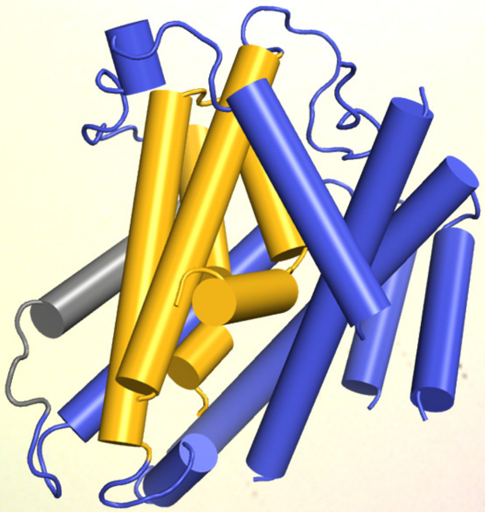
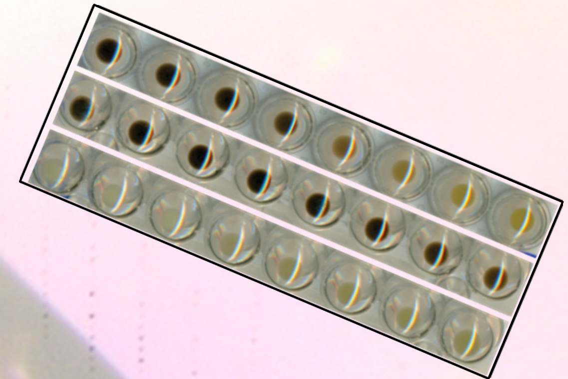




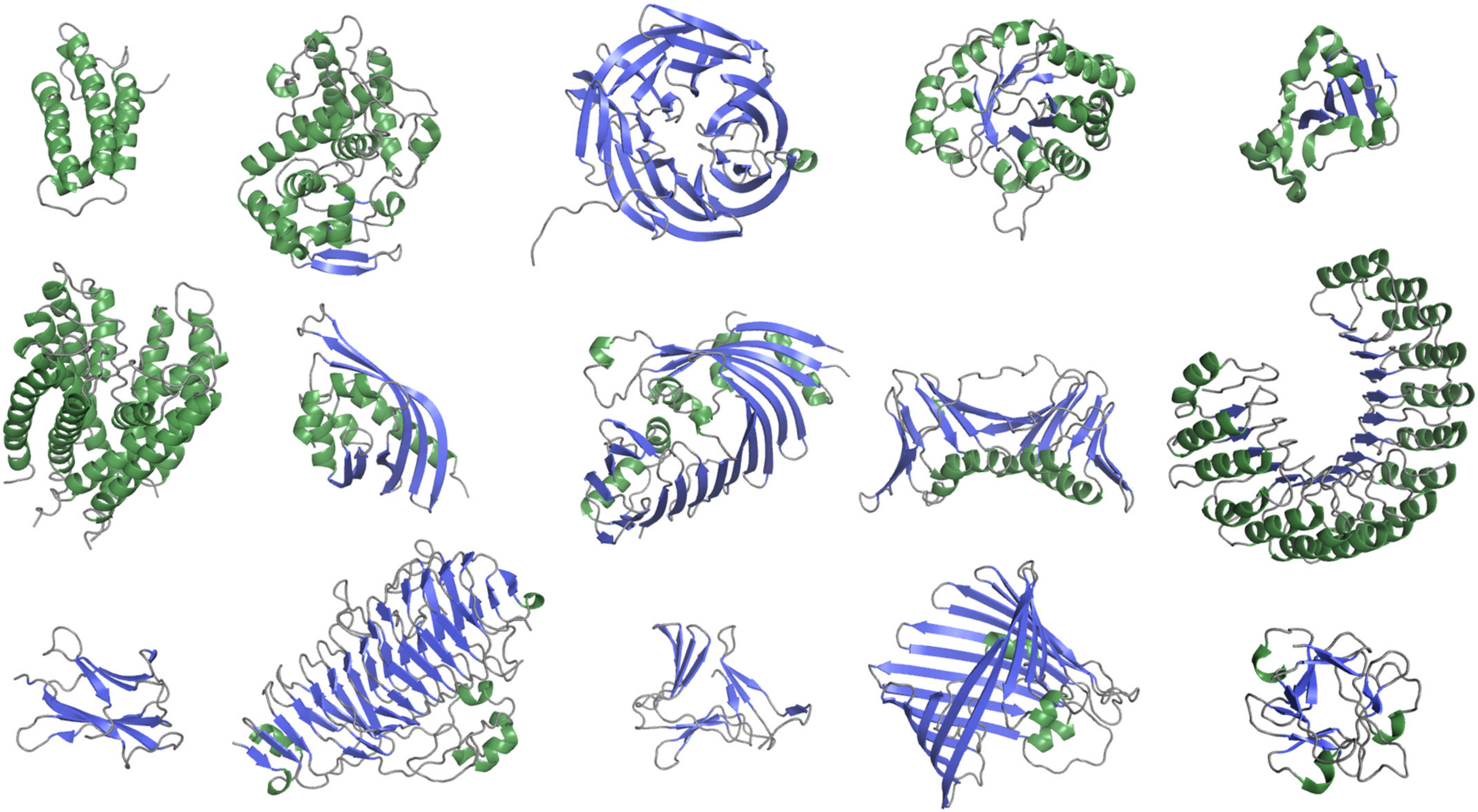
Panning for Precious Metals: How cells take up rare essential nutrients

Rachelle Gaudet
Professor of Molecular and Cellular Biology
Harvard University

What do we do in the Gaudet Lab?

- Proteins as nanomachines that carry out a majority of cellular functions
- We investigate what these nanomachines look like – how they are shaped and how the parts move as they work
- We’re interested in proteins that perform various functions at the surface of cells
  - They are sensors and entry/exit mechanisms

Why is important to know the structure of a protein?
"Form ever follows function" - Louis Sullivan



Why is important to know the structure of a protein?
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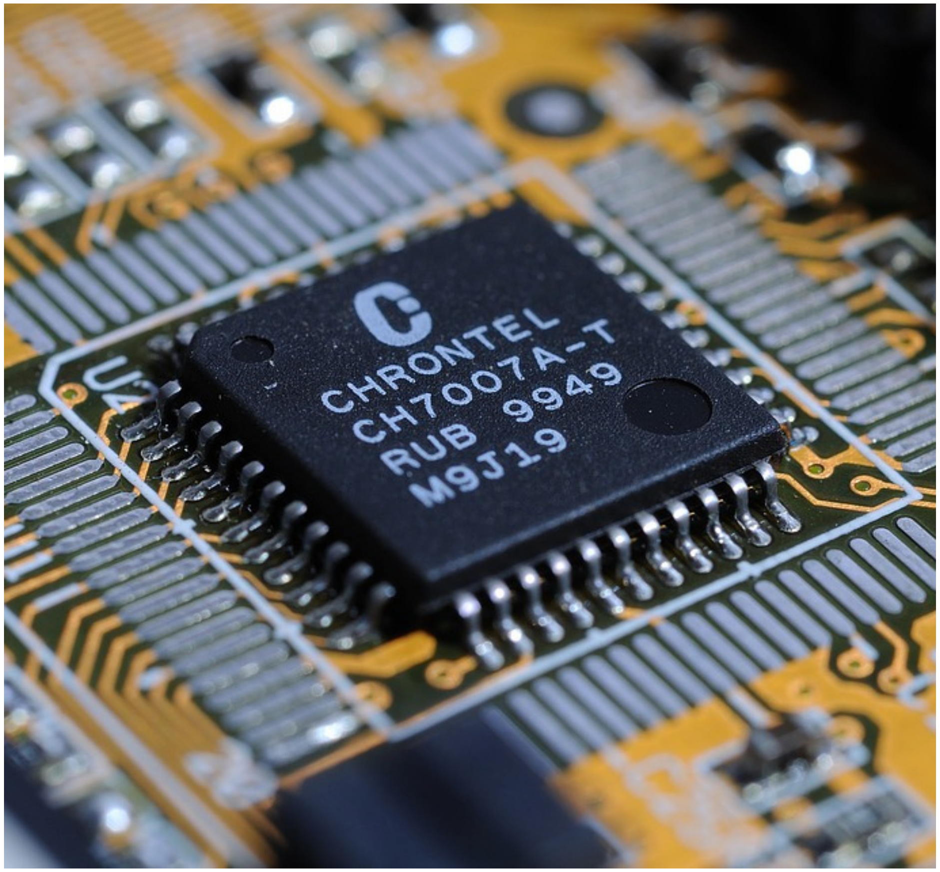
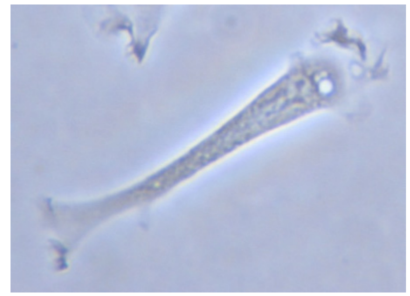


How big is a protein?

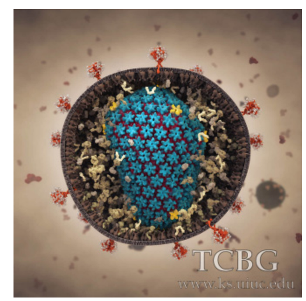
Zoom ~10x →

Zoom ~10x →

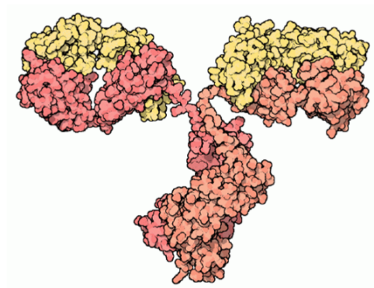


Zoom ~10x →

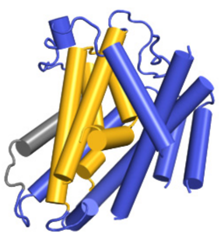
Zoom ~10x ↓



← **Zoom ~10x**



← **Zoom ~10x**

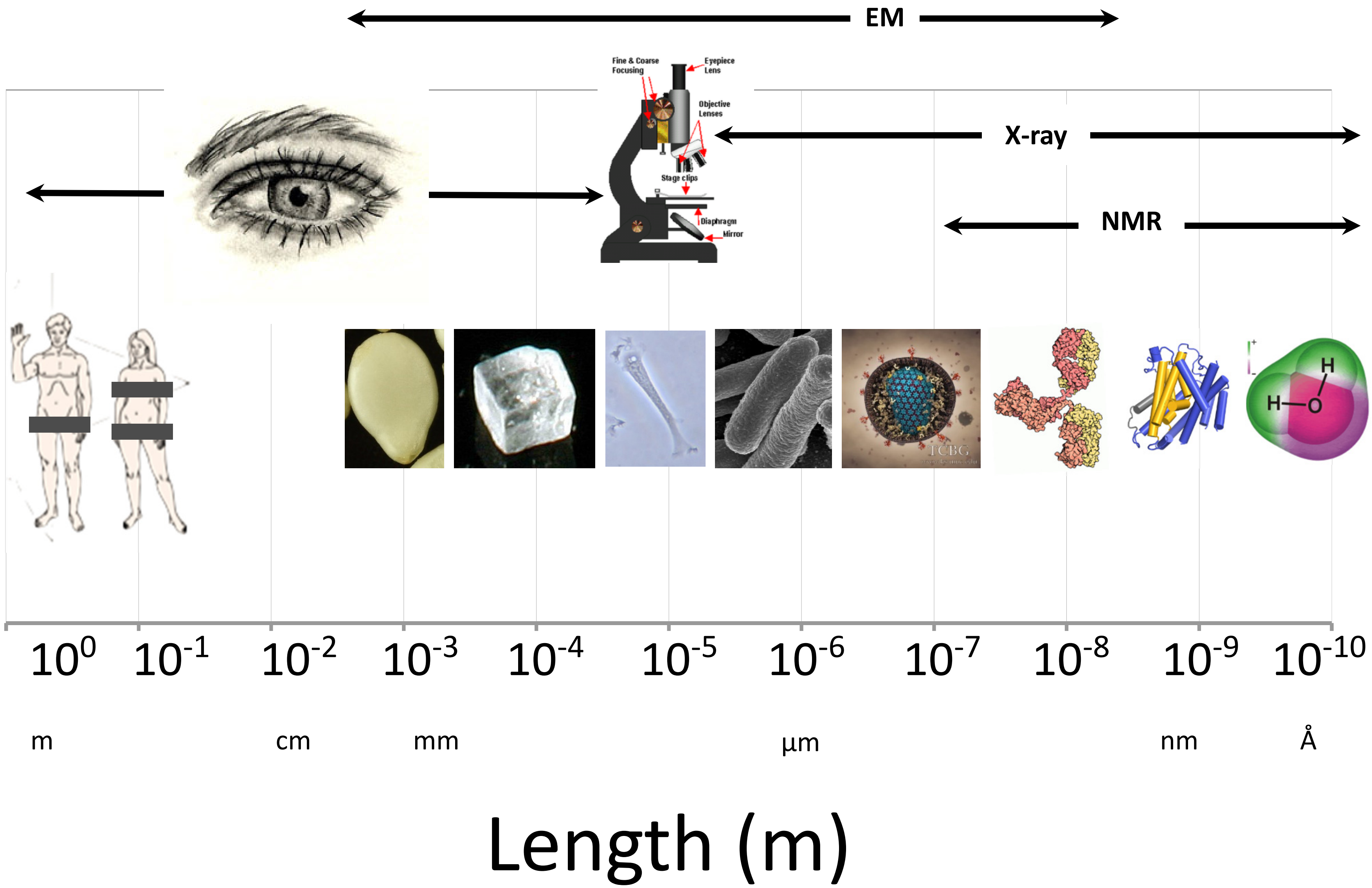


Zoom ~1 Million x (from first image)

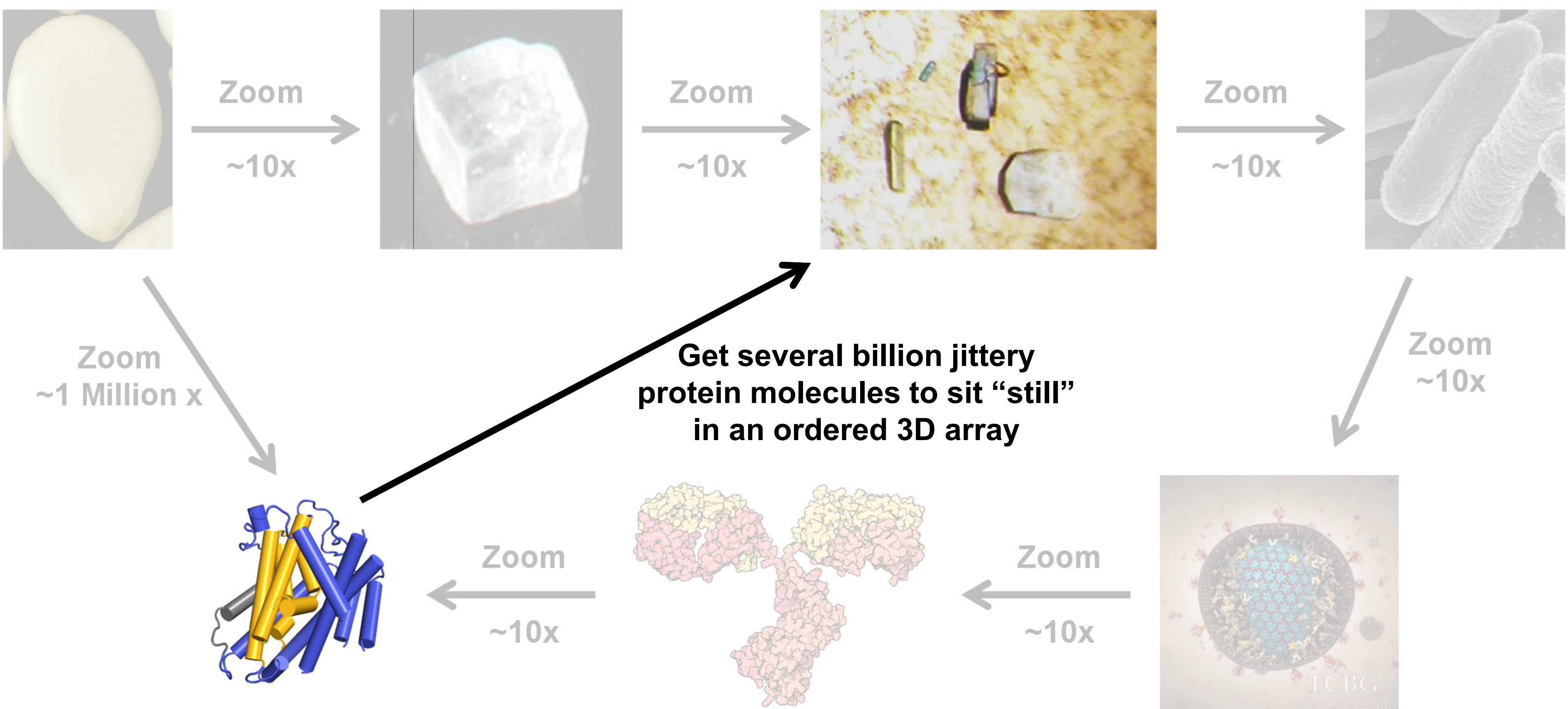
Two steps to “see” proteins:
(1) Generate protein crystals
(2) Use X-rays to generate image of protein from the crystals

Sources: Sanjay ach, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=2389127; Wikipedia commons; TCBG www.ks.uiuc.edu; www.rcsb.org

Powers of 10: A relative scale of lengths



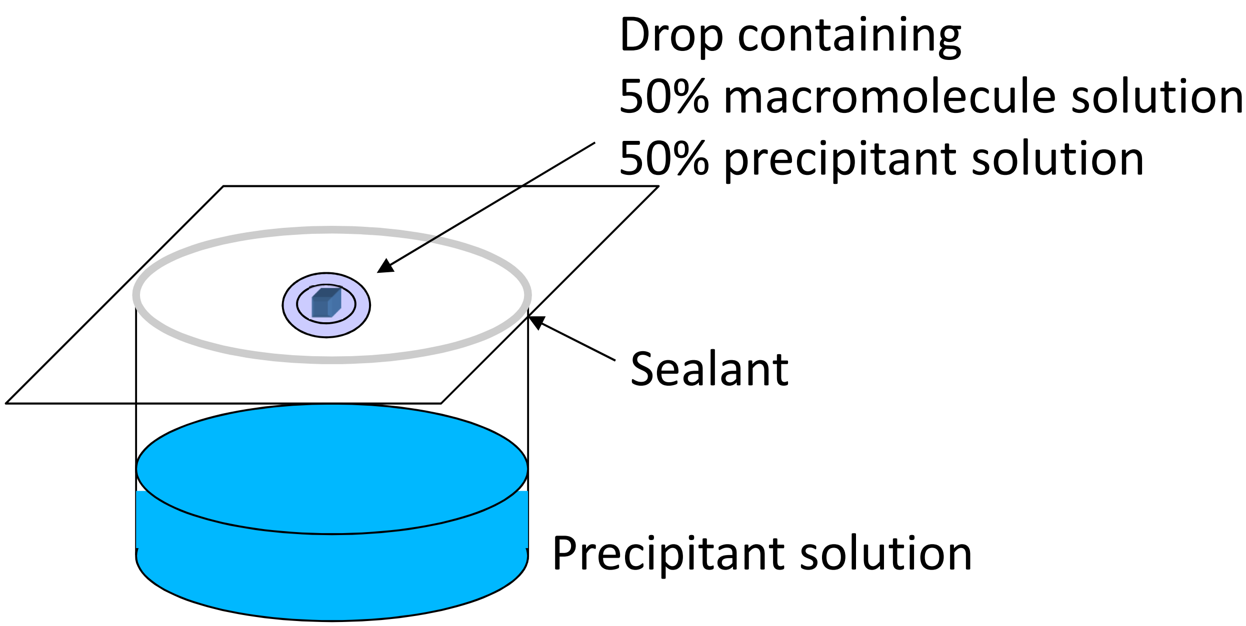
Coaxing proteins into crystals – to "see" them better

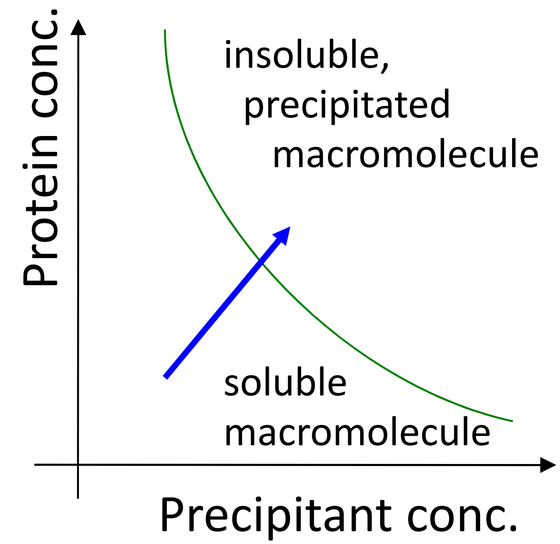


Sources: Sanjay ach, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=2389127; Wikipedia commons; TCBG www.ks.uiuc.edu; www.rcsb.org

Vapor diffusion setup for crystallization

Crystals required to increase the signal-to-noise to a detectable level

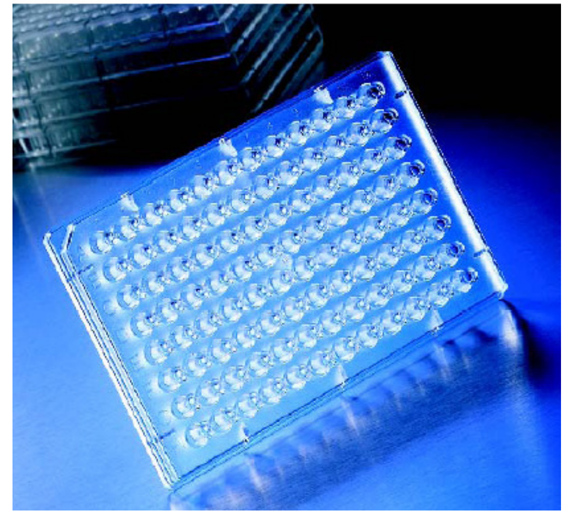




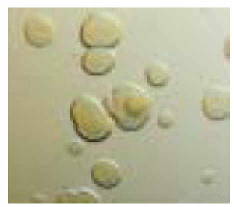
Over time, water leaves the drop and diffuses into the precipitant solution. The drop gets smaller and the macromolecule gets more concentrated in the presence of the precipitant.

