Validation of Antibodies on Protein Arrays

The Antibody Society Webcast series – Antibody Validation #8

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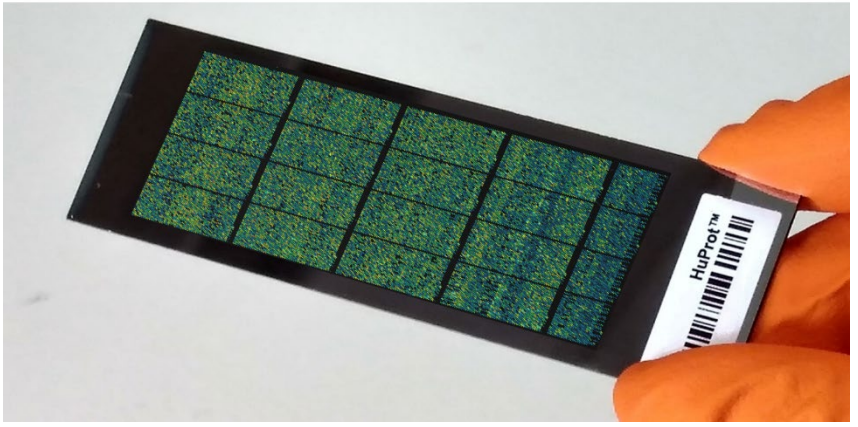
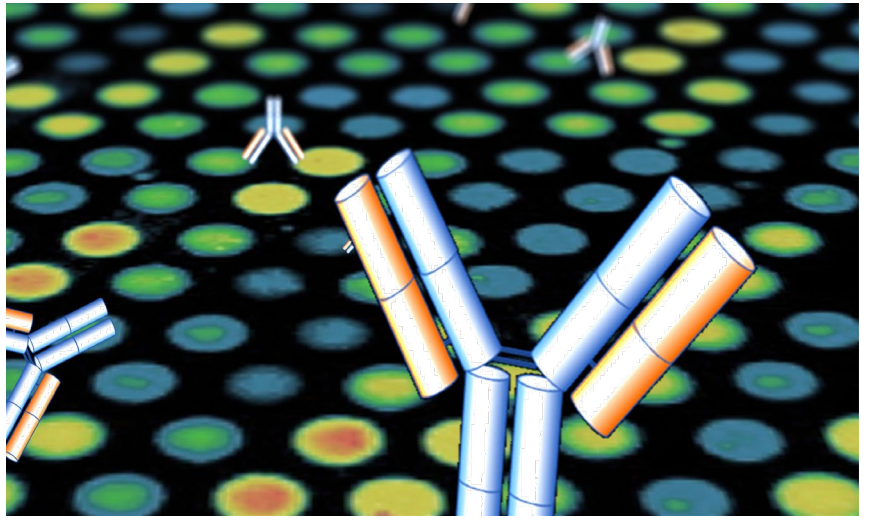
Antibody validation: problem and solution

- Use of antibodies depends on target specificity and selectivity. But they often cross-react
- This leads to misleading or erroneous data
- Validation of specificity is therefore critical for antibody reagents and therapeutics
- A solution for anti-protein antibodies: screen for binding to the widest range of proteins possible
- Efficient and economical using protein arrays





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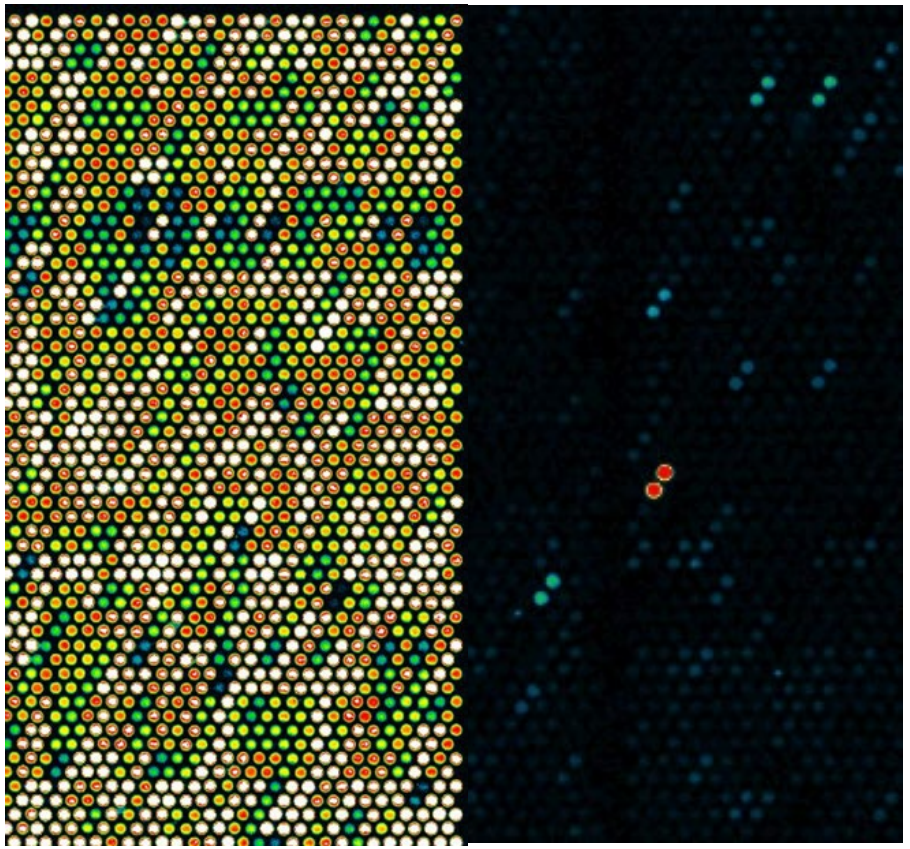
What can be learned from protein arrays for antibody validation?

