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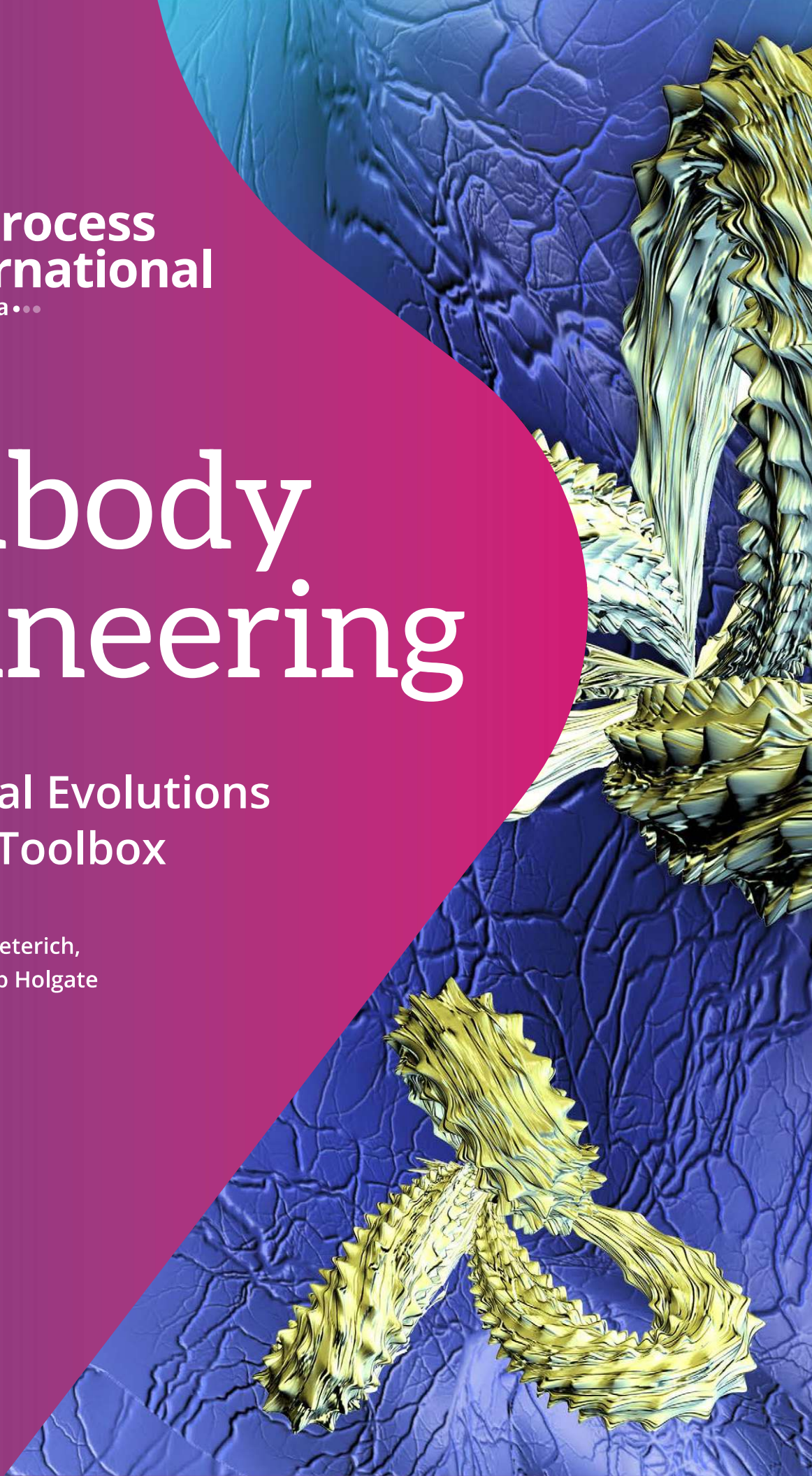
Antibody Engineering

Technological Evolutions Expand the Toolbox

by Cheryl Scott, Petra Dieterich, Thomas Cornell, and Rob Holgate



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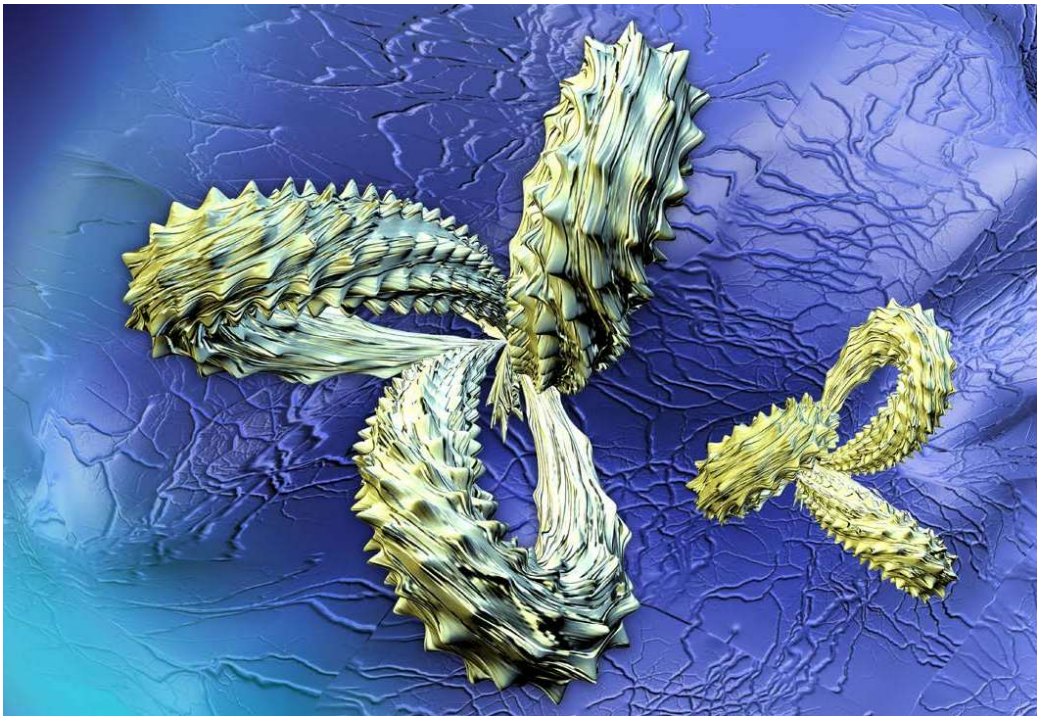
Antibody Engineering

