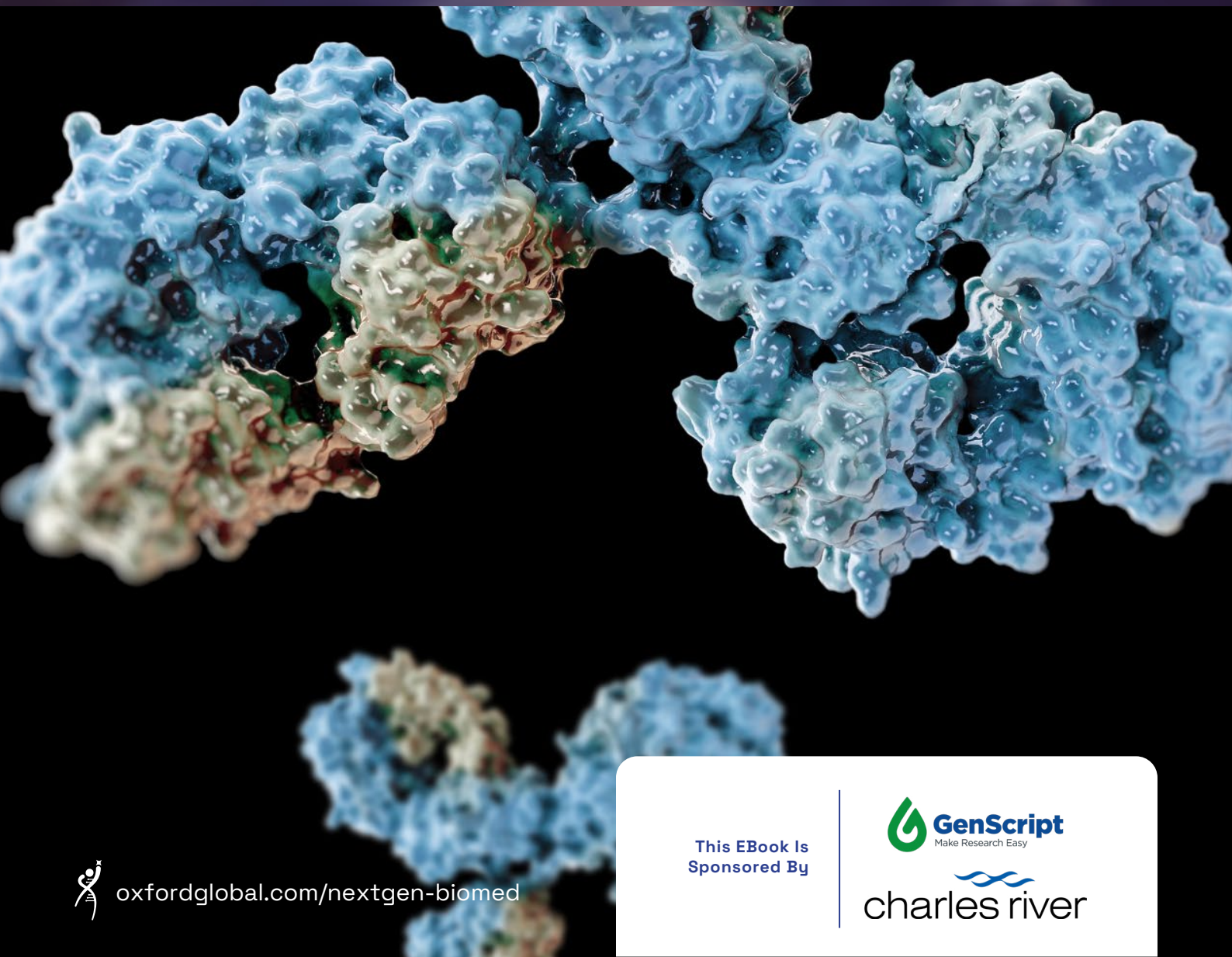
BEST PRACTICE EBOOK

The Future of Antibody Engineering

AI, Optimisation, and Stability

Harnessing AI and Optimisation for Next-Generation Therapeutics in Antibody Engineering



This EBook Is Sponsored By

GenScript
Make Research Easy

charles river

oxfordglobal.com/nextgen-biomed

Introduction

Welcome to The Future of Antibody Engineering eBook, a comprehensive exploration of the latest advancements and innovative strategies in the field of antibody engineering. This eBook delves into a variety of themes and topics that are crucial for understanding and advancing the science of antibodies.

The journey begins with an in-depth look at the mechanisms of influenza viruses and the innovative strategies to inhibit their entry into cells. This section highlights the importance of targeting the hemagglutinin-sialic acid interaction and explores the potential of receptor mimicry as a novel approach to combating influenza.

