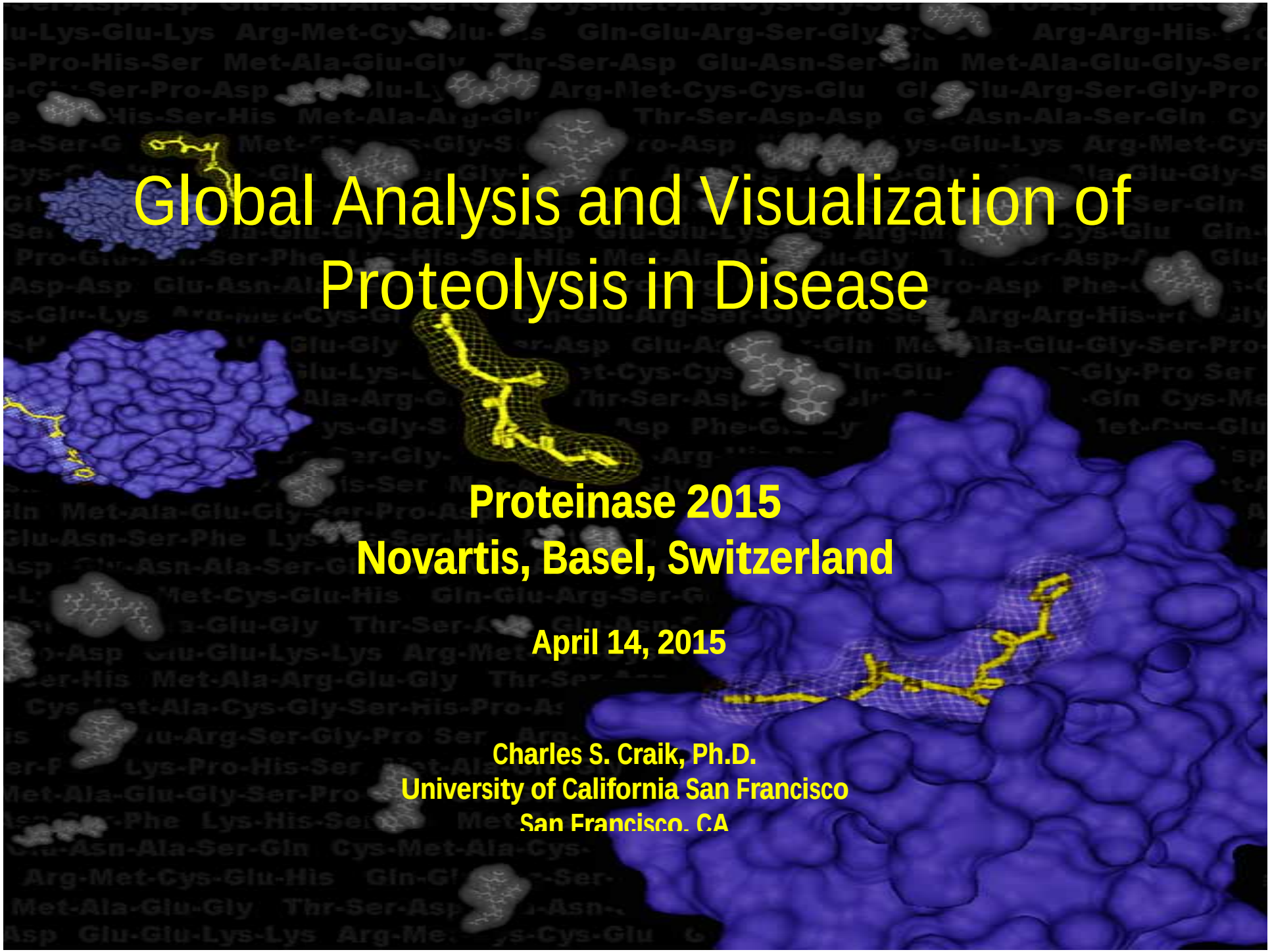
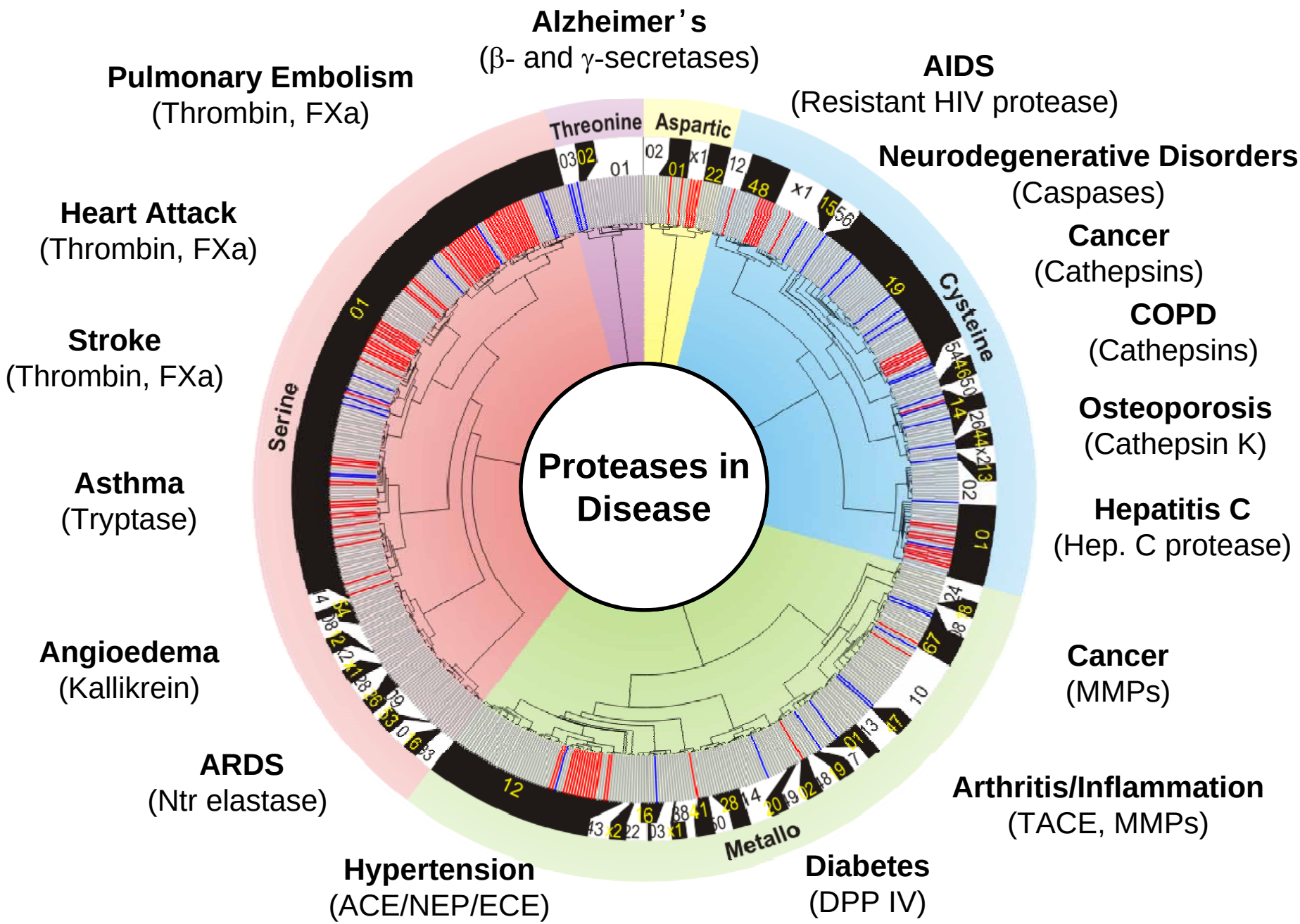
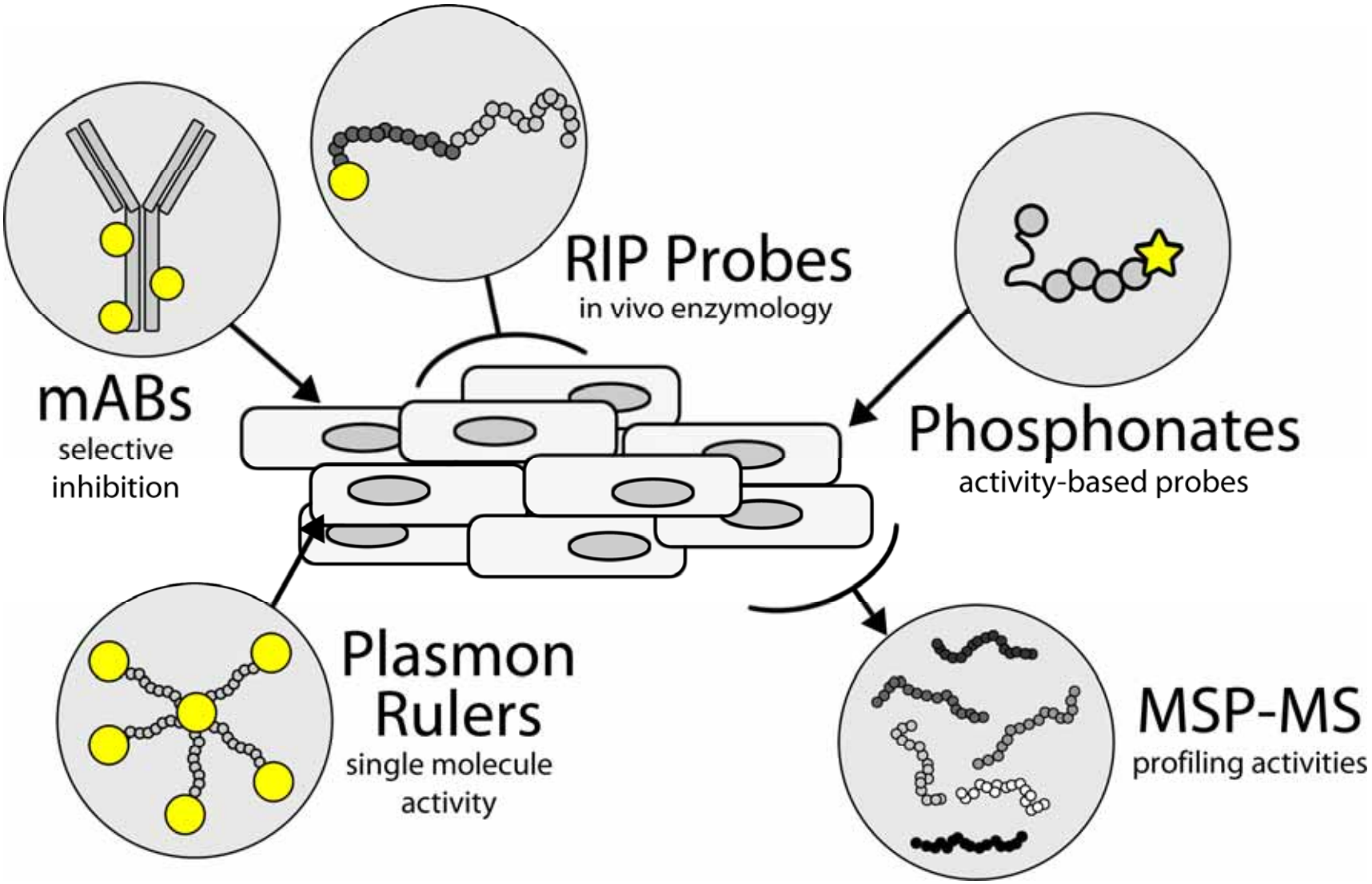

Global Analysis and Visualization of Proteolysis in Disease
Proteinase 2015
Novartis, Basel, Switzerland
April 14, 2015
Charles S. Craik, Ph.D.
University of California San Francisco
San Francisco, CA



http://www.unioviedo.es/degradome/tables/wheel.html

Probing Cell Function with Proteolysis



Melody Lee

Mike Page

RIP Probes
in vivo enzymology

mABs
selective
inhibition

Phosphonates
activity-based probes

Plasmon
Rulers
single molecule
activity

MSP-MS
profiling activities

Anthony O'Donoghue

Outline

- Description of MSP-MS and what it can do
- Two examples protease mediated molecular imaging
- Renewable antibodies to conformational states of enzymes and membrane proteins
- Targeting a conformational state of a target protease

For any enzyme:

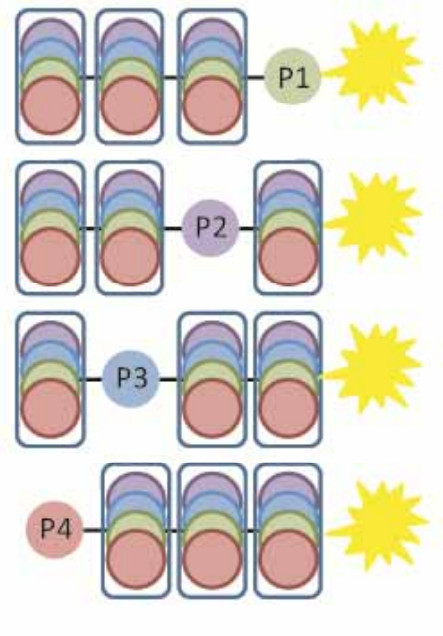
What is the chemical reaction?

