


Five Computational Developability Guidelines for Therapeutic Antibody Profiling

Matthew Raybould
Oxford Protein Informatics Group
University of Oxford

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Common Antibody Developability Issues

- Poor expression
- Aggregation
- High Viscosity
- Chemical/Physical Instability
- Polyspecificity
- Immunogenicity

- Many different ***in vitro*** assays to test for each of these issues
- However, the time/quantity of monoclonal antibody (mAb) needed to experimentally test for each of these is often prohibitive in early-stage development
- Therefore, desire to generate ***in silico*** assays that can rapidly filter out mAb drug candidates with poor developability

***in silico* developability assessment tools (2018)**

1. Various algorithms for "humanness" assessment *via* comparison to natural antibody sequences
2. Statistically-fit predictors of *in vitro* assay values (e.g. *CamSol*, *Developability Index*, FvCSP) or sites of post-translational modification

No publicly-available method that captured general developability

The Therapeutic Antibody Profiler:

A structure-based, *in silico* method for rapidly detecting mAbs with poor developability

Assumptions

- Many instances of poor developability are caused by the chemical properties **of a region of the antibody surface**.
- The most variable region between antibodies is the **Fv region**, so we analyse this region alone
- The best way to measure Fv surface properties is *via* a **structural representation**
- A set of these properties may offer some predictive power to identify more “drug-like” antibodies, ***cf*. Lipinski rules**
- We assume that therapeutics that have reached Phase-II of clinical trials have acceptable developability

Requirements

- We must be able to identify poor developability mAbs in a **high-throughput manner**
- This necessitates using **homology models** over *ab initio* models or crystal structures

Five properties:

1. CDRH3 or Total CDR length [aggregation, flexibility, topology]
2. Patches of Surface Hydrophobicity (PSH) across the CDR Vicinity [aggregation, viscosity, polyspecificity]
3. Patches of Surface Positive Charge (PPC) across the CDR Vicinity [poor expression, aggregation, viscosity, polyspecificity]
4. Patches of Surface Negative Charge (PNC) across the CDR Vicinity [poor expression, aggregation, viscosity, polyspecificity]
5. Structural Fv Charge Symmetry Parameter [aggregation, viscosity]

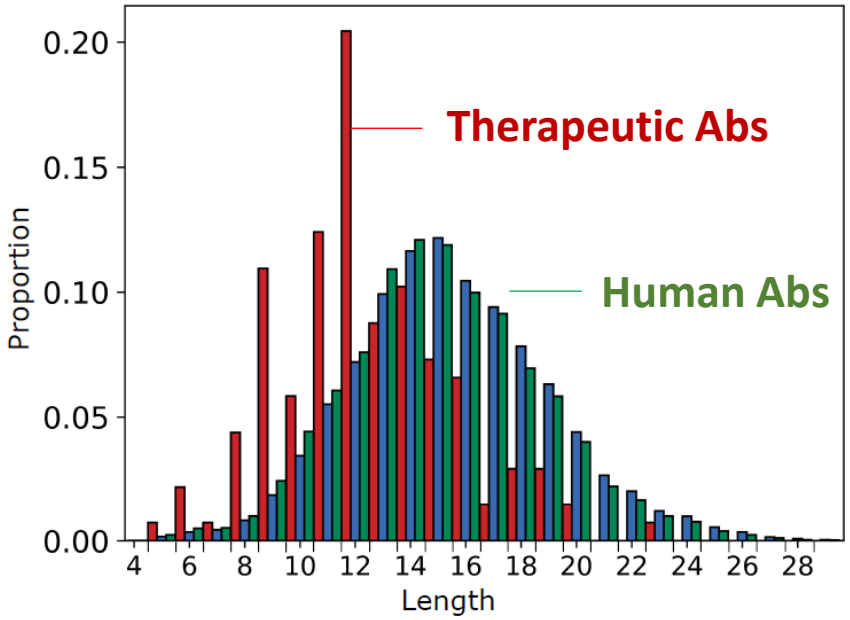
Datasets:

137 Post-Phase I Therapeutic Models[1]	14k Representative Human Antibody Models[2,3]	2 Datasets of MedImmune Developability Failures
Sets the **acceptable bounds** of the five properties	Provides a "**natural antibody comparison**"	Used to **validate** that we can selectively highlight mAbs with developability issues

