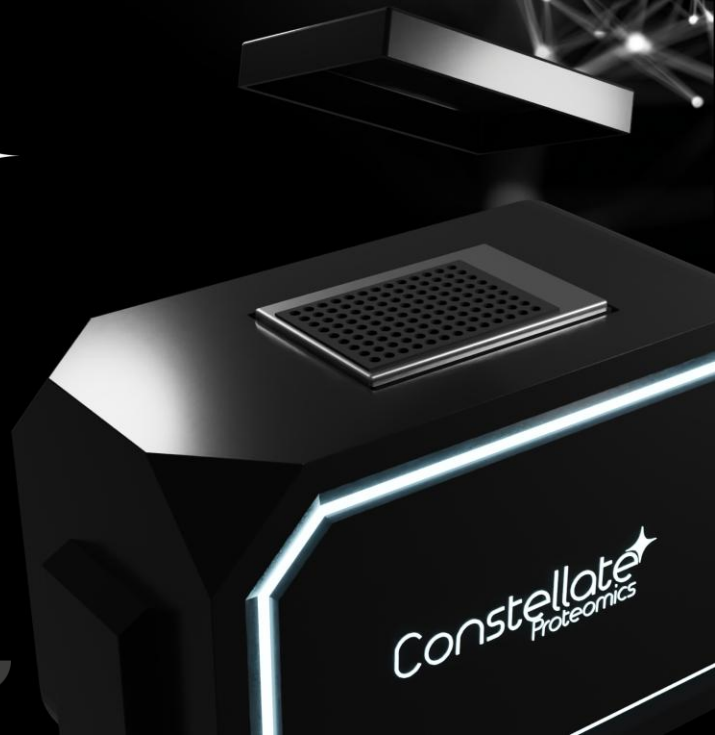
Acceleration of mAb cell line and USP development by single-molecule antibody quality analysis

Constellate Proteomics

info@constellate.pro - www.constellate.pro

TU Delft · imec istart · biotech booster · THEMATIC TECHNOLOGY TRANSFER ttt



IMPACT IN CELL LINE DEVELOPMENT

ACCELERATE CELL LINE DEVELOPMENT WITH EARLY-STAGE QUALITY ANALYSIS AND DECISION-MAKING

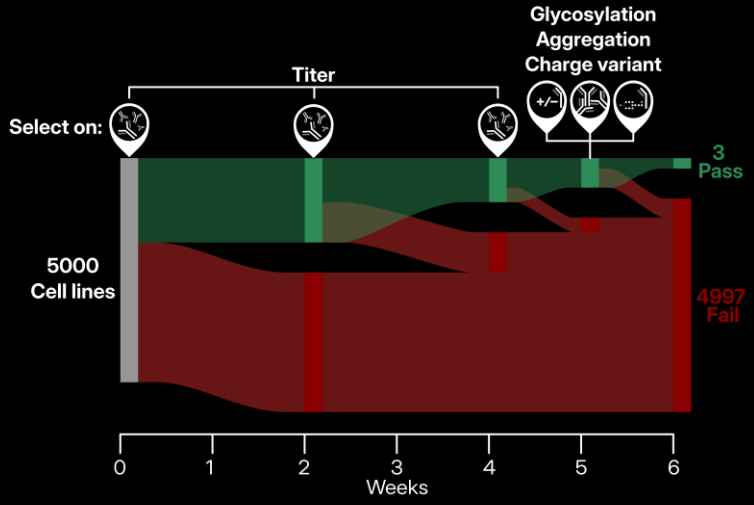
Within the first week of cell line development, our technology delivers 4-in-1 CQA readouts. This pulls ranking and selection of clones forward to the earliest stage of the workflow, where it has the highest impact on timelines and cost.

