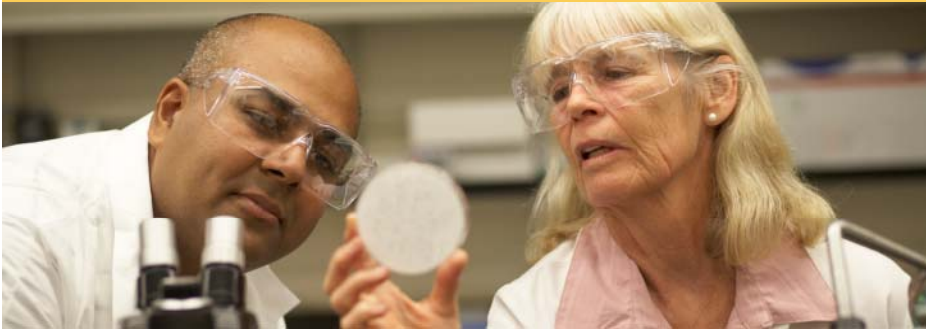


**SRI International**



# Detection of *Aspergillus* proteolytic activity for diagnostics

Amit Galande, Ph. D.  
Nov. 16, 2009

# The Group



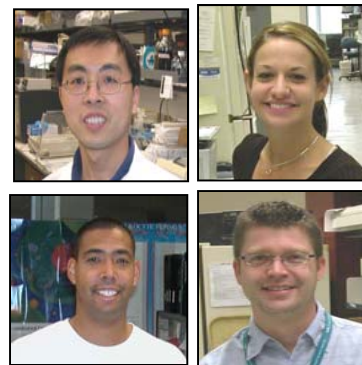
Amit Galande



- Douglas Watson
- Jennifer Miller
- Gabriela Molina
- Benben Song
- Patrick Kibler



David Askew



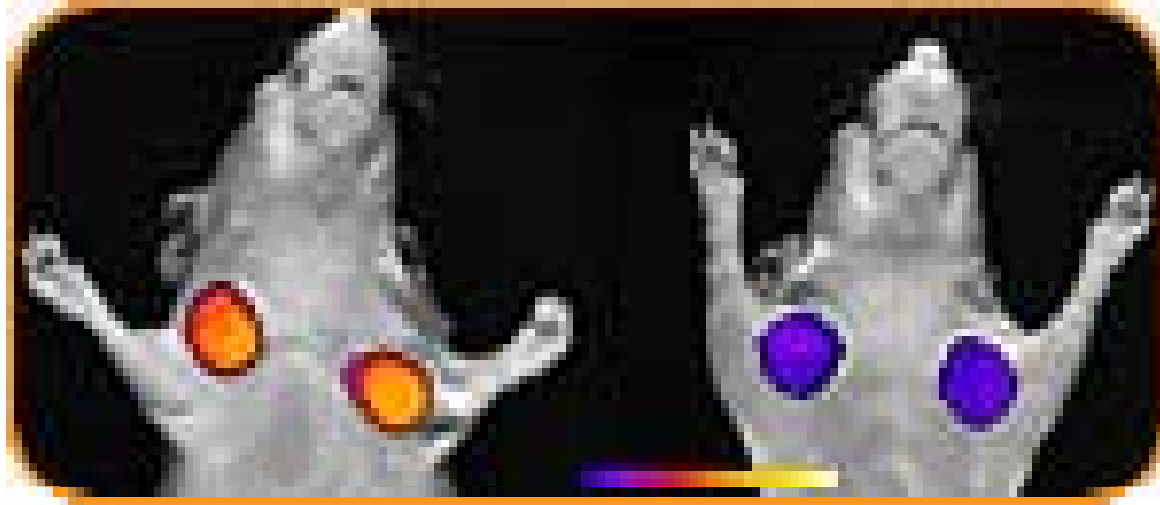
- Jerry Feng
- Daryl Richie
- Mike Miley
- Stephanie White



# Secreted proteases as biomarkers for Aspergillosis

- Absorptive mode of nutrient acquisition requires extensive secretion of hydrolases
  - Environmental niche
  - Host
- *A. fumigatus* has a highly developed secretory system
  - 1% of the genome encodes secreted proteases
- Evidence for protease secretion during infection
  - Metabolic studies
    - Af uses amino acids for a nutrient source in the host
  - Expression studies
    - Af protease genes expressed in vivo (mRNA/protein)
  - Virulence studies
    - Mutants with defects in the secretory system are hypovirulent
- Unique thermostable and thermophilic proteases are likely among secreted hydrolases in *A. fumigatus*

