Funneling while maintaining functional diversity:
Strategies for amplified lead selection

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IpA
IMMUNOPRECISE ANTIBODIES
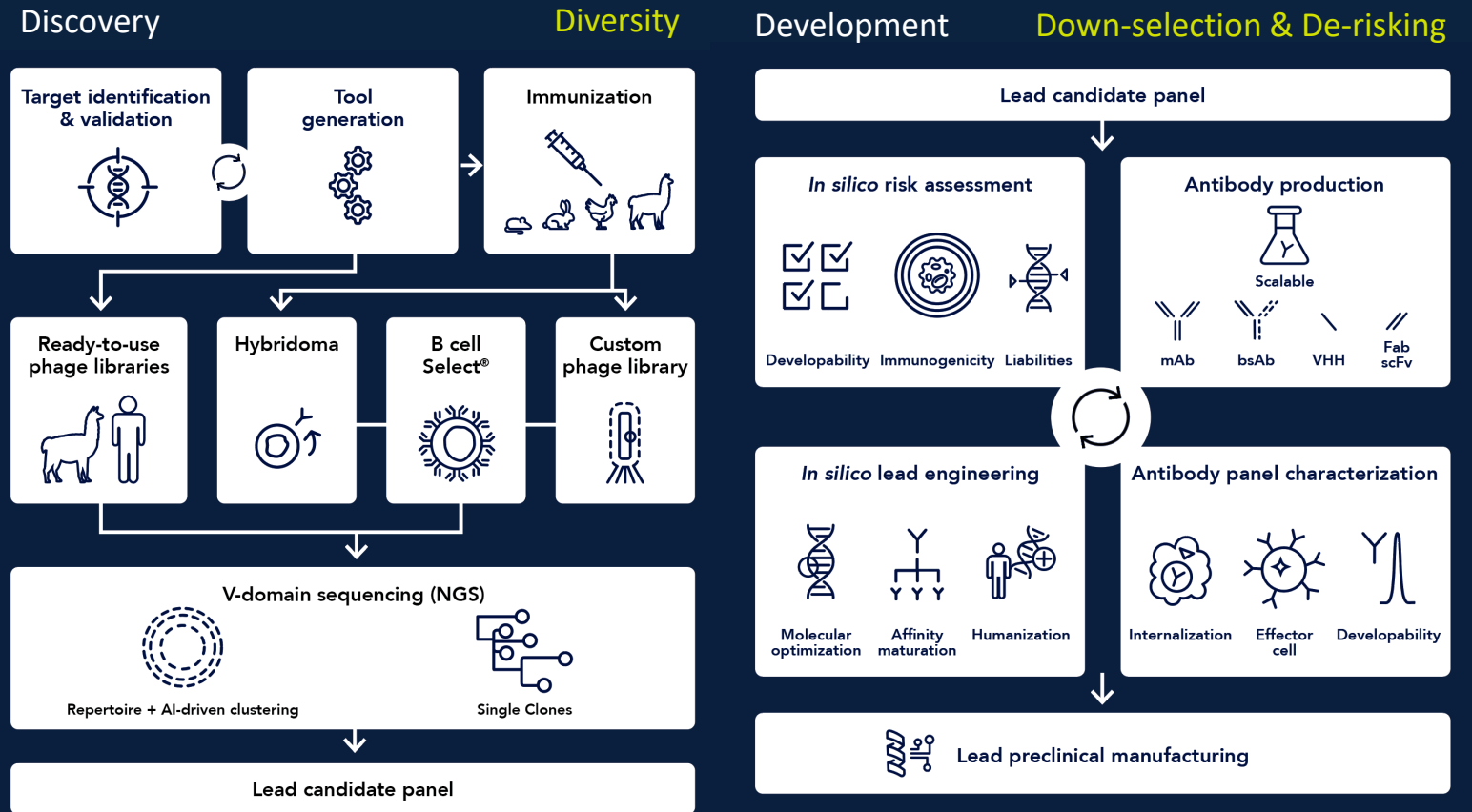
ENGINEERED for the scientific RACE

End-to-end antibody discovery and development
From research to the clinic

IpA

Fully-integrated lead generation workflow through a powerful integration of *in vitro, in vivo, in silico* technologies

Experienced experts

IPA Europe – Oss
IPA Europe – Utrecht
BioStrand Belgium - Diepenbeek

IPA USA – Fargo
IPA Canada – Victoria

Discovery   **Diversity**

Target identification & validation

Tool generation

Immunization

Ready-to-use phage libraries

Hybridoma

B cell Select®

Custom phage library

V-domain sequencing (NGS)

Repertoire + AI-driven clustering

Single Clones

Lead candidate panel

Development   **Down-selection & De-risking**

Lead candidate panel

In silico risk assessment

Developability   Immunogenicity   Liabilities

Antibody production

Scalable

mAb   bsAb   VHH   Fab scFv

In silico lead engineering

Molecular optimization   Affinity maturation   Humanization

Antibody panel characterization

Internalization   Effector cell   Developability
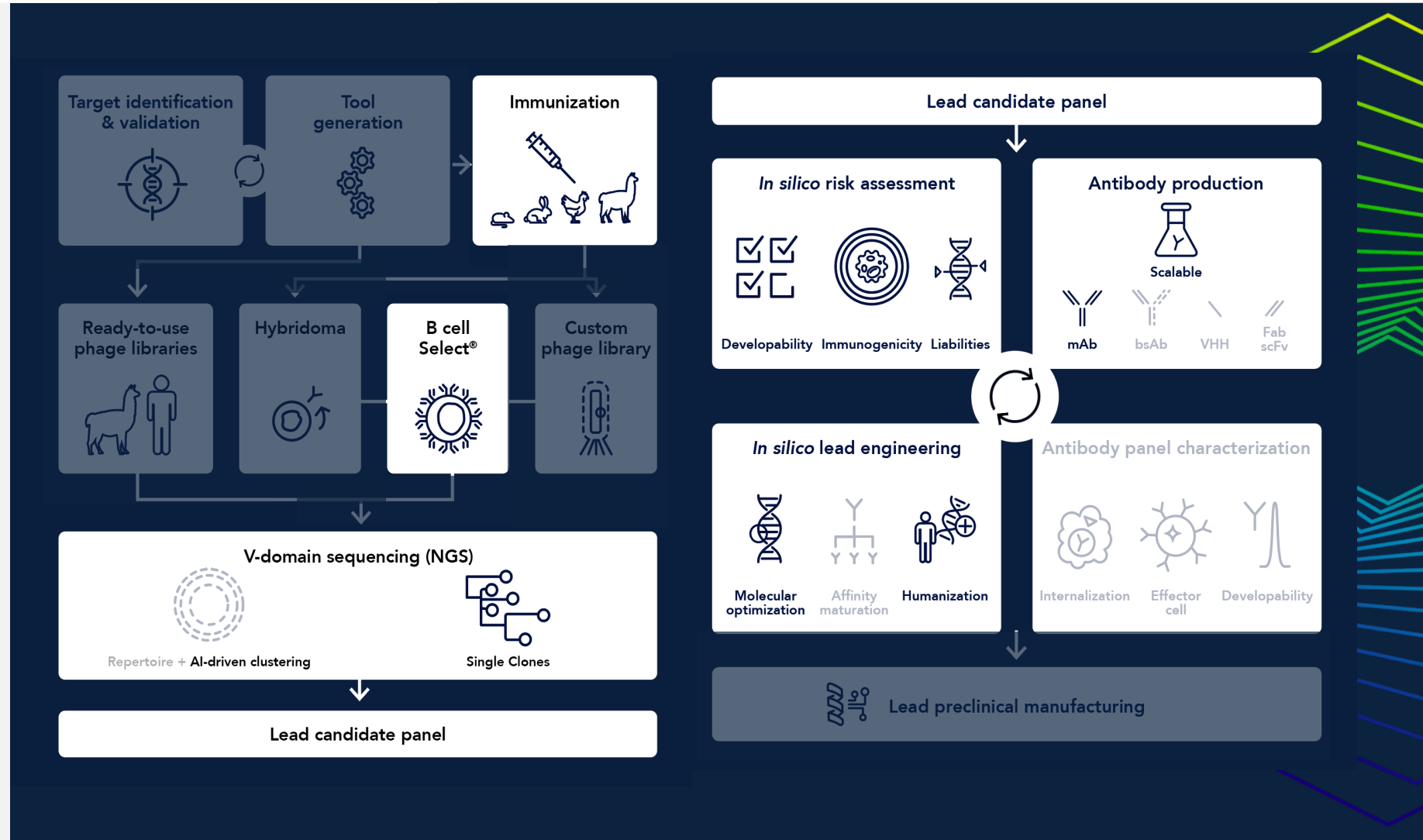
Lead preclinical manufacturing

Integrated end-to-end workflow – case study overview
The fusion of *in vitro, in vivo, in silico* technologies

