Using footprinting to probe gating events in zinc transporters

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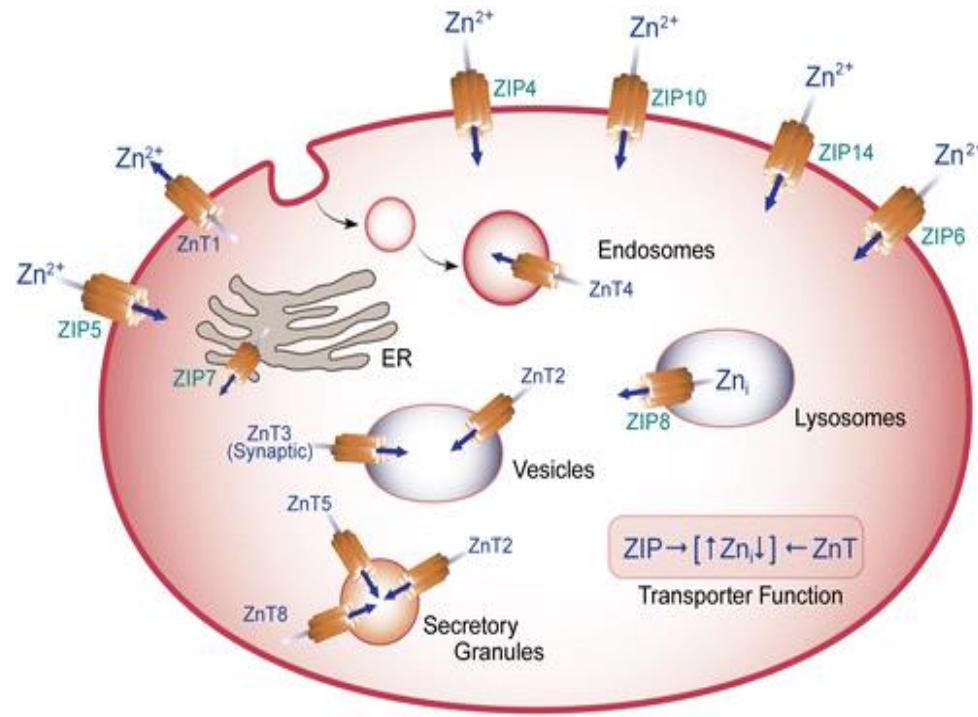
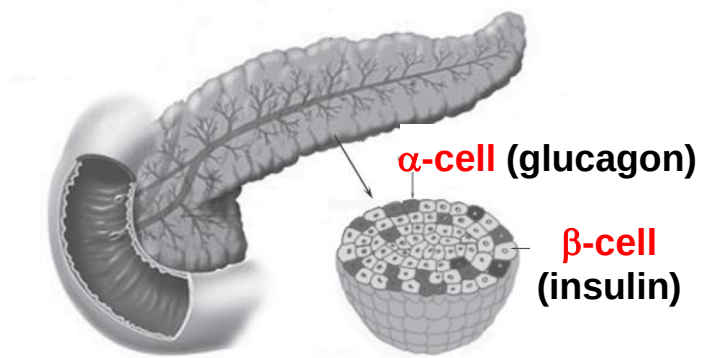
Zinc transporters and cellular zinc homeostasis

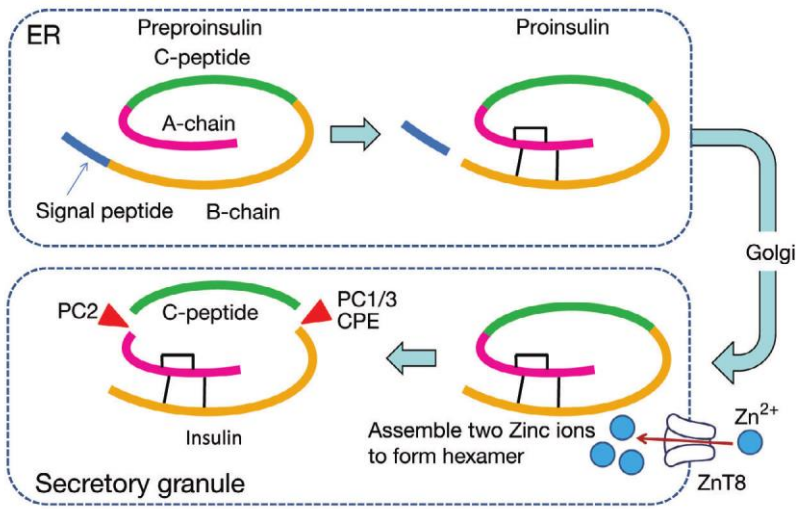
$[Zn^{2+}]_{total} \sim 0.1$ mM $[Zn^{2+}]_{cytosol} <$ pM $[Zn^{2+}]_{vesicle} > 10$ mM

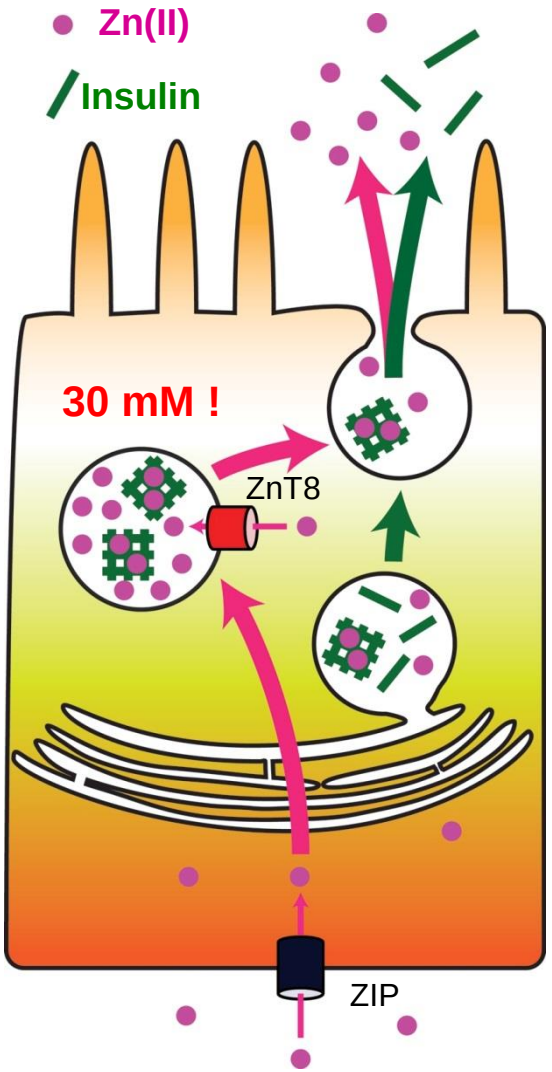


Lichten, LA and Cousins, RJ, (2009) .
Annual Review of Nutrition (29): 153-176

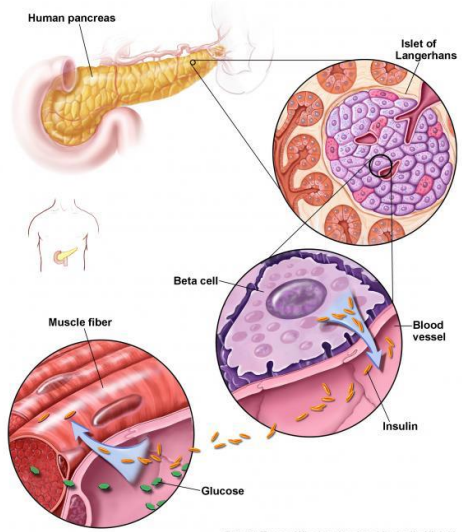
Zinc transporter ZnT8 mediates vesicular zinc enrichment in pancreatic beta-cells







Correlation between ZnT8 (SLC30A8) activity and T2D risk



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ARTICLES

A genome-wide association study identifies novel risk loci for type 2 diabetes

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Loss-of-function mutations in *SLC30A8* protect against type 2 diabetes

