# Towards a structural understanding of drug and peptide transport within the proton-dependent oligopeptide transporter (POT) family

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## Abstract

**One of the principal aims of modern drug design is the targeted delivery of drugs within the body, such as to the central nervous system, combined with their exclusion from the liver and kidneys, which break down foreign molecules and subsequently eliminate them. Many of the commonly prescribed drugs are transported into cells and across the plasma membrane via endogenous membrane transporters, whose principal roles are the uptake of essential nutrients for metabolism. In many cases, such drug transport is serendipitous as they are simply mistaken as 'natural' compounds. Many of these transporters could, however, be targeted more efficiently, improving drug absorption, distribution and retention. The molecular details of these drug–transporter interactions, however, are at best poorly understood, in large part through the absence of any high-resolution structural information. To address this issue, we recently determined the structure of a prokaryotic peptide transporter, $PepT_{So}$ from *Shewanella oneidensis*, which shares a high degree of sequence similarity and functional characteristics with the human PepT1 and PepT2 proteins. PepT1 and PepT2 contribute significantly to the oral bioavailability and pharmacokinetic properties of a number of important drug families, including antibiotics, antivirals and anticancer agents. The crystal structure of $PepT_{So}$ provides the first high-resolution model of a drug importer and provides the starting point for understanding drug and peptide transport within the human body.**

## Introduction

The principal route by which mammals obtain bulk quantities of amino acids is through the transport of di- and tri-peptides across the intestinal brush border membrane in the gastrointestinal tract [1]. This process is mediated through a large and diverse family of integral membrane proteins called the POT (proton-dependent oligopeptide transporter) family, also referred to simply as the peptide transporter or PTR2 family [2,3]. POT family transporters are found in all organisms, with the notable exception of the archaea, and use the proton electrochemical gradient to drive the uptake of di- and tri-peptides across cell membranes [4,5]. The human genome contains four members of the POT family, which belong to the SLC (solute carrier) 15 gene grouping and are called PepT1, PepT2, PHT2 and PHT1. PepT1 was identified in 1994 from a rabbit intestinal cDNA library, and was predicted to contain 12 TM (transmembrane) $\alpha$-helices [6,7]. Phylogenetic analysis identified the POT family as belonging to the much larger MFS (major facilitator superfamily) of secondary active transport proteins [8,9]. The closely related PepT2 protein was initially thought to be predominantly expressed in kidney cells, and is often referred to as the renal peptide transporter, although the tissue distribution of PepT2 is significantly broader, with confirmed reports of expression in the liver, lung, testis and central nervous system [10,11]. However, the extent of the physiological role of PepT2 in these diverse organs is still unclear. PepT1 and PepT2 recognize most proteinogenic di- and tri-peptides, which equates to some 300–400 dipeptides and >7000 tripeptides. The diversity of ligand recognition is remarkable for a transport protein, and PepT1 and PepT2 are some of the most promiscuous transport proteins identified to date.

Peptide transporters are of significant pharmacological importance being one of the principal routes of entry, distribution and retention of many different families of drugs, such as $\beta$-lactam antibiotics, antivirals and anticancer agents [12,13]. This characteristic has been ascribed to the similar stereochemical properties that many of these drug molecules have to naturally occurring peptides, with the main consensus being that these drugs are simply 'carried along for the ride'. This realization led to the development of specifically engineered peptide pro-drugs, molecules that can be attached to peptide scaffolds and subsequently utilize the widespread tissue distribution of peptide transporters to improve their pharmacokinetic properties, most notably oral bioavailability [14]. There are currently a number of peptide pro-drugs being routinely prescribed to patients, such as the antiviral valacyclovir, which takes advantage of endogenous peptide transporters to improve both efficacy and bioavailability over the original parent molecule [15].

Although the primary structure, substrate specificity and kinetics have all been investigated for mammalian

**Key words:** crystal structure, drug transport, major facilitator superfamily (MFS), peptide transport, $PepT_{So}$, protein-dependent oligopeptide transporter (POT).

