BioSpotlight

Venus rising

Analysis of protein-protein interactions (PPIs) in living cells has greatly benefited from the development of the bimolecular fluorescence complementation (BiFC) assay, which relies on the restoration of fluorescence when two nonfluorescent N- and C-terminal halves of a fluorescent protein are brought together through the interaction of the proteins to which they are fused. A limitation of this assay, however, has been self-assembly between the two nonfluorescent fragments, causing false positive fluorescence and a low signal-to-noise (S/N) ratio. In this issue, Y. Kodama and C.-D. Hu at Purdue University (West Lafayette, IN) report the identification of a mutation in the Venus protein, commonly used for the BiFC assay, that greatly reduces self-assembly of the N- and C-terminal fragments of this protein. Using a pair of N- and C-terminal fragments of Venus split at amino acid residue 155 (VN155 and VC155, respectively) for BiFC in COS-1 cells, the authors found that the false-positive fluorescence of a negative PPI pair was 20–50% of the fluorescence of a positive PPI pair, and that the S/N ratio ranged from 3 to 7. Using the crystal structure of Venus as a guide, the authors searched for candidate

mutations that would reduce the association of the 7th β -strand in VN155 with the 8th and 10th β -strands in VC155 mediated by backbone hydrogen bonding and possible side-chain hydrophobic interactions. Two mutations, V150L and I152L, were chosen to reduce the hydrophobicity of the 7th β -strand without disrupting its structure. When introduced into the VN155 background and tested by BiFC with VC155, the false-positive fluorescence was greatly reduced in both mutants and the S/N ratio was improved four-fold, although the overall fluorescence intensity was reduced compared to using wild-type VN155. Even though the I152L mutation had a somewhat lower S/N ratio than V150L, its BiFC intensity was about six-fold stronger (at 30% of the wild-type). This bright fluorescence was readily detectable 24 h post-transfection, and the high S/N ratio was maintained 48 h after transfection. Thus, the I152L mutation significantly improves Venus-based BiFC and will enable better visualization of PPI in living cells.

(See “An improved bimolecular fluorescence complementation assay with a high signal-to-noise ratio” on page 793.)

4-color FRET

Single-molecule Förster resonance energy transfer (FRET) is now commonly used to detect molecular associations and conformational changes among proteins. Measuring fluorescence emission between labeled single molecules can lead to valuable insights regarding protein-protein and protein-nucleic acid interactions, and the simultaneous or sequential use of multiple dyes enables studies of even more complex systems. Although both the detection of three distinct single-molecule fluorescence signals for multiple FRET couplings and the discrimination of four dyes during DNA sequencing has been achieved, until now, simultaneous detection of single molecule fluorescence using four colors had not been described. In this issue of *BioTechniques*, V. DeRocco and colleagues describe how to modify a two-color total internal reflection microscope (TIRM) to detect four spectral channels at once during quantitative FRET studies. In combination with an improved tris-nitrotriacetic acid (tris-NTA) dye labeling strategy, this instrument facilitates studies of two FRET pairs on a single complex or monitoring of relationships between conformational changes and colocalization events among binding partners in differing parts of a complex. The authors demonstrated the system by examining DNA bending induced by

Four-color single molecule TIRF microscope schematic. Simultaneous FRET and colocalization experiments were performed using the prism-type total internal reflection configuration.

yMutSa (a protein responsible for recognizing and initiating repair of base pair errors in newly synthesized DNA) and Munc-18 interacting with syntaxin and the SNARE complex using covalently and noncovalently bound fluorescent dyes. Under continuous illumination with two lasers, the authors were able to observe the two independent FRET pairs simultaneously. During these

studies, the authors were also able to demonstrate that tris-NTA dye conjugates could label His tags of recombinant proteins for single molecule studies, a finding that will significantly expand the options available to researchers when labeling proteins for FRET studies. The noncovalent attachment of the dye to the affinity tag may minimize structural or functional perturbations caused by attachment of other dyes or dyes that require point mutations for coupling to the protein of interest. Maybe best of all is the fact that the modifications to the TIRM are inexpensive and simple, resulting in a readily accessible instrument that extends the possibilities for FRET studies when examining complex molecular interactions.

(See “Four-color single-molecule fluorescence with noncovalent dye labeling to monitor dynamic multimolecular complexes” on page 807.)

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