Technological Evolutions Expand the Toolbox

by Cheryl Scott, Petra Dieterich, Thomas Cornell, and Rob Holgate



Computational advances and sophisticated molecular architectures are driving rapid transformation in the antibody engineering field. Digital platforms dramatically accelerate antibody design, as industry and academia work together to apply modeling for developability predictions and affinity optimization. Antibody derivatives offer new modes of action and improved bioavailability. Developers can prioritize safety and efficacy while integrating manufacturability assessment early early in development. Here, a literature review finds antibody engineering positioned at the forefront of precision medicine, and authors from Abzena provide a deep-dive example of platform engineering.

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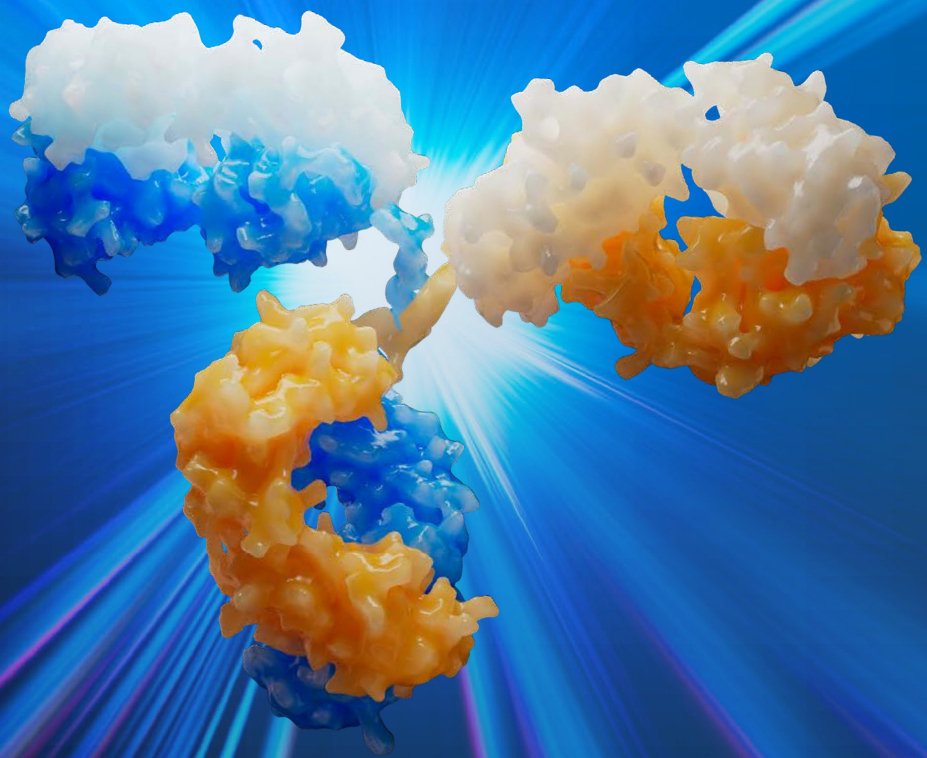

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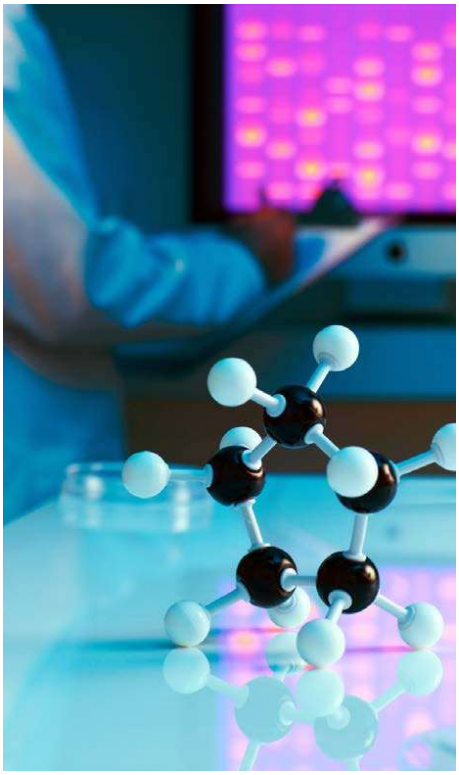
Fine-Tuning Structures and Behaviors

Antibody Design in an Age of Precision Engineering

Cheryl Scott

Back to Contents



Monoclonal antibodies (mAbs) and their derivatives continue to dominate the field of therapeutic proteins, and antibody engineering has emerged as a transformative accelerating factor in the development pipeline's growth. A global market already valued in hundreds of billions of US dollars is expanding as molecular engineering technologies yield increasingly sophisticated therapeutic modalities beyond traditional full-length immunoglobulin G (IgG) antibodies.