**Abbreviations used:** MFS, major facilitator superfamily; POT, proton-dependent oligopeptide transporter.

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peptide transporters, there is currently no structural information on these proteins, which has severely hampered further investigation into the molecular mechanism and the utilization of these proteins as 'Trojan horses' for drug delivery. To gain much needed insight into the molecular mechanism of transport, we determined the crystal structure of a prokaryotic member of the POT family, from the bacterium *Shewanella oneidensis*, $PepT_{So}$ [16]. $PepT_{So}$ shows a high degree of sequence conservation within the TM region to the mammalian PepT1 and PepT2 proteins, and associated functional data on both $PepT_{So}$ and other prokaryotic members of the POT family reveal similar kinetic properties supporting the potential for common elements in the mechanism of peptide transport [17–20]. The present review of the structure of $PepT_{So}$ outlines the main findings from our study and highlights the critical questions that require addressing if we are to fully understand and benefit from the function of these proteins as drug transporters.

## Structure of $PepT_{So}$

$PepT_{So}$ contains 14 TM helices (Figure 1; PDB code 2XUT), of which helices H1–H12 adopt the overall fold observed previously in other members of the MFS [21–24]. This fold is characterized by two six-helical bundles, formed by the interdigitation of helices H1–H6, that constitute the N-terminal half of the molecule and H7–H12 that form the C-terminal half. Both bundles are linked by an intracellular loop (partially disordered in the crystals of $PepT_{So}$) that come together within the membrane to form a V-shaped transporter, with both halves related by a pseudo-two-fold symmetry axis running perpendicular to the membrane plane. $PepT_{So}$ is the first 14-TM MFS transporter to have its crystal structure determined, and the additional helices, which we have termed HA and HB, fold within the intracellular loop connecting the N- and C-terminal bundles. These form a hairpin structure that packs against the side of the two six-helical bundles. This provides the first evidence that larger MFS members, some of which contain 18 TM domains, most likely all contain the 'core' 12-TM translocation domain observed previously with additional folded domains that sit to one side.

## The central cavity, an unusual polyspecific binding site

The structure reveals a previously unobserved ligand-bound occluded state for a member of the MFS, with an unidentified ligand situated within a large central cavity that is closed to both sides of the membrane (Figure 2). Many of the residues conserved between $PepT_{So}$ and the mammalian peptide transporters cluster around this cavity, that has approximate dimensions of 13 Å × 12 Å × 11 Å (1 Å = 0.1 nm). The central cavity is in many respects the main focus of investigation into these proteins. As mentioned previously, the peptide-binding site is unusually polyspecific, capable of recognizing many thousands of different di- and tri-peptide combinations. This remarkable characteristic is presumably why these transporters are capable of recognizing different drug molecules, many of which owe their favourable pharmacokinetic properties to recognition and transport via PepT1 and PepT2. Understanding the biochemical principles of this polyspecificity will be a significant achievement towards understanding and utilizing drug transport by these proteins.