What is its substrate and how does it recognize it?

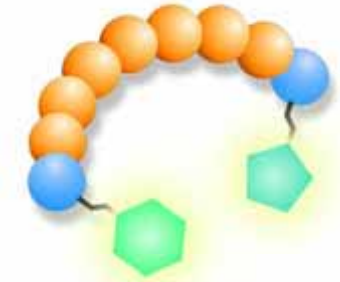
What is the biology associated with the activity?

There are multiple technologies to profile Protease Substrate Specificity

Non-Prime Only

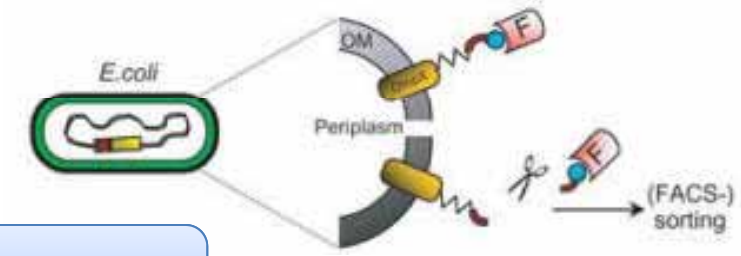


Combinatorial Library

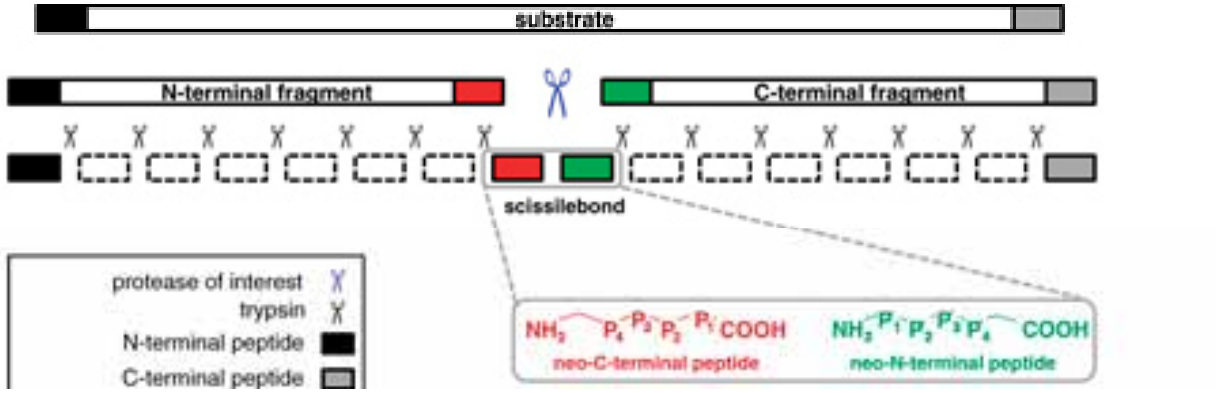


FRET Library

Prime and Non-prime



Peptide Display



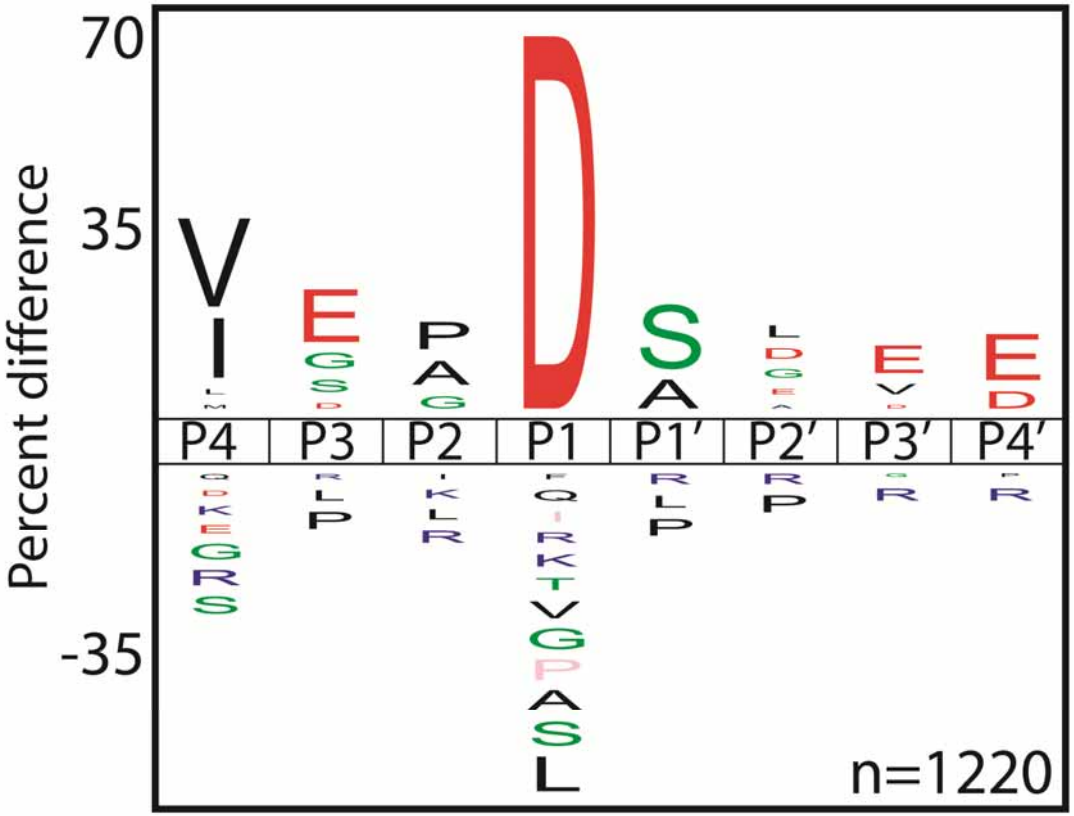
Proteome derived proteins and peptides

Ideal technology:

- is compatible with **endo-** and **exo-**acting proteases
- can probe **prime** and **non-prime** sites
- can profile **multiple** enzymes **simultaneously**
- is **quantitative**

Hypothesis: Substrate recognition is determined by two optimally positioned residues

V/I-E-*-*-D-S/A-*-*-E 43%

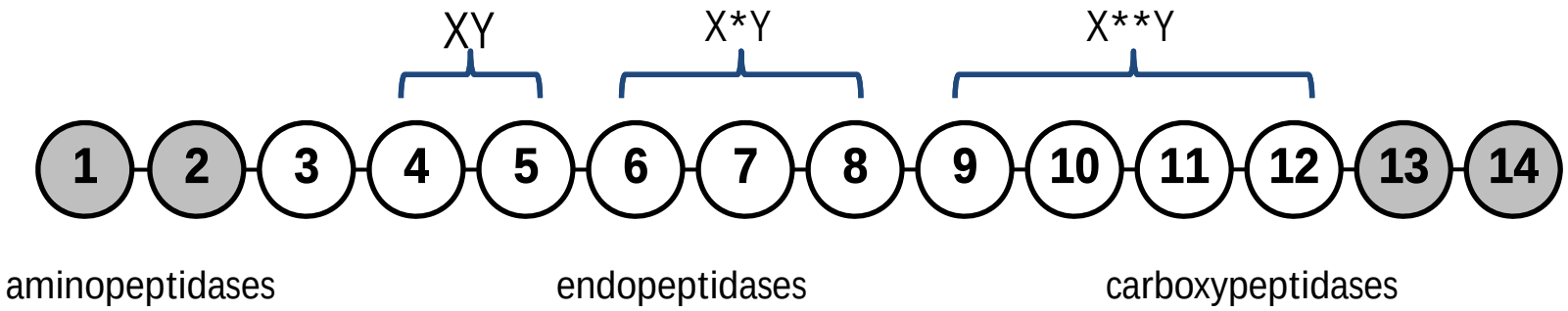


Granzyme B

Van Damme P., Mol Cell Proteomics (2009);
Van Damme P., Nat Methods (2010);
Plasman K., Mol Cel Proteomics (2011)

Multiplex Substrate Profiling by Mass Spectrometry (MSP-MS) Allows Global Substrate Profiling

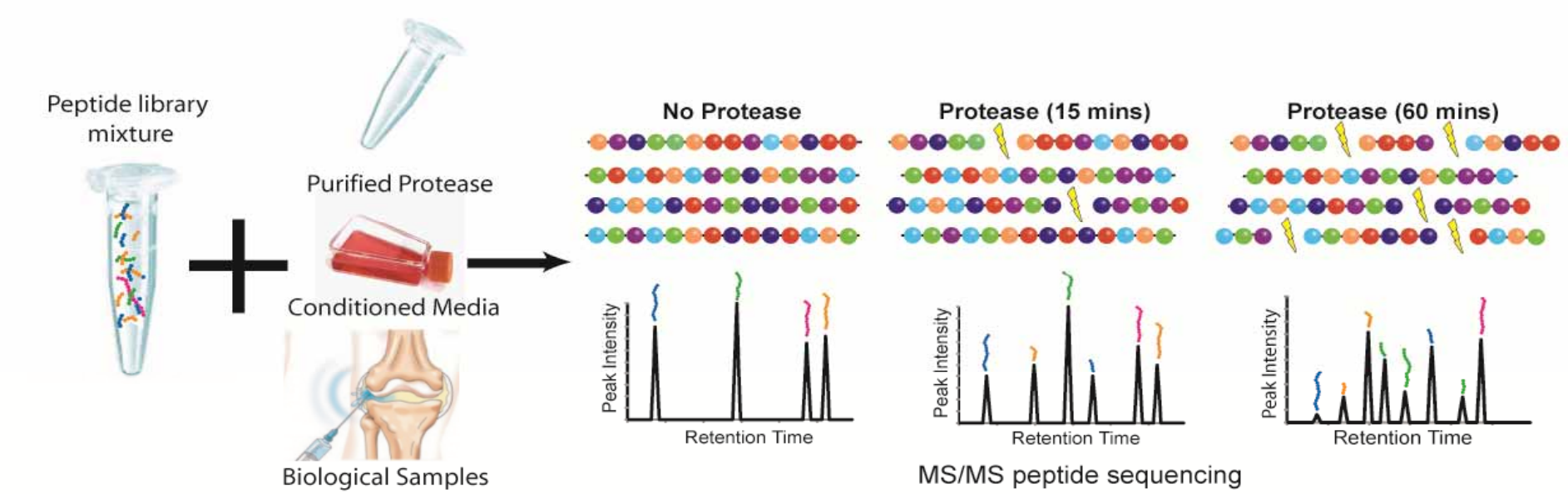
- Two-site hypothesis:
  - Substrate recognition for many proteases is dominated by **two optimally positioned residues**
  - Substrates can be recognized in a **linear epitope**



- 19 amino acids (no Cys, Met substituted for Nle)
- 228 14-mer peptides

O'Donoghue et al. *Nature Methods.* (2012).

