



The Abdus Salam  
International Centre  
for Theoretical Physics

# Looking for turn-on fluorescent probes for mucin glycoprotein detection

Light-matter Interactions from scratch: Theory and Experiments at the Border with Biology

24/11/2021

Cosmin Stefan Butnarusu, Alex Affricano, Carlotta Pontremoli, Nadia Barbero, Sonja Visentin



UNIVERSITÀ  
DEGLI STUDI  
DI TORINO



# Looking for turn-on fluorescent probes for mucin glycoprotein detection

## Agenda

---

- Introduction
- Aim
- Results
- Conclusion and final remarks
- Q&A

3'

2'

8'

2'

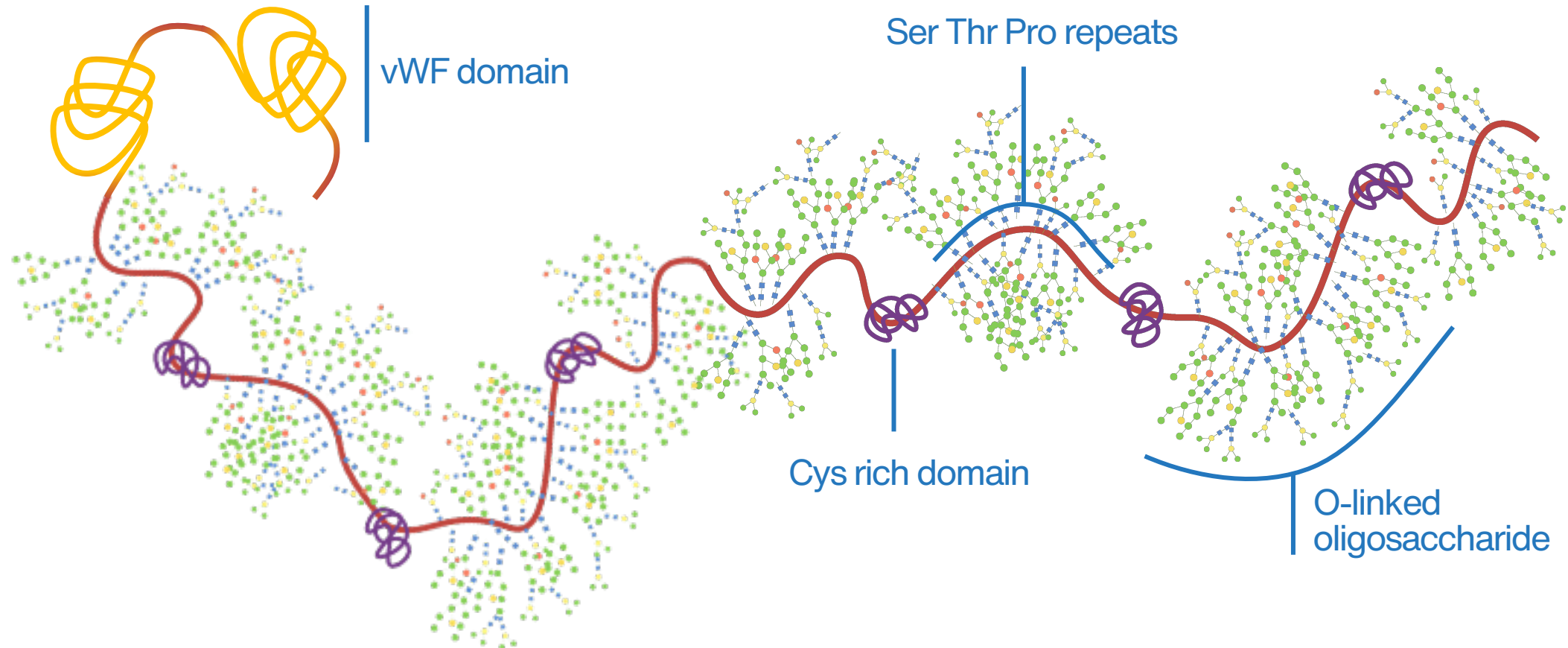
5'

# Introduction

Mucin, a complex glycoprotein

**Secreted** and **transmembrane** glycoprotein (up to **80%** O-linked oligosaccharides)

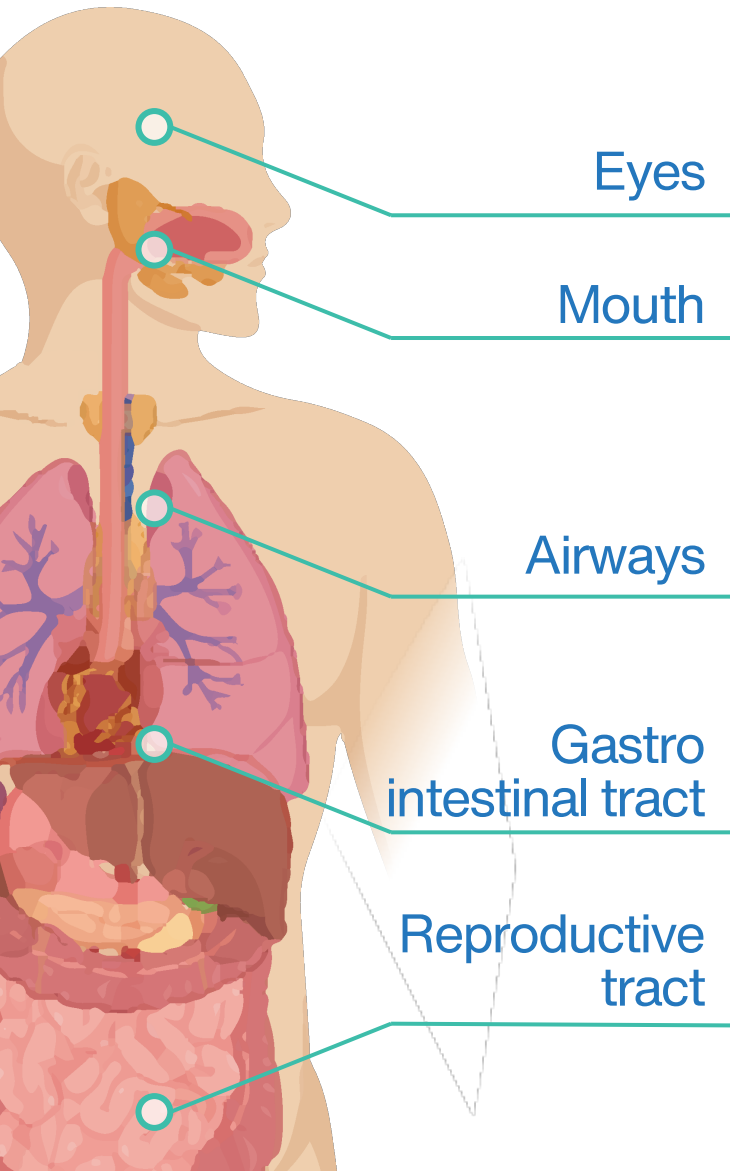
**640** kDa monomeric form



# Introduction

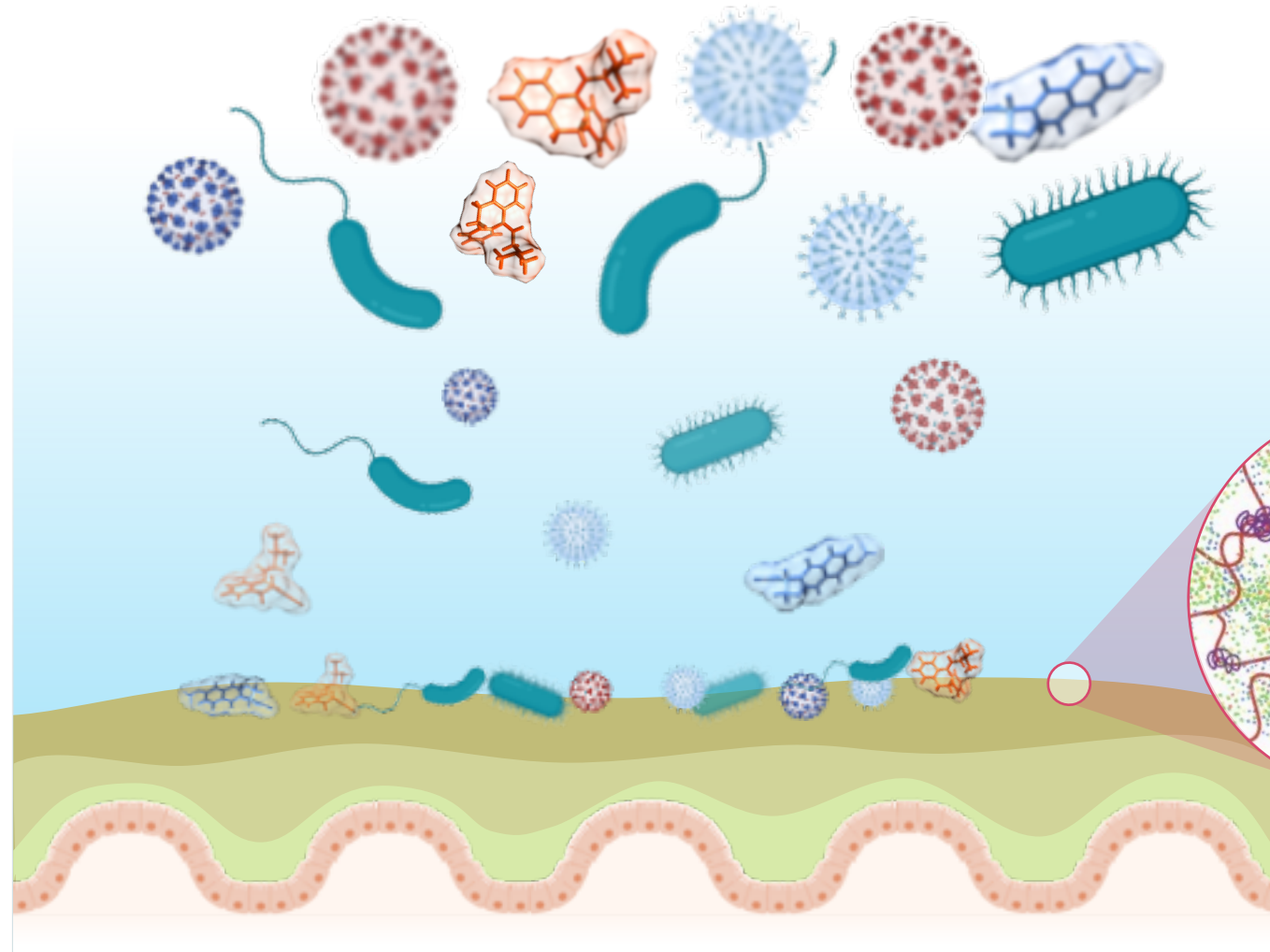
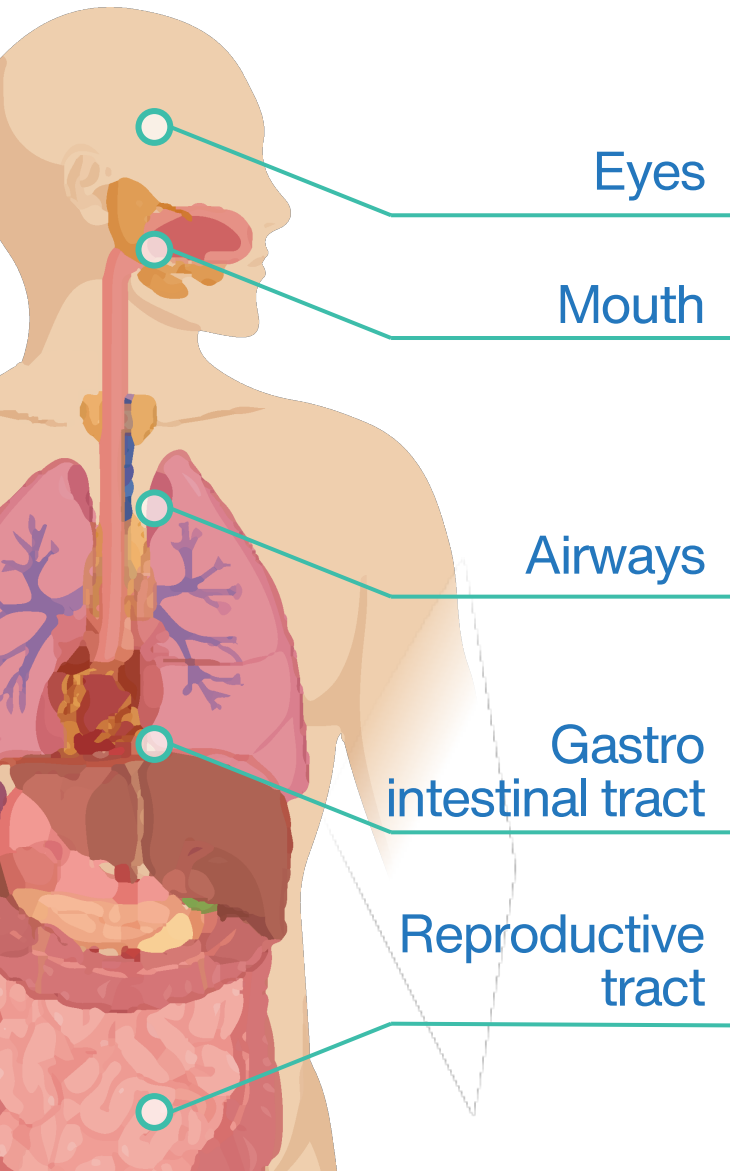
Mucin is the backbone of mucus

