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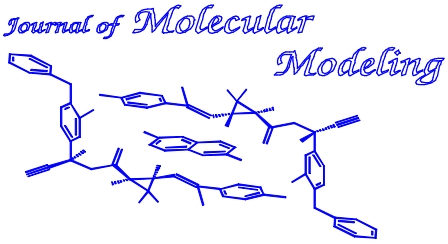

The Erythrocyte/Brain Glucose Transporter (GLUT1) May Adopt a Two-Channel Transmembrane α/β Structure.

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Abstract

There are two models of topology for the membrane domains of the erythrocyte/brain facilitative glucose transporter, GLUT1. The first is composed of 12 membrane-spanning α-helices, the second of 16 membrane-spanning β-strands. We have used Jähnig's and Eisenberg's methods to identify possible transmembrane segments (10 spanning α-helices and 4 β-strands). The topology proposed is more consistent with available experimental data from FTIR, CD and mapping experiment than the previous models . We suggest that GLUT1 might form two channels, one of which is responsible for glucose transport. This agrees with the theoretical and experimental arguments. Finally, an analysis of the mutation periodicity and of the mean hydrophobicity for the GLUT family is provided in order to evaluate the packing of the protein in the membrane.

Keywords: GLUT1, glucose transport, structure, transmembrane protein, α/β structure, modeling
Running Title: GLUT1 transmembrane α/β model
Abbrevations: B16: Fischbarg *et al.* model [4]; CD: Circular Dichroïsm; FTIR: Fourier Transform Infrared; h1-h12: the transmembrane segments of the model of Mueckler *et al.*[3]; HB: model described in this paper; hb1-hb14: the transmembrane segments (α and β) of our model; HB12: Mueckler *et al.* model [3]; RX: X-Rays**;** Sw: Swiss-Prot references

Introduction

GLUT1 is a membrane protein, present in both red blood cells and the brain that transports glucose through the lipid bilayer. It is one of a family of mammalian facilitative hexose transporters, GLUT1 to GLUT7, that are unequally distributed among cell types [1,31]. The primary sequences of these proteins are very similar (50-76% similarity between GLUT1 to GLUT5 human isoform) [1]. GLUT-proteins transport substrates other than glucose (e.g. GLUT1, GLUT2 and GLUT3 transport galactose, GLUT2 transports fructose) with specific kinetics [2]. GLUT5 is really a fructose transporter and the corresponding DNA of GLUT6 is a pseudogene. Very little is known about GLUT7.

GLUT1 gene (492 amino-acids) was sequenced in 1985 [3]. Two models for its topology have been proposed. The first is widely accepted and is based on a hydropathy analysis of the sequence [3]. The method used assigns, as membrane spanning domains, non-overlapping segments of 21 residues with an average hydropathy of more than 0.42 (consensus normalized scale). The model (named H12 in this paper) consists of twelve membrane-spanning α-helices (h1 to h12). More recently, Fischbarg et al. [4] suggested that this model is not consistent with the recognition of the region Ile386-Ala405 by an antibody on the extracellular side of the membrane. They used an algorithm detecting β-strands to generate a model composed of 16 β-strands (named B16), forming a porin-like structure (β-barrel). The algorithm uses a function equal to a level-headed sum of the average

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hydrophobicity (Kyte & Doollitle scale), the hydrophobic moment (id.) and the turn propensity (Chou & Fasman scale) and predicts β-strands where the function is greater than a threshold. However, this model appears to be incompatible with various experimental data (see below).

No three-dimensional structure has been published for the H12 model. Mueckler et al. suggested a channel formed by the five most amphipathic helices, with no defined role for the rest of the protein [3]. According to the B16 model, Fischbarg et al. proposed that GLUT1 works like a porin, i.e. forms an open channel whose entry is controlled by extramembrane loops [4].

The object of this paper is to identify transmembrane segments (topology) in agreement with the experimental data and to analyze the general frame of the transmembrane parts of GLUT1 (topography). We have used Jähnig's [5] and Eisenberg's [6] algorithms together with Chou & Fasman turn propensity [27] to identify membrane spanning domains. In order to analyze the topography of the protein, we developed a visual method based on the representation of the mutation periodicity and the mean hydrophobicity of protein alignment. Indeed, because GLUT1 is thought to form a channel, the residues pointing into the lumen of the channel should generally be more hydrophilic and better conserved than the residues facing the lipids. Moreover, we propose, on the basis of experimental data, that GLUT1 forms two channels.

Materials and methods

The software used is *PC-PROT+*: Protein Analysis (R. Brasseur), *WinMGM*: Molecular Graphic Manipulation (M.Rahman,[24]), WinDNA (M.Rahman) for hydro-phobicity analysis and WHEEL (Ph.Ducarme) for topography studies. CLUSTAL [25] was used for the alignments (Gap fixed=10; Gap vary.=10) and PhDhtm [33] for neural network based prediction The sequences of the GLUT family were obtained from the Swiss-Prot database (release 26, July 1993).

1. Sequence analysis methods

We analyzed the sequence of GLUT1 with the methods of Eisenberg [6, 28] and Jähnig [5]. These methods both seek stretches of amino acids sufficiently hydrophobic to span the membrane (i.e. it is known that protein residues within the membrane are statistically more hydrophobic than the extramembrane ones). In Jähnig's method, the hydrophobicity is averaged for a stretch of 19 (H_{19}) or 7 (H_7) residues corresponding to an α span and α β span, respectively. H_α 11 is a level-headed average function designed to seek amphipathic helices that can occur in membrane channel structures. In the Eisenberg method, in addition to mean hydrophobicity one calculates the hydrophobic moment, which is a measure of the homogeneity of the hydrophobicity in a segment (i.e. a high moment means that all hydrophobic residues are on the same side of the helix). In the plot of the hydrophobic moment versus mean hydrophobicity, several zones that correspond to a particular behavior of the segment (globular, transmembrane, surface, etc.) have been described.

Turn propensity was calculated as described in [27]. The method uses statistically derived tables to estimate the probability of a segment of 4 residues to be structured as a turn. The results obtained for GLUT1 alone were then confirmed by alignment of a consensus turn propensity function :

hydrophobic moment	**mutation moment**	**prediction method**
high(>1)	medium or low(<0.4)	use of hydrophobic moment only.
medium (<1 and >0.5)	low(<0.1)	use of hydrophobic moment only
medium or low(<0.5)	high(>0.4)	use of mutation moment only
low (<0.5)	medium (<0.4 and >0.1)	use of mutation moment only
medium (<1 and >0.5)	medium (<0.4 and >0.1)	*vectorial average* of normalized moments
high(>1)	high(>0.4)	*vectorial average* of normalized moments
low(<0.5)	low(<0.1)	results are not significative

Table 1. *Methodology used for prediction of the orientation of transmembrane segments. Empirical thresholds are based on the study of bacteriorhodopsin.*

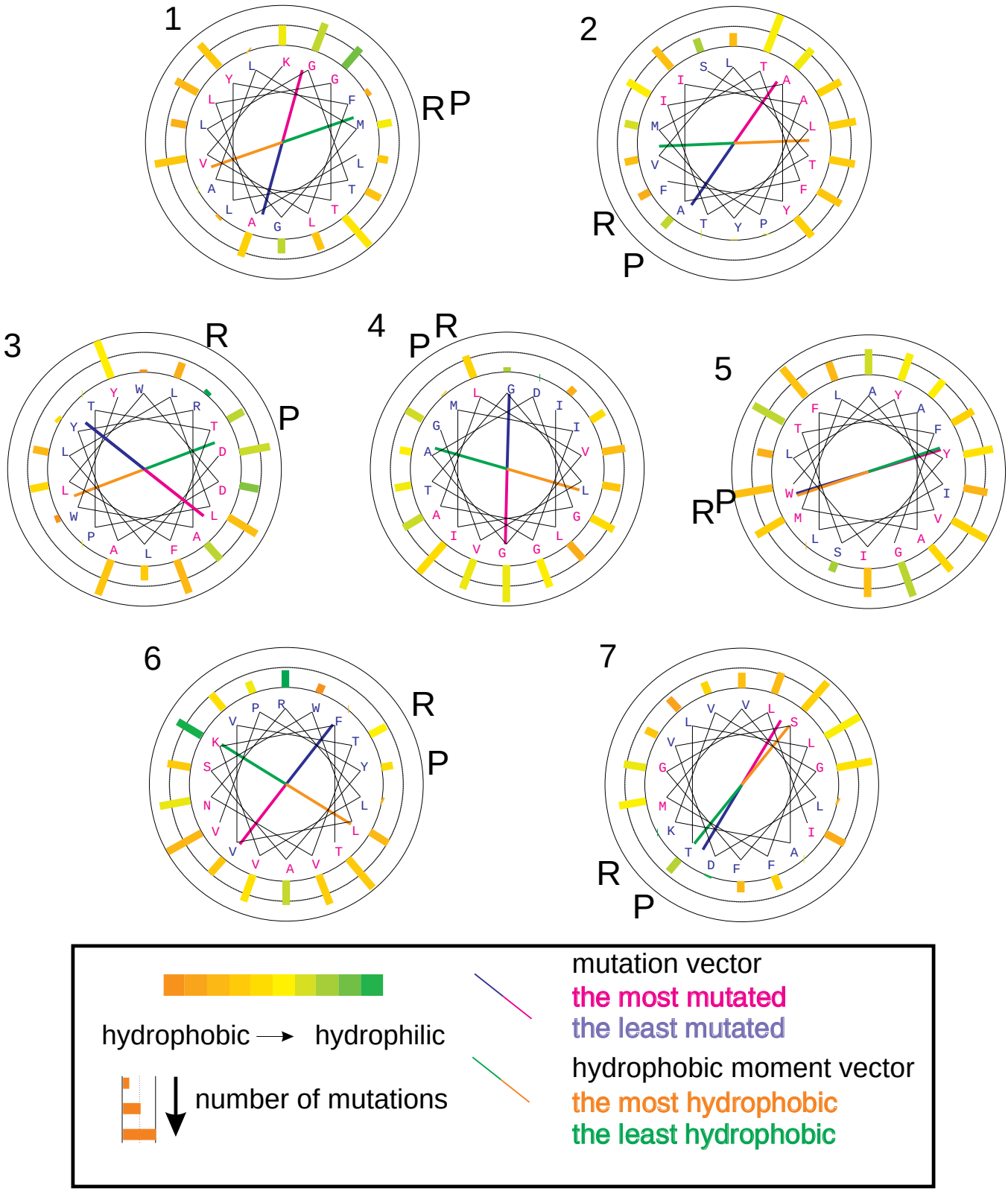


Figure 1. *Representations of the seven helices of the bacteriorhodopsin (see text for description). P is the prediction of the side which point to the lumen of the channel. R shows the lumen of the channel in the crystallographic structure*

Table 2. *Values of the hydrophobic and mutation moments for bacteriorhodopsin homologous sequences.*

Bacteriorhodopsin alignment		
helix	**mutation moment**	**hydrophobic moment**
1	.0281	.6899
2	.4100	.2921
3	.2550	1.5111
4	.4933	1.039
5	.8705	.03897
6	.4300	1.1933
7	.3670	1.7982

$f_n = \Pi_a (tp_{a,n})^{1/A}$ where $tp_{a,n}$ is the turn propensity of the position n in the sequence a and A the total number of sequences. The propensity was assigned to zero where the position n corresponds to a gap for one or more sequences in the alignment.

