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(Article begins on next page)

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A TURN-ON FLUORESCENT PROBE FOR MUCIN GLYCOPROTEINS DETECTION

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Introduction

Mucins are a family of long polymeric glycoconjugates having high molecular weight, produced by the gastrointestinal, respiratory, reproductive, pancreatic, hepatic and renal epithelium (Figure 1). Alterations or overexpression of mucins are associated with diseases like chronic obstructive pulmonary disease (COPD), asthma, cystic fibrosis and several types of cancer [1].
Particularly, in the last years, great attention was addressed to expression of mucins in various cancers such as pancreatic adenocarcinomas, colon and rectal cancer, breast cancer, ovarian cancer and gastric carcinoma.
The **early diagnosis** is a key factor for outcome, treatments, and healthcare. Thus, the identification and detection of specific and sensitive biomarkers has become extremely important in the last decades [2].

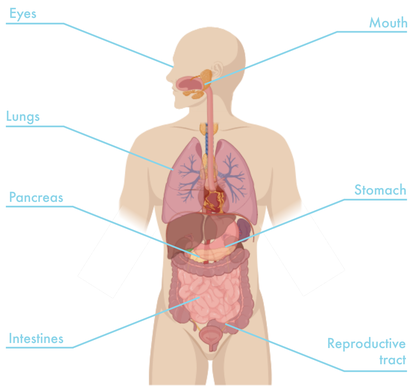
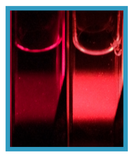


Figure 1. Mucins glycoproteins can be found in several tissues and organs in the human body.

Aims

Up until now, **fluorometric assays** have received remarkable attention due to their convenience, unparalleled sensitivity, simplicity, rapid implementation, noninvasive monitoring capability and usability in biological samples



Squaraine dyes exhibit a **fluorescence turn-on** when bound to proteins (Figure 2) and potentially could be employed as fluorescent markers for biological applications [3].

Herein we investigate from a spectroscopic point of view the interaction between porcine gastric mucin (PGM) and a series of squaraine dyes with different substitutions (Figure 3).

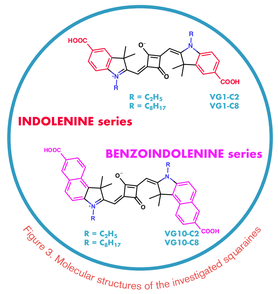


Figure 3. Molecular structures of the investigated squaraines

Experimental Part

- **Spectroscopic characterization**: UV/Vis absorption and steady-state fluorescence spectra of a constant concentration of the squaraine dye were recorded upon increasing the concentration of the PGM.
- **Time-domain lifetime** measurements of the PGM-Squaraine adducts.
- **Kinetic measurements** of the formation of Protein-Squaraine complexes.
- **Quantum yield** measurements.
- **Transmission electron microscopy** (TEM) characterization of the adducts.

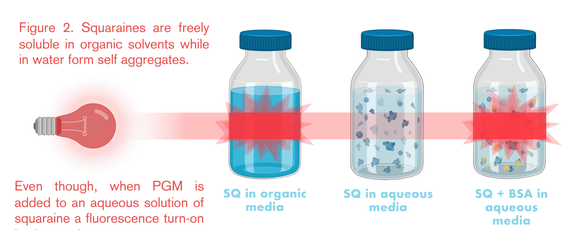


Figure 2. Squaraines are freely soluble in organic solvents while in water form self aggregates.
Even though, when PGM is added to an aqueous solution of squaraine a fluorescence turn-on is observed.

Results and Discussion

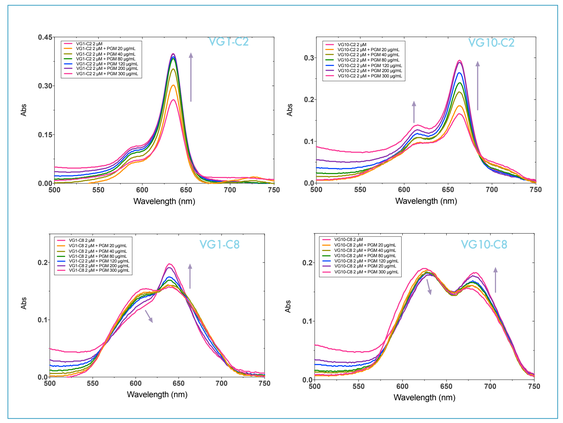


Figure 4. UV/Vis absorption spectra of the four squaraines upon increasing concentrations of PGM.

