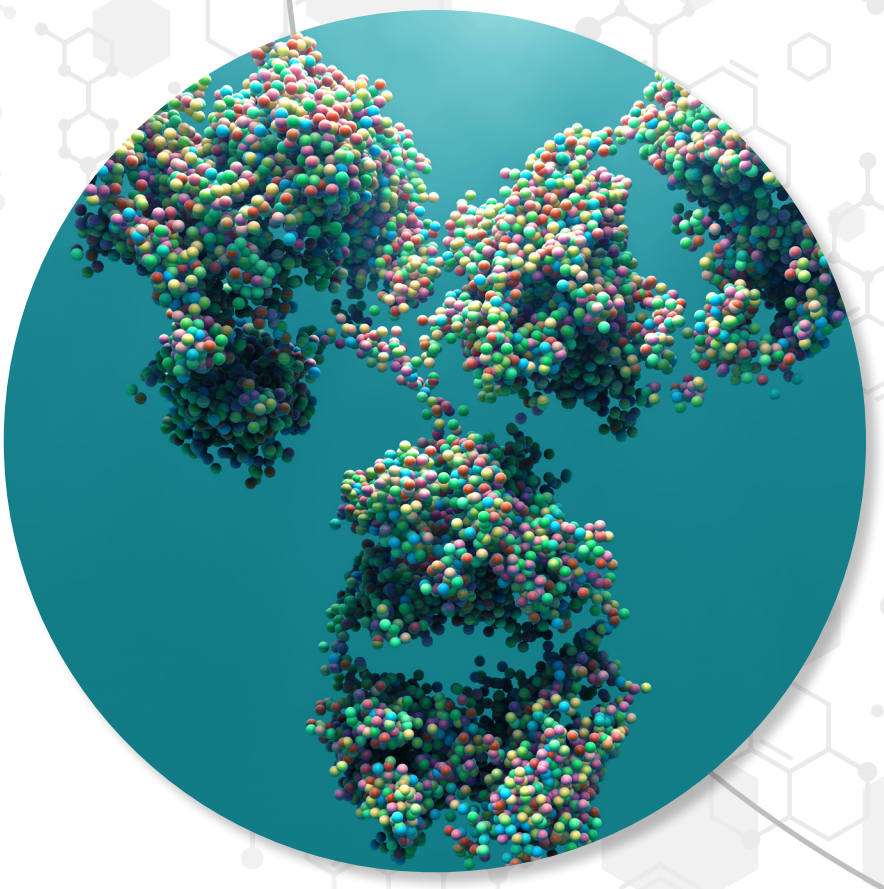


WHITE PAPER

The ‘Predict-First’ Approach -

How *In Silico* Strategies Can Enhance Efficiency in Monoclonal Antibody Bioprocess Development

Author: **Arghya Barman, PhD,** Associate Director of the Global Data Science team of FUJIFILM Biotechnologies

