Docking Studies Show That \(\beta\)-Glucose and Quercetin Slide through the Transporter GLUT1\(^*\)

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On a three-dimensional template model of GLUT1 (Protein Data Bank code 1SUK), a molecular recognition program, AUTODOCK 3, reveals nine hexose-binding clusters spanning the entire “hydrophilic” channel. Five of these cluster sites are within 3–5 Å of 10 glucose transporter deficiency syndrome missense mutations. Another three sites are within 8 Å of two missense mutations. \(\beta\)-Glucose binds to five sites in the external channel opening, with increasing affinity toward the center of the pore and then passes via a narrow channel into an internal vestibule containing four lower affinity sites. An external site, not adjacent to any mutation, also binding phloretin but recognizing neither \(\delta\)-fructose nor \(L\)-glucose, may be the main threading site for glucose uptake. Glucose exit from human erythrocytes is inhibited by quercetin \(\left(K_I = 2.4 \, \mu M\right)\) but not anionic quercetin-semiquinone. Quercetin influx is retarded by extracellular \(\beta\)-glucose \((50 \, \mu M)\) but not by phloretin and accelerated by intracellular \(\beta\)-glucose. Quercetin docking sites are absent from the external opening but fill the entire pore center. In the inner vestibule, Glu\(^{254}\) and Lys\(^{256}\) hydrogen-bond quercetin \(\left(K_I = 10 \, \mu M\right)\) but not quercetin-semiquinone. Consistent with the kinetics, this site also binds \(\beta\)-glucose, so quercetin displacement by glucose could accelerate quercetin influx, whereas quercetin binding here will competitively inhibit glucose efflux. \(\beta\)-d-Hexoses dock twice as frequently as their \(\alpha\)-anomers to the 23 aromatic residues in the transport pathway, suggesting that endocyclic hexose hydrogens, as with maltosaccharides in maltoporins, form \(\pi\)-bonds with aromatic rings and slide between sites instead of being translocated via a single alternating site.

The glucose uniporter GLUT1 (SLC2A1), a member of the major facilitator superfamily of solute transporters, has to date not been crystallized, but its three-dimensional structure has been modeled by templating it to that of Lac Y permease and glycerol 3-phosphate antiporter (GlpT) from Escherichia coli (1–3). The 12 transmembrane \(\alpha\)-helical domains of the monomeric GLUT protein are arranged around a central water-filled pore lined predominantly with uncharged hydrophilic and hydrophobic amino acids. The 15-Å-long, 8-Å-wide channel narrows near its midpoint (1, 2). Molecular dynamic simulations show that glucose binds close to this position within the pore, as expected of lactose binding to Lac Y permease (2). Glucose docks additionally in a cavity at the external entrance of the pore (3).

GLUTs transport other substrates besides hexoses (e.g. dehydroascorbate (4, 5) via GLUT1, -3, and -4 and glucosamine (6) via GLUT2). The flavonone, quercetin, is transported via GLUT4. Quercetin influx into GLUT4 is inhibited by high glucose or cytochalasin B concentrations (7). Conversely, quercetin inhibits glucose and ascorbate transport via GLUT1, -2, -3, and -4 (8–10).

This present study demonstrates that quercetin is also transported via GLUT1, and its uptake is accelerated by exchange with intracellular glucose. Our goal here is to deduce the transport mechanism of glucose and quercetin by correlating their transport with their binding properties determined using the molecular recognition program AUTODOCK 3 (11).

The conventional view of passive facilitated sugar transport is that sugar binds at a single centrally located site in GLUT that isomerizes between inward and outward facing conformations. The altered direction of ligand dissociation from the site mediates transport (12–15).

To explain accelerated exchange, where the maximal rate of glucose exchange with an intracellular ligand is faster than net flux, the glucose-loaded carrier must isomerize faster than the unliganded carrier. Glucose transport asymmetry is evident as a lower maximal rate and \(K_m\) for net uptake than for exit. Asymmetric transport by the mobile carrier requires that isomerization rates of the unloaded carrier are also asymmetric (16, 17). These assumptions require that all ligands should have the same maximal transport rate, since this is determined by the slow return rate of the unloaded carrier to complete the net transport cycle (18). However, differences have been observed between maximal rates of net influx of different sugars and their temperature coefficients or activation energies (19–21).

