Gating in the LeuT$_{Aa}$ Symporter: A Normal Mode Analysis Study

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Abstract

Opening and closing mechanisms of the extra- and intracellular gates of the Na^+/Cl^--dependent neurotransmitters that catalyze the symport of small molecules and ions across membranes remain elusive [1]. Large-scale conformational changes in a bacterial homologue, the LeuT$_{Aa}$ symporter [2], are studied using all-atom Normal Mode Analysis (NMA). Gating appears initiated by global counter-rotations of the intracellular and extracellular domains of LeuT$_{Aa}$ around the pore axis. As one half rotates clockwise, the other rotates anticlockwise, and vice versa. The stationary plane relative to which counter-rotation occurs passes through the center of LeuT$_{Aa}$ parallel to the membrane. The counter-rotations of the two halves are highly concerted and cooperative. On the extracellular side, the overall rotation of the peripheral TM helices affects the conformation of five of the inner TM helices: TM1, TM6, TM3, TM8 and TM10. They alternately straighten and bend near their midpoints. Straightening occludes and bending expands the extracellular pore. Loops and small helices on the extracellular surface of LeuT$_{Aa}$ undergo large-scale motions. The intracellular domain of LeuT$_{Aa}$ rotates concertedly around the pore axis essentially as a single unit. Relative to the pore axis, the radial location of the intracellular ends of TM1a-TM6b and TM3 and TM8 is not affected.

Introduction

Transporters are believed to possess two gates that provide alternating access to the binding site from opposite sides of the membrane bilayer [3]. In the crystal structure [2], the leucine molecule (Leu) at the binding site cannot access either the exterior or the interior. The side chains of F253 and Y108 primarily obstruct the pathway to the periplasm. Further towards the extracellular domain, the R30-D404 charged pair forms a water mediated salt bridge which stabilizes the extracellular gate in a closed conformation. It has been suggested [2] that when the extracellular gate opens, R30 and D404 separate, disrupting their interaction. On the intracellular side, there is ~20 Å span of ordered protein structure and no pathway is readily identified. Arguing from the architecture of LeuT$_{Aa}$ the following possible protein backbone movements have been suggested [2]:

1. "*the extracellular and cytoplasmic helical segments, that is TM1b-TM6a and TM1a-TM6b, respectively, might move relative to TM3 and TM8, helping to open and close the outward and inward gates*"
2. "*Movement in TM1 may be accompanied by a conformational change in the linker between TM1 and TM2*"
3. "*TM3 and TM8 may also shift their positions or rotate slightly. In addition, the extracellular and cytoplasmic entries could expand upon movement of surface-exposed elements, including the N terminus, IL1, EL2 and EL4*"
4. "*Finally, the surrounding transmembrane segments, TM2, TM7, TM10 and TM11, may accommodate movement of TM1, TM3, TM6 and TM8 because they have interruptions in the middle of their helical structures caused by Gly55 and Pro57 in TM2, Gly294 in TM7, Gly408 in TM10 and Pro457 in TM11*"

Computational Model

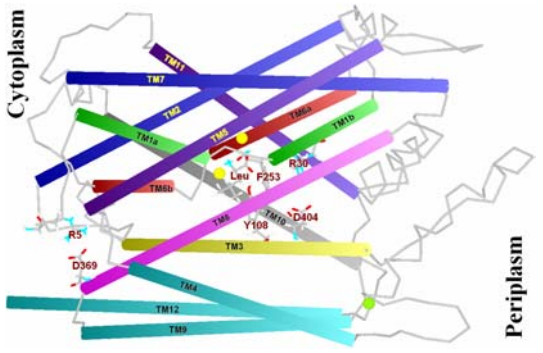
➢ We use the high-resolution (1.65 Å) 2A65 crystal structure of LeuT$_{Aa}$ [2], a molecular system of 8,204 protein atoms, 210 water molecules, Leu (22 atoms), two sodium ions and one chloride, totaling 8,859 atoms. E419 was protonated; in the crystal structure its side chain is near the E62 side chain (~2.6 Å). The bound Leu was treated as a zwitterion.