Next, we explore the Tumbler™ Multi-parameter Antibody Optimisation Platform, a highly customizable suite designed to enhance antibodies through humanisation, affinity maturation, and liability removal. This section showcases the power of combinatorial CDR shuffling and the integration of diverse libraries to create a vast array of antibody variants.

The eBook also delves into the realm of AI-driven optimisation of protein therapeutics. Here, we examine the use of machine learning and AI to explore design spaces and predict the properties of nano body molecules. This section underscores the importance of balanced data sets and the development of specialised protein language models for accurate property prediction.

Further, we discuss the advancements in machine learning methods for antibody design. This section highlights the integration of graphs, language models, and diffusion models to create novel proteins with practical applications. The transformative impact of these technologies on protein design is a key focus.

Lastly, the eBook addresses the critical importance of stability studies in human blood plasma and antibody formulation. This section emphasises the need for compatible formulations to prevent aggregation and ensure the safety and efficacy of biopharmaceutical products.

Throughout this eBook, you will find a blend of theoretical insights and practical applications, all aimed at pushing the boundaries of antibody engineering. We hope this collection of knowledge inspires and equips you to contribute to the ongoing advancements in this exciting field.

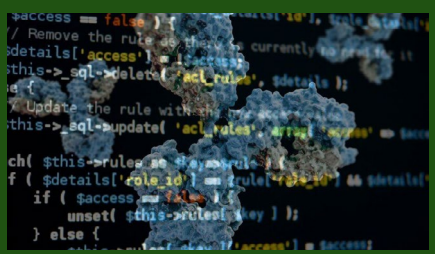
Tom Cohen
Senior Digital Editor, Oxford Global

Contents

Explore Our Comprehensive Content Portal

Immerse yourself in cutting-edge scientific content - from online Monthly Science Exchanges, best practice Online Symposiums to eBooks and landscape reports providing a unique perspective on the latest R&D trends and challenges.

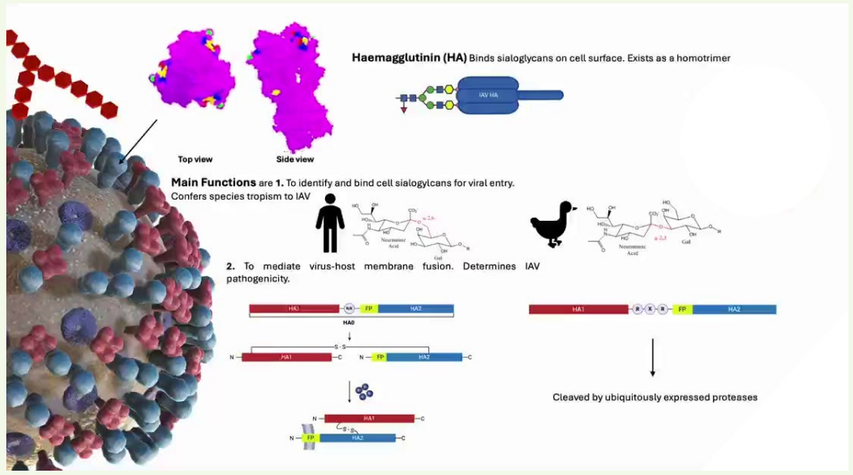
- Archon Biosciences Announces $20m of Seed Funding for its Nobel-Winning, AI-Driven Antibody Cage Technology
- Absci to Collaborate with the Memorial Sloan Kettering Cancer Center to Develop Biologics using AI
- Introducing the Development and Clinical Landscape of Bi and Multispecific Antibodies



On the Use of IgG1 Fcs to Inhibit IAV and IBV Cell Entry

Jid Purohoo, Post Doctoral Research Associate, Liverpool School of Tropical Medicine

Jid Purohoo, a molecular viral GIST and immunologist at the Liverpool School of Tropical Medicine, delivered a presentation on strategies to inhibit influenza A and B viral entry into cells. Purohoo began by providing a background on influenza viruses, explaining their structure as negative-sense, single-stranded RNA viruses enclosed in a protein envelope. These viruses have a segmented genome, allowing for reassortment and antigenic shift, which leads to the emergence of new strains and pandemics.



Purohoo highlighted the historical significance of influenza pandemics, starting with the Spanish Flu in 19. This pandemic demonstrated the virus's ability to cross species and cause widespread infection. Influenza A viruses are naturally found in aquatic birds, while influenza B viruses mostly circulate in humans. The presentation emphasised the pathogenicity of influenza, which causes respiratory disease that can be mild or severe, potentially leading to death if untreated. Current treatments include vaccines and antivirals, though these can become ineffective due to antigenic drift.

The presentation delved into the roles of hemagglutinin and neuraminidase in influenza infection. Hemagglutinin binds to sialic acid on cell surfaces, allowing the virus to enter cells and conferring species tropism. Neuraminidase cleaves sialic acid, facilitating the virus's movement through the mucin layer, scanning of the cell surface, and release of new viral variants. Purohoo explained that mutations in the hemagglutinin cleavage site can increase the virus's pathogenicity by enabling it to infect cells beyond the respiratory tract.

