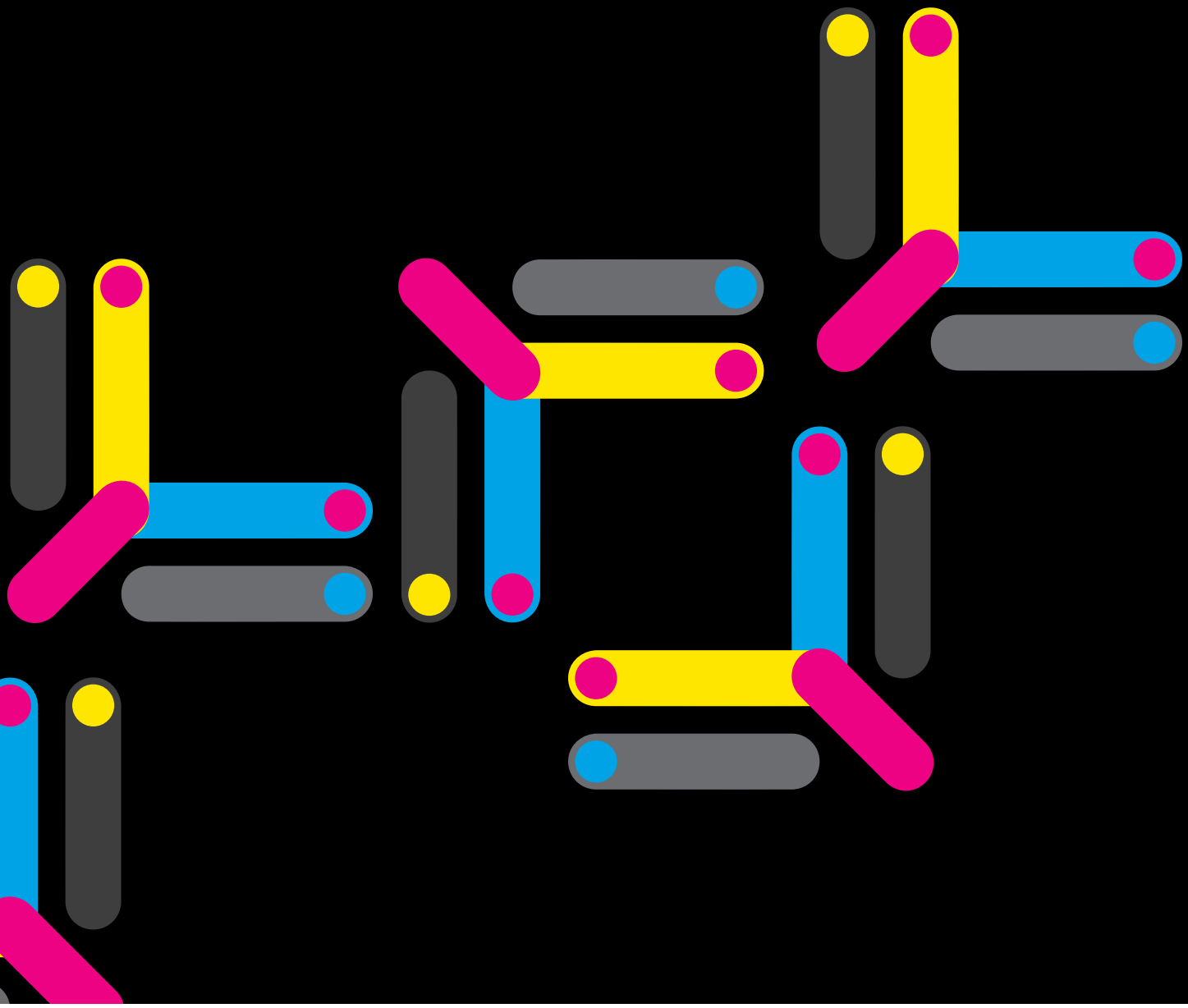


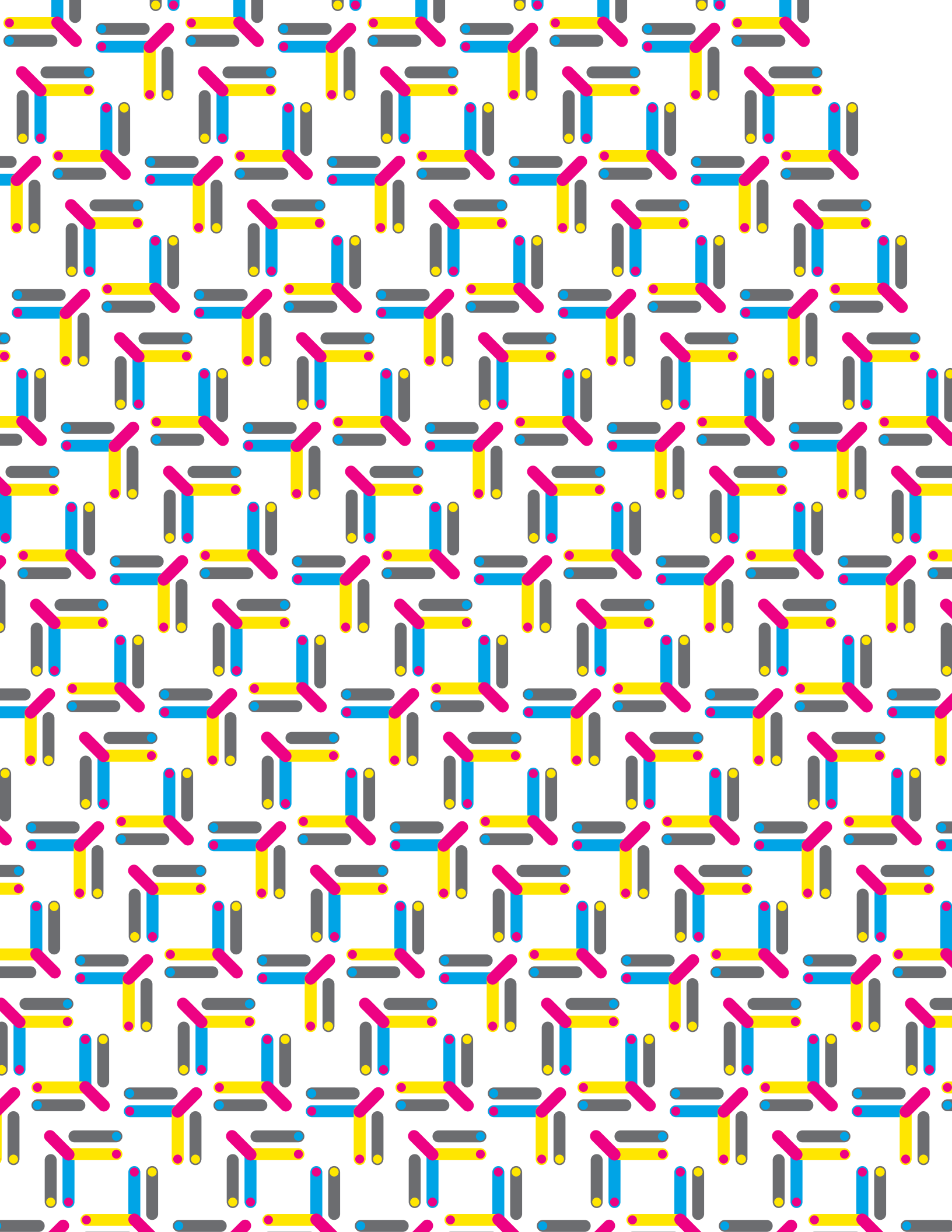
antibody engineering

Antibody production and purification

Engineering antibodies for developability

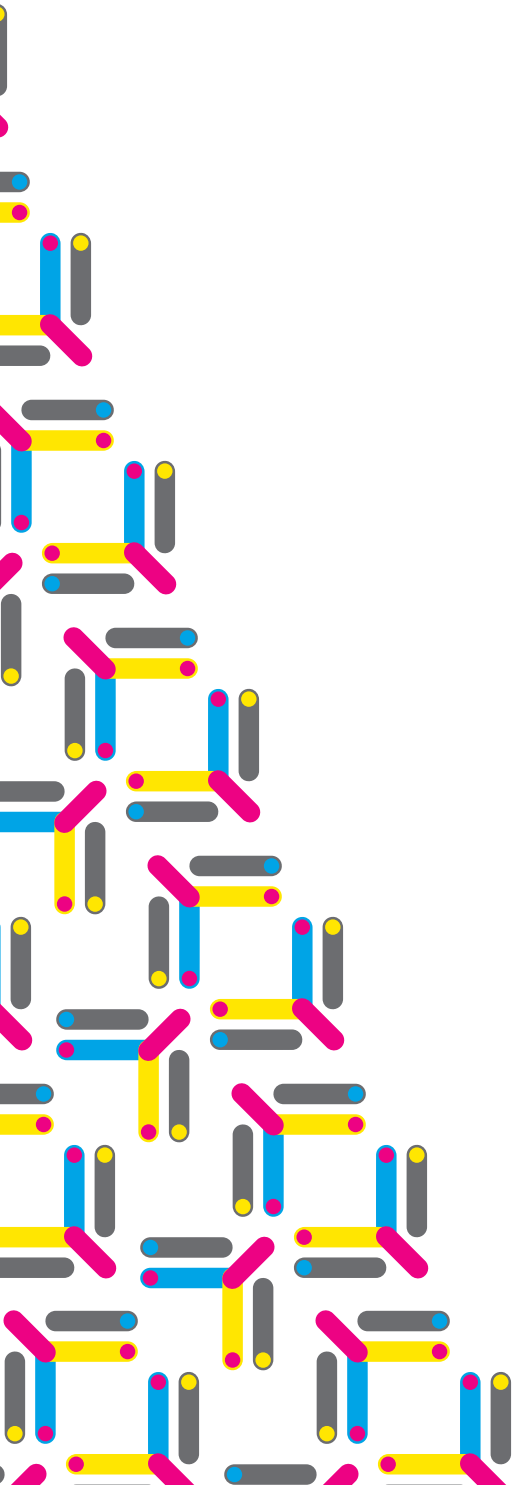
Antibody humanization

ATUM



antibody engineering

Engineering of better antibodies, improved cell lines and higher production yields requires efficient tools to navigate biological high dimensional sequence-function space. ATUM's integrated pipeline from oligonucleotide synthesis via gene design/gene synthesis/vector optimization all the way to transient and stable mammalian protein production enable the direct application of machine learning tools to maximize results.

