A Novel Split Reporter Uncages New Possibilities

Emma Sierecki

University of New South Wales, Sydney, New South Wales 2052, Australia

A new strategy for proximity-dependent unmasking of small molecules using an engineered enzyme biosensor system.

Split reporters have emerged as valuable tools for the study and manipulation of biological systems. The development of split reporters takes advantage of the properties of some proteins to become fully functional following the recombination of two or more protein fragments that have no activity on their own. In this issue of ACS Central Science, Jones et al.1 add a versatile new tool to this expanding toolbox by developing a new split esterase reporter.

The most common use of split reporters is for the detection and study of protein–protein interactions (PPIs). Protein-fragment complementation assays (PCAs) use the split reporters to inform on the interaction/proximity between two proteins. In these assays, proteins of interest are covalently linked to fragments of a split reporter. PPI induces recombination of the reporter and generation of a measurable signal (Figure 1A).

The last 20 years have seen the development of multiple split proteins with different outputs. Genetic changes, cell survival, enzymatic activities, and more can be used to detect and screen for PPI. Yeast two-hybrid screening, the most widely used PPI screening method, uses a split-transcription factor as a reporter and detects the transcription of a specific gene. Bimolecular fluorescence or luminescence complementation assays (BiFCs and BiLCs, respectively) rely on the recombination of a fluorescent protein2 or luciferase and its derivatives3 upon interaction between two proteins of interest. A variety of enzymes have been used as scaffolds for split reporters, providing a variety of read-outs, ranging from colorimetric tests as in the case of the split-β-lactamase to cell viability (split-β-galactosidase). Some split reporters are even compatible with molecular imaging techniques such as positron emission tomography,4 allowing detection of molecular events at the scale of the whole organism.

The newly developed split esterase reporter has the ability to provide multiple read-outs on its own.1 Indeed, upon recombination, the active esterase can unmask a variety of probes, with different properties. In this publication, Jones et al. exemplify this versatility by using fluorescence, luminescence, and cell death as read-outs of PPI. This variation of the output signal without modification of the reporter offers the possibility to study a given PPI at multiple scales, optimized for different systems. Fluorescence, for example, is one of the most used read-outs in cellular biology as it gives information on the localization of the protein within the cell. Multiple groups have fine-tuned BiFC to provide subcellular localization,2 including organelle-specific targeting of proteins, or even allow subdiffraction localization to be obtained. However, the use of green fluorescent protein and its derivatives is poorly amenable to in vivo imaging where autofluorescence and light penetration are limiting factors. Efforts have been deployed toward the generation of far-red split-fluorescence reporters for better tissue penetration. Luminescence-based split reporters, on the other hand, do not provide precise cellular localization but can be used in vivo. Both BiFC and BiLC report on interactions within a cell or between neighboring cells but do not provide information on signal propagation from cell to cell. By varying the substrate, the split esterase system could be used to understand the mechanisms and effects of a specific PPI from the sub-micro- to the macroscale.

Significant advance in our understanding of the role of biochemical networks has come from the multiplexing of PCAs. Competition between protein partners can be visualized using multicolor split reporters. These competition assays take advantage of the fact that a reporter scaffold can recombine with different fragments of proteins to produce signals (Figure 1B). This is the case for the cyan fluorescent protein that can be reconstituted by its own fragment and...
emit light at 480 nm, or it can bind to a fragment of the yellow fluorescent protein to produce a fluorophore that emits at 530 nm. Conversely, the fragment of the click beetle luciferase (green) can recombine either with its own scaffold and emit green light (540 nm) or with the scaffold of the click beetle luciferase (red) and emit luminescence in the red region (615 nm). Competition between proteins for binding to a common interactor can be measured by simply comparing fluorescence/luminescence in two detection channels. Multicolor reporters also offer the possibility to multiplex the analysis and detect multiple PPIs simultaneously. PCAs have also been extended to measure tripartite interactions either indirectly (PPI is reconstituted if the three components are interacting) or directly (the reporter is composed of three fragments) (Figure 1C). Finally, different split reporters generating different signals can be used in conjugation, where the signal from one reporter is used to activate the second split system (Figure 1D).

The versatility of the split esterase opens the door to combinatorial assays and offers perspectives in synthetic biology.