TITER

GLYCOSYLATION

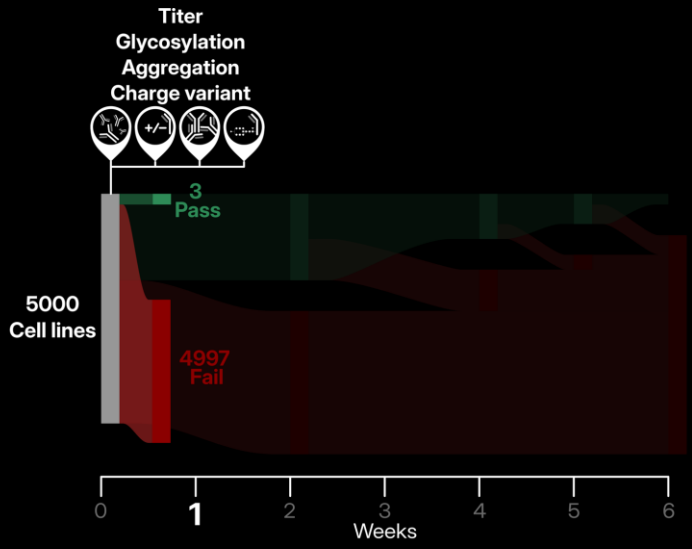
AGGREGATION
IN DEVELOPMENT

CHARGE VARIANTS
IN DEVELOPMENT



CONVENTIONAL METHOD

Most quality attributes are measurable after 5-6 weeks of cell line growth.



CONSTELLATE METHOD

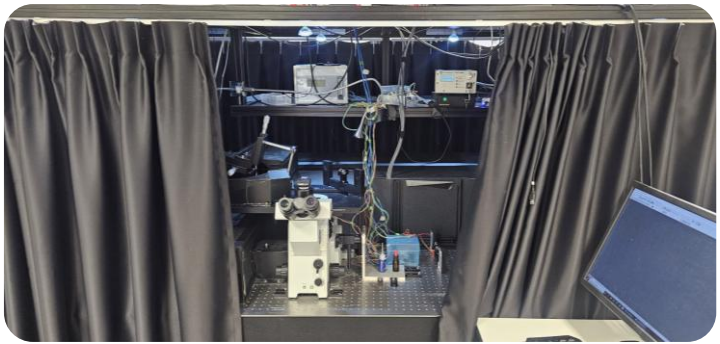
Earlier assessment of all quality attributes saves development time and cost.

WHERE ARE WE NOW

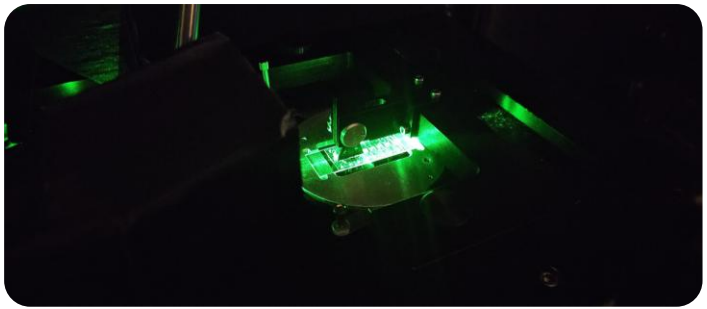
OUR PROOF OF CONCEPT SETUP ENABLES US TO ANALYZE SAMPLES IN-HOUSE

Our in-house analytical setup enables us to manually analyze crude antibody samples in minutes with a range of quantification starting at 10 picogram. Titer, fucosylation, deglycosylation and affinity can be measured within 1 second and 1 minute, respectively. We are now working on finalizing aggregation and charge variants analytical capabilities.

