ADME Sciences: Where are we now and where do we go next?

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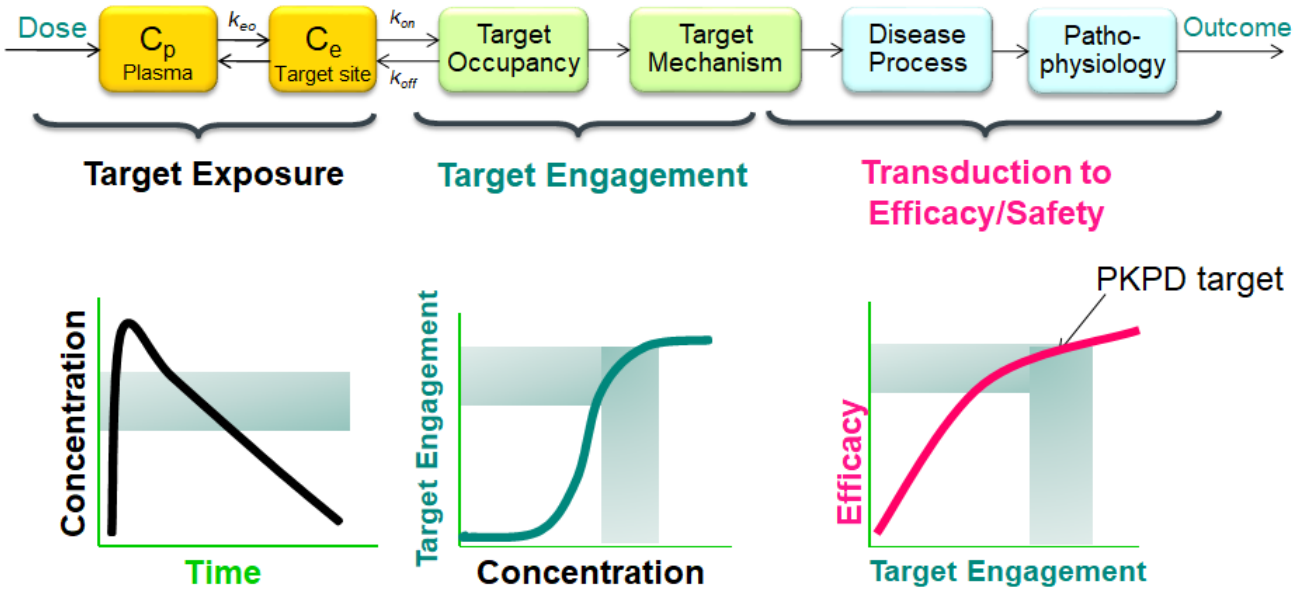
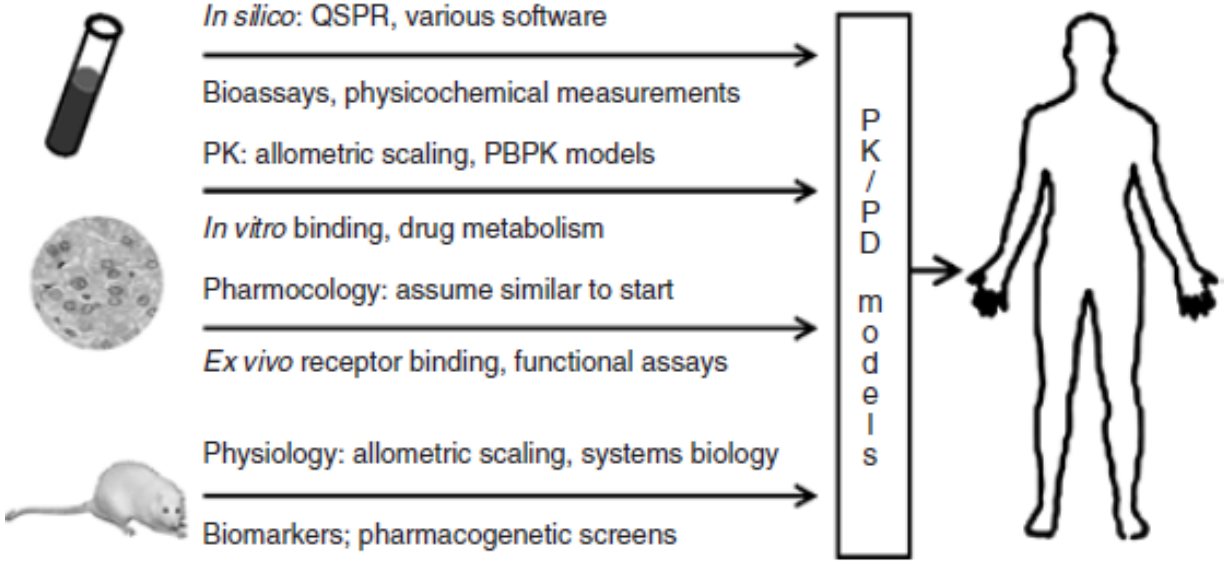
Gulf-Coast Consortium: The Future of ADMET

Houston, TX October 2023

ADME scientists of today and tomorrow are data/knowledge integrators seeking translation from preclinical to clinical and back again





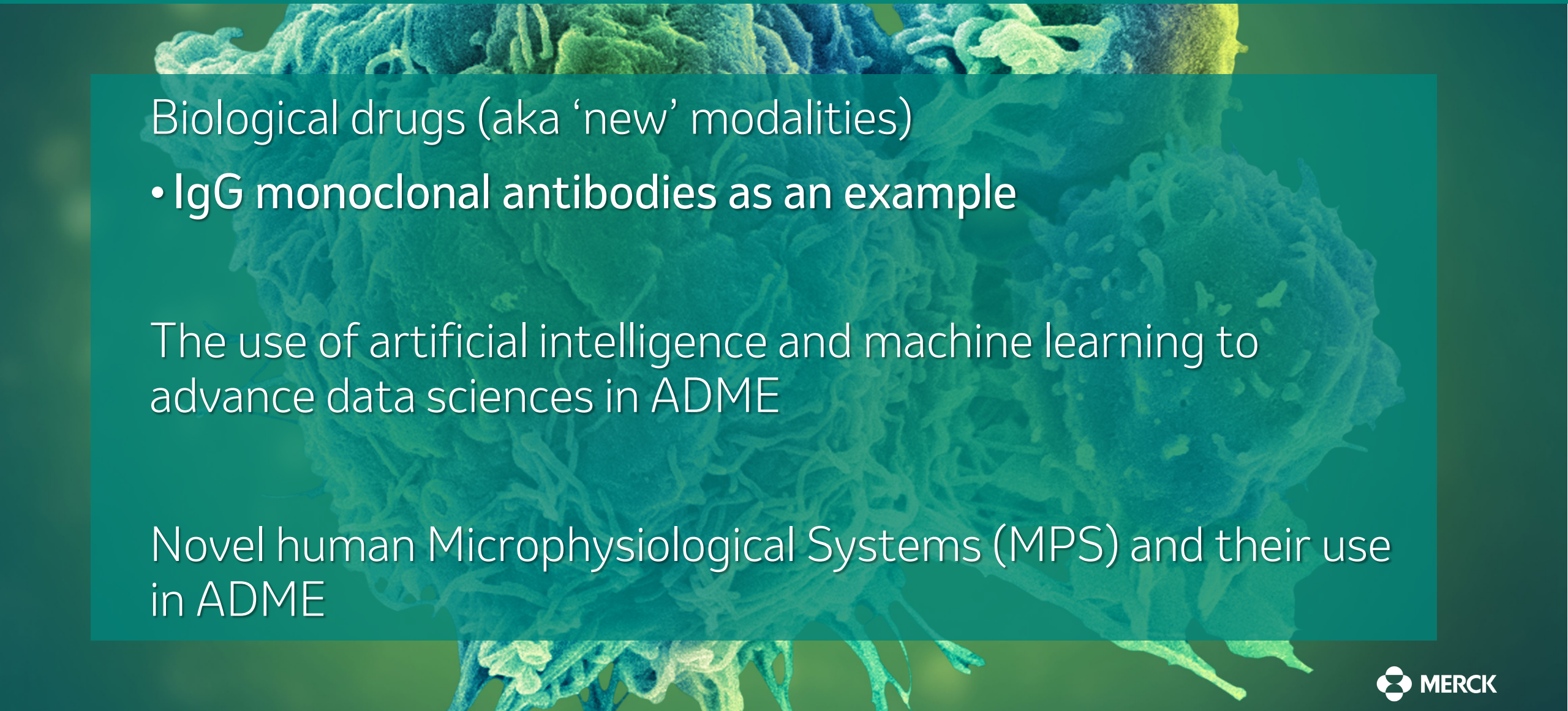
An overarching theme throughout this talk is the implicit desire to link biological, biophysical and other types of data into predictive translational models

Today's ADME scientist uses a suite of predictive translational tools to enable teams to answer tough questions

"..the integration of *in silico*, *in vitro* and *in vivo* preclinical data with mechanism-based models to anticipate the effects of new drugs in humans and across levels of biological organization."

Mager and Jusko CPT 83(6): 909-912

ADME Sciences: Where are we now and where do we need to go?



Biological drugs (aka ‘new’ modalities)

- **IgG monoclonal antibodies as an example**

The use of artificial intelligence and machine learning to advance data sciences in ADME

Novel human Microphysiological Systems (MPS) and their use in ADME

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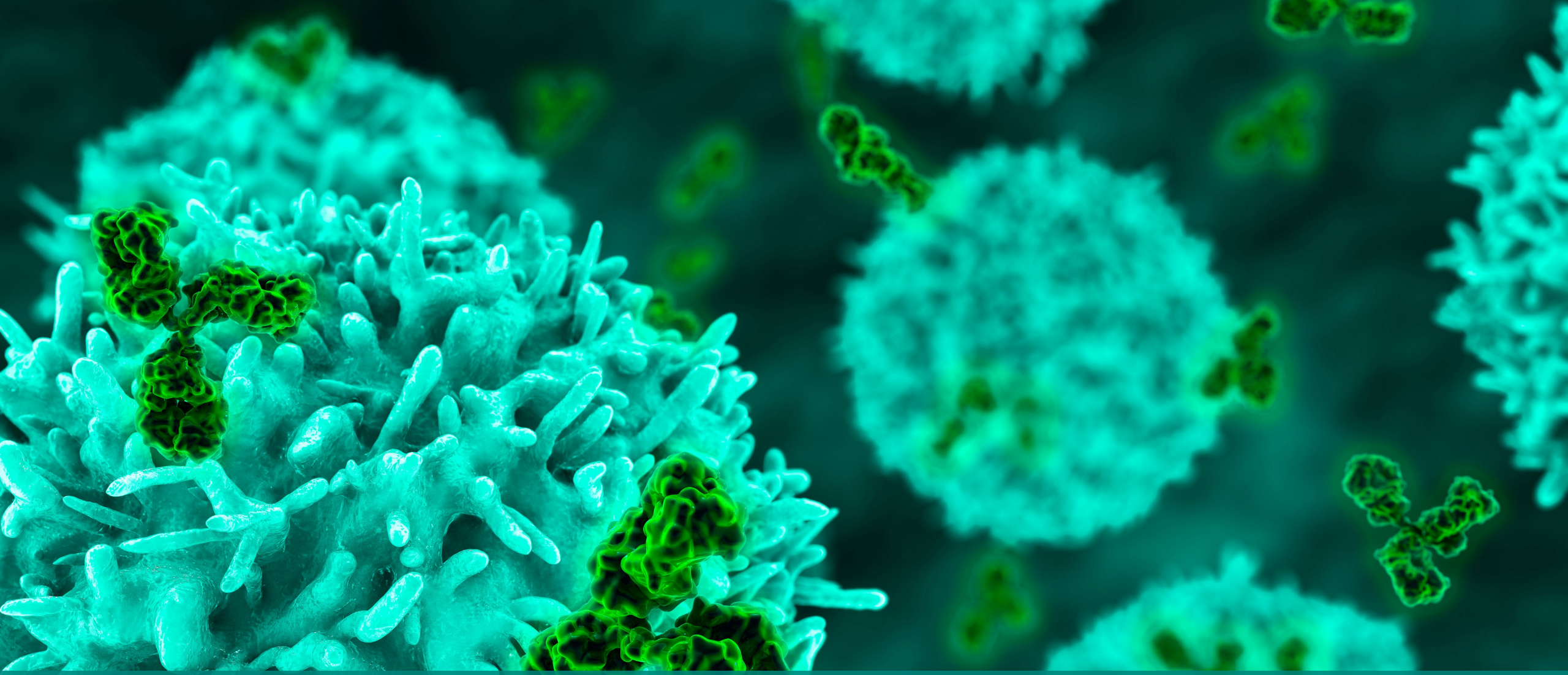


Biological drugs (aka ‘new’ modalities)

- IgG monoclonal antibodies as an example

The rate of approval for biologics drug is increasing

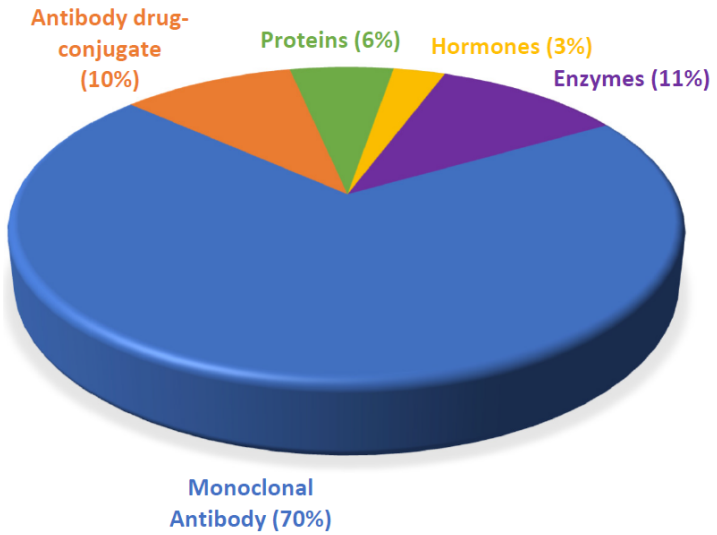


Figure 3. Percentage of new biopharmaceuticals approved by the Food and Drug Administration (U.S. FDA) from 2015 to 2021 [2,17–21].