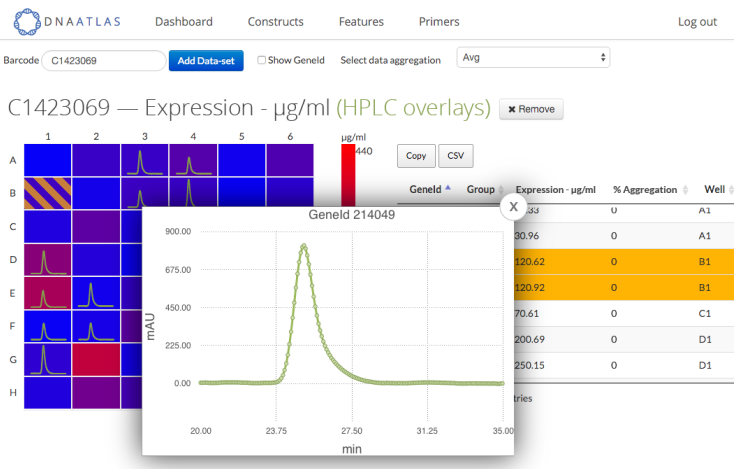


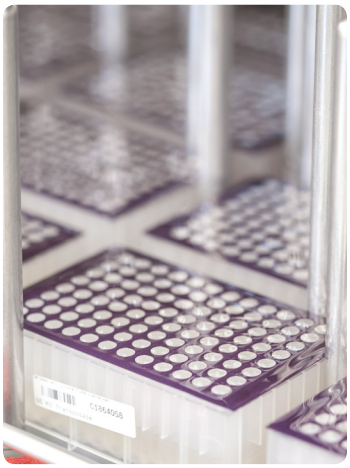
contents

ATUM

antibody production & purification

ATUM's Mammalian Antibody Production Service is based on our integrated manufacturing platform, all under one roof at our facility in sunny California. We combine our codon optimization algorithms, secretion signal toolbox, flexible expression vector configurations and high productivity CHO and HEK 293 cell lines to provide outstanding service. Each step is barcoded and time stamped to ensure an efficient, consistent, robust and trackable process.





Process

- Expression vector constructs are made with customer's variable heavy and light chain regions grafted into one of several available and validated scaffold sequences. The construct identity is confirmed by sequencing and transfection grade DNA is prepared.
- HEK 293 or CHO cells are transiently transfected in 1 ml 96-well (HTP) format, or up to 10l. Larger volumes available upon request.
- Cells are grown, harvested and proteins are purified using appropriate purification resin.
- Proteins are eluted, neutralized and desalted (if required).
- Second column purification (SEC, IEX, MMC) and buffer formulations are available per client request.

Protein Yield

Expected yields of typical Human IgG1 or Mouse IgG2a purified protein.

scale	format	yield
1ml	HTP, 96-well	~250 - 400µg (varies per protein)
10 - 25ml	HTP, up to 400 cultures per week	~2 - 10mg
1l	standard	100mg - 1g
2l	standard	scalable
5l	standard	scalable
>10l	standard	scalable

Analytics Package - Available options

The purified IgGs will be characterized by the following and a report will be provided.

- Concentration - A280
- Molecular weight - Reducing and non-reducing SDS-PAGE
- Aggregation status - SEC-HPLC analysis (% monomer; % HMW)
- Total glycan analysis - Mass spectroscopy
- Endotoxin testing

Optional Assays:

- Thermal stability - Tm analysis on full length antibody and/or Fab fragments
- Binding kinetics - biolayer interferometry (Octet® ForteBio)

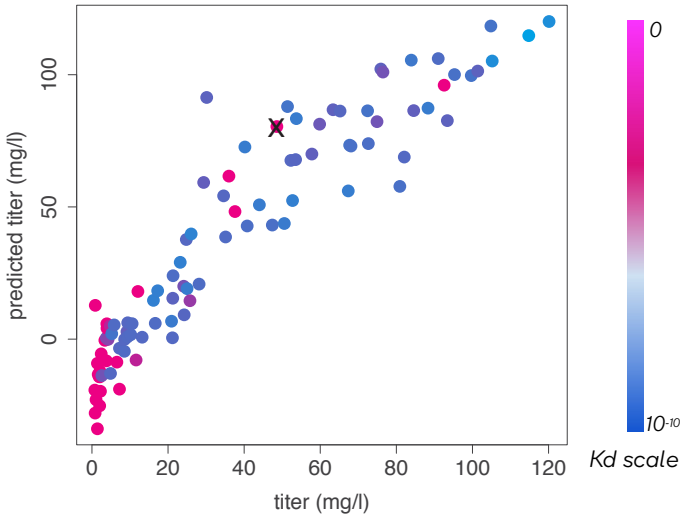
ATUM specializes in expression & purification of challenging proteins such as antibody-like proteins, bispecifics, membrane proteins and cytoplasmic proteins.

