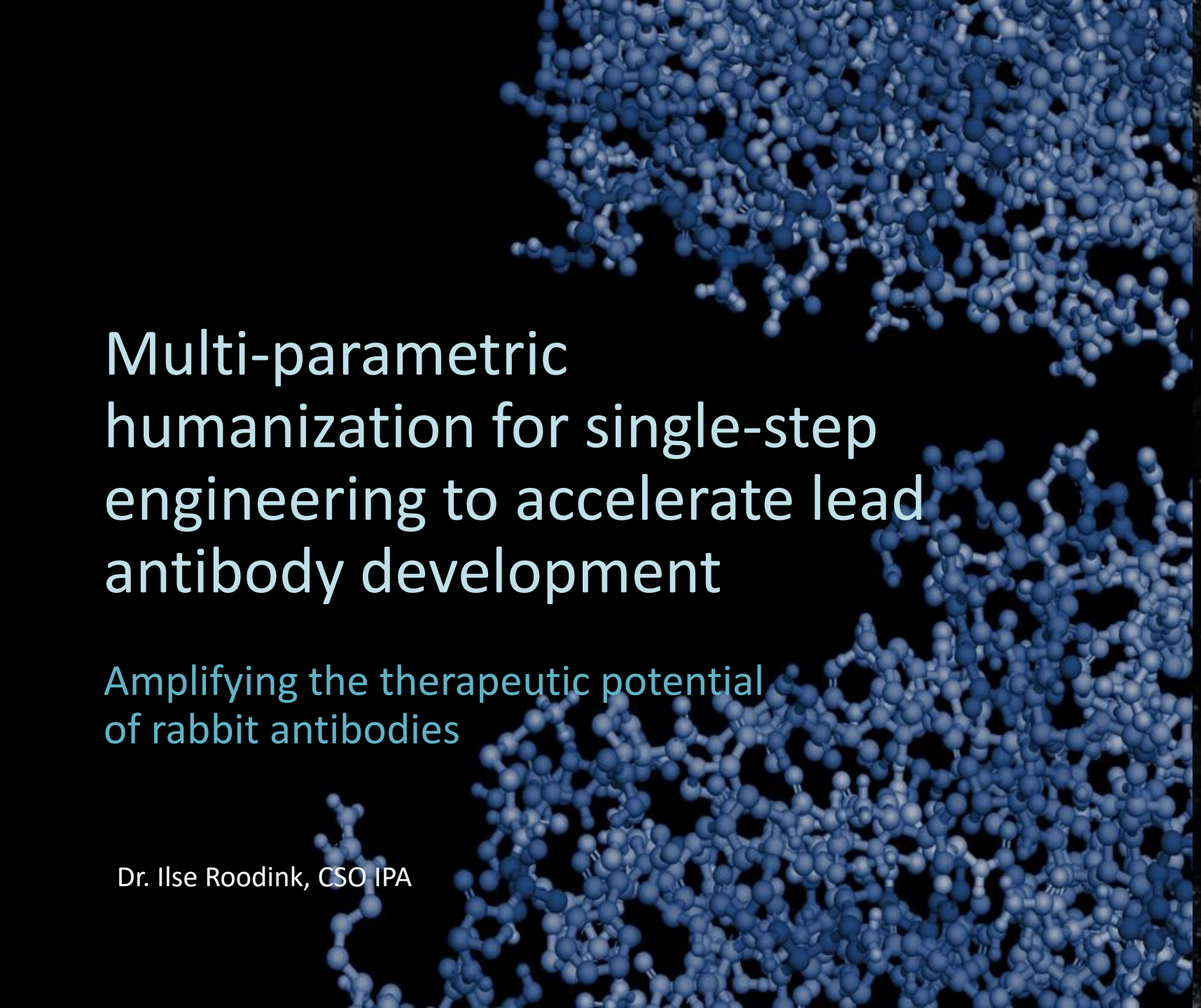

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IMMUNOPRECISE ANTIBODIES

Multi-parametric humanization for single-step engineering to accelerate lead antibody development

Amplifying the therapeutic potential of rabbit antibodies

Dr. Ilse Roodink, CSO IPA

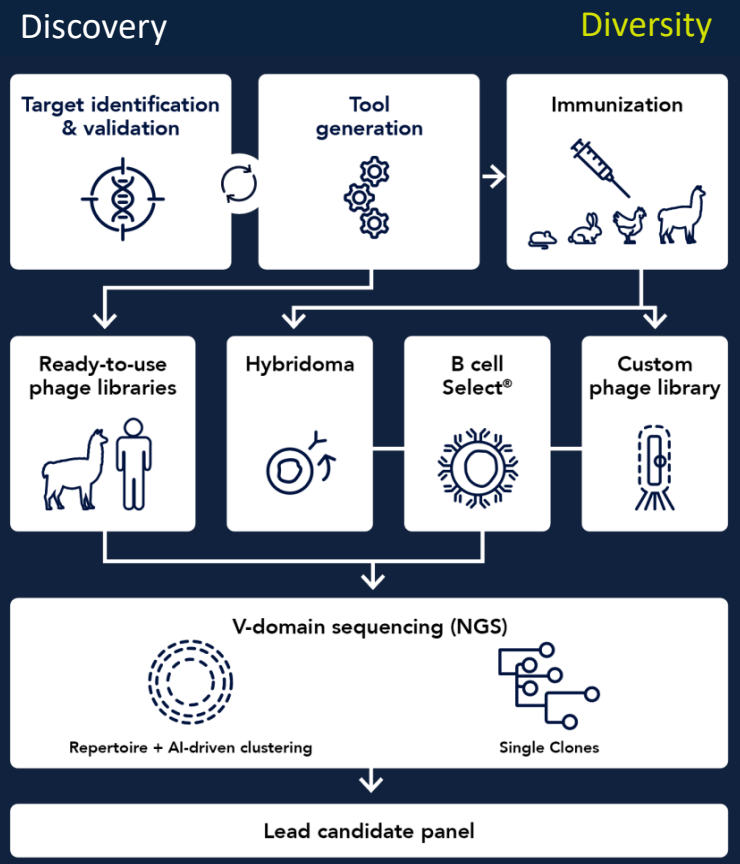
ENGINEERED for the scientific RACE

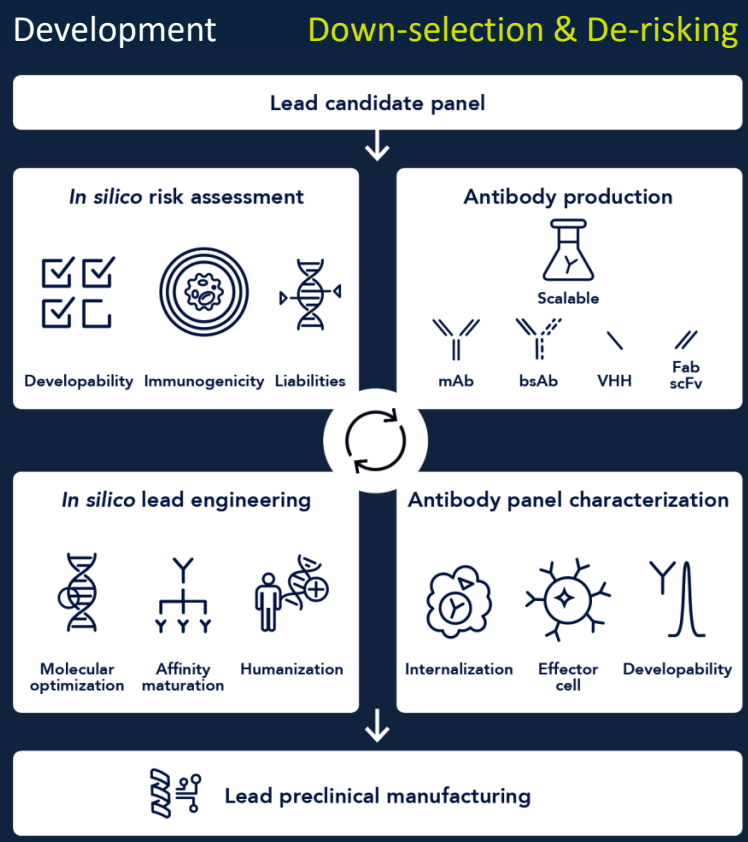
End-to-end antibody discovery and development

