Modeling of intestinal peptide transporter hPepT1 and analysis of its transport capacities by docking and pharmacophore mapping

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Background

- *The more recent strategies in medicinal chemistry involve the pharmacokinetic profiling of new molecules as soon as possible in the development pipeline with the clear aim to develop only drug-like compounds.[1]*
- *Such an early pharmacokinetic analysis requires the achievement of a maximum of useful and relevant molecular descriptors to allow a reliable prediction of the drug-likeness.*
- *Among the barriers determining the bioavailability of peptide-like molecules, the intestinal absorption plays a crucial role particularly for hydrophilic peptidomimetics, which rarely can be absorbed by passive permeation.*
- *This problem might be overcome increasing the affinity for the intestinal transporters which are involved in the absorption of digested dietary proteins.*
- *For peptide-like molecules, the ability to predict which compounds can be actively absorbed by intestinal carriers is an information even more relevant than the common physicochemical descriptors used in the pharmacokinetic screening (e.g. logP, log D, PSA).*

- *Among the intestinal carriers,[2] the apical proton-dependent oligopeptide transporters (POT) play a key role in the absorption of both digested dietary proteins and peptidomimetics; In humans, two members of POT are found, namely hPepT1 and hPepT2.*
- *The hPepT1 transporter is a 708 residues protein, whose transmembrane bundle is composed by 12 helices. It is mainly expressed in the small intestine, in the proximal tubules of the kidney as well as in pancreatic, liver and renal cells.*
- *The known SARs suggest that (a) the best size corresponds to that of di/tripeptides; (b) the charged termini and amide groups are not mandatory; (c) the binding is highly stereospecific preferring L-residues; (d) the hydrophobic side chains are mostly favored.*

Aim of the work

Aim of the work[3] was to generate a full-length model for the human intestinal transporter hPepT1 and to analyse the substrate recognition at an atomic level with a view to develop a computational strategy able to predict the affinity of new molecules for the hPepT1 transporter.

Homology model for hPepT1

The Figure shows the hPepT1 model, colored by segments, unveiling its typical folding with 12 transmembrane segments (TM1-12) and a large extracellular loop (EL5). The structural quality of the model is assessed by the significant percentage of residues which fall in the allowed regions of the Ramachandran’s plot (70.62%) with a marked preponderance of helix motifs.

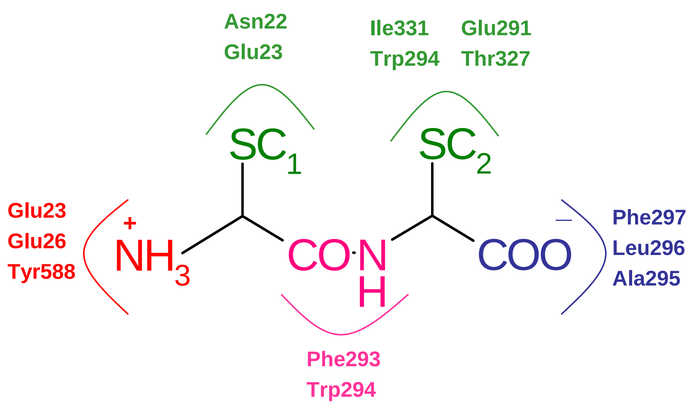
The **TM bundle** assumes an elliptical truncated conic shape, which is due to fact that the TM segments are far from being parallel and some segments are staggered with an angle of 30° in respect to the adjacent heli ces. The TMs arrangement does not agree the numerical order, but it is possible to recognize an internal group of helices (i.e. TM1, TM4, TM5, TM7, TM10), which line the central pore and bear the key residues for the binding, and an external set of TM segments (TM2, TM3, TM6, TM8, TM11, TM12), which define the boundary of TM bundle. Notably, the helices facing the central pore are clearly more hydrophilic than the external TM segments.

The **extracellular loop EL5** (red segment) fully covers the extracellular side and consists of two large domains connected by two hinge loops. The hinges may confer flexibility to the domains, which could assume closed or open conformations modulating the accessibility of the binding cavity. Such a flexibility is confirmed by the used template (sucrose phosphatase) which can assume two different states. Such template is a metalloenzyme which selectively recognizes some sugars. This suggests that also EL5 may bind sugars and/or metal ions involved in modulatory effects on hPePT1, as reported by experimental studies.

Docking analyses

Despite the clear heterogeneity among the docking results, a deeper analysis of all putative complexes allows the identification of the residues most frequently involved in the ligand recognition, which can be sum up as represented in the figure.