2. Alignment analysis method

In order to evaluate the packing of the transmembrane helices, we used an Edmunson-Wheel representation (i.e. a schematic view of the backbone with the helix normal to the drawing plane). On this representation, we superimposed in front of each residue heavy lines, the colors of which are representative of the mean hydrophobicity defined as :

$$|H_x| = \frac{1}{S}\sum_{i=1}^{S} H_{x,i}$$

where x is the position considered in the sequence, s, the sequence considered in the alignment, S, the total number of alignments and $H_{x,s}$, the hydrophobicity (normalized Fauchère scale) at the position x of the sequence s. A gradual scale from orange-red (hydrophobic) to green (hydrophilic) is used.

The lengths of the heavy lines are proportional to the number of mutations, M, corrected following the method of Donnelly *et al.*(see *treatment of outliers and ramps* in [29]) considering a window size of 18 for the corrections. These corrections enhance the legibility of the graphics. The green/orange-red vector shows the direction of the hydrophobic moment of Eisenberg (pointing as the orange-red side) and the blue/purple vector corresponds to what we call the mutation vector (pointing to the purple side). This vector is de-

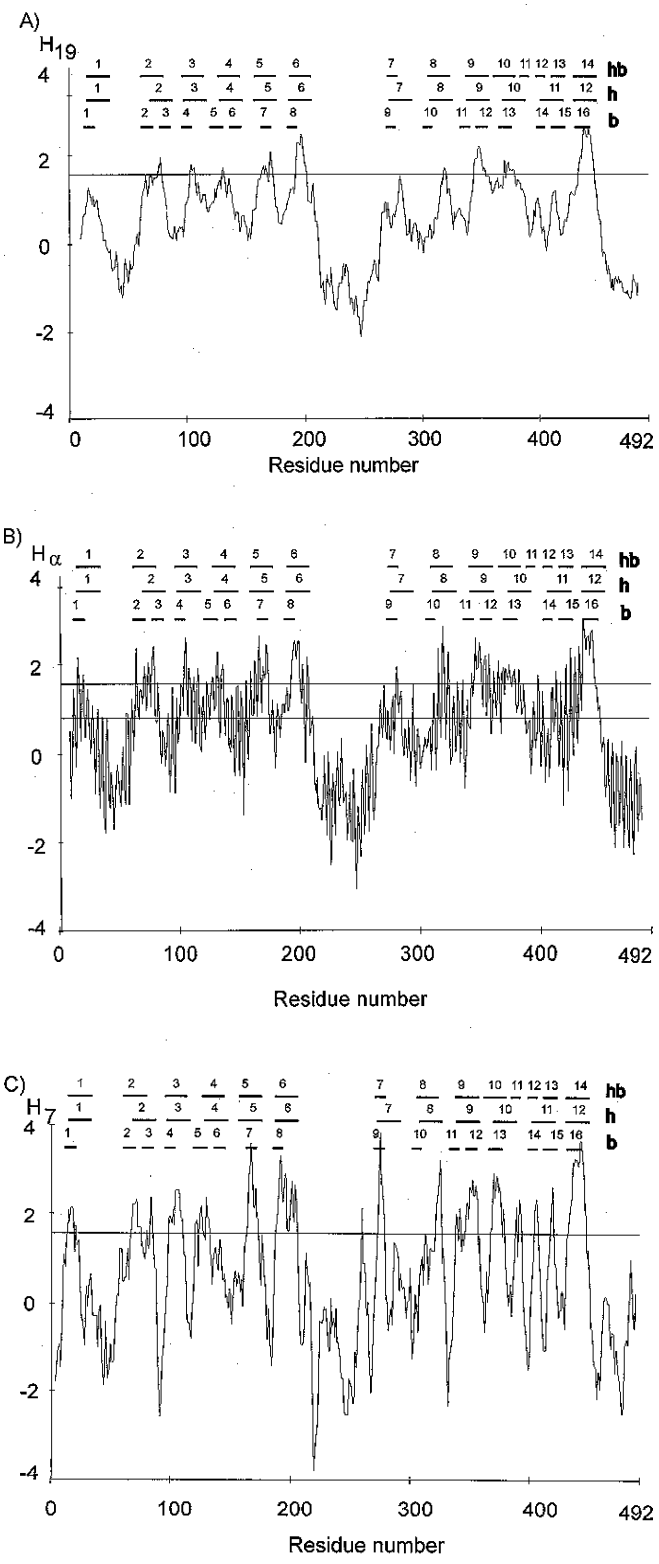


Figure 2. *Plots of Jähnig functions A)$H_{19,}$ B)H and C)H_7 applied to theGLUT1 sequence. Transmembrane segments predicted by model H12 (h1 to h12,[3]), B16 (b1 to b16, [4]) and HB (hb1 to hb14, model proposed in this paper) have been added for comparison. Threshold values are drawn on each plot.*

fined exactly as the hydrophobic moment, except here the hydrophobicity is replaced by the corrected number of mutations. Finally, residues poorly conserved (M>mean M for the helix) are shown in purple, the others (Mmean M for the helix) in blue.

We tested this representation on bacteriorhodopsin. In figure 1, the predicted and experimentally determined buried faces of the 7 helices are denoted by P and R respectively. We aligned the sequence of bacteriorhodopsin (Sw : BACR_HALHA) with three homologous sequences (Sw: BACS_HALHA, BACH_NATPH, BACH_HALSP). Predictions have been made following the methodology described in table 1. The exact values of the moments are shown in table 2.

Predictions are in very good agreement with the RX data from crystallization showing that the method, although very simple, seems reliable enough to be applied to proteins with unknown structures.

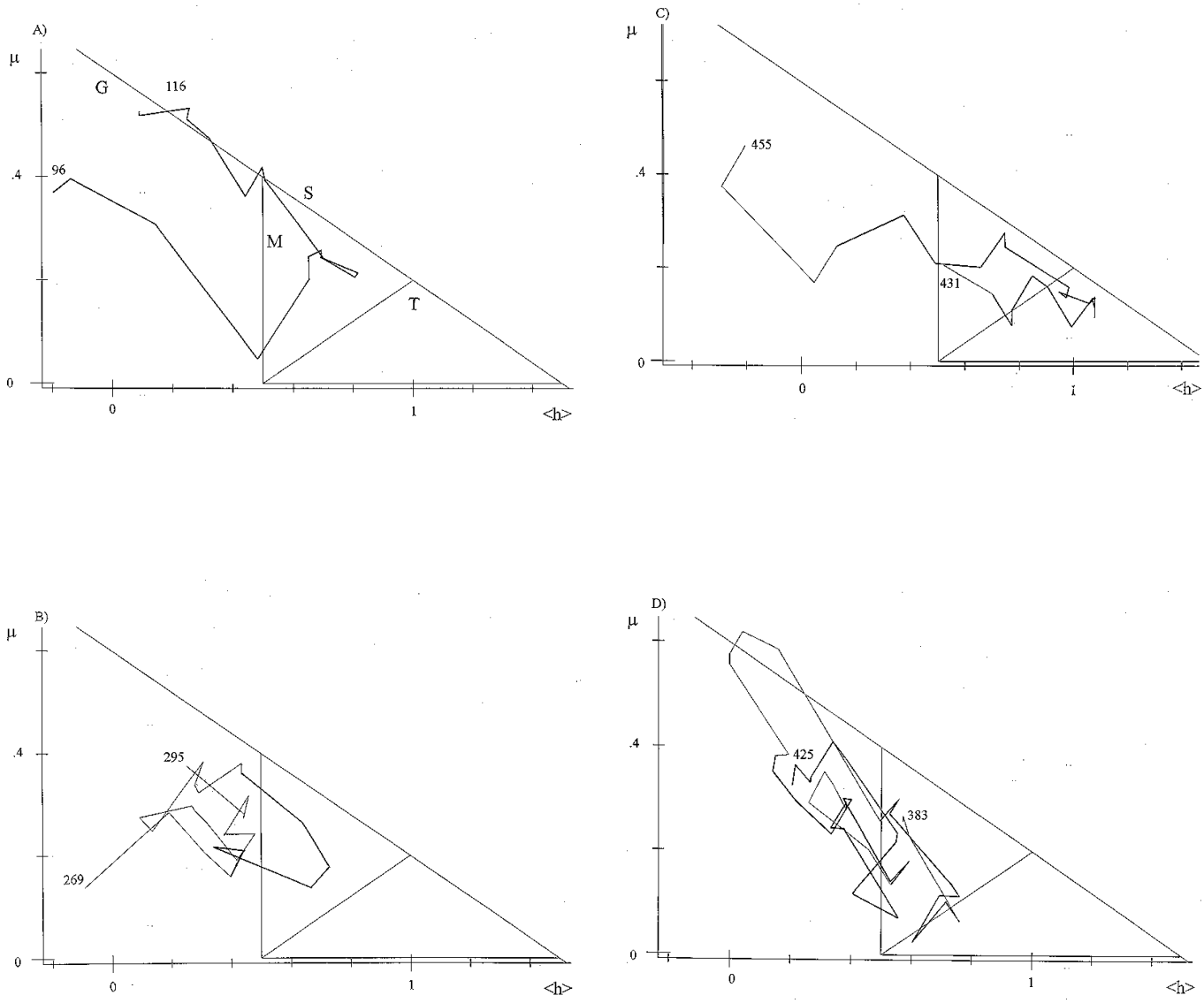


Figure 3. *Eisenberg's plots -hydrophobic moment (μ) versus hydrophobicity(<H>)- of predicted transmembrane segments A) hb3, B) hb 7, C) hb12, D) hb11, hb12 and hb13. The line connects the values of each residue along the primary sequence. Numbers of the two terminal residues of the segment considered are indicated. According to Eisenberg, segments buried in the membrane are expected to be located in the membrane or transmembrane zones of the plot (M or T, cf. fig 2A). G, S and R stand for globular, surface and receptor.*

```
  1     MEPSSKLTG   RLMLAVGGAV   LGSLQTGYNT   GVINAPQKVI   EEFYNQTWVH
                        MTMMM    MMM              SSS

 51     RYGESILPTT  LTTLWSLSVA   IFSVGGMIGS   FSVGLFVNRF   GRRNSMLMMN
                        MMMMM    MMMMMSMMMM   MTM   SS            M

101     LLAFVSAVLM  GFSKLGKSFE   MLILGRFIIG   VYCGLTTGFV   PMYVGEVSPT
        MMMMMMMMMS   SSS  SS       MMSMSSS   SMMMMMMMMM    MM

151     AFRGALGTLH  QLGIVVGILI   AQVFGLDSIM   GNKDLWPLLL   SIIFIPALLQ
                 M  MMMMMTTTTM   MTMMM            SSM   TTTTMMSMSM

201     CIVLPFCPES  PRFLLINRNE   ENRAKSVLKK   LRGTADVTHD   LQEMKEESRQ
        MMM                R   RRRRR R                   RRRRRRR

251     MMREKKVTIL  ELFRSPAYRQ   PILIAVVLQL   SQQLSGINAV   FYYSTSIFEK
        RRRR                       MMM

301     AGVQQPVYAT  IGSGIVNTAF   TVVSLFVVER   AGRRTLHLIG   LAGMAGCAIL
                    MM  MM MMM   MMMM           SS    M   MMMMMMMMMM

351     MTIALALLEQ  LPWMSYLSIV   AIFGFVAFFE   VGPGPIPWFI   VAELFSQGPR
        MMTMMMMMM        MMMMT   TTTTMMMMMM   MMMMMTMTMM   MMM SS

401     PAAIAVAGFS  NWTSNFIVGM   CFQYVEQLCG   PYVFIIFTVL   LVLFFIFTYF
             MMM          M  M   M            MMTMMTTTTT   TTTTTMMM

451     KVPETKGRTF  DEIASGFRQG   GASQSDKTPE   ELFHPLGADS   QV
                      SS S
```

