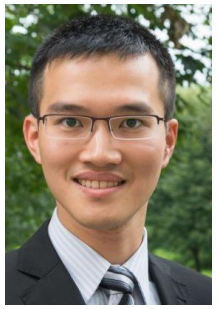
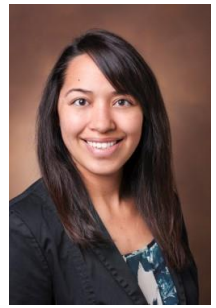
Renewable and Specific Affinity Reagents for Mapping Proteoforms in Human Tissues

Neil Kelleher & Jeannie Camarillo
Jim Wells & Kevin Leung

Northwestern Proteomics
Defining Key Molecules of Life

UCSF





Mapping protein-level biology with greater reproducibility and molecular precision

Validated Affinity Reagents to Link Spatial Protein Imaging and Targeted Proteoform Discovery

Targets of Interest

Protein 1

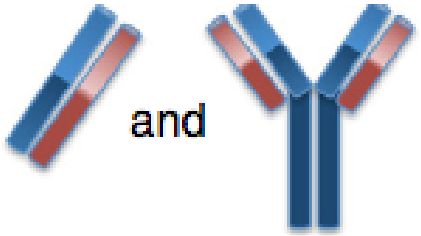
Protein 2

Protein 3

Protein 4

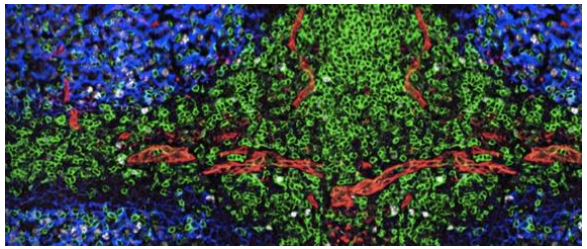
▪
▪
▪

Wells Lab:
Provide recombinant affinity reagents (rABs)



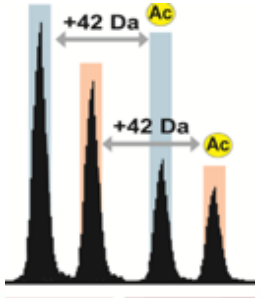
Stringent validation & reproducible production

HuBMAP Labs:
Use CODEX and other Ab-based imaging



Kelleher Lab:
Map proteoforms

PFR's 1xx
PFR's 2xx
PFR's 3xx
PFR's 4xx



HuBMAP
The Human BioMolecular Atlas Program

Next Year's Deliverables in 1 slide

- Highly validated affinity reagents, tagged for CODEX
- The proteoforms they pull down from human cells & tissues
- HuBMAP/CODEX images generated using recombinant binders

Collaborations in 1 slide

- Kelleher/Wells Lab interface (next slides)
- With TMC at UF (source tissues and CODEX)
- With TMC at Vanderbilt (CODEX)

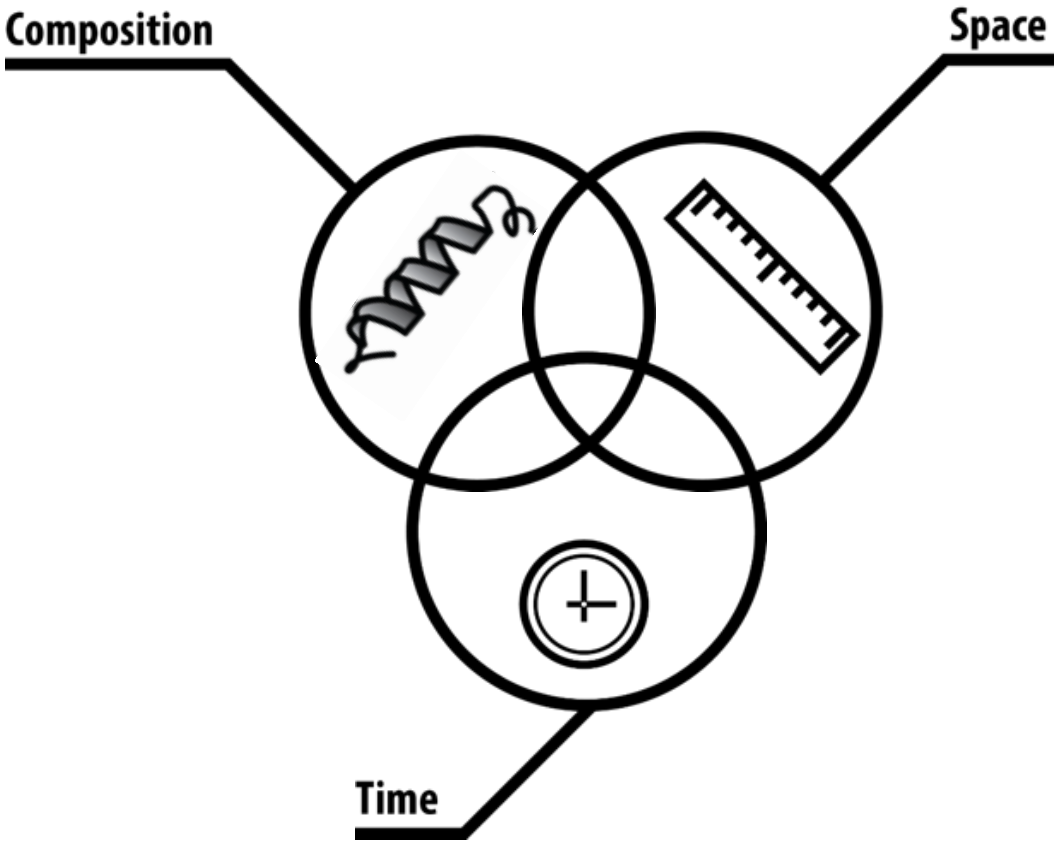
- Main barrier → being new to HuBMAP Consortium!

What should HuBMAP Do ?