FUJIFILM
Value from Innovation

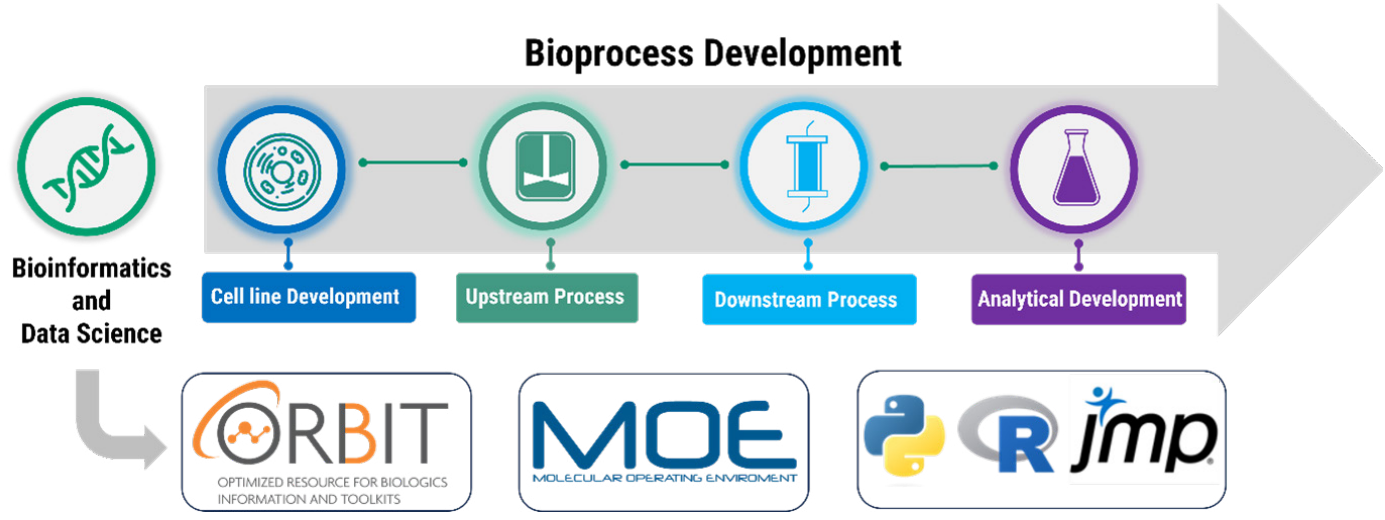
In today's rapidly advancing biopharmaceutical landscape, monoclonal antibodies (mAbs) have emerged as a cornerstone, representing a market that sees billions of dollars in annual sales worldwide with substantial growth predictions.[1] Accounting for over 60% of all biopharmaceutical revenues, these life-changing biologics are at the forefront of therapeutic innovation.[2] However, the path from discovery to large-scale production is burdened with complexity and challenges.

Bioprocess development plays a pivotal role in this journey, ensuring that each mAb meets rigorous standards of purity, quality, and efficacy. This intricate process presents numerous challenges that demand innovative solutions, particularly in optimizing clonal selection, maximizing yield, and ensuring consistent product quality.[3]

To address these challenges, the biotech industry is increasingly embracing state-of-the-art *in silico* methods, for example machine learning algorithms, digital twins, mechanistic modelling, and fluid dynamics which can assist in process development.4 At FUJIFILM Biotechnologies, the kojoX™ philosophy is foundational to transforming the biopharmaceutical industry by harmonizing processes and standardizing operations. Our kojoX approach ensures that precision and quality are integrated from the outset across all phases of development - we leverage our in-house cutting-edge bioinformatics to enhance, de-risk and expedite mammalian and microbial biologics process development services.[5] Within the kojoX ecosystem, from initial cell line development and protein character assessments, through to optimizing upstream/downstream processes and analytics, bioinformatics makes an important contribution and enables us to decode complex biological data. In addition to our existing bioinformatics analysis, we have investigated the cutting-edge computational tools to develop a new and enhanced approach tailored to both mammalian and microbial biologics development – ultimately allowing our partners to make better informed decisions and expedite speed to market.

Our strategy is rooted in recognizing the unique "molecular personality" of each biomolecule - a distinctive profile originating from sequence variability, structural characteristics, and physicochemical properties. Understanding the individual molecular characteristics has been pivotal in crafting our "Predict-First" approach (Figure

Figure 1. FUJIFILM Biotechnologies "Predict-First" approach in bioprocess development using bioinformatics and data science.



1). By leveraging proprietary bioinformatics pipelines and robust data science capabilities (To learn more about our in-house bioinformatics capabilities read our blog), we can anticipate potential bioprocessing challenges even before bioprocess development begins. This proactive strategy empowers our process scientists to pinpoint potential obstacles, gain critical insights, and devise approaches, that ensure a more streamlined, faster and successful development process.

Our "Predict-First" approach spans bioprocess development (Figure 1) and provides tangible improvements in bioprocessing in four key areas including: transfection recovery, forecasting productivity in bioreactors, assessing aggregation risks during viral inactivation, and sub-visible particles formation during formulation.