Screening for crystallization

- Trial-and-error process
  - Determining conditions *a priori* requires the 3D structure…
- Screen hundreds of conditions
- Best conditions optimized by interpolation and screening of additives



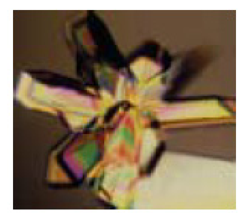
Clear drop



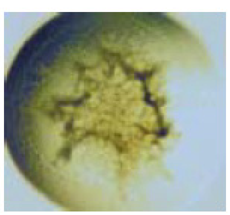
Quasi-crystals

Denatured protein

Micro-crystals

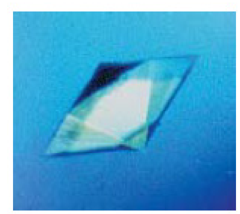


Crystals

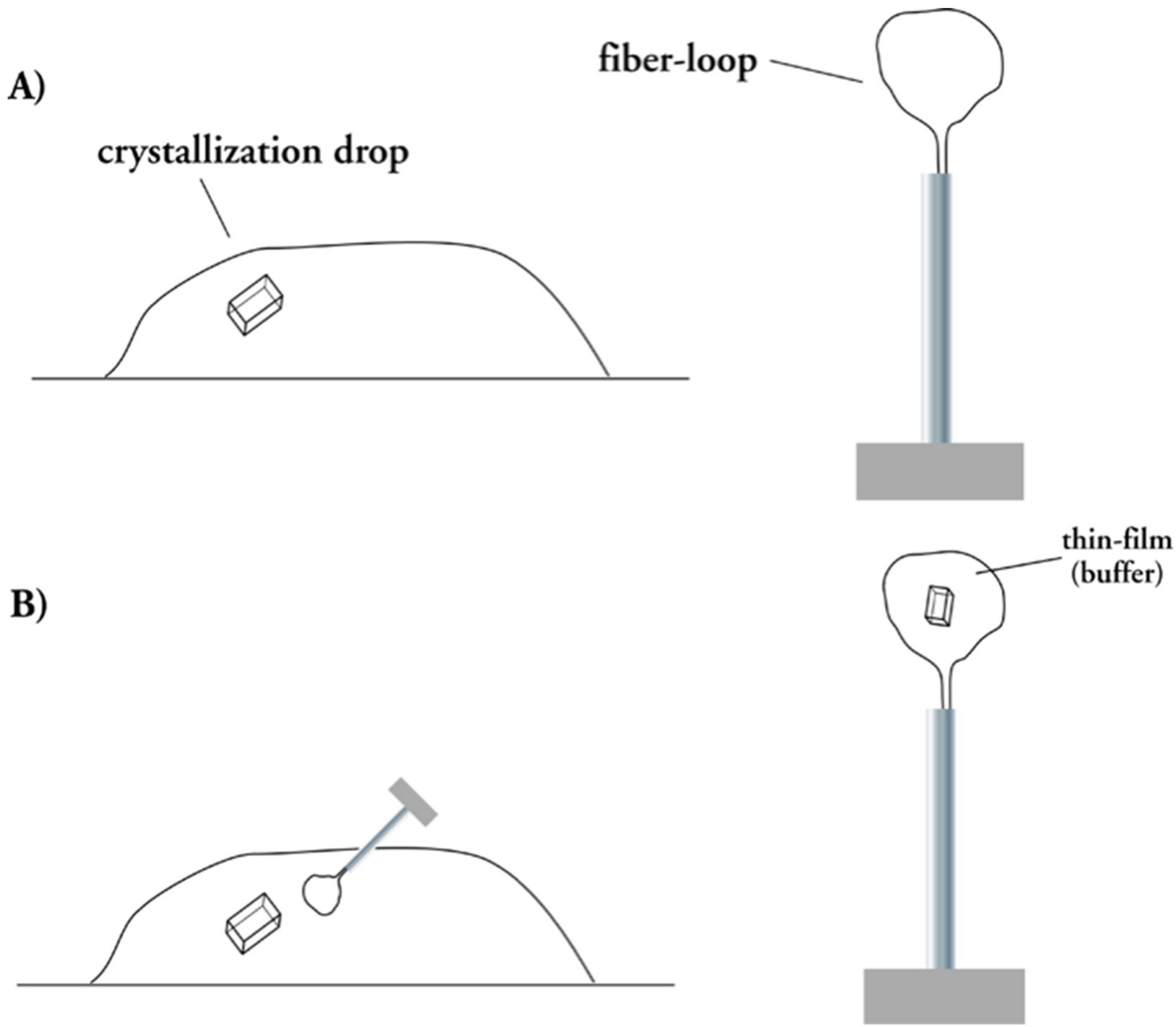


Precipitate

Needles

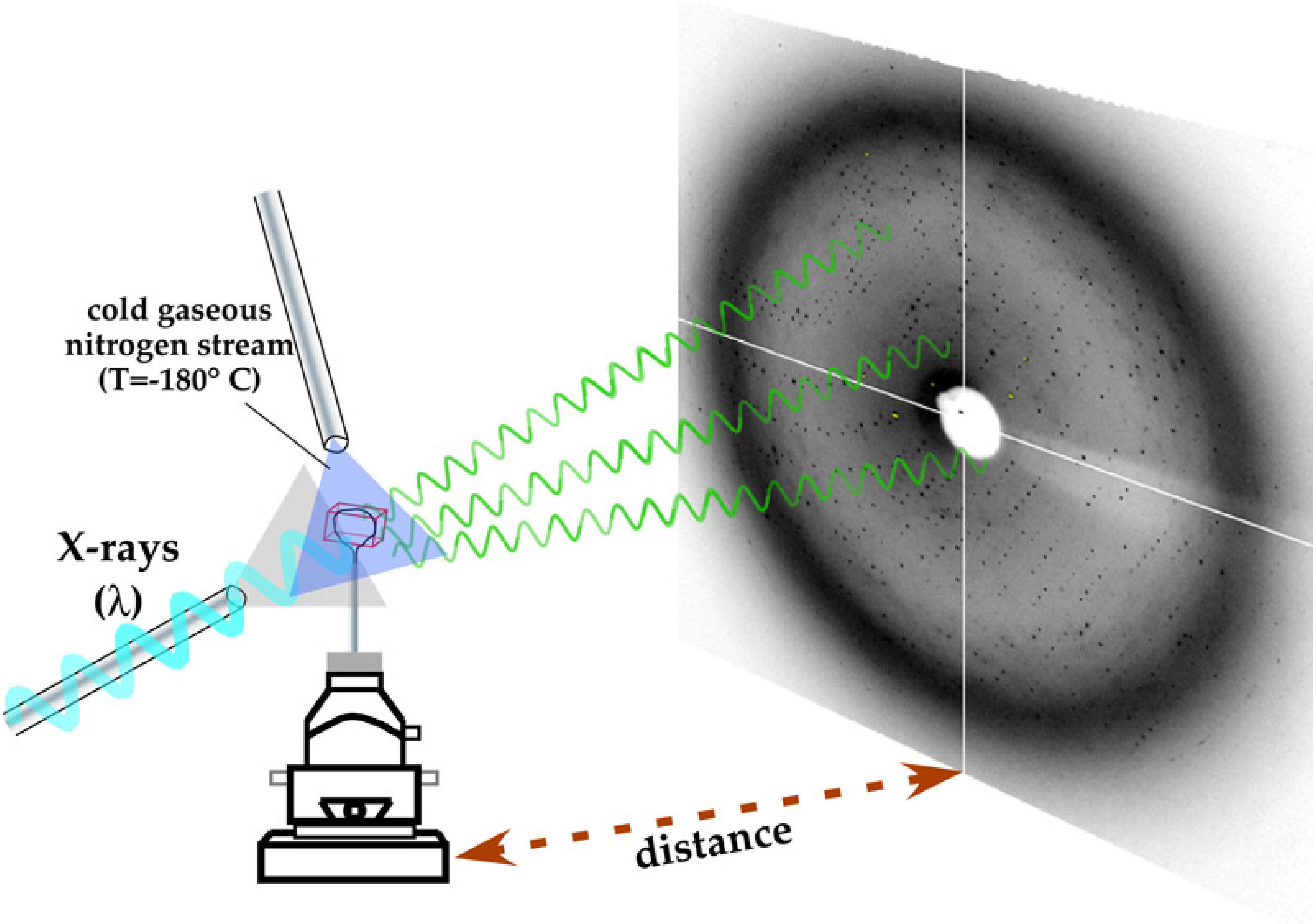


Handling of Macromolecular Crystals



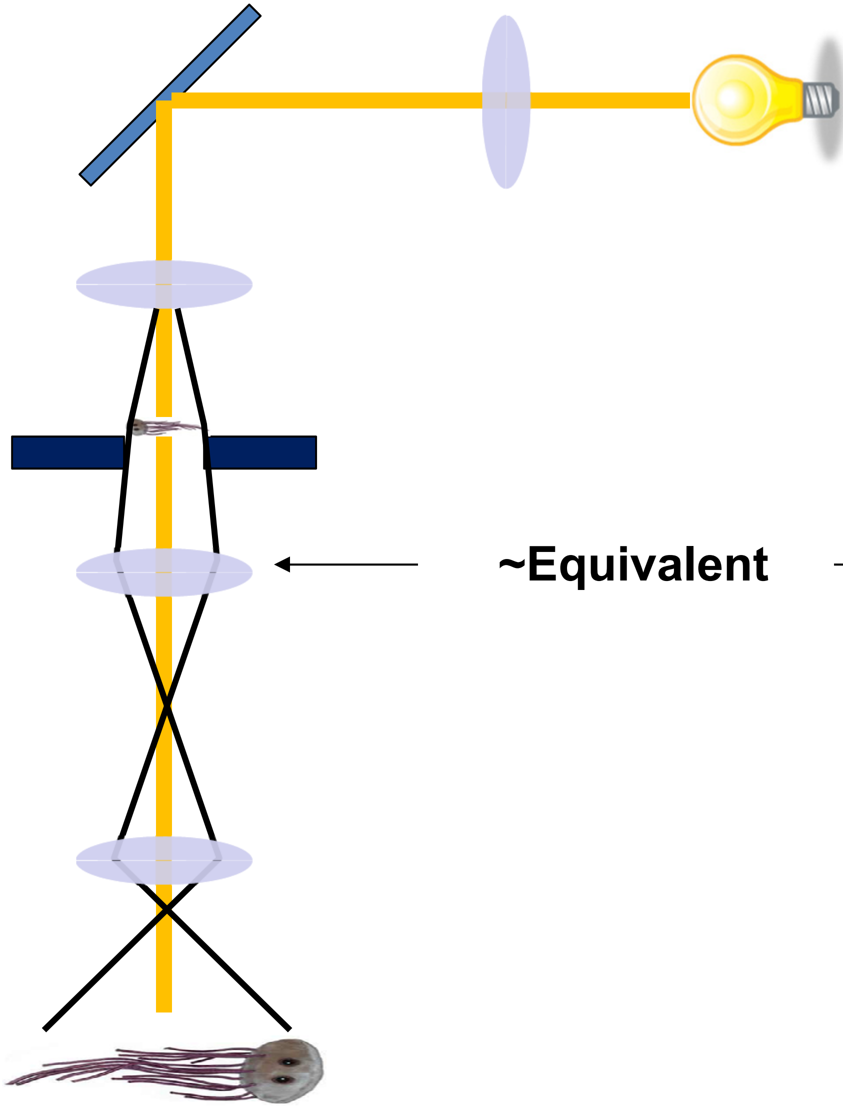
Courtesy of David Jeruzalmi

Diffraction data measurements

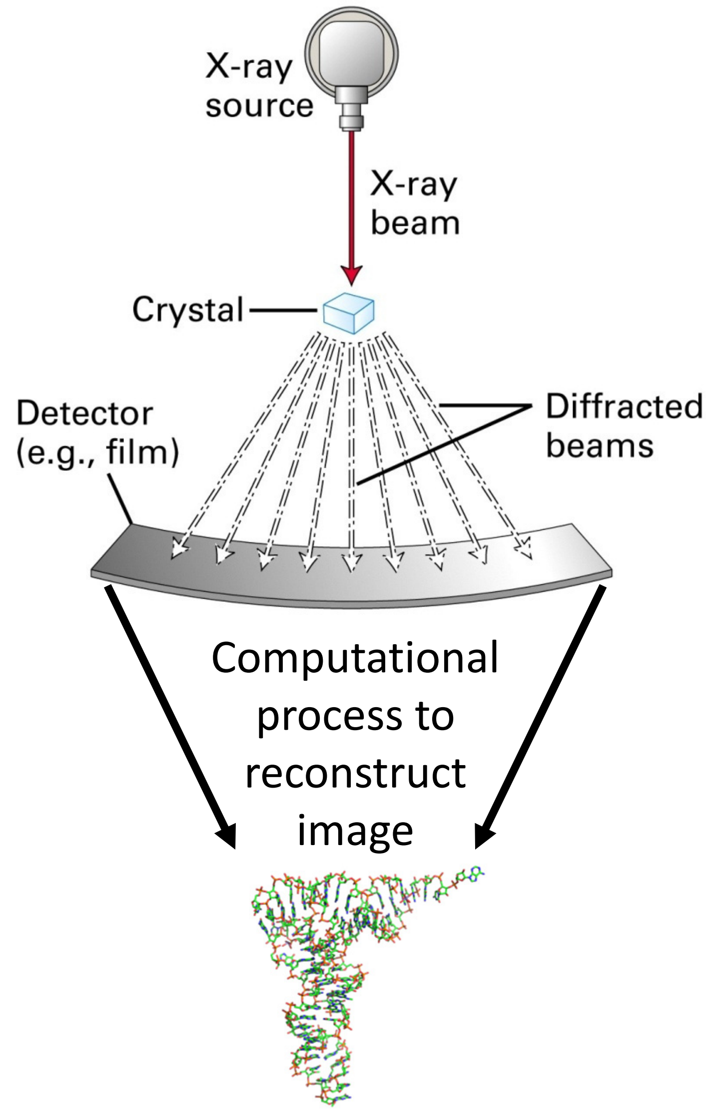


Courtesy of David Jeruzalmi

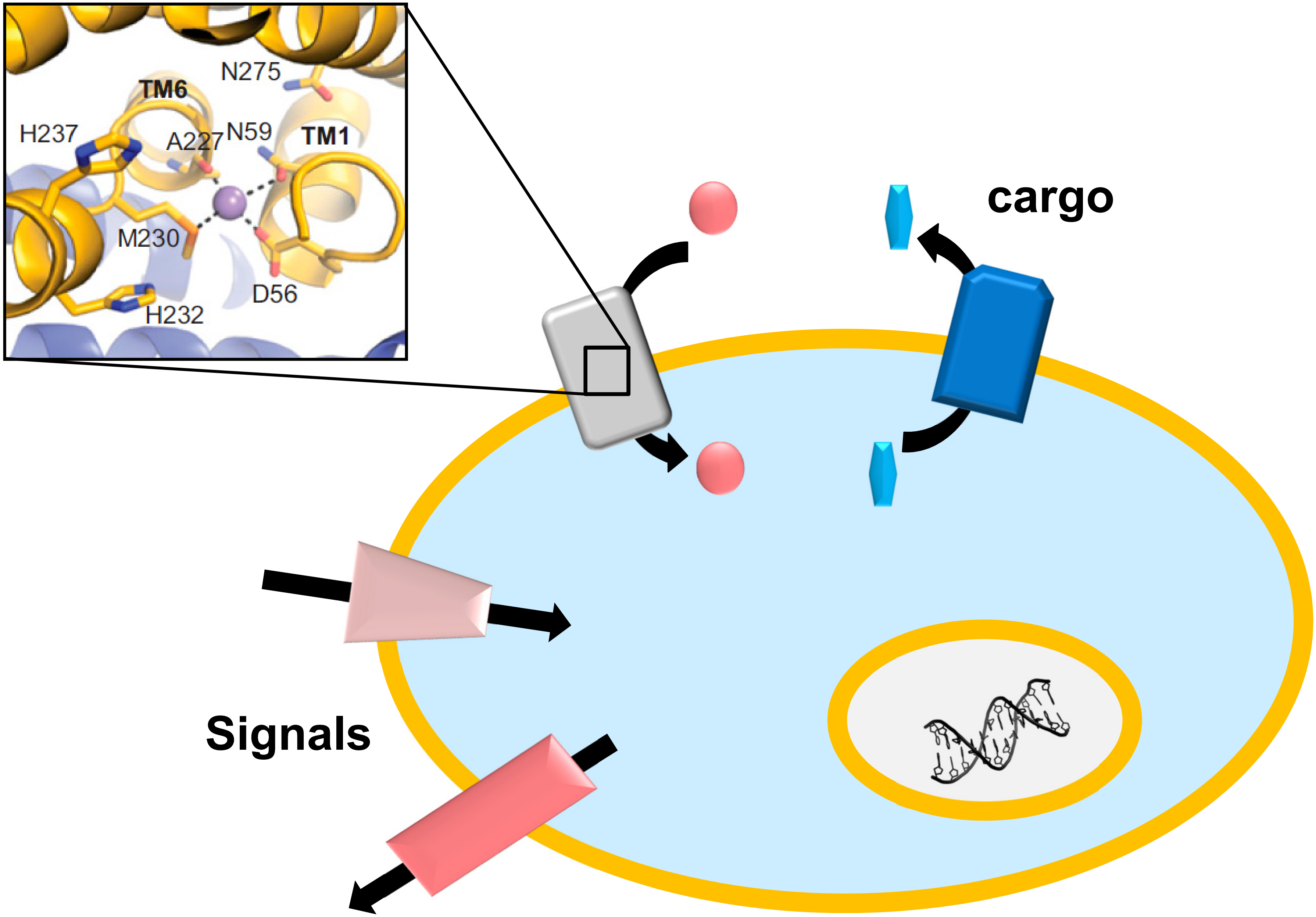
Light microscopy vs. crystallography



~Equivalent

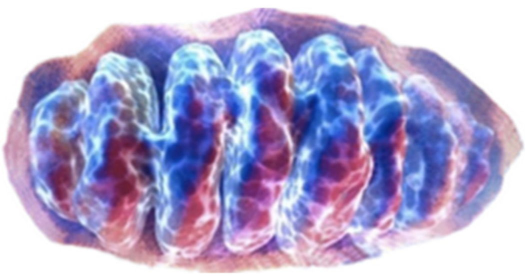


We want to understand membrane proteins at an atomic level

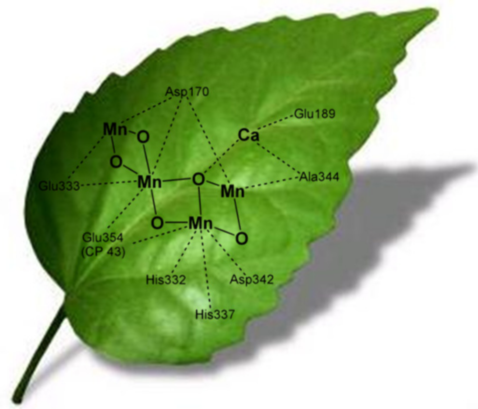


Homeostasis of divalent transition metals is essential for life

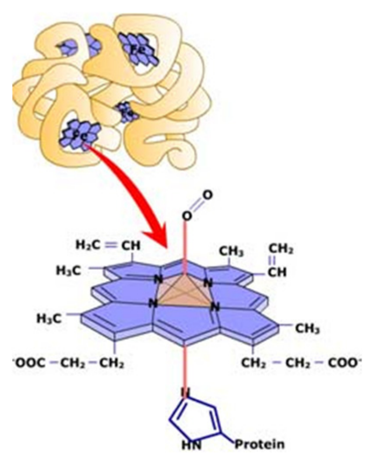
Electronic Transport Chain

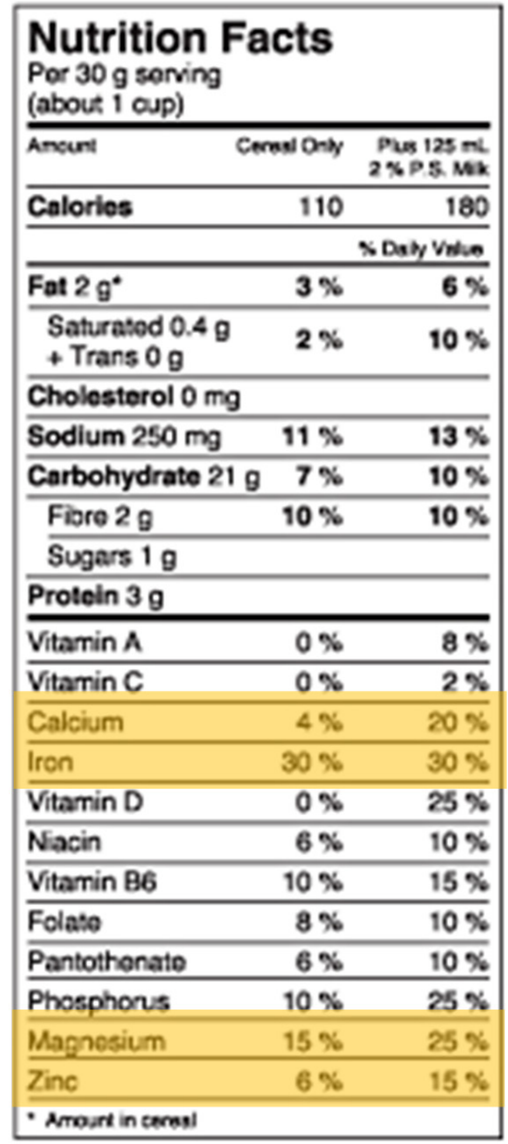


Photosynthesis PSII



Oxygen Transport





Nutrition Facts
Per 30 g serving
(about 1 cup)

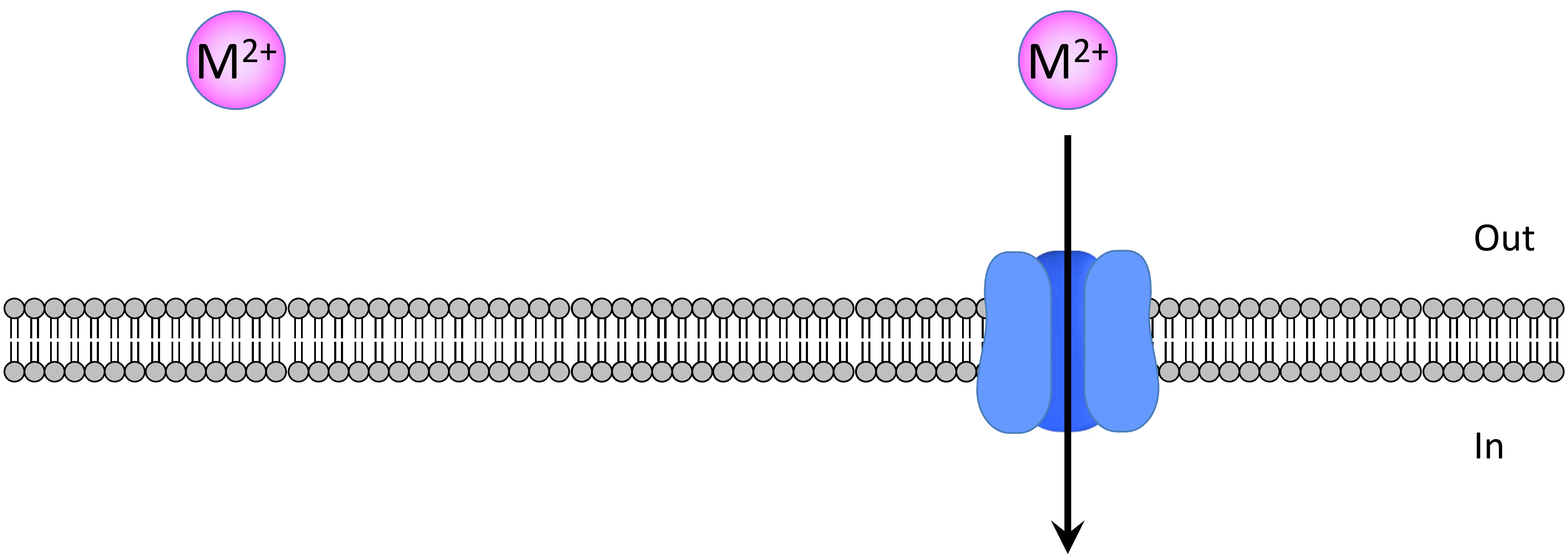
Amount	Cereal Only	Plus 125 mL 2 % P.S. Milk
Calories	110	180
		% Daily Value
Fat 2 g*	3 %	6 %
Saturated 0.4 g + Trans 0 g	2 %	10 %
Cholesterol 0 mg		
Sodium 250 mg	11 %	13 %
Carbohydrate 21 g	7 %	10 %
Fibre 2 g	10 %	10 %
Sugars 1 g		
Protein 3 g		
Vitamin A	0 %	8 %
Vitamin C	0 %	2 %
Calcium	4 %	20 %
Iron	30 %	30 %
Vitamin D	0 %	25 %
Niacin	6 %	10 %
Vitamin B6	10 %	15 %
Folate	8 %	10 %
Pantothenate	6 %	10 %
Phosphorus	10 %	25 %
Magnesium	15 %	25 %
Zinc	6 %	15 %

