Two Papers on Photoacoustic Dyes

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Overview of Project

- Looking for candidate dyes for photoacoustic neuro-imaging
  - Voltage and pH changes in neurons correlate to brain signal
  - Empirical characterization of photoacoustic response for variety of dyes
  - Photoacoustic imaging has attractive spatial and temporal resolution

Two Papers on Photoacoustic Dyes

- **Functional photoacoustic microscopy of pH (pH)**
  - Muhammad Rameez Chatni, Junjie Yao, Amos Danielli, et. al in *Journal of Biomedical Optics,* 2011
- **Design, Synthesis, and Imaging of an Activatable Photoacoustic Probe (probe)**
  - Jelena Levi, Sri Rajasekhar Kothapalli, Te-Jen Ma, et. al in *Journal of American Chemical Society,* 2010

pH: Relevance

- Evidence that pH changes are detectable via photoacoustic imaging, through tissue

pH: Goal

- Use a commercially available pH sensitive fluorescent dye (SNARF-5F) to measure absolute pH photoacoustically (a first!)
  - Limit of fluorescence is ~ 100 micrometers, and MRI is a non-optimal technique

pH: Procedure

- .1 mM SNARF-5F dye solutions in 6.78, 7.45, and 7.80 pH buffer solutions
- OR-PAM phantom (a)
  - .3mm ID silicone tubes, raster scanned at 581 and 594 nm, laser set to 60 to 80 nJ, covered in ~ 200 micrometer mouse skin tissue
- AR-PAM phantom (b)
  - Machined acrylic block with wells, raster scanned at 565 and 580 nm, 230 to 300 nJ laser energy, covered in ~2 mm chicken breast tissue

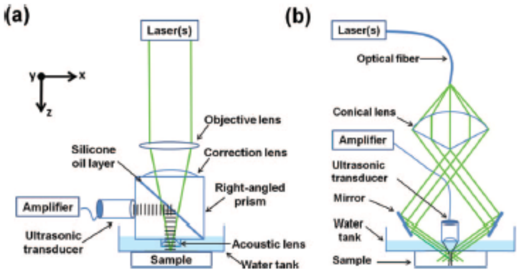
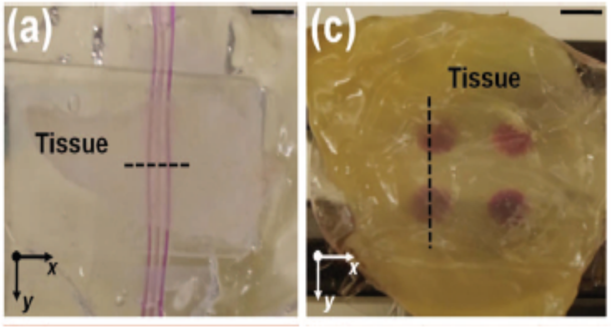


Fig. 1 Schematics of (a) OR-PAM system and (b) AR-PAM system.