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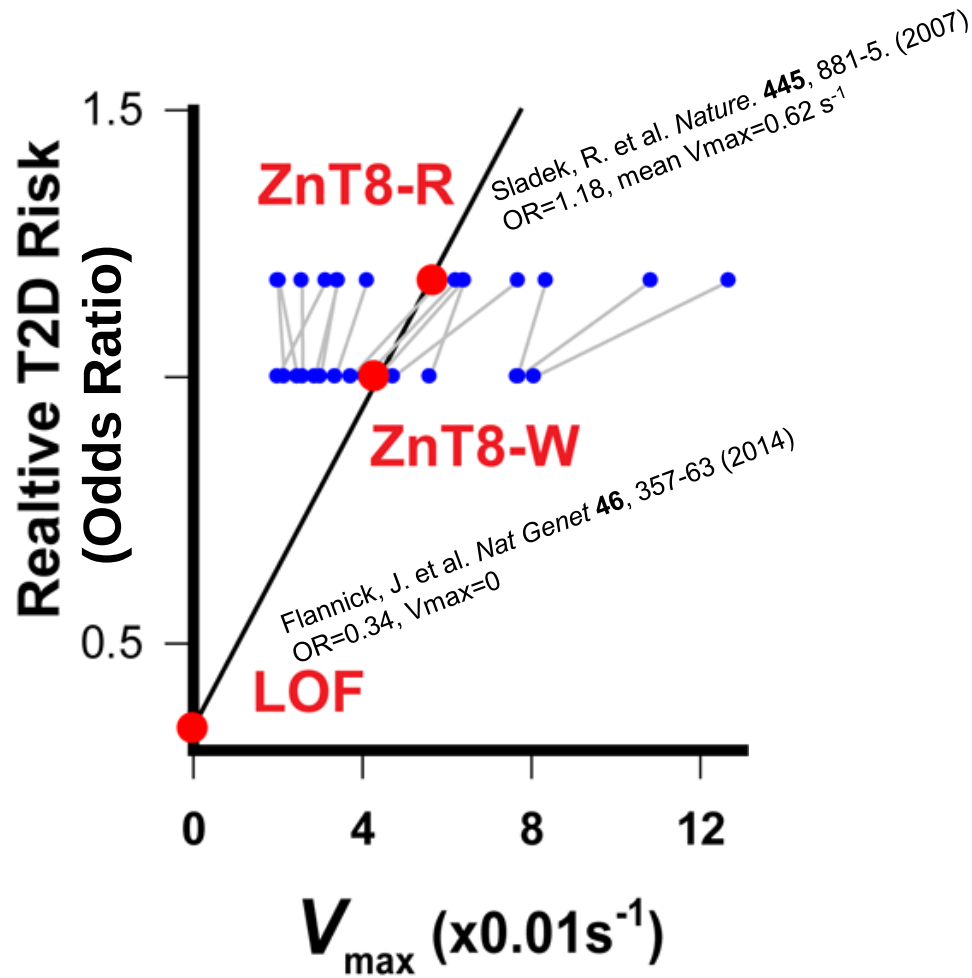
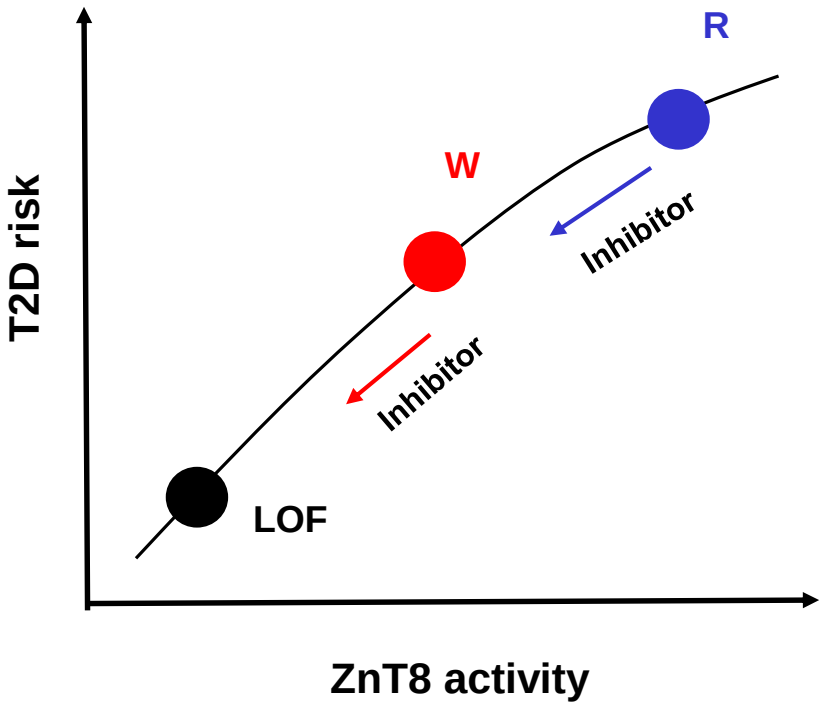
Pfizer, Massachusetts General Hospital, Broad Institute and Lund University.

R325W SNP: R-form, high risk, W-form, low risk allele



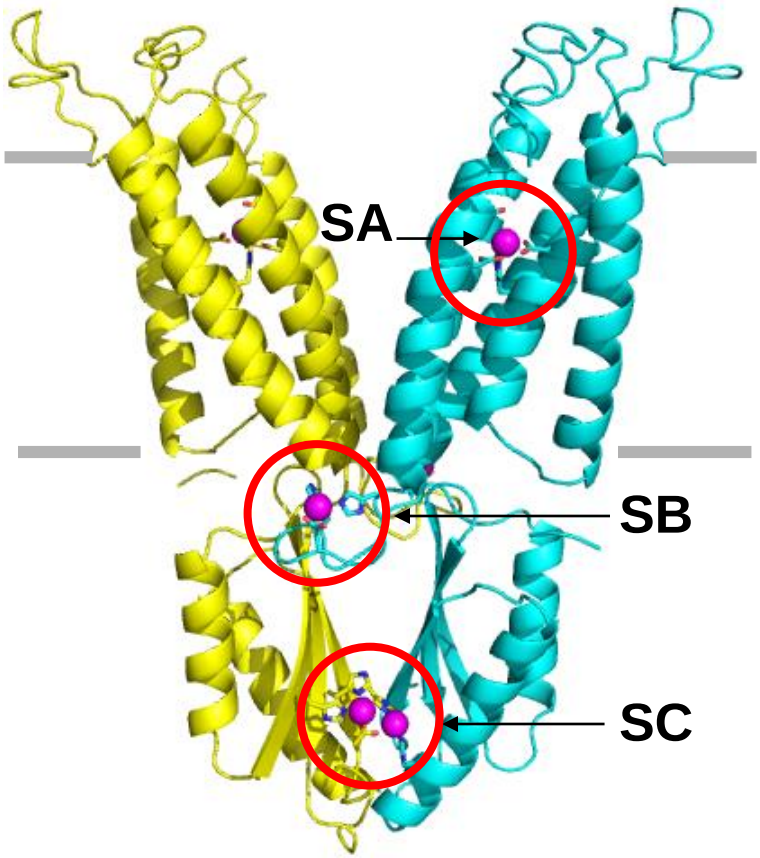
Merriman C, Huang Q, Rutter GA and D. Fu (2016) Unpublished data