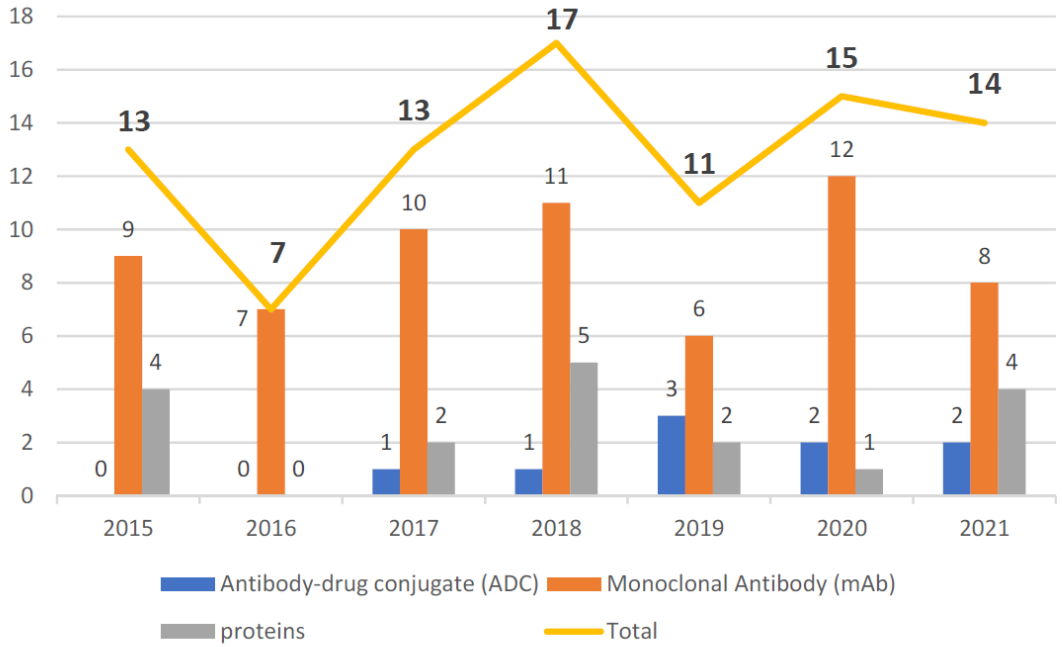


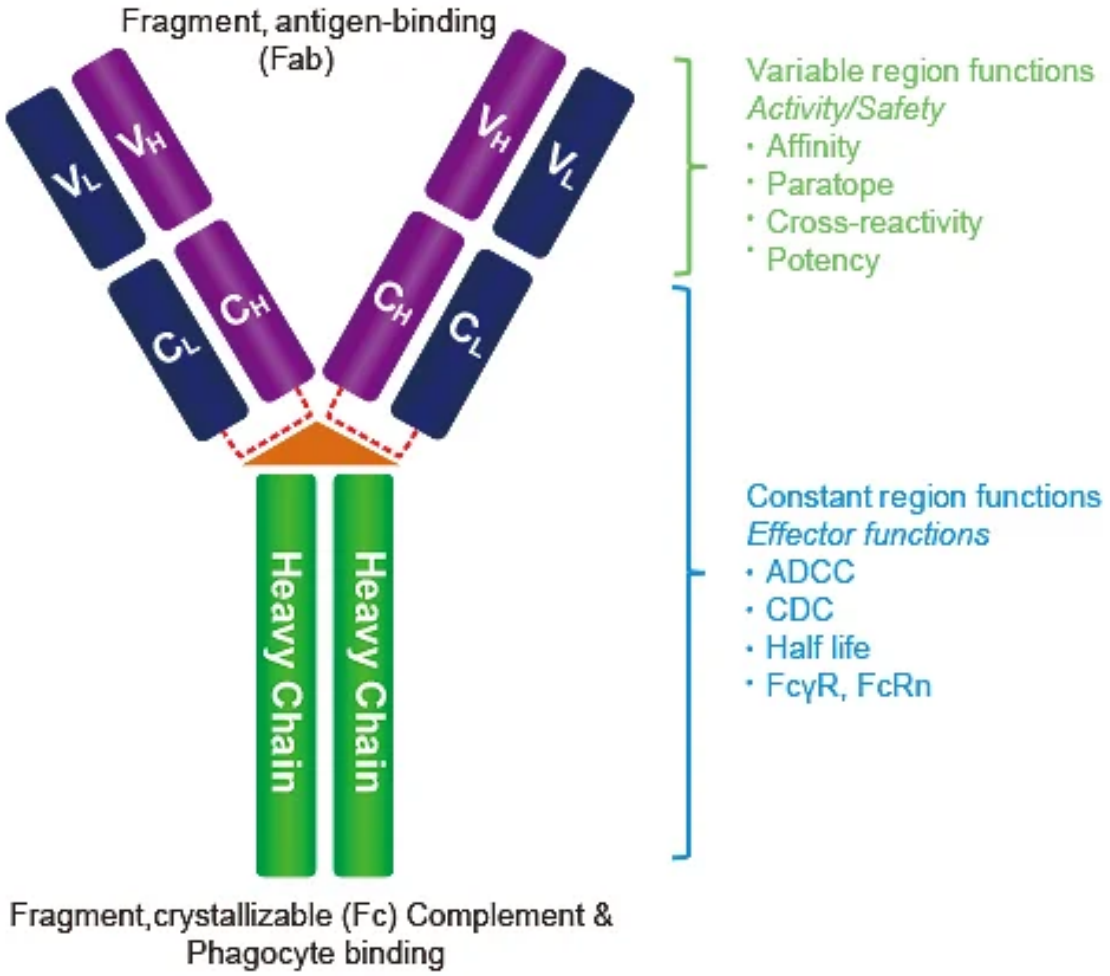
Figure 2. Biologicals approved by the Food and Drug Administration (FDA) from 2015 to 2021 [2,15–21].

- Biologic drugs produced by living systems
- Complex molecules not easily identified or characterized
  - Glycosylation and other post-translational modifications

Martins, A.C., et al. 2022. Trends and Perspectives of Biological Drug Approvals by the FDA: A Review from 2015 to 2021. *Biomedicines*, *10*(9), p.2325.

The anatomy and function of human Immunoglobulin G (IgG) antibodies

- IgG monoclonal antibody drugs have been successfully developed for a wide range of diseases including auto-immune/rheumatoid, cancer, hypercholesterolemia, migraine and Alzheimer's
- Two different regions of the antibody have different structure and function
- Fab portion of the antibody contains the complementarity determining region (CDR) and is responsible for binding to the target antigen



https://www.genscript.com/IgG-antibody.html

The Neonatal Fc receptor FcRN is critical for the disposition of IgG and their long plasma half-life

- Binding of the Fc portion of IgG to FcRN plays a major role in governing the long half-life of some monoclonal antibody drugs
- Protects the mAb from lysosomal degradation and recycles back to cell surface
  - pH sensitive binding
- Humanized FcRN knock-in mice needed to study

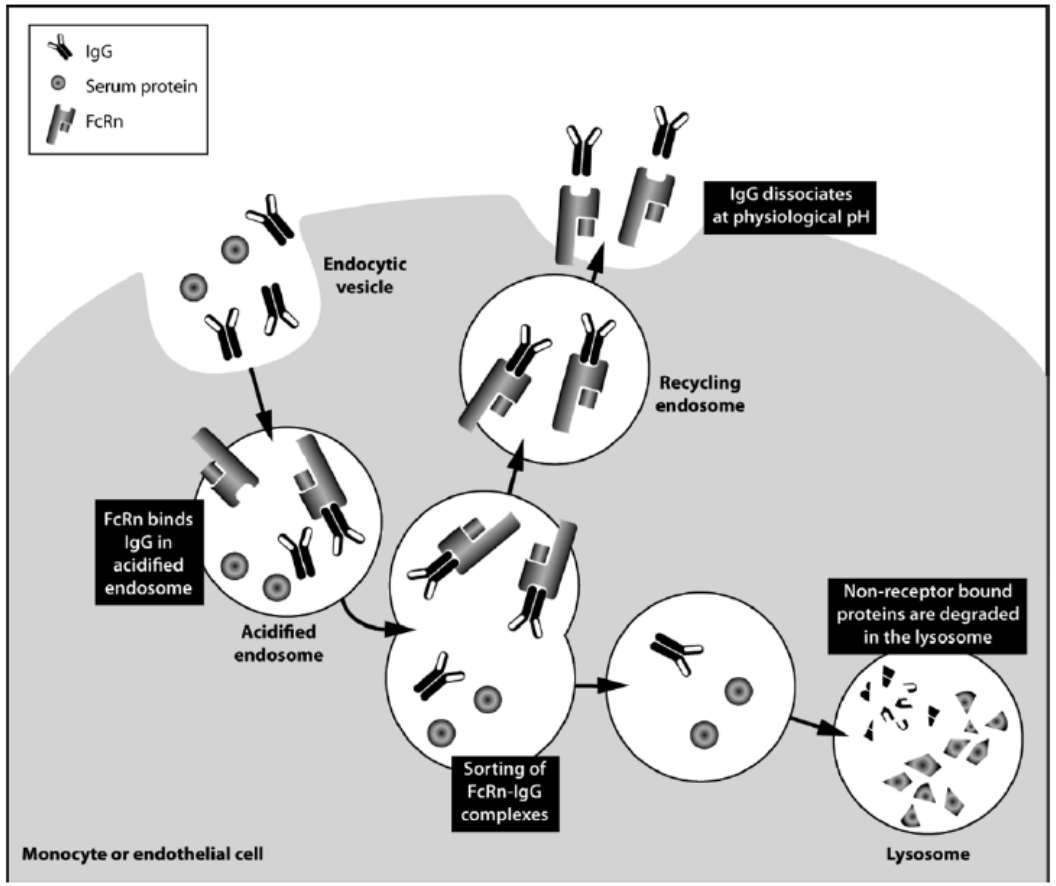
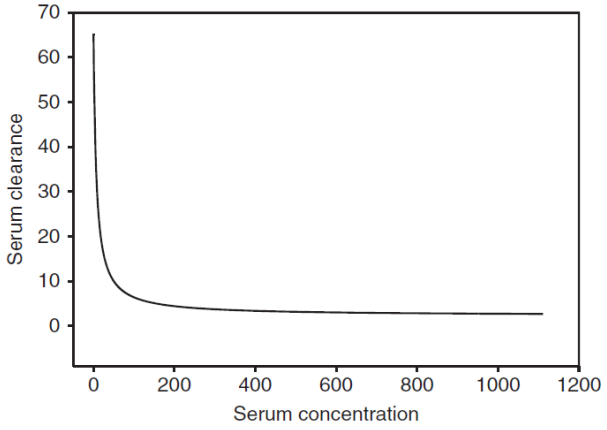


Figure 19.1 Following uptake into the cell, IgG binds to FcRn as the pH is decreased. After sorting, the IgG-FcRn complexes are returned to the cell surface where the IgG is released back into the interstitial space. Any IgG that does not bind FcRn upon entering the cell is destined for degradation. (Adapted from D.C. Roopenian and S. Akilesh. 2007. *Nature Rev. Immunol.* 7:715–725.)

Chapter 19 – C Gibson, P Sandhu and W Hanley. Therapeutic Monoclonal Antibodies: From Bench to Clinic. Edited by Zhiqiang An. John Wiley & Sons, Inc.

Target-Mediated Drug Disposition (TMDD) Is Commonly Observed for mAbs binding cell surface targets

- Binding of an antibody to a cell surface target will cause the bound complex to endocytose into the cell for degradation
  - As the mAb is consumed as it exerts its pharmacologic effect
- Causes non-linear pharmacokinetics and increased plasma clearance
  - The dynamics of the target can also influence PKPD and dose
- Opportunities for translational PBPK and QSP modeling



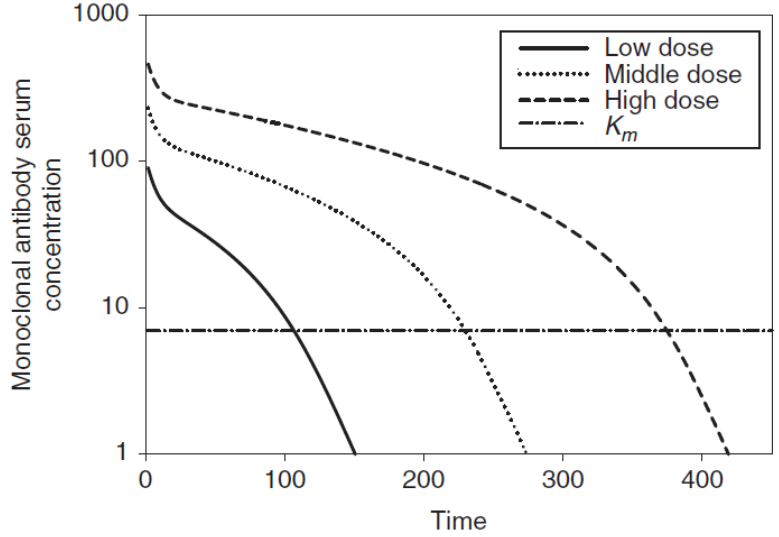


Figure 19.4 Simulated serum concentration-time profiles of a hypothetical monoclonal antibody, following three escalating doses, which behaves according to the pharmacokinetic model shown in Figure 19.2. For this example, the K_m was fixed to a value of 7 arbitrary concentration units.

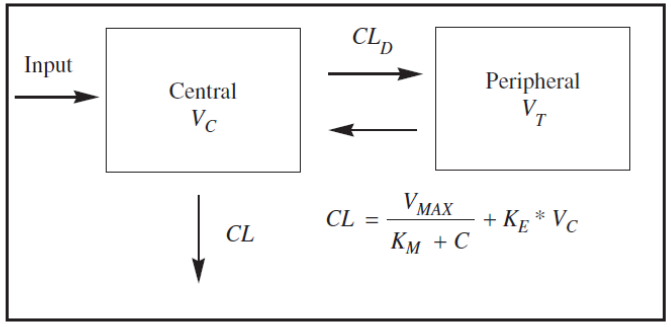


Figure 19.2 A classical two-compartment open model with parallel saturable (Michaelis–Menten) and first-order elimination occurring from the central compartment. CL = clearance, CL_D = distribution clearance resulting from drug movement between central and peripheral compartment, V_C = volume of central compartment, V_T = volume of peripheral compartment, K_E = first-order elimination rate constant, V_{MAX} = maximum rate of elimination from saturable pathway, K_M = serum concentration where saturable elimination rate is half maximal (Michaelis constant), C = serum concentration.

Chapter 19 – C Gibson, P Sandhu and W Hanley. Therapeutic Monoclonal Antibodies: From Bench to Clinic. Edited by Zhiqiang An
John Wiley & Sons, Inc.

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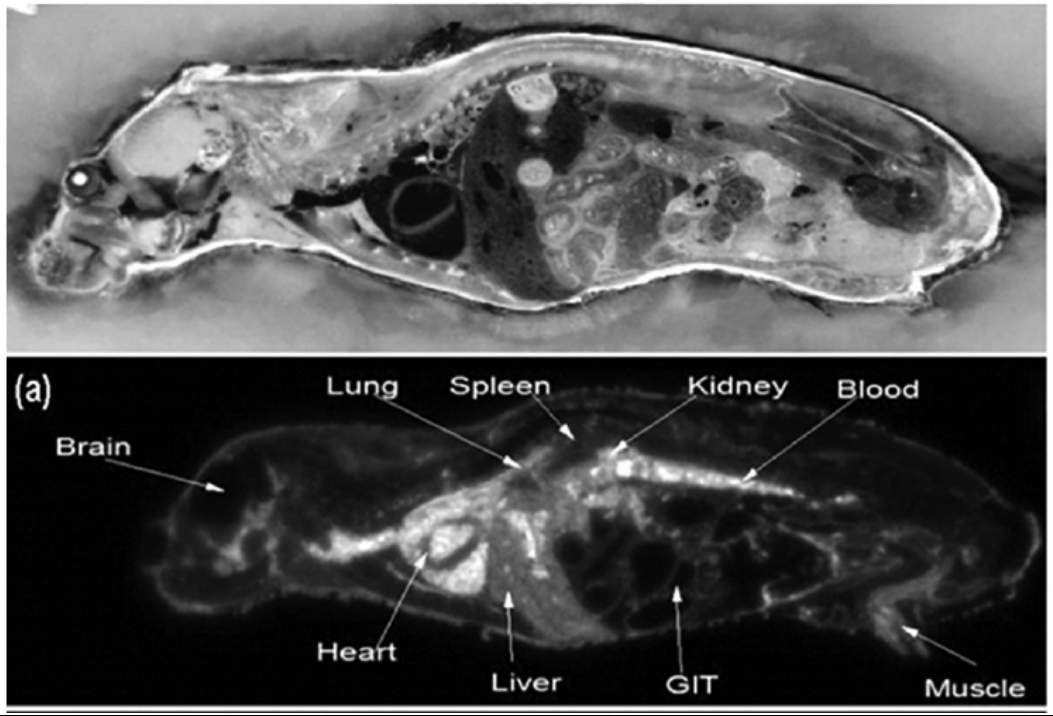
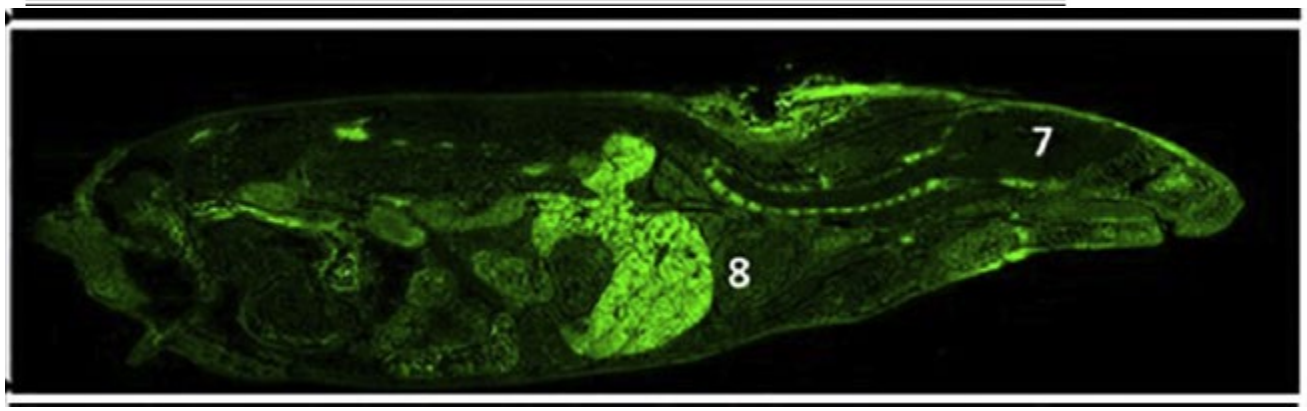
The Biodistribution of IgG Drug Candidates Have Been Extensively Characterized

- Biodistribution studies of biologics employ a variety of strategies to label the biologic (fluorescence or radiolabel) and study the whole body distribution
- Historically have used primates and transgenic mice
  - Lack of species cross-reactivity
- Studies like these of IgG distribution have been well characterized in non-clinical species and used to build predictive PBPK models
- Brain distribution of IgG is very low and is ~ 0.1-0.3% of the plasma concentration*
- Novel engineered and other tri-specific mAbs would need their in vivo biodistribution characterized





48 hrs post 8 mg/kg IV bolus dose anti-topotecan IgG1

Conner, K.P., Devanaboyina, S.C., Thomas, V.A. and Rock, D.A., 2020. *Pharmacology & therapeutics*, *212*, p.107574.