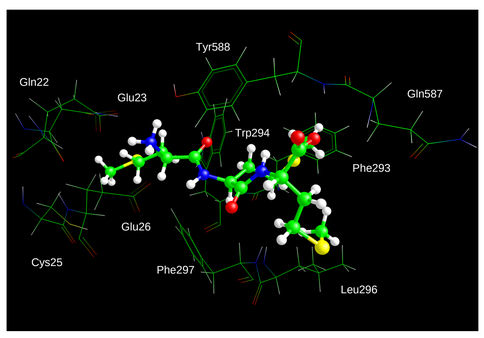
The residues which interact with the side chains are heterogeneous, justifying the ability of hPepT1 to interact with structurally diverse substrates. It is possible to recognize a set of residues involved in the interaction with the N-terminal side chain (SC1) such as **Asn22** (TM1), **Glu23**, and **Phe293**, while the C-terminal side chain (SC2) contact **Trp294**, **Ile331** (TM8), and **Glu291** (TM7) and **Thr327** (EL4).



The ammonium head probably plays the most critical role since it realizes a reinforced H-bond with **Tyr588** as well as ion-pairs with **Glu23** (TM1) and/or **Glu26** (TM1). Notably, the contact between Tyr588 and ammonium head characterizes the most affinitive ligands.

The carboxy terminus appears less involved in ligand recognition, since it stabilizes only H-bonds with the backbone of **Ala295** (TM7), **Leu296** (TM7), and **Phe297** (TM7) without forming strong ionic interactions

The central peptide bond can stabilize H-bonds with backbone atoms of **Phe293** and **Trp294**. Such interactions can be hindered by bulky side chains, and, thus, one can conclude that the contacts of the peptide groups could partially counterbalance the reduced interactions stabilized by small side chains.

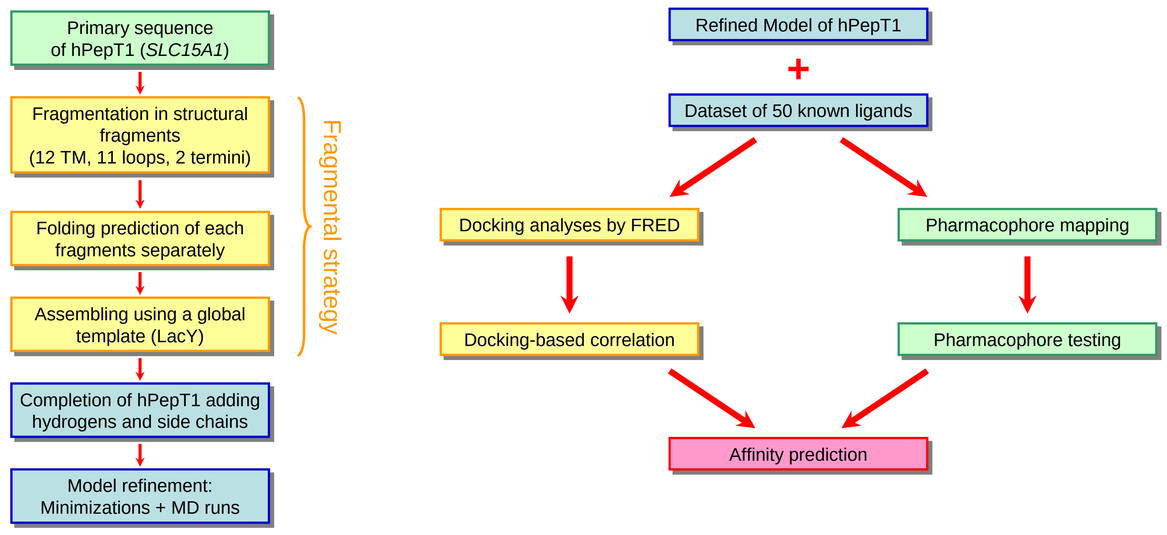


hPepT1-MetMetMet complex

The Figure shows that hPepT1 realizes a vast pattern of relevant contacts also with tripeptides involving all ligand’s functional groups:

- The ammonium head realizes ion-pairs with **Glu23** and **Glu26** plus the critical H-bond with **Tyr588**, suggesting that the contacts of ammonium head are constant and independent of the substrate’s length.
- The carboxylate forms H-bonds with the backbone atoms of **Tyr588** and **Gln587** (TM10) instead of **Ala295**, **Leu296**, and **Phe297** as shown by dipeptides.
- The peptide bonds stabilize H-bonds with the backbone atoms of **Phe293**, **Trp294**, **Leu296**, and **Phe297**.
- The side chains realizes hydrophobic contacts with **Cys25** (TM1), **Phe293**, **Trp294**, **Leu296**, **Phe297** plus a set of aliphatic residues.