Protein Arrays can address essential questions for validation of **antibody specificity and selectivity**:
Does the antibody:

- Recognize its nominal target?
- Cross-react with other proteins. If so, what is its relative binding to them?
- Working concentration affect its cross reactivity?
- Recognize native (conformational), denatured (linear) or modified epitopes?



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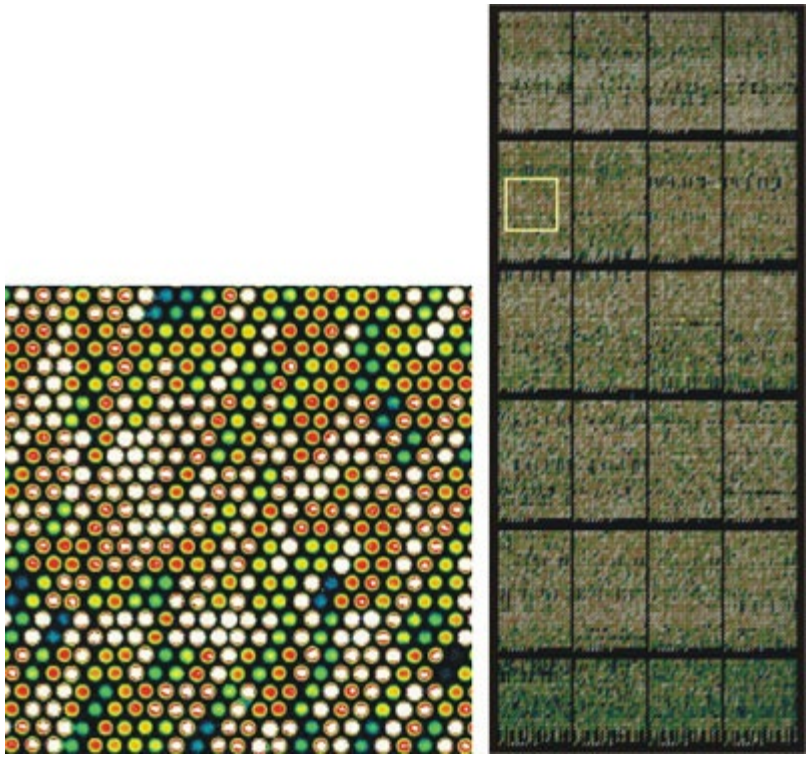
Features of protein arrays

- Miniaturised solid phase binding assays: with hundreds or thousands of surface-immobilised proteins
- Highly multiplexed, parallel screening of interactions
- Content expandable to proteome scale
- Low antibody consumption; high sensitivity
- Extensive data from single experiments



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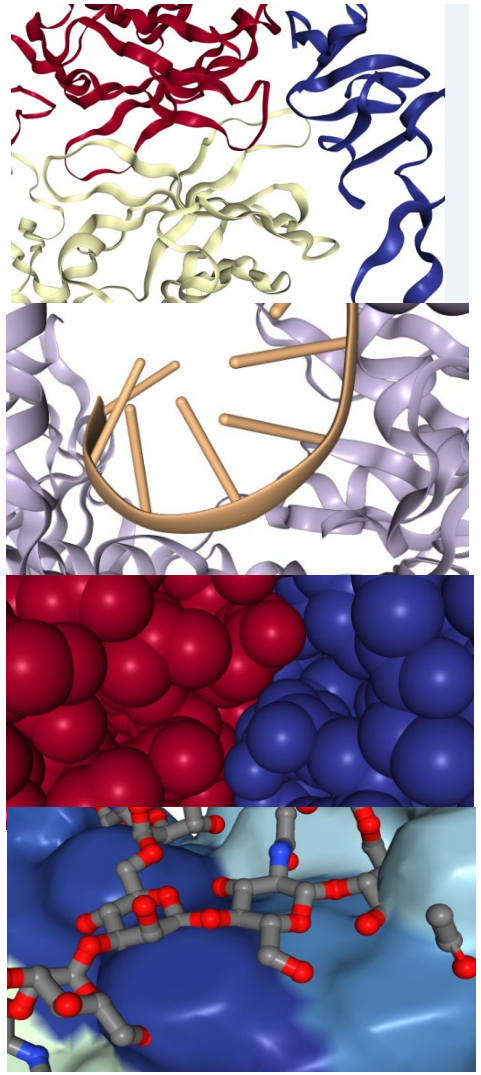
Screening applications of protein arrays

- Antibody and binder validation: format-independent definition of specificity and cross-reactivity (mAbs; recombinants; nanobodies; scFv; polyclonals; alternative scaffolds)
- Autoantibodies (plasma, sera): identify novel targets
- Protein-protein interactions
- Protein-DNA, -RNA, -small molecule interactions
- Protein target modifications
- Epitope mapping on peptide arrays

Note: Biomarker screens require antibody arrays (not covered in this presentation)



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Protein array technology issues

