone of tight junctions and determine their permeability

(Otani and Furuse, 2020)

Tetrameric Pore of Claudin-15
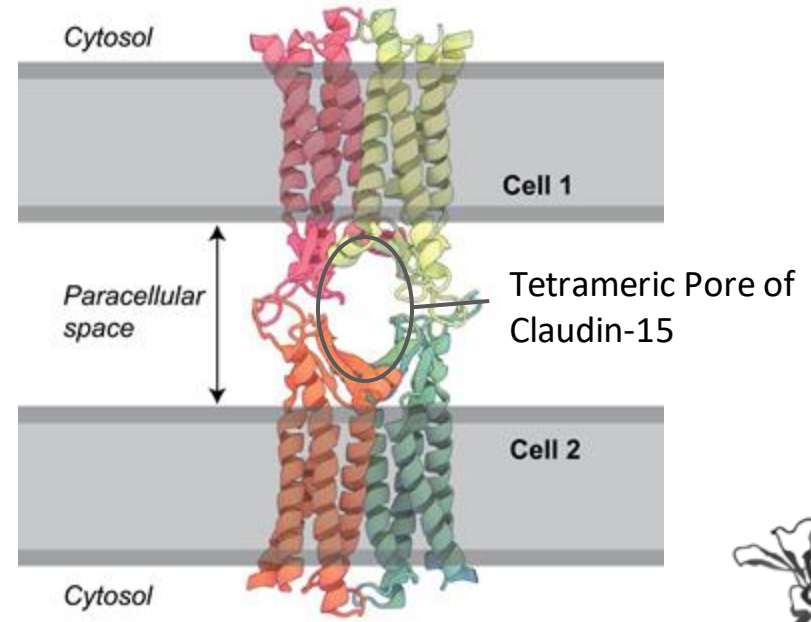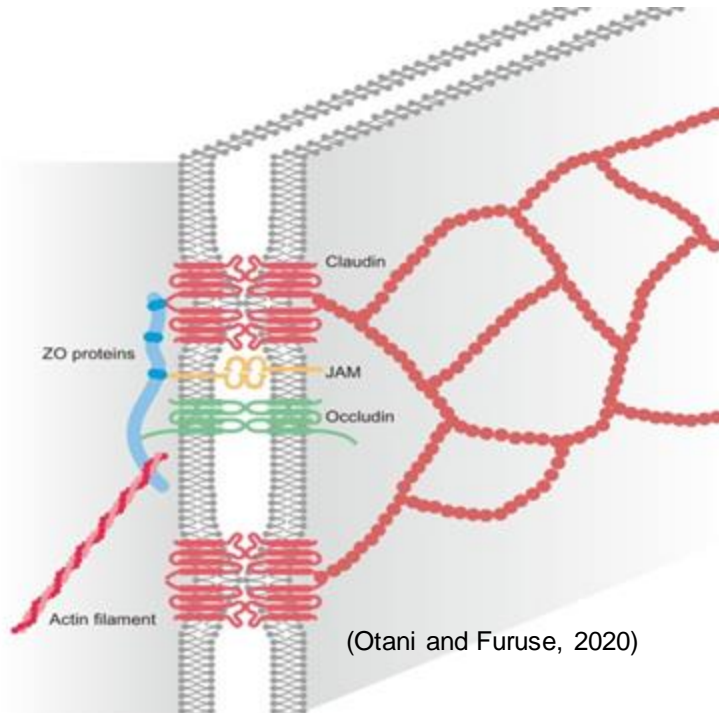
How do claudins polymerize to form strands?
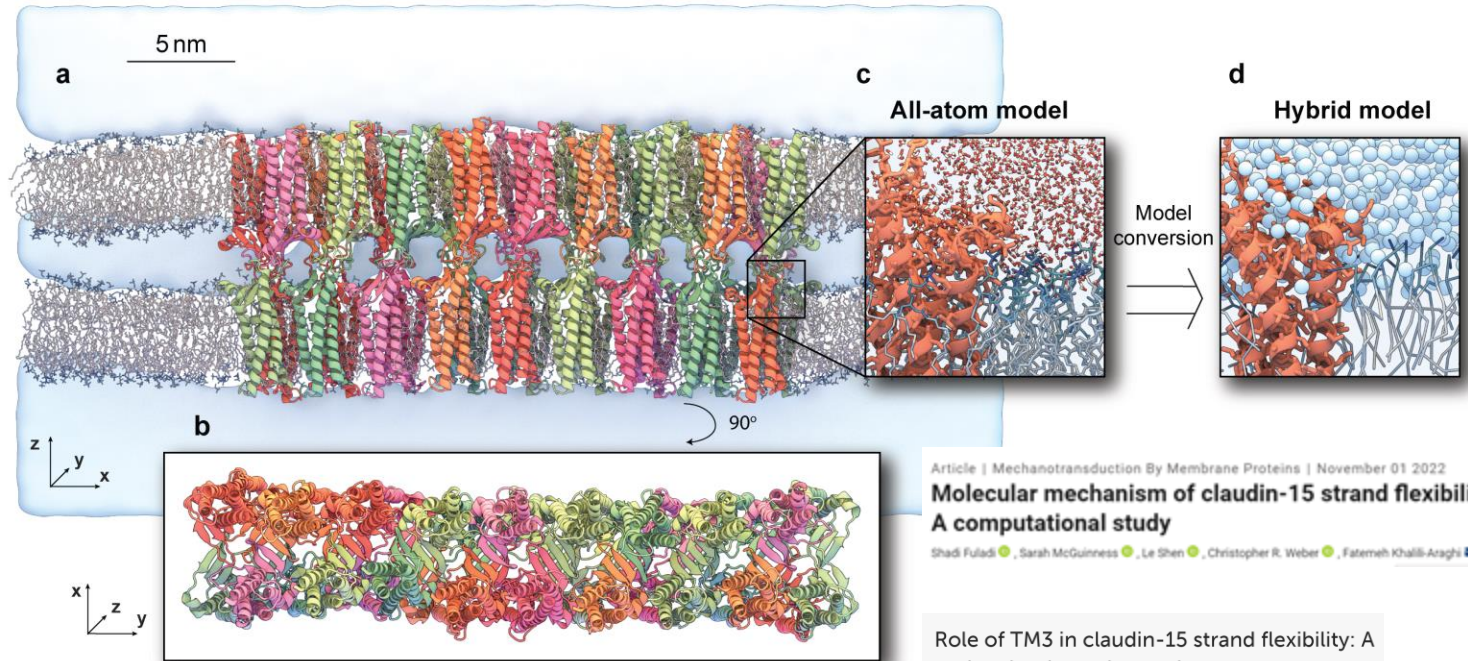
Article | Mechanotransduction By Membrane Proteins | November 01 2022
Molecular mechanism of claudin-15 strand flexibility: A computational study

Shadi Fuladi, Sarah McGuinness, Le Shen, Christopher R. Weber, Fatemeh Khalili-Araghi

Role of TM3 in claudin-15 strand flexibility: A molecular dynamics study

Shadi Fuladi[1], Sarah McGuinness[2], Fatemeh Khalili-Araghi[1*]

