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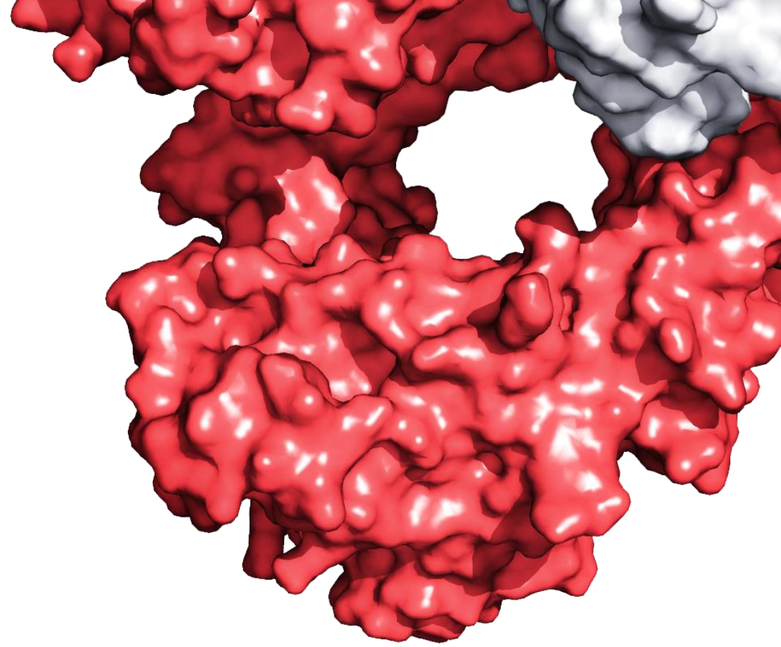
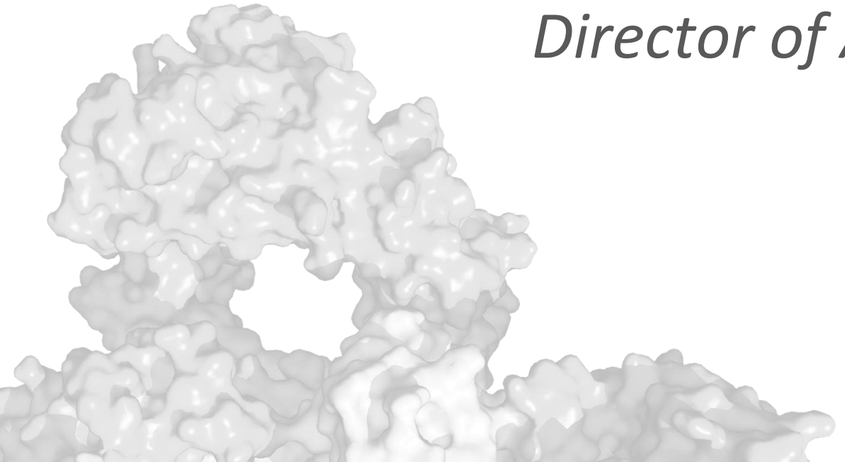


IPI - Recombinant Antibodies for the Advancement of Science

Deborah Moshinsky, PhD
Director of Antibody Characterization and Validation
May 6, 2025



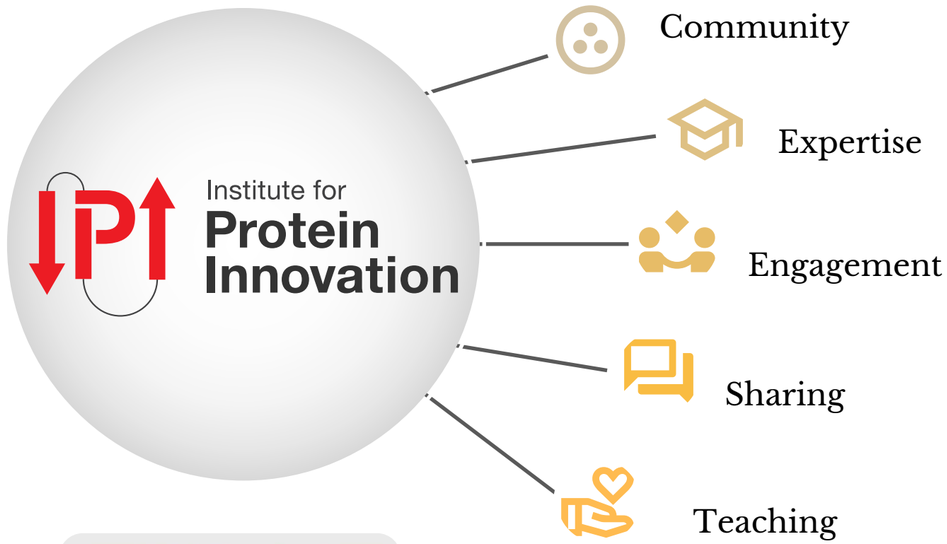
Outline of Presentation

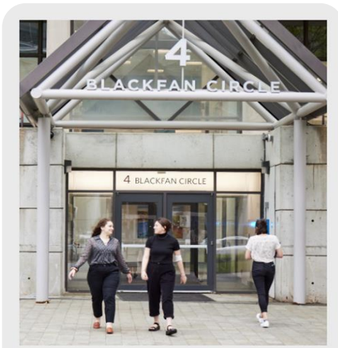
- Introduction to IPI
- IPI's Monoclonal Antibody Generation Program
  - Yeast Display Technology and Library
- Antibody Characterization and Validation
  - Antigen binding
    - Importance of High-Throughput Surface Plasmon Resonance (SPR)
    - Cell Display
  - Application Testing
    - External Collaborator and Internal
    - Immunofluorescence (IF), ELISA, Western Blot, Immunoprecipitation (IP) Mass Spec., Immunohistochemistry (IHC)
  - Cross-Reactivity Analysis
- Distribution through Addgene

The Institute for Protein Innovation (IPI)

IPI is a non-profit hybrid organization focused on protein science

- Co-founded in 2017 by Timothy Springer, PhD & Andrew Kruse, PhD
- Developing protein tools and knowledge about their use for the biomedical research community
- Dedicated to sharing science broadly and openly
- Located in Boston's Longwood Medical Area





Enabling New Biology Through Protein Innovation

High-Throughput Reagent **Antibody Discovery** using Display Technology
Goal: >1,000 recombinant mAbs for 200 targets per year

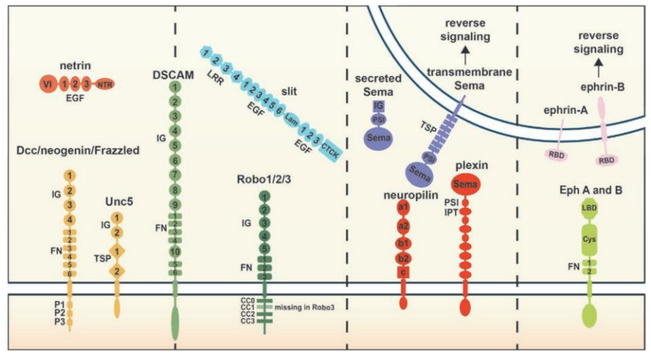
Campaigns against **Protein Families**

- Collaborate
- Build communities
- Disseminate reagents & knowledge

Focus in specific areas of **Neuroscience**

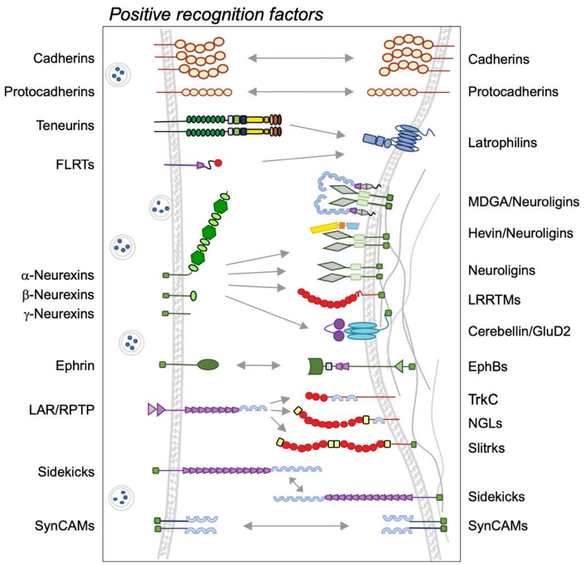
- Future expansion into other under-served research areas

Axon Guidance



Zhang et al. Current Topics in Developmental Biology

Synaptic Cleft



Chowdury et al Current Topics in Developmental Biology 2021

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Recombinant Monoclonal Antibody Platform Overview

Antigen Production in Mammalian Cells → **Yeast Display Selection** → **NGS and ML** → **Antibody Production & Purification** → **Antibody Characterization**