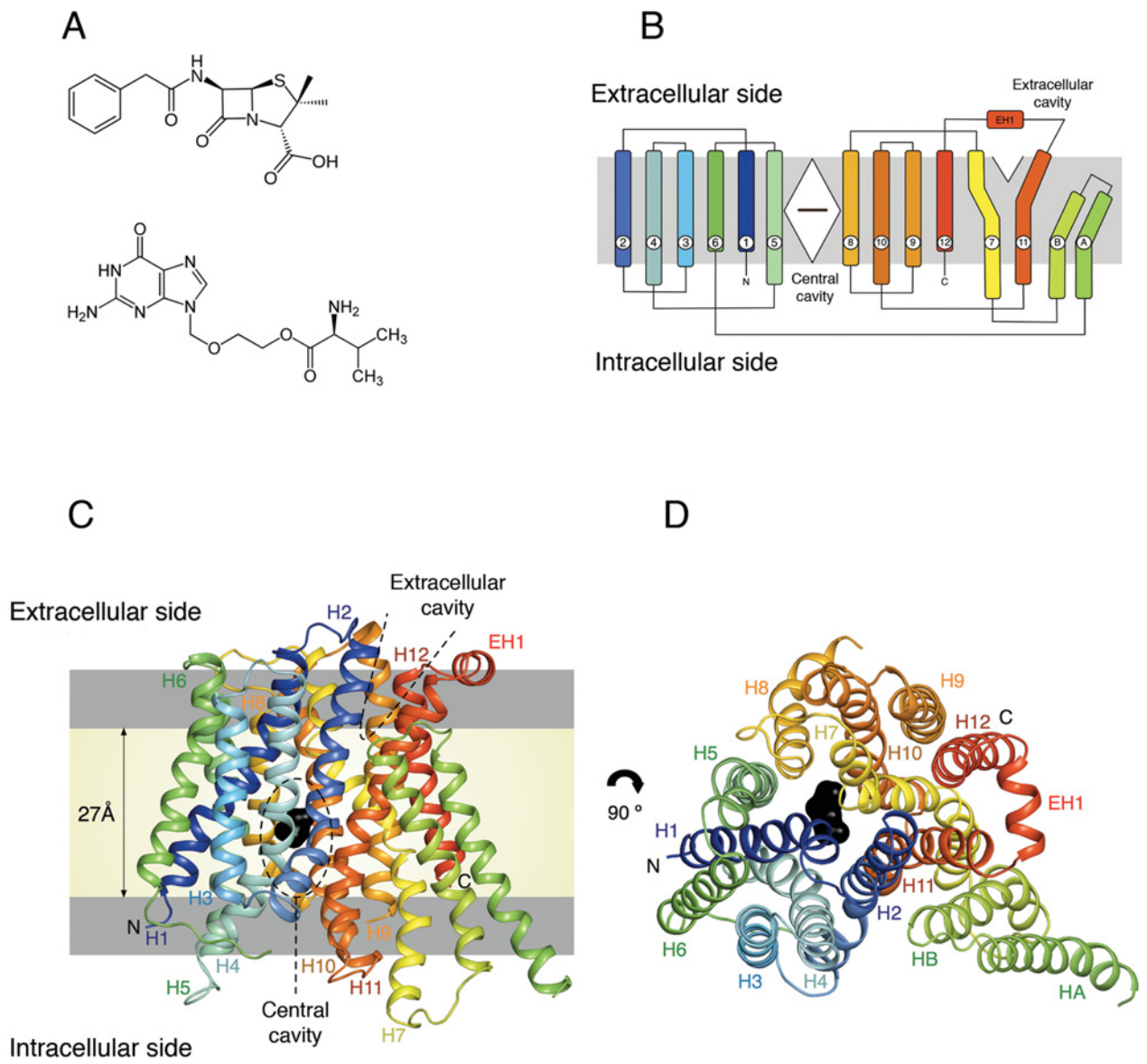
The cavity itself is striking for the number and placement of charged residues that cluster to opposite ends to create a potentially important dipole across the cavity. This orientation of charged residues suggests a possible mechanism of peptide recognition, with the N-terminus of peptides interacting with the strictly conserved glutamic acid residue ($Glu^{419}$) within the C-terminal six-helical bundle and the C-terminus interacting in some manner with the highly conserved cluster of positively charged residues, arginine and lysine ($Arg^{25}$, $Arg^{32}$ and $Lys^{127}$), on the opposing N-terminal bundle. The additional placement of three conserved tyrosine residues is also reminiscent of many antigen-recognition sites, where tyrosine and arginine play pivotal roles in providing a flexible environment for side-chain accommodation [25,26]. The overall dimensions and charge placement within this cavity may also explain the specificity that we observe within the POT family for di- and tri-peptides. The dimensions of the cavity are only sufficient to accommodate peptides of these lengths; larger tetrapeptides would be sterically restricted and single amino acids unable to interact with both halves of the transporter, and so presumably fail to trigger the required conformation changes to reorient the binding site across the membrane. The orientation of peptides during transport, however, remains to be determined, with the present structure merely providing hints as to one possible arrangement.

