Biological imaging enabled by organic fluorophore's supramolecular interactions

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Most organic dyes are only luminescent in dilute solutions and see their emission intensity dramatically quenched when their molecules interact with each other. But certain fluorophores present a different behavior: they are almost non-emissive in solution, and their emission intensity increases upon crystallization or when they form supramolecular complexes. This behavior results from the stiffening of the fluorophore backbone upon aggregation or complexation, which in turn blocks internal conversions enabling the dye's emission.

In the course of our investigation [1], a family of emissive dyes has been developed, based on a hydrogen-bonded azine backbone [2]. Excited with UV light, such dyes are non-emissive in dilute solution but very bright in the crystalline form. Moreover, varying the substituents on the aromatic rings changes the emission color from blue to orange (Figure 1).

Protein misfolding and aggregation occur under several stress conditions, and can be associated with aging and age-related diseases, such as Alzheimer's, Huntington, Parkinson's or prion disease. Fluorescence-based techniques are useful to study such protein aggregates, but developing more selective and brighter fluorescent probes for protein aggregation is still a challenge.

The introduction of cationic groups on the fluorophore's backbone turns them soluble in water, allowing their application in biological imaging [2]. One fluorophore is non-toxic to cells, and has been applied to the staining of protein aggregates. The fluorophore enters the cells upon incubation, and accumulates selectively inside the protein aggregates present, probably in between the beta-sheets, forming a supramolecular complex. In these conditions, the molecular motions of the probe are restricted, and the fluorophore changes from non-emissive in solution to highly luminescent in the aggregate, selectively lighting-up the protein aggregates.

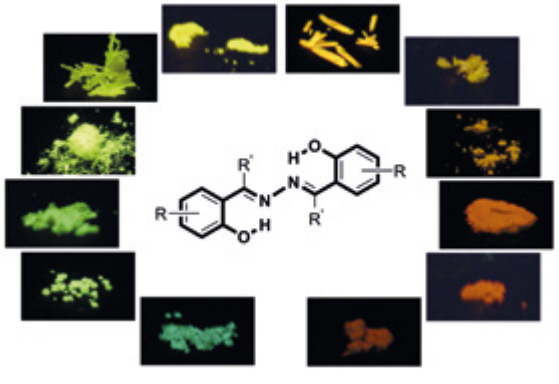

FIGURE 1
General structure of the family of fluorescent dyes, and photographs of their crystals or amorphous powders under UV light.

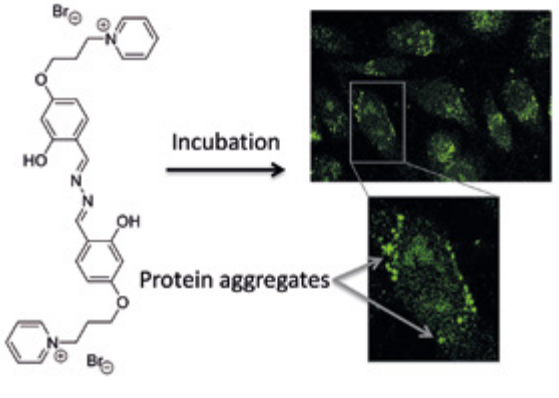

FIGURE 2
Structure of the fluorescent molecular probe used to detect protein aggregates, and fluorescence microscopy images of cells after incubation with the probe. The green dots are the protein aggregates.

[1] *"pAGE – Protein aggregation across the lifespan"*, Reference CENTRO-01-0145-FEDER-000003.
[2] *Tunable Color of Aggregation Induced Emission Enhancement in a Family of Hydrogen-Bonded Azines and Schiff Bases*, S. Guieu, F. Cardona, J. Rocha, A. M. S. Silva, Chem. Eur. J. 2018, 24, 17262-17267.
[3] *Fluorescent light-up probe for the detection of protein aggregates*, R. Nunes da Silva, C. C. Costa, M. J. G. Santos, M. Q. Alves, S. S. Braga, S. I. Vieira, J. Rocha, A. M. S. Silva, S. Guieu, Chem. Asian J. 2019, 14, 859-863.