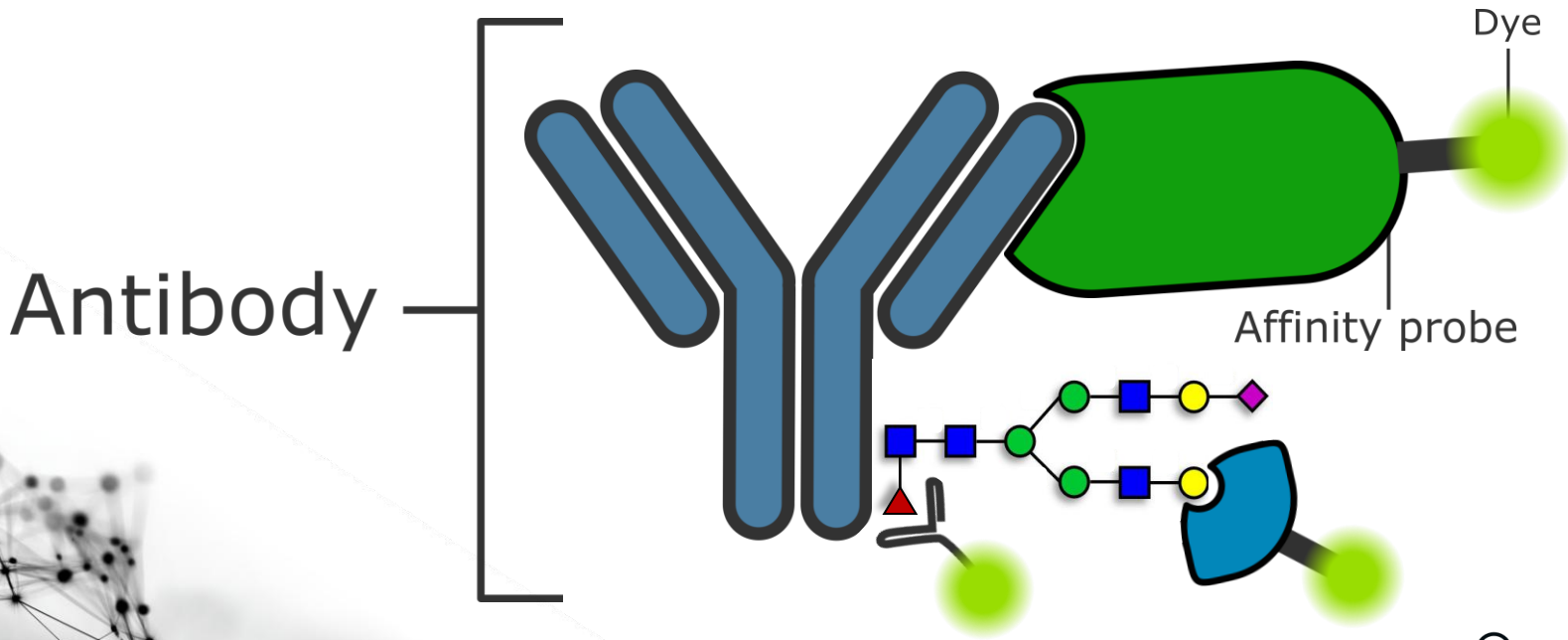








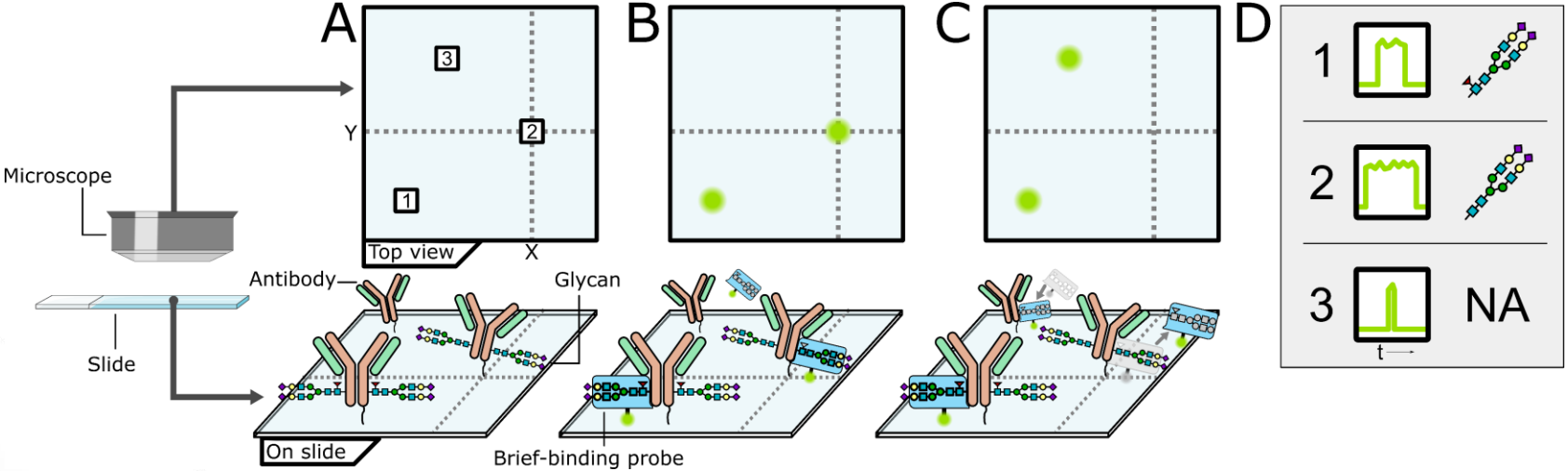
CQAs CAN BE DETERMINED BY OBSERVING BINDING TIME OF REVERSIBLY BINDING AFFINITY PROBES



