RESEARCH ARTICLE

REVISED Impact of pathogenic mutations of the GLUT1 glucose transporter on solute carrier dynamics using ComDYN enhanced sampling [version 2; peer review: 1 approved, 1 approved with reservations]

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Abstract

Background: The solute carrier (SLC) family of membrane proteins is a large class of transporters for many small molecules that are vital for cellular function. Several pathogenic mutations are reported in the glucose transporter subfamily SLC2, causing Glut1-deficiency syndrome (GLUT1DS1, GLUT1DS2), epilepsy (EIG2) and cryohydrocytosis with neurological defects (Dystonia-9). Understanding the link between these mutations and transporter dynamics is crucial to elucidate their role in the dysfunction of the underlying transport mechanism, which we investigate using molecular dynamics simulations.
Methods: We studied pathogenic and non-pathogenic mutations, using a newly developed coarse-grained simulation approach 'ComDYN', which captures the 'COMmon constraints DYNamics' between both states of the solute carrier protein. To guarantee the sampling of large conformational changes, we only include common constraints of the elastic network introduced upon coarse-graining, which showed similar reference distances between both conformational states ($\leq$1 Å difference).
Results: ComDYN is computationally efficient and sufficiently sensitive to capture effects of different mutations. Our results clearly indicate that the pathogenic mutation in GLUT1, G91D, situated at the highly conserved RXGRR motif between helices 2 and 3, has a strong impact on transporter function, as it blocks the protein from sampling both

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conformational states. In comparison, predictions from SIFT and PolyPhen only provided an impression of the impact upon mutation in the highly conserved RXGRR motifs, but yielded no clear differentiation between pathogenic and non-pathogenic mutations.
Conclusions: Using our approach, we can explain the pathogenicity of the mutation G91D and some of the effects of other known pathogenic mutations, when we observe the configurations of the transmembrane helices, suggesting that their relative position is crucial for the correct functioning of the GLUT1 protein. To fully understand the impact of other mutations in the future, it is necessary to consider the effect of ligands, e.g., glucose, within the transport mechanism.

Keywords
GLUT1 glucose transporter deficiency syndrome, Human glucose transporters, SLC transporter family, transport mechanism, molecular dynamics simulation, Martini force field, coarse-grained simulations, enhanced sampling method

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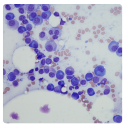

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REVISED **Amendments from Version 1**

In the revised version we have clarified the scope of the study, which aims to study the impact of mutations on the first part of the transporter mechanism without considering the impact of glucose or other ligands. We have renamed our method to ComDYN for "COMmon constraints DYNamics" as retaining only common constraints in the elastic network is the key ingredient for the enhanced conformational sampling. To study the complete pathway and elucidate the effect of all mutations in future studies, the simulations need to be extended towards the outward-open conformation and consider the impact of the ligand upon the transportation mechanism.

We would like to thank the reviewers again for providing us with useful comments and suggestions which helped improve our manuscript.

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Introduction

The solute carrier (SLC) transporter superfamily is known to play a key role in the transport of small molecules. The superfamily comprises 52 families, and at least 386 different transporter genes have so far been identified in humans (Hediger *et al.*, 2013; Higuchi *et al.*, 2018). This family of membrane proteins is a large class of transporters for many small molecules such as glucose that are vital for the cell, and can be found in all kingdoms of life. Of particular interest are the glucose transporters SLC2A1 from the SLC2 subfamily; GLUT1 mutations are associated with GLUT1 deficiency syndrome (GLUT1DS1 and GLUT1DS2), and some forms of spasticity (Dystonia-9) and epilepsy (EIG2) (Klepper *et al.*, 2016; Mongin *et al.*, 2016). Shedding light on the molecular mechanism of the transport function, enables us to understand the difference between pathogenic and benign mutations that have been observed in human subjects. Glucose transporter GLUT1 is built from 12 transmembrane helices (TMs) and exhibits a two-fold symmetry plane joining the two times six TM helices over a bridging helix on the cytoplasmic side of the membrane (see Figure 1A). The transport mechanism of glucose involves cycling through four states (Deng *et al.*, 2015): outward open (ligand bound or ligand free), outward occluded (O_o, ligand bound), inward open (I_o, ligand bound or ligand free), and inward occluded (ligand free), as summarized in Figure 1B. Throughout the SLC transporters, a highly conserved RXGRR-motif is found between TM2 and TM3 and between TM8 and TM9 at the intracellular side of the corresponding loops (Pao *et al.*, 1998; Sato & Mueckler, 1999). Several mutations at these anchor points are known to be disease-related, such as G91D and R92W which are known to cause GLUT1DS1 (Klepper *et al.*, 2001; Klepper & Voit, 2002), whereas R93W is associated with GLUT1DS2 (Joshi *et al.*, 2008). Between TM8 and TM9, R333W is also confirmed to be pathogenic (Klepper *et al.*, 2001; Klepper & Voit, 2002), while the clinical significance of R334Q is unknown, although it is likely to affect the protein function.

Here, we investigate the effect of different mutations on the dynamic of the human GLUT1 protein. As the dynamic response upon mutation may depend on the conformational state, we aimed to simulate the first part of the transport mechanism between the outward-occluded state (O_o) and inward-open state (I_o), as shown in Figure 1B. Hereby, we investigate the intrinsic dynamics of the transporter protein without the ligand. For GLUT1, however, a three-dimensional structure is only available for the I_o state; therefore, simulations of GLUT3, which is evolutionarily quite close to GLUT1, in the O_o state were analysed. GLUT3 is also suspected to be associated with neurological disorders such as Alzheimer (An *et al.*, 2018; Gu *et al.*, 2018; Simpson *et al.*, 1994; Szablewski, 2017). We expect that the intrinsic dynamics of the ligand-free states of the transporter provides new insight on the effects of the pathogenicity of certain mutations, without explicit consideration of the ligand-bound states.

