Periodic Report (630731 PerBioImage)

Results & discussion: Bioorthogonal chemistry is now permitting to site-selectively install modifications on individual proteins in complex biological mixtures, including in living organisms (Nature Chem 2016, 103). In this project, I have explored a conceptually novel bioorthogonal approach that combines the use of small, stable handles that may be labelled upon chemical activation in a temporal controlled manner. The initial approach was focused on a new photo triggered [2+2] cycloaddition bioorthogonal ligation between two alkene containing partners as well as the known inverse electron-demand Diels-Alder cycloaddition (IEDDA) reaction between the minimal alkene-tag and a tetrazine. First, we developed a method for the site-specifically installation of the unstrained *S*-allyl cysteine (Sac) amino acid into proteins (Angew Chem Int Ed 2016,14683). We found that the photo [2+2] cycloaddition reaction between Sac and an alkene moiety (maleimide) allows controlled labelling of Sac-tagged proteins in the test tube, but the use of such approach in live cells failed because of selectivity issues (unpublished). On the contrary, we have demonstrated that unstrained S-allyl handles precisely installed at predefined cysteine residues within the sequence of a protein are suitable chemical handles for IEDDA reactions with tetrazine dyes. This strategy allowed for selective labelling of proteins in live cells using a pre-targeting approach (Angew Chem Int Ed 2016,14683). The easy site-specific installation and the small size of the allyl handle, which is potentially less disruptive compared to non-canonical amino acids bearing bulky strained alkenes, is likely to be of a general benefit for other sensitive protein systems including antibodies used in pre-targeting approaches. As such, we believe that the simple site-specific labelling strategy disclosed here, which enables bioorthogonal live-cell imaging, will find significant use in the biological community, allowing imaging of specific targets with minimal effects on their intrinsic properties.

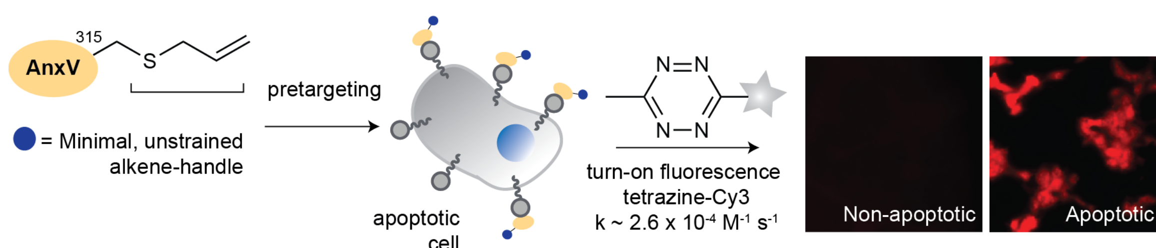

Figure 2. Pre-targeting of apoptotic cells with Anexin V-Sac followed by IEDDA labelling with fluorogenic Tz-Cy3. Specific labelling of apoptotic cells pre-targeted with Anexin V-Sac and subsequently labelled with Tz-Cy3. Cells were imaged 3 h after addition of the fluorogenic dye. Non-apoptotic cells were included as a control. Apoptotic cells are shown red. (Angew Chem Int Ed 2016,14683)

Future application: Current bioorthogonal reactions usually require the installation of bulky non-natural side-chains (norbornene or *trans*-cyclooctyne) and suffer from lack of selectivity (reaction

of biological nucleophiles) and metabolic stability. We have developed an approach that uses small handles that do not disturb a protein's structure and activity and that may be activated in a temporal manner to undergo a rapid, fluorogenic reaction in living systems. We are interested in probing and characterizing the uptake and intracellular processing pathways of key proteins involved in disease progression, namely interleukins in solid cancers and leukaemias, in order to guide the development of targeted strategies for intracellular drug-delivery. This reaction and approach can be generalized to follow protein/receptor complex internalisation and degradation pathways.