- Stringent validation of renewable binders as mapping reagents (distinguish denatured vs. native antigens)

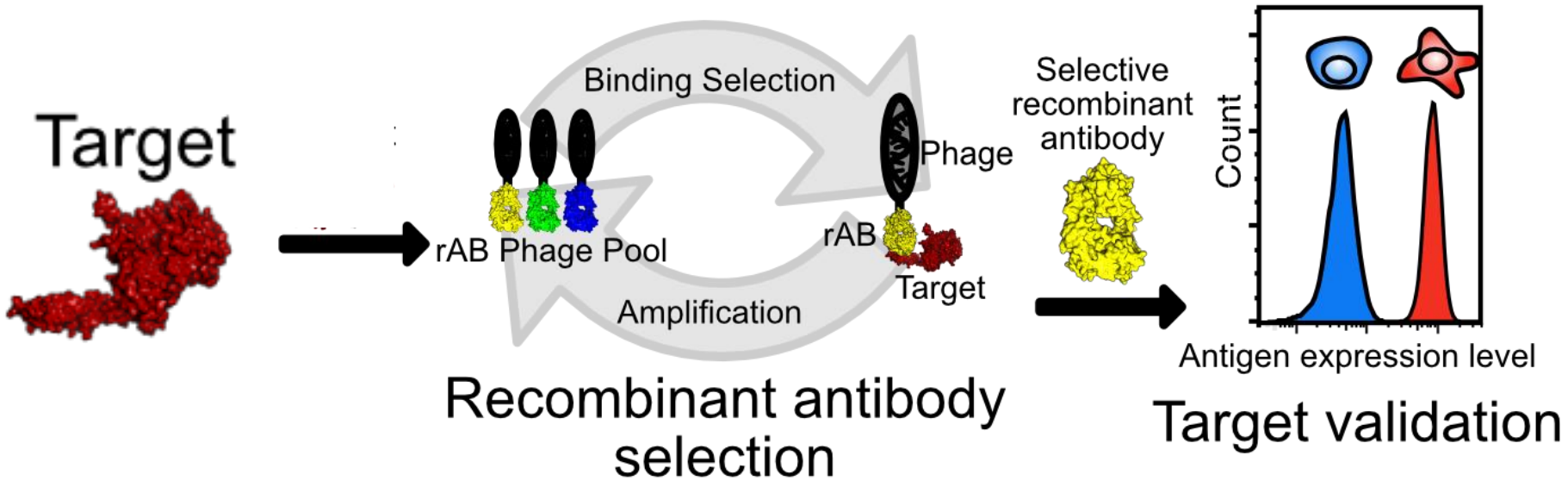
- Link spatial mapping of proteins with knowledge of their compositional space (‘proteoform-aware’ operations)

Mapping Biological Systems



Complementarity between **compositional**, **spatial** and **temporal** analysis

Generation and Validation of Recombinant Antibodies (Wells Lab)



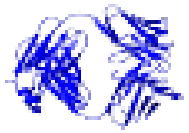
Recombinant Antibody (rAB):

- Cloned Fragment antigen-binding (Fab) domains
- Compatible with multiple frameworks (e.g. IgG1)
- Renewable

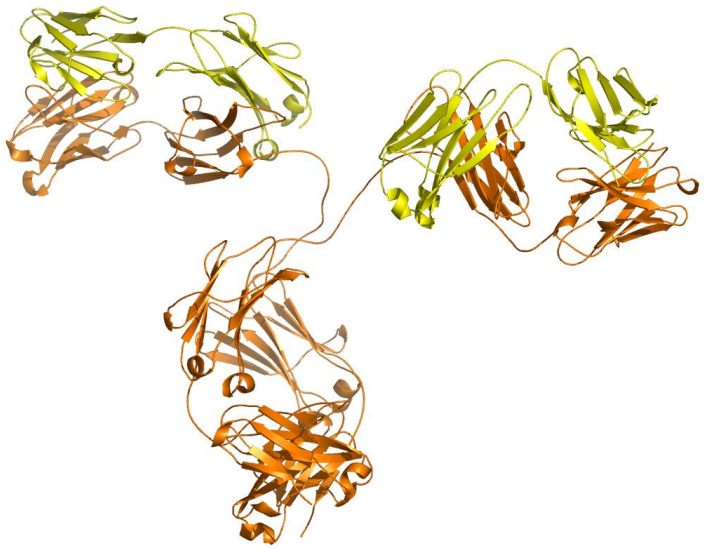
Recombinant Antibody Conversion for CODEX