## Insights into conformational flexibility

The occluded conformation observed for $PepT_{So}$ opens many intriguing avenues of investigation concerning the number and types of conformational states required for a complete transport cycle, both generally within the MFS and more specifically within the POTs. The extracellular occlusion is substantial, the result of close packing between helices H1 and H2 with H7 and H8, tightly sealing off the central cavity from the external environment. This interaction appears to be strengthened through a salt bridge interaction between a conserved arginine residue on helix H1 ($Arg^{32}$) and an aspartic acid residue on helix H7 ($Asp^{316}$), both of which are conserved in human PepT1 ($Arg^{32}$ is a lysine residue in PepT2) (Figure 3). Intriguingly, positioned close to this junction, but separated by the current position of helix H7, is a conserved histidine residue ($His^{61}$), which sits on helix H2 at the base of the extracellular cavity. Functional studies have identified the equivalent histidine residue in human PepT1 as the site of proton binding during the transport cycle [27]. The role of these residues in the transport cycle of $PepT_{So}$, however, remain to be determined. Although $PepT_{So}$ is in an occluded conformation, the central cavity is not tightly

**Figure 1** | **Structure of $PepT_{So}$**

(**A**) Chemical structures of penicillin G and valacyclovir, two commonly prescribed drugs that are recognized and transported across the gastrointestinal brush border membrane by PepT1. (**B**) $PepT_{So}$ topology. The central and extracellular cavities are shown as a closed diamond and open triangle respectively. A bound ligand in the central cavity is represented as a black horizontal bar. (**C**) $PepT_{So}$ structure viewed in the plane of the membrane. The two hydrophilic cavities present in the structure are outlined by broken lines. The hydrophobic core of the membrane (pale yellow) is distinguished from the interfacial region (light grey). N and C represent the N- and C-termini respectively. Bound ligand is shown in black. Helices are labelled. (**D**) View from the extracellular side of the membrane. Reproduced with permission from [16].