Split reporters have also found use beyond the detection of PPI. Indeed, the recent development of a new split reporter allows the performance of biotin identification of interactors of specific heterocomplexes, in cells. It could also be used to identify the interacting partners of a given protein in a specific subcellular compartment. Another approach, termed bimolecular complementation affinity purification, uses the recombination of the split-Venus reporter to identify specific interactors of a protein pair, using affinity-dependent pull-down followed by liquid chromatography−mass spectrometry. Incorporation of split reporters in synthetic biology offers exciting possibilities to engineer mammalian cells into biosensors. Split reporters can sense different cues and act as logic gates in a synthetic signaling circuitry. Prior to the development of the split esterase reporter offers great opportunity to combine this reporter with other PCAs, as the recombined esterase could unmask a substrate for a downstream reporter.
esterase, Dickinson and co-workers created a split polymerase and showed that it could be used as a small-molecule sensor in mammalian cells.\textsuperscript{10} The new split esterase can also easily be integrated into a genetic circuit. The proposed coupling with chimeric antigen receptor T-cell therapy is a fascinating perspective as it would add an activatable, \textit{in situ} drug delivery dimension to this very promising therapeutic development.

\section*{Author Information}
E-mail: e.sierecki@unsw.edu.au.

\section*{REFERENCES}
\begin{enumerate}
\item Jones, K. A.; Kentala, K.; Beck, M. W.; An, W.; Lippert, A. R.; Lewis, J. C. Development of a Split Esterase for Protein–Protein Interaction-Dependent Small-Molecule Activation. \textit{ACS Cent. Sci.} 2019, in press. DOI: 10.1021/acscentsci.9b00567
\item Pedelacq, J.-D.; Cabantous, S. Development and Applications of Superfolder and Split Fluorescent Protein. \textit{Int. J. Mol. Sci.} 2019, 20 (14), 3479.
\item Dixon, A. S.; Schwinn, M. K.; Hall, M. P.; Zimmerman, K.; Otto, P.; Lubben, T. H.; et al. NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions in Cells. \textit{ACS Chem. Biol.} 2016, 11 (2), 400–8.
\item Massoud, T. F.; Paulmurugan, R.; Gambhir, S. S. A molecularly engineered split reporter for imaging protein-protein interactions with positron emission tomography. \textit{Nat. Med.} 2010, 16 (8), 921–6.
\item Villalobos, V.; Naik, S.; Bruinsma, M.; Dothager, R. S.; Pan, M.-H.; Samrakandi, M.; et al. Dual-Color Click Beetle Luciferase Heteroprotein Fragment Complementation Assays. \textit{Chem. Biol.} 2010, 17 (9), 1018–29.
\item Urizar, E.; Yano, H.; Kolster, R.; Galés, C.; Lambert, N.; Javitch, J. A. CODA-RET reveals functional selectivity as a result of GPCR heteromerization. \textit{Nat. Chem. Biol.} 2011, 7 (9), 624–30.
\item Schopp, I. M.; Amaya Ramirez, C. C.; Debeljak, J.; Krebich, E.; Skribbe, M.; Wild, K.; et al. Split-BioID a conditional proteomics approach to monitor the composition of spatiotemporally defined protein complexes. \textit{Nat. Commun.} 2017, 8, 15690.
\item Geering, B.; Zokouri, Z.; Hürlemann, S.; Gerrits, B.; Ausländer, D.; Britschgi, A.; et al. Identification of Novel Death-Associated Protein Kinase 2 Interaction Partners by Proteomic Screening Coupled with Bimolecular Fluorescence Complementation. \textit{Mol. Cell. Biol.} 2015, 36 (1), 132–43.
\item Adamala, K. P.; Martin-Alarcon, D. A.; Guthrie-Honea, K. R.; Boyden, E. S. Engineering genetic circuit interactions within and between synthetic minimal cells. \textit{Nat. Chem.} 2017, 9, 431.
\item Pu, J.; Kentala, K.; Dickinson, B. C. Multidimensional Control of Cas9 by Evolved RNA Polymerase-Based Biosensors. \textit{ACS Chem. Biol.} 2018, 13 (2), 431–7.
\end{enumerate}