Issue	Steps and variables
Protein production	Prokaryotic, eukaryotic, cell free; tags, modifications; native, denatured; soluble, membrane
Surface	Functionalised glass, nitrocellulose, beads, hydrogel
Attachment chemistry	Covalent (epoxy), adsorption (nitrocellulose), tagged
Spotting	Inkjet, contact, spot dimensions, concentration
Incubation conditions	Native, denatured
Detection	Secondary Ab, streptavidin, directly labelled
Scanning	Fluorescence (10µm resolution), 2 or more channels
Data analysis	Grid alignment, numerical data extraction, processing (e.g. R, Excel), filtering, z-scores, ranking

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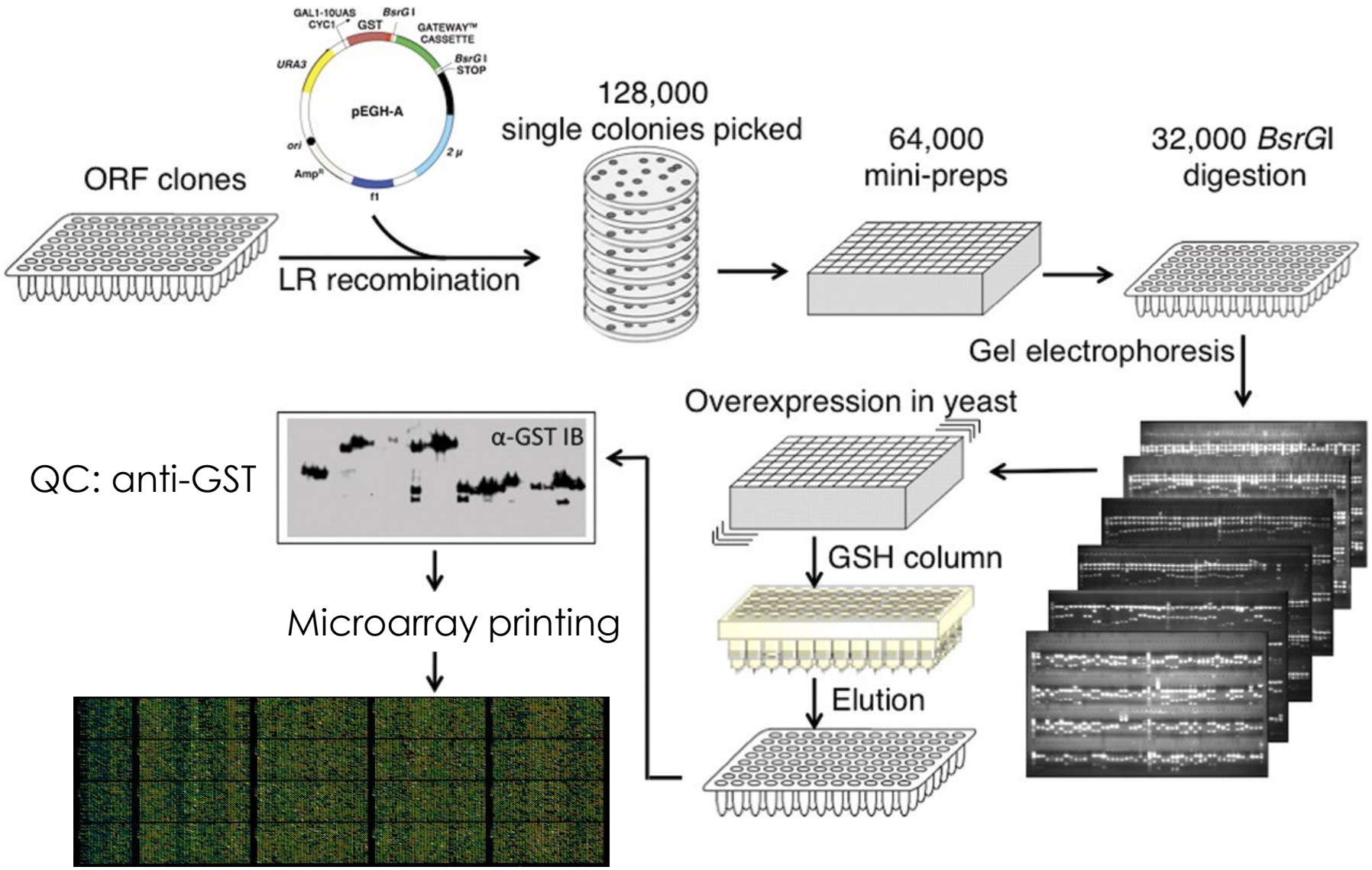
Construction of a human proteome array