- **A moving target**
  Opposing effects

- **Discovery**
  Diversity-focused

- **Functionality profiling**
  Epitope landscape-guided

- **Further development**
  Data-driven decision making

A moving target
Maximize therapeutic potential of moving targets

Restoring normal signaling

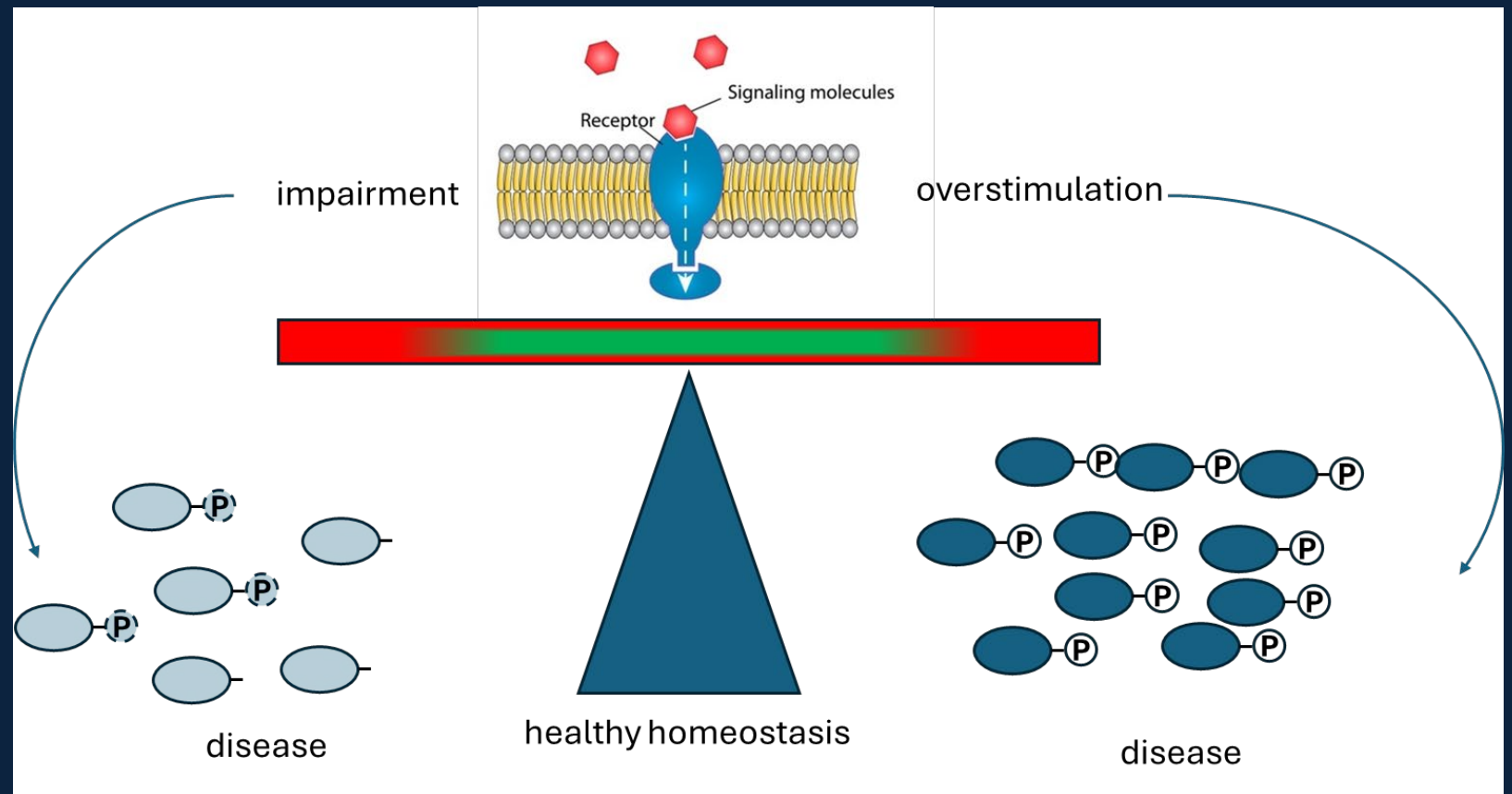- Shown to reduce pathological effects of disbalance in preclinical models

Dampening overstimulation

- Antagonistic molecule in clinical trials

Aim:

- Identify molecules inducing opposing effects on cellular function

Balanced signaling needed for prevention of disease

Diversity-focused discovery
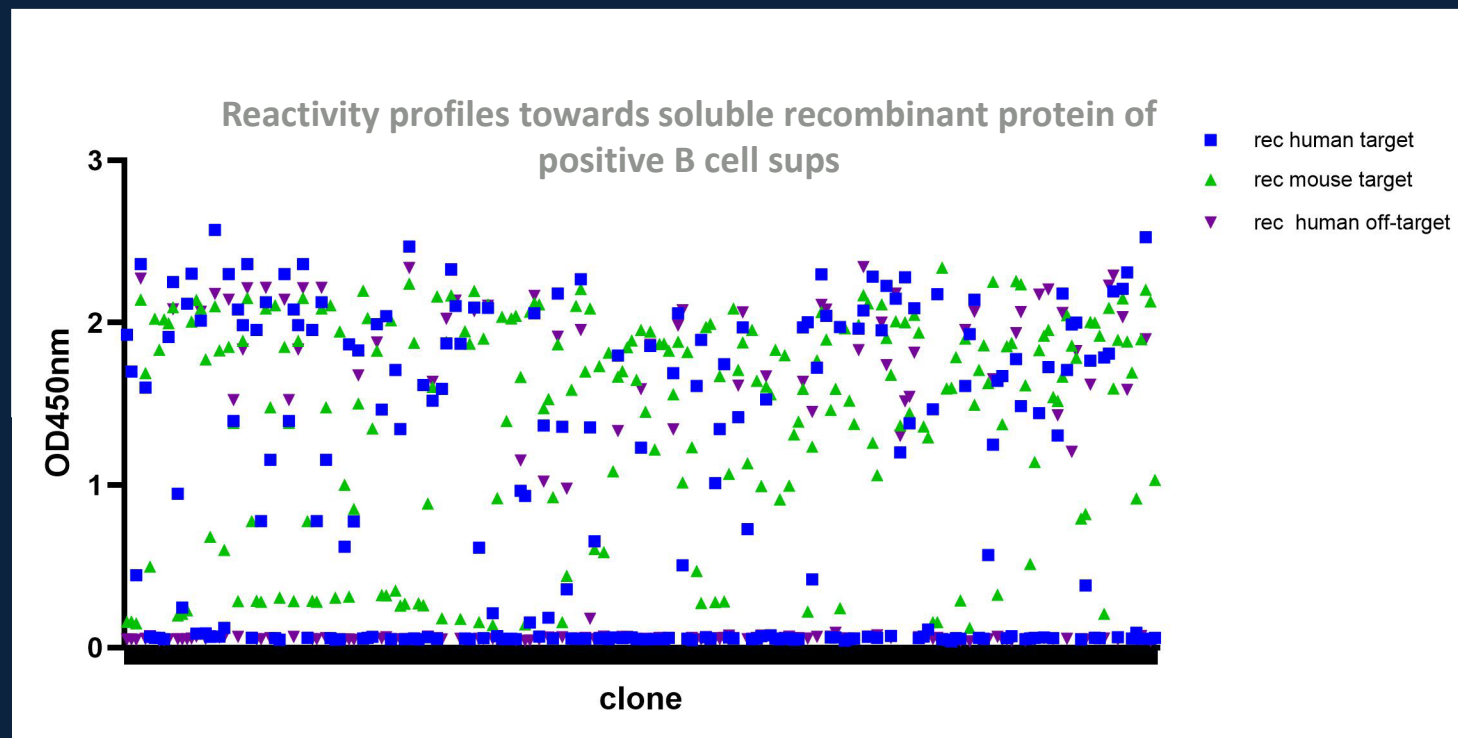## Discovery to hit unique epitopes

Animal immunization

- *In vivo* antibody maturation with diversity

- Alternating immunization

Robust B cell selection platform

- Target enrichment

- High throughput reactivity screening

- B cell clone v-domain sequencing

Triaging based on multiple binding data points

Reactivity profiles towards soluble recombinant protein of positive B cell sups

- rec human target
- rec mouse target
- rec human off-target

- Initial screening revealed 220 hits

- 48 sequence unique hits prioritized for confirmational screening

Diversity-focused discovery
AI-driven epitope binning, tackling the need for physical material facilitating early-stage insights
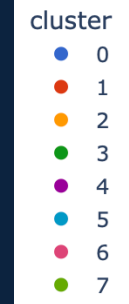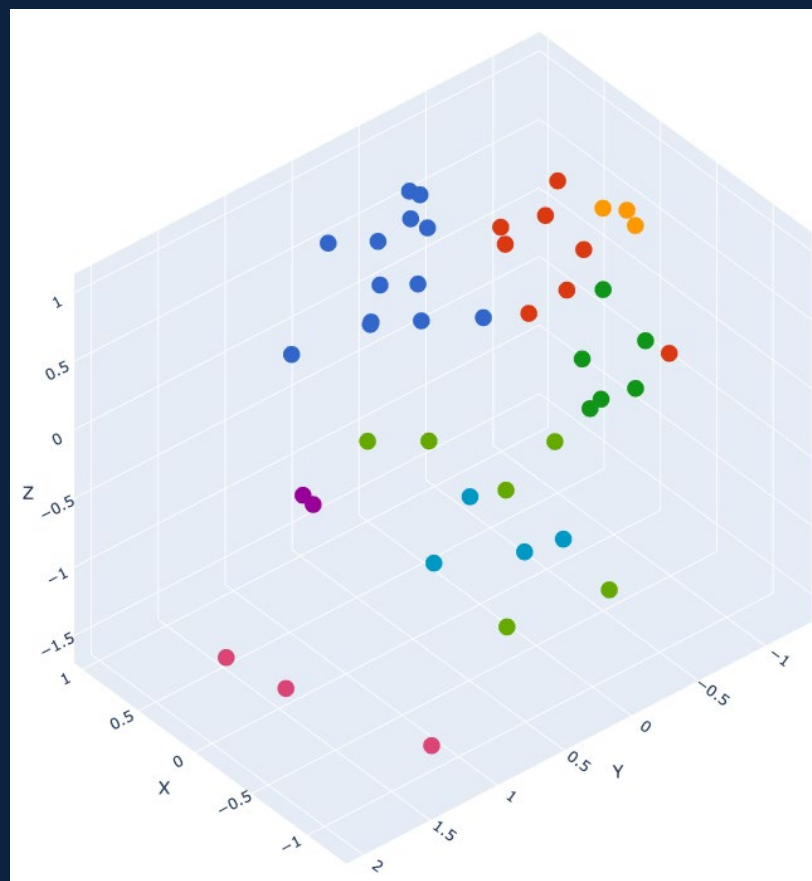
LENS^ai™ epitope binning