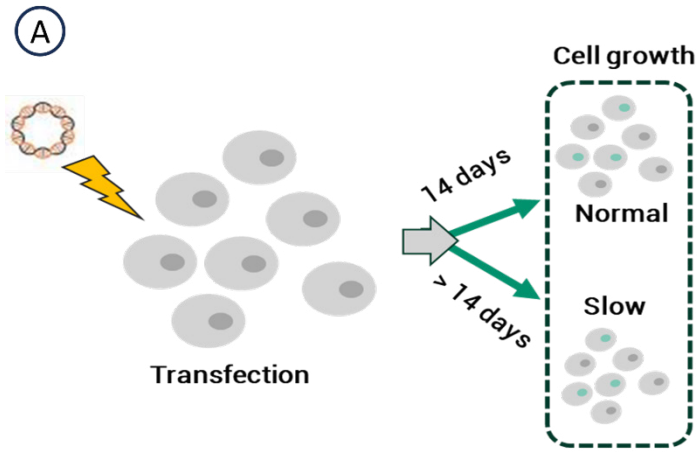
Forecasting Success: Predicting Transfection Recovery and Ambr®15 Titer Productivity in Cell Line Development

One of the challenges often faced during cell line development of monoclonal antibodies is slow recovery of the cell lines after transfection (Figure 2A). Slow recovery means that it takes longer for the viability of cells to increase after selection pressure is applied resulting in extended cell line development timelines.

To address this potential issue, an innovative approach using machine learning (ML) models to predict lines that may exhibit slow recovery post-transfection has been implemented and allows exploration of specific molecular traits that might contribute to slower cell recovery and growth. Our ML model analysis of the *in silico* molecular properties of biological molecules and forecasts extended recovery periods (Figure 2B). This advanced foresight enables our cell line development (CLD) scientists to take proactive measures and implement targeted strategies that reduce recovery time and enhance overall workflow efficiency.

Predicting product yields in bioprocessing is complex due to numerous influencing factors, and "molecular personality" can significantly contribute to this challenge. Our research suggests that the unique profiles of each molecule, determined through *in silico* analyses, plays a critical role in influencing titer predictions in Ambr® 15 bioreactors. Through a series of case studies, including a comprehensive molecule screening campaign, our in-house *in silico* models accurately forecasted the expected

Figure 2. (A) Normal versus slow recovery post-transfection. (B) A classification model was developed with transfection data. The model was found to predict the slow recovery based on the bioinformatics derived parameters with an accuracy of 86%.



B

Projects	Actual	Predicted
Project 1	High	Normal
Project 2	High	High
Project 3	Normal	Normal
Project 4	Normal	Normal
Project 5	Normal	Normal
Project 6	Normal	Normal
Project 7	Normal	Normal

product titer, as demonstrated in Figure 3. While we noted slightly higher numbers in the screening results, the ranking order remained consistent with experimental data. This consistency underscores the value of molecular personality assessments in refining bioprocess strategies and improving efficiency outcomes.

Unmasking the Risks: Prediction of mAbs Aggregation During Low pH Viral Inactivation

During the bioprocess development of mAbs, low pH viral inactivation (VI) is a commonly used method contributing to the viral safety profile of the biologic. However, this process can inadvertently cause aggregation of some mAbs, negatively impacting both process efficiency and the quality of the final product (Figure 4A). To maintain optimal performance and product integrity, it is vital to identify and address the factors that may contribute to these aggregation issues during the VI step.