---



# Introduction

Mucus is a protective barrier



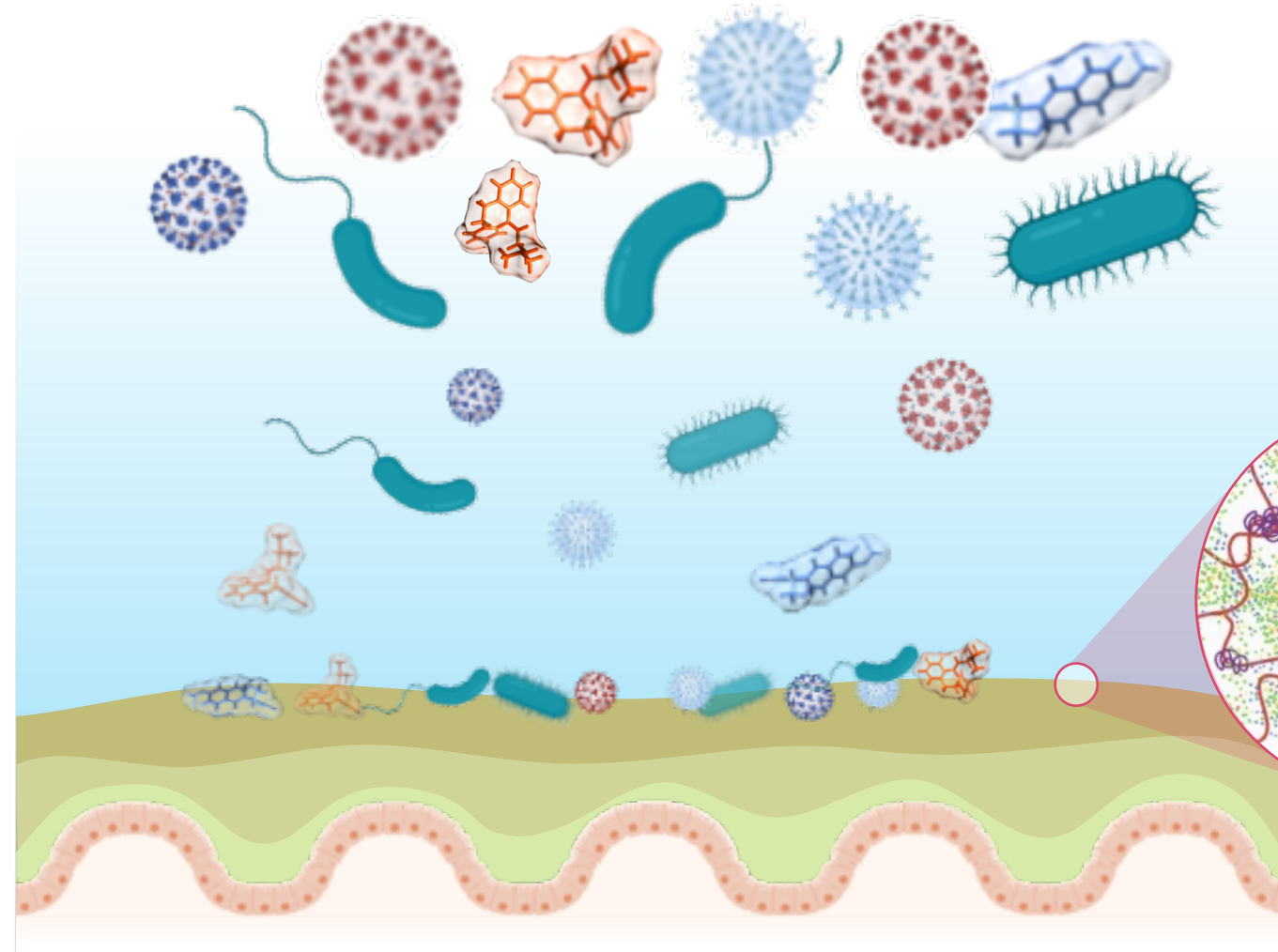
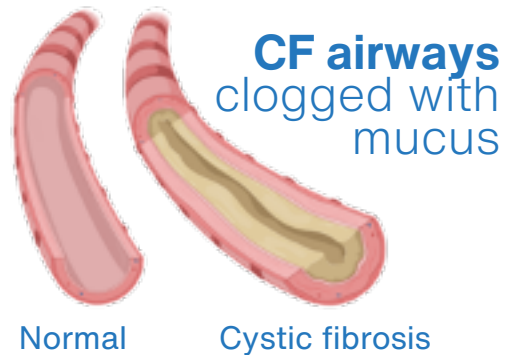
**Mucus** is a barrier against environmental threats

# Introduction

Mucin is the backbone of mucus

## Mucus-related disorders

- Cystic fibrosis
- COPD
- Asthma



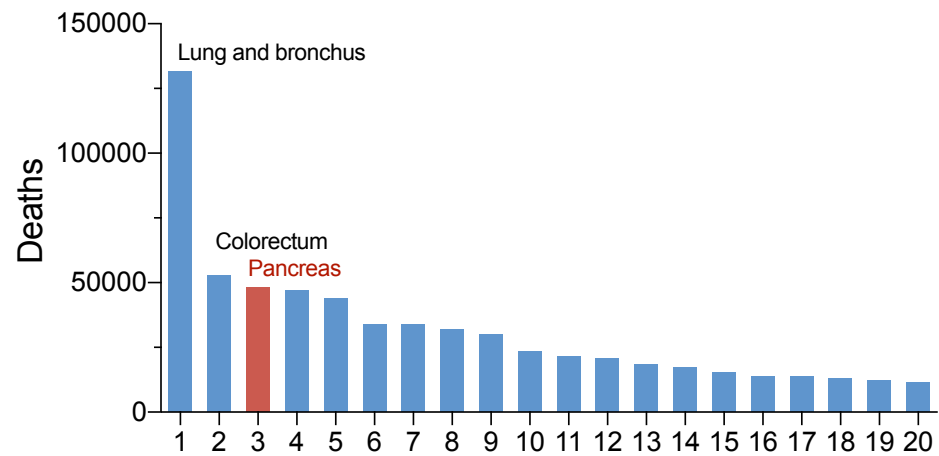
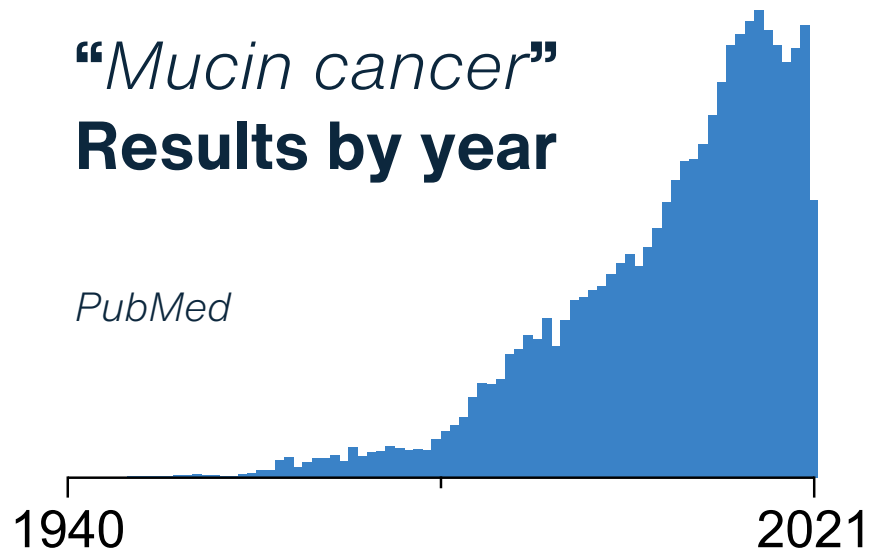
**Mucus** is a barrier against environmental threats

# Introduction

## Mucin and cancer

### “Mucin cancer” Results by year

PubMed

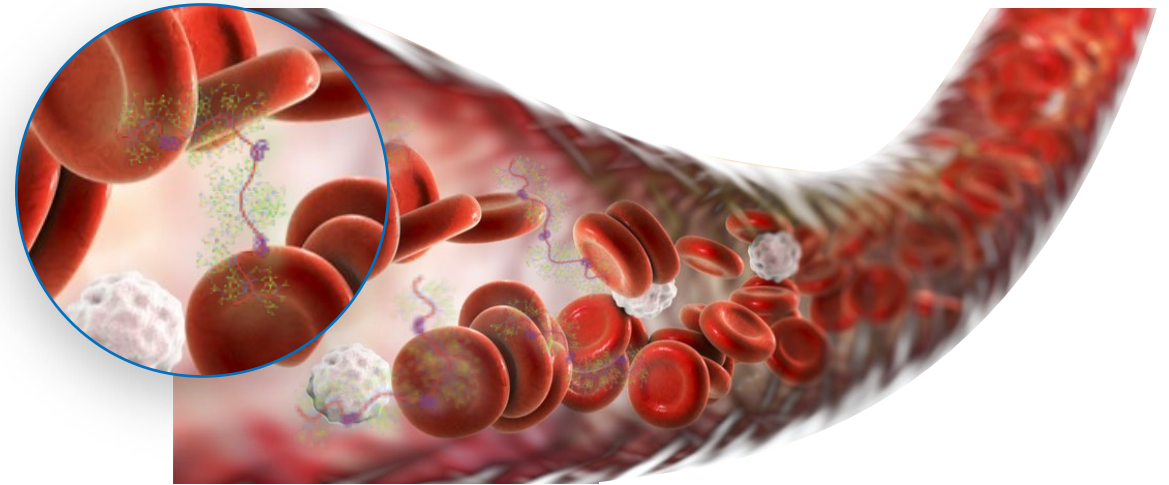
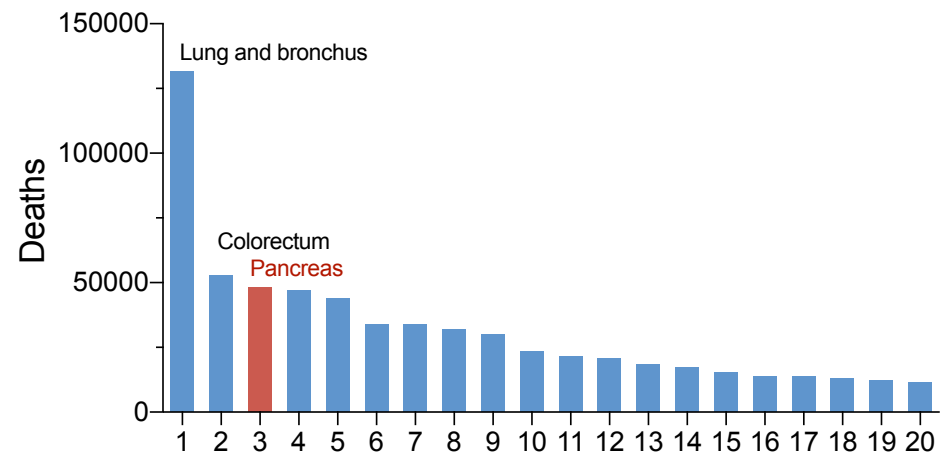
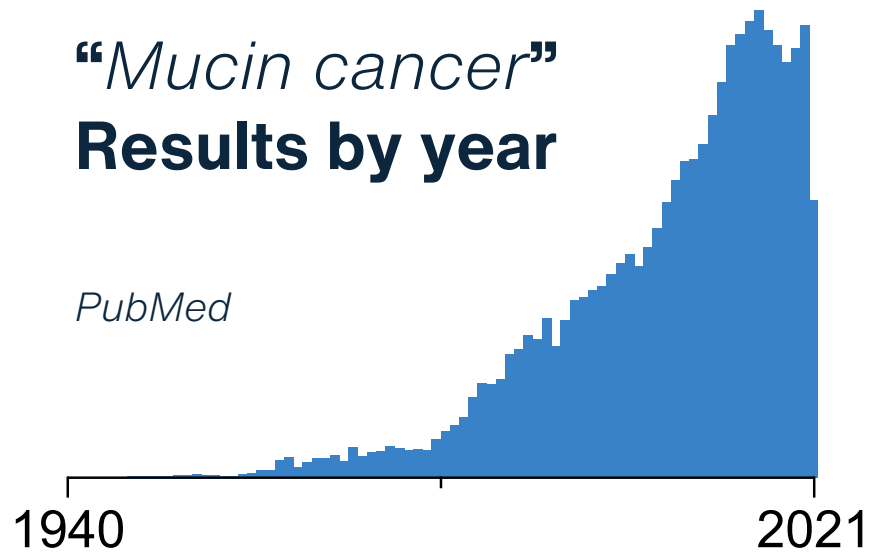