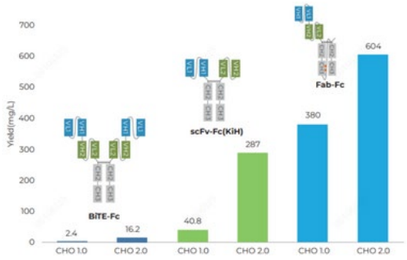
Purohoo discussed the reassortment potential of influenza, which has led to several pandemics, including the Asian flu in 1957 and the Hong Kong pandemic in 196. These pandemics resulted from the emergence of new strains through the reassortment of viral genome segments from different strains. The presentation also covered the infection mechanism of influenza, which involves binding to sialic acid, endocytosis, and release of viral genetic material into the cytoplasm, leading to cell death and the spread of the virus.

The core of Purohoo's research strategy focused on inhibiting influenza cell entry by synthesising crystallizable fragments that mimic cell receptors, preventing hemagglutinin from binding to sialic acid. This approach aimed to block the virus's entry point, rendering other viral determinants of pathogenicity irrelevant. Purohoo's team partnered with Genscript to synthesise high-quality crystallizable fragments, allowing for robust testing and further characterisation of their effectiveness against influenza.

The presentation concluded with an overview of the team's achievements and ongoing work. By collaborating with Genscript, they managed to produce a panel of glycosylated IgG1 crystallizable fragments that bind to various hemagglutinins, including those from influenza A and B viruses. The team planned to further characterise these fragments using live viruses to assess their robustness and effectiveness. Purohoo expressed optimism about the potential of their research to contribute to the development of new strategies for controlling influenza and invited collaboration from other researchers.

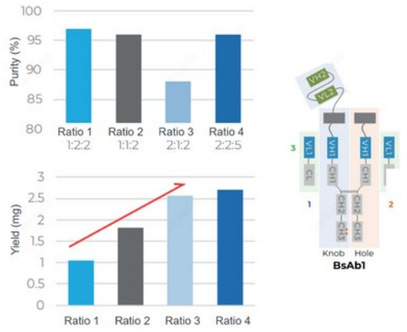
Overall, Purohoo's presentation provided a comprehensive overview of influenza viruses, their pathogenicity, and innovative strategies to inhibit their entry into cells. The research highlighted the importance of targeting the hemagglutinin-sialic acid interaction and demonstrated the potential of receptor mimicry as a novel approach to combating influenza.

Cell line and vector optimisation to improve the expression levels of BSAbs



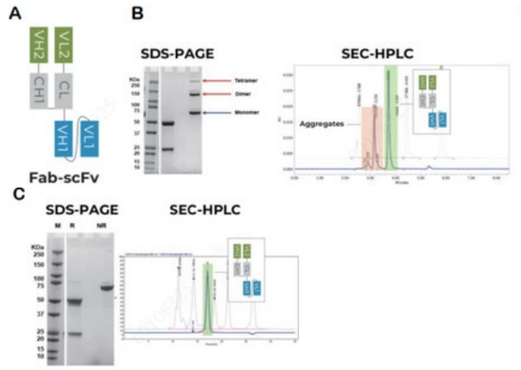
TurboCHO 2.0 has been upgraded from 1.0 to improve the yield of bispecific antibody production.

Plasmid ratio optimisation to improve purity & yield levels



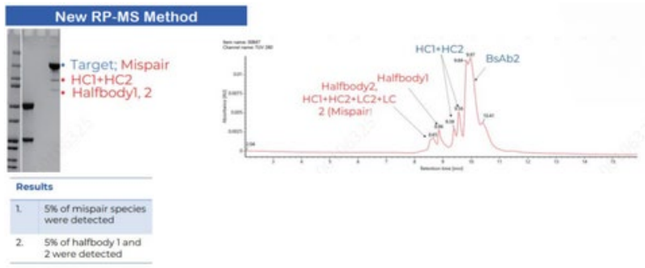
Chain ratio optimisation can identify the limiting factor & improve the yield of BsAbs.

Purification strategy to improve purity & yield



A) Common Fab-scFv construct. **B)** After one step CH1-purification, monomers & aggregates can be observed in both SDS-PAGE & HPLC. **C)** After one step Pro-G purification with modified elution conditions, high purity can be achieved in both SDS-PAGE & HPLC.

