

Electron transfer challenges common fluorescence technique

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Tryptophan is an amino acid, one of the building blocks of proteins. It is used extensively to study how proteins change their 3D structure, and also how they interact with other proteins and molecules. This is studied with a fluorescence technique called FRET, which measures the transfer of energy from tryptophan to another molecule. But in some cases, FRET data could be distorted because tryptophan transfers an electron instead of energy. Using a unique spectroscopic technique, scientists at EPFL have now confirmed for the first time that this is indeed the case. The study, which has far-reaching implications for the effectiveness of FRET, is published in *PNAS*.

FRET is often used with the amino acid tryptophan, which has the added advantage of being intrinsically fluorescent. For FRET, the [protein](#) to be studied is modified to contain an acceptor molecule at the right position. When the protein changes its 3D structure, tryptophan interacts with the acceptor molecule, and transfers energy to it. The result is a decrease in tryptophan's fluorescence emission, which can be linked proportionally to tryptophan's distance from the acceptor molecule. Since the protein's 3D structure is already known, FRET can tell us a lot about how it has changed.

But there are problems. The lab of Majed Chergui at EPFL has now shown conclusively that FRET readouts can be skewed by the transfer of electrons from tryptophans within proteins. The work builds on a previous paper published in *Science* (2013) on ferric myoglobins, the protein in our blood that carries oxygen within muscles. In that study, Chergui's team showed that tryptophan in myoglobin naturally transferred electrons to the protein's oxygen-binding molecule, which is called "heme". Because [electron transfer](#) also causes a decay in tryptophan's fluorescence, it can be mistaken as a positive signal, raising the question as to whether this was also the case in other proteins.

In the study discussed here, Chergui's lab looked at a more biologically relevant class of heme proteins, the ferrous myoglobins, which carry oxygen to muscles. The scientists studied them in their physiological state to see if tryptophan transferred electron to the myoglobin's oxygen-binding heme molecule. However, they had to work with the extremely short timeframes in which electron transfer takes place. To overcome this obstacle, the scientists developed a new, and, so far, unique spectroscopic tool, called "ultrafast UV two-dimensional spectroscopy".

The study confirmed that electron transfer indeed takes place between tryptophan and the heme molecule, producing an ionic form of the latter that matched theoretical predictions. In addition, their study demonstrated that electron transfer from tryptophan actually occurs more frequently than previously thought. Consequently, the systematic use of tryptophan fluorescence in FRET studies is called into question. "This discovery really creates problems for studies based on FRET analysis," says Majed Chergui. "If tryptophan transfers electrons in the tested protein, this could give out false readings that can be mistaken for conformational changes of proteins."

More information: Monni R, Al Haddad A, van Mourik F, Auböck G, Chergui M. Tryptophan-to-haem electron transfer in ferrous myoglobins. *PNAS* 20 April 2015. [DOI: 10.1073/pnas.1423186112](https://doi.org/10.1073/pnas.1423186112)

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