lightningHEK™ Developability Summary Report

Reference ID: 12345

August 14, 2025

Prepared for XXXX

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lightningHEK Background

lightningHEK™ is ATUM's high-throughput expression and liability screening platform that enables the rapid triage of hundreds or thousands of antibody candidates. The platform includes:

- sequence optimization of the targets of interest
- rapid linear DNA-based amplification
- transient protein expression in HEK
- protein purification
- titer and purity estimates
- binding assessment (optional), and
- developability assessment (optional)

lightningHEK allows for a rapid, high-throughput transition from sequence to protein expression in quantities sufficient for small-scale, high-throughput binding and liability assessments. Researchers can proactively identify developability and manufacturing risks across several parameters and downselect or rule out higher risk candidates before scaling up lead optimization studies.

lightningHEK Transient Expression Overview

Client-provided V_H/V_L sequences for mAbs or Fc fusions are ligated into a conserved antibody backbone optimized for expression. The constructs are transiently expressed in HEK 293, allowing for the generation of hundreds or thousands of purified candidates in approximately 10 business days (longer for customer provided antibody backbones).

Candidate antibodies are purified using a single-step protein A purification, confirmed by SDS-PAGE, and buffer exchanged into PBS. Titer is measured by Octet.

Clients may choose to perform some or all the additional analytical assays described on the following page.

All work is performed at ATUM in Newark, CA.

lightningHEK Developability Overview

Attribute	Assay	Method
Titer	Octet Binding	Protein A-coated tips are used to capture analyte. Concentration is measured against a standard curve generated with Herceptin.
Binding	Octet Binding	Human Fc-coated tips capture analyte; antigen at a single concentration is associated for 5 minutes, dissociation is measured for 10 minutes at 27˚C.
Aggregation	SEC-HPLC	Samples are run on a 300Å pore column, detected at 280 nm with PBS as the running buffer.
Thermal Stability (T_m)	Differential Scanning Fluorimetry (DSF)	Samples are diluted to 0.5 mg/mL in buffer and mixed with SYPRO Orange and heated in a thermal cycler from 40 - 95˚C at 0.5˚C/min. Melting temperature is defined as the temperature at which the negative derivative of fluorescence reaches a minima.
Self-association	AC-SINS	Normalized samples are affixed to gold nanoparticles. A plasma wavelength shift indicates sample self-association.
Polyspecificity	Ovalbumin binding	Normalized samples are measured using standard PAIA Biotech protocols.
Nonspecificity	Heparin Binding	
Surface Charge	CEX	
Hydrophobicity	HIC	
Identity	Mass Spec	Samples were normalized to 1 mg/ml or 0.5 mg/ml. Post normalization samples were deglycosylated, reduced and run on Waters SQD MS.

Client Sample List

Protein ID	Name / Molecule	Molecular Weight(kD)	Isoelectric Point	Chain Name	Chain Letter	Chain Ratio	Molecular Weight(kD)	Isoelectric Point	N-Glycans	Hydrophob. (GRAVY)
196837.14.A	Candidate 1	144.67	8.24	Candidate 1 _HC	H	2	48.97	8.36	1	-0.38
				Candidate 1 _LC	L	2	23.37	6.99	0	-0.39
196843.14.A	Candidate 2	146.53	8.09	Candidate 2 _HC	H	2	49.82	8.34	1	-0.40
				Candidate 2 _LC	L	2	23.45	6.34	0	-0.44
196849.14.A	Candidate 3	145.48	8.25	Candidate 3 _HC	H	2	49.31	8.59	2	-0.39
				Candidate 3 _LC	L	2	23.43	5.95	1	-0.45
196855.14.A	Candidate 4	145.30	8.25	Candidate 4 _HC	H	2	49.27	8.36	1	-0.33
				Candidate 4 _LC-reopt	L	2	23.38	6.91	0	-0.45
196861.14.A	Candidate 5	145.20	8.55	Candidate 5 _HC	H	2	49.11	8.36	1	-0.35
				Candidate 5 _LC-reopt	L	2	23.49	8.57	0	-0.47
196867.14.A	Candidate 6	145.58	8.23	Candidate 6 _HC	H	2	48.96	8.34	1	-0.44
				Candidate 6 _LC	L	2	23.83	6.91	0	-0.46

Client Sample List

Protein ID	Name / Molecule	Molecular Weight(kD)	Isoelectric Point	Chain Name	Chain Letter	Chain Ratio	Molecular Weight(kD)	Isoelectric Point	N-Glycans	Hydrophob. (GRAVY)
196873.14.A	Candidate 7	146.98	8.54	Candidate 7 _HC	H	2	49.93	8.68	1	-0.39
				Candidate 7 _LC	L	2	23.56	6.91	0	-0.43
196879.14.A	Candidate 8	147.70	8.08	Candidate 8 _HC	H	2	49.63	8.17	1	-0.45
				Candidate 8 _LC	L	2	24.22	6.91	0	-0.49
196885.14.A	Candidate 9	145.91	7.33	Candidate 9 _HC	H	2	49.52	8.18	1	-0.45
				Candidate 9 _LC	L	2	23.44	5.82	1	-0.41
196891.14.A	Candidate 10	145.37	8.54	Candidate 10 _HC	H	2	49.23	8.69	1	-0.40
				Candidate 10 _LC	L	2	23.46	6.91	0	-0.42
196897.14.A	Candidate 11	146.07	8.36	Candidate 11 _HC	H	2	49.09	8.61	1	-0.36
				Candidate 11 _LC	L	2	23.95	6.35	0	-0.44
196903.14.A	Candidate 12	146.58	8.43	Candidate 12 _HC	H	2	49.73	8.57	1	-0.43
				Candidate 12 _LC	L	2	23.56	6.99	0	-0.49