Methods

Starting structures

The crystal structures of both I_o and O_o states are available in the PDB; 5EQI for GLUT1 I_o state (Deng *et al.*, 2015), and 4ZW9 for GLUT3 O_o state (Kapoor *et al.*, 2016); these were used as starting points for the simulations, as we focus on the first part of the transport mechanism. The next step, i.e., to elucidate the complete pathway, would be to extend our work to the outward-open conformation which is also available in the PDB (4ZWC GLUT3 with maltose bound), and to study the effect of ligand binding on the transporter dynamics. However, this is beyond the scope of the present study. Based on the reported pathogenic mutations of GLUT1 in the conserved RXGRR motif region, we searched the corresponding positions of GLUT3 for additional mutations and included them in our study. Due to the high sequence identity (~70%) between the proteins, we intentionally did not build a homology model for one or the other protein. This is justified, as our main aim is to characterize the global opening and closing mechanism rather than to look into atomistic details such as protonation states. Moreover, we now avoid additional uncertainties about details of the structure as would be inevitably introduced during the homology modelling process. Our composite scheme using coarse-grained molecular dynamics with common constraints elastic network 'ComDYN', that keeps part of the unchanged position constraints between the two protein structures, is explained in the approach below and further details are supplied in the supporting methods, available in the deposited code (Feenstra, 2019b).

Molecular dynamics simulations

In this study, we employed molecular dynamics (MD) simulations using the GROMACS 4.0.5 programme package (Hess *et al.*, 2008). For efficiency reason, we investigated the applicability of the MARTINI coarse-grained (CG) force field (Arnarez *et al.*, 2015; de Jong *et al.*, 2013; Hsu *et al.*, 2017; Monticelli *et al.*, 2008; Periole *et al.*, 2009), which is about 500-fold faster than the full-atomistic GROMOS (May *et al.*, 2014). This speed-up is obtained at the expense of explicit description of hydrogen bonds in the protein, which necessitates the addition of an elastic network to maintain secondary structure and other tertiary contacts and thus overall protein stability; the elastic network is thus tailed to a particular conformational state of the protein. Here, we propose to modify the elastic network that is used in MARTINI based on our starting

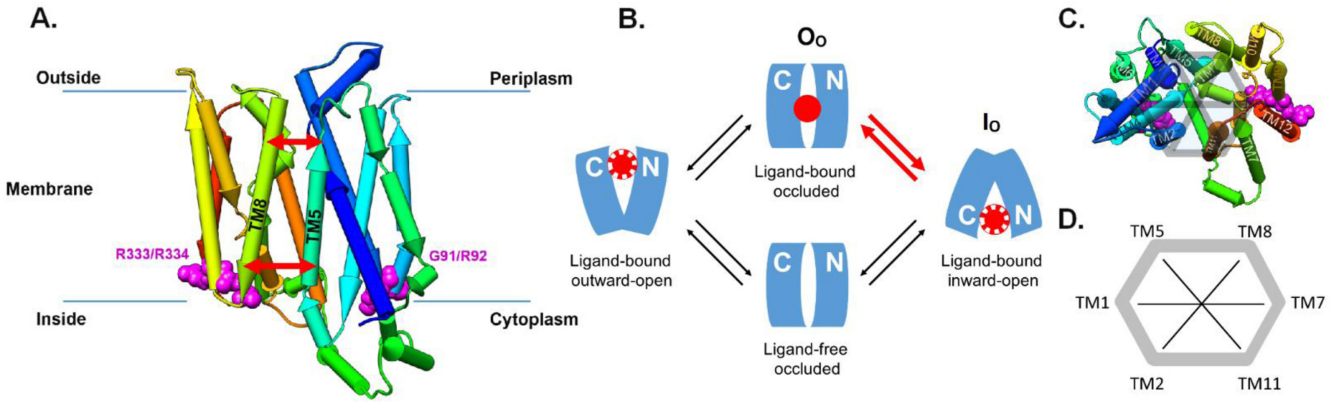

Figure 1. GLUT1 structural overview. (**A**) Pipe representation of the inward-open (I_O) conformation (PDB-ID: 5EQI; bound inhibitor removed) of GLUT1 situated in the lipid bilayer. Note that the protein structure has a two-fold rotational symmetry and the two conserved RXGRR-motifs are located at the junctions of the transmembrane (TM) helices 2 and 3 and TM8-TM9, around the R333/R334 and G91/R92 mutation sites shown in magenta. The red arrows symbolize the inside and outside distances. Note that we number the helices starting from TM1 at the N-terminus of the transporter (dark blue in the pipe representation). (**B**) Schematic cycle of the glucose transport mechanism between the four different open and occluded states as adapted from (Deng *et al.*, 2015). The bound glucose ligand is indicated as a red sphere. In the open states (inward or outward), glucose may be bound or unbound; this is represented using white dots. This work concerns the first part of the transport mechanism, i.e., the dynamics between the outward-occluded state (O_O) and inward-open state (I_O) as highlighted with red arrows. Note however, that we do not consider ligand binding within the scope of this work (see Discussion further below). (**C**) Pipe representation of the I_O conformation (PDB-ID: 5EQI) of GLUT1 viewing on the outward facing part of the transporter inside the periplasm. (**D**) Definition of the order parameters to follow the motion of the helices over the ComDYN simulations.

conformations, to include only common elastic network constraints that differed less than 1Å between the I_O and O_O states. Increasing the distance threshold for the common constraints would result in even fewer constraints in the ComDYN, increasing the risk of instability and possibly unfolding of the protein. Therefore, our chosen constraints allow transition between both states, while maintaining protein structure stability. This composite scheme will henceforward be referred to as ComDYN (COMmon constraints DYNamics). The detailed computational set-up is provided in the Supporting Methods (Feenstra, 2019b).

