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Assembly and Solution Structure of the Retromer Complex

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Retromer is a peripheral membrane protein complex that plays a critical role in a broad range of physiological, developmental and pathological processes by mediating retrograde transport of proteins from endosomes to the *trans*-Golgi (TGN) network. Wnt signalling, toxin transport and amyloid production in Alzheimer's disease are just some of the processes known to be regulated by retromer-mediated trafficking.

Mammalian retromer consists of a core heterotrimeric cargo-recognition subcomplex associated with a membrane-targeting dimer of sorting nexins. The core subcomplex consists of vacuolar protein sorting (VPS)26, VPS29 and VPS35 subunits that play different roles in complex stabilisation and in binding to transport cargo and regulatory proteins. The composition of the membrane-binding subcomplex is inconclusive but consists of a homo- or heterodimer of sorting nexins, SNX1, SNX2, SNX5 and SNX6. These BAR (Bin/Amphiphysin/Rvs)-domain proteins can induce the formation of high curvature membrane tubules through the formation of a polymerised helical coat.

We have used small-angle X-ray scattering (SAXS), X-ray crystallography, nuclear magnetic resonance (NMR) and isothermal titration calorimetry to elucidate a qualitative and quantitative model of retromer assembly [1]. In our proposed model, VPS35 forms an extended, gently curved structure composed of alternating HEAT-like helical repeats. VPS26 and VPS29 bind to distal ends through N- and C-terminal regions of VPS35, respectively, to form a stable trimeric core assembly. Results from thermodynamics experiments have shown that VPS29 and VPS26 bind to VPS35 completely independently of each other, confirming that VPS35 plays the role of central scaffold and VPS29 and VPS26 do not form any contact with each other. Intriguingly, the core trimeric complex is able to form a symmetric dimer, which may have implications for functional interactions *in vivo*.

Solution structures of SNX1 and SNX2 homodimers have been determined using SAXS. The SNX dimer associates with cellular membranes enriched in phosphatidylinositol 3-phosphate and 3,5-diphosphate and is believed to polymerise into a helical protein coat. NMR studies have confirmed that VPS29 coordinates the binding of the core subcomplex to this membrane remodelling complex. This coupling of cargo binding and membrane tubulating events enable retromer to recruit cargo molecules into a tubular endosome–to-Golgi transport carrier.

[1] S. Norwood, D. Shaw, N. Cowieson, D. Owen, R. Teasdale, B. Collins, *Traffic* **2010**, *12(1)*, 56-71.

Keywords: saxs, retromer, sorting nexin

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On-axis single-crystal raman, fluorescence and UV/Vis micro-spectroscopy at the MX spectrolab of the swiss light source

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By the in-situ combination of macromolecular crystallography (MX) with complementary optical spectroscopic techniques, the specificity of the information obtained from the crystallographic electron density maps can be greatly enhanced. Even at high resolution, e.g. the chemical state of co-factors and many structural ambiguities can only be resolved by the additional complementary information provided by UV/Vis absorption, fluorescence, Raman and Resonance Raman spectroscopy.

To exploit the strengths of these spectroscopic methods, a multi-mode micro-spectrophotometer (MS2) has been developed at the Spectroscopy Laboratory at beamline X10SA of the Swiss Light Source. An upgrade to the first installment of the spectrometer [1], which supported the UV/Vis absorption and fluorescence modes, the new version now is in regular user operation also for non-resonant and Resonance Raman operation. Raman spectroscopy in particular can provide specific information e.g. on ligand binding. Via difference Raman spectroscopy one can follow chemical changes such as ligand - active site interactions in soaking experiments. Resonant excitation enables monitoring of chromophore-related vibration bands.

The unique on-axis geometry of the instrument, with collinear alignment of both the X-ray and all optical axes, ensures the optimal overlap of the X-ray irradiated crystal volume and the area sampled by the spectrometer. Systematic errors from imperfect overlap of the sampled volumes can thus be avoided, which can become a problem in a more traditional off-axis alignment scheme. By utilizing exclusively reflective components for coupling and focusing elements, a broad spectral bandwidth down to 250 nm can be achieved. The MX SpectroLab, a separate off-line spectroscopy laboratory with a full goniometer setup is available for preparation of experiments on the beamline as well as for single crystal spectroscopy not requiring X-ray irradiation.

We present selected results obtained with the different modes of the spectrometer and will discuss the current instrument design as well as the final version of the spectrometer which is being designed to remain always online at the beamline.

[1] R.L. Owen, A.R. Pearson, A. Meents, P.Boehler, V. Thominet, C. Schulze-Briese *Journal of Synchrotron Radiation* **2009**, *16*, 173-182.

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Biophysical and structural characterization of the stress response protein CpxP

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Bacteria have evolved mechanisms to sense and adapt to continually changing conditions, allowing them to survive in a wide range of environments. In Gram-negative bacteria, such as *Escherichia coli,* the Cpx two-component signal transduction pathway senses misfolded envelope proteins and subsequently activates the expression of periplasmic proteases and folding factors to maintain protein integrity in the envelope. The Cpx stress response system consists of a membrane-localized sensor histidine kinase CpxA, the response regulator CpxR, and the novel periplasmic accessory protein CpxP. CpxP is a key regulator of the Cpx response and has been found to inhibit the pathway, likely through interaction with CpxA, and is required for the degradation of some misfolded proteins by the periplasmic protease DegP.