Highly scalable *in silico* clustering to triage hits based on predicted target binding region

- Syntactical and structural profiling

- Docking

- Atomic interactions of Ab-Ag complexes

Early insights in epitope landscape without the need for protein production

- 8 LENS^ai epitope clusters were identified within the prioritized hits

- Representatives of clusters picked for wet lab analysis

Diversity-focused discovery

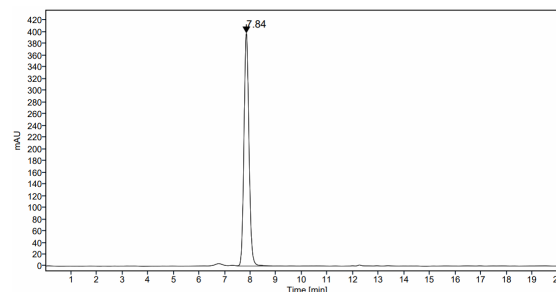*In vitro* epitope landscape profiling to triage mAbs for MoA analysis

## Recombinant production of selected candidates (rPEx®)

- Recombinant cloning, production and purification

- QC including HP-SEC and multiplex flow cytometry

High quality proteins to advance mode of action (MoA) analysis

Example HP-SEC chromatogram

Example Multiplex cell-based screening

- human target
- mouse target
- human off target

FL2-H

Antibody concentration (µg/ml)
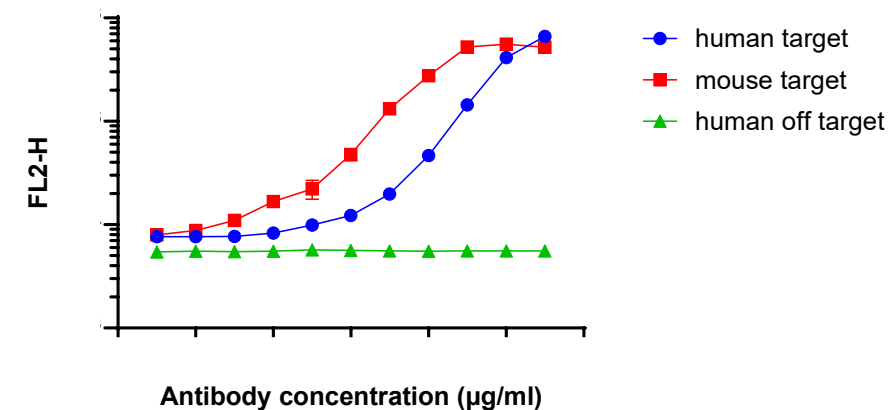
Recombinantly expressed mAbs passed QC

- Monomericity

- Confirmed binding

Diversity-focused discovery
In vitro epitope landscape profiling to triage mAbs for MoA screening
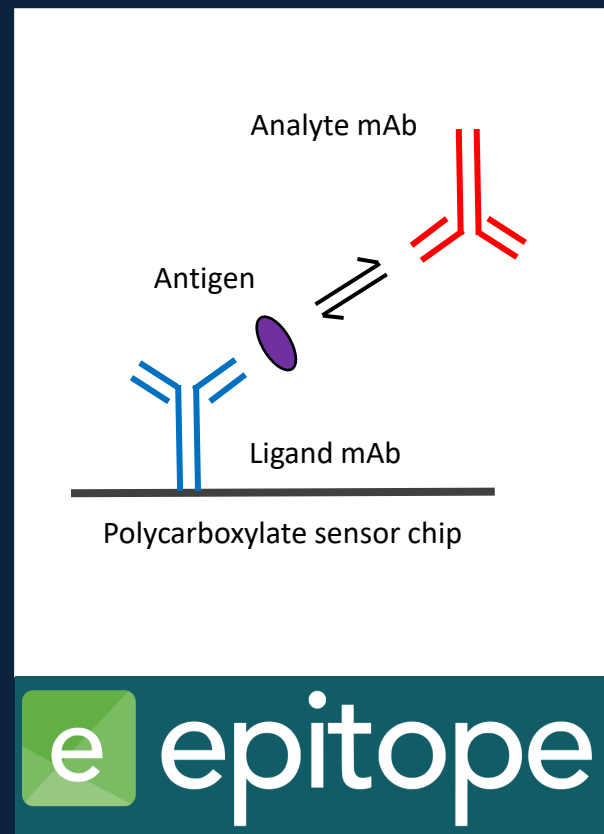
LSA-based epitope binning analyses
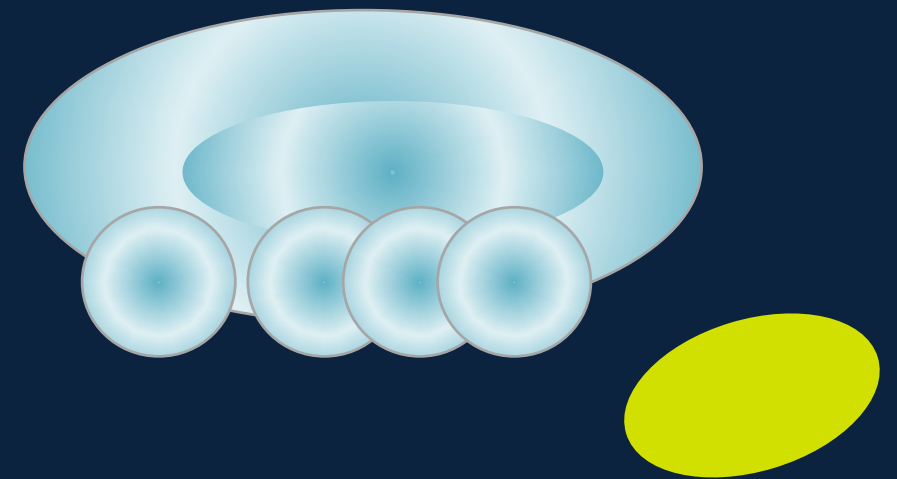
Competition-driven epitope landscape profiling

- Natural signaling molecule
- Clinical benchmark
- All-to-all candidates

HT Classical Binning - monovalent Ag

Analyte mAb

Antigen

Ligand mAb

Polycarboxylate sensor chip

Venn diagram

Clustering of antibodies into 7 'bins' based on the antigen epitope region

- Clinical benchmark falls into a bin in major cluster

- mAbs of major cluster prioritized for MoA analysis

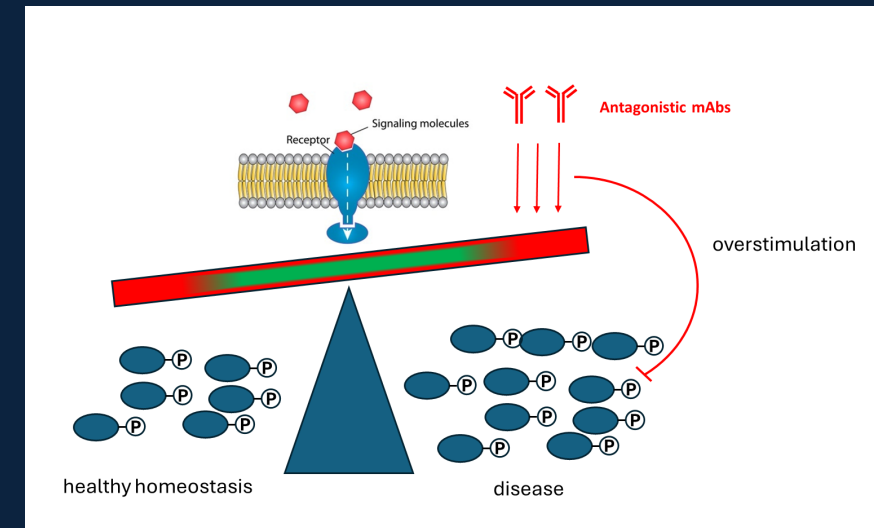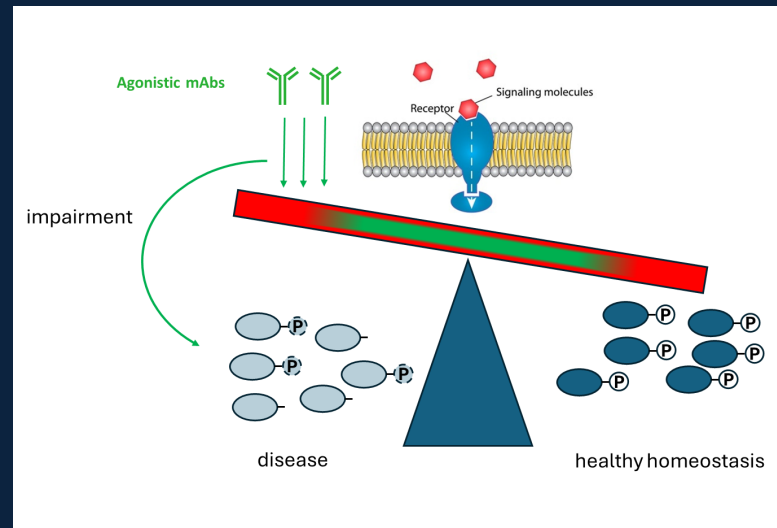# Functional Diversity: from sequence to epitope to MoA