QC analytical method to identify & quantify mispair species in BsAbs production

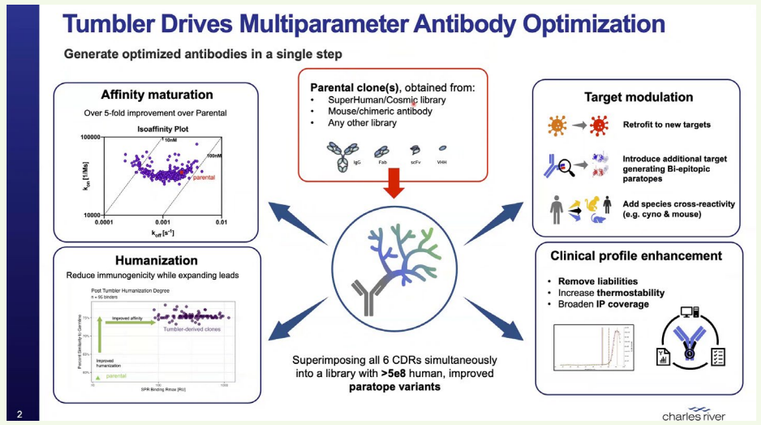


New RP-MS method works well for asymmetric BsAbs such as CrossMab. different halfbody and mispaired species can be identified & quantified.

- **GenScript's TurboCHO 2.0 system:** designed & optimised to maximise titer and shorten turnaround time
- **Large capacity:** 10,000+ targets/month of TurboCHO to accelerate protein and antibody therapeutics development & commercialisation globally
- **Fast delivery:** from gene sequence to purified antibody delivery starting from 5BDs
- **Easy scale-up:** from microgram to kilogram in the same CHO cell line to minimise surprises

Tumbler™ Multi-parameter Antibody Optimisation Platform: Humanisation, Affinity Maturation, Liability Removal, and More

Michali Izhaky, a bioinformatics scientist at Charles River Antibody Discovery, presented Tumbler, a highly customizable antibody optimisation suite. The presentation began with an introduction to Tumbler, highlighting its unique ability to mature antibodies, humanise them, modulate targets, and enhance clinical profiles, all in a single step. The process started with building a custom library centred around the starting clone or clones by shuffling parental CDRs with carefully designed CDR variants, as well as CDRs from in-house libraries. This results in a diverse Tumbler library containing 500 million to 1 billion unique variants of the starting clone.



Izhaky explained that Tumbler could drive, through panning selection, to achieve various antibody optimisation outcomes, including affinity maturation, humanisation, target modulation, and clinical profile enhancement. The process also involves removing liabilities, improving thermal stability, and broadening IP coverage. The presentation detailed the Tumbler library build process, which began by exploring the near and far paratope space through CDR shuffling. Parental CDRs, near sequence space CDR variants, and additional diversity from in-house SuperHuman® or Cosmic® libraries were grafted into a 100% germline human framework with a high developability profile. This combinatorial CDR shuffling approach produced a custom library with 500 million to 1 billion unique variants of the original antibody.

The presentation also highlighted the importance of leveraging the SuperHuman and Cosmic libraries within the Tumbler process. The SuperHuman library was constructed by sourcing CDRs from different B cell populations of 100 human donors to optimise for both affinity and diversity. The Cosmic library, built on the same principles as SuperHuman, further expanded diversity by enriching minor CDRs with liability-free oligo pools and an expanded framework selection. These libraries contributed to the custom Tumbler library diversity by exploring far sequence space and bridging the gap between parental sequences and in-house library CDRs.

Izhaky emphasised that Tumbler could optimise antibodies in as little as four months. The process began with a free pre-project risk assessment, followed by a structured timeline of library building, panning, and validation. The presentation included data and results from previous Tumbler campaigns, demonstrating its effectiveness in improving antibody affinities, humanising clones, and introducing cross-reactivity. For example, one campaign identified 257 unique binders, with 189 having improved affinities compared to the starting parental clone. Another campaign aimed to introduce cross- reactivity while affinity maturing the starting clones, resulting in 365 novel cross-reactive clones.

The presentation also covered sequence-activity relationship (SAR) analysis, which provides detailed insights into changes in CDRs, and therefore guides future development and engineering decisions for candidate clones. The SAR analysis quantifies and analyses changes in minor CDRs, providing further insights into how the library components shuffled effectively to accomplish specific engineering objectives. This analysis was optimal for powering machine learning and AI models for antibody engineering.

In conclusion, Izhaky showcased Tumbler as a powerful and flexible one-step multi-parameter antibody optimisation tool. Tumbler could turn one or a handful of leads into hundreds of improved variants through simultaneous affinity maturation, humanisation, cross-binding induction, off-target avoidance, clinical profile enhancement, and broadening of IP coverage.