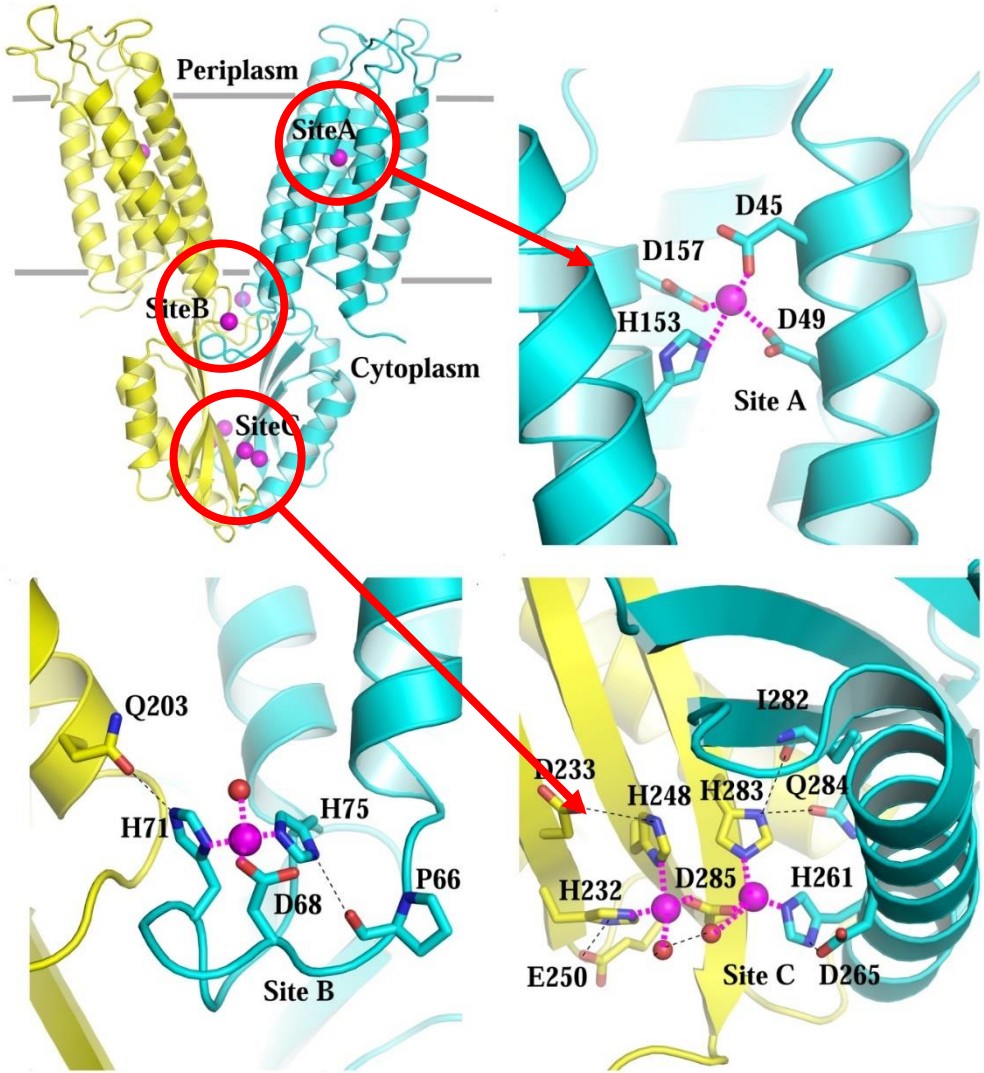
Human ZnT8 is a drug target for inhibition to reduce T2D risk in the general population



- **The transport mechanism**
- **Mechanism-based inhibitor development**

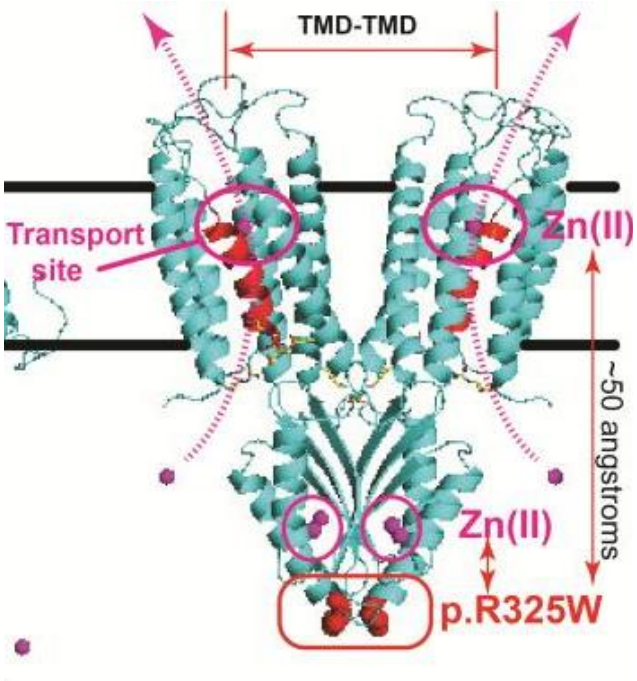
Crystal Structure of a bacterial ZnT8 homolog YiiP



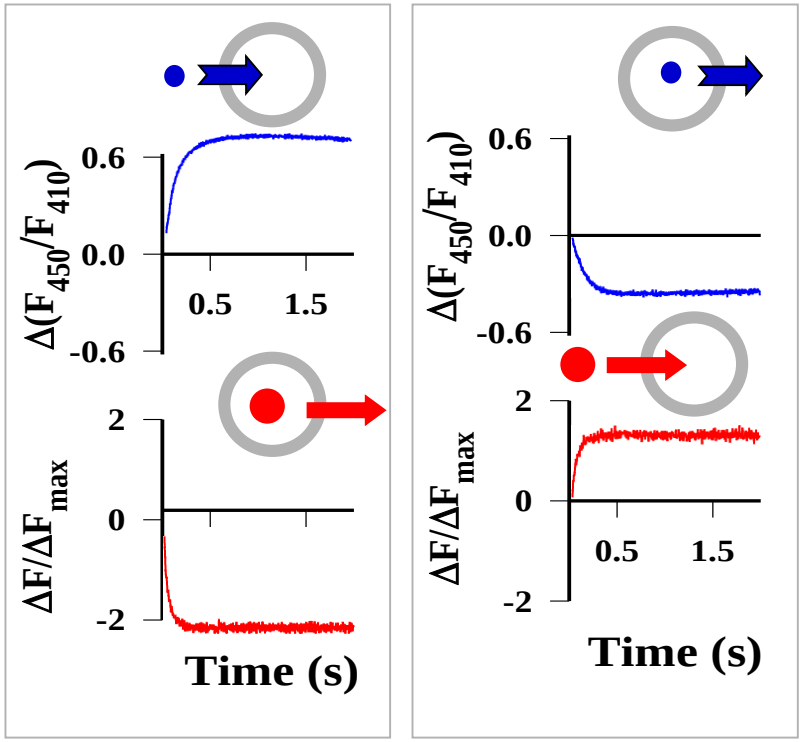


Lu, M. and Fu, D. (2007) *Science* 317: 1746
Lu M, Chai J, Fu D., (2009). *Nat Struct Mol Biol*. 16(10):1063

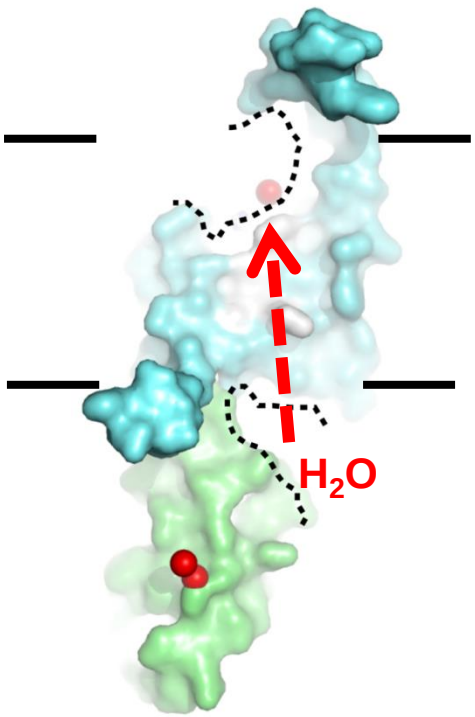
Energetics of conformational changes in YiiP