Custom functional screening for moving targets – assaying agonistic and antagonistic potential

Mode of Action profiling

- Assessment of pathway-specific transcription factor phosphorylation

- Incubation of endogenous expressor with mAb

Custom functional assay to allow for MoA profiling
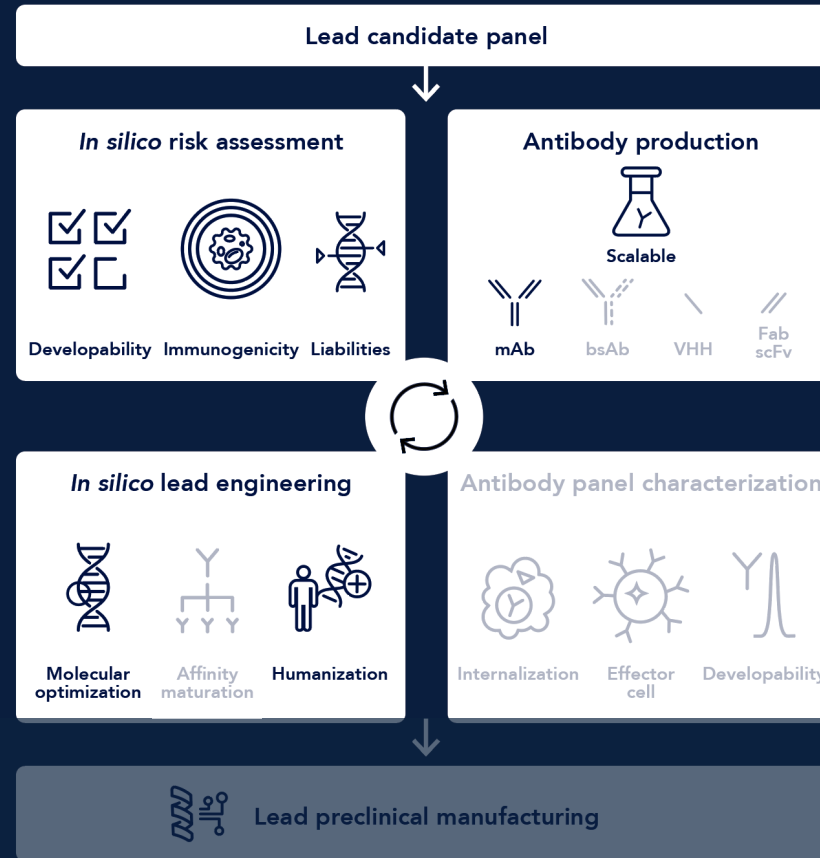

Epitope bin-dependent Mode of Action

- 10 candidates selected for further development

Integrated end-to-end workflow – case study overview
Throughput empowering data-driven down-selection

- A moving target

- Discovery

- Functionality profiling

- **Further development**

  - ✓ Scalable, integrated derisking workflow

HT antibody de-risking workflow to reduce time and cost

More informed decision making: integrated lead triage and optimization

Integrated *in silico*

Species-agonistic lead candidate humanization

Rodent
Rabbit
Chicken
Llama

Highly scalable

De-risking

Liability analysis
Immunogenicity screening
Developability profiling
Molecular optimization

Integrated / Interactive with humanization

In vitro

Recombinant optimized lead screening

Binding characterization (SPR, flow)
Functionality verification
Developability profiling

High-throughput platforms
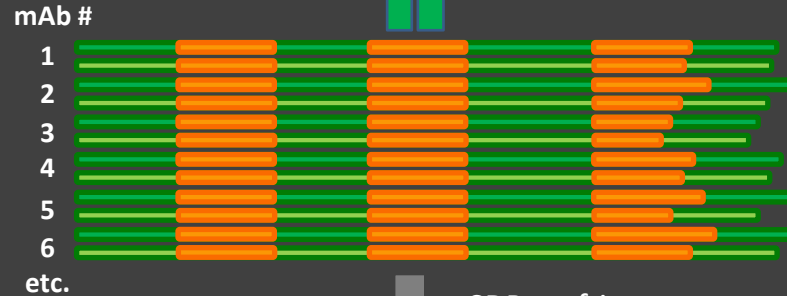
Scalable *in silico* humanization

Species agnostic workflow allowing for humanizing multiple lead candidates in parallel
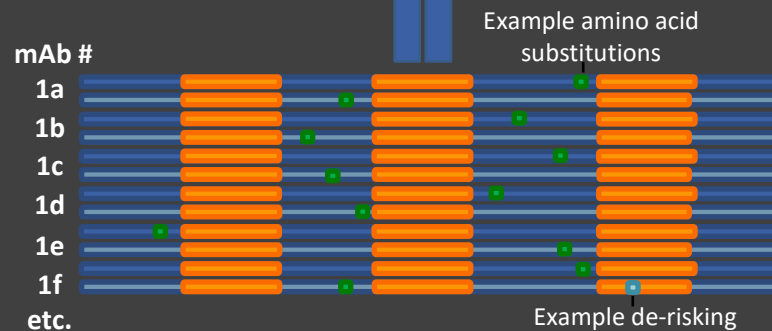
Scalable *in silico* humanization

- CDR grafting to prioritized human germlines

- Amino acid substitutions based on automated structural analysis

- Early de-risking addressing high risk liabilities

Animal mAb

mAb #
1
2
3
4
5
6
etc.

CDR grafting

Humanized mAb

mAb #
1a
1b
1c
1d
1e
1f
etc.

Example amino acid substitutions

Example de-risking

Highly scalable technologies advancing lead selection

10 parental lead candidates in parallel

- Design of up to 6 variants per parental v-domain

- High-throughput *in silico* risk assessment

  - Immunogenicity

  - Developability

    - AggScore

    - Solvent-exposed liabilities
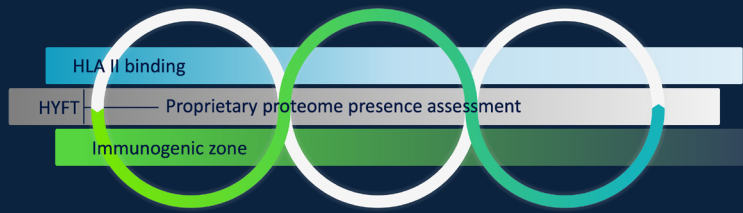
Highly scalable *in silico* risk assessment

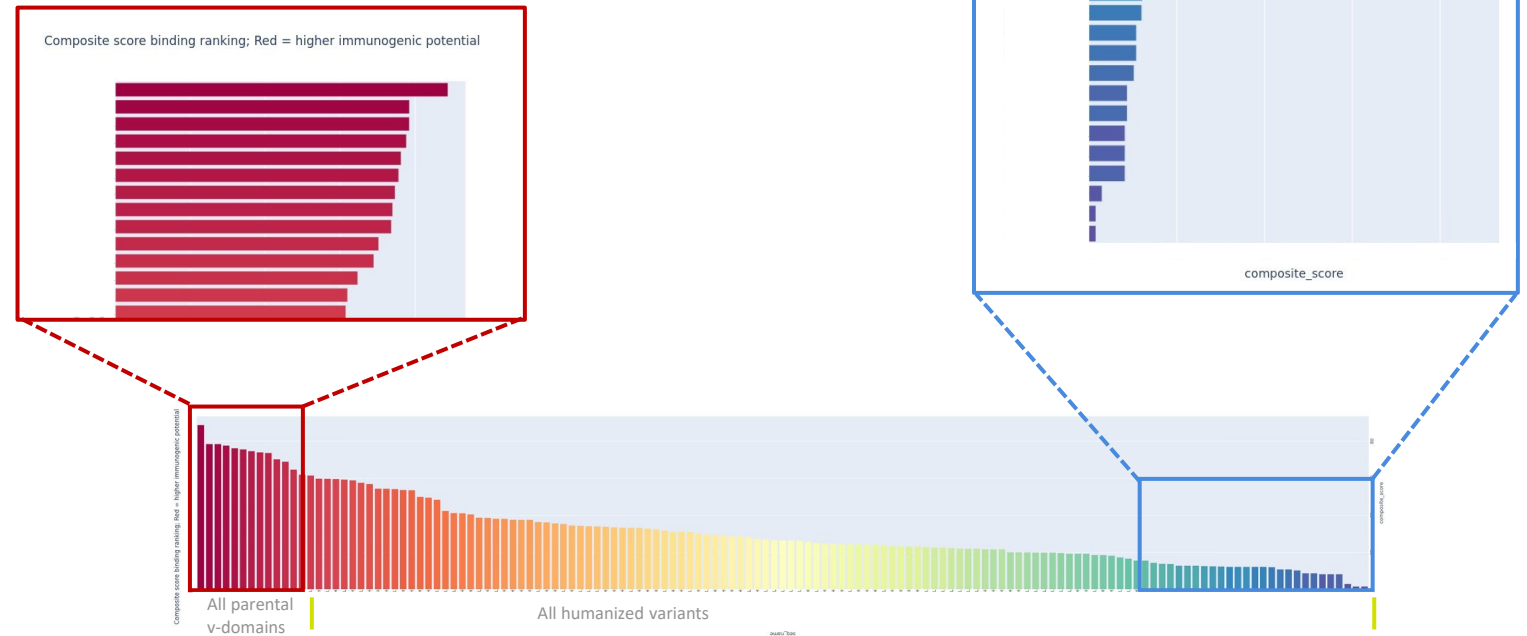LENSai immunogenicity analysis: Built for volume with detailed insights