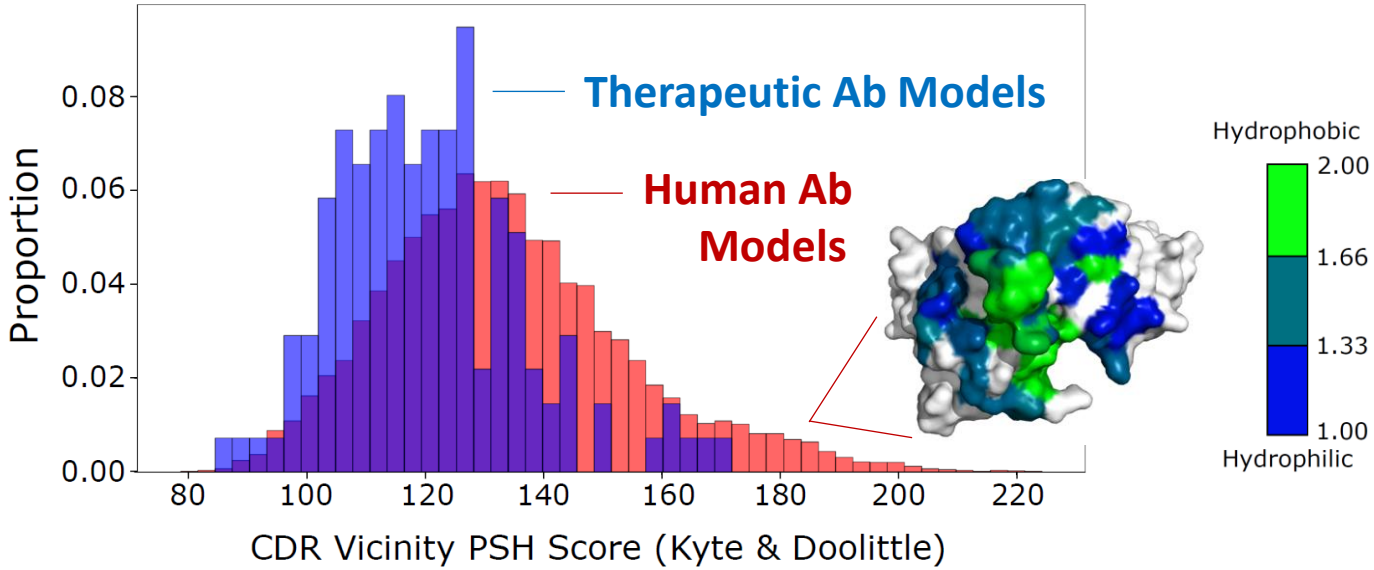
[1]Jain T, *et al*. (2017) Biophysical properties of the clinical-stage antibody landscape. *Proc Natl Acad Sci USA* 114(5):944–949.
[2]Vander Heiden JA, *et al*. (2017) Dysregulation of B cell repertoire formation in myasthenia gravis patients revealed through deep sequencing. *J. Immunol*. 198:1460–1473.
[3]Raybould, MIJ *et al*. (2019) Five computational developability guidelines for therapeutic antibody profiling. *Proc Natl Acad Sci USA* 116(10):4025-4030.

Comparisons: Therapeutics *vs*. Human Antibodies

CDRH3 Length



Patches of Surface Hydrophobicity (PSH)