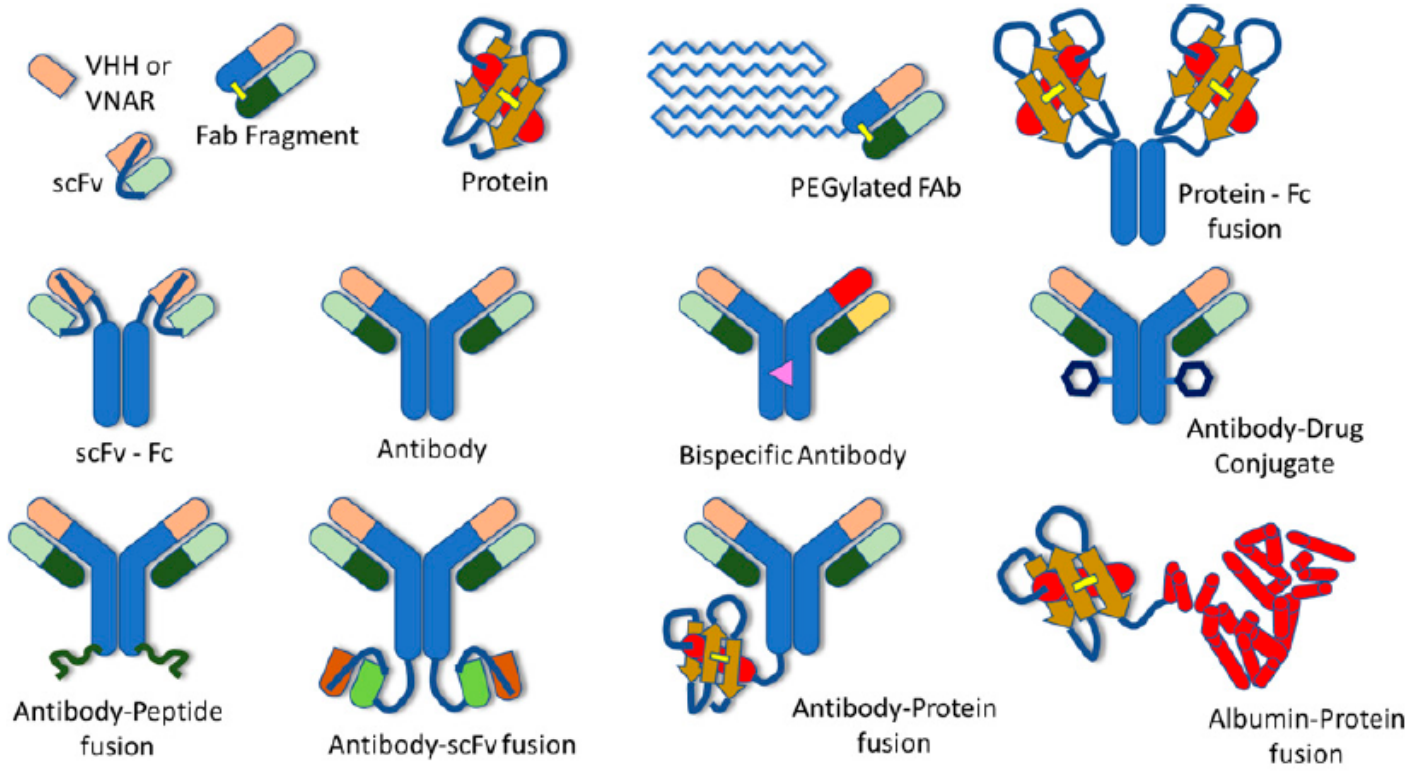
* Shah, D.K. and Betts, A.M., 2013, March. *MAbs* (Vol. 5, No. 2, pp. 297-305).

High Level Generalities of Small Molecule and Biologics ADME

ADME Properties	Small Molecules	Biologics
Absorption	Oral or S.C. – largely driven by solubility and passive permeability	IV, IM or SC – passive permeability negligible so driven by lymphatics
Distribution	Passive diffusion and active transport and non-specific tissue binding	Fluid-phase convection and endocytosis by the reticuloendothelial system, no passive flux. Binding to FcRN and target are critical.
Metabolism	Biotransformed by classical Phase 1&2 DME families such as CYP450 and UGT.	*Catabolism* occurs by proteolytic enzymes to smaller peptide fragments. Possible to have target-mediated drug disposition (TMDD)
Excretion	Can be excreted unchanged in the urine (active or passive) and bile/gut (active)	No unchanged excretion

Assays and approaches used to measure and model these are also different

There is a wide (and ever growing) range of biologics constructs being pursued as therapeutics



VHH – camelid antibody; VNAR – variable new antigen receptors; PEG – polyethylene glycol; scFv – single chain variable fragment; Fab – antigen binding fragment; Fc – crystallizable fragment

It should be noted that the diversity of molecules shown in this diagram is not exclusive, and represents known modalities to the authors currently in preclinical or clinical development. Not all these formats have been granted market authorisation.

The differences in molecular weight and overall physical chemical properties give each of these construct unique aspects to their disposition that requires study and characterization

Bolleddula, J., et al., 2022. Absorption, distribution, metabolism, and excretion of therapeutic proteins: current industry practices and future perspectives. *Drug Metabolism and Disposition*, *50*(6), pp.837-845.

Antibody Drug Conjugates (ADC) have complexity both of a biologic and small molecule and requires both to be optimized

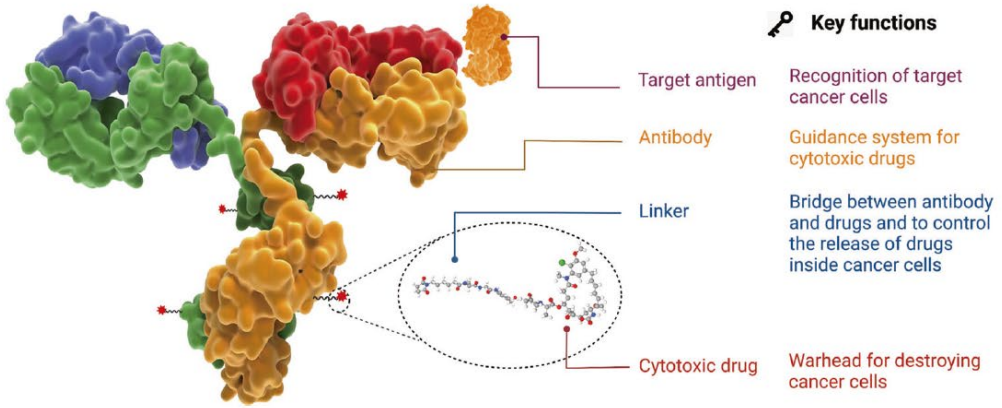
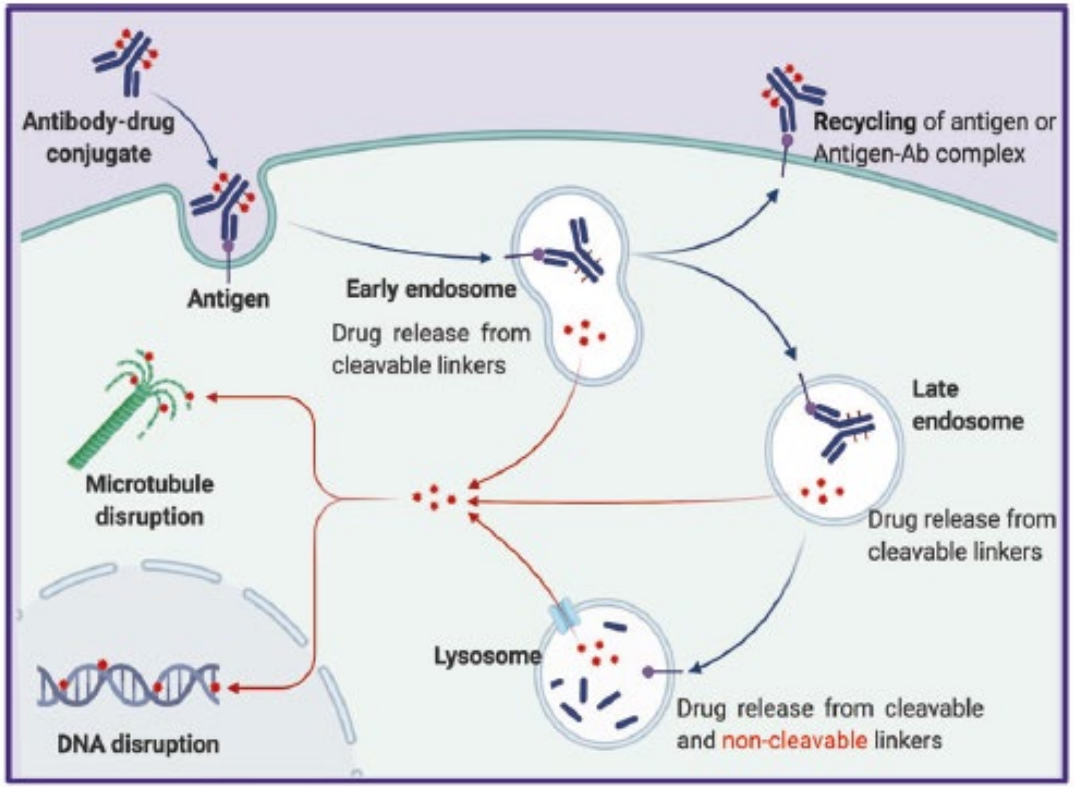
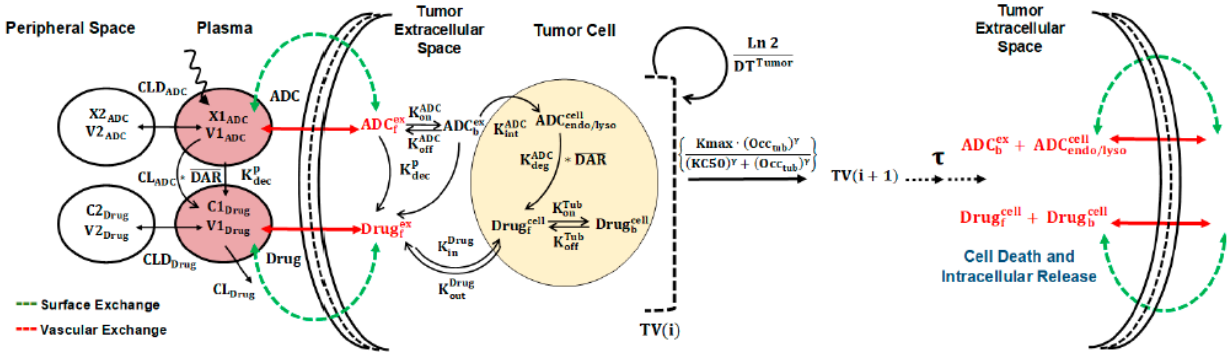


Fig. 2 The structure and characteristic of an ADC drug. The core components including target antigen, antibody, linker, cytotoxic drug along with their key functions are demonstrated.



Holistic QSP models of ADC disposition

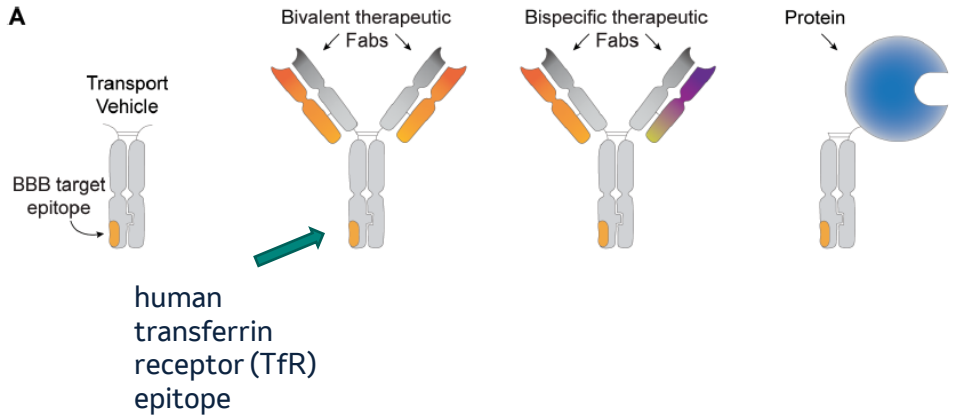


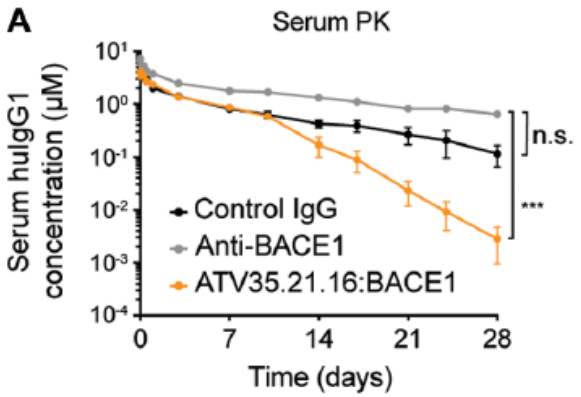
Plasma PK → Tumor Distribution → Single Cell Disposition → Single Cell Killing

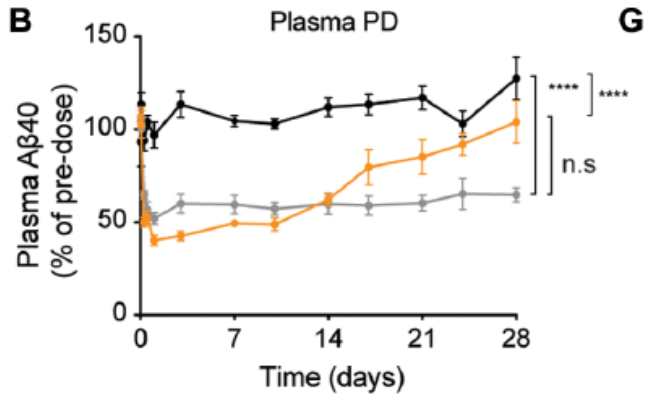
Fu, Z., Li, S., Han, S., Shi, C. and Zhang, Y., 2022. Antibody drug conjugate: the "biological missile" for targeted cancer therapy. *Signal transduction and targeted therapy*, 7(1), p.93.