LENSai immunogenicity analysis

Multidimensional assessment

- Combining humanness with HLA-II binding

- Ranking of virtually limitless quantities

- From global ranking to subsequence scoring

HLA II binding

HYFT — Proprietary proteome presence assessment

Immunogenic zone

Ranking of antibody sequences with detailed insights

Composite score binding ranking; Red = higher immunogenic potential

composite_score

All parental v-domains
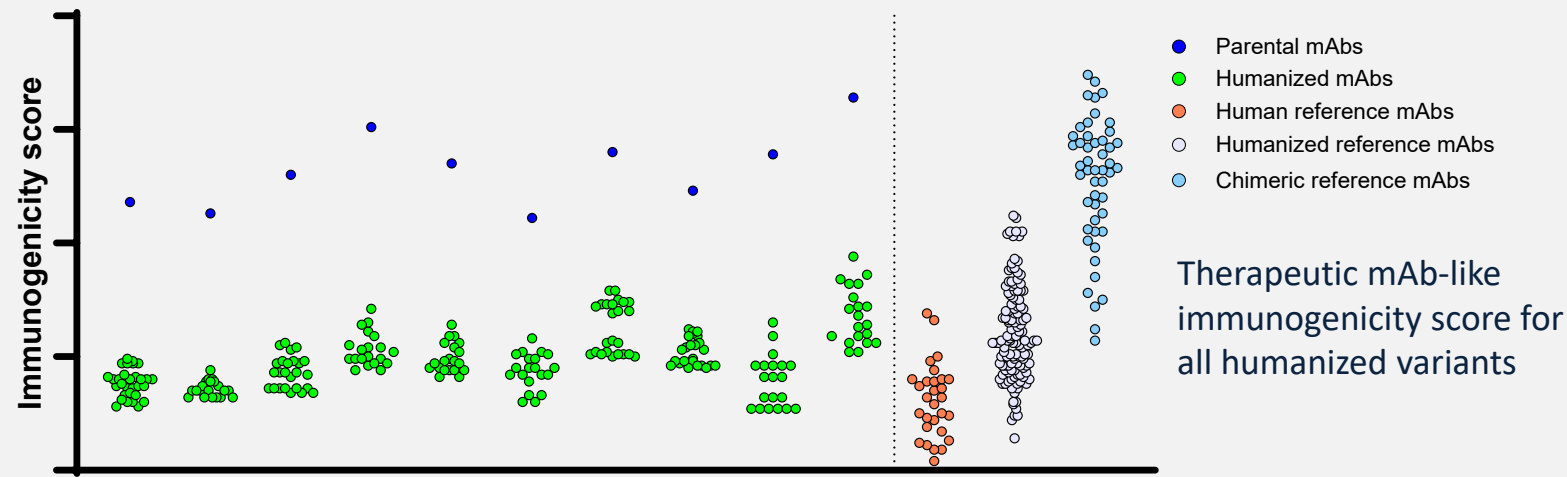
All humanized variants

Humanized v-domain variants showed lower immunogenicity scores compared to parental sequences

Highly scalable *in silico* risk assessment

LENS^{ai} immunogenicity analysis: Built for volume with detailed insights

LENS^{ai} Immunogenicity score of candidate and therapeutic mAb

- Parental mAbs
- Humanized mAbs
- Human reference mAbs
- Humanized reference mAbs
- Chimeric reference mAbs

Therapeutic mAb-like immunogenicity score for all humanized variants

Example heatmaps of immunogenic zones

Immunogenicity score

High — Low

High scoring v-domain

Low scoring v-domain

Highly scalable *in silico* risk assessment

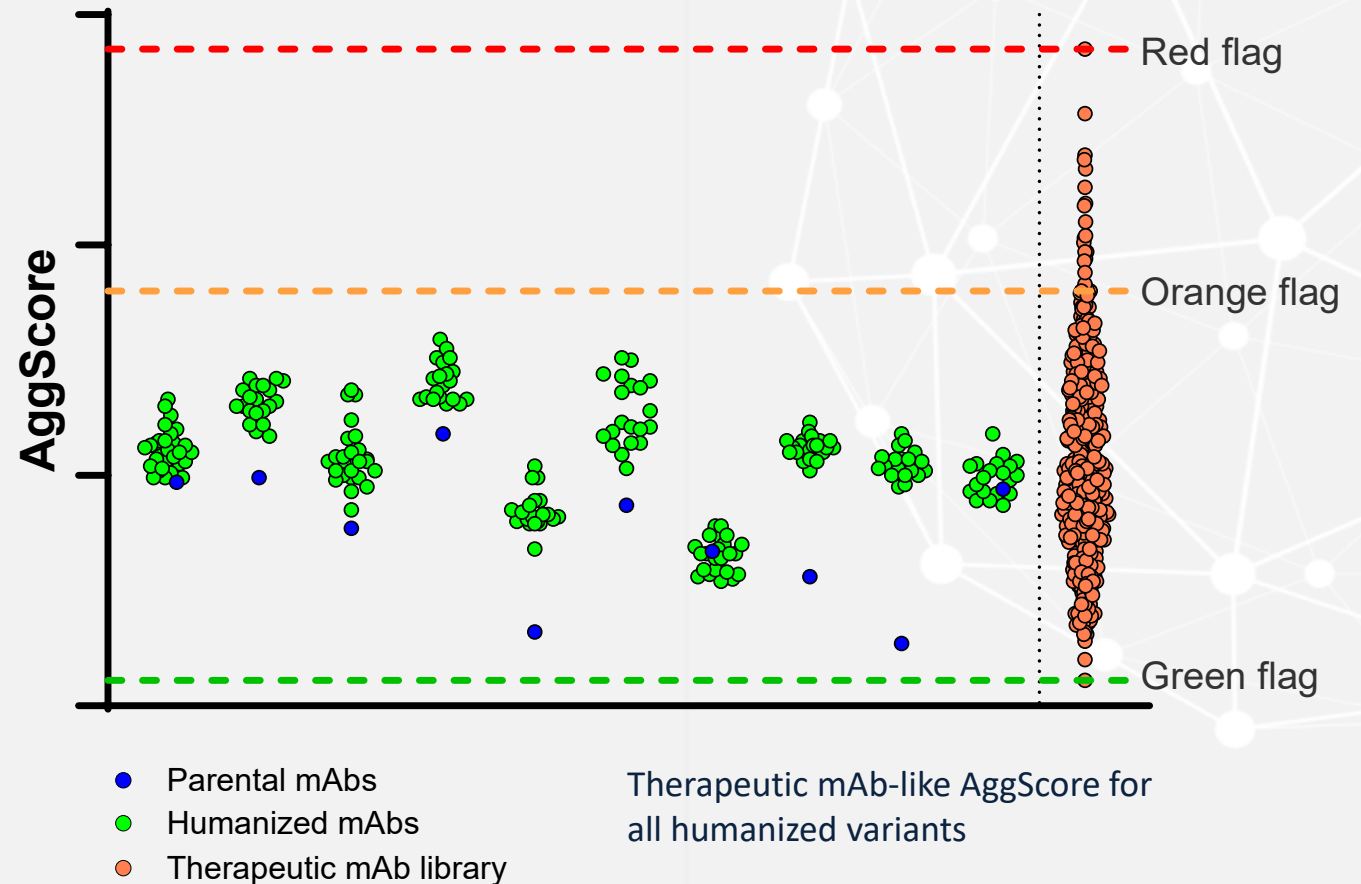*In silico* developability profiling enabling data-driven decision making

In silico biophysical characterization

Fv AggScore

- Scoring for aggregation-prone regions in antibodies based on hydrophobic and electrostatic patches
- Relative ranking towards a clinical benchmark mAb library