Core Engineering Methodologies: Humanization and affinity remain foundational targets, with computational modeling and directed evolution now helping companies to optimize binding kinetics and minimize immunogenicity of their drug candidates. Phage, yeast, and mammalian-cell display systems enable rapid screening of vast antibody libraries for enhanced specificity and potency.

Fragment engineering has gained significant traction in recent years, producing small-format antibody derivatives including single-chain variable fragments (scFvs), diabodies, and single-domain antibodies comprising just the variable domain of heavy-chain antibodies (V_HHs). Such engineered fragments offer improved tissue penetration and novel pharmacokinetic profiles that are particularly valuable for oncology and neurological applications. For manufacturing, fragments can solve some complexities while introducing new ones.

Multispecific antibodies represent another technological leap forward. Bispecific antibodies (bsAbs) enable simultaneous targeting of multiple antigens or cell types. Next-generation trispecific and tetraspecific formats are entering clinical development now, although their manufacturing remains challenging.

Advanced Engineering Approaches: *Fc engineering* focuses on modulating effector functions through strategic amino-acid substitutions on the crystallizable fragment (Fc) antibody

domain. Silencing Fc domains can eliminate unwanted immune activation in certain therapeutic contexts.

Goals such as antibody-dependent cellular cytotoxicity (ADCC) enhancement, complement-activation modulation, and half-life extension are achieved routinely in mAb development programs. Many mAbs are protected from degradation through optimized binding to neonatal Fc receptor (FcRn), a protein that binds to IgG antibodies and albumin, helping to keep mAbs in circulation.

Computational design is showing great promise as companies use machine-learning (ML) algorithms and structural biology insights to predict optimal mAb sequences. Artificial intelligence (AI) platforms now can design new antibodies, which could shorten discovery timelines down from years to months.

Current Goals and Applications: Key antibody-engineering objectives now include developing next-generation cancer immunotherapies — e.g., checkpoint inhibitors and immune-cell engagers. Autoimmune-disease treatment can benefit from precision-engineered antibodies that target specific inflammatory pathways while preserving essential immune functions. Infectious-disease applications have gained renewed focus in recent years, with rapid-response platforms demonstrated during COVID-19 enabling antibody development within weeks rather than years.

Industry Trends and Future Directions: Personalized medicine has been "just on the horizon" for decades now; many of the trends above could come together and finally make it a reality with patient-specific antibody engineering based on individual genetic and immunological profiles. The convergence of synthetic biology, AI/ML, and advanced manufacturing is positioning antibody engineering at the forefront of precision medicine.

Many of these trends could come together and finally make **PERSONALIZED MEDICINE** a reality, with patient-specific antibody engineering based on individual genetic and immunological profiles.

A Brief Literature Review

Publications over the past year in Informa's Taylor & Francis journal *mAbs* reveal a number of interconnected trends in mAb engineering. In reviewing this area of literature, I've discovered companies and research ins working to enhance binding specificity, improve product developability, and innovate new molecular formats.

Computational Design: Here as elsewhere in the biopharmaceutical industry, integration of AI and other computational methods is proving to be transformative. Authors at Regeneron Pharmaceuticals describe an ML model for predicting developability properties (**1**). And scientists at AstraZeneca and Texas A&M University have created a structure-based model for size-exclusion chromatography predictions (**2**). Working with Nvidia Corporation, researchers at A-Alpha Bio used language

Back to Contents

modeling to achieve state-of-the-art performance for guided affinity optimization (**3**). And rational design approaches combine structural insights with computational predictions, as shown in framework mutation studies at University College London, Birkbeck University of London, King's College London, and the University of Rome in Italy (**4**).

An industry–academic collaboration involving Exscientia (now part of Recursion), Thermo Fisher Scientific, Utrecht University (Netherlands), and the University of Oxford (UK) produced a comprehensive computational pipeline for discovery/design of therapeutic antibodies that incorporates physics- and AI-based methods for generation, assessment, and validation of candidates with improved developability against diverse epitopes through efficient "few-shot" experimental screening (**5**).

Affinity Optimization and Safety Enhancement: Efficacy and safety can go hand in hand with strategic modulation of binding affinities that improves mAb therapeutic windows. Cluster of differentiation (CD) protein complexes are important in immunology, and CD3 affinity attenuation has emerged as a key strategy for maintaining mAb efficacy while reducing the tendency to induce cytokine release. Swiss and German authors at Roche Innovation Center Zurich, the University of Basel, and Ludwig Maximilian University of Munich generated a series of T-cell bsAbs of CD3 binders with different affinities and tumor-antigen binders of either high or low affinities (**6**). They used the resulting library to reduce cytokine release while maintaining adequate efficacy through CD3-binder affinity attenuation.

Efficacy and safety can go hand in hand with **STRATEGIC MODULATION** of binding affinities that improves mAb therapeutic windows.

