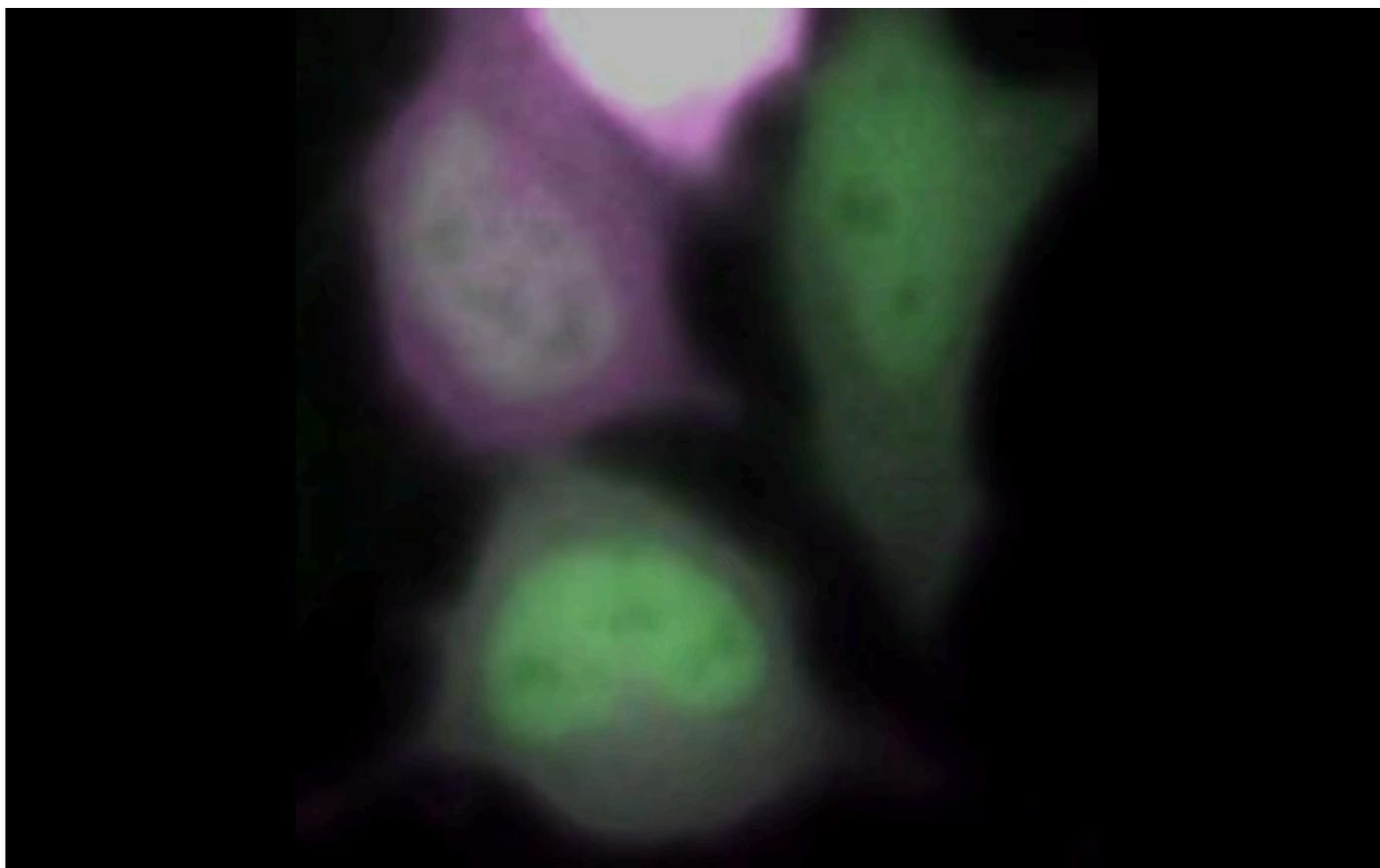


TOOLBOX

Glowing sensors shine new light on protein interactions

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Researchers may soon be able to easily visualize protein pairings in living cells through vibrant flashes of color. So-called ‘fluorescent protein exchange,’ a technique detailed 26 January in *Nature Methods*, may illuminate how autism-related proteins connect¹.

Maps of protein interactions in autism have shown that many proteins mutated in the disorder

interact with one another, helping to explain how mutations in different genes can lead to similar symptoms.

Among the various methods for investigating protein interactions, the new method is most akin to 'fluorescence resonance energy transfer,' or FRET, which is also used to glimpse protein connections in living cells. This method involves labeling proteins with fluorescent molecules that transfer energy to each other when they are close together, altering the intensity of their fluorescence.

But FRET can be finicky because the molecules transfer energy efficiently only when they are oriented just so. And due to the relatively small amounts of energy transferred, the resulting visual changes can be subtle.

The new system more reliably marks protein partnerships and leads to more dramatic shifts in hue. In this technique, two different protein tags compete for binding to a third protein, dubbed 'B,' and the resulting duet shines fluorescent red or green, depending on which tag wins.

When researchers engineer cells to express the red tag, the green tag and B, the three fuse in various combinations to specific cellular proteins. As a result, the relative proximity of each tag to B changes, leading to shifts in the ratio of red to green fluorescence. These changes in fluorescence tell researchers that certain protein pairs are coming together or separating.

As a proof of concept, the researchers trained the technique on a classic protein tango: that of the calcium-binding protein calmodulin and a binding domain called M13 that is important for sparking muscle contractions. These proteins pair up only in the presence of calcium.

The researchers fused a red tag to calmodulin and B to M13 while the green tag floated freely in the cell. When calmodulin and M13 bound, the coupling brought the red tag and B together, leading to a spike in red fluorescence.

A video accompanying the paper shows cells starting out green and then flashing red when calcium levels rise, and changing back to green as the cells pump out the calcium.

Researchers hoping to decipher key protein pairings in autism can design their own sensors by fusing the tags and B to the relevant protein suspects.

REFERENCES:

1. Ding Y. *et al. Nat. Methods* **12**, 195-198 (2015) [PubMed](#)