Analysis of transporter dynamics

Essential dynamics analysis was performed on the GLUT1 and GLUT3 simulations using the built-in analysis tools of GROMACS. To allow this comparison between these two homologous proteins, and allow for focusing on overall motions of the transporter region, we selected the structurally conserved helical segments, as summarized in Supporting Table S2, available as extended data (Feenstra, 2019a). Then, the covariance and eigenvalue calculation were performed on the ensemble of both wild-type systems, using the coordinates of the C-alpha atoms for the full atomistic (AT) simulations, and the backbone particle at the C-alpha position for the coarse-grained (CG) and ComDYN simulations.

To analyse the transporter dynamics from the ComDYN, we defined several order parameters as previously proposed by Nagarathinam *et al.* (2018) by measuring distances between adjacent TM helices, at the intracellular (in)- and extracellular (out)sides of the protein (see Figure 1B, C). For each of the ring of six central helices that make up the solute transporter region, TM2, TM1, TM5, TM8, TM7, TM11 (and back to TM2), we defined an 'inside' and 'outside' segment of ten residues (See Figure 1 and extended data, Supporting Figure S3 (Feenstra, 2019a)). Comparing the distances of the mutations to both wild types allows us to capture abnormal behaviour and identify the mutations that have the highest impact on the opening and closing mechanism of the apo-form of the transporter protein (see Results and Discussion).

To compare the distributions of sampled distances during the simulations, two metrics were employed: overlap and shift. 'Overlap' is the fraction of overlap between both distributions, as the integral of the minimum of both functions. 'Shift' quantifies the direction of change, and is obtained by taking the difference in the position of the peak of the two distributions from the ensemble of the simulations (typically, mutation version wild-type). Negative indicates a 'closing' motion, positive is 'opening'. This analysis was performed using the script 'calc_overlap.py', which may be found in the extended data.

Results and discussion

Prediction of mutation impact

Table 1 lists the mutants of GLUT1 and GLUT3 that we will consider here, which are situated in the conserved RXGRR-motif distal to the transporter region. The table lists the predicted impact upon mutation obtained from SIFT (Sim *et al.*, 2012) and PolyPhen-2 (Adzhubei *et al.*, 2010). Most mutations are classified as likely pathogenic by both methods, with the exception of GLUT1 R93Q, and GLUT3 R91C and R91H. However, these methods are trained on the dbSNP database which also includes these known mutations, so this should be no surprise. Moreover, these predictions do not allow us to gain any insights into the mechanism by which these mutations may affect transporter function.

Table 1. Overview of selected mutations of GLUT1 (PDB-ID: 5EQI) and GLUT3 (PDB-ID: 4ZW9) studied in this work and the impact predictions obtained from SIFT and PolyPhen. The pathogenic mutations in GLUT1 are underlined.

Protein	Mutation	Clinical significance *	SIFT	PolyPhen
GLUT1	G91D	(Klepper *et al.*, 2001; Klepper & Voit, 2002) GLUT1DS1 omim:606777	affect protein function	probably damaging
	R92Q	(Leen et al., 2010) dbSNP rs779073410 significance unknown	affect protein function	probably damaging
	R92W	(Schneider et al., 2009) GLUT1DS2 omim:612126	affect protein function	probably damaging
	R93Q	dbSNP rs80359815 significance unknown	tolerated	probably damaging
	R93W	(Joshi et al., 2008) GLUT1DS2 omim:612126	affect protein function	probably damaging
	R333W	(Klepper & Voit, 2002; Klepper et al., 2001) EIG12 omim:614847	affect protein function	probably damaging
	R334Q	ClinVar rs892715050 significance unknown	affect protein function	probably damaging
GLUT3	G89V	dbSNP rs758117298 significance unknown	affect protein function	probably damaging
	R90W	not reported in dbSNP (rs1270428275 R90T)	affect protein function	probably damaging
	R91C	dbSNP rs756172777 significance unknown	affect protein function	benign
	R91H	dbSNP rs145936296 significance unknown	tolerated	benign
	R331K	dbSNP rs770855736 significance unknown	affect protein function	probably damaging
	R331S	dbSNP rs749200071 significance unknown	affect protein function	probably damaging
	R331W	not in dbSNP significance unknown	affect protein function	probably damaging

* References given to literature describing clinical appearance, OMIM entries, and dbSNP entries given, if available.

Verification of constraining approach

Firstly, we want to verify if our common constraints-based approach for coarse-grained MD simulations (ComDYN) is able to sample the intermediate states between the I_o and O_o states. Including only elastic network constraints that differed less than 1Å between the I_o and O_o states in ComDyn, resulted in 1025 constraints (39.9% of the 2568 in the Martini elastic network) for the O_o state and 978 (42.2% of 2315) for the I_o state. We performed essential dynamics (ED) analysis (Amadei *et al.*, 1993; Van Aalten *et al.*, 1997) to compare AT, traditional CG MARTINI, and ComDYN simulations, as described in the Methods. Figure 2 shows 2D plots of the first two (largest) ED eigenvectors, representing the extracted correlated motions over the ensemble of our simulations. The first eigenvector (EV1) represents the major conformational changes between the O_o and I_o states, as also indicated by the RMSD values between starting states. The changes observed along EV2 may also be genuinely part of the state transition, however we cannot exclude the possibility that some of these conformational changes may relate to differences between the forcefields used. The sampling of the different states, inward-open and outward-occluded, in AT simulation hardly converges due to their limited time scales. The regular CG simulations reach much longer timescales, and already sample more intermediate conformations, but there is no overlap. The ComDYN simulations, on the other hand, also sample many states intermediate to the inside-open and outside-open starting states, compared to the other simulations. This shows that improved sampling of large conformational transitions may be attained using this approach of CG and ComDyn simulations.

