**University of Leicester**

**Future 50 PhD Scholarship**

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| **Project Reference** | RI LISCB Gooptu |

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| **Additional Supervisor** | Dr Pietro Roversi, LISCB and University of Milan, Italy |

**Section 2 – *Project Information***

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| **Project Title** | Assigning fate in protein misfolding: structural and cellular studies of alpha1-antitrypsin interactions with ER chaperones |
| **Project Highlights:** | 1. | An interdisciplinary project of clinical relevance defining interactions of ER chaperones with alpha1-antitrypsin using structural biology |
| 2. | Understanding how differences in structural interactions of a single ER chaperone with alpha1-antitrypsin in different misfolding or conformational states leads to re-entry into the folding pathway in one case and lysosomal degradation in another. |
| 3. | Probing whether enhancing these interactions are beneficial or harmful in cell models of disease. This will guide new strategies for treating alpha1-antitrypsin deficiency and other diseases mediated by misfolding of proteins along the secretory pathway. |
| **Project Summary**  |
| Alpha1-antitrypsin is the most abundant circulating antiprotease. Its function relies upon an ability to undergo dramatic conformational change conferred by folding to a metastable native structure - a conformation that represents a kinetically-trapped intermediate rather than the most stable state. α1-antitrypsin folds within the endoplasmic reticulum (ER). As a folding compartment, the ER contains chaperone mechanisms to prevent the accumulation of trapped folding intermediates that usually represent misfolded states. Moreover, the fold is inherently prone to alternative stabilisation in pathological states such as the α1-antitrypsin polymer, seen in the context of mutations that cause disease, e.g. the Z (Glu342Lys) variant. Despite these challenges, wild-type (M) α1-antitrypsin is synthesised, folded and secreted at high rates (~2 g/d) by liver cells. However in the presence of the Z mutation only a small proportion of synthesised α1-antitrypsin folds correctly and is secreted (<15%). Homozygous individuals are prone to severe, early-onset liver (childhood and adult cirrhosis, cancer) and lung (emphysema) disease due to a combination of loss- and toxic gain-of-function consequences of the events within the ER. In this project we propose to dissect these events in terms of interactions of α1-antitrypsin with ER chaperones, using structural and cellular biology. The supervisors and a co-supervised student have previously determined structures of polymeric α1-antitrypsin and the ER chaperones UGGT1, PDI and EDEM by electron (cryo)microscopy (EM/cryoEM) (Fig. 1). UGGT1 facilitates the recycling of misfolded glycoproteins like α1-antitrypsin for further folding attempts, yet also interacts with α1-antitrypsin polymers in a recently described process of ER-to-lysosome-associated-degradation (ERLAD). Conversely PDI and EDEM cooperate to target terminally-misfolded monomeric protein for export to the cytoplasm for degradation by the proteasome. The project aims to purify complexes of disease-mutant α1-antitrypsin in complex with these chaperones from cells and/or reconstitute from individual components and determine their structures by cryoEM. We will discuss the degree to which these processes worsen or improve responses in cell models of disease using overexpression, inhibitor, knockdown and/or knockout methods, to understand whether strategies for the development of new drugs should aim to enhance (collaboration with Richard Doveston) or block these interactions. |