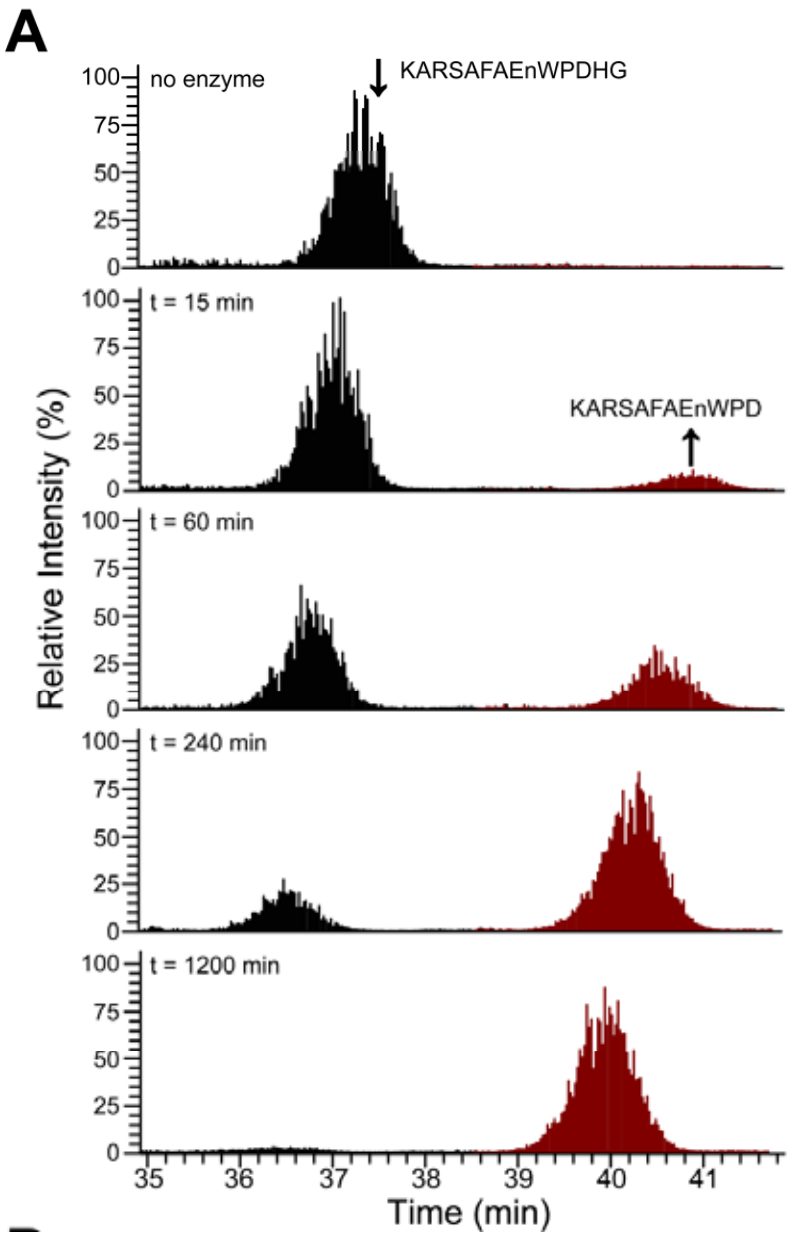
Multiplex **s**ubstrate **p**rofiling by **m**ass **s**pectrometry (MSP-MS) provides proteolytic signatures



O'Donghue et al. *Nature Methods* (2012)

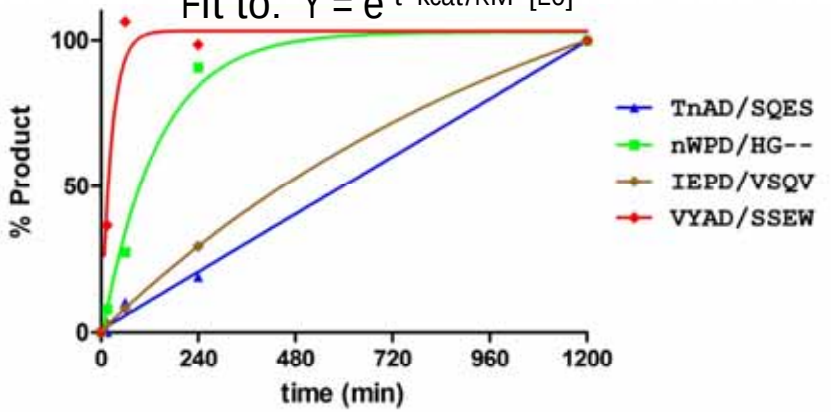
Granzyme B cleaves multiple peptides and kinetic values can be obtained from progress curves

Integrate Peak area from
extracted ion chromatograms



Progress curve

Fit to: $Y = e^{-t*kcat/KM*[E0]}$

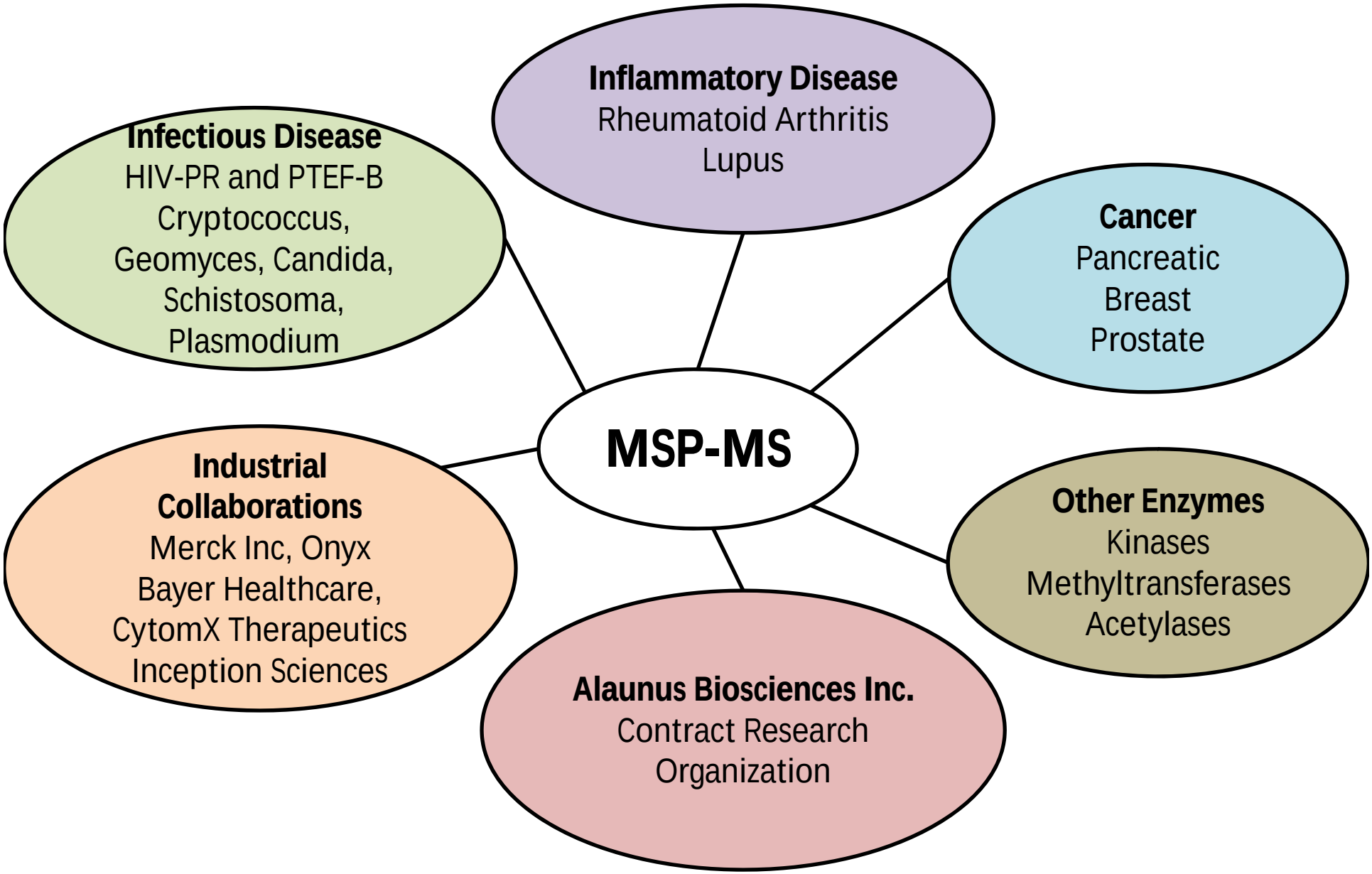


Substrate	k_{cat}/K_m (s^{-1} M^{-1})
KARSAFAEnWPD/HG	45,600
SFIEPD/VSQVKHLE	31,300
GWKTnAD/SQESARD	4,070
KHPLETVYAD/SSEW	**127,000**
Previously Published	
[1]abz-VVAD/SSMESK-dnp	116,000
[2]Ac-IEPD/WGA-NH_2	52.7

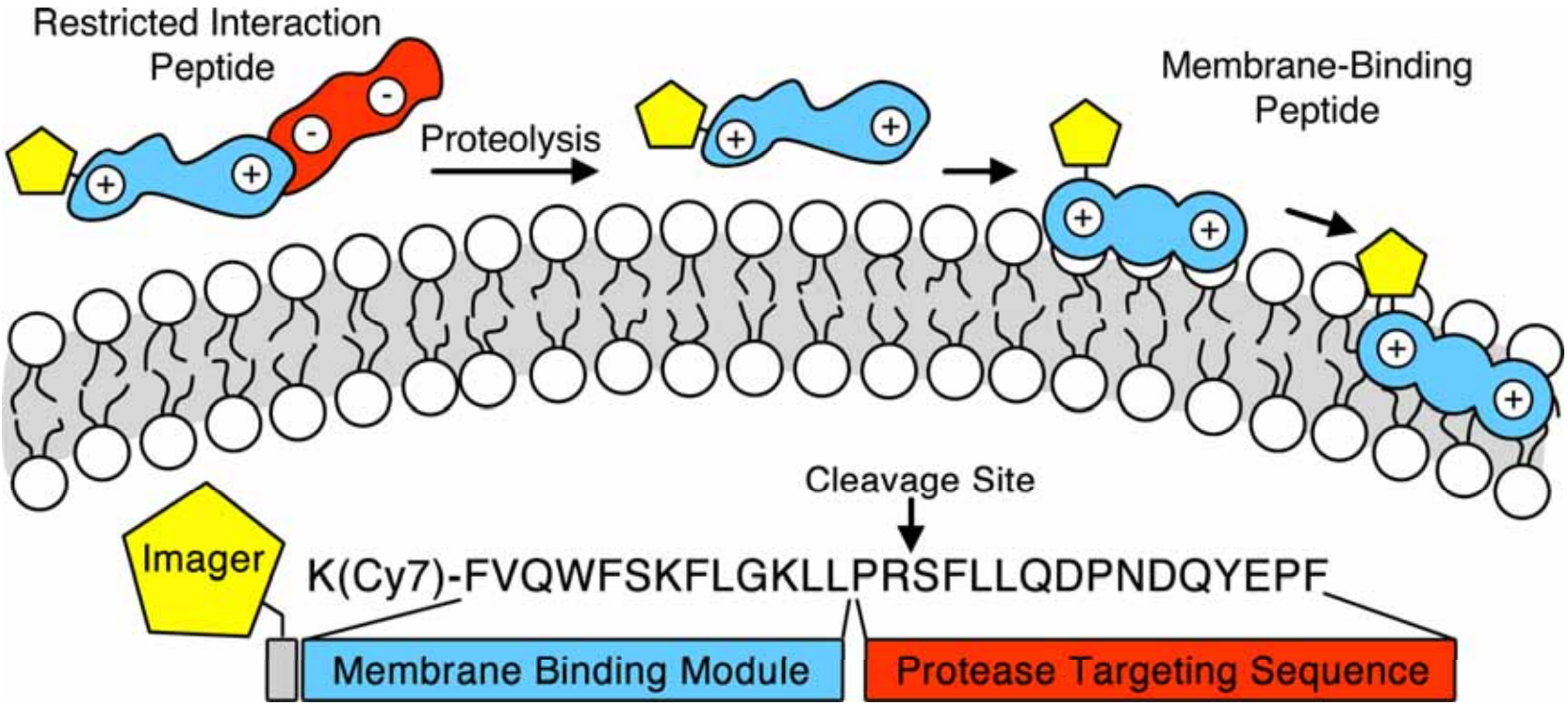
[1]Sun et al. (2001) JBC 276, 15177.