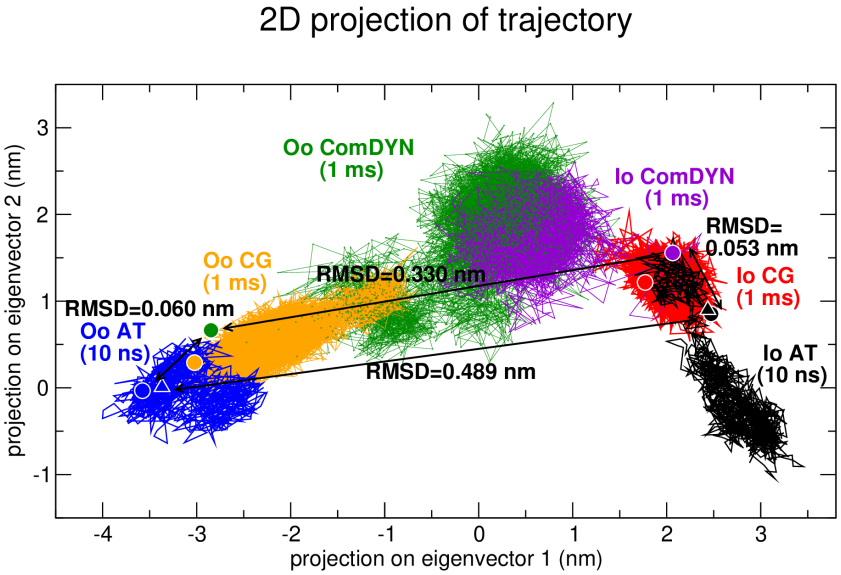

Figure 2. Two-dimensional essential dynamics plot of the simulations. In this projection, eigenvector 1 corresponds to changes from O_O (left) to I_O (right), showing also the overlap and differences between the AT (full atomistic), the CG (coarse-grained), and the ComDYN (common constraints CG) simulations. Note that there is considerable overlap in the sampling, but that the time-scale of the AT simulations only samples conformations around the I_O and O_O states and the elastic network in the CG simulations also limits the visited conformations, while the ComDYN samples a large number of conformations between both states.; I_O, inward open state (PDB-ID: 5EQI); O_O, outward occluded state (PDB-ID: 4ZW9). Spheres indicate the respective starting conformations for each method, triangles to show the corresponding crystal structures; black arrows and labels indicate the RMSD between the starting conformations of AT and ComDYN simulations.

Probing conformational changes

To probe the degree of the conformational changes during ComDyn simulations of the wild types and the mutants in more detail than done with the ED analysis, two distances were used to describe the opening and closing of the periplasmic and cytoplasmic sides of the transporter. Nagarathinam *et al.* (2018) studied a bacterial homolog of GLUT1 and GLUT3, and analysed the movement between TM5 and TM8. In the extended data, Figure S4 (Feenstra, 2019a), we can see that the distributions obtained from our ComDYN simulations, resemble those reported by Nagarathinam *et al.* (2018), providing an independent validation that our ComDYN approach is able to sample biologically relevant conformational states for large scale motions, such as those involved in the glucose transporter mechanism. Nevertheless, there are differences between the distance distributions in our work and that of Nagarathinam *et al.* (2018), which could, apart from obvious differences comparing human glucose transporters with a bacterial multidrug transporter (see also extended data, Figure S4 and Table S3 (Feenstra, 2019a)), also arise from differences in the sampling protocols applied in the two studies.

Therefore, in addition to the TM5-TM8 distances, we extended the analysis to other helices along and across the rim that make up for the entire SLC transporter architecture, allowing us to monitor changes in their position (see Figure 1C, D). For each of these order parameters, we calculated the distance at the inside and outside of the protein with respect to the membrane. Using this analysis, we can immediately observe the changes occurring between the inward-open and outward-occluded states. We see several distances changing significantly during this process: TM1/TM2(in), TM1/TM5(out), TM1/TM7(out), TM1/TM8(in), and TM5/TM11(in) are all closing, while TM1/TM2(out), TM1/TM5(in), TM1/TM7(in), TM2/TM11(in), TM2/TM8(out), and TM5/TM11(out) are opening (see extended data, Table S3 and Figure S4 for more details (Feenstra, 2019a)); these motions are also schematically summarised in extended data, Figure S3 (Feenstra, 2019a). Table 2 summarises the overlap and shift between the sampled distributions of distances between the wild type and each of the mutants for TM5 and TM11 that exhibited the strongest effects and conformational changes during the simulations (the complete table of the distributions for all order parameters are available in the extended data, Table S3 (Feenstra, 2019a)). The corresponding conformational distributions from the ComDYN simulations, calculated as a function of the inner and outer distances between TM5 and TM11 are given in Figure 3.