engineering antibodies for developability

test learn

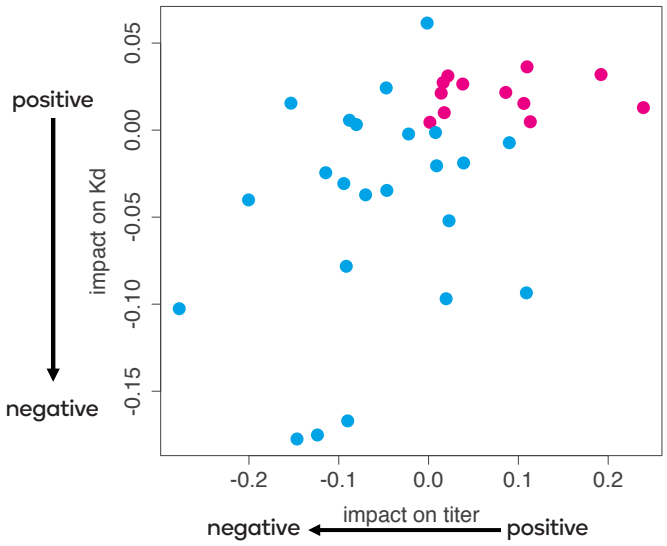
Phylogenetic and structural modeling identifies key residues affecting affinity, stability, expression yield, aggregation and humanization. The functional data derived from physical testing is modeled against the systematically varied infolog variants and used to generate predictions of new variants with enhanced developability properties.

Modeling titer



A total of 96 systematically designed variants of antibody X. The X-axis denotes the observed expression yield. The Y-axis denotes the predicted expression yield. The diagonal distribution represents the accuracy of the model. We have here color coded the binding affinity of the antibody variants. Parent (mouse) variant is denoted as 'X'.

Impact of sequence variables



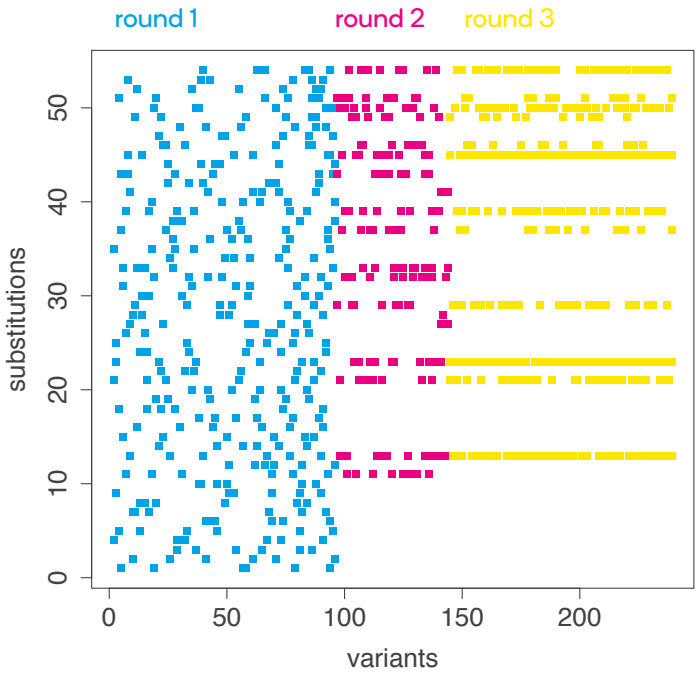
Amino acid substitutions used for humanization distributed by their relative contribution towards titer (X axis) and binding (Y axis). Substitutions contributing positively in both dimensions are denoted in pink.

ATUM's proprietary Design of Experiment (DoE) technology enables systematic exploration of sequence-function relationships, identifying and quantifying amino acid substitutions and their relative contribution in multiple different functional dimensions. Assessing the sequence-function relationship and the amino acid substitutions relative independence provide guidance for generating predictive and testable models of target protein performance, metrics of its humanness, and developability. We typically test a total of 48-400 antibody variants over 1-4 iterations.