Figure 3e: *Summary of the analysis shown in figures 3a-d. Globular residues correspond to a white space.*

Results and discussion

1. Analysis of the GLUT1 primary sequence.

• Hydropathy

Function H_{19} of Jähnig's method is used to identify segments able to form hydrophobic membrane-spanning α-helices (figure 2A, which also shows transmembrane segments predicted previously). Only nine stretches scored higher than the threshold specified by Jähnig (H_{19} = 1.6, Kyte&Doolittle scale). The predicted transmembrane -helices corresponding to these peaks were named hb2 to 6, hb8 to 10 and hb14. Other previously predicted α-helices gave hydrophobic peaks but scored below the threshold value (especially h1 and h11).

According to Jähnig's method, the sequence was further analyzed with the H_{α} function (figure 2B). This plot shows that the segment corresponding to h1 oscillates between the two critical values (0.8 and 1.6) and could be an amphipathic or a weakly hydrophobic helix. This segment was thus named hb1. Segment h11 could also be considered as a transmembrane amphipathic helix. However, this structure has not been retained because of H_7, the turn propensity function and the Eisenberg's plot described below.

H_7 analysis (figure 2C) divided the segment including h11 into three highly hydrophobic peaks. These regions formed

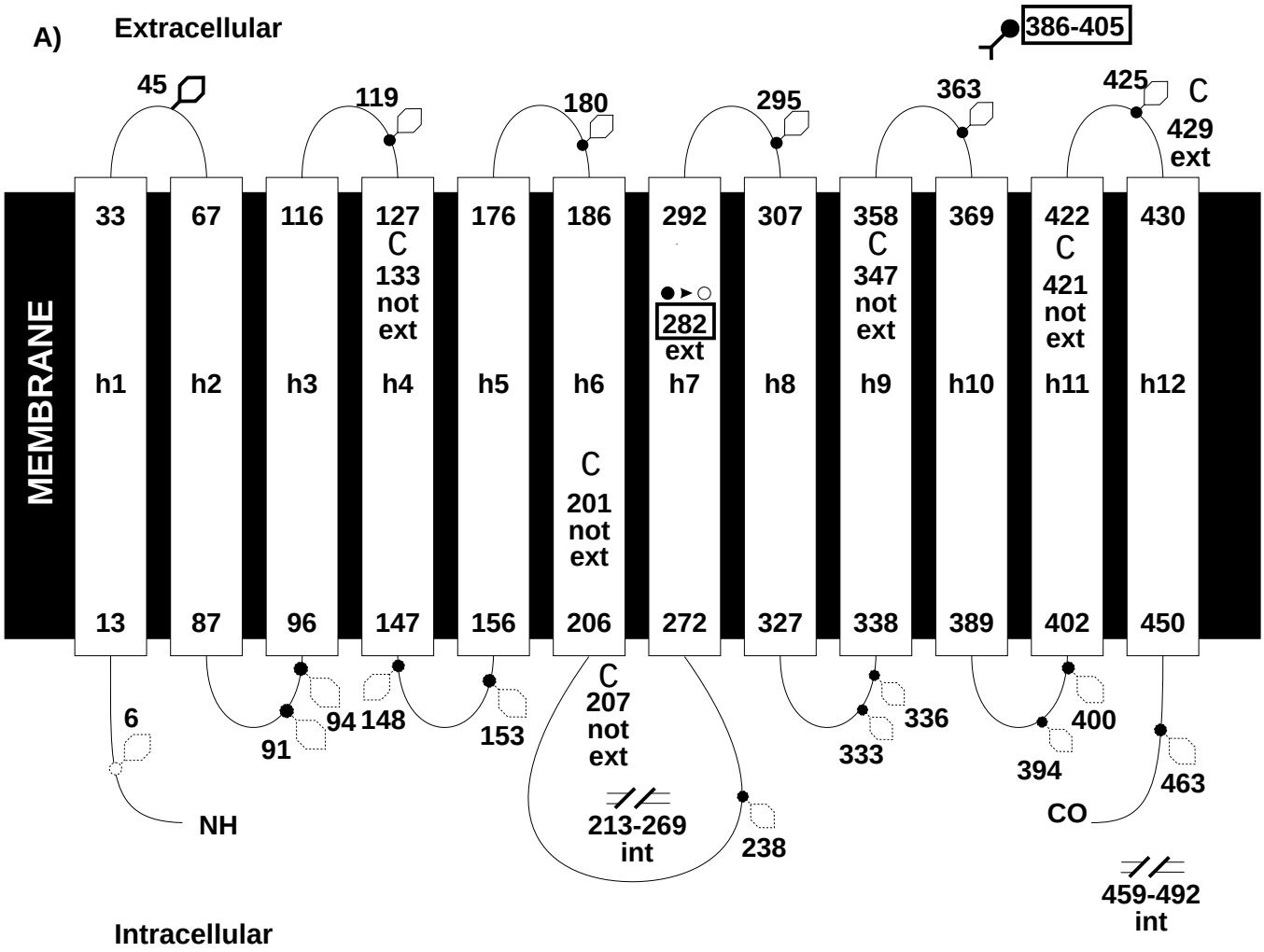


Figure 4a. *Membrane insertion model H12 [3]*

β-strands (named hb11, hb12 and hb13) and not an α-helix. The segment surrounding h7 gave two peaks (figure 2C). The segment Phe^{213}-Arg^{269} is intracellular [7] and therefore the first N-terminal of these two peaks cannot be a membrane-spanning segment. The second peak was considered as a membrane-spanning -strand and named hb7 . Replacement of residue Gln^{282} by Leu reduces the affinity for the outside-specific ligand 2-*N*-4(1-azi-2,2,2-trifluoroethyl)benzoyl-1,3-bis(D-mannos-4-yloxy)-2-propylamine (ATB-BMPA) and has little effect on the transport activity [8]. Gln^{282} is likely, therefore, to be extracellular. This is in line with the occurrence of a β-strand.

We used Eisenberg's plots to confirm this model. This method confirms that segments corresponding to h2, h3 (see figure 3A), h4 to h6, h8 to h10 and h12 (3C) can all form transmembrane helices. Only 3 residue of segment h7 (3B) appeared to be in the transmembrane region, the rest being assigned as globular. The region surrounding h11 (3D) formed 3 clusters of (trans)membrane residues separated by two segments which lie some distance from the (trans)membrane region in the plot. Consequently regions h7 and h11 are unlikely to be structured as helices. The segment hb1 includes a few membrane (M) residues (3E) but the plot is ambiguous and therefore we cannot confirm that this is a transmembrane helix.

These analyses of GLUT1 led us to propose the model HB depicted by figure 4C. The exact limits of the segments hb1 to hb14 (our model) are based on Jähnig, Eisenberg and Edmunson-Wheel plots (data not shown) with the exception of segments hb7, hb12 and hb13, three of the -strands proposed by Fischbarg *et al* [4]. The H12 and B16 models are shown in figures 4A and 4B. Besides hb2 and hb10, the α-helices of our model (4C) are very similar to those of the H12 model (4A). It is worth noting that part of the segment, Pro^{383}-Pro^{387}, which is thought to be important for the protein flexibility, [9, 10] forms an intracellular loop in our model.