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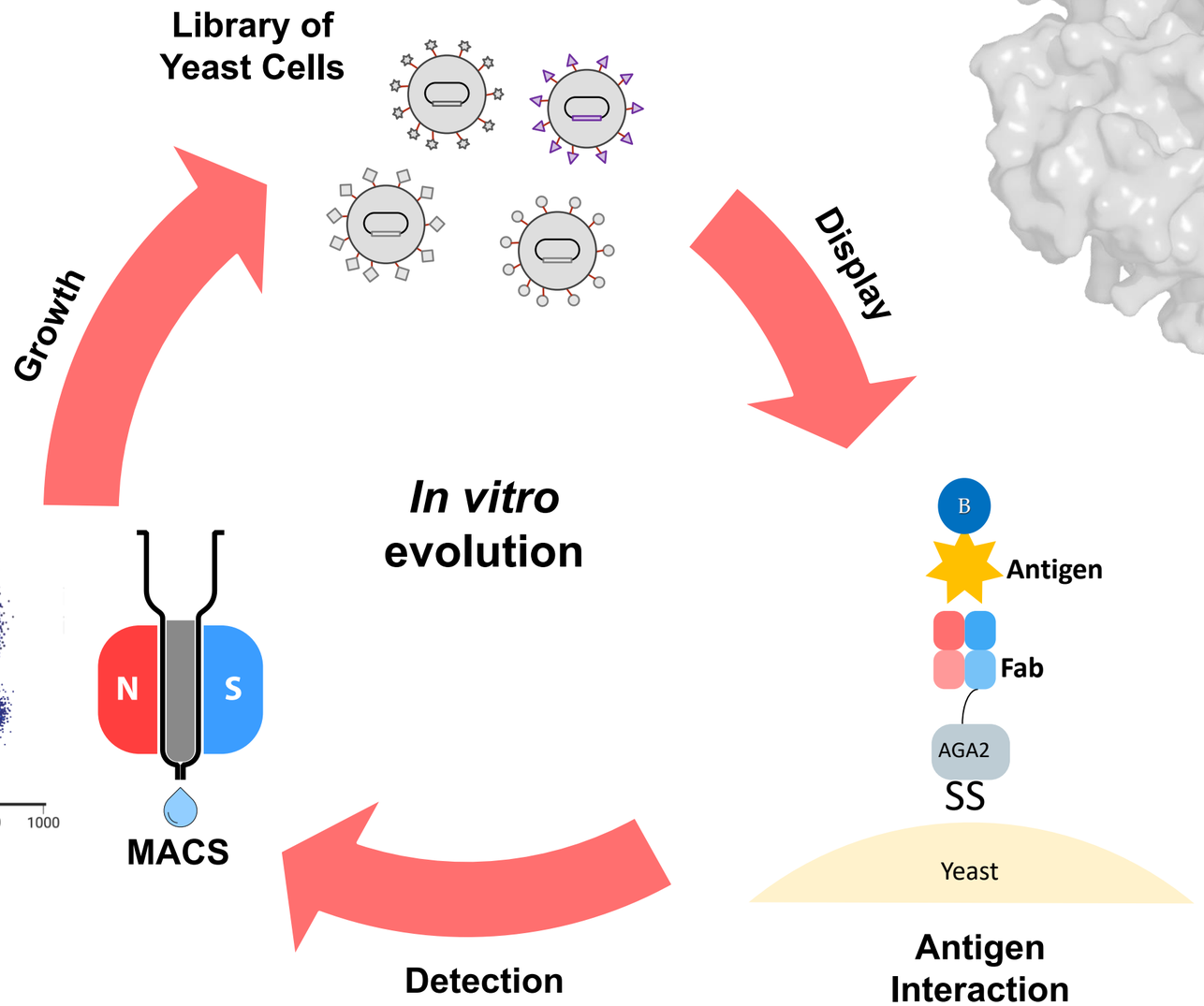
IPI Utilizes Yeast Display for Ab Discovery

- 3 to 6 rounds of selection
- Down to **4 nM [target]**
- **Off-target** deselections
- **96** campaigns/researcher/year



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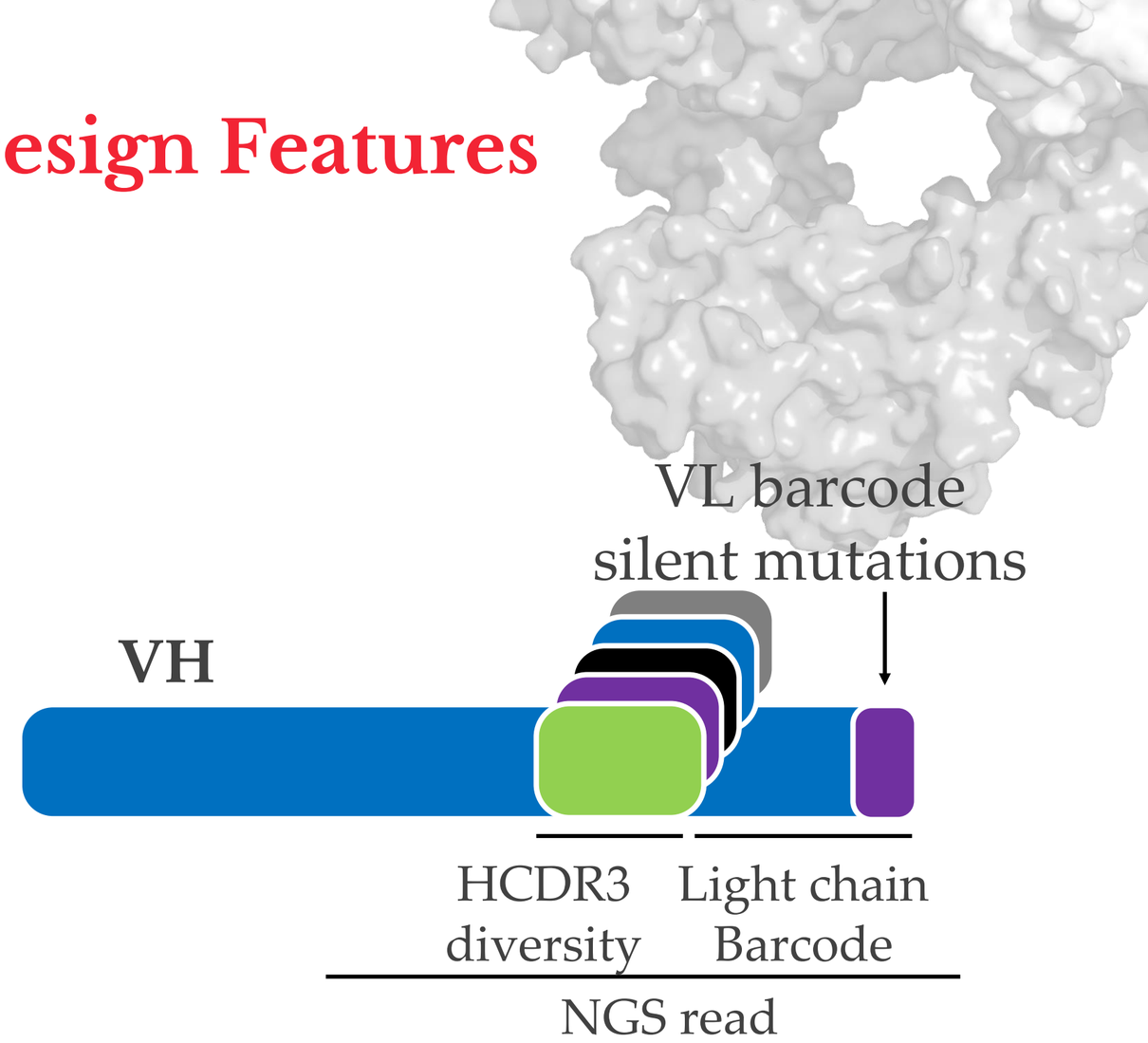
IPI Library 3.1 – Design Features

- 5 VH and 4 VL germlines
- HCDR3 is the only region containing diversity (**>2x10^{10} transformants**)
  - 8 x10^{9} sequences
- Amino acid frequencies **mimic human naive HCDR3**
- Sequence **liabilities removed**
- HCDR3 length: 11 to 17 amino acids
- Rational design enables AI/ML approach for Ab selection



Working on other formats (VHH, biparatopics)

The Reproducibility Crisis

- Reproducibility of scientific research questioned
  - E.g. 47 of 53 landmark cancer research papers not reproducible*
- Poorly characterized antibodies a major driver
  - ~$1 billion each year is wasted on antibodies that don't work
  - Many anecdotal stories
    - David Rimm ended a $2M program in melanoma
      - New batch of antibody could not reproduce original results
- Ab characterization efforts have increased
  - Ab providers' application testing
  - YCharOS - Ab Characterization through Open Science
    - Commercial Ab testing in WB, IP, IF
      - Remove inactive Abs from market

BLAME IT ON THE ANTIBODIES

Antibodies are the workhorses of biological experiments, but they are littering the field with false findings. A few evangelists are pushing for change.