Fv charge symmetry, CDR AggScore, pI, CDR + and – patch energy, CDR length

Fv AggScores of candidate and therapeutic mAb

- Red flag
- Orange flag
- Green flag

AggScore

- Parental mAbs
- Humanized mAbs
- Therapeutic mAb library

Therapeutic mAb-like AggScore for all humanized variants

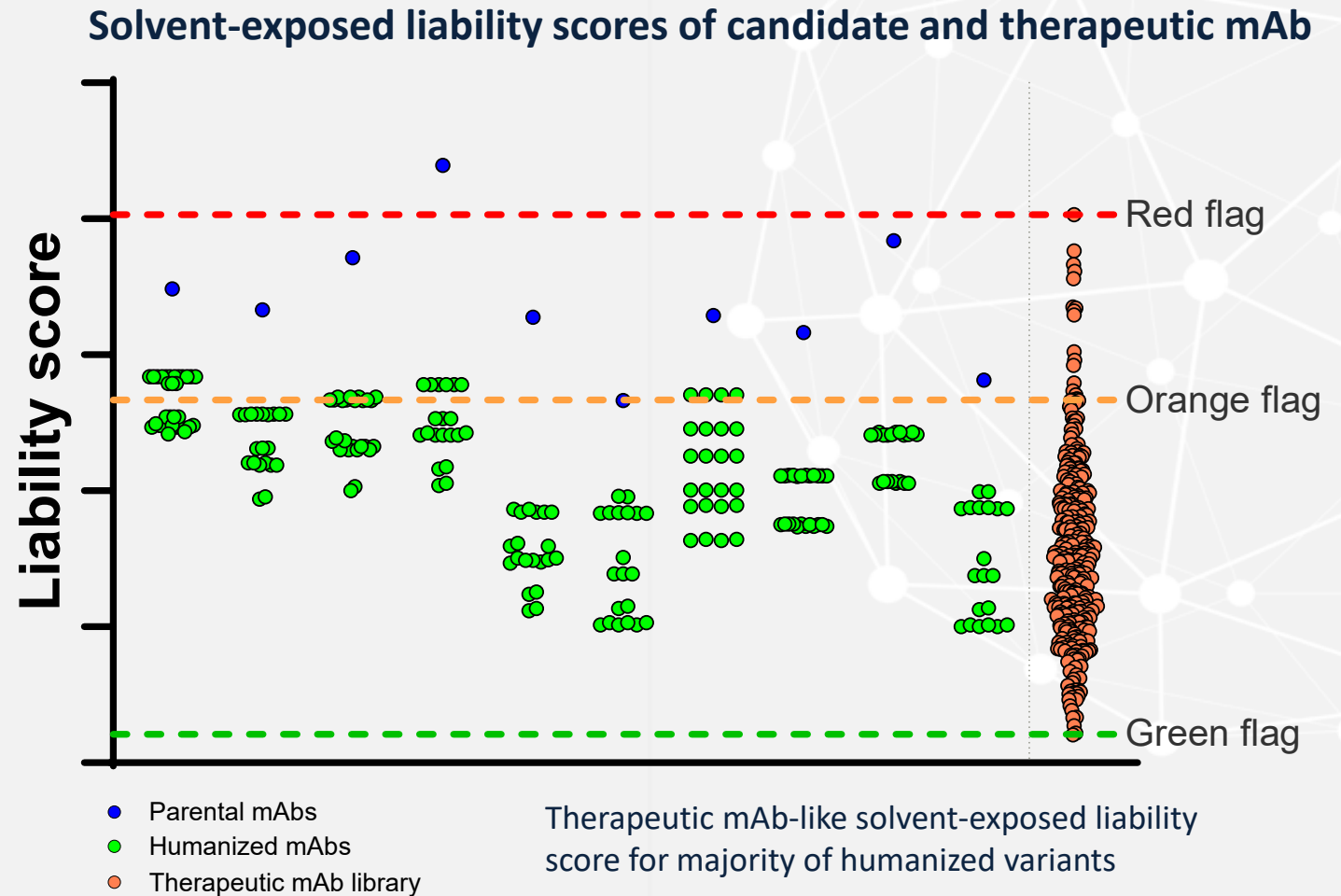# Highly scalable *in silico* risk assessment
In silico developability profiling enabling data-driven decision making

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In silico structure-based liability analysis

Liability score

- Scoring for solvent-exposed sequence liabilities
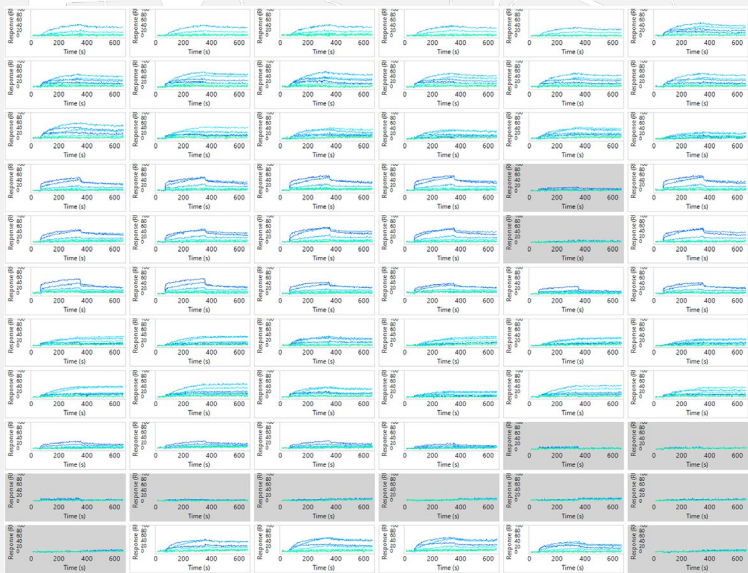- Relative ranking towards a clinical benchmark mAb library

Solvent-exposed liability scores of candidate and therapeutic mAb

Liability score

- - - Red flag
- - - Orange flag
- - - Green flag

- Parental mAbs
- Humanized mAbs
- Therapeutic mAb library

Therapeutic mAb-like solvent-exposed liability score for majority of humanized variants

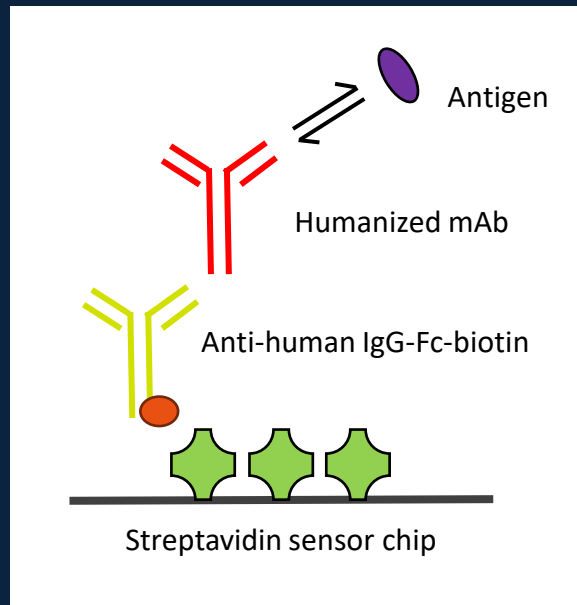# High-throughput *in vitro* affinity screening of humanized variants

Empowering diversity-driven discovery during lead candidate development
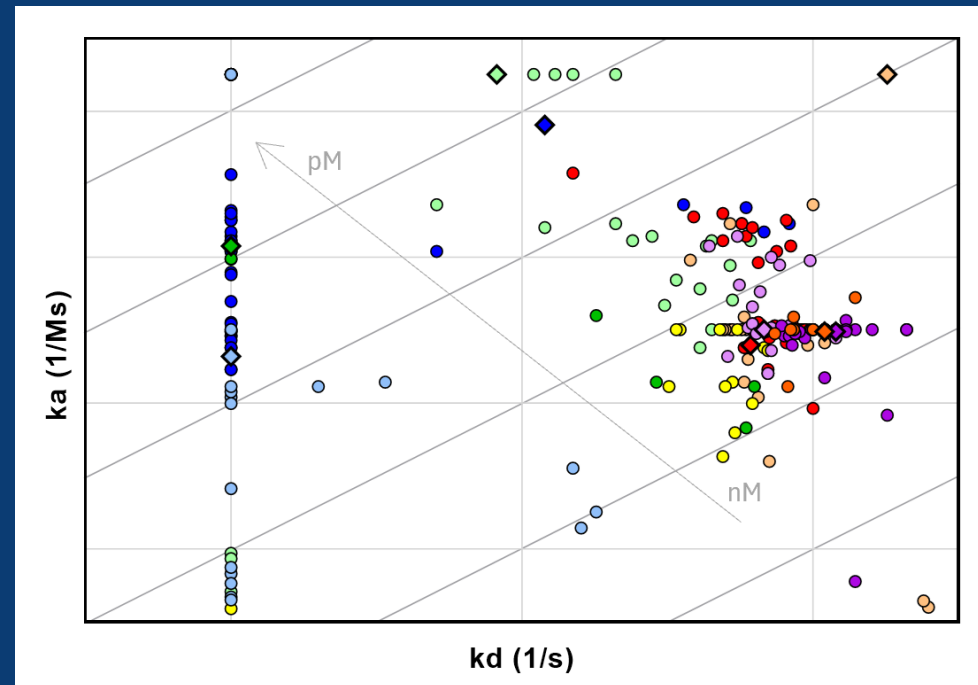