An academic collaboration involving the Technical University of Denmark, the University of Innsbruck (Austria), the Scripps Research Institute (California), The University of Costa Rica, and Denmark's BioInnovation Institute focused on exploiting pH-dependent binding for enhanced selectivity (**7**). The authors show how pH sensitivity can be introduced into mAbs through the substitution of selected residues at the heavy–light-chain interface, which enhanced pH-dependence more effectively than would histidine substitutions alone. Scientists at German company YUMAB worked with researchers at the University of Heidelberg, the University of Stuttgart and affiliated Stuttgart Research Centre for Systems Biology (Germany), the University of Tsukuba (Japan), and the University of Portsmouth (UK) to demonstrate successful transferrin-receptor–mediated transcytosis, also using pH-sensitive binding modalities (**8**). And authors at the University of Michigan show how avidity-based selectivity can be leveraged to distinguish between cells that express antigens at high and low levels. The

team used that approach to screen antibody mixtures for maximized therapeutic windows (**9**).

Developability-Focused Engineering: To prevent late-stage manufacturing process-development delays, the industry has turned its attention toward early consideration of developability properties and engineering approaches to address related problems. For example, a team at AstraZeneca addressed formulation viscosity at high mAb concentrations through rational protein engineering using hydrogen–deuterium exchange mass spectrometry to identify self-interaction hotspots on mAb structures (**10**). And Merck scientists worked with researchers at Germany's Technical University of Darmstadt in studying stability optimization through aligning isoelectric point (pI) profiles across variable domains to mitigate charge asymmetries (**11**). Aggregation propensity is being predicted and minimized using both sequence and structure-based approaches in multiple other developability studies.

Fc Engineering and Effector Function Modulation: Fc-region optimization continues to be a major focus. Combinatorial Fc modifications are explored for complementary functionality, as demonstrated using human immunodeficiency virus (HIV-1) mAb libraries in work at the Massachusetts Institute of Technology and affiliated Ragon Institute (United States), the Hannover Medical School Cluster of Excellence (Germany), Helmholtz Center for Infection Research (Germany), and the TWINCORE Institute for Experimental Infection Research (a joint venture of Hannover and Helmholtz) (**12**). Japan's Chugai Pharmaceutical is establishing methods for predicting human pharmacokinetics of Fc-engineered mAbs for FcRn binding enhancement (**13**). And effector function tuning has been achieved through various approaches, including the development of effectorless variants for specific applications.

Humanization and Immunogenicity Reduction: Although mAb humanization is a well-known strategy, a number of teams are working to develop advanced approaches. Authors from GSK and the University of Oxford created an open-source computational tool designed to offer experimental-like humanization of heavy and light chains in seconds using convolutional neural networks (**14**). Immunogenicity assessment is being integrated into the mAb design process, with multiple studies emphasizing the importance of reducing immunogenic risks as early as possible.

Multispecific Antibody Architectures: Developers are moving beyond relatively simple bsAb formats onto ever more sophisticated architectures. For example, trispecific antibodies are emerging as powerful tools, with authors at AstraZeneca

Developers are **MOVING BEYOND** relatively simple bispecific antibody formats onto ever more sophisticated architectures.

demonstrating synapse-gated and affinity-tuned designs that achieve conditional dual tumor-associated antigen targeting while sparing healthy cells (**15**).

A *biparatopic antibody* is a type of bsAb that binds to two different, nonoverlapping epitopes of the same antigen — a dual-binding mechanism that increases binding affinity and specificity and can be engineered to act as either an agonist (activating a receptor) or an antagonist (blocking a receptor). Such approaches are gaining traction, as shown by authors at Ichnos Glenmark Innovation with a candidate that uses two adjacent anti-CD38 arms to increase antibody density and avidity (**16**).

Other Antibody Formats and Scaffolds: Meanwhile, some researchers are exploring alternative antibody architectures beyond the usual IgG. V_HHs are optimized for therapeutic applications, for example, with scientists at Merck Healthcare KGaA and the Technical University of Darmstadt providing comprehensive insights into camelid-derived single-domain antibodies (sdAbs) (**17**). Others at Specifica LLC (part of IQVIA) and the New Mexico Consortium are developing humanized V_HH libraries for enhanced developability (**18**).

Hybrid antibody formats are emerging as well, including an IgE–IgG1 hybrid (IgEG), combining functionalities of both isotypes, described by authors from King's College London, Epsilogen, and Guy's Hospital (**19**). An alternative multispecific scaffold based on IgA has been developed by Merck Healthcare and Austria's University of Natural Resources and Life Sciences, with their trispecific SEED antibodies for neutrophil-mediated cell killing (**20**).

Evolving Designs for an Evolving Field

Increasing knowledge of immunology, expanding options among molecular-engineering methods, and rapidly advancing computational technologies are driving antibody engineering toward sophisticated approaches that prioritize antibody safety, efficacy, and developability from the earliest stages of product design. The field is moving beyond relatively simple binding optimization to embrace complex, multispecific architectures that can achieve unprecedented levels of selectivity and therapeutic potential.