Jeong J S et al. Mol Cell Proteomics 2012

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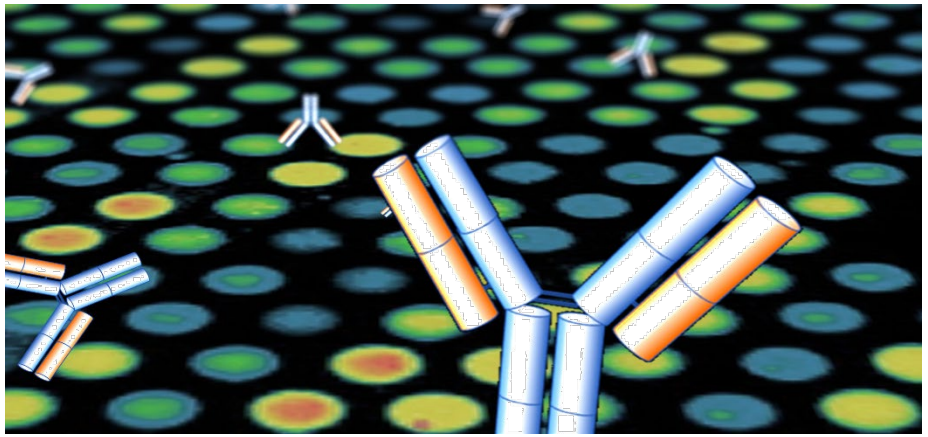
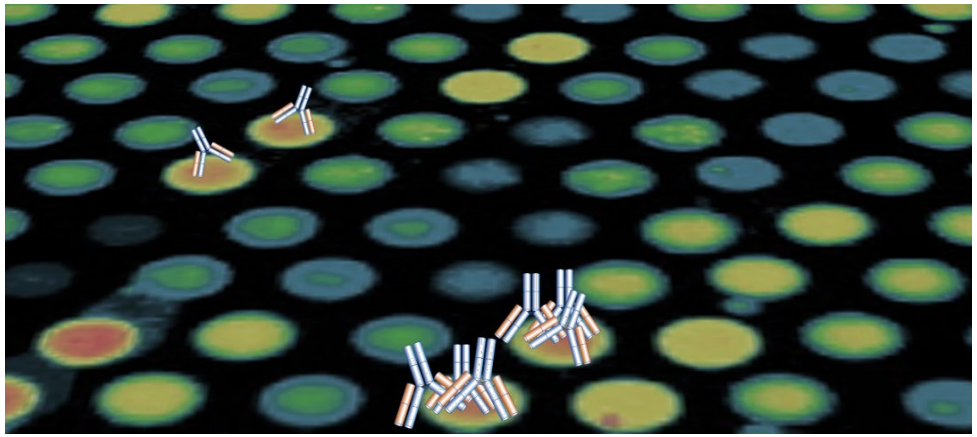
Workflow for antibody validation on protein arrays



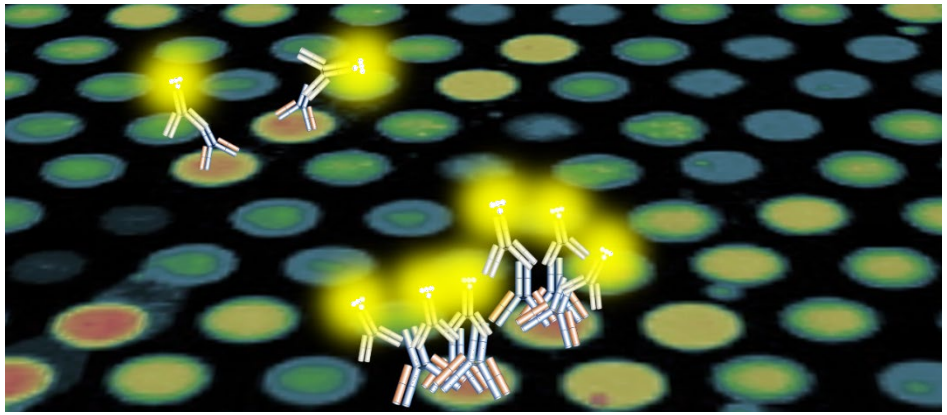
Incubate test antibody on array

Wash



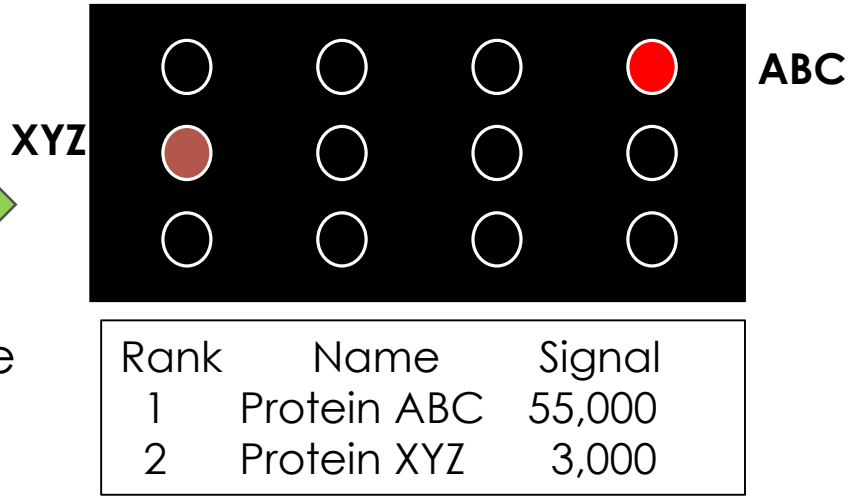
Antibody binds to specific and x-reactive spots