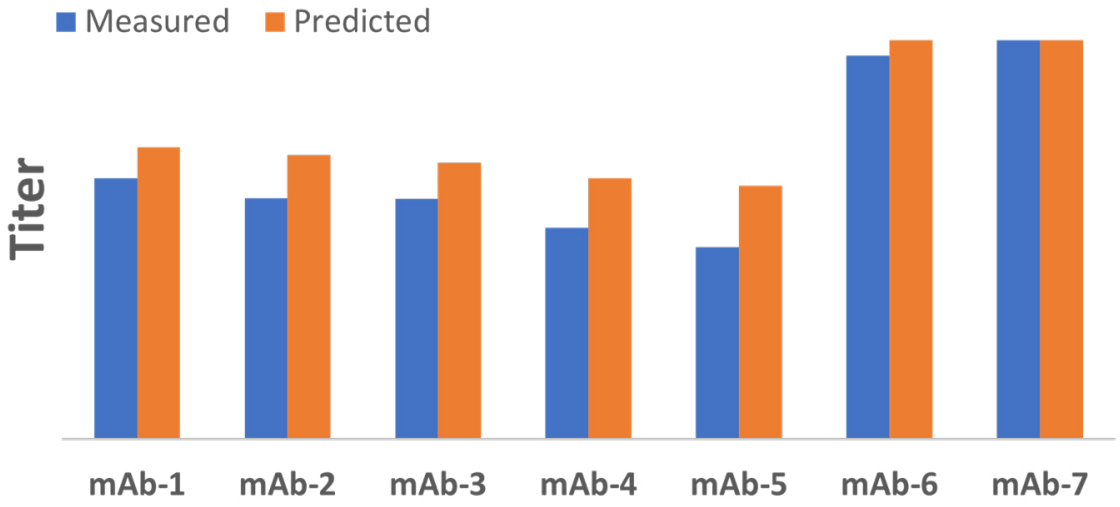
To gain deeper insights into aggregation during the viral inactivation (VI) step, we adopted a predictive approach that examines the molecular characteristics of mAbs to pinpoint those at higher risk in this step. Our analysis uncovered that various structural and surface properties of these molecules serve as indicators of their propensity to aggregate during the VI stage. Notably, we identified that the IgG4 class of mAbs is particularly prone to aggregation when certain surface patch parameters exceed a specific threshold. However, these parameters did not effectively predict aggregation tendencies for the IgG2 class of molecules (see Figure 4B).

Particle Watch: Early Insights into Formulation Development by Predicting Formation of Sub-visible Particles

Formulation is a critical aspect of bioprocessing and formulation scientists working with mAbs can encounter significant challenges related to sub-visible particles (particles usually within 1 µm to ~100 µm), which can impact the safety, efficacy, and stability of the final product.[6] These particles, which are not easily detectable with standard visual inspection methods, can form during handling and storage, often as a result of protein aggregation or interactions with excipients.

Addressing these challenges requires a deep understanding

Figure 3. Predicted vs measured Ambr® 15 titers of mAbs. mAb1 – mAb5 was a screening campaign. mAb and mAb7 are two different molecules.