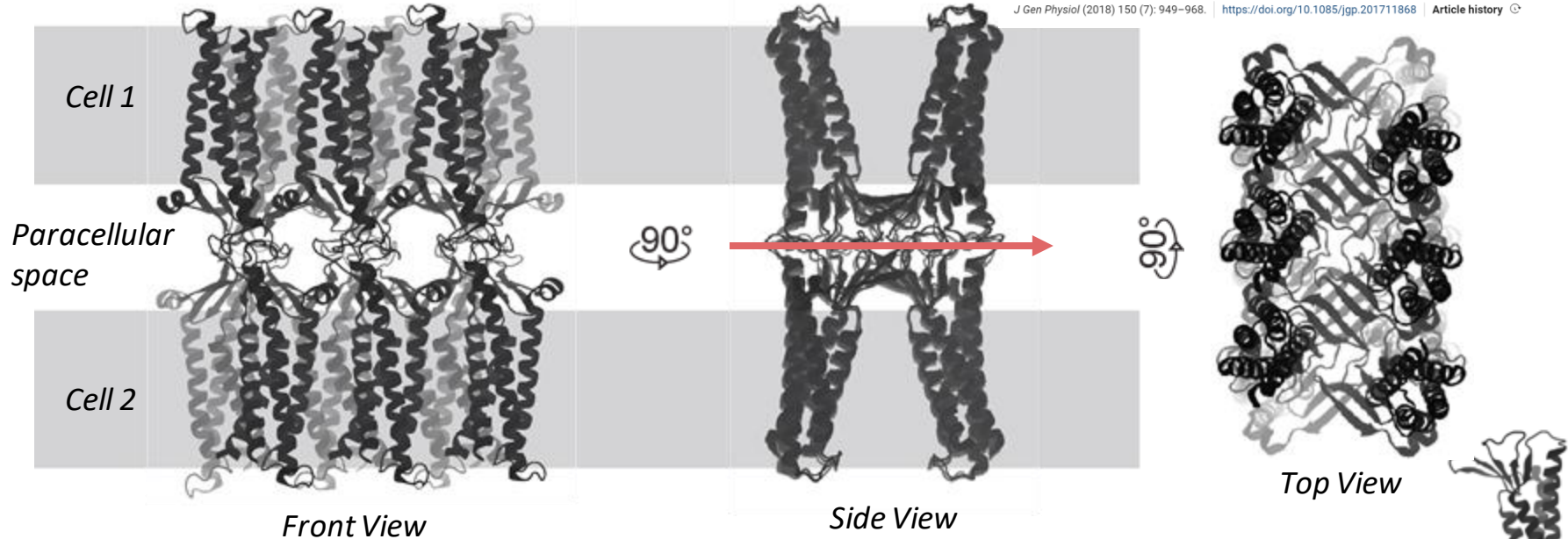# Claudin-15 is a size and charge selective paracellular channel

Molecular determination of claudin-15 organization and channel selectivity

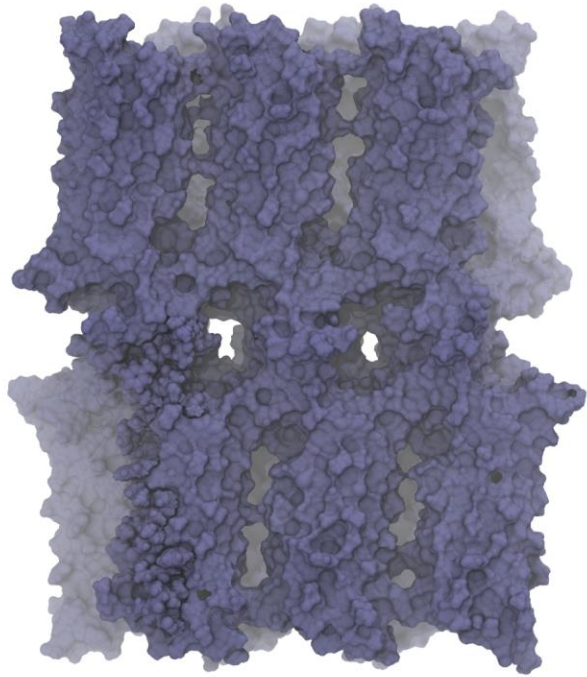Priyanka Samanta, Yitang Wang, Shadi Fuladi, Jinjing Zou, Ye Li, Le Shen, Christopher Weber, Fatemeh Khalili-Araghi

J Gen Physiol (2018) 150 (7): 949–968. https://doi.org/10.1085/jgp.201711868

Cell 1

Paracellular space

Cell 2

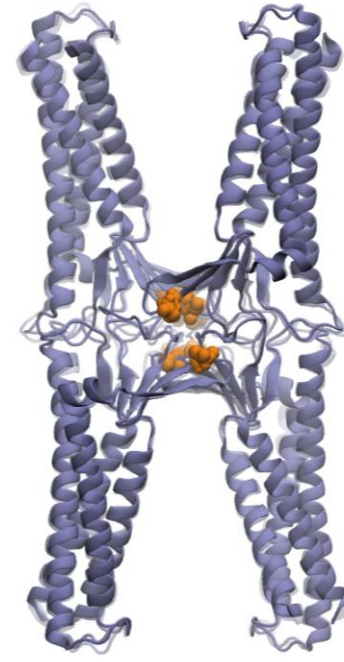*Front View*

Side View

Top View

90°

90°

Site of claudin-15 selectivity filter at D55

Front View, Surf Representation
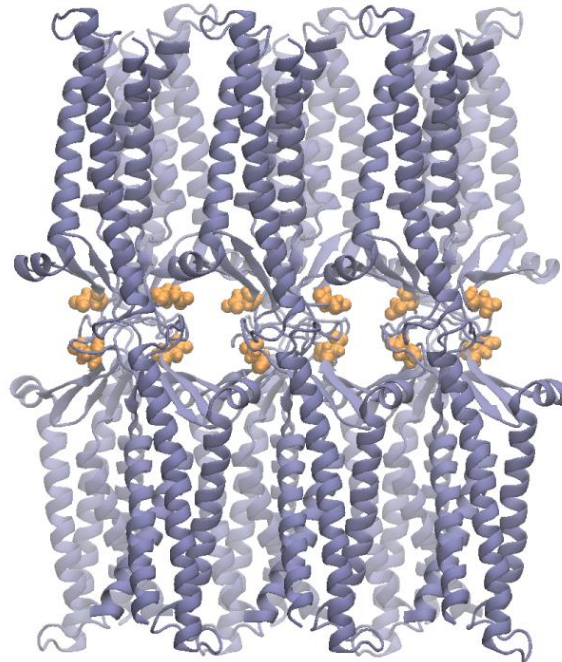
Front View, NewCartoon Representation

Side View
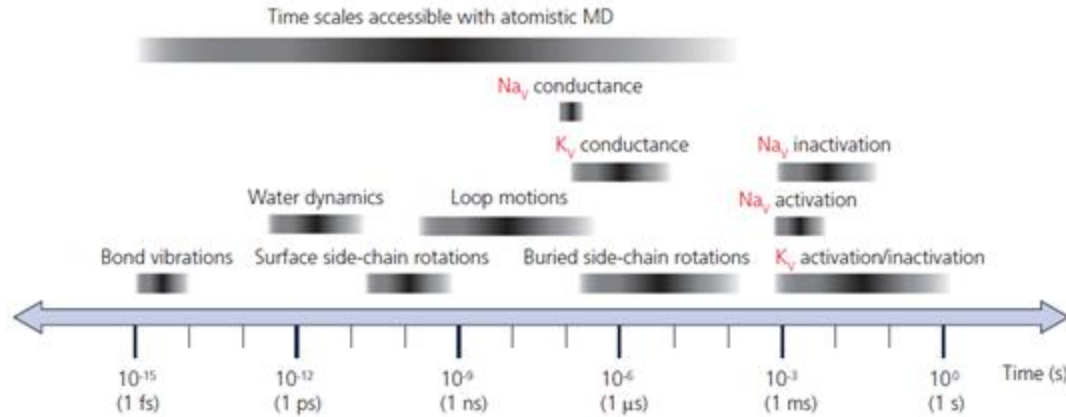
Using MD to investigate ion channels

Non-bonded Interactions

$$\sum_i \sum_{j \neq i} 4\epsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^6 \right] + \sum_i \sum_{j \neq i} \frac{q_i q_j}{\epsilon r_{ij}}$$

Bonded Interactions

$$\underbrace{\sum_{bonds} k_i^{bond} (r_i - r_0)^2}_{U_{bond}} + \underbrace{\sum_{angles} k_i^{angle} (\theta_i - \theta_0)^2}_{U_{angle}} +$$

