A New Approach Methodology (NAM) for Specificity Testing

FDA ISTAND qualification of the Membrane Proteome Array to evaluate off-target binding of MAb-based therapeutics

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New Approach Methodologies (NAMs)

1 in 3
MAbs tested are polyspecific

We identified off-target binding in 33% of lead candidate MAbs and in 15% of clinically approved antibodies, highlighting a critical need for improved specificity testing

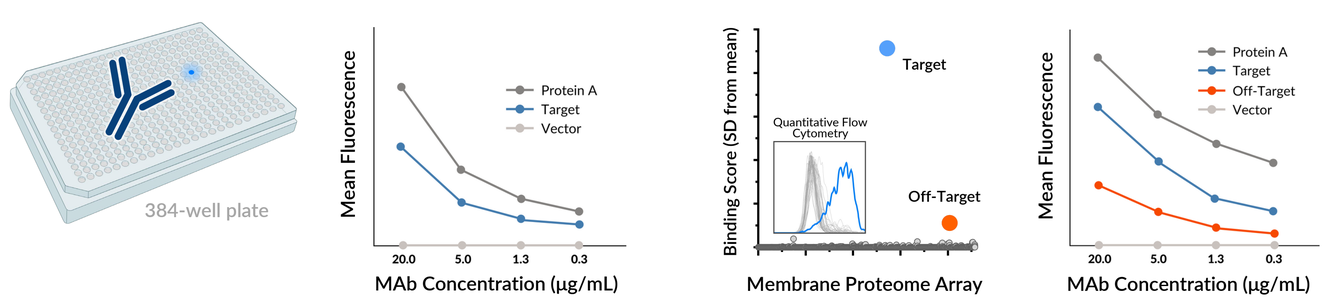
Scientifically validated NAMs provide an opportunity to better predict drug safety in humans while also accelerating drug development and regulatory review

Norden et al., 2024, *mAbs*

Membrane Proteome Array™ (MPA)

Better Specificity Screening Can De-Risk Antibody Development

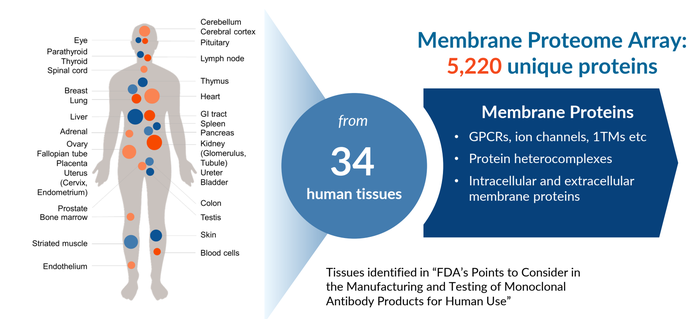
Cell-Based Protein Array *~6,000 native human membrane proteins* → **Assay Setup** *in unfixed cells by flow cytometry* → **Test Specificity** *across the membrane proteome* → **Validate Interactions** *using antibody titration analysis*



- Compatible with all biotherapeutic modalities
- Proteins screened under unfixed conditions to maintain native conformations
- MPA screening enables confident lead selection and better specificity data for IND
- Optional additional 1,200 native secreted proteins screened by ELISA

Assay Validation for DDT Qualification

1. Library Based on FDA-Recommended Tissues for Specificity Testing



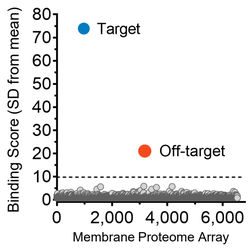
94% coverage
of membrane proteome

Key features:
- ✓ *V5 tag to confirm expression*
- ✓ *Heterocomplexes*
- ✓ *GPI-linked proteins*
- ✓ *Sex-specific proteins included*
- ✓ *Developmental stages represented*
  - *Fetal proteins*
  - *Placental proteins*

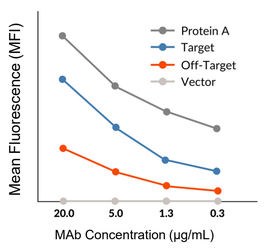
- All targets are tested for binding while expressed within human cells
- Proteins screened under unfixed conditions to maintain native conformations

2. Quantitative Data to Analyze Results and Justify Interpretations

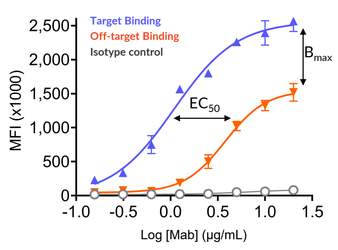
Primary Screen
Z-score (SD from mean)