Impact of mutations on dynamics

Not all mutations have a high impact on the overall dynamics (extended data, Table S3 and Figure S4 (Feenstra, 2019a)). However, in GLUT1, the reported pathogenic mutation G91D has a profound effect on the dynamics of the protein (i.e., a low overlap and large shift, see Table 2). Also when we consider the distance of TM5-TM11, as shown in Figure 3, the strongest effect is observed for the pathogenic G91D mutant: its distribution varies strongest from the wild types, and hardly visits the inward-open and outward-occluded states. Furthermore, Figure 3

Table 2. Overview of the changes in inside and outside distances in the CG ComDYN simulations, between transmembrane helix 5 (TM5) and TM11. Figure 3 shows the corresponding distance plots for some of the mutants. These are quantified using the fraction of overlap between both distributions in sampling distributions of sampled conformations, calculated as the integral of the minimum of both functions, i.e., the volume (normalized to a maximum of one), given in %, of the wild type and mutant (small value is large change), and the shift of the peak location (in nm including the direction; positive is to larger distances). The pathogenic mutations are underlined. All the large shifts (absolute above 0.15 nm) and small overlaps (below 0.6 nm) are set to bold. For a visual aid, the shifts are set in italic.

GLUT1		**G91D**	**R333W**	**R334Q**	**R92Q**	**R92W**	**R93Q**	**R93W**
Inside	Overlap	**4%**	**24%**	**47%**	60%	64%	**46%**	62%
	Shift	***0.84***	***0.34***	*-0.05*	*-0.02*	*-0.02*	***0.28***	*-0.03*
Outside	Overlap	**20%**	69%	70%	70%	**48%**	70%	61%
	Shift	***0.25***	*-0.13*	*-0.13*	*0.02*	***-0.23***	*-0.12*	***-0.18***
GLUT3		**G89V**	**R331K**	**R331S**	**R331W**	**R90W**	**R91C**	**R91H**
Inside	Overlap	**25%**	67%	**9%**	**7%**	**6%**	56%	**12%**
	Shift	***0.27***	*-0.06*	***-0.28***	***-0.35***	***-0.32***	*-0.12*	***0.30***
Outside	Overlap	**28%**	57%	50%	53%	50%	62%	**15%**
	Shift	***0.37***	*0.11*	*0.14*	***0.21***	*0.12*	***0.33***	***0.58***

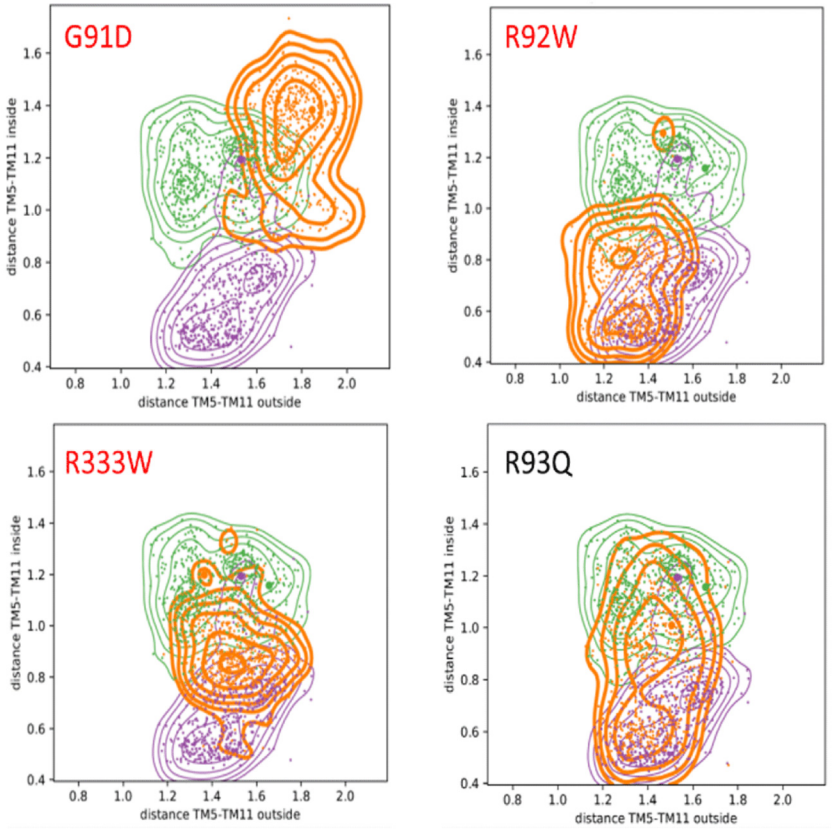

Figure 3. Distance plots of the inner and outer distances along the order parameter TM5-TM11 in nm over the complete time span of the CG ComDYN simulations (see Figure 1D). Colour code: wild types I_O in purple (PDB-ID: 5EQI), O_O in green (PDB-ID: 4ZW9), mutants in orange. Pathogenic mutants are highlighted in red. It should be noted that in contrast to the benign R93Q mutant, the pathogenic mutants do not sample the I_O and O_O states during the simulation, which strongly indicates that the mutation blocks the proper opening and closing mechanism. Corresponding plots for all mutations are in the extended data, Figure S4. The corresponding quantification of these plots provided as shift and overlap are given in Table 2.

shows that for the pathogenic R92W and R333W mutations, only one state or small parts from both can be accessed. For the benign mutant R93Q, in contrast, it can be seen that both states, inward-open and outward-occluded, are sampled thoroughly during the simulations. Assuming that the relative distance between the two helices is crucial for the correct functioning, this strongly suggests that the pathogenic mutations directly affect the opening and closing mechanism of the GLUT1 transporter.