$$\underbrace{\sum_{dihedrals} k_i^{dihe} [1 + \cos(n_i \phi_i + \delta_i)]}_{U_{dihedral}}$$
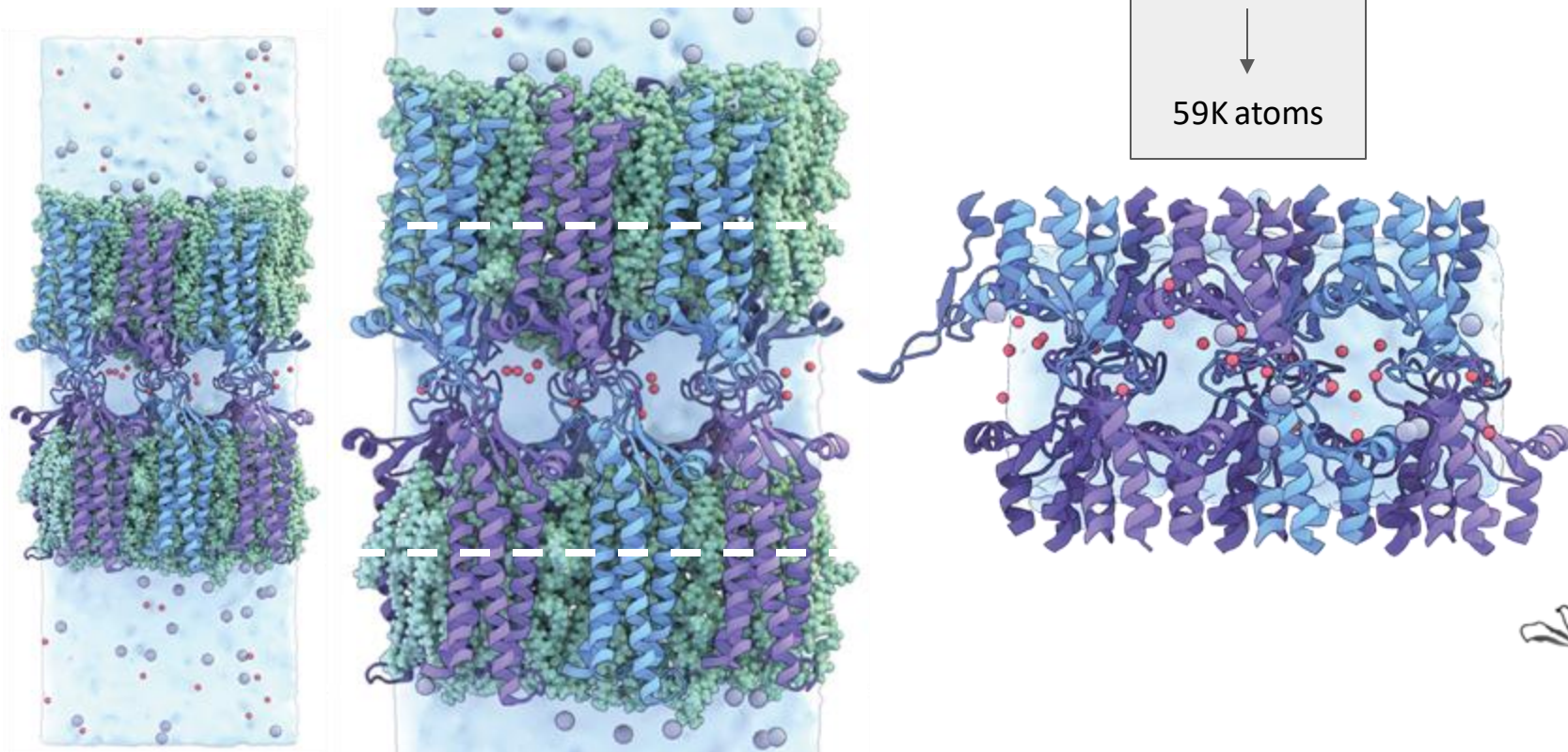
The Journal of **Physiology**

CHARMM
Chemistry at HARvard Macromolecular Mechanics
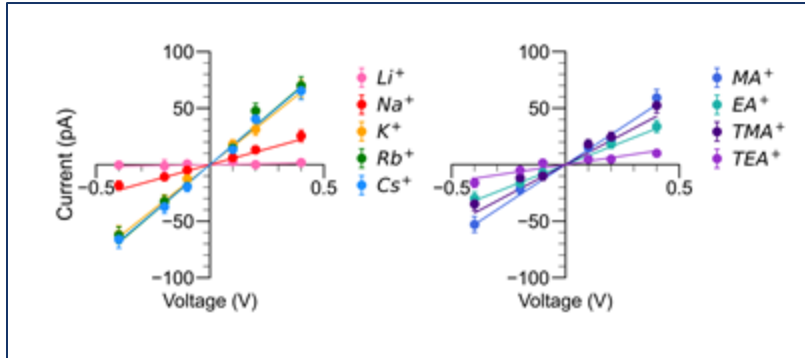
NAMD
Scalable Molecular Dynamics

Figure 3. Time scales accessible with atomistic MD simulations and those related to ion channel function (gating and permeation, estimated based on IUPHAR/BPS data; Southan *et al.* 2016) as well as protein and solvent dynamics (adapted from Lindahl, 2008; Zwier & Chong, 2010; Harvey & De Fabritiis, 2012) The fastest molecular motions in a simulated system are bond and angle vibrations (on the fs time scale) and serve as an upper limit of MD time step. A lower time limit of ion channel activation/inactivation transition at ~1 ms practically coincides with an upper time limit of atomistic MD simulations.

(DeMarco et al., 2019)

Generating the reduced model

341K atoms

↓

59K atoms

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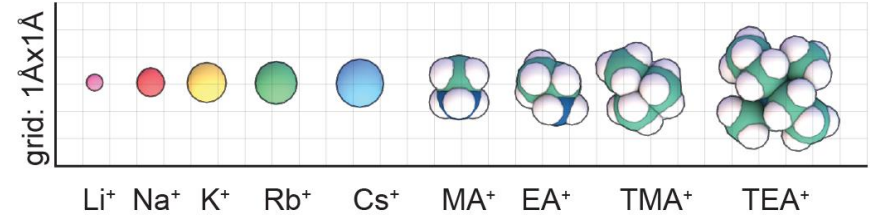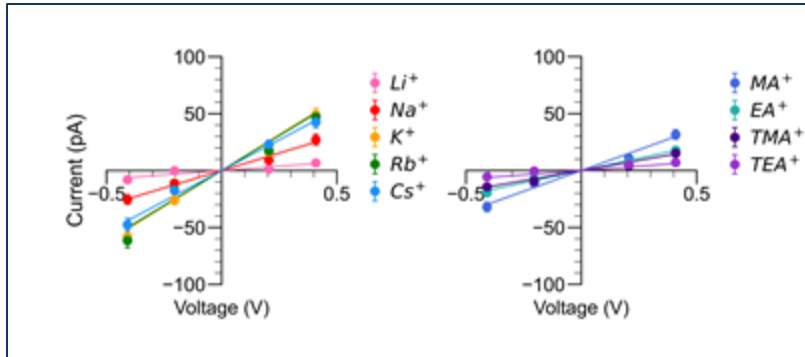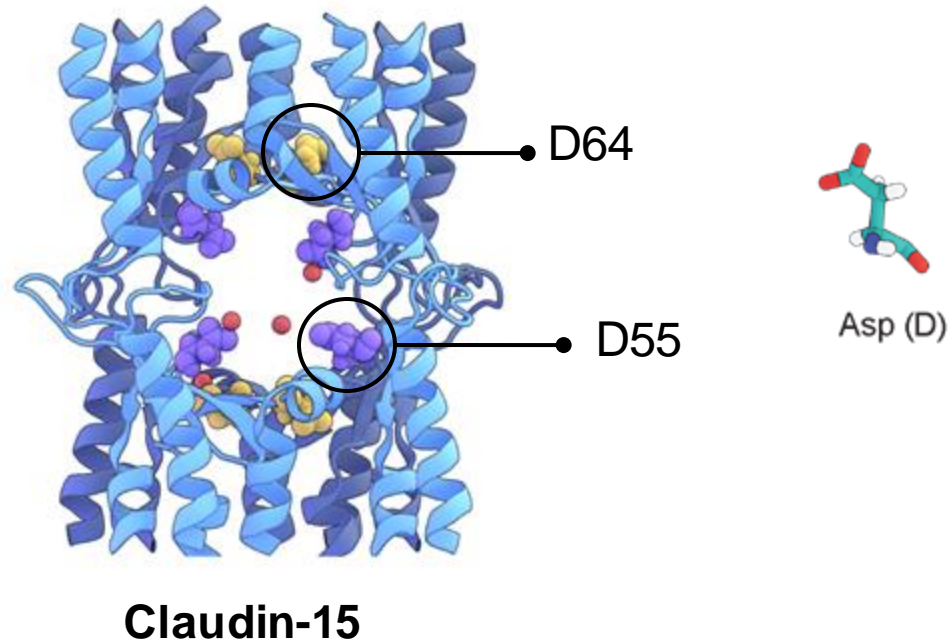# Full and reduced models demonstrate congruence