1. Fab-phage

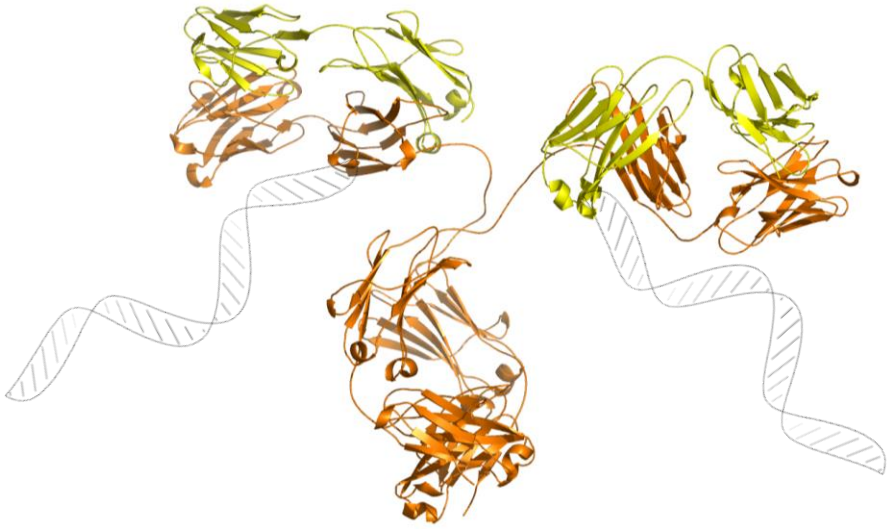
2. Fab



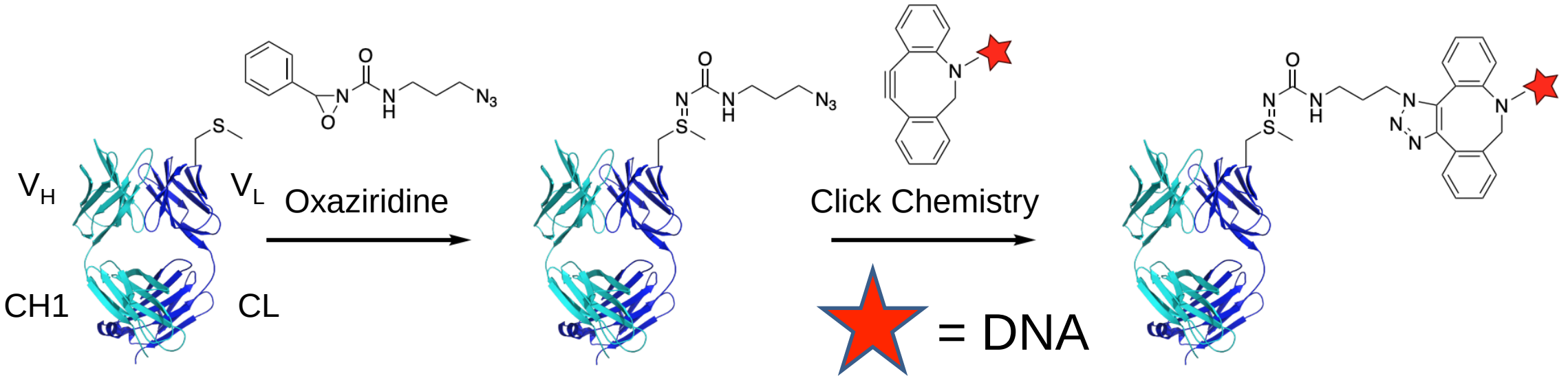
3. IgG



4. DNA barcoded

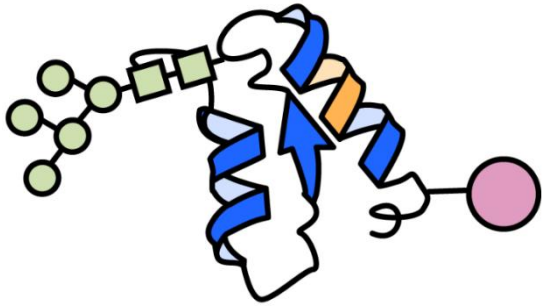


Site-specific Labeling of Methionine on Recombinant Antibodies by Oxaziridine Labeling



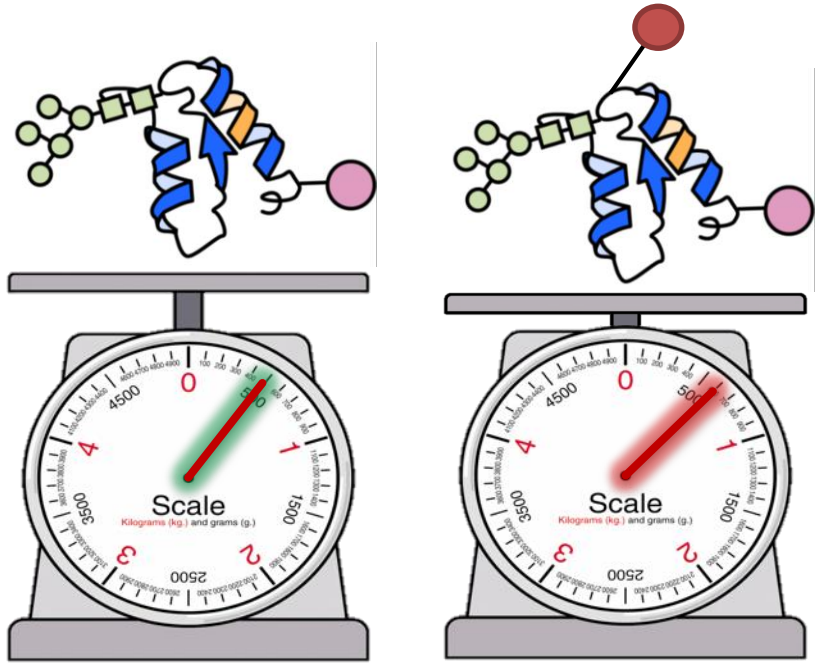
Lin, S. *et al.* 2017 *Science* 355, 597-602.
Elledge *et al.* 2019, bioRxiv 748160.