BY MONYA BAKER

274 | NATURE | VOL 521 | 21 MAY 2015

* Begley, C. G. & Ellis, L. M. *Nature* **483,** 531–533 (2012)

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IPI's Quality Standards

- Antigens and antibodies are produced in-house
- Antibodies undergo QC and application testing
  - Size exclusion chromatography (SEC) for aggregation status
  - Mass Spec MW analysis for sequence verification
  - Stability analysis
  - Antigen binding
    - Surface Plasmon Resonance (SPR) using purified antigen
    - Flow cytometry on cells expressing exogenous antigen ectodomain (cell display)
  - Collaborator application testing
    - Immunohistochemistry (IHC), Immunofluorescence (IF), ELISA
    - Specialized testing (e.g. knockouts, primary neurons)
    - Leaders in the field
  - Internal IPI application testing – ongoing initiative
    - IF
    - ELISA, Western Blot, Immunoprecipitation (IP) Mass Spec., and IHC to be implemented

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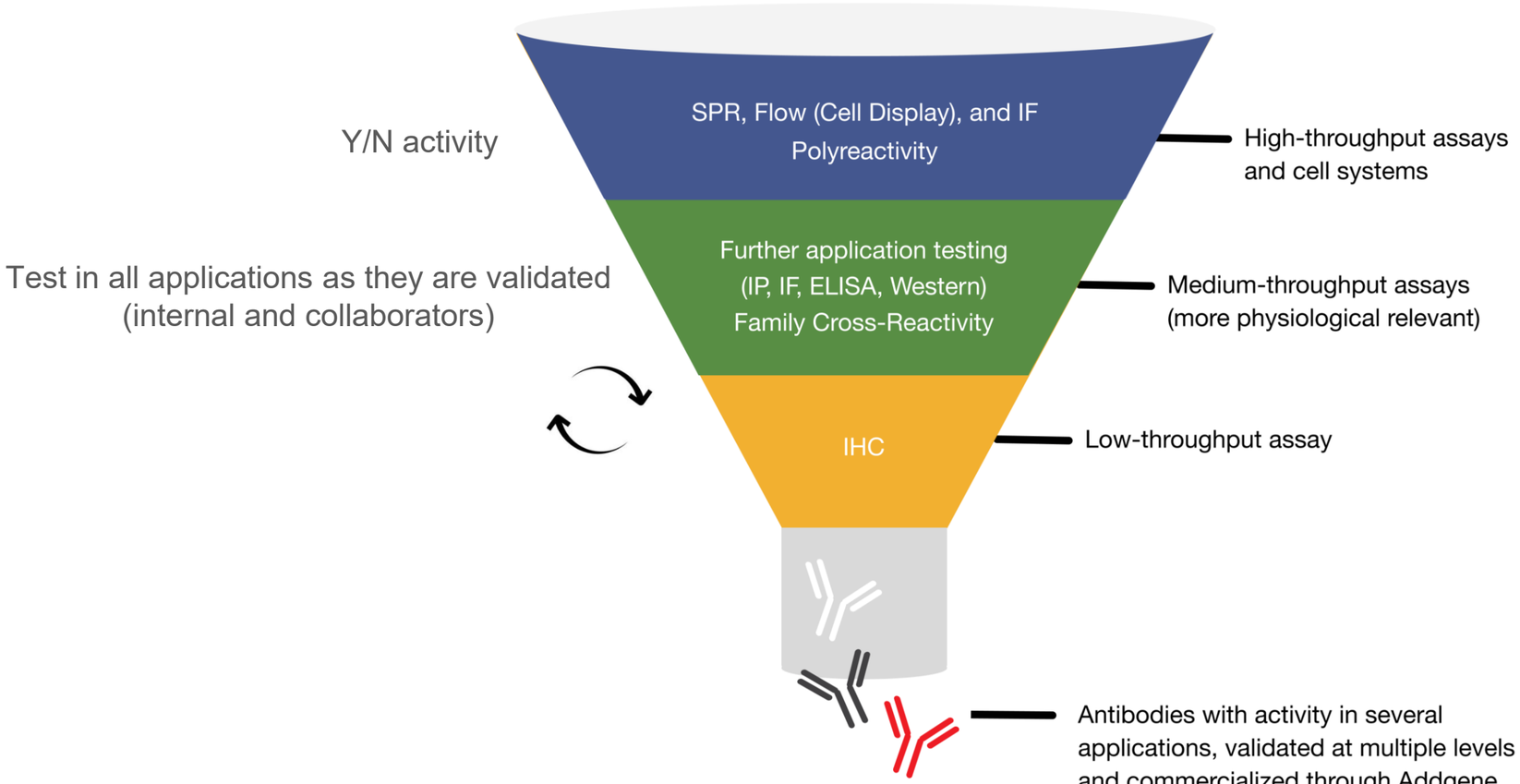
Antibody Characterization and Validation Testing Funnel

~24 antibodies per target; hundreds of targets



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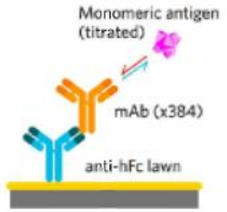
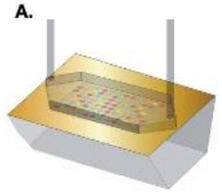
SPR Testing Workflow

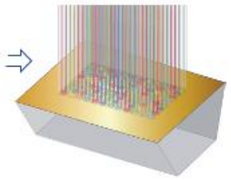
carterra



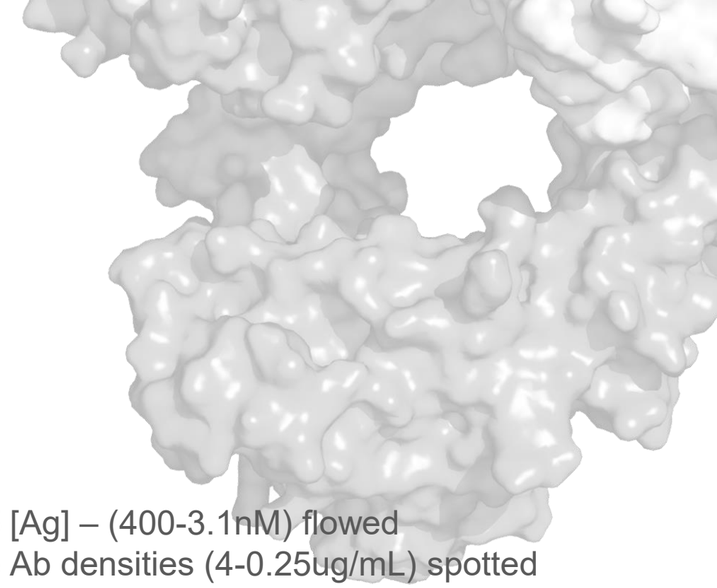
Capture kinetics



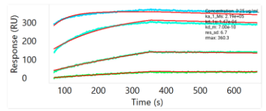
Single flow cell

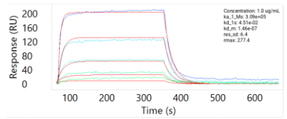


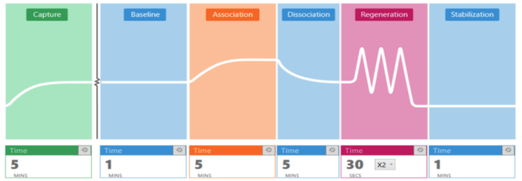
96-channel printhead



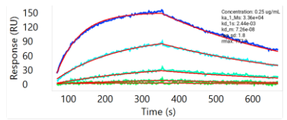
- 5 [Ag] – (400-3.1nM) flowed
- 3 Ab densities (4-0.25ug/mL) spotted
- K_D and off-rate determinations

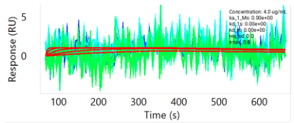






Criteria	Premium	Strong	Weak	Fail (non-binders)
SPR Signal (R.U.)	> 100	> 75	> 50	< 20
Dissociation Rate (K_d, s^{-1})	$\leq 10^{-3}$	$\leq 10^{-2}$	$\geq 10^{-2}$	n/a
Dissociation Constant (K_D, nM)	≤ 10	$10 < K_D < 100$	Any K_D	n/a