Full Model (341,000 atoms) at 213mM X$^+$
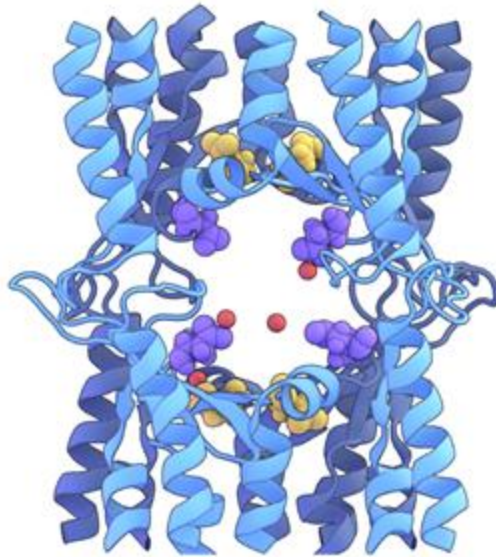

Reduced Model (59,000 atoms) at 153mM X$^+$

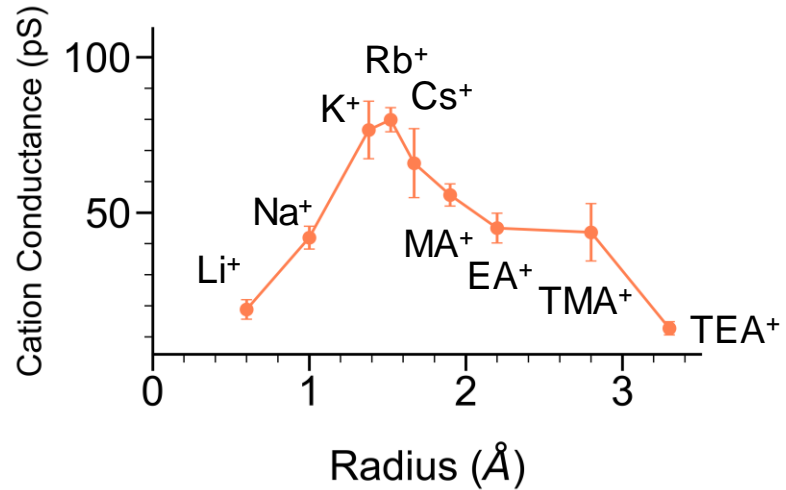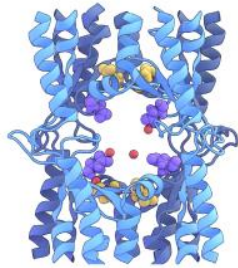# Claudin-15 selectivity filter is negatively charged tetrameric cage

Claudin-15

D64

D55

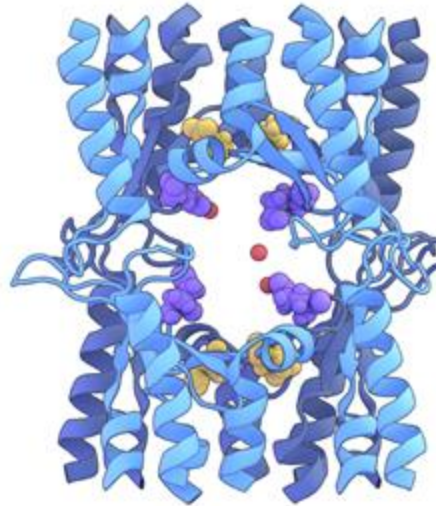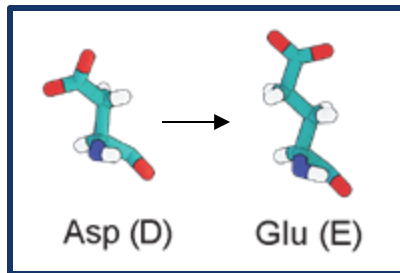Asp (D)

The selectivity profile of claudin-15

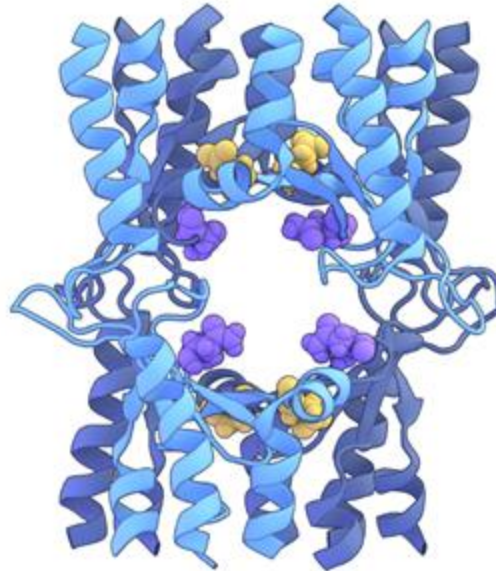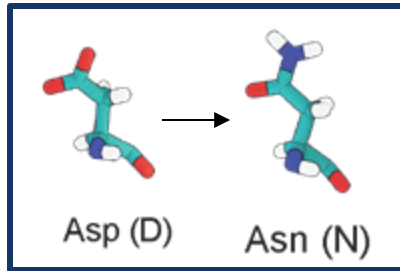**Claudin-15**

D55E mutation alters claudin-15 selectivity profile

WT

Asp (D) → Glu (E)

D55E
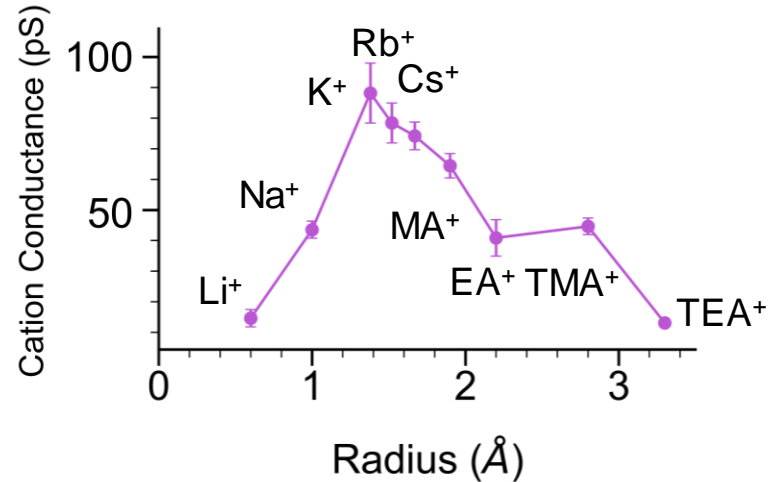
D55N mutation alters claudin-15 selectivity profile
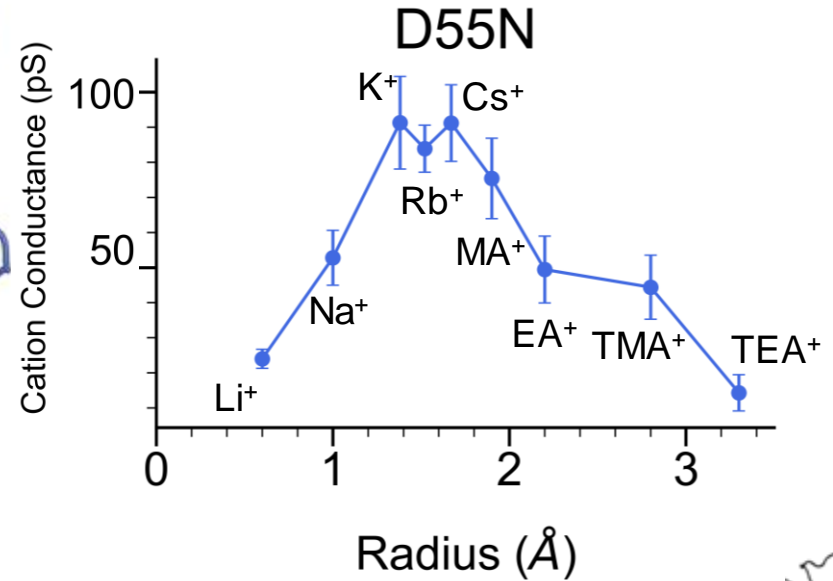
Comparing *in silico* with *in vitro* selectivity profiles