[2]Harris et al. (1998) JBC 273, 27364.

MSP-MS is applicable to studying enzymes in a wide variety of diseases



Restricted Interaction Peptides (RIPs)

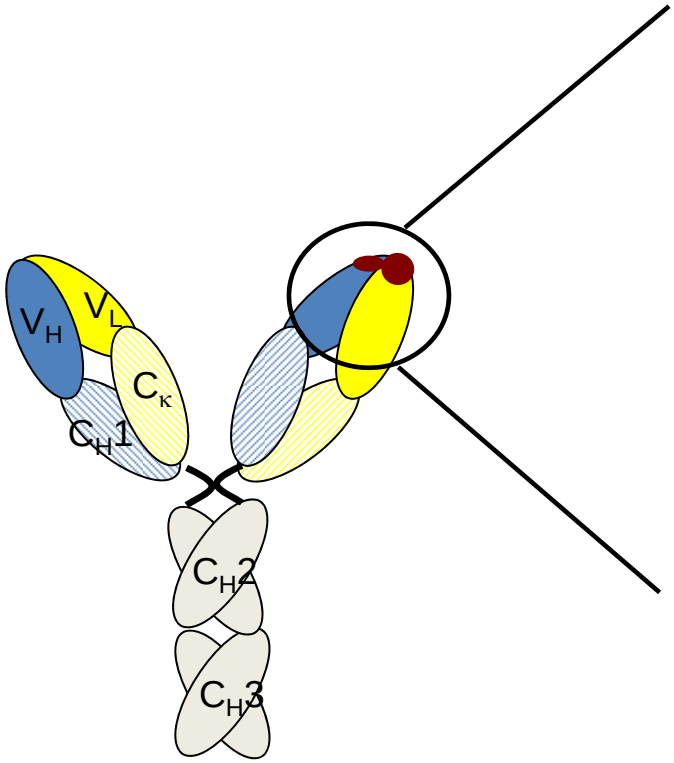


Affinity from Bigger Targeting Sequence
+ Imaging Agent
+ Membrane Insertion Peptide
Protease-dependent Interaction

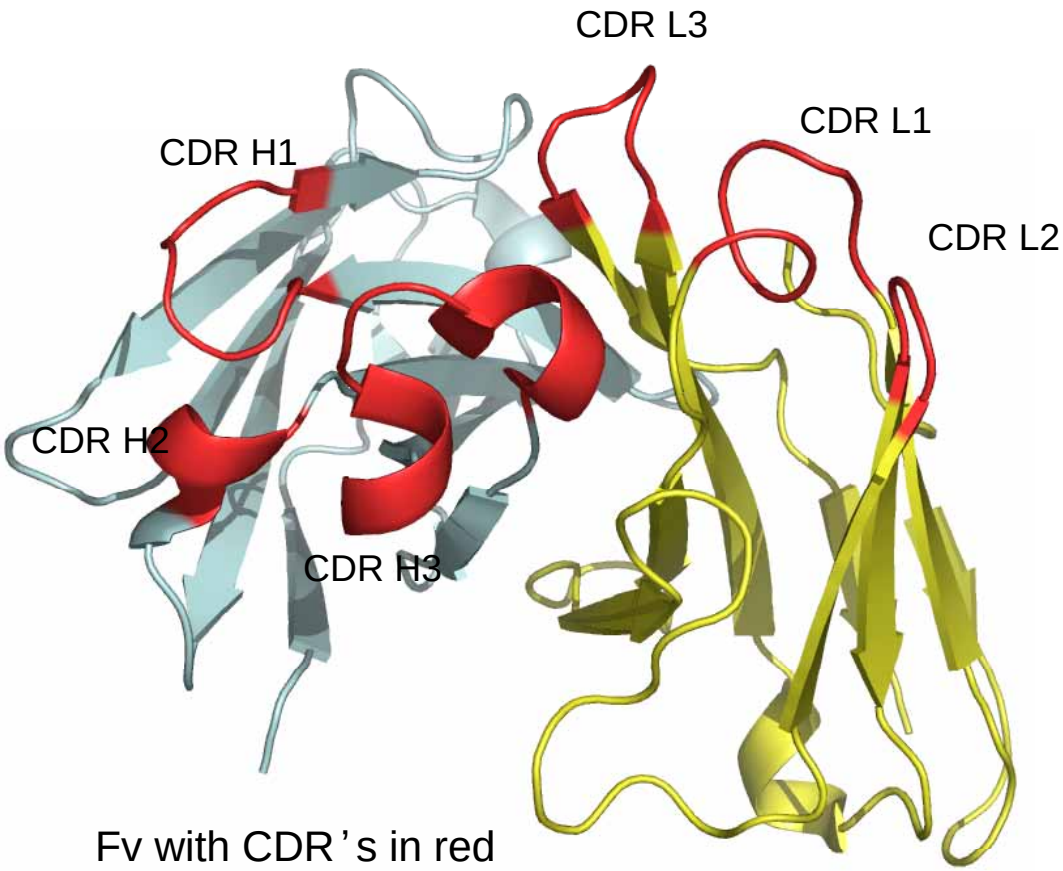
Highly specific probes are needed to dissect the complex biology of proteases and validate them as possible therapeutic or imaging targets.

Could also serve as a proof of principle for other extracellular enzymes and families of closely related proteins.

Antibodies differentiate between highly homologous antigens and can recognize conformational epitopes



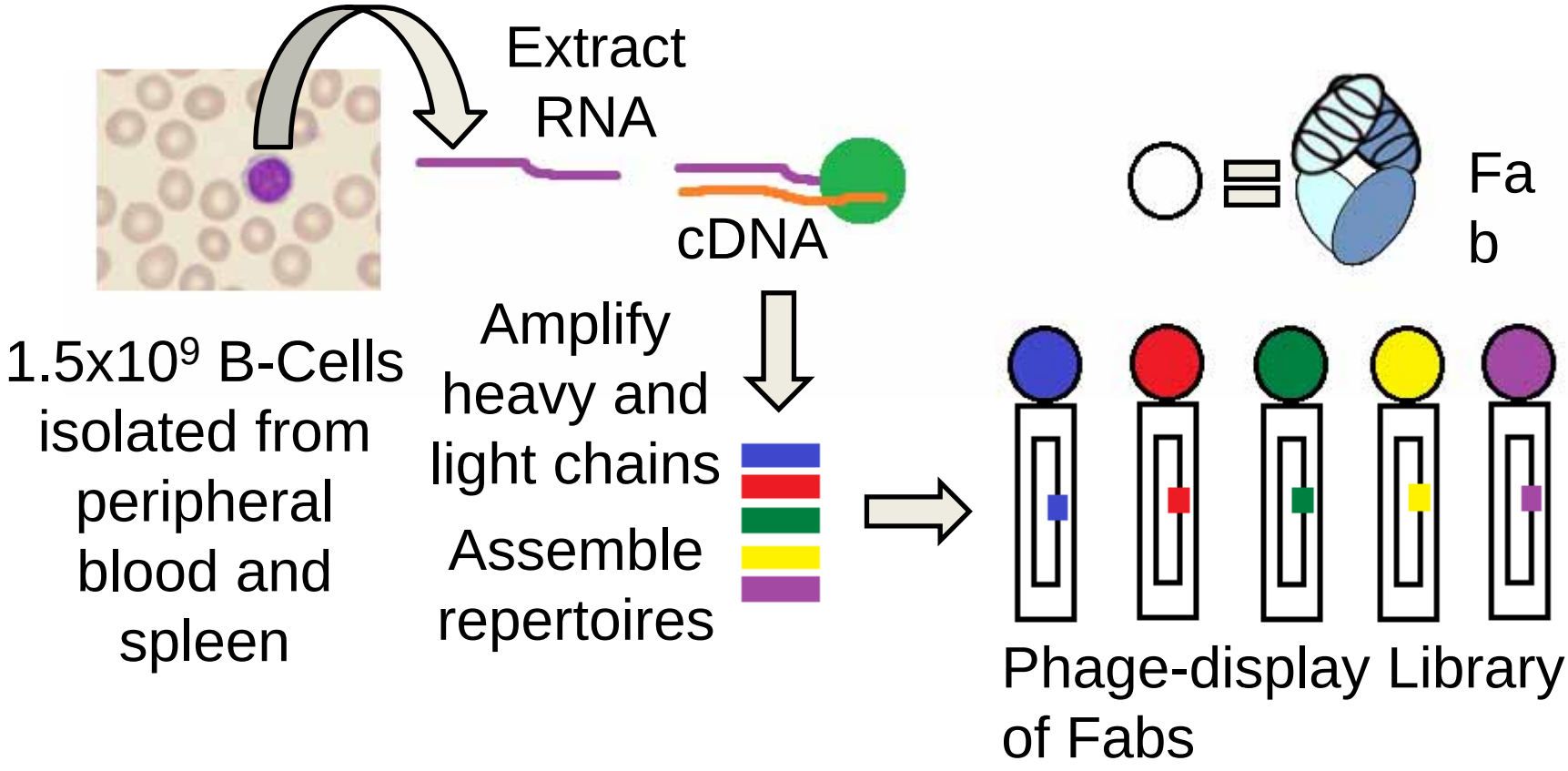
IgG- 150 kD



Fv with CDR's in red

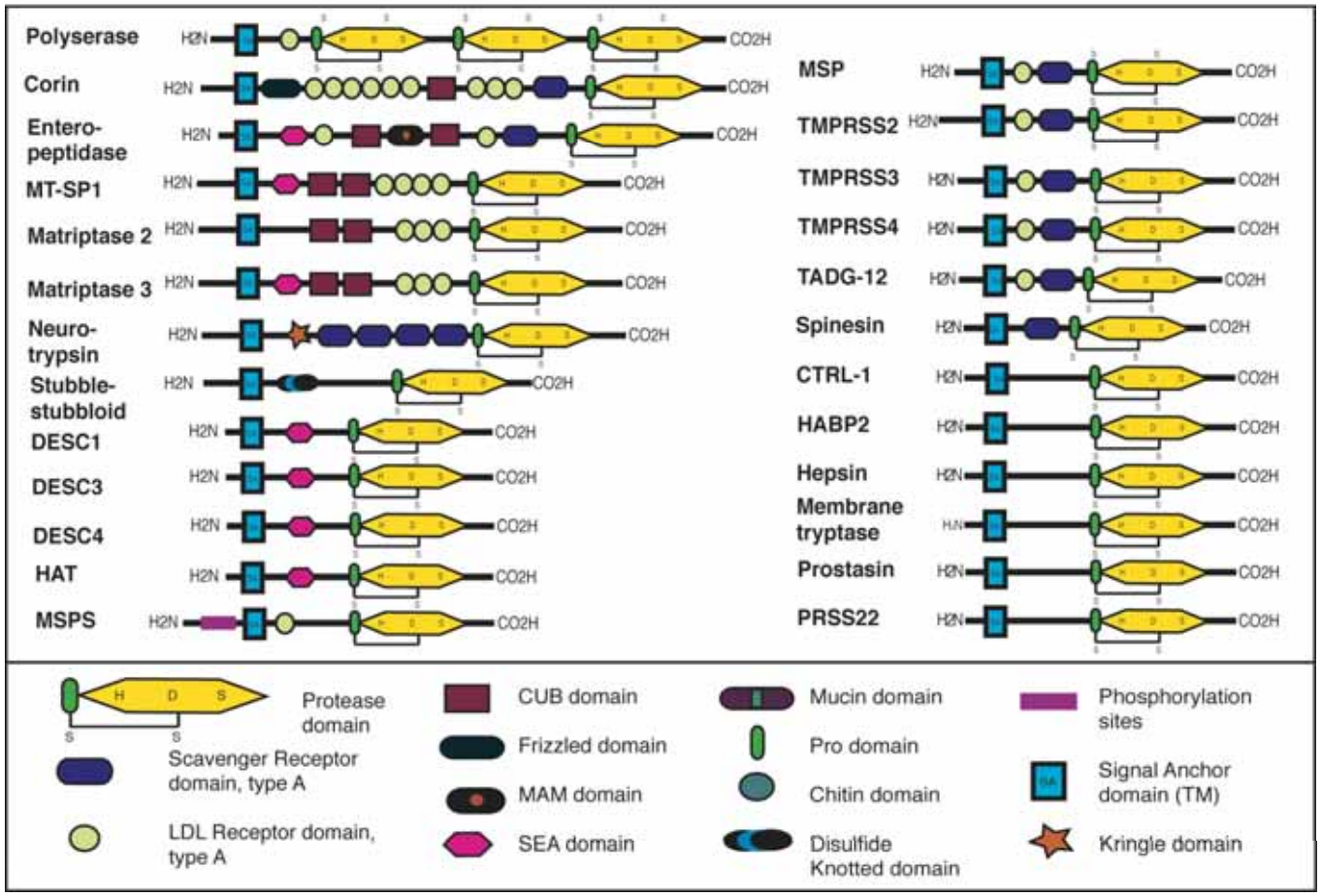
Farady et al, *J. Mol. Bio.* **369** (2007)
Farady et al. *J. Mol Bio* **380** (2008)
Schneider, et al. *J. Mol Bio* **412** (2012**)**