Computational methods[4]



Docking-based affinity prediction

Docking results suggest that the most affinitive compounds have a distance between charged termini about equal to 6 Å. **Δdistance** defines the difference between the distance value of a given ligand and the optimal distance (6.02 Å) as evidenced by the most affinitive ligand. Such descriptor reflects the significant role of the contacts stabilized by the charged termini

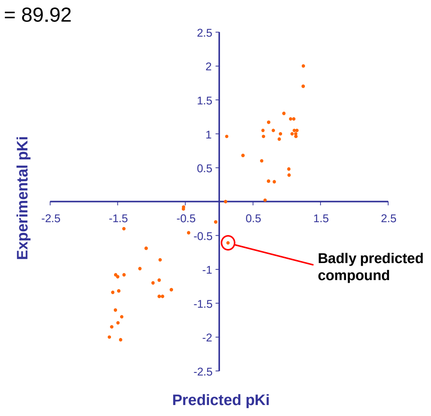
Zapbind accounts for the ionic interactions. Despite the known predilection of hPepT1 for hydrophobic ligands, the role of Zapbind score emphasizes the relevance of the polar interactions mostly realized by the ligand’s charged groups.

Given the beneficial role of the interaction between Tyr588 and ammonium head, we introduced a binary descriptor (**Int_Tyr588**), which is equal to 1 for substrates which realize such a reinforced H-bond and 0 otherwise. Such a descriptor markedly enhances the predictive power of the equation.

$$pKi = 0.235\ \Delta distance - 7.912\ 10^{-3}\ Zapbind + 1.534\ Int\_Tyr588 - 0.480$$

$n = 50;\ r^2 = 0.85;\ s = 0.45;\ F = 89.92$

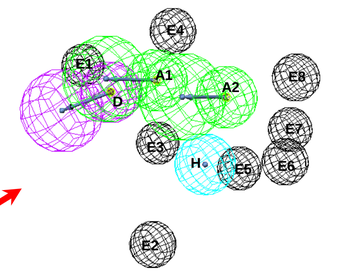
The figure confirms the goodness of the affinity predictions and, when considering the classification in more affinitive (pKi > 0) from less affinitive ligands (pKi < 0) as defined by Cartesian axes, one can note that equation is able to successfully discriminate among the docked ligands and only one derivative is incorrectly predicted giving a false positive (as evidenced by red circle). The badly predicted compound is the tripeptide Gly-His-Lys that, in fact, gave a good docking pose (as exemplified by H-bond with Tyr588).



Pharmacophore mapping

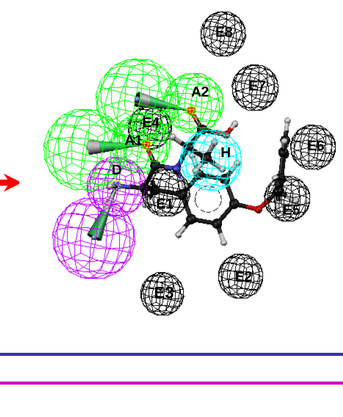
To confirm the docking results, enriching our knowledge about the hPepT1 binding, HypoRefine was exploited to derive a SAR pharmacophore model for hPepT1 ligands.

The selected model consists of one hydrophobic region (H), two hydrogen-bond acceptors (A1–A2), one hydrogen-bond donor (D) and eight excluded volume sites (E1–E8) in a specific three-dimensional arrangement.



The Figure shows the pharmacophoric regions mapped on docking pose of most affinitive derivative (Tyr-(OBzl)-Ala).

- The ammonium head occupies the **H-bond donor region (D)** which overlaps Tyr588, Asp23 and Asp26
- the carboxylate and the peptide bond map the **H-bond acceptor regions (A1-A2)** corresponding to Phe293, Trp294, Leu296, and Phe297
- **the hydrophobic region (H)** corresponds to C-terminal side chain, suggesting that an hydrophobic residues is really beneficial in such position.
- **the excluded volumes (E1-E8)** are mostly located near to C-terminus, while the N-terminus appears less sterically constrained.



Conclusions

The congruity of the obtained complexes and the agreement between docking results and pharmacophore mapping afford an encouraging validation for the here described hPepT1 which can be successfully used to predict the affinity of new molecules. The fragmental strategy appears a fertile methodology to model any transmembrane protein and the combined approach docking search plus pharmacophore mapping allows to deeply explore the molecular recognition at an atomic level.

References

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4. *Pedretti, A.; Villa, M.; Pallavicini, M.; Valoti, E.: Vistoli, G. J Med Chem. 2006, 49, 3077-3085.*