* Amount in cereal

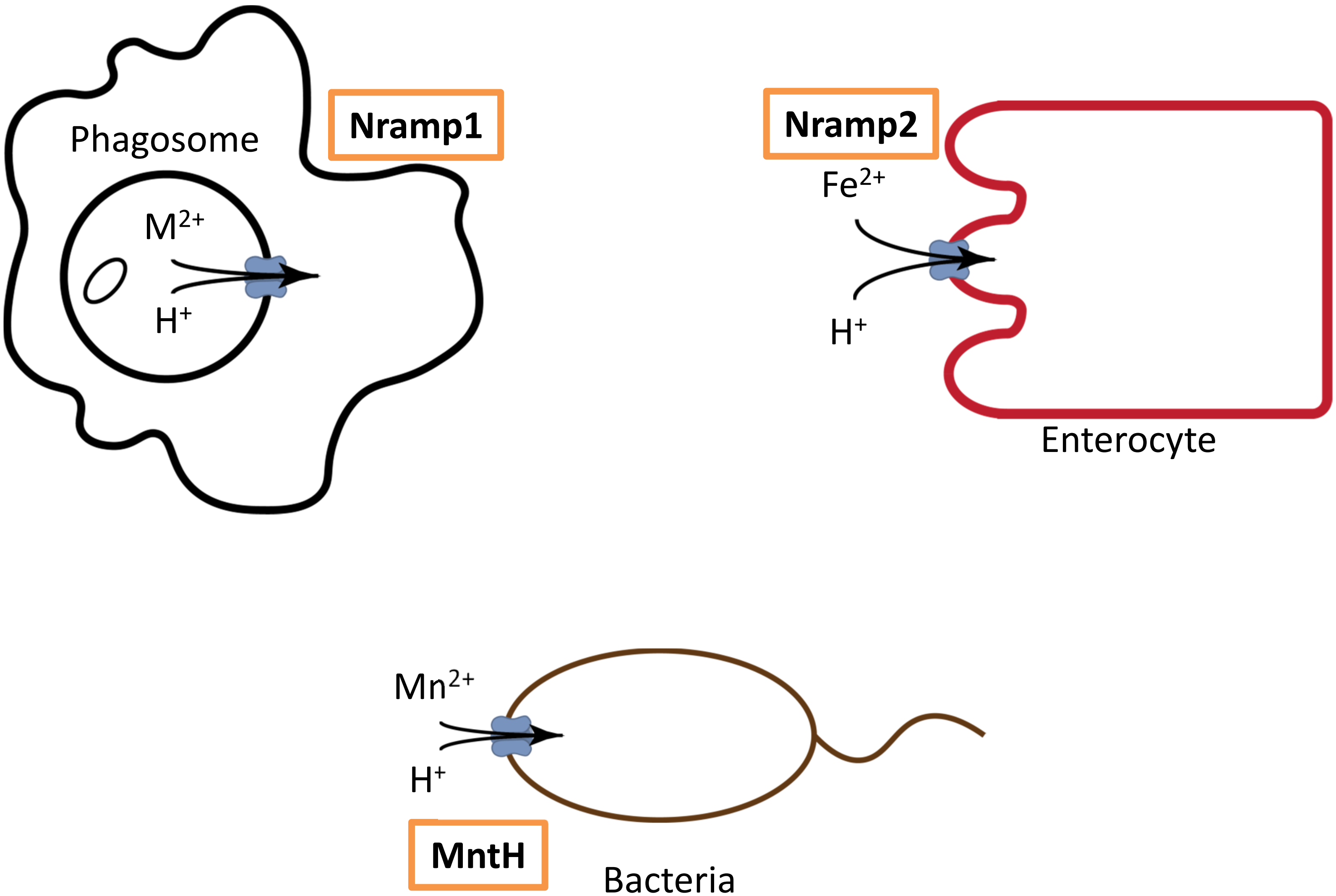
Images: Microbiwiki, Home-air-purifier-expert, Wikipedia

Nramps are divalent metal transporters

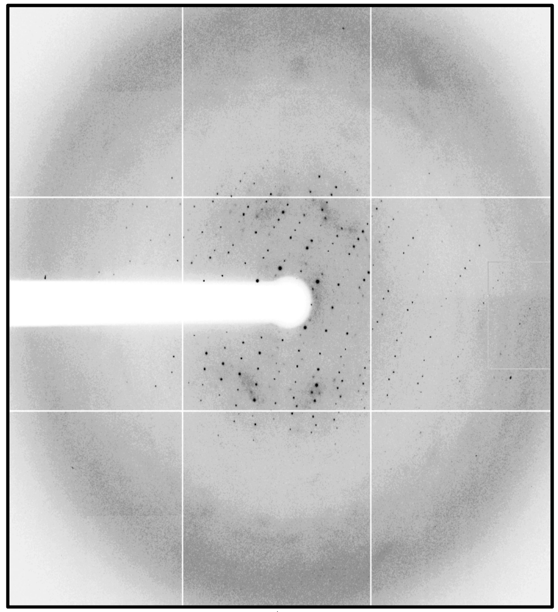
- Natural Resistance Associated Macrophage Protein
- Some version of Nramp is present in most living organisms
- Import essential metals like iron, manganese, cobalt

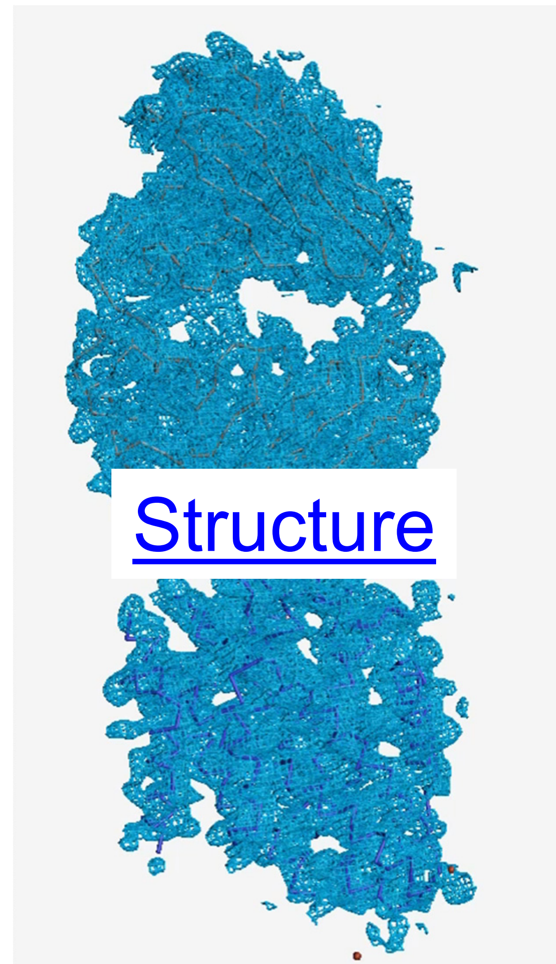


Nramps are transition divalent metal transporters

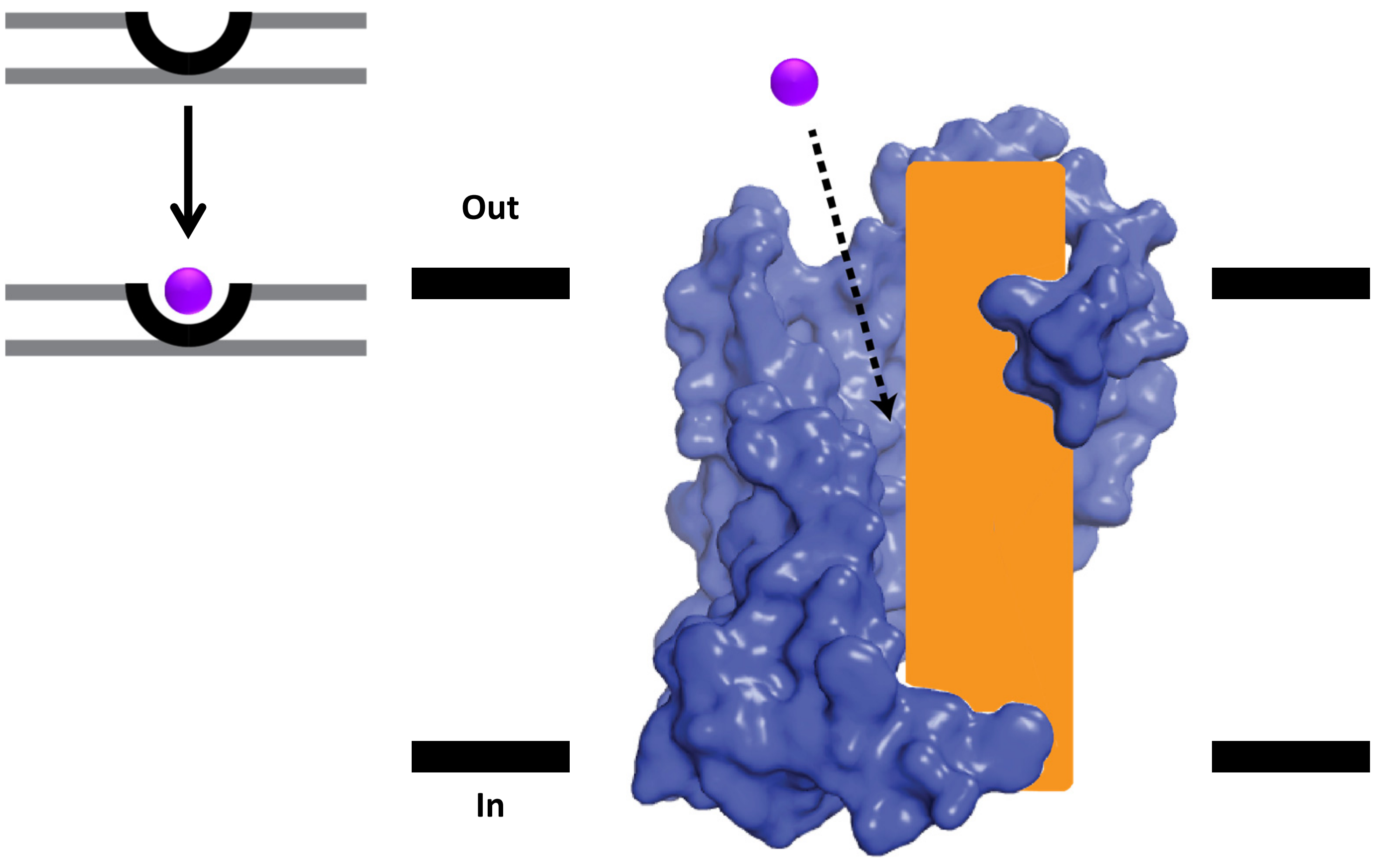


Structure of an Nramp transporter



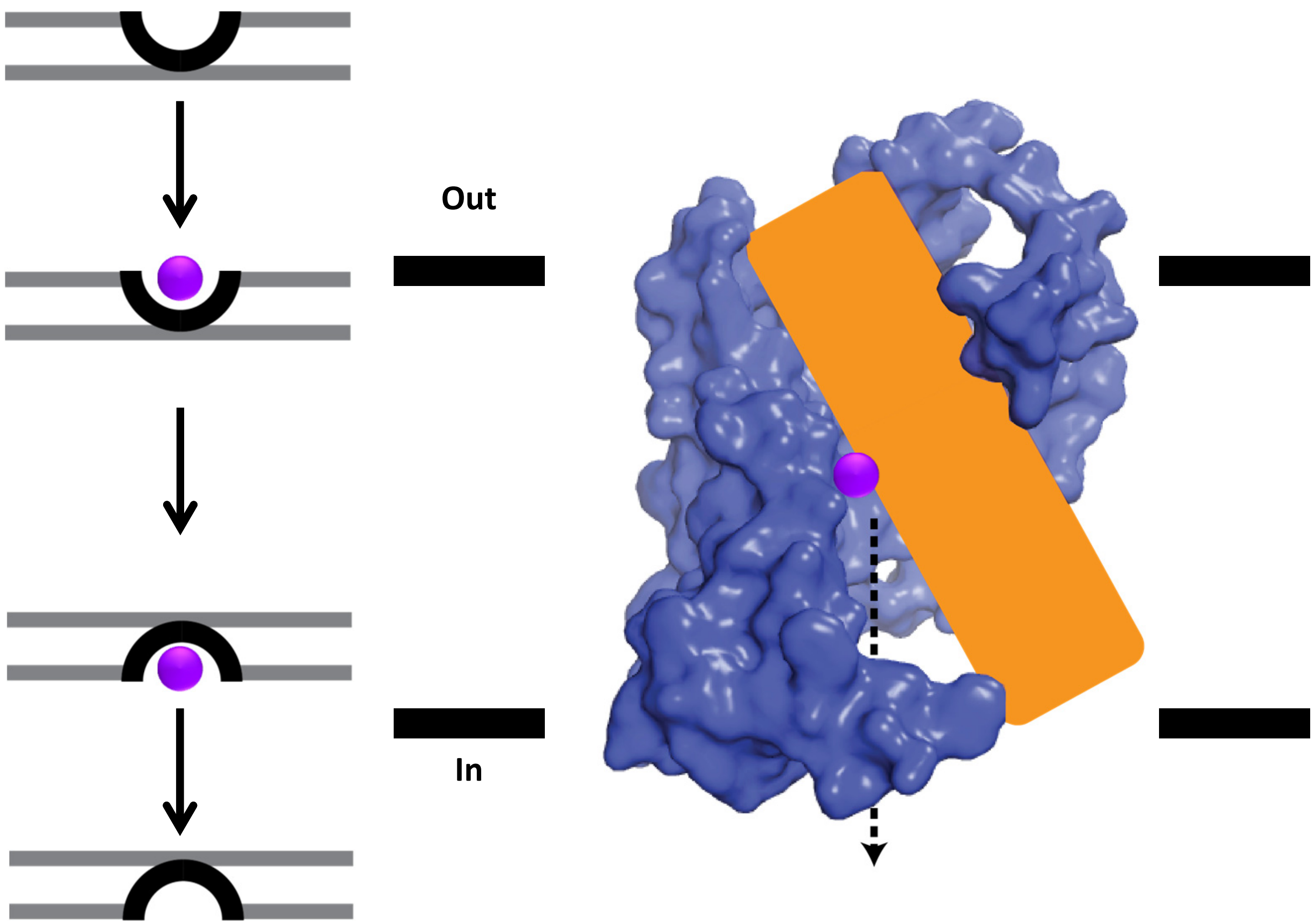


The bundle "rocks" within the scaffold to change substrate accessibility



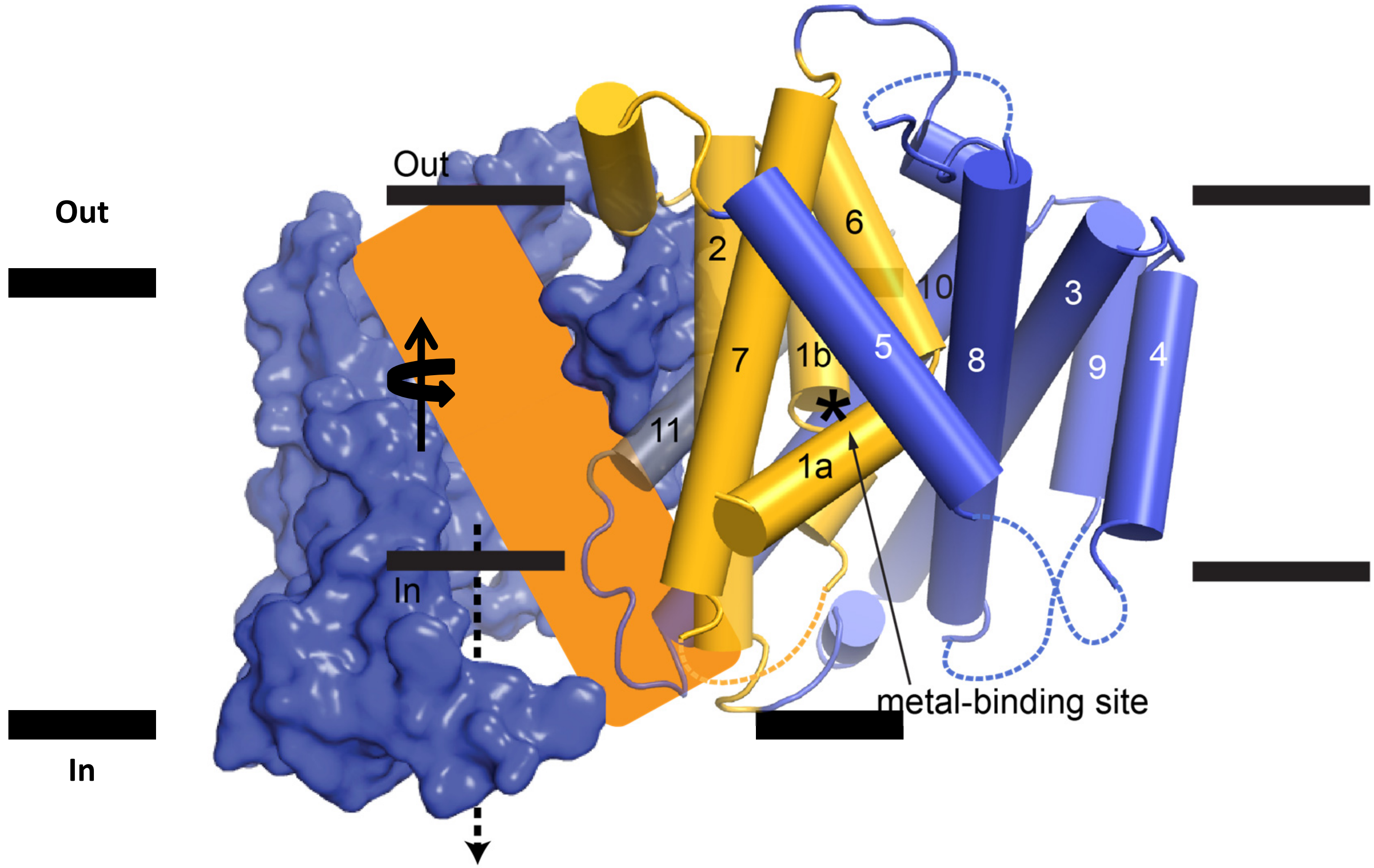
Forrest and Rudnick, Physiology (2009)

The bundle "rocks" within the scaffold to change substrate accessibility



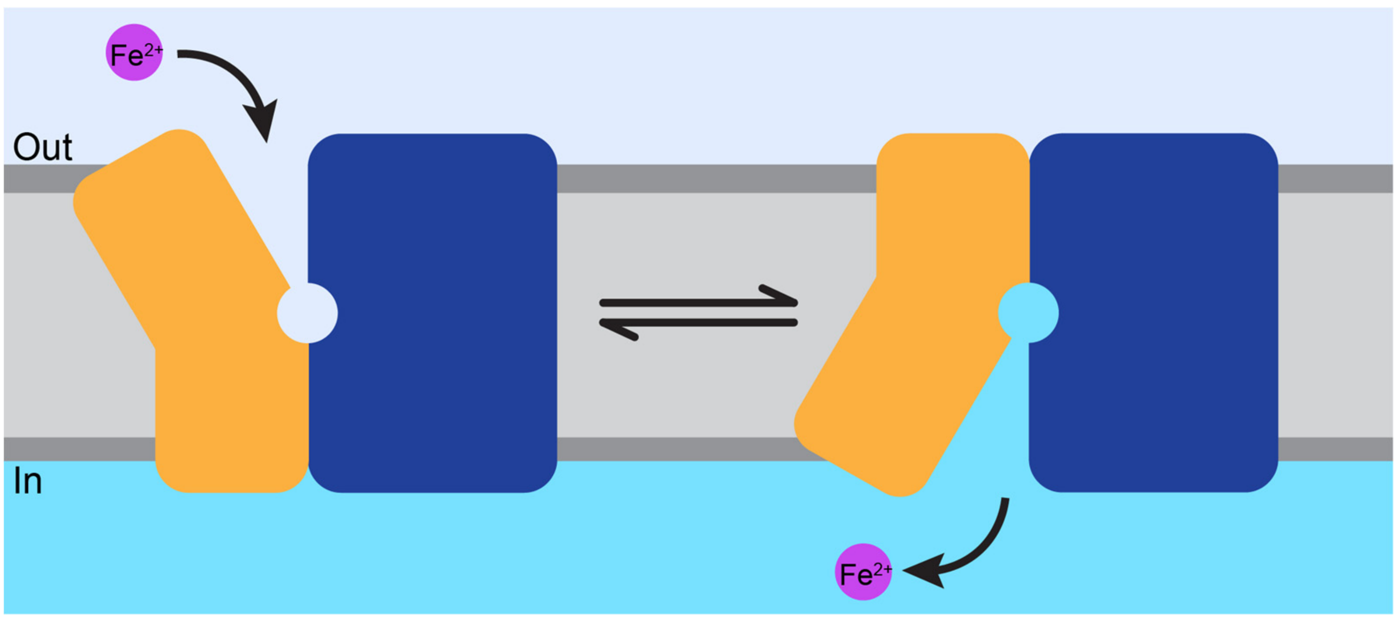
Forrest and Rudnick, Physiology (2009)

The bundle "rocks" within the scaffold to change substrate accessibility

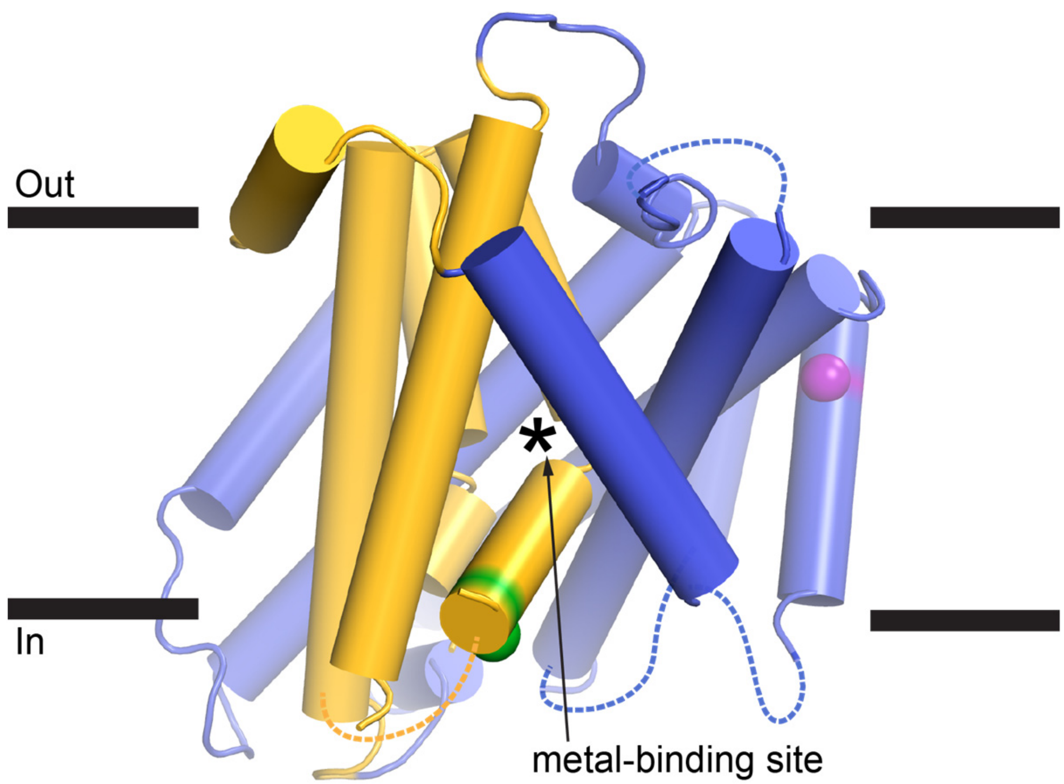


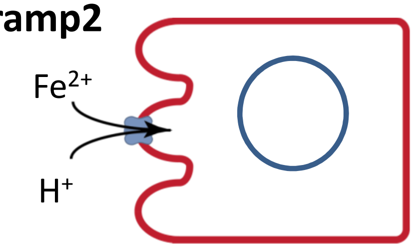
Forrest and Rudnick, Physiology (2009)

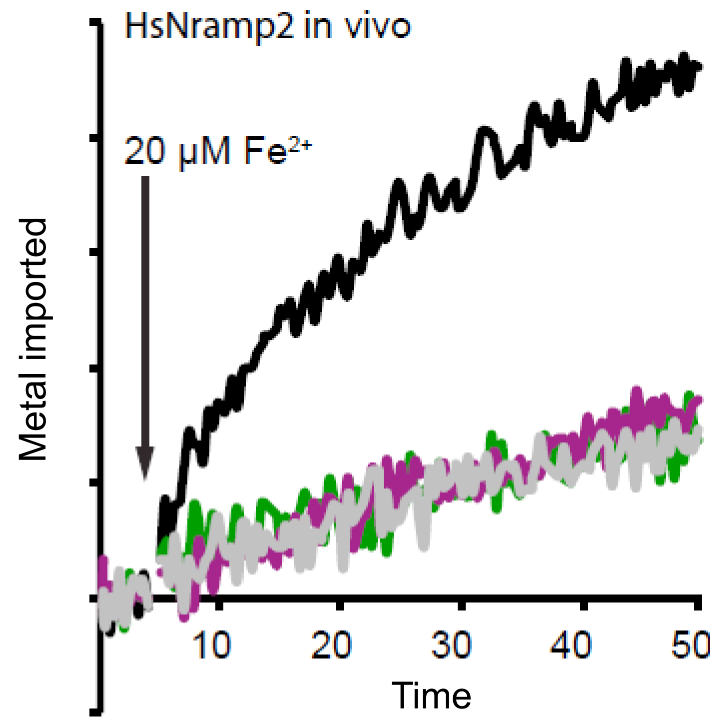
Simplified model of metal transport



How do mutations in Nramp2 cause anemia?