Incubate, wash



Binding revealed with labelled 2ry reagent

Scan image



Rank	Name	Signal
1	Protein ABC	55,000
2	Protein XYZ	3,000

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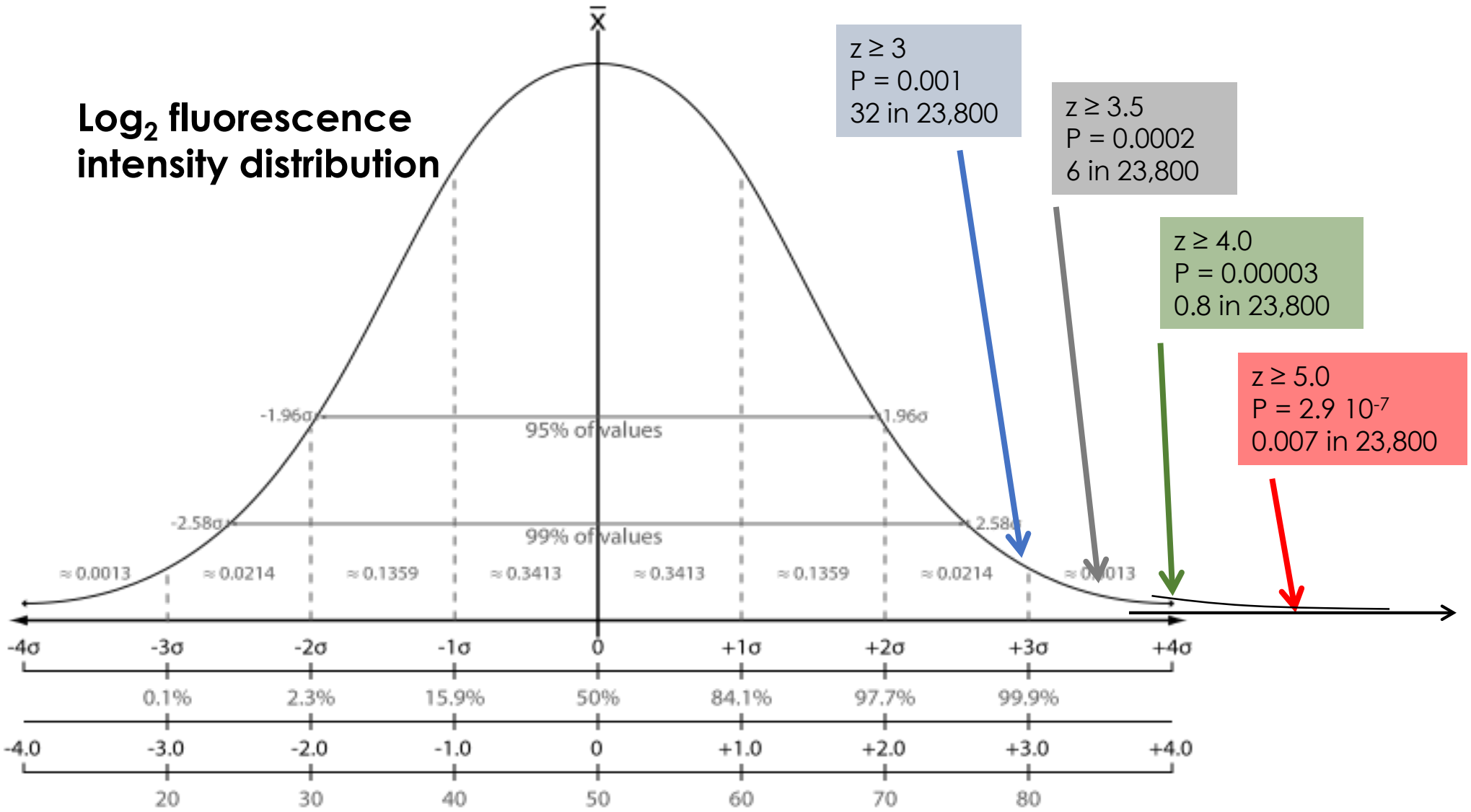
Protein array data analysis: z score



Source: Wikipedia

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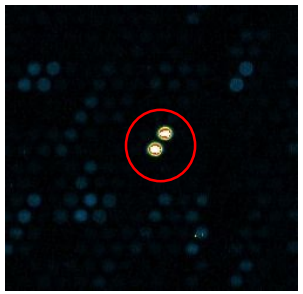
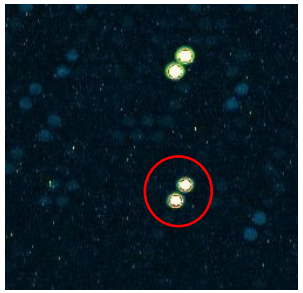
Antibody validation screening examples