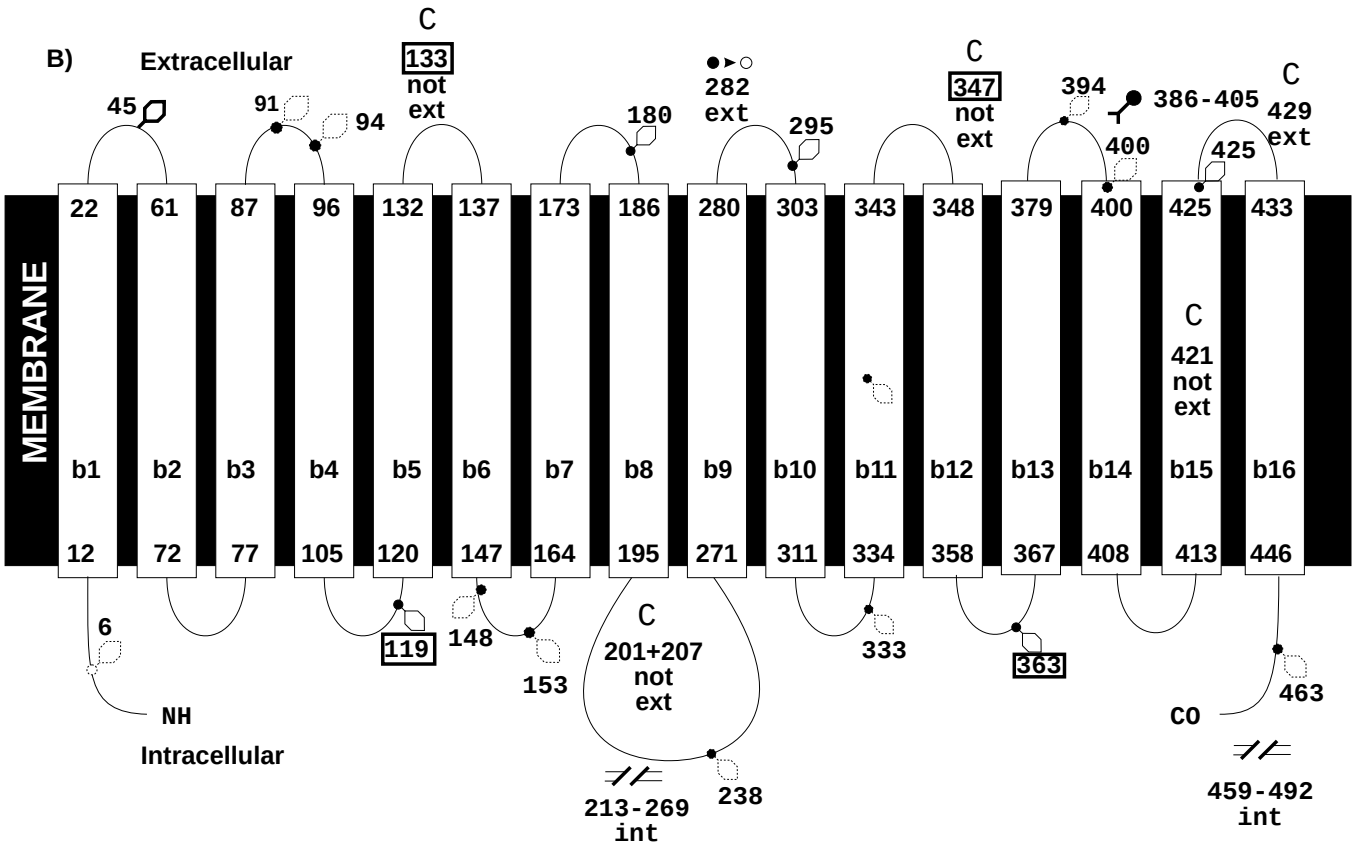


Figure 4b. *Membrane insertion model B16 [4]. See also figure 4c.*

• Turn propensity

In the turn propensity plot of GLUT1 (figure 5A), four very high peaks from residues 381 to 441 can be noted. This profile matches our topology model, each peak corresponding to a short loop separating the transmembrane segments. This is an interesting point because this region of the protein seems to be critical for transport [1]. A consensus turn propensity calculation (figure 5B) was performed on the GLUT-protein alignment as described in material and methods. These high turn propensity regions were found in all the isoforms of the alignment, suggesting that they are a general property of the GLUT family.

2. Confrontation of the topological models with experimental data.

• Secondary structure

FTIR and CD studies disagree about the exact ratio of α to β structures in GLUT1[11, 12, 13, 14] but the most recent studies [12,14] suggest that it is predominantly α-helical with a significant proportion of β-strands. Moreover, papain digestion, associated with FTIR, suggests that the intramembranous part of GLUT 1 is largely α-helical although some β-strands are present [7]. This is partially confirmed by a new deconvolution algorithm which suggests the presence of transmembrane α-helices (by opposition to extramembrane helices) [14]. The H12 and B16 models are incompatible with these observations because they are *all alpha* or *all beta* models. Conversely, from a qualitative point of view, the HB model matches these data. If we assume that the two large loops (Pro^{36}-Thr^{60} between hb1/hb2 and Pro^{208}-Pro^{271} between hb6/hb7), the carboxy- and the amino-terminal segments are mostly α-helical, the proportion of α-helical structure in our model is about 65%. The transmembrane proportion of β-strands is about 8%.

• Mapping experiments

We superimposed several mapping experiments on the three topological models so as to evaluate their quality (figure 4). HB is the only model fully compatible with the experimental data despite none of the data being used to construct the model. This verification is a strong argument in its favor. The B16 model does not match the experimental results for positions 119 and 363, which are glycosylated after insertion of a glycosylation site [16], suggesting that these sites are extracellular. Moreover Cys^{133} and Cys^{347} are not extracellular [26]. The H12 model cannot explain the recognition of segment 386-405 by an antibody on the extracellular side of the membrane and does not agree with the localization of Gln^{282} in the extracellular space.

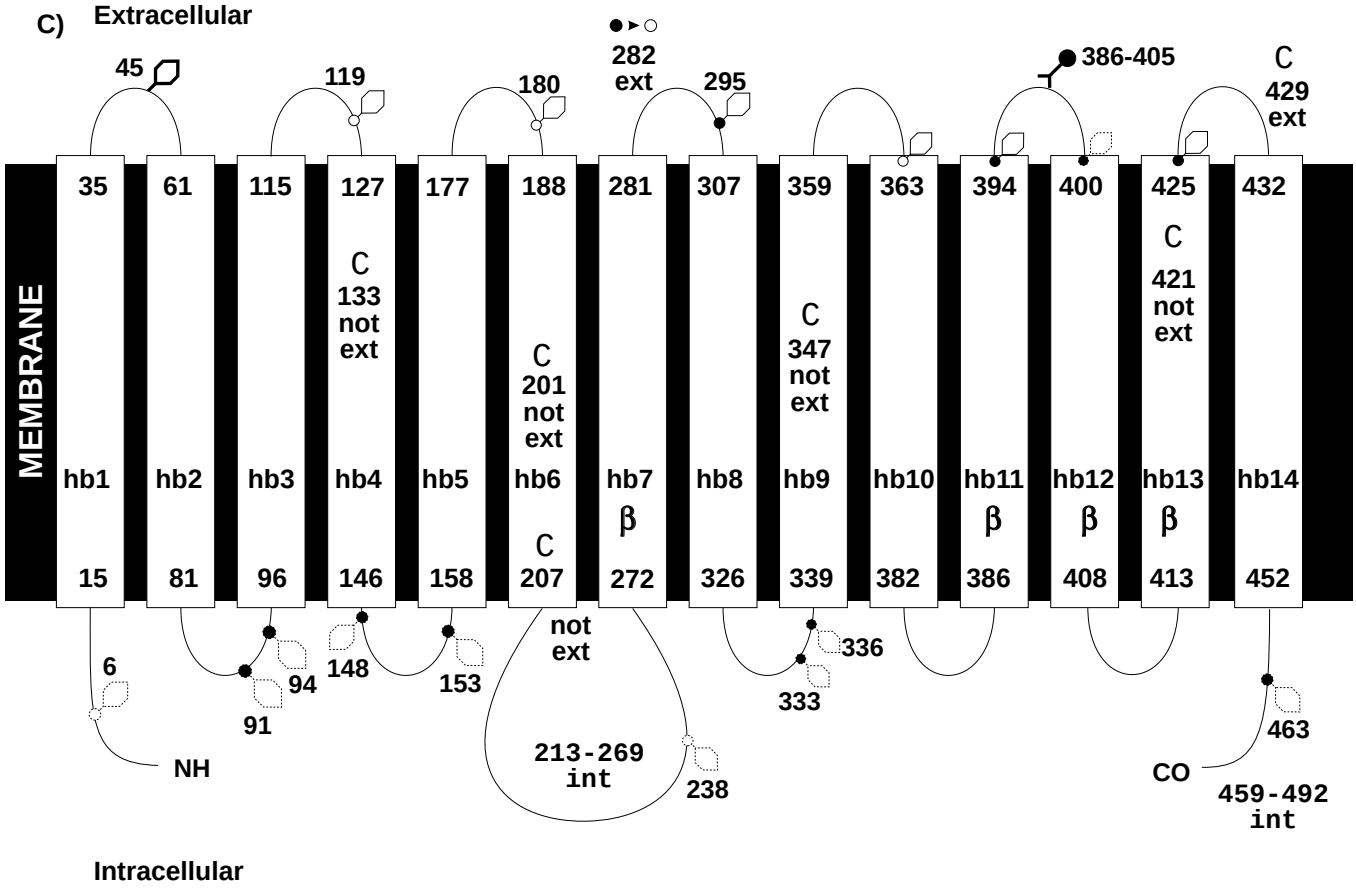


Figure 4c. *Membrane insertion model HB [this paper].The exact segment limits for model H12 are those supplied by SwissProt databank (GTR1_HUMAN, release of sept.93). Mapping experiments are indicated with the residues involved. Boxed segments are those inconsistent with the model.*

• Extension of the model to other proteins

The hydropathy analysis was performed on several isoforms of GLUT1 (GLUT2-4) in different species and on other transporters belonging to the same family as GLUT1 (Sugar Transporter Family) (samples in figure 6). Although GLUT3 and GLUT4 show strong similarity(except for hb4), all the other protein plots are clearly different and lead to distinct predictions. This suggests that the structure of these proteins may also differ. We do not think that this works against the α/β–model because there is no reason why all these transporters should have the same structure. Although it is generally admitted that similar sequences correspond to the same structure, recent studies have shown that one mutation can turn the prions structure from α to β [32]. Thus, it may be possible that the GLUT1,3 and 4 proteins, although sharing the biggest part of their structures with similar proteins, have some widely modified, particular domains in common.

• Neural network prediction

We used a neural network-based prediction program (PhDhtm,[33]) in order to confirm the HB model (figure7). This program considers only two states for the structure : helix (H) and loop (L) and provides a *reliability index* for the prediction (0-9). Helix hb 4 should not be considered because it is clear from the hydropathy plots that this part of the transporter is not similar in GLUT1, 3 and 4. Except for the segments hb1, hb11, hb12 and hb13, the prediction is in good agreement with our model and Mueckler's model. The helical segment predicted near hb7 is too small (12 residues) to span the membrane. This may suggest that the loop following this segment lies in the lumen of the channel (which is thought to be hydrophilic) or that this part of the molecule is not structured as an helix. The predicted helix corresponding to the three β-strands of hb11, hb12 and hb13 is shorter (15 residues) and has lower reliability than the other predicted transmembrane helices. This is not consistent with our model, but as the prediction program knows only two structural states we must keep in mind that, if this part of the protein is actually structured in β-strands, the prediction may be false. Finally, hb1 is not predicted at all. This may be due to the hydrophilic property of this segment already shown above.

3. Topography analysis

• Hypothesis of the double channel.

The construction of the topographical model required an overall framework of the protein to be determined, in order to identify which transmembrane segments are associated. Since the protein is largely accessible to water -80% of deuterium/hydrogen exchange occurs rapidly [12] - most of the transmembrane segments are presumably involved in the channel. Moreover, GLUT1 does not let small ions cross the membrane so that there must be a steric or electric gate. Since the external loops of our model are too short to form an efficient gate for a single channel constructed with the 14 transmembrane segments (about 40 Å in diameter), such a channel is incompatible with the experimental data. However, a channel made from the C-terminal part of the protein (Ile^{272}-Val^{492}, referred to as channel 2) would have a diameter just large enough for glucose and hence be more efficient in stopping ions. Besides, the glycerol facilitator of *E.Coli* (GlpF) has a similar length (281 residues). This suggests that channel 2 contains enough residue to form an effective facilitator.