From research to clinic

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

Experienced experts

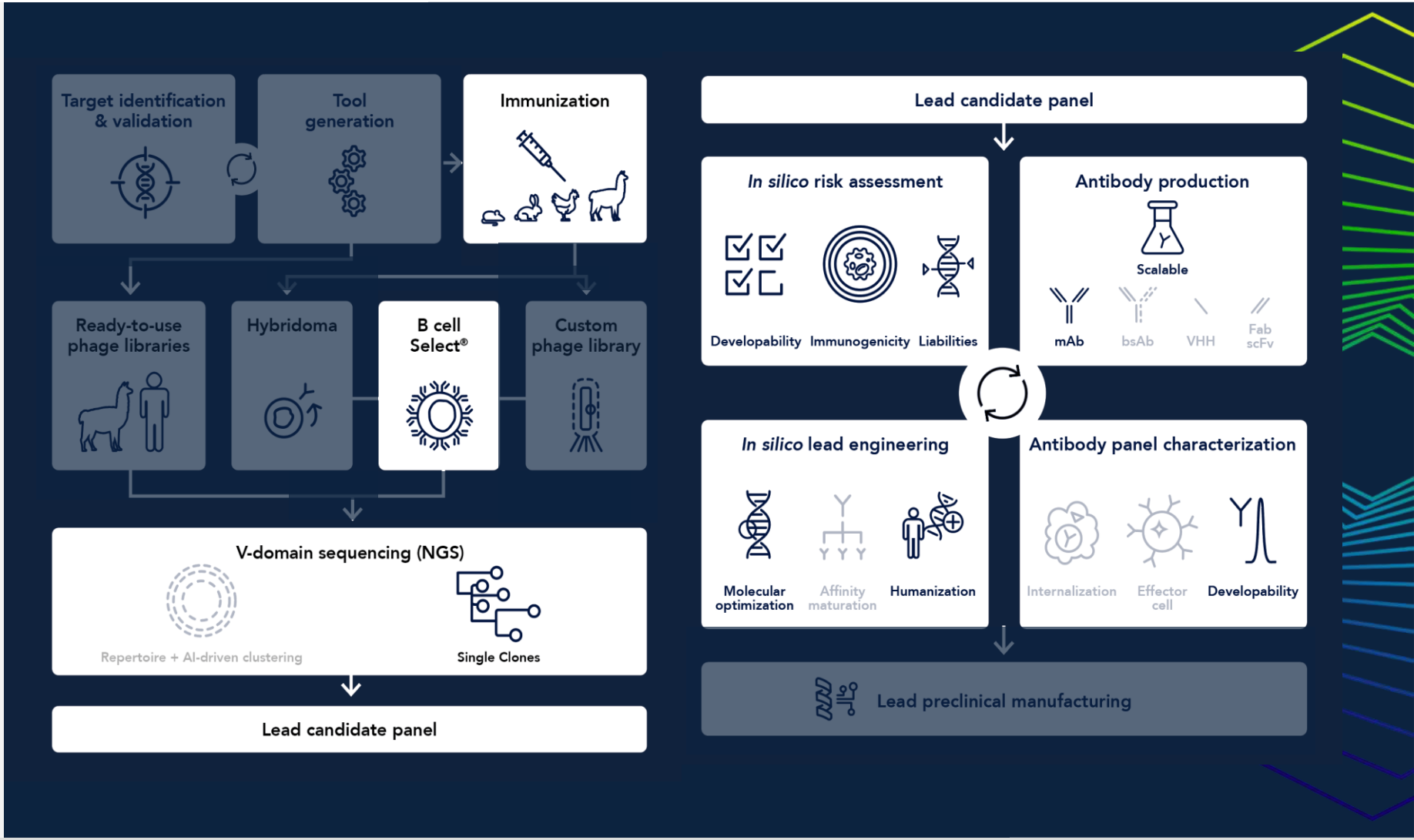




Integrated end-to-end workflow – case study overview

Empowering diversity-focused discovery and data-driven down-selection

- 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

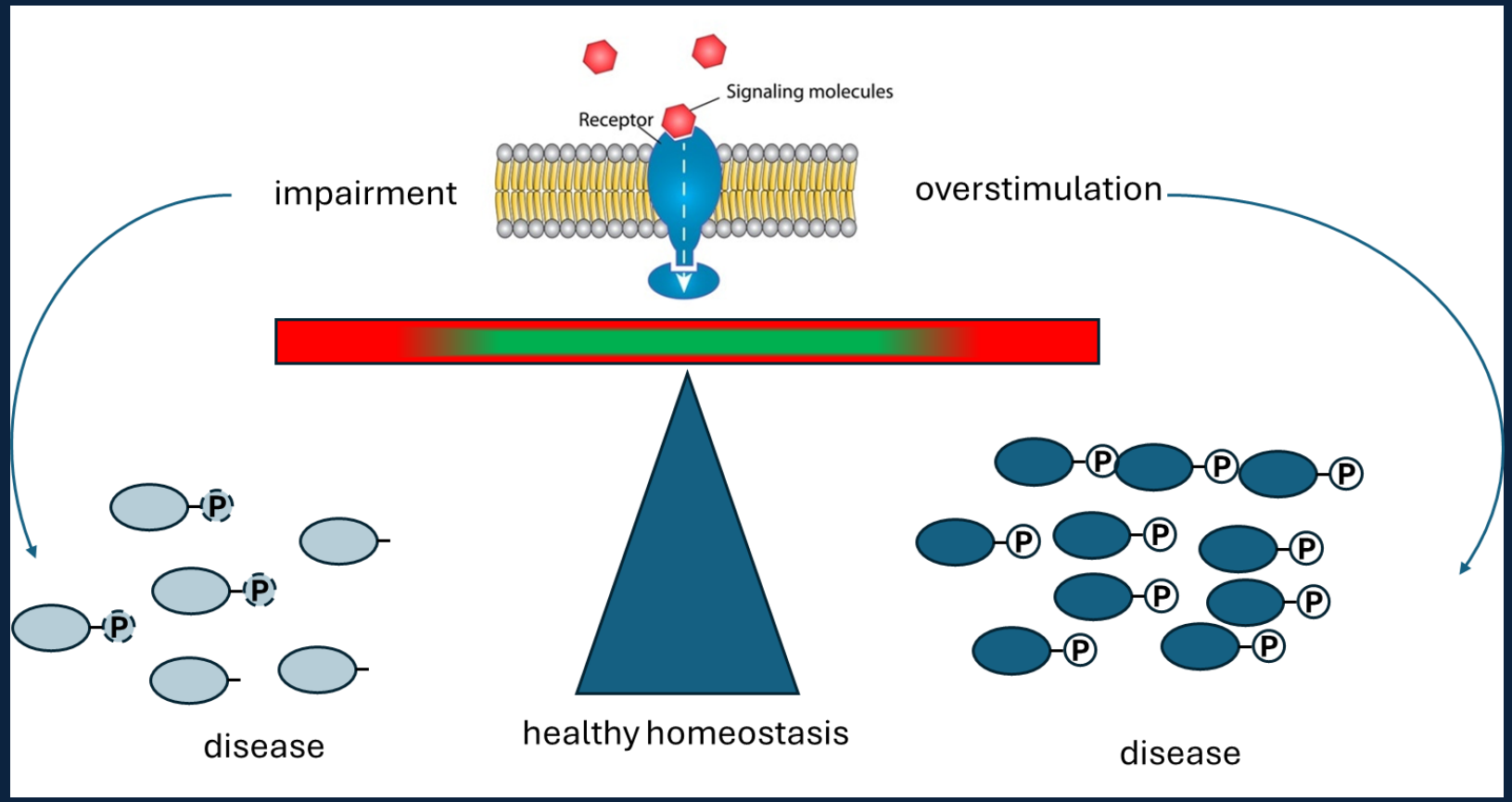
Dampening overstimulation

- 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

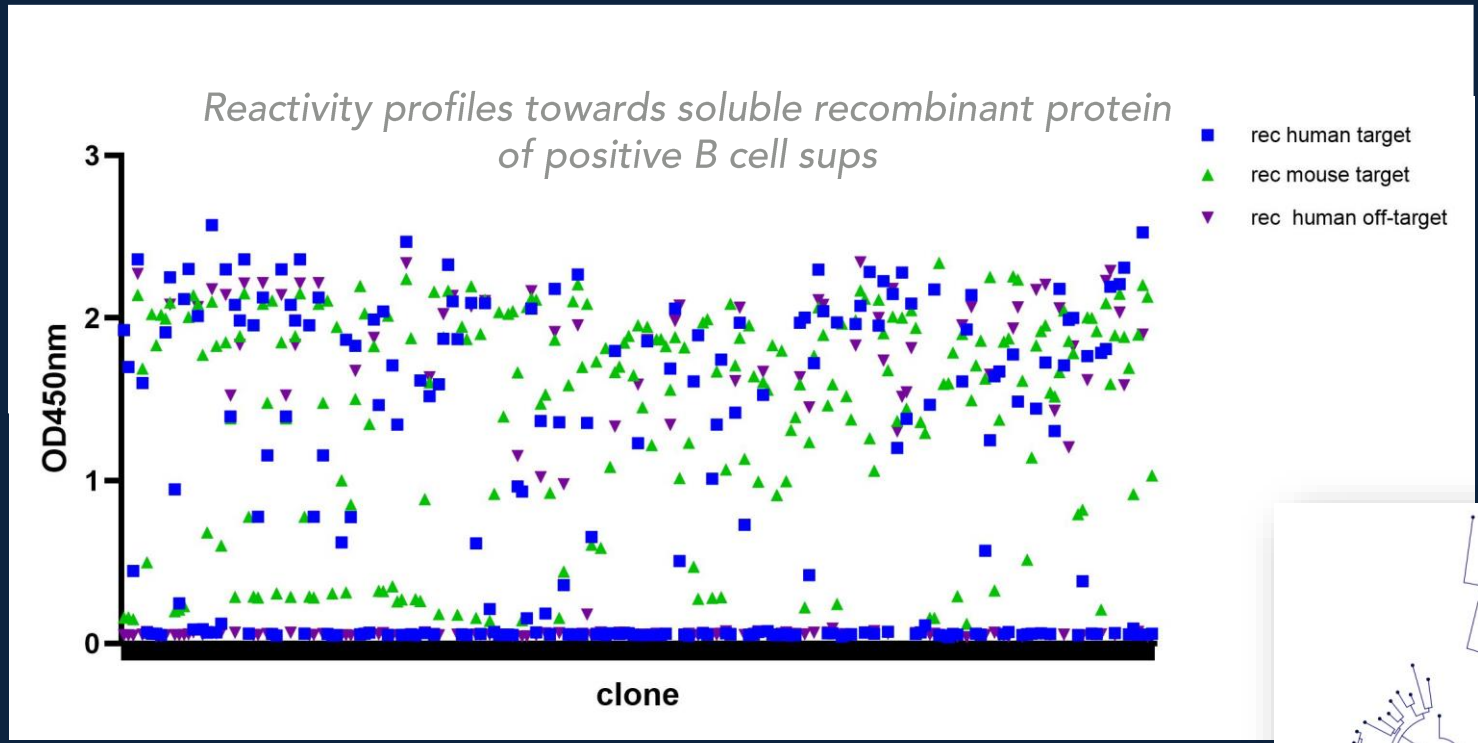
Rabbit immunization

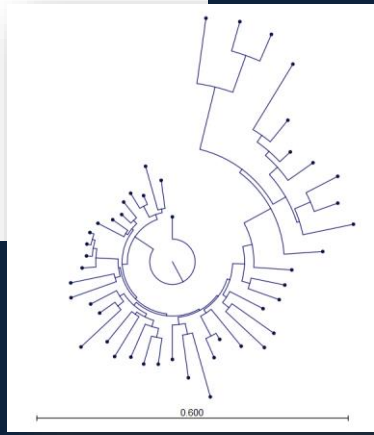
- *In vivo* antibody maturation with diversity
- Alternating immunization

Robust B cell selection platform

- Target enrichment
- High throughput reactivity screening
- B cell clone v-domain sequencing

Triaging based on multiple binding data points





- Initial screening revealed 220 hits
- 48 prioritized for confirmational screening and v-domain sequencing