Increasing knowledge of immunology, expanding molecular-engineering options, and advancing computational technologies are driving antibody engineering toward sophisticated approaches that prioritize safety, efficacy, and developability in **PRODUCT DESIGN.**

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Back to Contents

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Back to Contents

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Back to Contents

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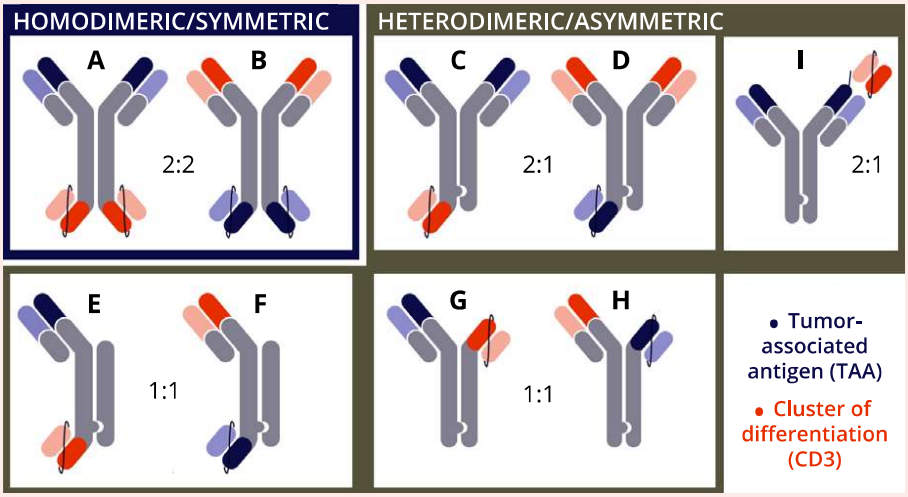
Robust Platform Technologies Can Streamline Next-Generation T-Cell Engager Development

Petra Dieterich, Thomas Cornell, and Rob Holgate

Antibody-based drug modalities such as T-cell engagers (TCEs) and antibody–drug conjugates (ADCs) are making a substantial impact in oncology and are expected to progress further over the next decade. TCEs can enhance the effectiveness of a patient's innate immune system in a controllable, measurable, and dose-dependent way. They represent an approach to bridging the gap between classical chemotherapy and bespoke cell therapies such as chimeric antigen receptor (CAR) T cells. Providing a cost-effective way to engage the immune system, TCEs could make immunotherapy a first-line therapy option.

Figure 1: Example bispecific T-cell engager (TCE) construct design matrix features two different specificities — one targeting CD3 and the other targeting a tumor associated antigen (TAA). Designs use silenced crystallizable fragment (Fc) domains with knobs-into-holes (KiH) heterodimerisation technology where appropriate.



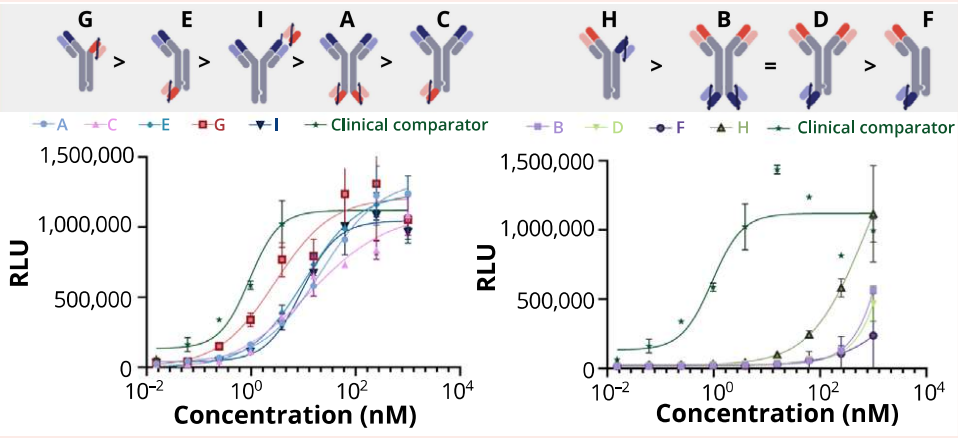
Bridging the Gap

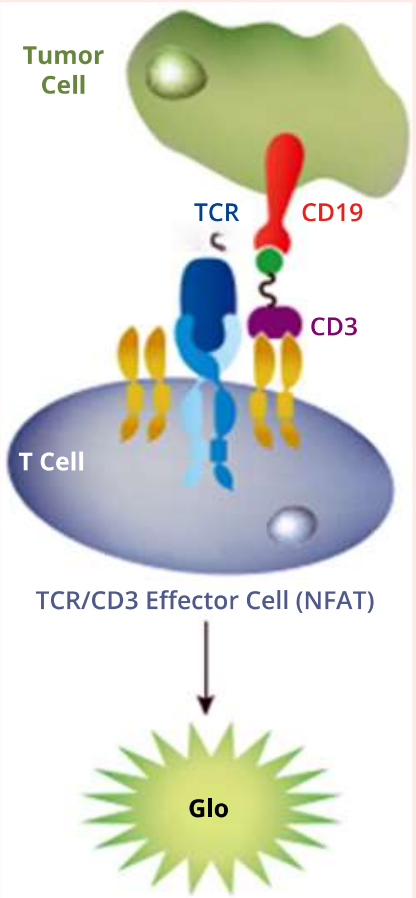
Immunotherapy has received widespread attention as a therapeutic approach not only to fight cancer, but also more broadly to address infectious diseases as well as autoimmune and inflammatory conditions. One specific modality within this field uses bispecific antibody (bsAb)–based immune-cell engagers, with each antibody arm engineered to present different binding domains. Such molecules are making inroads as a potential option for both active and passive immunotherapy.

