Watching proteins move and interact within living cells

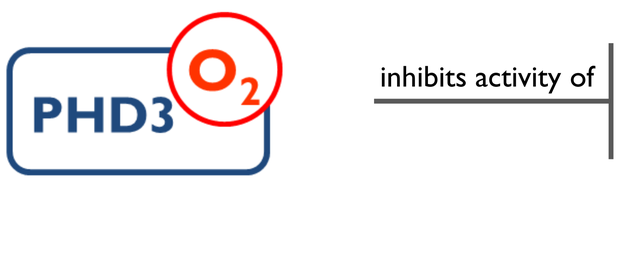
Amelie Schober*, Marco Marcello, Edurne Berra, Violaine Sée
* schobera@liv.ac.uk
Center for Cell Imaging, Institute of Integrative Biology, University of Liverpool in cooperation with CIC bioGUNE, Bilbao

Abstract

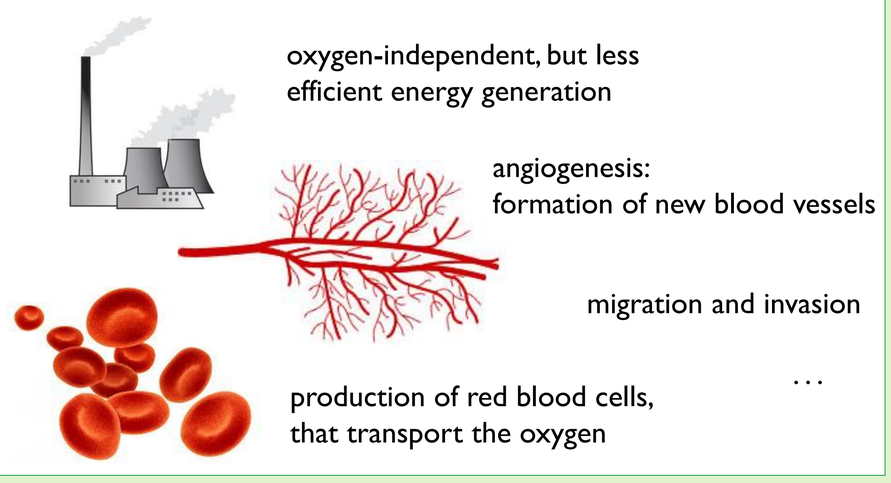
Signalling pathways in the cell rely on the interaction of two or more proteins with each other. Deregulation of those interactions can have deleterious effects on cells and can be the cause of diseases such as cancer. Understanding which proteins interact, how they interact and what kind of modifications are implicated is therefore of great interest. Classical approaches to address these questions are based on bulk cell analysis and do not account for cell-to-cell variability and dynamic changes. Here we applied two modern single live cell imaging techniques in order to understand how the SUMOylation of a protein involved in oxygen sensing affects its interaction with HIF-1α, an important regulator of gene expression that has also been shown to be over-activated in many tumour tissues.

The proteins of the focus of interest

PHD3
(prolyl-hydroxylase domain containing protein 3):
Oxygen sensor of the cell: in the presence of sufficient oxygen PHD3 targets HIF-1α for degradation



HIF-1α (hypoxia inducible factor 1α):
In the absence of sufficient oxygen HIF-1α switches on a variety of processes that allow the cell and organism to survive



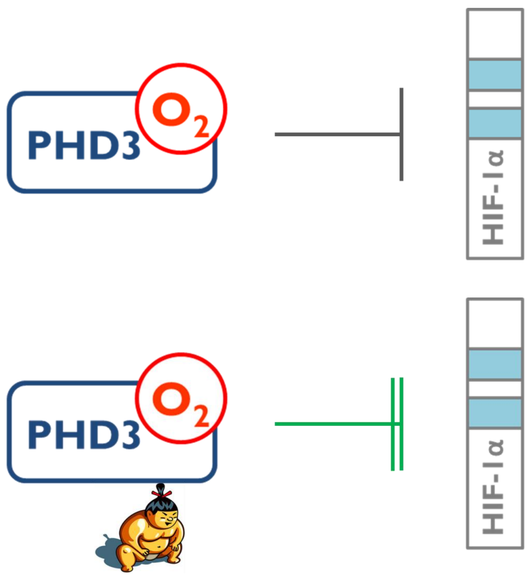
SUMO

small **u**biquitin like **mo**difier

SUMO is a small protein that can be attached to an other protein thereby affecting its localisation, stability, activity or interaction with other proteins