Furthermore, most of GLUT1 mutants affecting glucose transport map in the C-terminal part of the protein [1]. The N-terminal part of GLUT1 should also form an amphipathic structure as indicated by the high deuterium/hydrogen exchange mentioned above [12]. As GLUT1 is thought to come

Table 3. *Values of the hydrophobic and mutation moments for the alignment of figure 7.*

GLUT1, 3 and 4 alignment		
helix	**mutation moment**	**hydrophobic moment**
hb14	.4290	.3931
hb10	.2287	.3751
hb9	.4361	.3944
hb8	.2716	1.5021
hb6	.3766	.7690
hb5	.1694	1.6267
hb4	.1626	1.8152
hb3	.4186	2.2679
hb2	.2396	1.3135
hb1	.1397	.4279
h7	.0374	1.7248
h11	.1696	1.5135

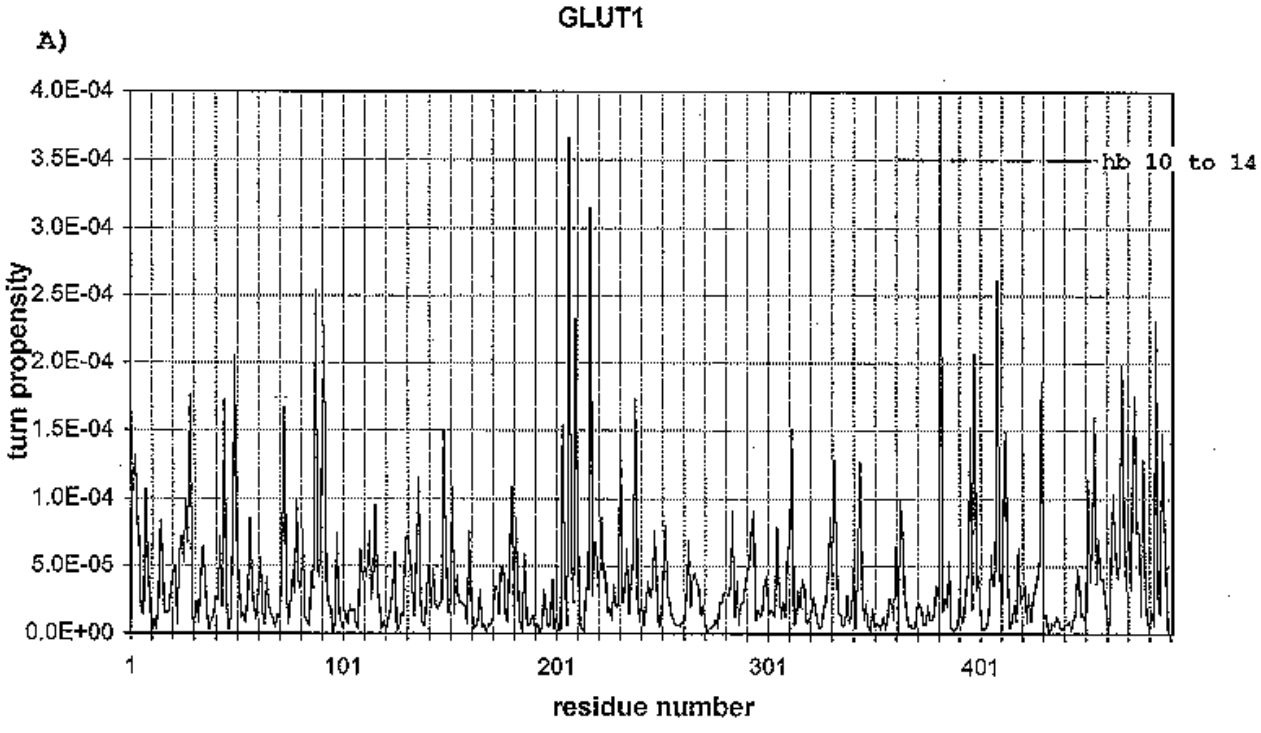


Figure 5 a. *Turn propensity function of GLUT1(27). The threshold value established by Chou and Fasman for predicting a turn is 0.75 E-04. Transmembrane segments in our model have been added around the high propensity regions (see text).*

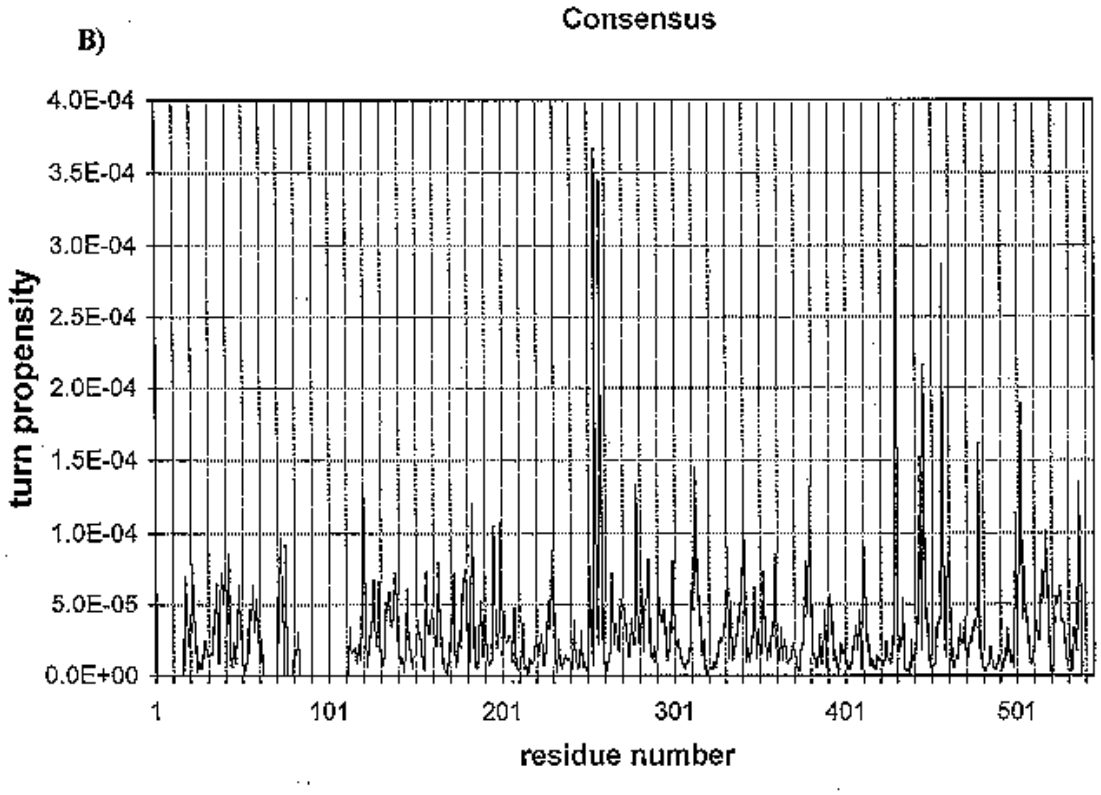


Figure 5b. *Consensus turn propensity function for the alignment of figure 7. Compared to fig. 4A, several peaks are reduced (e.g. near residues 220) whereas four high peaks remain between residues 380 to 440.*

from a genetic duplication [17] and as hydropathy plots show a symmetry between both halves of the protein, the N-terminal (Met^{1}-Cys^{207}) part of the molecule could also form a channel (named channel 1). This channel, in the case of the proline/betaine cotransporter of E.*coli* (swprot : PROP_ECOLI), which also belongs to the sugar transporter family and probably has a similar global structure, may also transport a substrate. Channel 1 of GLUT1 may thus be a degenerate form of a cotransport channel still interacting with the C-terminal part of the molecule but which does not transport cosubstrate. Another possibility (not exclusive) is that channel 1 serves as a water channel. Indeed, rat glucose transporters GLUT1, GLUT2 and GLUT3 allow the passage of water through the membrane [30]. The large charged loop between helices hb1 and hb2 (Pro^{36}-Thr^{60}) may thus act as an electric filter preventing small ions passing through the channel. Such a structure (a water channel associated with a glucose channel) may act as a osmotic regulator.

Cope *et al.* [18] showed that when the N- or C-terminal halves of GLUT1 are expressed in a Sf9 cell membrane, they do not recognize ATB-BMPA or cytochalasine B. In cells producing both amino- and carboxy-terminal halves, the ligand labeling is restored. This strongly suggests that the two parts of the molecule can fold independently. This seems incompatible with the model B16 which is a porin-like structure and with the model H12 where the channel is composed of helices of both the first and the second parts of the molecule (h3, h5, h7, h8 and h11[3]). This experiment also suggests that the two channels strongly interact because the amino-terminal part of the molecule, which does not bind ligands, restores ligand binding to of the carboxy-terminal part [18]. Thus, the functions of the two halves of the protein are likely to be coupled. This could explain why the mutation of Gln^{161} in the N-terminal part of the molecule strongly reduces the rate of conformational change [22] even if this residue is not included in channel 2, which transports glucose.

• Topographic analysis of the GLUT family alignment

We aligned the available sequences of GLUT1, 3 and 4 (figure 8). Representations of the 10 helices of our model and of helices h11 and h7 of Mueckler's model are shown in figure 9. Exact values of the moments are given in table 3. Following the methodology described in table 1, the side of the helices predicted to point to the lumen of the channel are denoted by P.

helix hb1: Mutation vector (MV) is medium and the hydrophobic vector(HV) is low. This may reflect that helix hb1 is surrounded by other helices so that there are no really privileged direction. This is in line with the hydrophobicity plots.