# Introduction

## Mucin and cancer

### “Mucin cancer” Results by year

PubMed



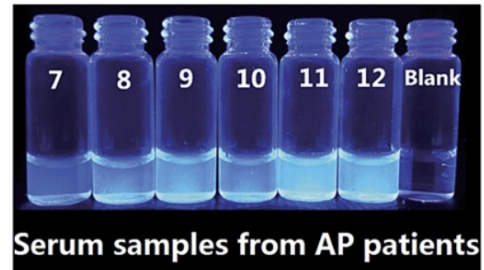
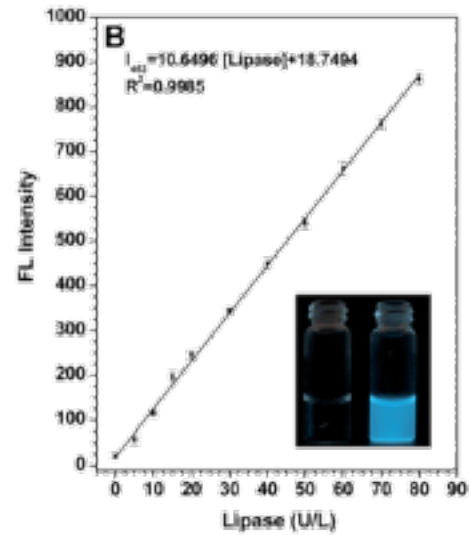
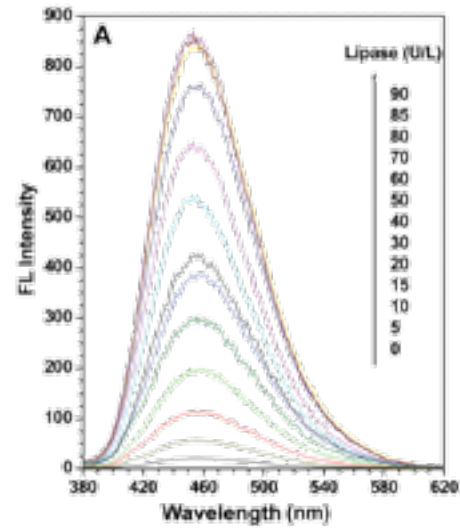
**Mucin in pancreatic cancer:** a well established but promising family for diagnosis, prognosis and therapy

*J Cell Mol Med. 2020;24:10279–10289.*



# Introduction

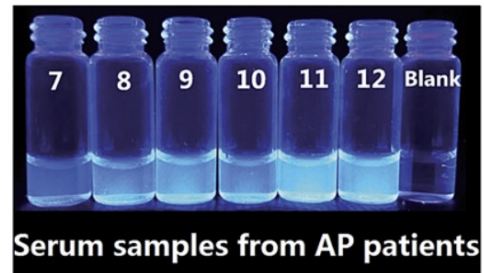
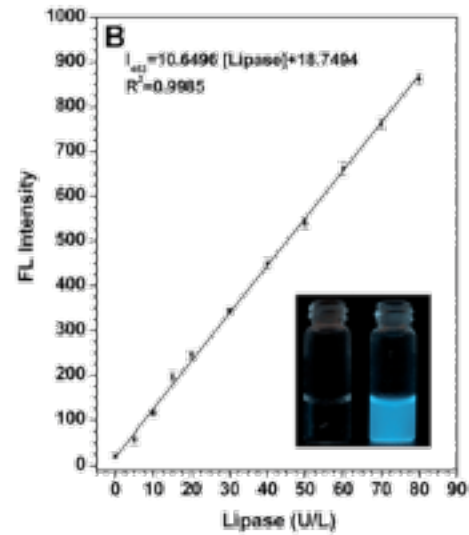
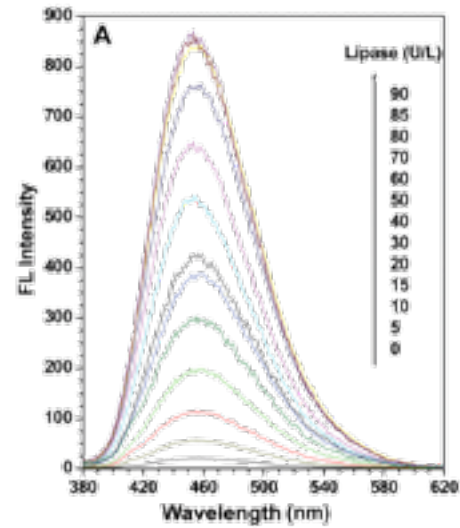
## Mucin as biomarker for pancreatic cancer



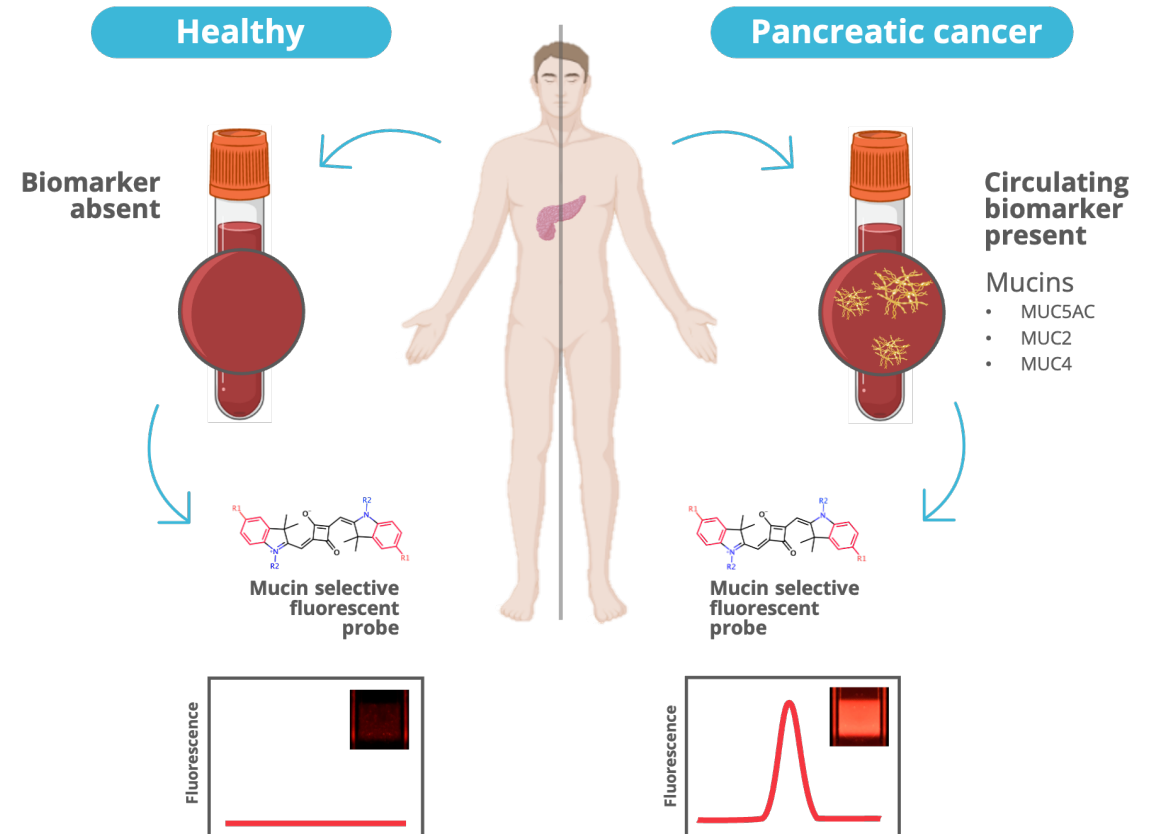
*Chem. Sci.*, 2017, 8, 6188–6195

# Aim

## Fluorometric detection of mucin



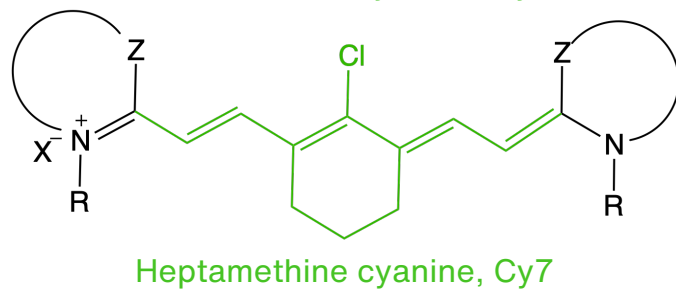
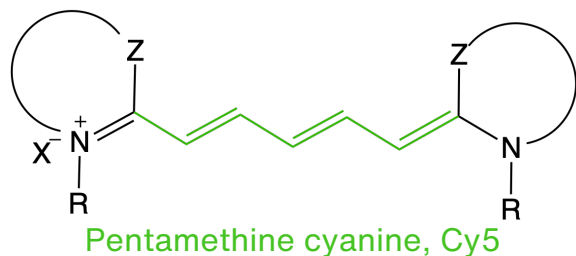
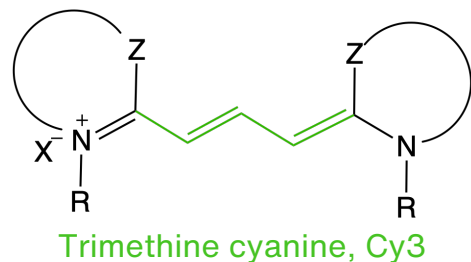
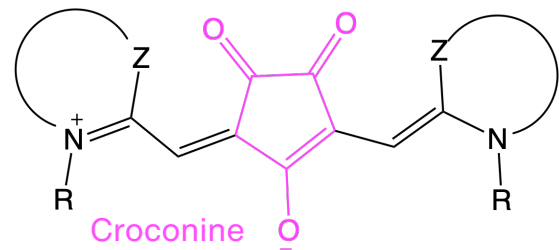
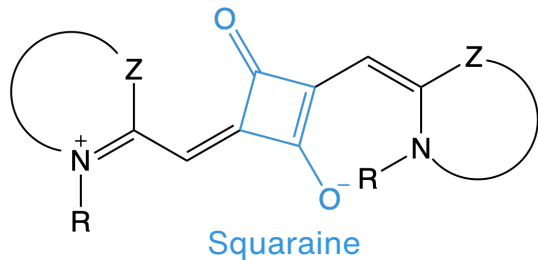
## Liquid biopsy



Chem. Sci., 2017, 8, 6188–6195

# Experimental

## Polymethine dyes

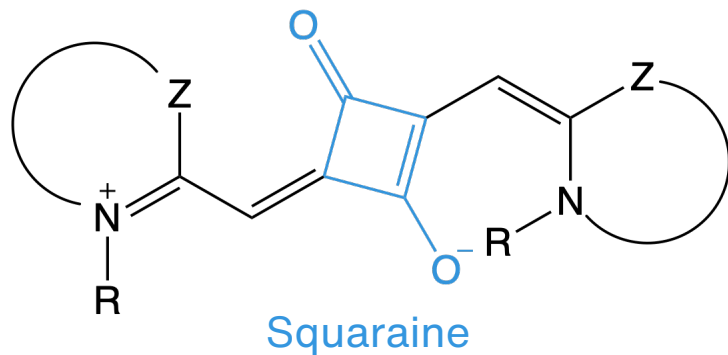


## Excellent photophysical properties

- Sharp and intense Abs and emission
- High photostability
- Easy tuning of photophysical properties (towards NIR)
- Wide applications (solar cells, light emitting diode, data storage, photoconductivity, histological probes)