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AFFINITY PROBES ENABLE CQA ANALYSIS OF ANTIBODIES

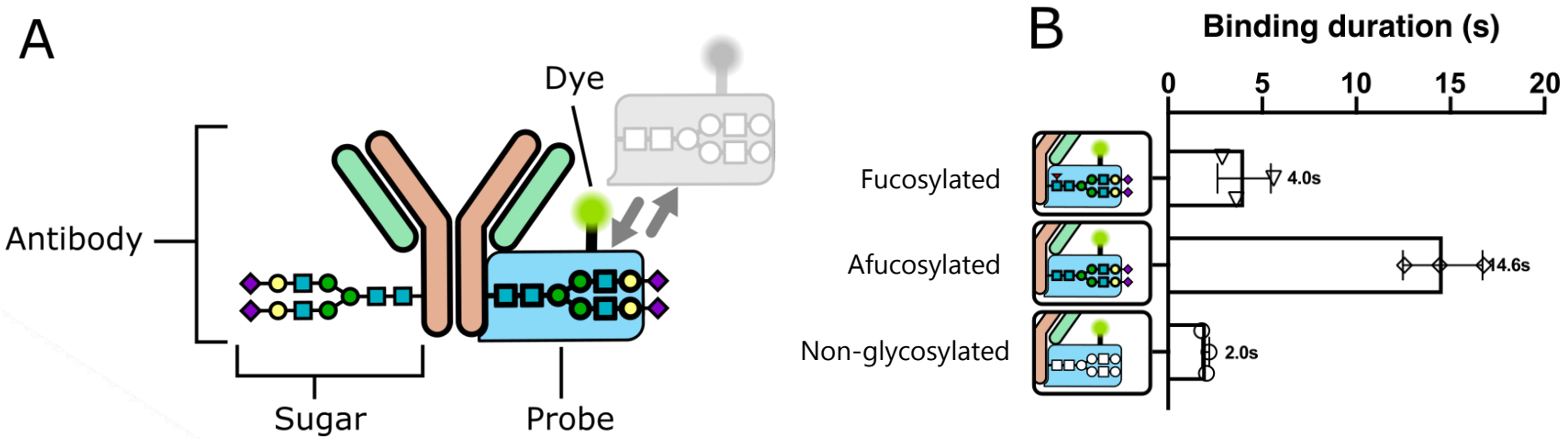
Patents: WO2024049290A1



Antibodies are immobilized on the surface of proprietary 96-well plates engineered for single-molecule TIRF microscopy. Fluorescently labeled affinity probes are then introduced, binding transiently to the immobilized antibodies and generating blinking fluorescence signals captured by the microscope camera. Each probe is subsequently washed away, and the next probe in the panel is introduced sequentially. This cycle repeats until all probes in the assay have been applied and their signals recorded.