A Fully Human Natural Antibody Repertoire Was Generated from Naïve B-Cells



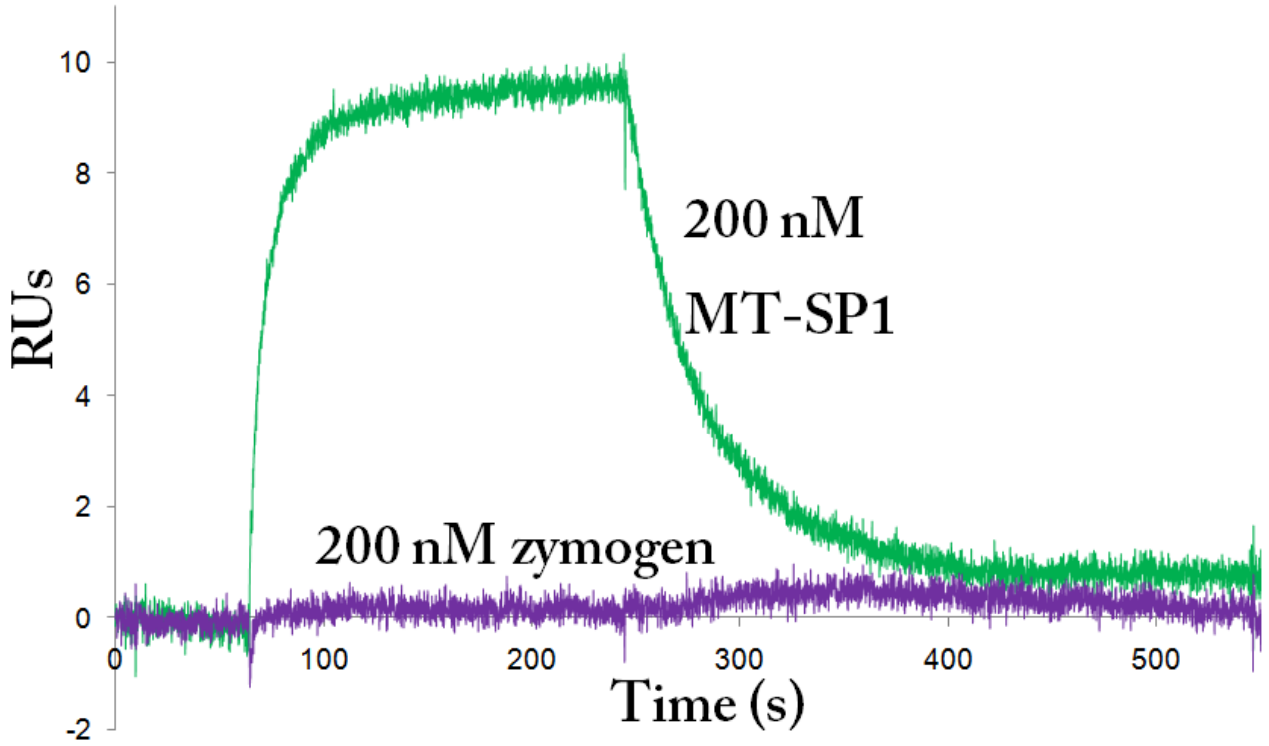
Duriseti, S. et al, JBC 2010

The type II transmembrane serine proteases (TTSPs) constitute a large family of promising targets

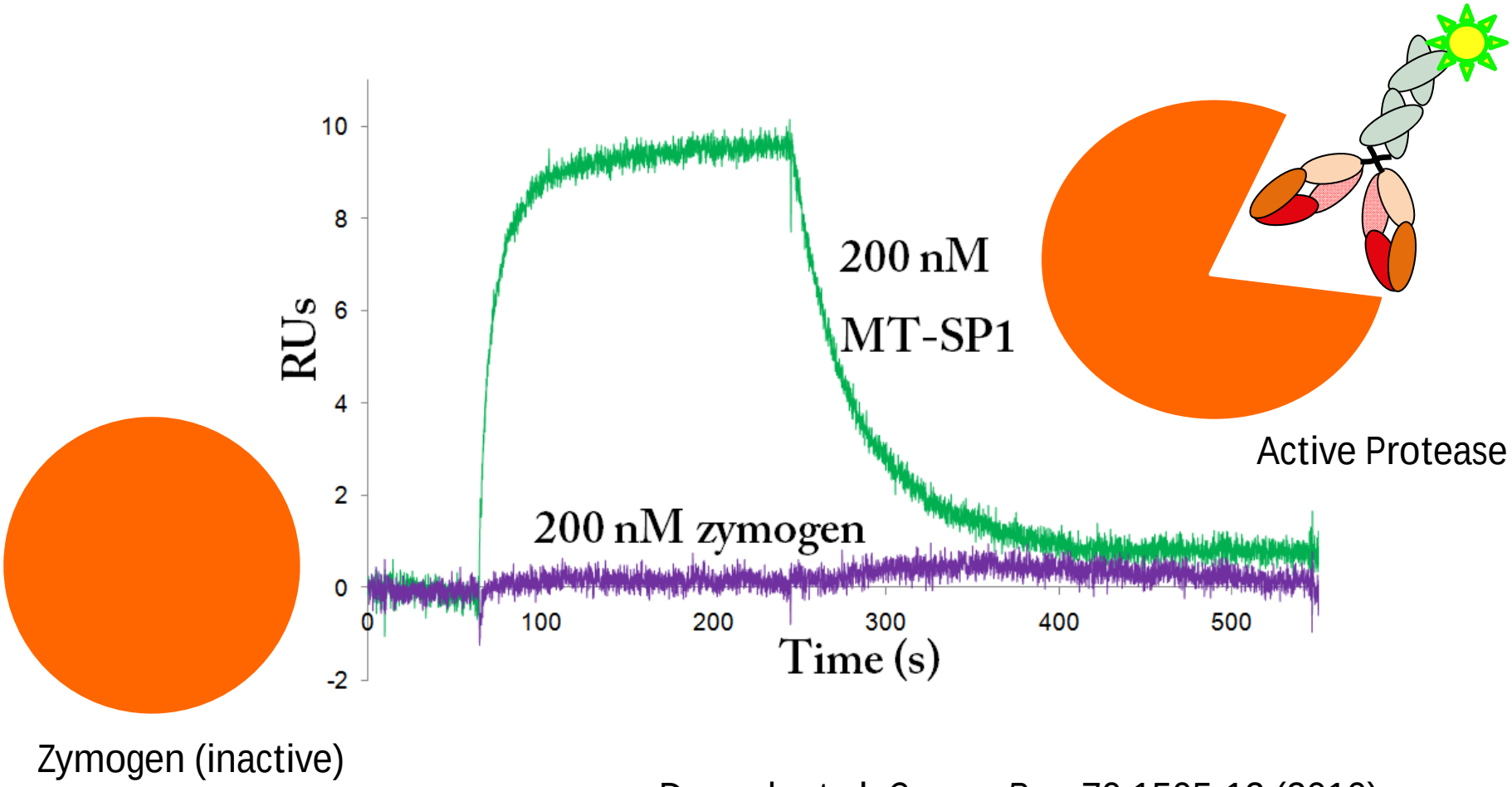


Bugge et al 2009, 20011

Anti MT-SP1/Matriptase Antibody is specific for the active form of the protease

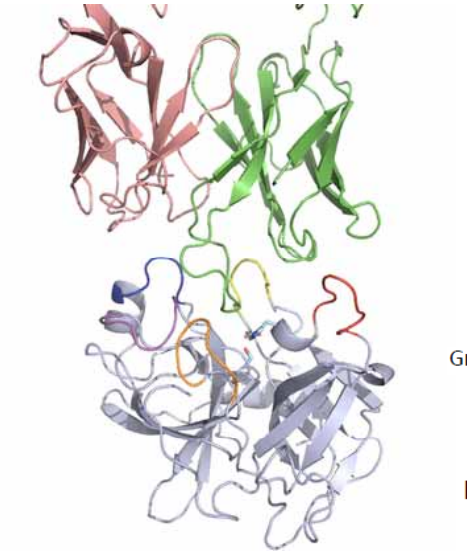


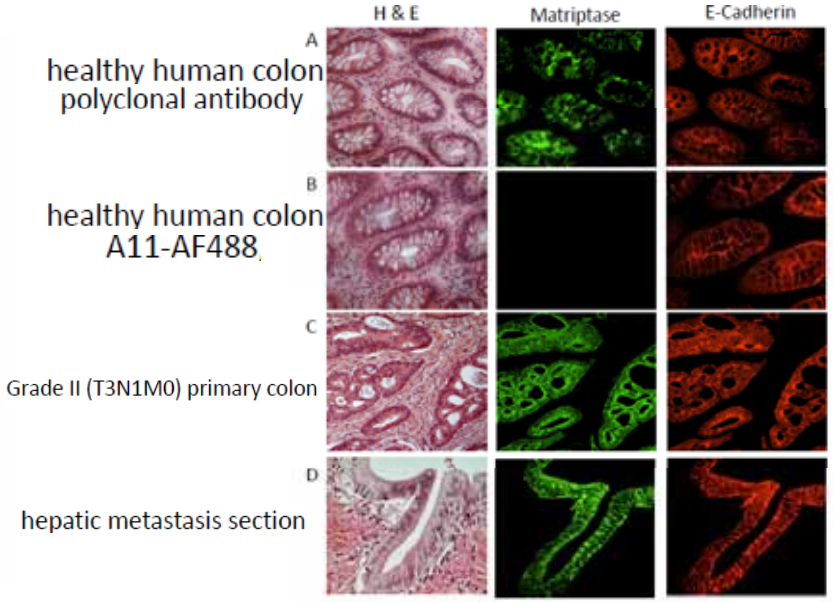
Anti MT-SP1/Matriptase Antibody is specific for the active form of the protease



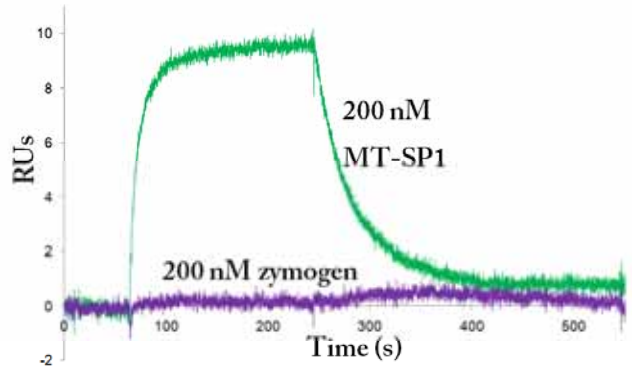
Darragh et al, *Cancer Res.* 70,1505-12 (2010)