# Proteases as targets for diagnostics in cancer

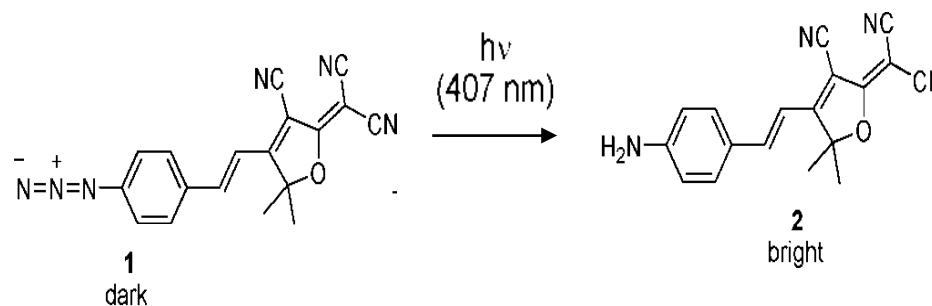
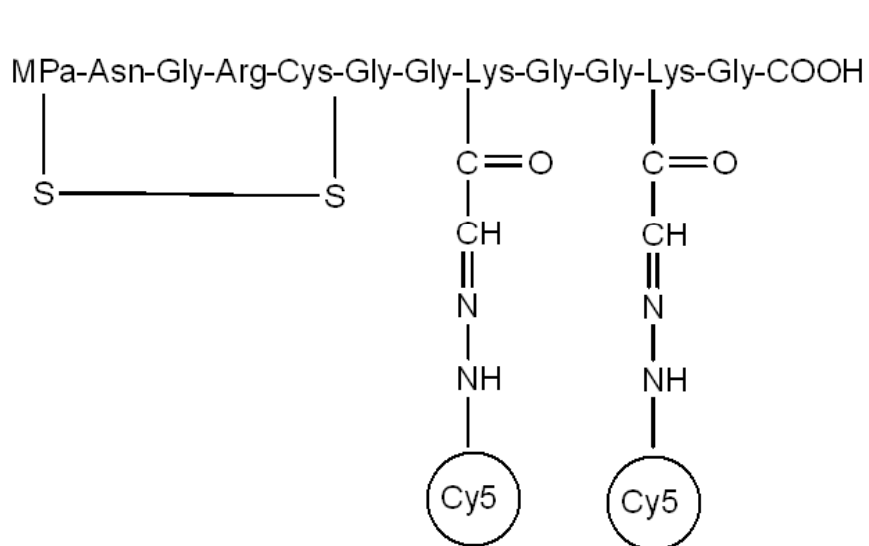
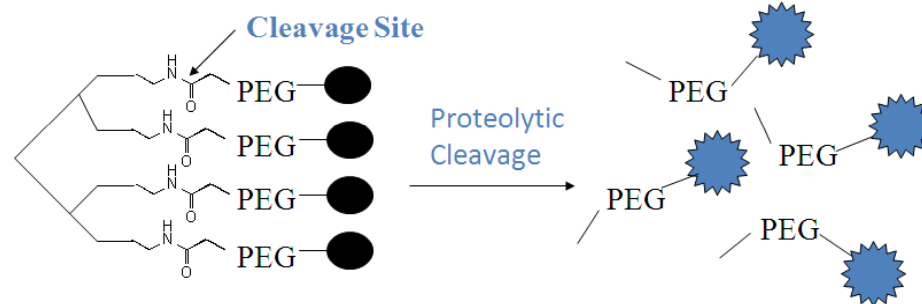
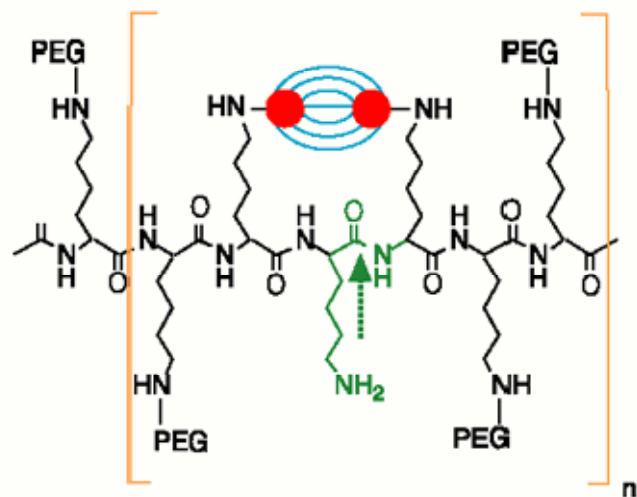


Fluorescence signal from the overexpression of MMP-2

No fluorescence signal following administration of MMP-2 inhibitor

Bremer, C. et al. *Nature Med.* 2001, 743

# Examples of "Smart" Fluorescence probes



Galande, A. K. et al. *J. Med. Chem.* 2006, 4715  
Galande, A. K. et al. *Bioconjugate Chem.* 2006, 255

