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BIOPHYSICS GROUP - STRUCTURAL BIOLOGY

Many key physiological processes are controlled at a molecular level by large multi-protein complexes. These complexes are often prone to disease-producing mutations. Research in the lab focuses on '*pushing the limits*' of structural techniques to reveal structure and movement in several large dynamic protein complexes, including:

(i) The Troponin complex – the 'ON' switch for muscle contraction,

(ii) The metamorphic CLIC ion channel family

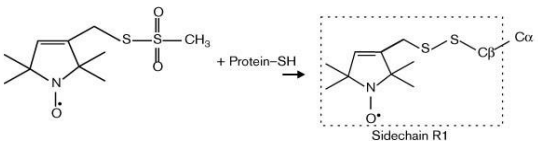

Figure 1: "Spin Labeling" - the attachment of a spin label to the side chain of a cysteine residue that has been introduced into a specific site on the protein by site-directed mutagenesis.

Due to the large size and the dynamic nature of these two protein complexes, their structures are often difficult to determine by conventional biophysical methods. The focus of our group is to therefore use '*reporter-probe*' based spectroscopic methods to study these challenging protein systems. We use site-directed labeling methods to attach small fluorescent or magnetic chemical labels to targeted regions of interest on the protein complex (Fig. 1). This enables the structure and dynamics of the proteins to be revealed using spectroscopic techniques including Electron Paramagnetic Resonance (EPR), Nuclear Magnetic Resonance (NMR) and Fluorescence Spectroscopy.

We are also exploring new labeling technologies – namely **Nanodiamonds as biological probes**. Nanodiamonds are promising labels for tracking and imaging of proteins down to the single molecule level.

THE CONTROL OF MUSCLE CONTRACTION BY THE TROPONIN COMPLEX

There are many debilitating and even fatal cardiovascular and skeletal diseases that arise from defects in muscle proteins. This project aims to understand the molecular basis for some of these disorders in the large muscle protein complex called 'Troponin'. There are now more than 60 mutations in Troponin linked to heart disease (Fig. 1). However, understanding how these often single point mutations can result in the disease state is still experimentally challenging due to the large size and dynamic nature of the complex. In this project, we are using novel EPR and NMR methods to construct a dynamic molecular movie of Troponin function to better understand why mutations lead to disease states.

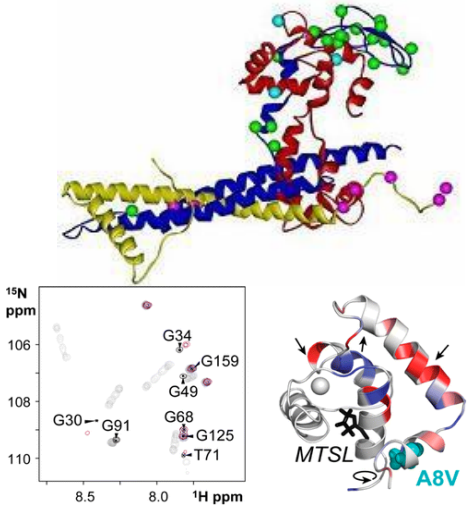

Figure 2: Structural model of Troponin Tn showing the locations of several mutations that cause Familial Hypertrophic Cardiomyopathy (FHC) and NMR methods used to map conformational changes (from ref. 3).

STRUCTURE OF THE METAMORPHIC CHLORIDE INTRACELLULAR ION CHANNELS - CLICS

Chloride ion channels are involved in diverse physiological processes and channel malfunction can lead to severe diseases. This project examines the structure and conformational changes of a unique chloride ion channel, called 'CLIC' (Chloride Intracellular Ion Channel). CLIC proteins

are highly unusual in that they can reversibly transit between a soluble and active membrane channel form (Fig 3). We use x-ray crystallography, EPR, fluorescence spectroscopy and electron microscopy methods to 'track the view the structural gymnastics CLICs undergo as they interact with membranes to form ion channels.

