EPSRC Centre for Doctoral Training (CDT) in Molecular Sciences for Medicine (MoSMed)

CRISPR-mediated tagging of endogenous proteins for structural mapping of interactions

Newcastle University (Biosciences Institute), Durham University

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Project overview/context

Structural biology offers atomic views of proteins with interacting partners, and when protein samples are isolated from their native states, allows mapping of physiologically relevant interactors. The emerging technology of CRISPR knock-in enables site-specific tagging of endogenous proteins to facilitate purification from native cells, towards the realm of high-throughput structural biology of multi-protein complexes. This project aims at developing a workflow that couples CRISPR endogenous tagging with cryo-EM structural biology, to study supramolecular complexes associated with genetic defects, where high-resolution protein-protein interaction maps would yield insight into the poorly understood disease mechanism and inform potential therapeutic targets.

Research Project

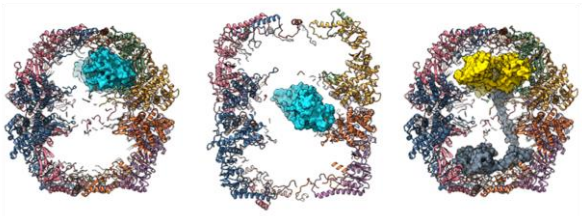
Background and Rationale

The essentiality of protein-protein interactions to the cell's functional landscape is underscored by the saying that "*no protein is an island entire of itself …*" [1]. It is of utmost importance to study proteins in the context of their physiologically relevant interaction partners, which shape their cellular functions.

Structural biology offers an atomic mapping of protein-protein interactions, particularly within multi-component supramolecular complexes. When carried out using endogenous samples in the relevant cellular context, it has untapped potential in revealing novel interactors that illuminate uncharted biological functions.

Ground-breaking CRISPR-Cas9 technology has made possible the site-specific knock-in of sequences to a gene, adding fusion tags to the corresponding protein. This 'endogenous tagging' approach, coupled to affinity purification (ETAP), creates a powerful toolkit to isolate the target and associated proteins, and will become the *tour de force* for structural biology of protein complexes.

Our pilot allows the pulldown of novel client proteins (blue, yellow surface) of a folding chaperone (ribbon), revealed by cryo-EM



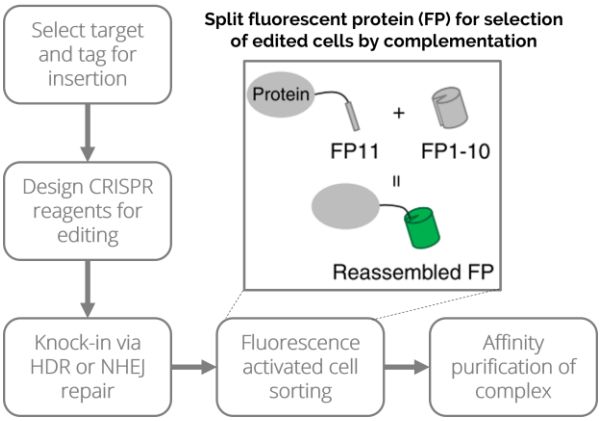
We recently piloted the ETAP approach in the isolation of a 1 MDa folding machine TRIC for structure determination by cryogenic electron microscopy (cryo-EM), and through this uncovered novel client proteins recruited by TRIC [2].

Vision and Workplan

This project aims to build upon our pilot and develop a streamlined ETAP workflow for systemic structural biology of complexes [3]. Using as examplar two chaperone/co-chaperone complexes for study, efforts will be focused on

- optimising the CRISPR knock-in efficiency
- using split-fluorescent protein complementation for selection and enrichment of CRISPR-edited cells
- surveying diverse range of tags to be appended, at different protein locations, for maximum yield

- characterising the isolated complexes by cryo-EM, mass spectrometry and biophysical assays.



Further reading

[1] Kumar A & Snyder M (2002) Protein complexes take the bait. *Nature* 415:123

[2] Kelly JJ et al (2022) Snapshots of actin and tubulin folding inside the TRiC chaperonin. *Nat Struct Mol Biol* 29:420

[3] Zhao J et al (2022) Structural insights into the human PA28-20S proteasome enabled by efficient tagging and purification of endogenous proteins. *PNAS* 119: e2207200119

Further Information

Email wyatt.yue@newcastle.ac.uk
Visit https://www.staff.ncl.ac.uk/yuelab/
Follow us on @TheYueLab

How to Apply

If applying to a **Newcastle project**, you must apply through the University's **Apply to Newcastle Portal**. Once registered select **'Create a Postgraduate Application'.**

Use 'Course Search' to identify your programme of study:

- search for the 'Course Title' using the programme code: **8207F**
- select **'PhD Molecular Sciences for Medicine (SNES)'** as the programme of study

You will then need to provide the following information in the 'Further Questions' section:

- a 'Personal Statement' (this is a mandatory field) - upload a document or write a statement directly into the application form. Please include the full title of the studentship, the studentship code, and how your interests and experience relate to the project.
- the relevant studentship code **(for example: mos23_14)** in the 'Studentship/Partnership Reference' field. If you wish to apply for additional studentships, please make sure to add the relevant studentship reference each time, before submitting each separate application. For example, you may wish to apply for mos23_13 AND mos23_14. **You must include the relevant code for your application to be considered.**
- when prompted for how you are providing your research proposal - select 'Write Proposal'. You should then type in the title of the relevant research project. You do not need to upload a research proposal.
- An up to date CV.
- Please upload all documents in PDF format.

Training & Skills

Through this project the student will gain exposure and experience at the interface of cutting-edge biotechnology, biochemical investigation, and target discovery. The supervisory team will provide multi-disciplinary training and access to facilities in the growing fields of genome editing, structural biology and protein interactomes. The student will be supported in writing manuscripts and presenting findings at international conferences. These training skills and experience will be highly valuable for career development in discovery science and translational research.

Equality, Diversity and Inclusion (EDI)

Within the MoSMed CDT we are committed to building a diverse community based on excellence and commitment. To that end in our recruitment of Doctoral Researchers we welcome applications from outstanding candidates of all backgrounds regardless of ethnicity, disability, gender identity, sexual orientation and will consider all applications equally based on merit.

Should you have any queries regarding the MoSMed application process to Newcastle University please contact Craig Hinds, the MoSMed CDT Manager: **mosmed.cdt@newcastle.ac.uk**

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