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PROBES ALLOW IDENTIFICATION AND COUNT OF 3 ANTIBODY GLYCOFORMS

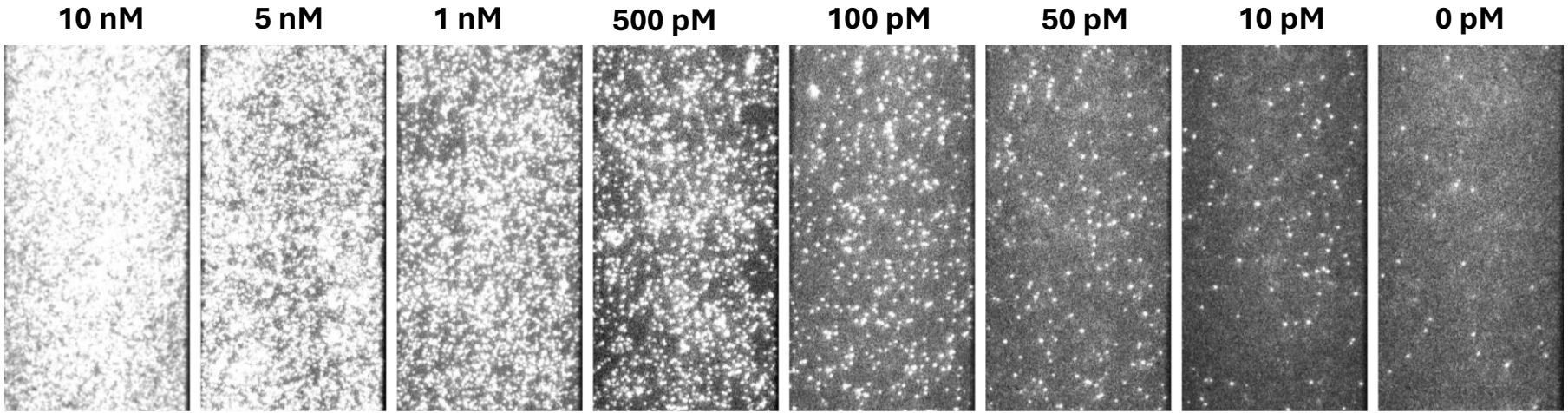


Fluorescently labeled affinity probes bind reversibly to defined regions of the antibody molecule, such as the Fc glycan. Each probe exhibits a characteristic binding duration that corresponds to a specific antibody quality attribute. Using our in-house developed fucosylation probe, the platform discriminates between fucosylated, afucosylated, and non-glycosylated antibodies based on these distinct dwell times (see whitepaper for details).

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PROOF-OF-CONCEPT DATA

CONCENTRATION IS CALCULATED BY MOLECULE COUNTING



Fields of view acquired with our in-house single-molecule TIRF microscope setup, spanning antibody concentrations from 10 nM to 0 pM. Each bright spot represents an individual antibody molecule immobilized on the quartz surface. Transient binding of a fluorescently labeled affinity probe generates a blinking signal per molecule, which the microscope records as a video and our software algorithm converts into a quantitative readout.

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INSTRUMENT (IN DEVELOPMENT)

COSMO™ - COMPACT OBSERVATORY SYSTEM FOR MILLISECOND OMICS

Our automated high-throughput analytical instrument paired with proprietary 96-well cartridge kits. Any developer can acquire quality data within minutes, plug-and-play. Currently in development; we are using our proof-of-concept system at TU Delft to demonstrate the technology's capabilities on real workflows.

PROPRIETARY 96-WELL CARTRIDGES

ANALYTICAL INSTRUMENT*



Portable prototype expected Q2 2027 for on-site demonstration at your facilities.

A PLUG & PLAY BIOLOGICS CQA ANALYSIS DEVICE

USPs

Ultrasensitive
works on picograms

All CQAs
One sample, multiple assays

Zero prep
Works on unfiltered samples

Automated
Works for everyone

TECHNOLOGY

SINGLE-MOLECULE ANALYSIS MADE EASY, BUILT ON 20 YEARS OF EXPERIENCE

COSMO™ uses single-molecule TIRF microscopy to measure four CQAs (titer, glycosylation, aggregation, charge variants) from a single small-volume sample of unpurified cell culture supernatant. Antibodies are captured on the plate surface, then fluorescently labeled affinity probes are flowed in sequentially. A laser illuminates the surface via total internal reflection fluorescence; exciting only probes bound within ~100 nm of the plate. Each probe binds and dissociates reversibly, producing discrete blinking events that the camera records. Spot counts yield titer; binding kinetics (dwell time, on/off rates) distinguish molecular variants. Once a probe completes its read, it is washed out and replaced with the next, fully automated on the cartridge. We call this the RAYmAb™ technology.

Quick results
1 s - 2 min per attribute

Low Limit of Detection
1 picogram

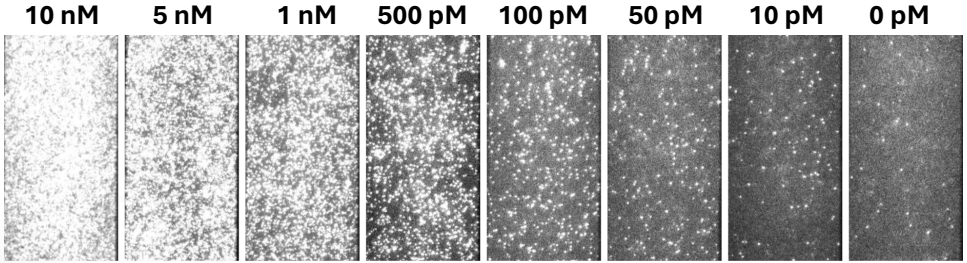
For small samples
3-5 µL per assay

All-in-one
Many CQAs, 1 sample

No sample prep
Works on crude medium

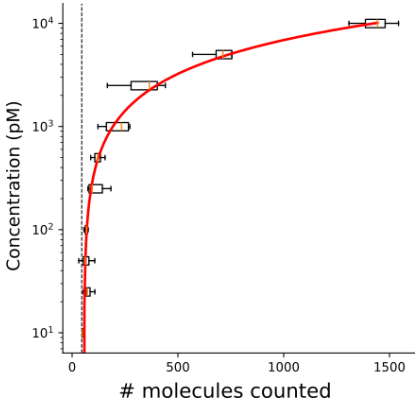
0.97 R2 Coefficient of fit (0 - 1, 1 is perfect fit)