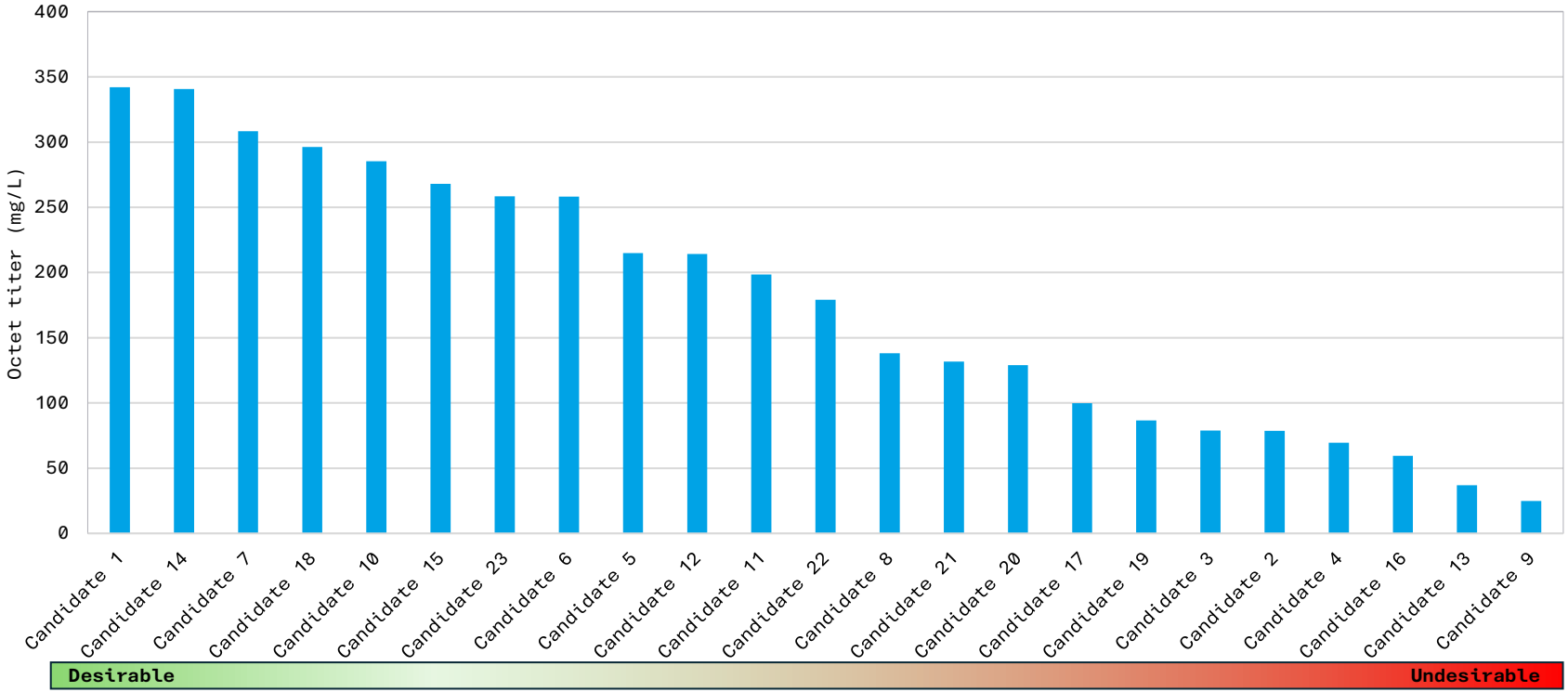
Client Sample List

Protein ID	Name / Molecule	Molecular Weight(kD)	Isoelectric Point	Chain Name	Chain Letter	Chain Ratio	Molecular Weight(kD)	Isoelectric Point	N-Glycans	Hydrophob. (GRAVY)
196909.14.A	Candidate 13	147.61	8.36	Candidate 13 _HC	H	2	49.67	8.35	1	-0.44
				Candidate 13 _LC	L	2	24.13	7.78	0	-0.46
196915.14.A	Candidate 14	143.96	8.46	Candidate 14 _HC	H	2	48.61	8.60	1	-0.41
				Candidate 14 _LC	L	2	23.37	6.91	0	-0.44
196921.14.A	Candidate 15	145.79	8.53	Candidate 15 _HC	H	2	49.66	8.58	1	-0.39
				Candidate 15 _LC	L	2	23.24	7.77	0	-0.47
196933.14.A	Candidate 16	146.63	8.35	Candidate 16 _HC	H	2	49.57	8.58	1	-0.45
				Candidate 16 _LC	L	2	23.74	6.48	0	-0.36
196939.14.A	Candidate 17	146.14	8.53	Candidate 17 _HC	H	2	48.92	8.59	1	-0.41
				Candidate 17 _LC	L	2	24.15	7.77	0	-0.41
196949.14.A	Candidate 18	145.27	8.68	Candidate 18 _HC	H	2	49.14	8.69	1	-0.36
				Candidate 18 _LC	L	2	23.49	8.27	0	-0.47