Diversity-focused discovery

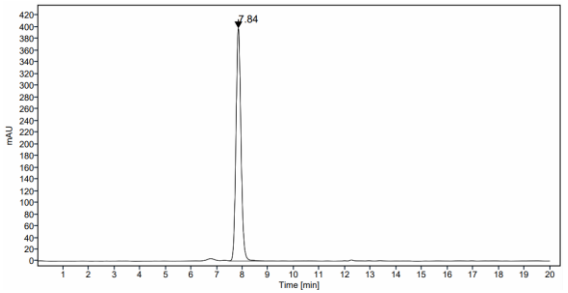
In vitro epitope landscape profiling to triage mAbs for MoA analysis

Recombinant production of selected candidates (rPEx®)

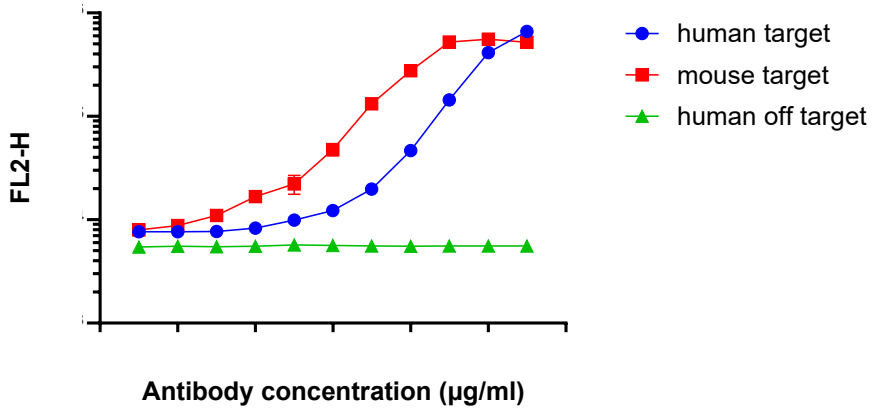
- Recombinant cloning, production and purification
- QC including HP-SEC and multiplex flow cytometry

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

Example HP-SEC chromatogram



Example Multiplex cell-based screening



Recombinantly expressed mAbs passed QC

- Monomericity
- Confirmed binding

Diversity-focused discovery

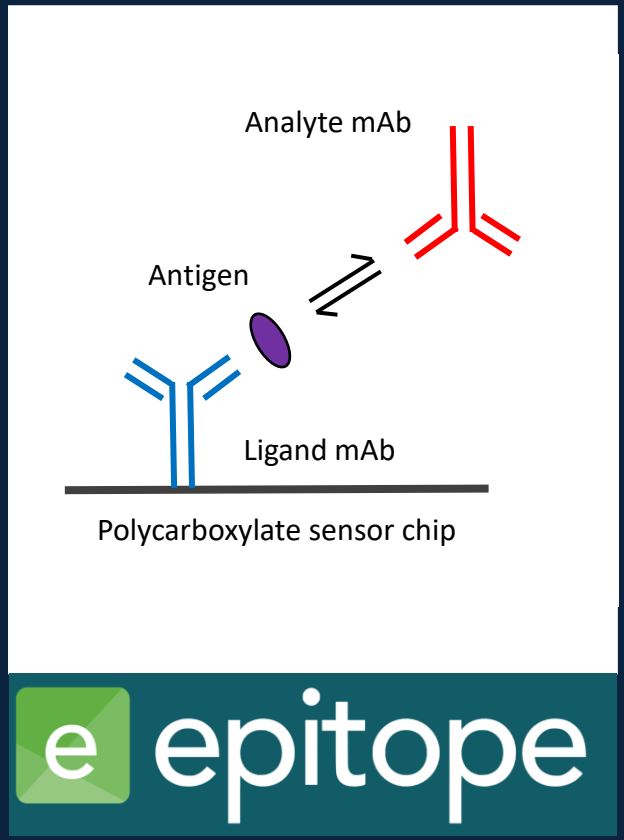
In vitro epitope landscape profiling to triage mAbs for MoA screening

SPR-based epitope binning analyses

Competition-driven epitope landscape profiling

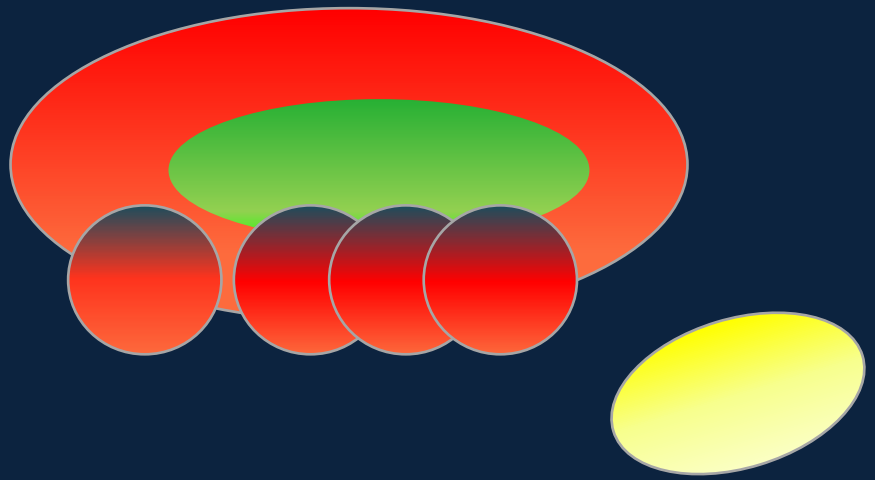
- Natural signaling molecule
- Clinical benchmark
- All-to-all candidates