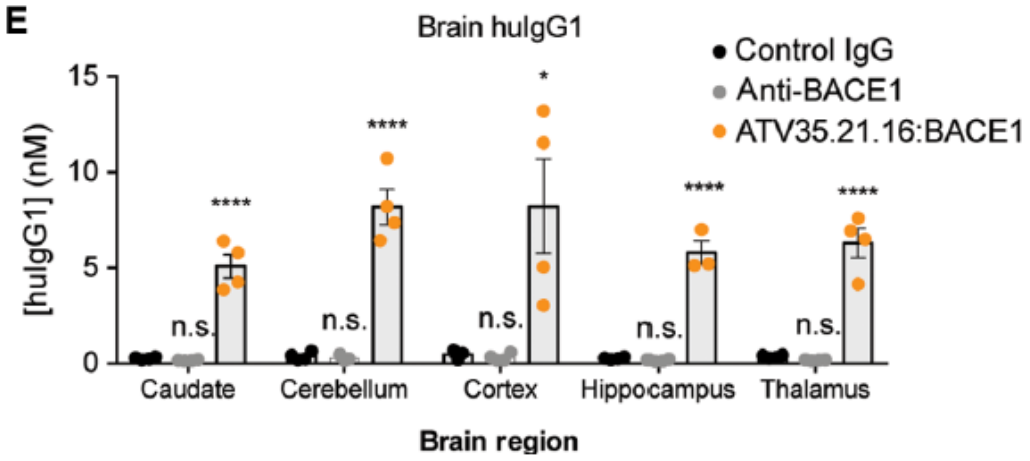
Singh, A.P., Guo, L., Verma, A., Wong, G.G.L. and Shah, D.K., 2019. A cell-level systems PK-PD model to characterize in vivo efficacy of ADCs. *Pharmaceutics*, *11*(2), p.98.

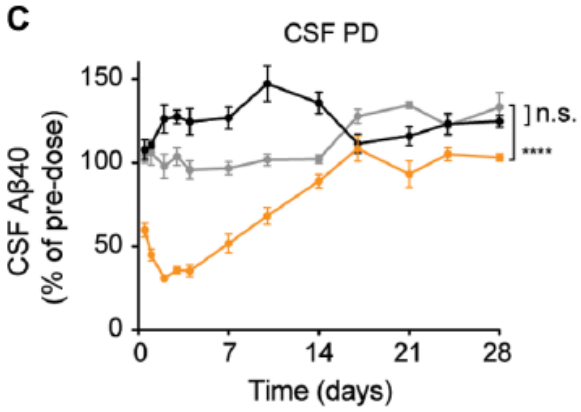
Trojan Horse Approaches Utilizing Receptor-Mediated Transcytosis to Boost CNS Exposure and Pharmacodynamics of Biologics

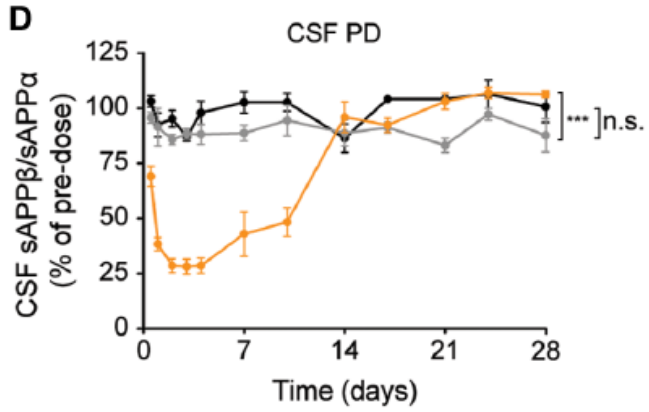












PKPD of an inhibitory mAb against BACE1 using TfR-mediated RMT

- Single 30 mg/kg IV dose to cynomolgus monkeys (a cross-reactive species)

Substantial boost in brain PKPD with TfR ATV clone relative to parental mAb

- Also has increased plasma clearance due to TfR-mediated TMDD

Kariolis, M.S., et al., 2020. *Science translational medicine*, *12*(545), p.eaay1359.

Trojan Horse Brain Shuttles To Boost CNS Exposure of Biologics Introduce New Complexities And Opportunities

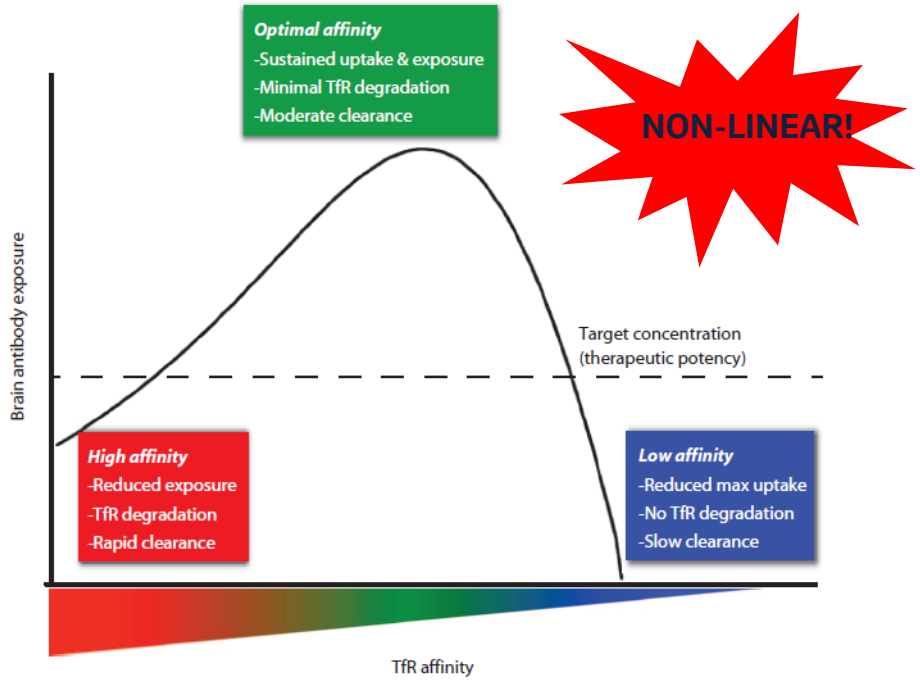
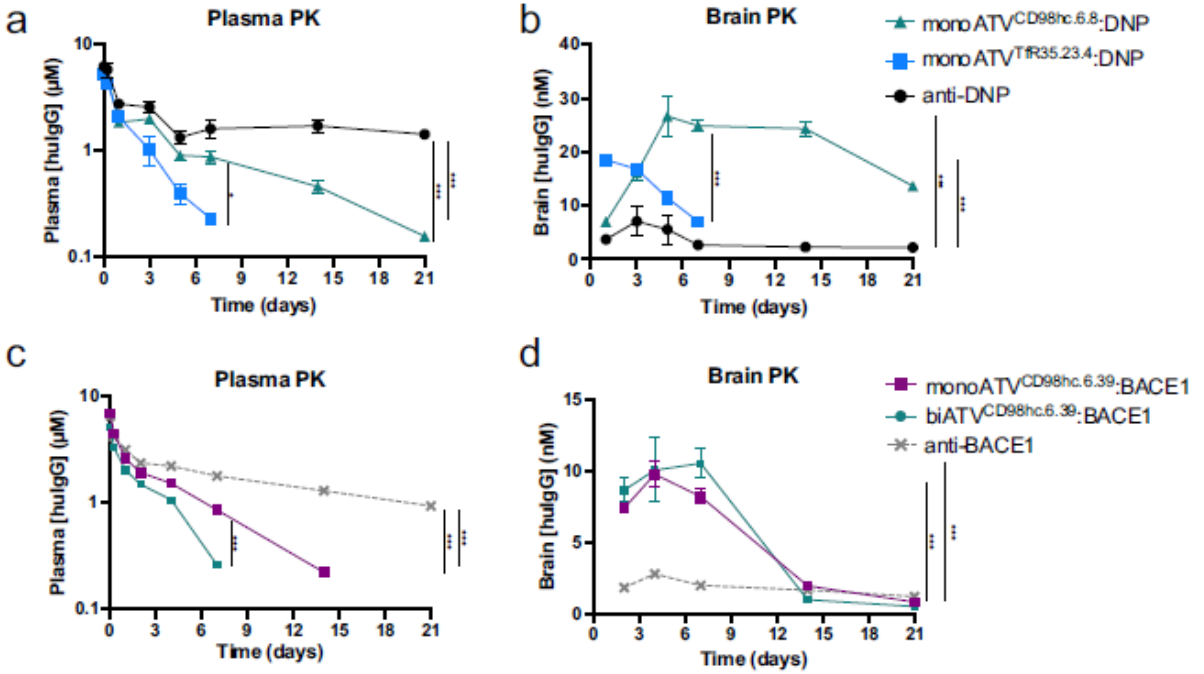


Fig. 5. Relationship between TfR affinity and sustained antibody uptake by brain tissue. Data across multiple species and across multiple TfR antibody affinities suggested a model relating TfR affinity (*x* axis) to brain antibody exposure (*y* axis). Monovalent anti-TfR bispecific antibodies with high affinity (red) show reduced sustained exposure in both brain and periphery as a result of rapid clearance and TfR degradation. Low-affinity binding to TfR (blue) results in reduced maximal brain uptake; however, TfR protein levels are not altered, and both systemic and brain antibody concentrations are sustained as a result of slow clearance. An optimal affinity for TfR (green) shows sustained presence of antibody in brain above therapeutic target concentrations (threshold depends on the potency of the therapeutic arm, dashed line). Minimal TfR degradation and moderate anti-TfR antibody clearance are observed with an optimal TfR affinity. The exact optimized TfR affinity depends on the species/model being investigated and possibly other properties of the selected TfR antibody (for example, TfR expression and anti-TfR antibody epitopes).



- DNP antibodies (dinitrophenol, antigen not present in vivo) prepared with either transferrin receptor (TfR) or CD98hc showed distinct PK within the CNS
- Open the possibility of designing antibodies/fusions that can penetrate the BBB better and target specific cells within the CNS once inside

Yu, Y.J., et al. 2014. *Science translational medicine*, *6*(261), pp.261ra154-261ra154.

In vitro assays used to study the disposition of biologics

The exquisite target binding selectivity of some biologics compromises the translational value of in vivo studies done in non-binding species

- Often has equated with increased use of non-human primates and transgenic animal models

The development of human relevant in vitro assays to triage ADME properties of new biologics drug candidates is essential

- Consistent with the principals of the 3R's - replacement, reduction, and refinement of animals used in research, teaching, testing, and exhibition
- Allow's for iterative rounds of protein engineering for optimization prior to in vivo studies

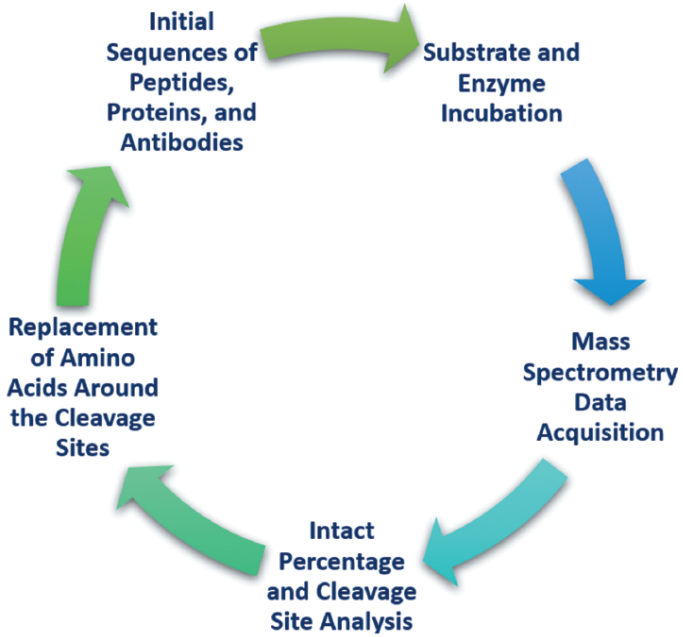


Figure 1. A generic workflow for identifying the cleavage sites and engineering the sequence to improve proteolytic stability. Initially, a peptide, protein, or antibody is selected based on potency for further optimization, including improving proteolytic stability. The selected molecule is incubated with a peptidase or a mixture of peptidases at certain pH, temperature, and duration. The samples are processed and analyzed by LC-MS. From the acquired MS data, the percentage of the intact molecule at various time points is calculated comparing to time 0. Meanwhile, the degradants are identified to provide cleavage site information. Based the cleavage sites, replacement of amino acids around the cleavage sites is performed, generating a new molecule for next round of testing and engineering.

In vitro proteolysis assays used in biologics discovery include the use of various types of models

- Whole blood and tissue homogenate (e.g. liver and kidney), pancreatin
- Purified subcellular fractions like lysosomes
- Purified proteases and peptidases

Lai, X., Tang, J. and ElSayed, M.E., 2021. Recent advances in proteolytic stability for peptide, protein, and antibody drug discovery. *Expert Opinion on Drug Discovery*, *16*(12), pp.1467-1482.

Probing for protein intrinsic conformational stability using a novel thermolysin assay

Key questions we want to address

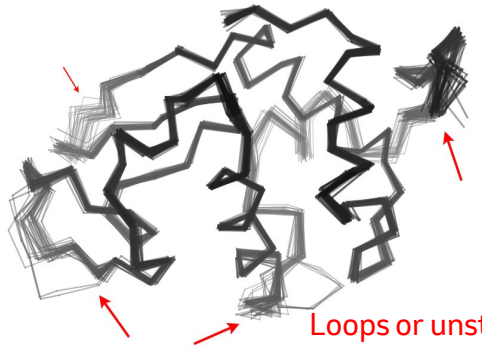
- **Can the assay allow us to triage the molecules based on their stability?**
- **Will it allow us to predict the stability of molecules *in vivo?***

As proteins traffic throughout the tissues/cells or interact with their binding partners, they may undergo region specific local unfolding events (conformational dynamics) providing different and additional regions of the primary sequence that are accessible to proteolysis

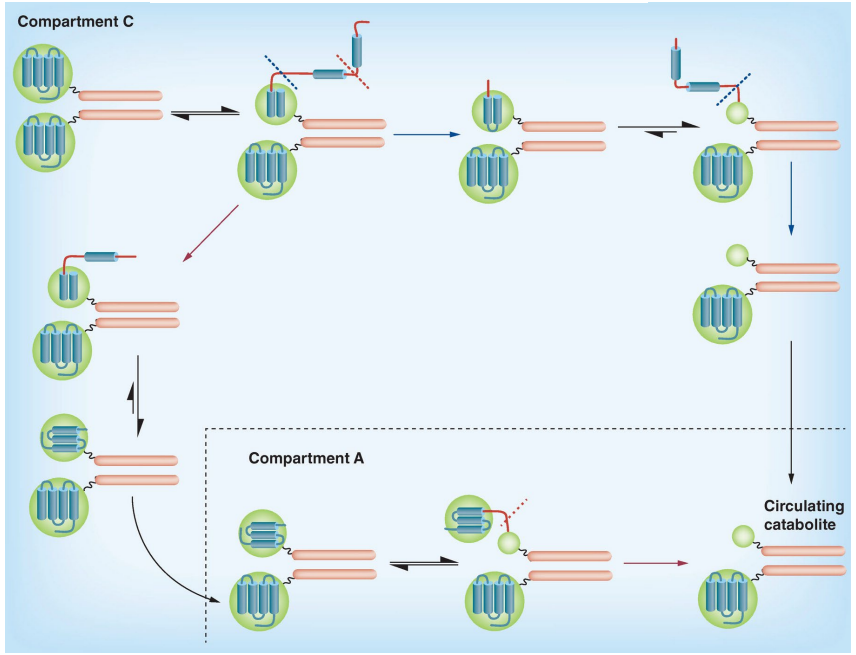
Thermolysin:

- Bacterial metalloprotease
- Can detect exposed hydrophobic patches
- Cleaves peptide bonds at N-terminal side of hydrophobic amino acids
- Is sensitive to detect the conformational instability of proteins *in vitro*

Protein breathing/motions



Loops or unstructured region



Bioanalytical approaches to assess the proteolytic stability of therapeutic fusion proteins

Josh T Pearson & Dan A Rock

Pearson, Josh T., and Dan A. Rock. "Bioanalytical approaches to assess the proteolytic stability of therapeutic fusion proteins." *Bioanalysis* 7.23 (2015): 3035-3051.