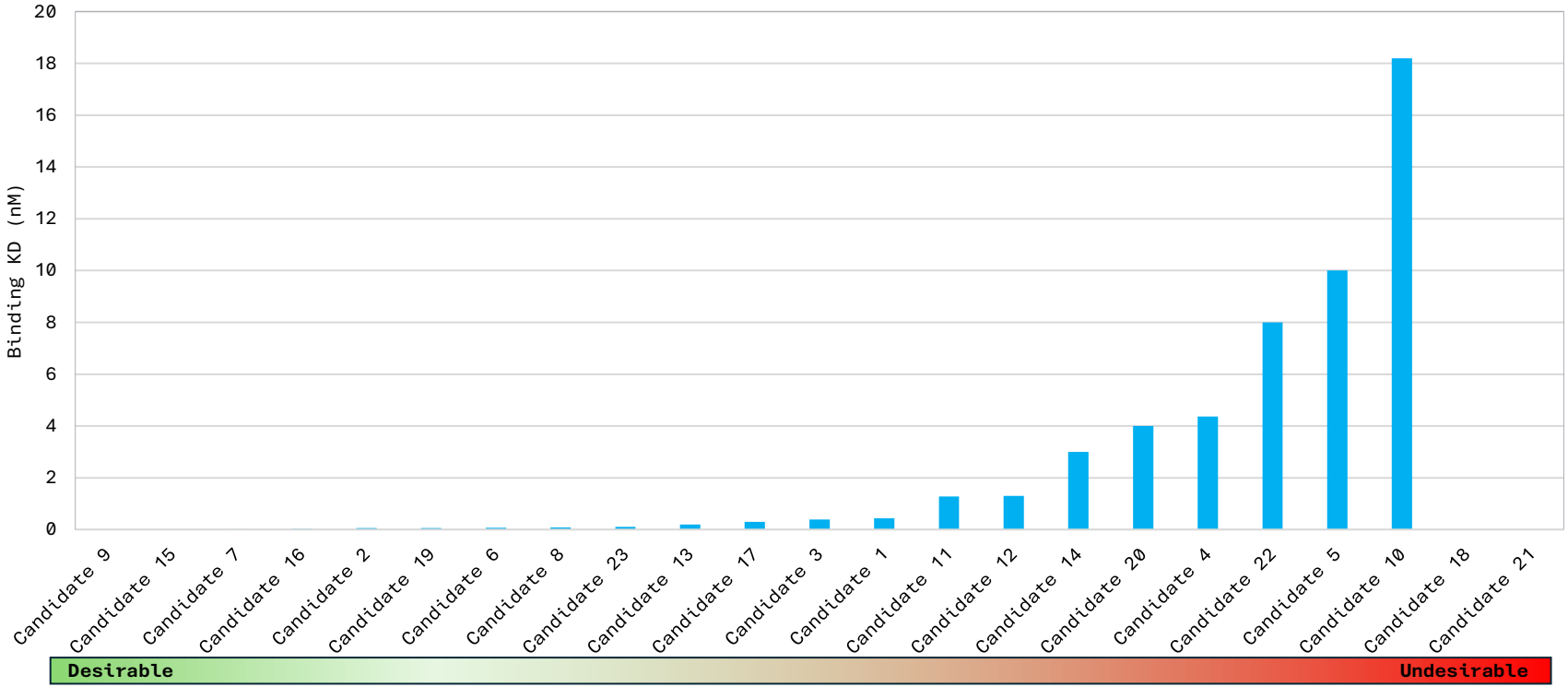
Client Sample List

Protein ID	Name / Molecule	Molecular Weight(kD)	Isoelectric Point	Chain Name	Chain Letter	Chain Ratio	Molecular Weight(kD)	Isoelectric Point	N-Glycans	Hydrophob. (GRAVY)
196955.14.A	Candidate 19	145.59	8.53	Candidate 19 _HC	H	2	49.34	8.68	1	-0.40
				Candidate 19 _LC	L	2	23.45	6.90	0	-0.45
196961.14.A	Candidate 20	146.93	8.09	Candidate 20 _HC	H	2	49.56	8.17	1	-0.43
				Candidate 20 _LC	L	2	23.91	6.99	0	-0.43
196967.14.A	Candidate 21	144.11	8.46	Candidate 21 _HC	H	2	48.73	8.60	2	-0.37
				Candidate 21 _LC	L	2	23.33	6.99	0	-0.42
19906.41.A	Candidate 22	144.60	8.66	Candidate 22 _HC	H	2	49.24	8.67	1	-0.42
				Candidate 22 _LC	L	2	23.06	8.26	0	-0.40
53609.50.A	Candidate 23	145.48	8.36	Candidate 23 _HC	H	2	49.33	7.64	1	-0.36
				Candidate 23 _LC	L	2	23.41	8.75	0	-0.47