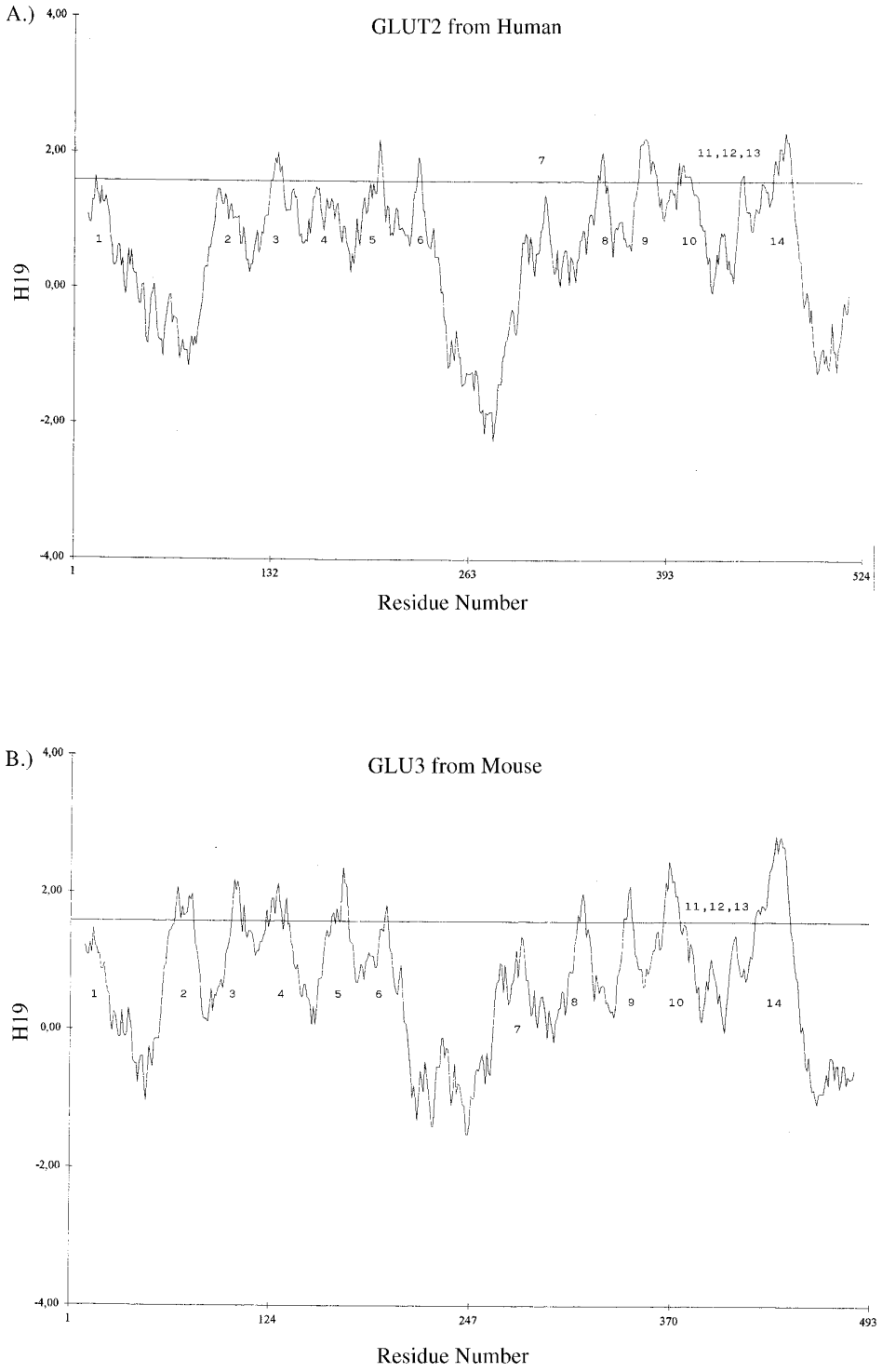
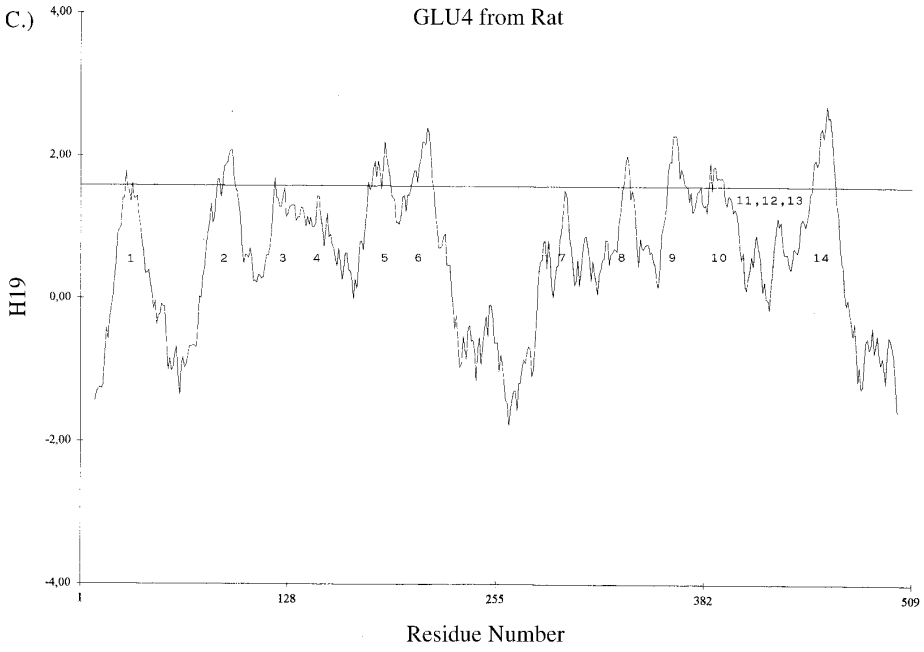
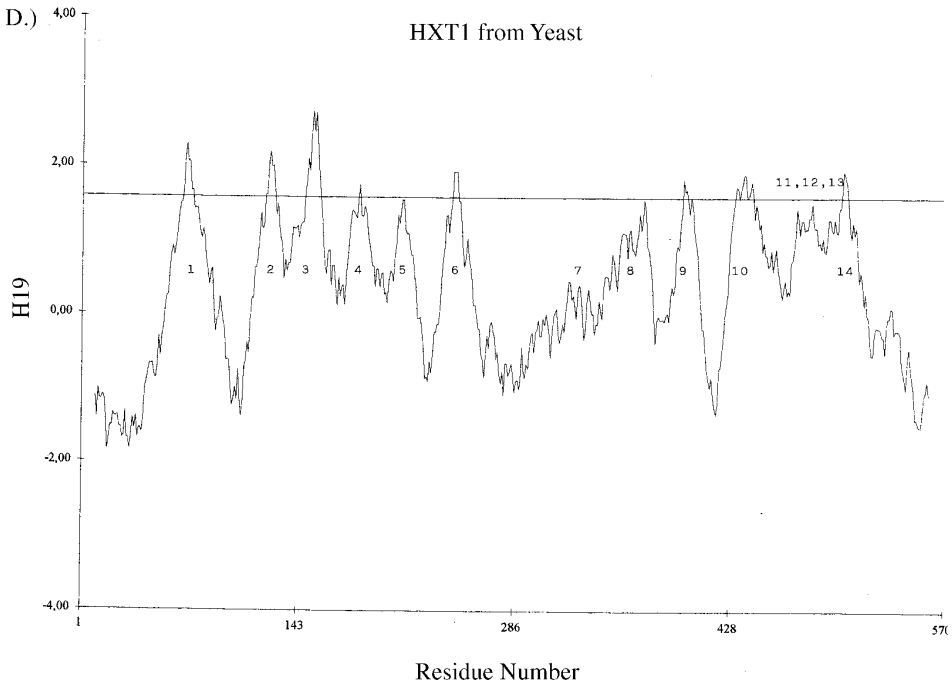


Figure 6. *Plots of Jähnig functions of A) GLUT2 from human, B)GLUT3 from mouse, C) GLUT4 from mouse and D) HXT1 from yeast.*

helix hb2: MV is low (the helix is very well conserved) and does not agree with the high HV. This suggests that hb2 plays an important functional role and may be part of a channel.
helix hb3: MV and HV are high and consistent. These are the typical properties corresponding to pore-forming helix.
helix hb4 and hb5 and hb8: MV is medium and HV is high. These helices seem to make part of the pore but without any important functional role.
helix hb6: MV and HV are medium.
helix hb9 and hb 14: HV low but MV high. This suggests that hb9 and hb14 play a particular structural role, because although they are almost entirely hydrophobic, one face of these helices is especially conserved.





helix hb10 : HV low and MV medium. Same as hb9.

At the moment we cannot provide a topographic model for the C-terminal channel because we have no idea of what a α/β-transmembrane structure could look like. No transmembrane α/β–structure has been identified by X-ray crystallography and this proposal may therefore seem speculative. However, transmembrane structures are so poorly documented that α/β-structures cannot be excluded. Moreover, both experimental and theoretical analyses suggest that such a structure is found in the acetylcholine receptor [15]. If such a structure does exist, it is more likely to be folded, at least in part, in the cytoplasm before being inserted as the insertion of a non H-bonded -strand in the membrane is energetically very unfavorable. We do not know how such a process would take place so we can hardly provide a model for a α/β-structure.

```
                 ....,....1....,....2....,....3....,....4....,....5....,....6
        AA      |MEPSSKKLTGRLMLAVGGAVLGSLQFGYNTGVINAPQKVIEEFYNQTWVHRYGESILPTT|
                              AAAAAAAAAAAAAAAAAAAA
PhDhtm predict. |LLLLLLLLLLLLLL.................LLLLLLLLLLLLLLLLLLLLLLLLLLL.|
        Rel htm |99999999999975310000000001444578899999999999999999999998751|

                 ....,....7....,....8....,....9....,....10...,....11...,....12
        AA      |LTTLWSLSVAIFSVGGMIGSFSVGLFVNRFGRRNSMLMMNLLAFVSAVLMGFSKLGKSFE|
                  AAAAAAAAAAAAAAAAAAAA                AAAAAAAAAAAAAAAAAAAA
PhDhtm predict. |.HHHHHHHHHHHHHHHHHHHHHHHH......LL.....HHHHHHHHHHHHHHHH.....H|
        Rel htm |35678888877777777777776642102466410046777877777777766000006|

                 ....,....13...,....14...,....15...,....16...,....17...,....18
        AA      |MLILGRFIIGVYCGLTTGFVPMYVGEVSPTAFRGALGTLHQLGIVVGILIAQVFGLDSIM|
                        AAAAAAAAAAAAAAAAAAAA            AAAAAAAAAAAAAAAAAAAA
PhDhtm predict. |HHHHHHHHHHHHHHHHHHHH........LLLLLL....HHHHHHHHHHHHHHHHH.....|
        Rel htm |67777777777777656665400012345677531005677777887777776533430|

                 ....,....19...,....20...,....21...,....22...,....23...,....24
        AA      |GNKDLWPLLLSIIFIPALLQCIVLPFCPESPRFLLINRNEENRAKSVLKKLRGTADVTHD|
                          AAAAAAAAAAAAAAAAAAAA
PhDhtm predict. |.....HHHHHHHHHHHHHHHHHHHH.....LLLLLLLLLLLLLLLLLLLLLLLLLLLLLL|
        Rel htm |00004567777877777777776540014678788999999999999999999999999|

                 ....,....25...,....26...,....27...,....28...,....29...,....30
        AA      |LQEMKEESRQMMREKKVTILELFRSPAYRQPILIAVVLQLSQQLSGINAVFYYSTSIFEK|
                                              BBBBBBBBBB
PhDhtm predict. |LLLLLLLLLLLLLLLLLLLLLLLLLL...HHHHHHHHHHHH.................L|
        Rel htm |99999999999999999999999876412566777776655420100000000000246|

                 ....,....31...,....32...,....33...,....34...,....35...,....36
        AA      |AGVQQPVYATIGSGIVNTAFTVVSLFVVERAGRRTLHLIGLAGMAGCAILMTIALALLEQ|
                        AAAAAAAAAAAAAAAAAAAA            AAAAAAAAAAAAAAAAAAAA
PhDhtm predict. |LLLLLL....HHHHHHHHHHHHHHHHHH....LLLLLLL..HHHHHHHHHHHHHHHHHH..|
        Rel htm |67777520246777777778787776520446776665105677788877777766522|

                 ....,....37...,....38...,....39...,....40...,....41...,....42
        AA      |LPWMSYLSIVAIFGFVAFFEVGPGPIPWFIVAELFSQGPRPAAIAVAGFSNWTSNFIVGM|
                   AAAAAAAAAAAAAAAAAAAA   BBBBBBBBB     BBBBBBBBB    BBBBBBBBB
PhDhtm predict. |.HHHH...HHHHHHHHHHHHHHHHHHHHHHHHH.......LL......HHHHHHHHHHHHH|
        Rel htm |45677000877777777777777777777765400003455430004667776766666|

                 ....,....43...,....44...,....45...,....46...,....47...,....48
        AA      |CFQYVEQLCGPYVFIIFTVLLVLFFIFTYFKVPETKGRTFDEIASGFRQGGASQSDKTPE|
                  BBBBB       AAAAAAAAAAAAAAAAAAAA
PhDhtm predict. |HH....HHHHHHHHHHHHHHHHHHHHHHH...LLLLLLLLLLLLLLLLLLLLLLLLLLL|
        Rel htm |65000066777777777777778888775003678899999999999999999999999|

                 ....,....49...,....50...,....51...,....52...,....53...,....54
         AA      |ELFHPLGADSQV|

PhDhtm predict. |LLLLLLLLLLL|
         Rel htm |99999999999|
```