SUMO's effect on PHD3

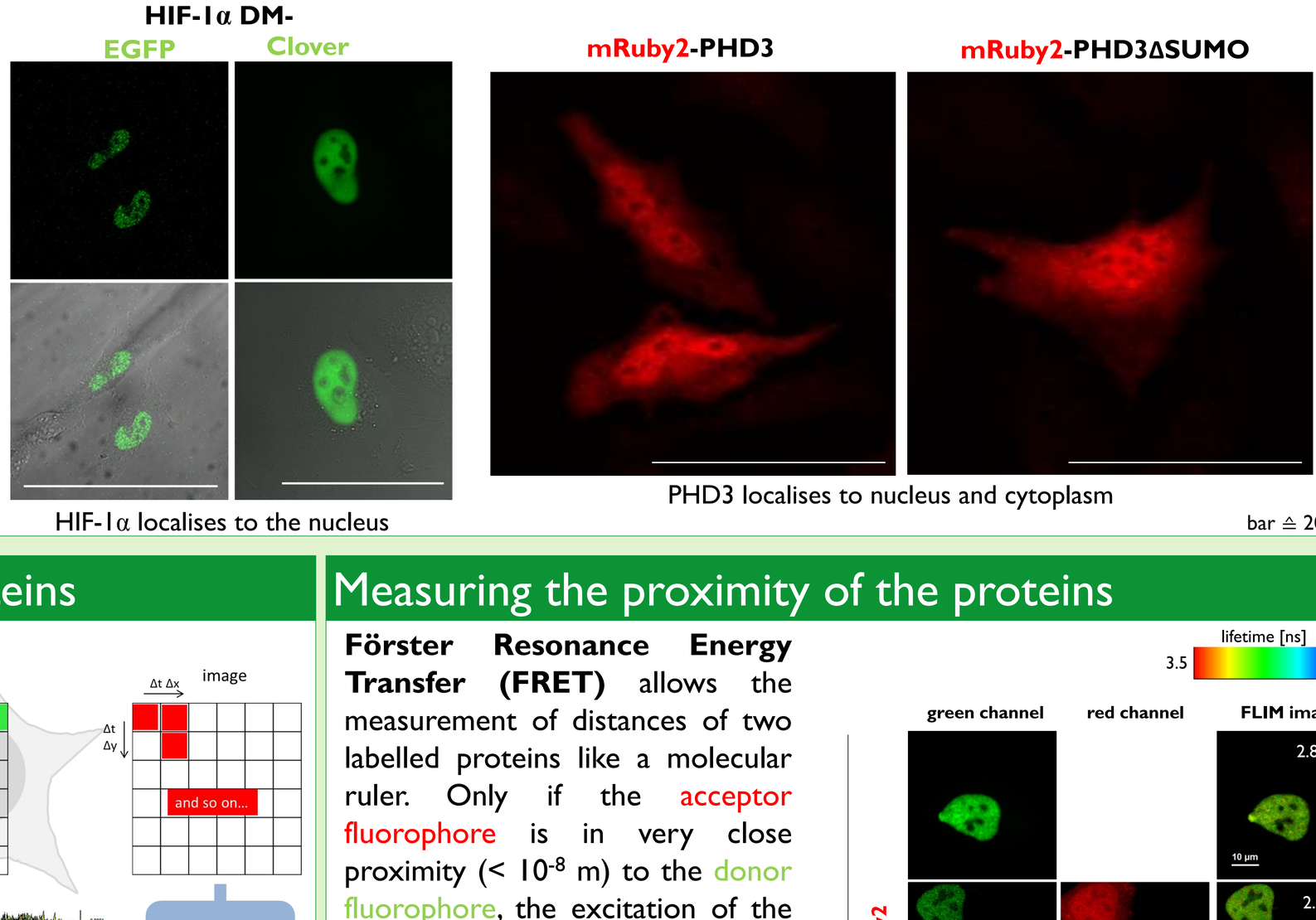
SUMOylation of PHD3 leads to a greater inhibition of HIF-1α's activity → HOW?