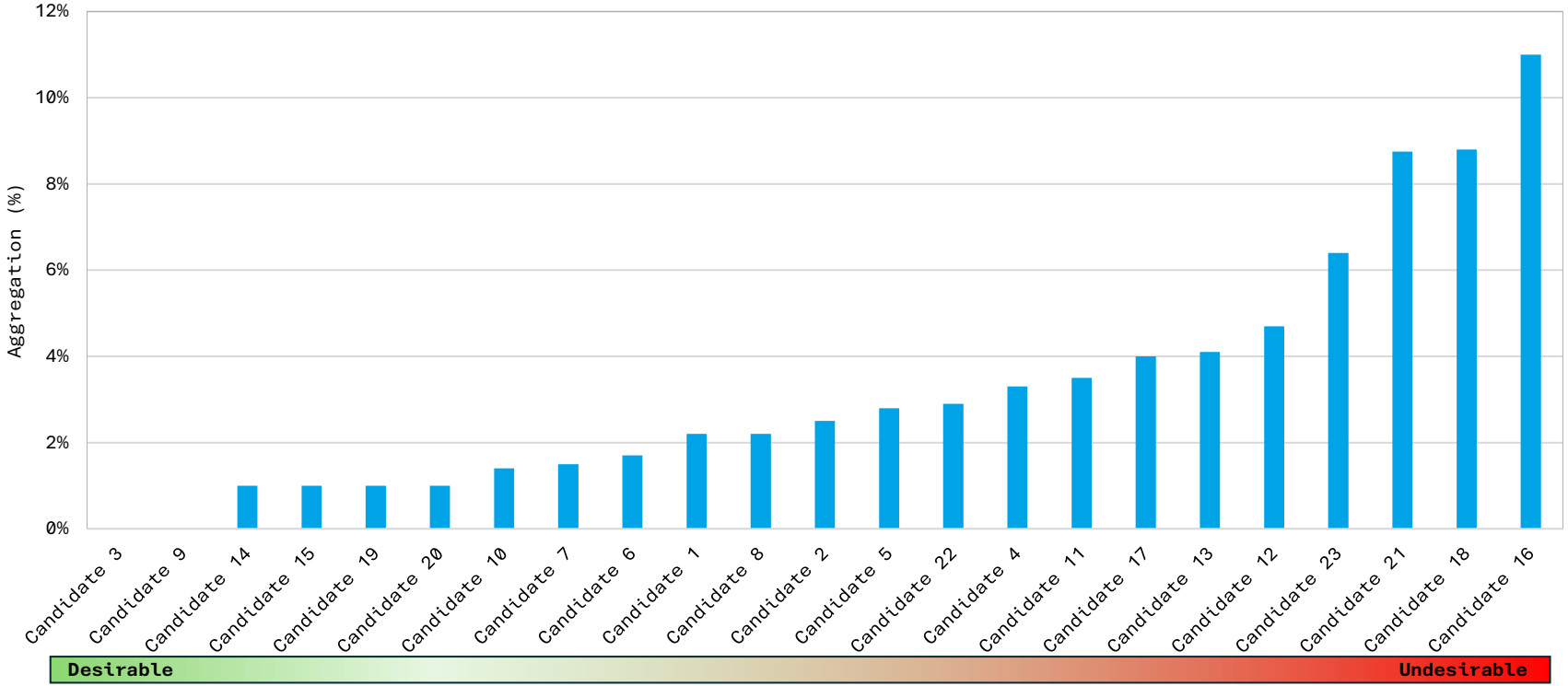
Titer (proA Octet)



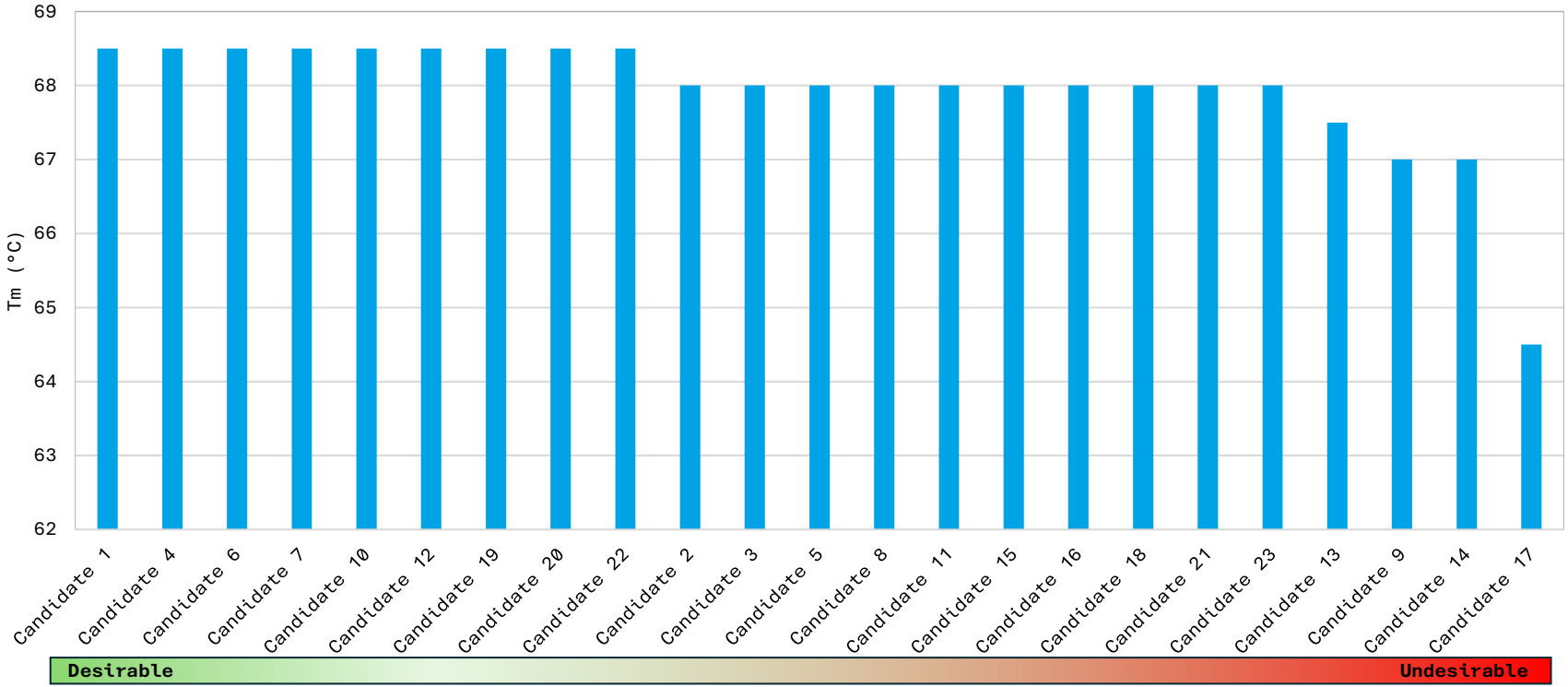
Binding (Octet)