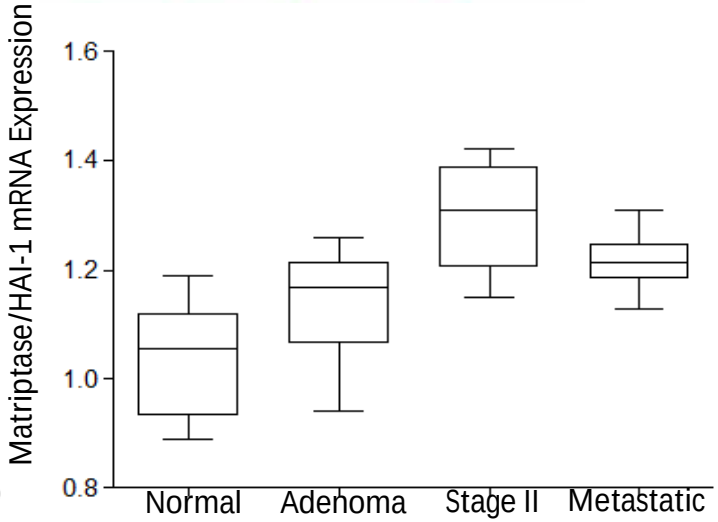
Antibody Based Probes to Extracellular Targets Provide Cellular Information





HT29-Colon



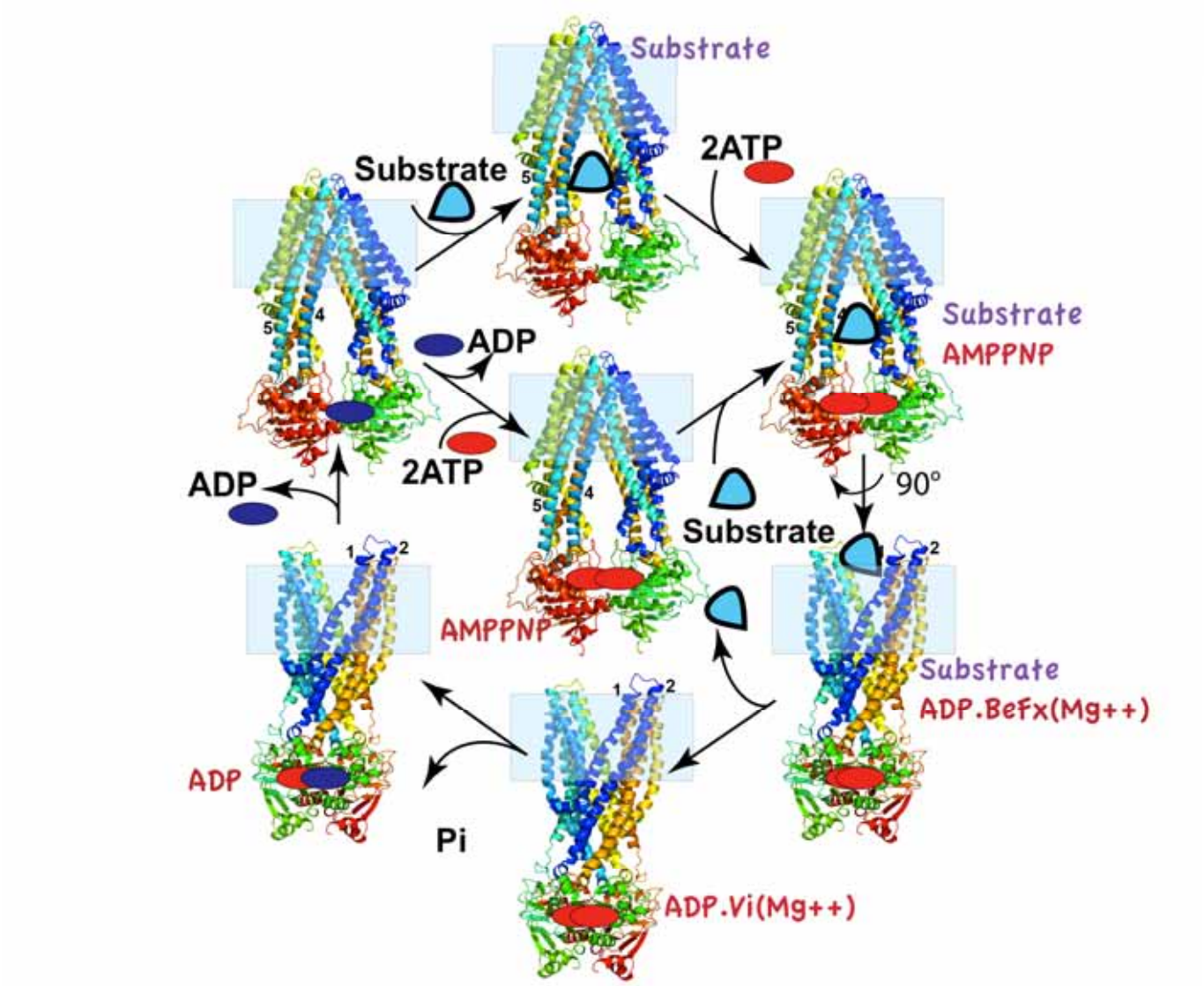


PDX-Colon

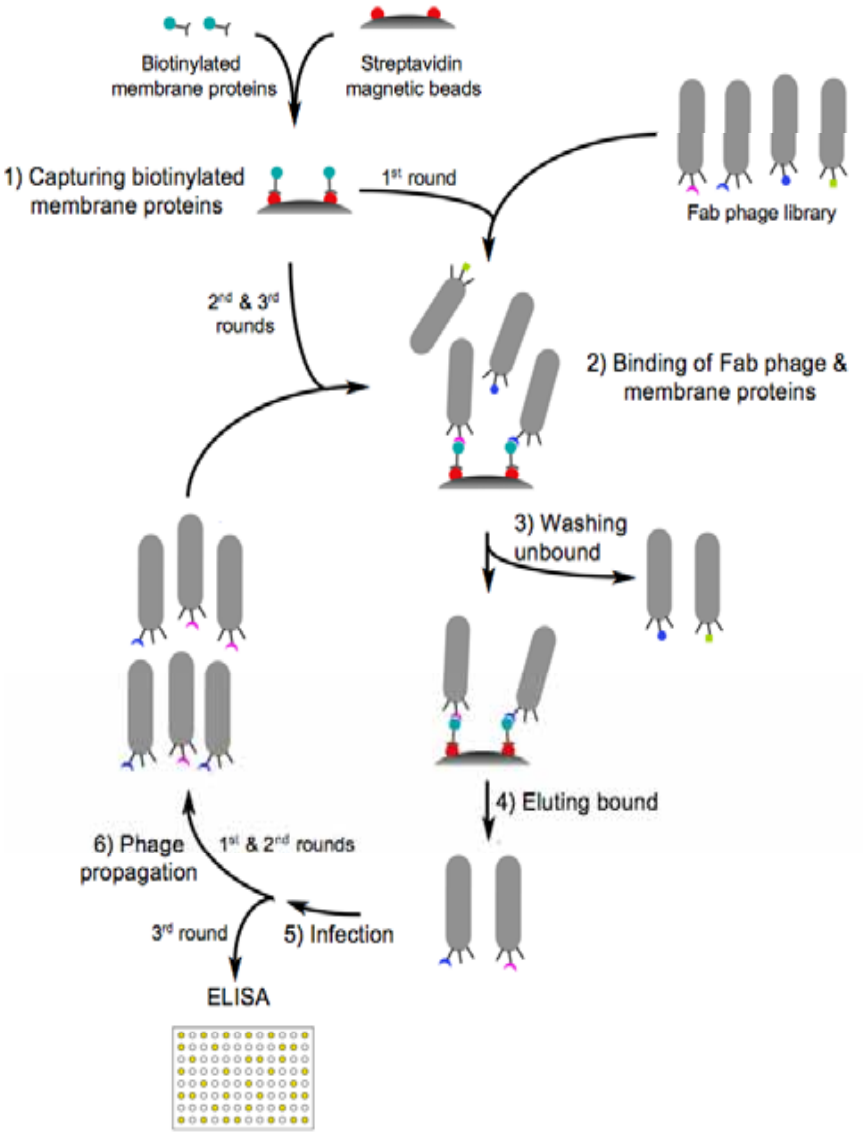
Schneider E, et al. *J. Mol Bio* **412** (2012)
LeBeau AM, et al. *PNAS* **110** (2013)

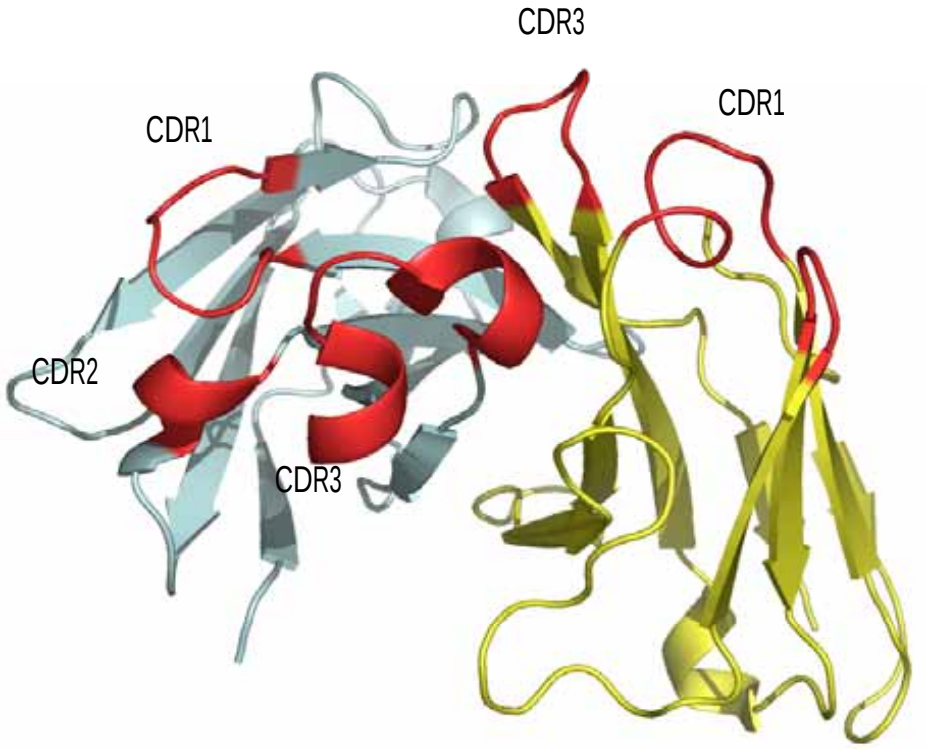
Can we expand this approach to other proteins and in particular membrane proteins?

