Building molecular transport machines regulated by autoinhibition and ligand-induced activation.

Supervisory team:
Main supervisor: Dr Mark P Dodding (University of Bristol)
Second supervisor: Prof Derek N Woolfson (University of Bristol)

Host institution: University of Bristol

Project description:

All life exploits molecular machines. These can be put to a multitude of complex tasks that include enabling cells to maintain and adapt their shape, move components around, and divide to make new cells. Here, we will address two key challenges in the growing field of synthetic biology: How do we build molecular machines? And, how do we integrate them into natural systems? The answers to these questions will have many future applications as we engineer biology to obtain fundamental understanding of natural biological systems, and how we translate this knowledge in the future for biotechnology applications in the emerging area of engineering biology.

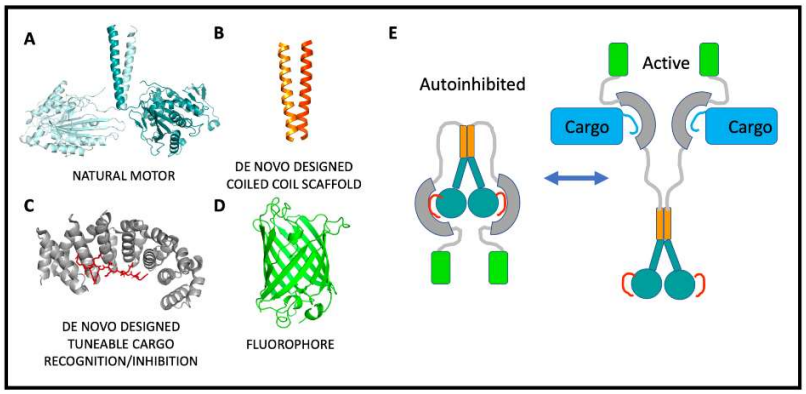

Figure 1. Key building blocks and overall design of a self-regulated, ligand-activated motor protein. A) Kinesin-1 dimeric motor domains, PDB:3KIN. (B) Dimeric *de novo* designed coiled coils (Woolfson lab), PDB: 4DZM (C) *De novo* designed tuneable cargo recognition/inhibition (Plückthun lab), PDB: 5AEI. (D) Fluorophore, GFP, PDB:1EMA. (E). A possible design of a de novo designed cargo-activated kinesin motor.

The activities of molecular machines have to be tightly controlled. We have studied how this control is achieved in natural systems. Often, this occurs when one part of the machine interacts with another part - essentially jamming the mechanism. This jam can be unblocked on specific cues, and so allows activity to be triggered. This jamming phenomenon is known as 'autoinhibition' and the cue is known as 'ligand-induced activation'.

We want to learn how to build self-regulated molecular machines that can be activated by specific cues introduced through rational protein design and engineering. We are inspired by natural transport machines - the cytoskeletal motors - which often use such mechanisms. We will focus on the kinesin family of microtubule motors where we have particular expertise.

To do this, we will take component parts from natural systems and combine them with parts that we will design de novo or from scratch (Figure 1). These will include kinesin ATPase motor domains (A), de novo designed coiled coils from the Woolfson lab (B), designed armadillo repeat proteins (dArmRPs, C) for cargo recognition and autoinhibiton, and fluorophores for cell imaging (D). The key challenge will be making these components work together in cells. This will require fine tuning of interactions between the natural and designed components. We will move on to use the system to incorporate sophisticated regulatory mechanisms such as phosphorylation, and ask how we can combine motors of opposite directionality or that utilise other cytoskeletal tracks such as myosins and actin.

The project will combine expertise from the Dodding and Woolfson labs at Bristol, in cell biology & motor proteins, protein design & synthetic biology, respectively. As such, the student will develop skills in computational protein design, molecular biology, cell biology, structural biology and advanced cell imaging techniques.