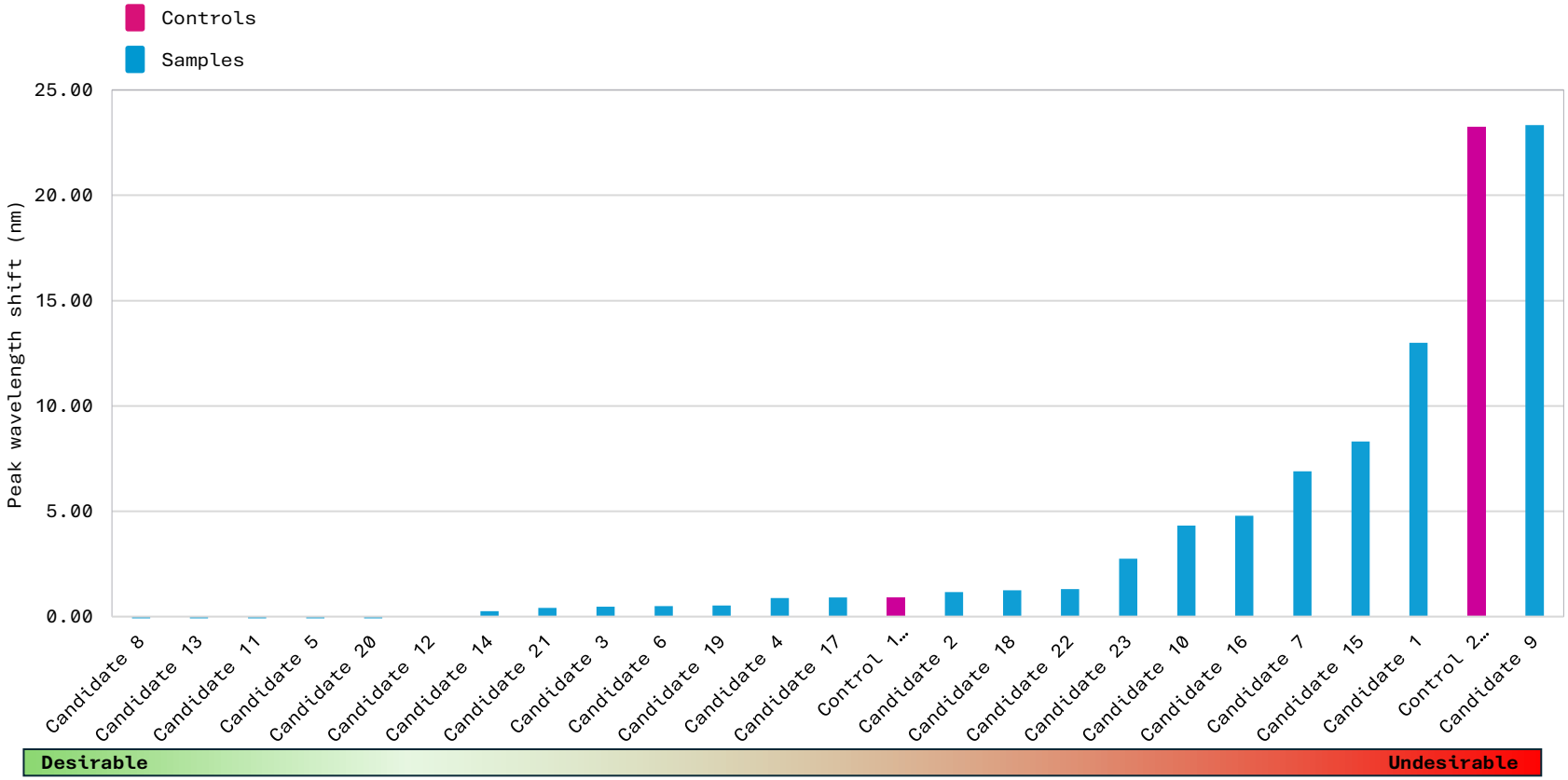
Aggregation (HPLC-SEC)