Figure 7. *Transmembrane helices prediction from PhDhtm (33) are in purple. H:helix, L:loop, (.):no prediction, Rel htm lines indicate the reliability of the prediction from 0 to 9. Transmembrane segments of our model are in red.A: structure, B: structure. The sequences submitted to PhDhtm are those from fig.8. GLUT1sequence is shown (AA).*

```
* :=>  match across all seqs.
. :=>  conservative substitutions

GTR1_HUM M———————EPSSKKLTGRLMLAVGGAVLGSLQFGYNTGVINAPQKVIEEFYNQ
GTR1_BOV M———————EPTSKKLTGRLMLAVGGAVLGSLQFGYNTGVINAPQKVIEEFYNQ
GTR1_MOU M———————DPSSKKVTGRLMLAVGGAVLGSLQFGYNTGVINAPQKVIEEFYNQ
GTR1_RAB M———————EPSSKKVTGRLMLAVGGAVLGSLQFGYNTGVINAPQKVIEEFYNQ
GTR1_RAT M———————EPSSKKVTGRLMLAVGGAVLGSLQFGYNTGVINAPQKVIEEFYNQ
GTR3_HUM M————————GTQKVTPALIFAITVATIGSFQFGYNTGVINAPEKIIKEFINK
GTR3_MOU M————————GTTKVTPSLVFAVTVATIGSFQFGYNTGVINAPETILKDFLNY
GTR4_HUM MPSGFQQIGSE—DGEPPQQRVTGTLVLAVFSAVLGSLQFGYNIGVINAPQKVIEQSYNE
GTR4_MOU MPSGFQQIGSDVKDGEPPRQRVTGTLVLAVFSAVLGSLQFGYNIGVINAPQKVIEQSYNA
GTR4_RAT MPSGFQQIGSE—DGEPPQQRVTGTLVLAVFSAVLGSLQFGYNIGVINAPQKVIEQSYNA
         *                  ..*  *..*.  *..**.*****.******.....  *

GTR1_HUM TWVHRYG——ESILPTTLTTLWSLSVAIFSVGGMIGSFSVGLFVNRFGRRNSMLMMNLL
GTR1_BOV TWVQRYG——EPIPPATLTTLWSLSVAIFSVGGMIGSFSVGLFVNRFGRRNSMLMMNLL
GTR1_MOU TWNHRIG——EPIPSTTLTTLWSLSVAIFSVGGMIGSFSVGLFVNRFGRRNSMLMMNLL
GTR1_RAB TWIHRYG——ERILPTTLTTLWSLSVAIFSVGGMIGSFSVGLFVNRFGRRNSMLMMNLL
GTR1_RAT TWNHRYG——ESIPSTTLTTLWSLSVAIFSVGGMIGSFSVGLFVNRFGRRNSMLMMNLL
GTR3_HUM TLTDKGNAPPSEVL——LTSLWSLSVAIFSVGGMIGSFSVGLFVNRFGRRNSMLIVNLL
GTR3_MOU TLEERLEDLPSEGL——LTALWSLCVAIFSVGGMIGSFSVGLFVNRFGRRNSMLLVNLL
GTR4_HUM TWLGRQGPEGPSSIPPGTLTTLWALSVAIFSVGGMISSFLIGIISQWLGRKRAMLVNNVL
GTR4_MOU TWLGRQGPGGPDSIPQGTLTTLWALSVAIFSVGGMISSFLIGIISQWLGRKRAMLANNVL
GTR4_RAT TWLGRQGPGGPDSIPQGTLTTLWALSVAIFSVGGMISSFLIGIISQWLGRKRAMLANNVL
         *   . .    . .    **.**.*.**********.** .*.. ...**...**  *.*

GTR1_HUM AFVSAVLMGFSKLGKSFEMLILGRFIIGVYCGLTTGFVPMYVGEVSPTAFRGALGTLHQL
GTR1_BOV AFVSAVLMGFSKLGKSFEMLILGRFIIGVYCGLTTGFVPMYVGEVSPTELRGALGTLHQL
GTR1_MOU AFVAAVLMGFSKLGKSFEMLILGRFIIGVYCGLTTGFVPMYVGEVSPTALRGALGTLHQL
GTR1_RAB AFVSAVLMGFSKLAKSFEMLILGRFIIGVYCGLTTGFVPMYVGEVSPTALRGALGTLHQL
GTR1_RAT AFVSAVLMGFSKLGKSFEMLILGRFIIGVYCGLTTGFVPMYVGEVSPTALRGALGTLHQL
GTR3_HUM AVTGGCFMGLCKVAKSVEMLILGRLVIGLFCGLCTGFVPMYIGEISPTALRGAFGTLNQL
GTR3_MOU AIIAGCLMGFAKIAESVEMLILGRLLIGIFCGLCTGFVPMYIGEVSPTALRGAFGTLNQL
GTR4_HUM AVLGGSLMGLANAAASYEMLILGRFLIGAYSGLTSGLVPMYVGEIAPTHLRGALGTLNQL
GTR4_MOU AVLGGALMGLANAVASYEILILGRFLIGAYSGLTSGLVPMYVGEIAPTHLRGALGTLNRL
GTR4_RAT AVLGGALMGLANAAASYEILILGRFLIGAYSGLTSGLVPMYVGEIAPTHLRGALGTLNQL
         *  .. .**. .   * *.*****..** ..** .*.****.**..** .***.***..*

GTR1_HUM GIVVGILIAQVFGLDSIMGNKDLWPLLLSIIFIPALLQCIVLPFCPESPRFLLINRNEEN
GTR1_BOV GIVVGILIAQVFGLDSIMGNQELWPLLLSVIFIPALLQCILLPFCPESPRFLLINRNEEN
GTR1_MOU GIVVGILIAQVFGLDSIMGNADLWPLLLSVVFVPALLQCILLPFCPESPRFLLINRNEEN
GTR1_RAB GIVVGILIAQVFGLDSIMGNEDLWPLLLSVIFVPALLQCIVLPLCPESPRFLLINRNEEN
GTR1_RAT GIVVGILIAQVFGLDSIMGNADLWPLLLSVIFIPALLQCILLPFCPESPRFLLINRNEEN
GTR3_HUM GIVVGILVAQIFGLEFILGSEELWPLLLGFTILPAILQSAALPFCPESPRFLLINRKEEE
GTR3_MOU GIVVGILVAQIFGLDFILGSEELWPGLLGLTIIPAILQSAALPFCPESPRFLLINKKEED
GTR4_HUM AIVIGILIAQVLGLESLLGTASLWPLLLGLTVLPALLQLVLLPFCPESPRYLYIIQNLEG
GTR4_MOU AIVIGILVAQVLGLESMLGTATLWPLLLALTVLPALLQLILLPFCPESPRYLYIIRNLEG
GTR4_RAT AIVIGILVAQVLGLESMLGTATLWPLLLAITVLPALLQLLLLPFCPESPRYLYIIRNLEG
         .**.***.**..**. ..*. .*** **. . .**.**   **.******.* *  .. *.

GTR1_HUM RAKSVLKKLRGTADVTHDLQEMKEESRQMMREKKVTILELFRSPAYRQPILIAVVLQLSQ
GTR1_BOV RAKSVLKKLRGTADVTRDLQEMKEESRQMMREKKVTILELFRSAAYRQPILIAVVLQLSQ
GTR1_MOU RAKSVLKKLRGTADVTRDLQEMKEEGRQMMREKKVTILELFRSPAYRQPILIAVVLQLSQ
GTR1_RAB RAKSVLKKLRGNADVTRDLQEMKEESRQMMREKKVTILELFRSPAYRQPILSAVVLQLSQ
GTR1_RAT RAKSVLKKLRGTADVTRDLQEMKEEGRQMMREKKVTILELFRSPAYRQPILIAVVLQLSQ
GTR3_HUM NAKQILQRLWGTQDVSQDIQEMKDESARMSQEKQVTVLELFRVSSYRQPIIISIVLQLSQ
GTR3_MOU QATEILQRLWGTSDVVQEIQEMKDESVRMSQEKQVTVLELFRSPNYVQPLLISIVLQLSQ
GTR4_HUM PARKSLKRLTGWADVSGVLAELKDEKRKLERERPLSLLQLLGSRTHRQPLIIAVVLQLSQ
GTR4_MOU PARKSLKPLTGWADVSDALAELKDEKRKLERERPMSLLQLLGSRTHRQPLIIAVVLQLSQ
GTR4_RAT PARKSLKRLTGWADVSDALAELKDEKRKLERERPLSLLQLLGSRTHRQPLIIAVVLQLSQ
          *   *. * *  **   ..*.*.*  .. .*. ...*.*.   .. **.. ..******
```

```
GTR1_HUM QLSGINAVFYYSTSIFEKAGVQQPVYATIGSGIVNTAFTVVSLFVVERAGRRTLHLIGLA
GTR1_BOV QLSGINAVFYYSTSIFEKAGVQQPVYATIGSGIVNTAFTVVSLFVVERAGRRTLHLIGLA
GTR1_MOU QLSGINAVFYYSTSIFEKAGVQQPVYATIGSGIVNTAFTVVSLFVVERAGRRTLHLIGLA
GTR1_RAB QLSGINAVFYYSTSIFEKAGVQQPVYATIGSGIVNTAFTVVSLFVVERAGRRTLHLIGLA
GTR1_RAT QLSGINAVFYYSTSIFEKAGVQQPVYATIGSGIVNTAFTVVSLFVVERAGRRTLHLIGLA
GTR3_HUM QLSGINAVFYYSTGIFKDAGVQEPIYATIGAGVVNTIFTVVSLFLVERAGRRTLHMIGLG
GTR3_MOU QLSGINAVFYYSTGIFKDAGVQEPIYATIGAGVVNTIFTVVSLFLVERAGRRTLHMIGLG
GTR4_HUM QLSGINAVFYYSTSIFETAGVGQPAYATIGAGVVNTVFTLVSVLLVERAGRRTLHLLGLA
GTR4_MOU QLSGINAVFYYSTSIFESAGVGQPAYATIGAGVVNTVFTLVSVLLVERAGRRTLHLLGLA
GTR4_RAT QLSGINAVFYYSTSIFELAGVEQPAYATIGAGVVNTVFTLVSVLLVERAGRRTLHLLGLA
         *************.**. *** .* *****.*.*** **.**...**********..**.

GTR1_HUM GMAGCAILMTIALALLEQLPWMSYLSIVAIFGFVAFFEVGPGPIPWFIVAELFSQGPRPA
GTR1_BOV GMAGCAVLMTIALALLERLPWMSYLSIVAIFGFVAFFEVGPGPIPWFIVAELFSQGPRPA
GTR1_MOU GMAGCAVLMTIALALLERLPWMSYLSIVAIFGFVAFFEVGPGPIPWFIVAELFSQGPRPA
GTR1_RAB GMAACAVLMTIALALLEQLPWMSYLSIVAIFGFVAFFEVGPGPIPWFIVAELFSQGPRPA
GTR1_RAT GMAGCAVLMTIALALLEQLPWMSYLSIVAIFGFVAFFEVGPGPIPWFIVAELFSQGPRPA
GTR3_HUM GMAFCSTLMTVSLLLKDNYNGMSFVCIGAILVFVAFFEIGPGPIPWFIVAELFSQGPRPA
GTR3_MOU GMAVCSVFMTISLLLKDDYEAMSFVCIVAILIYVAFFEIGPGPIPWFIVAELFSQGPRPA
GTR4_HUM GMCGCAILMTVALLLLERVPAMSYVSIVAIFGFVAFFEIGPGPIPWFIVAELFSQGPRPA
GTR4_MOU GMCGCAILMTVALLLLERVPAMSYVSIVAIFGFVAFFEIGPGPIPWFV-AELFSQGPRPA
GTR4_RAT GMCGCAILMTVALLLLERVPSMSYVSIVAIFGFVAFFEIGPGPIPWFIVAELFSQGPRPA
         **  *...**..* * .    **...* **. .*****.********. ***********

GTR1_HUM AIAVAGFSNWTSNFIVGMCFQYVEQLCGPYVFIIFTVLLVLFFIFTYFKVPETKGRTFDE
GTR1_BOV AIAVAGFSNWTSNFIVGMCFQYVEQLCGPYVFIIFTVLLVLFFIFTYFKVPETKGRTFDE
GTR1_MOU RIAVAGFSNWTSNFIVGMCFQYVEQLCGPYVFIIFTVLLVLFFIFTYFKVPETKGRTFDE
GTR1_RAB AVAVAGFSNWTSNFIVGMCFQYVEQLCGPYVFIIFTVLLVLFFIFTYFKVPETKGRTFDE
GTR1_RAT AVAVAGFSNWTSNFIVGMCFQYVEQLCGPYVFIIFTVLLVLFFIFTYFKVPETKGRTFDE
GTR3_HUM AMAVAGCSNWTSNFLVGLLFPSAAHYLGAYVFIIFTGFLITFLAFTFFKVPETRGRTFED
GTR3_MOU AIAVAGCCNWTSNFLVGMLFPSAAAYLGAYVFIIFAAFLIFFLIFTFFKVPETKGRTFED
GTR4_HUM AMAVAGFSNWTSNFIIGMGFQYVAEAMGPYVFLLFAVLLLGFFIFTFLRVPETRGRTFDQ
GTR4_MOU AMAVAGFSNWTCNFIVGMGFQYVADRMGPYVFLLFAVLLLGFFIFTFLKVPETRGRTFDQ
GTR4_RAT AMAVAGFSNWTCNFIVGMGFQYVADAMGPYVFLLFAVLLLGFFIFTFLRVPETRGRTFDQ
          .**** .***.**..*. *. ..   *.***..*. .*. *. **...****.****..

GTR1_HUM IASGFRQGGA—SQSDKTPEELFHPLGA——DSQV
GTR1_BOV IASGFRQGGA—SQSDKTPEELFHPLGA——DSQV
GTR1_MOU IASGFRQGGA—SQSDKTPEELFHPLGA——DSQV
GTR1_RAB IASGFRQGGA—SQSDKTPEELFHPLGA——DSQV
GTR1_RAT IASGFRQGGA—SQSDKTPEELFHPLGA——DSQV
GTR3_HUM ITRAFEGQAHGADRSGKDGVMEMNSIEPAKETTTNV
GTR3_MOU IARAFEGQAHSG—KGPAGV-ELNSMQPVKETPGNA
GTR4_HUM ISAAFHRTPSLLEQEVKPSTEL-EYLGP——DEND
GTR4_MOU ISAAFRRTPSLLEQEVKPSTEL-EYLGP——DEND
GTR4_RAT ISATFRRTPSLLEQEVKPSTEL-EYLGP——DEND
         *. .*         .        . . .     ..
```