In the case of bispecific TCEs (e.g., Amgen's BiTE technology), their engineered antibody-based structures are designed literally to "bridge the gap" between a patient's own immune T cells and a target tumor-cell receptor. Their different antigen-binding sites

Back to Contents

Figure 2: T-cell activation reporter bioassay uses tumor target cells and cluster of differentiation 3 (CD3) T-cell receptor (TCR) effector cells expressing nuclear factor of activated T-cells (NFAT, Promega) to measure the activity of bispecific designs.



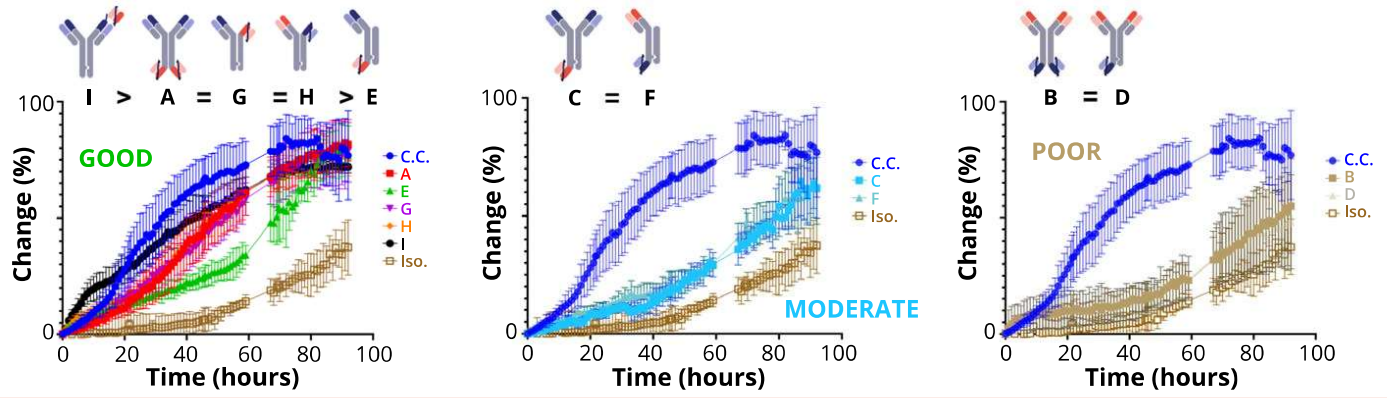


crosslink receptors to activate the innate immune system for killing cancer cells.

By the middle of 2024, nine such TCE therapeutics had been approved, and some analysts predict that the market could exceed US$20 billion in value by 2030 (**1**). Amgen's Blincyto (blinatumomab) is a first-generation BiTE product that received FDA approval in 2014, initially for second-line treatment of Philadelphia-chromosome–negative relapsed lymphoblastic leukemia. It was approved also for use in combination with chemotherapy and in postremission therapy. Such TCEs have the potential to be used as first-line therapies due to their potential reduction in toxic side effects compared with traditional chemotherapy alone. Despite showing good efficacy, however, the first-generation BiTE immunotherapies and similar bispecific antibodies often have been hampered by on-target, off-tumor toxicities that lead to immune-related adverse events.

Smart TCE Engineering and Integrated Analytical Method Development: Deciding where to start when it comes to TCE design can be daunting. Through complex engineering, bsAbs can be designed to cover substantial structural diversity, offering incredible flexibility beyond what's possible with traditional monoclonal antibodies (mAbs). With that flexibility comes the need for broadened exploration of amino-acid sequencing and complex selection criteria to find optimal leads. Successful therapeutics require the correct symmetry, avidity, spacing, and affinity — as well as potential requirements for inclusion and silencing of crystallizable fragment (Fc) moieties — all of which are vital to ensure efficacy and prevent off-target toxicity. Also important to consider during lead selection is the additional complexity of bispecifics, which increases the chance

Figure 3: Results from tumor-cell killing assay using purified human peripheral blood mononuclear cells (PBMCs), Cytolight Red–stained tumor cells, and Cytotox Green reagent (an indicator for dead cells), monitored using an Incucyte live-cell imaging system (Sartorius). As T cells are recruited and cancer cells are killed, the red and green stains mix, and the appearance of a yellow color is monitored. In these example results, four designs (I, A, G, and H) show a strong ability to recruit and kill cancer cells, whereas two others (B and D) show limited recruitment activity. CC = clinical comparator; Iso. = isotype.



for production of undesirable by-products during their manufacture.

A drug developer's goal is to identify the most appropriate drug candidate both in terms of efficacy and manufacturability. Decision-makers must bear in mind that even the most efficacious molecule cannot advance into clinical development if it cannot be made consistently to the required purity and scale. To that end, we have developed a toolbox of protein-engineering technologies that produce bi- and multispecific constructs while controlling critical quality attributes (CQAs), including structural consistency and purity.

Figure 1 highlights a potential TCE matrix designed to probe multiple stoichiometries and spacing with a minimal number of designs and ensure that most functionality issues and format types will be considered. Examples include homo-/heterodimerization, proximal/spatial arrangement of arms, reformatting of fragment antigen-binding (Fab) regions into single-chain fragment-variable (Fv) structures, and so on.