Top-down Mass Spectrometry (TDMS): Measuring Whole Protein Molecules



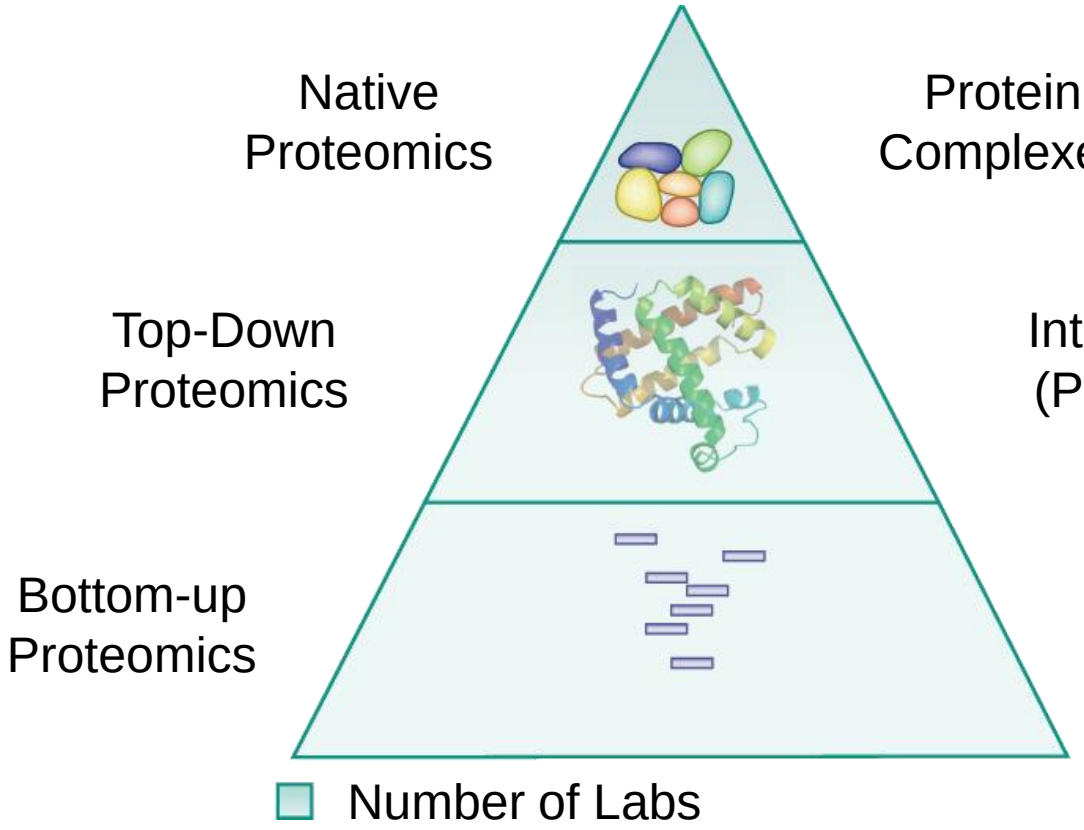
A Proteoform

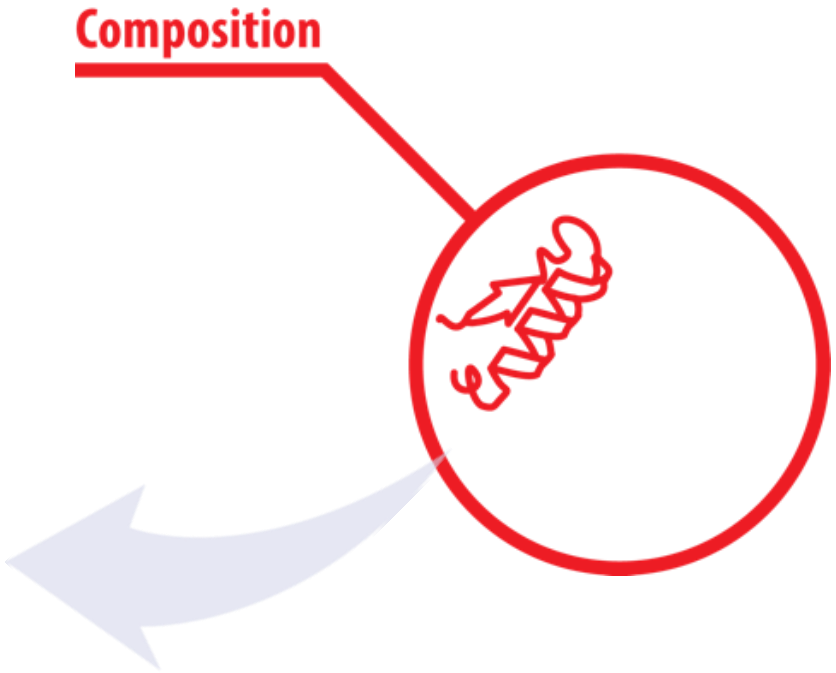
A distinct molecular form of a protein from a single gene



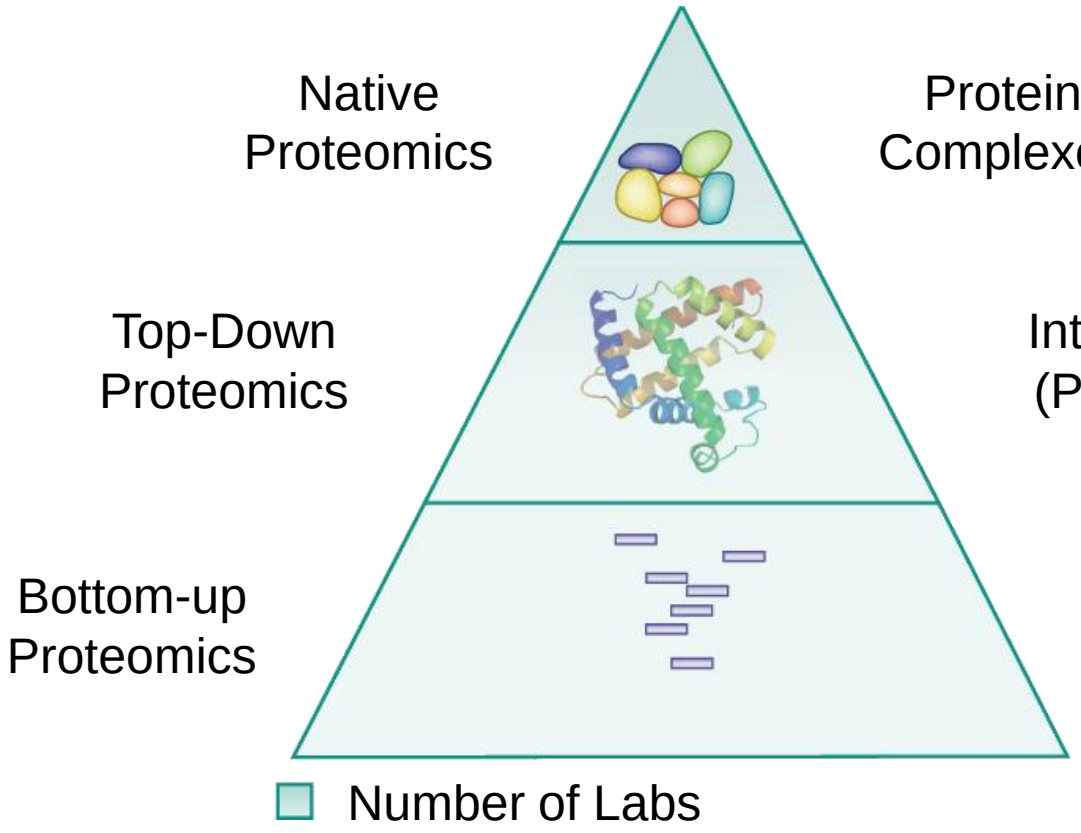
Top-Down Mass Spectrometry measures intact proteins