# Experimental

## Polymethine dyes

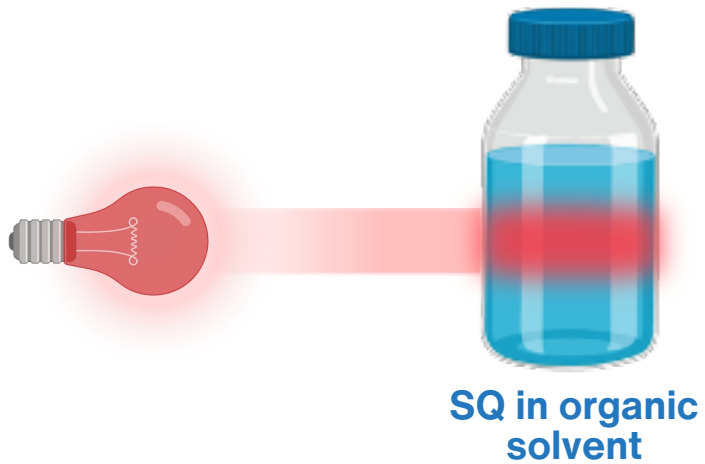
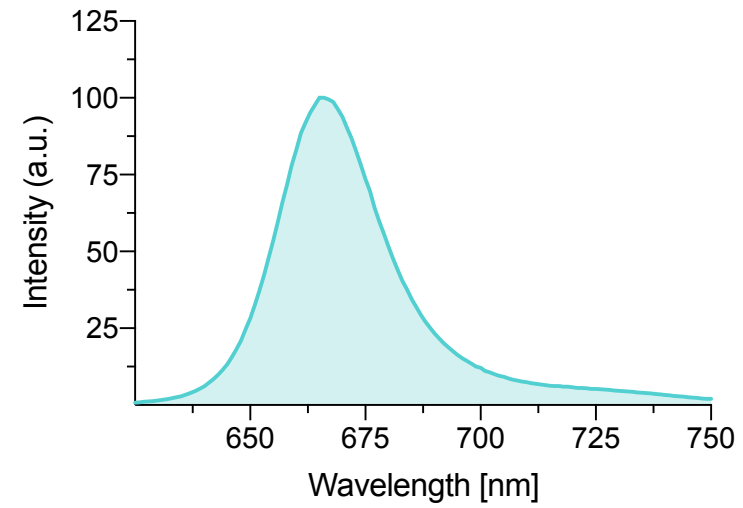
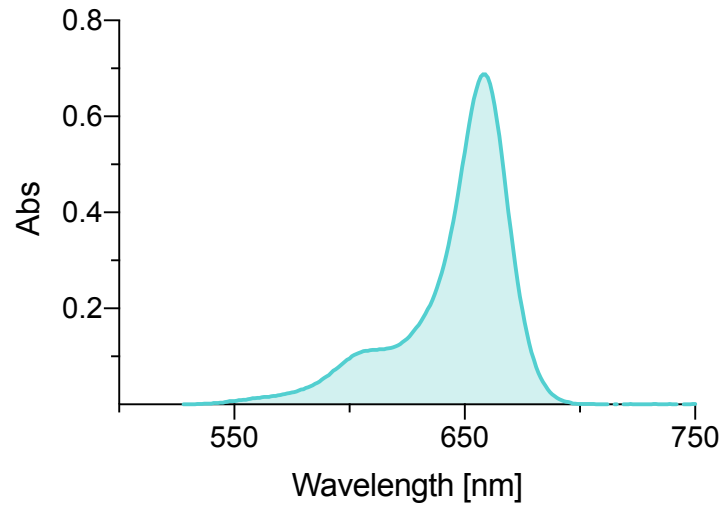


## Excellent photophysical properties

- Sharp and intense Abs and emission
- High photostability
- Easy tuning of photophysical properties (towards NIR)
- Wide applications (solar cells, light emitting diode, data storage, photoconductivity, histological probes)

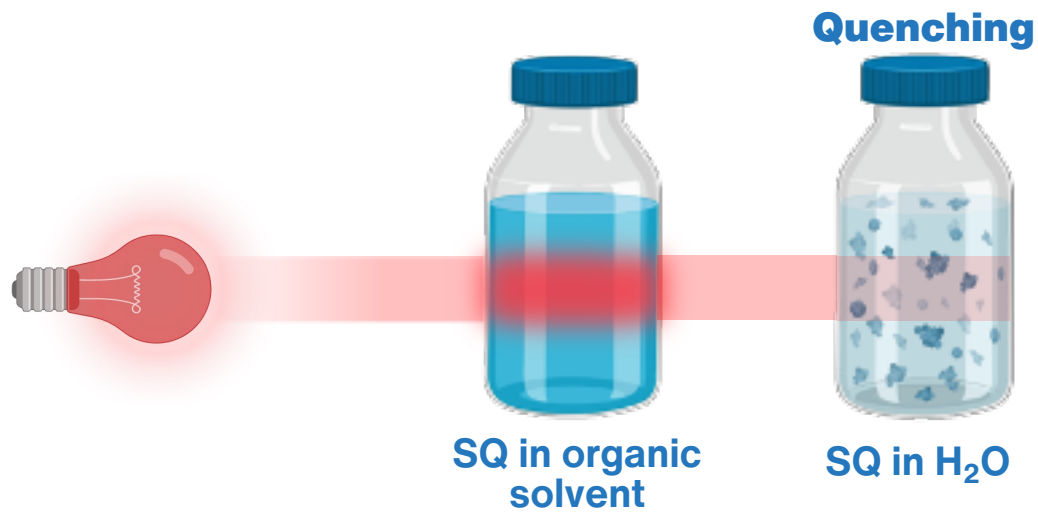
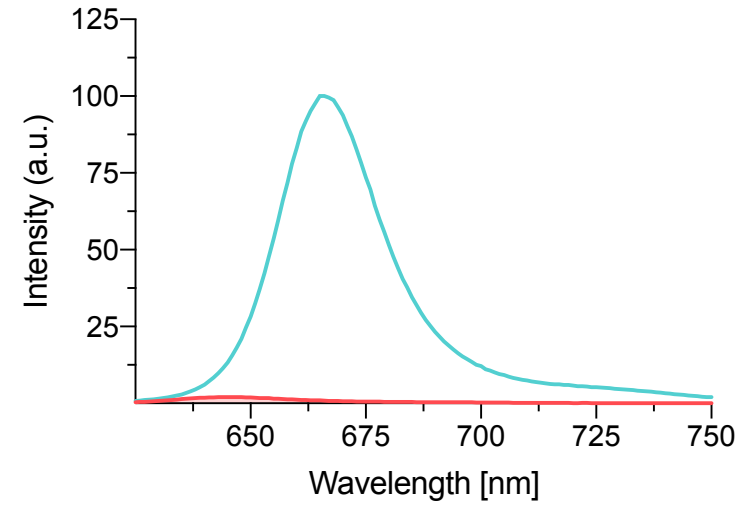
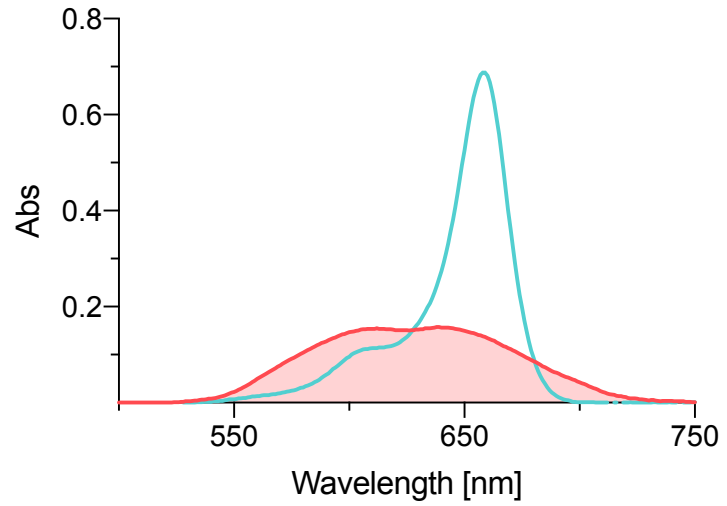
# Experimental

## Squaraine dyes as turn-on fluorescent sensors



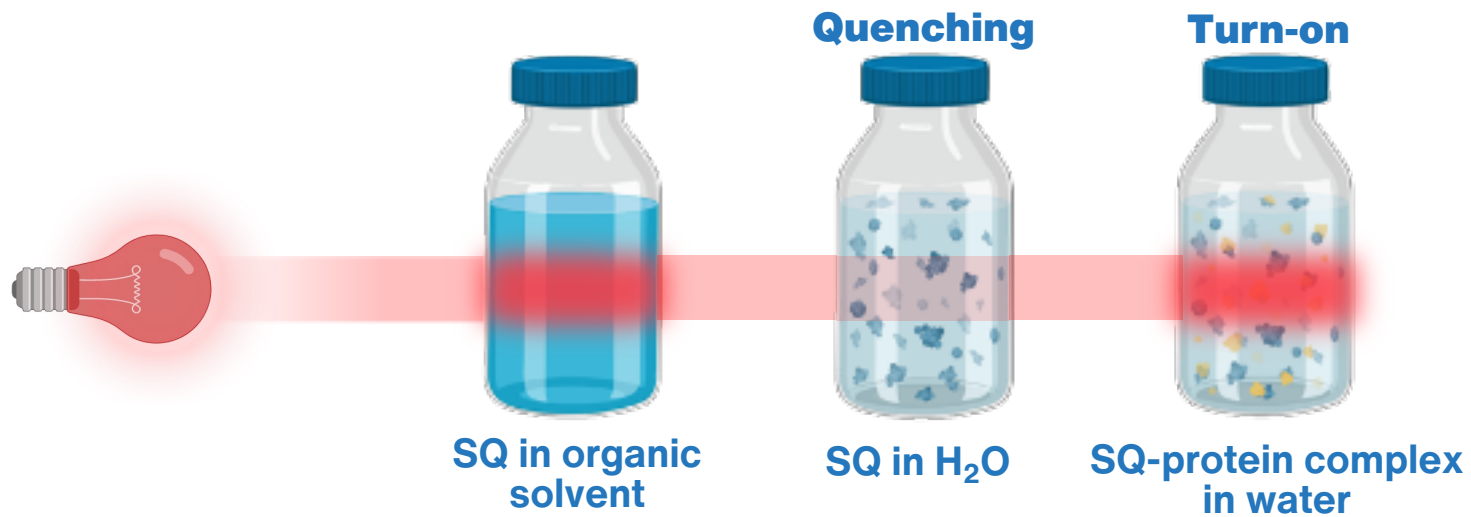
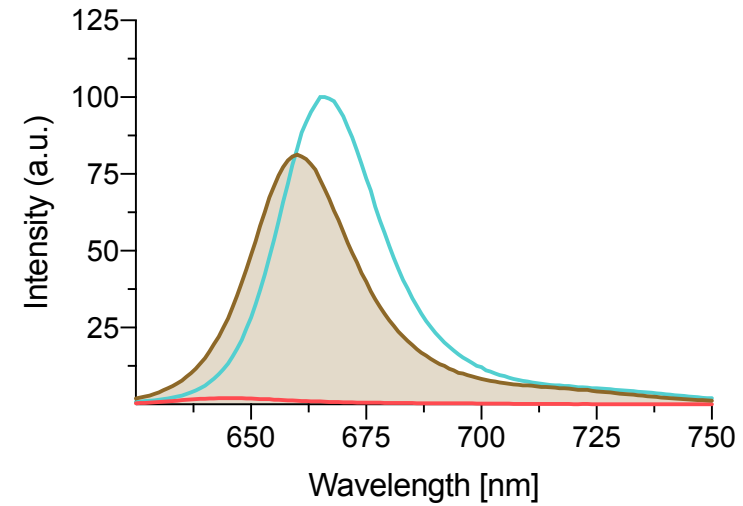
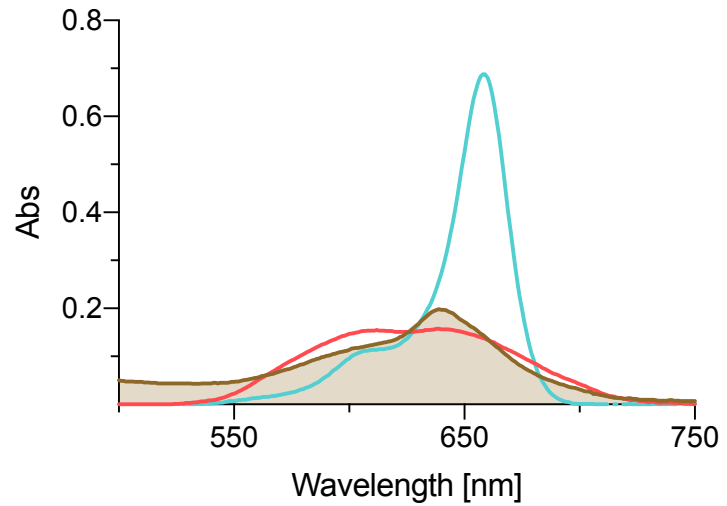
# Experimental