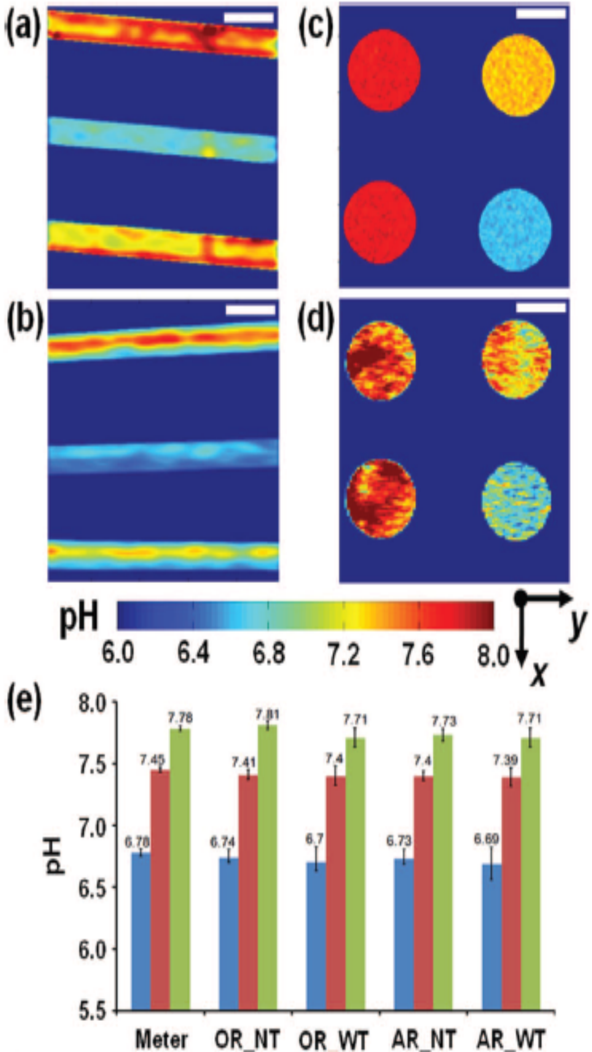


pH: Results

- SNARF-5F has different absorption spectrum when dissociated, dissociation directly related to pH via Henderson-Hasselbach eq.
- Imaged pixels were unmixed between two wavelengths and averaged for a tube or well

pH: Results

- First column is OR-PAM, second is AR-PAM
- First row is without tissue, second row is with tissue
- No more than ~1% error



pH: Assessment

- (At the time) a novel and promising result
- Dual wavelength imaging
- Drawbacks
  - Realism of phantoms
  - Low laser energy

probe: Relevance

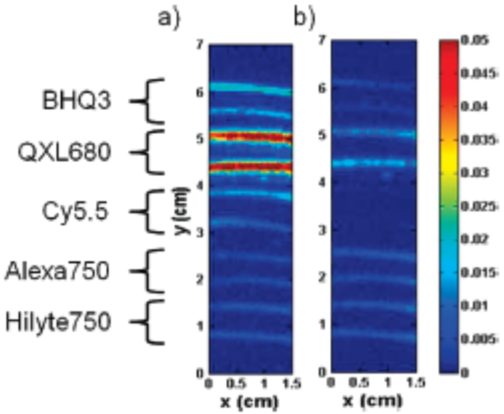
- Bespoke photoacoustic dye design to observe biological activity
- Detection of small concentration of dye activation
- Data on 5 chromophores

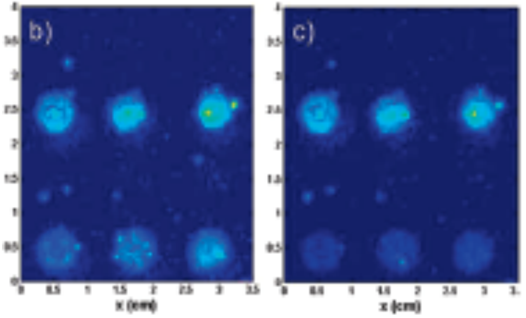
probe: Goal

- Design a dye for photoacoustic imaging that will be changed by a biological process, resulting in an observable signal change
    - Use well studied cellular protease and protein substrate, pick chromophores to attach to substrate
    -