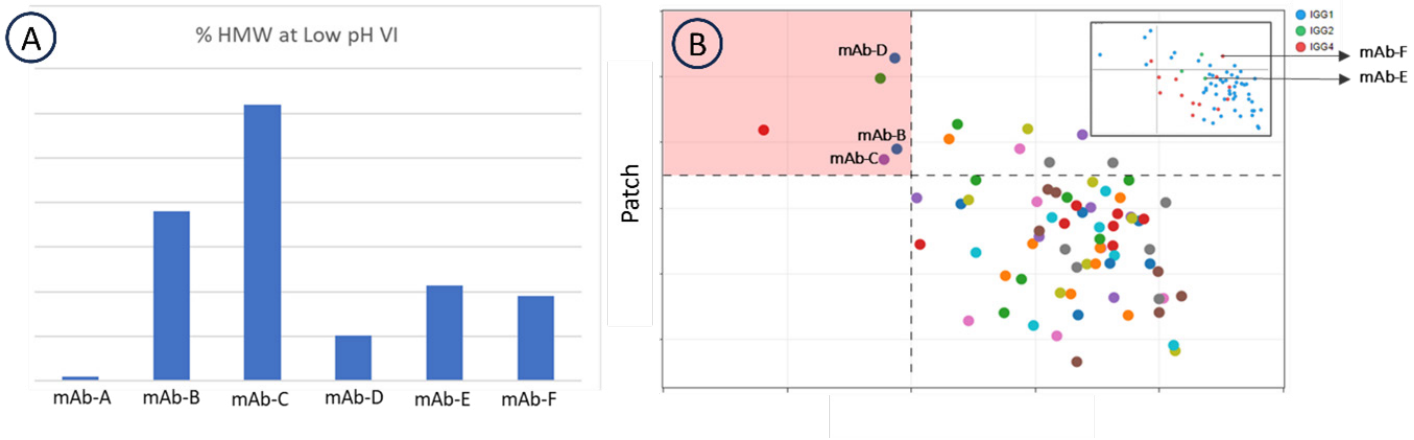


of the physicochemical properties of mAbs, as well as the formulation development process. Having prior insight into a molecule's potential to form sub-visible particles can greatly assist development teams in foreseeing possible stability concerns during the development phase. This foresight enables the strategic use of advanced analytical techniques to precisely monitor and quantify these particles so that innovative formulation strategies can be proactively explored and tailored to minimize the presence of such particles, enhancing the overall stability and efficacy of the therapeutic mAb product. FUJIFILM Biotechnologies has created a proprietary predictive tool to identify molecules susceptible to forming sub-visible particles, that calculates an aggregation score from computationally predicted properties of the mAbs forecasting the likelihood of particle formation in the formulation buffer (Figure 5A). A lower aggregation score indicates a higher probability of subvisible particle formation (Figure 5B). Notably, our predictions align closely with experimental observations, underscoring the reliability of our predictive methodology – ultimately the approach brings benefits of minimizing delays and risks of re-working ineffective formulations.

Smart Screening: Harnessing *In Silico* Manufacturability Assessment for Multi Candidate Screening

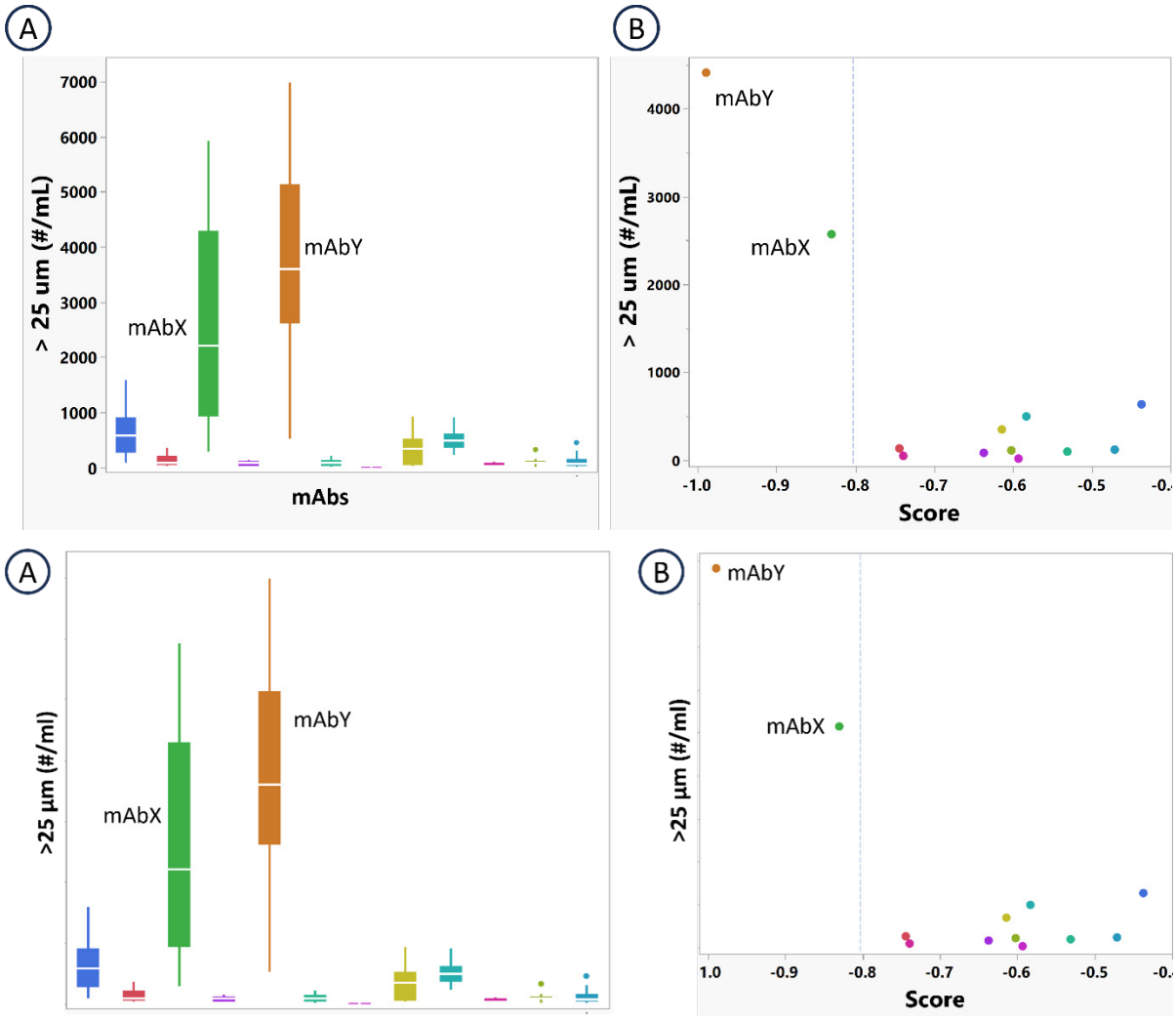
The development and manufacturing of mAbs can be challenging due to their physical and chemical instability. Various factors, such as charge heterogeneity, post-translational modifications, and aggregation, can have a detrimental effect on their function and increase the risk of immune response.[7] To overcome these challenges, it is crucial to identify the liabilities of these molecules. Understanding their limitations is essential for the development of robust processes, analytical methods, and formulations. At FUJIFILM Biotechnologies, we recognize the value in de-risking candidate selection and expediting the development process to accelerate bringing therapies to market more rapidly. By adopting an *in silico* manufacturability assessment, our partners leverage data-driven bioinformatics techniques to identify the most viable candidates faster, reducing the time and effort in bioprocess development.

