High throughput approaches for in silico developability, production and characterization in Antibody Discovery

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FairJourney Biologics

The development of high throughput production and characterization workflows is an important asset for antibody discovery. Such approaches provide a time and cost-effective solution to screen antibody libraries from a functional and biophysical perspective. The inclusion of these approaches early in the discovery process enables a full understanding of panels of hundreds of antibodies.

Enabling the development of better antibodies, faster

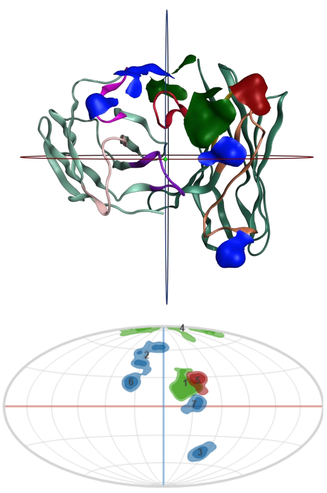
The Workflow

Antigen Production | Immune Libraries | Naïve Libraries

Immunization and Library Generation → Phage and/or Mammalian Display Selections → Primary Screening and Analysis of Diversity and of CMC Liabilities

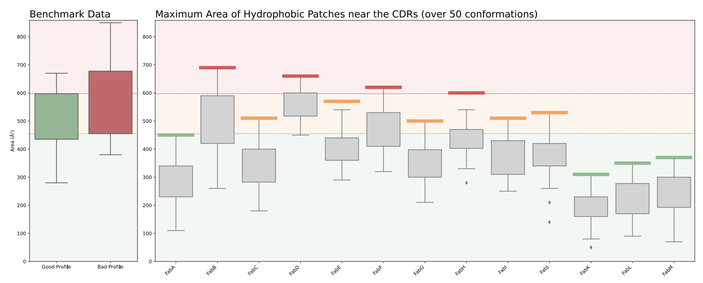
Antigen Binding and functionality:
- ELISA
- FACS
- SPR
- Cell Based-Assays

Step 1 – *In silico* Analysis Example



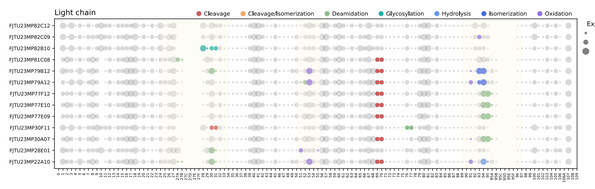
Developability and liability identification *in Single structure patches near CDRs*

Provides a birds-eye view of the size and position of hydrophobic and charged patches at a single simulation frame.



Key in-silico descriptors - over structure ensembles

Compares the distribution of key structure-based metrics against a dataset of known antibody profiles.



Liability exposure map - over structure ensembles

Provides an overview of the count, sequence position and exposure levels of different liability motifs.

Secondary Screening and Selection of Lead Panel → Small scale production

- Species X-reactivity
- Binning
- SPR (off-rate)
- Blocking Assays in ELISA, FACS, HTRF, SPR
- *In silico* liability assessment and molecular properties prediction

Step 2 – DNA Preparation

- Codon optimization from mammalian expression
- Recloning into optimized production vectors
- Recloning into various antibody formats
- Time saving for later stage

Step 3 – Expression and purification

- 96 and 24 well plates available
- Affinity Purification (multiple resins available, e.g. Protein A and G, IMAC)
- High recovery buffer exchange protocol
- QC: concentration determination; µCE-SDS

Step 4 – Functional Characterization Example

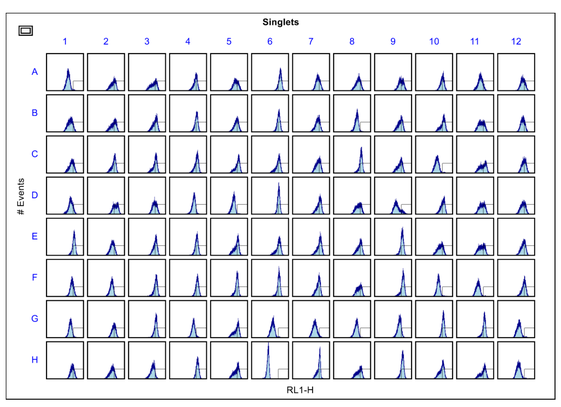
Characterization for lead selection → Scale up production → Lead Identification