Zn^{2+}/H^{+} Antiport



No pH titratable sidechains

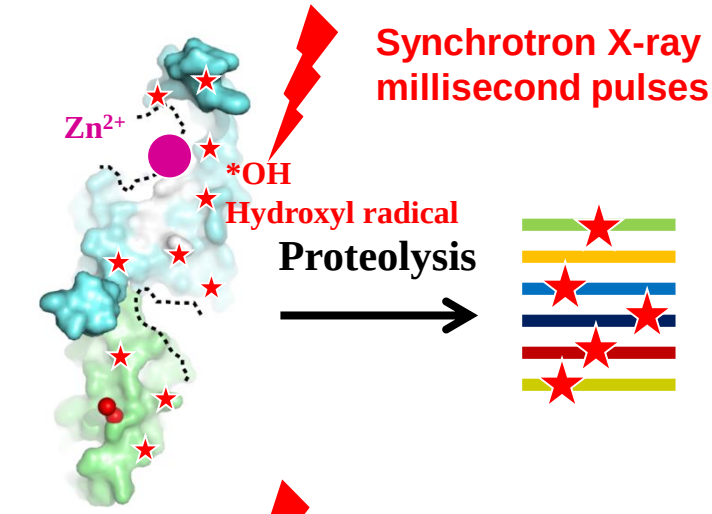


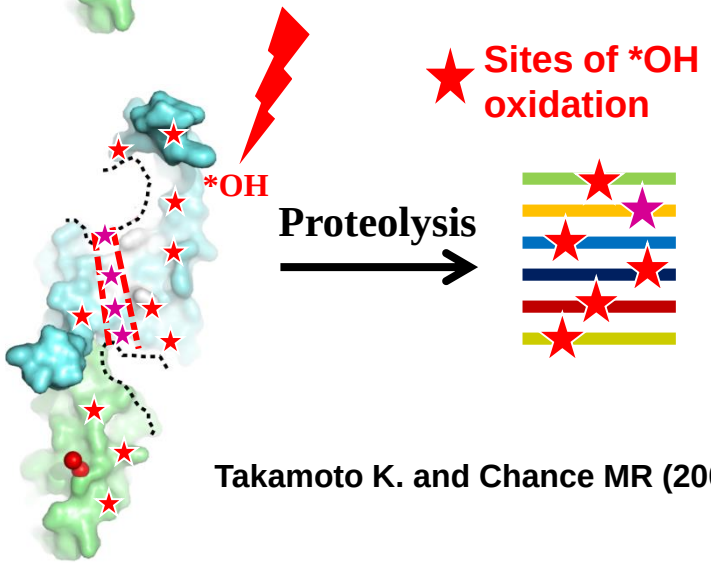
● Zn^{2+} ● H^{+}

Chao, Y., Fu, D. (2004) *JBC* 279(13): 12043

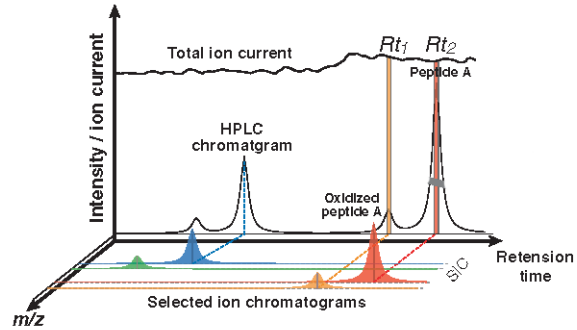
Probing the translocation pathway by water radiolysis

Irradiation → Quenching → proteolysis → LC-MS → Targeted MS/MS → Accessibility

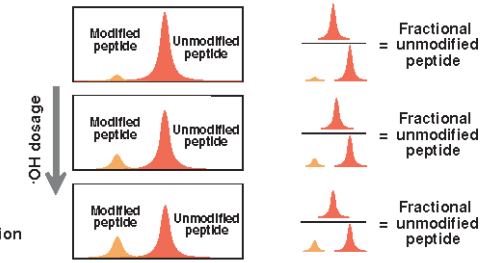




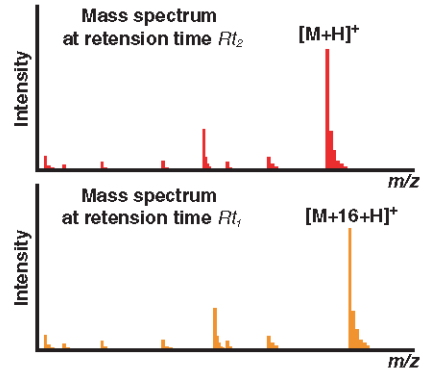
LC-MS

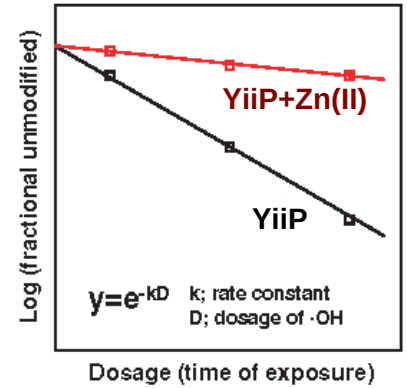


Does response plot



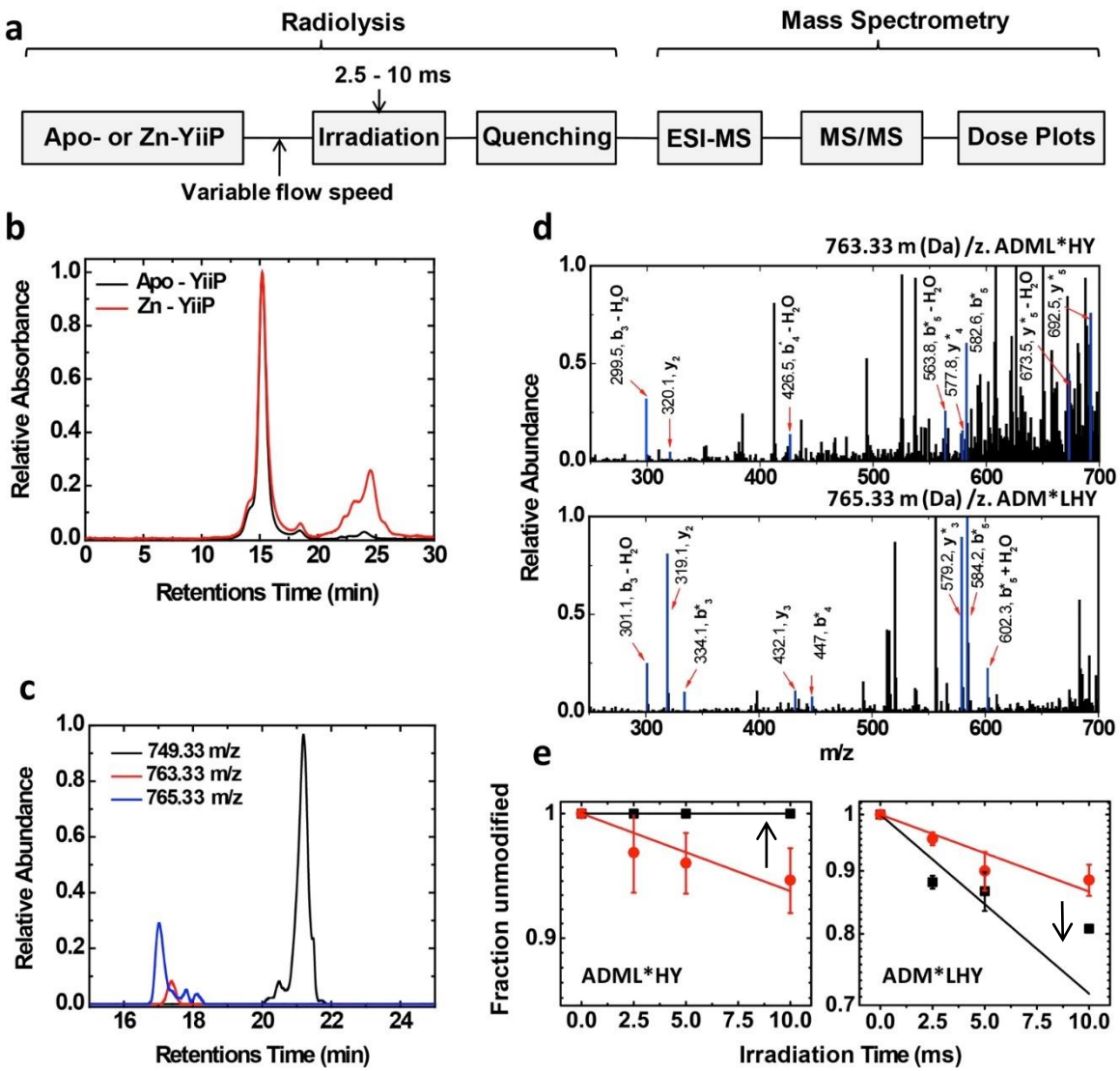
MS/MS

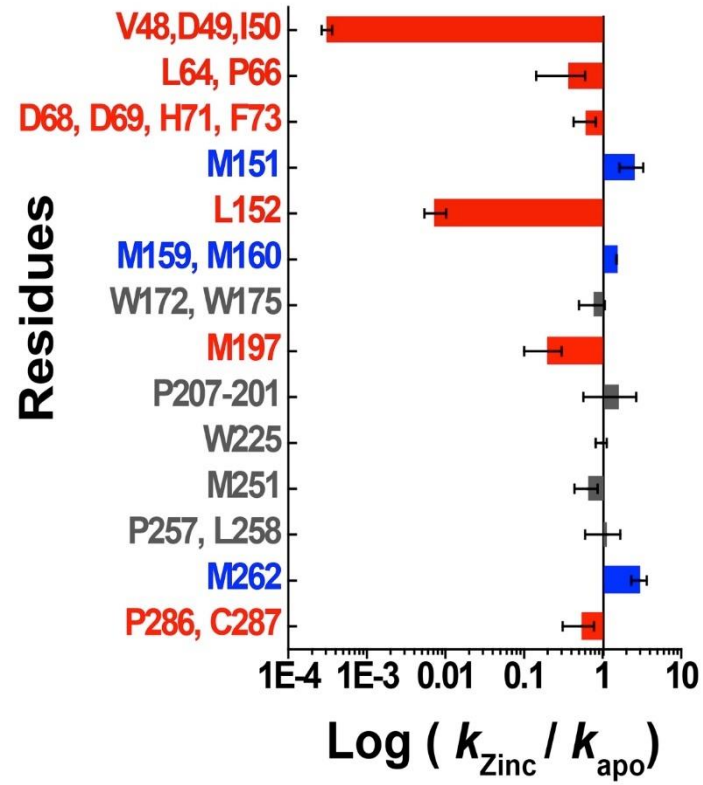




Takamoto K. and Chance MR (2006) Annual Review of Biophysics and Biomolecular Structure 35: 251-276