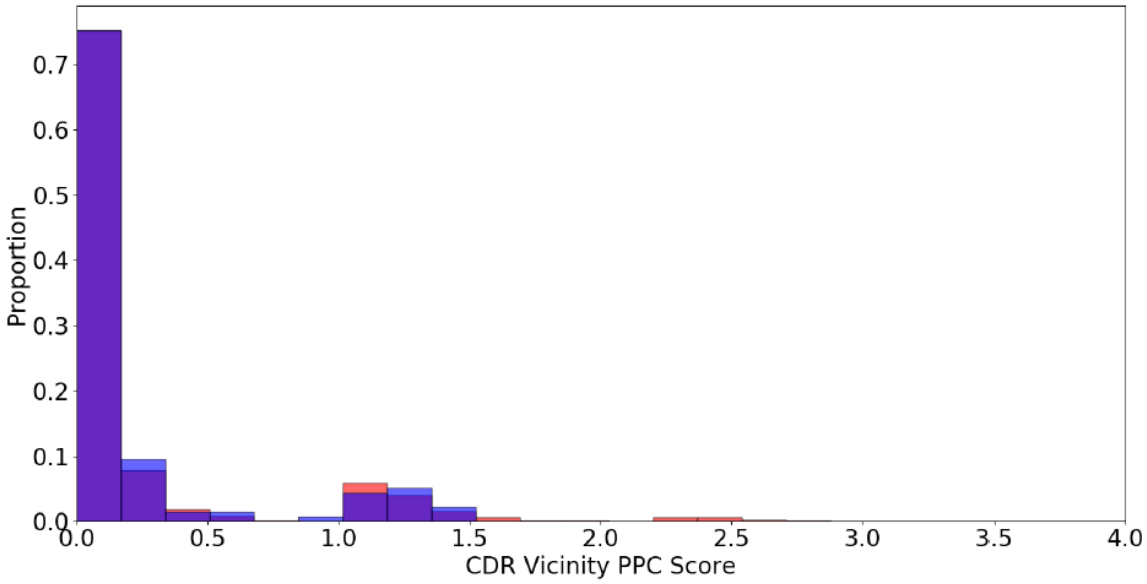


$$\sum_{R1\ R2} \frac{H(R1,S)\ H(R2,S)}{r_{12}^2}$$

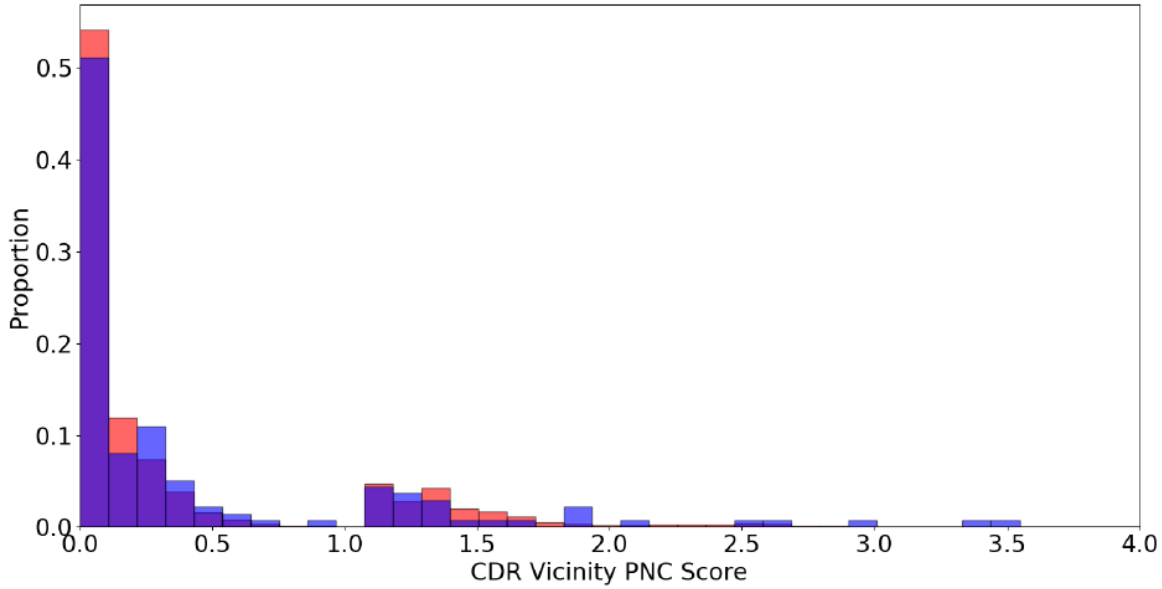
- Therapeutics tend to have shorter CDRH3s and smaller patches of surface hydrophobicity than human antibodies

Comparisons: Therapeutics *vs*. Human Antibodies

Patches of Surface Positive Charge (PPC)



Patches of Surface Negative Charge (PNC)