Mutations in GLUT3 show similar behaviour in TM dynamics compared to those in GLUT1. Here, two mutants with strong abnormal behaviour can be identified: G89V and R91H (Table 2; extended data Figure S4 (Feenstra, 2019a) shows the corresponding distance distribution plots). Additionally, similar to the observations for pathogenic mutations on GLUT1, these mutations no longer sample intermediate states associated with the transport function, unlike the wild type and many of the other mutations. This strongly suggests that the corresponding mutations between GLUT1 and GLUT3 also have the same direct blocking effects on the opening and closing mechanism of the GLUT3 transporter. However, it should be noted that we cannot make any conclusions about the clinical significance of these GLUT3 mutants, as none have been reported to be pathogenic. A next step to elucidate the complete pathway would be to extend the analysis to the outward-open conformation, and include ligand-bound states (Delemotte, 2019), which may shed further light on some of the currently still unexplained pathogenic mutations.

Conclusion

Using extensive ComDYN simulations of GLUT1 and GLUT3 wild type and several clinically relevant mutations, we provide an effective way to study dynamic effects of mutations on the molecular mechanism of human glutamate transporter proteins. Without using full-atomistic details, we were able to get insight into the opening and closing mechanisms, which may account for the (dys)function of the SLC family caused by pathogenic mutations around the conserved RXGRR-motif. Through these mutations (especially G91D, R92W and R333W in GLUT1), we observe that the distances between TM5 and TM11 across the rim of the solute carrier structure are affected the strongest. For this reason, we chose them as our order parameter to explain the abnormal behaviour in the dynamics of the transporter opening and closing mechanism for some of the observed mutants. It should be mentioned that this does not provide an ultimate order parameter to explain all the effects of the pathogenic mutations, but allows us to better understand some of the effects caused by these pathogenic mutations. Comparing atomistic (AT), coarse-grained MARTINI (CG), and ComDYN simulations, our work shows that our CG ComDyn simulations are sufficiently accurate to sample the intermediate states between the conformational states and capture some of the effect of the mutations on the dynamic and function of these transporter proteins. This helps to elucidate the effects of pathogenic mutations on the structure and dynamics of the GLUT1 and GLUT3 transporters which were previously not understood.

Data availability

Underlying data

Crystal structures for GLUT1 I_O state (Deng *et al.*, 2015) and for GLUT3 O_O state (Kapoor *et al.*, 2016) were obtained from the Protein Data Bank, under accession numbers 5EQI and 4ZW9, respectively.

Extended data

Open Science Framework: ComDYN. https://doi.org/10.17605/OSF.IO/F82H5 (Feenstra, 2019a).

The following extended data are available:

- Data.tgz. data files accompanying analyses performed in this study.
- Table S1. Summary of the molecular composition of simulated systems.
- Table S2. Structurally conserved helical segments between 4ZW9 and 5EQI.
- Table S3. Wild-type and Mutant simulations compared by Overlap and shift between TM helix distance distributions.
- Figure S1. Sequence alignment between *E. coli* multidrug transporter MDFA, and human glucose transporters GLUT1 and GLUT3.
- Figure S2. Pipe representation of the inward-open conformation of the transporter.
- Figure S3. Schematic view of the observed pore mechanism going from the inward-open state to the outward-occluded state.
- Figure S4. Distribution of inside and outside helix distances for all examined mutants in GLUT1 and GLUT3.

Software availability

Scripts used to setup and analyze the ComDYN simulations available from:

https://github.com/ibivu/ComDYN.

Archived source code at time of publication: https://doi.org/10.5281/zenodo.2591477 (Feenstra, 2019b).

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Current Peer Review Status: ✔ ?

Version 2

Reviewer Report 22 July 2022

https://doi.org/10.5256/f1000research.133928.r140632

✔ **Lucie Delemotte**
Department of Applied Physics, Science for Life Laboratory (SciLifeLab), KTH Royal Institute of Technology, Stockholm, Sweden

The manuscript is improved and describes accurately the insights made possible by the newly developed ComDYN method.

I noticed two small issues that should be resoved:

1. In the abstract, a reference is made to "both states of the protein". However, it should be clarified which two states given that this transporter cycles through more than 2 states during its conformational cycle.

2. In the conclusion, "human glutamate transporter proteins" should be replaced by "human glucose transporter proteins".

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Membrane proteins, ion channels, molecular dynamics simulations, enhanced sampling

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 08 May 2019

https://doi.org/10.5256/f1000research.20307.r47253

?

Jocelyne Vreede
Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Amsterdam, The Netherlands

The paper reports a molecular dynamics simulation study of the glucose transporter GLUT1 using all-atom and coarse grained force fields, in combination with a conserved elastic network. Using essential dynamics analysis and comparison of various distances the authors compared the dynamics of the wild type protein to pathogenic variants in the outward occluded and inward open states. This procedure enabled the prediction of the effect of mutations on the dynamics of GLUT1 and GLUT3.

The manuscript requires a few clarifications to improve my understanding:

- As I am not very familiar with the mechanism of glucose transporters, I would like a few sentences describing how these proteins work, thus giving more context to the different conformational states I_o and O_o. Also in the conclusion, a bit more context as to how the states interconvert and the impact of the mutations on these transitions would aid my understanding tremendously.

- The manuscript reports results on mutations at positions 92, 93, 333 and 334, which are highlighted in Fig. 1. Only mutations 92 and 93 are discussed in the introduction. For more context, the mutations at 333 and 334 should be discussed in the introduction as well.

- What motivates the cut-off of 1 angstrom for including constraints in the elastic network? Would 1.5 angstrom or 2 angstrom work as well?

- As essential dynamics analysis is performed on both all-atom and coarse grained simulations, I assume only C-alpha positions are included. Is this assumption correct?