Another explanation for sugar transport asymmetry is that hexoses diffuse between recognition sites in the transport pathway independently of protein conformational changes. This multisite model accounts for saturation and inhibition kinetics and relaxes the requirement for uniform maximal rates of hexose transport (26).

Attention has been drawn to the structural similarity between GLUT1 and maltoporins (3). Large numbers of aromatic amino acids line the “greasy pore” of maltoporin and the central pore of the GLUT1. Maltosaccharides are guided through maltoporin by slippage between hydrophobic axial hydrogen atoms projecting from the planar surface of the glycosidic ring and \(\pi\)-electrons of aromatic amino acid side chains in one of the maltoporin strands lining the pore. Hydrogen bonds between OH or endocyclic oxygen groups on both sides of the pyranose ring and
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hydrophilic porin tracks constitute the other two support rails required to guide the maltosaccharide train through the porin (27, 28).

Docking and sequence comparisons here reveal that glucose transport across GLUT1 resembles sugar transport across maltoporin in that multiple docking sites provide the nodal points in a network spanning the pore length. The homologies between maltoporin and GLUT1 sequences (29) may exemplify the phenotypic convergence required of any structure that facilitates slippage of pyranose ligands through a narrow orifice rather than an ontological relationship.

GLUT1 deficiency syndrome (GLUT1-DS) is caused by an inadequate energy supply to brain with failure of growth and development of the central nervous system resulting from diminished glucose transport across the blood brain barrier via defective endothelial GLUT1. The children affected tend to develop microcephaly, epileptic seizures, and ataxia. The major biochemical signs of GLUT1-DS are low cerebrospinal fluid glucose and lactate concentrations typically accompanied by low rates of glucose transport in the subjects’ erythrocytes as a result of defective GLUT1 present in these cells (30, 31). Early introduction of a ketogenic diet may ameliorate this condition by supplying the brain with an alternative energy source.

Sixteen missense mutations resulting in amino acid substitutions at 12 sites have been observed in 6 of the 10 exons of GLUT1 (30, 31). When mapped on the three-dimensional GLUT1 structure, these mutations are close to all but one of the hexose docking sites revealed by AUTODOCK 3.

In this paper, it is shown that one of the glucose binding sites in the inner vestibule of GLUT1 that also binds uncharged quercetin is negatively charged. Docking studies reveal that this site is likely to involve Glu254 and Lys256 that hydrogen-bond to quercetin and glucose. The site is situated appropriately to allow for both retardation of net glucose and quercetin transport, so that displacement of quercetin from it by intracellular glucose explains the observed acceleration of quercetin influx.

This new model of transport via GLUT1 built on previous transport, structural, and modeling studies (1, 2, 3, 7, 10, 20, 22, 23–28) delineates how glucose and quercetin may slide via a pore structure and is clearly differentiated from the conventional alternating carrier description of glucose transport. It offers an explanation for the widely dispersed mutation sites affecting GLUT1-DS (30, 31).

**EXPERIMENTAL PROCEDURES**

Fresh human erythrocytes obtained by venepuncture from a healthy donor after informed consent and approval by the King’s College London Research Ethics Committee, were washed three times in isotonic saline by repeated centrifugation and resuspension.

**Materials**—Phosphate-buffered saline tablets, D-glucose, quercetin, phloretin, ascorbate, ferricyanide, and HgCl$_2$ were all purchased from Sigma. Stopping solution consisted of phosphate-buffered saline with 10 mM HgCl$_2$ and 4 °C was used to register the addition of 200 μl of stopping solution. The cell suspensions were twice washed by centrifugation at 4 °C in fresh stopping solution and then resuspended in 400 μl of phosphate-buffered saline and left for 24 h at 4 °C to allow intracellular quercetin to leak into the supernatant. The cells were then recentrifuged, and the supernatants were transferred to a 96-well plate. Quercetin in the supernatants was determined by the absorption at 380 nm with a microplate spectrophotometer.