HT SPR-based affinity determination

- Crude small-scale recombinant production sup
- High-throughput

Kinetics using monovalent Ag

Antigen

Humanized mAb

Anti-human IgG-Fc-biotin

Streptavidin sensor chip

In-depth insights in kinetic parameters

ka (1/Ms)

pM

nM

kd (1/s)

Case study highlights

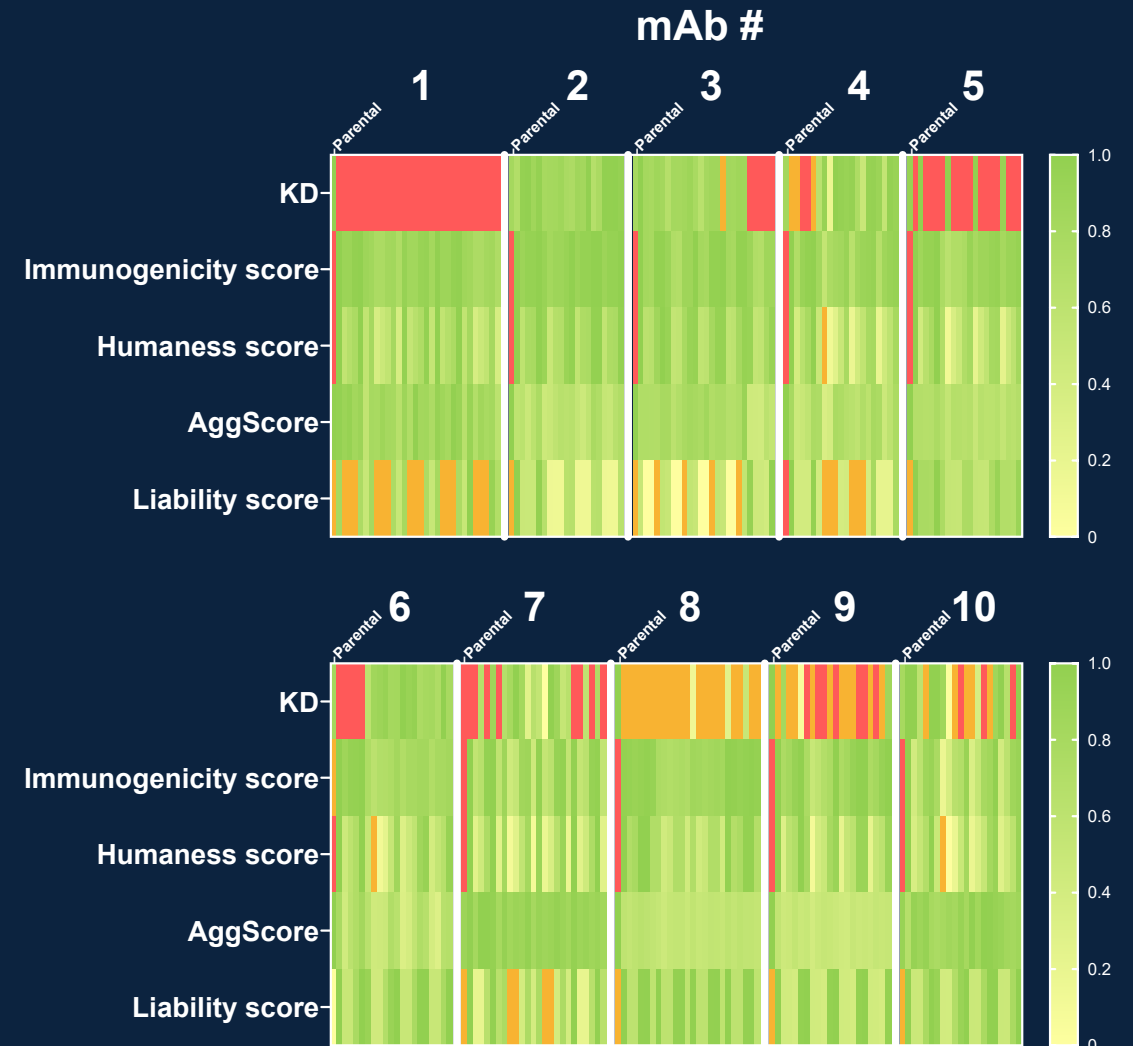Integrated end-to-end discovery and development

Synergy between *in silico* and *in vitro* technologies

Diversity-focused antibody discovery

- Multiple data points at an early-stage

- HT methods/technologies facilitating triaging
  for MoA screening
  - Binding, sequencing, epitope landscape profiling

Advancing and de-risking lead development

- Scalable/efficient lead candidate humanization

- More informed decision making
  - Highly scalable *in silico* assessments, high-throughput
    in vitro characterizations

Case study highlights
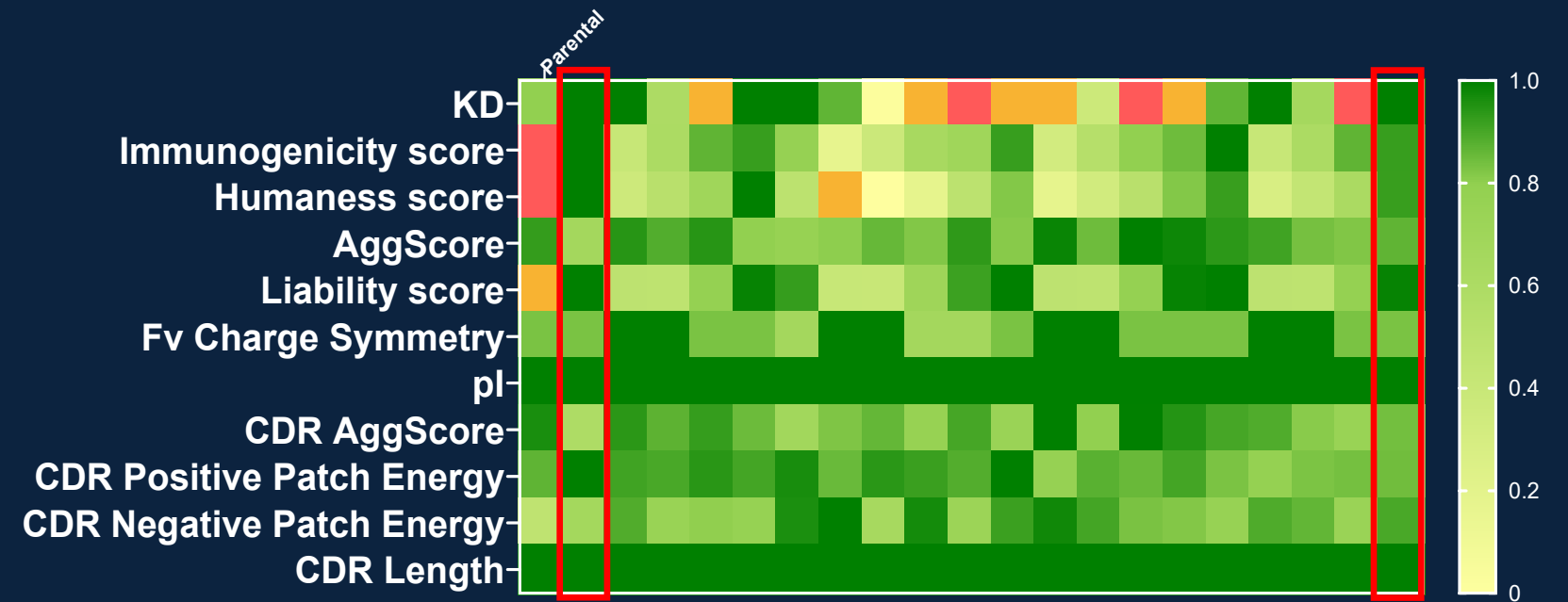## Integrated end-to-end discovery and development