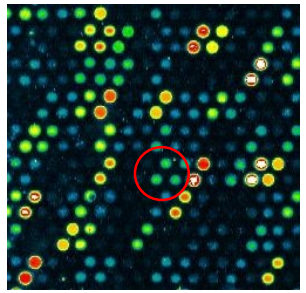
Image detail



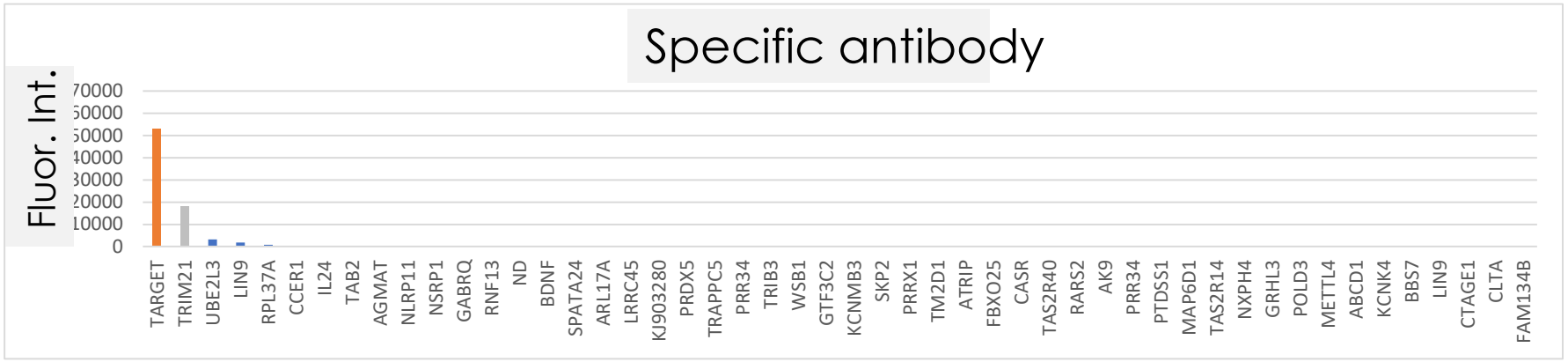
Ab 1

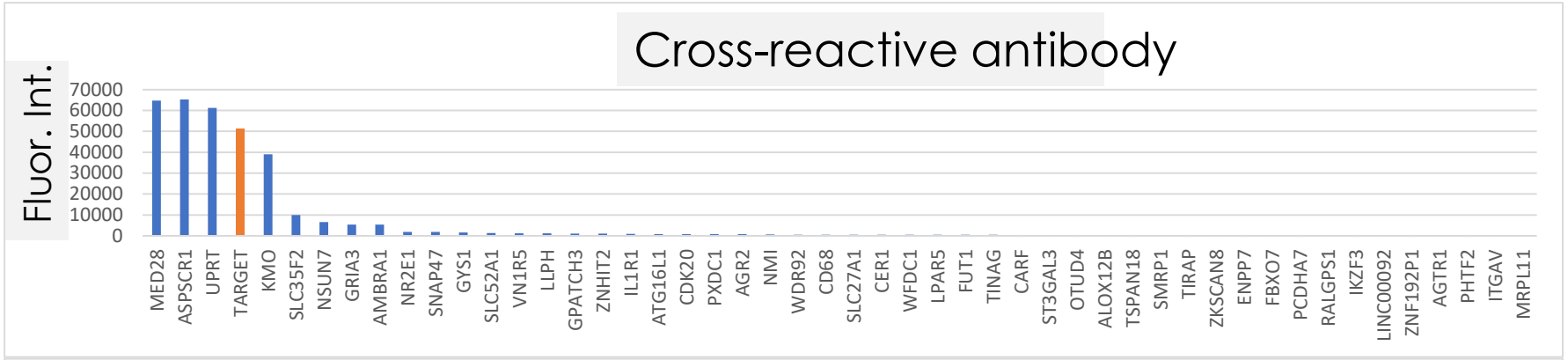


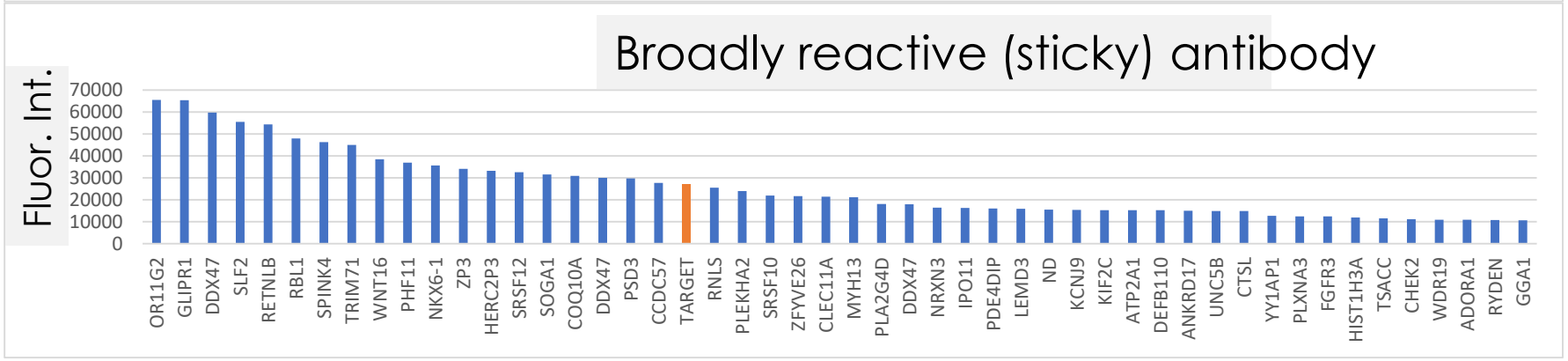
Ab 2



Ab 3
Target circled







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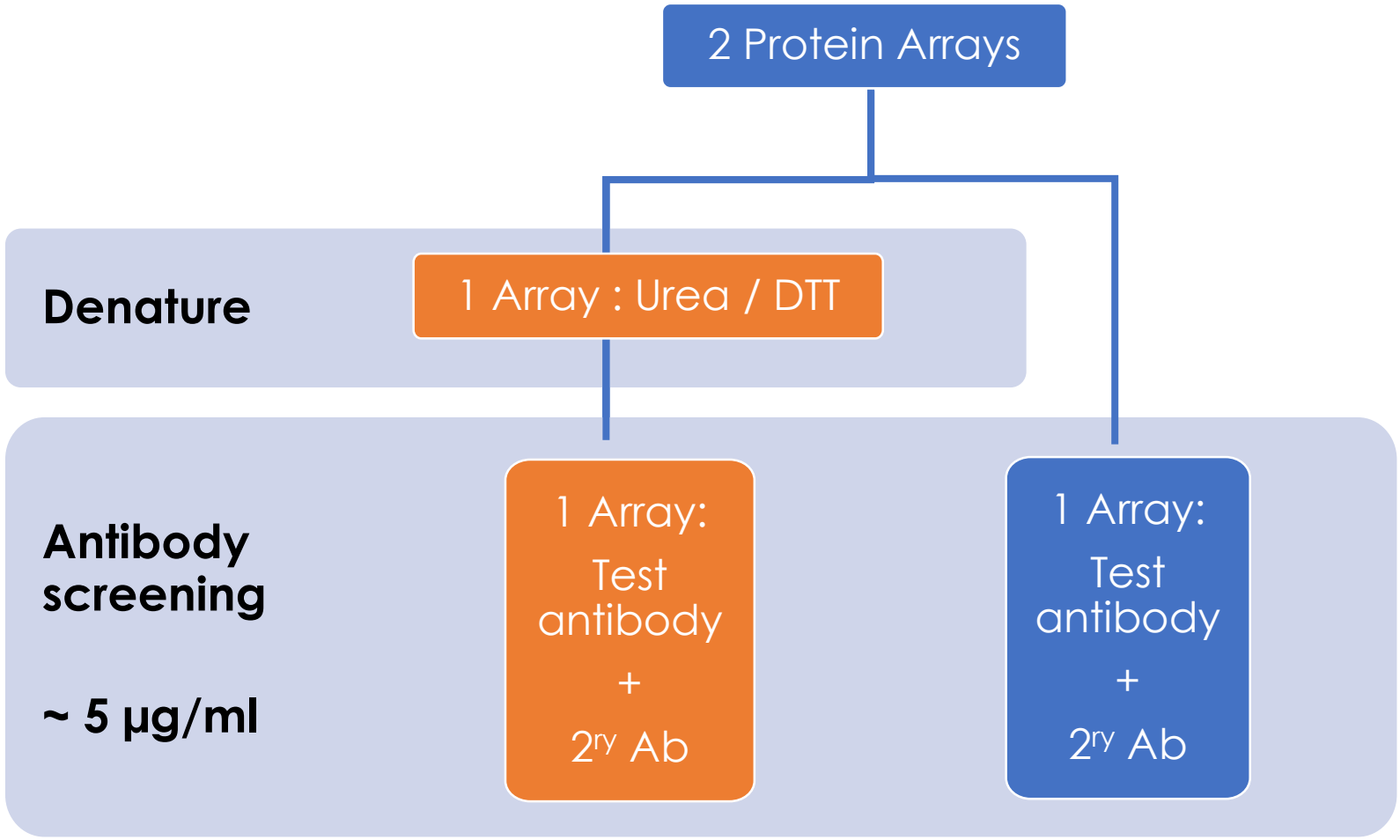
Antibody screening on denatured arrays

- Arrayed proteins can be denatured by treatment with 8M urea/DTT, or heat
- Antibodies are tested on native and denatured arrays in parallel