## Squaraine dyes as turn-on fluorescent sensors



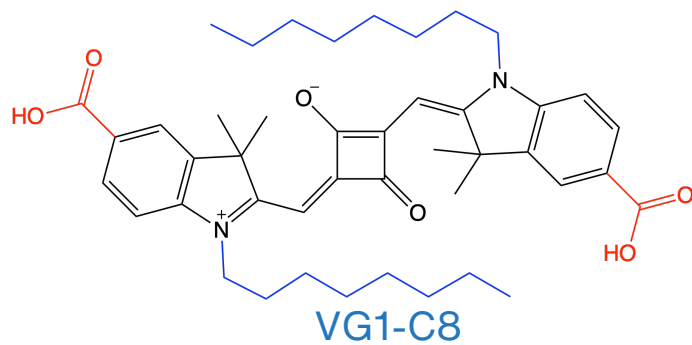
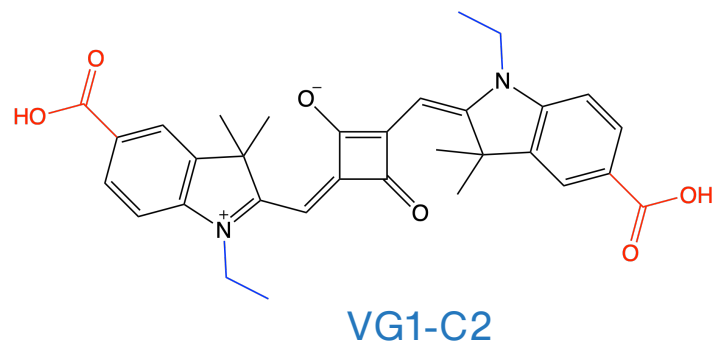
# Experimental

## Squaraine dyes as turn-on fluorescent sensors

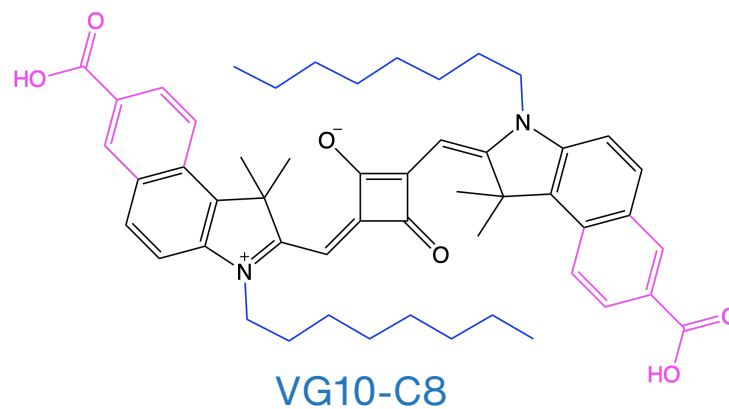
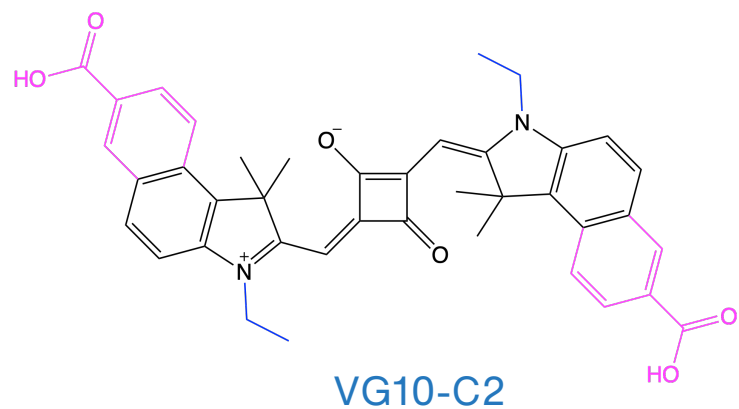