- What do the two eigenvectors shown in Fig. 2 mean? My interpretation is that EV1 is the transition from the O_o to the I_o state, and that EV2 is the transition from the all-atom to coarse grained-constrained description. If this interpretation is correct, would the conclusion be correct that the dynamics sampled in the different force fields overlap?

- The O_o and I_o states as sampled with the consdyn and the AT approach seem quite different in Fig. 2. What could be the explanation for this difference?

- Snapshots of the conformations at the maxima of the probability histograms would help my understanding of the differences as introduced by the mutations.

- I do not understand how the overlap in the distributions in Table 2 is computed.

- What is the unit of the shift in Table 2 and of the distances in Fig. 3?

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular simulation of proteins and DNA

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 30 May 2022

K. Anton Feenstra

We thank the reviewer for the attention and time spent on our work, and respond to each of their comments in detail below:

1. Reply: We have added a short explanation of the basics of the SLC glucose transport and how the process cycles through the various states in the Introduction, and now also emphasize the main changes arising from the pathogenic mutations in the Conclusion.

2. Reply: We have added a discussion on the mutations at the R333 and R334 positions in the Introduction.

3. Reply: The 1Å cutoff retains 40% of the constraints, which is already relatively little. Shorter cut-offs would yield more restricted sampling. A wider cut-off would not necessarily provide better sampling, but would very likely lead to too large conformational changes or even unfolding of the transporter protein. This is now explicitly mentioned in the methods section under *Molecular Dynamics*.

4. Reply: Indeed, the ED analysis uses C-alpha for AT and backbone particles at C-alpha

position for CG. We have now clarified this in the *Analysis of transporter dynamics* section in the Methods.

5. Reply: Indeed, transitions between Oo and Io are visible on EV1 in Figure 2; we have now added this explicitly in the figure caption. The sampled states do overlap between the different forcefield approaches. Moreover, as we also discuss in our reply to question 3 of Reviewer 2, the CG sampling partly overlaps the AT sampling on both sides of the Oo to Io transition, and partly with the ComDYN sampling more to the middle of the transition. Furthermore, the Oo ComDYN sampling overlaps considerably with the Io ComDYN sampling. We have also added the starting conformations in Fig 2 as an aid to the reader to better navigate this projected sampling space. We cannot exclude that EV2 may represent conformational changes due to differences between the forcefields, however as we are also sampling far longer timescales in the CG and ComDYN simulations, these differences may also genuinely be part of the transition between the Oo and Io states.

6. Reply: Both ComDYN simulations start at or near the conformations from the AT simulations, but this is not quite visible in the plot in Fig 2. We have now added spheres to indicate the respective starting conformations, as well as triangles to show the corresponding crystal structures. The CG simulations sample much longer timescales, so allow conformational transition that cannot be reached during the sampling time of the AT simulations. We have now clarified this in results, Section "Verification of constraining approach".

7. Reply: The outcome of such a visualization would not be informative for a protein of this size. The differences in conformation (namely the distances between the transmembrane helices) are small and therefore difficult to visualize. Superpositioning snapshot does therefore not help to understand the differences occurring through the mutations. To capture molecular motions during our simulations, we use the defined inter-helix distances (cf. Figure 1D.) as order parameters, which are directly interpretable.

8. Reply: 'Overlap' is the fraction of overlap between both distributions of sampled conformations, calculated as the integral of the minimum of both functions, i.e., the volume (normalized to a maximum of one) that represents the amount of sampling between two distributions. We have now clarified this in the section *Analysis of transporter dynamics* in Methods, with a reference to the relevant python script and now provide the 'overlap' in percentage to make this explicit.

9. Reply: They are all in nanometers (nm), we have added this now explicitly to the table and figure captions.

Competing Interests: No competing interests were disclosed.

Reviewer Report 15 April 2019

https://doi.org/10.5256/f1000research.20307.r46135

Lucie Delemotte
Department of Applied Physics, Science for Life Laboratory (SciLifeLab), KTH Royal Institute of Technology, Stockholm, Sweden

This paper describes a computational study of the effect of mutations on GLUT transporter dynamics. The work consists of atomistic and coarse grained simulations, including some using a protocol called ConsDYN which imposes constraints on distances conserved across conformational transitions. The study identifies that several pathogenic and other mutations modify the structural ensemble visited by the protein.

While the topic is important and computational methods are well-suited to answer the question, I have reservations about the study design and the conclusions reached:

1. A pathogenic mutation modifies the function of the protein such that cellular and organism function are altered. Glucose transporters carry out their function, i.e. importing sugars, via an alternating access cycle in which the transporter transits between outward-open and inward-open states via occluded states; whereas sugar binding from the extracellular medium promotes a transition to the inward facing state, sugar release to the inside intracellular medium promotes a return to the outward facing state. Other than the intrinsic dynamics of interconversion between states, the fact that the sugar modifies the stability of states and the kinetics of interconversion is key for function. Thus pathogenicity of a mutation could be due to many factors: among others, sugar binding, unbinding, (de)stabilization of one or more states on the functional cycle, modification of the rate of conversion between states, in the presence and/or absence of sugar. In this paper, the authors investigate the effect of mutations on the dynamics of interconversion between states. I believe the assumption should be spelled out more clearly, and the omission of all other possible effects on the sugar transport cycle should be explained.

   Relatedly, were the simulations performed in the presence or absence of sugar? Comparing both cases could lead to increased insights.

2. Why was the analysis limited to outward occluded and inward open states, when high-resolution structures of other states are available? If only two states should be considered, why not consider the inward open and outward open since they are the two end-points of the transport cycle?

