**A novel approach to combat multidrug-resistant (MDR) gram-negative bacterial infections: human Oligopeptide Transporter 2 (hPepT2)-targeted design of new and safer polymyxin antibiotics**

**Yining Luo1**, Xukai Jiang2, Jian Li3, Fanfan Zhou1,\*

1Molecular Drug Development Group, Sydney Pharmacy School, The University of Sydney, Sydney 2006, Australia;

2National Glycoengineering Research Center, Shandong University, Qingdao 266237, China;

3Biomedicine Discovery Institute, Infection & Immunity Program, Monash University, Melbourne 3800, Australia;

**Background and aims.** The emergence of multidrug-resistant (MDR) gram-negative bacterial infections requires new antibiotics. Polymyxins are the last-line treatment for these ‘superbugs’; however, their clinical usage is greatly limited due to nephrotoxicity. We previously reported that human Oligopeptide Transporter 2 (hPepT2) mediates the renal reabsorption of polymyxins causing their toxic accumulation. Nevertheless, how hPepT2 and polymyxins interact remains unclear.

To facilitate the rational design of new and safer polymyxin antibiotics, we explore the structure-interaction relationship (SIR) of hPepT2 and polymyxins. Using bioinformatic prediction, we identified specific amino acid residues and transmembrane domains (TMs) of hPepT2 that may be involved in polymyxin binding, which will be further explored in this study.

**Methods.** Alanine scanning mutagenesis has been adopted to generate hPepT2 mutants in the related regions. Uptake assay and fluorescence imaging have been utilised to assess the function of hPepT2 and its mutants in mediating the uptake of glycosarcosine (a typical hPepT2 substate) and MIPS-9541 (an approved fluorescence probe of polymyxins) in over-expressing HEK293 cells. Kinetic parameters of hPepT2 and its mutants were also derived. Biotinylation and immunoblotting were employed to investigate the cell surface and total cell expression of hPepT2 and its mutants.

**Results.** Our data showed that several hPepT2 mutants are associated with significantly reduced transport uptake of MIPS-9541. Importantly, D215 residue has a remarked reduced binding affinity to polymyxin and other function-less mutants have impaired protein expression or transporter turn-over rate compared to hPepT2.

**Conclusion/Discussion.** We investigate the SIR model of hPepT2 and polymyxin for the first time. D215 residue is critical for the binding of polymyxins with hPepT2. Our findings provided important information to elucidate the interaction of polymyxin and hPepT2, which will guide us to design new antibiotics with reduced nephrotoxicity that may combat MDR infections.

**References:**

(1) Lu, X. et al. (2016) JAC 71: 403–412

(2) Luo, Y. et al (2023) Pharmaceutics 15: 2517