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Macrocyclic peptides as the novel chemical probes for modulating the function of the Retromer endosomal trafficking complex

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Maintenance of appropriate levels of endocytic trafficking and subsequent sorting in endosomes is essential for every aspect of cellular life. The evolutionarily conserved Retromer complex (composed of VPS35-VPS26-VPS29) is a central hub responsible for this process in endosomal compartments in all eukaryotes. It is known that mutations in Retromer complex can cause late-onset Parkinson's disease, and can also be hijacked by viral and bacterial pathogens during cellular infection. Seeking tools to modulate Retromer function would provide new avenues in understanding Retromer function and the associated diseases. Here we employed the random nonstandard peptides integrated discovery (RaPID) approach to identify a group of macrocyclic peptides capable of binding to Retromer with high affinity and specificity. Our crystal structures show that five of the macrocyclic peptides bind to Vps29 via a di-peptide Pro-Leu sequence. Interestingly, these peptides structurally mimic known interacting proteins including TBC1D5, VARP, and the bacterial effector RidL, and potently inhibit their interaction with Retromer in vitro and in cells. Further analysis using cryo-electron microscopy (CryoEM) and mutagenesis showed that a unique macrocyclic peptide binds Retromer at the interface between Vps35 and Vps26 subunits and can act as a molecular chaperone to stabilise the complex with minimal disruptive effects on Retromer's ability to interact with its accessory proteins. Finally, using reversible cell permeabilization approach, we demonstrate that both the Retromer inhibiting and stabilizing macrocyclic peptides can specifically co-label Vps35-positive endosomal structures, and can be used as baits for purifying Retromer from cells and subsequent proteomic analyses. We believe these macrocyclic peptides can be used as a novel toolbox for the study of Retromer-mediated endosomal trafficking, and sheds light on developing novel therapeutic modifiers of Retromer function.

Primary author(s) : Dr CHEN, Kai-En (The University of Queensland, Institute for Molecular Bioscience); Ms GUO, Qian (The University of Queensland, Institute for Molecular Bioscience); Dr CUI, Yi (The University of Queensland, School of Biomedical Sciences); Ms KENDALL, Amy (Department of Biological Sciences Center for Structural Biology, Vanderbilt University,); Dr YANG, Zhe (The University of Queensland, School of Biomedical Sciences); Dr HILL, Timothy (The University of Queensland, Institute for Molecular Bioscience); Prof. SUGA, Hiroaki (Department of Chemistry, Graduate School of Science, the University of Tokyo); Prof. FAIRLIE, David (The University of Queensland, Institute for Molecular Bioscience); Prof. JACKSON, Lauren (Department of Biological Sciences Center for Structural Biology, Vanderbilt University); Prof. TEASDALE, Rohan (The University of Queensland, School of Biomedical Sciences); Dr PASSIOURA, Toby (Sydney Analytical, the University of Sydney); Prof. COLLINS, Brett (The University of Queensland, Institute for Molecular Bioscience)

Presenter(s) : Dr CHEN, Kai-En (The University of Queensland, Institute for Molecular Bioscience)

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