The presentation highlighted Tumbler's ability to generate novel insights into sequence-activity relationships, and emphasised that clients own

all data generated during a Tumbler project, including the library itself, royalty-free. Since its launch, Tumbler had completed nearly 20 customised antibody optimisation campaigns across various therapeutic domains, including immunology, oncology, cardiovascular disease, neurodegenerative disease, and rare diseases. The presentation concluded with an invitation for inquiries about antibody optimisation with Tumbler or standard discovery programs at Charles River Antibody Discovery.

charles river

Tumbler® Multi-parameter Antibody Optimization Platform

Highly Customizable Antibody Optimization

Antibodies are increasingly used in drug development due to their ability to target a wide range of antigens. However, many antibody-based drug candidates require additional engineering for optimal therapeutic efficacy in humans. This can include affinity maturation, humanization, and stability enhancement. Here, we introduce the Tumbler® platform, a CDR-shuffling approach for customizable antibody optimization validated across a diverse set of targets.

This method utilizes diversity from Charles River's in-house libraries and near-parental sequence space CDR variants, grafted into a 100% germline human framework, to minimize redundancy and liabilities and maximize functional diversity. The approach fuels successful affinity maturation, induction of cross-binding, and antibody humanization campaigns, as well as provides valuable insights about sequence-activity relationships.

Antibody engineering projects can start from one or several parental clones that originate from various sources, including animal immunization or phage display discovery. More than 500 million variants can be generated from an original antibody, with separate panning arms allowing for different engineering objectives to be explored simultaneously.

Antibody Humanization and Affinity Maturation

The Tumbler® library is interrogated under unique strategies to enrich for clones that meet desirable criteria and accomplish engineering goals. First, the platform can optimize leads originating from non-human sources, providing a developable selection of hits with a greatly reduced risk of immunogenicity in humans. Figure 1 illustrates the degree of humanization and off-rate of the original parental sequence compared to final optimized clones for 95 binders. We simultaneously humanized nonhuman clones while improving binding from micromolar to nanomolar affinities.

The Tumbler® platform also enables affinity maturation. We have used the platform to identify 257 unique binders, 189 with improved affinities compared to the parental clone and 28 demonstrating over five-fold affinity improvement (Figure 2).

Other successful project outcomes include cross-reactivity induction and liability removal. Tumbler® offers a robust and flexible antibody engineering solution to help accelerate therapeutic candidates through the drug development process.

View the full poster →

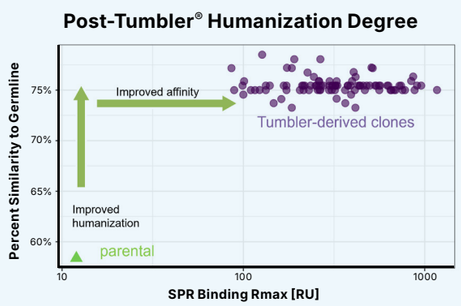


Figure 1

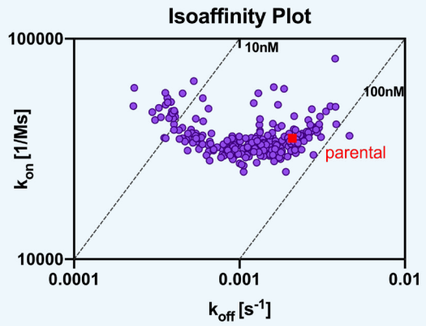
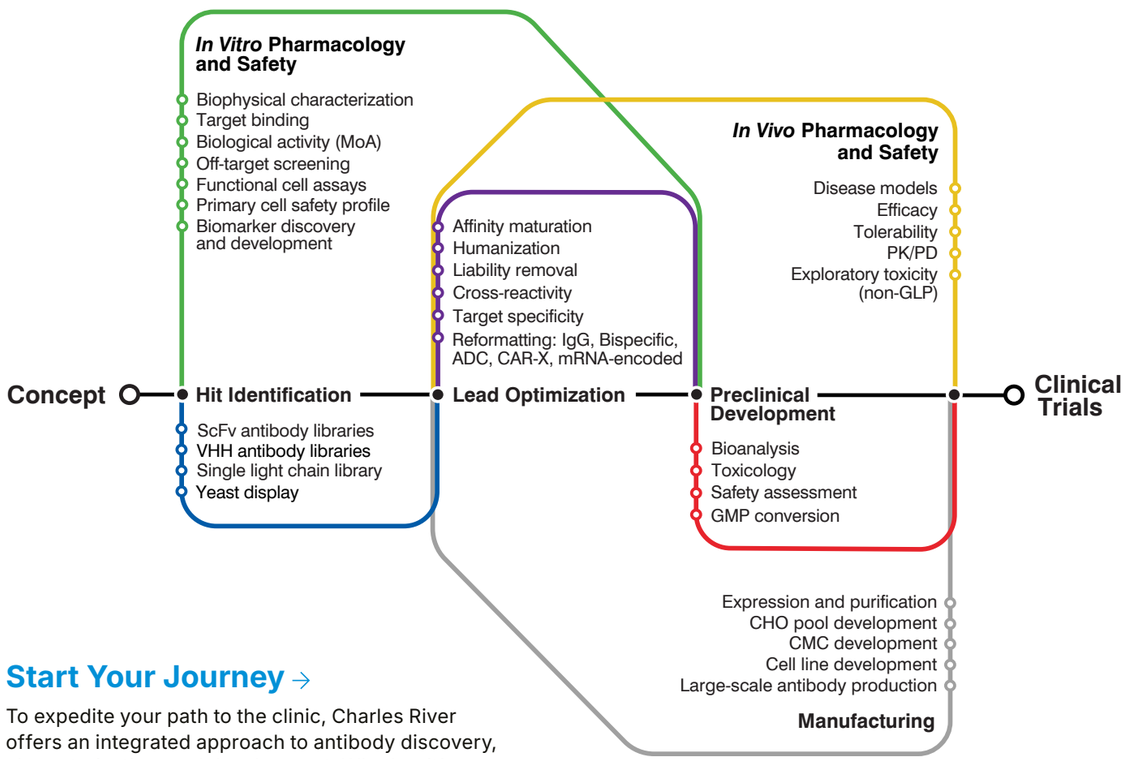


