FITC Substitution Doubles the Dynamic Range of a Fluorescein-tagged Oligoribonucleotide pH Sensor

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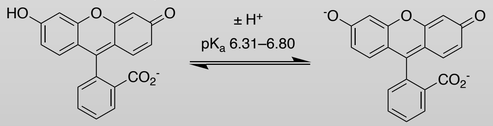
Introduction

- Within the physiologic pH range of 4.0-7.5, the pH sensitivity of the xanthene dye fluorescein originates primarily from the equilibrium between the monoanionic and dianionic species.
- Since the pK_a corresponding to this equilibrium sits well within the range of biologically relevant pH values, fluorescein-biomolecule conjugates have found use as pH sensors.
- The pK_a itself is sensitive to the electrostatic environment around the dye and can be perturbed by judicious alteration of the biomolecule.



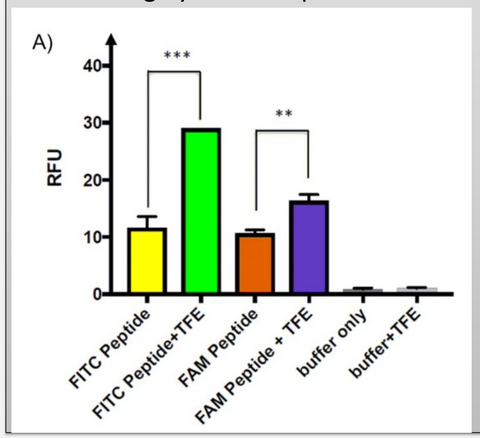
λ_{max} = 453, 472 nm
ε = 29,000–32,600 $M^{-1}cm^{-1}$
Φ = 0.36–0.37
Modestly fluorescent

λ_{max} = 490 nm
ε = 76,900–87,600 $M^{-1}cm^{-1}$
Φ = 0.92–0.95
Very fluorescent

Anal. Chem. **2007**, *79*, 6775-6782.

Background and Motivation

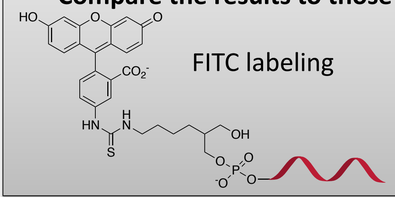
- We have found that FAM (6-carboxyfluorescein) -labeled pH probes are adequately sensitive in buffer but not in cells.
- The thiourea linker arising from FITC (fluorescein isothiocyanate) labeling is a known fluorescein quencher in peptides. We hypothesized that this quenching could work synergistically with the intrinsic pH sensitivity of fluorescein to increase the sensitivity.
- If the thiourea can perform this function in a labeled oligo, this heightened sensitivity may allow us to deploy a variety of oligo-based highly sensitive pH sensors in live cells.

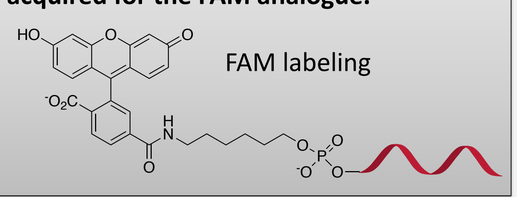


ChemBioChem **2019**, *20*, 40-45.

Objectives

- **Synthesize a 5'-FITC-(2'-OMe-U)$_5$-3' model oligoribonucleotide.**
- **Evaluate its pH sensitivity.**
- **Compare the results to those acquired for the FAM analogue.**





Synthesis and Workup

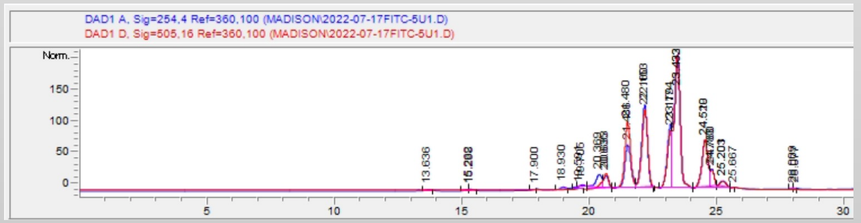
Automated Solid-phase Oligonucleotide Synthesis