HT Classical Binning - monovalent Ag



epitope

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

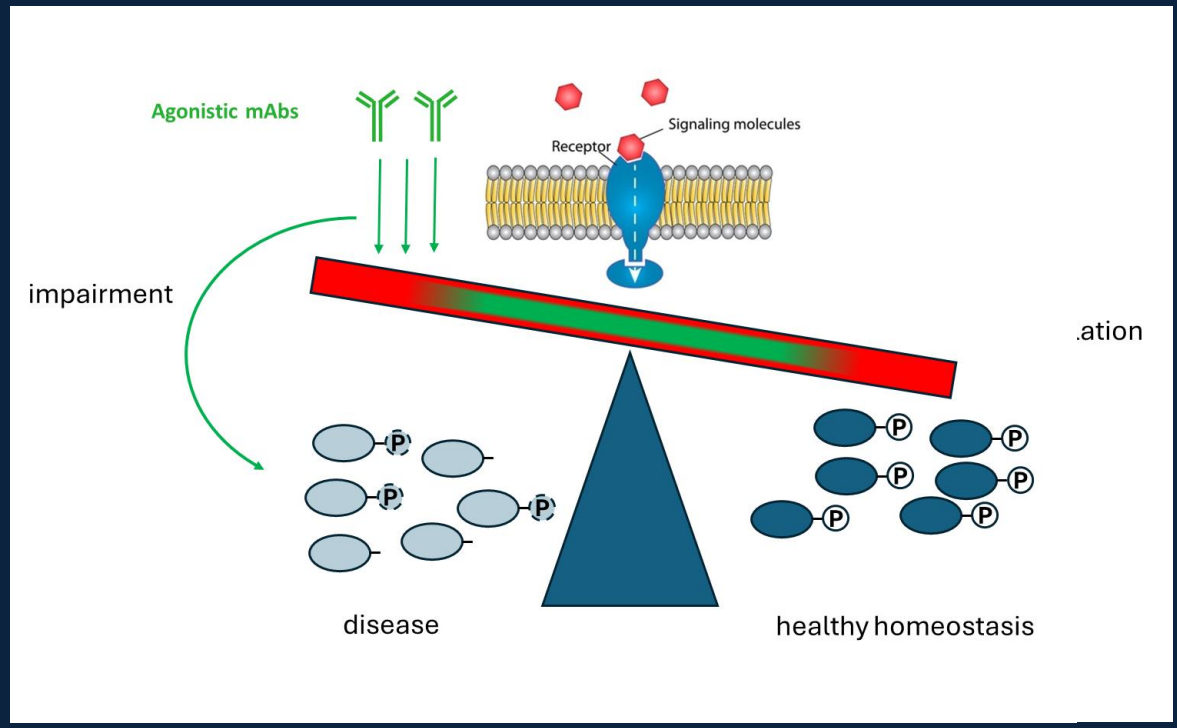
Functional Diversity: from sequence to epitope to MoA

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

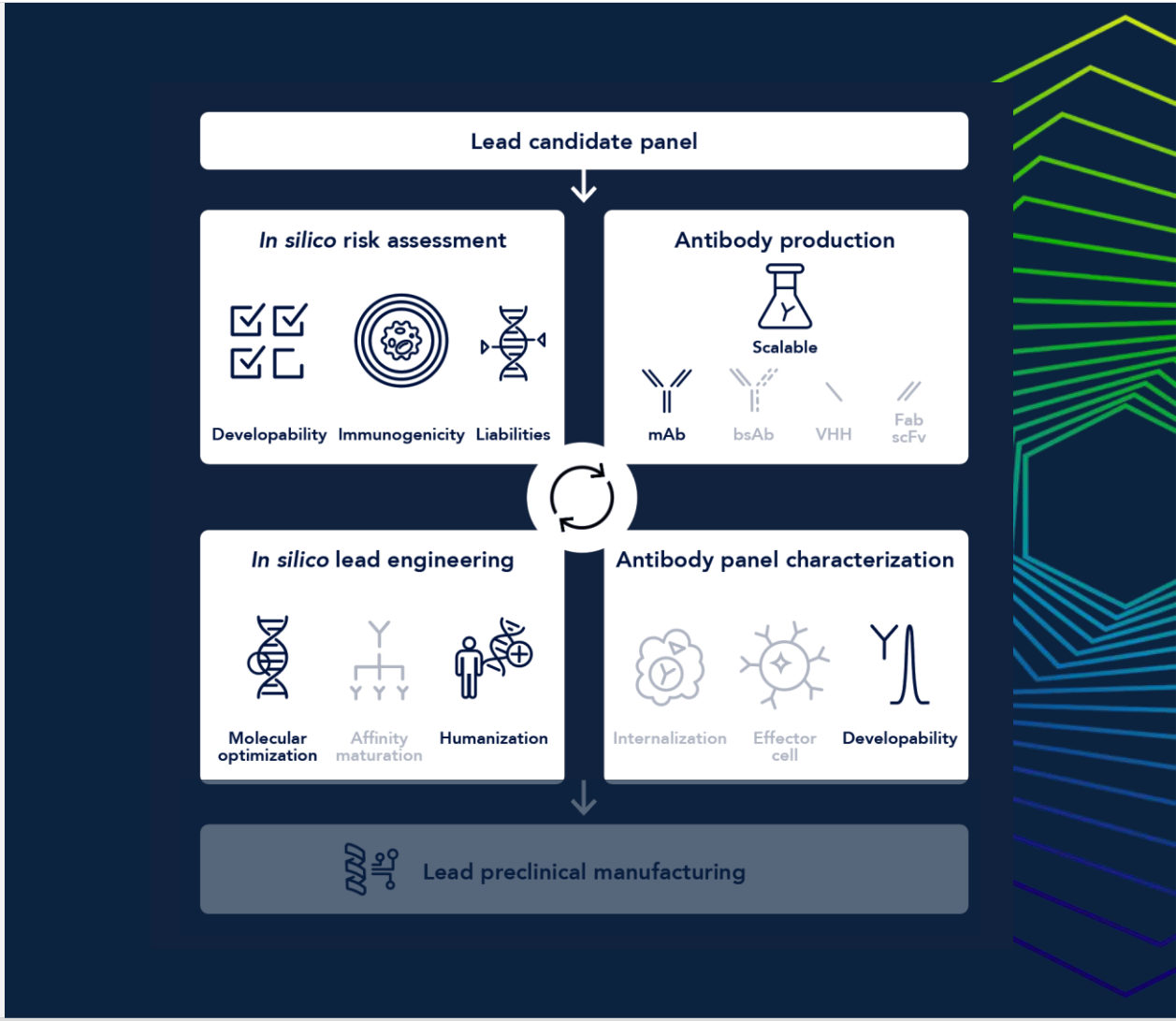
Integrated end-to-end workflow – case study overview

Throughput empowering data-driven down-selection

- A moving target
- Discovery
- Functionality profiling
- **Further development**

  Scalable, integrated de-risking workflow

Integrated *in silico*		*In vitro*
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**	**Recombinant optimized lead screening** Binding characterization (SPR, flow) Functionality verification Developability profiling **High-throughput platforms**

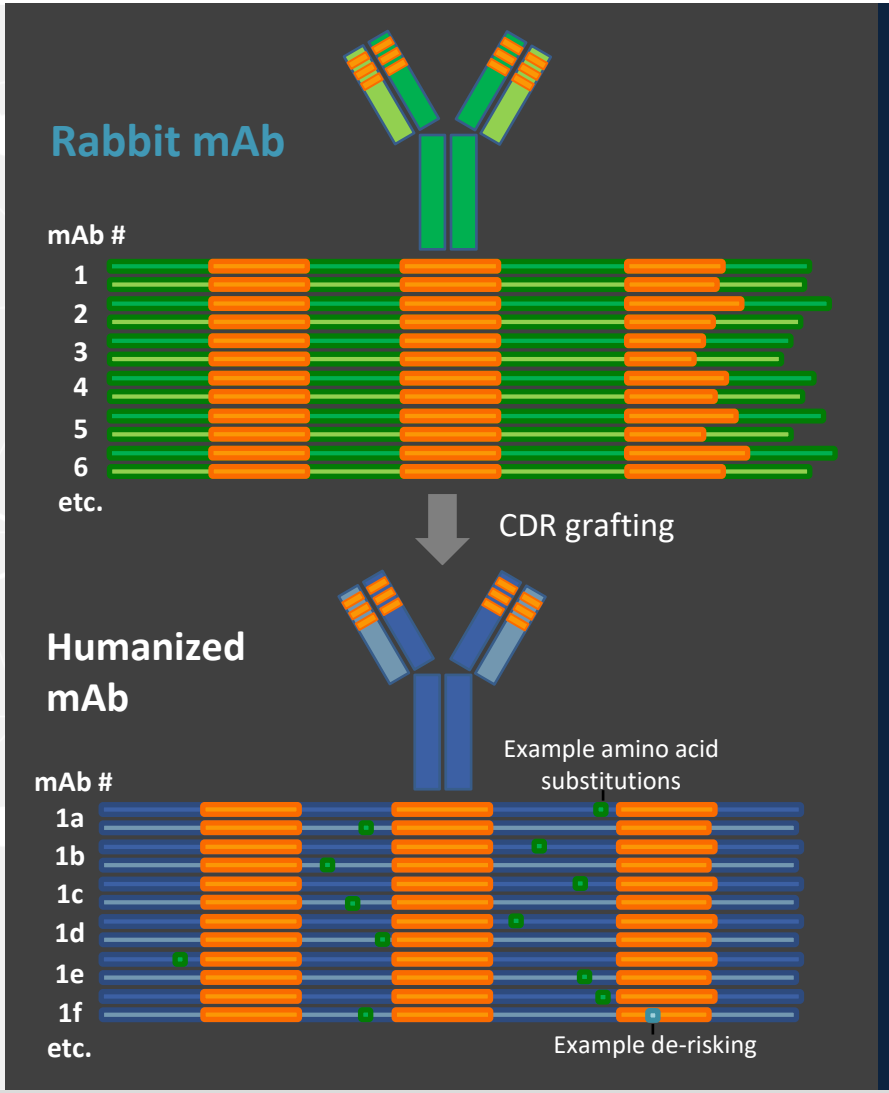


Multi-parametric humanization for single-step engineering

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 advanced structural analysis
- Early de-risking addressing high risk liabilities