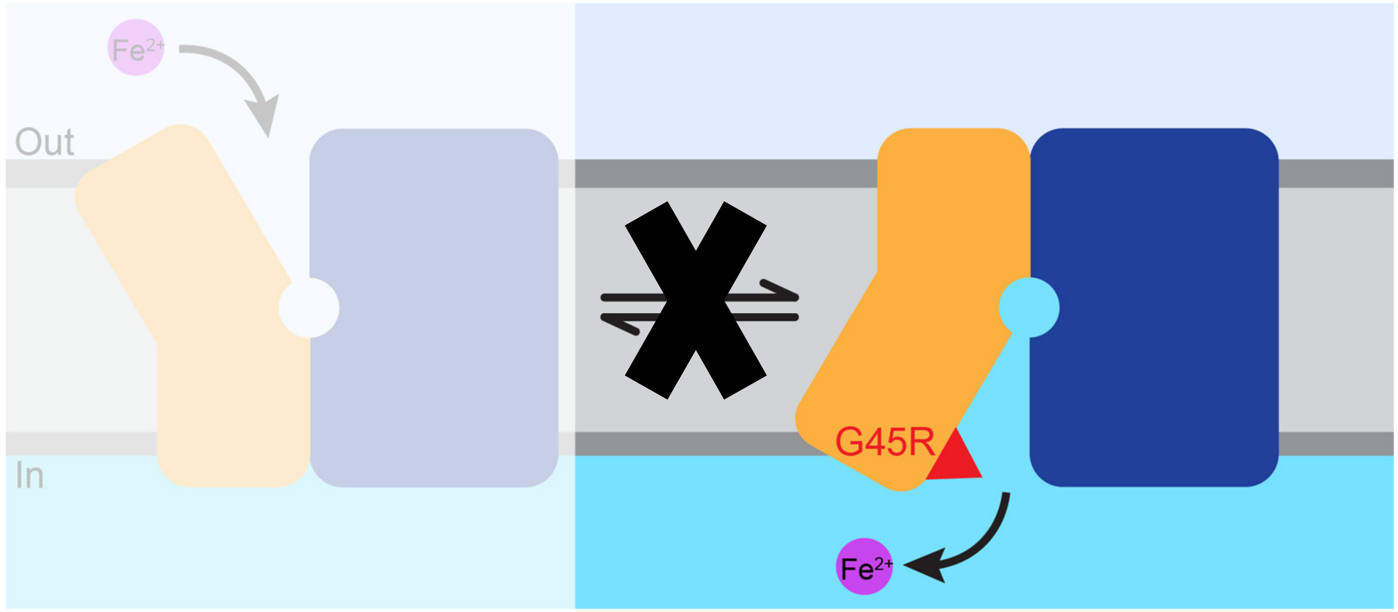




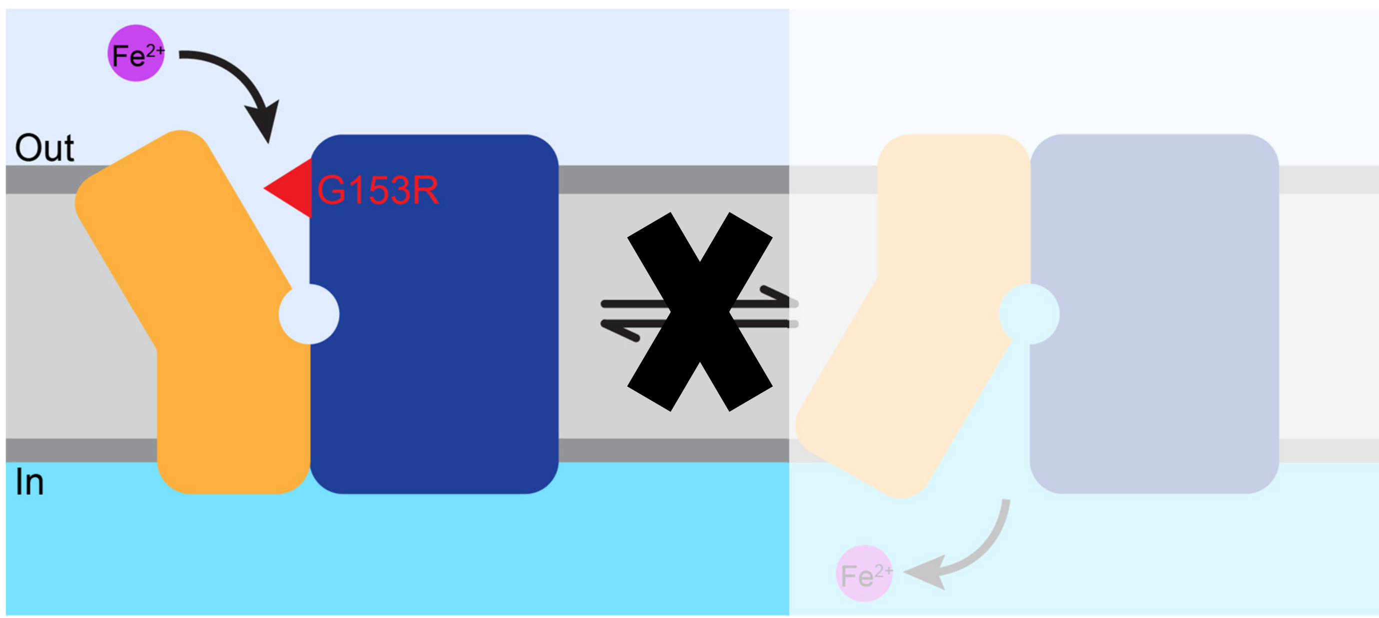
WT G45/75R G153/185R No protein

Mutations lock the conformation of the transporter

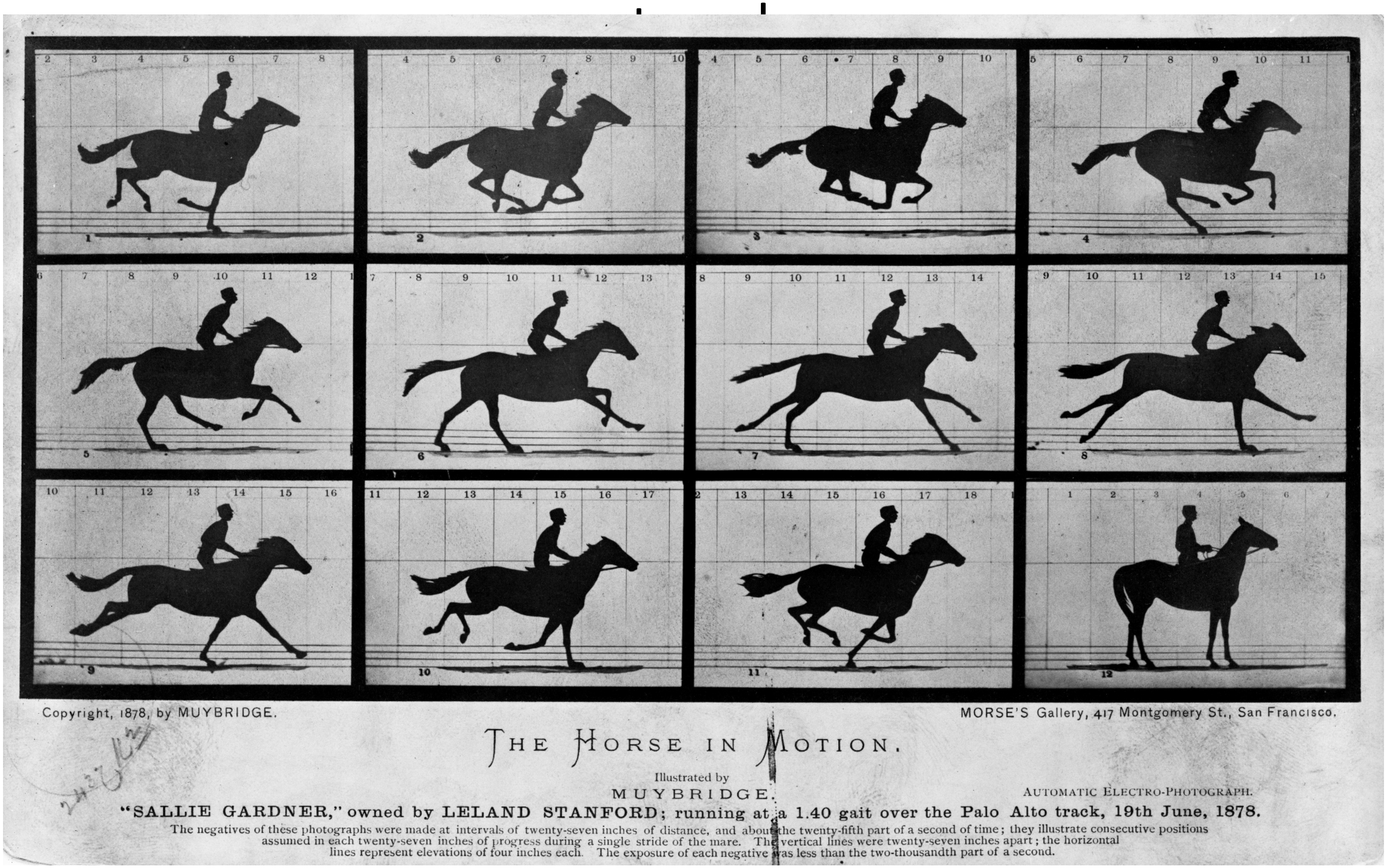
Mutation 1:



Mutation 2:

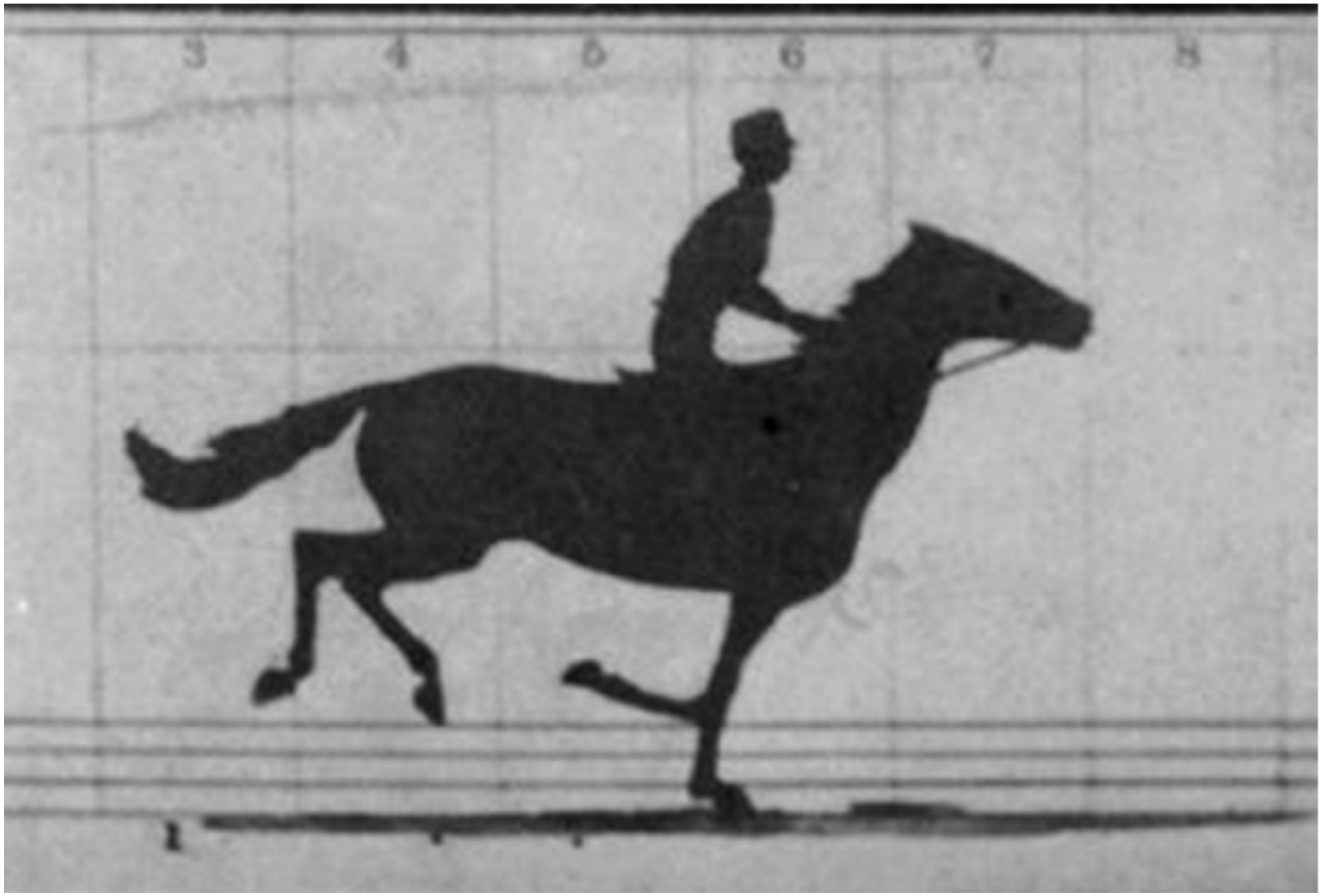


From several still images – reconstruct a movie of protein



Eadweard Muybridge Library of Congress Prints and Photographs Division
Public Domain, https://commons.wikimedia.org/w/index.php?curid=57260211

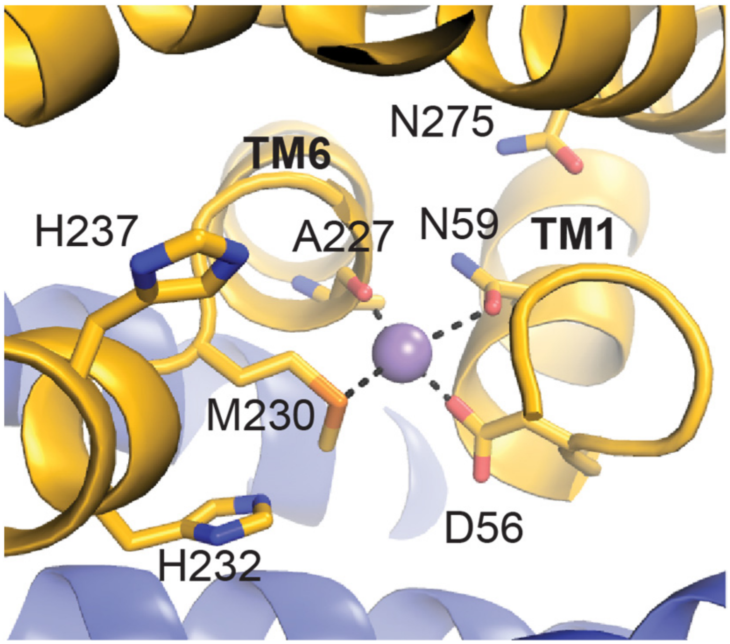
From several still images – reconstruct a movie of protein at work



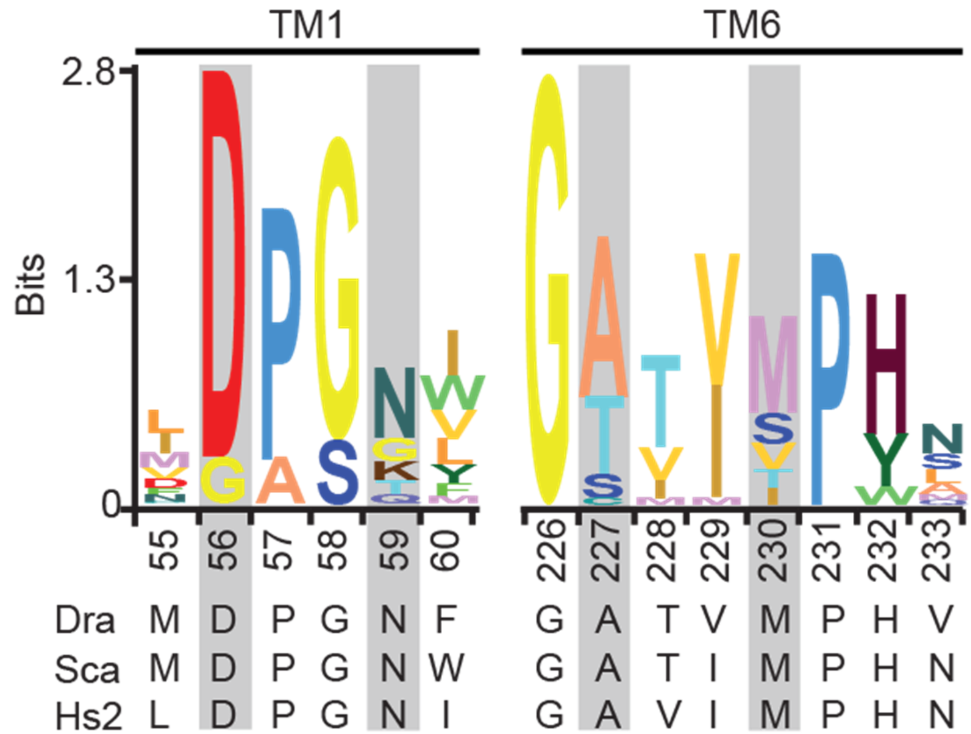
Eadweard Muybridge, Animation: Nevit Dilmen – Public Domain
Library of Congress Prints and Photographs Division http://hdl.loc.gov/loc.pnp/cph.3a45870

Nramp's metal site includes a methionine

Looking in from the intracellular vestibule:



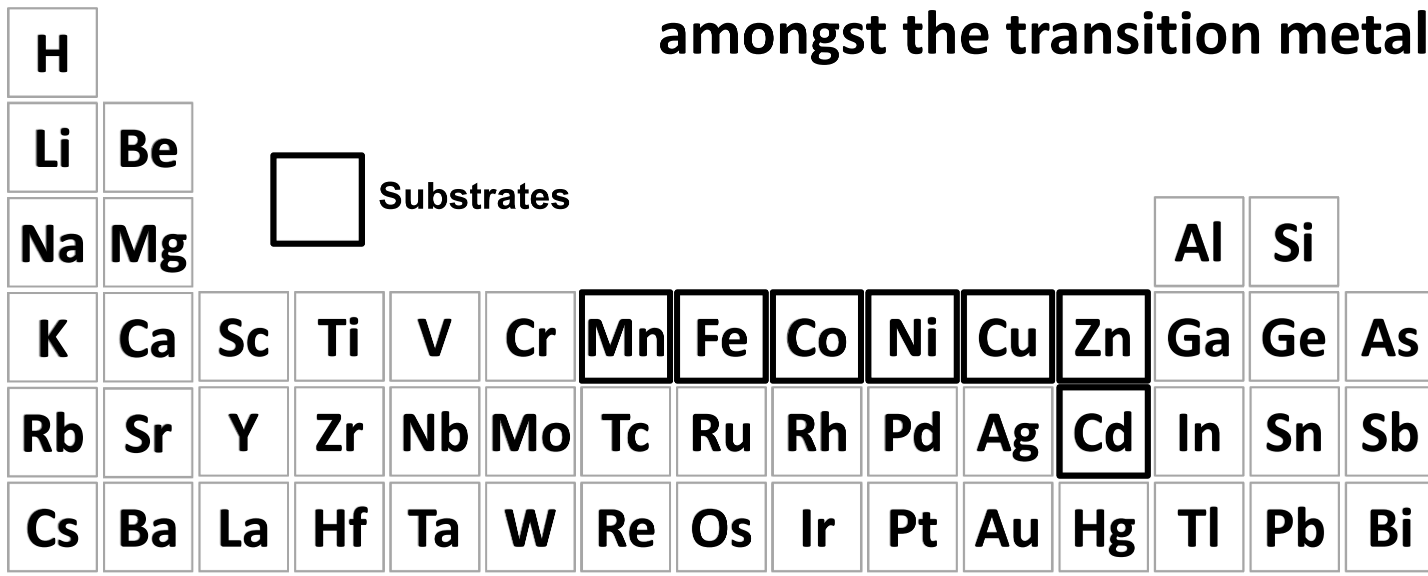
Sequence conservation over ~2,700 sequences is high:



S. capitis **Nramp structure from: Ehrnstorfer et al., NSMB, (2014), 21(11).**

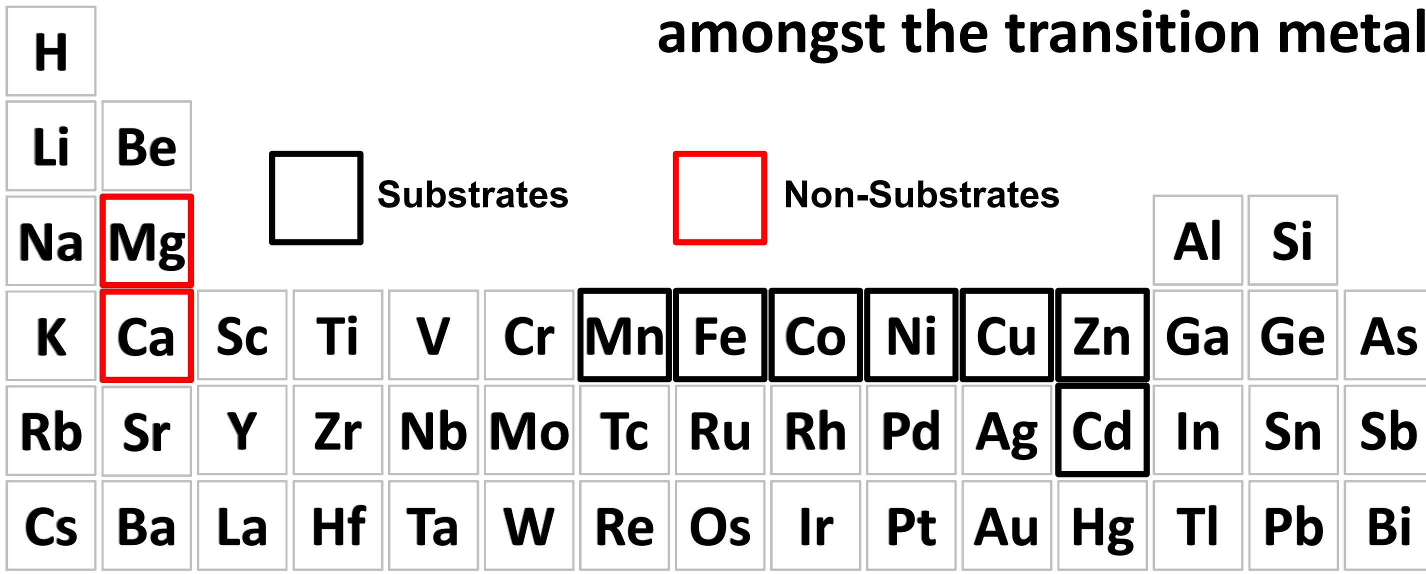
Nramps transport a range of transition metals

Nramps are not very discriminatory amongst the transition metals.