Figure 4. (A) Extent of High Molecular Weight (HMW) formation of mAbs during low pH viral inactivation stage. mAbA serves as a reference showing low percent of aggregate formation. (B) zone defined by the higher threshold of surface patches shows clustering of the mAbs forming aggregate during the VI stage. No data available for the unlabelled mAbs in the red zone. mAb-B and mAb-F belongs to the IgG4 class and mAb-E belongs to the IgG2 class.



Our *in silico* manufacturability assessment utilizes molecular descriptors of the mAbs and calculates manufacturability scores based on these descriptors to rank candidates (Figure 6A). In multiple instances, our predictions have effectively identified lead candidates. For example, in one case study, the *in silico* manufacturability score for mAb1 was relatively high compared to mAb2, indicating that mAb2 was the more suitable candidate (a lower score implies lower risk, Figure 6B). Subsequent product quality analysis, including the percentage of IgG in size exclusion chromatography (SEC), %HMW, %Man5, total IgG in non-reducing CE, and %Acidic species, revealed that mAb2 exhibited better overall product quality compared to mAb1 (Figure 6C). Based on these findings, mAb2 was selected as the lead candidate, fully aligning with the computational prediction.

Figure 5. (A) Sub-visible particles observed during formulation development for different mAbs. On average, mAbX and mAbY showed higher number of particles (> 25 µm number of particles / mL) versus other mAbs. (B) The predicted aggregation score for mAbX and mAbY was calculated to be more negative compared to the other mAbs and correlates with the total number of sub-visible particles.