Three Strategies for Protein Measurement





Three Strategies for Protein Measurement



Native-mode Top-down MS

Denatured-mode TDPs

Emerging Theme:
Higher levels of protein measurement connect tightly to underlying biology

Key Deliverables – Highly Validated Affinity Reagents

Current Targets with Binders

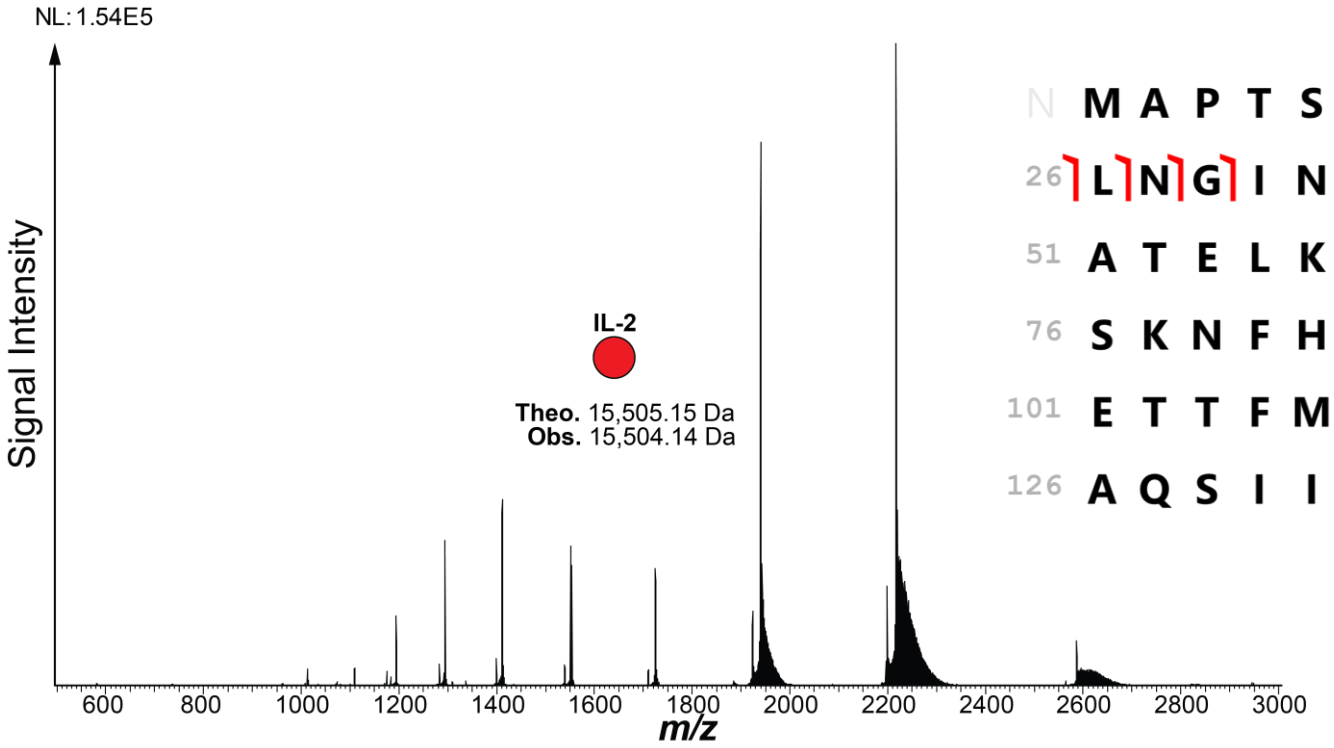
Target protein	Size (kDa)
IL-2	~20 kDa
CD25	~30 kDa
Layilin	~30 kDa
ROR1	104 kDa
FLT3	105 kDa
CD13	120 kDa
PDGFRA	123 kDa

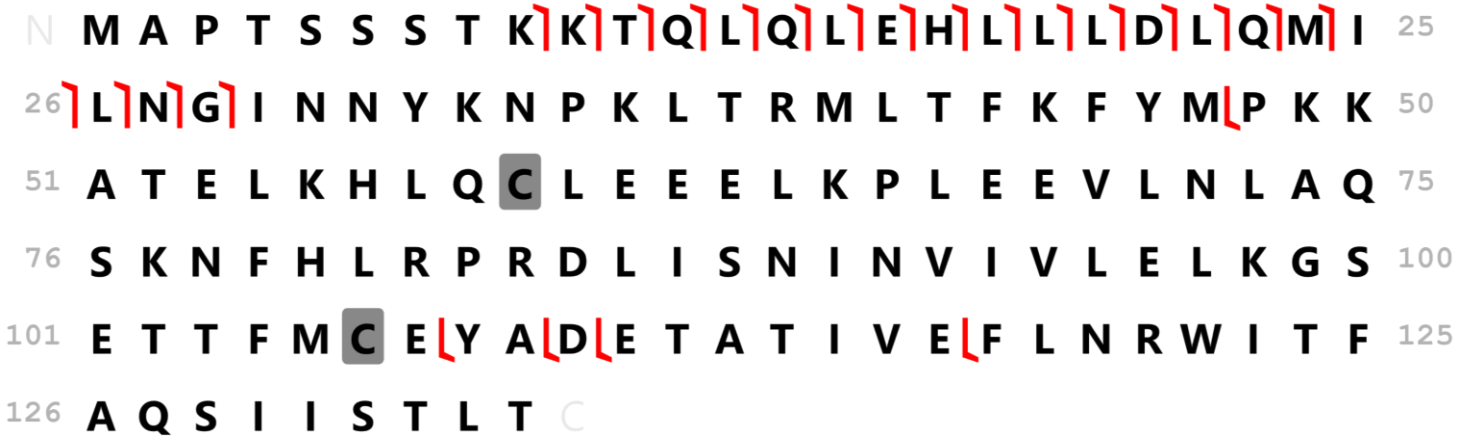
Targets Selected by HuBMAP Labs

Target protein	Size (kDa)
CD4	~51 kDa
CD8	~24 kDa
CD20	~33 kDa
PV-1	~51 kDa
Kim1/HAVCR	~39 kDa
N-Gal/Lipocalin	~23 kDa
PD-1	~32 kDa
PD-L1	~33 kDa
PD-L2	~31 kDa