CIC bioGUNE Bilbao Núñez O'Mara et al., submitted

Making the proteins visible

The attachment of a fluorescent protein to the proteins of interest allows the visualisation of the proteins within a living cell using a fluorescence microscope.



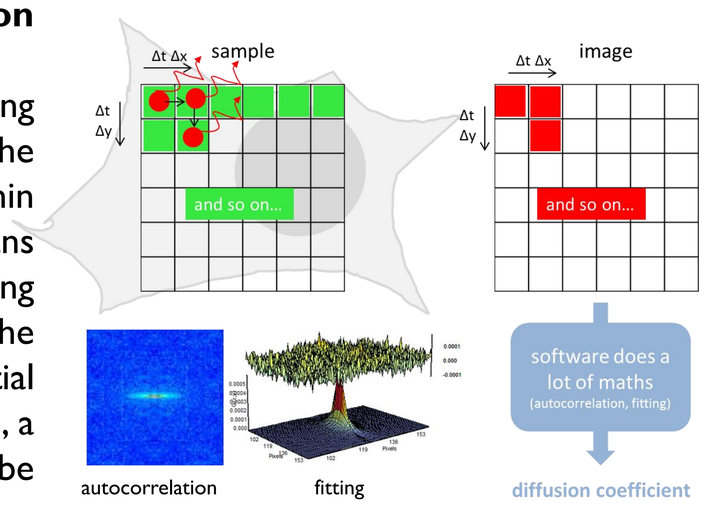
HIF-1α localises to the nucleus

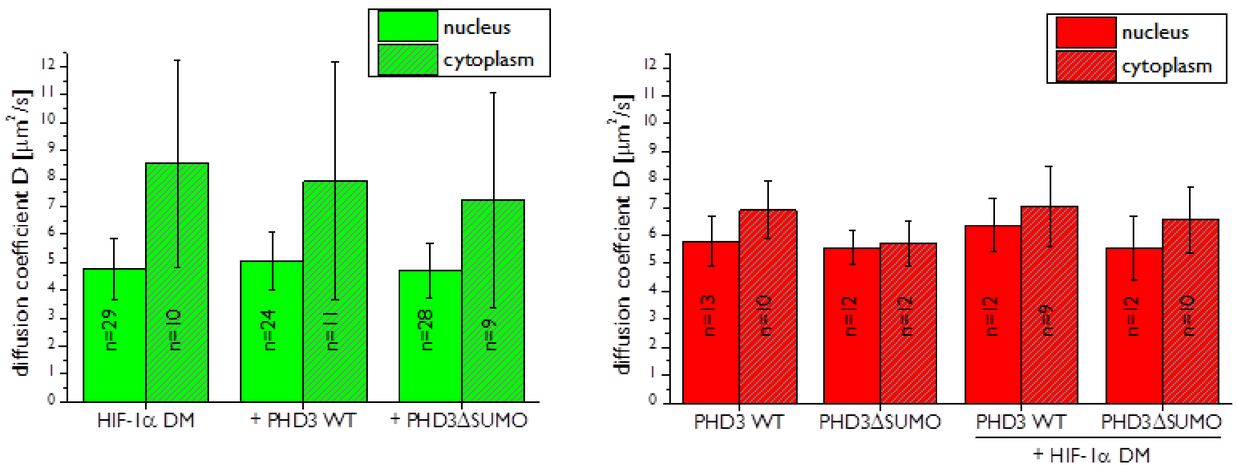
PHD3 localises to nucleus and cytoplasm

bar ≙ 20 nm

Measuring the movement of the proteins

Raster Image Correlation Spectroscopy (RICS)
With a fluorescence laser scanning microscope we can measure the velocity of a labelled protein within a living cell. While the laser scans the sample pixel by pixel, a moving fluorophore creates a trace on the image. With the temporal and spatial information inherent to the image, a diffusion coefficient can be calculated.