UV/Vis absorption spectroscopy

Addition of increasing concentrations of PGM to a constant concentration of squaraine results in a **disaggregation effect** with a greater amount of solubilized squaraine (Figure 4).

Steady-state fluorescence spectroscopy

Squaraine molecules are almost non emissive when they are suspended in water, however a gradual addition of protein (such as BSA or PGM) gave an **enhancement** in fluorescence intensity (turn-on), (Figure 5).

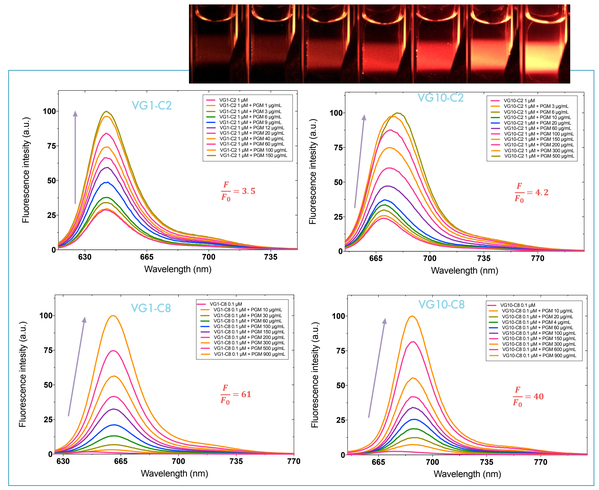


Figure 5. Steady-state fluorescence spectra of VG1-C8 upon increasing concentrations of PGM.

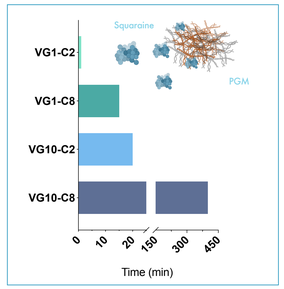


Figure 6. Kinetic behavior of the squaraine-PGM complexes formation.

Kinetics of interaction

The bulkier the dye's molecular structure the slower the interaction (Figure 6).

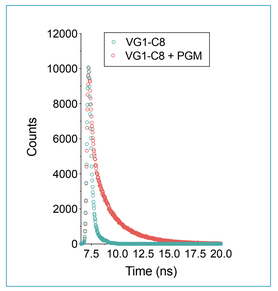


Figure 7. Time-domain lifetime of VG1-C8 alone and in presence of PGM.

Lifetime measurements

An increase in fluorescence lifetime was observed in presence of PGM (Figure 7).

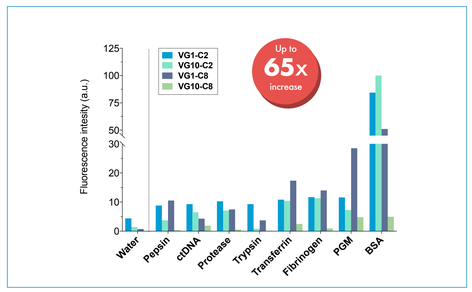


Figure 8. Maximum of the fluorescence intensity of the four squaraines alone and in presence of different proteins.

Fluorescence "turn-on"

The addition of the proteins to a water solution of squaraine generally yielded a significant increase of the fluorescence intensity (Figure 8).

Take home message

Squaraine dyes have a **structure-relationship influence** on the kinetic interaction with PGM.

Squaraine showed a significant **increase of fluorescence intensity** when PGM was added probably due to the interactions established with the hydrophobic domains of the protein.

Protein-dyes adducts could be employed as potential probes or photosensitizers for different applications (bioimaging, photodynamic therapy, etc).

Have a look also at our last works about mucin

We developed an airway CF mucus model based on mucin in an alginate network. The hydrogel is proposed to specifically model the chemical-physical properties of CF mucus.

For more info
scan QR code

We have studied the interaction between mucin and several drugs used in the treatment of cystic fibrosis.

For more info
scan QR code

15TH INTERNATIONAL WORKSHOP ON CARCINOMA-ASSOCIATED MUCINS
29th July – 2nd August 2019 – Robinson College, Cambridge, UK.

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[1] S. K. Behera, et al., Exploring the role and diversity of mucins in health and disease with special insight into non-communicable diseases. *Glycoconj. J.*, 2015, 32, 575–613.
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