Figure 2

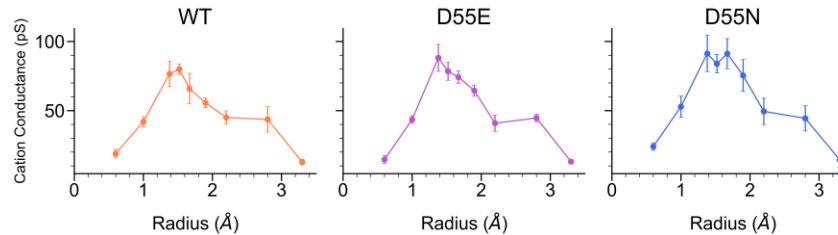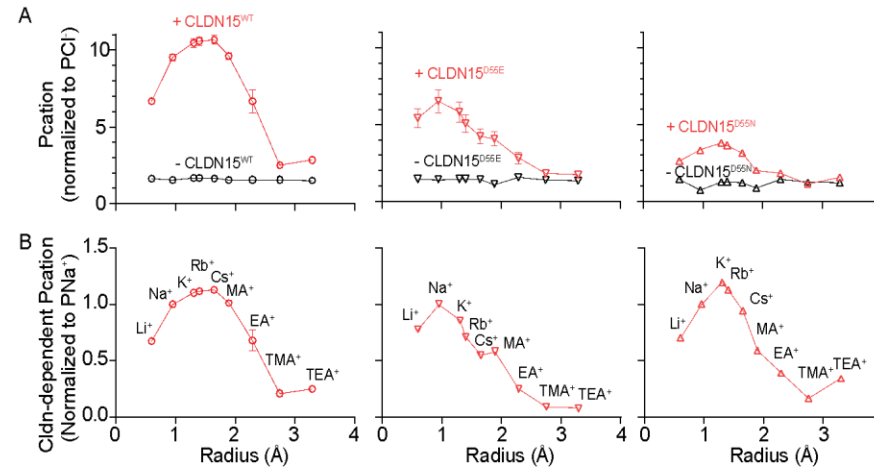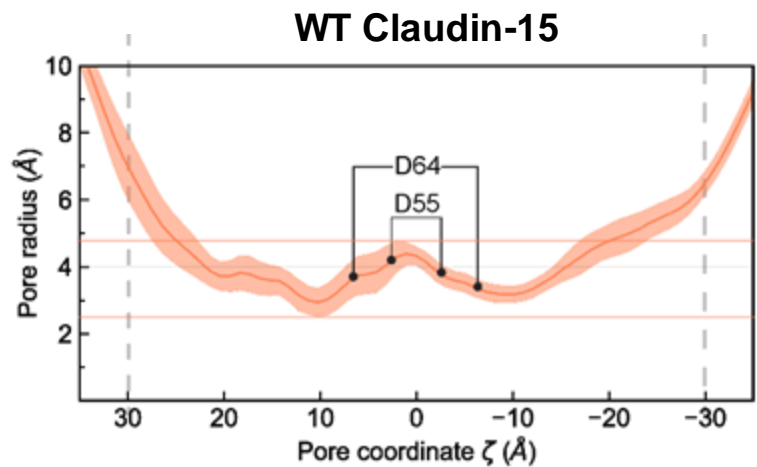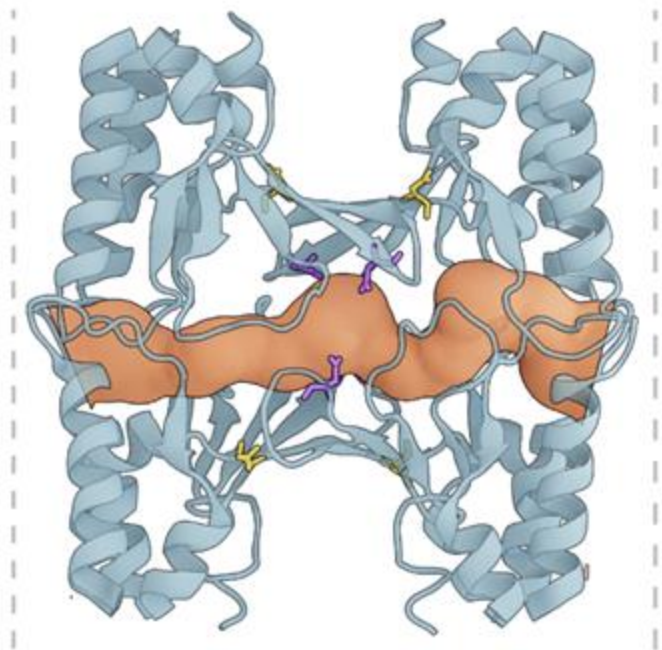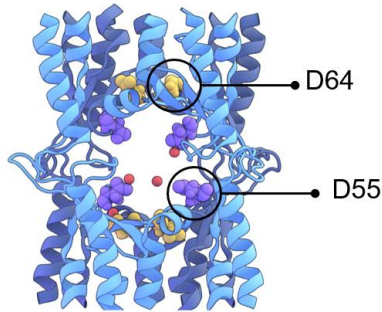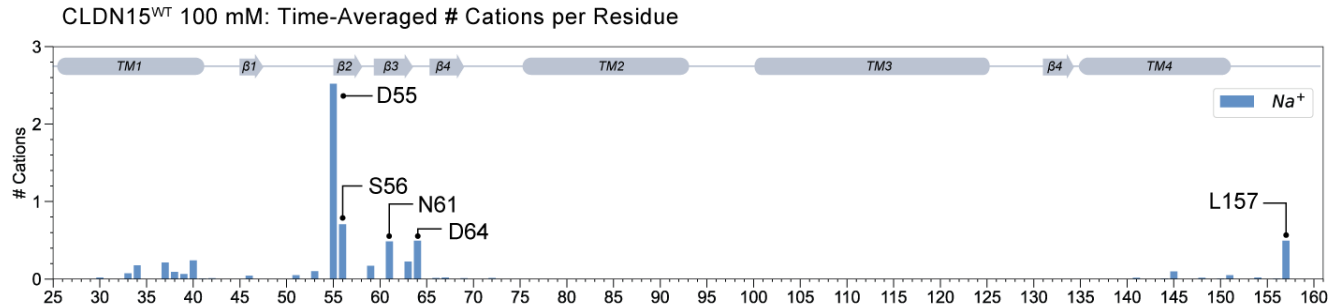# Claudin-15 pore radius calculations