**Docking Studies**—Docking with the various ligands was carried out using AUTODOCK 3 (11). This estimates the energy of ligand binding to the site and its $K_i$ on a rigid three-dimensional construction of the protein and allows for rotations of the ligand bonds where applicable.

Data on the position, frequency, and affinity of the ligand docking across the entire GLUT1 surface are obtained and used to map the amino acid residues within 3 Å of the ligand binding sites and later the coincidences and differences between different ligands.

The three-dimensional data files were collected from the following sources: GLUT1 (1SU5) and maltoporin (1AF6) from the Protein Data Bank (via the Macromolecular Structure Database on the World Wide Web at www.ebi.ac.uk/msdb/). Protein Data Bank files of sugars were from “SWEET” on the World Wide Web at www.dzkz-heidelberg.de/spec (supported by the German Research Council) and from Daresbury Chemical Data base Service via “CrystalWeb” at www.cds.dl.ac.uk/cweb. Other Web tools used were the Dundee PRODRG2 Server at www.davapc1.bioch.dundee.ac.uk/prodrg. Molecular displays were created by Swiss-PdbViewer (available at ca.expasy.org/spdbv/) and RasMol (available at openrasmol.org/).

The default settings of AUTODOCK were normally used, with the exception of the run and the population size. Between 80 and 100 runs were usually performed. Docking involved a grid of 101 points in three dimensions with a spacing of 0.375 Å centered on the GLUT1 molecule. When restricting the docking to sites with sparse ligand binding, the cube side was reduced to 51 units with the same spacing and centered at the coordinates of the region under consideration.

**Sequence Homologies between Maltoporin Ligand Binding Strands and the Central Pore Region of GLUT1**—Homologies between glucose binding sequences in GLUT1 and the ligand binding strands of maltoporin were obtained as follows: the 492 amino acids of GLUT1 (1SU5) were split into 20-mers, with an overlap of 10 amino acids. The program, FASTA (29) was used to identify and evaluate the partial matches between each of the 20-mers and sequences in one of the maltoporin subunits (1AF6). The searches were then restricted to those matching the pore region GLUT1, as predicted from the templated model of GLUT1 (3), and the docking data for D-glucose α- and β-anomers were obtained.

**Coincidence between D-Glucose Docking Sites and GLUT1-DS Mutation Sites**—The sites of GLUT1-DS mutations (30, 31) were mapped on the three-dimensional structure of GLUT1, and their coincidence was estimated by layering this map onto others with the α- and β-D-glucose docking sites obtained with AUTODOCK. The distances of the mutations from the hexose clusters were obtained using the measuring tools available with Swiss-PdbViewer “Deepview.”

**RESULTS**

**Quercetin Inhibition of Glucose Transport**—Inhibition of glucose transport by quercetin and other flavonols decreases when the pH is raised above 7.5 (32). Quercetin, like genistein and estradiol, inhibits the maximal glucose exit rates without significant effect on glucose affinity at the external site, indicating that it acts on glucose transport at the endofacial surface (10, 33). Quercetin takes part in redox reactions with...
both ferricyanide and ascorbate to form the anion quercetin semiquinone (34, 35).

The addition of ferricyanide or ascorbate to quercetin solutions prevents quercetin from inhibiting glucose transport. Neither ferricyanide, nor ascorbate alone, has any effect on glucose transport. This indicates that quercetin-semiquinone does not inhibit glucose transport (Fig. 1, A and B) and corroborates the view that negatively charged quercetin does not inhibit glucose transport (36).

Quercetin Uptake into Red Cells at 4 °C—High glucose concentrations or cytochalasin B inhibits quercetin uptake into adipocytes via GLUT4 (7). External glucose (50 mM) inhibits the quercetin uptake (25 μM) at 4 °C (Fig. 1C). However, when 50 mM glucose is preloaded in the internal solution and the external solution is maintained nominally glucose-free, the quercetin uptake rate is accelerated. This acceleration of quercetin uptake in the infinitetrans mode confirms that glucose and quercetin share a common transport path. Although phloretin (100 μM) inhibits glucose influx by more than 90%, it has no effect on quercetin influx with or without intracellular glucose present (not shown).