Figure 2

Experience Charles River Antibody Discovery



Start Your Journey →

To expedite your path to the clinic, Charles River offers an integrated approach to antibody discovery, characterization, and development. Whether it's an end-to-end antibody discovery need, or a standalone antibody service, Charles River can power your innovations to translational success.

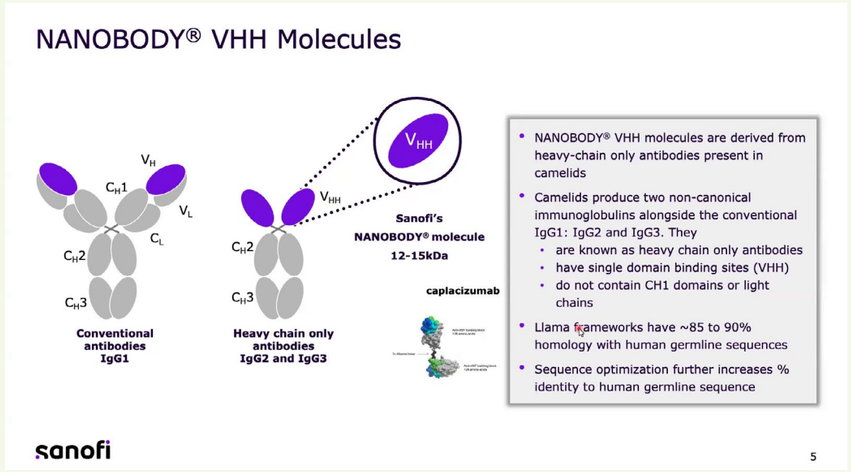
Learn more at criver.com/antibodies

criver.com/contact-us | askcharlesriver@crl.com

charles river

Towards Biologics by Design: AI-Driven Optimisation of Next Generation Protein Therapeutics

Norbert Furtmann, the head of AI innovation for the nano body platform within the large medical research department at Sanofi, delivered a comprehensive presentation on the computational optimisation of protein therapeutics. The talk focused on the application of digital technology to support and guide the discovery and optimisation of nano body-based therapeutics.



Furtmann began by introducing nano bodies, which are single-domain antibodies derived from camelids. These nano bodies, also known as heavy chain-only antibodies, are much smaller than conventional antibodies due to their single-domain binding site and lack of light chains. The research strategy at Sanofi aimed to deliver first and best-in-class medicines by addressing complex disease conditions using multi-targeting approaches. Nano body building blocks were utilised for this purpose, combining different targets in a single molecule connected by flexible linkers.

The design of multi-targeting nano bodies presented significant challenges due to the vast design space and the complexity of the molecules. Furtmann emphasised the importance of automation and computational tools, including machine learning and AI, in exploring these design spaces and predicting the properties of nano body molecules. The collection of thermal stability data for nano bodies was crucial, and balanced data sets were necessary for building predictive models.

Specialised protein language models were used to represent nano body sequences for property prediction, showing better performance than general models. Furtmann highlighted the development of digital tools for predicting the thermal stability of nano bodies. The data set consisted of 5,902 nano bodies with thermal stability measurements, with only a fraction classified as having poor stability. The goal was to balance the data set to improve the predictive models.

Furtmann discussed the use of different types of descriptors to describe a sequence, including one-hot encoding, amino acid properties, and structural models. Protein language models, which generate vectorised descriptions of protein sequences, were also utilised. The presentation highlighted the importance of avoiding data leakage by ensuring that the training and test data sets were diverse and did not overlap.

The evaluation of different protein language models showed that nano body-specific models performed better than general models in predicting thermal stability. The best model was a hybrid approach that combined embeddings from nano body-specific protein language models with sequence-based descriptors. This hybrid model demonstrated high correlation and low error in predicting thermal stability.