Figure 8. *Alignment of glucose transporters belonging to the sugar transporter family by CLUSTAL (Gap fixed=10; Gap vary.=10). The different species considered are HUMan, MOUse, RAT, RABbit and BOVine. The asterisk (*) marks perfectly conserved residues, the point (.) marks conservative substitutions.*

• Mechanism of transport

Pawagi & Deber suggested that glucose produces a change in the conformation of the region surrounding Trp^{388} [9] which leads to the transfer of this residue from an aqueous domain to or near the membrane. Although this region is rich in proline, directed mutagenesis suggests that the cis/trans isomerization of proline residues is not critical for transport [19]. In our model, Trp^{388} lies in segment hb11 and poly-Pro segment separates helix hb10 from β-strand hb11. We suggest that segment hb11 could initiate the molecular movement induced by glucose and transmit it, via the poly-Pro segment to helix hb10 which is considered as forming together with segment hb9 an hydrophobic region closing the outward portion of the channel during glucose transport. On the one hand, Holman and Rees [20] have reported that Trp^{388} is one of two which may be located near the ligand binding site. On the other hand a multi-β-structure, contrary to α-helices associations, could be very sensitive to exchange of hydrogen bonds which is thought to occur at the beginning of glucose translocation [21, 23]. Hydrogen bonds could be

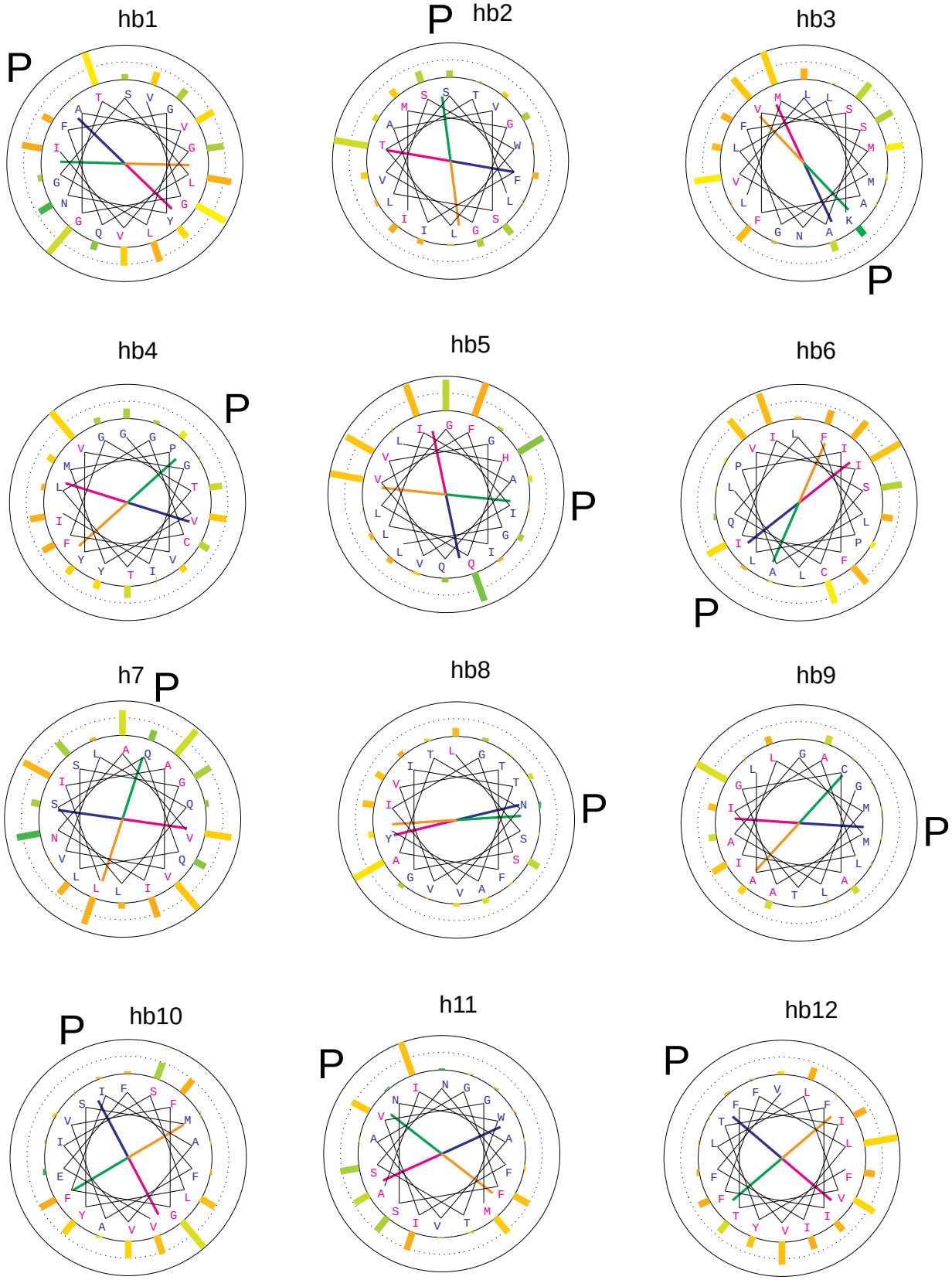


Figure 9. *Representation of the 10 helices of our model (hb) and helices 7 and 11 of Mueckler's model (see text for description). P is the prediction of the side which point to the lumen of the channel.*

formed with the backbone of the protein and not with the side chains. This could explain why only a few residues capable of hydrogen bonding have been reported to be critical for glucose transport [22]. It may seem improbable that glucose could initiate such a movement in the membrane by interacting with a β-strand. However, hb11 has very special properties : it is highly hydrophobic, surrounded by very short loops, one of which has high conformational flexibility and could interact through its charge, with the next transmembrane segment. Segment hb14 is particularly rich in Ala (Arg^{400}Pro Ala Ala Ieu Ala Val Ala Gly^{407}). These amino-acids have particular hydrophobic properties: as its accessible surface is small it does not strongly influence the hydropathic environment. Thus segment hb14 could move without causing an important modification of energy. This could be critical for the working of a channel such as GLUT1.

Conclusion

In this paper we have presented a new model for GLUT1 that seems to agree better than previous models with experimental evidence. The use of this model together with the earlier models may help in the conception of new experiments or theoretical analyses. In fact, as the three models are based on different methods which each have their own limitations, they should be regarded as tools and not as exact predictions. In this perspective the new hypothesis suggested by our model are that:

a) GLUT1 is composed of 14 transmembrane segments.
b) it is an α/β-protein.
c) it forms two channels instead of one.
d) molecular movement could be initiated by a β-strand and transmitted via a poly-P segment to the hydrophobic cleft formed by helices hb10 and hb9.

Availability

A public version of WHEEL is available for academic purposes at: http://www.fsagx.ac.be/info_faculte/info_bp/presentation_bp.html.

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