Reserve Slides

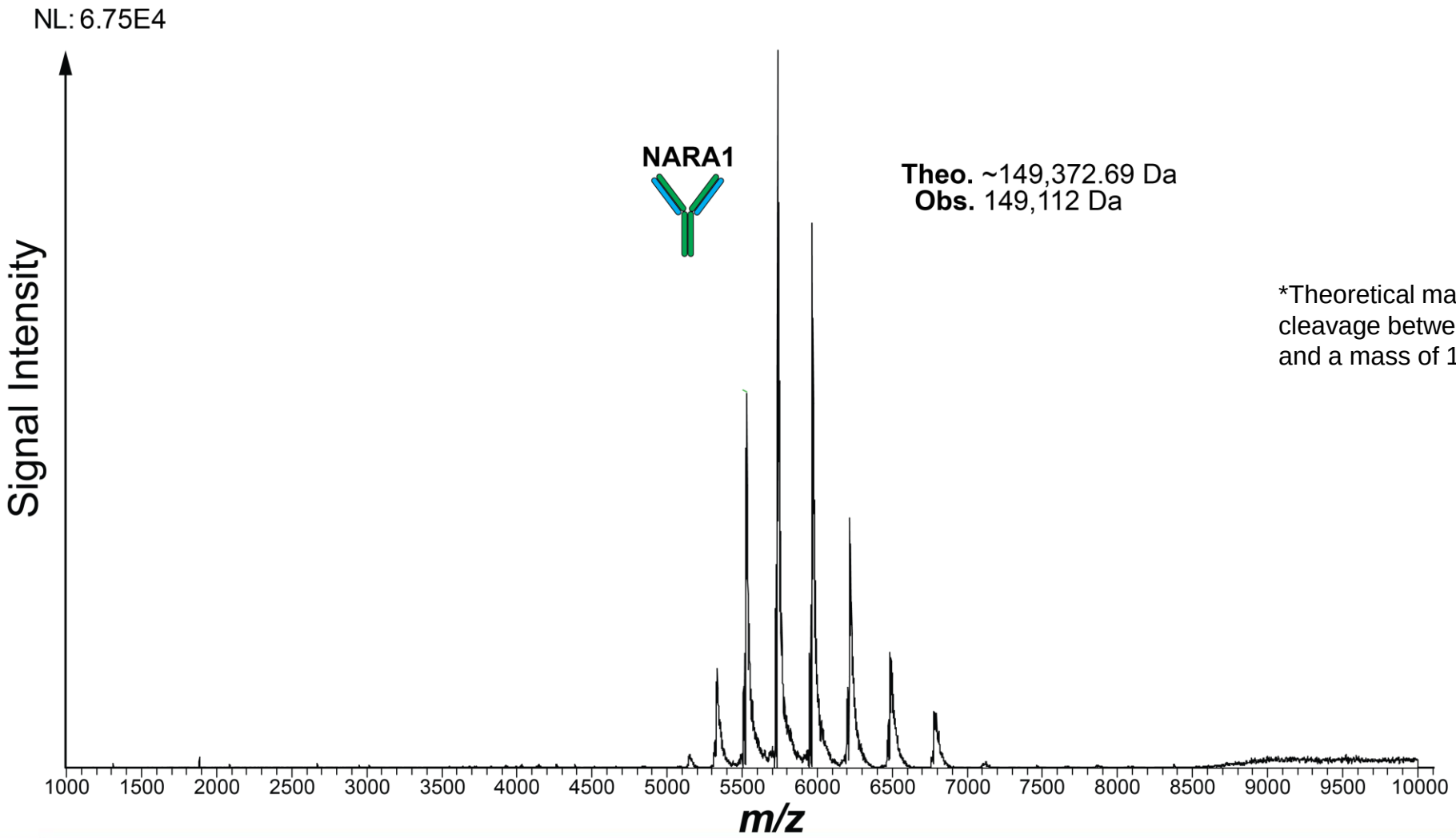
IL-2 Confirmation of Molecular Composition by Top-down MS