Functional Characterization
- Kinetics
- Binning
- Cell Based-Assays

Biophysical Characterization
- AC-SINS
- Poly ELISA
- T_M/T_{agg}
- HIC score
- SEC-HPLC
- CE-SDS
- DLS
- Heparin column

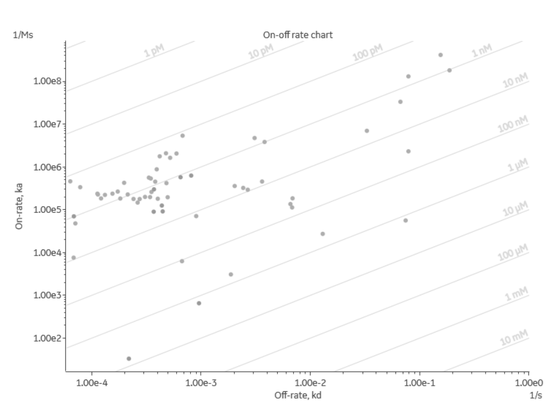
Functional Characterization
- Kinetics
- Functional assays

Biophysical Characterization
- Size and charge profiling
- Accelerated stability
- Pre-formulation studies



Cell based assays: Flow Cytometry (iQue®)

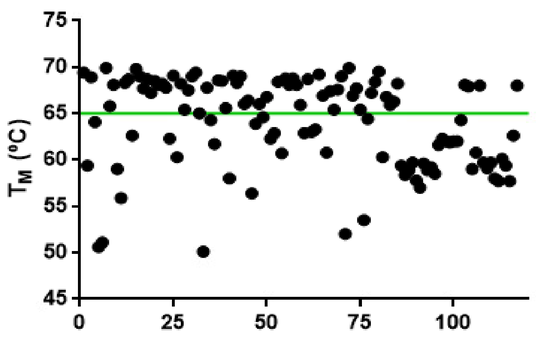
Determination of candidate clones binding specificity to target; internalization amongst other functional assays.



SPR on 8K+: Surface Plasmon Resonance

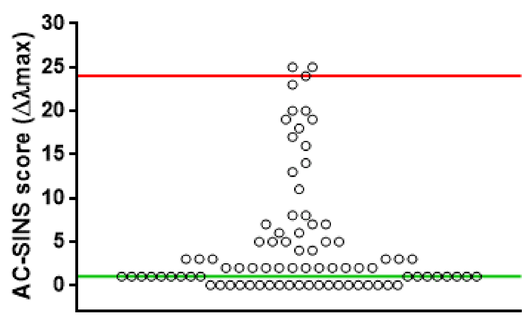
On and off rate determination and affinity calculation, allow the ranking of candidate clones based on kinetics of the interaction to target.

Step 5 – Biophysical Characterization Example



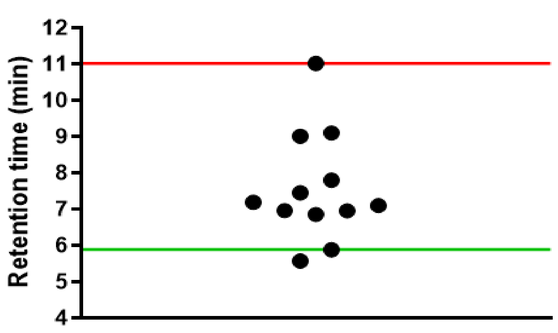
T_M: Melting Temperature determination by DSF

Protein unfolding allows the ranking of the stability of proteins and compare different formulations to find the most favourable conditions.



AC-SINS: Affinity-capture self-interaction nanoparticle spectroscopy

AC-SINS assay has shown to predict viscosity, solubility issues and in vivo clearance helping predicting the antibody developability profile.



Heparin Affinity Chromatography

Heparin Affinity Chromatography allows the assessment of a major contributor for antibody PK: unspecific charge-based glycocalyx interactions that lead to increased pinocytosis and degradation. Thereby, correlating retention time with poor PK profiles.

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