

STRUCTURAL STUDIES OF SECONDARY MULTIDRUGS TRANSPORTERS

Jodaitis Léni¹, Hutchin Andrew¹, Jaumaux Félix¹, Debruycker Vincent¹, Overtus Marie¹, Roth Aurélie¹, Steyaert Jan², Uchanski Tomasz², Fiselage Marcus², Cedric Govaerts¹

¹ : S.F.M.B, Université Libre de Bruxelles, Blvd, du Triomphe, Bâtiment BC, local 1C4, 208, B-1050 Bruxelles, Belgium.

² : Structural Biology Brussels, Vrije Universiteit Brussel (VUB), Pleinlaan 2, B-1050 Brussels, Belgium.
E-mail : Leni.jodaitis@ulb.be

We are interested in understanding the molecular basis of multidrug transport. By combining functional and biophysical approaches we intend to characterize structure and dynamics of these transporters. Over the last years we have focused our efforts on two main targets. LmrP from *L. lactis* and QacA from *S. Aureus*, both are exporters with remarkable diversity in substrate and extensive functional characterization. Several lines of evidence demonstrate that both must visit different conformations to extrude their ligands. Our goal is to characterize all the conformations at high resolution to decipher the molecular basis of transport.

Different conformations of QacA and LmrP have already been solved by X-ray Crystallography in our lab. We used nanobodies to stabilize specific conformations of the proteins. Unfortunately, we miss the inward open state of LmrP.

Thanks to Single Molecule FRET experiments, we identified one nanobody (nb) that is able to stabilize the inward open state of LmrP. We propose to use such conformational stabilizers in crystallography and/or in high resolution cryo-EM.

Cryo-electron microscopy (cryo-EM) is now a high-resolution structural technique but the individual particles must be large enough to generate sufficient contrast (at least ~100 kDa). One strategy the inclusion of a medium-size proteins such as MFS transporters (~40-50 kDa) is to artificially increase their size while preserving their native structure. By rigidly fusing a nanobody on a larger protein scaffold, the laboratory of Jan Steyaert (VUB, Brussels), has developed a novel technology, termed *megabodies* (*Mb*), that may answer this challenge. Indeed, as nanobodies recognize a single conformational epitope, the use of megabodies offers a direct strategy to increase the mass of the protein of interest. In addition, they provide strong asymmetry due to the shape of the megabody, which would be of great use during particle orientation and classification.

Based on previously isolated LmrP-specific nanobodies, we have obtained a first set of LmrP-specific megabodies which have confirmed to be specific binder by ELISA. Size-exclusion chromatography has confirmed the formation of a stable complex. Initial Cryo-EM of LmrP-Mb complexes shows the presence of individual particles of about 10nm. The first set of 2D classification and 3D refinement are promising even though they do not allow structure solving due to the complex mobility.

STRUCTURAL STUDIES OF SECONDARY MULTIDRUGS TRANSPORTERS

Jodaitis Léni¹, Hutchin Andrew¹, Jaumaux Félix¹, Debruycker Vincent¹, Overtus Marie¹, Roth Aurélie¹, Steyaert Jan², Uchanski Thomas², Fiselage Marcus², Cedric Govaerts¹

¹ : S.F.M.B, Université Libre de Bruxelles, Blvd, du Triomphe, Bâtiment BC, local 1C4, 208, B-1050 Bruxelles, Belgium.

² : Structural Biology Brussels, Vrije Universiteit Brussel (VUB), Pleinlaan 2, B-1050 Brussels, Belgium.
E-mail : Leni.jodaitis@ulb.be

We are interested in understanding the molecular basis of multidrug transport. By combining functional and biophysical approaches we intend to characterize structure and dynamics of these transporters. Over the last years we have focused our efforts on LmrP from *L. lactis*, an exporter with remarkable diversity in substrate and extensive functional characterization. Several lines of evidence demonstrate that LmrP must visit different conformations to extrude its ligands. Our goal is to characterize all the conformations at high resolution in order to decipher the molecular basis of transport.

We have solved the structure of LmrP in presence of a substrate by X-ray crystallography at 2.9Å resolution. In those conditions, the protein was stabilized in the outward-open state. We now intend to characterize the other states in order to understand transport.

We are attempting to stabilize the other conformations using nanobodies. Single Molecule FRET experiments have identified nanobodies (nb) that for instance stabilize the inward-open state. We propose to use such conformational stabilizers in high resolution cryo-EM to resolve the missing states.

Cryo-electron microscopy (cryo-EM) is now a high-resolution structural technique but the individual particles must be large enough to generate sufficient contrast (at least ~100 kDa). One strategy the inclusion of a medium-size proteins such as MFS transporters (~40-50 kDa) is to artificially increase their size while preserving their native structure. By rigidly fusing a nanobody on a larger protein scaffold, the laboratory of Jan Steyaert (VUB, Brussels), has developed a novel technology, termed *megabodies* (*Mb*), that may answer this challenge. Indeed, as nanobodies recognize a single conformational epitope, the use of megabodies offers a direct strategy to increase the mass of the protein of interest. In addition, they provide strong asymmetry due to the shape of the megabody, which would be of great use during particle orientation and classification.

Based on previously isolated LmrP-specific nanobodies, we have obtained a first set of LmrP-specific megabodies which have confirmed to be specific binder by ELISA. Size-exclusion chromatography has confirmed the formation of a stable complex. Initial Cryo-EM of LmrP-Mb complexes shows the presence of individual particles of about 10nm. The first set of 2D classification and 3D refinement are promising even though they do not allow structure solving due to the complex mobility.