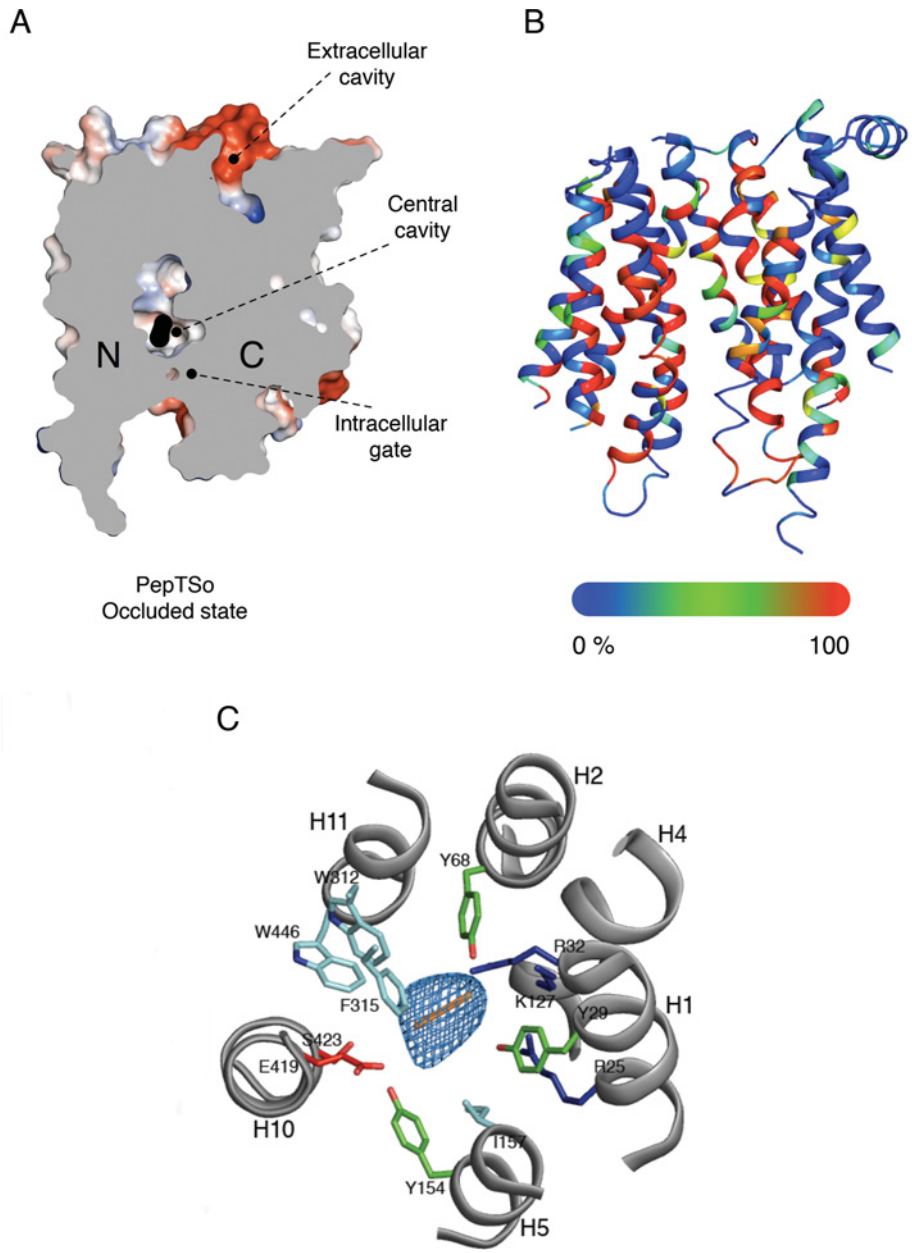



sealed on the cytoplasmic side of the molecule. Indeed, the cytoplasmic occlusion is the result of only side-chain interactions between helices H4 and H5 and H10 and H11 (Figure 3).

To understand how this conformation could 'open' to release the ligand into the cytoplasm, we carried out a detailed comparison with the inward open structure of the lactose permease structure LacY. The main difference was clearly identified as residing within the C-terminal domain, the average distances between the helices that make up the N- and C-terminal helix bundles being 2.8 and 4.4 Å respectively [16]. The C-terminal six-helical bundle of MFS transporters is made up of two continuous inverted repeats, constructed of helices H7–H9 and H10–H12. Another set of repeats also exists, however, but are discontinuous and which we have chosen to call sub-bundles. These are helices H7, H11 and H12 (sub-bundle C1) and H8, H9 and H10 (sub-bundle C2) (Figure 3). When the comparison between $PepT_{So}$ and LacY

**Figure 2** | **Analysis of the central cavity**

(**A**) Electrostatic surface representation showing the location of the hydrophilic cavities in $PepT_{So}$ in a section through the protein volume. The N- and C- terminal six-helical bundles are labelled. (**B**) $PepT_{So}$ coloured according to residue conservation with mammalian peptide transporters. (**C**) Peptide-binding site. The central cavity is viewed from above on the extracellular side of the membrane. Conserved residues between $PepT_{So}$ and the mammalian peptide transporters are labelled and coloured according to side-chain type, arginine and lysine (blue), glutamic acid and serine (red), tyrosine (green) and tryptophan, phenylalanine and leucine (cyan). A dipeptide-sized $C_\alpha$ baton (orange) is fitted as a size reference into the $mF_O-DF_C$ electron density observed in the central cavity (blue mesh), contoured at $4\sigma$.