➢ The three independent minimization runs were performed on the Leu-LeuT$_{Aa}$ system. One additional minimization run was performed on the molecular system devoid of Leu (Water-LeuT$_{Aa}$), replacing it by seven waters for a total of 8,858 atoms. To remove steric clashes and to relax the molecular systems, ~2,000 minimization steps were performed via steepest descent with a random step length; finally, the molecular systems were well tuned via conjugate gradient with guaranteed descent [4]. All degrees of freedom (bond lengths, bond angles, torsion and improper torsion angles) in both LeuT$_{Aa}$, Leu and the waters were variable.

➢ The geometry of all four molecular systems reached an absolute largest component of the gradient $<5\cdot10^{-10}$ kcal mol^{-1} Å^{-1}. The RMSD for 512 alpha carbons between the crystal and minimized LeuT$_{Aa}$ structures is 1.06 Å; the RMSD for all 8,204 protein atoms is 1.33 Å; and the RMSD for the full 8,859 atom system (including waters) is 1.49 Å.

➢ All-atom NMA was carried out using the LAPACK library and BLAS routines; the global translational and rotational modes were removed using the Eckart conditions.

➢ The LeuT$_{Aa}$ systems were described with the all-hydrogen CHARMM22 topology and parameter set, with NMA calculations carried out in vacuum.



Minimized LeuT$_{Aa}$ structure with two Na^+ ions (yellow spheres) and Cl^- ion (green sphere) at their binding sites

For clarity, crystallographic water molecules are suppressed. Leu is shown at its binding site in the center of LeuT$_{Aa}$. Y108 and F253 (close to Leu) and R30 and D404 are shown on the right (periplasmic side). R5 and D369 (far from Leu) are shown on the left (cytoplasmic side). These residues form salt bridges across the Leu pathway, and are believed [2] to play an important role in gating. TM helices are represented as cylinders, with extracellular and intracellular ends shown in light and dark colors, respectively. TM helices are grouped as follows: **1)** "V-shaped" TM1 and TM6 are green and red, respectively. They are broken at the Leu binding site and oriented relatively antiparallel. **2)** TM3 is yellow, TM8 is pink and TM10 is grey. These abut the unwound parts of TM1 and TM6. TM1, TM6, TM3, TM8 and TM10 form the protein pore for symport of Leu and sodium ions, and comprise the inner TM helices. **3)** TM5 & TM11 are violet and TM7 & TM2 are blue. These helices surround TM1 and TM6, and are perimeter TM helices. **4)** TM4, TM9 and TM12 are cyan and form another group of perimeter TM helices surrounding the inner TM3, TM8 and TM10. The figure was generated using our MCICP code.

Leu-LeuT$_{Aa}$	
n	ν, cm^{-1}
1	-3.14E-05
2	-2.41E-05
3	-1.75E-05
4	-8.60E-06
5	2.26E-05
6	3.28E-05
7	3.67947
8	4.25554
9	4.37963
10	4.73024
11	4.83067
.....	
26575	3688.62
26576	3689.91
26577	3711.14

Eigenvalues of the minimized LeuT$_{Aa}$ systems

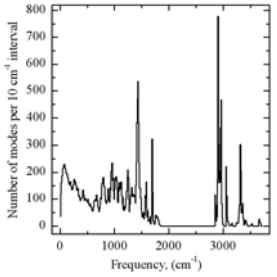
The first six eigenvalues are near zero and other eigenvalues are positive indicating a minimum.

The lowest-frequency eigenvalue (7th) was nearly equal in the three Leu-LeuT$_{Aa}$ systems: 3.68, 3.66 and 3.57 cm^{-1}, respectively. This eigenvalue is 3.74 cm^{-1} for the Water-LeuT$_{Aa}$ system. The eigenvalues for Leu-LeuT$_{Aa}$ from one run are shown on the left-hand side and those for Water-LeuT$_{Aa}$ are shown on the right-hand side.