Docking Studies on GLUT1: Hexoses—D-Glucose affinity for the GLUT1 transport system is ∼5 times higher than either D-mannose or D-galactose; D-fructose has a very low affinity and L-glucose has negligible affinity for GLUT1 (37, 38). The locations of amino acids with similar selectivity to the above were obtained using docking studies with D-glucose, D-galactose, D-mannose, and D-fructose ligands on the GLUT1 three-dimensional template. In aqueous solution, hexoses coexist as α- and β-anomers in a 34:66% ratio, respectively (39). β-D-Glucose is transported faster than α-D-glucose by human erythrocytes (40). Consequently, docking of α- and β-anomers of D-glucose, D-galactose, D-mannose, and L-glucose was studied.

Docking reveals 10 clusters of sites for α- and β-D-glucose anomers on GLUT1. The clusters were identified, initially by visual inspection of the docking output of AUTODOCK 3 (Fig. 2A) and then confirmed with K-means cluster analysis (Table 1). Nine clusters are in the central hydrophilic pore region and another in a side pocket accessible from the endofacial vestibule. The α- and β-D-glucose affinities increase with depth in the external vestibule (clusters 1–5; Kᵢ = 0.34–0.11 mM; Table 1). The increase in affinity with depth favors sugar movement from the external solution to the bottom of an energy well at cluster 5 (yellow) at the narrowest part of the channel before emergence into the internal vestibule. A similar affinity gradient to that found here with D-glucose has been observed in the maltoporin pore with maltosaccharides (27). The clusters in the internal vestibule (clusters 6–9) all have lower affinities (see Table 1). The cluster (green cluster 10) in the side pocket has the highest affinity, Kᵢ = 0.04 mM. All but the red (cluster 1) and green (cluster 10) clusters are either coincident with or close to missense mutations found in GLUT1-DS (30, 31) (see “Discussion” and Table 4).

Assuming that the rates of ligand movement between the cluster positions have an inverse relationship with separation distance, the more isolated clusters will rate limit sugar flow. The root mean squared distances between each cluster and its three nearest neighbor clusters were determined (Table 1). One cluster (red 1) in the external vestibule and three clusters in the internal vestibule (clusters 7–9) are more isolated than the others; cluster green 10 in a side pocket is in the most isolated position.

Types of Interaction between GLUT1 and Transported Sugars—The kinds of interaction between hexoses and GLUT1 are quantified by examining the frequency of amino acid types located within 3 Å of the docking positions of α- or β-hexose anomers (Table 2). The templated...
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### TABLE 2

#### The types of hexose GLUT1 interaction obtained by the estimating the percentage of interactions with five categories of amino acids

The total number of interactions for each hexose anomer varied from 300 to 900. Quercetin is included for reference.

| Kinds of interaction | α-D-Galactose | β-D-Galactose | α-D-Glucose | β-D-Glucose | α-D-Mannose | β-D-Mannose | Quercetin |
|----------------------|---------------|---------------|-------------|-------------|-------------|-------------|-----------|
| Hydrophilic          | 42.8          | 10.6          | 34.2        | 22.8        | 38.4        | 34.4        | 33.3      |
| Acidic               | 2.9           | 8.0           | 0.0         | 5.1         | 7.4         | 10.9        | 0.2       |
| Basic                | 34.1          | 19.7          | 12.3        | 7.9         | 26.8        | 22.4        | 20.8      |
| Aromatic             | 11.1          | 27.0          | 6.8         | 13.9        | 7.2         | 10.9        | 14.8      |
| Nonpolar             | 9.0           | 34.6          | 46.7        | 50.9        | 20.1        | 21.5        | 30.9      |

### TABLE 3

#### GLUT1-maltoporin homologies

Column 2, positions of homologies in GLUT1 and Lam B, respectively; column 3, Smith-Waterston score (29); column 4, percentage identity; column 5, number of amino acid overlaps; column 7, amino acid similarities between GLUT1 and Lam B.