probe: Procedure

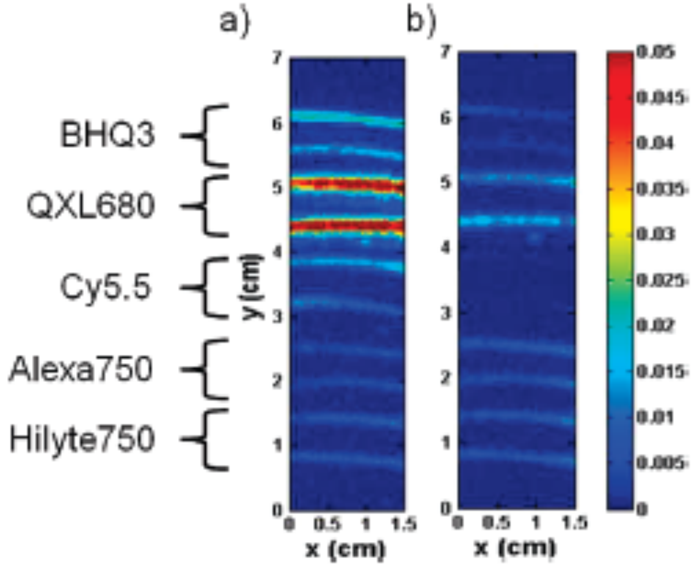
- First, dye selection
  - Polyethylene tubes in agar, filled with dye and imaged
- Second, cleavage in solution
  - Protein substrates built with BHQ3-Alexa750 pair or QXL680-Hilyte750 pair
  - Solutions of cleaved and uncleaved substrates tested in tubes
- Third, test in cell with protease
  - Cultivated cells suspended in agar, mixed with probe, and put in agar wells





probe: Results

chromophore	$\lambda_{ex/em}$ (nm)	ε (mol/cm · g)	Φ (%)
BHQ3	672	42 700	
QXL680	679	110 000	
Cy5.5	675/695	250 000	0.23
Alexa750	749/775	290 000	0.12
Hilyte750	754/778	275 000	0.12