IpA

Synergy between *in silico* and *in vitro* technologies

Data-driven lead selection

- Combining scalable engineering and detailed risk insights

- Start at advanced

- Avoid extensive engineering

Empowering the value of hit diversity by highly scalable *in silico*-driven de-risking
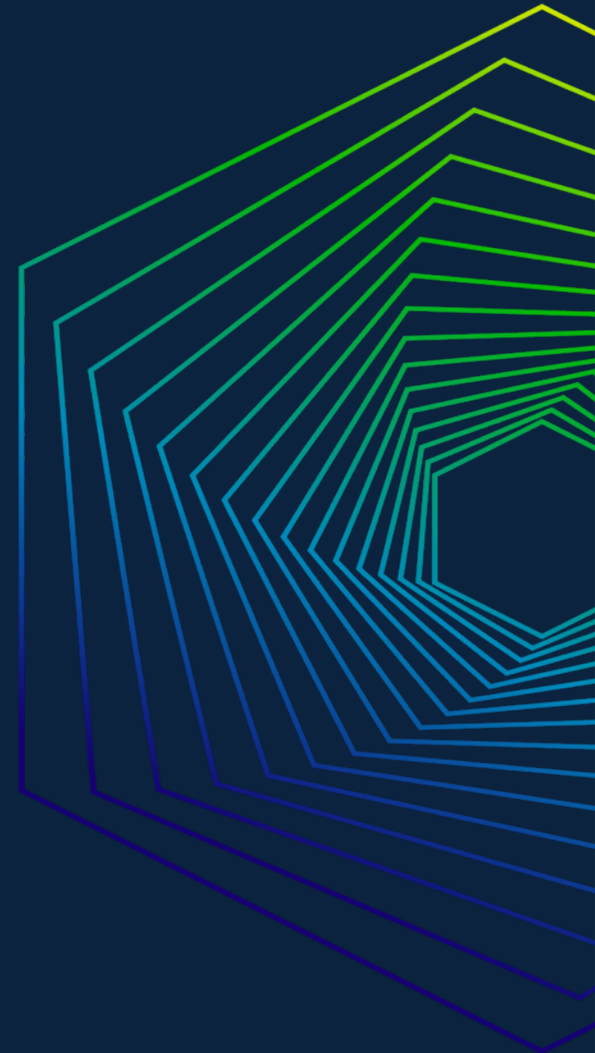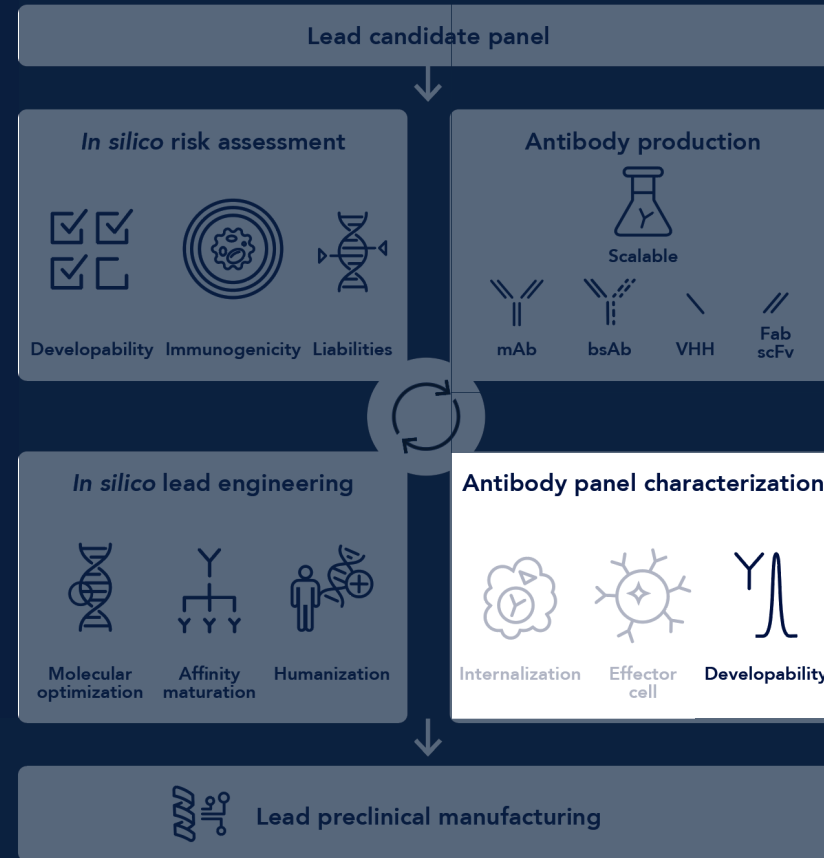
Details mAb #10

Post case study analysis
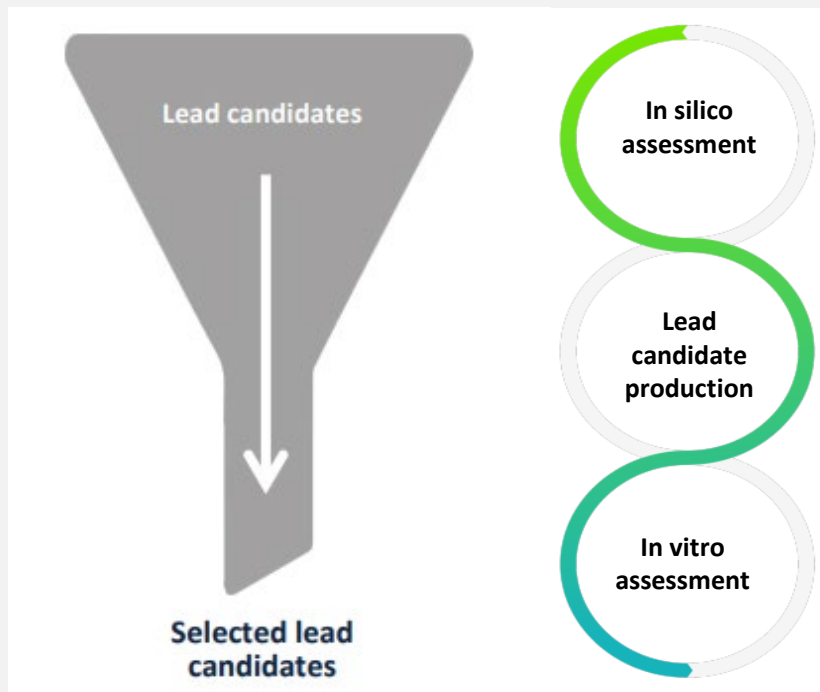## From research to clinical success

- A moving target

- Discovery

- Functionality profiling

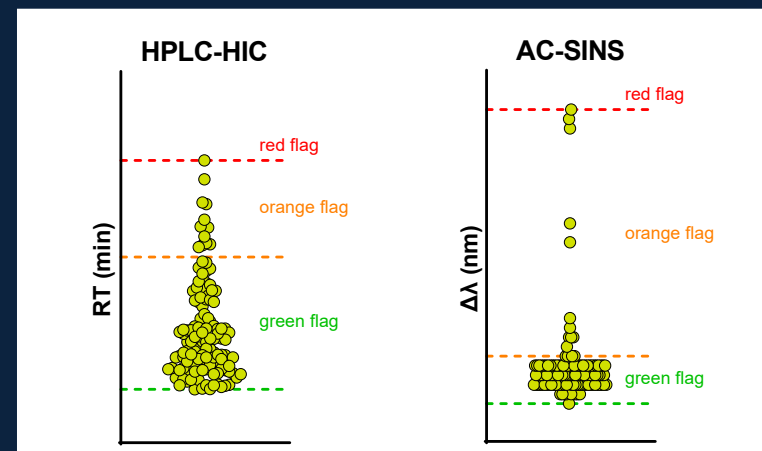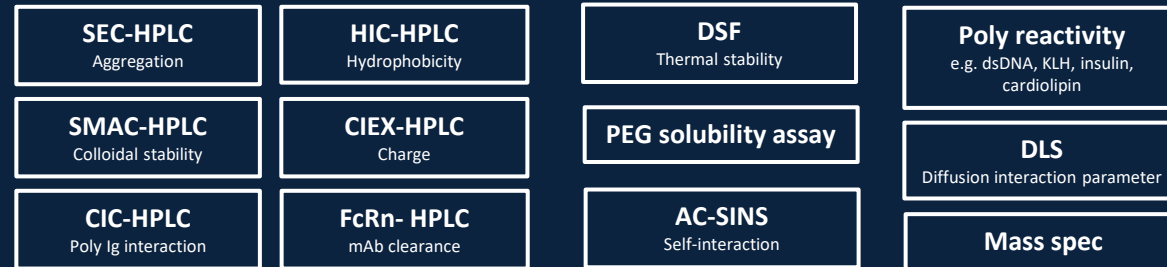- Further development:
  The story continues

High-throughput developability profiling
Empowering diversity to mitigate risk for clinical development

Informed triaging: combining multiple data points to identify most suitability lead for clinical development

Lead candidates

Selected lead candidates

In silico assessment

Lead candidate production

In vitro assessment

- **High-throughput profiling of physicochemical properties**
- **Rank candidates against >125 clinical mAbs (CHO-expressed)**

SEC-HPLC Aggregation	**HIC-HPLC** Hydrophobicity	**DSF** Thermal stability	**Poly reactivity** e.g. dsDNA, KLH, insulin, cardiolipin
SMAC-HPLC Colloidal stability	**CIEX-HPLC** Charge	**PEG solubility assay**	**DLS** Diffusion interaction parameter
CIC-HPLC Poly Ig interaction	**FcRn- HPLC** mAb clearance	**AC-SINS** Self-interaction	**Mass spec**

HPLC-HIC

RT (min)

red flag
orange flag
green flag

AC-SINS

Δλ (nm)

red flag
orange flag
green flag

The fusion of *in silico* and wet lab

More informed decision making to amplify lead selection

Customized program design

> Target insights

> Therapeutic lead requirements

Diversity-focused discovery

> Functional diversity: From sequence to epitope to MoA

> HT technologies empowering triaging of large antibody panels

Data-driven decision making

> Early engineering combined with in-depth risk assessment

> Highly scalable *in silico* technologies matched with HT *in vitro* techniques

In conclusion,

In today's ever-evolving clinical landscape, uniting the scalability of *in silico* models with the experimental depth of high throughout *in vitro* studies amplifies therapeutic lead generation with precision by advancing and enabling data-driven decision making.
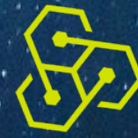
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Advanced antibody technologies providing speed without sacrificing quality

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