| Glu | LamB | SW score | % identity | aa overlap | Amino acid homologies |
|-----|------|----------|------------|------------|-----------------------|
| 1   | 152  | 100      | 25.8       | 25.8       | GLUT1                 |
| 2   | 45-49 | 100      | 27.7       | 27.7       | LamB                  |
| 3   | 137-145 | 100 | 55.5       | 55.5       | GLUT1                 |
| 4   | 148-156 | 100 | 45.5       | 45.5       | LamB                  |
| 5   | 157-163 | 100 | 25.0       | 25.0       | GLUT1                 |
| 6   | 290-296 | 100 | 25.0       | 25.0       | LamB                  |
| 7   | 270-276 | 100 | 25.0       | 25.0       | GLUT1                 |
| 8   | 291-115 | 100 | 30.0       | 30.0       | LamB                  |
| 9   | 312-318 | 100 | 30.0       | 30.0       | GLUT1                 |
| 10  | 327-333 | 100 | 30.0       | 30.0       | LamB                  |
| 11  | 377-383 | 100 | 30.0       | 30.0       | GLUT1                 |
| 12  | 491-507 | 100 | 30.0       | 30.0       | LamB                  |
| 13  | 413-423 | 100 | 30.0       | 30.0       | GLUT1                 |

Amino Acids with Selectivity for α-Glucose, β-Galactose, and β-Mannose in GLUT1—The selectivity pattern of GLUT1 amino acids for glucose, galactose, and mannose is observed by comparing the sugar affinities of every amino acid in GLUT1 within 3 Å of a docked ligand and mapped on GLUT1. Amino acids at the exofacial opening of the hydrophilic pore prefer α-glucose > 2β-D-galactose (shown as red) (Fig. 3A). These glucose > galactose sites surround the dark blue cluster 3 (Fig. 2, A and B, Table 2). The amino acids favoring α-glucose > 2× mannose, shown in dark blue, surround clusters 4 and 5, and those favoring α-glucose > 2× galactose or mannose surround clusters 6 and 7.

Selectivity for α-Glucose over α-Fructose—Discrimination of GLUT1 for glucose in preference to fructose is thought to be due mainly to the QLS motif in TM 7 at the endofacial end of the pore. This motif is present in GLUT1, -3, and -4 but absent from fructose transporters, GLUT2 and GLUT5 (45). This assumption accords with the view that a single centrally located binding site for hexoses acts as discriminator and gate.

The purple cluster 4 and blue glucose-docking site 3 are 4.5 and 5.2 Å, respectively, from Ser364 (Fig. 2, A and B, Table 1). D-Fructose binds with similar affinities to α-glucose in all of the clusters except clusters 1 and
2 in the external cavity and at the docking cluster 7 in the large endofacial vestibular cavity. The absence of fructose docking from clusters 1, 2, and 7 was confirmed by search repetitions with narrower windows in AUTODOCK.

Selectivity for D-Glucose over L-Glucose—L-Glucose, like fructose, does not dock in cluster 1. The pattern of GLUT1 discrimination against L-glucose shows a ring of amino acids with a high docking frequency and affinity for D-glucose surrounding the rim of the external opening of the pore from which α- and β-L-glucose docking are absent (Fig. 3B). In the internal vestibule, other sites fail to dock with L-glucose anomers.

Coincidence of Glucose Cluster Sites with Mutation Sites in GLUT1 Deficiency Disease—A map of the 10 GLUT1-DS missense mutation sites on GLUT1 overlaid on the D-glucose cluster map shows that five mutations are within 5 Å of five separate glucose-docking clusters (Fig. 4, Table 4). Two adjacent clusters (blue 3 and purple 4) have two mutations in common, and additionally each is close to another unshared mutation. Since all of these mutations decrease 3-O-methyl glucose uptake into human erythrocytes by 50–60%, it can be deduced that at least five of the nine separate docking sites within the central transport pathway have functional importance for glucose transport. Three other glucose-docking sites are more distant (7.5–10 Å) from mutations (Table 4), so these cannot be linked with any confidence at present to docking sites. Only one site (red 1) has no mutations associated with it (see "Discussion"). The high affinity site (green cluster 10) in the side pocket has one mutation 11.5 Å distant form the nearest sugar in the green cluster. Recently, another functional missense mutation, G75W (31), has been observed in GLUT1. It maps close to gray cluster 6 and causes a 64% reduction in glucose transport into the subjects’ erythrocytes.