<u>Throughput</u>
384 antibodies/week
for up to 16 antigens

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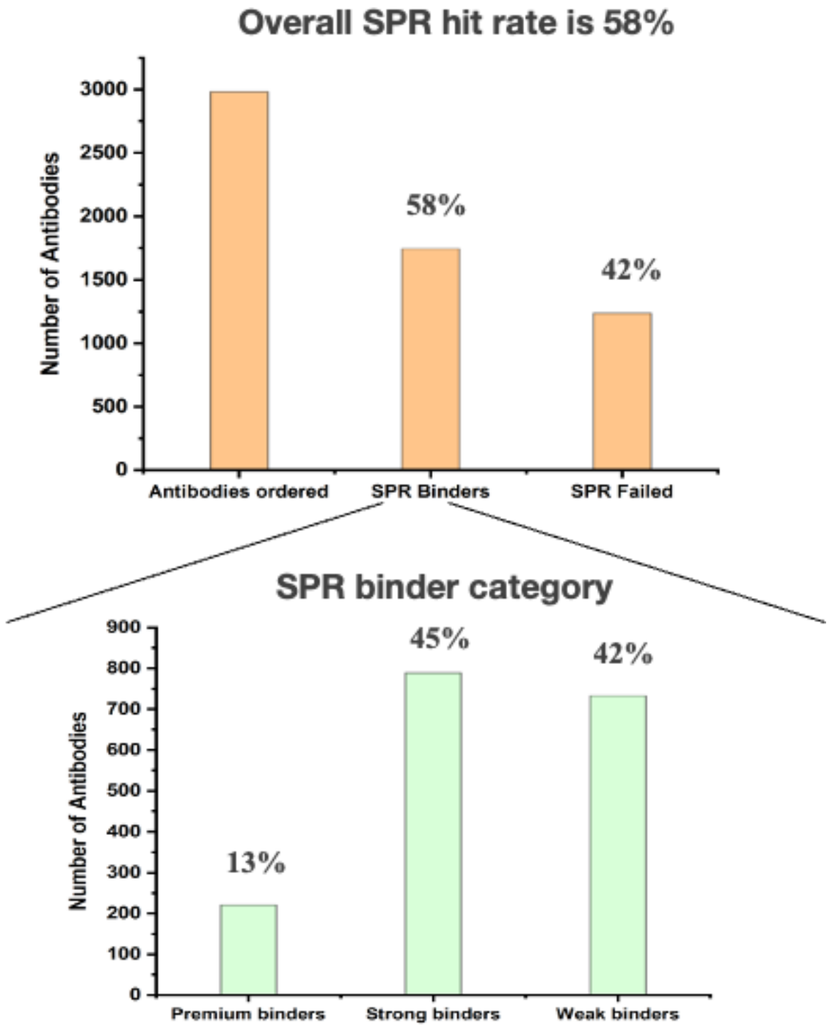
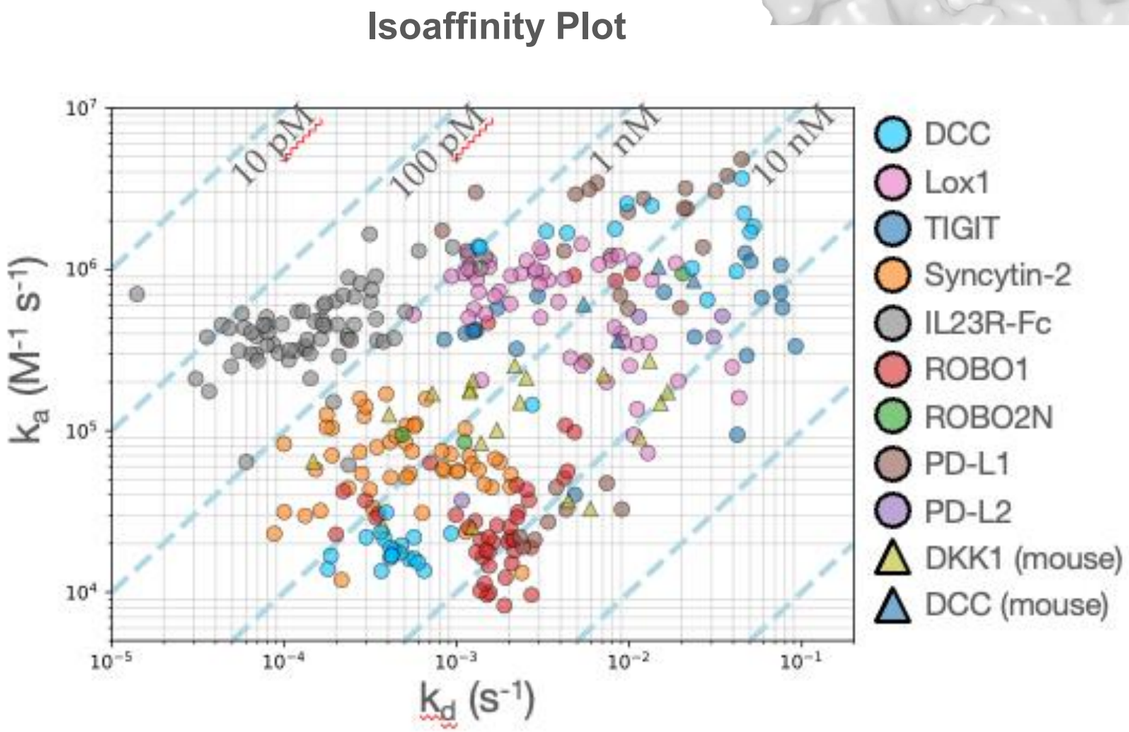
SPR Data for Selected Targets





Antibodies bind their antigen target with high affinity and suitable off-rate

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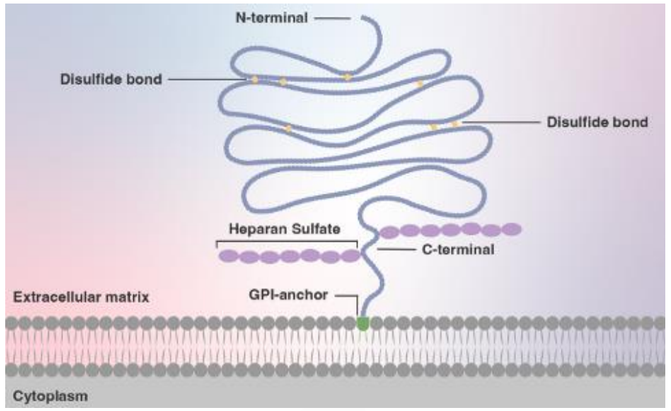
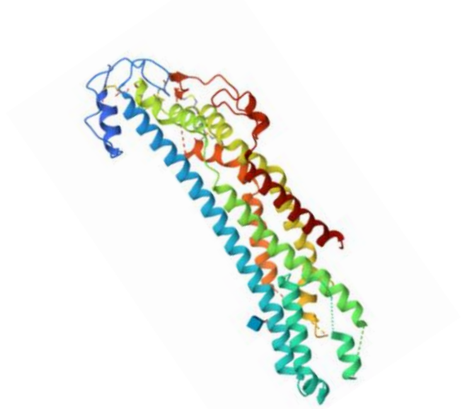
SPR Characterization for Glypican (GPC) Antibodies

Human and Mouse GPC1-6

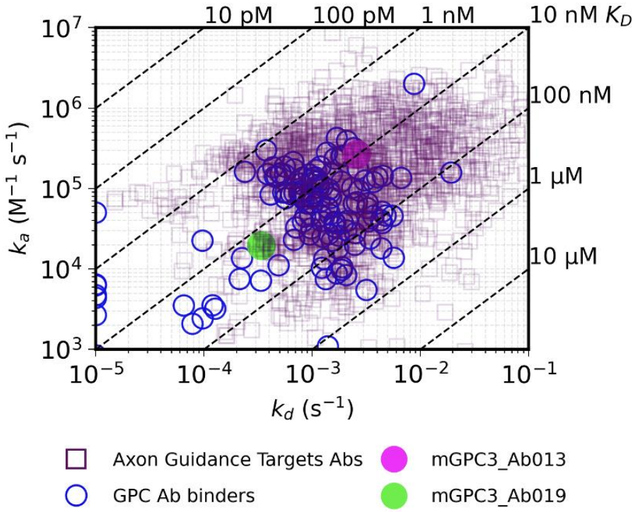




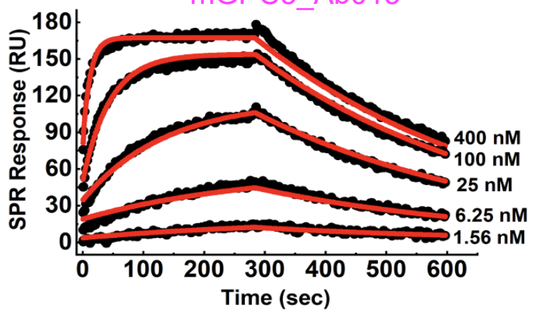
Crystal structure of N-glycosylated, C-terminally truncated human glypican-1

•PDB DOI: https://doi.org/10.2210/pdb4ACR/pdb

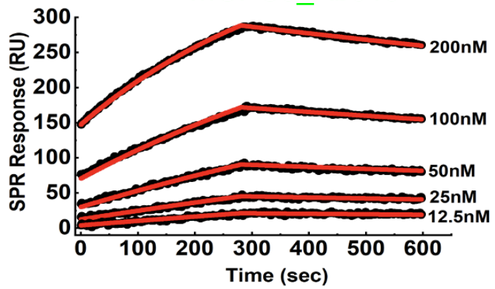
Isoaffinity Plot



mGPC3_Ab013



mGPC3_Ab019



Antibodies bind glypican targets with high affinity and suitable off-rate

- Do they bind antigen with high affinity when expressed on cells?