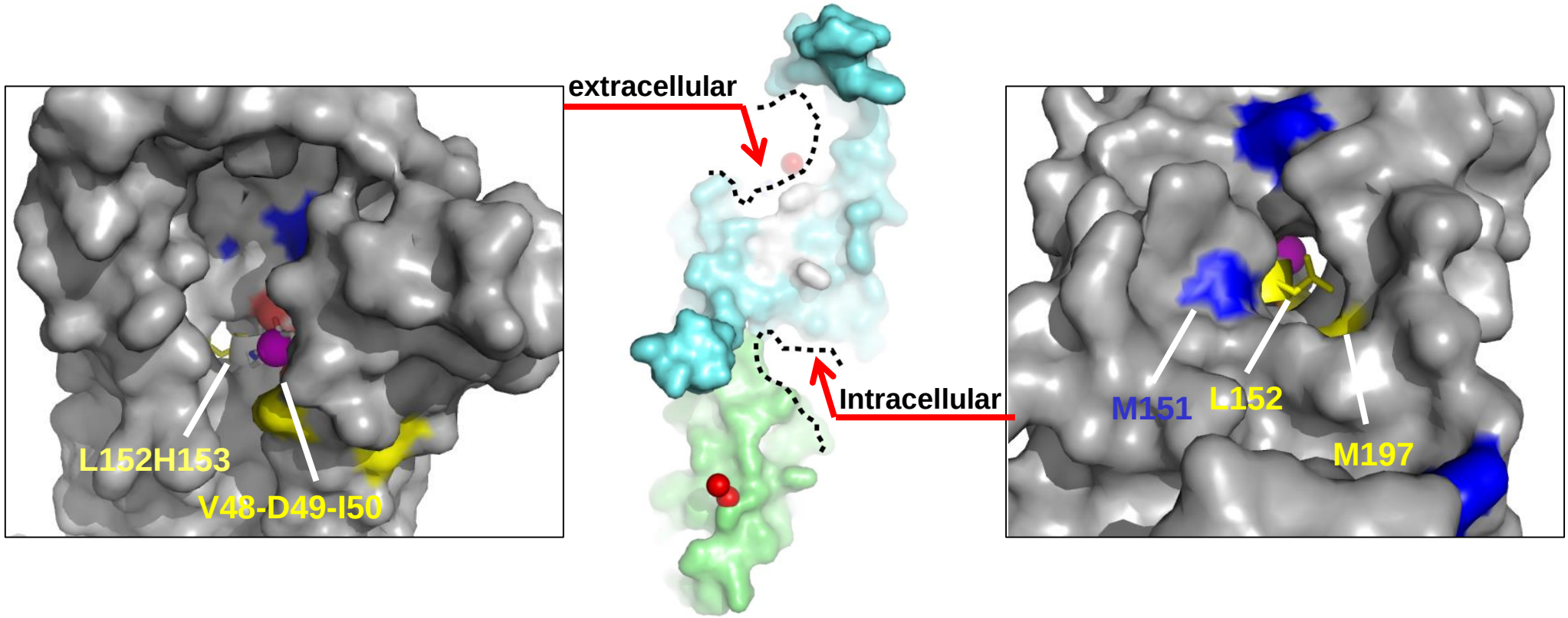
Radiolysis and bottom-up MS analysis of YiiP





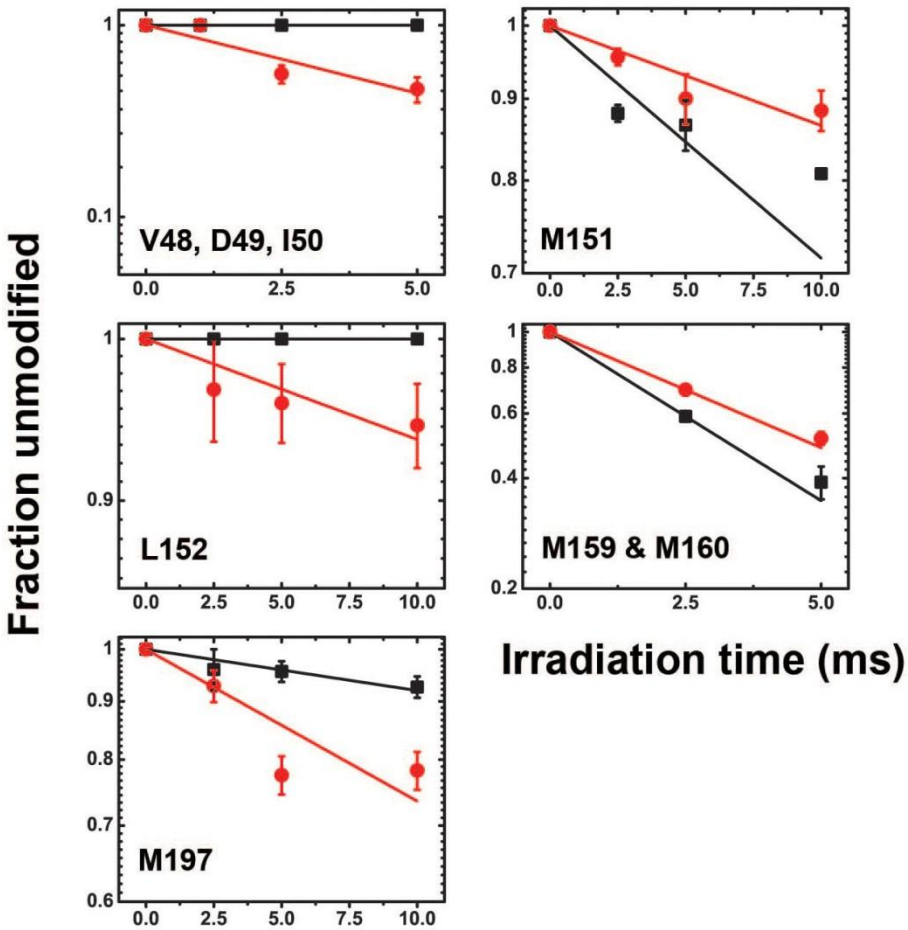
Gupta, S., Chai, J., Cheng, J., D'Mello, R., Chance, MR, Fu, D. *Nature*, 512: 101-104 (2014)