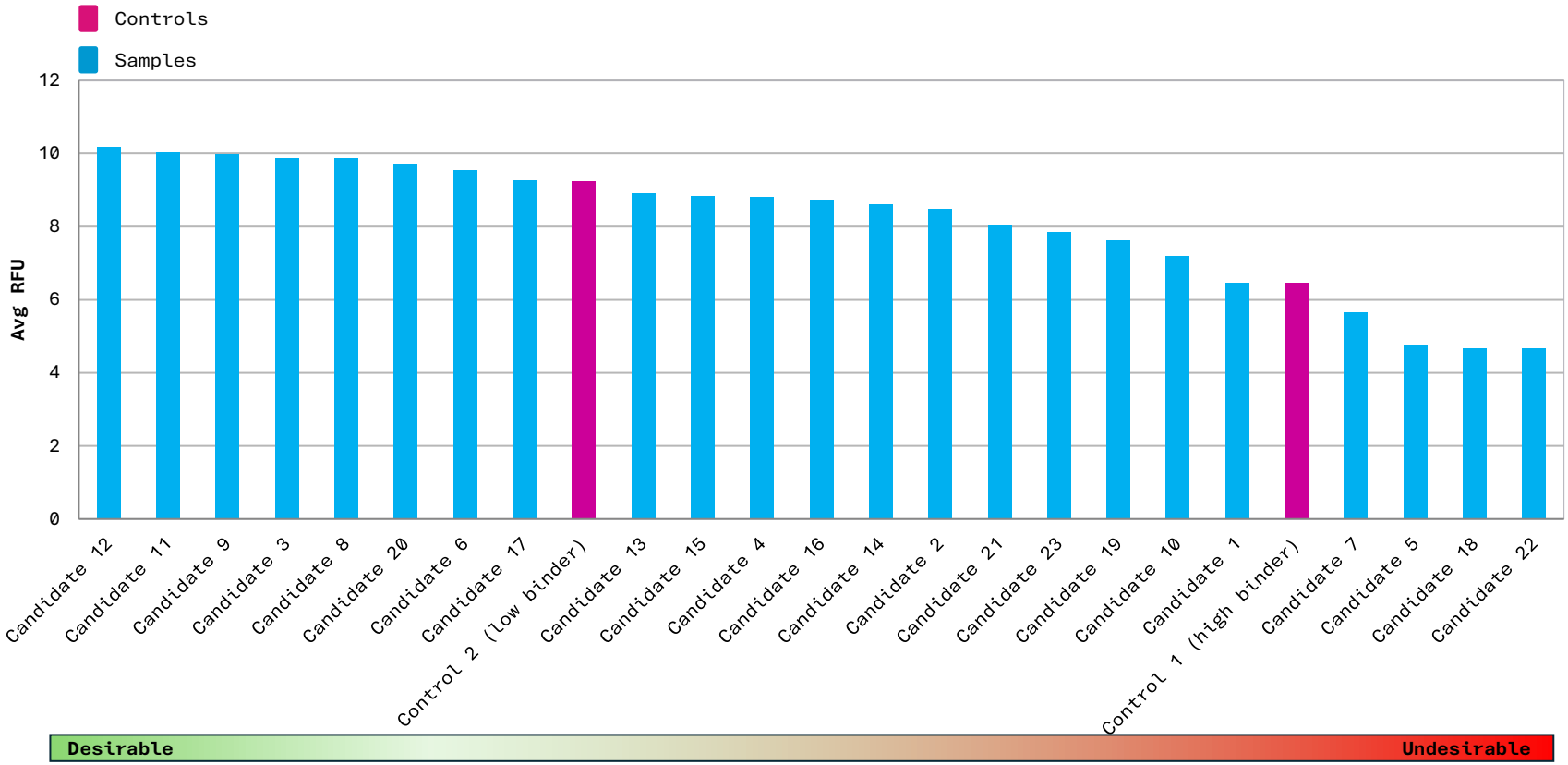
Thermal stability (T_m) (DSF)



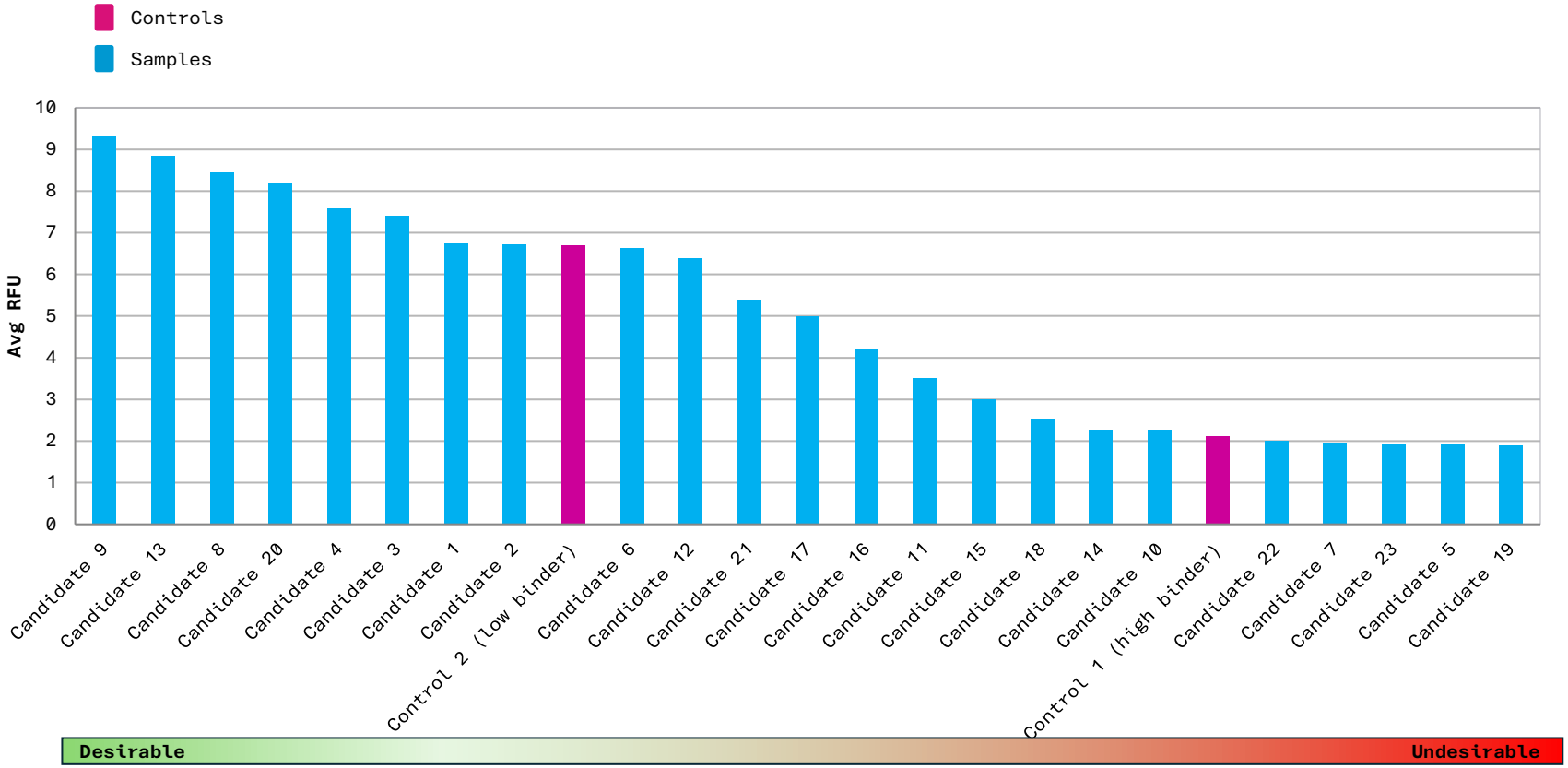
Self-association (AC-SINS)