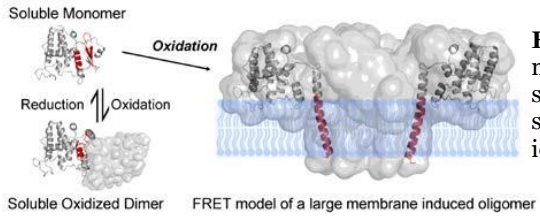

Figure 3: Insertion of CLIC into the membrane bilayer. Structural studies, using site directed mutagenesis and single probe studies, will help us understand this unusual ion channel family (from ref. 5)

NANODIAMONDS AS BIOLOGICAL PROBES

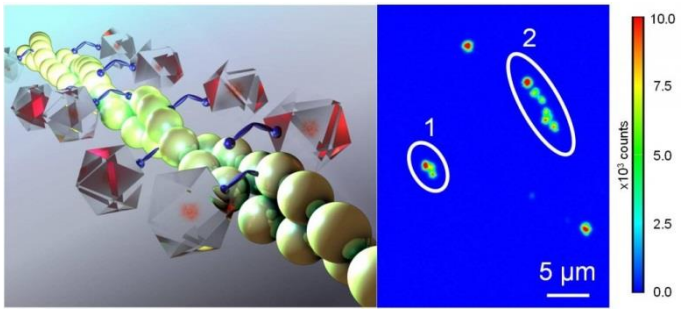

Figure 4: Artistic view of nanodiamonds conjugated to a biological filament. Single molecule imaging of nanodiamonds (from ref 1).

Nanodiamonds (< 100nm) have emerged from primarily an industrial and mechanical applications base, to potentially underpinning sophisticated new technologies in quantum science and biology. In addition to their unique chemical and physical stability, they can have colour centres whose properties make them attractive bio-labels for imaging and tracking.

The bright and stable photoluminescence, as well as the straightforward surface functionalisation for targeting to biological structures, has allowed us to begin to probe cellular processes down to the single-molecule scale; one of the primary goals of biomedical science and, ultimately, therapeutics. We are exploring applications ranging from using nanodiamonds as superior biological markers to, potentially, developing novel bottom-up approaches for the fabrication of hybrid quantum devices that would bridge across the bio/solid-state interface.

Projects in our lab would suit students keen to work at the interface of biology, chemistry and physics with backgrounds in any of the following: molecular biology, biochemistry, protein chemistry, physical chemistry (spectroscopy), organic chemistry, nanotechnology, synthetic biology or computational chemistry.

Recent selected publications

1. Bradac C, Say JM, Rastogi ID, Cordina NM, Volz T, Brown LJ (2015) Nano-assembly of nanodiamonds by conjugation to actin filaments. *Journal of Biophotonics* doi: 10.1002/jbio.201500167
2. Geiselmann M, Juan ML, Renger J, Say JM, Brown LJ, et al. (2013) Three-dimensional optical manipulation of a single electron spin. *Nature Nanotechnology*, 8: 175–179
3. Cordina NM, Liew CK, Gell DA, Fajer PG, Mackay JP, Brown LJ (2013) Effects of calcium binding and the hypertrophic cardiomyopathy mutation A8V on the dynamic equilibrium between closed and open conformations of the regulatory N-domain of isolated cardiac Troponin C. *Biochemistry*, 52:1950-1962
4. Say JM, Vreden C, Reilly D, Brown LJ, et al. (2011) Luminescent Nanodiamonds for Biomedical Applications Biophysical Reviews. *Biophysical Reviews*, 3:171-184
5. Goodchild SC, Howell MW, Littler DR, Mandyam RA, Sale KL, Mazzanti M, Breit SN, Curmi PM, Brown LJ (2010) Metamorphic response of the CLIC1 chloride intracellular ion channel protein upon membrane interaction. *Biochemistry*, 49:5278-5289
6. Bradac C, Gaebel T, Naidoo N, Sellars M, Twamley J, Brown LJ, et al. (2010) Observation and control of blinking nitrogen-vacancy centres in discrete nanodiamonds. *Nature Nanotechnology*, 5:345-349

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