In the final part of the presentation, Furtmann showcased the performance of the hybrid model. The model achieved a Spearman correlation of about 0.72 and an error of about 4 degrees in predicting thermal stability. The model was tested on a new data set consisting of 550 data points, including wild types and mutational variants, and showed a correlation of about 0.6 for wild type predictions.

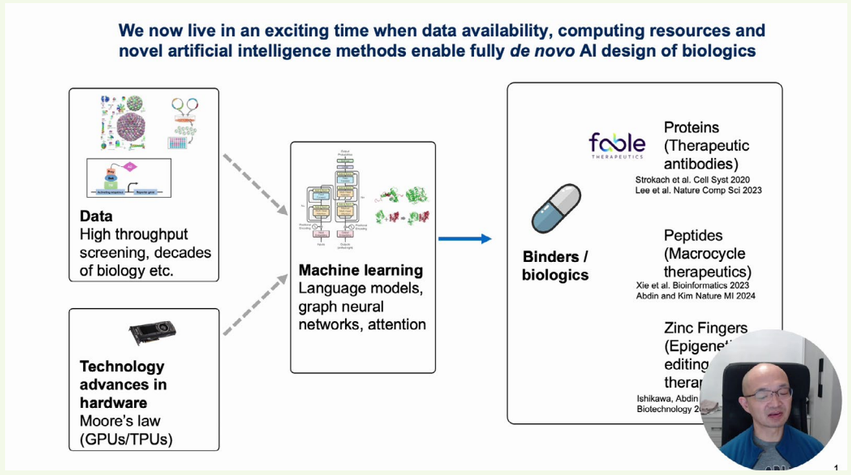
Overall, Furtmann's presentation provided a detailed overview of the computational optimisation of nano body-based therapeutics at Sanofi. The use of automation, machine learning, and AI in exploring design spaces and predicting properties of nano body molecules was emphasised. The development of specialised protein language models and the importance of balanced data sets for building predictive models were key takeaways from the talk.

Machine Learning Methods for Antibody Design

Philip Kim, a professor at the University of Toronto and the Chief Technology Officer and Co-founder of Fable Therapeutics, delivered a presentation on the advancements in protein design, particularly focusing on the integration of machine learning and AI in the field. He highlighted his contributions and the work done at Fable Therapeutics, an antibody AI design company.

In recent years, the field of protein design had seen significant progress due to the availability of extensive data and powerful computers. This combination enabled the use of AI and machine learning for custom therapeutic design. Kim emphasised that the advancements in protein design were largely due to the confluence of decades' worth of data on protein interactions and structures, along with the advent of powerful computing resources. These developments allowed for the realisation of the dream of custom-designed therapeutics.

Kim discussed his work in three areas: proteins, antibodies, and peptides, with a primary focus on proteins. He outlined several key events that were necessary for machine learning and AI to design biologics effectively. One of the most critical advancements was the invention of a good representation of 3D protein structures for deep learning models, primarily through the use of graphs. Kim claimed credit for introducing graphs to the field, which allowed for a much better representation of protein structures for computational models.



Graphs played a crucial role in encoding protein structures, leading to the development of the first inverse folding model using graph neural networks. This model significantly improved the accuracy of protein design by encoding the entire protein structure into a graph, allowing the model to learn constraints and generate sequences that would fold into real proteins. Kim noted that this advancement fuelled much of the subsequent progress in the field.

In addition to graphs, language models, particularly protein language models, were instrumental in understanding protein structures and interactions. These models aided in docking models and enhanced design precision. Kim also discussed the advent of diffusion models, which revolutionised protein design by enabling the generation of novel protein backbones. These backbones could then be sequenced and folded into real proteins.

Kim highlighted the practical applications and validation of these advancements. The new proteins created using these methods folded correctly and exhibited realistic properties, validated both computationally and experimentally. At Fable Therapeutics, foundation models for structure and sequence optimisation were developed, showing high performance in generating realistic protein loops and antibodies with good properties.

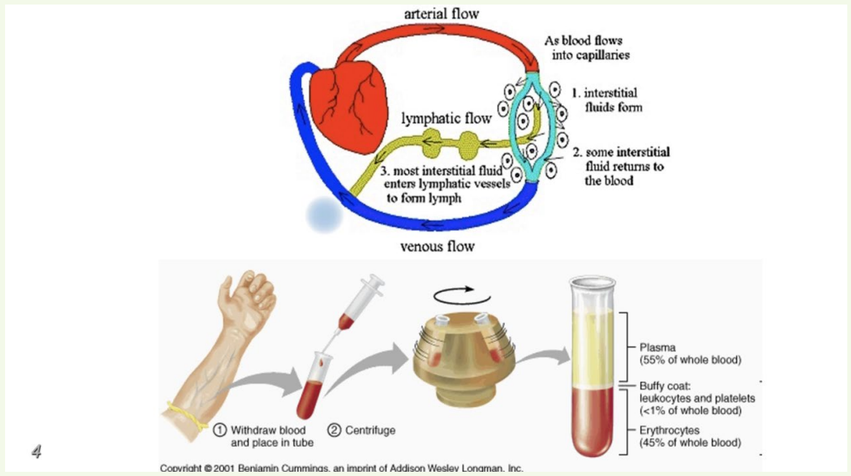
Looking ahead, Kim emphasised the importance of staying updated with technological advancements. He highlighted ongoing improvements in model generations and their applications in protein design. He also mentioned the development of antibody SGM, a diffusion model for antibody generation, which outperformed previous models in sequence recovery and RSD benchmarks.