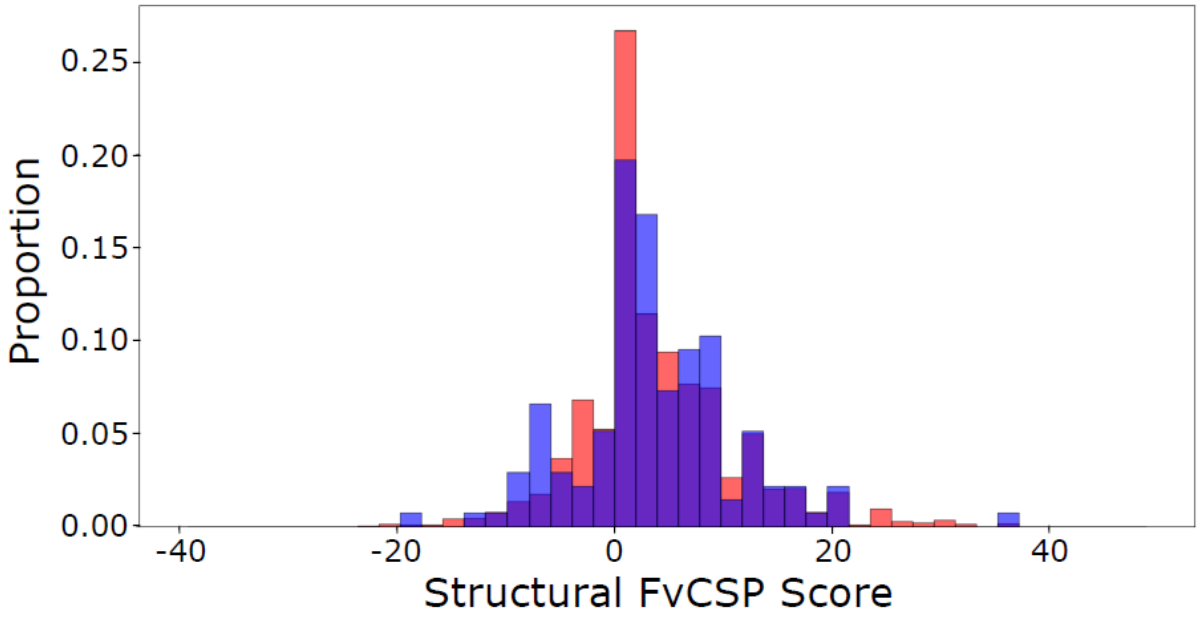
$$\sum_{R1\ R2} \frac{|Q(R1)|\ |Q(R2)|}{r_{12}^2}$$

Blue: Therapeutic Antibody Models
Red: Human Antibody Models

- Therapeutics and human Abs have similar sizes of positive charge and negative charge patches

Comparisons: Therapeutics *vs*. Human Antibodies

Structural Fv Charge Symmetry Parameter (SFvCSP)



$$\left[\sum_{R_H} Q(R_H)\right]\left[\sum_{R_L} Q(R_L)\right]$$

Blue: Therapeutic Antibody Models
Red: Human Antibody Models

- Both therapeutic and human antibodies have an aversion to strongly oppositely-charged VH and VL chains