# Experimental

## VG-series of SQ



Indolenine  
squaraines



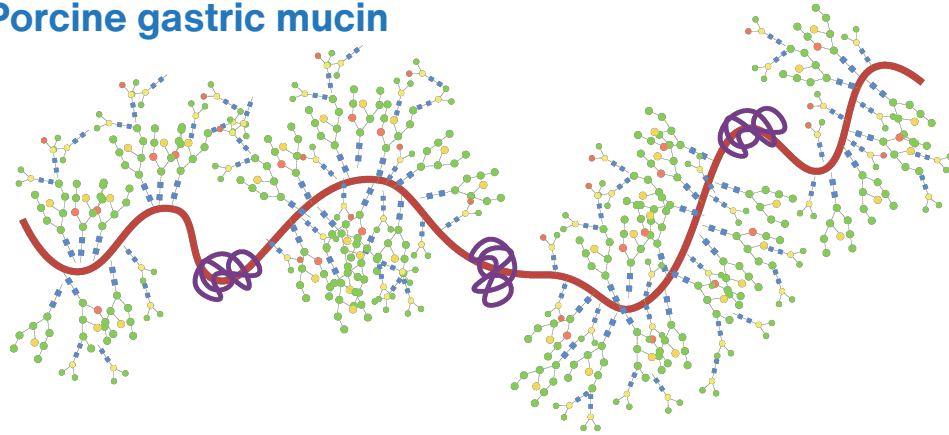
Benzoindolenine  
squaraines



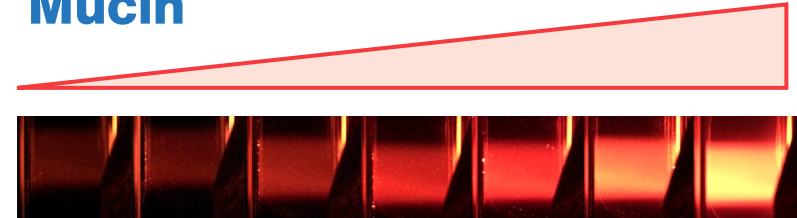
# Results

## Fluorescence turn-on induced by mucin

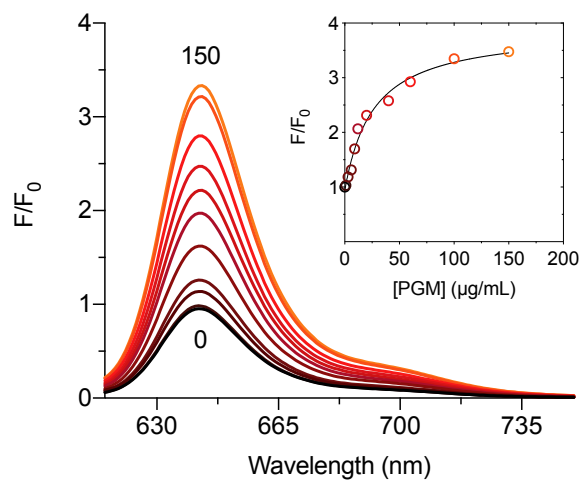
### Porcine gastric mucin



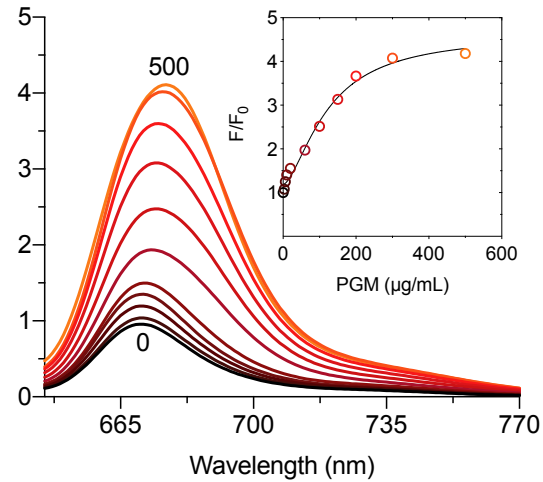
### Mucin



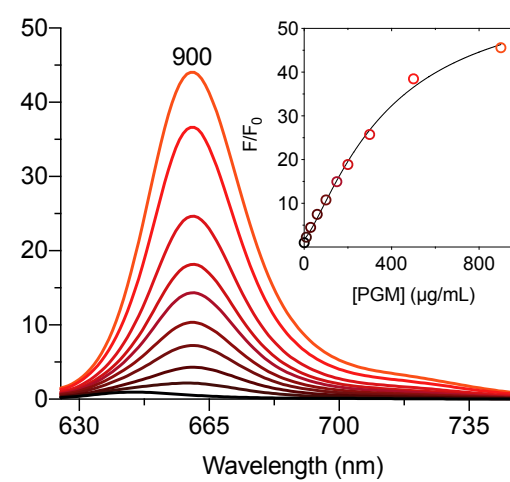
### VG1-C2



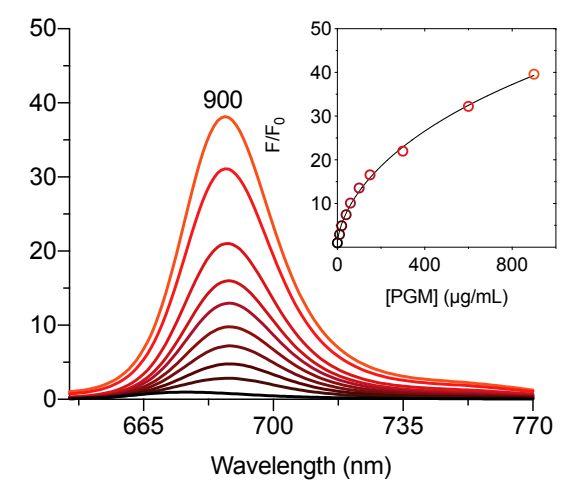
### VG10-C2



### VG1-C8

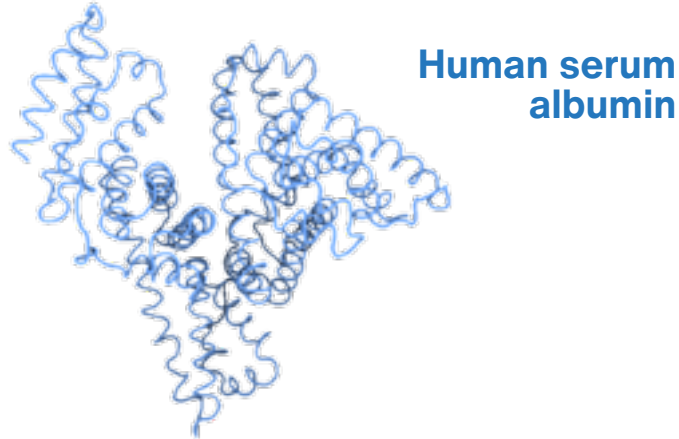


### VG10-C8



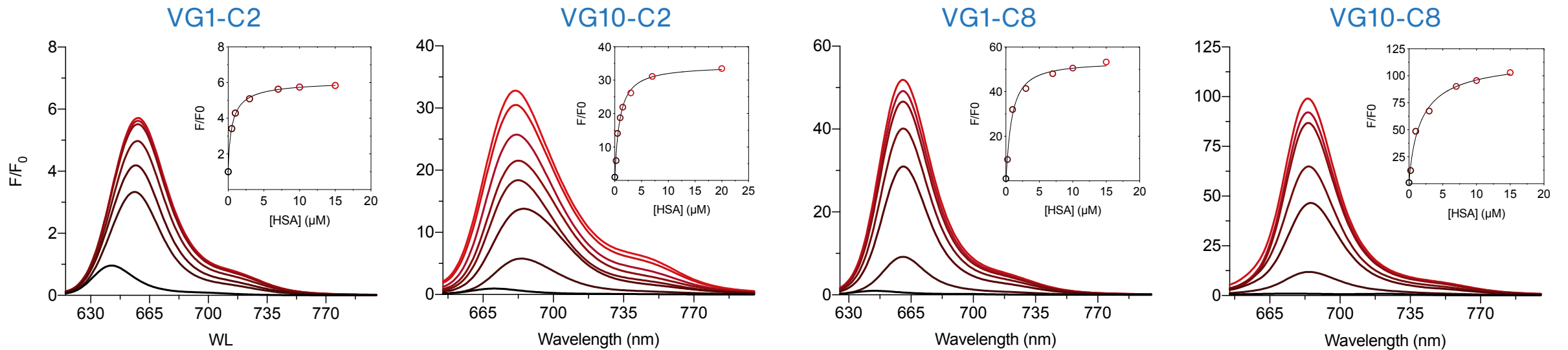
# Results

## Fluorescence turn on induced by albumin



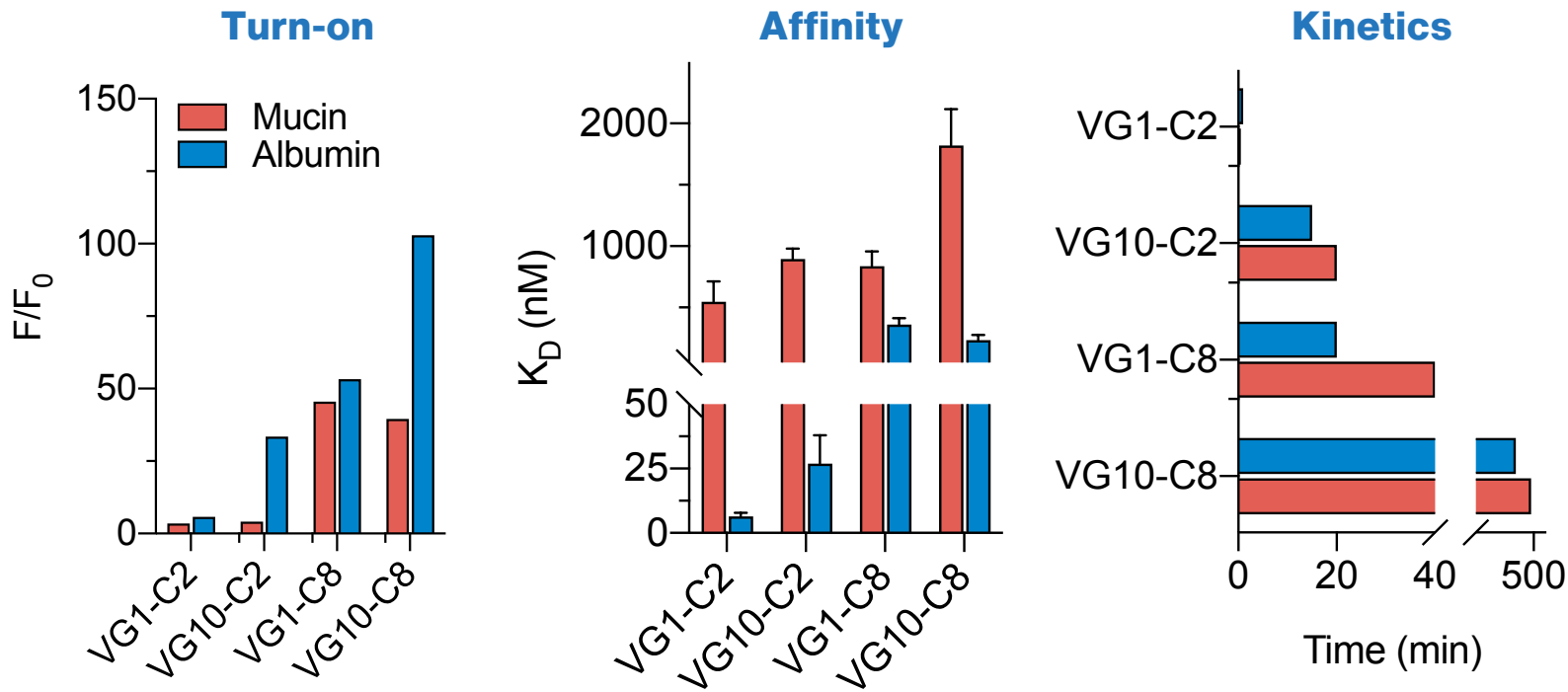
**No selectivity for mucin**

Higher turn-on signals induced by albumin



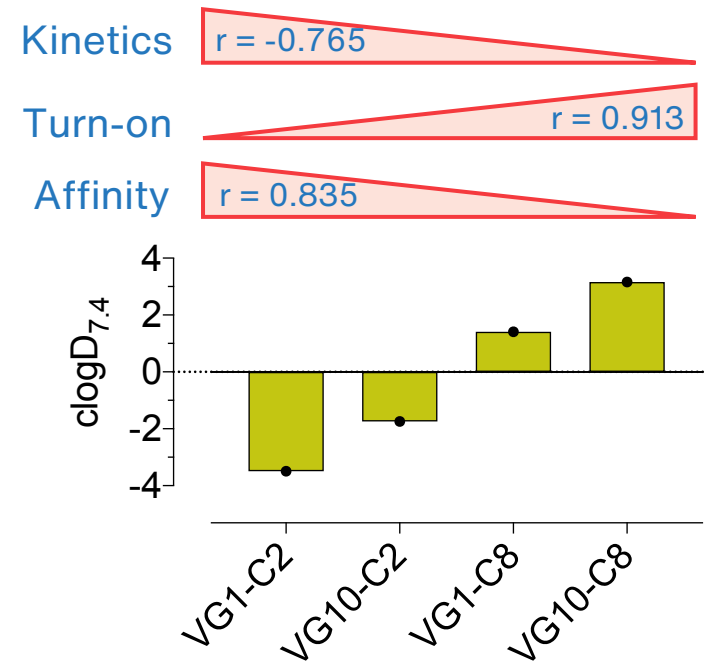
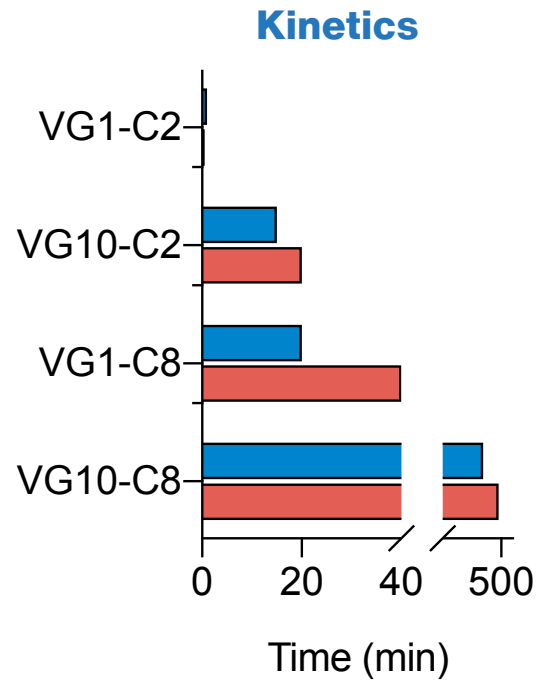
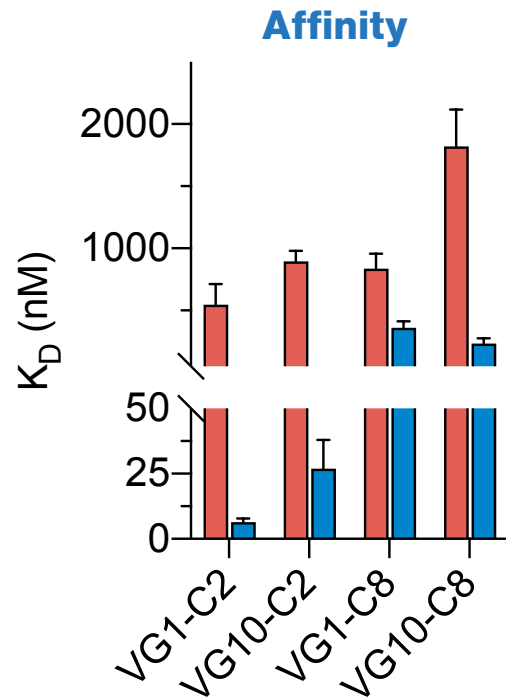
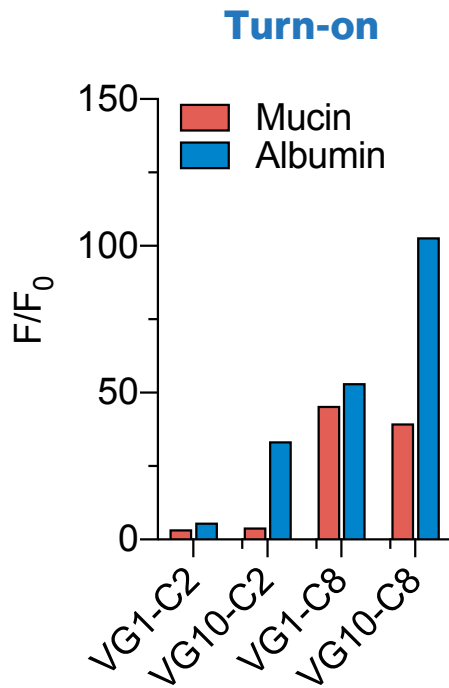
# Results