8% Deviation of glycoform-specific concentration measurements (95% confidence interval)



A concentration gradient of antibodies with a specific quality profile shows up as a decreasing number of individual dots under the microscope. We count the dots.

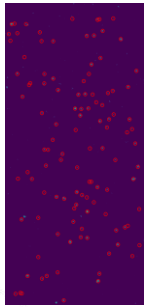
The counted number of dots is related to the concentration of antibodies in solution.



PROOF-OF-CONCEPT DATA

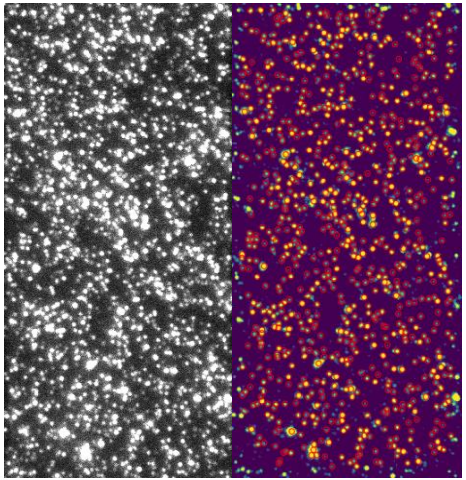
TITER FROM CRUDE SUPERNATANT VALIDATED BLIND AGAINST PARTNERED CDMO GROUND TRUTH

Validated blindly against CDMO ground truth on real CLD and USP samples. Single-molecule counting delivered accurate titer directly from provided crude supernatants.



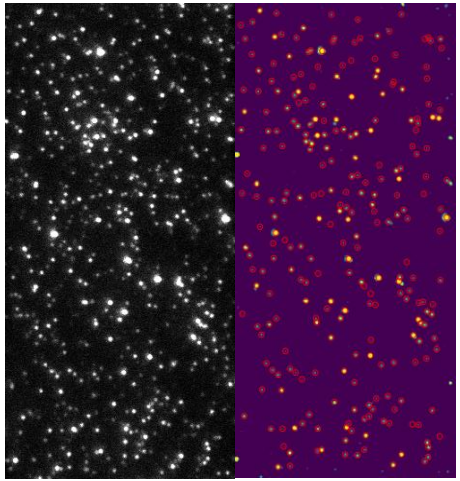
Count of molecules: 111

Background signal



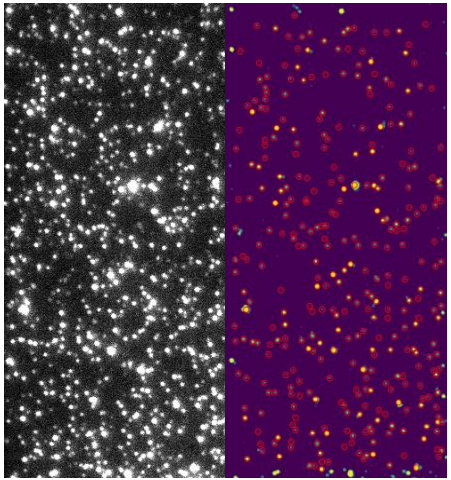
Count of molecules: 861

Clone 2 : 4.9 mg/mL



Count of molecules: 266

Clone 3 : 4.0 mg/mL



Count of molecules: 339

Clone 5 : 4.3 mg/mL

Constellate Proteomics

IMPACT IN USP DEVELOPMENT

DAILY BIOREACTOR CQA MONITORING IN USP DEVELOPMENT

In traditional USP, CQA data only arrives after harvest and purification, locking developers into long reactive cycles. COSMO measures CQAs at multiple points during the run, directly from unpurified supernatant, multiple CQAs at once. Bad conditions are dropped early, promising ones are doubled down on, and CPPs can be steered toward target CQAs while the Ambr or bioreactor is still running.