Validation

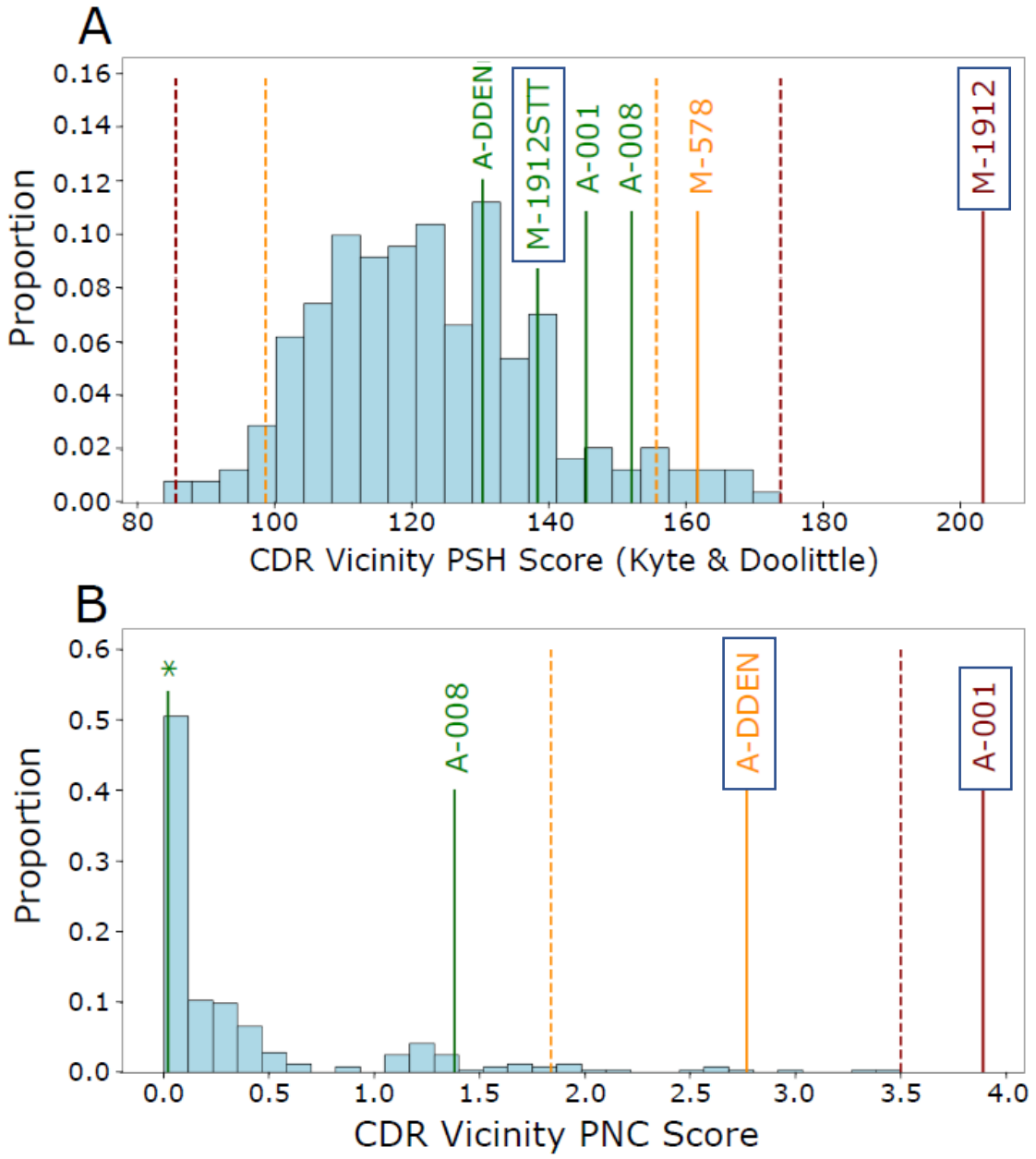
- Found a further 105 post-Phase I therapeutic sequences, as "developable antibodies"
- Only 8/105 were assigned by TAP to have a property outside the existing distributions. Most (except PPC) were minorly adjusted:

Property	Red Threshold (137 Phase-II+ therapeutics)	Red Threshold (242 Phase-II+ therapeutics)
Total CDR Length (Lower)	39	39
Total CDR Length (Upper)	59	**60**
PSH (Lower)	85.64	**83.34**
PSH (Upper)	168.30	**173.85**
PPC	1.51	**3.16**
PNC	3.50	3.50
SFvCSP	-19.50	**-20.40**

Validation

M-1912 aggregated uncontrollably during development, and exhibited extremely high values in our CDR Vicinity PSH metric.
M-1912STT resolved the issue.

A001 had prohibitively poor expression levels, and exhibited extremely high values in our CDR Vicinity PNC metric.
A-DDEN fixed the issue (backbone engineering)



TAP Developability Guidelines

Values based on 242 clinical-stage therapeutic antibodies as of Feb' 2019

	(Bottom 5%/Top 5%)	(Below/Above)
Metric	Amber Flag Region	Red Flag Region
Total CDR Length	$39 \leq L \leq 43$ $54 \leq L \leq 60$	$L < 39$ $L > 60$
PSH, CDR Vicinity	$83.84 \leq PSH \leq 100.71$ $156.200 \leq PSH \leq 173.850$	$PSH < 83.84$ $PSH > 173.850$
PPC, CDR Vicinity	$1.25 \leq PPC \leq 3.16$	$PPC > 3.16$
PNC, CDR Vicinity	$1.84 \leq PNC \leq 3.50$	$PNC > 3.50$
SFvCSP	$-20.40 \leq SFvCSP \leq -6.30$	$SFvCSP < -20.40$