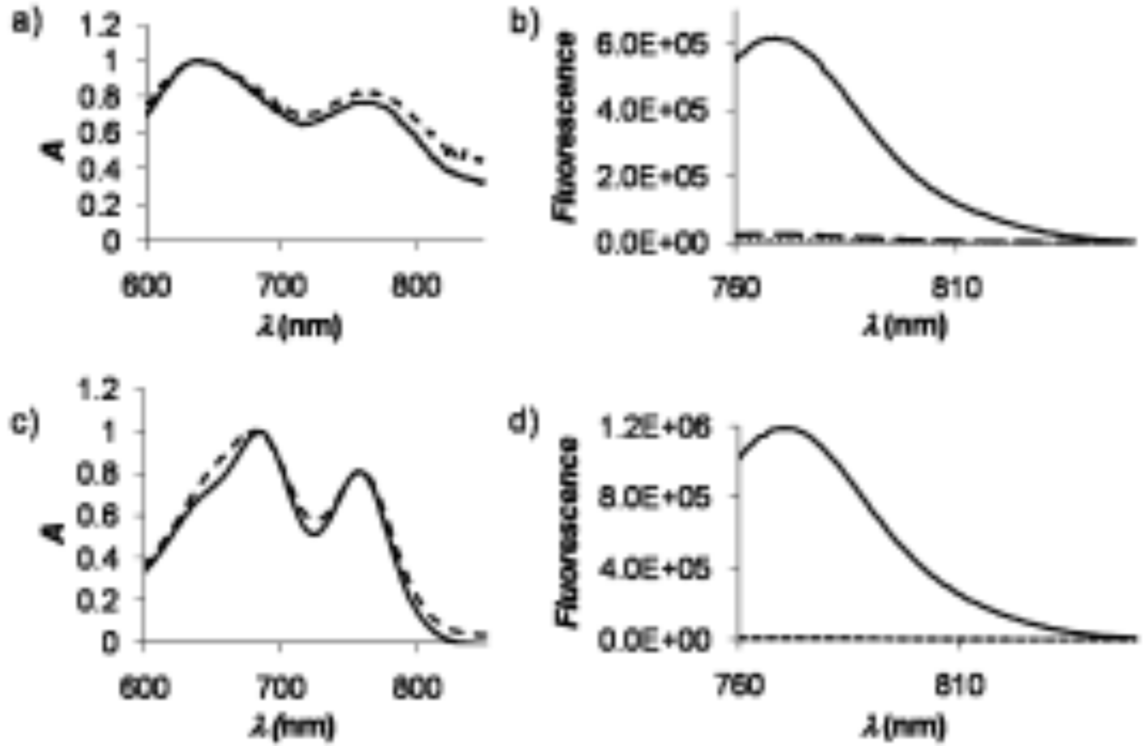
- (a) 675 nm
- (b) 750 nm

Beer Lambert Law

$A = \varepsilon * c * l$

probe: Results

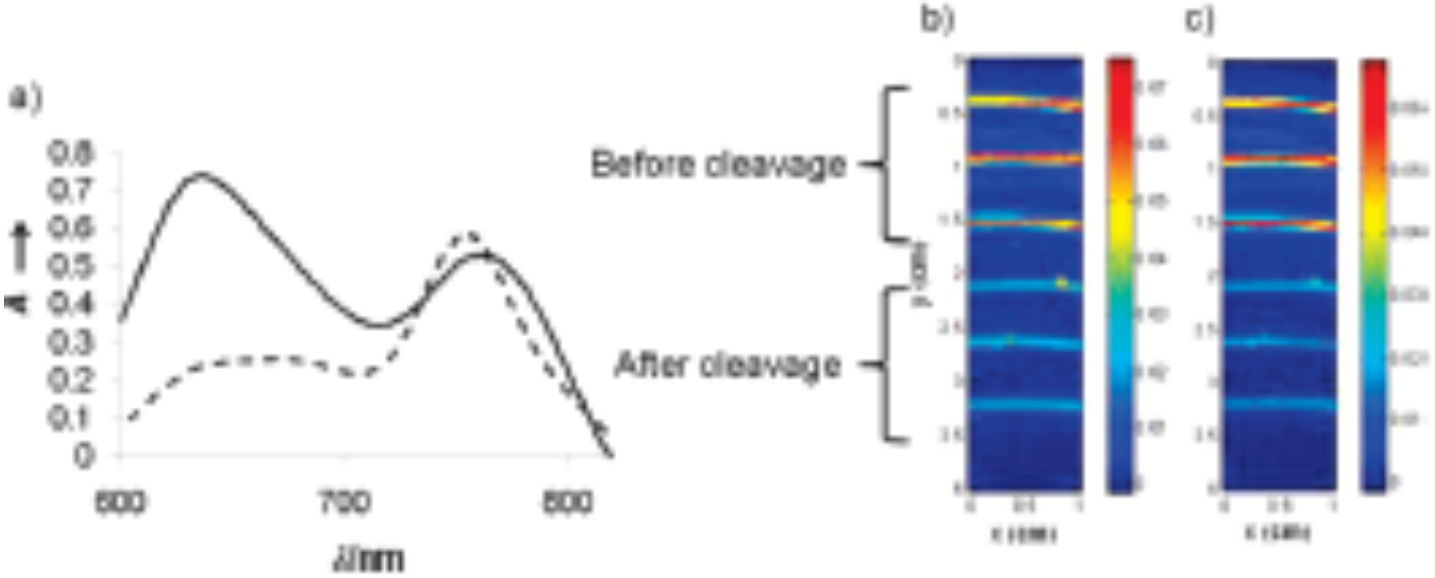
- First row, B-A protein
- Second row, Q-H protein