WT Claudin-15

r_{avg} = 3.88 Å

Mutation to binding site reduces pore radius

WT

D55N

D55E

r_{avg} = 3.88 Å

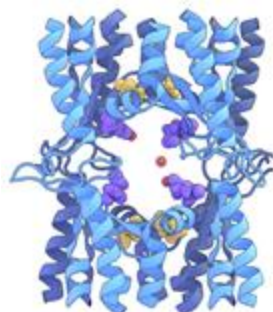r_{avg} = 3.77Å

r_{avg} = 3.51 Å
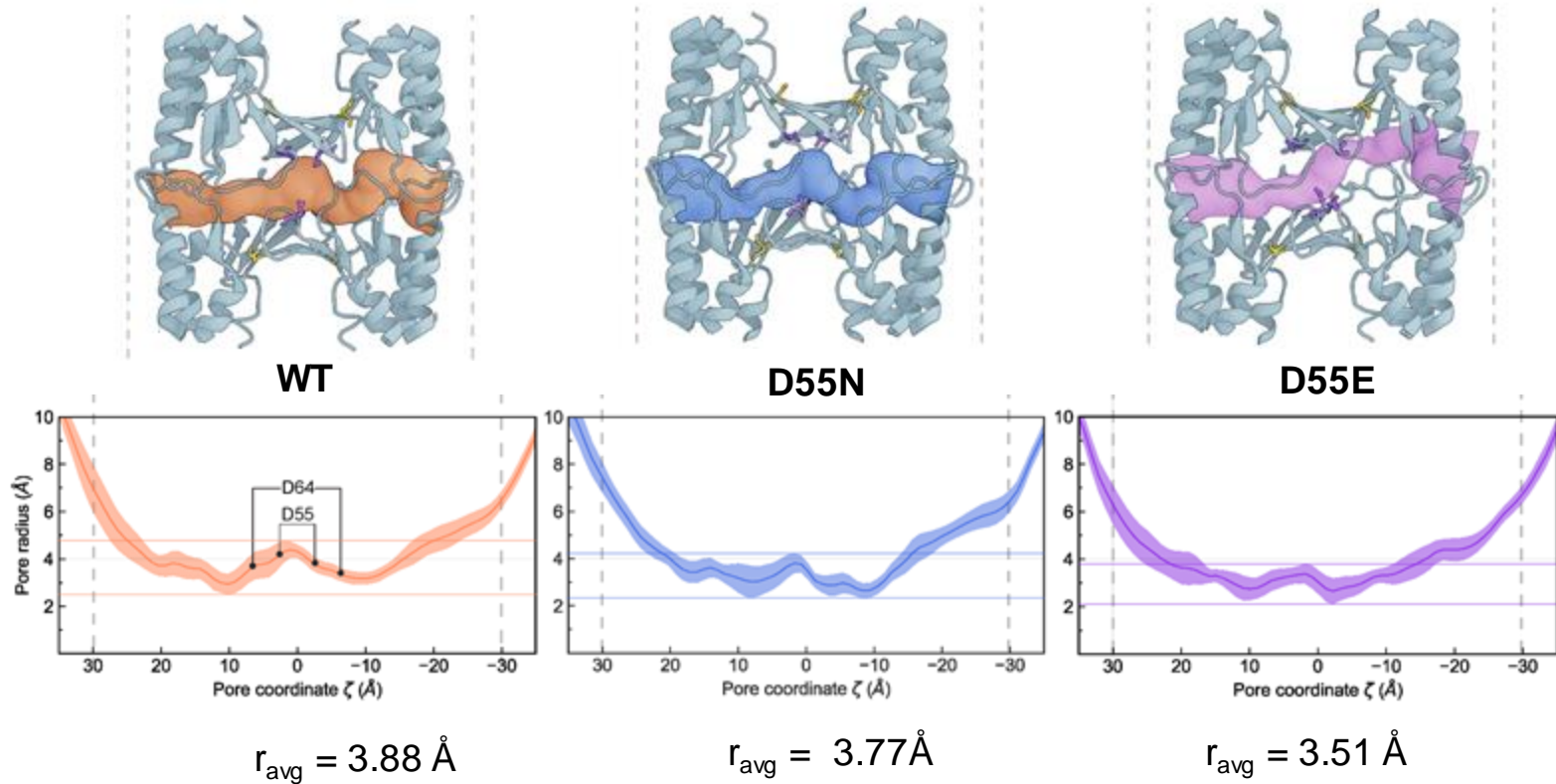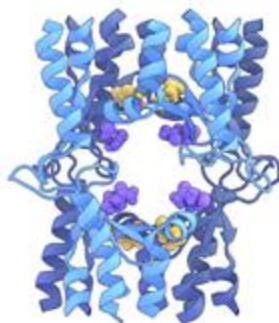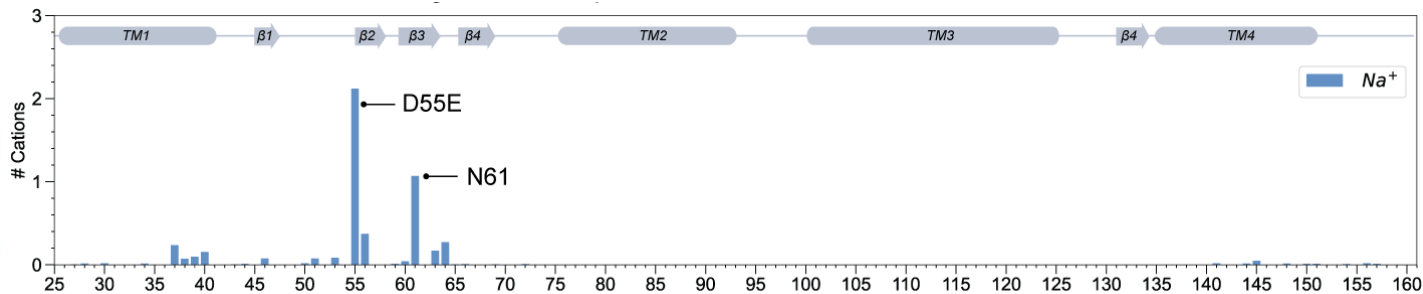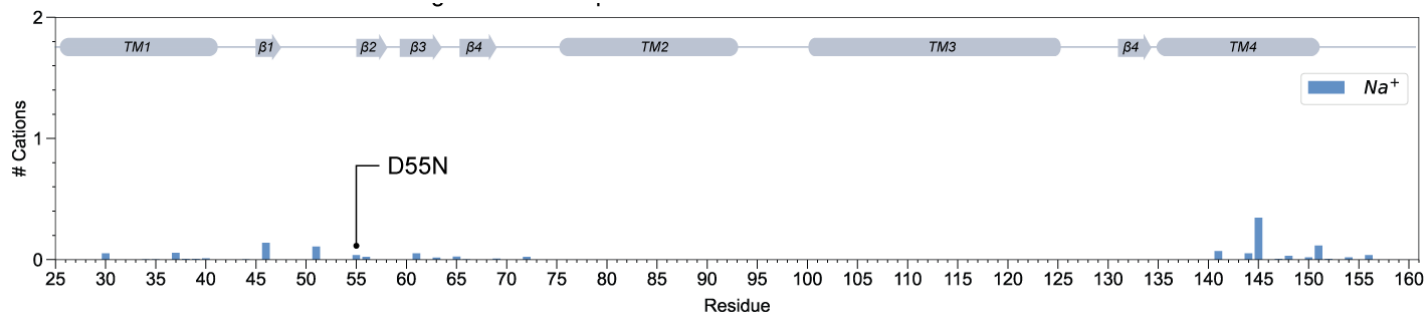
Interactions of cations with pore-lining residues

Claudin-15

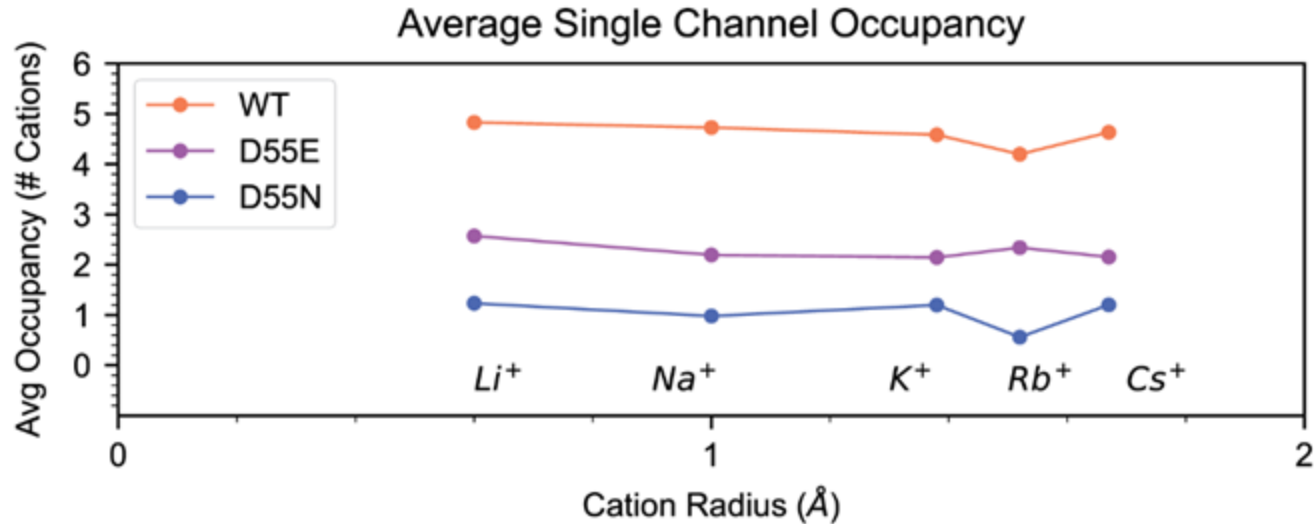# Interactions of cations with pore-lining residues of D55 mutants