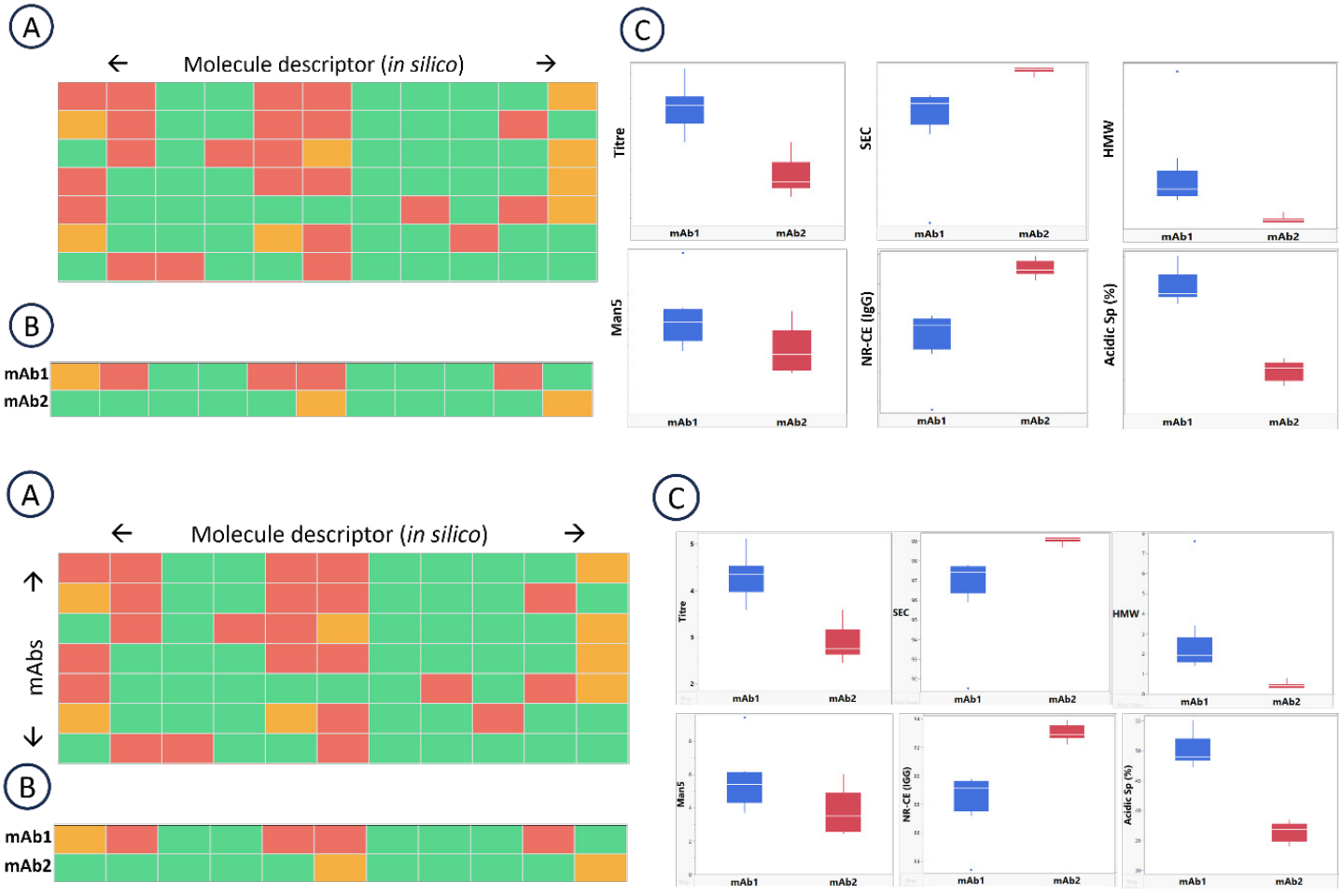


Conclusion

Advanced computational methods coupled with bioinformatics can be used to navigate the complexities of bioprocessing by anticipating challenges before they arise. FUJIFILM Biotechnologies "Predict-First" methodologies can be applied over a wide range of activities in biologics development: from predicting transfection recovery, to forecasting productivity, and identifying aggregation risks – to streamline and expedite processes and enhance product quality. In the longer term, by refining predictive models, employing other aspects of AI, and integrating emerging technologies, our experts aim to extend the utility and impact of the "Predict-First" approach, driving greater efficiency and innovation in biotherapeutic production.

- Supply chain agility, redundancy, and resilience, with seamless technology transfers across an integrated global network
- Optimized process efficiencies and improved productivity

Figure 6. (A) Traffic light scoring matrix based on the calculated and predicted descriptors of mAbs (red – high risk, amber – medium, and green – low risks). (B) Scoring matrix for the mAb1 (high score) and mAb2 (low score). (C) Measured product quality attributes for mAb1 and mAb2.



Beyond infrastructure, kojoX represents a cultural shift in how biomanufacturing is approached. By removing traditional hierarchies and empowering both employees and customers, FUJIFILM Biotechnologies fosters a mindset of continuous improvement and operational excellence. Rather than reinventing processes for each project, proven solutions are encapsulated into reusable modules that seamlessly support multi-site, multi-product operations — without compromising the unique needs of individual clients and therapies.