Affinity maturation techniques can introduce complexities that can result in poor pharmacokinetics

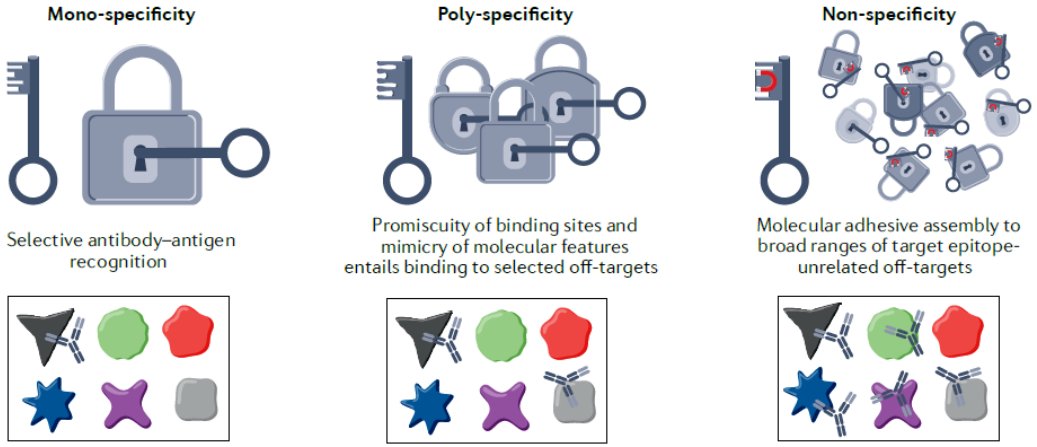


Fig. 1 | Definitions of mono-specificity, poly-specificity and non-specificity. Ideal mono-specific antibody–antigen recognition is often compared to a lock-and-key mechanism, as only the intended target is recognized. Poly-specificity is the selective recognition of off-targets based on the flexibility of the paratope. This is indicated by a less well-defined key or molecular mimicry of target epitope features shown by padlocks with the same keyhole but altered appearances, all of which facilitate the applicability of one key to multiple locks. Non-specificity describes the binding of an antibody to a variety of off-targets unrelated to the target epitope that resembles a magnetic key attracting all ferromagnetic locks. This 'stickiness' is a crucial hindrance to antibody development. The origins of this behaviour have remained elusive to date, spurring this Review of existing literature in which we highlight the contributions of so-called surface patches to generating non-specificity.

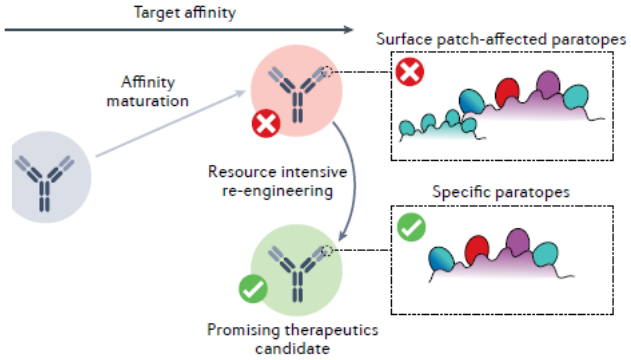


Fig. 7 | Affinity maturation commonly yields potent but difficult-to-develop candidates. Both high affinity and specificity are crucial for antibody therapeutic candidates as drug molecules. Common maturation approaches are often unable to improve target affinity without comprising specificity. This frequently leads to antibodies that are affected by surface patches, which require resource-intensive engineering to recover suitable therapeutic candidates.

Reviews Chemistry | Volume 6 | December 2022 | 844–861

Non-specificity can cause the mAb to bind to a variety of receptors (e.g. scavenger receptors) and increased endogenous endocytosis in the reticuloendothelial system *resulting in rapid plasma clearance*

Non-specificity also promotes mAb self-aggregation to larger complexes which exacerbates the non-specific clearance and can cause anti-drug antibodies (ADA)

Ausserwöger, H., et al. 2022. Non-specificity as the sticky problem in therapeutic antibody development. *Nature Reviews Chemistry*, *6*(12), pp.844-861.

When amino acid sequences go wrong

When a set of amino acids with similar physical-chemical properties reside close to each other they cause patches

- These surface patches can be either hydrophobic or hydrophilic and can drive interactions with cell surface membranes and proteins
- The increased cell surface interactions will result in increase clearance from the blood

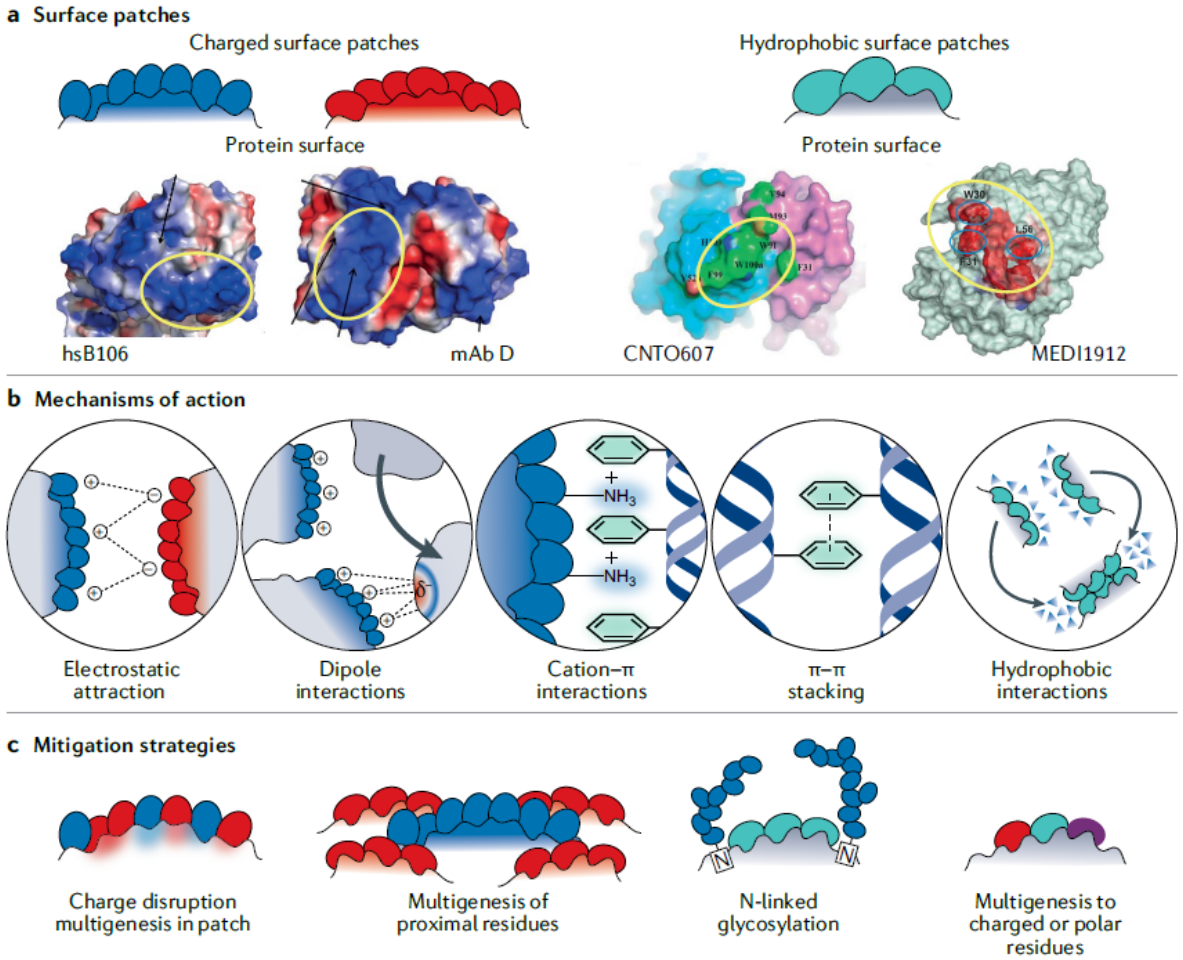


Fig. 4 | Surface patches in literature and their modes of action for non-specific binding and common mitigation strategies. Surface patches are regularly found in the literature as key contributors to non-specific binding and appear as local accumulations of residues with similar physicochemical properties on the protein surface (part **a**). This allows for surface patches to engage in common supramolecular assembly interactions (part **b**) and usually can only be mitigated by disruption or masking of the patch features (part **c**). In part **a**, structural models of representative surface patch-affected antibodies were adapted as follows: hsB106, adapted from ref.[123] CC-BY 4.0 (https://creativecommons.org/licenses/by/4.0/); mAb D, adapted with permission from ref.[64] Taylor & Francis Ltd; MEDI1912, adapted from ref.[66] CC-BY 4.0 (https://creativecommons.org/licenses/by/4.0/); CNTO607, adapted with permission from ref.[82] Oxford University Press.

Ausserwöger, H., et al. 2022. Non-specificity as the sticky problem in therapeutic antibody development. *Nature Reviews Chemistry*, *6*(12), pp.844-861.

Polyspecificity Assay To Identify Antibody Clones at Risk of High In Vivo Clearance

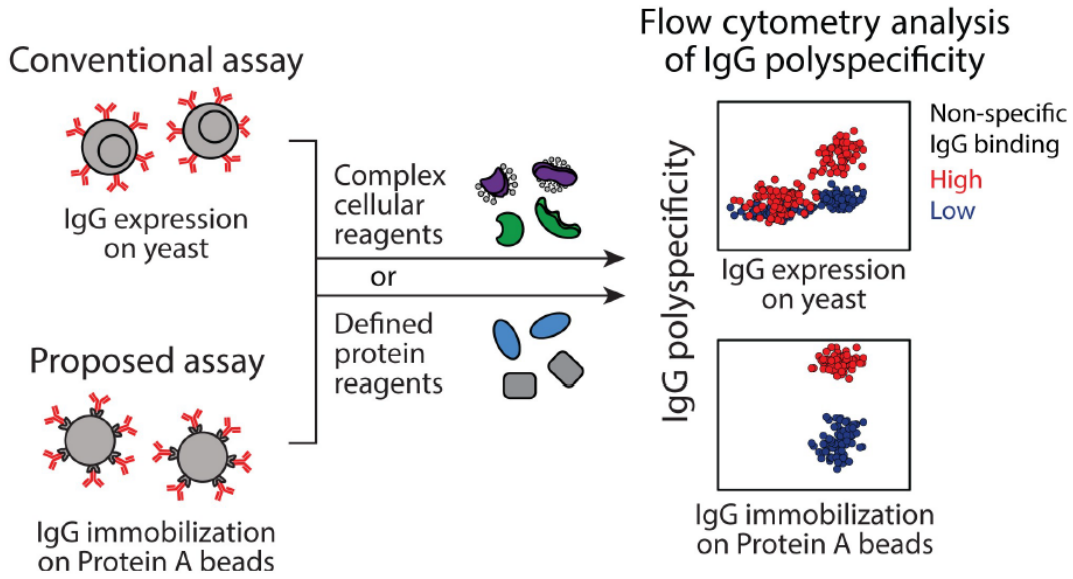


Figure 1. **Overview of the PolySpecificity Particle (PSP) assay for evaluating antibody nonspecific interactions.** In the PSP assay, mAbs are immobilized on micron-sized magnetic beads coated with Protein A and then the conjugates are incubated with different types of polyspecificity reagents. The levels of antibody nonspecific interactions are evaluated via fluorescence detection using standard flow cytometry methods. The primary advantages of the PSP assay relative to the conventional PolySpecificity Reagent (PSR) assay are that the PSP assay does not require proprietary technologies for displaying full-length IgGs on yeast and it is compatible with the soluble IgGs and other soluble Fc-fusion proteins (including bispecific and multispecific antibodies).

PSR data collected on mAbs with varying degrees of polyspecificity and in vivo clearances

- Used to correlate to in vivo clearance
- Also integrated with other types of data (e.g. biophysical) to train predictive models for risk of high in vivo clearance

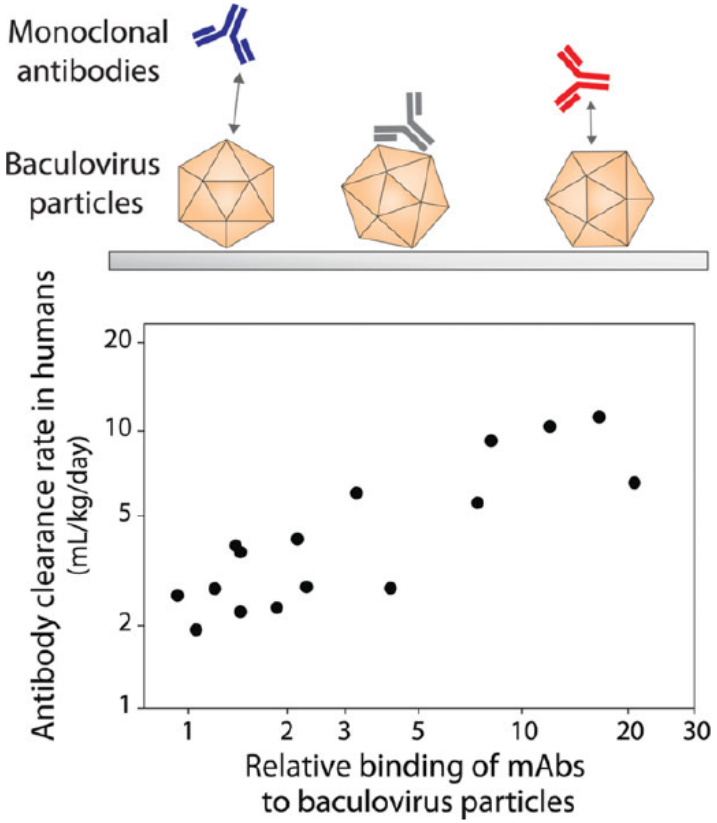
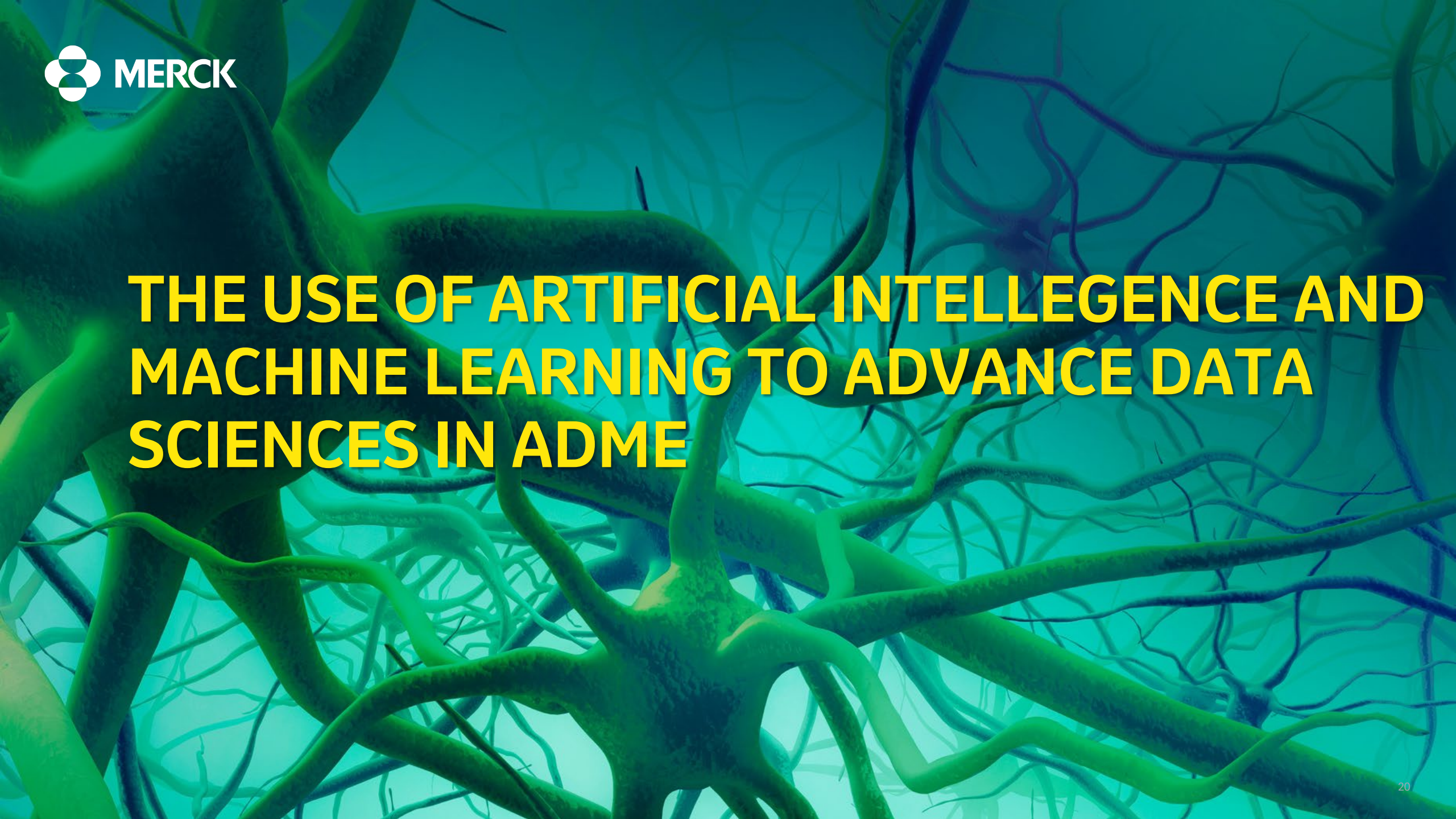


Figure 4. Nonaffinity interactions between monoclonal antibodies and baculovirus particles are correlated with the rate of antibody clearance in humans. Baculovirus particles are immobilized in microtiter plates, and the relative binding of antibody variants is evaluated. Increased antibody binding is correlated with increased rate of antibody clearance. The data are reproduced from a previous report.[14]

Makowski, E.K., et al., 2021, January. Highly sensitive detection of antibody nonspecific interactions using flow cytometry. In *MAbs* (Vol. 13, No. 1, p. 1951426). Taylor & Francis.