Nramps discriminate against divalent alkaline earth metal ions

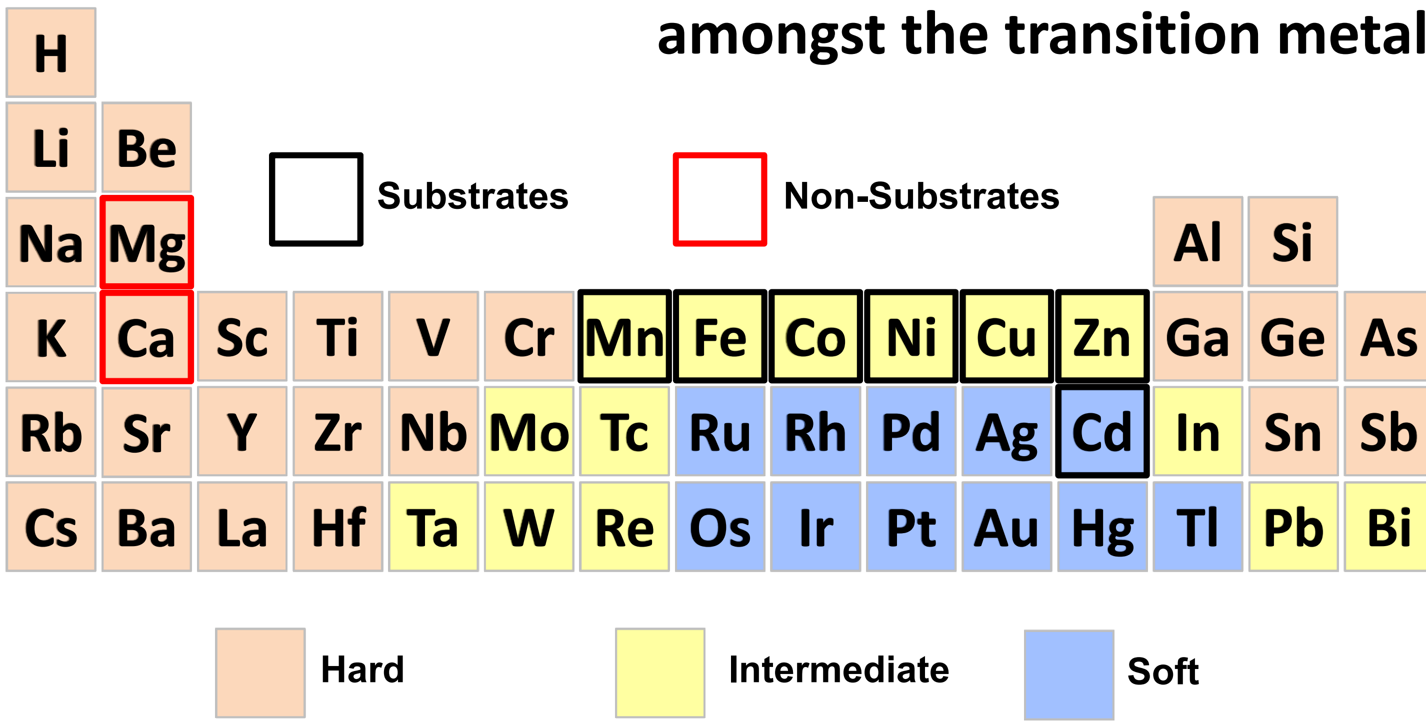
Nramps are not very discriminatory amongst the transition metals.



Nramps *do* discriminate against alkaline earth metal ions, which are orders of magnitude more abundant.

The hard soft acid base theory offers a potential mechanistic explanation

Nramps are not very discriminatory amongst the transition metals.



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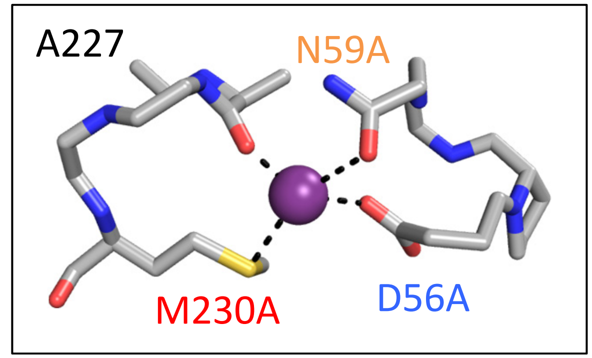
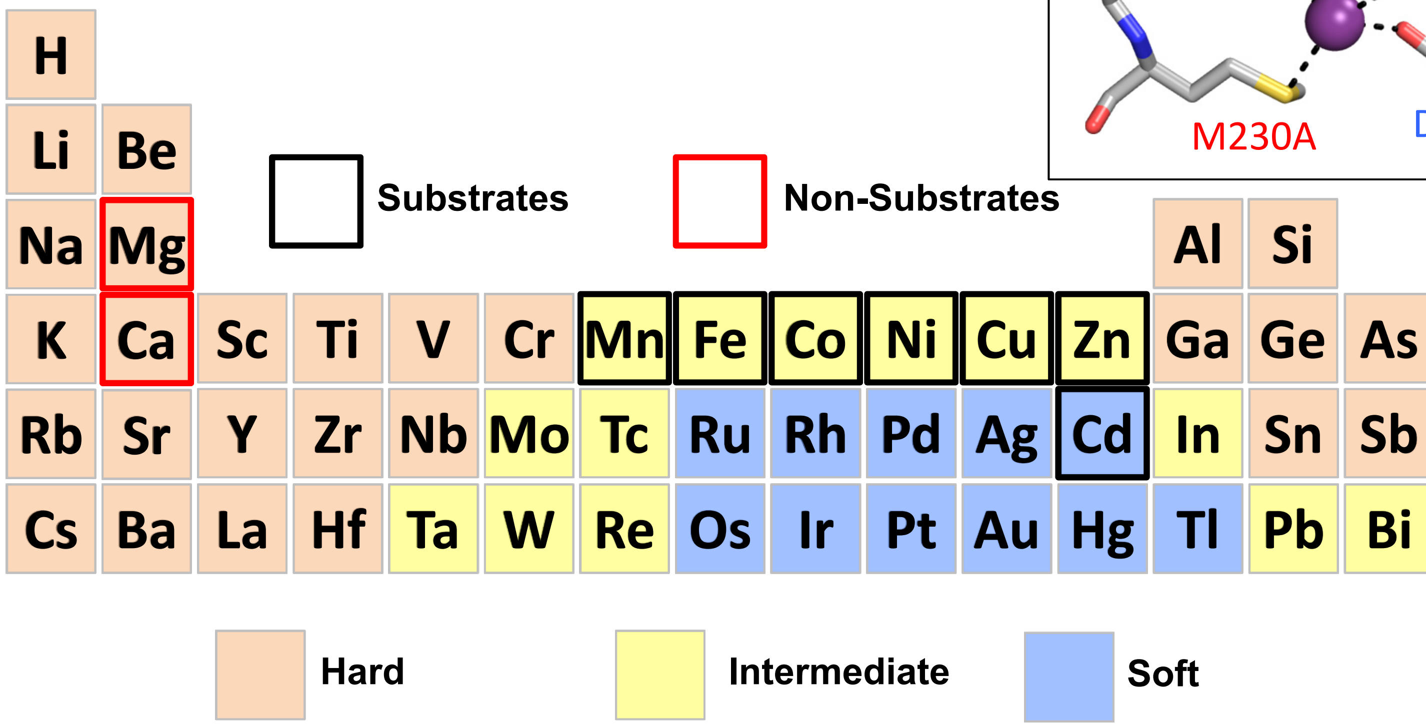
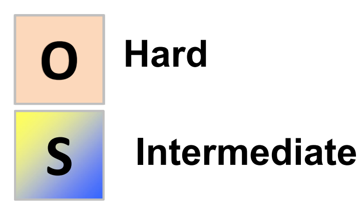
The hard soft acid base theory offers a potential mechanistic explanation

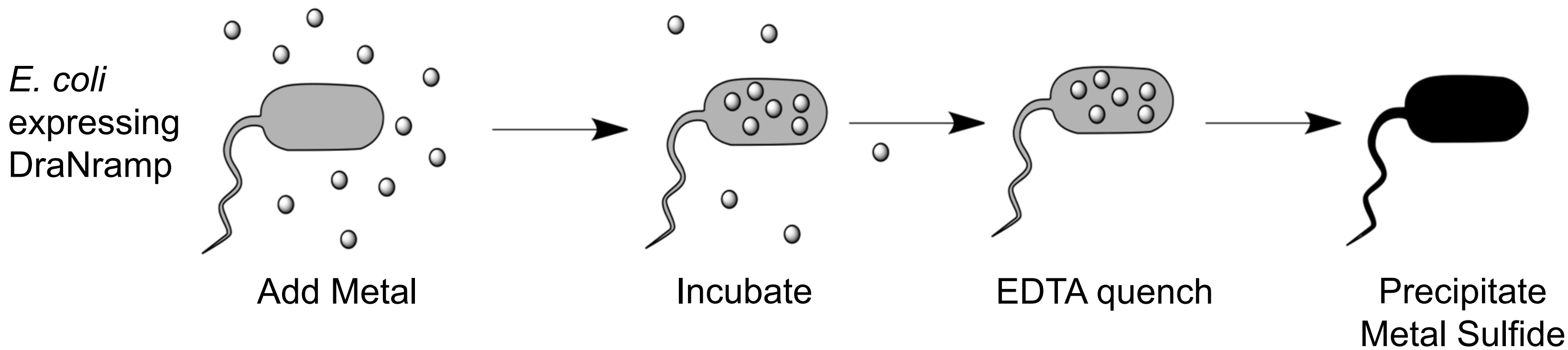


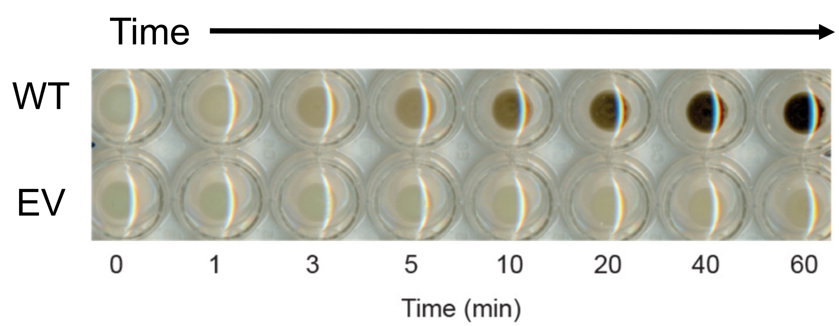


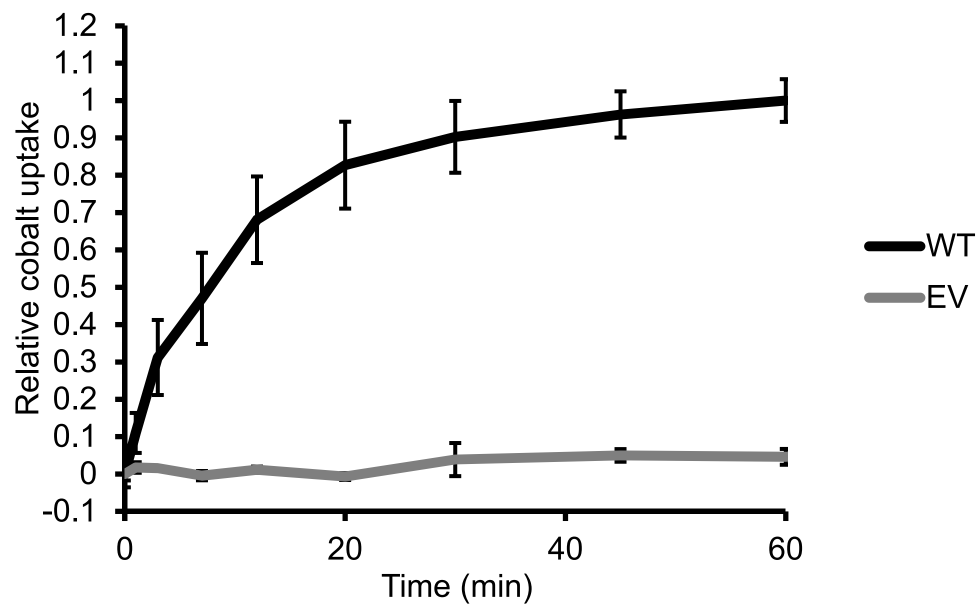
Intermediate base ligand like sulfur will favor transition metals



In vivo metal-uptake assay to assess transport function of DraNramp

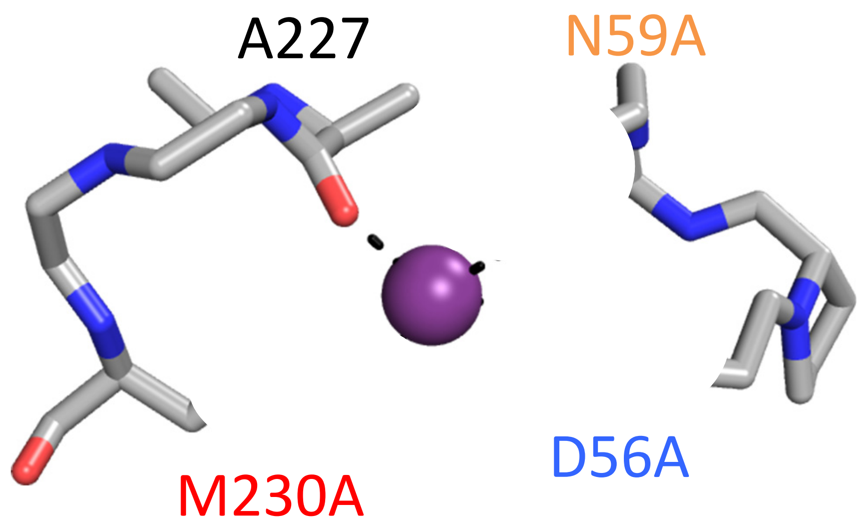


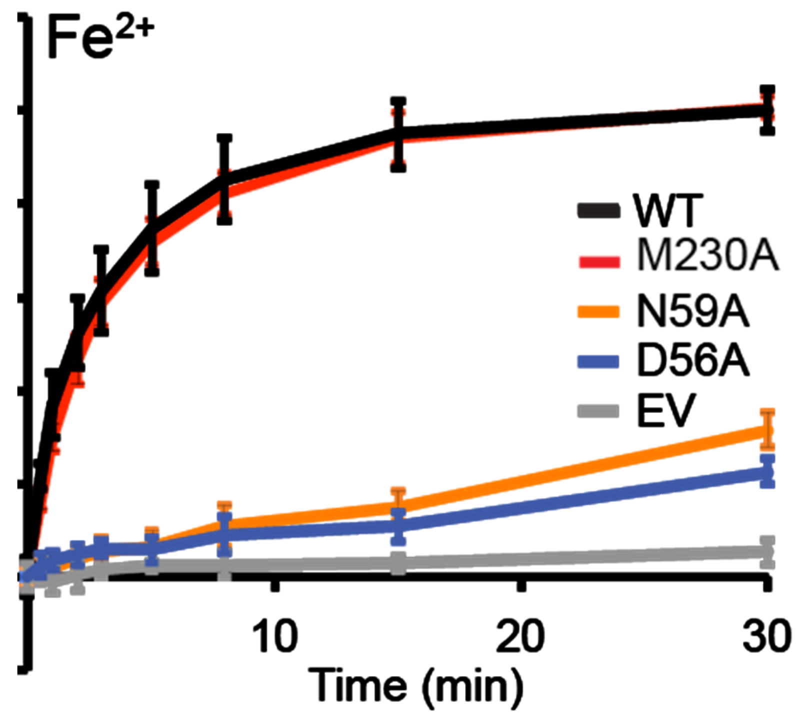




Anne McCabe and Aaron Bozzi

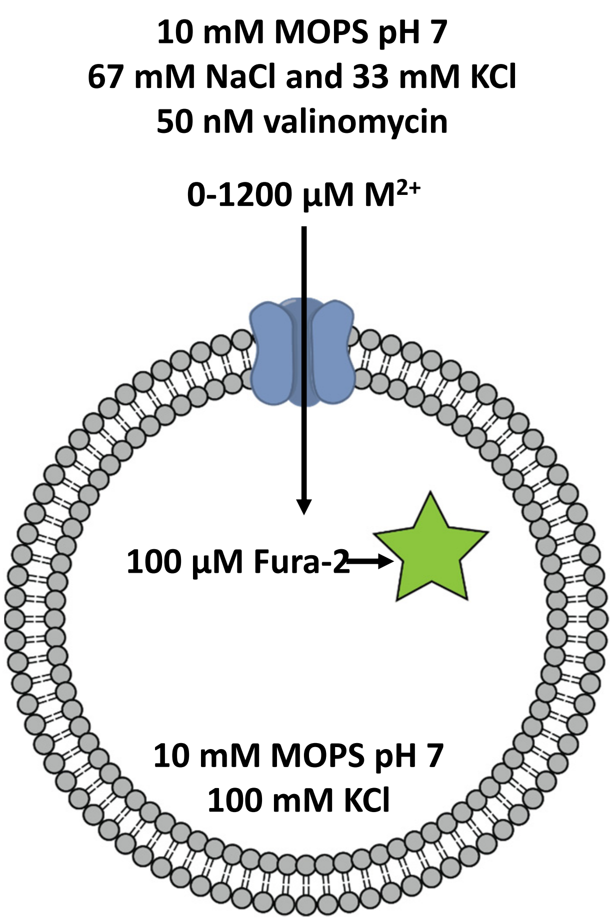
Conserved methionine is not required for Co^{2+} or Fe^{2+} uptake