Resolution 120,000

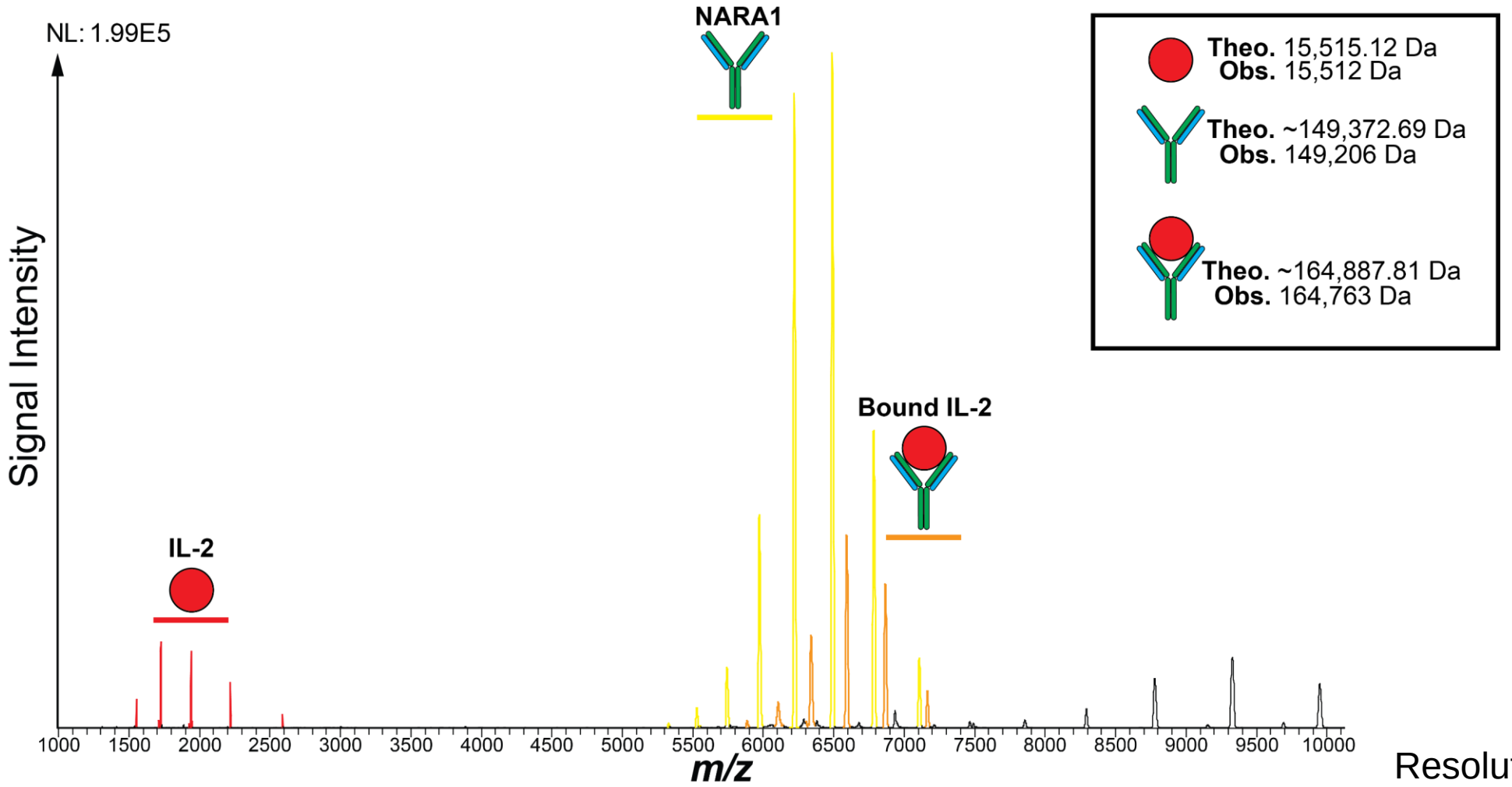
rAB Binder for IL-2, in IgG1 Framework



Theoretical mass assumes signal peptide cleavage between residues 20/21, 16 disulfides, and a mass of 1463 Da for each g0 glycosylation

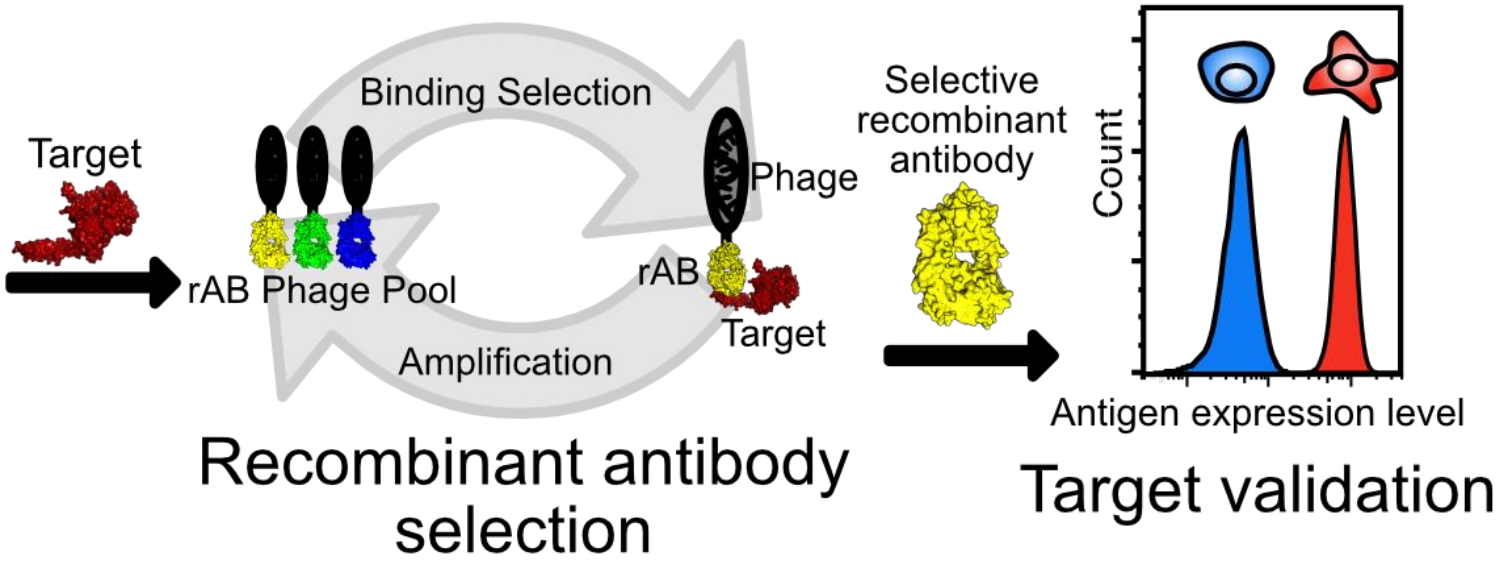
Resolution 15,000

rAB Binder for IL-2, in IgG1 Framework



Resolution 3,725

Generation and Validation of Recombinant Antibodies (Wells Lab)