Quercetin and Quercetin Semiquinone Docking—Quercetin and quercetin semiquinone share many docking sites with D-glucose in GLUT1; however, unlike glucose, quercetin does not dock in discrete clusters. Instead, a multitude of possible docking positions for both quercetin and quercetin semiquinone entirely fill the central hydro-
Quercetin is hydrogen-bonded to Glu254 and Lys256 (Fig. 5A). Six hexose clusters (clusters 4–9) coincide with quercetin binding sites in the central pore and internal vestibule, but since quercetin does not bind in the external vestibule, no docking coincidence occurs between glucose and quercetin at glucose clusters 1–3. Quercetin is hydrogen-bonded to Glu234 and Lys236 ($K_i = 18 \, \mu M$) in the linker between TM6 and 7, and to Gln397 in the linker between TM10 and 11 and coincides with glucose cluster 8 (Table 1, Fig. 5B) and a cytochalasin B binding site (3), thus explaining its transport inhibition by cytochalasin B in GLUT4 (7). Quercetin-semiquinone does not hydrogen-bond here (Fig. 5A).

This quercetin binding site satisfies all of the characteristics predicted by kinetics: it is positioned at the internal surface of GLUT1, hence appropriately sited to competitively inhibit glucose exit without altering external glucose affinity; it hydrogen-bonds with a glutamate residue, which repels anionic quercetin semiquinone; high intracellular glucose concentrations will displace quercetin from the site, leading to accelerated quercetin uptake, and the site is spatially adjacent to the cytochalasin B binding site, thus explaining its transport inhibition by cytochalasin B in GLUT4 (7). Quercetin-semiquinone does not hydrogen-bond here (Fig. 5A).

**Phloretin Docking**—Phloretin has several high affinity docking positions on GLUT1. It is observed here that phloretin binding coincides with the red cluster 1 in the external opening and the yellow cluster 5 in the pore neck of the endofacial vestibule (Fig. 4), as was shown previously (3). There are also other high affinity phloretin binding sites in clusters 3, 4, 5, 8, and 9 and the high affinity green site 10 in the side pocket.

**DISCUSSION**

The validity of all docking sites discovered here depends on that of the three-dimensional template of GLUT1 (3) and the docking methodology of AUTODOCK (11). Using AUTODOCK rather than molecular dynamic simulations (3) provides a more comprehensive view of the available ligand docking sites and additionally generates quantitative data on fit reliability by assigning an affinity to each docking position. A limitation of the AUTODOCK program, to date, is that it assumes that docking occurs on a rigid protein framework, although in the current version, AUTODOCK 3, rotation of bonds within the docking ligands is permitted. Rigidity of the protein framework does not detract from the validity of docking sites revealed here; inclusion of additional degrees of freedom into the docking process can only increase the possibilities for site recognition. The program operator has no capacity to steer the selection of the docking sites, except by exclusion from the grid frame, within the protein. Since the docking sites are all selected on the basis of ligand affinities that are determined from the sum of the free energies obtained from the distances between the variable ligand postures on the grid and the fixed adjacent protein chemical structures (namely van der Waals, coulombic, hydrogen bond, solvation, and rotatable bond torsion forces), there is no chance of a cluster fit being obtained by "chance" (11). However, since AUTODOCK does not obtain its fits by dynamic simulation (i.e. by ligands traversing along the possible tracks within the protein) but by scanning the available grid points on the entire protein surface, it can obtain docking sites in cavities normally inaccessible from the external solution. Fortunately, this problem does not arise with hexose or quercetin binding to GLUT1, because it has a very open structure, so all 10 hexose clusters and the quercetin sites within the hydrophilic pore are in continuity with the external surface of the protein. The docking positions of the hexose clusters are all discrete, as determined by the K means statistic (Table 1), so their close positioning to GLUT1-DS mutation sites is significant. Since quercetin-docking sites are widespread over the entire central and endofacial vestibular surface, the location of point mutations affecting quercetin transport is less assured.