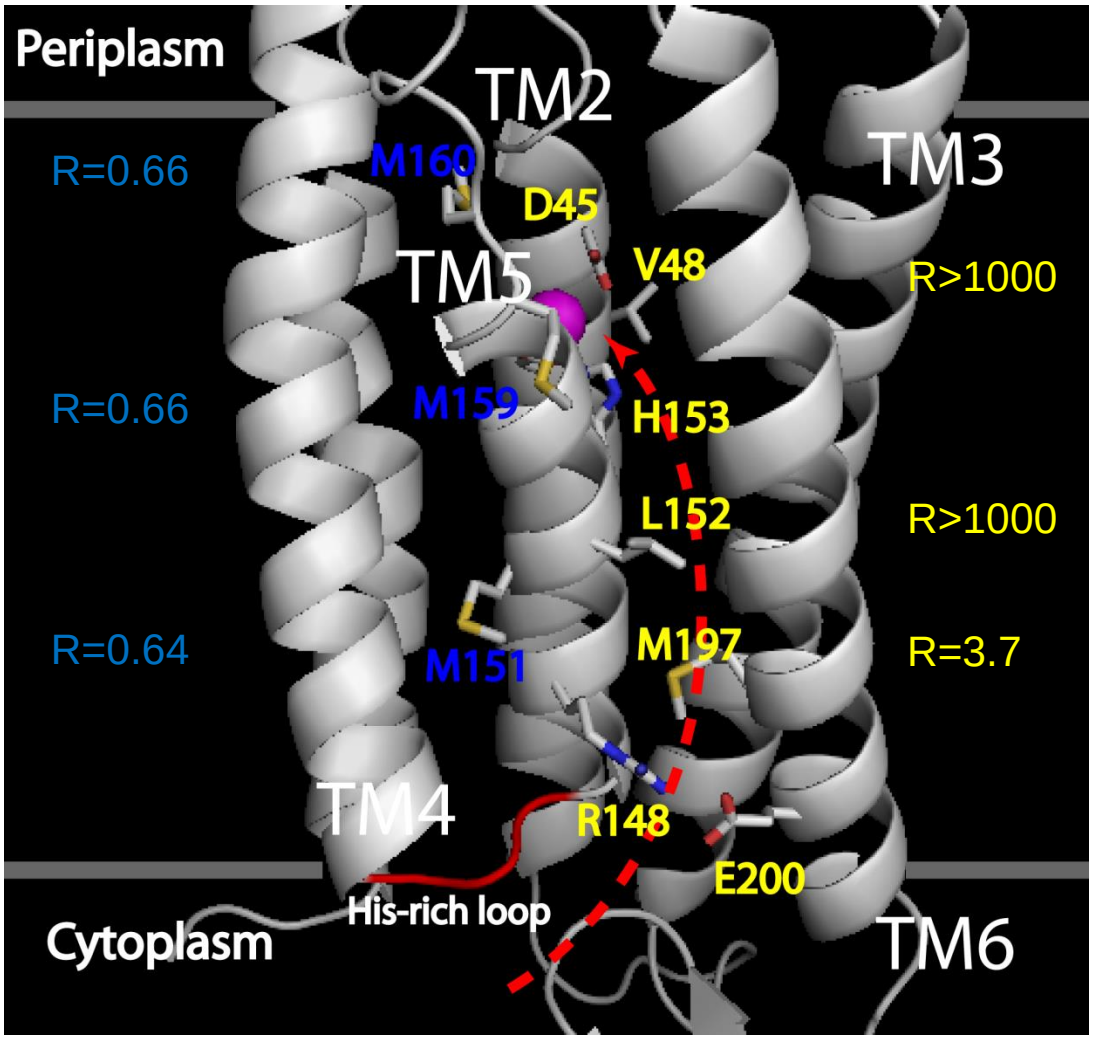
Identification of an inter-cavity gating residue



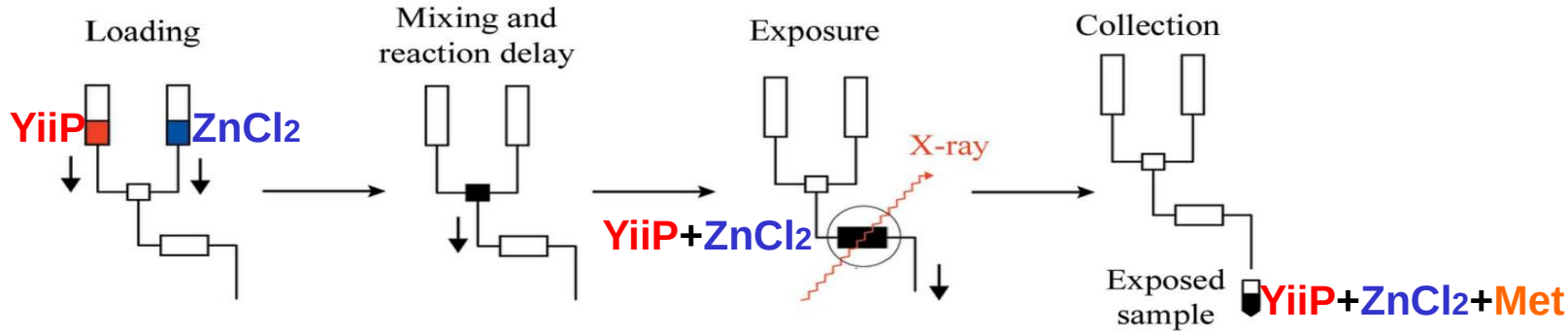
Surface representation of two solvent-filled cavities viewed along the arrows as indicated. L152 and H153 are removed from surface drawing to reveal an opening between the two cavities. D45 is also removed to reveal the bound zinc ion in the extracellular cavity.