Highly scalable technologies advancing lead selection

Multiple lead candidates in parallel

- Scalable *in silico* design of variants
- High-throughput *in silico* risk assessment
  - Immunogenicity
  - Developability
- High-throughput *in vitro* characterizations
  - Kinetics
  - Developability

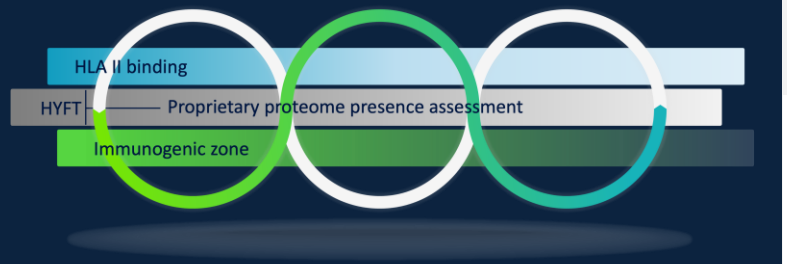
Highly scalable *in silico* risk assessment

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

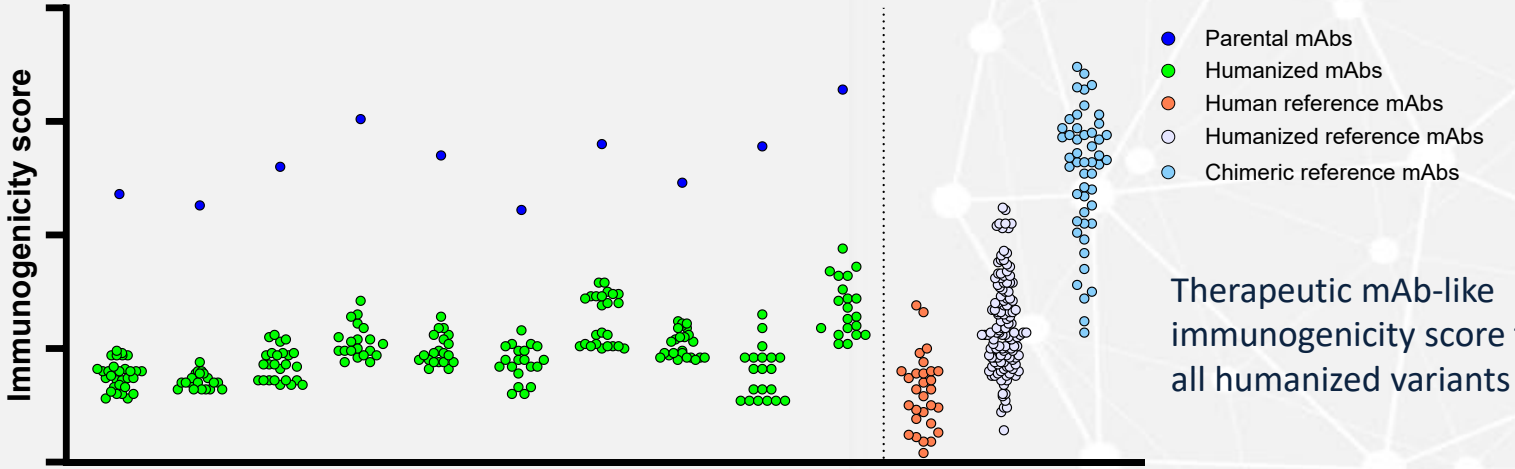
LENS^{ai} immunogenicity analysis

Multidimensional assessment

- Combining humanness with HLA-II binding
- Limitless quantities
- From global ranking to subsequence scoring

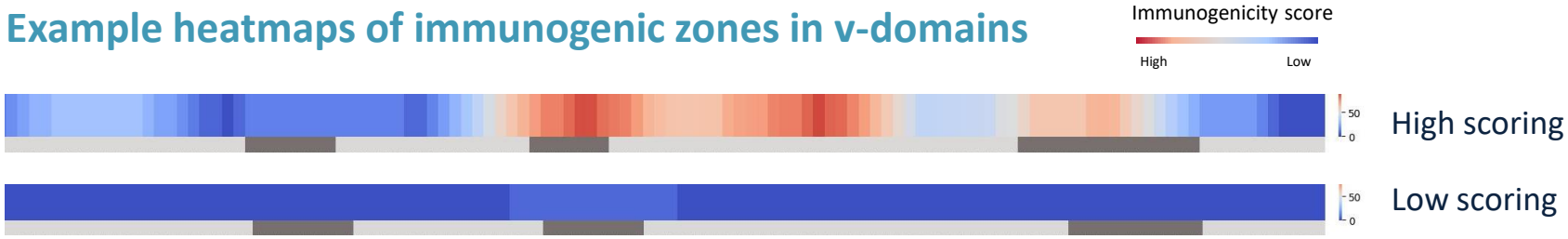


LENS^{ai} Immunogenicity score of candidate and therapeutic mAb



Therapeutic mAb-like immunogenicity score for all humanized variants

Example heatmaps of immunogenic zones in v-domains



Highly scalable *in silico* risk assessment

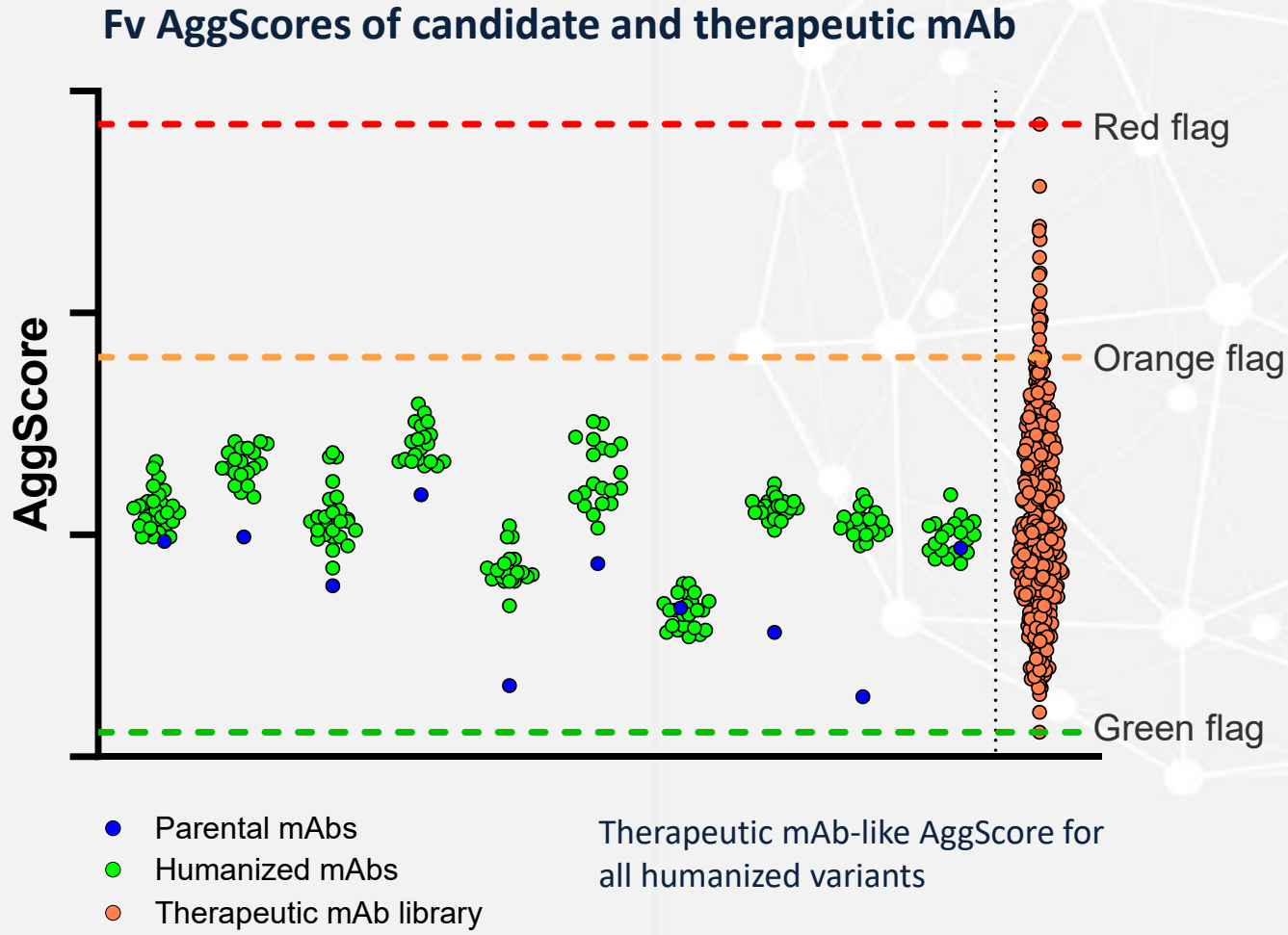
In silico developability profiling enabling data-driven decision making