D55E

D55N

Occupancy of claudin-15 channel by cations

Average Single Channel Occupancy

Using the TACC Analysis Portal (TAP) to visualize Na$^+$ interactions with the claudin-15 binding site
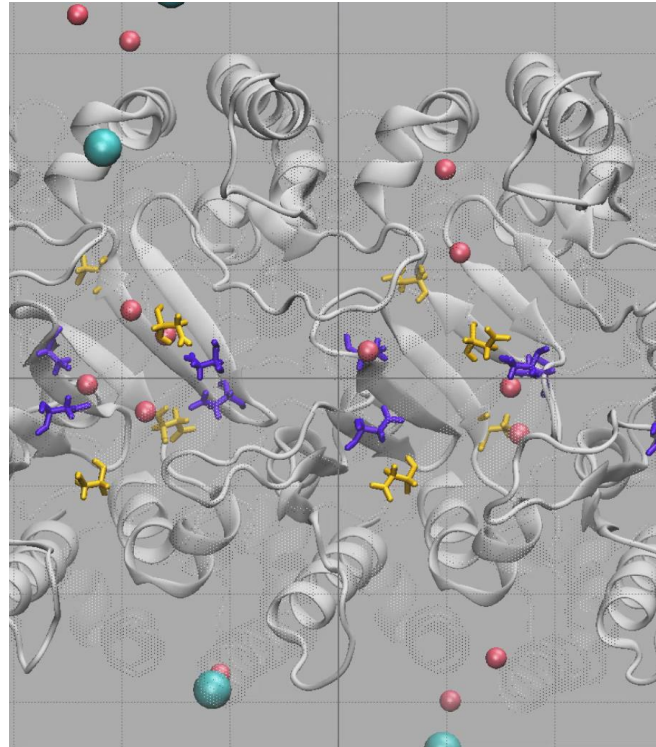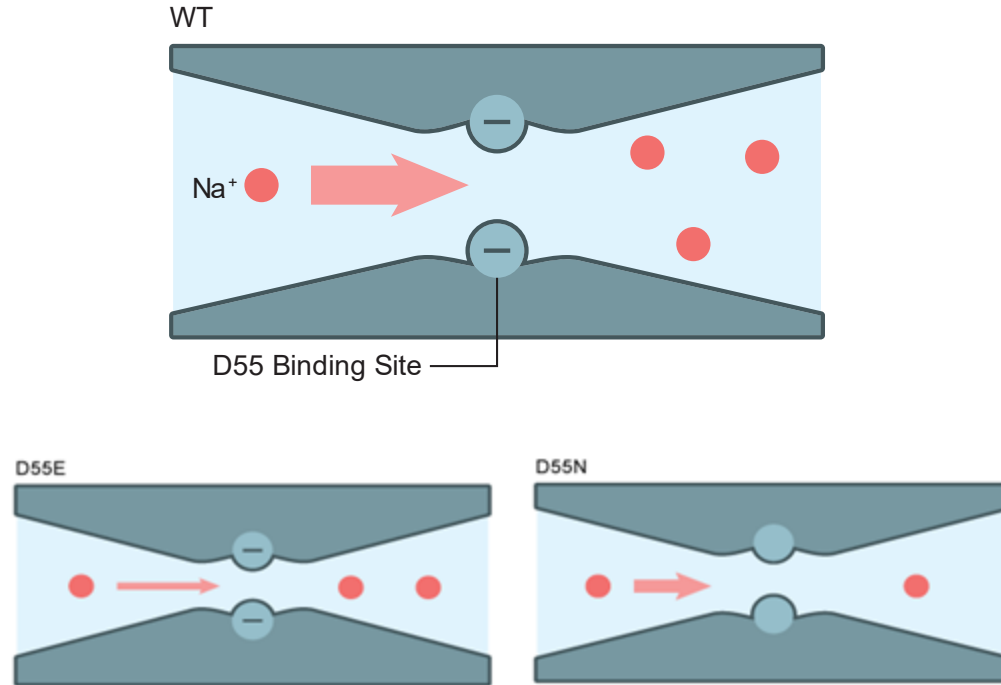

Claudin-15 (-0.4V)

Asp55
Asp64
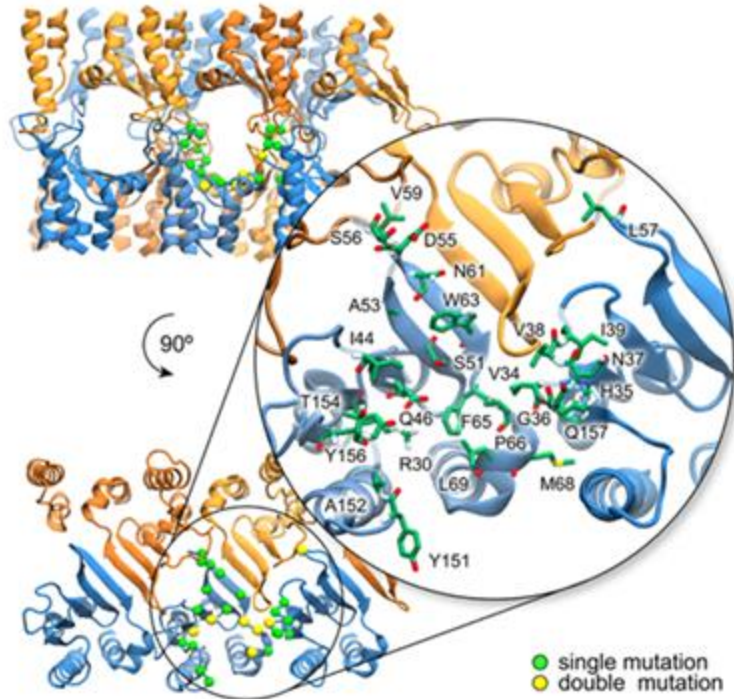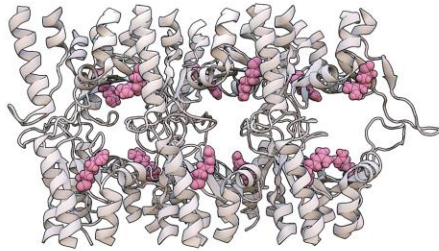
Na$^+$
Cl$^-$

0   ns

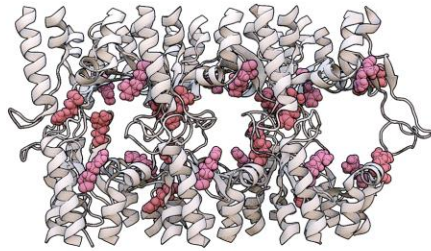# Ion diffusion is driven by charge and volume of pore
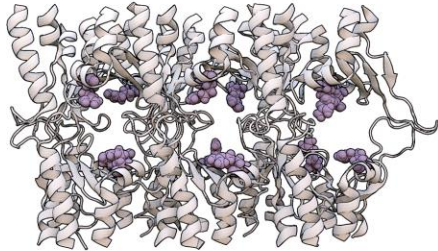
Mutagenesis of pore-lining residues

Probing pore–lining residues in parallel

E46R

E46R-L57R

L69R
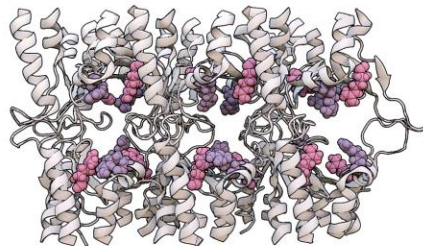
L69R-E46R

Single Mutants	Double Mutants	Triple Mutants
A53E/R	D55N-E46R	D55N-E46R-L57R
D55E/K/N	D55N-L57R	D55N-L69R-E46R
D64K	D55N-L69R	D55N-L69R-L57R
E46K/R	D55N-N61R	E46K-D55K-D64K
F65E/R	E46R-L57R	
L57R/	L69R-L57R	
L69E/R	L69R-E46R	
N61D/E/R	R30E-F65R	
R30E/N		
S51E/R		
V59E/R		
W63D/E/R		