These metrics could be rapidly calculated:

- During early-stage discovery
- During *in silico* affinity maturation

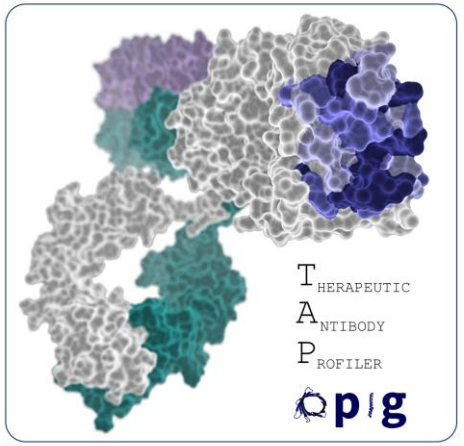
to help select mAbs more amenable to therapeutic development

NB: Metric values for therapeutics can change as model quality improves

Notes

- The TAP thresholds are now set by *c.* 400 CSTs in Phase-II+ development. We actively track these in Thera-SAbDab (http://opig.stats.ox.ac.uk/webapps/therasabdab). Thresholds have proven robust to the addition of more data.
- Typical runtime for TAP is < 30s/antibody on a single core (if all loops are homology-modellable)
- The TAP metrics were chosen to be developability-linked and interpretable. With sufficient “negative” data, they could be more systematically derived. As could the amber/red threshold percentile values
- The TAP metrics are **guidelines**, not strict rules. They could change over time with advances in process development
- These principles could be extended to **other classes of protein therapeutics**

The Therapeutic Antibody Profiler is described in our paper in PNAS[1]

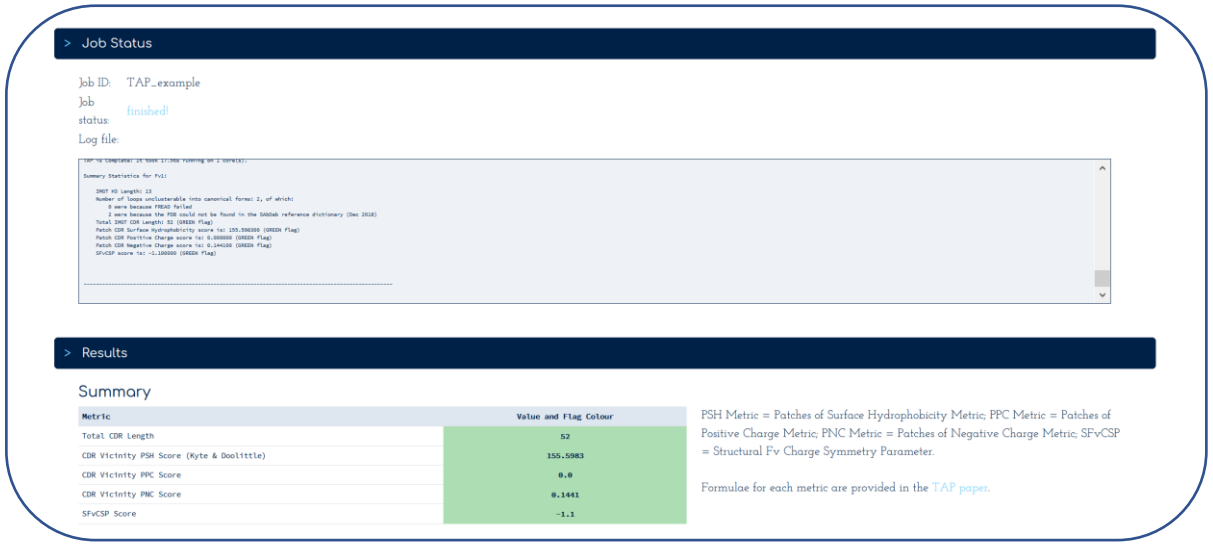


[1]Raybould, MIJ *et al*. (2019) Five computational developability guidelines for therapeutic antibody profiling. *Proc Natl Acad Sci USA* 116(10):4025-4030.