Geng, S.B., et al. 2014. *Journal of pharmaceutical sciences*, *103*(11), pp.3356-3363.


MERCK
THE USE OF ARTIFICIAL INTELLEGENCE AND MACHINE LEARNING TO ADVANCE DATA SCIENCES IN ADME

Do you remember when we were stuck with just ‘natural’ intelligence?

How Artificial Intelligence is Revolutionizing Drug Discovery

March 20, 2023 Matthew Chun Artificial Intelligence, Biotechnology, Matthew Chun, Pharmaceuticals

By Matthew Chun

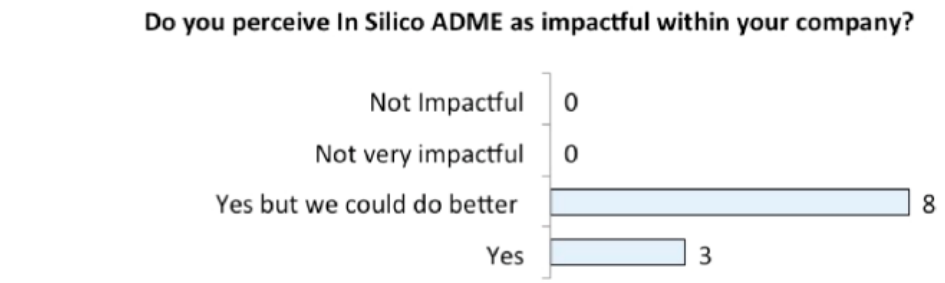
In recent months, generative artificial intelligence (AI) has taken the world by storm. AI systems like ChatGPT and Stable Diffusion have captured the imagination of the masses with their impressive

- **Target identification:** At the target identification phase of drug discovery, AI is being trained on large datasets, including omics datasets, phenotypic and expression data, disease associations, patents, publications, clinical trials, research grants, and more to understand the biological mechanisms of diseases and to identify novel proteins and/or genes that can be targeted to counteract those diseases. Combined with systems like AlphaFold, AI can go even further than mere target identification by predicting the 3D structures of targets and accelerating the design of appropriate drugs that bind to them.
- **Molecular simulations:** AI is also being used to reduce the need for physical testing of candidate drug compounds by enabling high-fidelity molecular simulations that can be run entirely on computers (i.e., *in silico*) without incurring the prohibitive costs of traditional chemistry methods.
- **Prediction of drug properties:** Some AI systems are being used to bypass simulated testing of drug candidates by predicting key properties such as toxicity, bioactivity, and the physicochemical characteristics of molecules.
- ***De novo* drug design:** While traditional drug discovery has historically involved the screening of large libraries of candidate molecules, AI is shifting this paradigm too. Some systems are capable of generating promising and never-before-seen drug molecules entirely from scratch.
- **Candidate drug prioritization:** Once a set of promising “lead” drug compounds has been identified, AI is used to rank these molecules and prioritize them for further assessment, with AI approaches outperforming previous ranking techniques.
- **Synthesis pathway generation:** Going beyond theoretical drug design, AI is also being used to generate synthesis pathways for producing hypothetical drug compounds, in some cases suggesting modifications to compounds to make them easier to manufacture.

https://blog.petrieflom.law.harvard.edu/2023/03/20/how-artificial-intelligence-is-revolutionizing-drug-discovery/

Quantitative Structure Activity Relationship (QSAR) Developed Using Machine Learning of Individual ADME Properties Have Been Used in Biopharma for >10 years

- QSAR is the science of using statistical and AI/ML approaches to relate a biological activity of a molecule directly to its chemical structure
- Building a QSAR model occurs in 4 steps
  1. Data set building and curation
  2. Model building and final model selection
     - Uses a wide array of molecular (e.g. MW, PSA, HBD) and substructure (e.g. atom pairs and heterocycles) to find patterns correlating with the biological endpoint
     - Often involving different 'training' and 'validation' data sets
  3. Model deployment and usage
  4. Ongoing model refinement with new data



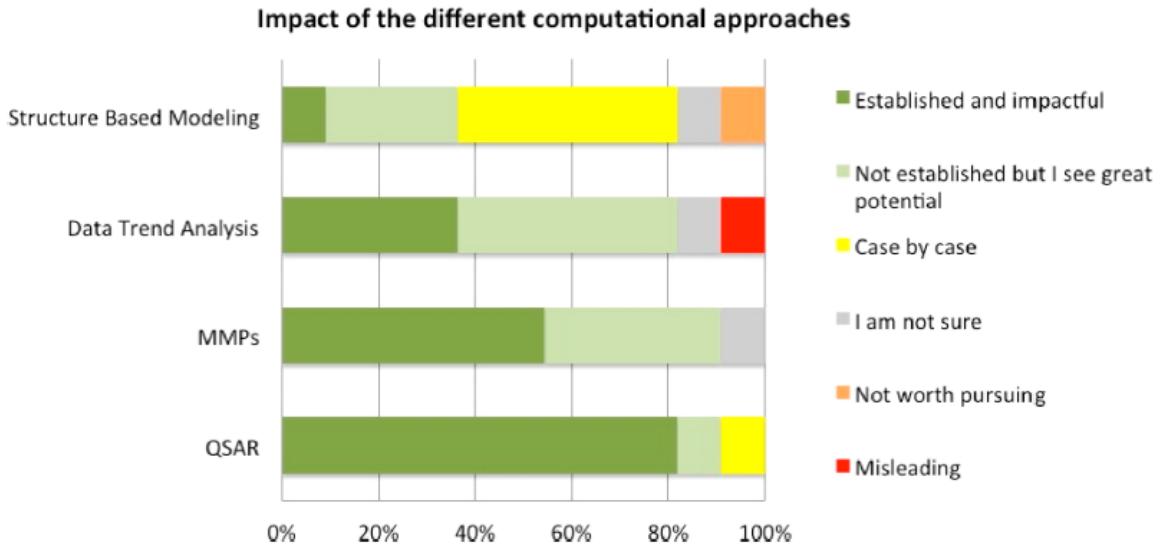
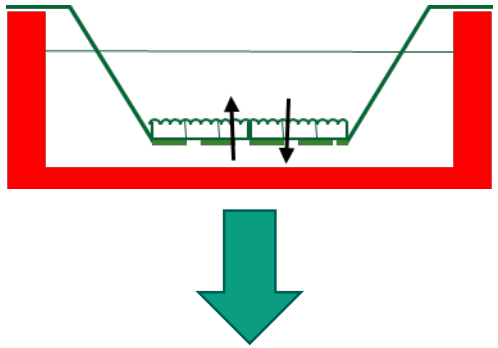


Figure 2. Results of the survey among industrial scientists involved in *in silico* ADME.

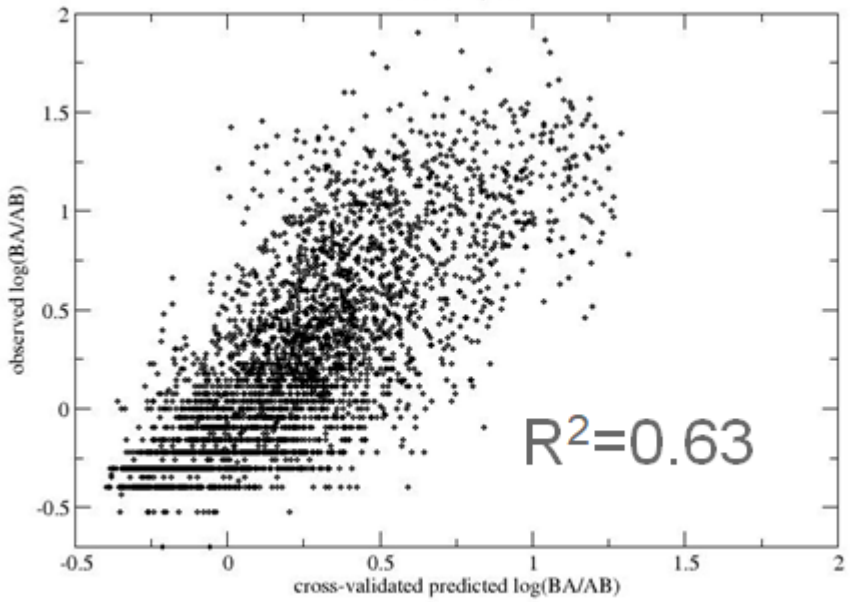
Lombardo, F., et al., 2017. *Journal of Medicinal Chemistry*, *60*(22), pp.9097-9113.

Illustrative Example Using In Vitro Pgp Efflux Ratio QSAR Model To Prioritize Study of Molecules Devoid of Active Efflux

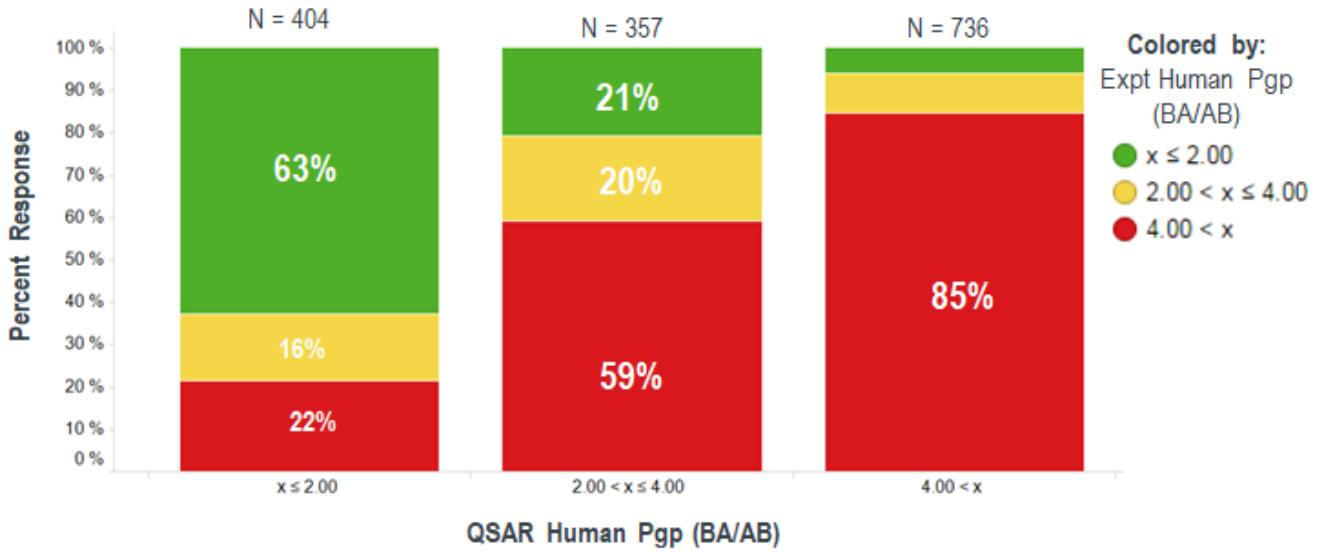
Monolayer cell model over-expressing human PgP



Model Build/Selection/Validation



Deployment and Usage



Model capable of categorizing molecules either as non-substrate, potential substrate and substrate with reasonable accuracy

Can be used with new candidate chemical structure to predict their Pgp efflux potential enabling synthesis/purification and experimental testing only on those structures in either the non-substate or potential substate category

- Termed ‘categorical enrichment’

AI/ML Approaches Extending Beyond Prediction of Individual ADME Parameters Into Integrative Analysis

Innovations in new AI/ML algorithms and approaches such as neural net technologies can enable better prediction of ADME endpoints directly from chemical structure, opening the possibility to using them in modeling and simulation of things like dose, exposure and regimen

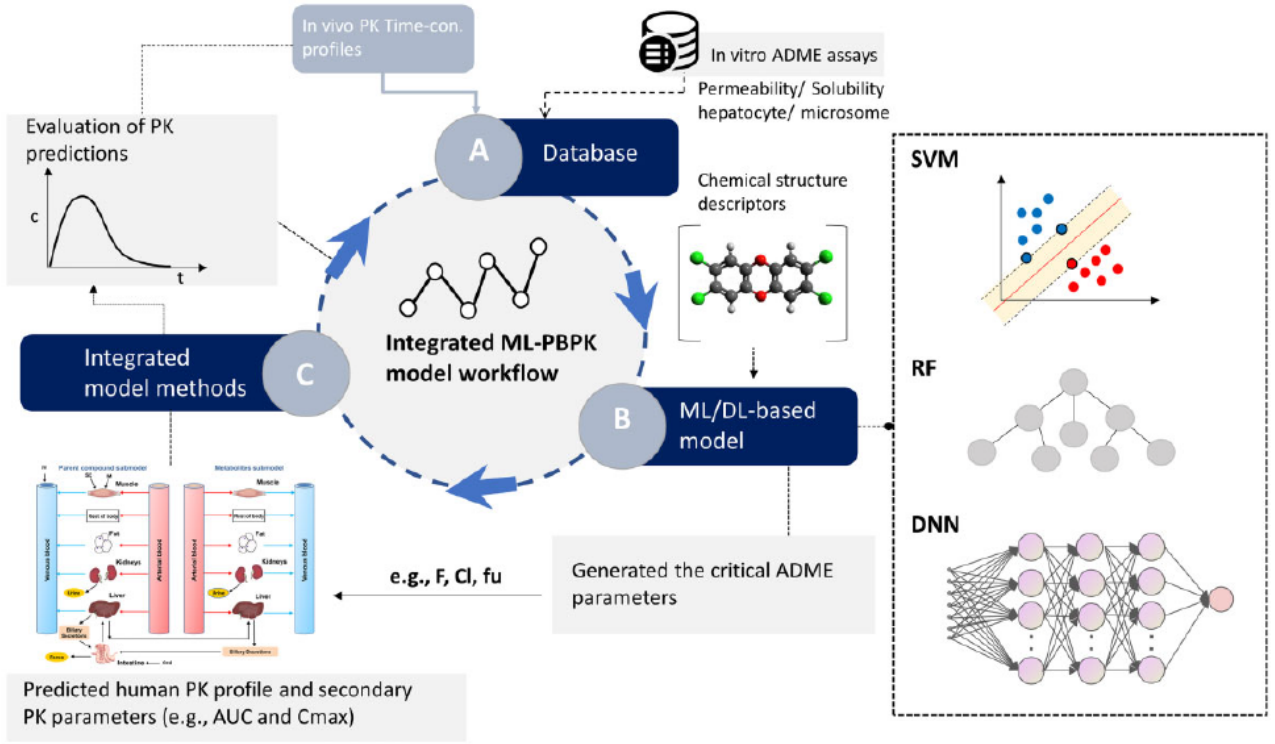
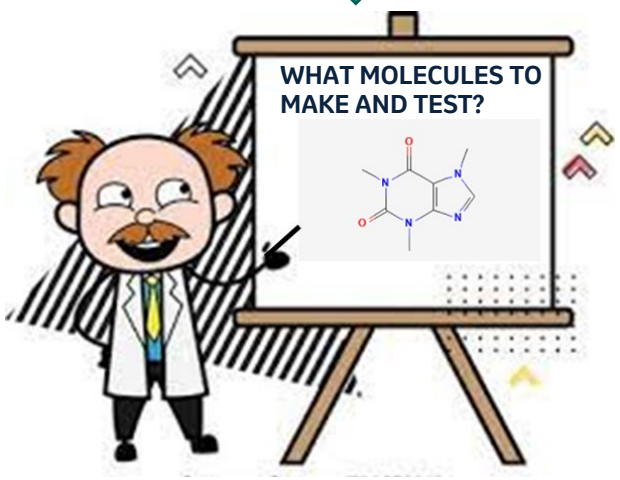