Synthesis is 3'–to–5'

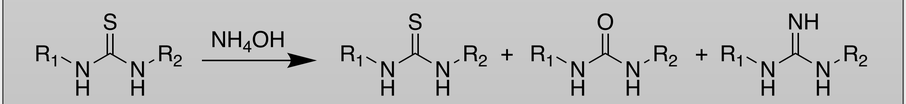
FITC — 2'-OMe-U — 2'-OMe-U — 2'-OMe-U — 2'-OMe-U — 2'-OMe-U-RNA CPG (controlled pore glass)

Workup and Purification

- 30% aqueous NH_4OH for 2h with agitation to deprotect and cleave
- Evaporation of excess base by venting and vacuum centrifugation
- Collect and syringe filter sample, washing the CPG with 1:1 MeOH:TEAA (0.1 M) to maximize recovery
- Semi-preparative reverse-phase HPLC
- Lyophilization

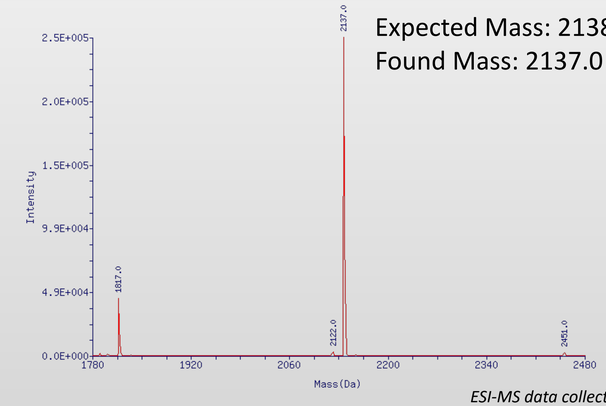


Representative HPLC trace of crude 5'-FITC-(2'-OMe)U$_5$-3'.



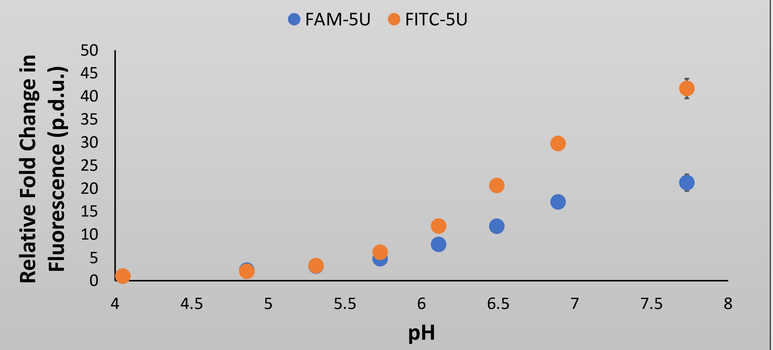
Side reactions at thiourea during deprotection/cleavage complicate the purification. The peak at 23.5 min is the intended product.

Characterization and Evaluation



Expected Mass: 2138.58 Da
Found Mass: 2137.0 Da

ESI-MS data collected by Novatia, LLC.



5'-FITC-(2'-OMe)U$_5$-3' is more pH-sensitive than the FAM analogue.

Future Directions

- Improve the synthesis of FITC-labeled oligos to increase recovery.
- Confirm the mechanism of fluorescence quenching and determine if the observed effect is as pH-dependent as it appears to be.
- Develop and deploy a FITC-labeled oligo to monitor pH in live cells.

References

Lavis, L. D.; Rutkoski, T. J.; Raines, R. T. Tuning the pKa of fluorescein to optimize binding assays. *Anal. Chem.* **2007,** *79*, 6775-6782.

Johnson, O. T.; Kaur, T.; Garner, A. T. A conditionally fluorescent peptide reporter of secondary structure modulation. *ChemBioChem* **2019**, *20*, 40-45.