Software Availability

- Free OPIG Webserver

  (http://www.opig.stats.ox.ac.uk/webapps/tap)



If data is IP-sensitive…

- Vagrant VirtualBox
- Coming Soon: Singularity Container

SAbBox

enquiries to: opig@stats.ox.ac.uk

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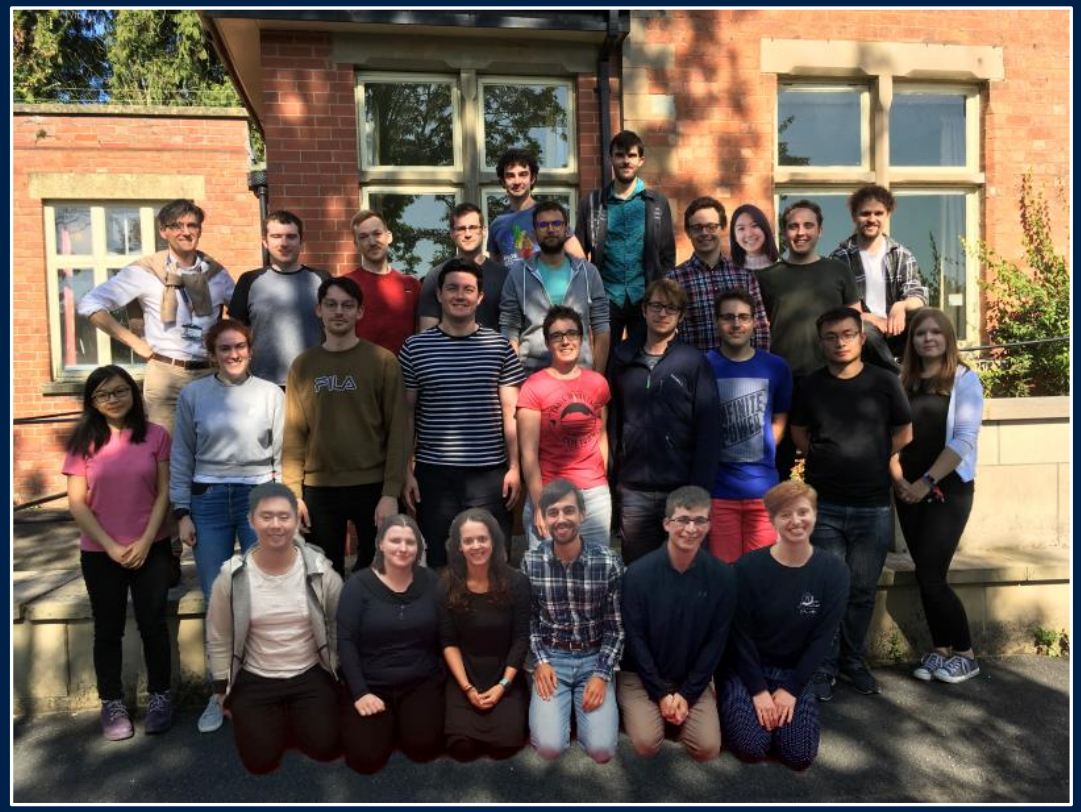
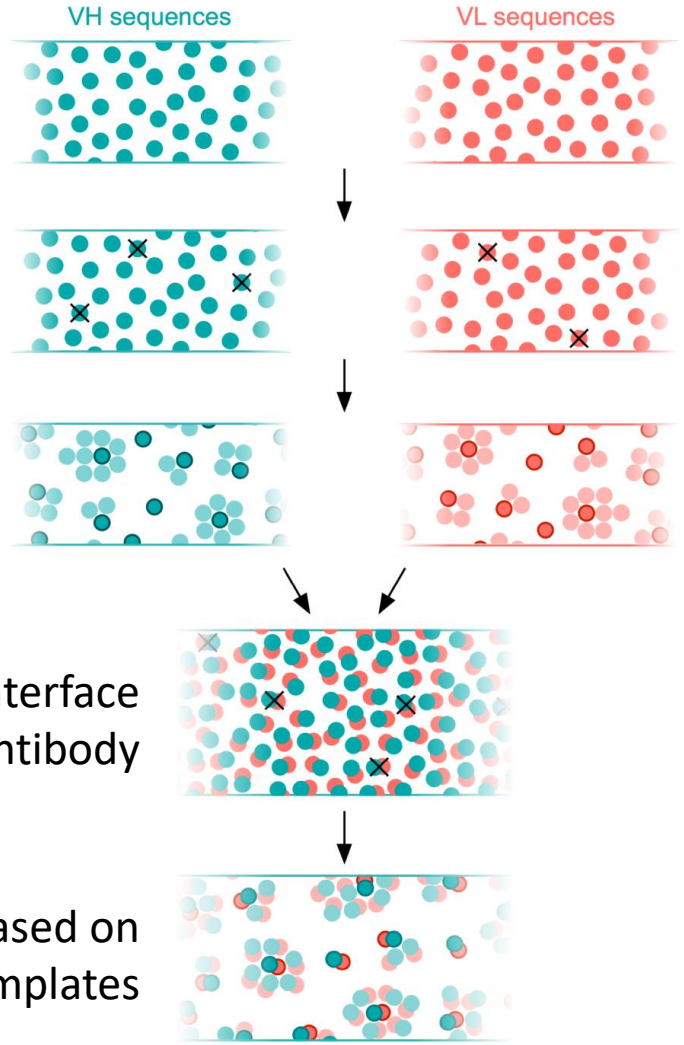