probe: Results

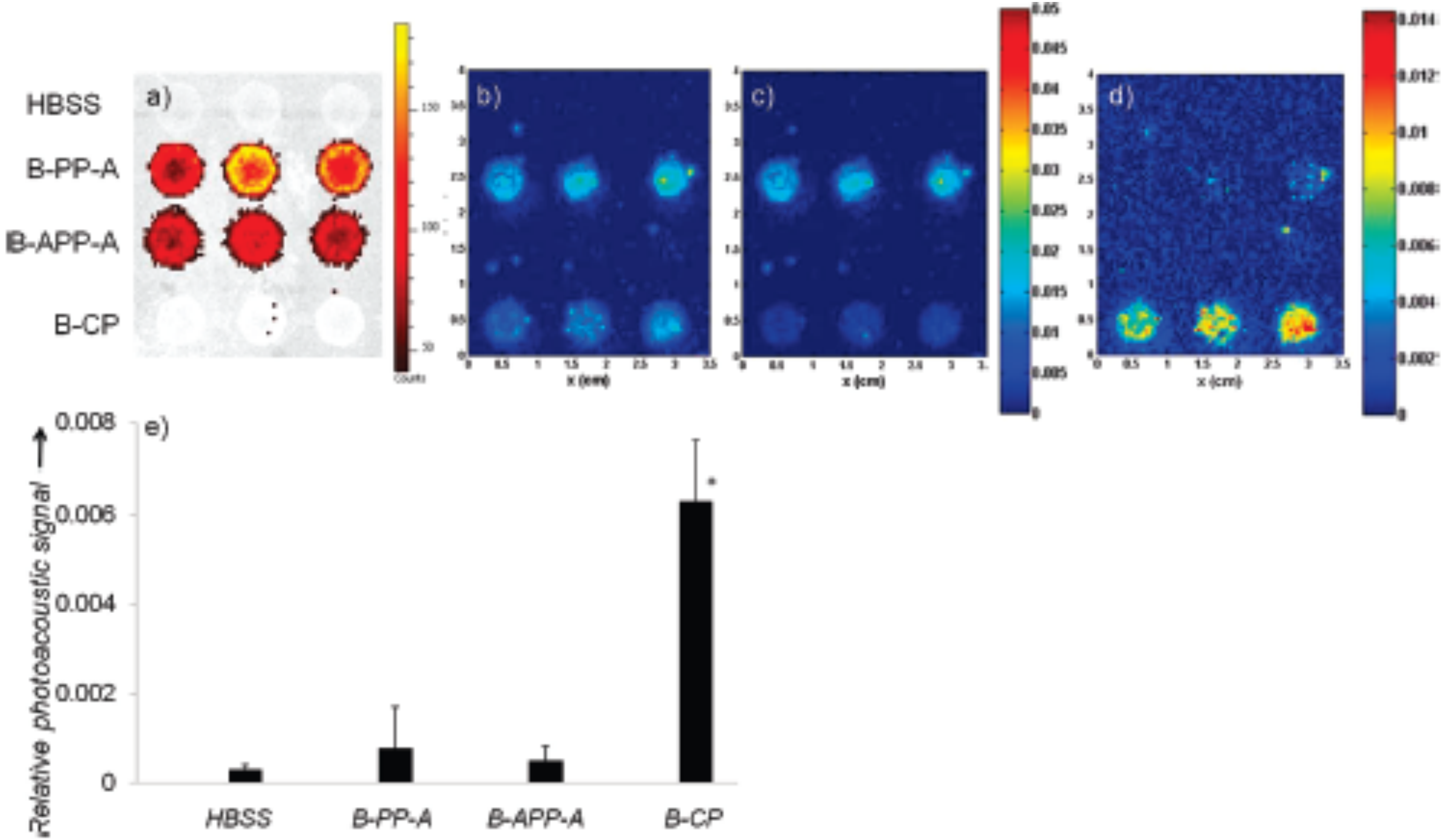
- “We have found that the photoacoustic signal did not correlate with the absorbance and fluorescence of the molecules, as the highest photoacoustic signal arose from the least absorbing quenchers…”

probe: Results



- (B-A) One peak disappears post cleavage|b/c 675/750nm

probe: Results



probe: Assessment

- Walks through process of designing photoacoustic probe-dye
- Again, dual wavelength imaging
- Drawbacks
  - Realism of phantoms
  - Constrained wavelength characterization