# Our Approach:



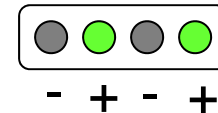
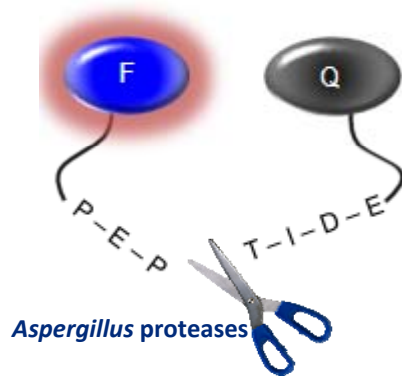
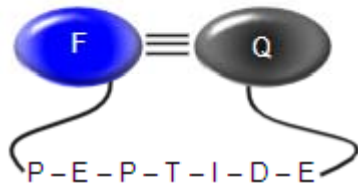
Apex 396: Synthesis of combinatorial libraries of fluorescence probes



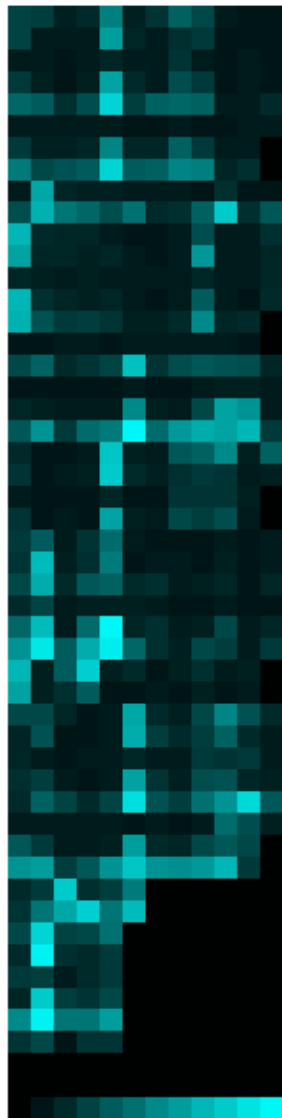
HT Analyst: High-throughput screening of fluorescence probes



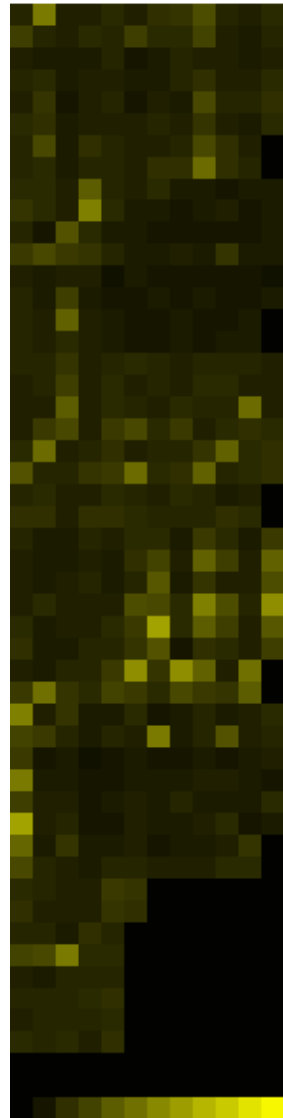
ABI 4800: Mass spectrometry-based deconvolution of fluorescence probes



# Preliminary Data



A. fumigatus

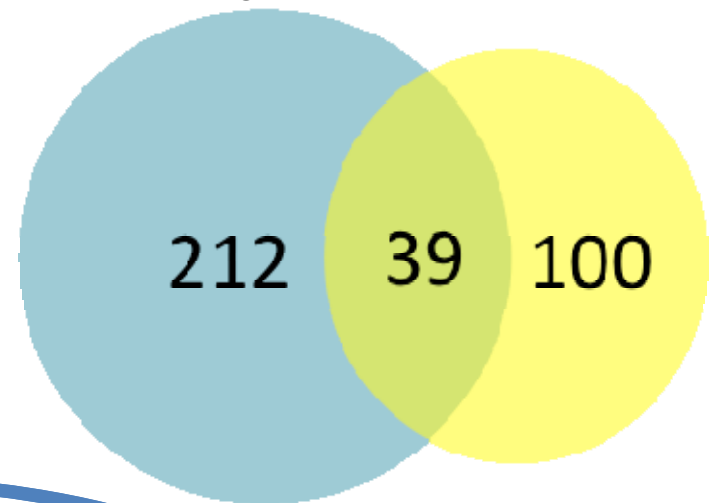


Human serum



Candidates

Number of wells cleaved by:  
A. fumigatus      Human serum



*A. fumigatus* diagnostic candidates:

Fold change	Number of wells	Percent
2 to 4	58	11.3
4 to 6	24	4.7
6 or more	10	2.0

Data generated and slide created by Douglas Watson



## Next Steps:

- Test additional chemical and biological combinatorial substrate libraries to improve specificity of diagnostic fluorescence probes
- Test additional FRET and fluorophore-quencher pairs to improve sensitivity of diagnostic fluorescence probes
- Screen our best candidates with biological samples from infected animals in collaboration with IAAM
- Optimize our best candidates with biological samples from human patients in collaboration with AsTec