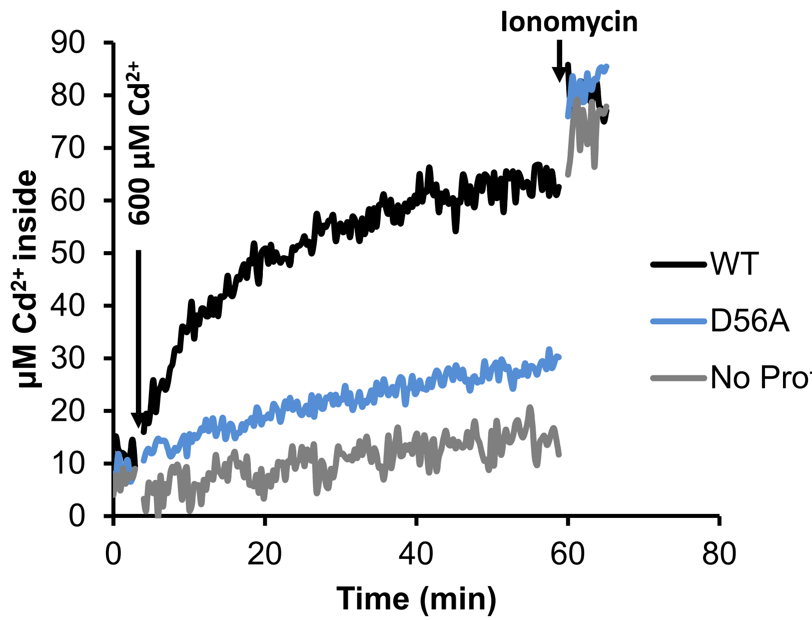


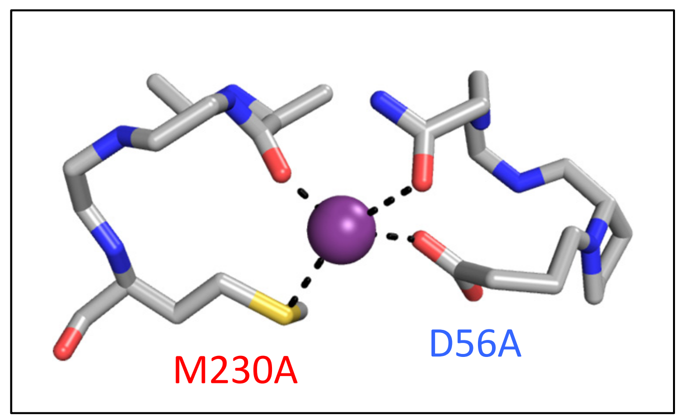


Aaron Bozzi

In vitro assay allows for the direct monitoring of transport

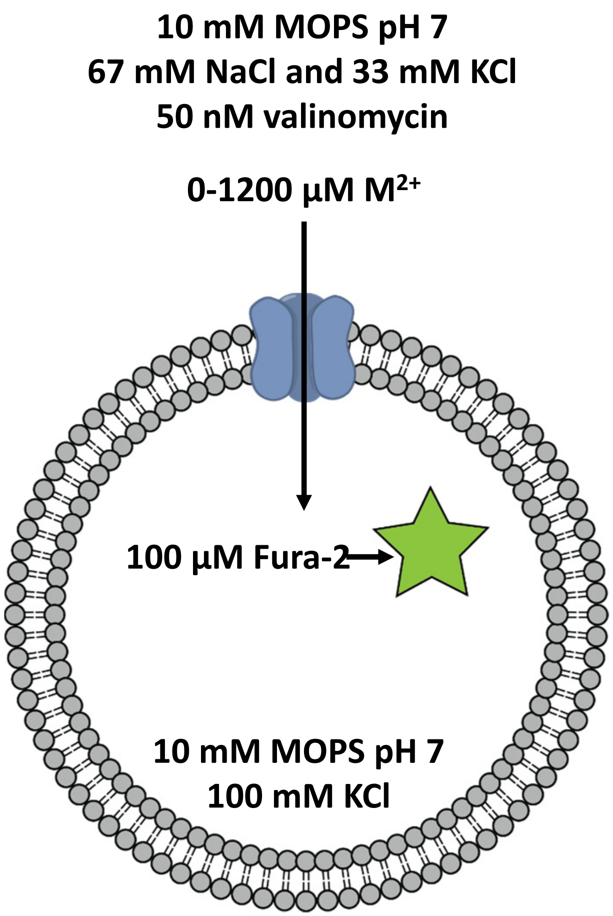


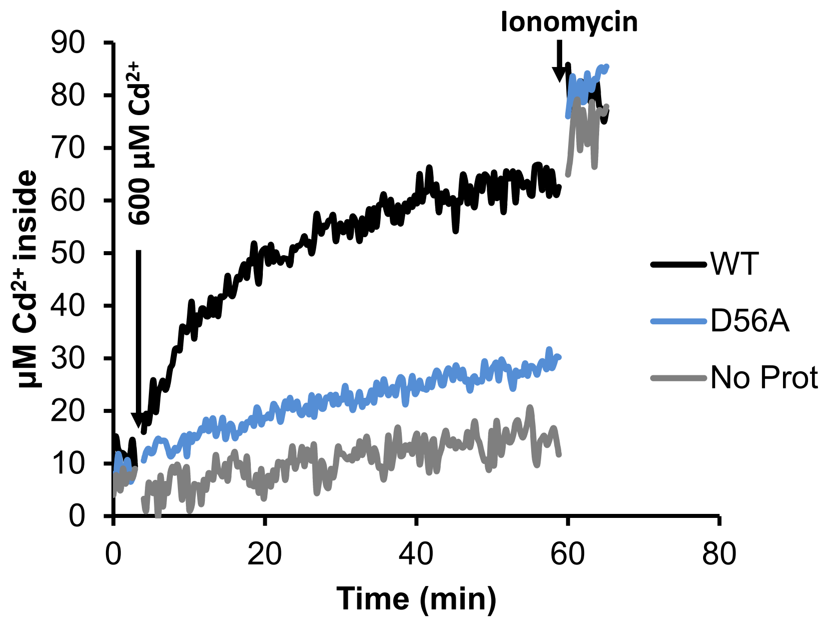


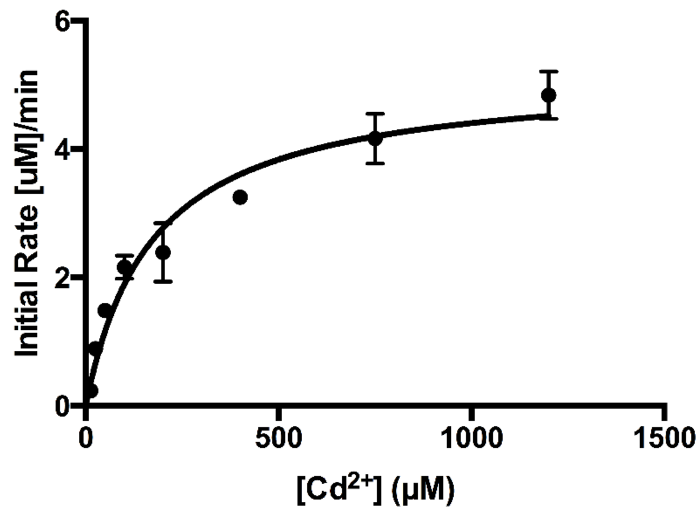


Lukas Bane and Aaron Bozzi

In vitro assay allows for the direct monitoring of transport



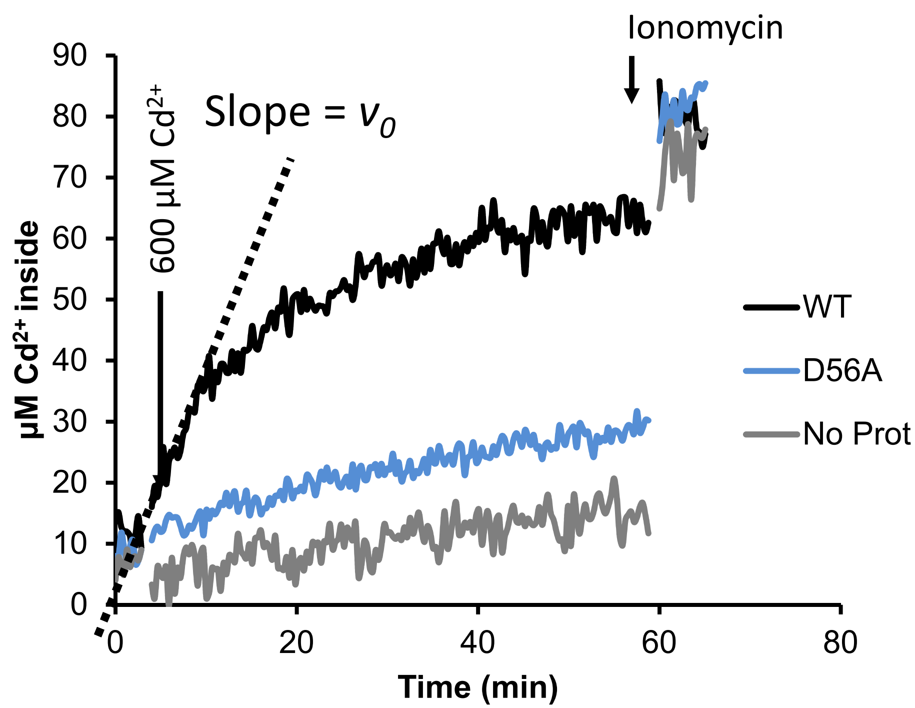




Lukas Bane and Aaron Bozzi

From kinetic data to enzyme kinetic constants

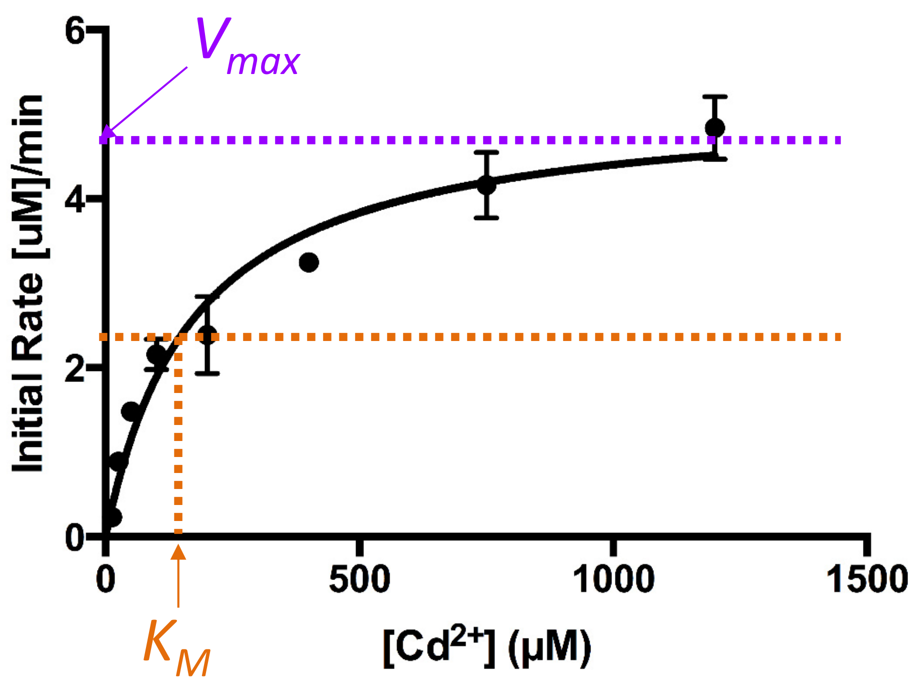
Step 1: getting initial velocity (v_0) from time course plots



- Initial velocity (v_0) is the rate of transport in the linear phase of the time course
- Fit the data to a line to get the slope
- Repeat this process with data at different concentrations of substrate (here, Cd^{2+})

From kinetic data to enzyme kinetic constants

Step 2: from the initial velocity (v_0) vs. substrate concentration, obtain kinetic constants, K_M and V_{max}

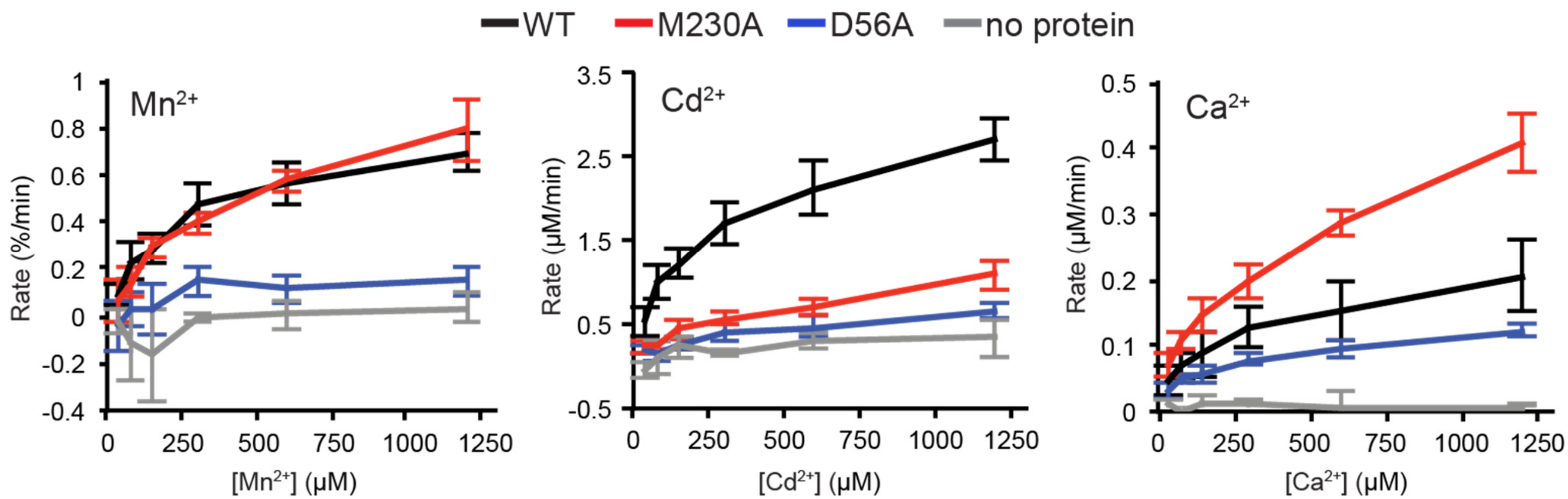


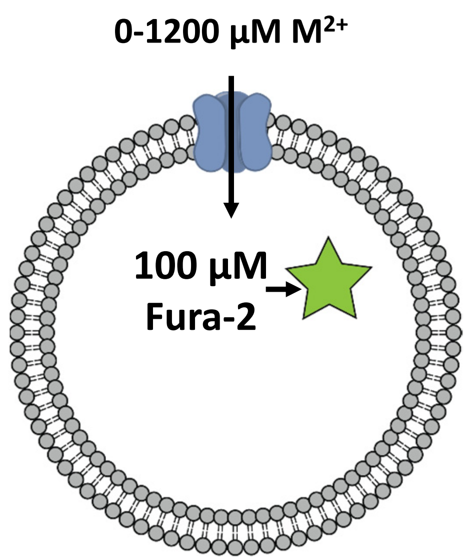
Plot initial velocity (v_0) vs substrate concentration

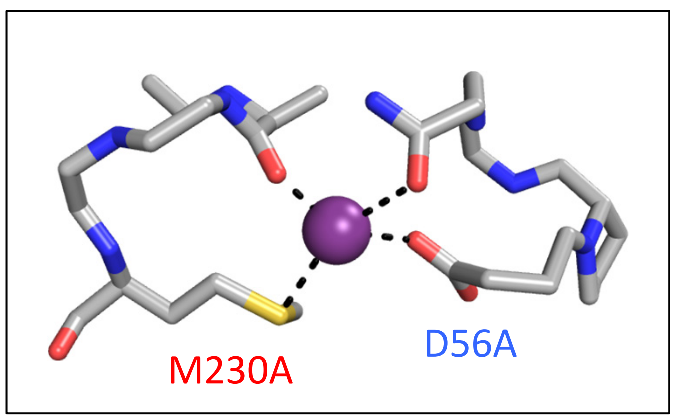
V_{max} is the maximal velocity (transporter is saturated with substrate)

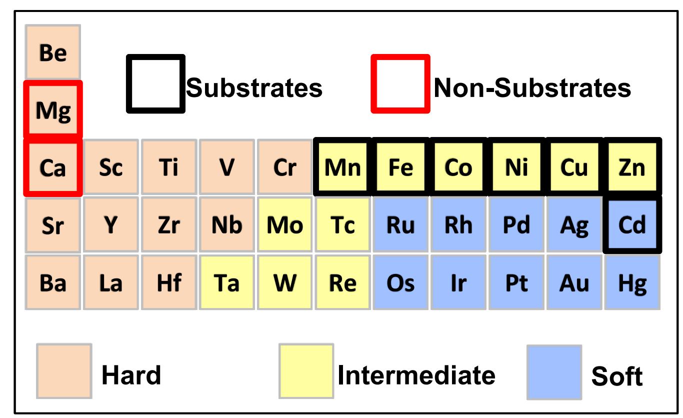
K_M is the concentration of substrate at which the velocity is half maximal

Metal-binding site methionine prevents enables cadmium but prevents calcium (and magnesium) import



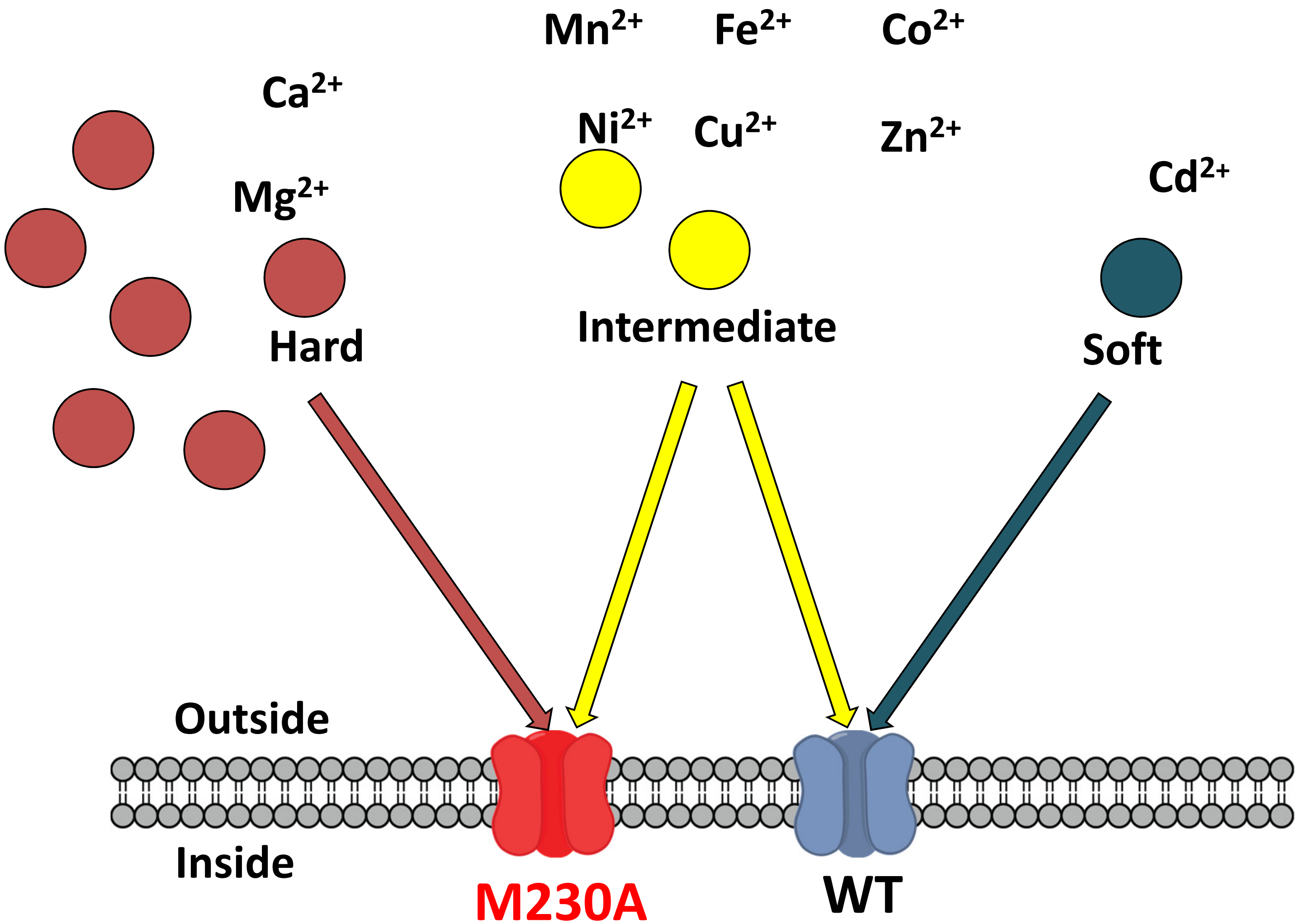


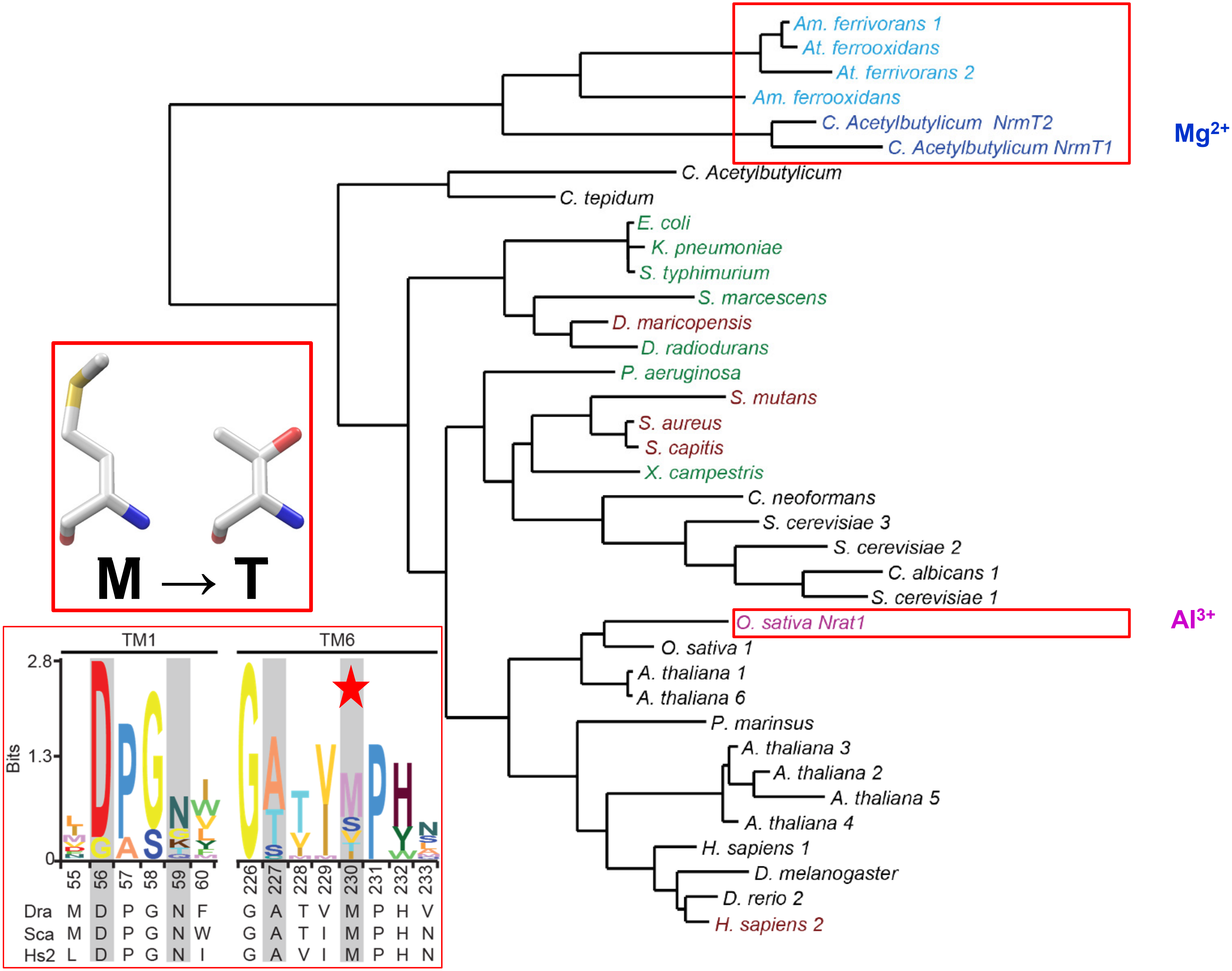




Lukas Bane and Aaron Bozzi

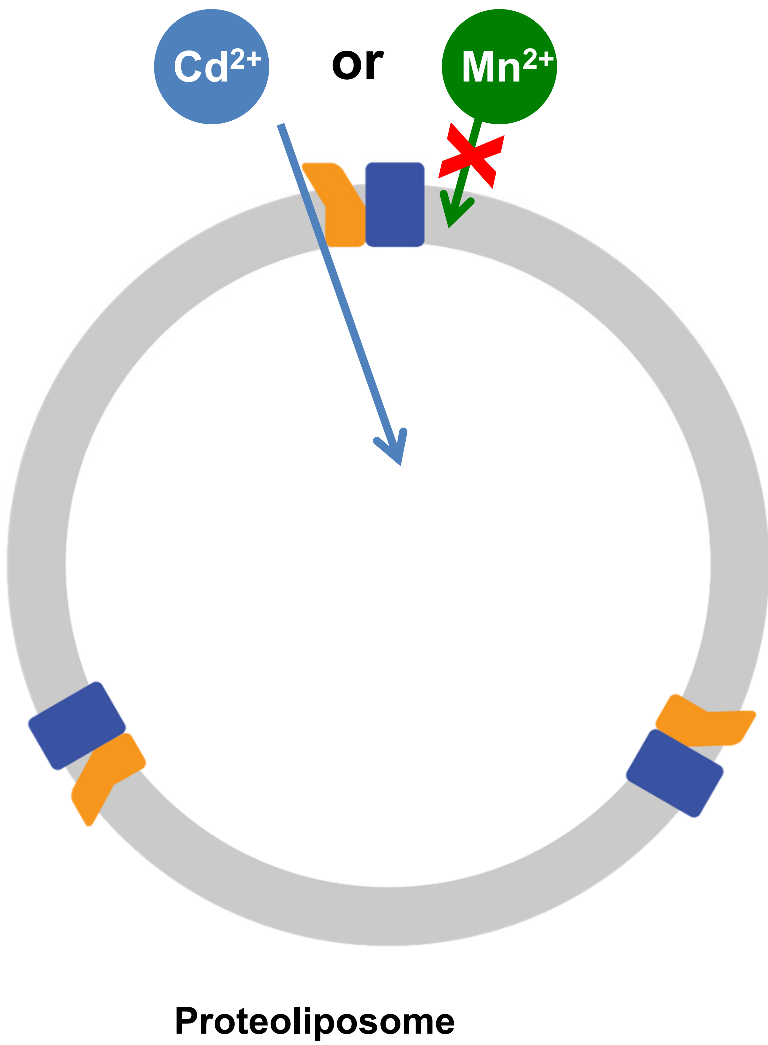
M230 likely represents an evolutionary trade-off

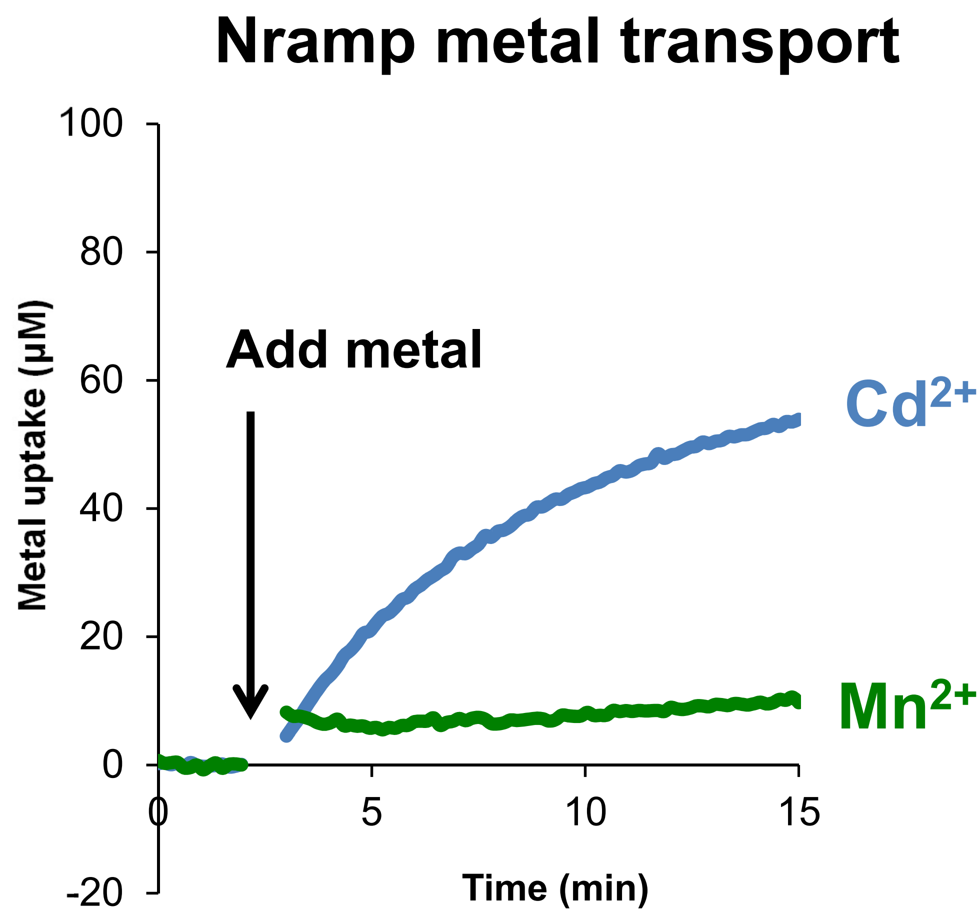




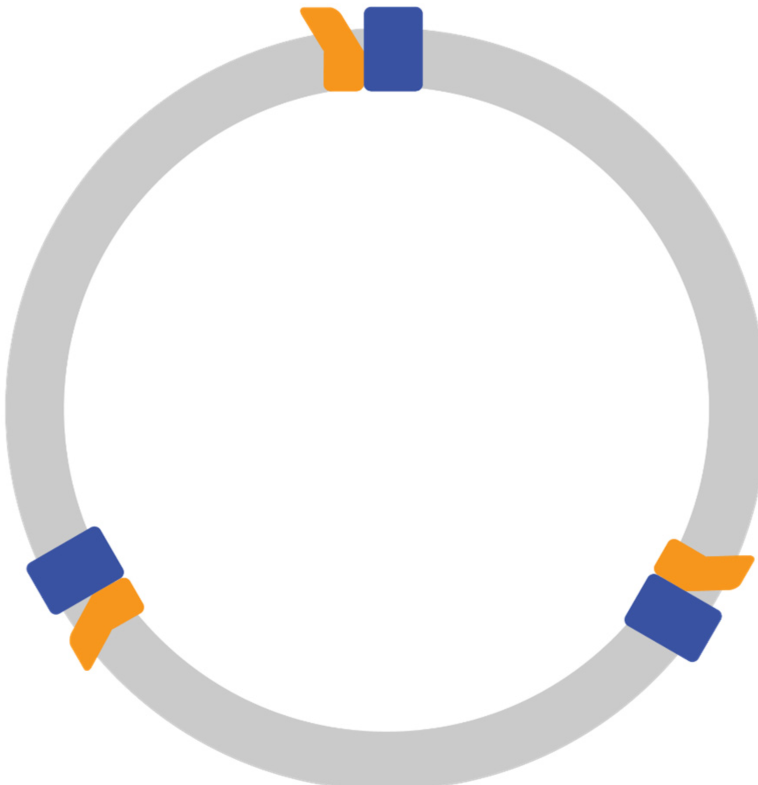
Am. ferrivorans 1
At. ferrooxidans
At. ferrivorans 2
Am. ferrooxidans
C. Acetylbutylicum NrmT2
C. Acetylbutylicum NrmT1
Mg2+
C. Acetylbutylicum
C. tepidum
E. coli
K. pneumoniae
S. typhimurium
S. marcescens
D. maricopensis
D. radiodurans
P. aeruginosa
S. mutans
S. aureus
S. capitis
X. campestris
C. neoformans
S. cerevisiae 3
S. cerevisiae 2
C. albicans 1
S. cerevisiae 1
O. sativa Nrat1
Al3+
O. sativa 1
A. thaliana 1
A. thaliana 6
P. marinsus
A. thaliana 3
A. thaliana 2
A. thaliana 5
A. thaliana 4
H. sapiens 1
D. melanogaster
D. rerio 2
H. sapiens 2
M → T
TM1
TM6
Bits
2.8
1.3
0
55 56 57 58 59 60
226 227 228 229 230 231 232 233
Dra M D P G N F G A T V M P H V
Sca M D P G N W G A T I M P H N
Hs2 L D P G N I G A V I M P H N

Why can't we measure Mn^{2+} transport in vitro?