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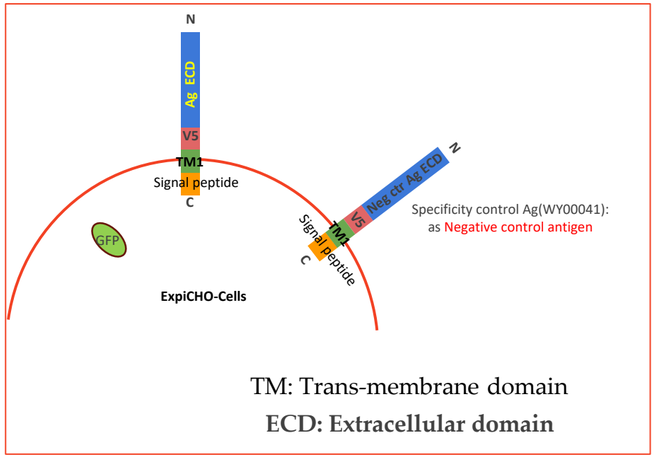
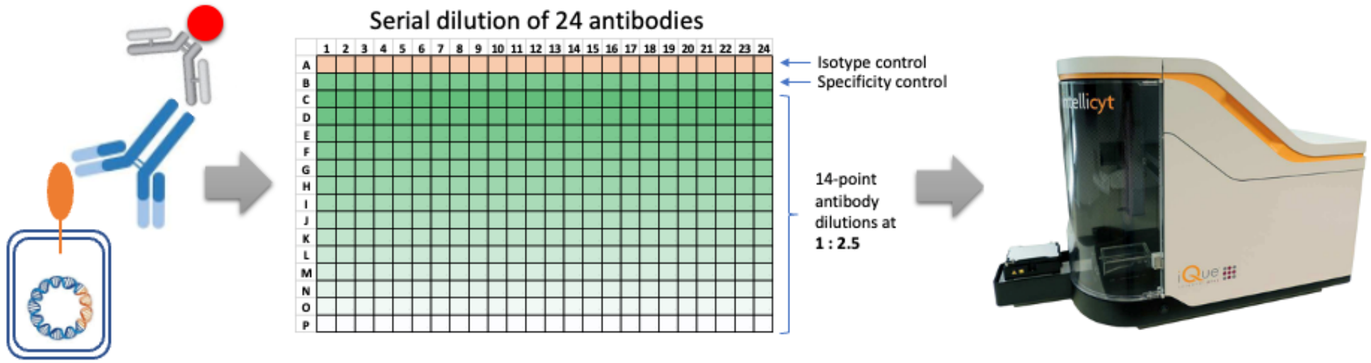
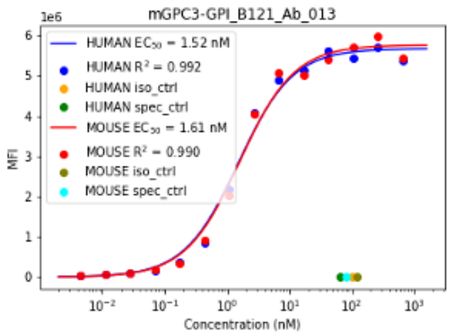
Flow Cytometry (Cell Display) Testing

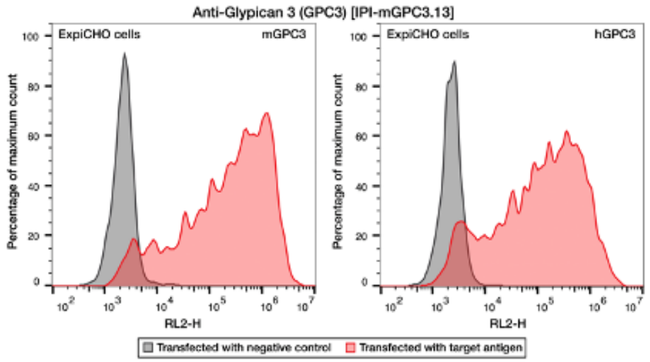


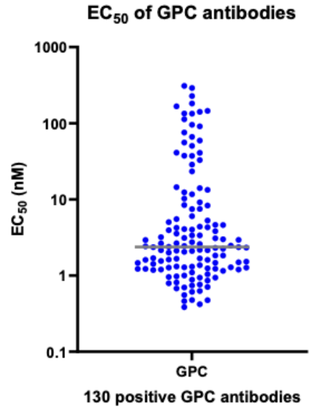
Workflow



IPI-mGPC3.13 Results







Antibodies bind antigen with high affinity when expressed on cells

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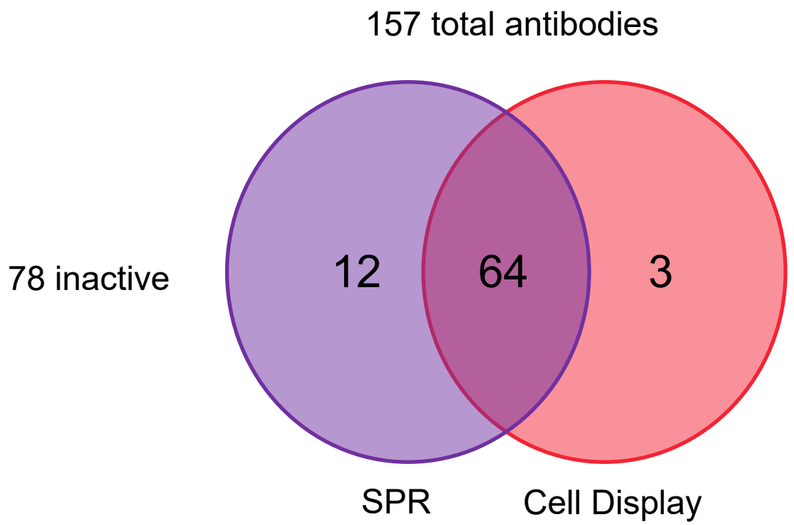
Overlap between SPR and Cell Display Results

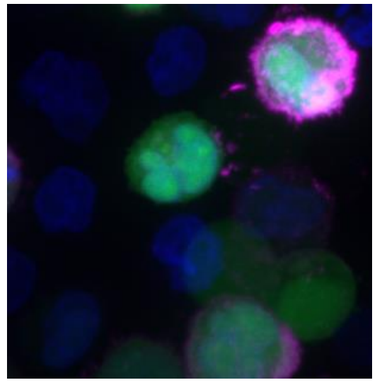


- 64 total antibodies bind glypican targets in SPR and on cells
- What is the significance?
  - Do they work in common research applications?
    - Internal IF testing
    - External collaborator IHC and IF testing

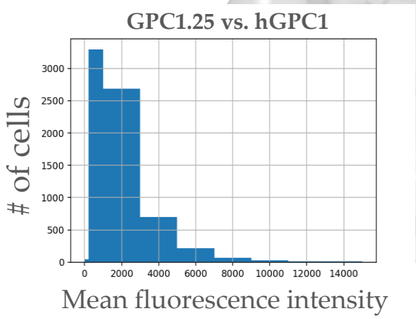
Immunofluorescence Testing of Glypican Antibodies – Exogenous Antigen Expression

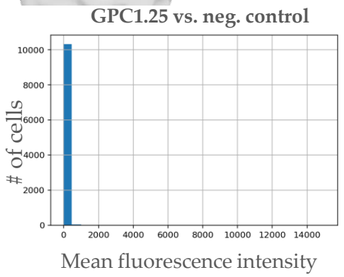
- Transfect CHO cells with antigen construct + GFP
- Affix cells to 96-well glass bottom plates
- Fix cells with 4% PFA
- Permeabilize and block cells
- Incubate with Ab then Alexa 647-labeled secondary
- Incubate with DAPI
- Image using Molecular Devices ImageXpress confocal HT
  - Positives – Abs where green cells have far-red staining on the membrane
  - Negatives – Abs lacking far-red staining on the membrane

hGPC1 + IPI-GPC1.25

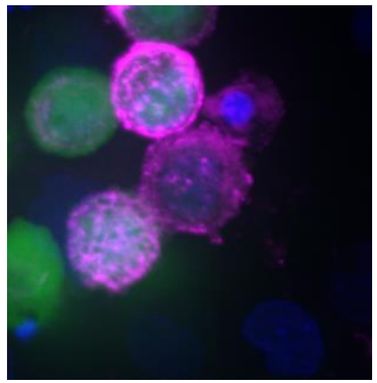


antibody
GFP
DAPI

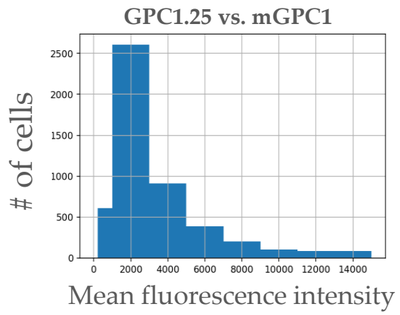




mGPC1 + IPI-GPC1.25



antibody
GFP
DAPI



Representative Images (40X Magnification)

Antibodies work in IF using a transfected CHO cell system

- Do they work for IF on cells expressing endogenous antigen?