With this new level of global modularity, kojoX is not just enhancing efficiency — it is transforming the way biotherapeutics and advanced therapies are developed, manufactured, and delivered. By providing customers with everything they need to advance treatments from concept to commercialization, FUJIFILM Biotechnologies is redefining what's possible in biopharmaceutical manufacturing — and ensuring that medicines are delivered to the market faster and more reliably than ever before.

References

1. https://www.biospace.com/monoclonal-antibodies-industry-is-rising-rapidly

2. Biopharmaceutical benchmarks 2022. https://doi.org/10.1038/s41587-022-01582-x

3. Challenges and Emerging Technologies in Biomanufacturing of Monoclonal Antibodies (mAbs). https://doi.org/10.5772/intechopen.108565

4. A roadmap to AI-driven in silico process development: bioprocessing 4.0 in practice. https://doi.org/10.1016/j.coche.2021.100692

5. https://fujifilmdiosynth.com/knowledge-center/resource-library/bioinformatics-a-vital-precursor-to-successful-biomanufacturing/

6. Monoclonal antibody and protein therapeutic formulations for subcutaneous delivery: high-concentration, low-volume vs. low-concentration, high-volume. https://doi.org/10.1080/19420862.2023.2285277

7. Blueprint for antibody biologics developability. https://doi.org/10.1080/19420862.2023.2185924

About FUJIFILM Biotechnologies

FUJIFILM Biotechnologies, a subsidiary of FUJIFILM Corporation, is a world-leading contract development and manufacturing organization (CDMO) for the development and manufacture of biologics, advanced therapies, and vaccines. The company operates a global network with major locations in the United States of America, the United Kingdom and Denmark, offering end-to-end services including drug substance, drug product, and finished goods services. It is also building a new manufacturing site in Holly Springs, North Carolina, USA, scheduled to be operational in 2025. FUJIFILM Biotechnologies has over thirty years of experience in developing and manufacturing drug substance of recombinant proteins, monoclonal antibodies, vaccines, among other large molecules, viral products and medical countermeasures expressed in a wide array of microbial, mammalian, and host/virus systems. We have drug product filling capabilities to support both clinical and commercial demands. Our finished goods services, supported by more than 15 years of experience, can accommodate commercial products for more than 65 countries around the world. The company offers a comprehensive list of services from cell line development using its proprietary pAVEway™ microbial and ApolloX™ cell line systems to process development, analytical development, clinical and FDA-approved commercial manufacturing. For more information, go to: www.fujifilmbiotechnologies.fujifilm.com.

About the Author

ARGHYA BARMAN, PHD
Associate Director of the Global Data Science

Arghya Barman, PhD, Associate Director of the Global Data Science team of FUJIFILM Biotechnologies brings deep expertise in bioinformatics and computational research, playing a key role in advancing science and innovation within the organization. Arghya uses advanced machine learning and AI to address complex bioprocess development challenges and enhance biomanufacturing. He has authored numerous research articles and holds patents in the field. Argyhya holds a PhD from the University of Miami in Computational Chemistry.

Partners for *Life*

"Partners for Life" represents a transformative approach to development and manufacturing, emphasizing relationships founded on trust and transparency — founded in people-centric values, transformative science and innovation, and unprecedented delivery.

CDMO LEADERSHIP AWARDS2024

Contact us to discuss your science.

USA

College Station, Texas
3940 Fujifilm Way
College Station, TX 77845
+1 979 431 3500

Holly Springs, North Carolina
100 Biotechnology Avenue
Holly Springs, NC 27540
(Coming online 2025)

Research Triangle Park, North Carolina
101 J Morris Commons Lane
Morrisville, NC 27560
+1 919 337 4400

Thousand Oaks, California
2430 Conejo Spectrum Street
Thousand Oaks, CA 91320
+1 805 699 5579

DENMARK

Hillerød
Biotek Allé 1
3400 Hillerød
+45 7741 6000

UNITED KINGDOM

Teesside
Belasis Avenue
Billingham, TS23 1LH
+44 1642 363511

JAPAN

Tokyo
R7 Building, 7-12-2 Roppongi
Minato-ku, Tokyo 106-0032
+81 3 6871 7736

FUJIFILM Biotechnologies

fujifilmbiotechnologies.fujifilm.com

FSC

MKT-0194-WP-rev01