Validation Assay
Mean Fluorescence



Enhanced Binding Analysis
EC_{50} and B_{max}

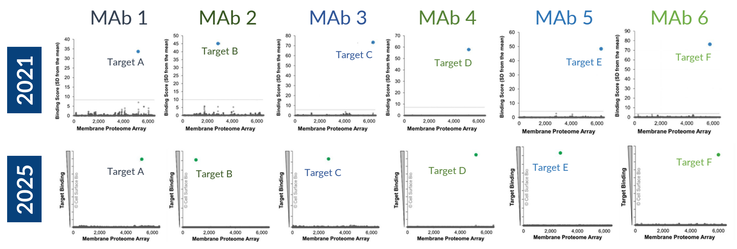


3. Analytical Validation for Full Qualification Package (FQP)

Reproducibility

Intermediate Precision Analysis
- Across days, concentrations, lots, operators

Internal Controls Over Time
- 2 MAbs tested repeatedly over years



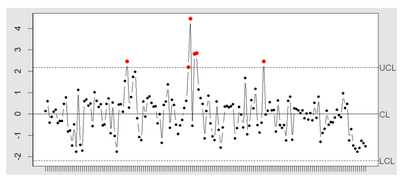
Quality control

Assay Validation

Specific target identification:
- False-negative rate <1% calculated in 2 different ways
- False-positive hits eliminated in validation

Sensitivity:
- Conditions optimized for high sensitivity

Control Charting & Thresholds



<1% background binding
<50,000 MFI background binding
S:N Maximized

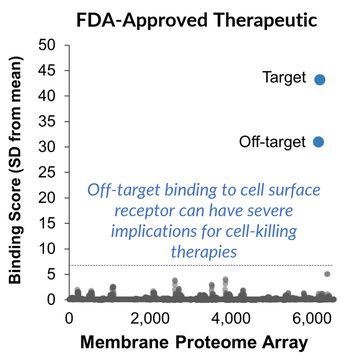
Assay Controls
- ✓ ProA control for 1°
- ✓ MAb controls for 2°
- ✓ Isotype control
- ✓ V5 expression
- ✓ GFP transfection
- ✓ 2 cell types
- ✓ Negative controls
- ✓ Replicates
- ✓ Sensitivity controls
- ✓ Titratable dilutions

QMS

ISO 9001

Clinical Validation: Comparison to Tissue Cross-Reactivity

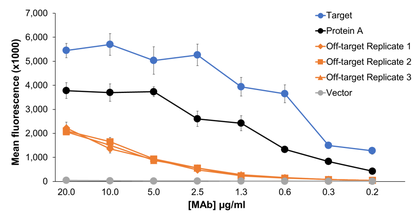
1. MPA Identified Off-Target Binding in Clinical Antibodies



Clinical MAbs Screened on the MPA				
	All	Approved	Phase 2/3	Withdrawn
# MAbs	83	40	25	18
Off-target	15	6	5	4
Rate	**18%**	**15%**	**20%**	**22%**

Norden et al., 2024, *mAbs*

Enhanced Binding Analysis



- Clinical-stage and FDA-approved MAbs were tested on the MPA
- The MPA identified polyspecificity at all stages of clinical development, underscoring the critical need for improved specificity testing

2. MPA Superior Over TCR in Identifying Off-Targets

# MAbs	MPA result	TCR result
7	Off-target binding	Clean
	4 have FDA black box warnings 2 have FDA warnings of severe side-effects 1 shows no toxicity, but MPA off-target is in brain	
3	Clean	Unexpected cross-reactivity
	In all 3 cases no off-target toxicity was identified in NHP or human studies	
17	Clean	Clean
8	Clean	Inconclusive (additional cytoplasmic staining, technical limitations, variability, false-positive staining)

- An unbiased selection of 35 FDA-approved antibody therapeutics were screened on the MPA and results were compared to TCR results obtained from public BLA reviews
- Ambiguous TCR results highlight technical limitations and the lack of actionable data resulting from many TCR studies

MPA identified off-targets missed in TCR studies. Preliminary data suggests MPA results are better correlated with clinical toxicity

MPA in Final Review as an FDA-Qualified Drug Development Tool

- FDA guidance recommends the use of advanced, **human-relevant**, in vitro assays (NAMs) to assess antibody specificity and drug safety
- MPA technology in final stages of **DDT qualification** (FQP submitted)
- MPA's ongoing process enhancements incorporate direct feedback from the FDA through its **ISTAND program**

Why Choose the MPA

TRUSTED – Successfully screened 2,000+ MAbs using **ISO 9001** certified quality processes; trusted by **hundreds of customers**

BIOLOGICALLY RELEVANT – The *only* screening platform that assesses its entire library under **native conditions**

ACCEPTED – Data accepted by FDA, EMA, NMPA; **100+ IND submissions** filed with MPA data

QUANTITATIVE – **Flow cytometry** provides sensitive and quantitative measurements

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