CONVENTIONAL METHOD

6-12 WEEKS

- **DoE design + bioreactor runs**
  CPP ranges set. Conditions run sequentially.
- **Harvest + purification**
  Protein A per condition. 2–4 days per batch.
  BOTTLENECK — sequential, material-intensive
- **CQA analytical panel**
  Glycan, charge variant, SEC, HCP per condition.
  2 weeks per DoE round
- **Statistical analysis**
  CPP-CQA response modelling. Design space draft.
- **Iterate or scale up**
  New DoE round if gaps found. 6–12 weeks total.
  Comparability data: post-scale harvest only

VS

CONSTELLATE METHOD

4-8 WEEKS

- **AI/ML-model + bioreactor runs**
  Continuous multi-parameter learning replaces sequential DoE logic
- **At-line micro-sampling + COSMO assay**
  CQA trajectory per condition — no purification.
  CPP-CQA response surface visible mid-run
- **Real-time design space mapping**
  Response surface built as conditions run, not after.
  Daily curves feed AI model, all parameters at once, not endpoint-only
- **Scale-up with inline CQA comparability**
  COSMO confirms CQA match ambr to pilot to mfg scale.
  No separate comparability harvest needed
- **Design space + PAR submission-ready**
  CPP-CQA linkage documented with run-time data.
  Analytical overhead compressed to days, not weeks

DAILY BIOREACTOR CQA MONITORING

ANALYTICAL OVERHEAD COMPRESSED TO DAYS, NOT WEEKS

REACH BIOSIMILARITY FASTER BY STEERING TO DESIRED CQAS

COMPARISON

ONLY CONSTELLATE COMBINES SENSITIVITY, SPEED AND VERSATILITY

Constellate uniquely enables sensitive, all-in-one measurement of the four most important CQAs, directly in unpurified samples within minutes. In comparison, other technologies screen one CQA per assay at maximum, with higher sample requirements, making them unapplicable in the earlier stages of cell line development and too costly for mid-stage USP.

	Constellate Proteomics	ELISA (ThermoFisher Scientific)	Octet (Sartorius)	Valita Titer (Beckman Coulter)	HPLC ProA (Agilent)	LC-MS (Bruker)	PAIA Biotech
CQA Readout*	**T G+ A C** ***in 1 assay***	T	T G- *in separate assays*	T A *in separate assays*	T	G+	T G- A *in separate assays*
Time	**1 s (titer) 2 min (CQA)**	4-8 h	1 h	15 min	5-10 h	2-7 days	30 min
Cost/sample ***	**€10**	€15	€10-20	€15	€40	€50	€15
Min. sample	**5 µL**	50-100 µL	40-200 µL	5 µL	10-50 µL	50 µL	5 µL
Sensitivity	**10 pg/µL**	50 pg/mL	500 ng/mL	2.5 µg/mL	20 µg/mL	5-50 ng	100 ng/mL
Labor	**Low**	High	Low	Low	High	High	Low
Purification need	**No**	No	No	No	Yes	Yes	No

****CQA Readout legend:* ***T*** *= titer,* ***G*** *= glycosylation profile,* ***A*** *= aggregation,* ***C*** *= charge variants. For glycosylation profiles; G- measures one glycofeature per assay,* ***G+*** *measures multiple glycofeatures (e.g. Sia, Fuc, Man) per assay.*

*** Average indication, costs may vary across brands, instruments, kits, CQAs, and more.*



Disclaimer

Currently, our patented technology (WO2024049290A1) is only available on the proof-of-concept setup in our laboratory yet.

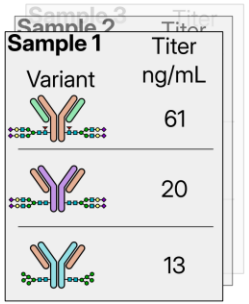
Our in-development benchtop device, the COSMO™, will bring our technology scalable to any other facility worldwide. Until that time, you are welcome to follow the process at ours, at any time.