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



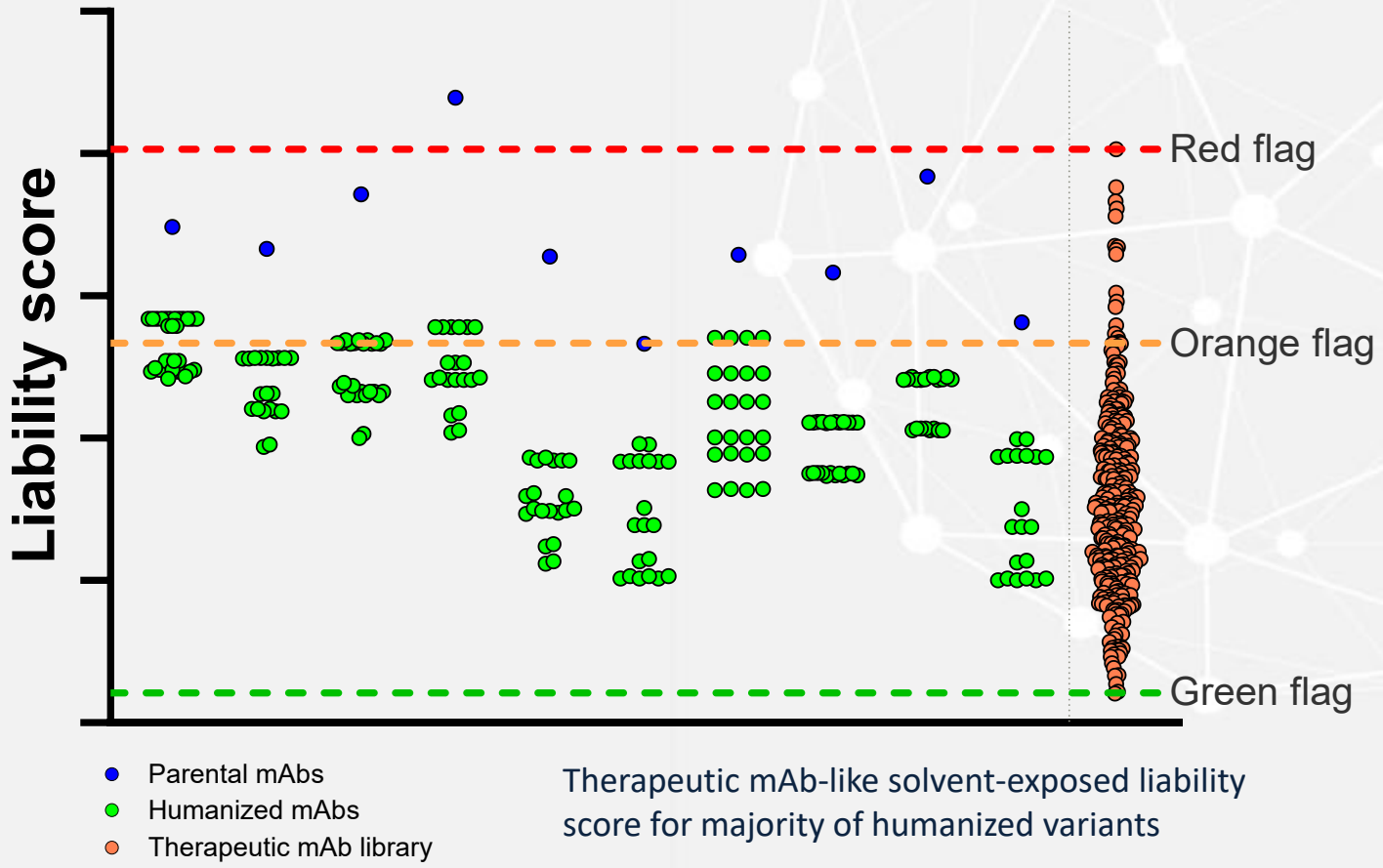
Highly scalable *in silico* risk assessment