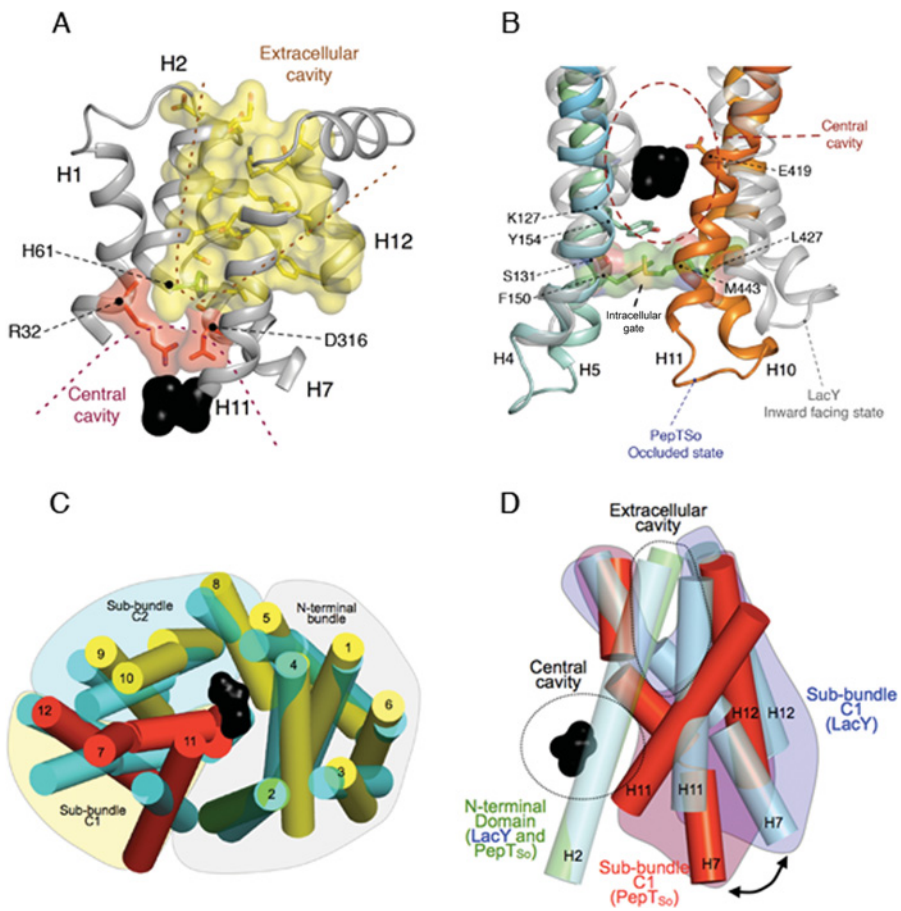



is made, the largest movement occurs in the helices that make up sub-bundle C1, which is displaced by an approximate rotation of 11°. Movement of helices H7 and H11 in this way creates a simple gating mechanism that simultaneously opens the central cavity and closes the extracellular one.

The occluded structure of $PepT_{So}$ therefore provides strong evidence that the six-helical bundles of MFS transporters may not act as quasi-rigid bodies, as postulated previously [21,28], but may contain mobile gates that open and close during the transport cycle.

**Figure 3** | **Comparison between $PepT_{So}$ and LacY**

(**A**) Extracellular cavity viewed in the membrane plane. The central and extracellular cavities are isolated from each other by a putative extracellular gate formed by the packing of helices H7 and H8 against helices H1 and H2. Residues in the central and extracellular cavity are highlighted in red and yellow respectively. $His^{61}$, part of the proposed proton–substrate coupling machinery is shown in green. Bound ligand is shown as a black CPK (Corey–Pauling–Koltun) model of a dialanine peptide. (**B**) Intracellular gate viewed in the membrane plane. Residues forming the gate are shown as stick models with transparent CPK surfaces. LacY helices (grey) are superimposed on to $PepT_{So}$. (**C**) Superimposed TM helices of $PepT_{So}$ and LacY viewed from the intracellular side of the membrane. $PepT_{So}$ helices are labelled and shown in yellow except for helix H2 (green) and helices H7, H11 and H12 (red), which form sub-bundle C1. The N-terminal six-helical bundle and the C-terminal sub-bundles C1 and C2 are highlighted. LacY helices are shown in cyan. Bound ligand is shown as a black CPK model of a dialanine peptide. Helices HA and HB have been omitted for clarity. (**D**) Rotation of sub-bundle C1 between the occluded conformation of $PepT_{So}$ and inward open conformation of LacY.




## Future directions

The ability of peptide transporters to recognize a wide array of different drug molecules combined with their location in a variety of different organs such as the gastrointestinal tract, kidneys and central nervous system make them attractive targets for the engineered delivery of future drug molecules. This goal can only be achieved if we gain a significantly broader understanding of their biochemistry. We are currently using the crystal structure of $PepT_{So}$ as a starting point for these investigations. Fundamental questions remain as to how peptide transport is coupled to the inward movement of protons, which residues are involved in binding peptides and how polyspecificity is achieved within the binding pocket. Structural biology has an important role to play in answering these questions. Further structures of peptide transporters in different conformational states along the transport cycle, combined with further biophysical and functional data on ligand binding will enable the molecular mechanism of drug transport to be determined, with significant advantages to pharmaceutical science.

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