

Supplementary Information

Analytical workflows to unlock predictive power in biotherapeutic developability

Markos Trikeriotis, Sergey Akbulatov, Umberto Esposito, Athanasios Anastasiou, Oksana I. Leszczyszyn

Table S1. Properties of the monoclonal antibodies used in this study

Name and abbreviation	Isotype	PDB ID ^a	Extinction coefficient ^b	Monomericity (%) ^c	Theoretical pI ^d		
					mAb	Fab	Fc
Atezolizumab (mAb-1)	IgG1	5X8L (Fab)	1.62	97.2	7.68	7.90	7.05
Golimumab (mAb-2)	IgG1	5YOY (Fv)	1.48	99.4	7.97	8.26	6.95
Ixekizumab (mAb-3)	IgG4	6NOV (Fab)	1.39	99.3	7.43	7.95	6.04
Lampalizumab (mAb-4)	IgG1	4D9Q (Fab)	1.41	98.8	6.67	6.22	7.11
Rituximab (mAb-5)	IgG1	2OSL (Fab)	1.64	98.4	8.12	8.48	7.11

^a In parenthesis the fragment type that corresponds to each PDB structure. The Protein Data Bank can be found at: <https://www.rcsb.org/>

^b Calculated by Evitria SA ($L \times g^{-1} \times cm^{-1}$ at 280 nm)

^c Reported by Evitria SA

^d Calculated using Prot pi, Protein Tool (Zurich University of Applied Sciences; <https://www.protpi.ch/Calculator/ProteinTool>)

Primary sequences of the studied mAbs

Atezolizumab (mAb-1)

Heavy chain

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWRQAPGKGLEWVAVWISPYGGSTYYADSVKGRFTISADTSKNTAYLQ
MNSLRAEDTAVYYCARRHWPGGFDYWGGTLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Light chain

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISLQPEDFATY
YCQQYLYHPATFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Golimumab (mAb-2)

Heavy chain

QVQLVESGGGVVQPGRSLRLSCAASGFISSYAMHWVRQAPGNGLWVAFMSYDGSNKKYADSVKGRFTISRDNKNTLYL
QMNSLRAEDTAVYYCARDRIAAGGNYYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Light chain

EIVLTQSPATLSLSPGERATLSCRASQSVYSYLAWYQQKPGQAPRLLIYDASNRTGIPARFSGSGSGTDFTLTISLQPEDFAVYY
CQQRSNWPPFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Ixekizumab (mAb-3)

Heavy chain

QVQLVQSGAEVKKPGSSVKVSCASGYSTFDYHIHWVRQAPGQGLEWMGVINPMYGTDDYNQRFKGRVTITADESTSTAYM
ELSSLRSEDTAVYYCARYDYFTGTGVYWGQGLTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI
SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQE
GNVFSCSVMHEALHNHYTQKSLSLSLG

Light chain

DIVMTQTPLSLSVTPGQPASISCRSSRLVHSRGNTYLHWYLQKPGQSPQLLIYKVSNRFIGVPDRFSGSGSGTDFTLKISRVEAE
DVGYYCSQSTHLPFTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE
QDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Lampalizumab (mAb-4)

Heavy chain

EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYADDFKGRFVFSLDTSVSTAYL
QISSLKAEDTAVYYCEREGLVNNWGGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMEALHNHYTQKSLSLSPGK

Light chain

DIQVTQSPSSLSASVGDRTITCITSTDIDDDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISLQPEDVATY
YCLQSDSLPYTFGGQTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Rituximab (mAb-5)

Heavy chain

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYM
QLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPK
PKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK

Light chain

QIVLSQSPAILASAPGEKVTMTCRASSSVSYIHWFFQQKPGSSPKPWYATSNLASGVPVRFSGSGSGTSYSLTISRVEAEDAATYY
CQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Salt Effect calculations

To generate the salt effect plot the change on the T_{agg2} and T_m values must be calculated to give ΔT_{agg2} and ΔT_m respectively. The aggregation temperature shift, ΔT_{agg2} , was calculated as:

$$\Delta T_{agg2} = T_{agg2} \text{ (low salt)} - T_{agg2} \text{ (high salt)}$$

where T_{agg2} (low salt) and T_{agg2} (high salt) refer to the value of T_{agg2} at NaCl concentration 0 mM and 150 mM respectively.

The unfolding temperature shift, ΔT_m includes the combined shift of the $T_m(C_H2)$, $T_m(C_H3)$ and $T_m(Fab)$, and was calculated as:

$$\Delta T_m = |\Delta T_m(C_H2)| + |\Delta T_m(C_H3)| + |\Delta T_m(Fab)|$$

where the ΔT_m for each domain or region was the difference between the respective T_m at NaCl concentration 0 mM (low salt) and 150 mM (high salt); for example for C_H2 :

$$\Delta T_m(C_H2) = T_m(C_H2)(\text{low salt}) - T_m(C_H2)(\text{high salt})$$

All the results of these calculations are listed on Table S2 below.

Table S2. Salt effect results for all mAbs

mAb	pH	ΔT_m (°C)	ΔT_{agg2} (°C)
mAb-1	4	13.2	11.0
mAb-1	5	8.1	8.3
mAb-1	6	3.5	1.5
mAb-1	7	2.2	-0.9
mAb-1	8	1.9	0.3
mAb-2	4	12.2	13.5
mAb-2	5	4.2	12.8
mAb-2	6	2.0	0.7
mAb-2	7	0.5	-2.6
mAb-2	8	1.1	-2.8
mAb-3	4	12.1	15.8
mAb-3	5	3.9	8.7
mAb-3	6	2.3	-2.9
mAb-3	7	1.9	-7.0
mAb-3	8	2.3	-5.7
mAb-4	4	9.8	17.9
mAb-4	5	4.9	1.9
mAb-4	6	4.0	N/A ^a
mAb-4	7	2.7	N/A ^a
mAb-4	8	7.0	-1.5
mAb-5	4	9.8	15.3
mAb-5	5	5.6	12.5
mAb-5	6	2.5	1.9
mAb-5	7	0.6	-1.2
mAb-5	8	0.4	-1.6

^a The ΔT_{agg2} for this sample was not possible to determine because it was heavily aggregated at low salt conditions

Additional Supplementary Files

ESM_2.xls Input parameters for Machine Learning

ESM_3.xls Biophysical characterisation results for the 5 mAbs