Supplementary Slides

Making a set of “representative human antibody” models

Next-Generation Sequencing data



Keep chains with homology-modellable CDRs

Sequence cluster to reduce complexity

Pair antibodies with high interface identity to a solved antibody

Structurally cluster based on most homologous CDR templates

Designed to capture as much sequence & structural diversity as possible within the “modellable space”

Protocol used in TAP metric comparison described in PNAS 116(10):4025-4030

Most recent protocol described in PLoS Comput. Biol. 17(3):e1008781

Splitting Therapeutics by Kappa/Lambda LCs

Table S5. TAP values across kappa and lambda models.

Dataset	TAP Metric	Kappa Subset ($\mu \pm \sigma$)	Lambda Subset ($\mu \pm \sigma$)
242 CST Models	PSH	120.89 ± 15.10	142.03 ± 19.09
	PPC	0.21 ± 0.47	0.53 ± 0.56
	PNC	0.38 ± 0.64	0.60 ± 0.77
	SFvCSP	3.82 ± 7.38	1.67 ± 7.87
14,072 VdH Ig-seq Models	PSH	131.27 ± 21.41	141.68 ± 17.82
	PPC	0.17 ± 0.40	0.52 ± 0.73
	PNC	0.27 ± 0.48	0.74 ± 0.83
	SFvCSP	4.56 ± 7.44	0.84 ± 6.48
19,019 UCB Ig-seq Models	PSH	125.40 ± 18.56	139.66 ± 17.88
	PPC	0.11 ± 0.31	0.31 ± 0.53
	PNC	0.22 ± 0.40	0.65 ± 0.88
	SFvCSP	3.67 ± 5.30	0.12 ± 5.24

Models containing Lambda light chains seemed inherently less 'developable' than those containing kappa light chains

(90% of CSTs involve **kappa light chains)**

- Consistent with DeKosky et al. (Lambda L3's much more hydrophobic than Kappa L3's)

DeKosky BJ, et al. (2016) Large-scale sequence and structural comparisons of human naïve and antigen-experienced antibody repertoires. Proc Natl Acad Sci USA 113(19):E2636–E2645.

Splitting Therapeutics by Species Origin

Table S8. 242 CST TAP values split by species origin.

TAP Metric	101 Human ($\mu \pm \sigma$)	108 Humanized ($\mu \pm \sigma$)	30 Chimeric ($\mu \pm \sigma$)	3 Mouse ($\mu \pm \sigma$)
Total CDR Length	48.68 ± 4.09	47.80 ± 3.42	46.77 ± 3.55	46.33 ± 1.25
PSH	127.76 ± 18.56	120.90 ± 14.20	115.73 ± 15.58	117.26 ± 9.44
PPC	0.29 ± 0.58	0.20 ± 0.36	0.26 ± 0.55	0.05 ± 0.06
PNC	0.34 ± 0.56	0.50 ± 0.75	0.30 ± 0.63	0.50 ± 0.50
SFvCSP	4.06 ± 7.44	3.13 ± 7.80	3.29 ± 5.99	7.58 ± 6.75

- Appears that the more human mAbs have larger patches of hydrophobicity than mouse mAbs
- We also split by clinical progression (P2, P3, Approved) and drug campaign status (active/discontinued) but found no significant differences in TAP metric values.