## SAR: mucin vs albumin



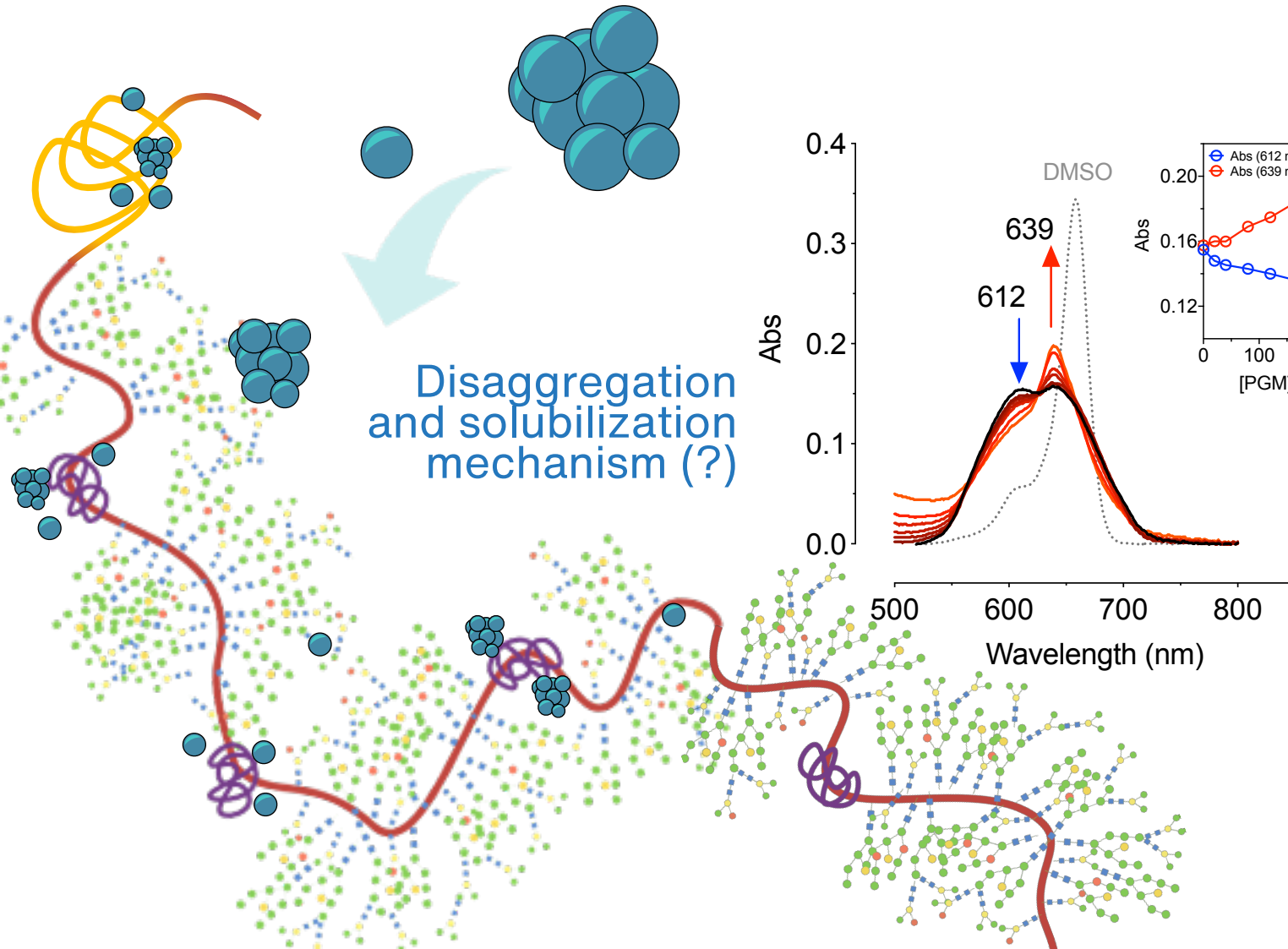
# Results

Squaraine lipophilicity may influence selectivity toward mucin



# Results

Squaraine lipophilicity may influence selectivity toward mucin



Kinetics

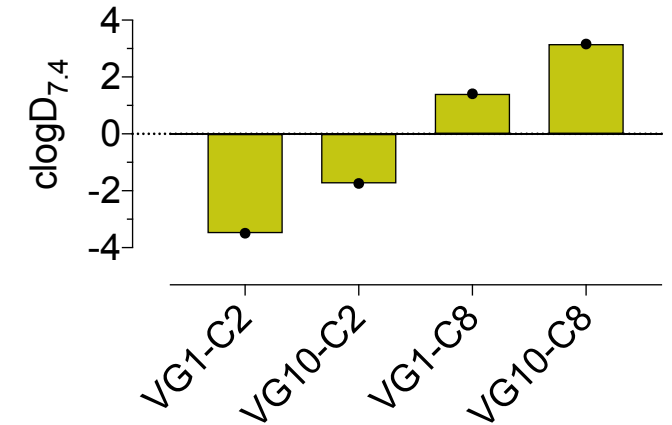
$r = -0.765$

Turn-on

$r = 0.913$

Affinity

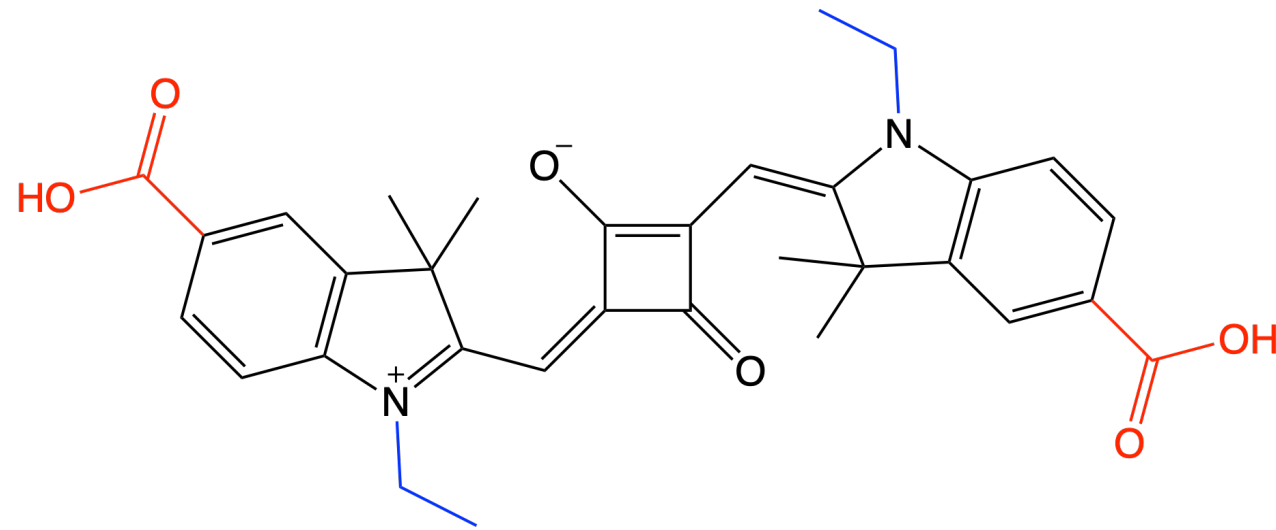
$r = 0.835$



# Results

## The lipophilicity hypothesis

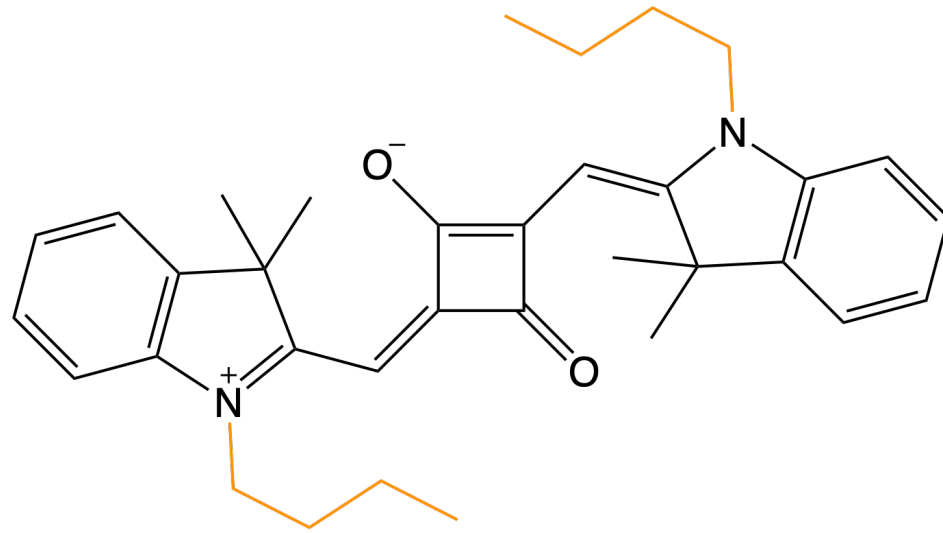
---



# Results

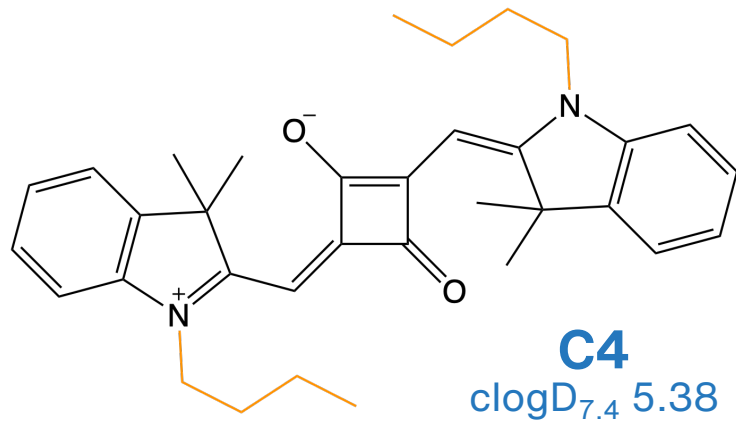
## The lipophilicity hypothesis

---

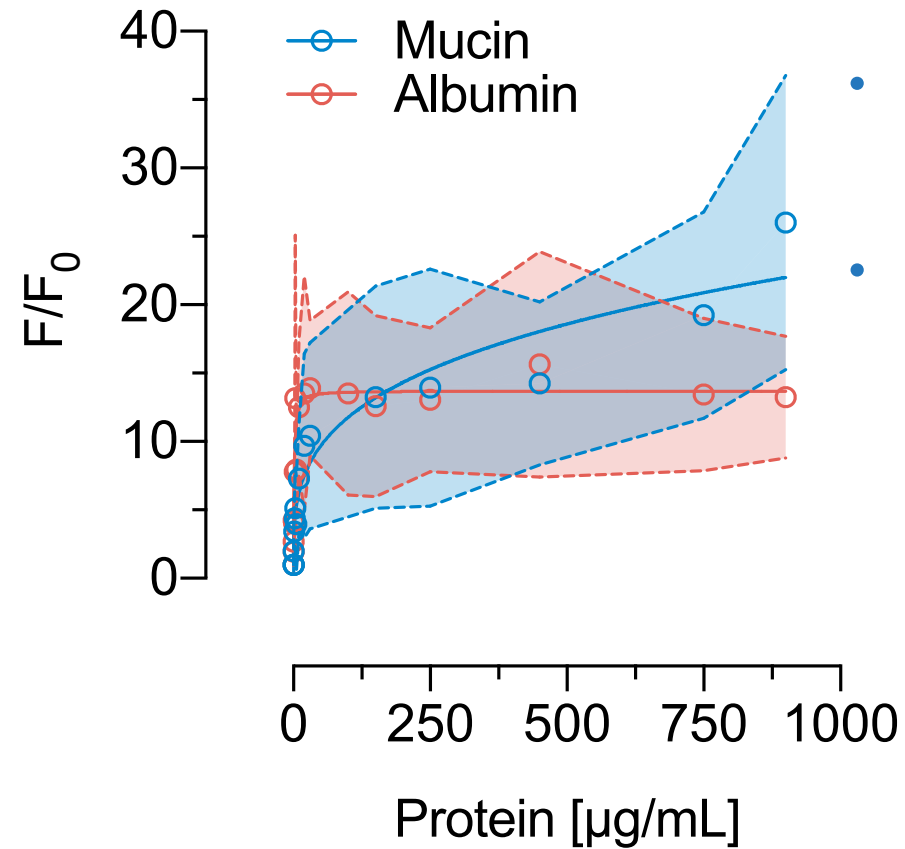


# Results

## The lipophilicity hypothesis



Lipophilicity

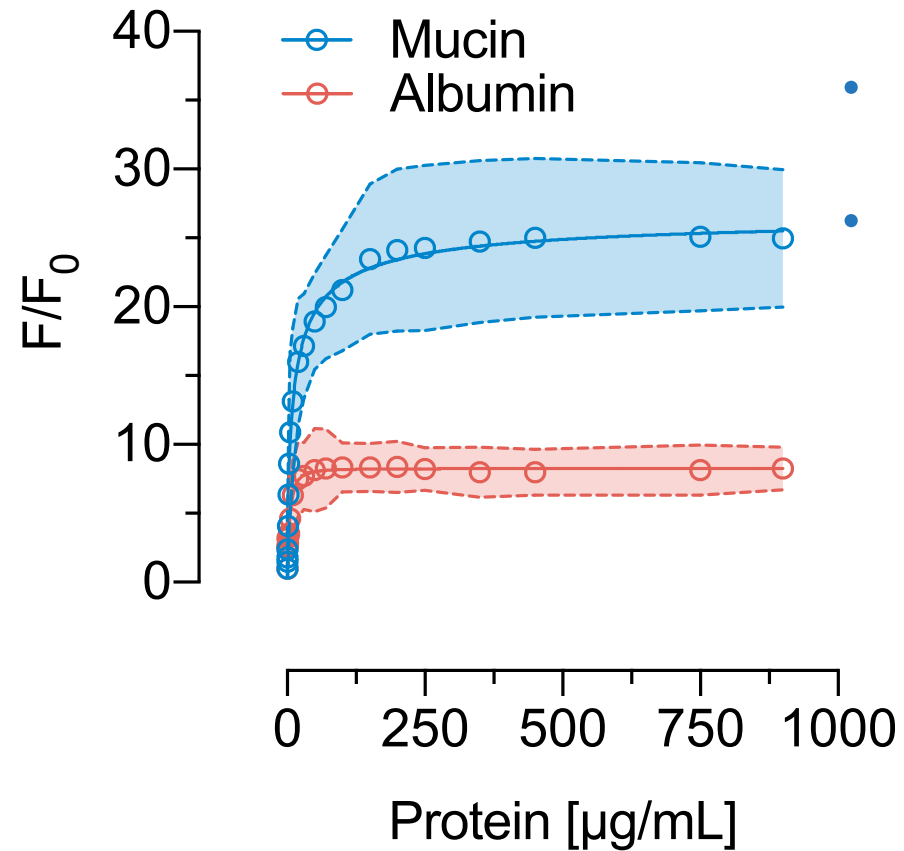
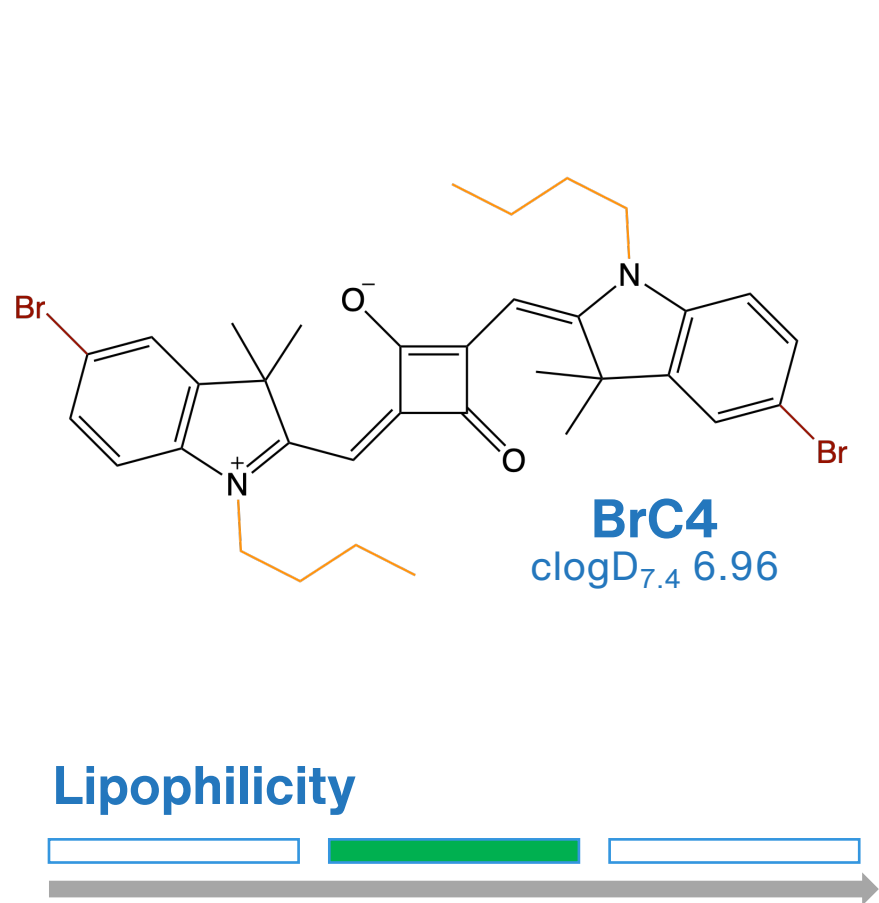


