

## Fluorescent labelling of specific protein targets *in vivo* and *in vitro*

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**Keywords:** protein labelling, protein detection, fluorescent labelling reagents, protein expression monitoring, real-time protein analysis, protein localization, protein-protein interaction

### Background

Several protein labelling techniques have been developed that involve the use of fluorescent dyes bearing reactive functional groups, such as succinimidyl esters or maleimides, known to react with amines or thiols. However, these techniques are typically non-specific since many such functional groups exposed on the surface of any protein may be labelled.

The genetic fusion of target proteins to fluorescent proteins, such as the green fluorescent protein (GFP), is the most specific and broadly applied approach for following individual protein dynamics in living cells and even for determining cellular localization of proteins on a whole genome scale. However, these large fluorescent proteins may alter turnover or localization of the target proteins to which they are fused. An alternative approach entails fusing tetracysteine-containing short peptides to target proteins and reacting these with organoarsenic-based fluorogens. But these compounds can be toxic and only allow labelling of one protein at a time. Our technology effectively overcomes the limitations of these methods.

### Technology

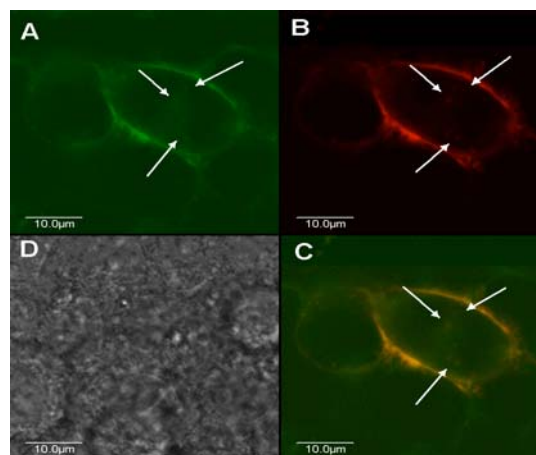
The technology consists of novel compounds containing two maleimide groups attached via a tether to a fluorescent core, whose latent fluorescence is quenched until their maleimide groups undergo a specific thiol addition reaction. Complementary proteins are designed bearing two cysteine residues appropriately positioned to react with our novel fluorogens.

Maleimide groups are known to react selectively with thiols *via* addition reactions involving their C=C double bond. They are also known to quench fluorescence in their conjugated form, but not as their

thiol adduct products. Thus, the fluorogens were prepared bearing two maleimide groups, so their latent fluorescence would only be realized upon their reaction with two thiol equivalents. Furthermore, the positioning of maleimide groups is such that they are separated by a precise distance. The resulting fluorogen reacts rapidly and specifically with compounds presenting two sulfhydryl groups separated by the appropriate distance.

### Results

Recombinant proteins containing tag peptides of 20 amino acids, which include the two target cysteine residues, have been efficiently and selectively labelled *in vitro* and *in vivo* in living cells. Kinetic studies show that our fluorogens react  $10^4$  times faster with our target sequence than with glutathione, eliminating concerns of background reactivity.



Labelling and hormone-induced endocytosis of ErbB1 receptors. **A:** Confocal images of HEK 293 cells expressing ErbB1 receptors tagged with dC10 sequence and labelled with dM10-fluorescein expressed on 293 cells. Arrows indicate endosome particles. **B:** same cell as A, with ErbB1 receptors bound to rhodamine-labelled EGF. **C:** Superposition of A and B showing overlap of rhodaminefluorescein signals. **D:** Phase-contrast image of the same field.

## Applications

The technology allows for protein localization and protein-protein interaction studies in living cells. More specifically, this technique provides detection of the following:

- proteins in appropriate sub-cellular compartments or organelles (localisation, turnover, trafficking)
- protein-protein interactions *in vivo* and *in vitro* in any cell type
- proteins in physiological cellular conditions detectable in real-time
- proteins in-gel, eliminating protein staining and western blot procedure.

## Competitive Advantages

This technology possesses several advantages:

- High specificity and sensitivity: maleimide groups react selectively with thiols
- Safer – maleimide groups are less toxic than the organoarsenic-based fluorogens prevalently used
- No false positive results – any background reaction with proteins bearing only one cysteine residue would not lead to increased fluorescence since the monothiolated fluorogen does not fluoresce

- Small tag (~2 kD) – unlikely to disrupt protein function or protein-protein interaction
- Multiplex labelling – different fluorogen/target sequence pairs (based on varied distances between the maleimide groups and Cys residues) allows simultaneous labelling of different proteins with different colours
- Flexibility – Currently, a choice of 27 fluorogens are available from a same tagged protein. But, virtually any fluorophore could be attached to dimaleimide moieties of different dimensions.

## Patent Status

US Patent application filed (Q2/2005)

## Business Opportunity

Univalor is seeking an exclusive or a non-exclusive license agreement.

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