UNIVERSITY OF LIVERPOOL

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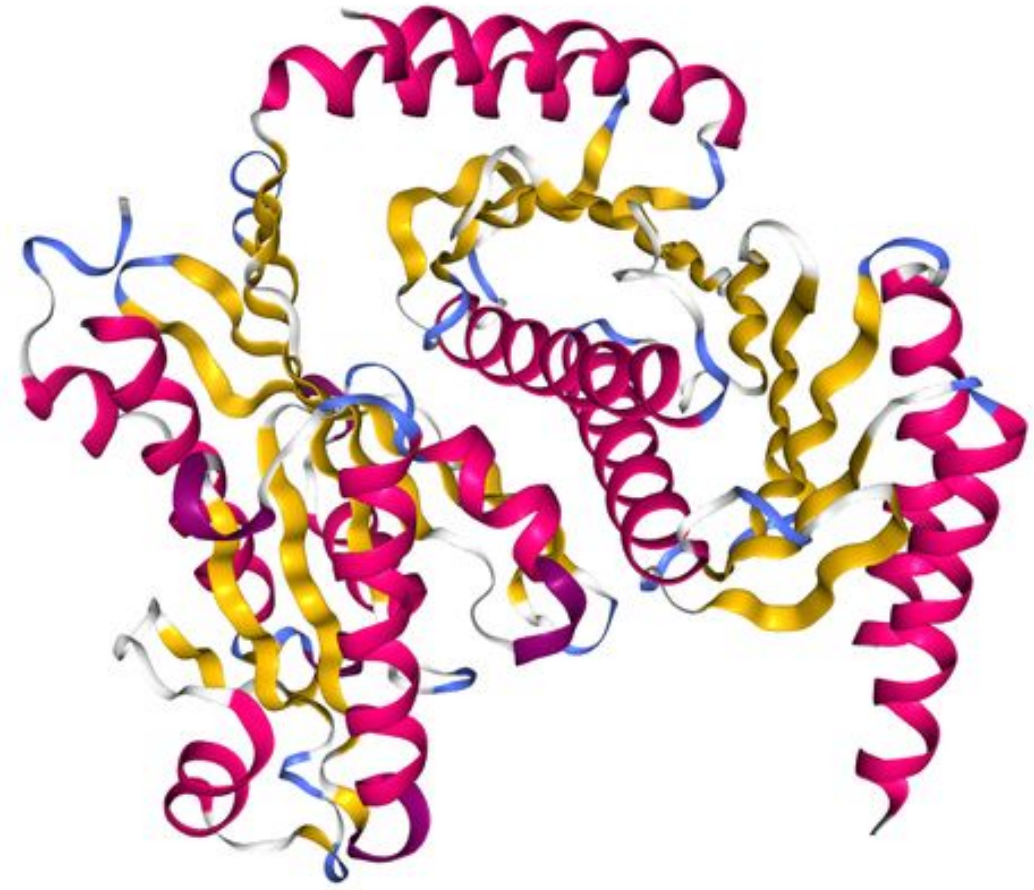
A Multiplexible Single-colour Probe for Monitoring GTPase Activation

A series of novel genetically encoded indicators which can be expressed in cells to monitor GTPase activity



Please note, header image is purely illustrative. Source: PDB ID 6IZW, doi:10.1093/bioinformatics/bty419, CC0

About **University of Liverpool**

By facilitating access to our expertise, facilities and networks, the University of Liverpool offers the means to transform ideas into creative solutions, improved performance, new technologies, strategies, applications, products or skills.

Background

Small GTPases are part of the systems that cells use to specify membrane function, and as such they are critical to very many cellular processes. This includes cell migration and invasiveness (two key facets of metastatic cancers), immune cell activation, cellular signalling cascades (such as those caused by the activation of growth factor receptors) and membrane sorting and remodelling (processes which are defective in a large series of congenital and idiopathic human disorders).

Tech Overview

A team at the University of Liverpool has developed a series of novel genetically encoded indicators which can be expressed in cells. These constructs monitor the activity of small GTPases by inducing an increase in fluorescent signal upon small GTPase activation. Unlike other systems which monitor the activity of small GTPases, this system requires no specialised microscopy (as would be required by currently available probes of small GTPase activity which rely on measuring Fluorescence Resonance Energy Transfer (FRET)). The probes can also be co-expressed with other fluorescent markers (of GTPase activity or otherwise).

The team at Liverpool has also developed a series of ratiometric probes (allowing the distinction between membrane-located and activated GTPases), probes which are selective for both activation and posttranslational modification, probes which de-excite themselves so that the localization of the initial activating signal is shown and probes which are tagged to specific organelles, to determine organelle-specific activation.

In transfected cells/cell lines, using antibodies to different surfaces of the fluorophore, the team can isolate those molecules where the fluorophore is reconstituted (ie where the probe is activated), and by the means of immunoprecipitations, isolate the fraction of active vs inactive probe to assay signalling pathway activation. The activation-sensitive anti-fluorophore antibody can also be used to selectively immunoprecipitate organelles/complexes containing active probe, or label these domains with nanogold particles in electron microscopy.

Benefits

The team can express fusion proteins of these probes and use the bacterially-purified protein as a single-component assay of GTP/GDP binding in plate assays.

This system can also be produced in a photoactivatable form, which allows GTPase activity to be monitored by F-PALM superresolution microscopy, allowing extremely high resolution images of GTPase activation in cells.

Applications

This technology can be used to develop a multitude of cell lines in which the GTPase activity can be scrutinised in cell signalling pathways at rest, following activation or in response to treatment/modulation of cells. This technology would be useful for basic research and in drug discovery.