3. The ConsDYN method can be an interesting way to promote conversion between states

using coarse grained simulations. However, whereas the stated aim on p5 is "to check that ConsDYN is able to sample both I_o and O_o states", Figure 2 reveals that ConsDYN simulations only sample intermediate states, instead of bridging between states. I would thus disagree with the conclusion according to which the method allows to capture the "conserved dynamics" and to "sample between the conformational states".

Would lowering the force constant of the constraints imposed lead to further exploration of the landscape?

The method also seems to have a serious conceptual drawback, in that it assumes that when switching from a state to another, common contacts are conserved, and additional ones are formed in either states. This does not seem to be a general feature of conformational changes in biological molecules and should be discussed.

4. Table 2 reports changes in distance between helices in the presence and absence of mutations, including pathogenic ones. Whereas the pathogenic G91D seems to cause major changes to the dynamics of the transporter, the other pathogenic mutations only alter some of the distance distributions. It does not appear that applying this methodology and measuring the difference in distance distributions as is done in Table 2 allows to predict pathogenicity. I thus disagree with the conclusions: "the distances between TM5 and TM11 can be used as order parameters to elucidate abnormal behavior in the dynamics of the transporter" and "ConsDYN simulations capture the effect of the mutations on the dynamic and function of the transporter proteins".

Minor comments:

1. The authors refer to a "channel" in the title and later in the manuscript. Do they mean the transporter lumen? In an alternating access mechanism, a channel is never observed.

2. p5: The difference between Nagarathinam *et al.* (2018) and this work is ascribed to differences in the transporter protein studied (bacterial vs human) but the authors cannot rule out that the differences can come from differences in the sampling protocol.

3. Were the CG or AA simulations of I_o and O_o analyzed in Figure 3, Table 2 and SI figures?

4. p6: "outer distances between TM5 and TM7 are given in Figure 3" should be replaced by "outer distances between TM5 and TM11 are given in Figure 3".

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Membrane proteins, ion channels, molecular dynamics simulations, enhanced sampling

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 30 May 2022

K. Anton Feenstra

We thank the reviewer for the attention and time spent on our work, and respond to each of their comments in detail below:

1. Reply: All the simulations were carried out without glucose or any other ligand, we only consider the ligand-free states of the transporters. This implies that the pathogenicity of a mutant might already be stated through the intrinsic dynamics of the transporter without explicitly considering the effect of the mutation in the ligand-bound state. This does indeed not exclude that pathogenicity linked to a specific mutant might arise from a dysfunction of the binding mechanism. In this study we focus on the first part of the transport mechanism of SLC transporters, and introduce the ComDYN method, which is designed to capture conformational changes between different states. We have now added this point to the manuscript and explicitly mention that, to guarantee the study of all the relevant effects, simulations including the ligand-bound states might yield additional insight on the structure and dynamic of different mutants. However, this would only be feasible using full-atomistic simulations, since sugars are not well parametrized in the Martini force field. As this would pose strong limits on the time scales we could practically attain in our simulations, it is out of the scope of the current study.

2. Reply: We want to focus on the first part of the transport mechanism: moving from the inward open to the outward occluded state. The next step to elucidate the complete pathway will be to extend this to the outward-open conformation which is available in the PDB. For the present work, we focus on the impact of some mutations on the intrinsic dynamics of the protein (without considering the impact of any ligand) and show in this case study that ComDYN is able to sample these large conformational changes between states. We have now clarified this point in the manuscript.

3. Reply: We mostly agree, we have rephrased “sample both IO and OO states” to “sample the intermediate states between IO and OO states” throughout the manuscript.

4. Reply: A lower force constant would indeed lead to more sampling, just like a wider cut-off which we discuss in our response to question 3 of Reviewer 1, but it would not necessarily lead to a better sampling or better exploration of the landscape. At low force constants for the constraints, in the MARTINI forcefield it becomes increasingly likely that too large conformational changes become permissible, even up to the point of unfolding of the transporter proteins.

5. Reply: The elastic network used in the MARTINI forcefield captures all residue contacts in the tertiary structure. In the case of SLC about 40% of contacts are conserved between both states, in our definition of less than 1 Å difference in the elastic network distance. Except in the case of (complete) unfolding, the vast majority of these contacts will exist in multiple conformational states of a solute carrier protein. However, it should be noted that we used the word ‘conserved’ in two different meanings: the specific evolutionary conservation, and the more general meaning of preservation, in this case of the contacts between two conformational states. We now clarified this throughout the text by referring to ‘common constraints’, and also changed the name ConsDYN to ComDYN accordingly.

6. Reply: Indeed, from the dynamics observed we cannot predict pathogenicity. However, we can ‘elucidate’ the known pathogenicity, in the sense of clarifying where previously effects were not understood. We have made the limitations of the method more explicit in the manuscript. In particular, this sentence now reads “For this reason, we chose them as our order parameter to explain the abnormal behavior in the dynamics of the transporter opening and closing mechanism for some of the observed mutants.” In future approaches, sugar binding will certainly need to be considered in order to fully understand the altered dynamics and behavior of the pathogenic mutants.

Minor comments:

1. Reply: Indeed, we have now changed the title and appropriate sections in the text to refer to ‘transporter’ in stead of ‘channel’.

2. Reply: Indeed we cannot. We have clarified this in the text.

3. Reply: The CG ComDYN simulations are analyzed in Figure 3, Table 2 and SI figures. We now mention this explicitly in the captions of Fig 3 and Table 2 and Figure S3.

4. Reply: We thank the reviewer for pointing out this mistake, which we have now corrected.

Competing Interests: No competing interests were disclosed.