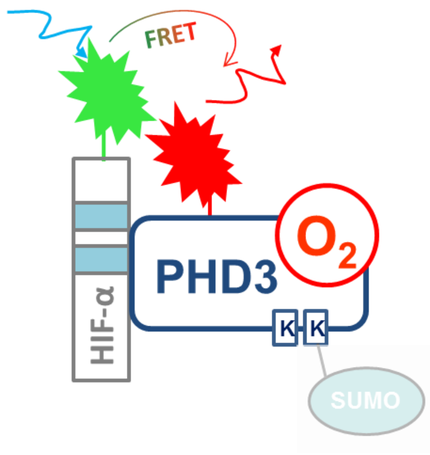


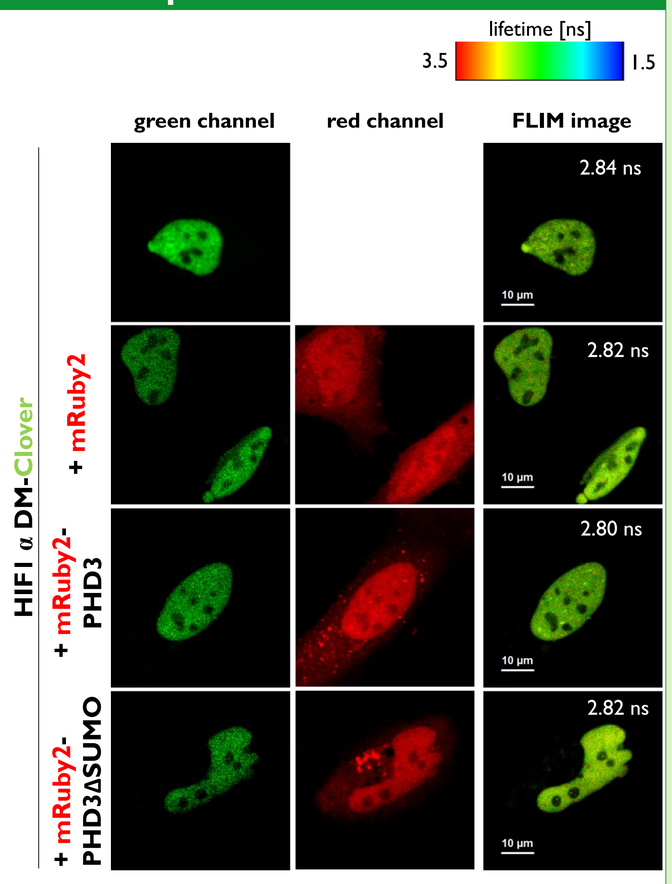


Neither PHD3 nor PHD3ΔSUMO change HIF-1α DM's diffusion coefficient and vice versa, suggesting that they might be part of the same complex regardless of PHD3's SUMOylation state.

Measuring the proximity of the proteins

Förster Resonance Energy Transfer (FRET) allows the measurement of distances of two labelled proteins like a molecular ruler. Only if the acceptor fluorophore is in very close proximity (< 10^{-8} m) to the donor fluorophore, the excitation of the donor is transferred onto the acceptor. This results in a decrease of the fluorescence lifetime of the donor, that we can measure using a time-correlated single photon counting system.





We could not measure a clear decrease of HIF-1α DM-Clover's lifetime in the presence of neither mRuby2-PHD3 nor -PHD3ΔSUMO. As it is known that PHD3 does bind to HIF-1a DM, the system established so far requires optimisation.