In silico developability profiling enabling data-driven decision making

In silico structure-based liability analysis

Liability score

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

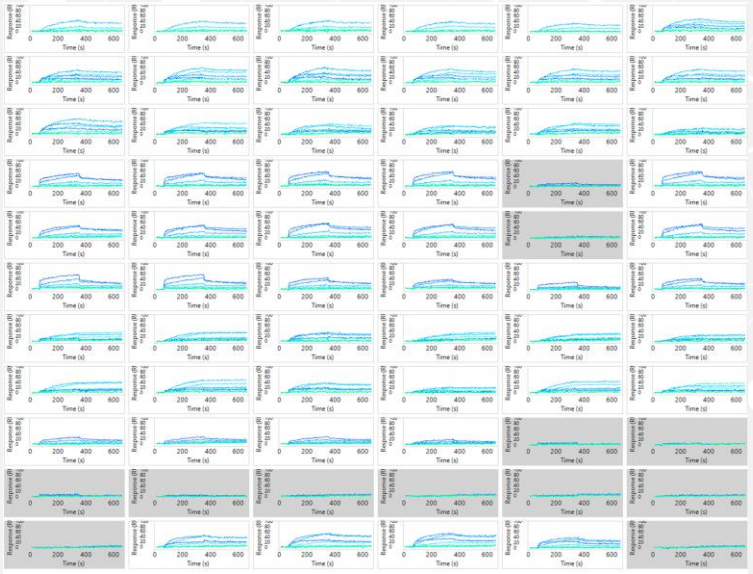


High-throughput *in vitro* affinity screening of humanized variants

Empowering diversity-driven discovery during lead candidate development

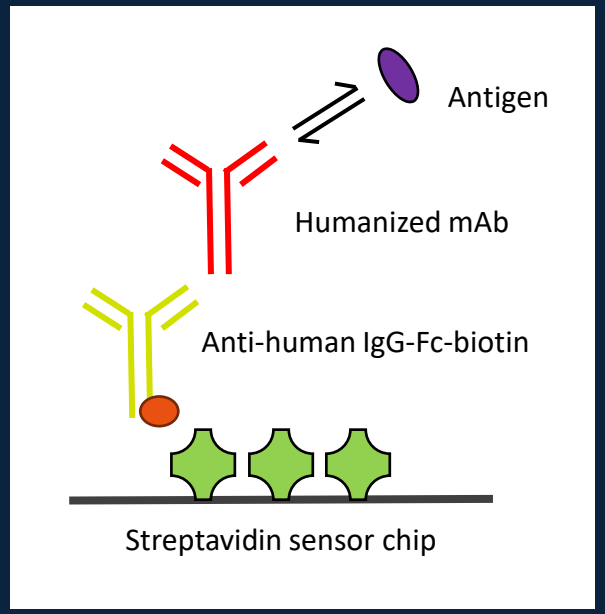
HT SPR-based affinity determination

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



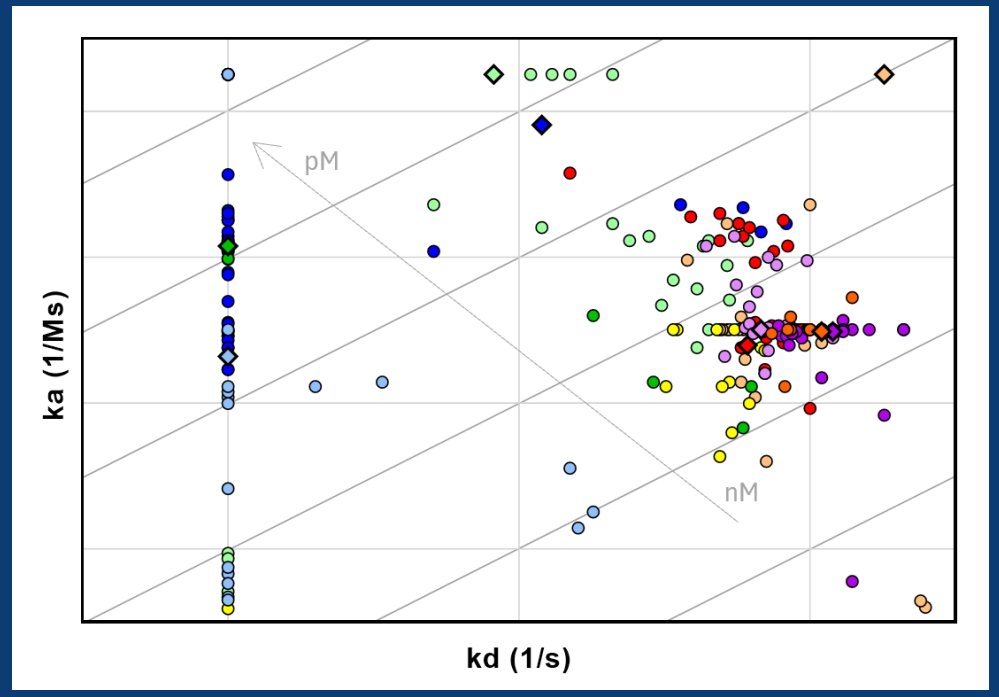
carterra

Kinetics using monovalent Ag



kinetics

In-depth insights in kinetic parameters



High-throughput *in vitro* profiling of physicochemical properties

Empowering diversity to mitigate risk for clinical development

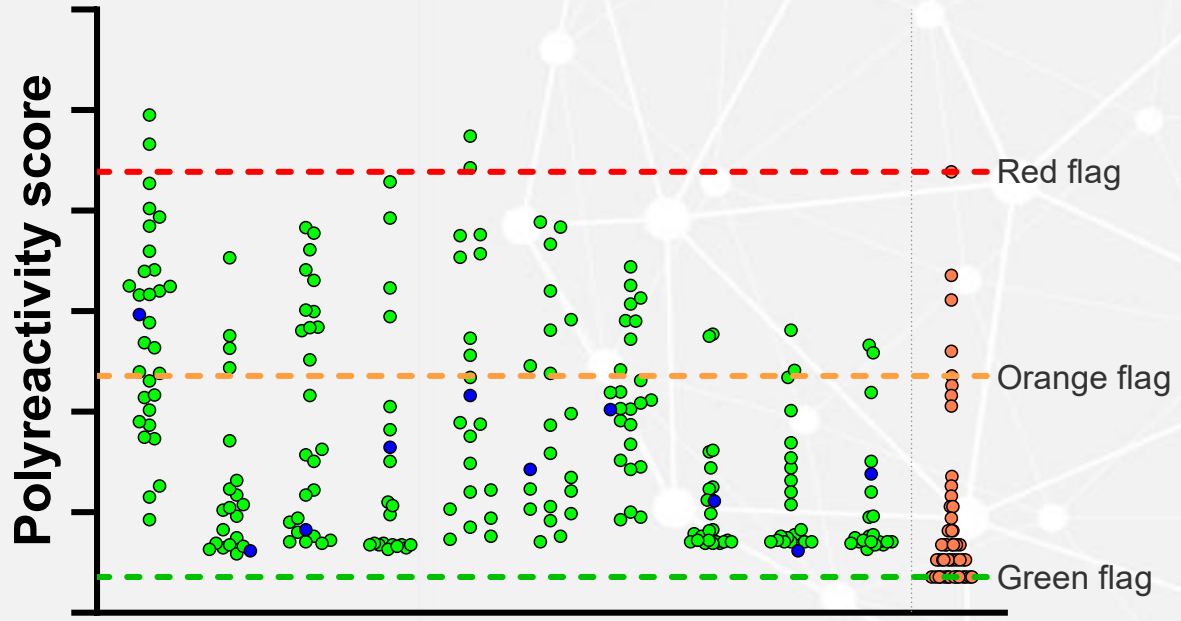
HT *in vitro* developability profiling

More informed decision making for clinical development

- 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, LPS, BSA
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**

Polyreactivity scores of candidate and therapeutic mAb



- Parental mAbs
- Humanized mAbs
- Therapeutic mAb library

Therapeutic mAb-like polyreactivity scores for significant set of humanized variants

Case study highlights

Integrated end-to-end discovery and development

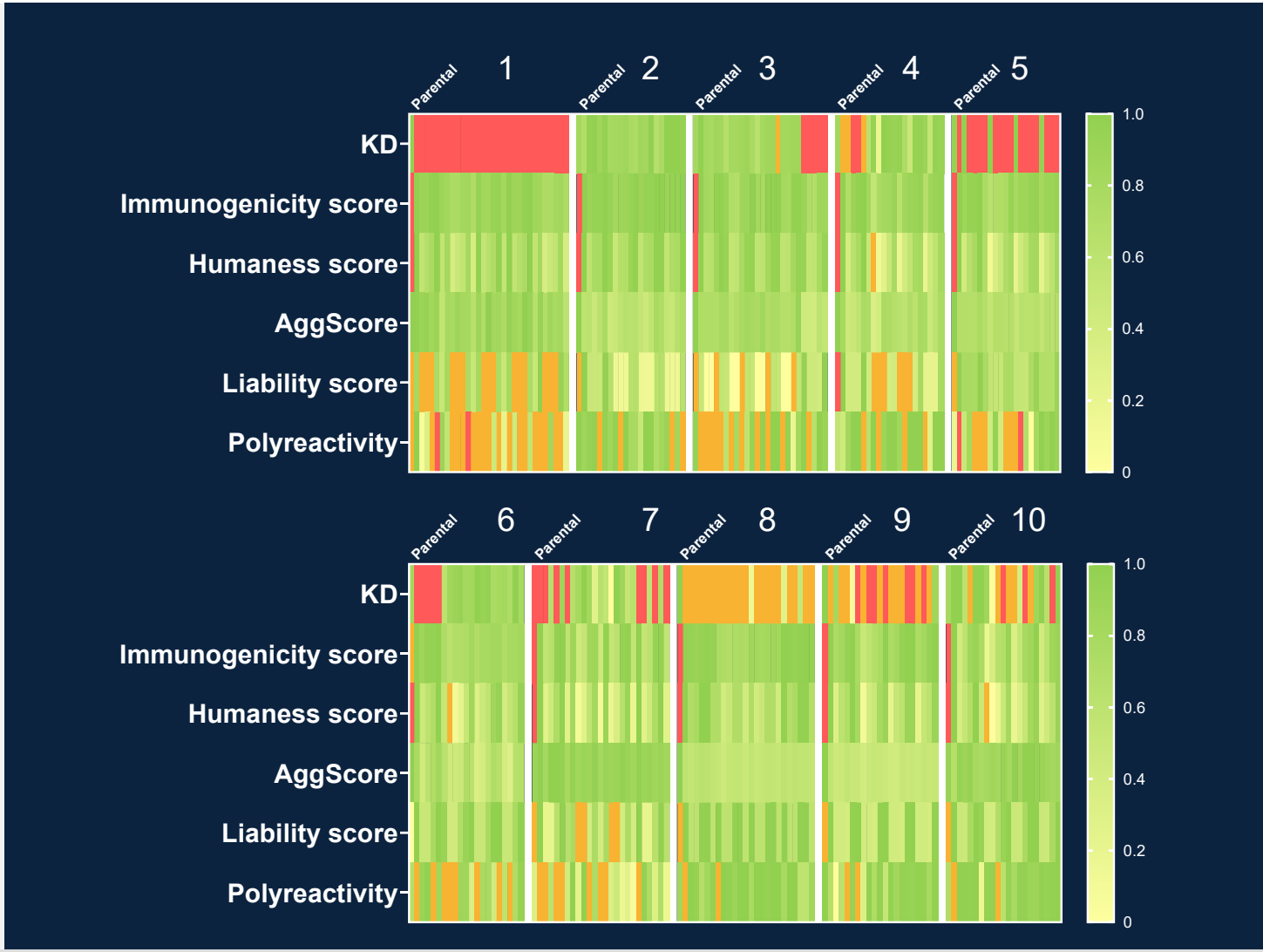
Synergy between *in silico* and *in vitro* technologies

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

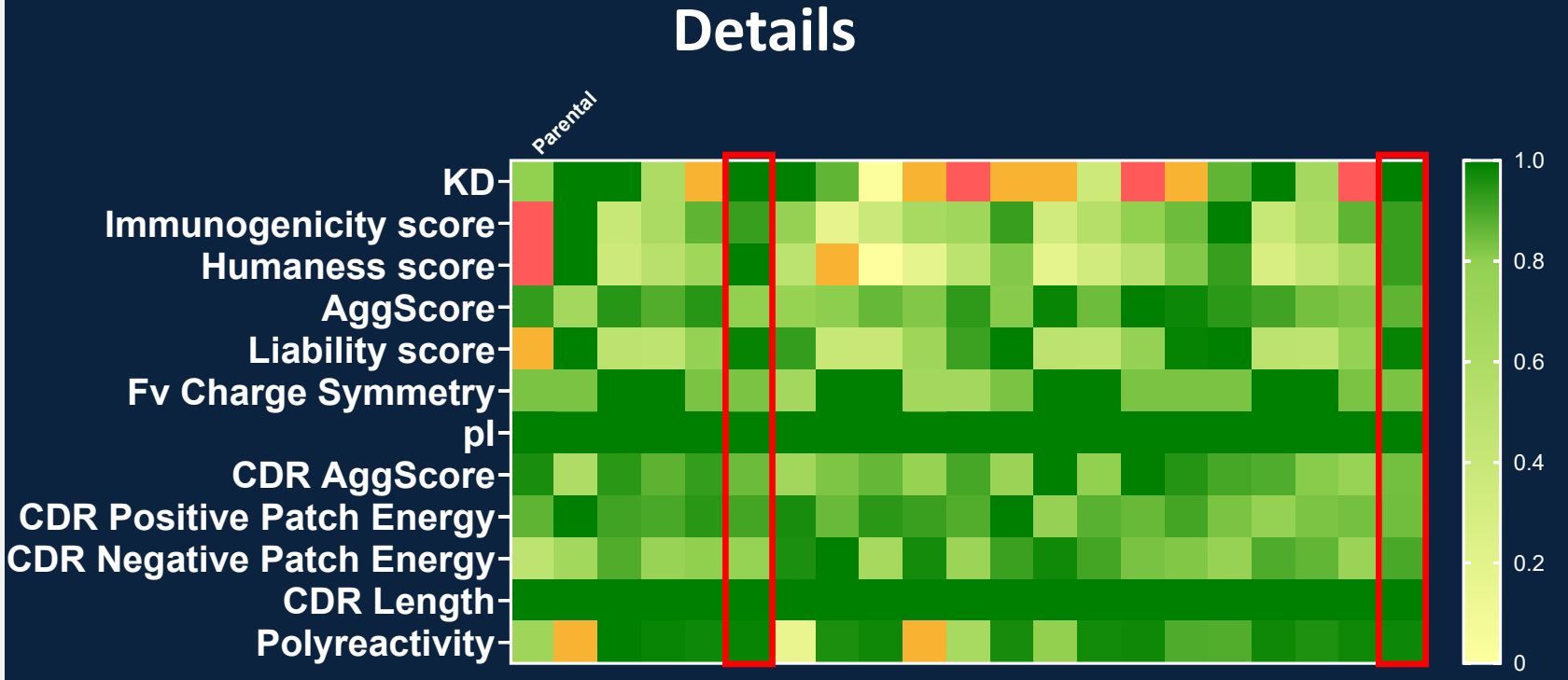
Integrated end-to-end discovery and development