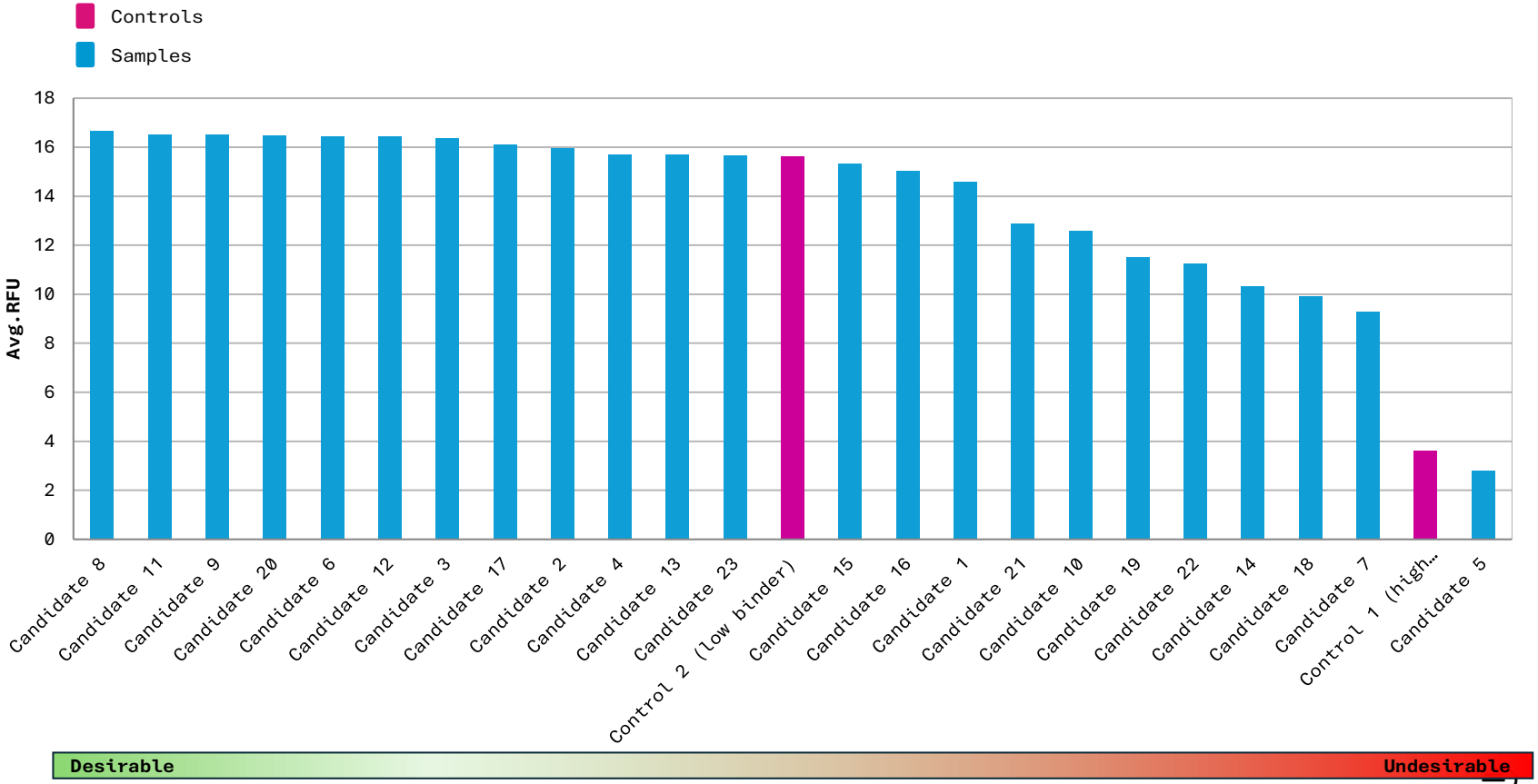
Polyspecificity (plate-based assay for Ovalbumin binding)