learn improve

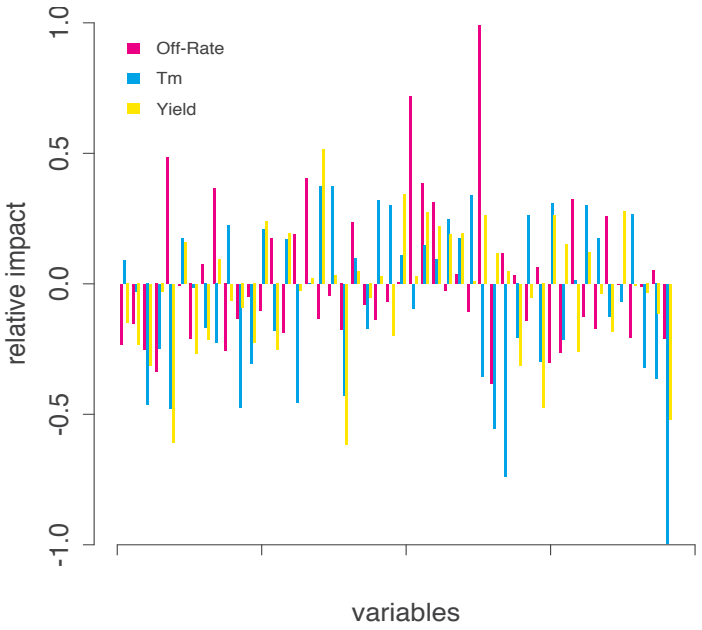
The results from 12 independent antibody humanization experiments impart predictive design strategies for future antibody humanizations.

Efficient search of space



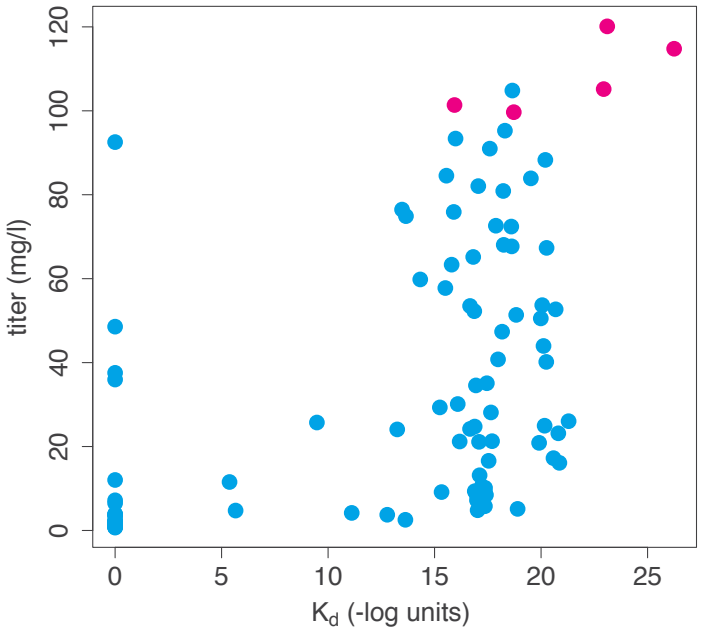
Reduction of tested amino acid substitutions over three iterations of antibody engineering for undisclosed biological activity. The X-axis denotes each tested mAb variant, Y-axis denotes presence/absence of substitutions. First round (R1) in blue tests 54 amino acid substitutions present in 96 mAb variants for a total space of $2^{54} = 10^{16}$ possible variants. Second round (pink) constitutes 48 mAb variants narrowing the search to 18 substitutions ($2^{18} = 10^{5}$). Third round (yellow) constitutes 96 mAb variants further narrowing the search to 12 substitutions (2^{12} = 4,000 variants).

Weights distribution plot



Relative contribution of 50 amino acid substitutions in mAb scaffold; binding off-rate (red bar), Tm (blue bar) and expression yield (yellow bar).

Predicting and validating new variants



improve validate

Sequence function information for binding (X-axis) and expression yield (Y-axis) derived from antibody engineering round 1 (blue) was used to build models and to predict 5 new improved variants (pink) which were engineered and validated.

