Name	DR JUSTIN E. MOLLOY
Position	Senior Group Leader
Year joined *(Crick or founder institute)*	2002

Career History

1979-1983: BSc Hons, Physiology, University of York
1984-1987: PhD Biophysics, University of York
1988-1990: NATO Post-Doctoral Fellowship, University of Vermont, USA.
1991-1995: Post-Doctoral Scientist Department of Biology, University of York
1995-2002: Royal Society University Research Fellow, York
2000-2002: Reader in Biology, York.
2002-2015: Head of Division of Physical Biochemistry, MRC NIMR
2005-2015: Head of Structural Biology Group, MRC NIMR
2015- present: Senior Group Leader, The Francis Crick Institute
2015- present: Honorary Professor: KCL & UCL

Major Awards, Honours and Prizes

1988: NATO Post-Doctoral Fellowship
1995: Royal Society University Research Fellowship
1998: Society of Experimental Biology, President's Medal
2000: European Biophysical Societies Association, Annual Prize for Biophysics
2003: Koerber Stiftung, European Science Prize
2007: IEEE Premium Award

Membership of external committees, editorial boards, review panels, SABs etc

Wellcome Trust, Expert Review Group (panel 8) (2011-2015)
Dresden MPI CBG SAB (2009-2016)
Hungary NRDI Grant panel (2016-2019)
BBSRC ALERT Panels (2016/17/18)
STFC (2015-2019) Access Panel (Panel Chair 2016-2019)

Sense Board of Directors (2013-)
Sense Chairman (2018-)

Journal of Microscopy Editorial Board (2009-2018)
Journal of General Physiology Editorial Board (2018-)

Lab Name	***Single Molecule Enzymology Laboratory***

Research programme and achievements

The principal goal of the lab is to understand the mechanical properties of protein-protein and protein-DNA interactions at the single molecule level, including force production by acto-myosin, protein and organelle motility within living cells, and DNA processing and packaging mechanics. We use laser-based optical methods to observe, track and manipulate individual molecules either in isolated preparations or within living cells. Over the past five-years the focus of my research has moved from studies of cytoskeletal proteins (actin and myosin) to studies of DNA processing and packaging systems.

With Holder (Crick) and Kursula (U. Bergen) we used *in vitro* motility assays, optical-trapping nanometry and ATPase assays, to show that **myosin 14** (*MyoA*) (which is responsible for the motorised invasion of malarial parasites into human red blood cells) requires two cognate light chains to produce full force and movement both in live merozoites and at the single molecule level. With Peter Rosenthal's group we solved the atomic structure of the acto-myoA complex to atomic resolution (paper in revision).

With Peckham (U. Leeds) we showed human **myosin 10**, which is important for filopodial formation and cancer metastasis, moves along actin in a hand-over-hand processive manner both *in vitro* and within living cells, and by using truncation mutants we identified the key functional regions of the protein by imaging individual GFP-tagged molecules within live mammalian cells.

With Mulvihill (U. Kent) we studied the role of **myosin I** in endocytosis using our TIRF imaging to reveal the timing of molecular events during the endocytic cycle.

Our work on **rolling circle plasmid replication** by RepD/PcrA/DNAPol1 showed that negative DNA supercoiling is required for initiation of replication by RepD and this ensures the plasmid is topologically intact (i.e. undamaged) before replication can commence.

Current and future work
With partners at Astra-Zeneca (Flocco and Maia-De-Oliviera) and UCL (Hoogenboom) our AZ-funded post-doc has shown that **Poly ADP-Ribose Polymerase enzyme (PARP)** (an important target for cancer therapeutics) compacts and cross-links damaged and undamaged DNA in a drug-dependent fashion. During this project, we have also developed new tools to manipulate and image single DNA molecules. In future work, we aim to increase our understanding of how PARP recognises and binds DNA damage and test if PARP inhibitors (i.e. drugs like olaparib) block DNA transactions driven by DNA helicase and RNA polymerase, thought to underlie synthetic lethality.