Five of the identified positions of the nine hexose clusters in the central pore of GLUT1 are corroborated by their adjacency with mutations observed in GLUT1-DS, and two others are within medium range of GLUT1-DS mutations, where small protein conformation shifts would bring these sites into range of the cluster sites and thus alter transport function. The only cluster site (red 1) not adjacent to a mutation site is the external phloretin-binding site, which also fails to bind D-fructose, L-glucose, or quercetin.
CONCLUSIONS

The new experimental findings are that quercetin and glucose are both transported via GLUT1, that quercetin uptake is accelerated by intracellular glucose, and that phloretin, unlike cytochalasin B (7), does not inhibit quercetin transport. This indicates that the transport pathways of glucose and quercetin are similar but not identical. The inhibition and acceleration of quercetin uptake by extracellular and intracellular glucose respectively is evidence that these ligands at least partly share the same transport pathway.

The docking characteristics of D-glucose and quercetin differ in several ways. Glucose dock at 10 isolated clusters in the external, middle, and inner vestibules of GLUT1, whereas quercetin docking is spread widely over the middle and inner vestibules only. The red site 1 on the outer rim of the external vestibule appears to have a special role in D-glucose recognition, since neither L-glucose nor D-fructose dock at this site, but phloretin does. The absence of phloretin-dependent inhibition of quercetin uptake supports the view that quercetin is transported by binding directly with the central sites and unlike glucose can bypass the external recognition sites. This is new evidence favoring a multithreaded access of ligands into GLUT1, rather than via a single alternating site.

It can be deduced that a key binding site for quercetin-glucose-cytochalasin B interaction is likely to be an anionic amino acid within the inner vestibule of GLUT1. Docking shows that the site is adjacent to Glu254 and Lys256 on the large endofacial linker between TM helices 6 and 7 that lie close to a Walker ATP binding site and the cytochalasin B site (23, 33). These data lend support to the view that quercetin is transported by binding directly with the Walker ATP binding site and the cytochalasin B site (23, 33). The presence of four discrete D-glucose cluster sites within the endofacial vestibule supports previous binding studies suggesting an ATP-dependent sequestration of multiple glucose molecules with GLUT1 (3, 24).

Additionally, the docking studies reveal nine glucose/hexose docking clusters in the central hydrophilic pore spanning GLUT1. The affinities of hexoses are estimated by the program, and comparisons between the affinities of different hexose epimers show that sugar selectivity is apparently distributed over a number of sites. Only a few cluster sites exhibit high stereospecificity; most of the sites bind D-fructose and D- and L-glucose with similar affinities. Surprisingly, the QLS 279–281 and QLS 283–285 in TM 7, although identified from homology studies of various GLUT isoforms to be likely stereoselective motifs for D-glucose (45), are close, but not coincident, to clusters 3 and 4.

These docking studies have uncovered further evidence suggesting that sugars slide between nodal sites of a network in a complex pore, namely, the sequence homologies between the transport pathways of GLUT1 and maltoporin, including large numbers of aromatic amino acids in the GLUT1 pore (Table 3, Fig. 2B) and the higher frequency of binding of planar B-β-hexose anomers than α-anomers (Table 2) by forming π-bonds with the aromatic residues in the pore (43, 44). Further evidence for this awaits detailed molecular dynamic simulations.

The proximity of five of the hexose cluster sites within 3–5 Å of 10 glucose transporter deficiency syndrome missense mutations (Fig. 4 and Table 4) is both corroborations of the positions of these sites and an explanation of the otherwise enigmatic dispersal of function-reducing missense mutations within GLUT1. The wide dispersal of these mutation sites does not easily accommodate to a single transport site mechanism but does accord with the view that they are mainly situated at key positions along the transport pathway (30, 31).