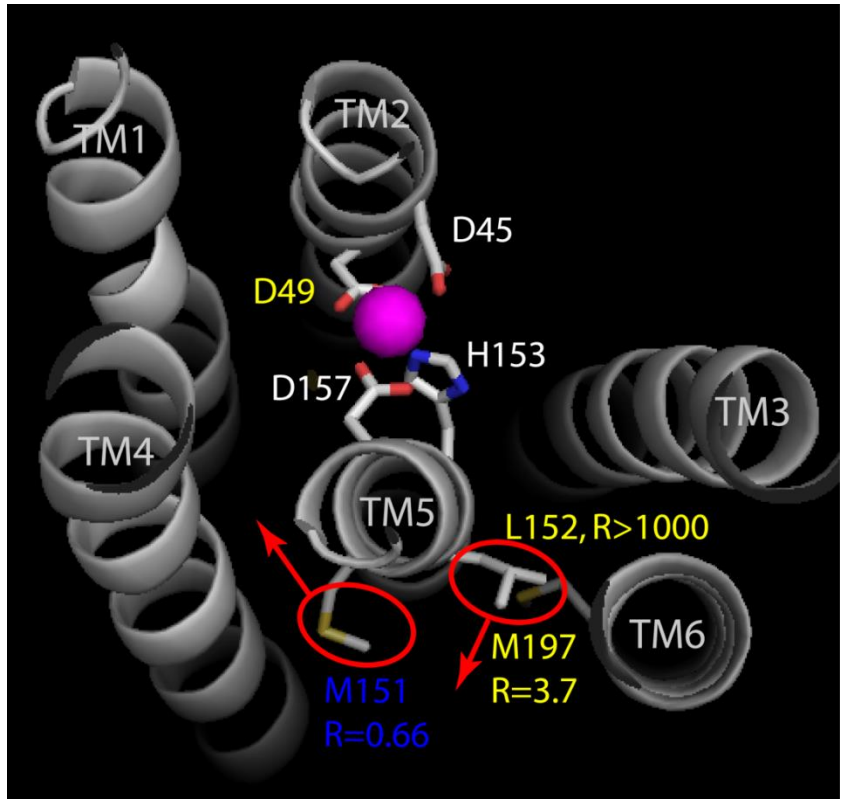
A Zn^{2+}/H^{+} translocation pathway

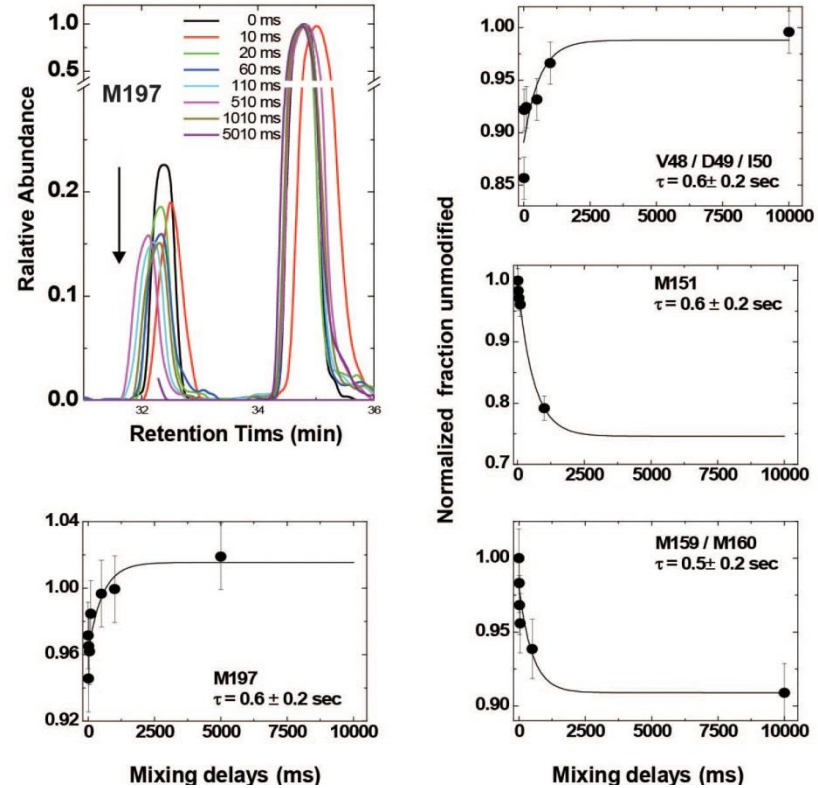




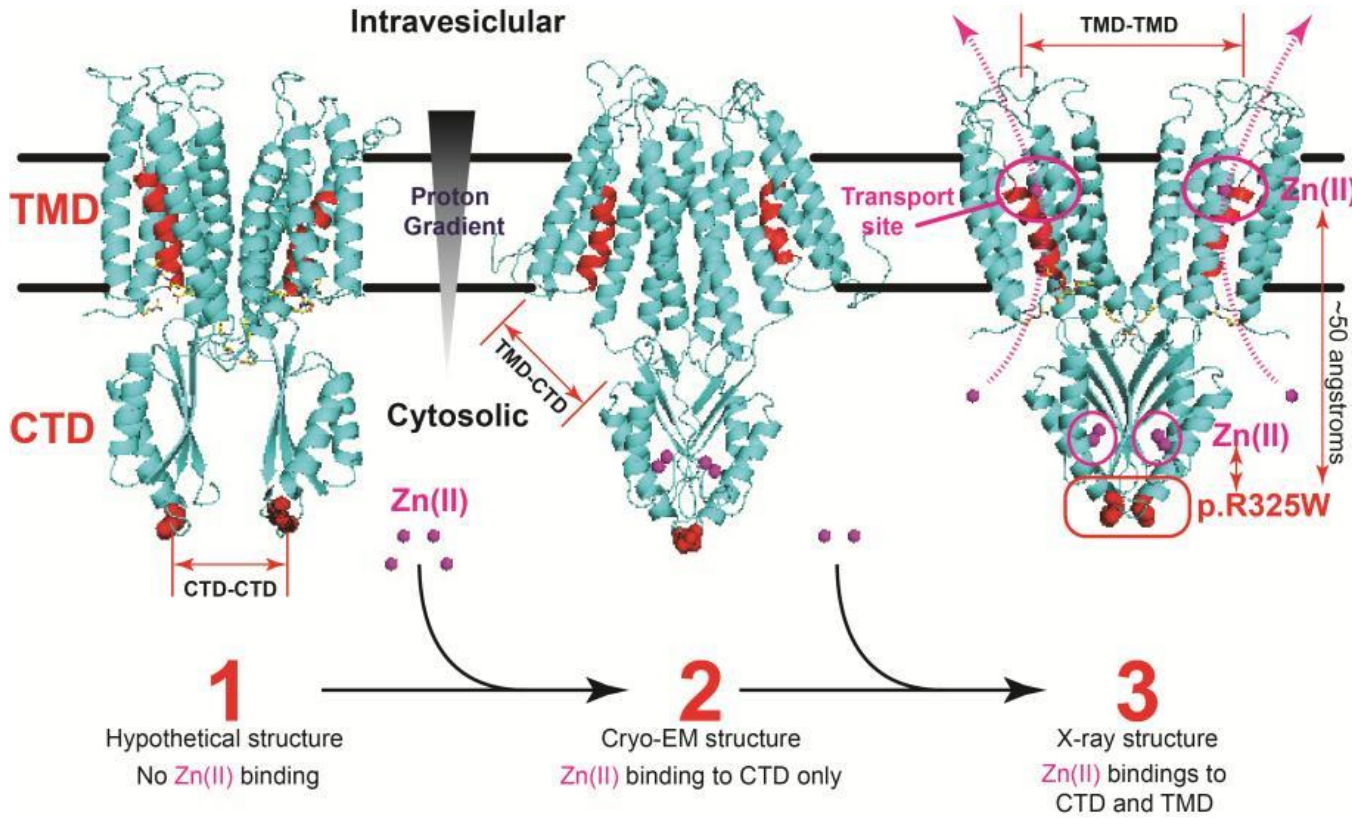
Kinetics of TM5 rigid-body rotation



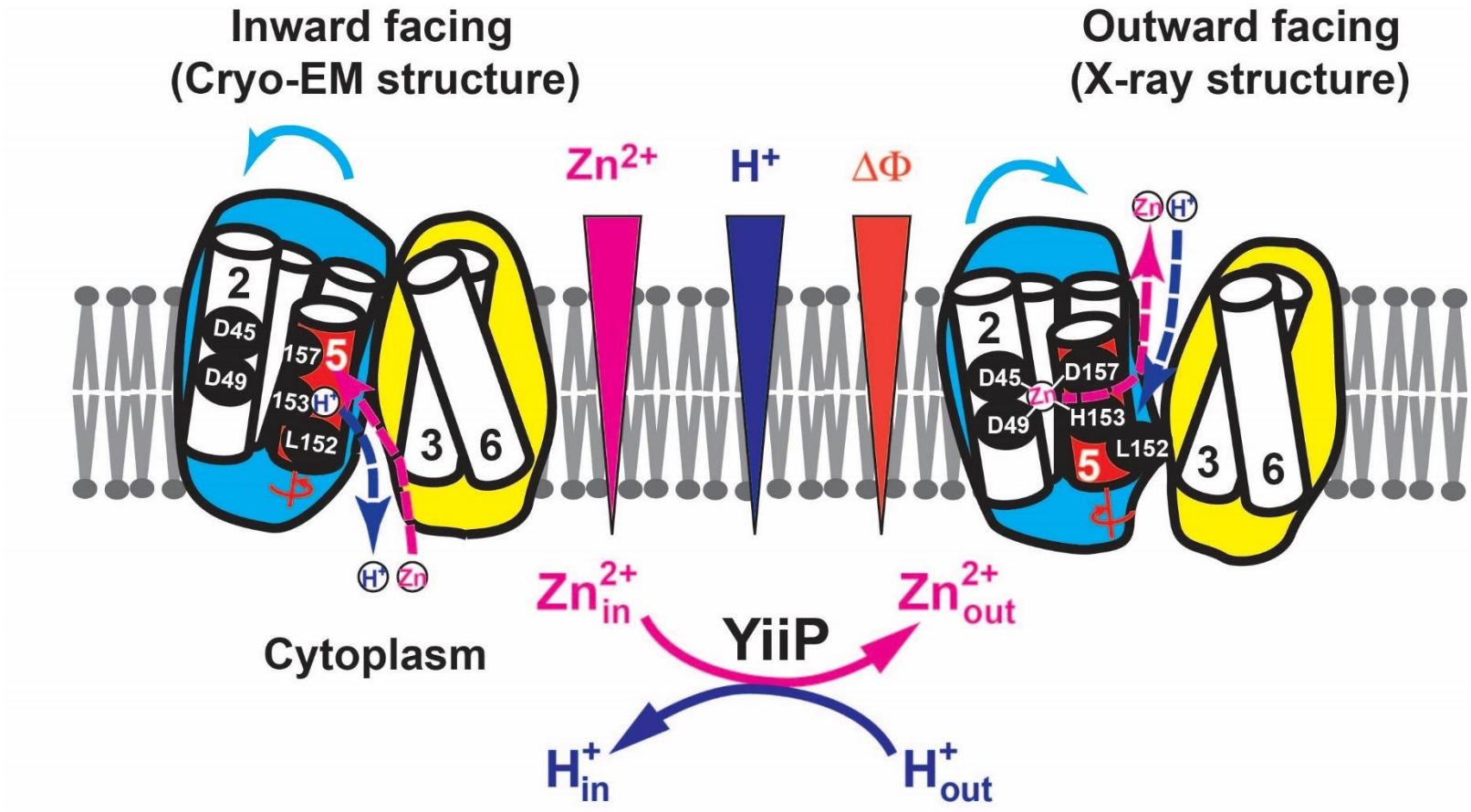




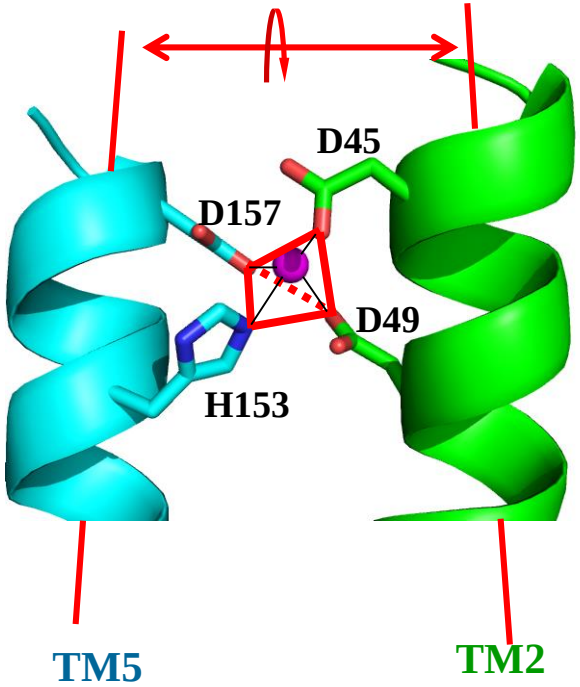
TM5 delivers the power stroke of conformational changes