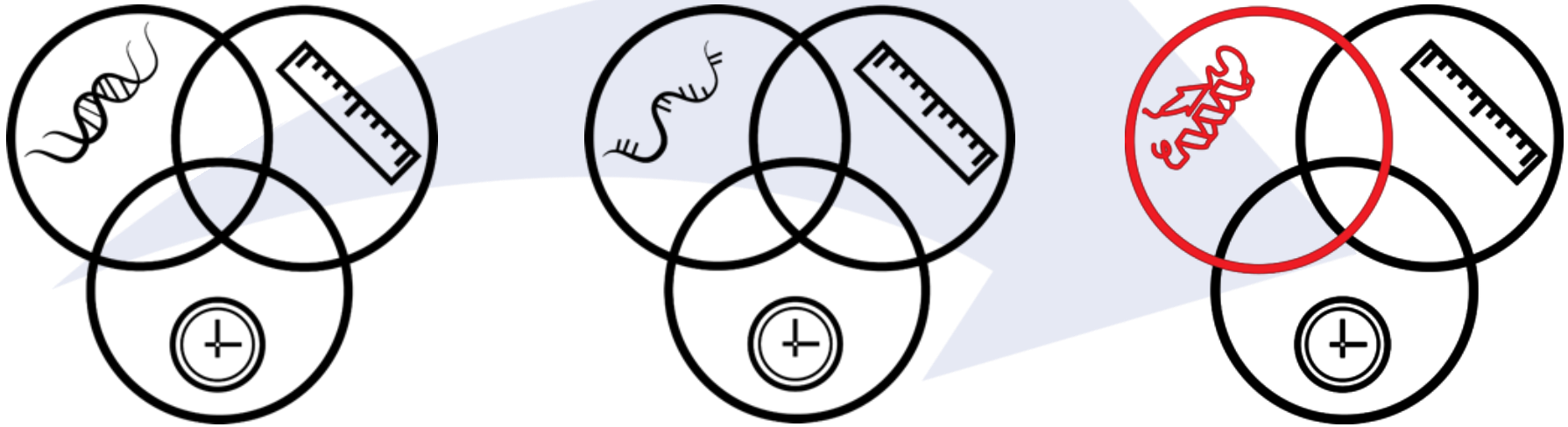
Validation of rAB's will use:

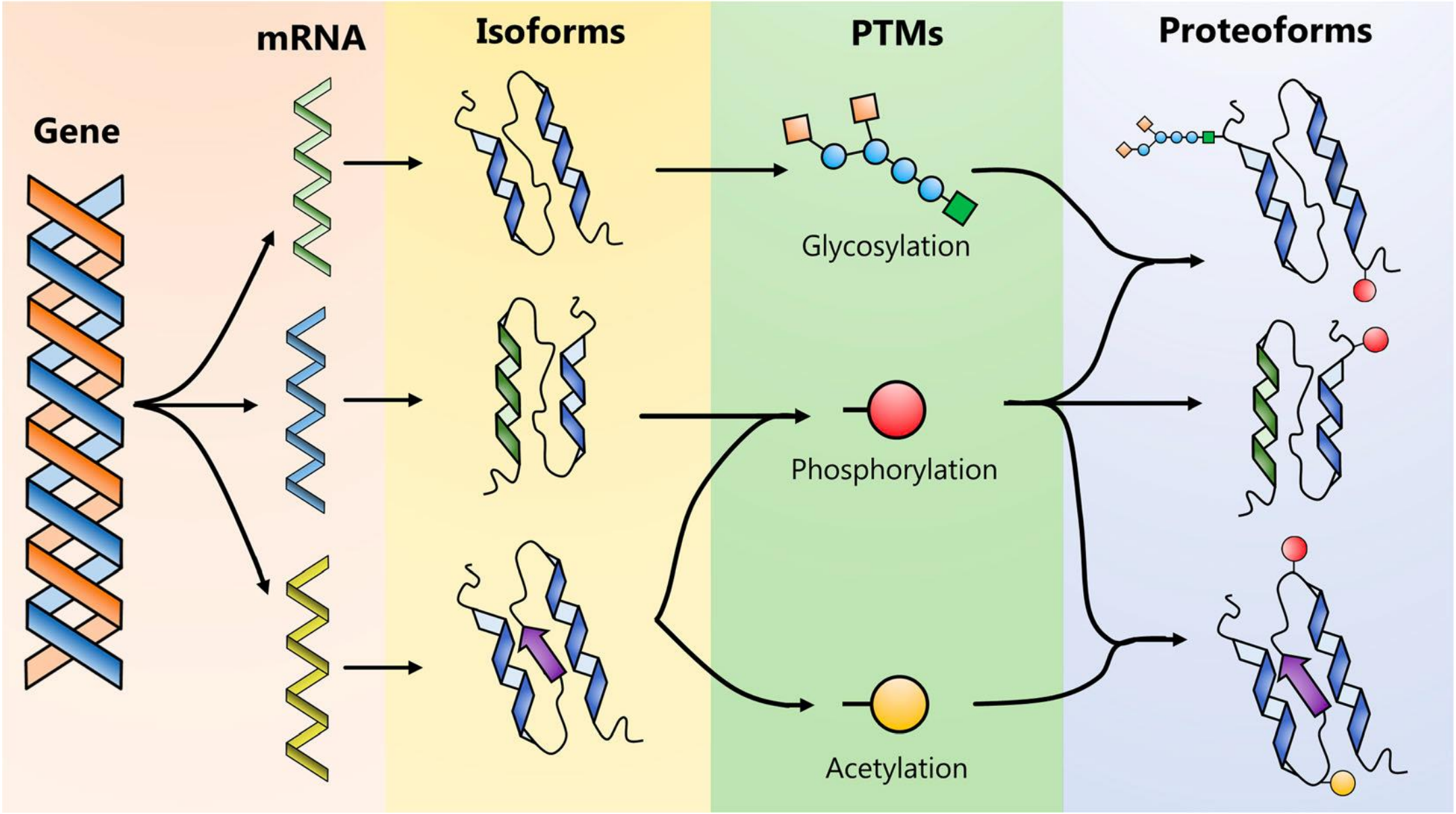
- Competition ELISA against recombinant, native antigen
- Determine K_d (or EC50) – typically <<10 nM
- Direct & complete characterization of rAB by Top-down Mass Spec (TDMS)
- Identification of target by Immuno-precipitation, bottom-up MS, and IP-TDMS (recombinant & endogenous target from cells & tissues)

Mapping Biological Systems

DNA **RNA** **Protein**

A Largely Unmapped Frontier




Gene
mRNA
Isoforms
PTMs
Proteoforms
Glycosylation
Phosphorylation
Acetylation

Alternative splice forms yield different protein molecules