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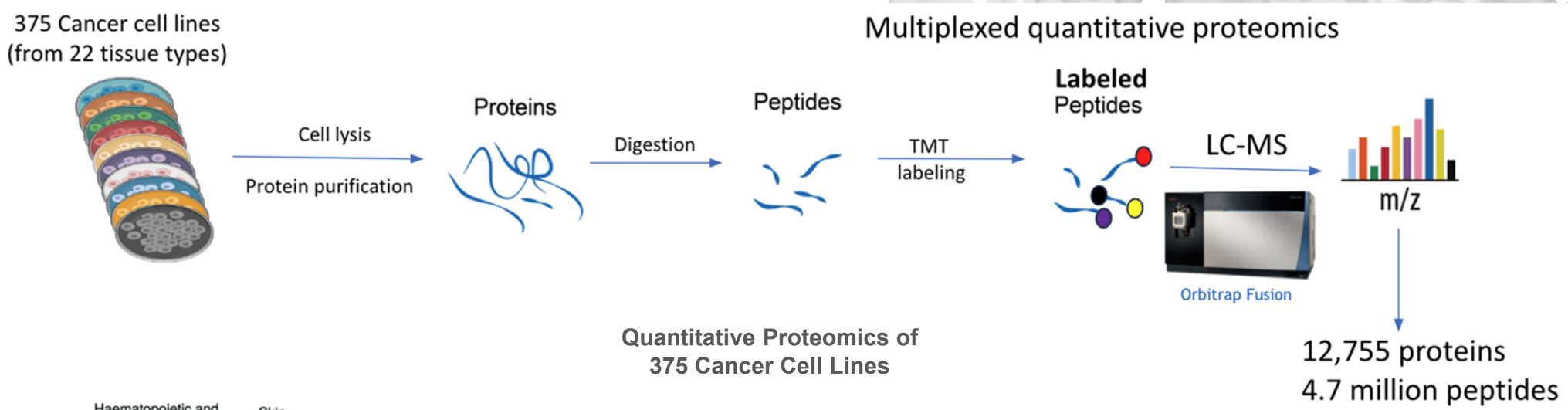
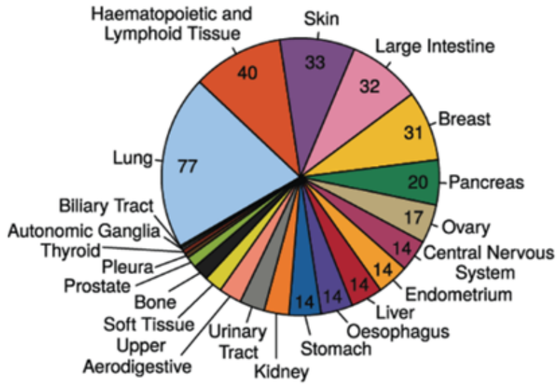
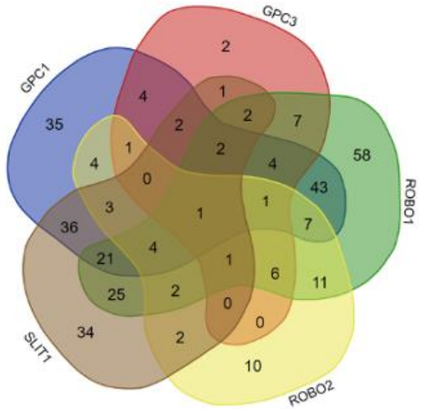
‘Omics Data-Mining Identifies Cell Lines with Endogenous Antigen



Quantitative Proteomics of 375 Cancer Cell Lines





Venn diagram example of 5 IPI targets across cell lines

Top candidate cell lines

Cell Name	Tissue type	IPI antigens
RD	Soft Tissue	FZD4; FZD7; GPC1; GPC3; GPC4; LTBP3; NBL1; NLGN2; NRP1; ROBO1; SLIT1; TGFB1; TGFB2
U118MG	CNS/Brain	FZD7; GPC1; GPC4; LTBP3; NBL1; NLGN1; NRP1; NTN1; ROBO1; SLIT1; TGFB1; TGFB2
TC71	Bone	FZD4; FZD7; LTBP3; NBL1; NLGN1; NLGN2; NRP1; ROBO1; ROBO2; TGFB1; TGFB2; TGFB3
KYSE410	Esophagus/Stomach	FZD7; GPC1; GPC3; GPC4; LTBP3; NRP1; ROBO1; SLIT1; TGFB1; TGFB3
NCIH196	Lung	FZD7; GPC1; GPC4; LTBP3; NBL1; NLGN2; NRP1; ROBO1; TGFB1; TGFB2

David P. Nusinow and Steven P. Gygi “A Guide to the Quantitative Proteomic Profiles of the Cancer Cell Line Encyclopedia”
bioRxiv preprint doi: https://doi.org/10.1101/2020.02.03.932384
Publication doi: https://doi.org/10.1016/j.cell.2019.12.023

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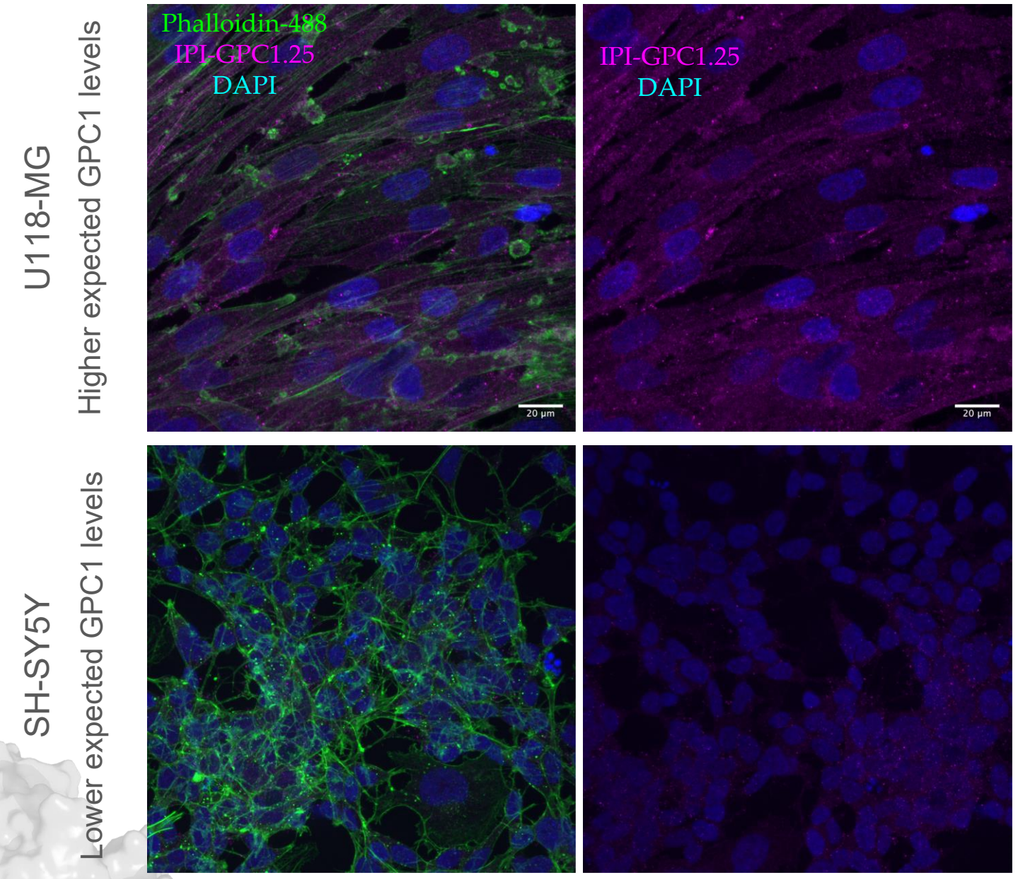
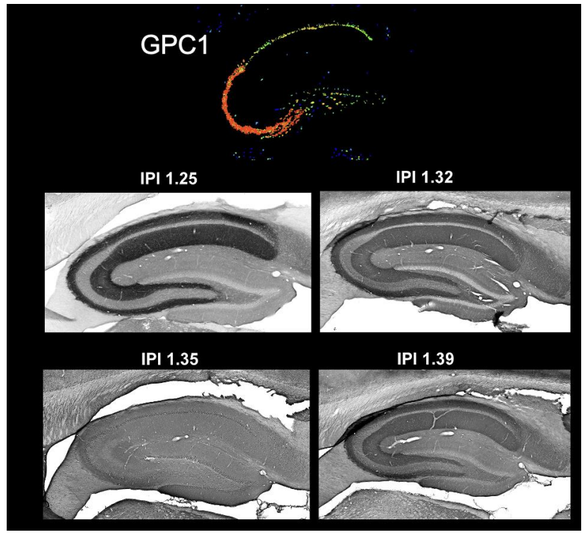
IF and IHC Testing of Glypican Antibodies – Endogenous Antigen Expression





Courtesy of Karl Murray & Jim Trimmer (UC Davis NeuroMab)