My lab has established a new partnership with two KCL labs (Booth and Garcia Manyes, Chemistry and Physics resp.) and our co-funded PhD student is using single molecule mechanical methods (magnetic tweezers and AFM) to study the folding pathway of prototypical **membrane transporter** proteins (LeuT and XyLe) in the complex physico-chemical environment of lipid membranes.

My other PhD student is studying the mechanical properties of **archaeal chromatin** (with Werner, UCL). They have found that archaeal chromatin is much more labile than eukaryotic chromatin and readily assembles and disassembles under relatively small forces and torques. Epigenetic marks would therefore be easily lost and therefore unlikely to be important in archaea – unless other mechanisms exist.

In a new departure, with Steve Gamblin's group we intend to study the mechano-chemistry of the **human chromatin remodelling** enzyme *BAF*, which is critical to maintenance of chromatin structure and epigenetic marks. We want to know how BAF moves, spaces, displaces and replaces nucleosomes on DNA.

Research outputs

Khan, S., Downing, K.H., and Molloy, J.E. (2019). ***Architectural Dynamics of CaMKII-Actin Networks*****. Biophys J 116, 104-119. DOI: 10.1016/j.bpj.2018.11.006**
This publication built on our earlier work (Khan & Molloy, J.E., 2016. Biophys J. 111, 395-408) showing how CaMKII acts as a signalling hub that crosslinks actin in a calcium-calmodulin dependent manner. The work shows how the postsynaptic signalling molecule, CaMKII, may bring about synaptic tagging (structural basis of memory).

Baker, K., Gyamfi, I.A., Mashanov, G.I., Molloy, J.E., Geeves, M.A., and Mulvihill, D.P. (2019). ***TORC2-GAD8 dependent myosin phosphorylation modulates regulation by calcium*****. eLife 8, 1-42. DOI: 10.7554/eLife.51150**
Our contribution to this work was to show how the light chains of MyoI bind and unbind in a phosphorylation-dependent manner during the endocytic cycle. The timing of each endocytic event (which lasts about 14 seconds) is highly regulated and we identified different phases of the event at individual endocytic sites using TIRF imaging.

Toleikis, A., Webb, M.R., and Molloy, J.E. (2018). ***oriD structure controls RepD initiation during rolling-circle replication*****. Scientific Reports 8. DOI: 10.1038/s41598-017-18817-6**
In this study we showed that negative supercoiling is required for RepD nicking activity to be activated. By controlling the degree of DNA torque using magnetic tweezers we were able to establish the exact energetic requirement for nicking activation by mechanical force.

Green, J.L., Wall, R.J., Vahokoski, J., Yusuf, N.A., Mohd Ridzuan, M.A., Stanway, R.R., Stock, J., Knuepfer, E., Brady, D., Martin, S.R., et al. (2017). ***Compositional and expression analyses of the glideosome during the Plasmodium life cycle reveal an additional myosin light chain required for maximum motility*****. J. Biol. Chem. 292, 17857-17875. DOI: 10.1074/jbc.M117.802769**
Our work here complemented that of the Holder Group, that identified the essential and regulatory light chains of MyoA and showed they are essential for merozoite invasion of red blood cells, we used biophysical and biochemical methods to study purified bacculuo-virus SF9-expressed MyoA in vitro which supported and strengthened the in vivo studies.

Baboolal, T.G., Mashanov, G.I., Nenasheva, T.A., Peckham, M., and Molloy, J.E. (2016). ***A combination of diffusion and active translocation localizes myosin 10 to the filopodial tip*****. J. Biol. Chem. 291, 22373-22385. DOI: 10.1074/jbc.M116.730689**
Here, we examined a panel of myosin 10 deletion mutants to show how different structural modules affected the intrafilopodial motility of individual myosin 10 molecules as they moved within the filopodium of live mammalian cells. We proposed that myosin 10 is targeted via a successive reduction in dimensionality from 3D -> 2D ->1D with transition rates that lead to rapid protein targeting.