The lowest-frequency eigendirections are nearly identical for all the three Leu-LeuT$_{Aa}$ and Water-LeuT$_{Aa}$ systems. The overlap is near ~1.

Main observation: replacing Leu at its binding site in the LeuT$_{Aa}$ transporter by water molecules has no effect on the lowest-frequency normal modes.

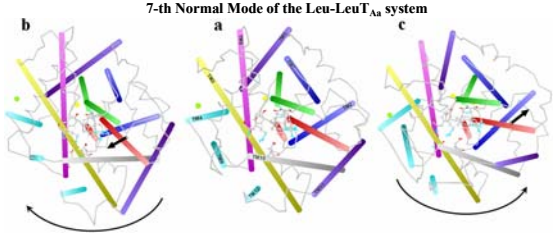
Water-LeuT$_{Aa}$	
n	ν, cm^{-1}
1	-2.91E-05
2	-1.70E-05
3	-5.98E-06
4	2.27E-05
5	2.82E-05
6	3.88E-05
7	3.73598
8	3.94494
9	4.66048
10	5.36380
11	5.58800
.....	
26572	3689.76
26573	3691.40
26574	3717.73



Frequency Spectrum of the Leu-LeuT$_{Aa}$ System

- the frequency spectrum is the same in all four runs
- the region < 600 cm^{-1} describes concerted motions of secondary structure elements (α-helices and large groups of amino acids)
- the region from 600 to 1840 cm^{-1} corresponds to internal vibrations of single amino acids
- the region > 2850 cm^{-1} corresponds to hydrogen-heavy atom vibrations

7-th Normal Mode of the Leu-LeuT$_{Aa}$ system

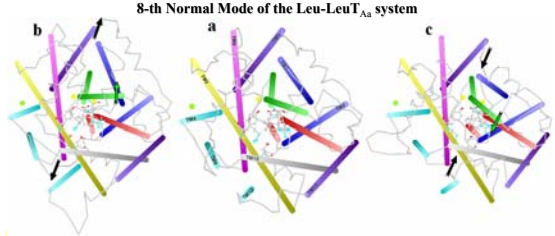


View from the extracellular side in a cylinder representation: (**a**) the minimized Leu-LeuT$_{Aa}$ system; (**b**) and (**c**) displacement along the 7th all-atom NM in the "negative" and "positive" directions.

Main observations:

- Overall highly concerted and cooperative counter-rotations (black curved arrows) of the intracellular and extracellular domains around the pore axis. In counter-rotation the extracellular half of LeuT$_{Aa}$ rotates clockwise, while the intracellular half rotates anticlockwise, and vice versa. The stationary plane relative to which the rotation occurs passes through the center of LeuT$_{Aa}$ parallel to the membrane.
- Extracellularly, overall rotation of the peripheral helices reconfigures the inner helices (TM1, TM6, TM3, TM8 and TM10). The V-shaped TM1 (green) and TM6 (red) helices alternately straighten (close) and bend (open) at their midpoint breaks (black straight arrows). Similarly TM8 (pink) and TM3 (yellow) also alternately bend (open) and straighten (close) near their midpoints. Straightening TM1, TM6, TM3 and TM8 occludes the extracellular mouth, and bending expands it. Loops and small helices on the extracellular side undergo large-scale rotational motion.
- The intracellular domain of LeuT$_{Aa}$ rotates concertedly around the pore axis nearly as a rigid unit. The radial location of the intracellular ends of TM1a, TM6b, TM3 and TM8 relative to the pore is not affected.

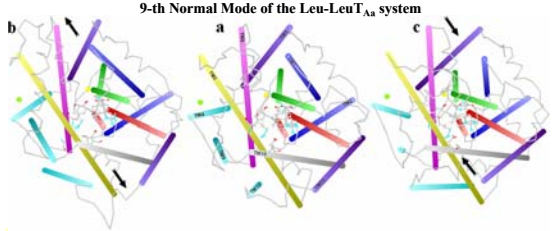
8-th Normal Mode of the Leu-LeuT$_{Aa}$ system



Main observations:

- The extracellular mouth undergoes large-scale expansion and contraction: the extracellular ends of the peripheral TM5 (violet) and TM7 (blue) alternately approach and separate from the diagonally opposed peripheral TM12 (cyan) and inner TM10 (grey) (black arrows). This motion affects the inner TM1b (green) displacing it off and collapsing it on the inner TM10 (green arrows). TM6a (red) is less affected.
- Expansion or contraction of the intracellular mouth is not observed. The relative configuration of the inner helices is unaffected and the intracellular domain remains nearly rigid.