Conformational states of the Pumping Cycle of an ATP Binding Cassette (ABC) transporter



Optimized phage display panning procedure for membrane proteins



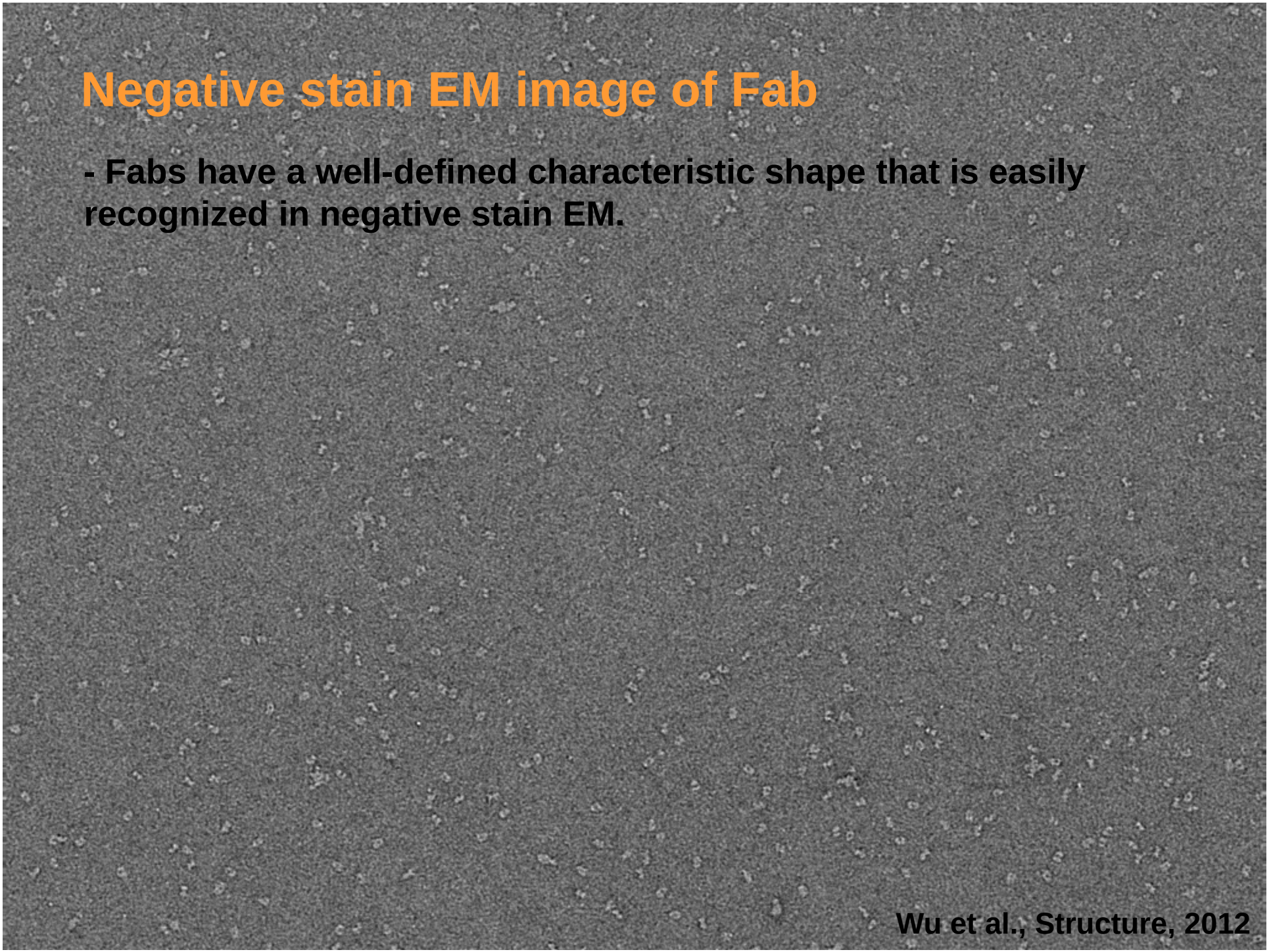


Fv with CDR's in red

- Kim et al. "Rapid identification of recombinant Fabs that binds to membrane proteins" Methods (2011) **55**, 303-309.

Negative stain EM image of Fab

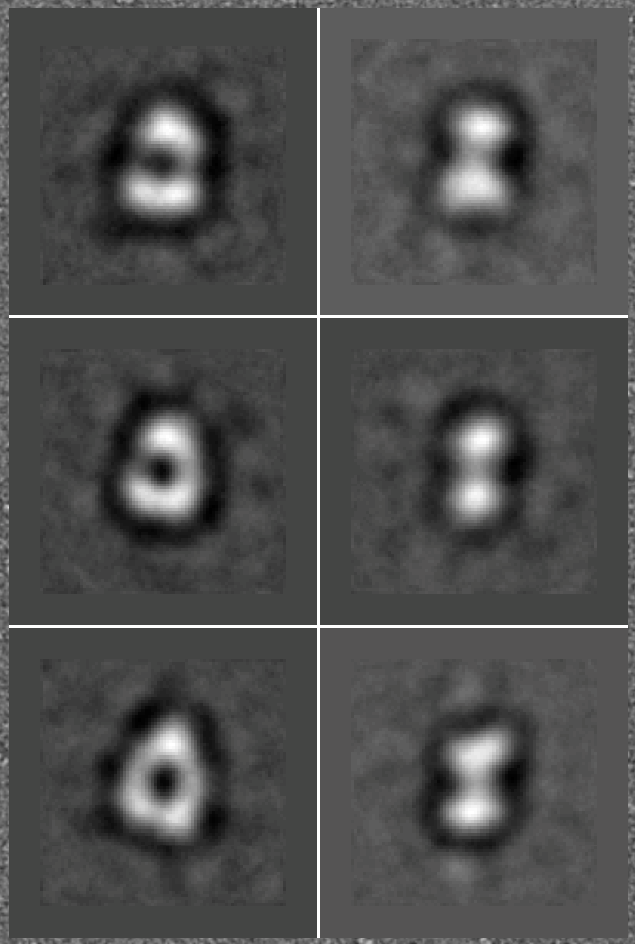
- Fabs have a well-defined characteristic shape that is easily recognized in negative stain EM.



Wu et al., Structure, 2012

Negative stain EM image of Fab

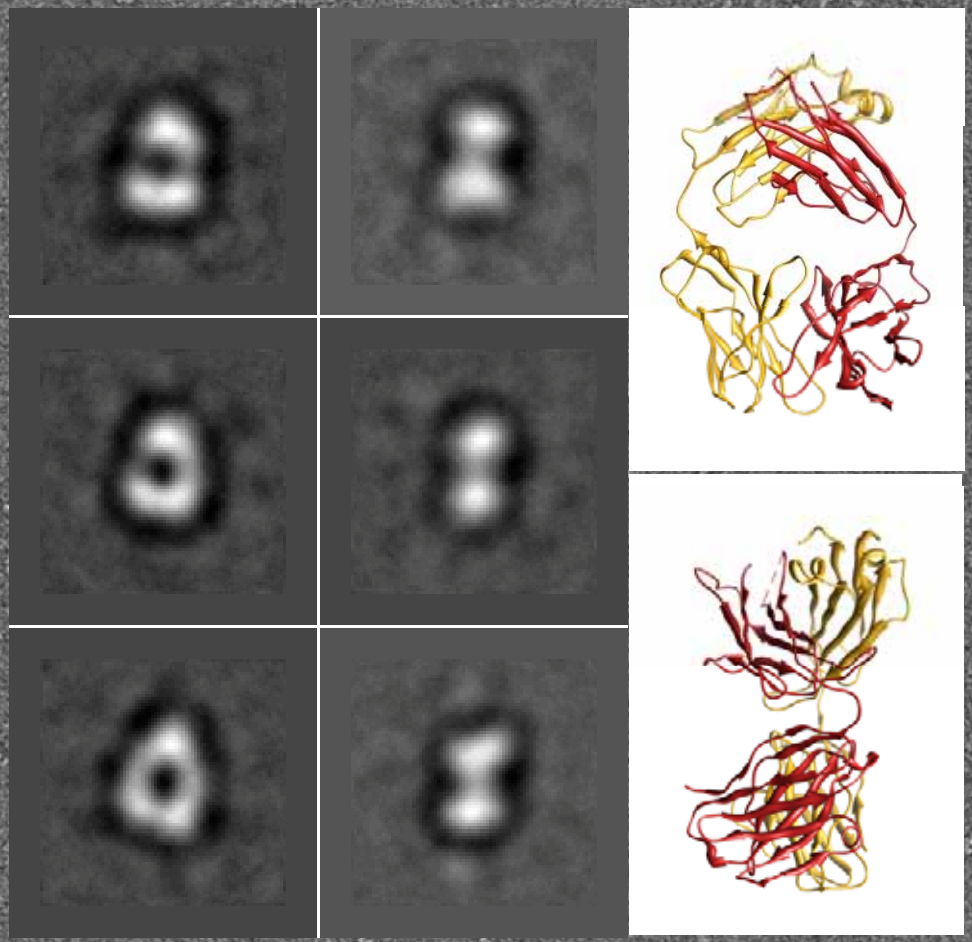
- Fabs have a well-defined characteristic shape that is easily recognized in negative stain EM.