What’s different in living cells?



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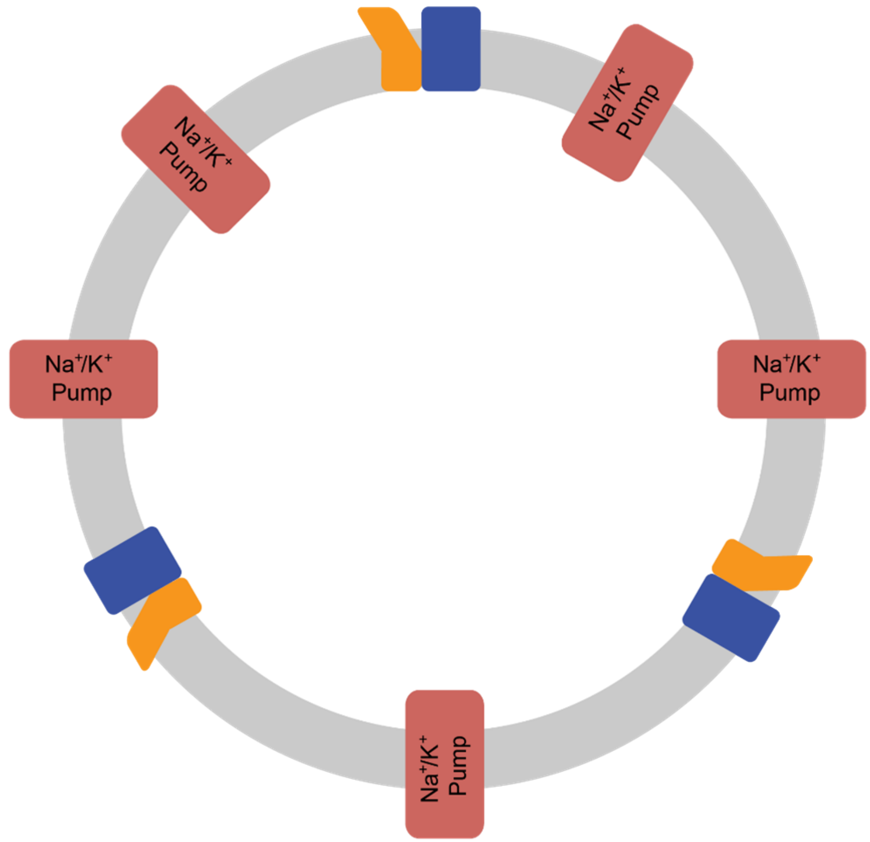

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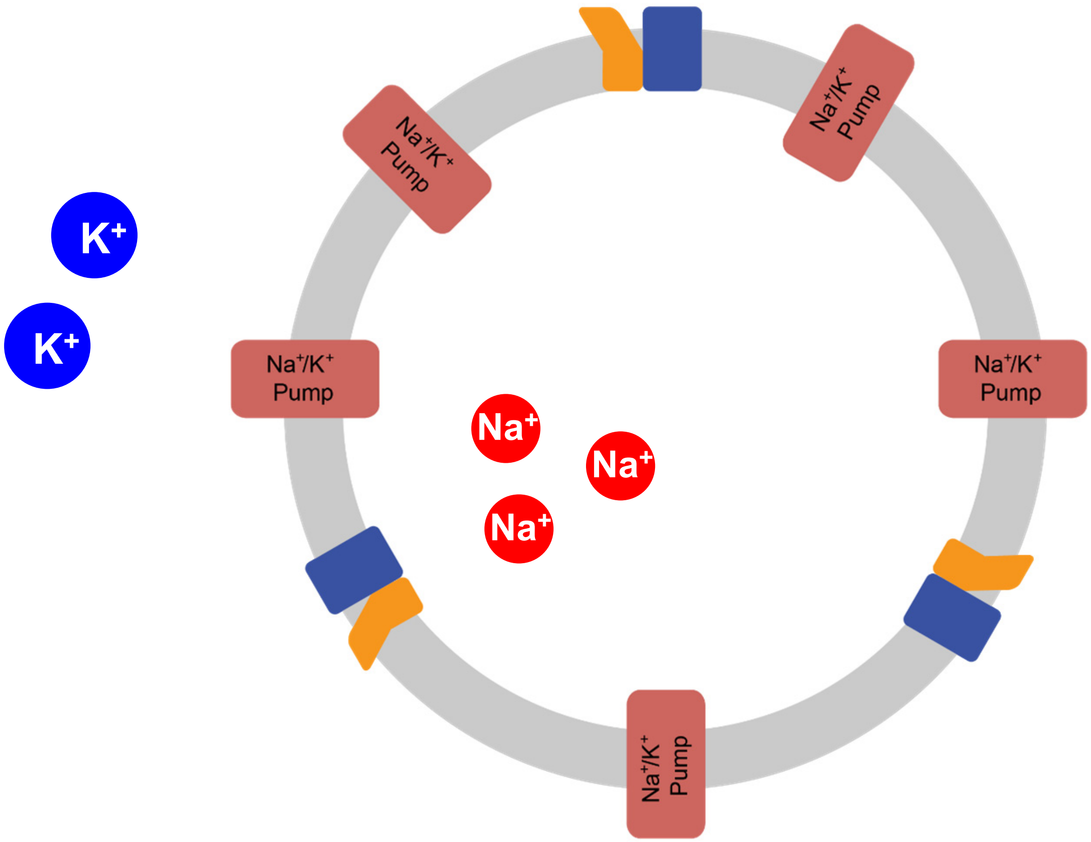

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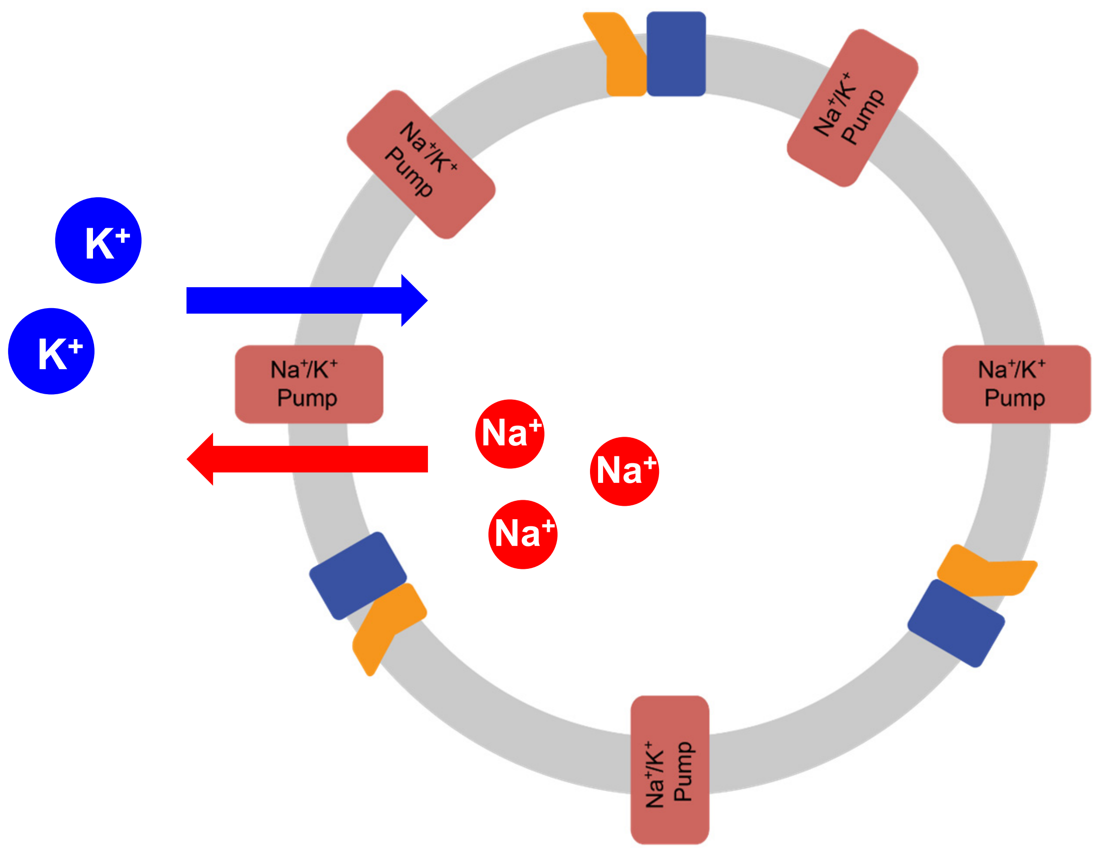

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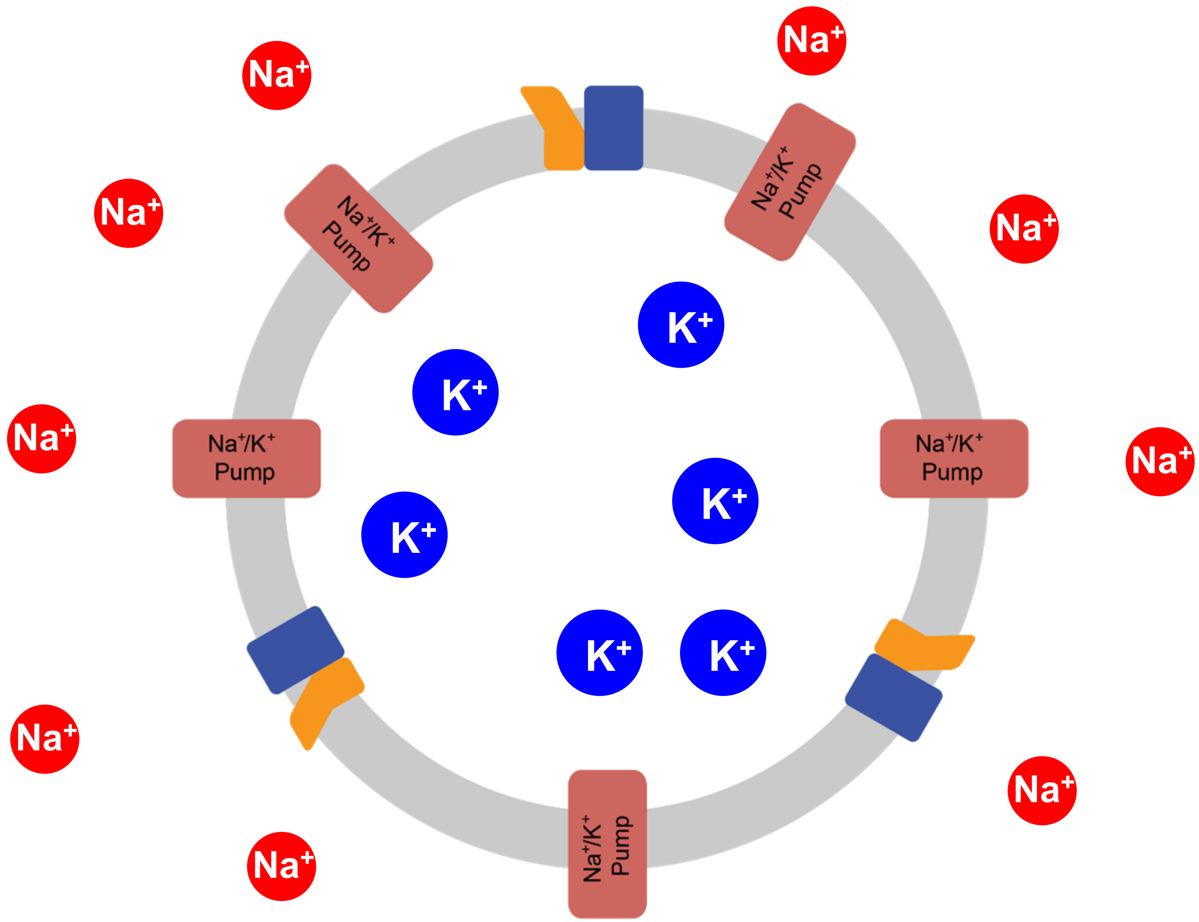

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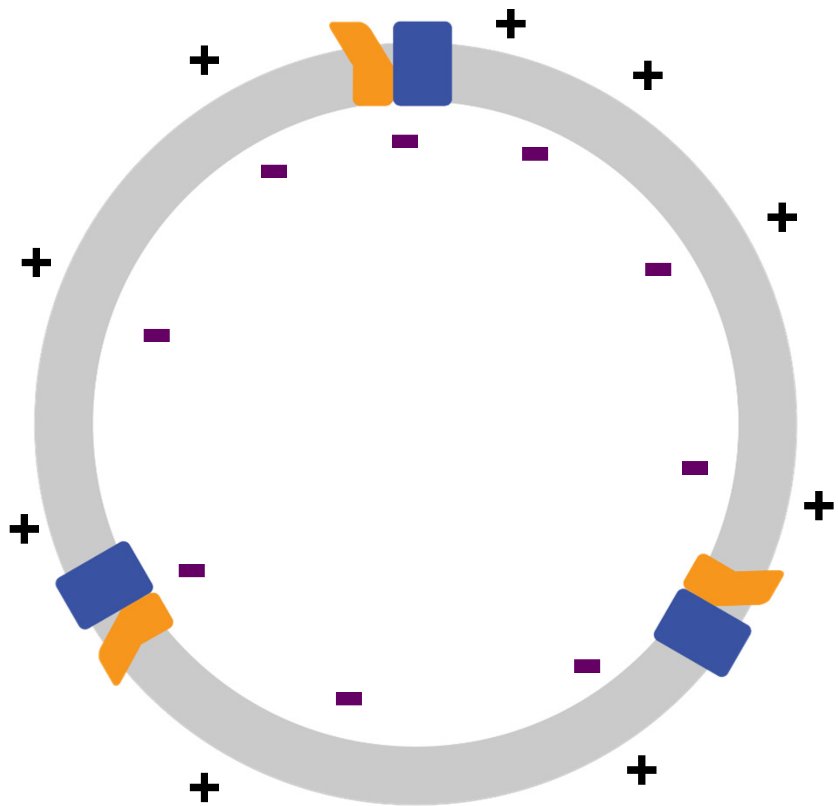
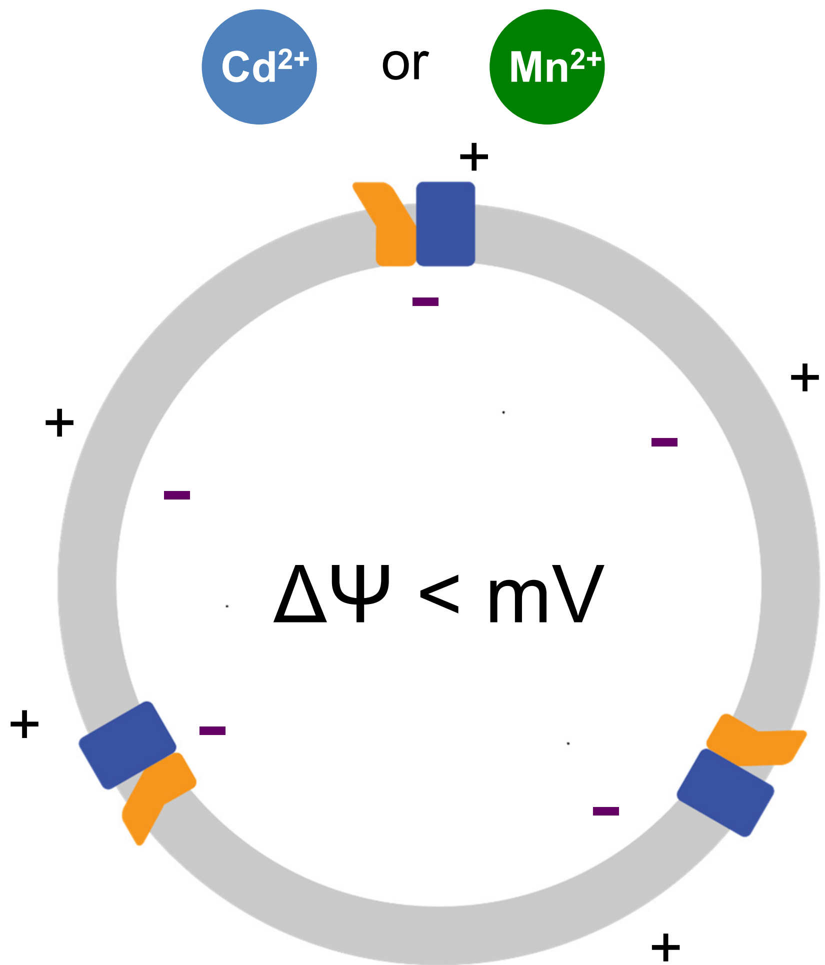


Membrane potential (ΔΨ) ≈ -140 mV in bacteria

Can we observe a membrane potential effect in vitro?



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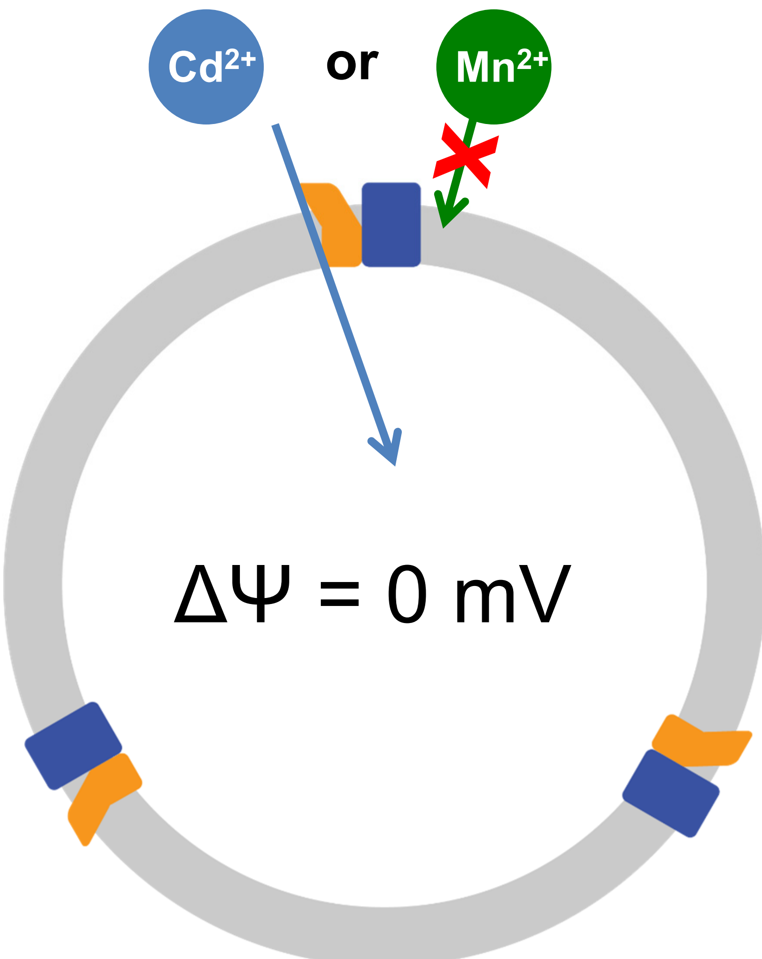