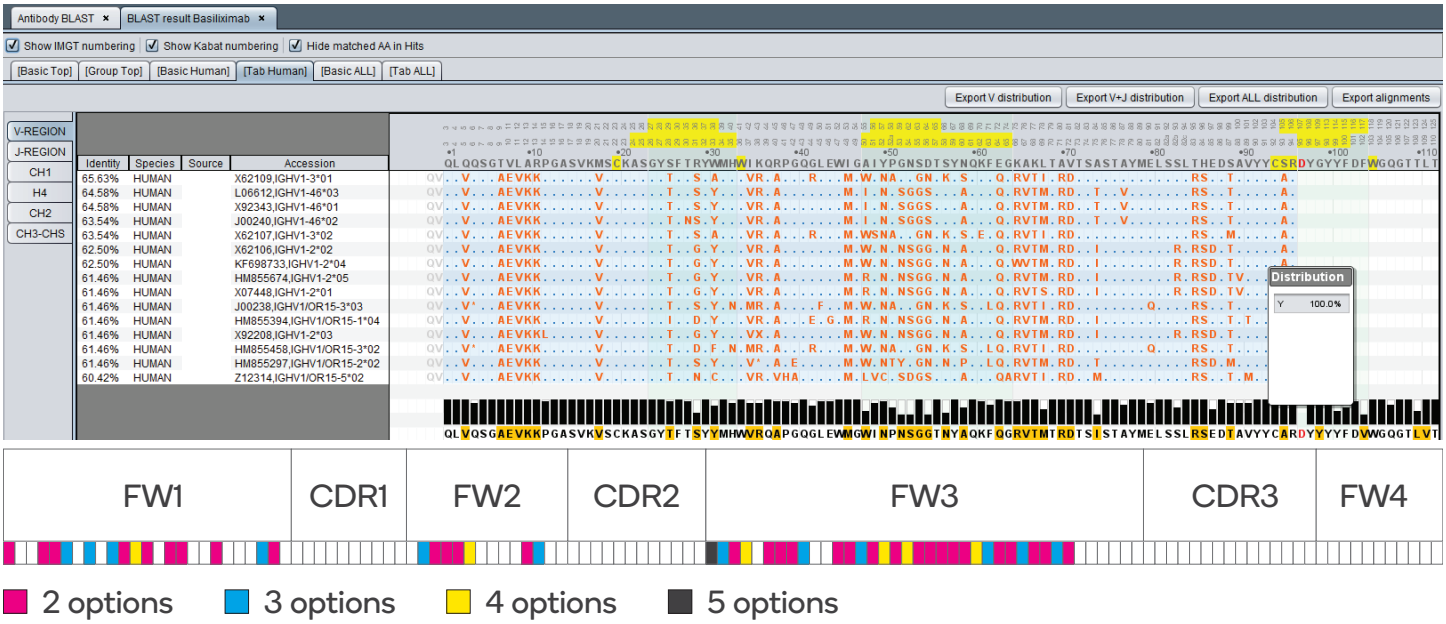
antibody humanization

ATUM's antibody humanization platform combines advantages of both rational and empirical antibody humanization approaches. Humanized antibodies are designed using our proprietary humanization and optimization algorithm and made by gene synthesis and transient protein expression in mammalian cells.

design build

Traditional humanization approaches and CDR grafting can be drastically improved by applying DoE and machine learning methodologies to generate a small number of humanized molecules with improved developability profiles – example expression titer, aggregation propensity, stability, polyspecificity profile, Tm, in conjunction with retained or enhanced affinity.

- ATUM uses homology modeling between the nonhuman and human V genes, followed by in silico CDR grafting.
- Sequences are analyzed for the presence of known T cell epitopes, N-linked glycosylation sites, unpaired cysteine residues, potential amino acid modification sites and other sequence liabilities.
- Humanized antibodies will be designed consistent with the current World Health Organization (WHO) humanization standards (INN). Specifically, the V-gene of the humanized heavy and light chain must align most closely with a human V-gene sequence, and be >85% identical to a human V-gene sequence.
- Antibody genes will be cloned and expressed in ATUM's expression vectors that have been optimized for cell line, isotype and signal sequences to obtain maximal yields.



Using a combination of Machine Learning for sequence space functional exploration and empirical data for identifying and quantifying the sequence-function correlation, we typically design and experimentally validate 24 to 96 antibodies or antibody-like constructs.

Analytics options

The purified IgGs will be characterized by the following and a report will be provided.