Figure 1. An emerging research paradigm to integrate machine learning and artificial intelligence approaches with physiologically based pharmacokinetic modeling. A, A database consisting of *in vivo* PK profiles and *in vitro* ADME assays (eg, permeability) is obtained from the literature, which is then used for the establishment of ML/DL-based model. B, ML/DL algorithms (eg, SVM, RF, and DNN) can be used to estimate ADME parameters by training with chemical descriptors and the properties of the molecules. These ADME parameters can be used as input parameters (eg, F, Cl, fu) for the development of a generic PBPK model. C, The ML-generic PBPK model can be used to generate the secondary PK parameters including AUC, C_{max}, and Vd and subsequently be evaluated with *in vivo* PK data. Once a PBPK model is evaluated to be adequate or acceptable at Step C, it can then be used to generate simulated time-concentration data, which in turn can be incorporated into existing databases or become a new database for Step A. Abbreviations: ADME, absorption, distribution, metabolism, and excretion; AUC, area under the curve; Cl, clearance; C_{max}, maximum plasma concentration; DL, deep learning; DNN, deep neural network; F, bioavailability; ML, machine learning; PBPK, physiologically based pharmacokinetic; PK, pharmacokinetic; RF, random forest; SVM, support vector machine; Vd, volume of distribution.

Chou, W.C. and Lin, Z., 2023. Machine learning and artificial intelligence in physiologically based pharmacokinetic modeling. *Toxicological Sciences*, *191*(1), pp.1-14.

Future state of ADME data science – *automation* of model predictions for efficacy and toxicity endpoints – prediction of therapeutic index at the synthetic design stage

MRL AI/ML Computational Chemistry



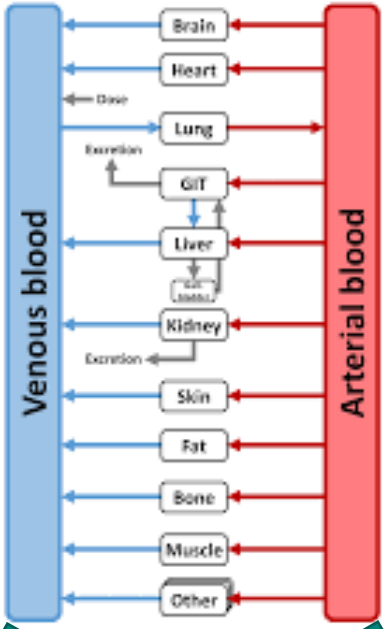
Tox/safety endpoints

distribution

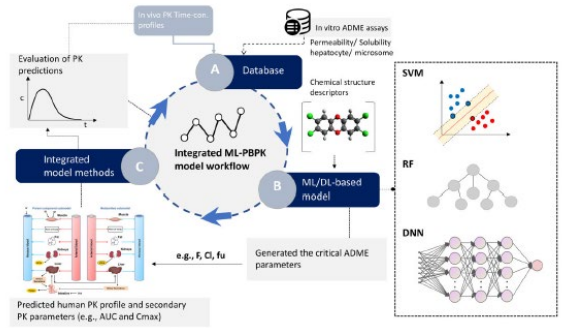
pharmacodynamics

elimination

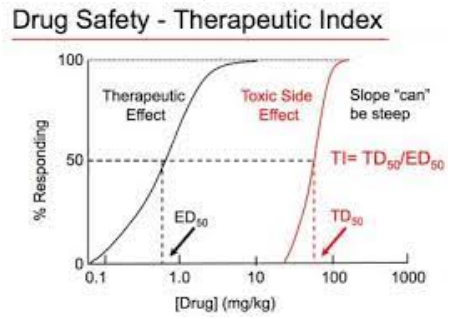
absorption

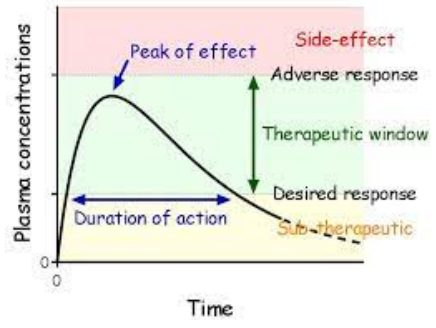


Integrative Translational Pharmacometrics Models



Toxicological Sciences, 2023, 191(1), 1–14





Prediction of safe low dose molecules devoid of dangerous side effects and drug-drug interactions

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NOVEL HUMAN MICROPHYSIOLOGICAL SYSTEMS (MPS) AND THEIR USE IN ADME

Human Cellular, Microphysiological and Organ-on-a-Chip Technologies Identified by the FDA as Being Critical to Developing Animal Alternatives

DOI: 10.1111/aor.14503

Artificial Organs

WILEY

NEWS

FDA Modernization Act 2.0 allows for alternatives to animal testing

Jason J. Han

Abstract

On December 29, 2022, President Biden signed into law the FDA Modernization Act 2.0. The bill essentially refutes the Federal Food, Drug, and Cosmetics Act of 1938, which mandated animal testing for every new drug development protocol. While for the past century,

organs such as kidneys, lungs, and livers. Induced pluripotent stem cells also show promise in this domain. Last but not least, organoids are small, self-organized three-dimensional tissue cultures that serve as in vitro miniaturized and simplified organ models.

The FDA started the New Alternative Methods Program in June of last year to support the development and regula-

Why do we need animal alternatives?

- Human drug toxicity is not always predicted by in vivo animal studies
  - Rodents predicted ~43% of human toxicities
  - Non-rodent species (dog, monkey) predicted ~63%

- There have been several in vitro formats referred to as ‘Microphysiological Systems MPS’ including
  - 3D spheroids static co-cultures, static micropatterned/printed, single/multiorgan on chip

- In support of the 3R’s principals - replacement, reduction, and refinement of animals used in research, teaching, testing, and exhibition

- MPS models will enable the testing of candidate drugs in human relevant in vitro systems prior to in vivo studies (non-clinical or clinical)

Fabre, K., et al. 2020. *Lab on a Chip*, *20*(6), pp.1049-1057.

Sneddon, L.U., et al. 2017. Considering aspects of the 3Rs principles within experimental animal biology. *Journal of Experimental Biology*, *220*(17), pp.3007-3016.

Microphysiological Systems in ADME

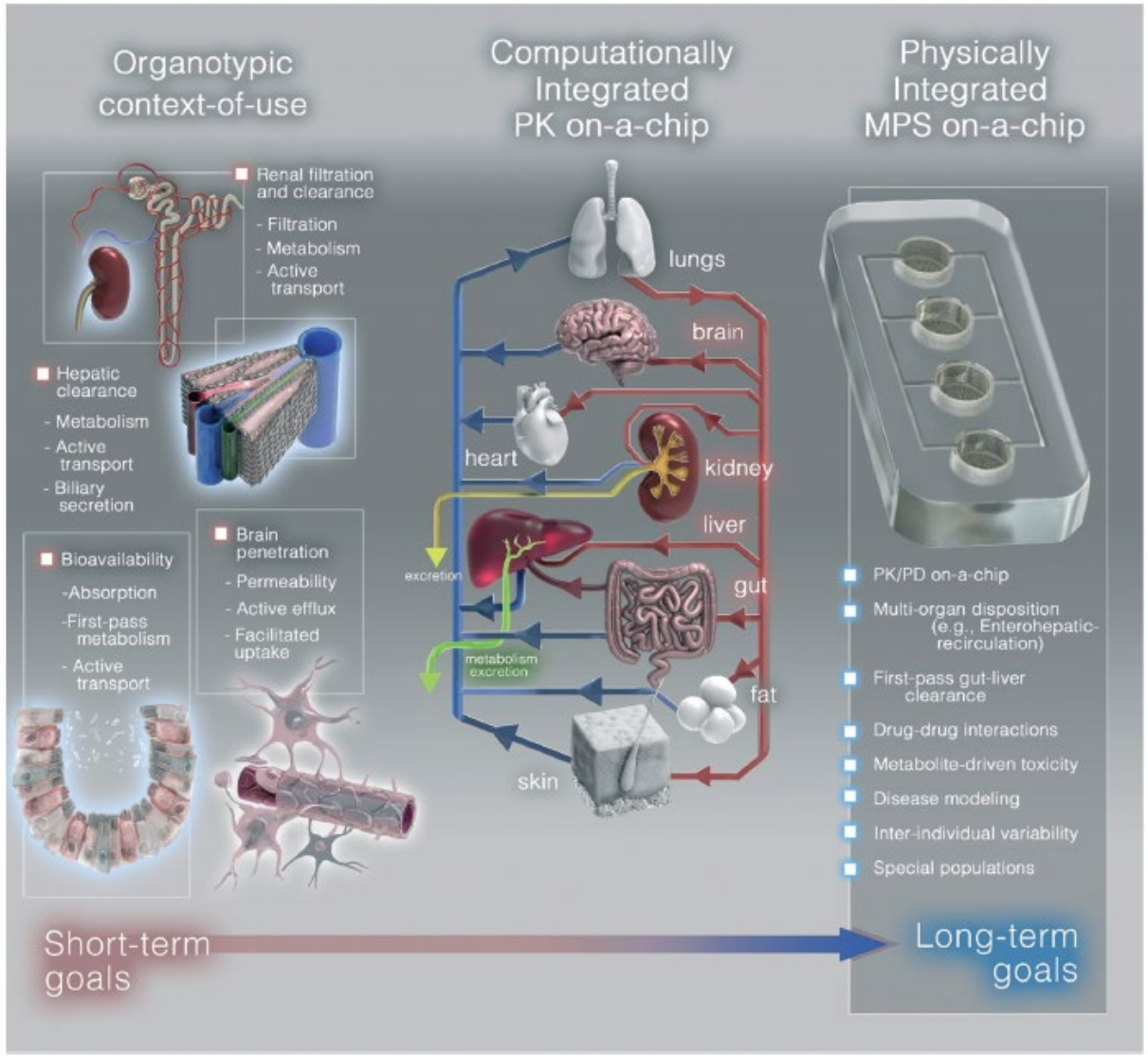


Fig. 1 A schematic to highlight potential applications of MPS systems in the ADME discipline. In the short-term, the focus should be to develop well characterized and validated organotypic models to recapitulate underlying processes (*e.g.* metabolism, transport) that define intrinsic clearance of drugs in organs of distribution and elimination. Such endpoints obtained from organotypic models could then be computationally integrated using physiologic parameters and mechanistic models to determine whole body PK. Finally, the long-term vision of such efforts would be to develop a highly evolved multi-organ chip model by establishing physiologic flow between organs to produce organ-organ interactions which will allow for the study of inter-dependent PK, PK/PD and TK/TD relationships *in vitro*. Illustration by Victor O. Leshyk.

- Static and flow through microphysiological systems models are being developed for human liver, blood-brain-barrier, kidney, gut and heart
- Complex 3D multicellular architecture resembling in vivo anatomy/histology

Fowler, S., et al. 2020. *Lab on a Chip*, *20*(3), pp.446-467.

Development of Flow-Through Gut-on-a-Chip Models

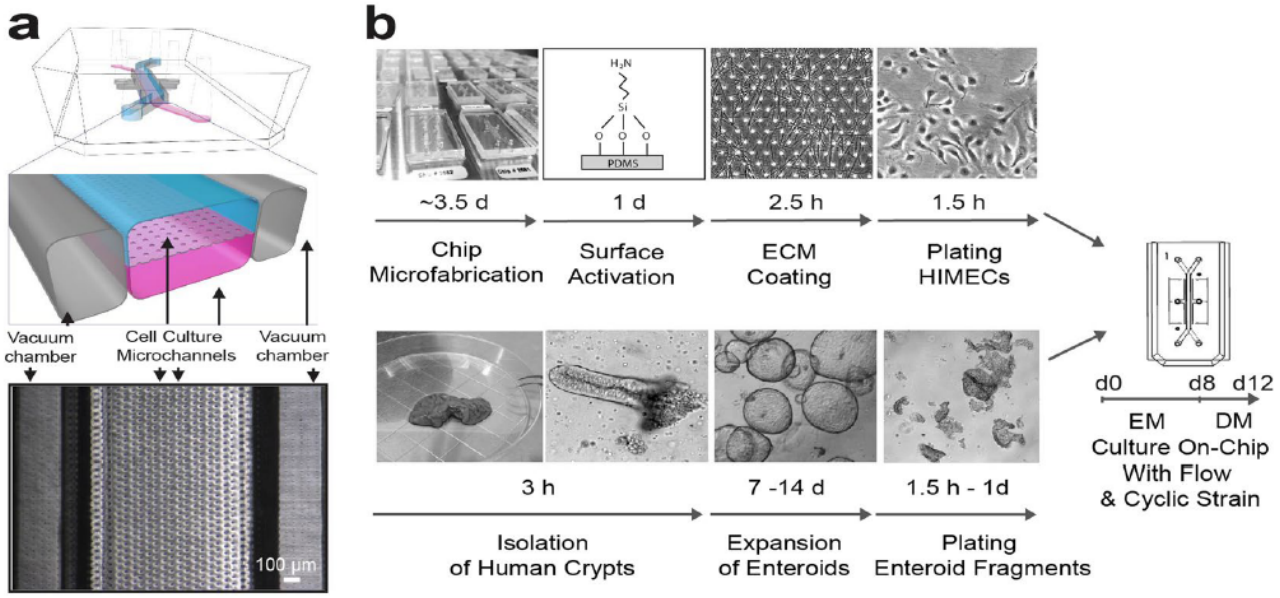


Figure 1. Fabrication of the primary human Intestine Chip. (**a**) A schematic cross-sectional view (top) and a phase contrast micrograph of the chip viewed from above (bottom) showing the upper (epithelial; blue) and lower (microvascular; pink) cell culture microchannels separated by a porous, ECM-coated, PDMS membrane sandwiched in-between. The membrane is elastic and can be extended and retracted by the application of cyclic vacuum to the hollow side chambers. This actuation causes outward deflection of the vertical side walls and lateral extension of the attached horizontal, porous elastic membrane, which induces mechanical deformation of the adherent tissue layers cultured in the central channels. (**b**) Schematic representation of the step-by-step procedure involved in the establishment of microfluidic co-cultures of primary human intestinal epithelium and intestinal microvascular endothelium in the Intestine Chip.