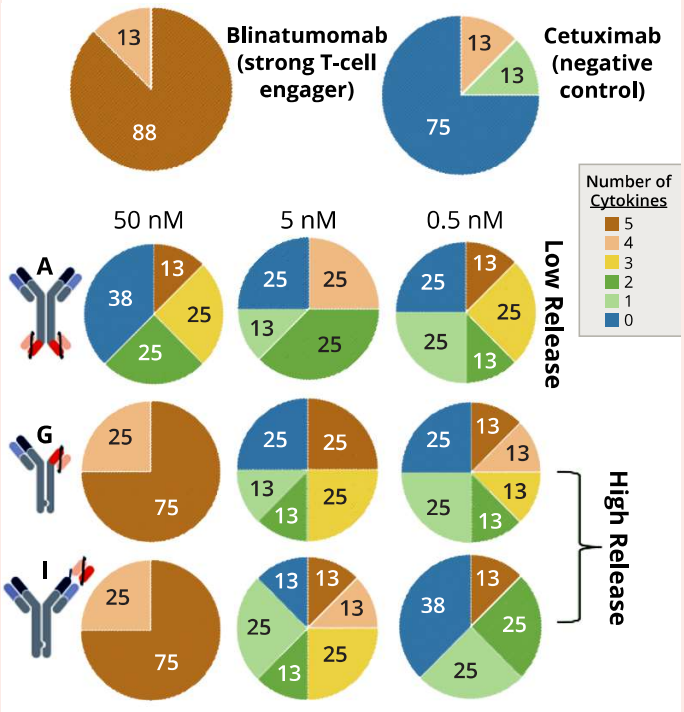
Designing a matrix panel is only the first stage. To tease out the optimum format for further development from a larger panel requires a robust expression platform, an effective bioassay, and a good analytical strategy. By focusing on biophysical characteristics initially, developers can assess a breadth of parameters (e.g., yield, purity, and thermal stability) readily after expression and purification, and the results can provide useful indicators for design selection. In-depth characterization of individual formats — using liquid chromatography with mass spectrometry (LC-MS), for example — also may be required to confirm such attributes as correct chain pairing.

Early biophysical data undoubtedly will be useful, but such information must be functional. Do molecular designs show the

expected mechanism of action (MoA), and if so, how well are they functioning? To ensure a molecule's efficacy for its therapeutic application, that key question needs to be answered. For TCEs in particular, good "first-pass" assays include those measuring T-cell recruitment (Figure 2) and ability to kill human peripheral blood mononuclear cells (PBMCs) (Figure 3). The latter type offers a real-time dynamic binding measurement to deconvolute cell-activation data. If a clinically validated TCE such as blinatumomab serves as a control, then the assay can demonstrate comparative cancer-cell killing. The examples in Figures 2 and 3 indicate that formats A, G, H, and I are most efficacious in cancer-cell destruction, whereas formats B and D show limited cancer-cell toxicity.

For regulators to allow a therapeutic candidate to enter clinical trials, the drug's developer must demonstrate that it is safe to inject into humans. The safety profile of TCE molecules can be assessed using whole-blood cytokine screens to measure the stimulation of cytokines after incubation of samples with human blood. They indicate the potential for a molecule to cause an unwanted, exaggerated cytokine release in vivo. Thus was it determined that format A was the molecule least likely to cause such an undesirable side effect (Figure 4).

Figure 4: Cytokine-release profile (measured by Luminex assay from R&D Systems) following incubation of test samples with human whole blood; design A showed the lowest propensity to induce cytokine release.



A data package including physicochemical attributes alongside bioassay results and safety assessments can deliver a comprehensive overview to enable informed selection among molecular formats (Figure 5). Format A shows the most favorable manufacturability attributes related to yield, purity, and stability. It also scored highly in cancer-cell killing with a correspondingly low potential for unwanted cytokine release.

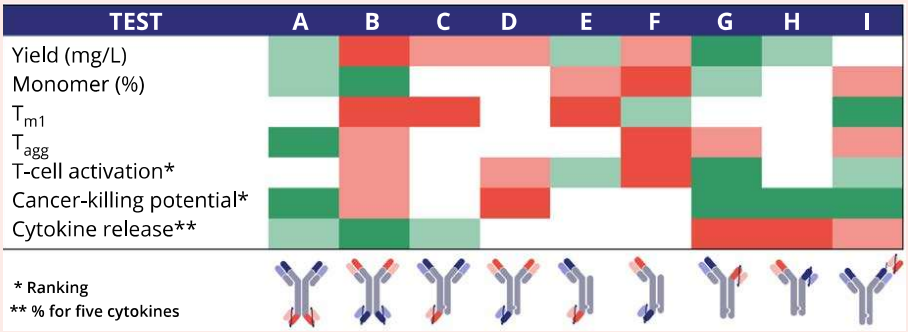
Manufacturing Tomorrow's TCEs

As with the broader field of bsAbs, producing the next generation of bispecific and multispecific TCEs will require a multidisciplinary approach. In addition to applying engineering expertise and computational insights to selection of optimal formats, developers must ensure that such molecules will be developable (by maximizing functionality and manufacturability while minimizing immunogenicity and off-target binding).

Back to Contents

Our company's approach to simplifying development and scale-up enables teams to address the multiple challenges associated with TCE development. Central to that is the appreciation that TCEs are complex molecules with a multipronged MoA, so a one-format-fits-all approach cannot apply. Combining rational design as outlined above with an effective triaging approach ensures effective and efficient candidate discovery and development.