- Concentration - A280
- Molecular weight - Reducing and non-reducing SDS-PAGE
- Aggregation status - SEC-HPLC analysis (% monomer; % HMW)
- Total glycan analysis - Mass spectroscopy
- Endotoxin testing

Optional Assays:

- Thermal stability - Tm analysis on full length antibody and/or Fab fragments
- Binding kinetics - biolayer interferometry (Octet® ForteBio)

Deliverables (~6 weeks)

- Amino acid sequence information for each expressed IgG variant.
- Purified chimeric and humanized IgGs.
- Process and characterization report for each IgG delivered.

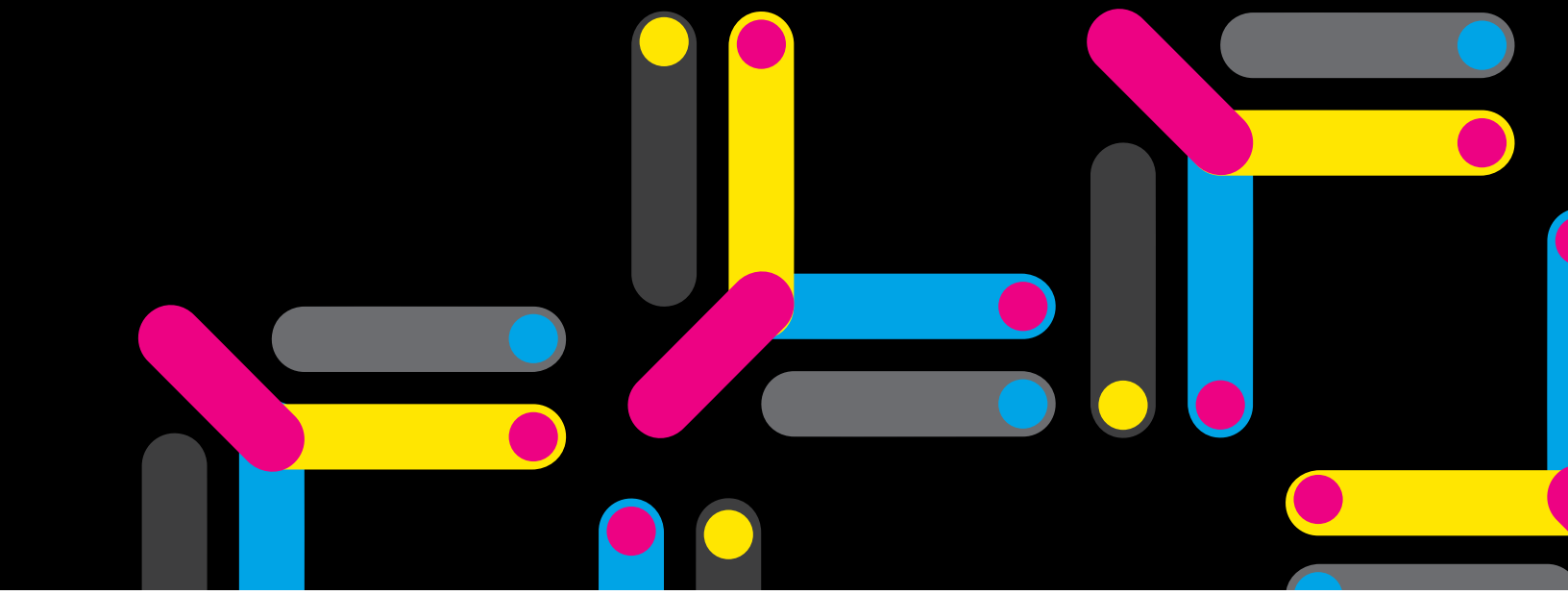
hybridoma purification & sequencing

ATUM offers sequencing of your hybridoma cell line to determine sequence of cDNA encoding the antibody variable heavy (VH) and variable light (VL) domains. Antibody sequence information is essential for monoclonal antibodies (mAbs) engineering and humanization, function optimization, database banking and patent applications.

ATUM provides:

- Determination of variable heavy and light chain sequences
- Species and IgG sub-type confirmation
- Follow on services: Combine antibody sequencing with our antibody engineering, humanization and antibody expression services

research. create. break through.



ATUM

+1 877 DNA TOGO
+1 650 853 8347
info@atum.bio