3 of 4 GPC1 antibodies are active in collaborators' IHC

Antibodies are active in IF and IHC with endogenous antigen

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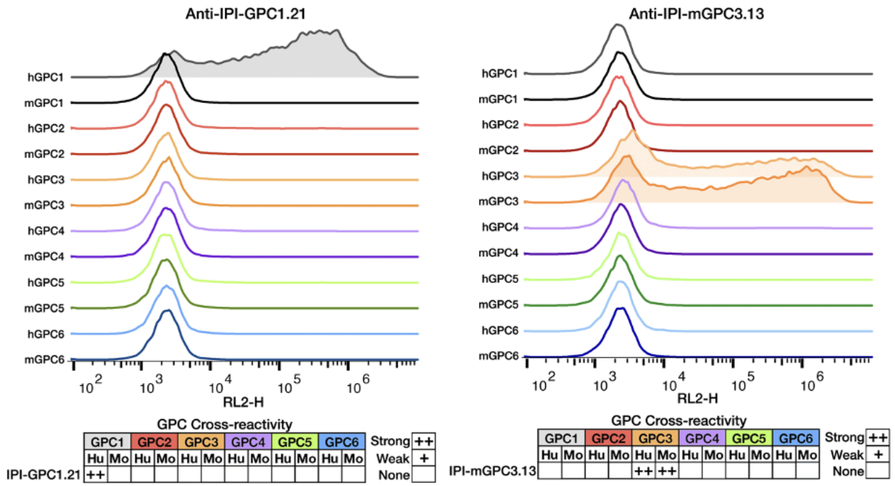
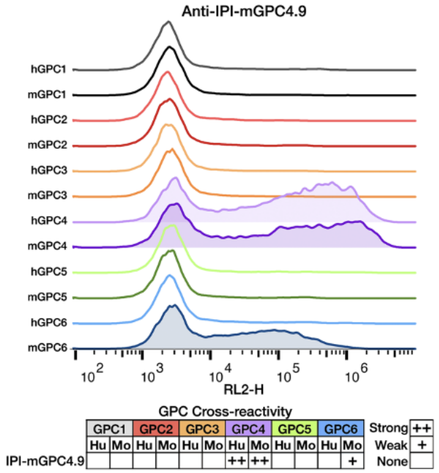
Cross-Reactivity Testing and Path Forward





- Cross-reactivity analysis
  - Enabled by the focus on protein families
  - Flow cytometry data on GPC1, 3, and 4 antibodies against all family members (Hu, Mo)
  - Can be assessed by Flow, SPR, or IF
- Further application testing (Future)
  - ELISA, IP/MS, Western Blotting, internal IHC
  - Epitope Binning
  - Cells and tissues
  - Knockdown and Knockout
- Validated antibodies distributed through Addgene

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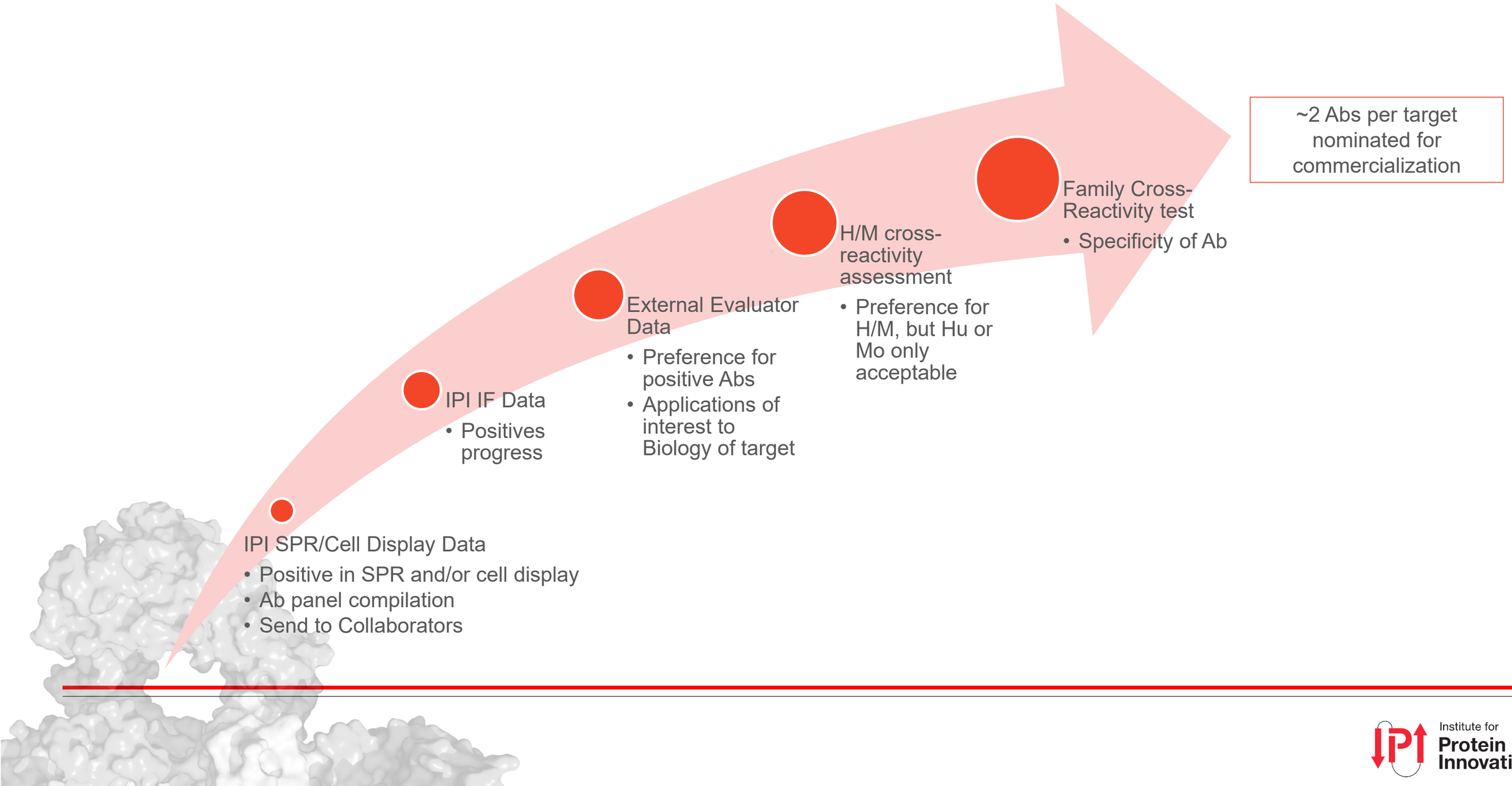
Antibody Selection Workflow for Commercialization



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Distributing Antibodies Through Addgene

Browse / Institute for Protein Innovation (IPI) / Anti-V5 [IPI-SV5-Pk1]

Anti-V5 [IPI-SV5-Pk1]

(Antibody #218107)

Print

Purpose

Anti-V5 tag chimeric recombinant antibody with fused human variable and rabbit constant domains; binds to GKPIPNPLLGLDST sequence.

Depositing Lab

Institute for Protein Innovation (IPI)

Publication

Randall et al J Gen Virol. 1987 Nov;68 (Pt 11):2769-80. doi: 10.1099/0022-1317-68-11-2769.

(How to cite)

Recommended Applications

Western Blot

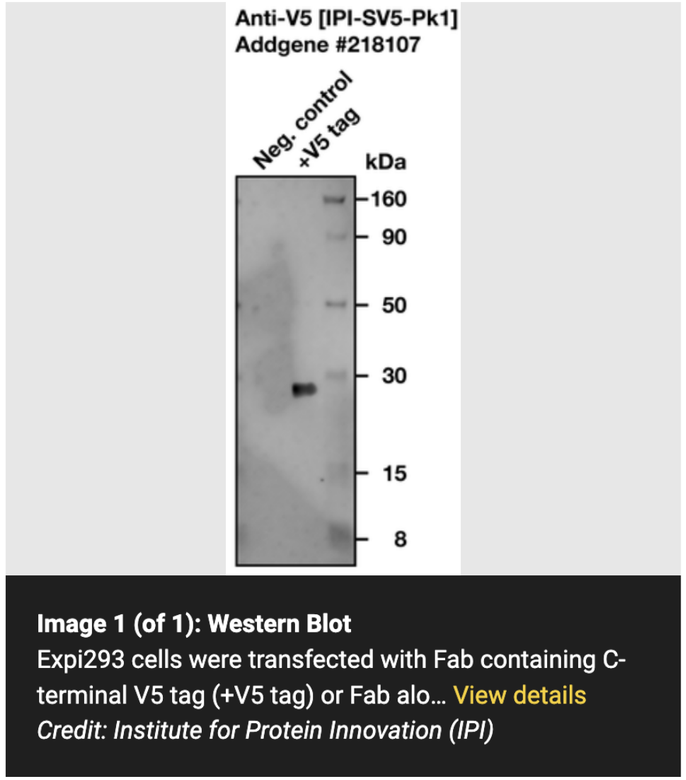

Image 1 (of 1): Western Blot

Expi293 cells were transfected with Fab containing C-terminal V5 tag (+V5 tag) or Fab alo... View details

Credit: Institute for Protein Innovation (IPI)

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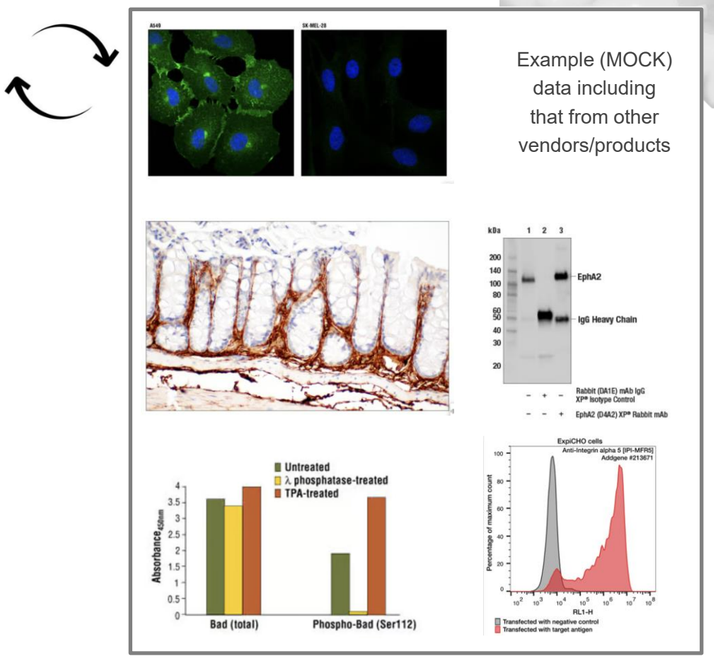
IPI Antibody Collection

This antibody was developed by the Institute for Protein Innovation (IPI).