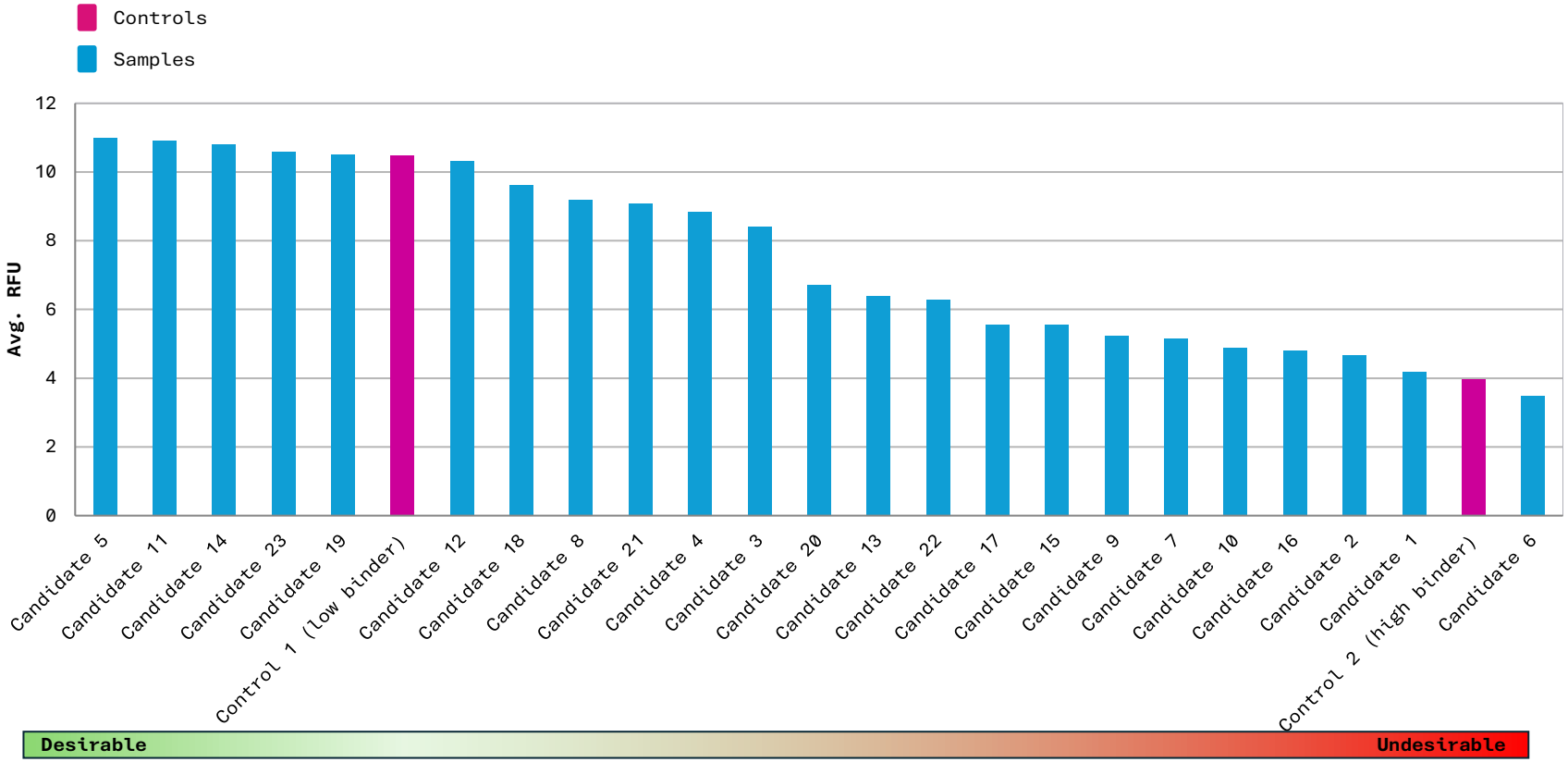
Nonspecificity (plate-based assay for Heparin binding)



Surface charge (plate-based CEX assay)



Hydrophobicity (plate-based HIC assay)



References

Heparin chromatography as an in vitro predictor for antibody clearance rate through pinocytosis. MAbs 2020; 12(1), e1683432

Assessment and incorporation of in vitro correlates to pharmacokinetic outcomes in antibody developability workflows. MAbs 2024; 16(1), 2384104

Establishing *in vitro in vivo* correlations to screen monoclonal antibodies for physicochemical properties related to favorable human pharmacokinetics MAbs 2018; 10(2): 244–255

https://www.paiabio.com/developability

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or
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Analytics Overview

Discovery/Engineering

- Titer measurement
  - HPLC
  - Octet
- Epitope Binning
  - Octet
- Identity and purity
  - SEC-HPLC
    - mCE-SDS
    - MS Identity
- Hydrophobicity
  - RP-HPLC
  - HIC-HPLC
- Charge Heterogeneity
  - cIEX - HPLC
  - cIEF *
- Aggregation propensity
  - AC-SINS *
  - DLS (Hydrodynamic radius, k_D, A_2) *
- Thermostability
  - Tm (DSF)
  - Tagg (DLS)
- Polyspecificity
  - BVP-ELISA

Stability

- pH stress
- Thermal stress
- Freeze thaw stress
- Agitation stress

Readout:

- SEC-HPLC
- µCE-SDS

Manufacturability

- Formulation study
- Concentration study

Readout:

- SEC-HPLC
- µCE-SDS
- DLS (Hydrodynamic radius, k_D, A_2)

- HCP Analysis
- HCDNA Analysis
- Glycan Analysis *

Activity

- Binding Kinetics
  - Octet, SPR
  - ELISA
- Cell Based Assays
- FcγRI, FcRn interaction
  - Octet, SPR
- Enzyme Kinetics

* Inquire for more details