A role for protein symmetry in moving small molecules across cell membranes

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Strategies for crossing membrane barriers

channels

transporters

lipid membrane

~3 nm

10^6 to 10^9 s^{-1}

10^{-1} to 10^3 s^{-1}

primary active transport

secondary active transport

neurotransmitters

ions

amino-acids

sugars

H^+

Na^+

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Biological roles of secondary transport

3-4% of human genome & ~15% of bacterial genomes encode membrane transporters (~20-30% encode membrane proteins)

Glucose uptake

Mitochondrial ATP/ADP exchange

Termination of synaptic signals
A Typical Secondary Transport Cycle (Alternating Access)

~50 atomic structures
~20 unique proteins
9 unique folds
Membrane Protein Folds Containing Repeats

Aquaporins (Channels)

Leucine Transporter, LeuT

Cl⁻/H⁺ Exchangers

Na⁺/H⁺ Exchangers

Glutamate Transporters

Hypothesis: repeats are important for alternating access
Inverted-topology repeat structures can be very similar

Aquaporin

RMSD
~ 1.0 Å

~5nm
LeuT is related to the neurotransmitter:sodium symporters (NSS)

Serotonin
Dopamine
GABA
Norepinephrine

Outward-facing state

Yamashita et al
Nature 2005
Inverted-topology repeats in LeuT are not identical

Repeat A

Repeat B

LeuT Core 10 TMs

TM 3-5 on TM 8-10
RMSD = 3.4 Å
Why is a difference between repeats important?
Difference between repeats is responsible for formation of extracellular pathway in LeuT

Forrest et al, PNAS 2008

Model

Template

~7% seq ID

Forrest et al, PNAS 2008
"Swapped" model of LeuT consistent with alternating access

LeuT Outward-Facing (Gouaux)  LeuT Inward-Facing Model (Forrest et al, PNAS 2008)

"Rocking-bundle" mechanism

Model Viewed from Cytoplasm

Pore-lining residues

Substrate

Gary Rudnick
Yuan-Wei Zhang

Forrest et al, PNAS 2008

Model Viewed from Cytoplasm

Substrate

Model Viewed from Cytoplasm

Pore-lining residues

Forrest et al, PNAS 2008
"Swapped" model of LeuT consistent with alternating access

LeuT Outward-Facing (Gouaux)

LeuT Inward-Facing Model (Forrest et al, PNAS 2008)

"Rocking-bundle" mechanism

Mhp1 Outward-Facing (Weyand et al, Nature 2008)

Mhp1 Inward-Facing (Shimamura et al, Nature 2010)
Membrane Protein Folds Containing Repeats

Aquaporins

LeuT

Cl⁻/H⁺ Exchangers

Na⁺/H⁺ Exchangers

Glutamate Transporters

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Glt\textsubscript{Ph} is an aspartate transporter related to neuronal glutamate transporters (EAATs)

Yernool \textit{et al.} \textit{Nature} 2004

Outward-Facing State
Differences in the 4TM+HP repeat of Glt$_{Ph}$

Crisman et al, PNAS 2009

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A repeat-swapped model of the Glt$_{ph}$ protomer is inward-facing

Model
Template

~10% seq ID

Outward-facing X-ray
Inward-facing Model

Crisman et al, PNAS 2009

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A repeat-swapped model of the Glt$_{Ph}$ protomer is inward-facing

Outward-facing X-ray Structure (Reyes et al. Nature 2009)

"Swapped" Model (Crisman et al. PNAS 2009)

C$_\alpha$-RMSD TM helices = 5.4 Å
Pseudo-symmetry and the art of transport

Repeats provide a mechanism for generating 2 degenerate, symmetry-related conformations of one protein

EmrE

NSS

MFS

EAAT

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