- **Similar response to mucin and albumin**
- **No selectivity for mucin**



# Results

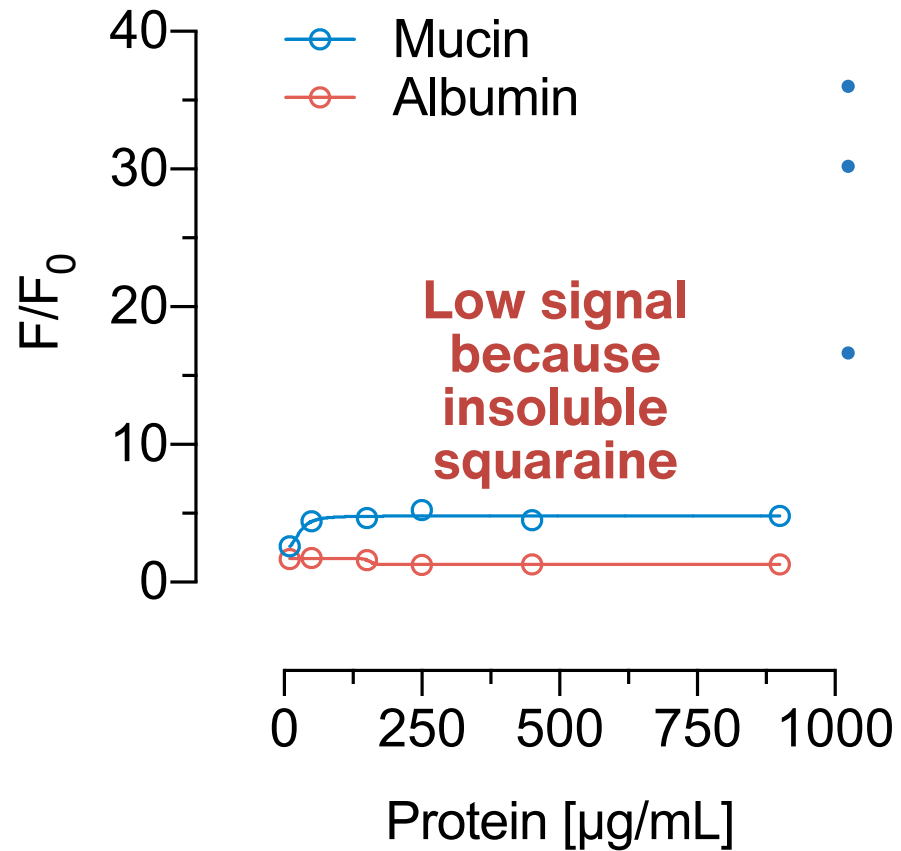
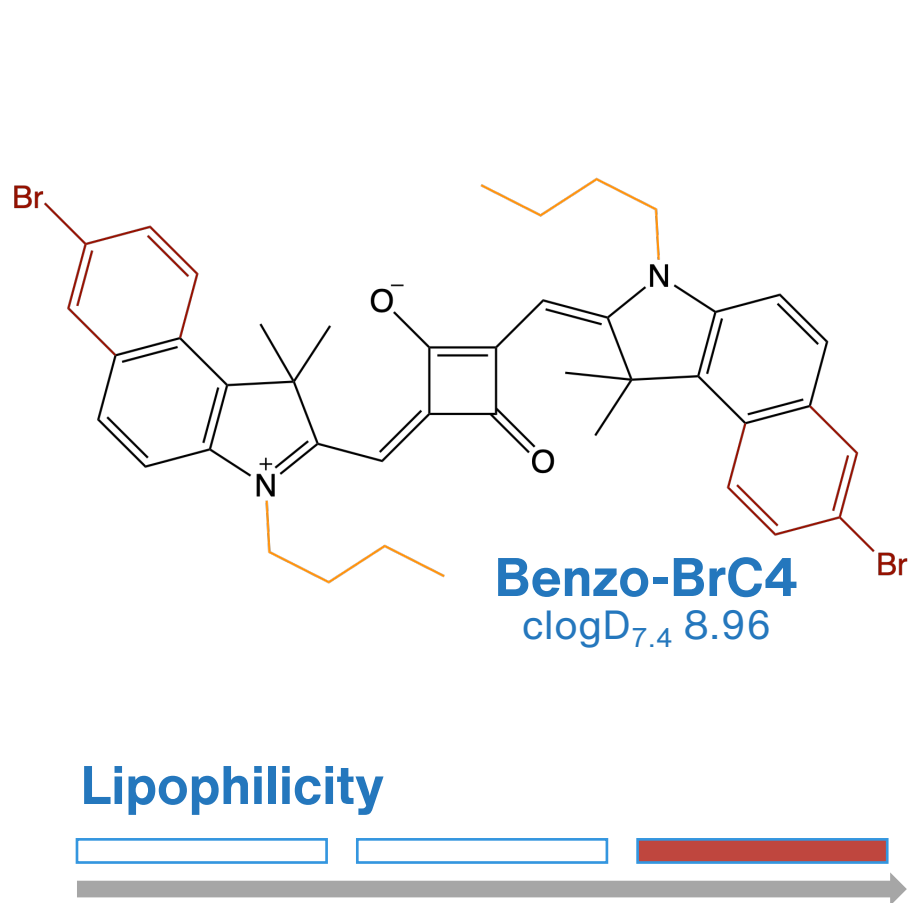
## The lipophilicity hypothesis



- Higher turn-on response to mucin
- More selective for mucin than albumin

# Results

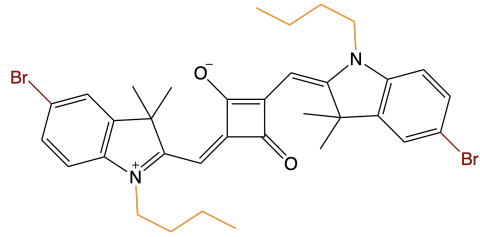
## The lipophilicity hypothesis



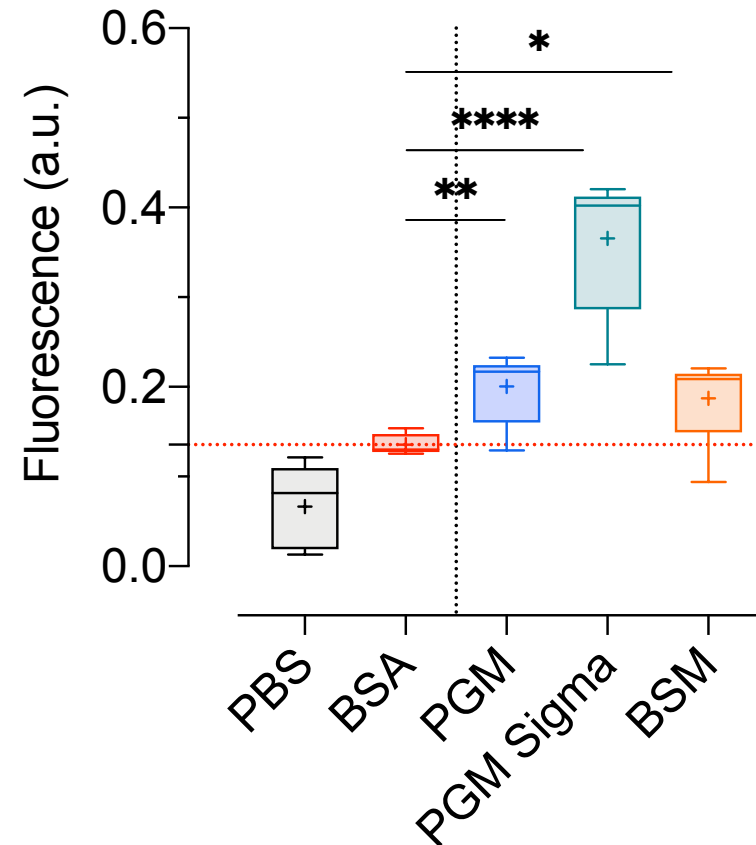
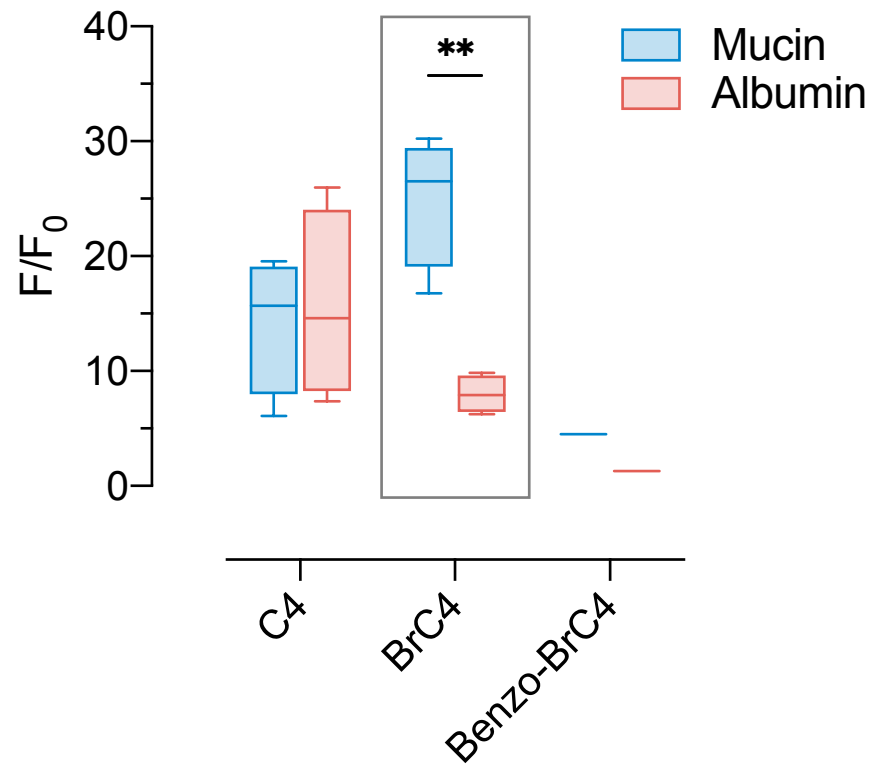
- **To much lipophilic**
- **Insoluble in water** (even in the presence of proteins)
- **Similar response with mucin and albumin**

# Results

BrC4 is more selective for mucins



**BrC4** is the best turn-on performer with mucin



- Turn-on tests on commercial and lab-purified mucins
- BrC4 is more selective for mucins

# Conclusions

---

Squaraine BrC4 is a good candidate to be used as turn-on fluorescent probe for mucin

## Next steps

Modulation of BrC4 structure to increase mucin selectivity

Tests with positively charged squaraines and natural dyes

# Acknowledgements

---



**Prof. Sonja Visentin**

---

Department of Molecular  
Biotechnologies and Health  
Sciences



**Prof. Nadia Barbero**



**Carlotta Pontremoli**



**Prof. Claudia Barolo**

---

Department of Chemistry



UNIVERSITÀ  
DEGLI STUDI  
DI TORINO

Thank you  
for your attention