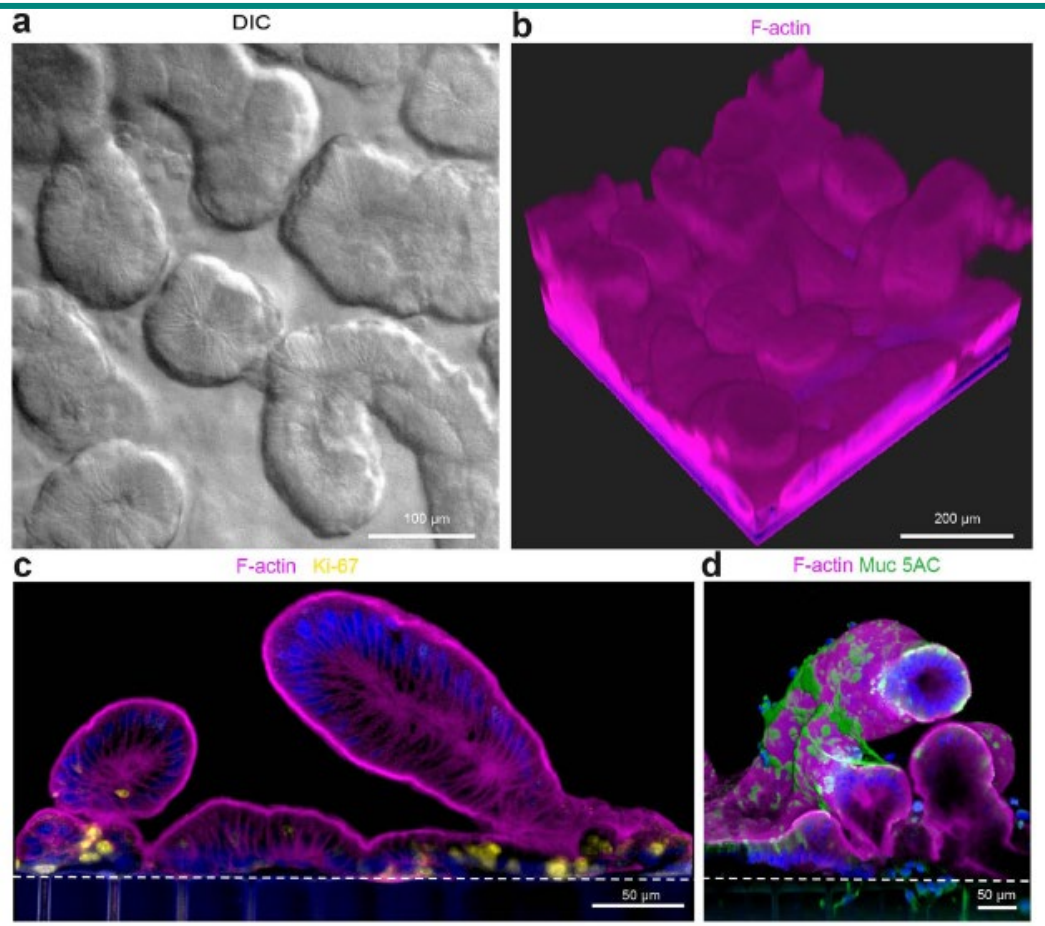


Figure 2. Morphological analysis of Organ Chips lined by primary duodenal organoid-derived epithelial cells in the absence of endothelial cells. (**a**) Microscopic views showing the finger-like potrusions of the primary intestinal epithelium cultured on-chip for 12 days under continuous flow (60 µl hr^{-1}), when viewed from above by DIC imaging. (**b**) Representative 3D reconstruction of confocal immunofluorescence micrographs of organoid-derived intestinal epithelium grown on-chip (magenta, F-actin; blue, DAPI-stained nuclei). (**c**) Representative vertical cross sectional view of confocal microscopic images showing intestinal epithelium immunostained for F-actin (magenta) and Ki67 (yellow). (**d**) Representative vertical cross sectional, confocal, micrographic views through the intestinal epithelium-membrane interface of the intestinal epithelium grown on-chip when immunostained for F-actin (magenta) and Muc5AC (green), and nuclei with DAPI (blue) (in c and d, white dashed lines indicate upper surfaces of the porous matrix-coated membrane).

Allows for experiments on bioavailability, gut wall metabolism/catabolism and pharmacology in the gut

Kasendra, M., et al. 2018. Development of a primary human Small Intestine-on-a-Chip using biopsy-derived organoids. *Scientific reports*, *8*(1), p.2871.

Microphysiological Models Attempting to Recapitulate the Complex Biology and Anatomy of the Human Blood Brain Barrier

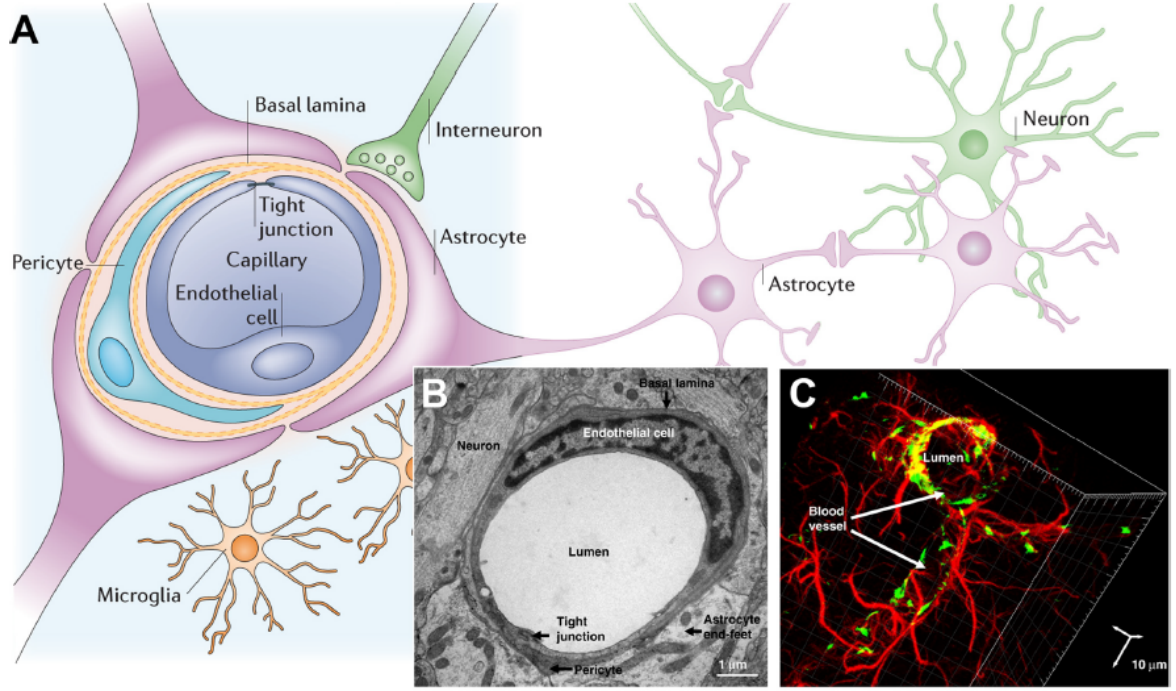
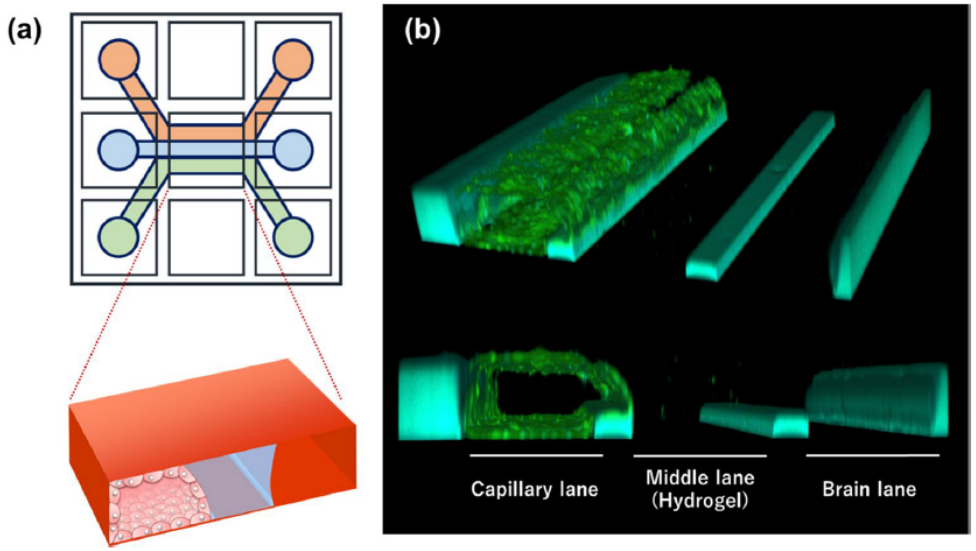


FIGURE 1. **Anatomical structure of the NVU.** *A*, a schematic representation of a capillary cross-section within a single neurovascular unit demonstrates the following important features: 1) Specialized brain endothelial cells line cerebral vessels. 2) Tight junctions between endothelial cells restrict paracellular diffusion and effectively "seal" the vessels. 3) A continuous basal lamina/basement membrane encases endothelial cells. Pericytes are embedded within this matrix, situated between endothelial cells and astroglial endfeet. 4) Astrocytes are centrally positioned within the brain parenchyma. These cells extend processes that communicate with local neurons and synapses and also extend foot-like processes that encase cerebral vessels. Astrocytes are therefore ideally localized to sense and respond to both neuronal and vascular activity. 5) Resident microglia use long cellular processes to survey their microenvironment and can quickly respond to insults at or near the NVU. 6) Local interneurons innervate cerebral vasculature and can induce vessels to change their tone based on incoming neuronal afferent signals (28) (adapted with permission from Macmillan Publishers Ltd.: Abbott *et al.* (2006) *Nat. Rev. Neurosci.* **7,** 41–53 (97), © Macmillan Publishers Ltd.). *B*, electron micrograph of a capillary cross-section in rat brain. *C*, 3D reconstruction of immunofluorescent NVU images taken on a confocal microscope demonstrating von Willebrand Factor reactivity (endothelial cells) and glial fibrillary acidic protein reactivity (astrocytes) outside the vascular wall (*panels B* and *C* reprinted from Weiss *et al.* (2009) *Biochim. Biophys. Acta* **1788,** 842–857 (98), with permission from Elsevier, © Elsevier).

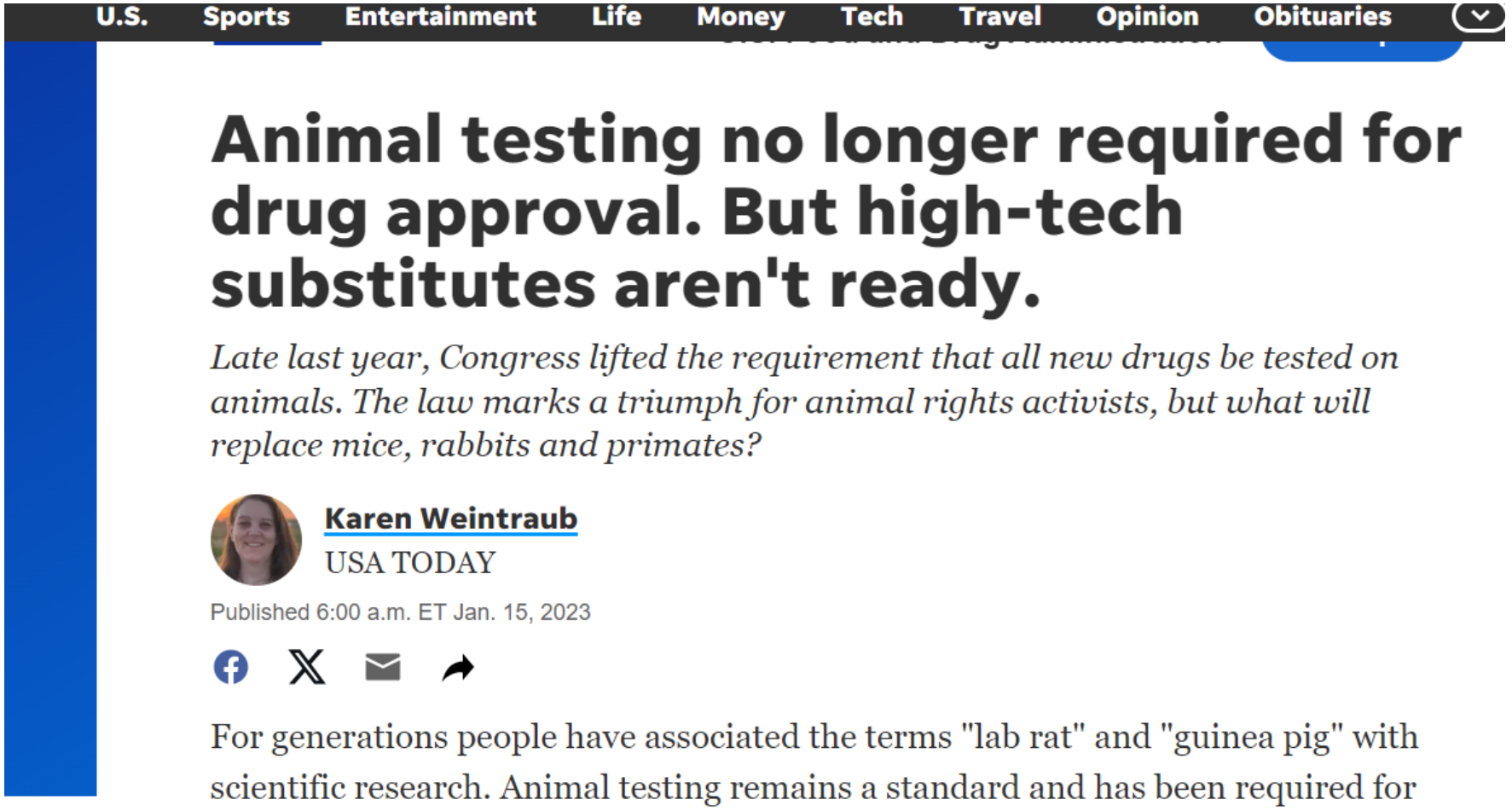
McConnell, H.L., et al. 2017. The translational significance of the neurovascular unit. *Journal of biological chemistry*, *292*(3), pp.762-770.

Fig. 2 Overall view of OrganoPlate® 3-lane. (**a**) Illustration of the OrganoPlate® 3-lane. The plate consists of 40 clusters of 9 wells, each divided into three microfluidic lanes. There are 40 of these nine-well clusters in one plate, allowing for high-throughput studies. Red lane, capillary lane; blue lane, middle (hydrogel) lane; green lane, brain lane. (**b**) Cell membrane staining of hiPS-BMECs seeded on the OrganoPlate® 3-lane. Green, cell membrane via CellMask staining; light blue, polymers that make up the walls and guides of the plate.



Kurosawa, T., et al. 2022. Construction and functional evaluation of a three-dimensional blood–brain barrier model equipped with human induced pluripotent stem cell-derived brain microvascular endothelial cells. *Pharmaceutical Research*, *39*(7), pp.1535-1547.

It is all very exciting, but what is the hold up?



U.S. Sports Entertainment Life Money Tech Travel Opinion Obituaries

Animal testing no longer required for drug approval. But high-tech substitutes aren't ready.

Late last year, Congress lifted the requirement that all new drugs be tested on animals. The law marks a triumph for animal rights activists, but what will replace mice, rabbits and primates?

Karen Weintraub
USA TODAY

Published 6:00 a.m. ET Jan. 15, 2023

For generations people have associated the terms "lab rat" and "guinea pig" with scientific research. Animal testing remains a standard and has been required for

Scientists working in ADME have a whole new set of challenges but with new tools and technologies to enable translation and problem solving

Developments in AI/ML

Established Mechanistic Core ADME Sciences/Toolkits of Today

Advances in protein engineering and fusion design of novel biologics

Novel Microphysiological systems (MPS)

Thank You for the invitation to speak with you today

Chris Gibson

Pharmacokinetics, Dynamics, Metabolism and Bioanalytics (PDMB)

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