In conclusion, Kim's presentation underscored the transformative impact of machine learning and AI on protein design. The integration of graphs, language models, and diffusion models had significantly advanced the field, enabling the creation of novel proteins with practical applications. Kim's contributions and the work at Fable Therapeutics demonstrated the potential of these technologies to revolutionise therapeutic design and improve patient outcomes.

Beyond Antibody Engineering: The Importance of Stability Studies in Human Blood Plasma and Antibody Formulation

Tudor Arvinte, Professor at Therapeomic, Basel, focused on the critical importance of stability studies in human plasma and blood for the development of antibody formulations and biopharmaceuticals. The speaker emphasised that these studies are essential for predicting and understanding aggregation phenomena, which can significantly impact the efficacy and safety of biopharmaceutical products.

The presentation began with an overview of the significance of stability studies, highlighting that they are crucial for ensuring the chemical and physical stability of biopharmaceuticals. However, the speaker also pointed out that other factors, such as ease of application, manufacturing processes, minimal side effects, and the absence of aggregation after in vivo application, are equally important.



A case study on Herceptin was presented to illustrate the impact of using different diluents on aggregation. It was revealed that using 5% dextrose as a diluent instead of sodium chloride led to significant aggregation, which would have caused clinical trial failures. The speaker explained that this unexpected result prompted further experiments, which showed that Herceptin, when mixed with human plasma, forms strong aggregates. This finding underscored the importance of compatibility studies with human plasma during product development.

The presentation also discussed the proposed aggregation model for antibodies in human plasma. The model highlighted the role of lipoproteins and the formation of large globular structures when antibodies are mixed with plasma. This model was supported by data from experiments on Herceptin and Avastin, which showed strong aggregation phenomena when these antibodies were mixed with human plasma.

Further, the speaker presented studies on Filgrastim and PEG Filgrastim, which are not antibodies but still provided valuable insights into the aggregation of biopharmaceuticals. These studies indicated that the formulation buffer is the main cause of aggregation and that strong effects on erythrocytes were observed. The findings emphasised the need for biobetters formulations that do not induce plasma and erythrocyte aggregation, thereby reducing side effects and improving therapy outcomes.

The implications of these findings for drug development were also discussed. The speaker pointed out that infusion studies showed that solutions do not mix quickly, leading to local aggregation and potential side effects. This highlighted the need for compatible formulations that can prevent such issues.

In conclusion, the presentation emphasised that aggregation in plasma and blood is a general phenomenon influenced by formulation, concentration, pH, and donor variations. The speaker stressed that good formulations could prevent aggregation and fused erythrocytes, although a general solution for all biological fluids is still lacking. The importance of using aggregation studies with IV diluents, human plasma, and blood was underscored, supported by FDA publications. The presentation concluded with a call for continued research and development to address these challenges and improve the safety and efficacy of biopharmaceuticals

Report Conclusion

We hope that this eBook has provided a comprehensive exploration of the latest advancements and innovative strategies in the field of antibody engineering. From the mechanisms of influenza viruses and the innovative strategies to inhibit their entry into cells, to the AI-driven optimisation of protein therapeutics and the critical importance of stability studies in human blood plasma, this eBook has covered a wide range of topics essential for advancing the science of antibodies.

The insights and practical applications discussed throughout this eBook aim to inspire and equip researchers and professionals to push the boundaries of antibody engineering. As we continue to explore new frontiers in this exciting field, the knowledge shared in this eBook will serve as a valuable resource for driving innovation and improving therapeutic outcomes. Thank you for joining us on this journey through the cutting-edge world of antibody engineering.

Explore Our Comprehensive Content Portal

Immerse yourself in cutting-edge scientific content - from online Monthly Science Exchanges, best practice Online Symposiums to eBooks and landscape reports providing a unique perspective on the latest R&D trends and challenges.

- Archon Biosciences Announces $20m of Seed Funding for its Nobel-Winning, AI-Driven Antibody Cage Technology
- Absci to Collaborate with the Memorial Sloan Kettering Cancer Center to Develop Biologics using AI
- Introducing the Development and Clinical Landscape of Bi and Multispecific Antibodies