- Results can be compared with WB or IHC to identify cross-reactive bands or off-target staining



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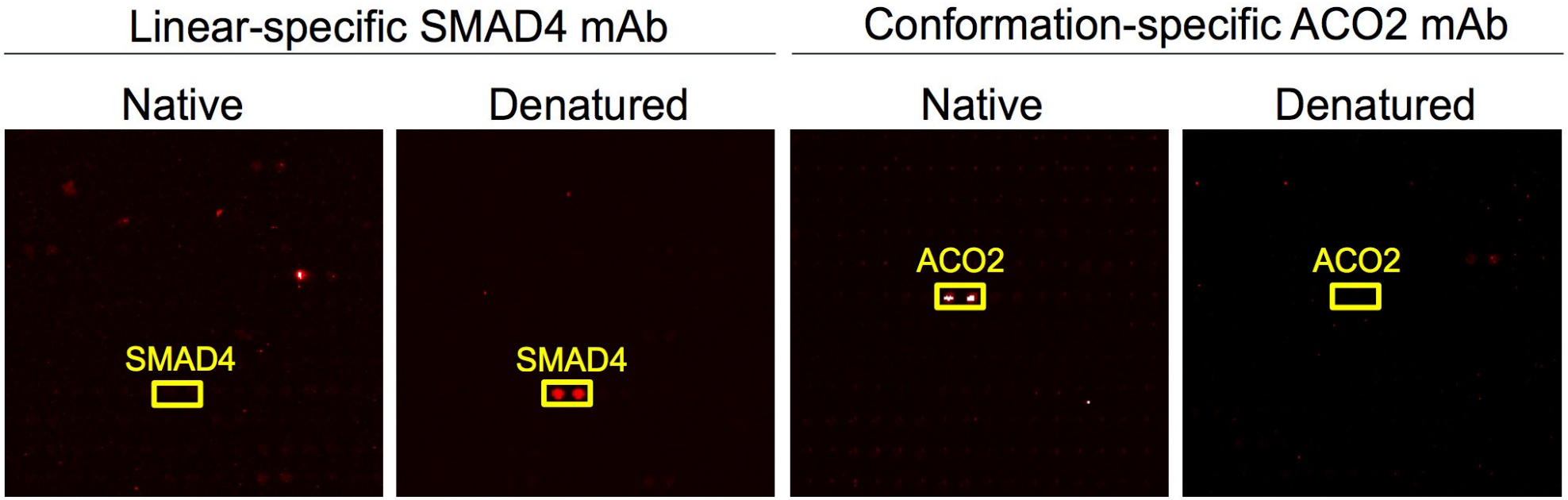
Antibody screening on denatured arrays: examples



Hu et al., CDI Laboratories & Johns Hopkins School of Medicine

- Anti-SMAD4 binding after array denaturation is consistent with linear epitope
- Anti-ACO2 binding to a conformational epitope is lost by array denaturation