Figure 5: Summary of combined characteristics used to assess example T-cell engager formats; parameters highlighted in green indicate molecules with favorable attributes, and those labeled in red indicate undesirable properties. Overall, format A appears to have the most favorable characteristics overall, so it would be the recommended choice to progress into further testing and characterization. T_{agg} = aggregation temperature, T_{m1} = melting temperature.



The TCEs field is moving fast. Joining them in the spotlight, with more than 200 different molecules currently in development, are other immune-targeting molecules such as natural killer (NK)–cell engagers and tri- and tetra-specific antibody formats. The therapeutic applications of different immune-cell engagers appear to be limited only by the time it takes to translate current scientific understanding into new disease treatments. Development pipelines across the biopharmaceutical industry are full of novel formats and complex engineered biologics, and it has yet to be seen whether the field will consolidate towards a few defined strategies.

TCEs provide an exciting option for bridging the gap between chemotherapy and cell therapy. The obstacle to their wide-scale adoption across healthcare systems remains industry's ability to develop, manufacture, purify, and analyze these complex biologics both efficiently and cost effectively. Coupling comprehensive characterization capabilities with cost-efficient platform strategies can inform lead selection and manufacturing at scale to help us further exploit the power of the human immune system.

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Back to Contents

Reclaiming Research Time: How Direct Tube Printing Is Modernizing the Antibody Lab

By Rusty Bishop, PhD, Chief Commercial Officer, TubeWriter

TubeWriter

In antibody engineering, precision drives progress, but something as simple as handwriting labels can quietly cost hundreds of hours of research a year. Rusty Bishop, PhD, shares how direct tube printing is transforming sample management, freeing scientists from the tedium and frustrations of stickers, smudges, and rework.

The Labeling Problem No One Talks About

Antibody discovery has evolved rapidly; automation, analytics, and AI are accelerating everything from clone selection to protein purification. Yet one step in the lab remains untouched by innovation: labeling.

Most scientists still mark samples with Sharpies or sticky labels that peel, smear, or fall off in freezers. A single missing label can derail a week's worth of antibody expression or characterization work. Still, because "that's how it's always been done," few teams question the hours lost to handwriting and relabeling.

A Scientist's View from the Bench

Before I joined TubeWriter, I spent years as a bench scientist. I know what it's like to spend nights labeling cryovials before an experiment or digging through a freezer looking for a sample ID that's fallen off. We would even hold "labeling nights," where teams gather around pizzas to tag thousands of tubes before a big project.

In the twenty years since I first stepped into a lab, nearly every process at the bench has evolved. Automation, analytics, and AI now drive discovery and purification—but labeling has stayed the same. It's still the quiet step that slows scientists down, a routine no one questions but everyone feels. Those small frustrations translate into measurable losses. Between aliquots, replicates, and controls, even a modest lab can label thousands of tubes a day. Each one takes precious seconds—seconds that add up to hours. It's a drain on productivity that no one budgets for, yet every scientist feels.

From Sticky Label Frustration to Durable, Reliable Print

That's where TubeWriter changes the story. The system replaces handwriting and stickers with direct printing, applying UV-curable ink directly onto any labware. The print cures instantly, bonding to plastic or glass for a durable, legible label that withstands freezing, thawing, and solvent exposure. Unlike industrial robotics, TubeWriter is built for everyday lab work—flexible, fast, and user-friendly. TubeWriter can label up to 1,000 tubes an hour, turning hours of manual effort into minutes of automation.

Designed for Modern Antibody Engineering

Antibody discovery relies on accuracy, traceability, and reproducibility. From screening and clone selection to purification and QC, every tube tells part of the story. TubeWriter brings precision and permanence to that workflow—no more guessing at smeared handwriting or reprinting labels mid-run.

The system adapts to almost any format: PCR strips, conical tubes, cryovials, NMR tubes, and even metal-capped vials. Labs can batch-print racks before experiments or print on demand at the bench. Either way, it integrates seamlessly into existing processes.

Bringing Precision Back to the Bench

Seeing TubeWriter in action often sparks an instant reaction—relief. Relief that the labeling bottleneck is gone. Relief that every sample is now clearly, permanently marked. For scientists, it's more than a convenience; it's a return to doing real science without interruption.

Because modernizing the lab isn't just about speed, it's about trust in every step of the process. With direct printing, scientists can ensure that each antibody, each sample, and each discovery starts with one simple truth: it's labeled right the first time.

Meet the Expert

Rusty Bishop, PhD, serves as CCO at TubeWriter. Trained as a biochemist, he brings both lab experience and commercial insight to improving everyday research processes. His work centers on helping scientists reclaim time and consistency in sample management through smarter labeling solutions.

Why Direct Print Labeling

Fast: Print up to 50 tubes in 3 minutes, that's 80% faster than manual labeling

Durable: Resistant to -80C, liquid nitrogen, and solvents. Your labels last as long as your samples.

Versatile: Prints text, barcodes, images on tubes, vials, slides, plates, and any other labware

Your New Reality

- Reclaim valuable research hours
- Keep every sample traceable and secure
- Run a more efficient and productive lab

Request a Free Sample

Scan to see how direct printing survives the toughest antibody workflows from freezing, thawing, solvents, and all.

Discover more at www.tubewriter.com