**BBSRC MIBTP Studentship Project**

**September 2023**

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| **Additional Supervisor** |  |

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| **Project Title** | Biophysical, Crystallographic, and Cryo-ET Studies of the Gal-3-Fibrosome |
| **Project Summary**  |
| **Background:** The Gooptu Group at the University of Leicester explores the biology of healthy and dysfunctional ageing across multiple scales, from a molecular structural perspective1,2.  Inflammation and scarring (fibrosis) can arise in response to mechanical and biochemical challenges. Exaggerated responses underlie deleterious age-related changes to organ function, and so healthy ageing requires them to remain well regulated. We have shown that the lattice-forming protein galectin-3, and cell surface membrane protein ligands such as CD98, integrins and the transforming growth factor (TGF-)β receptor (TGFβR) mediate these responses in a range of cell types2. Moreover, our data are consistent with their organisation into a dynamic, pro-inflammatory and pro-fibrotic cell surface complex we term the ‘gal-3-fibrosome’ (animated illustration in 3). Understanding the structural basis of this complex and its constituent intermolecular interactions will allow us to modulate its activity to promote healthy ageing. The group has existing collaborations with the Dafforn group (University of Birmingham) and Membrane Protein Lab (Diamond Light Source and Research Complex at Harwell) whose expertise and training input are key to this work. **Objectives:** 1 - To understand whether gal-3-fibrosome composition varies with different insults, cell and tissue contexts. 2 - To further define gal-3-fibrosome organisation by structural and biophysical methods. 3 – To validate findings in functional studies using cell models of pro-inflammatory and pro-fibrotic behaviour. **Methods:** The work will use SMALP nanodisc, advanced fluorescence microscopy, GFP/nanobody pull-down, western blot and mass spectrometry methods to evaluate gal-3-fibrosome composition and interactions in endogenous and over-expression states.  Detergent and amphipol membrane protein extraction methods will complement these to purify individual proteins and sub-complexes of interest for X-ray crystallographic or single particle electron microscopy (EM) studies.  Correlative light and EM (CLEM) approaches with cryo-focused-ion-beam (Cryo-FIB) milling approaches will be used to perform cryo-electron tomography (Cryo-ET) of the gal-3-fibrosome *in situ*. Cell biology experiments will validate findings from molecular and structural studies, and test strategies to modulate the gal-3-fibrosome and so promote healthy ageing. Techniques that will be undertaken during the project:The work will use:  * Membrane protein extraction methods: SMALP nanodiscs, detergent and amphipol approaches
* Fluorescent fusion protein expression and co-expression
* Advanced fluorescence microscopy, including proximity ligation assay, fluorophore localization imaging with photobleaching (FLImP), super-resolution microscopy (SIM, STORM)
* Interactomics using GFP/nanobody pull-down, western blot and mass spectrometry methods
* Membrane protein X-ray crystallography
* Single particle electron microscopy (EM) studies (negative stain and cryo-EM).
* *In situ* correlative light and EM (CLEM) approaches with cryo-focused-ion-beam (Cryo-FIB) milling approaches to perform cryo-electron tomography (Cryo-ET) of the gal-3-fibrosome.
* Tissue culture and mammalian cell model studies with mutagenized constructs, or k.o. (CRISPR) and/or k.d. (siRNA) approaches, and where feasible pharmacological inhibition studies in an *ex vivo* tissue model.
* We propose to undertake CLEM, Cryo-FIB and Cryo-ET studies via the student’s co-registration at the Harwell/Diamond campus and involving the eBIC facility there. Gooptu group members are already working with all of other methods mentioned.  These are undertaken at the University of Leicester, or via collaborations we have initiated with the 2nd supervisors or externally (Maddy Parsons, Nikon Imaging Centre, KCL).

BBSRC Strategic Research Priority: Understanding the Rules of Life - Structural Biology |
| **References** |
| (\*denotes joint first authorship):  1. Gooptu\*, Faull\*, Elliston\* *et al*.

The structural basis for Z α1-antitrypsin polymerization in the liver.  *Science Advances 2020, 6(43):eabc1370* 1. Stylianou\*, Rushwan\*, Wang\* *et al*.

CD98 is critical for a conserved inflammatory response to diverse injury stimuli relevant to IPF exacerbations and COVID pneumonitis *Biorxiv 2022* [*https://doi.org/10.1101/2022.08.12.503154*](https://doi.org/10.1101/2022.08.12.503154) 1. <https://www.youtube.com/watch?v=PXfZr1KXjP8>
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