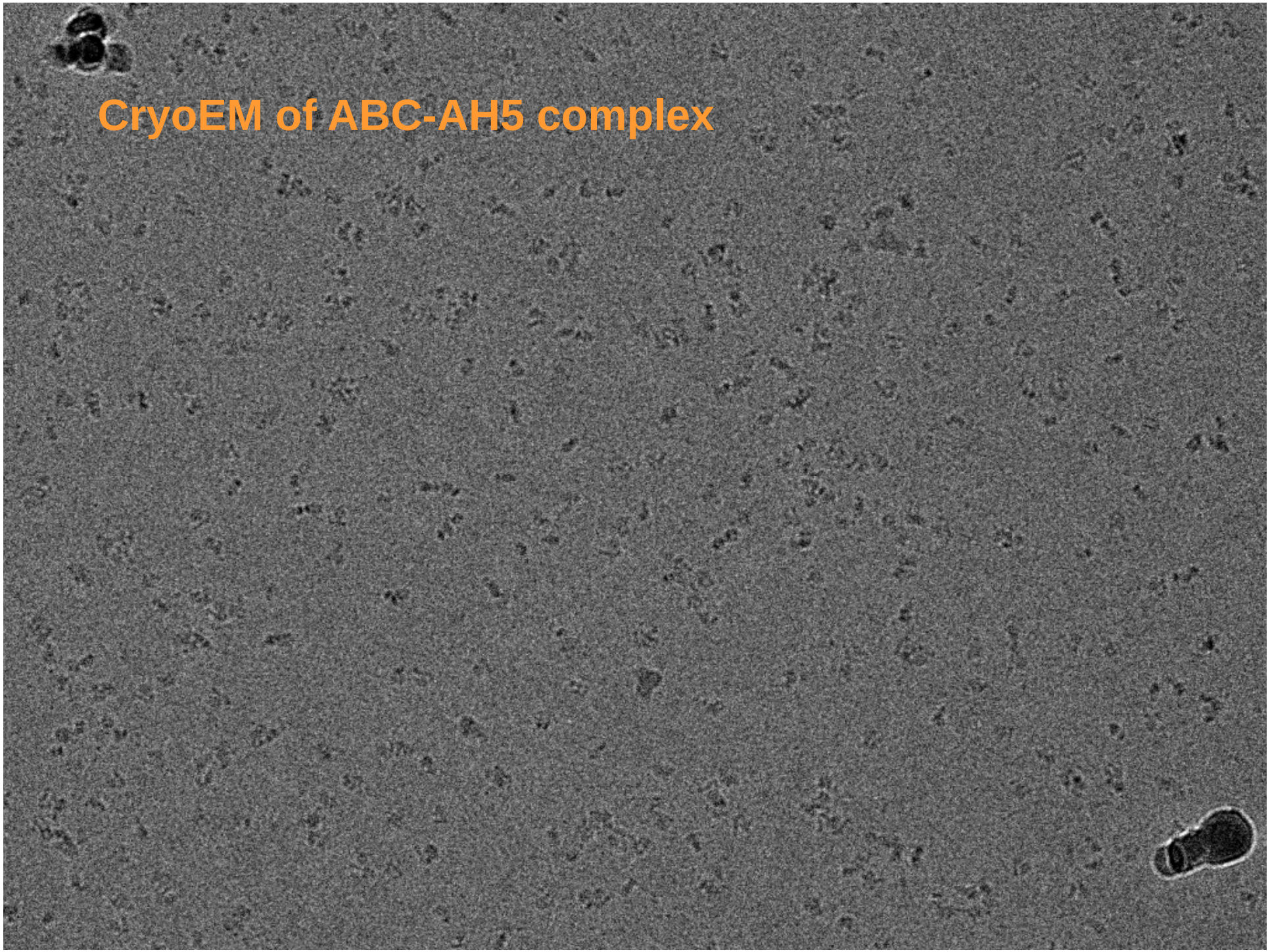
Wu et al., Structure, 2012

Negative stain EM image of Fab

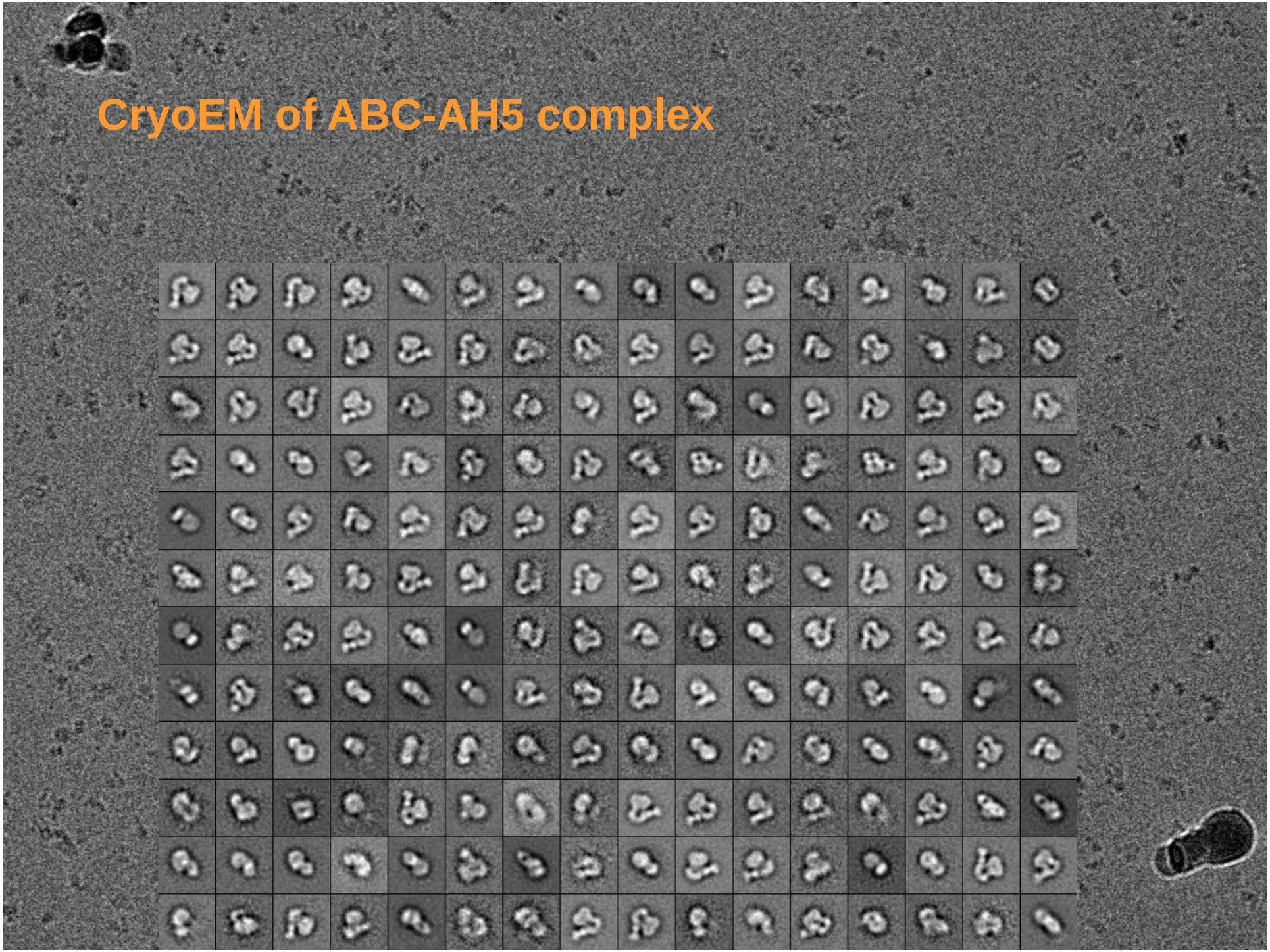
- **Fabs have a well-defined characteristic shape that is easily recognized in negative stain EM.**

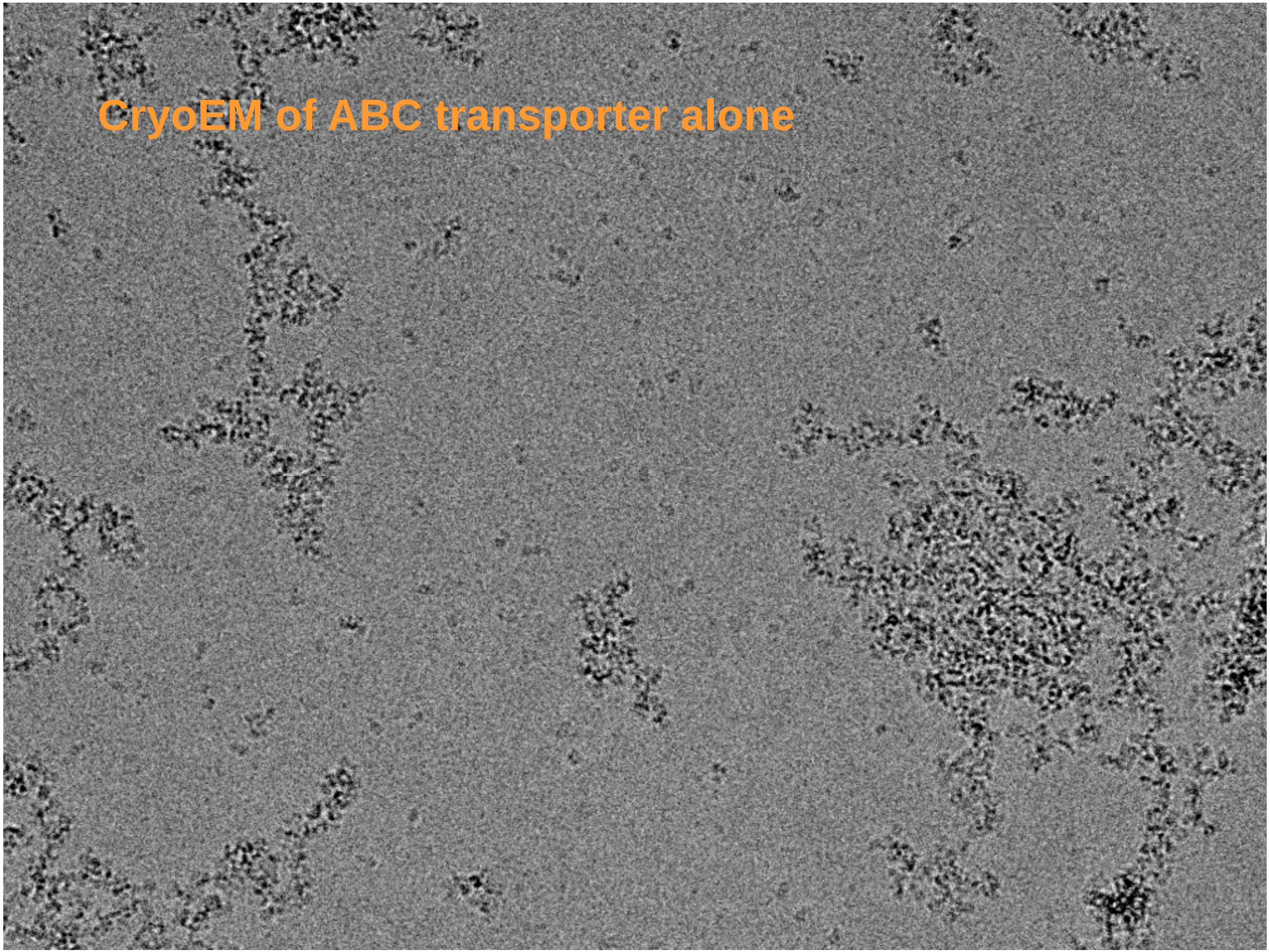


Wu et al., Structure, 2012

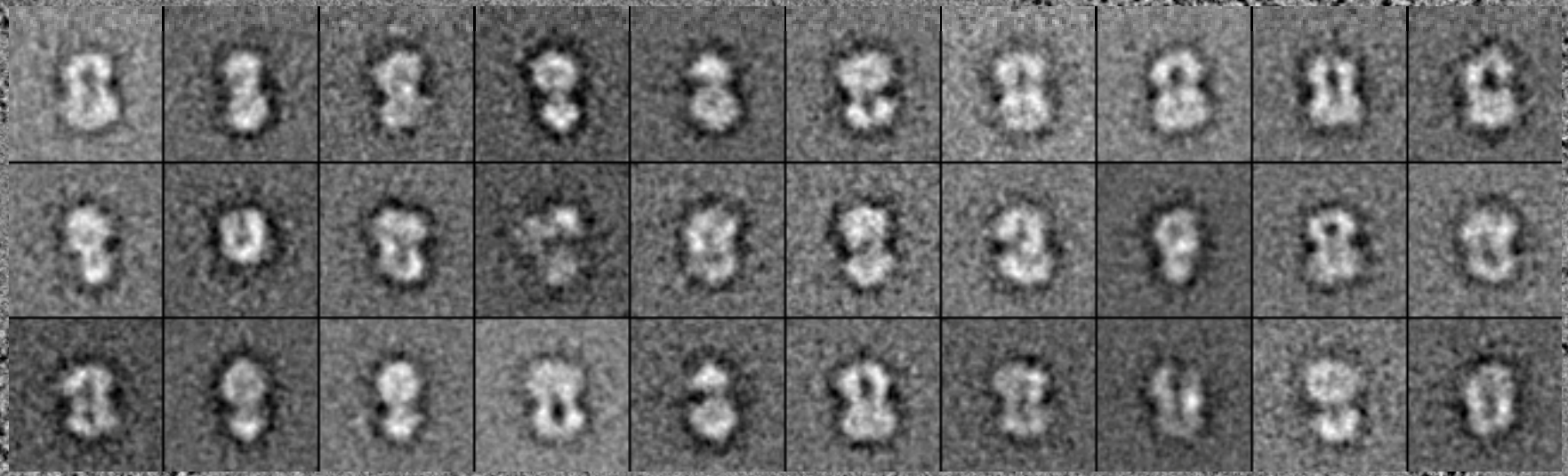

CryoEM of ABC-AH5 complex

CryoEM of ABC-AH5 complex




CryoEM of ABC transporter alone

CryoEM of ABC transporter alone

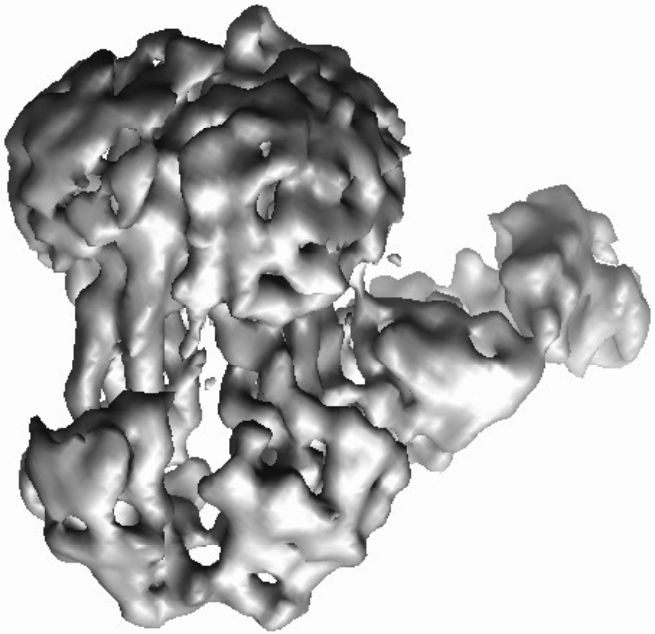


Summary of TmrAB by single particle cryoEM

- Established a procedure to identify and characterize Fabs that are suitable to facilitate structure determination of small proteins, including integral membrane proteins.

- Combining the Fab approach with the novel cryoEM technologies enabled sub-nanometer resolution 3D reconstructions of small integral membrane proteins.

- 3D reconstruction of ABC exporter TmrAB has a different conformation compared with crystal structures of other homologous ABC exporters in the apo state.



Kim et al, Nature, 2015.

Many Thanks

Anthony O'Donoghue

JungMin Kim

Melody Lee

Tajon Cheryl

Michael Page

Kimberly Kirkwood

Yifan Cheng

Henry Van Brocklin

Chris Farady

Matthias Hebrok

UCSF
University of California
San Francisco

CTSI
at UCSF

THE UNIVERSITY OF CALIFORNIA
LET THERE BE LIGHT
1868

qb3
ucb·ucsc·ucsf

American Heart Association
Learn and Live

NATIONAL INSTITUTES OF HEALTH