Learn about the IPI Collection



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Summary

- IPI Produces High Quality Recombinant Antibodies to Further Scientific Research
  - Extensive Antibody Characterization and Validation
    - High-Throughput Surface Plasmon Resonance (SPR)
    - Cell Display
    - Application Testing
  - Glypican Antibody Validation
    - IPI IF data
      - Exogenous and Endogenous Expression
    - Collaborator IHC data
    - Cross-Reactivity Analysis
- Antibody Distribution through Addgene
- Contact us if you'd like to collaborate on an antibody discovery project!

IPI@PEGS2025

Difficult-to-Express Proteins

Mastering the Expression, Purification, and Production of Challenging Proteins

Tools and protocols for improving functional protein production

9:05 am High-Throughput Protein Expression Screening and Production of Cell-Surface Protein Ectodomains

Rob Meijers, PhD, Head, Biological Discovery, Institute for Protein Innovation

Cell-surface receptors pose challenges in expression and purification due to low levels, misfolding, and instability. We introduce a high-throughput ELISA fluorescence approach to rapidly assess multiple recombinant constructs. Utilizing small-scale expression, enzymatic biotinylation, and C-terminal His-tag capture, this approach efficiently prioritizes constructs for large-scale production. We also tested several codon optimization schemes using a minimally designed expression vector. Testing truncation constructs across various protein families demonstrated its effectiveness, significantly saving time in identifying optimal candidates for downstream applications

Cambridge Healthtech Institute's 4th Annual

Maximizing Protein Production Workflows

Accelerating Delivery of High-Quality Recombinant Proteins to Support R&D

Developing optimized protein-process roadmaps (**Friday May16th**)

8:30 am Strategies for High-Throughput Protein Production

Anita Ghosh, PhD, Senior Scientist, Antigen Production, Institute for Protein Innovation

The production and purification of recombinant native mammalian proteins in microgram to milligram quantities is a complex and time-consuming process, often presenting a significant bottleneck in protein-based applications. Despite these challenges, the Antigen Engineering and Production Group at IPI effectively supports both the high-throughput antibody discovery and validation platforms. This presentation highlights the key strategies we employ to overcome large-scale antigen production challenges while achieving "high-throughput-like" efficiency.

Cambridge Healthtech Institute's 4th Annual

Machine Learning Approaches for Protein Engineering

Putting Theory into Practice and Streamlining Biologic Development

Benchmarking & Automation (**Friday May 16th**)

11:00 am Harnessing Language Models for Antibody Prioritization

André A. R. Teixeira, PhD, Senior Director, Antibody Platform, Institution for Protein Innovation

We are leveraging protein language models to prioritize antibody candidates with superior biophysical properties, streamlining the path from discovery to functional reagents. By applying these models to high-throughput data from our integrated antibody platform—including antigen design, yeast display, sequencing, and characterization—we can rapidly select leads with enhanced developability. This talk will highlight examples and comparisons between different models and languages.

Posters:

Monday/TuesdayA047: High-Throughput Discovery of Recombinant Antibodies Against the Semaphorin Family,
Presented by Rebecca Hershman, Institute for Protein Innovation

Tuesday/Wednesday B047: A Flexible Automated Workstation for our Antibody Discovery Pipeline,
Presented by Curtis Walton, Institute for Protein Innovation

Tuesday/Wednesday B046: High Throughput Development of High-Affinity Fabs and VHH Using Minimalistic Libraries,
Presented by Deepash Kothiwal, Institute for Protein Innovation

Thursday/Friday C050: High-Throughput Characterization and Validation of Reagent Antibody Tools for Neurexin-Neuroligin Receptor Families,
Presented by Murali Anuganti, Institute for Protein Innovation

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André Teixeira
Curtis Walton
Mike Walden

Haisun Zhu
Shaotong Zhu
Anita Ghosh
Deepash Kothiwal
Nick Hollmer
Karunya Rajaraman

Mina Abdollahi
Fabbia Hossain
Chang Yang
Nirakar Basnet
Filmawit Belay
Rebecca Hershman

Miriam Li
Min-Ahn Kieu
Mahesh Vangala
Swethika Balasankar
Robert May
Leo Fletcher

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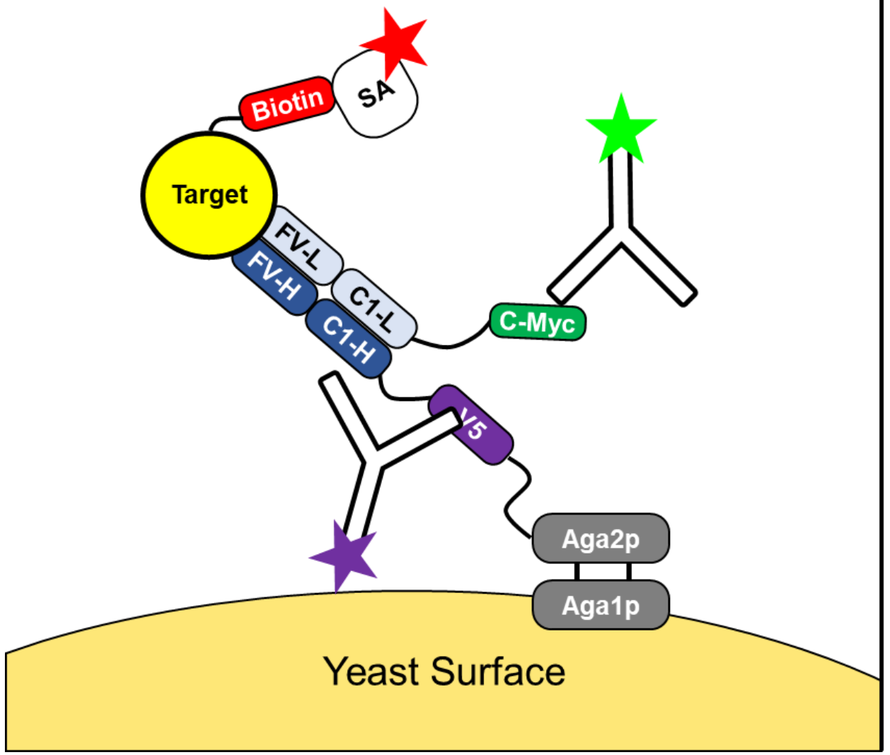
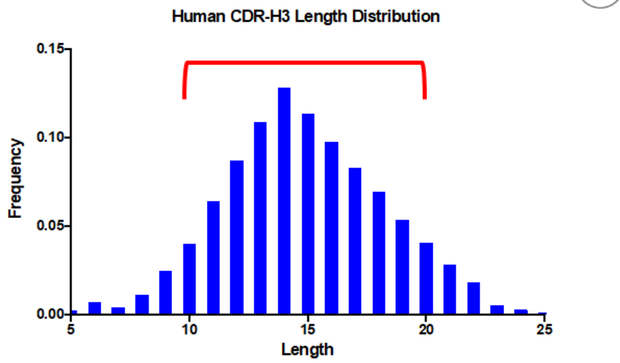
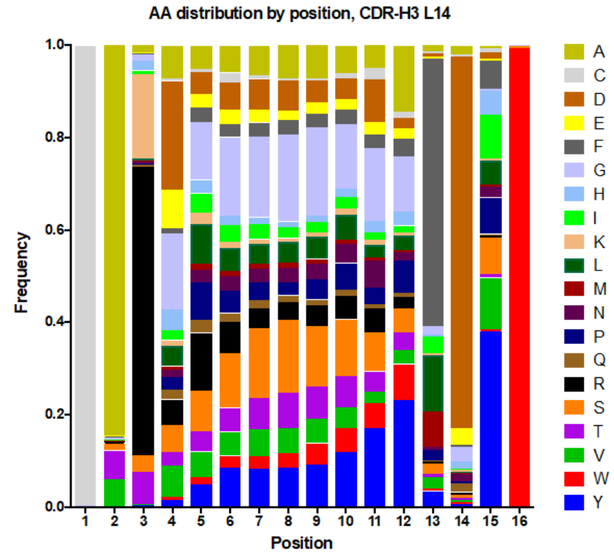
Back-up Slides

Antibody library design

Yeast antibody library
>1.5x10^{10} transformants
CDR-H3 Length 10-20
18 VH/VL pairs







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