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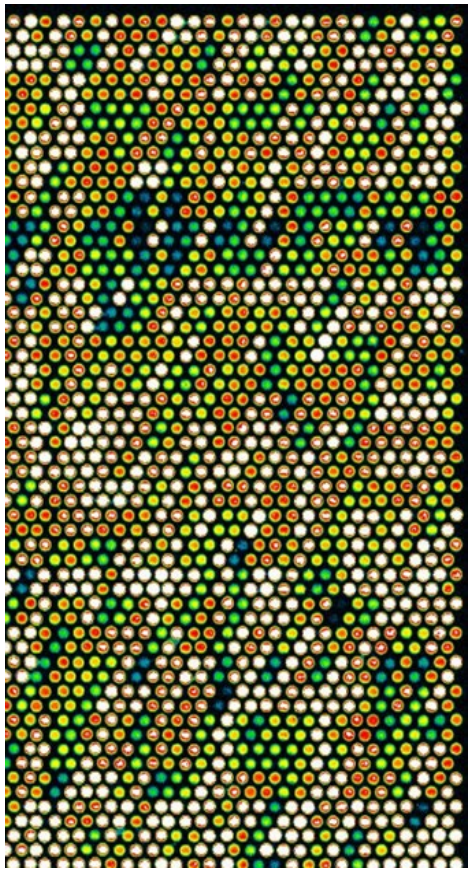
Benefits of antibody validation on protein arrays

- Sensitive screening of binding against hundreds or thousands of proteins in parallel
- Confirmation of primary antibody target specificity
- Identification of cross-reactive and off-target protein binding with ranking by relative binding strength
- Detection of linear and conformational epitopes
- Data to facilitate decision on use of antibody reagents in particular applications
- Data to guide use of a therapeutic Ab



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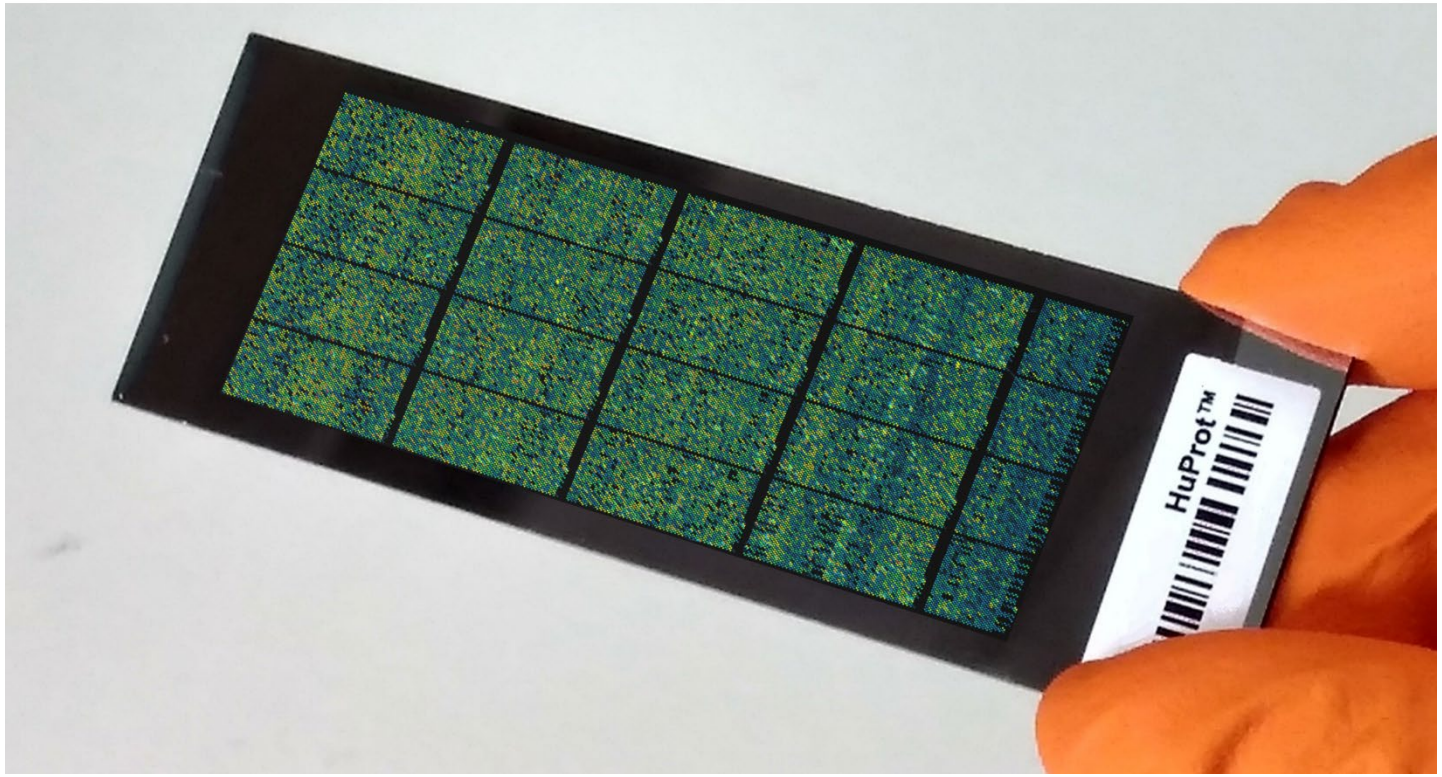
Questions?



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