

Fluorescent biosensors for cell regulation pathways

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Short Abstract - We are developing a “toolkit” of fluorescent probes (biosensors) for quantifying the protein regulation pathways of living cells at high resolution. Biosensors for membrane protein trafficking, intracellular protease activity, protein modification and protein-protein interactions would be very useful for basic research and drug discovery. Our new biosensors are genetically encoded for expression in and on cells and have an advantage in versatility over fluorescent proteins like GFP. The new biosensor paradigm is based on combining an engineered protein with an engineered fluorescent dye that has a large sensitivity to its local molecular environment.

Live cell health and function is controlled by interactions between thousands of types of proteins that are parts of regulatory pathways. Biosensors for membrane protein trafficking, intracellular protease activity, protein modification and protein-protein interactions would be very useful for basic research and drug discovery. The fluorescent biosensors must be sensitive and non-perturbing and the fluorescence signals should be readily obtained with imaging microscopes, flow cytometers and plate readers.

We are developing a “toolkit” of fluorescent probes (biosensors) for quantifying the protein regulation pathways of living cells at high resolution. The new biosensors are genetically encoded for expression in and on cells and have an advantage in versatility over fluorescent proteins like GFP. The new biosensor paradigm is based on combining an engineered protein with an engineered fluorescent dye that has a large sensitivity to its local molecular environment [1]. The fluorophore provides a readout signal by intensity or wavelength change. The combined product can be targeted to particular regions of cells by labeling cellular proteins of interest.

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Several biological applications have benefited from the biosensor toolkit. For example, with our collaborators we have shown that the membrane surface population (exclusively) of various proteins can be genetically labeled and quantified in real time during the modulation of cell pathways that regulate membrane insertion and removal of the proteins. We are currently studying trafficking of the chloride transport system involved in cystic fibrosis, the ADβ2 receptor, and the glucose transport protein. The technology is different from existing genetic fluorescent

protein labeling in which every copy of the protein is labeled and visualized regardless of its location. Large internal stores of GFP labeled membrane protein produce large signals from cells that prevent quantification of the protein that is exclusively at a specific site, such as the surface or in a recently endocytosed vesicle. The capability of following the membrane appearance, location, endocytosis of several proteins simultaneously with multicolor photostable biosensors will prove valuable for dissecting the timing and regulation of pathways that control membrane protein localization.

As another example, we have developed a quasi-generic biosensor for quantifying protease activity inside and outside of living cells. By placing a specific protease sequence in a key region of the sensor its specificity for a particular protease can be obtained. We have demonstrated, for example, genetic labeling of cells with a caspase 3 biosensor that becomes fluorescent only when cytoplasmic protease becomes active. This technology is being extended to matrix metalloproteases where the sensor can be placed on the external surface of cells to study inflammatory action of nearby cells and cancer related protease activity.

References

[1] Szent-Gyorgyi C, et al (2008) Fluorogen activating proteins: Technology for imaging and assaying live cells. *Nature Biotechnology*, **26**, 235-240.