Claudin-15 Mutants: Single Channel Conductance Normalized to Ion Concentration
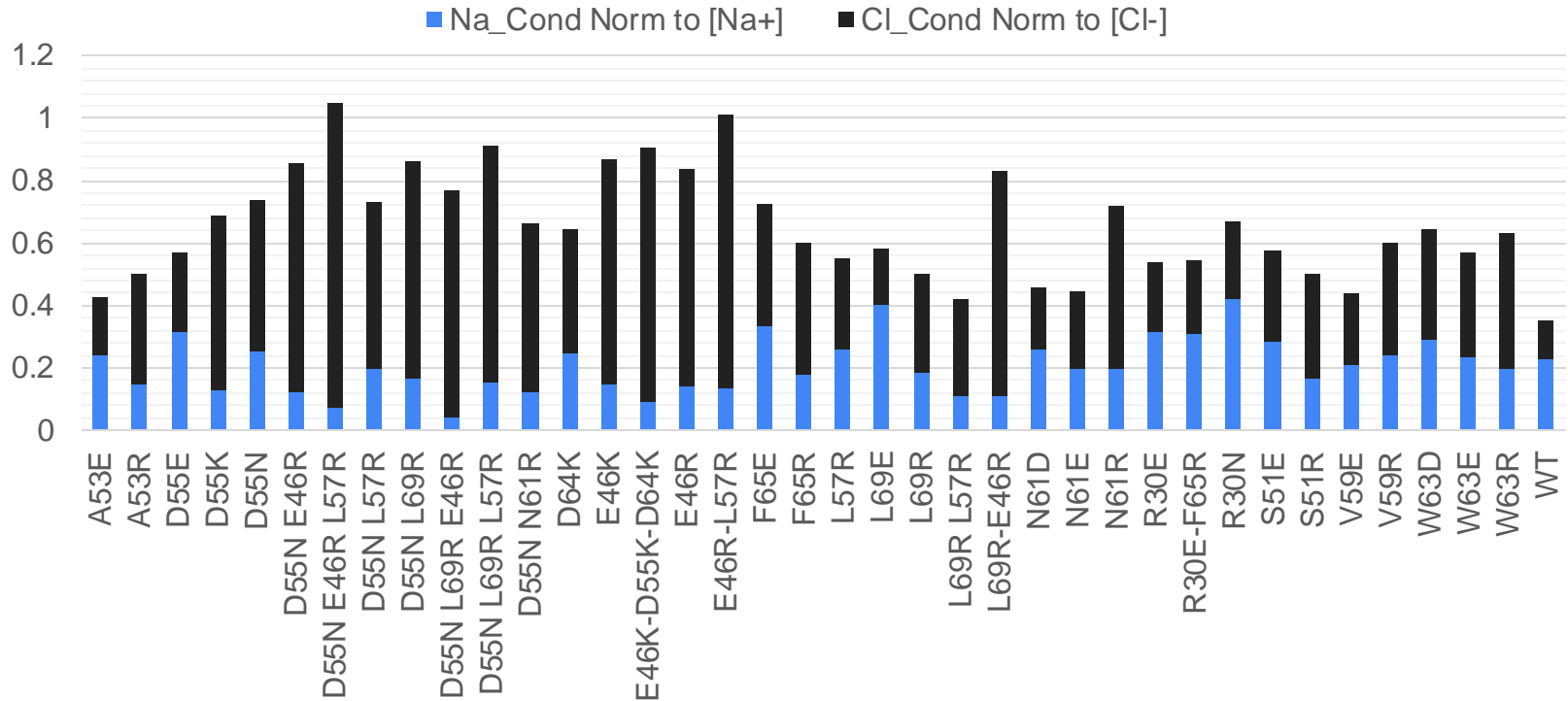
Computational and scientific visualization workflow
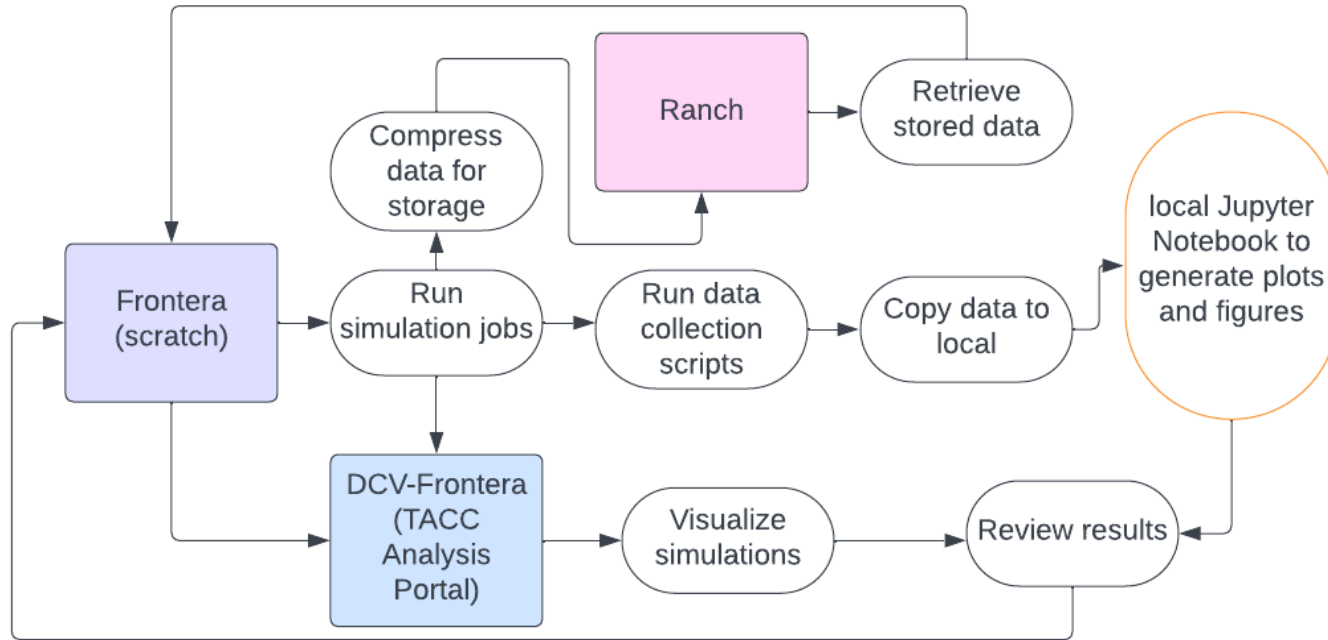
Big picture goals

- Scientific Goals

  - Characterize roles of pore-lining residues in claudin-15 ion conduction

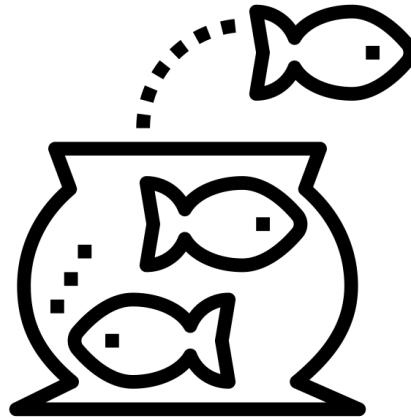 - Engineer claudin-15 channel with selectivity reversal

  - Screening for small molecule inhibitors *in silico*

- Computational Goals

  - Integrate elements of automation

  - Improve quality and efficiency of visualization workflows

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# Challenges and limitations

Created by Becris
from Noun Project

Conclusion

- We have determined the charge and selectivity of the claudin-15 pore.

- The reduced model reliably reflects experimental permeability profiles, allowing us to conduct scanning mutagenesis on claudin-15.

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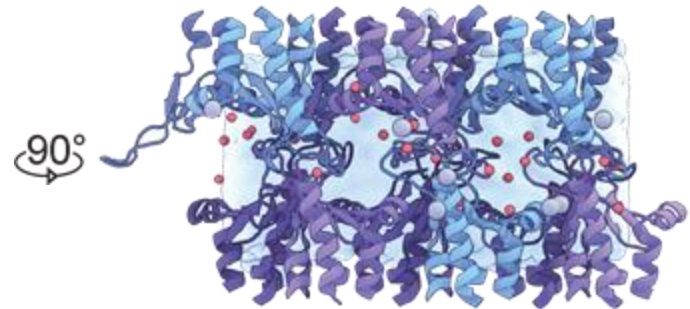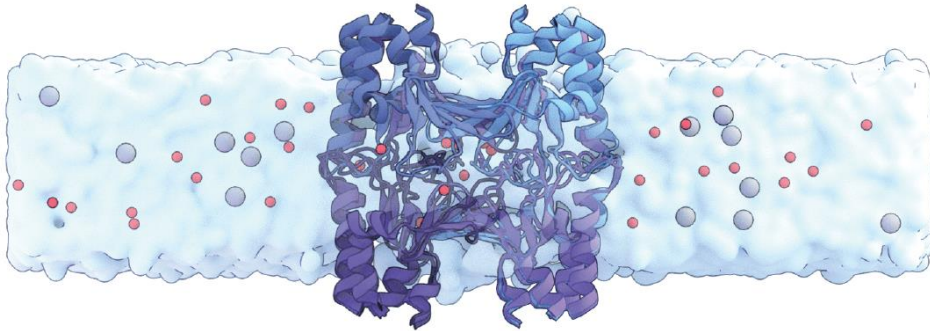
Acknowledgements

The Khalili Group (UIC)

Advisor:
Fatemeh Khalili-Araghi, PhD

Shadi Fuladi, PhD (graduated)

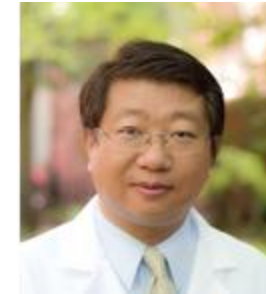Computational Biophysicist at Schrödinger

Sukanya Konar, PhD

Sarah McGuinness

Collaborators (UChicago):

Chris Weber, MD PhD

Le Shen, MD PhD