9-th Normal Mode of the Leu-LeuT$_{Aa}$ system



Main observations:

- The extracellular ends of TM10 (grey), TM11 (violet) and TM12 (cyan) alternately move relative to those of TM5 (violet), TM7 (blue) and TM8 (pink) resulting in a large-scale expansion and contraction of the extracellular mouth. TM3 (yellow) bends at its middle. This motion has almost no effect on the V-shaped inner TM1b-TM6a.
- The intracellular domain undergoes a rigid motion, and its mouth is nearly unaltered.

Pore of the Perturbed Leu-LeuT$_{Aa}$ System

The pore is blocked on both the extracellular and intracellular sides. By perturbing to an RMSD of 3.5 Å along low-frequency NMs in the "negative" and "positive" directions, the change in the distance, Δd_a and Δd_p, between the F253 & Y108, R30 & D404 and R5 & D369 residues was monitored:

		7-th NM		8-th NM		9-th NM	
	d_0, Å	Δd_a, Å	Δd_p, Å	Δd_a, Å	Δd_p, Å	Δd_a, Å	Δd_p, Å
R30&D404 NH2&OD1	5.4	+1.0	-1.0	-0.5	+1.1	-0.1	+0.1
F253&Y108 CZ&CE1	4.1	-0.1	+0.1	+0.3	-0.3	-0.3	+0.3
R5&D369 NH2&OD1	2.8	+0.07	+0.05	-0.2	-0.2	+0.4	-0.2

Main observations:

➢ in perturbation to an RMSD of 3.5 Å along the low-frequency NMs in either direction, the Leu-LeuT$_{Aa}$ pore remains closed on both sides

➢ in perturbation along the 7-th and 8-th NMs, the distance between R30 & D404 changes noticeably, by ~1 Å; the distance between F253 & Y108 and R5 & D369 is nearly unaffected for all three low-frequency NMs

Conclusions

- NMA identifies the intrinsic directionality of conformational changes in LeuT$_{Aa}$ for initiating a gating transition. The large-scale motions of the protein backbone along the low-frequency normal modes dictated by the LeuT$_{Aa}$ architecture are unique: 1) highly concerted and cooperative overall counter-rotations of the intracellular and extracellular LeuT$_{Aa}$ domains around the pore axis; 2) large-scale expansion and contraction of the extracellular mouth.
- In the periplasmic domain, overall rotation of the perimeter TM helices affects the conformation of the inner (TM1, TM6, TM3, TM8 & TM10) helices in agreement with suggestion 4 (see Introduction). The inner helices undergo relative motion (suggestion 1) alternately straightening and bending at their midpoint breaks. Straightening occludes and bending expands the extracellular pore entry (suggestion 3). Loops and small helices on the extracellular side undergo large-scale rotational motion.
- The intracellular domain of LeuT$_{Aa}$ rotates concertedly around the pore axis nearly as a whole. The radial location of the ends of TM1a-TM6b, TM3, TM8 and TM10 relative to the pore is unaffected. Cytoplasmicly, NMA does not reveal potential conformational changes in the protein backbone.
- Large-scale protein backbone displacements along low-frequency NMs do not directly lead to opening of either inner or outer gates. Perturbation along low frequency NMs does not reveal the alternating mechanism [3] of LeuT$_{Aa}$ functioning. The V-shape of TM1 and TM6 changes symmetrically, not alternately (see movies [5]). Normal mode tracking [6] is needed to see how closing the extracellular gate might couple to opening the intracellular gate.

References

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