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



Fusion of *in silico* and wet lab empowers multi-parametric approaches

More informed decision making to amplify lead selection

Customized program design

> Target insights

> Therapeutic lead requirements

Diversity-focused discovery

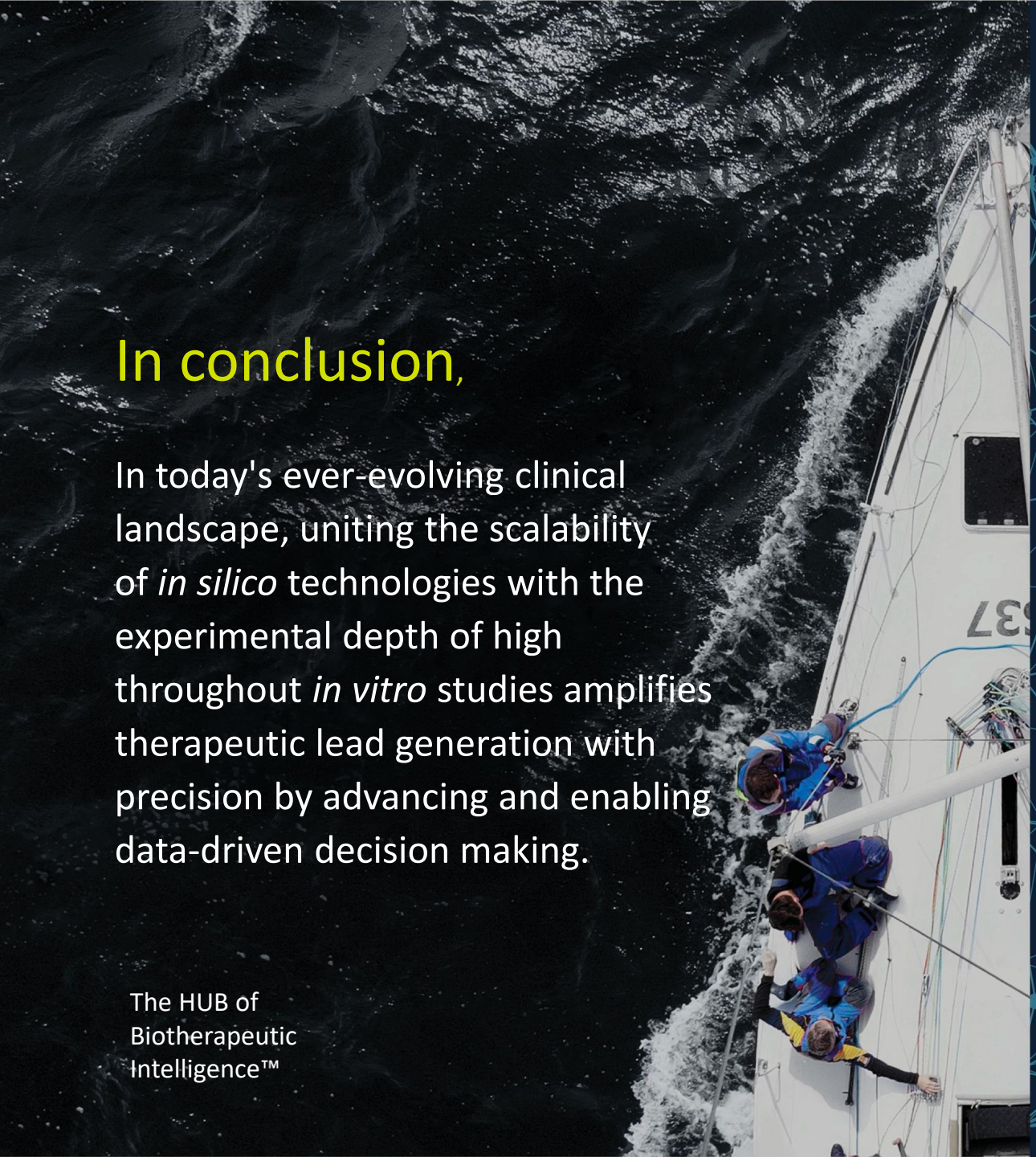
> Functional diversity: From sequence to epitope to MoA

> 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* technologies with the experimental depth of high throughout *in vitro* studies amplifies therapeutic lead generation with precision by advancing and enabling data-driven decision making.

The HUB of Biotherapeutic Intelligence™

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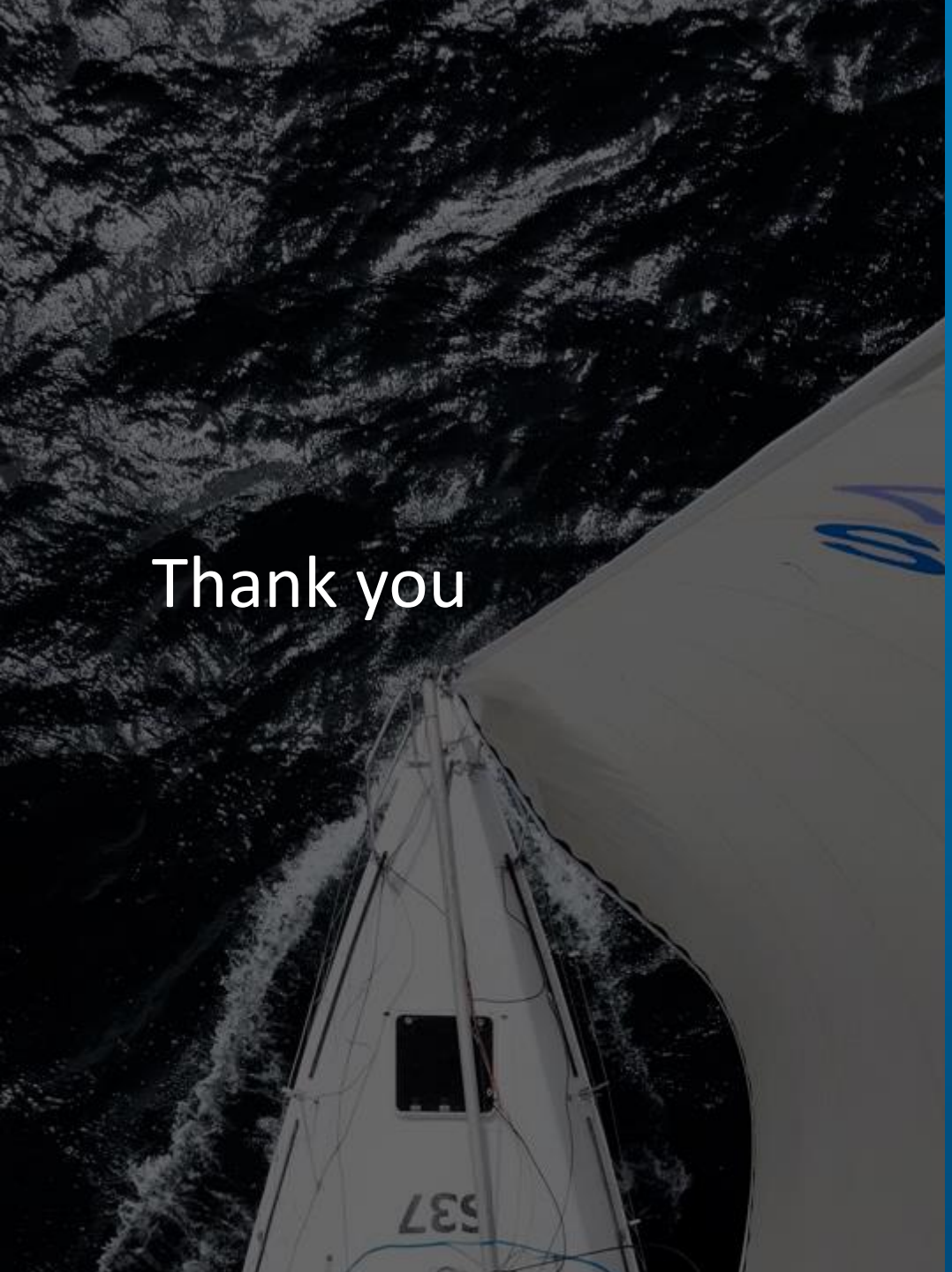
Advanced antibody technologies providing speed without sacrificing quality



Thank you

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