[MS3-04] A structural and biophysical comparison between the extra-cellular domains from the mammalian peptide transporters, PepT1 and PepT2.

Beale, J , Bird, L.,

Newstead, S. and Owens, R. University of Oxford, Oxford, OX1 3QU, UK

The primary focus of my research is in understanding the structural and functional differences between the extra-cellular domains (ECDs) of two human peptide transporters, PepT1 and PepT2. PepT1 is an integral membrane protein that couples the uptake of di- and tri- peptides across the brush border membrane of the small intestine to the proton electro-chemical gradient and is the main route through which dietary protein is absorbed. PepT2 shares 46 % sequence similarity to PepT1 and is expressed in the kidneys, lung and central nervous system. Many commonly prescribed drugs, such as penicillin, are peptide mimetic and therefore PepT1 and PepT2 play a direct role in their transport and pharmacokinetic properties; although the mechanism by which these drugs are recognised is poorly understood. To understand how these two transporters function crystal structures of two prokaryotic peptide transporters have been determined in our group. Although these proteins share ~ 30% sequence similarity to human PepT1 and PepT2 in their respective transmembrane helices (TMs) an additional 20 kDa ECD inserted between helices 9 and 10 is absent in the bacterial homologues. This additional ECD is not essential for transport and currently has no known function. PepT1 and PepT2 ECDs and TMs share 14 % and 56 % sequence similarity respectively. This suggests a conserved function in the TM domain, perhaps regulated by different mechanisms in the ECD. To investigate the role of this domain we present here the crystal structure of the mouse PepT1 and rat PepT2 ECD determined to 2.22 and 2.44 Å resolution respectively. Analysis of the domains indicates that the ECDs share a common fold despite the low sequence similarity. We are currently pursuing both in vitro biophysical, computational and in vivo cell based assays to determine a functional role for the ECD in regulating peptide transport and how this process differs between PepT1 and PepT2.