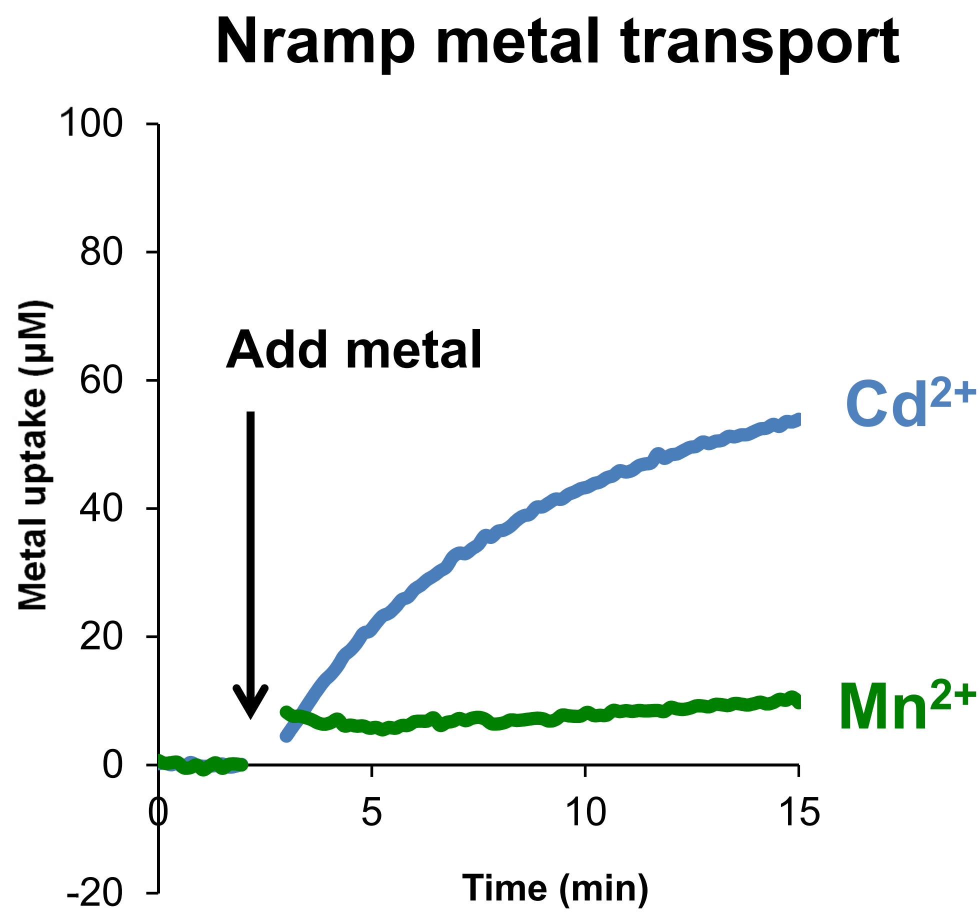

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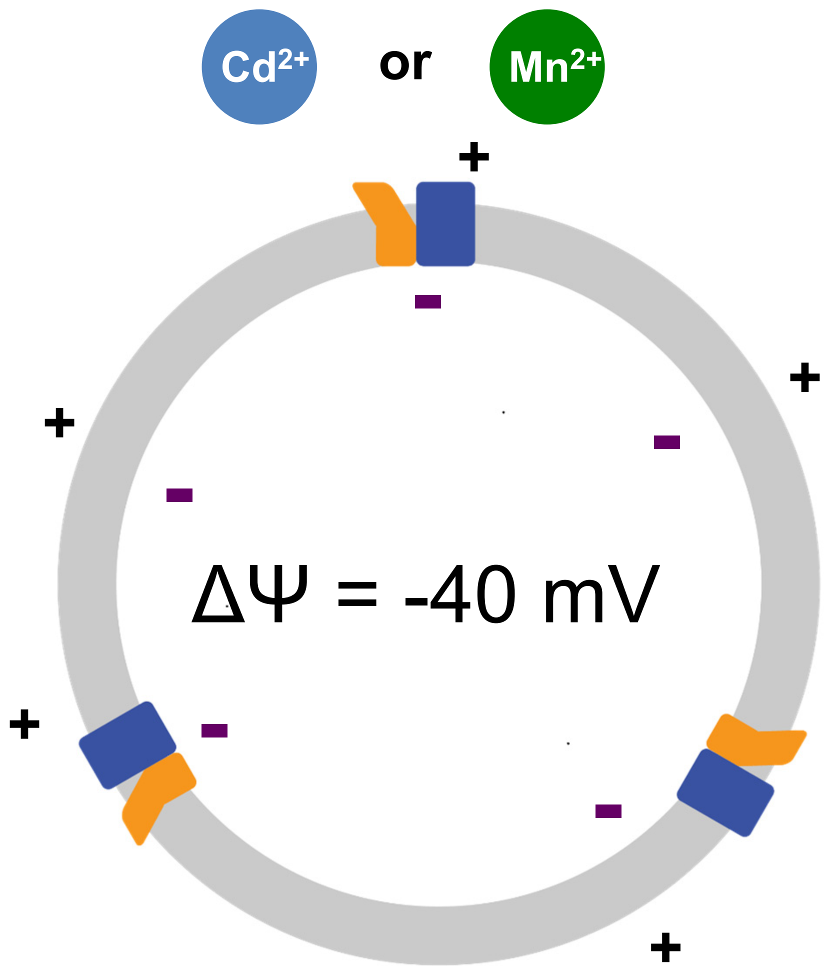

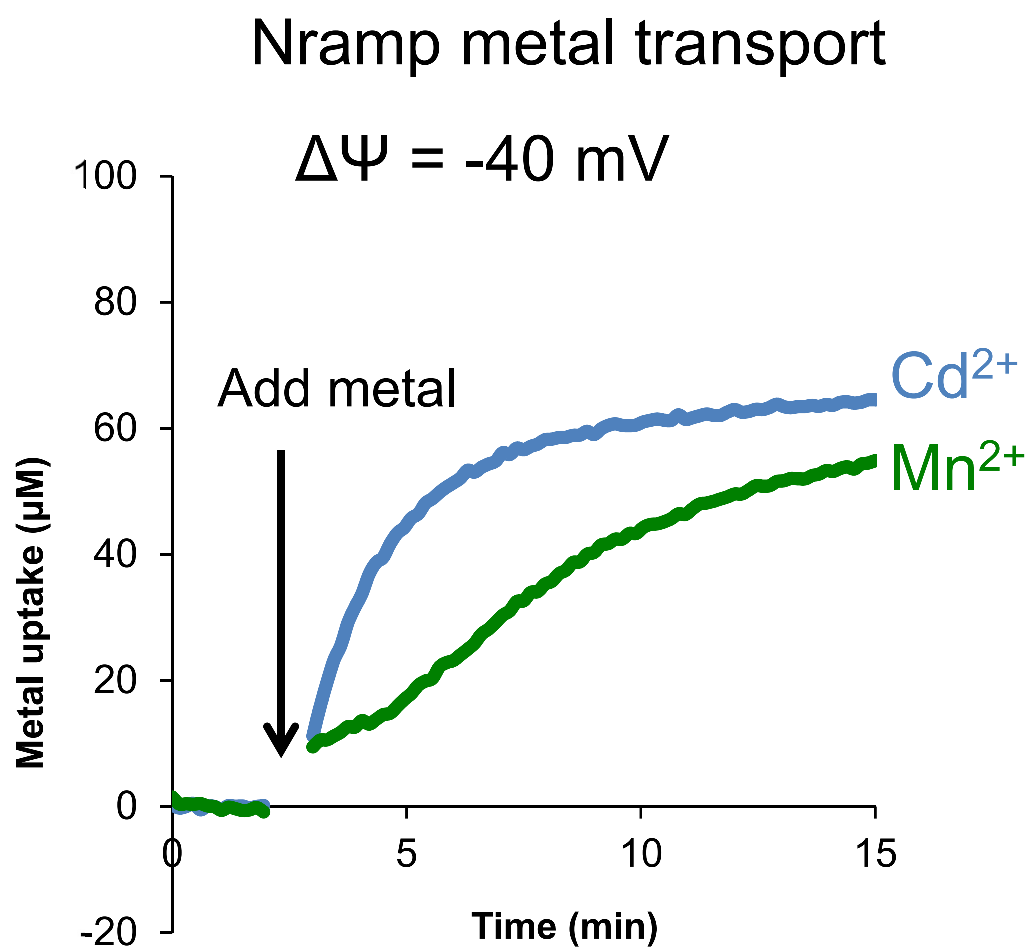

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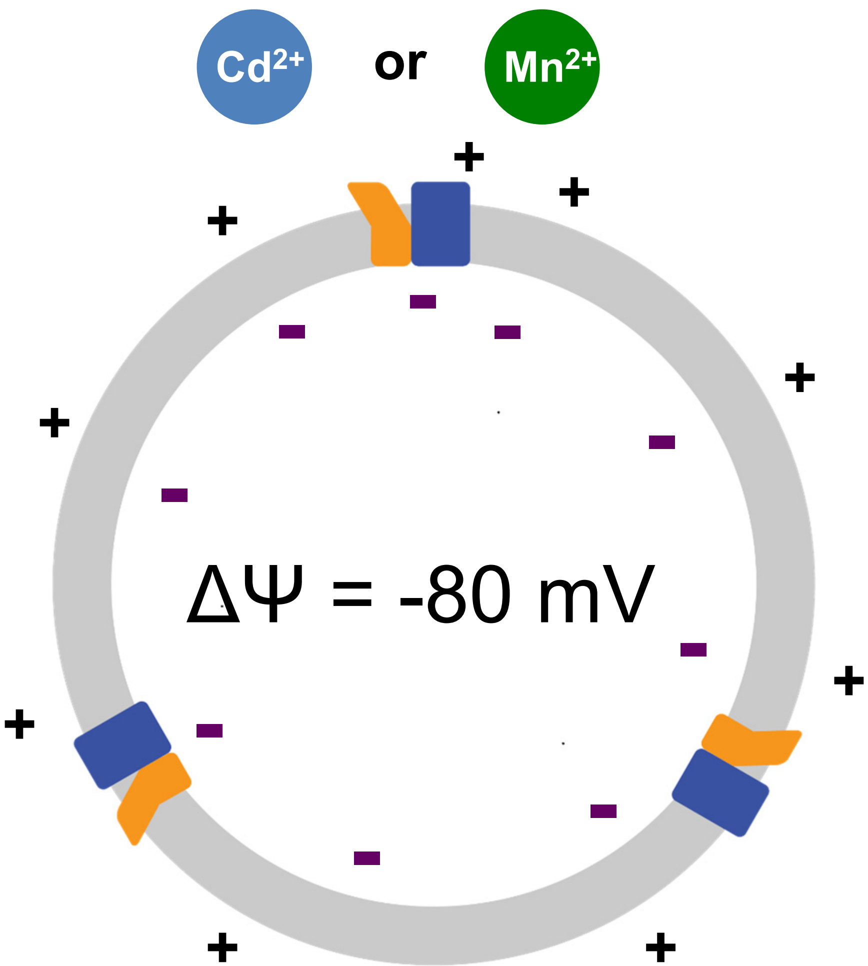
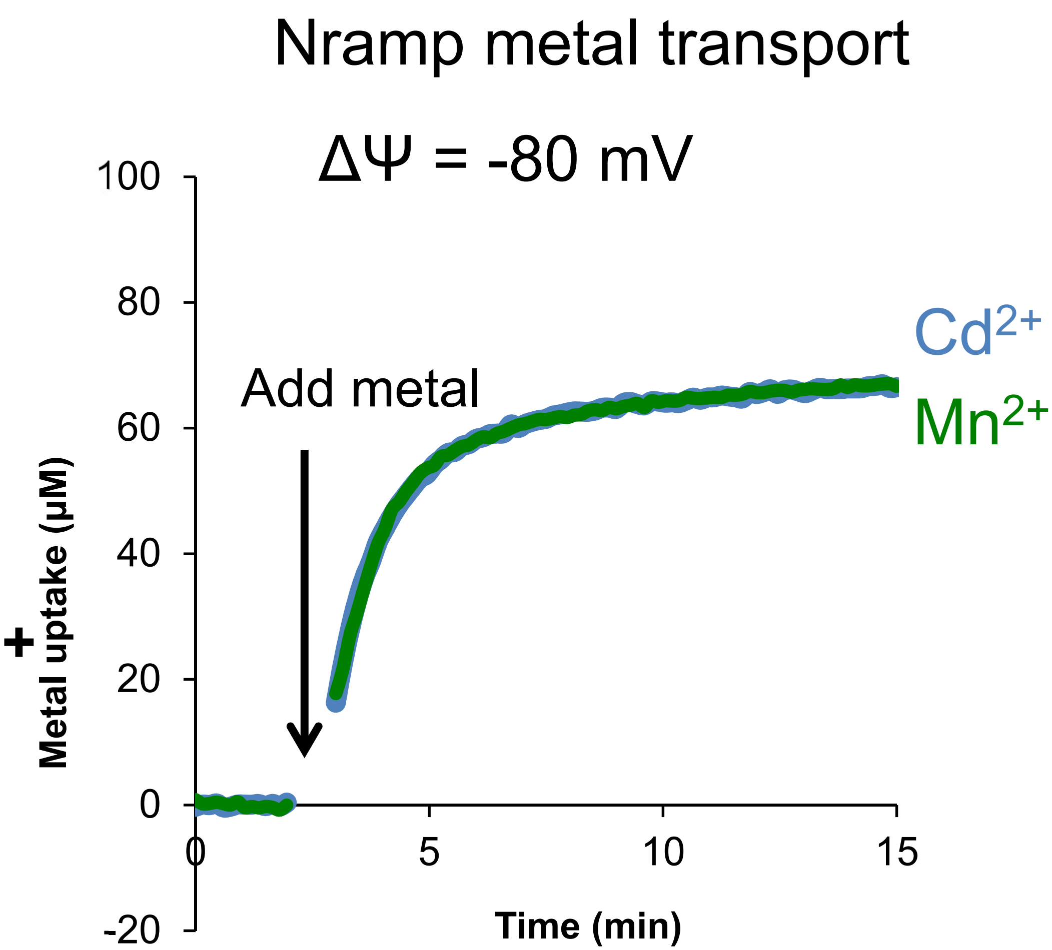
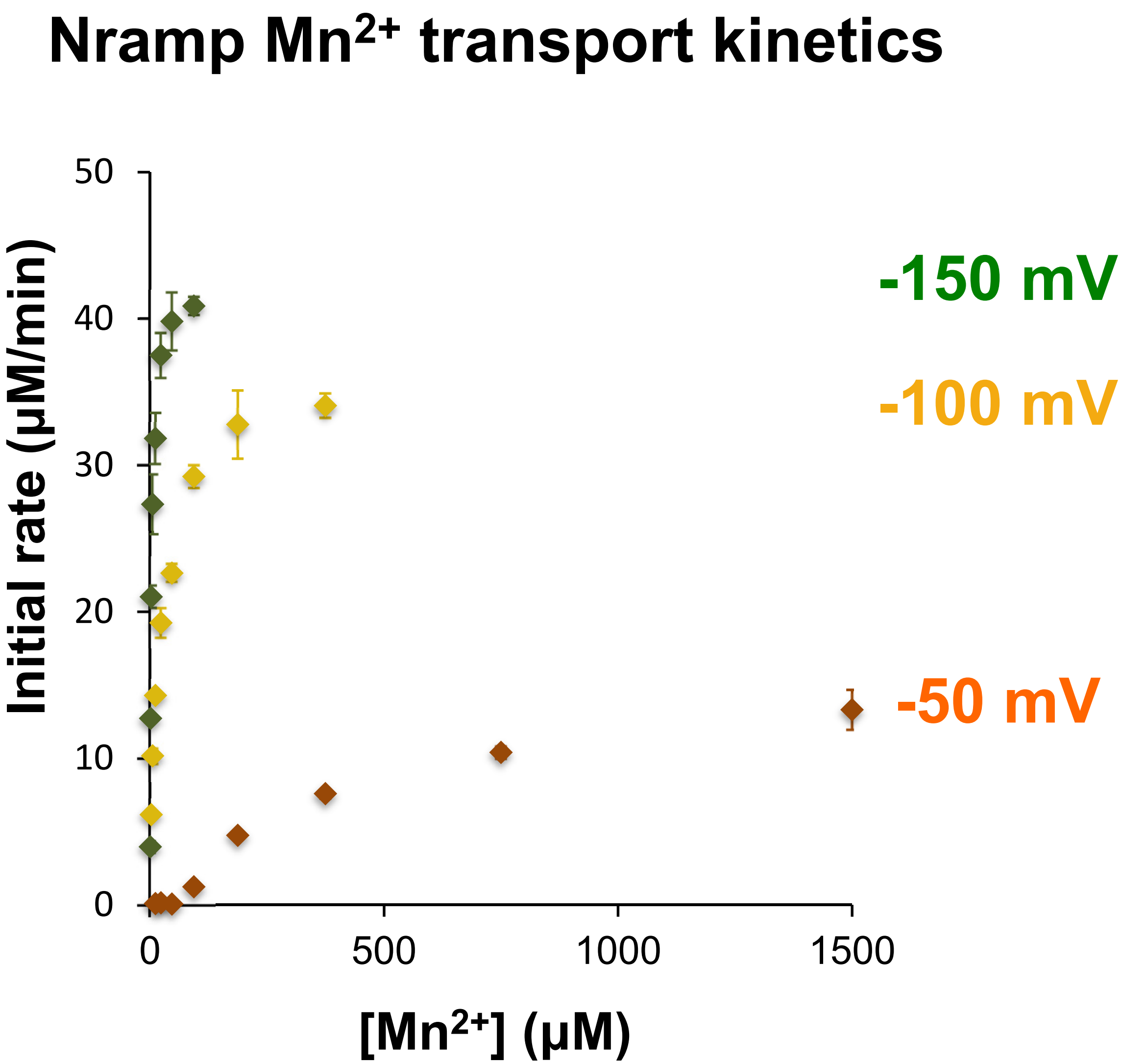
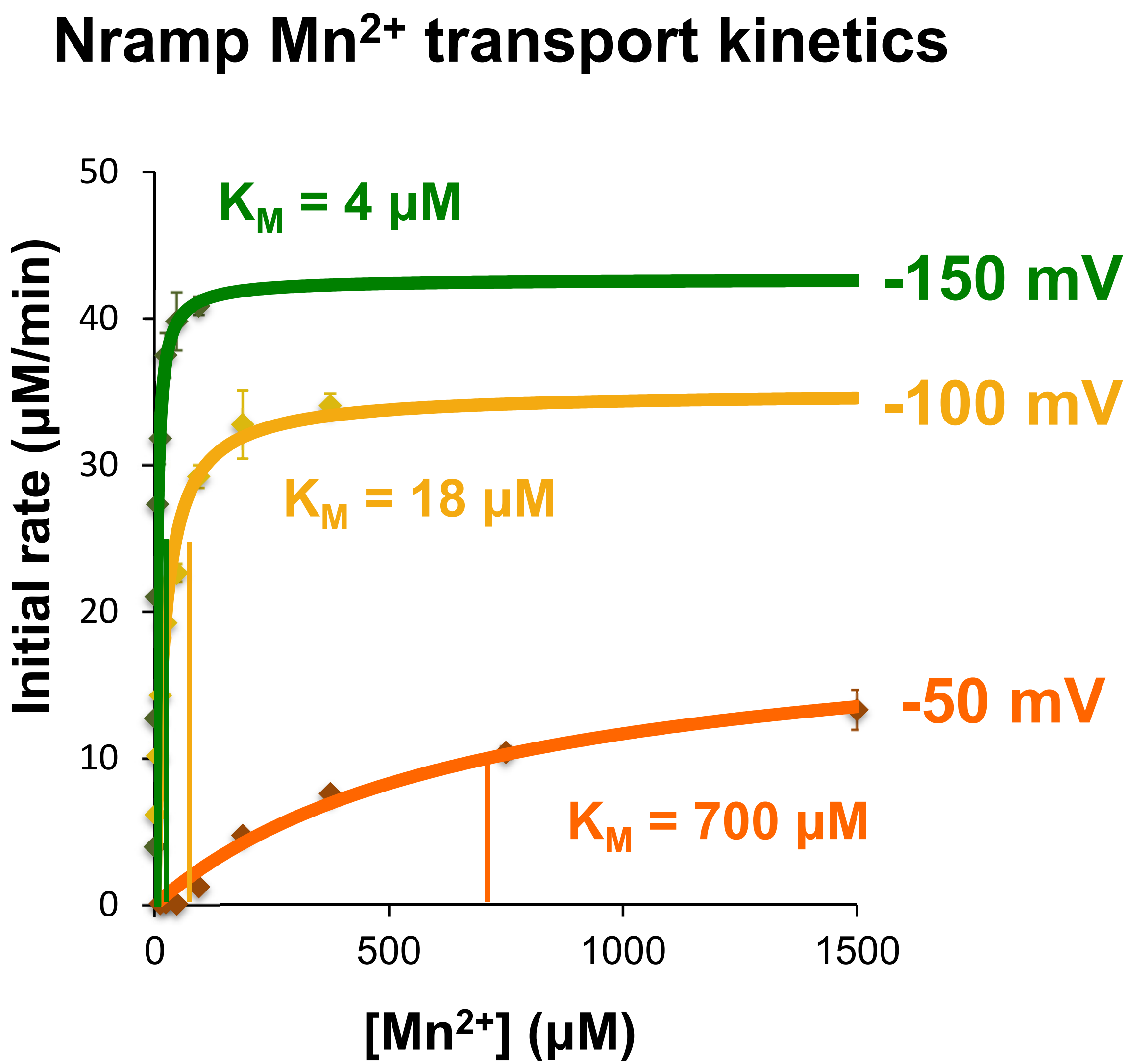


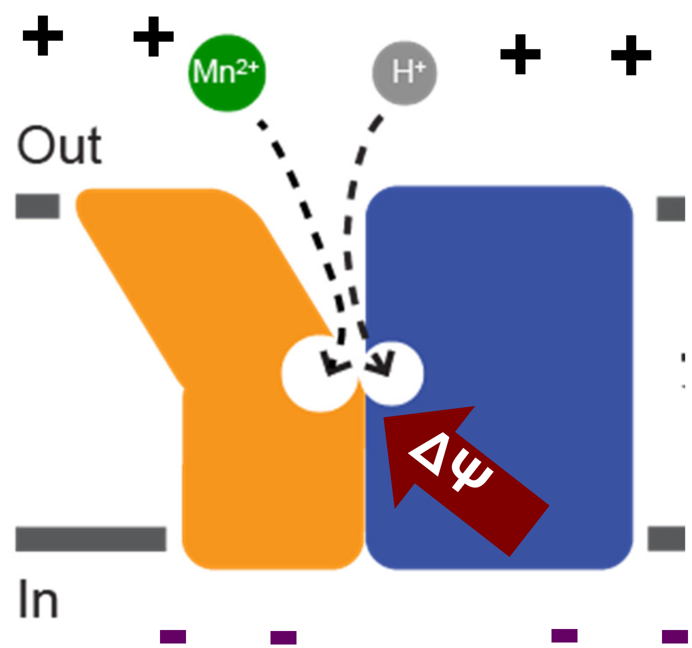


Membrane potential perturbs apparent metal binding affinity



Membrane potential perturbs apparent metal binding affinity



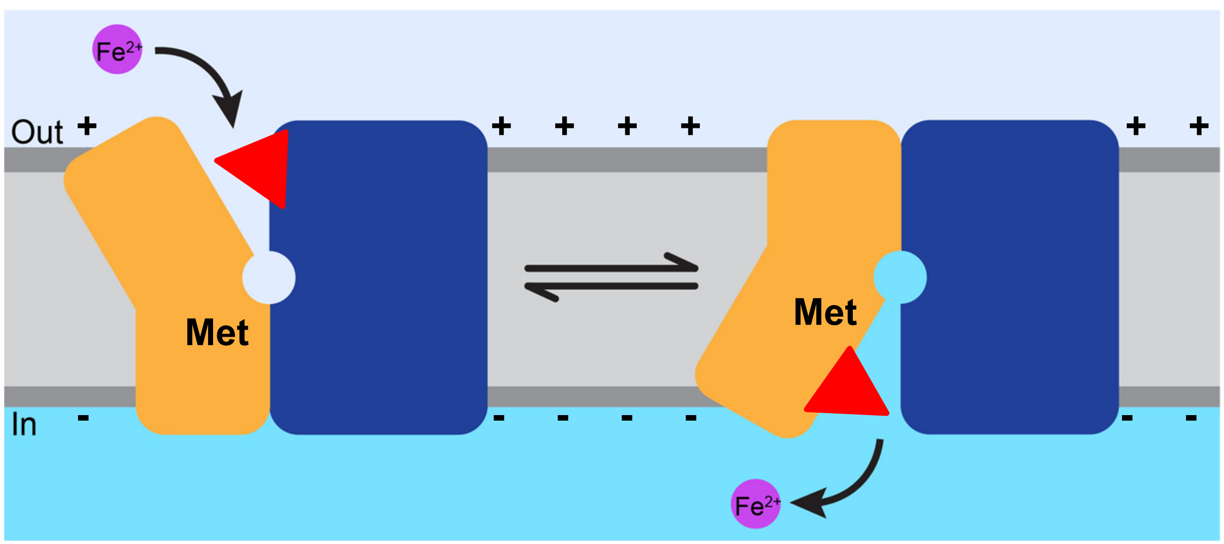


Mechanistic insights into Nramps

- Conserved methionine prevents calcium and magnesium transport but promotes cadmium uptake

- Anemia-causing mutations change the conformational dynamics of Nramps and impair transport

- Transporter shows voltage dependence



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