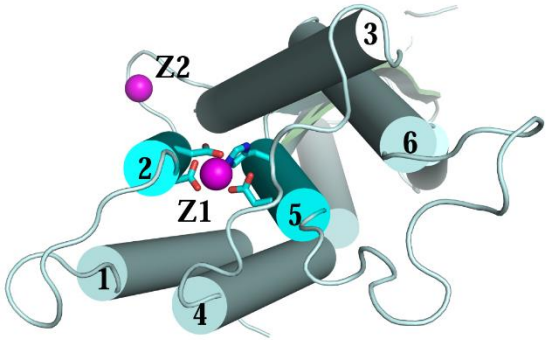


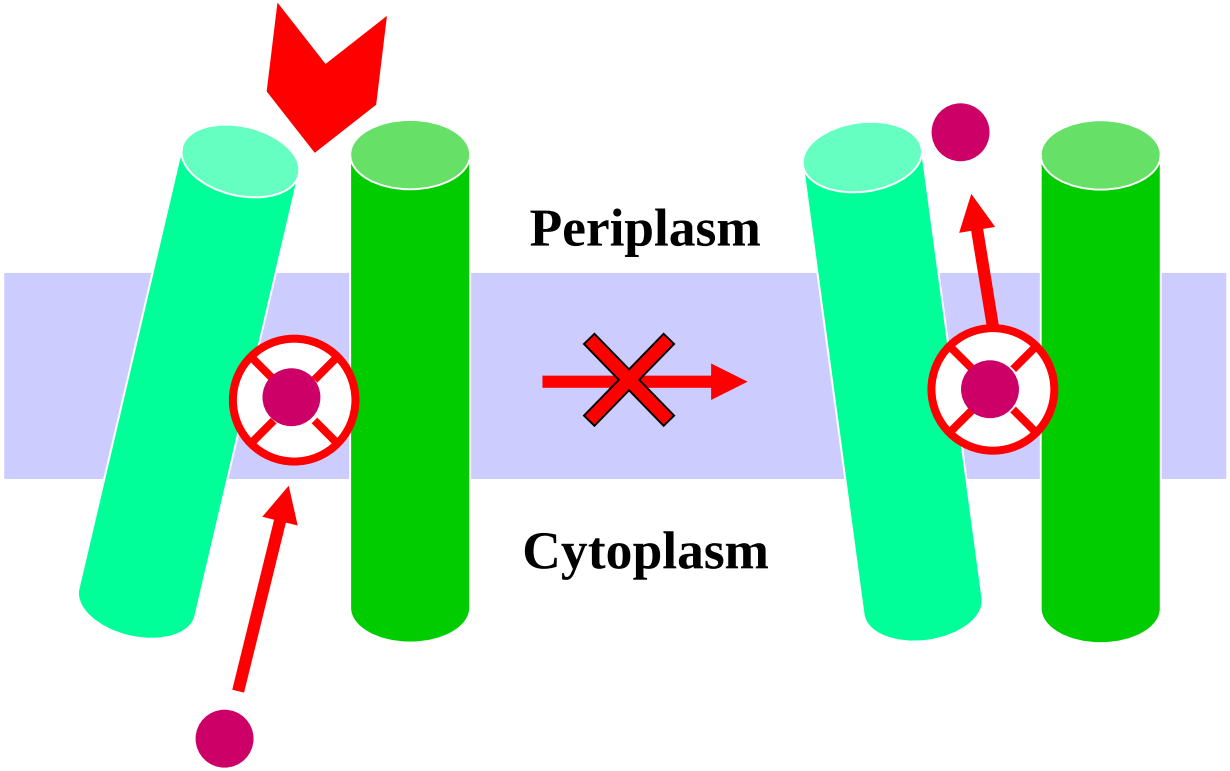
A structural model for Zn(II)/proton antiport



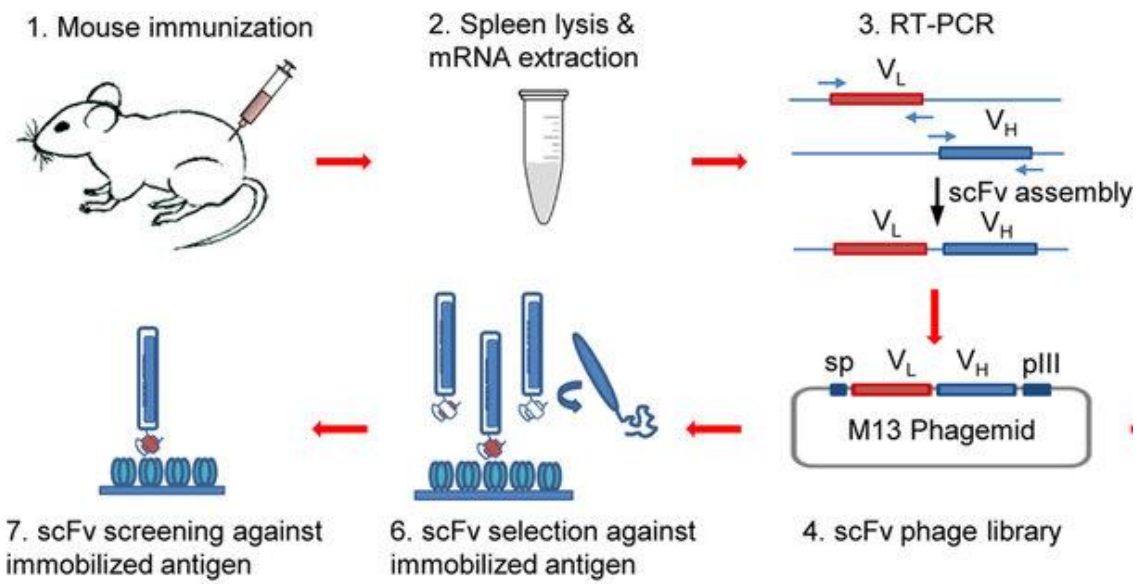
How to inhibit ZnT8 by mAb binding?

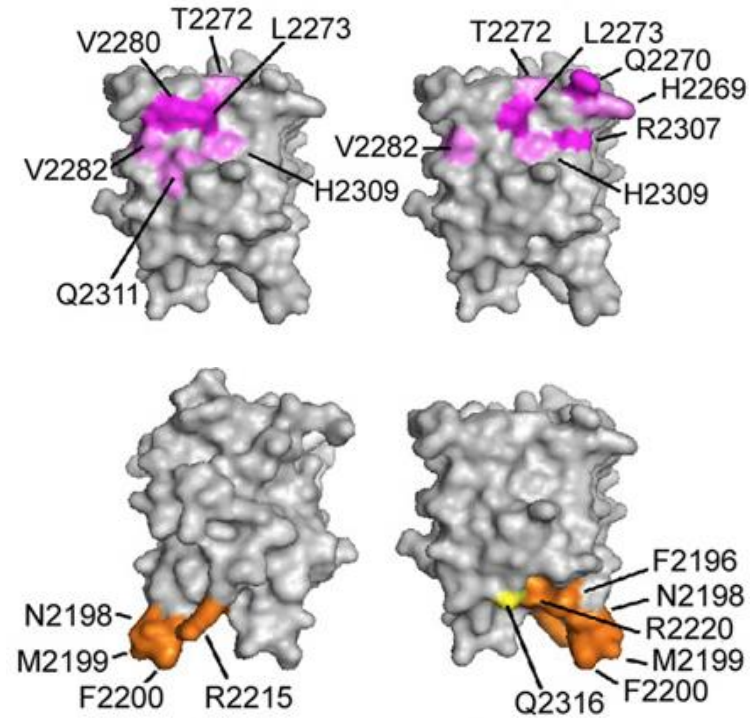






mAb development and epitope footprinting





Acknowledgments

Y. Wei, Y. Chao, biochemistry of zinc transporter YiiP
M. Lu, YiiP crystal structure
S. Gupta and J. Chai, structural dynamics of YiiP
C. Merriman and Q. Huang, human ZnT8

Collaboration
Mark Chance, BNL & Case Western Reserve University

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