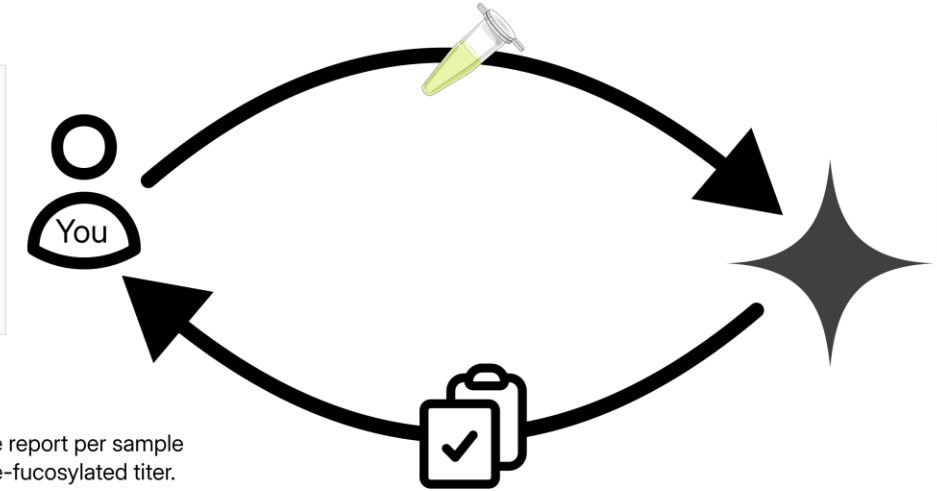
PILOT PROPOSAL

SEND US YOUR PILOT CLD AND/OR USP SAMPLES & RECEIVE YOUR QUALITY DATA THE NEXT DAY

1. SHIP

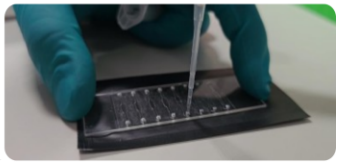
Send up to 12 samples of minimum 10uL unfiltered, flash-frozen medium.

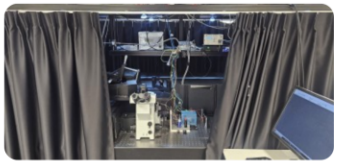




2. SCAN

Our team analyzes the samples 1-2 days after shipment arrival.





3. SELECT

Receive a ranking and one report per sample with total Ab titer and core-fucosylated titer. Select your best candidate.

Note
Need more samples analyzed?
Got non-antibody target proteins? Contact us!

WHAT'S IN IT FOR YOU

LET'S PARTNER UP & ACCELERATE ANTIBODY DEVELOPMENT TOGETHER

What's in it for you?

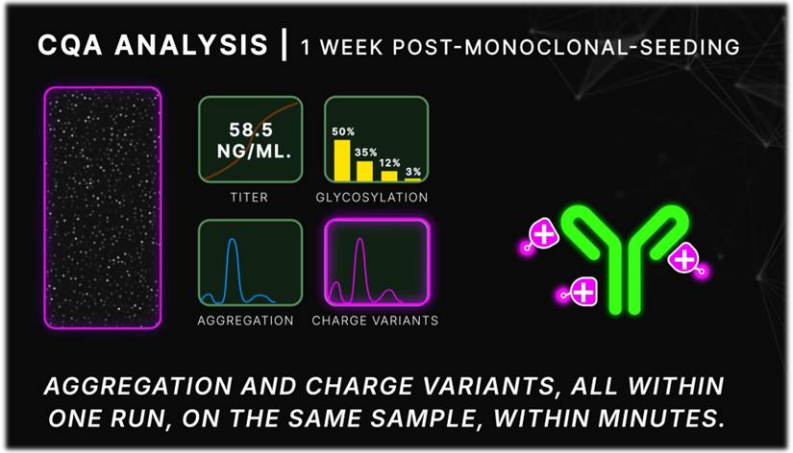
- **Rank clones in week one:** four-CQA profiles directly from unfiltered supernatant, weeks before harvest-dependent analytics catch up.
- **Steer USP cycles while they run:** CQA readouts within minutes turn DoE rounds from reactive into adaptive, so bad conditions are dropped and CPPs corrected before the next bioreactor batch starts.
- **Decisions on real data, not proxies:** single-molecule sensitivity reveals quality differences others miss, so resources only go to candidates that hold up at scale.

What's in it for us?

Real-world samples to prove robustness and build traction with industry and investors.

We are an early-stage spin-out from TU Delft, preparing for commercial launch. To get there, we need diverse, challenging samples from active development programs. Your toughest clones stretch our technology, and your feedback shapes the v1 COSMO product.

Watch our 1-min trailer by clicking on the image below:



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Plan a meeting here or scan the QR-code.

BOOK A MEETING: