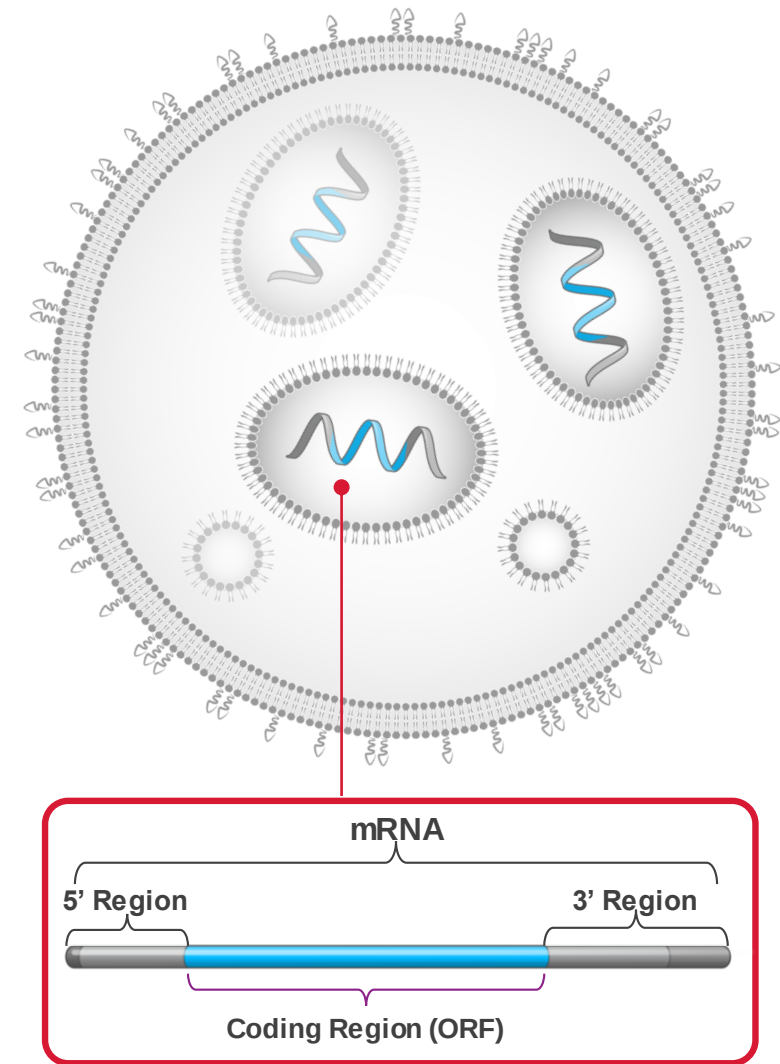




Annual Science Day

June 2, 2020

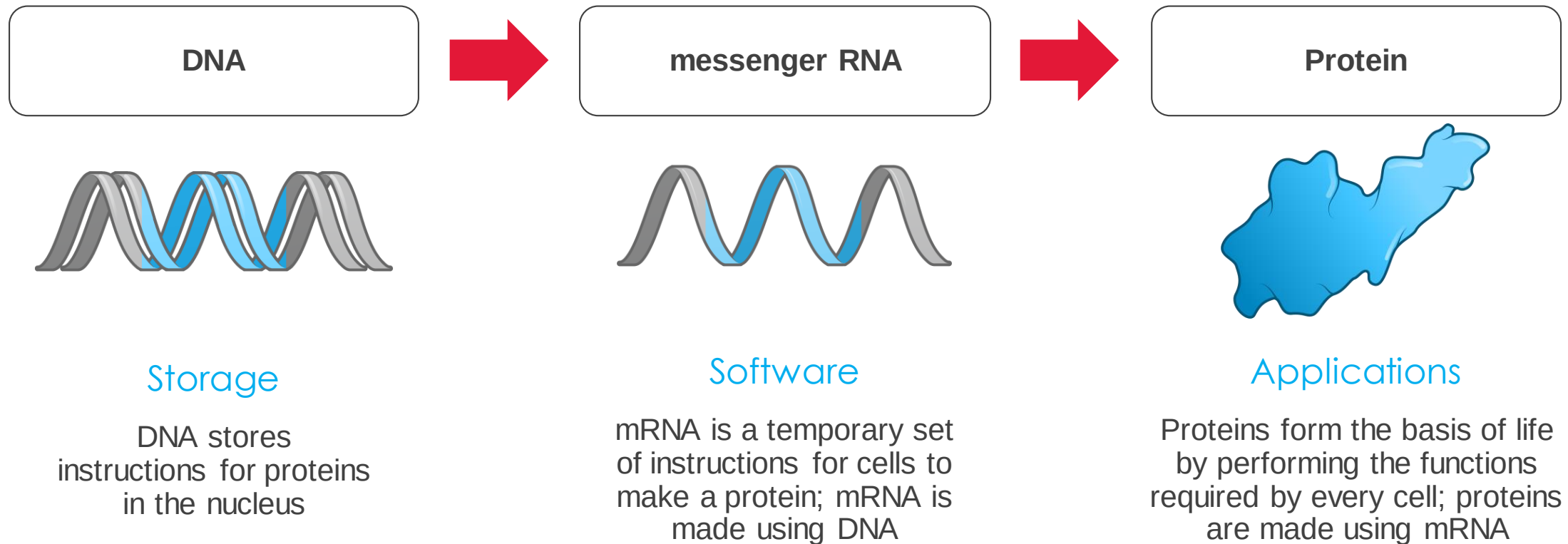


Forward-looking statements and disclaimers

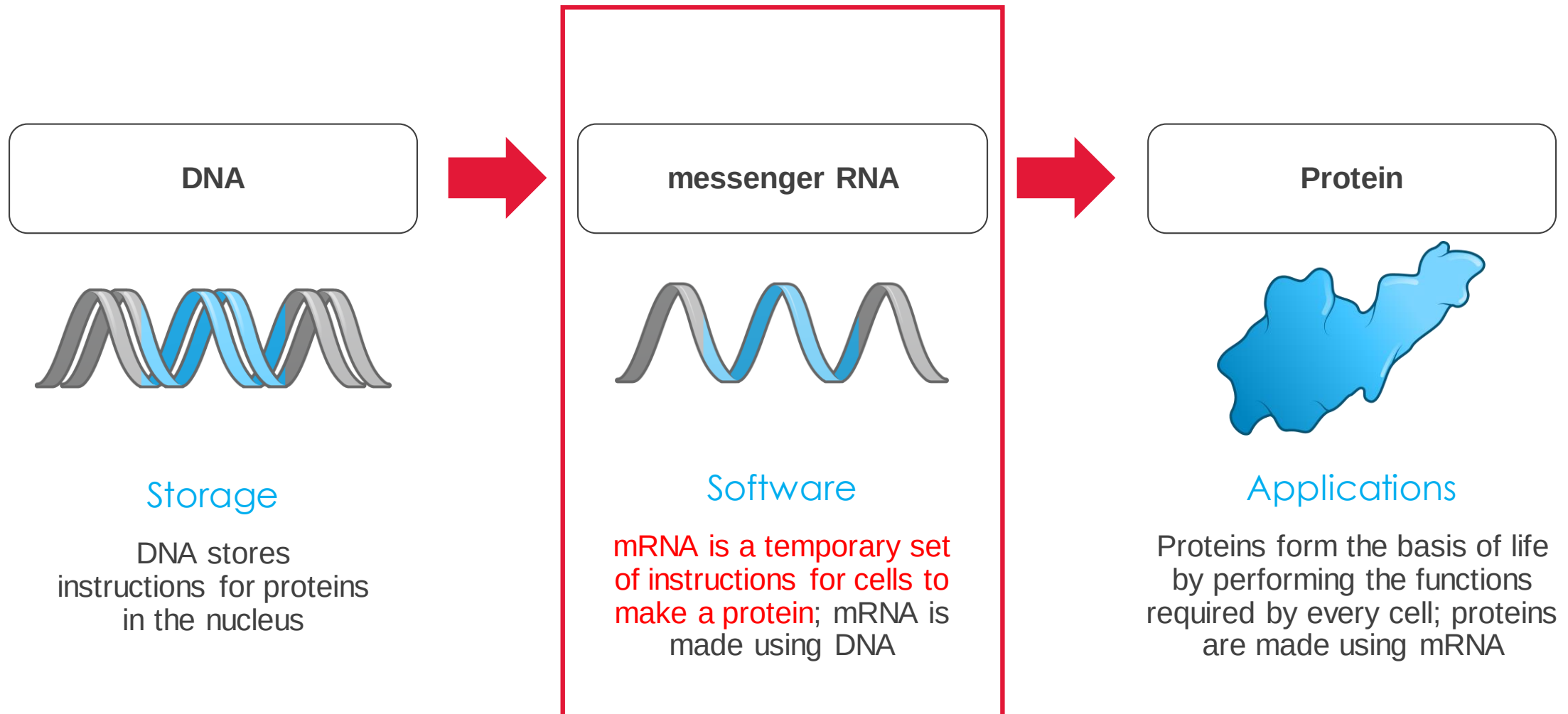
This presentation contains forward-looking statements within the meaning of the Private Securities Litigation Reform Act of 1995, as amended, including regarding the Company's mRNA and encoded protein engineering efforts, the Company's new squaramide-based LNP and its expected use with mRNA-3745 and other new development candidates, the ability of mRNA medicines to potentially modulate pharmacologic effects, the scientific and clinical potential of the Company's newly developed lipid nanoparticles; the ability of the Company's proprietary lipid nanoparticles to drive optimal immune response in primates, the potential for the Company's mRNA platform to significantly accelerate early clinical trials and transform HIV vaccine science and development efforts, and the presentation of research and data at the Company's upcoming Science Day. In some cases, forward-looking statements can be identified by terminology such as "will," "may," "should," "could," "expects," "intends," "plans," "aims," "anticipates," "believes," "estimates," "predicts," "potential," "continue," or the negative of these terms or other comparable terminology, although not all forward-looking statements contain these words. The forward-looking statements in this presentation are neither promises nor guarantees, and you should not place undue reliance on these forward-looking statements because they involve known and unknown risks, uncertainties, and other factors, many of which are beyond Moderna's control and which could cause actual results to differ materially from those expressed or implied by these forward-looking statements. These risks, uncertainties, and other factors include, among others: the fact that there has never been a commercial product utilizing mRNA technology approved for use; potential adverse impacts due to the global COVID-19 pandemic such as delays in regulatory review, manufacturing and supply chain interruptions, adverse effects on healthcare systems and disruption of the global economy; and those other risks and uncertainties described under the heading "Risk Factors" in Moderna's most recent Quarterly Report on Form 10-Q filed with the U.S. Securities and Exchange Commission (SEC) and in subsequent filings made by Moderna with the SEC, which are available on the SEC's website at www.sec.gov. Except as required by law, Moderna disclaims any intention or responsibility for updating or revising any forward-looking statements contained in this presentation in the event of new information, future developments or otherwise. These forward-looking statements are based on Moderna's current expectations and speak only as of the date hereof.

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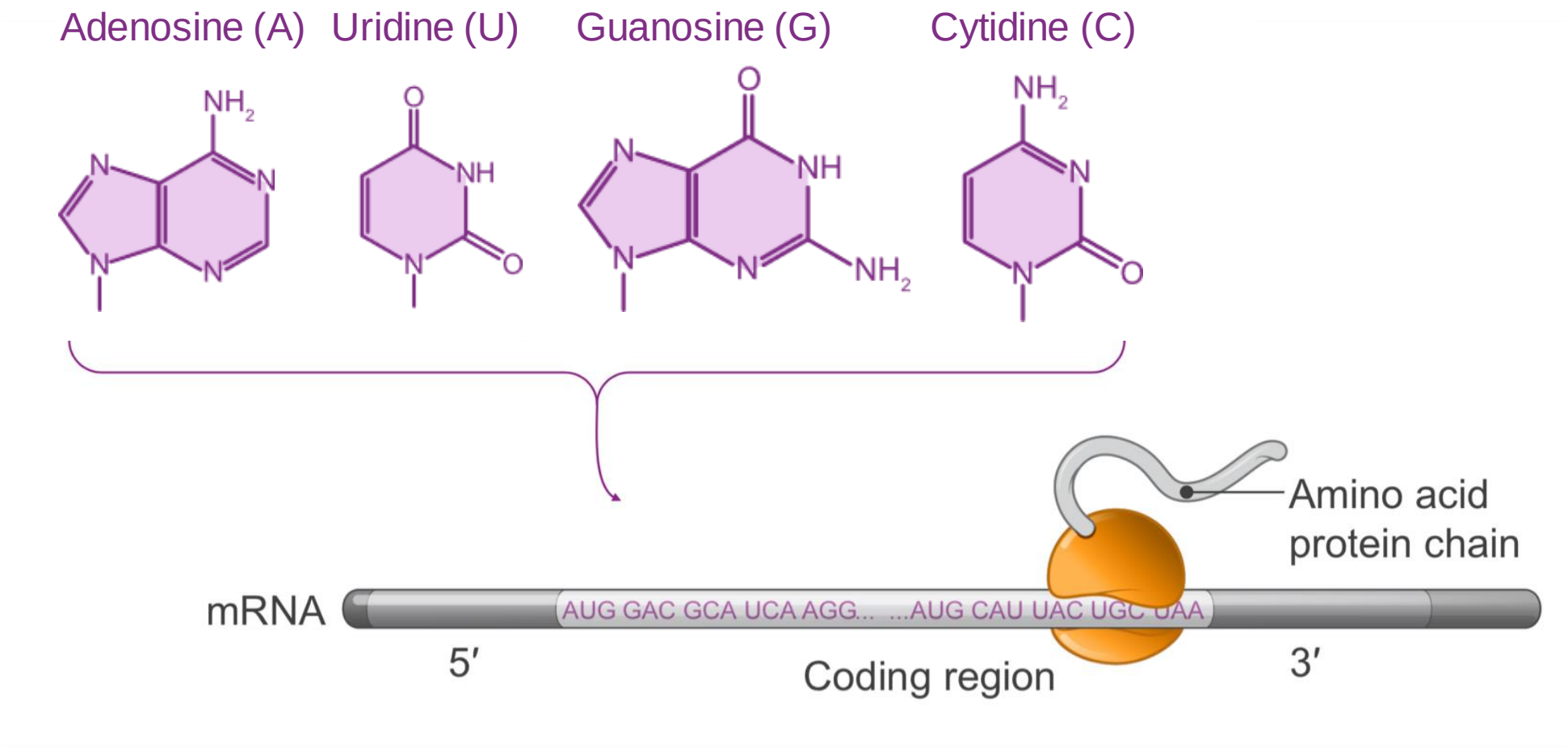
Central dogma of molecular biology



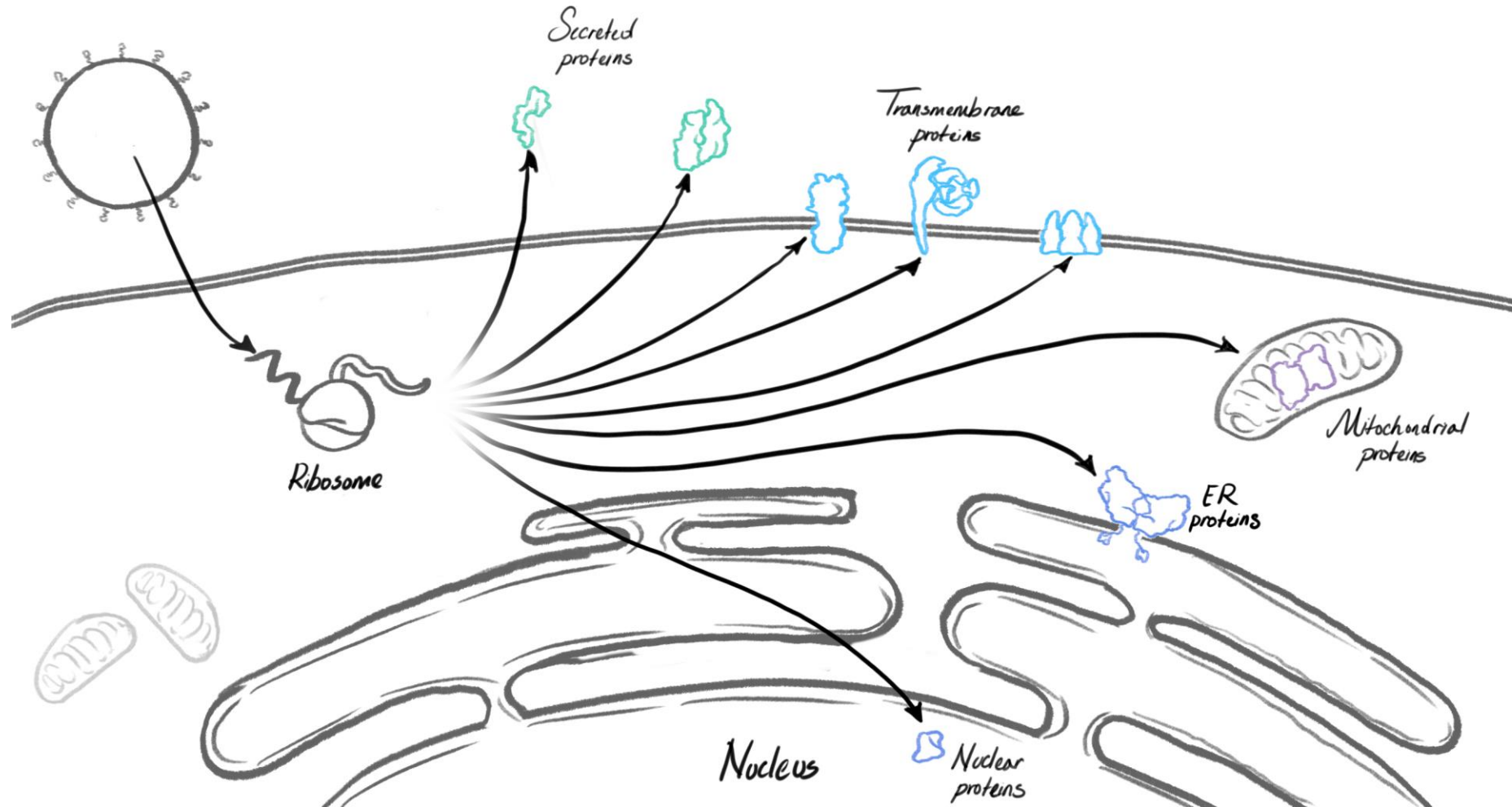
mRNA as a new class of medicine



mRNA is a temporary set of instructions for cells to make a protein

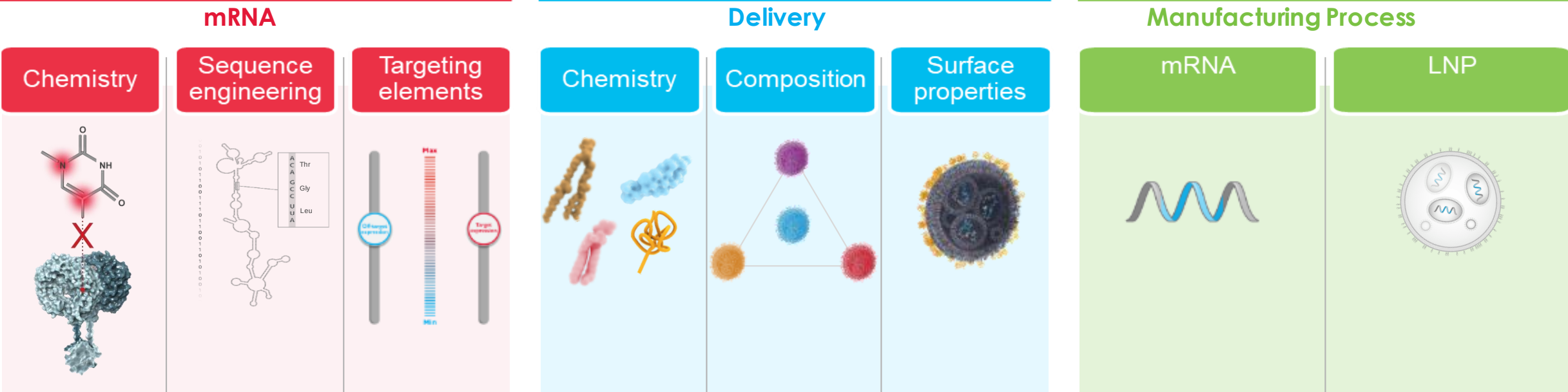


mRNA as a potential new class of medicines

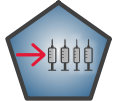


1. Large product opportunity
2. Higher probability of technical success
3. Accelerated research and development timelines
4. Greater capital efficiency over time vs. recombinant technology

Our platform



Prophylactic vaccines



Cancer vaccines



Intratumoral immuno-oncology



Localized regenerative therapeutics

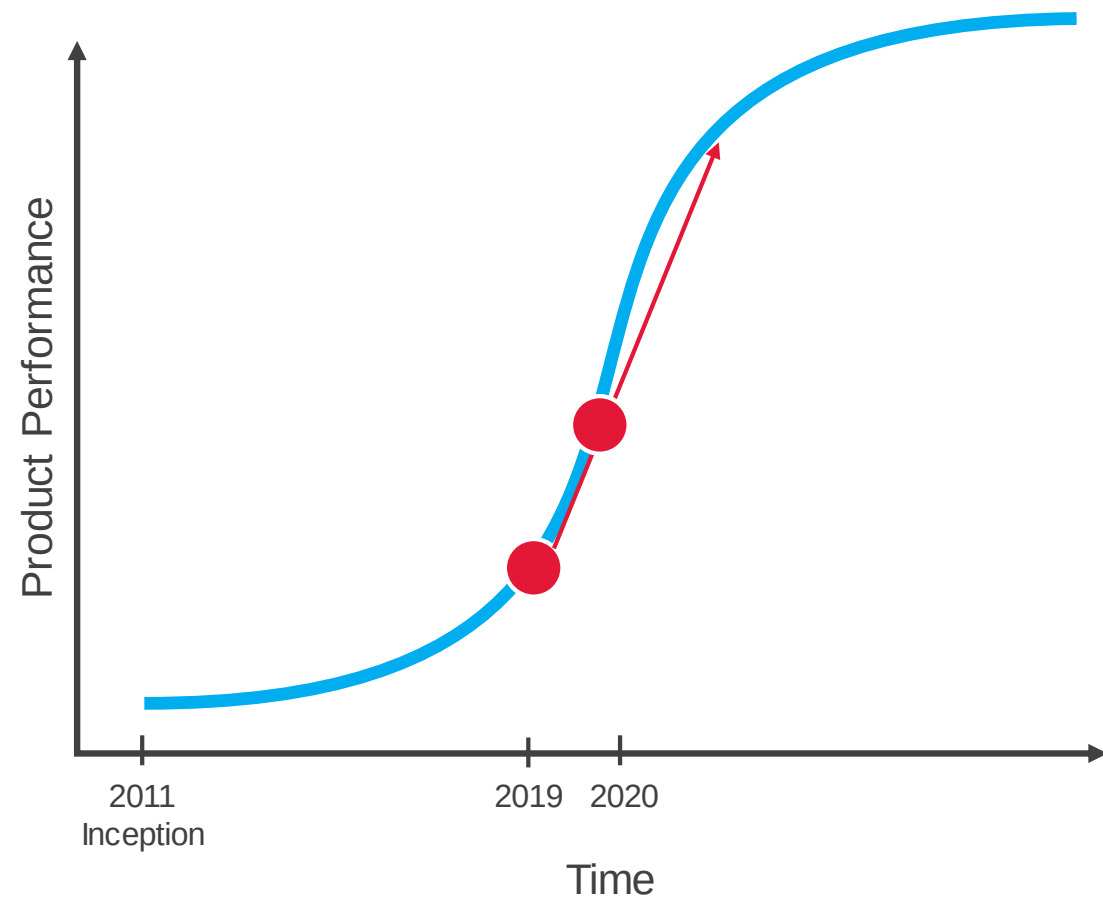


Systemic secreted & cell surface therapeutics

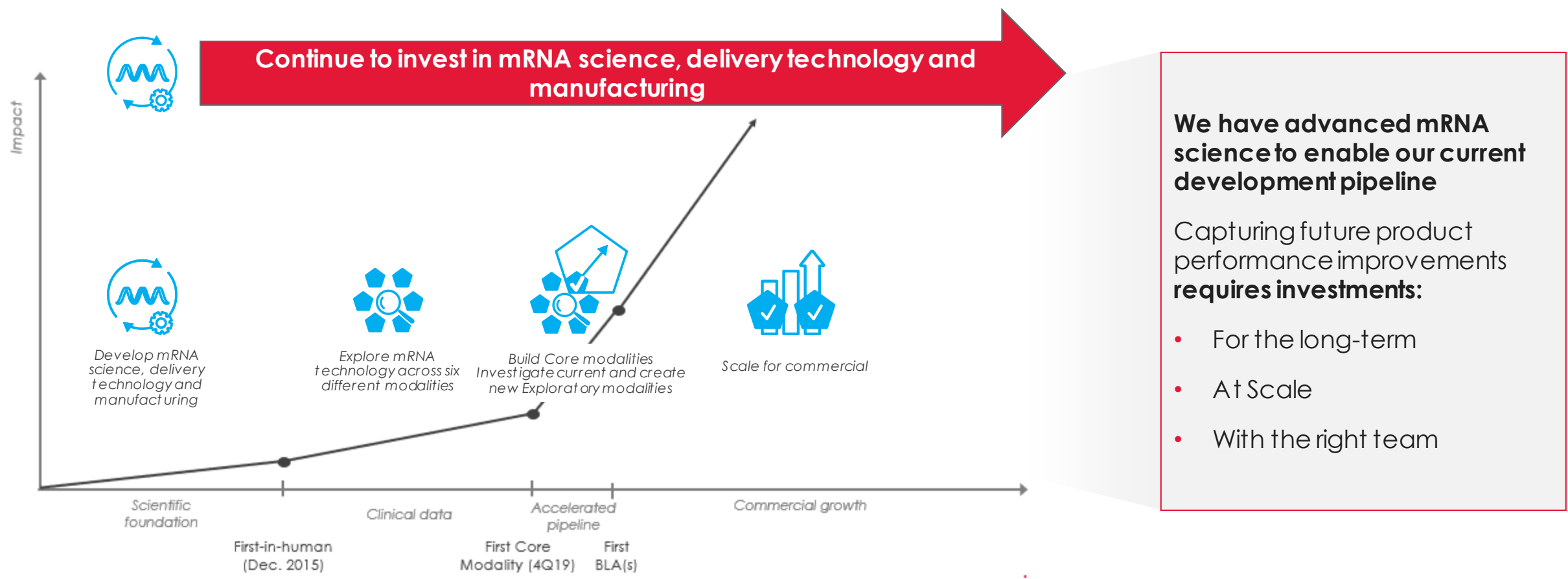


Systemic intracellular therapeutics

Our commitment to be the best at mRNA science is core to who we are: A 20-year journey



Our commitment to be the best at mRNA science is core to who we are



Stephen Hoge, M.D.

President



As the President of Moderna, Stephen Hoge leads our platform R&D, therapeutic area research, and pre-clinical development efforts. He joined Moderna in late 2012 from McKinsey & Company where he was a partner in the healthcare practice. Prior to McKinsey, Dr. Hoge was a resident physician in New York City.

He holds an M.D. with thesis from the University of California, San Francisco and a B.S. in neuroscience from Amherst College.

Melissa J. Moore, Ph.D.

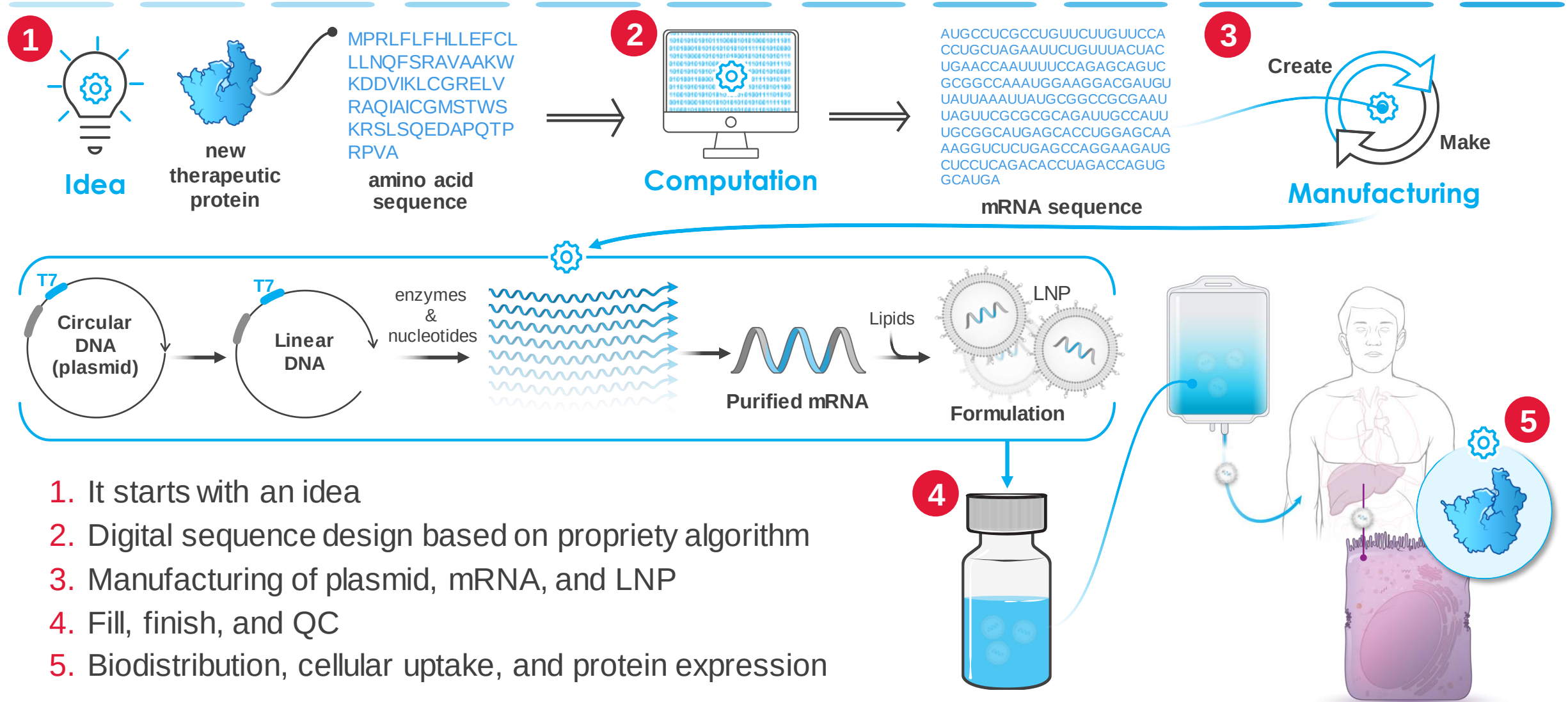
Chief Scientific Officer, Platform Research



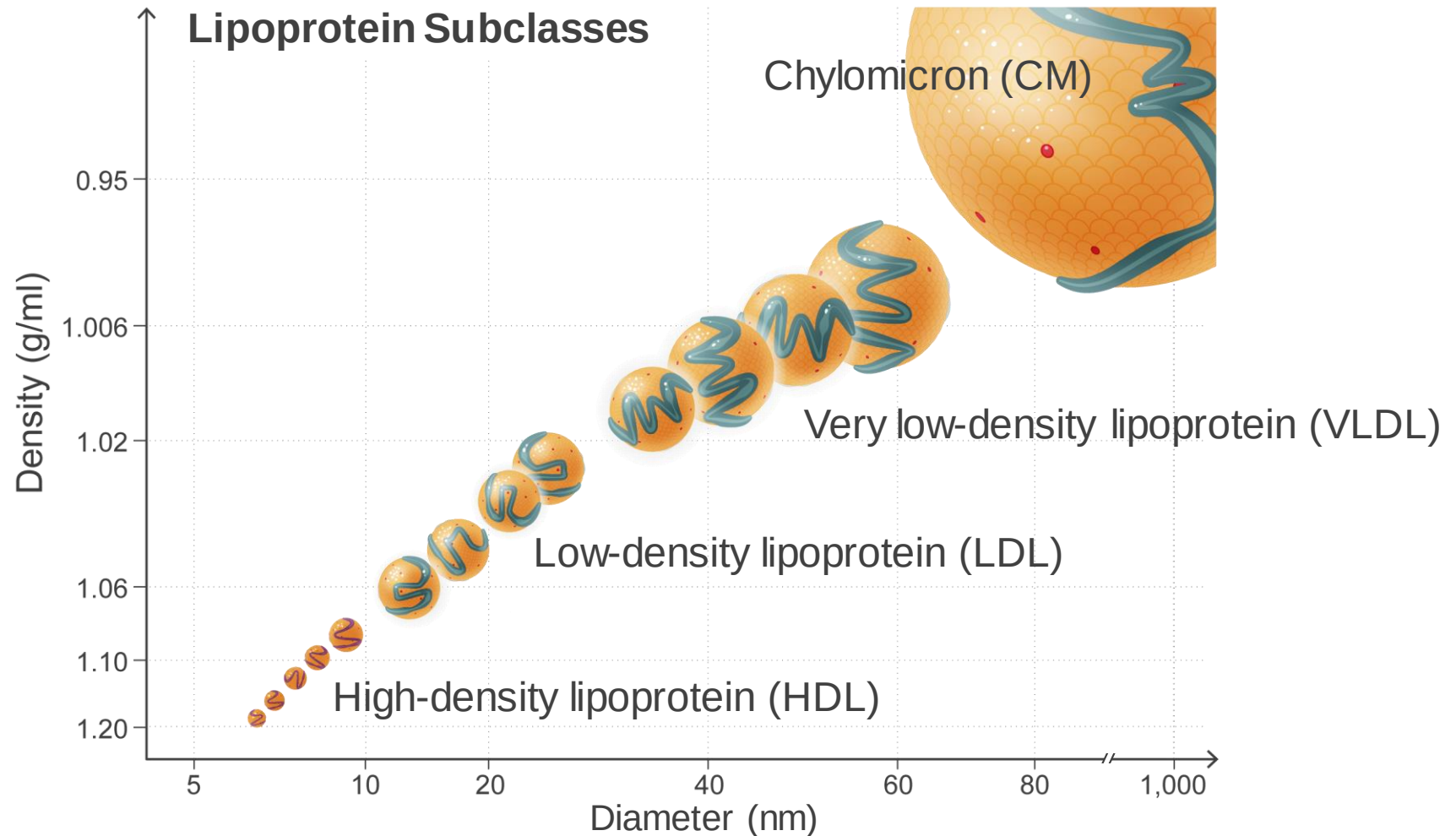
In her role as Chief Scientific Officer of Moderna's Platform Research, Dr. Moore is responsible for leading mRNA biology, delivery and computation science research at Moderna. She joined Moderna in 2016 from the University of Massachusetts Medical School (UMassMed), where she served as Professor of Biochemistry & Molecular Pharmacology, Eleanor Eustis Farrington Chair in Cancer Research and a long-time Investigator at the Howard Hughes Medical Institute (HHMI). At UMassMed, Dr. Moore was a founding Co-Director of the RNA Therapeutics Institute (RTI) and was instrumental in creating the Massachusetts Therapeutic and Entrepreneurship Realization initiative (MasTERi), a faculty-led program intended to facilitate the translation of UMMS discoveries into drugs, products, technologies and companies. Dr. Moore is an elected member of both the National Academy of Sciences (2017) and the American Academy of Arts and Sciences (2019).

Dr. Moore's 23-year career in academic research focused on the roles of RNA and RNA-protein (RNP) complexes in regulating gene expression, and touched on many human diseases including cancer, neurodegeneration and preeclampsia. She began working on RNA metabolism during her postdoctoral training with Phillip A. Sharp, Ph.D. at MIT, where she also received her Ph.D. in Biological Chemistry working under Christopher T. Walsh, Ph.D. Dr. Moore holds a B.S. in Chemistry and Biology from the College of William and Mary.

From idea to expression

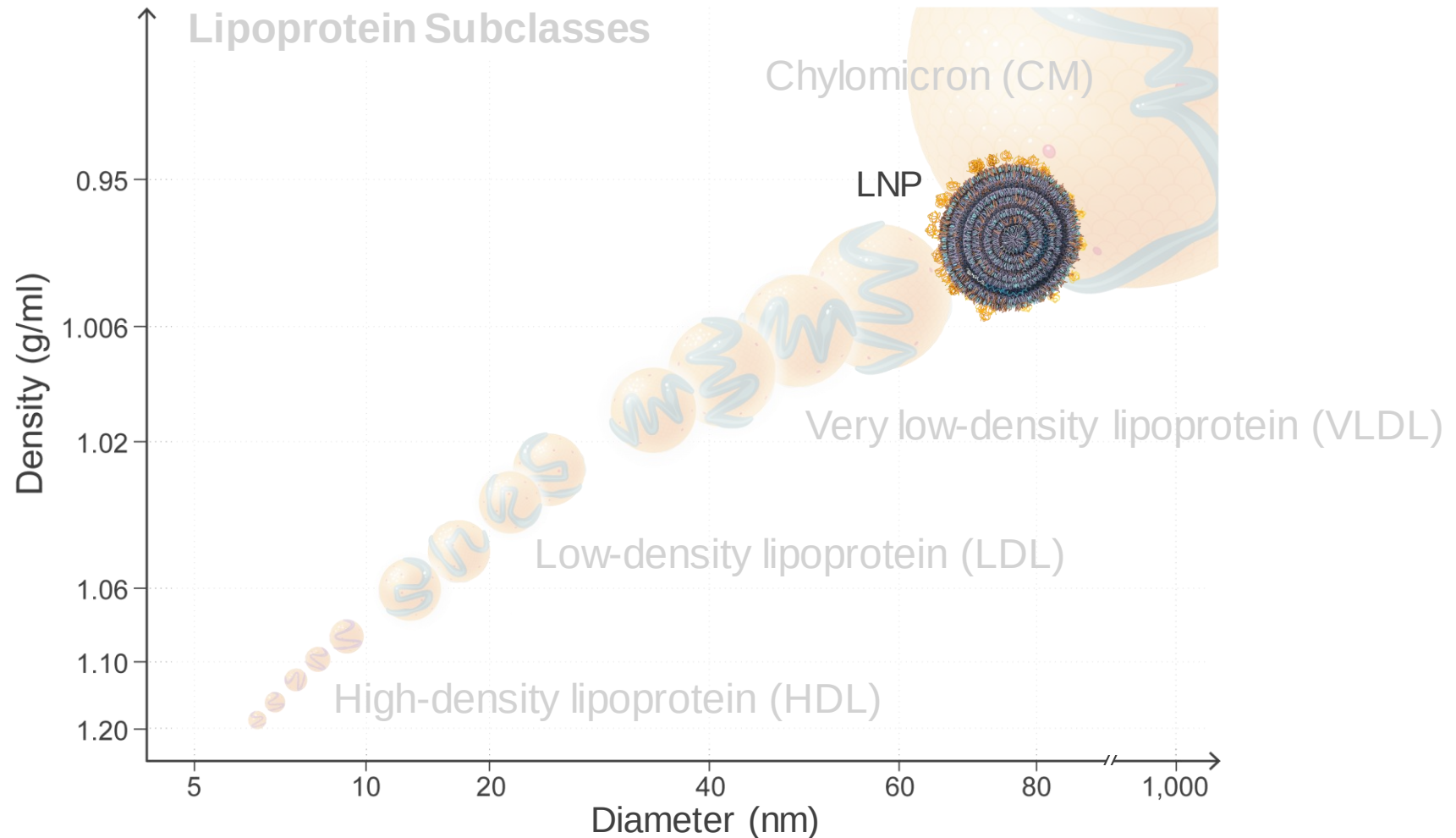


LNPs are akin to endogenous lipid transport complexes



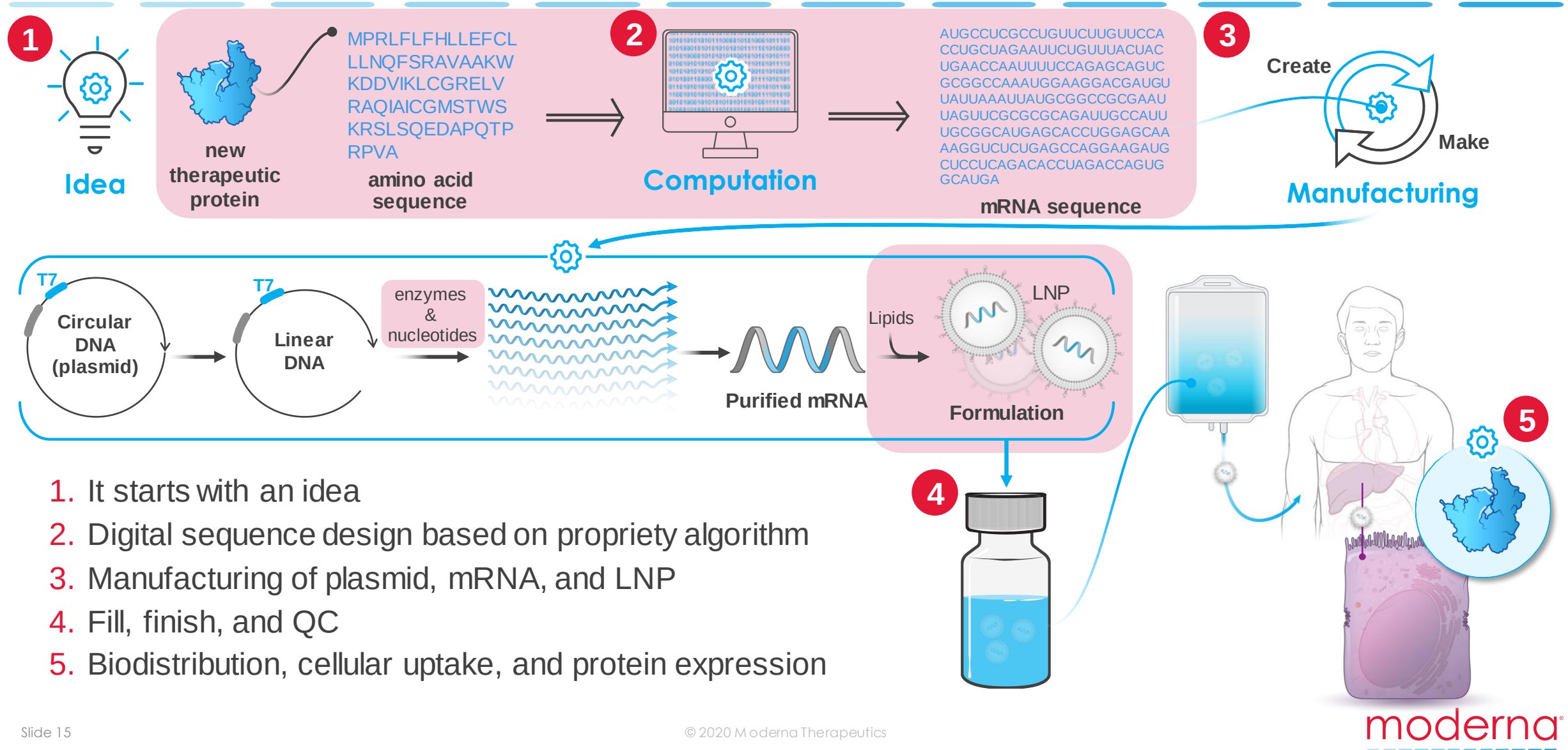
Modified from Katsuyuki Nakajima. "Remnant Lipoproteins: A Subfraction of Plasma Triglyceride-Rich Lipoproteins Associated with Postprandial Hyperlipidemia." *Clin Exp Thromb Hemost* (2014).

LNPs are akin to endogenous lipid transport complexes



Modified from Katsuyuki Nakajima. "Remnant Lipoproteins: A Subfraction of Plasma Triglyceride-Rich Lipoproteins Associated with Postprandial Hyperlipidemia." *Clin Exp Thromb Hemost* (2014).

Where does Platform Research fit in?



2018 Science Day Themes and Publications

- Development of proprietary ionizable lipids for improved systemic mRNA delivery
- Development of proprietary ionizable lipids with improved tolerability for mRNA vaccines
- Use of miRNA target sites to create mRNAs with "off switches"
- Translation initiation and "leaky scanning"
- How artificial intelligence can help us design better coding sequences

A Novel Amino Lipid Series for mRNA Delivery: Improved Endosomal Escape and Sustained Pharmacology and Safety in Non-human Primates

Staci Sabnis,¹ E. Sathyajith Kumarasinghe,¹ Timothy Salerno,¹ Cosmin Mihai,¹ Tatiana Ketova,¹ Joseph J. Senn,¹ Andy Lynn,¹ Alex Bulychev,¹ Iain McFadyen,¹ Joyce Chan,¹ Örn Almarsson,¹ Matthew G. Stanton,^{1,2} and Kerry E. Benenato¹

Sabnis ... Benenato (2018) Mol Ther

Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines

Kimberly J. Hassett,¹ Kerry E. Benenato,¹ Eric Jacquinet,¹ Aisha Lee,¹ Angela Woods,¹ Olga Yuzhakov,¹ Sunny Himansu,¹ Jessica Deterling,¹ Benjamin M. Geilich,¹ Tatiana Ketova,¹ Cosmin Mihai,¹ Andy Lynn,¹ Iain McFadyen,¹ Melissa J. Moore,¹ Joseph J. Senn,¹ Matthew G. Stanton,^{1,2} Örn Almarsson,¹ Giuseppe Ciaramella,^{1,3} and Luis A. Brito¹

Hassett ... Brito (2019) Mol Ther Nuc Acids

MicroRNAs Enable mRNA Therapeutics to Selectively Program Cancer Cells to Self-Destruct

Ruchi Jain,¹ Josh P. Frederick,² Eric Y. Huang,³ Kristine E. Burke,⁴ David M. Mauger,⁵ Elizaveta A. Andrianova,¹ Sam J. Farlow,² Summar Siddiqui,⁶ Jeffrey Pimentel,⁶ Kahlin Cheung-Ong,² Kristine M. McKinney,^{3,*} Caroline Köhrer,¹ Melissa J. Moore,¹ and Tirtha Chakraborty^{1,†}

Jain ... Chakraborty (2018) Nuc Acid Ther

2019 Science Day Themes and Publications

- Why we incorporate modified nucleotides into our mRNAs
- Coding sequence design: Codon optimality vs RNA secondary structure
- 5'-UTR design
- The physical and computational methods we employ to understand LNP structure
- An early glimpse into our Immune LNP

Impact of mRNA chemistry and manufacturing process on innate immune activation

Jennifer Nelson^{*†}, Elizabeth W. Sorensen^{*‡}, Shrutika Mintri[§], Amy E. Rabideau, Wei Zheng, Gilles Besin^{||}, Nikhil Khatwani[¶], Stephen V. Su[#], Edward J. Miracco, William J. Issa, Stephen Hoge, Matthew G. Stanton^{**}, John L. Joyal^{††}

Nelson ... Joyal (2020) Science Advances

mRNA structure regulates protein expression through changes in functional half-life

David M. Mauger[¶], B. Joseph Cabral^{¶,1}, Vladimir Presnyak[¶], Stephen V. Su^{¶,2}, David W. Reid[¶], Brooke Goodman[¶], Kristian Link[¶], Nikhil Khatwani^{¶,3}, John Reynders^{¶,4}, Melissa J. Moore^{¶,5}, and Iain J. McFadyen^{¶,6}

[¶]Platform Research, Moderna, Inc., Cambridge, MA 02139

Mauger ... McFadyen (2019) PNAS

Human 5' UTR design and variant effect prediction from a massively parallel translation assay

Paul J. Sample^{¶,1,5}, Ban Wang^{¶,1,5}, David W. Reid^{¶,2}, Vlad Presnyak^{¶,2}, Iain J. McFadyen^{¶,2}, David R. Morris[¶] and Georg Seelig^{¶,1,4*}

Sample ... Seelig (2019) Nature Biotech

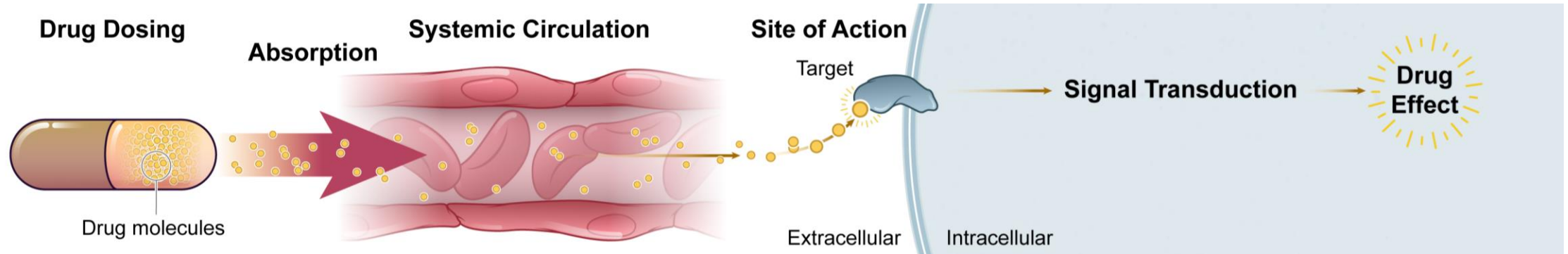
2020 Science Day agenda

Introduction	<i>Stéphane Bancel, CEO</i>	8:00-8:10 AM	10 min
Chapter 1 Extending Pharmacology - Controlling the duration of our pharmacologic effect - Enhancing mRNA and protein half-lives	<i>Melissa Moore, Ph.D., Chief Scientific Officer, Platform Research</i>	8:10-8:45 AM	35 min
Chapter 2 T7 Enzyme Engineering to Reduce dsRNA Production	<i>Amy Rabideau, Ph.D., Principal Scientist, Process Development</i>	8:45-9:05 AM	20 min
Chapter 3 New LNPs for Liver Delivery	<i>Kerry Benenato, Ph.D., Senior Director, Platform Chemistry</i>	9:05-9:25 AM	20 min
Chapter 4 Impact of LNP Size on Immunogenicity	<i>Kimberly Hassett, Ph.D., Senior Scientist, Formulation Science</i>	9:25-9:40 AM	15 min
Break		9:40-9:50 AM	10 min
Chapter 5 Our Progress Toward Creating a mRNA Vaccine for HIV - Vaccine design to elicit broadly neutralizing antibodies against HIV - NIH-Moderna HIV-1 vaccine	<i>Andrea Carfi, Head of Research, Infectious Disease</i> <i>William Schief, Ph.D., Scipps, International Aids Vaccine Initiative (IAVI)</i> <i>Paolo Lusso, M.D., Ph.D., National Institute of Allergy and Infectious Diseases (NIAID)</i>	9:50-10:50 AM	60 min
Conclusion	<i>Stephen Hoge, M.D., President</i>	10:50-11:00 AM	10 min
Q&A		11:00-11:30 AM	30 min



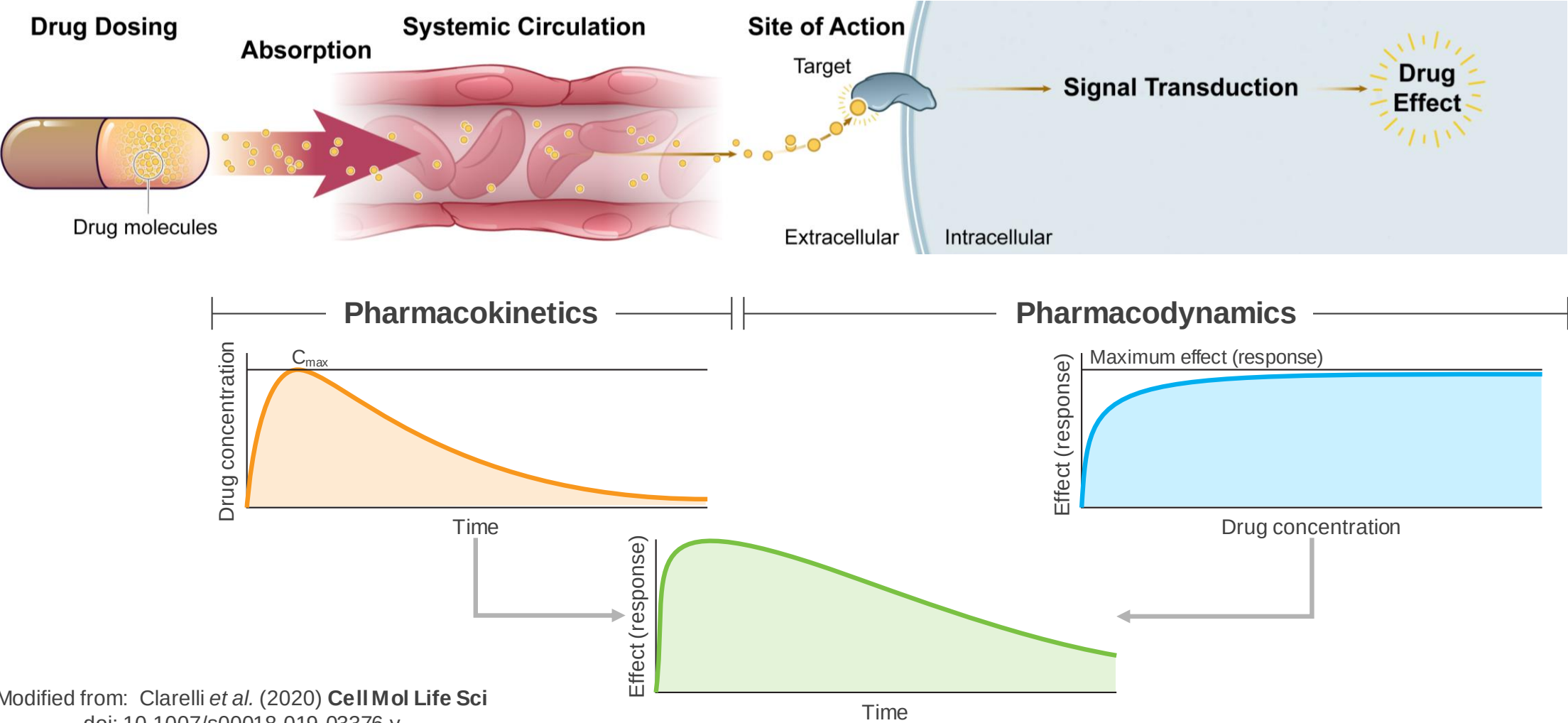
What controls the duration of
our pharmacologic effects?

Factors controlling duration of effect by traditional small molecule drugs are well understood



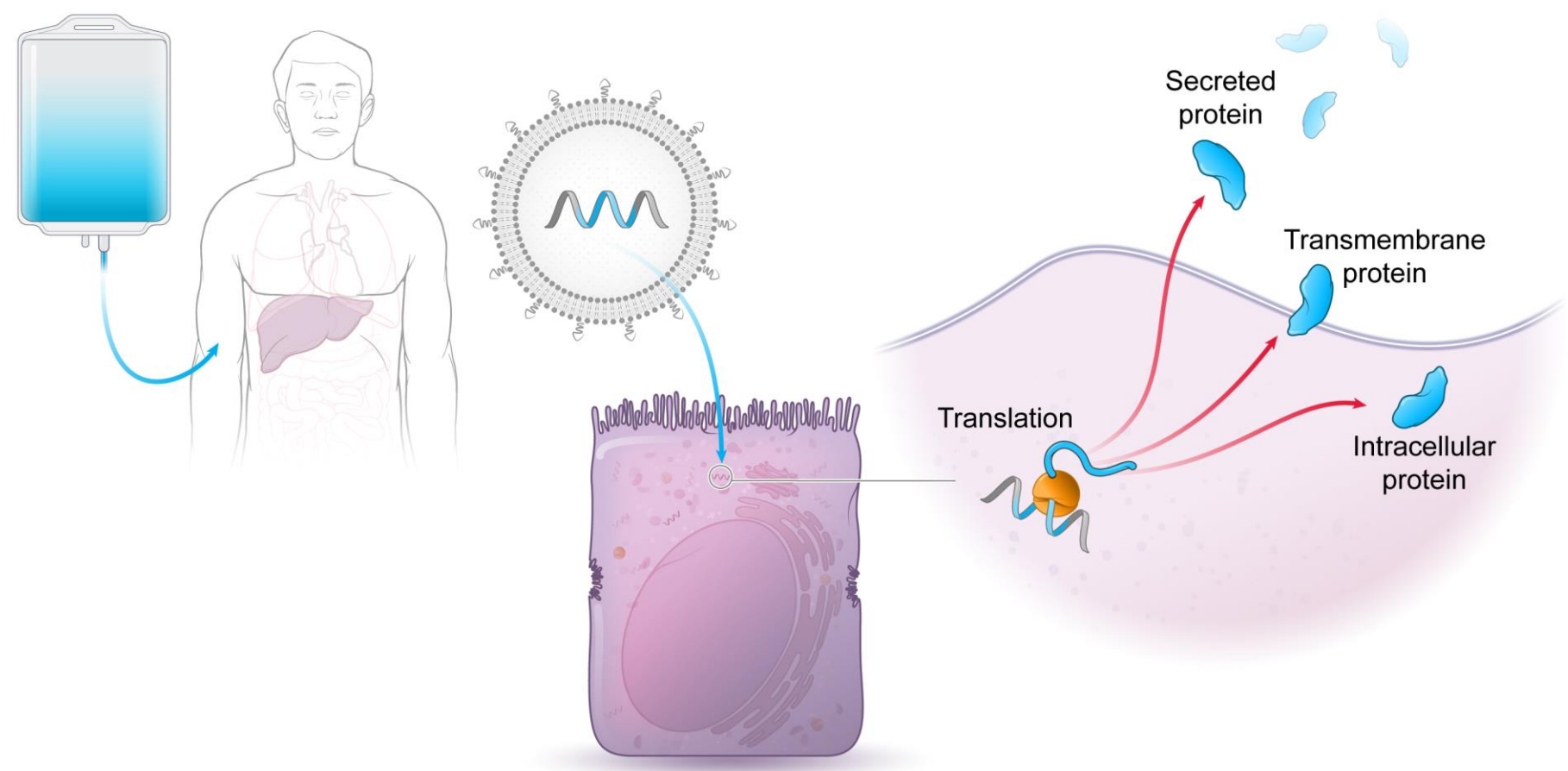
Modified from: Clarelli *et al.* (2020) *Cell Mol Life Sci*
doi: 10.1007/s00018-019-03376-y

Factors controlling duration of effect by traditional small molecule drugs are well understood

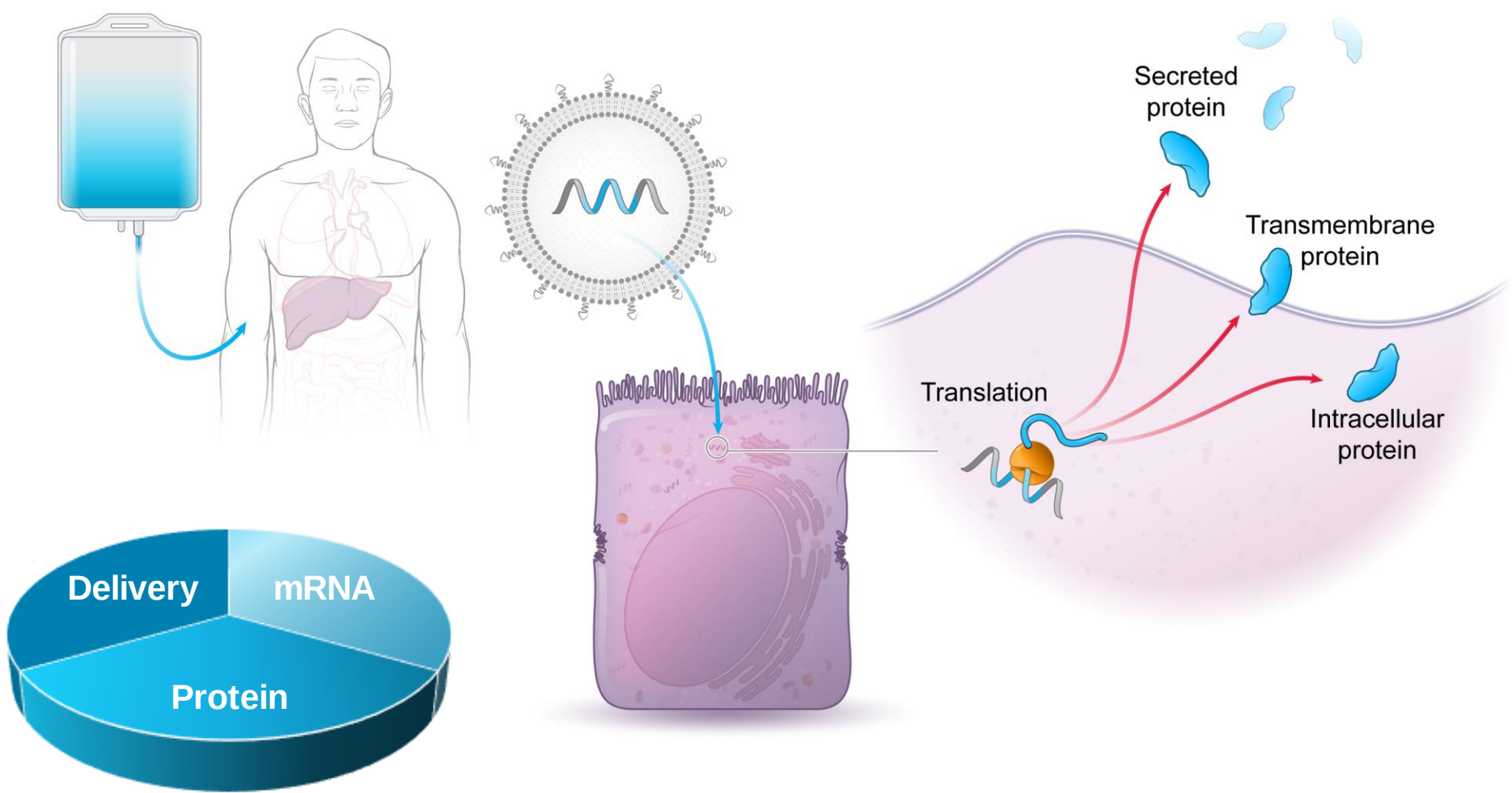


Modified from: Clarelli *et al.* (2020) *Cell Mol Life Sci*
doi: 10.1007/s00018-019-03376-y

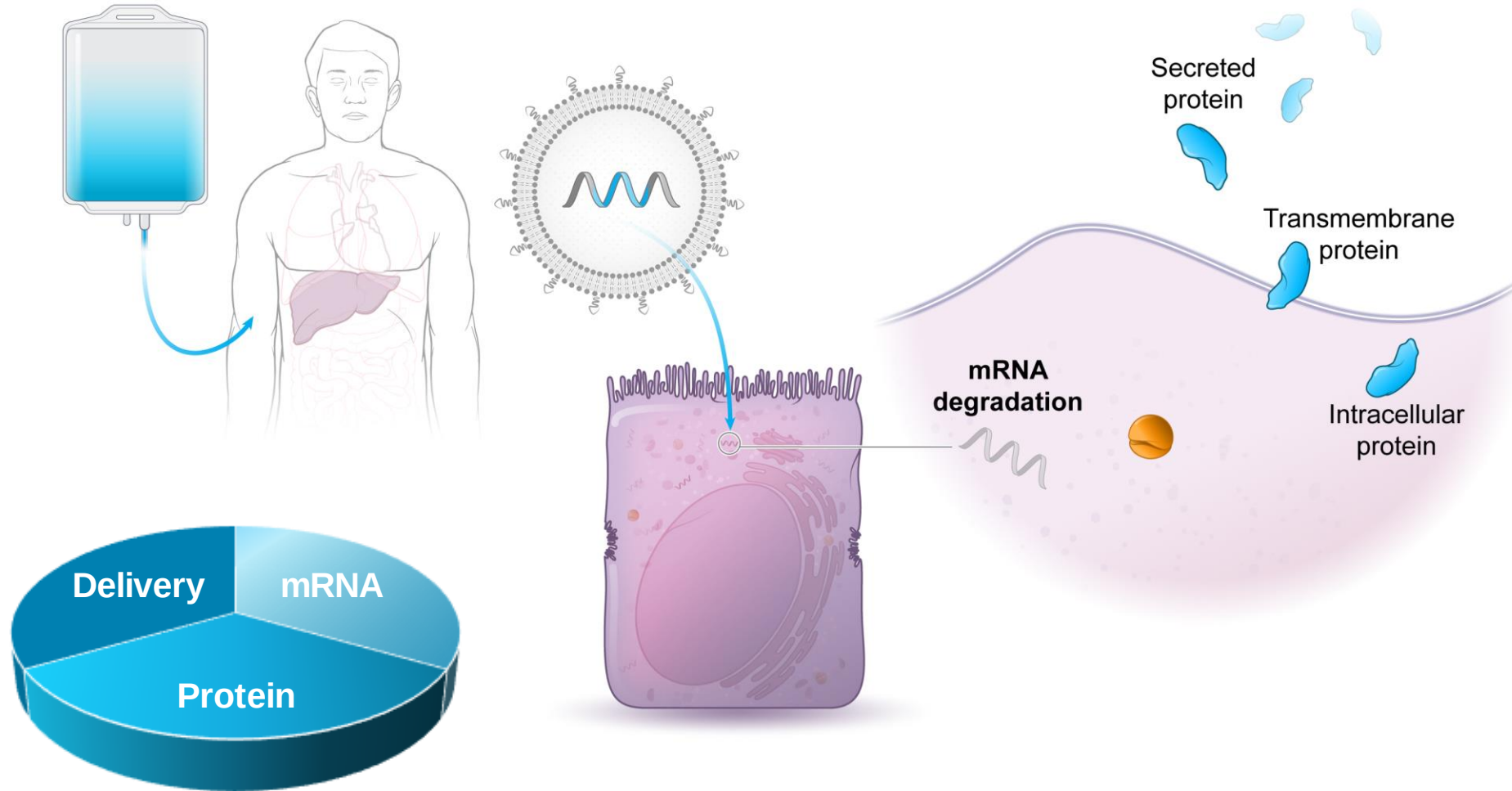
But what about mRNA medicines?



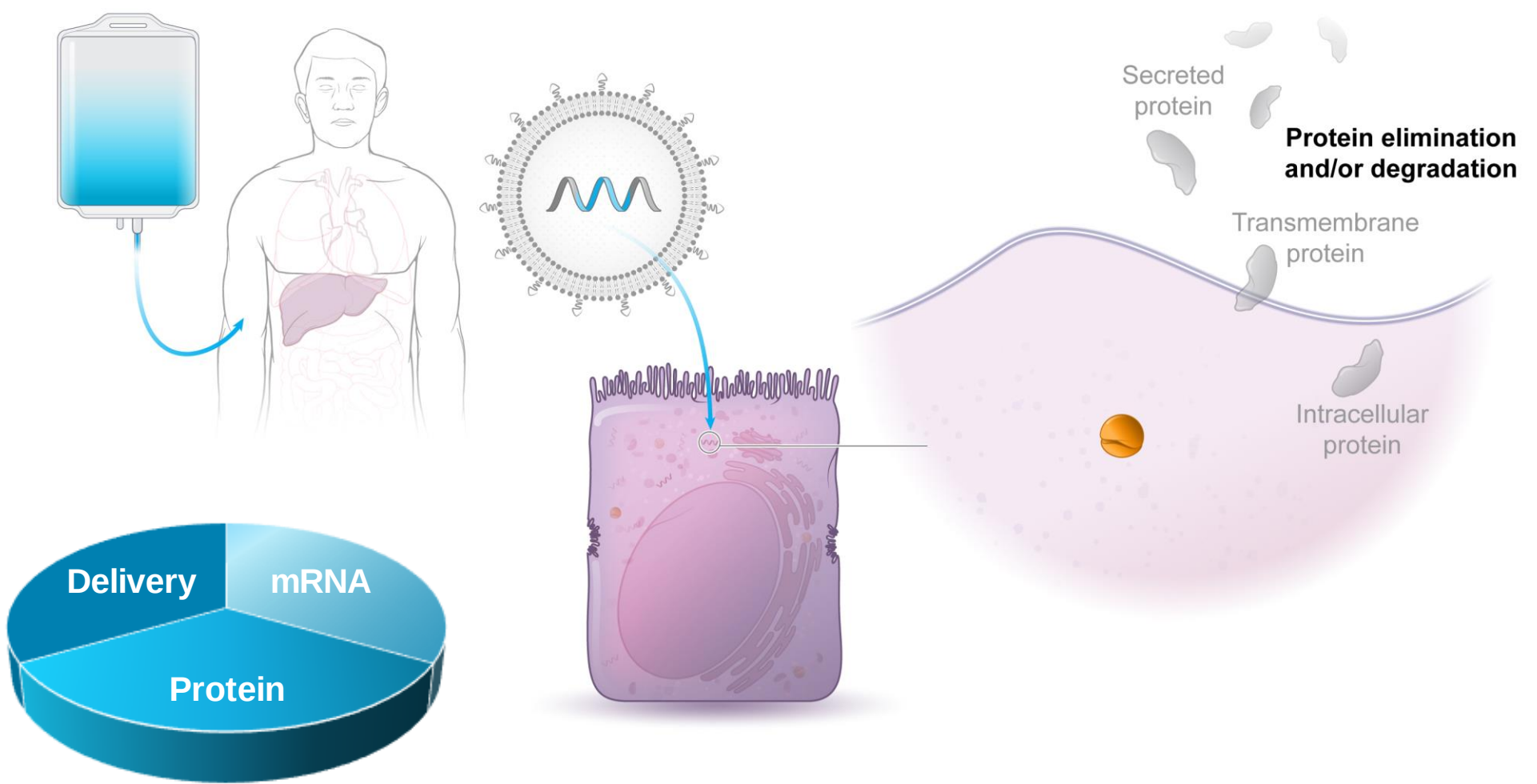
But what about mRNA medicines?



But what about mRNA medicines?



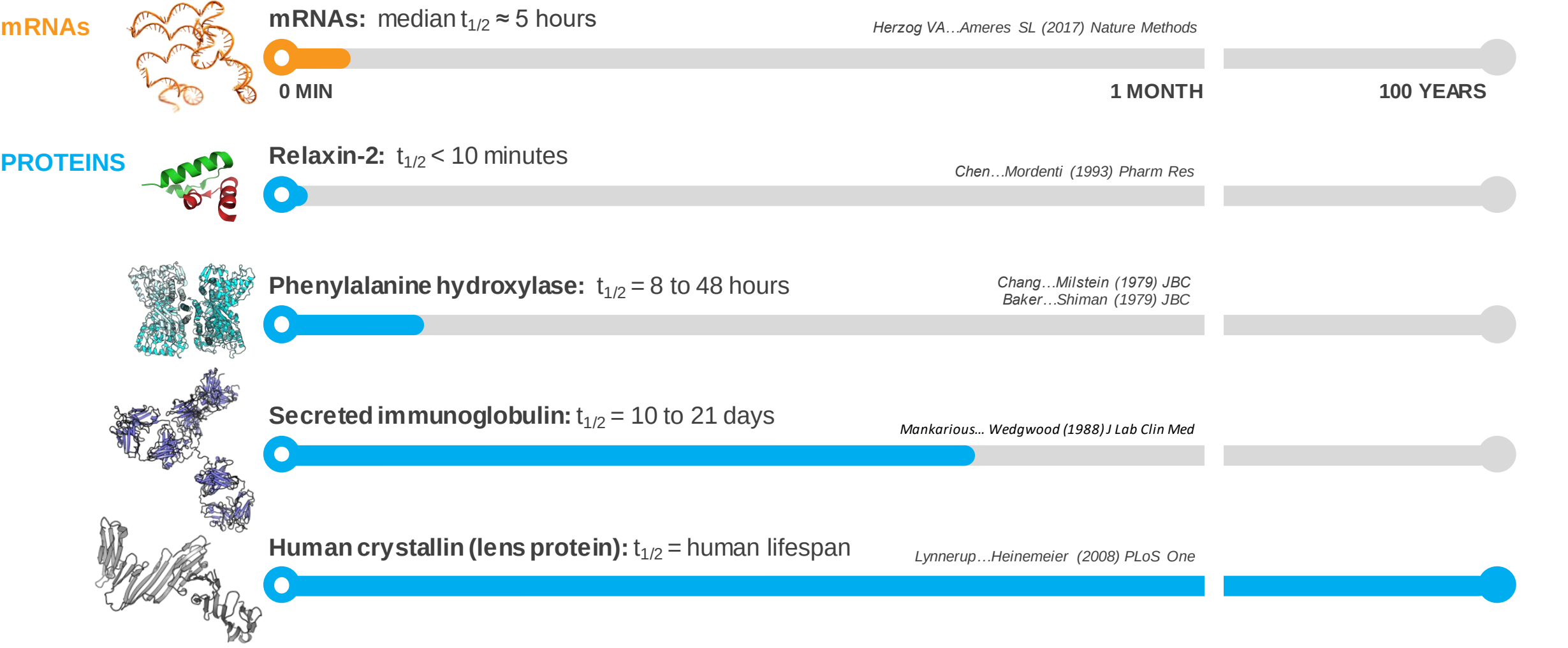
But what about mRNA medicines?





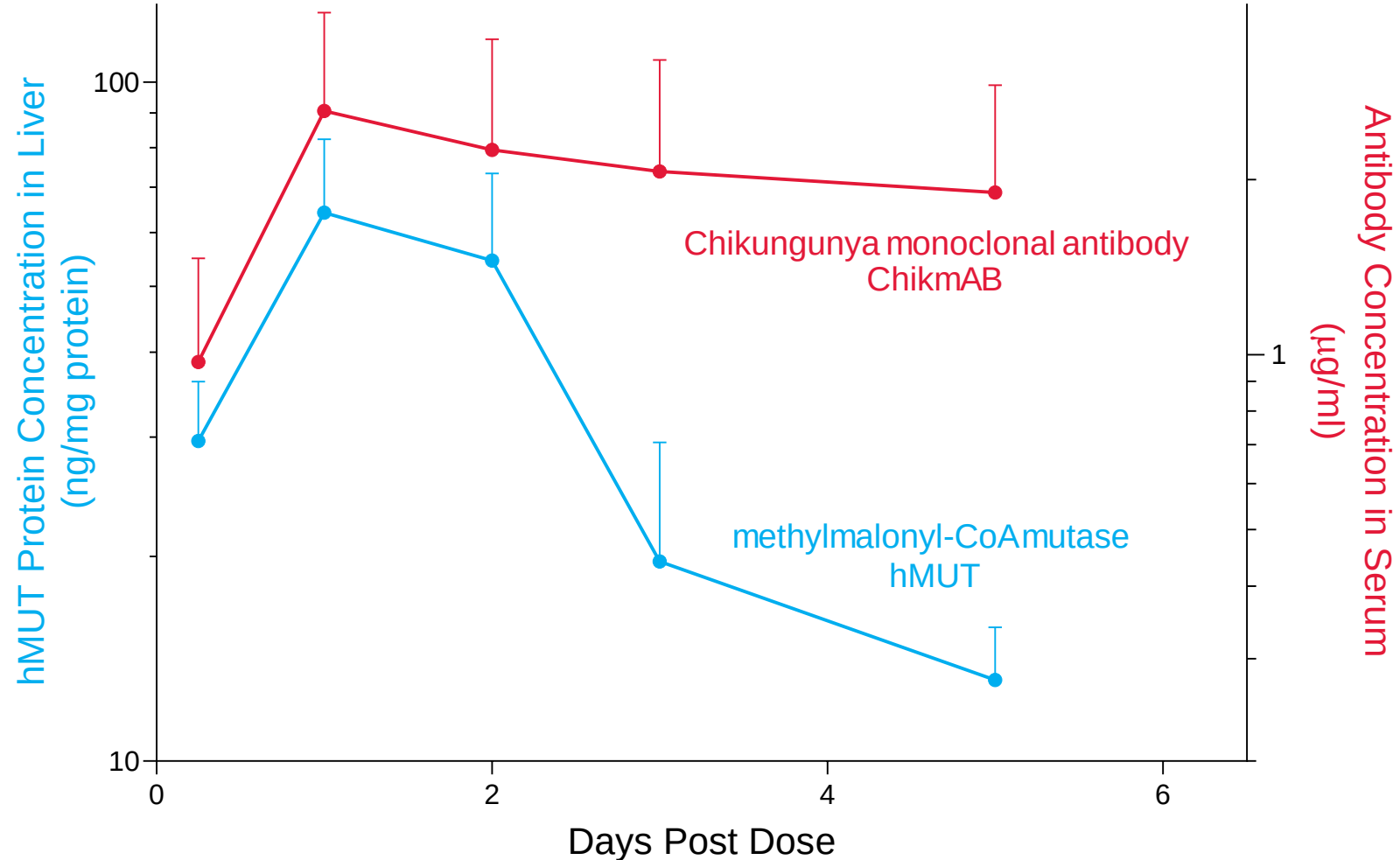
How mRNA and protein half-lives interplay
to modulate pharmacologic effects

What are typical endogenous mRNA and protein half-lives?



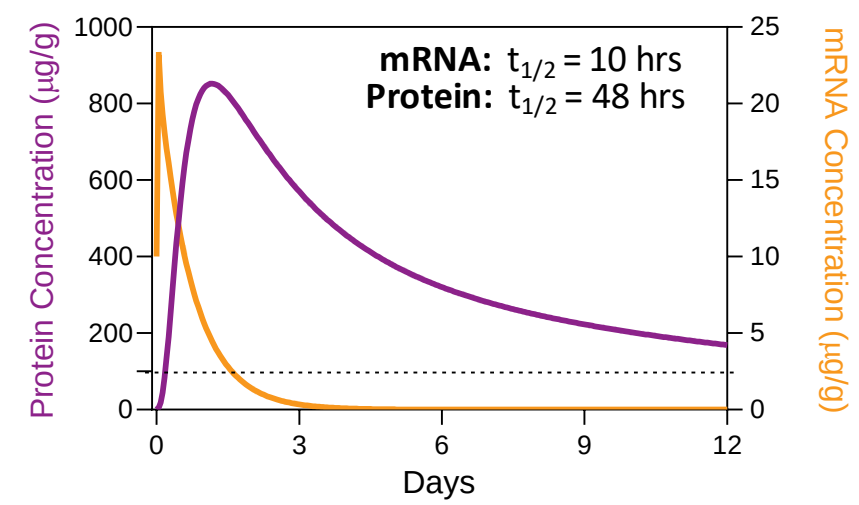
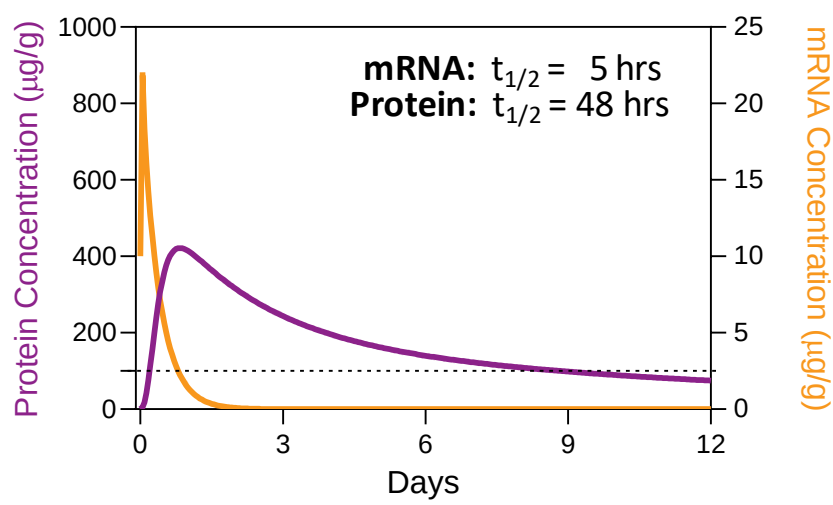
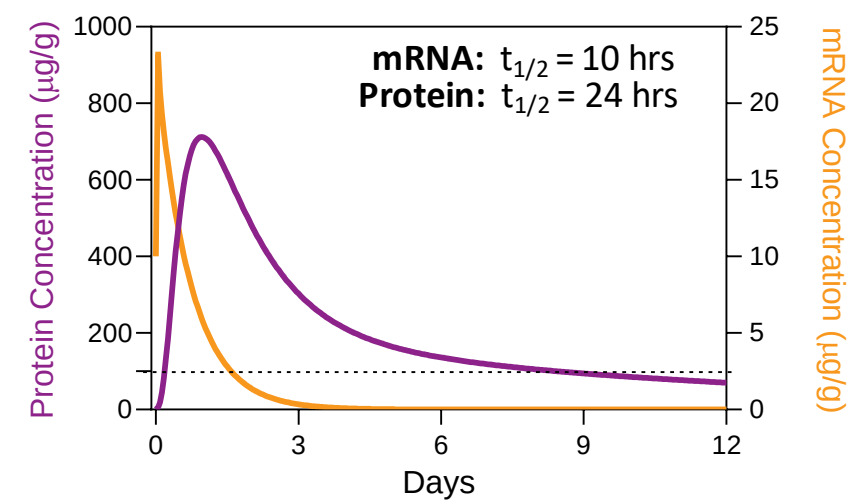
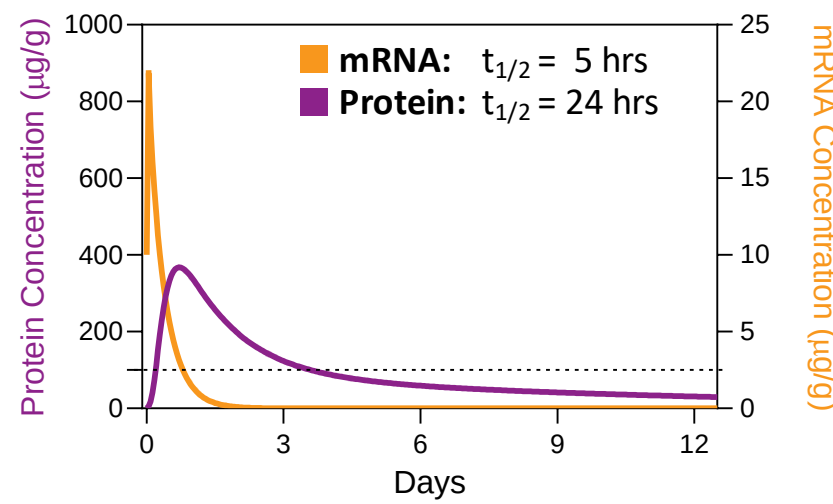
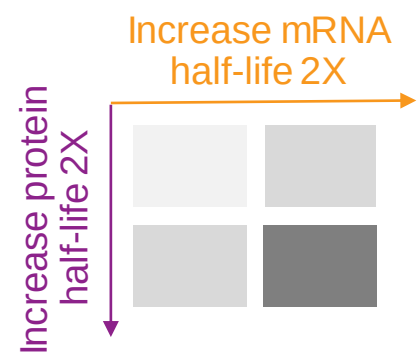
Examples of our therapeutic mRNA and protein half-lives (t_{1/2})

t _{1/2}	hMUT (mouse)	ChikmAb (NHP)
mRNA	5 hours	11 hours
protein	24 hours	26 days

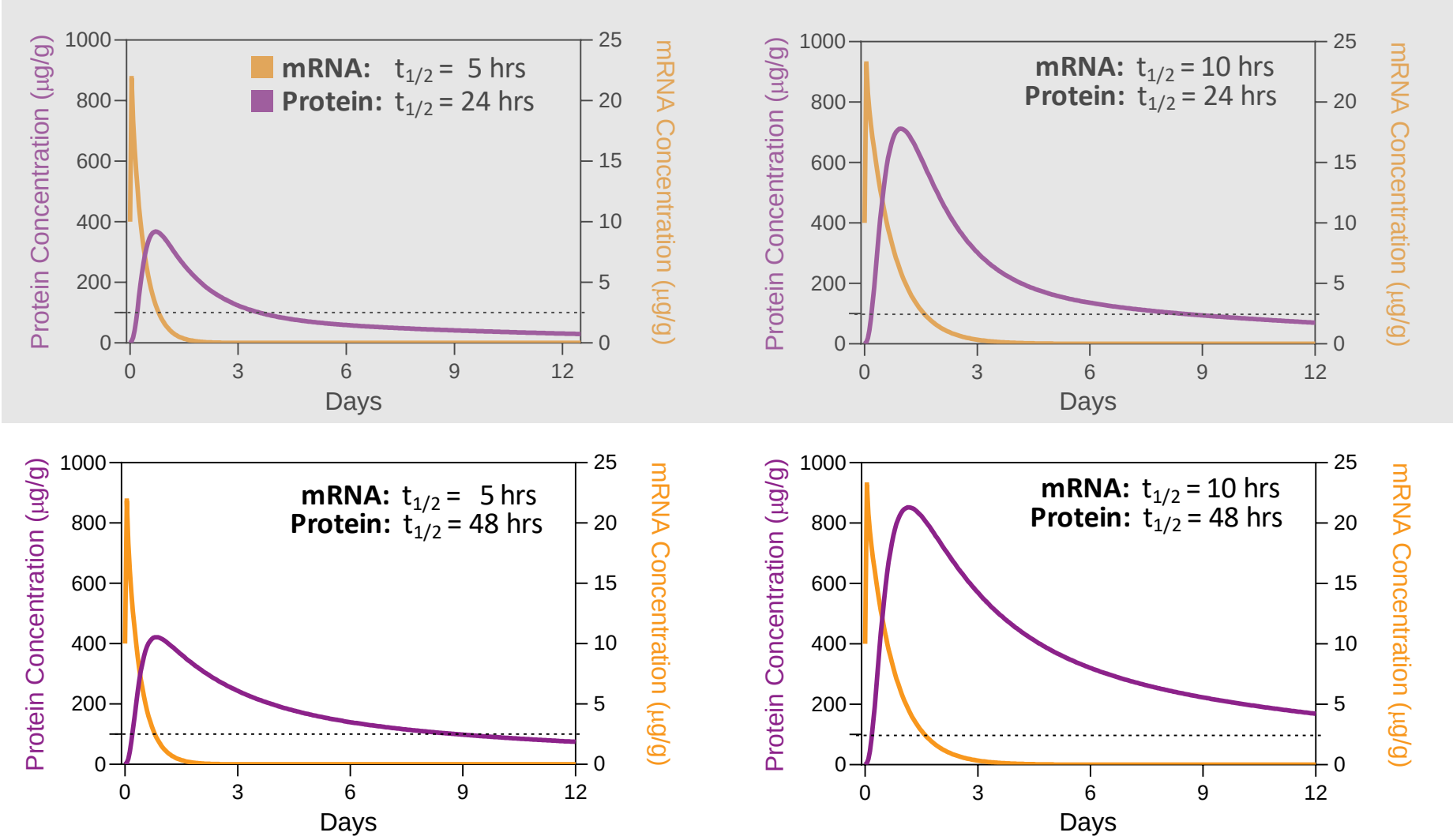
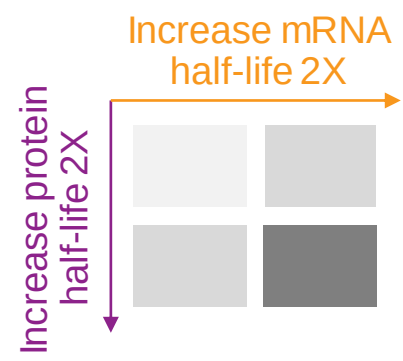


An *et al.* 2017 **Cell Rep** doi: 10.1016/j.celrep.2017.11.081
Kose *et al.* 2019 **Sci Immunol** doi: 10.1126/sciimmunol.aaw6647

Duration of response is a function of both mRNA and protein half-life



How can we extend mRNA half-life?



One way to increase mRNA half-life is to maximize codon optimality and secondary structure in the coding sequence

mRNA structure regulates protein expression through changes in functional half-life

David M. Mauger², B. Joseph Cabral^{3,1}, Vladimir Presnyak², Stephen V. Su^{2,2}, David W. Reid², Brooke Goodman², Kristian Link⁴, Nikhil Khatwani^{2,5}, John Reyniers^{2,6}, Melissa J. Moore^{2,5}, and Iain J. McFadyen^{2,6}

²Platform Research, Moderna, Inc., Cambridge, MA 02139

Edited by Joseph D. Puglisi, Stanford University School of Medicine, Stanford, CA, and approved October 8, 2019 (received for review May 9, 2019)

Messenger RNAs (mRNAs) encode information in both their primary sequence and their higher order structure. The independent contributions of factors like codon usage and secondary structure to regulating protein expression are difficult to establish as they are often highly correlated in endogenous sequences. Here, we used 2 approaches, global inclusion of modified nucleotides and rational sequence design of exogenously delivered constructs, to understand the role of mRNA secondary structure independent from codon usage. Unexpectedly, highly expressed mRNAs contained a highly structured coding sequence (CDS). Modified nucleotides that stabilize mRNA secondary structure enabled high expression across a wide variety of primary sequences. Using a set of eGFP mRNAs with independently altered codon usage and CDS structure, we find that the structure of the CDS regulates protein expression through changes in functional mRNA half-life (i.e., mRNA being actively translated). This work highlights an underappreciated role of mRNA secondary structure in the regulation of mRNA stability.

mRNA therapeutics | modified nucleotides | translation | RNA structure | SHAPE

Messenger RNAs (mRNAs) direct cytoplasmic protein expression. How much protein is produced per mRNA molecule is a function of how well the translational machinery initiates and elongates on the coding sequence (CDS) and the mRNA's functional half-life. Both translational efficiency and functional half-life are driven by features encoded in the primary mRNA sequence. Synonymous codon choice directly impacts translation, with highly expressed genes tending to include more "optimal" codons (1, 2). Conversely, "nonoptimal" codons can increase ribosomal pausing and decrease mRNA half-life (3, 4). Other mRNA sequence features that reportedly correlate with protein output are dinucleotide frequency in the CDS (5) and the effect of codon order on locally accessible charged tRNA pools (6). Because these effects are interdependent on mRNA sequence, teasing apart their individual contributions to protein output is difficult and often controversial (7, 8).

In addition to dictating encoded protein identity, its primary sequence also determines an mRNA's propensity to form secondary and tertiary structure (9). Transcriptome-wide RNA structure characterization is beginning to reveal global relationships between the structure content in different mRNA regions and protein expression (10, 11). Multiple studies have shown that secondary structure in the 5' untranslated region (5' UTR) generally reduces translation initiation efficiency and therefore overall protein output (10–13). But, the extent to which CDS and 3' untranslated region (3' UTR) secondary structure impacts protein output, however, is much less understood.

One way to alter RNA secondary structure is to change the primary sequence. In the CDS, however, primary sequence changes necessarily alter codon usage, confounding any effects that might be attributable to changes in mRNA structure alone. An alternate means to affect secondary structure without changing codons is to incorporate modified nucleotides (nt) that maintain the same Watson-Crick base-pairing relationships (e.g., pseudouridine [P] for U) but have small effects on local secondary structure. Such

modified nucleotides can either stabilize (14) or destabilize (15) base pairs and hence overall mRNA structure.

Here, we combined computational sequence design with global modified nucleotide substitution as tools to investigate the separate impacts of mRNA primary sequence and structural stability on protein output. We find that differences in the innate thermodynamic base pair stability of 2 modified uridine nucleotides, N¹-methylpseudouridine and 5-methoxy-uridine, induce global changes in mRNA secondary structure. These structural changes in turn drive changes in protein expression. As expected, our data confirm that reduced secondary structure within a 5' leader region (the 5' UTR and first ~10 codons of the CDS) correlates with high protein expression. Surprisingly, we also find that high protein expression correlates with increased secondary structure in the remainder of the mRNA (the rest of the CDS and the 3' UTR). We validated this finding by designing an enhanced gene fluorescent protein

Significance

Despite widespread recognition that RNA is inherently structured, the interplay between local and global mRNA secondary structure (particularly in the coding region) and overall protein expression has not been thoroughly explored. Our work uses 2 approaches to disentangle the regulatory roles of mRNA primary sequence and secondary structure: global substitution with modified nucleotides and computational sequence design. By fitting detailed kinetic expression data to mathematical models, we show that secondary structure can increase mRNA half-life independent of codon usage. These findings have significant implications for both translational regulation of endogenous mRNAs and the emerging field of mRNA therapeutics.

Author contributions: D.M.M., B.J.C., V.P., K.L., J.R., M.J.M., and I.J.M. designed research; D.M.M., B.J.C., V.P., S.V.S., D.W.R., B.G., and N.K. performed research; V.P., S.V.S., B.G., K.L., and N.K. contributed new reagents/analytic tools; D.M.M., B.J.C., V.P., S.V.S., D.W.R., B.G., and N.K. analyzed data; and D.M.M., B.J.C., V.P., M.J.M., and I.J.M. wrote the paper.

Competing interest statement: All authors are employees (or ex-employees) in the case of S.V.S., J.R., and B.J.C.) of Moderna, Inc.

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Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, <https://www.ncbi.nlm.nih.gov/geo/> (accession no. GSE119170).

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1908052116/-/DCSupplemental.

First published November 11, 2019.

www.pnas.org/cgi/doi/10.1073/pnas.1908052116

PNAS | November 26, 2019 | vol. 116 | no. 48 | 24075–24083

Codon optimality

Faster translation

High tRNA concentration

Slower translation

Low tRNA concentration

mRNA secondary structure

Weak secondary structure

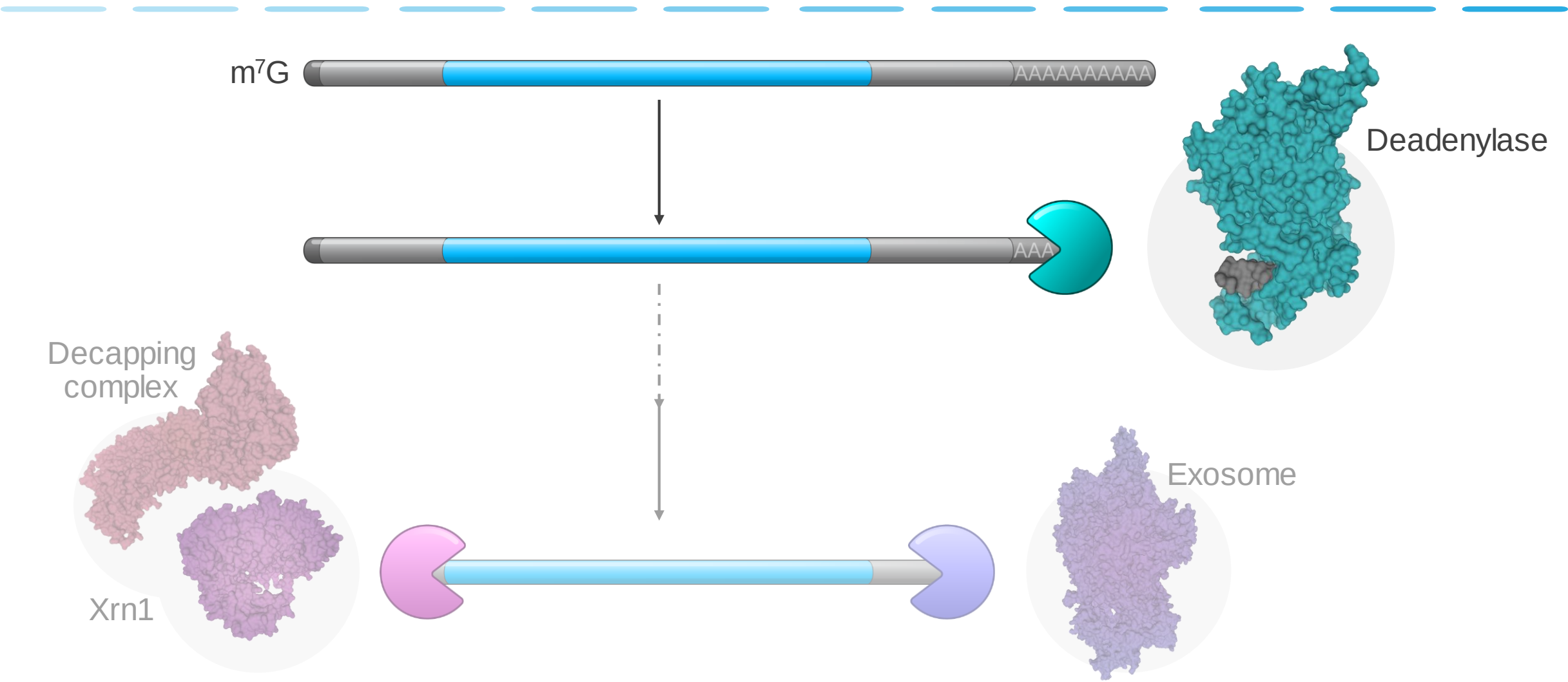
Strong secondary structure

fast

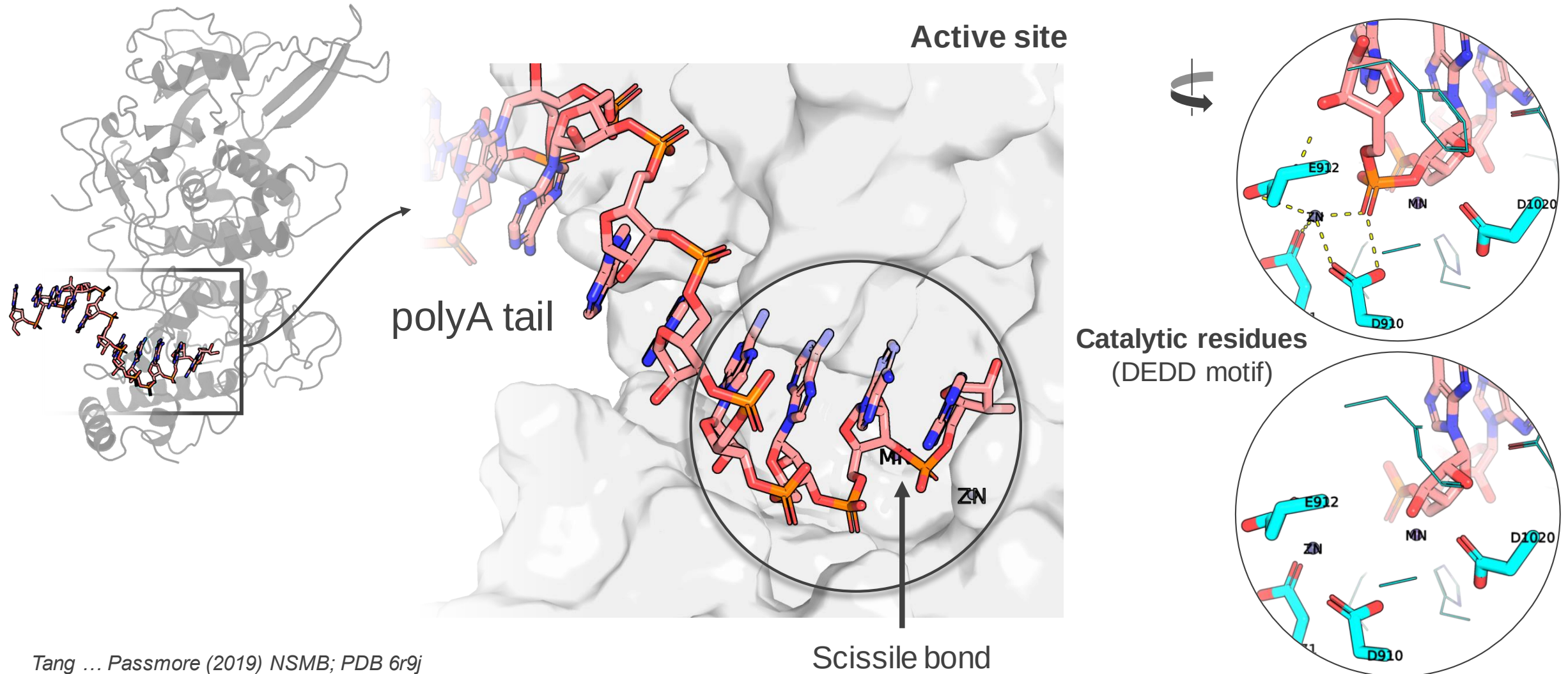
slow

after Gorochoowski ... Roubos (2014) NAR

mRNA decay in cells starts with deadenylation

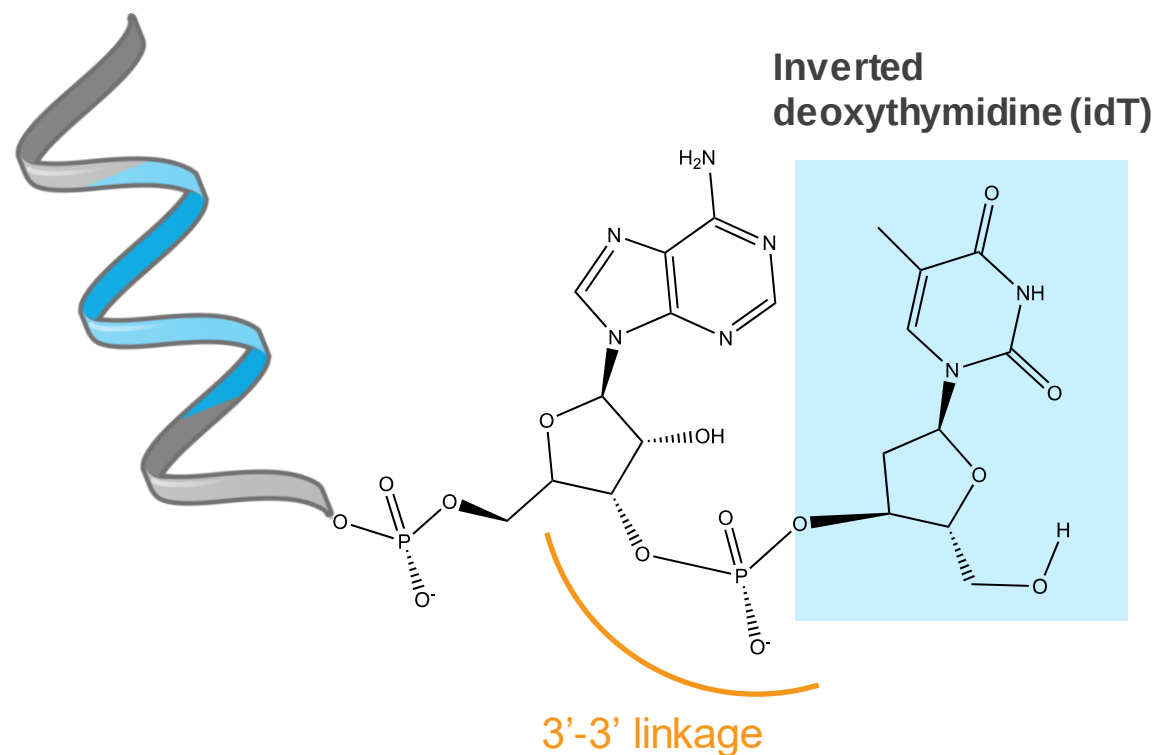
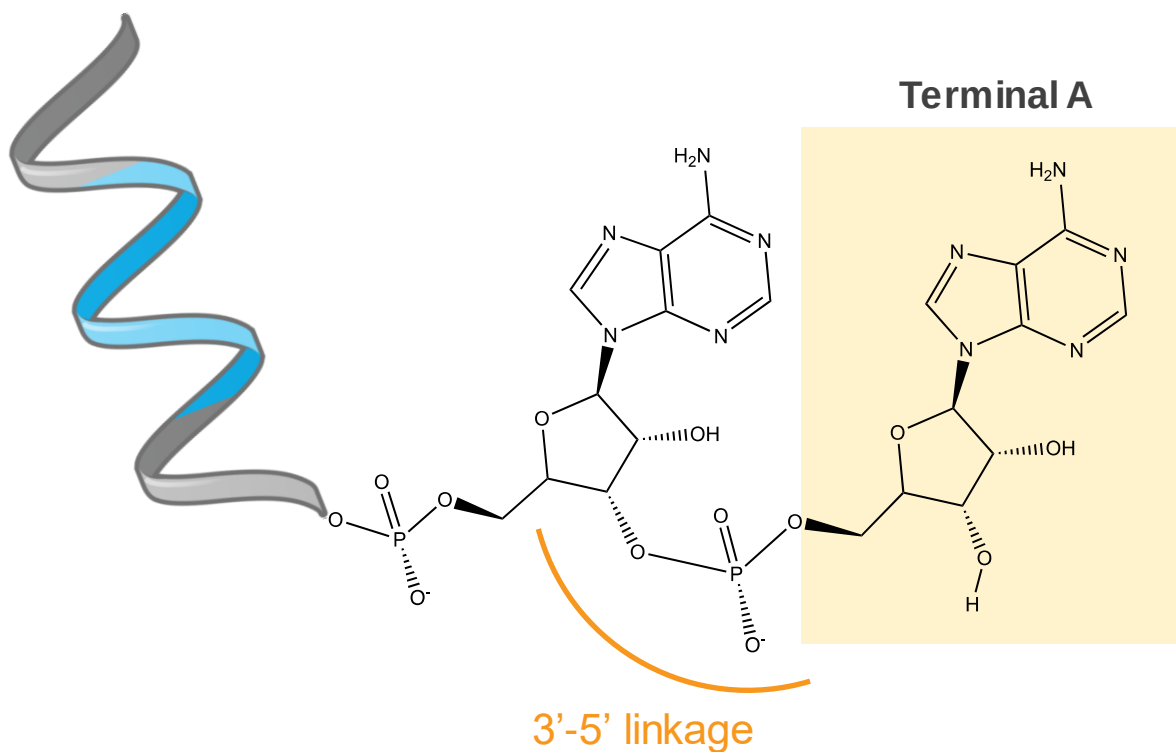


The deadenylase uses two active site metal ions to cleave off the terminal adenosine



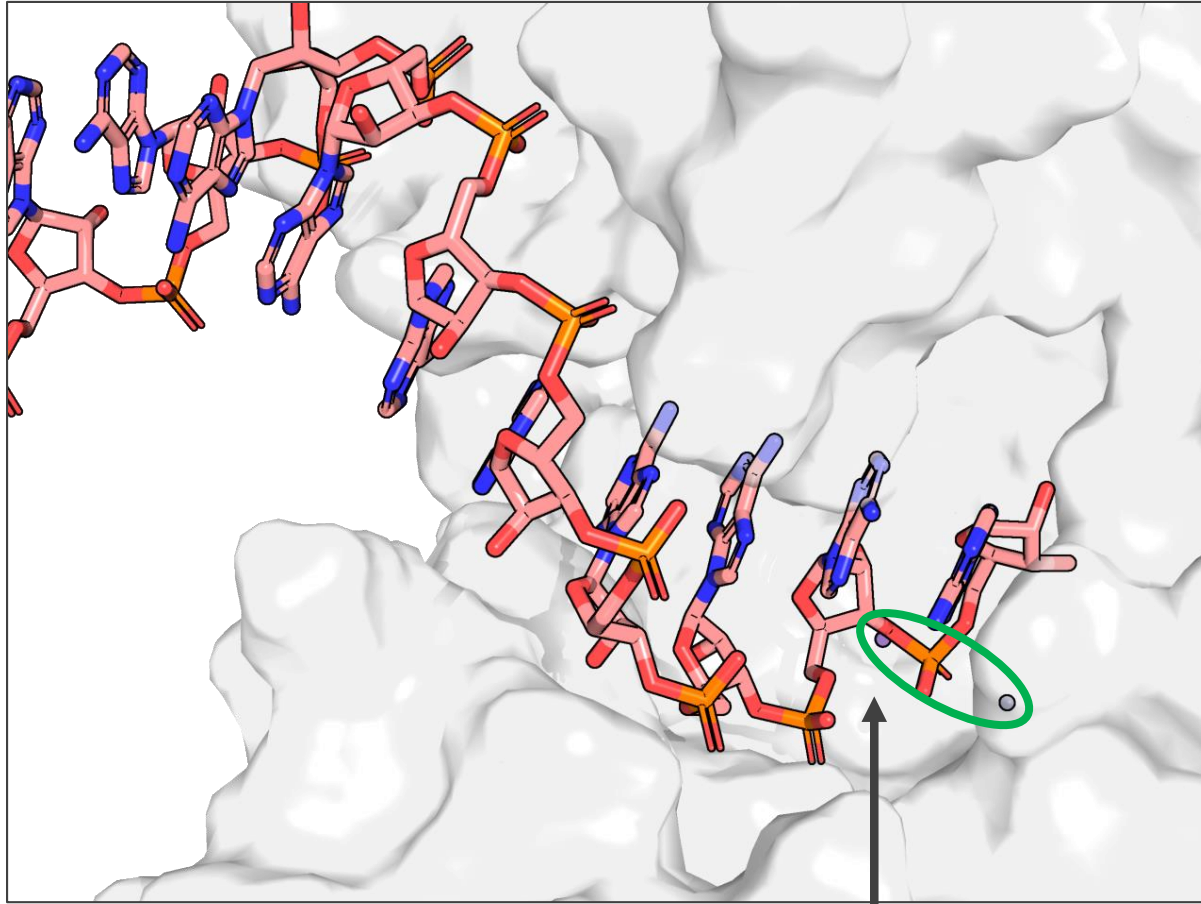
Tang ... Passmore (2019) NSMB; PDB 6r9j

Adding a terminal inverted dT (idT) changes the tail geometry



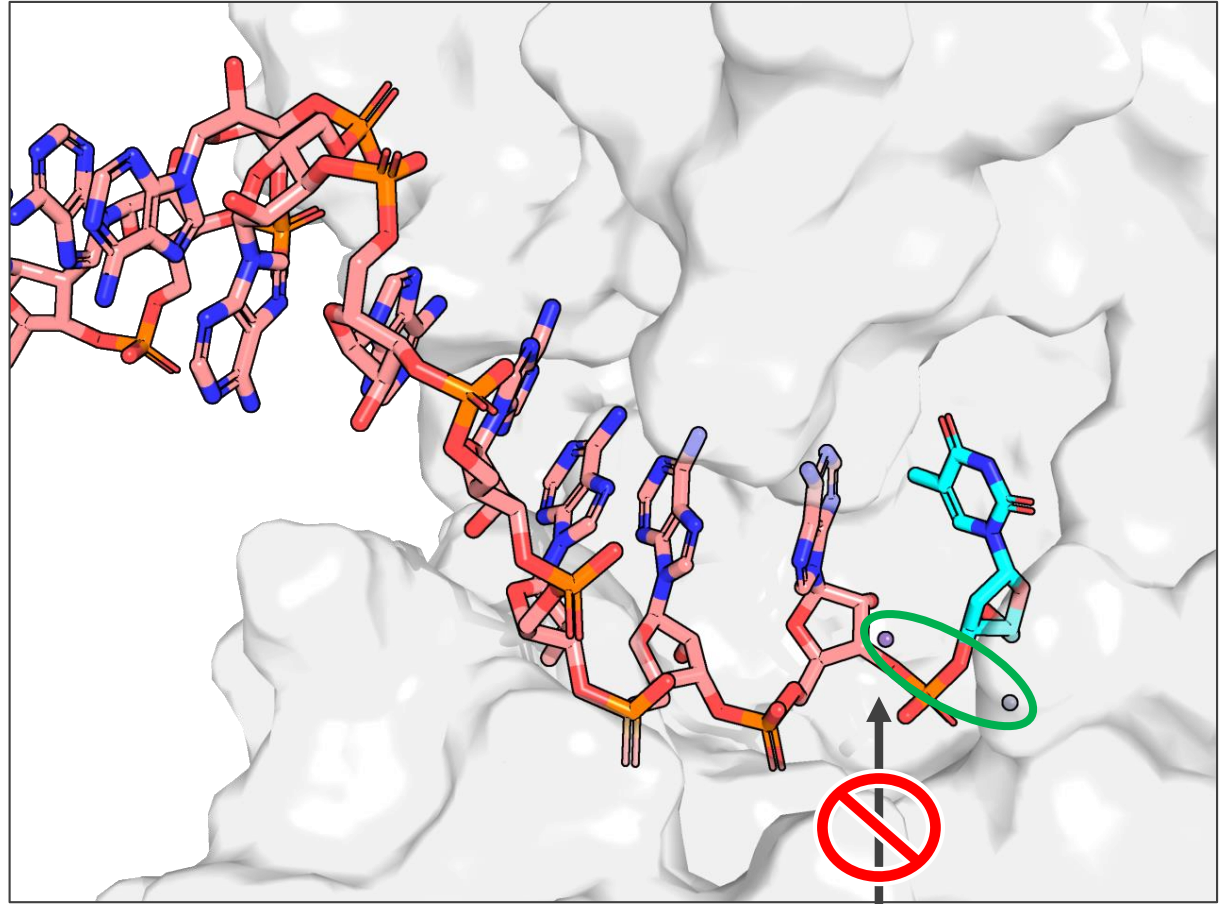
A terminal idT alters the enzyme-substrate interaction and prevents deadenylation

Standard poly(A)



Scissile bond

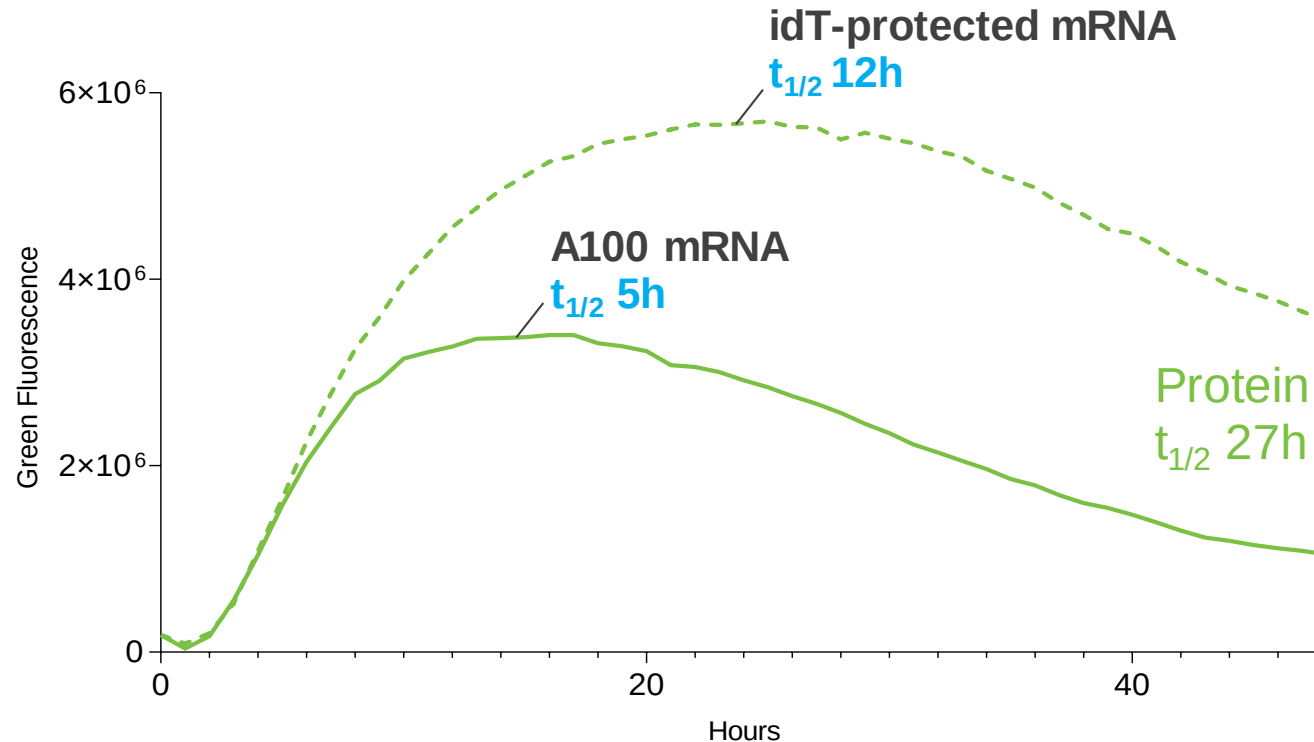
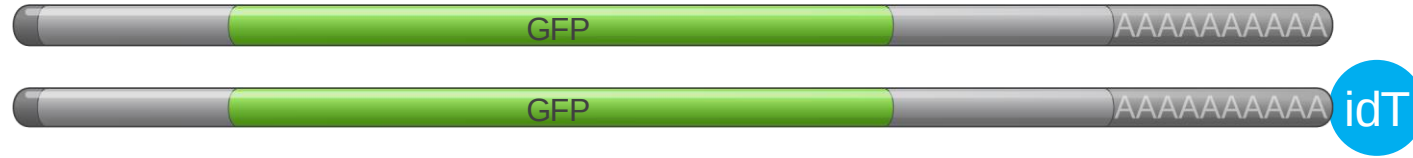
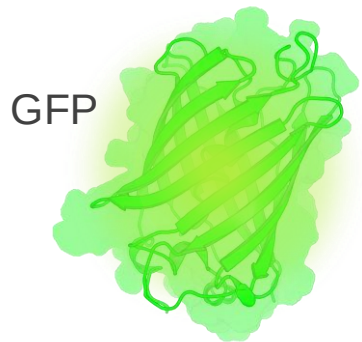
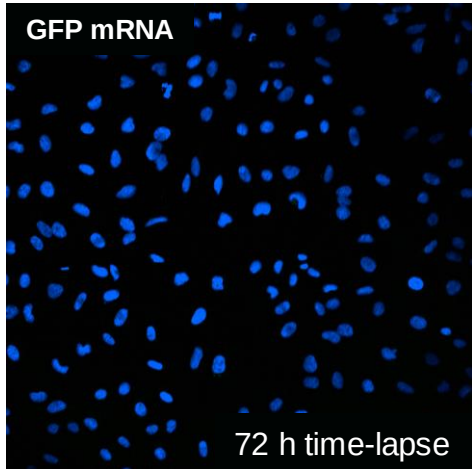
Standard poly(A) + idT



Scissile bond

idT provides a ~2-fold half-life extension in cultured cells

GFP Nuclei



$$\frac{dD}{dt} = -k_1 D$$

$$\frac{dR}{dt} = k_1 D - k_2 R$$

$$\frac{dP}{dt} = k_3 R - k_4 P$$

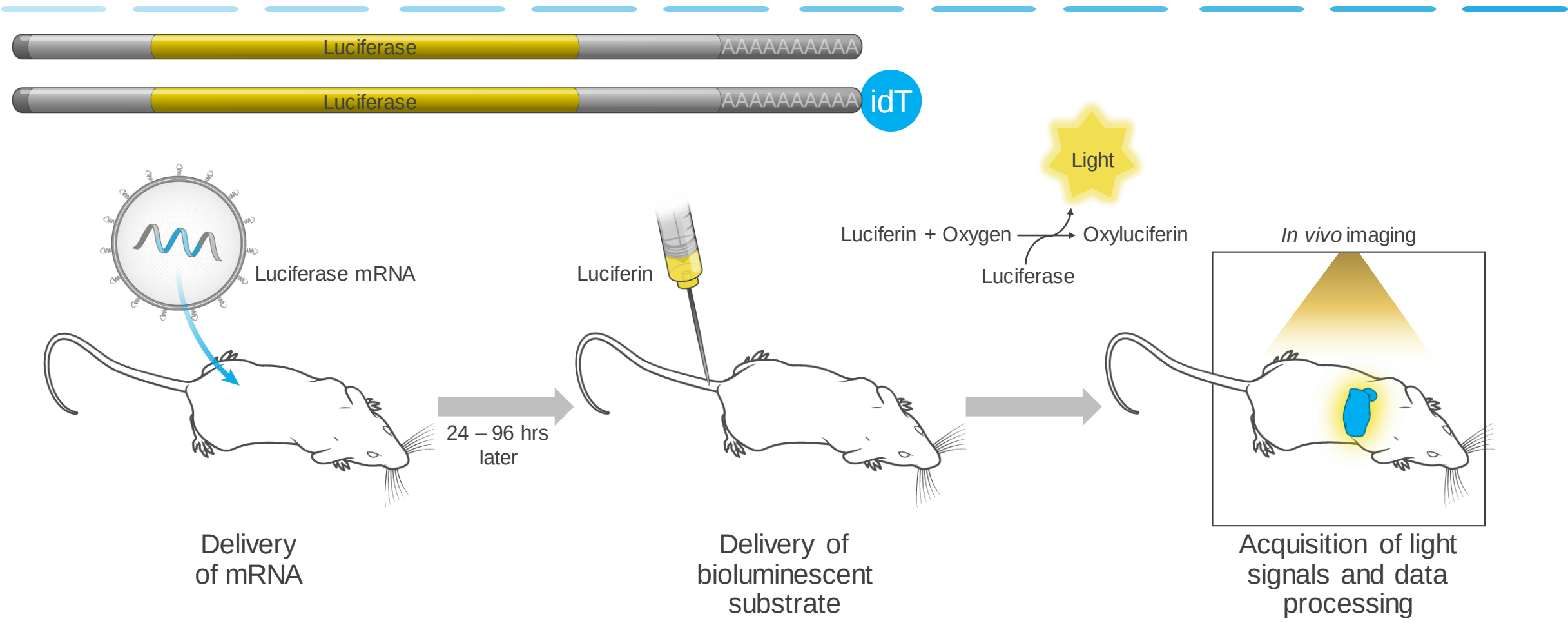
mRNA half-life:

$$t_{1/2,R} = \log(2) / k_2$$

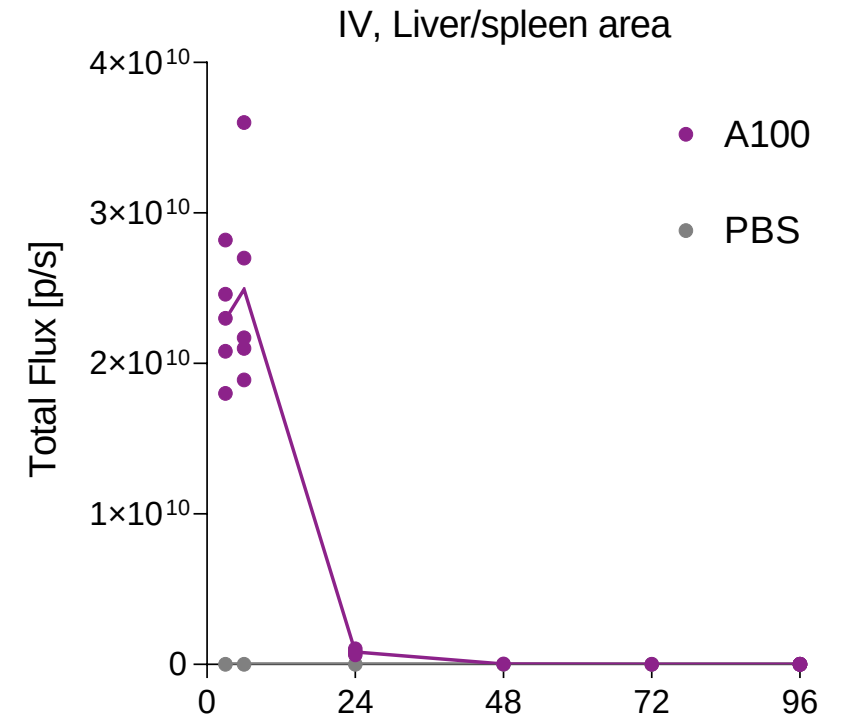
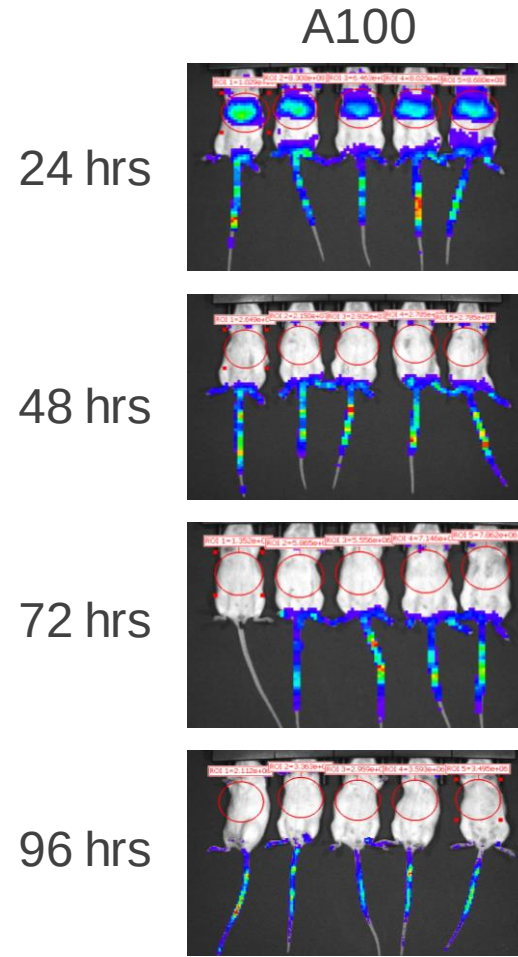
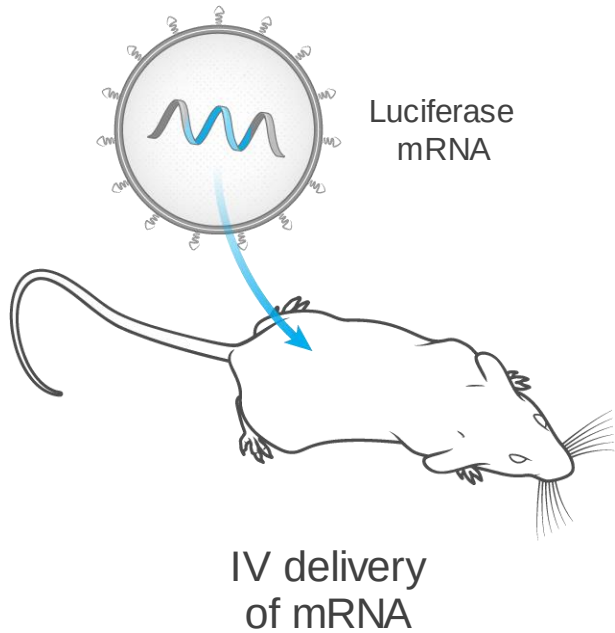
protein half-life:

$$t_{1/2,P} = \log(2) / k_4$$

For *in vivo* studies, we can use a luciferase reporter

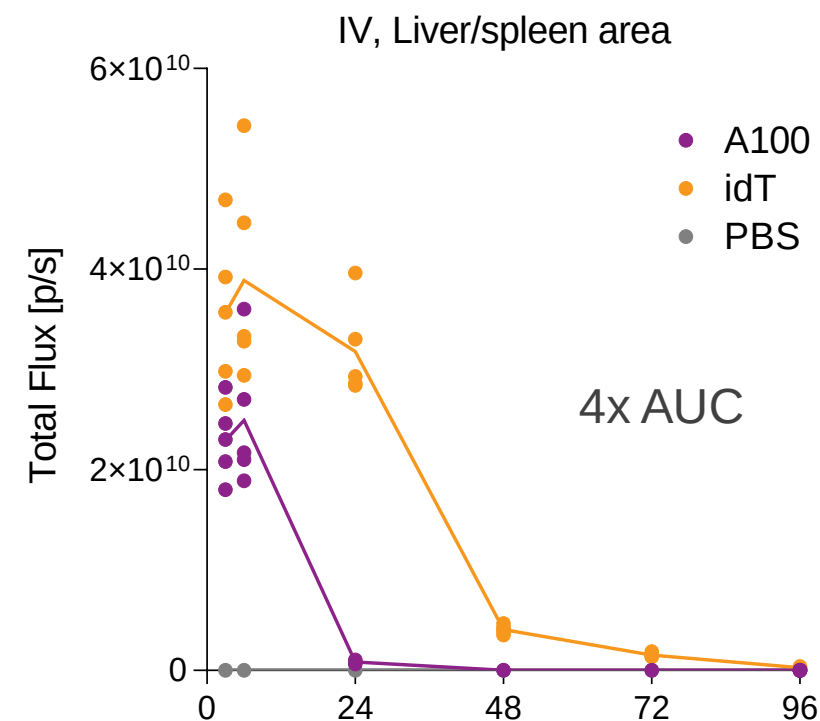
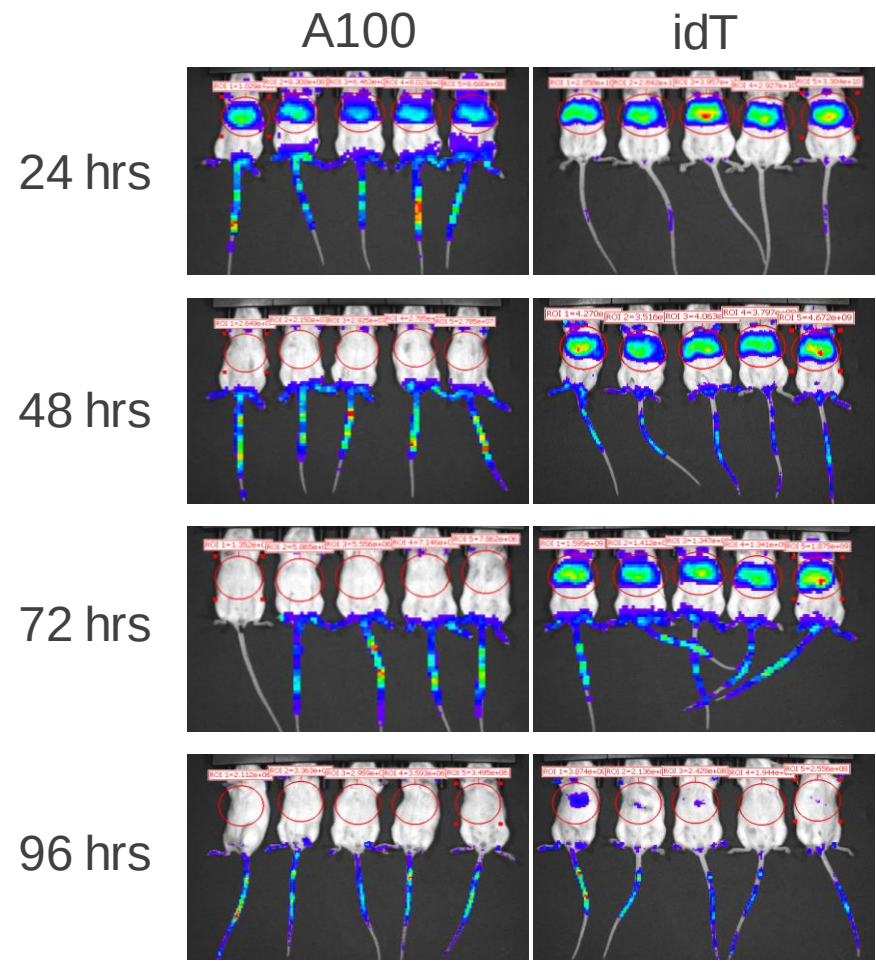
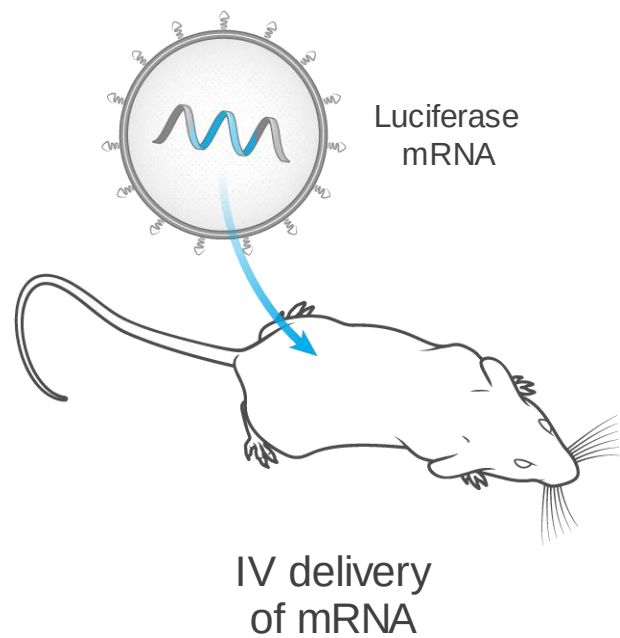


idT luciferase mRNA gives a 4-fold enhancement of protein activity



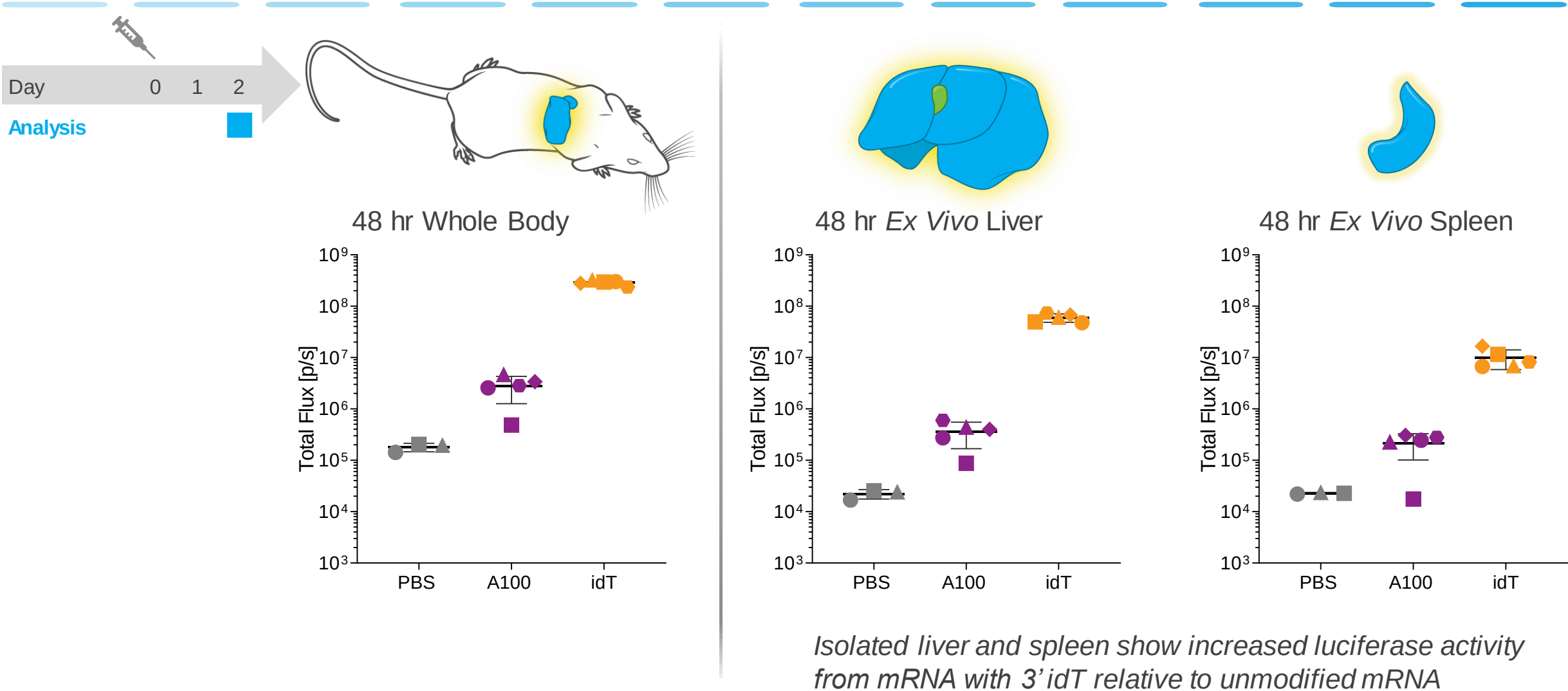
idT, inverted deoxythymidine; IV, intravenous

idT luciferase mRNA gives a 4-fold enhancement of protein activity

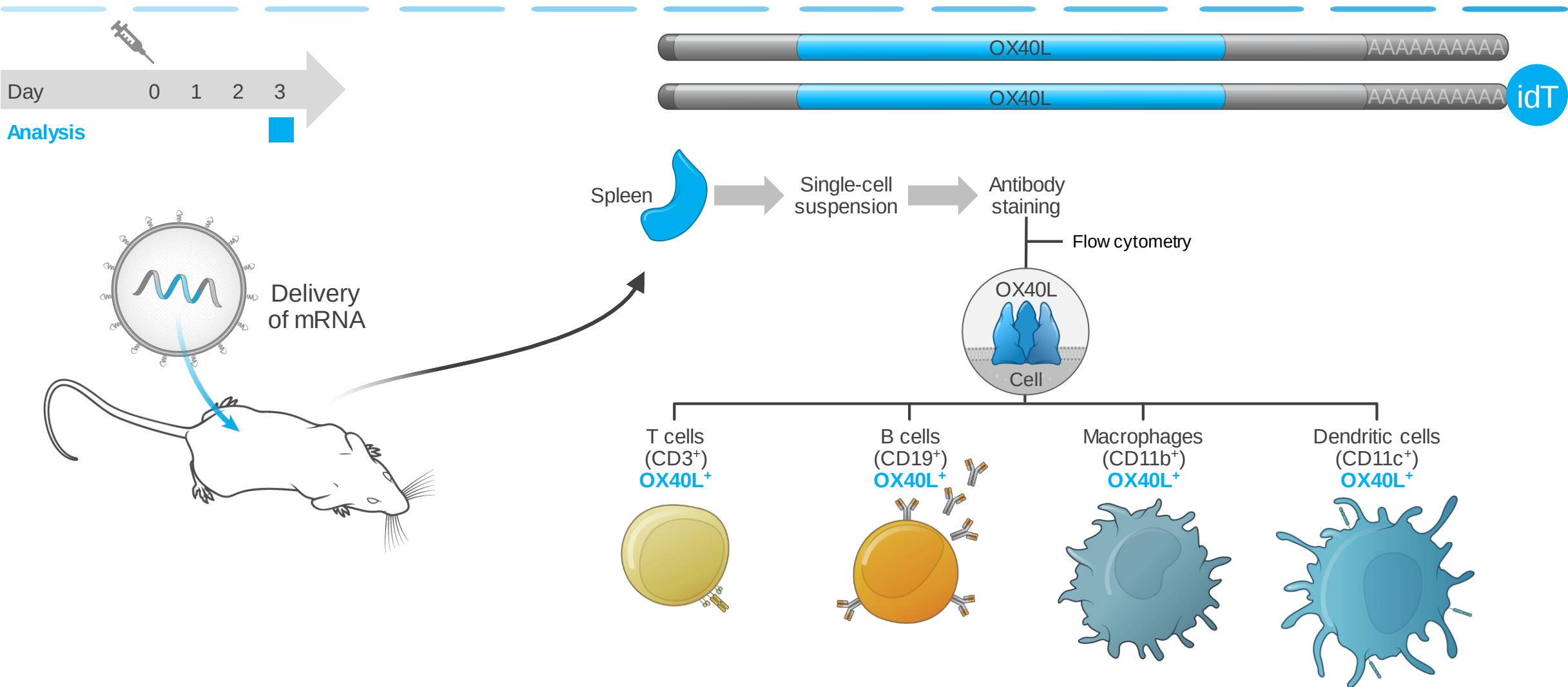


idT, inverted deoxythymidine; IV, intravenous

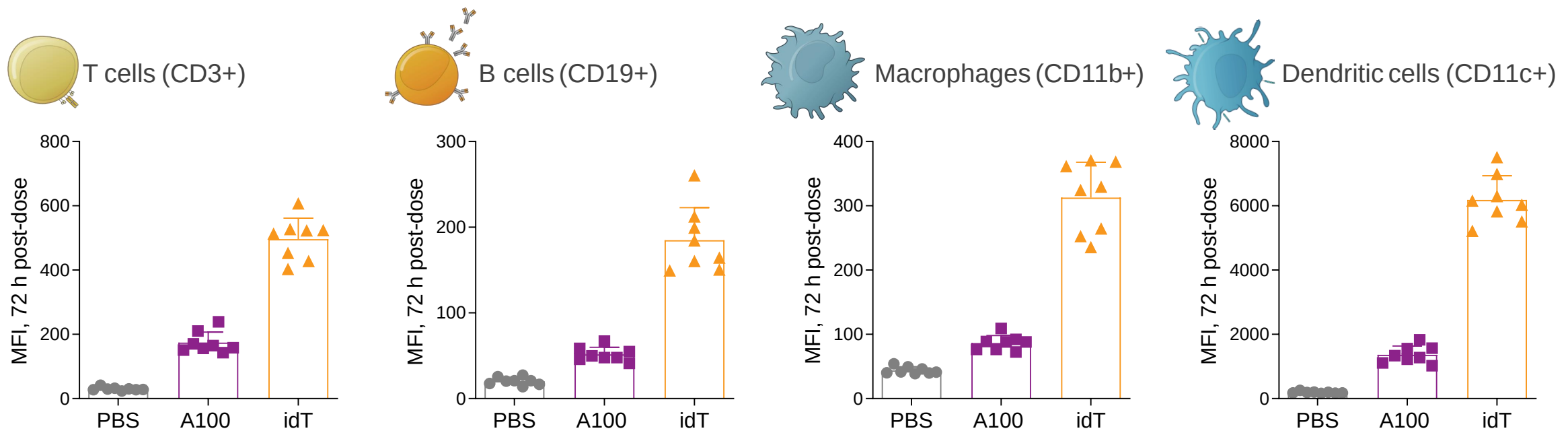
Substantial enhancement in luciferase expression in both liver and spleen at 48 hours



OX40L reporter for immune cell expression



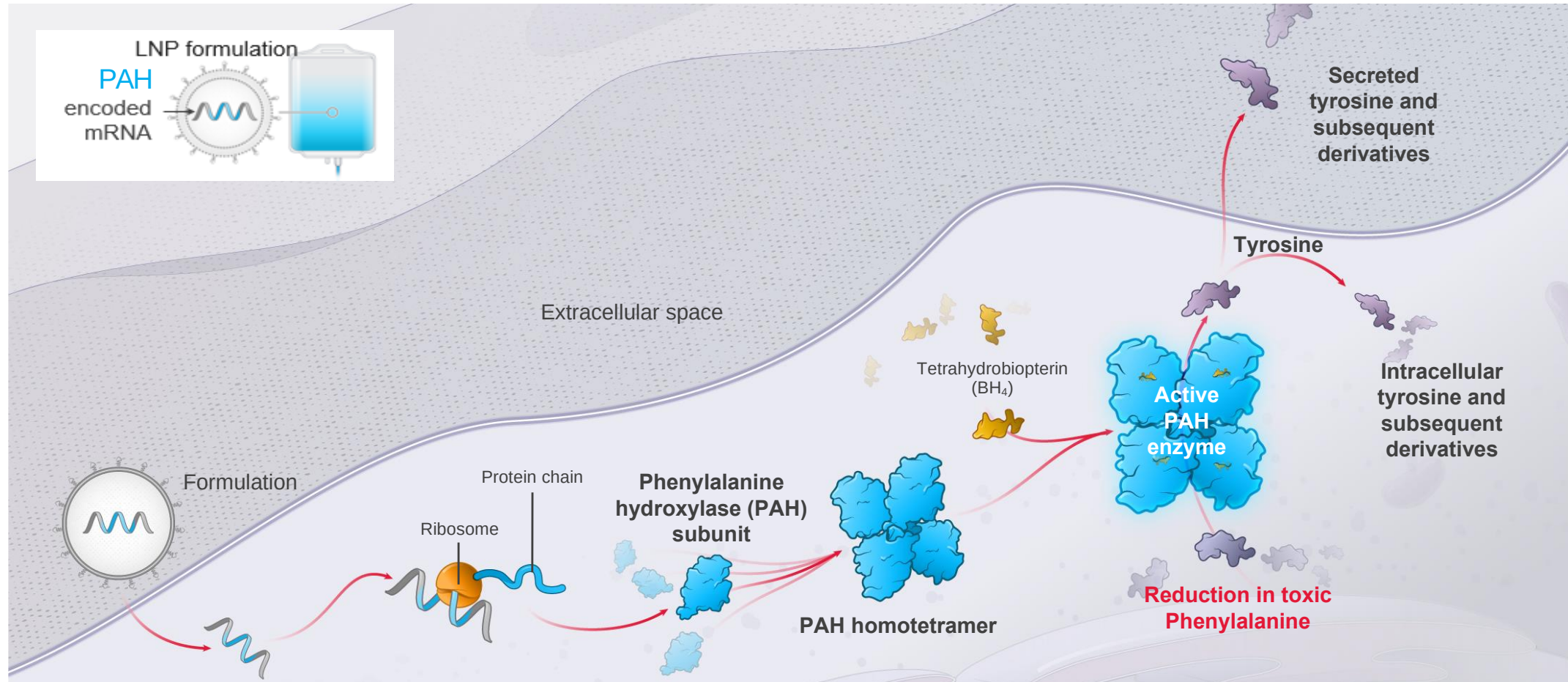
idT also enhances protein expression in sorted splenic immune cells



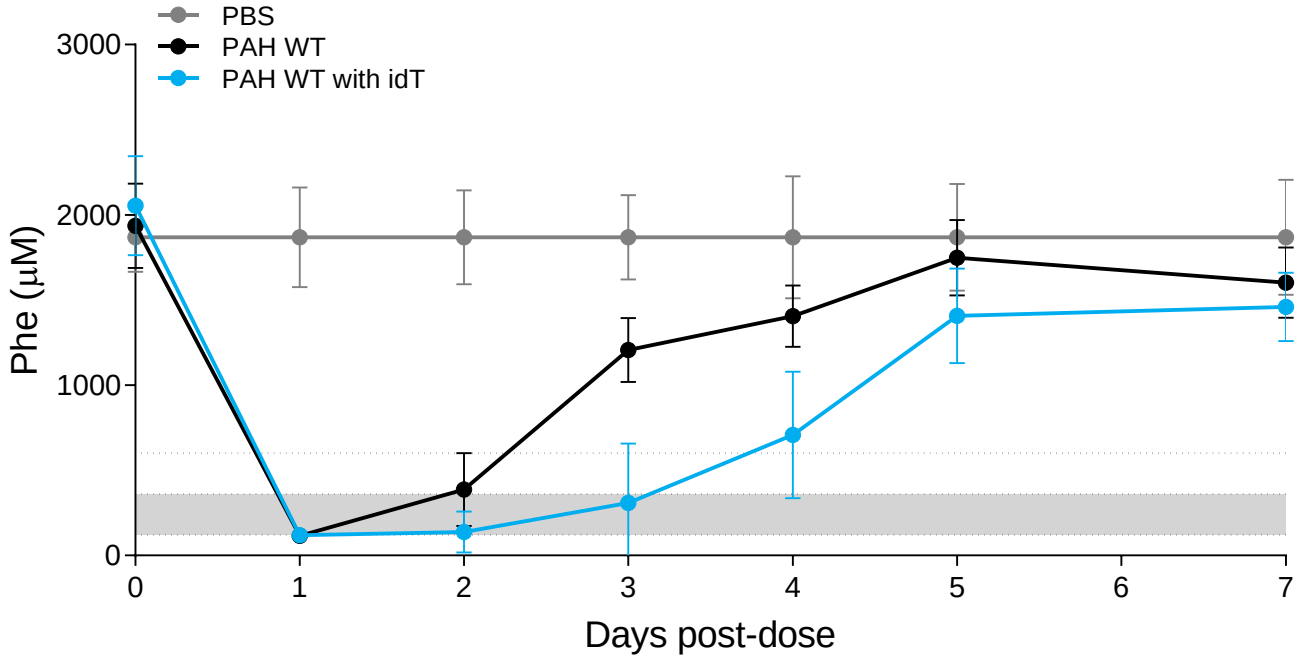
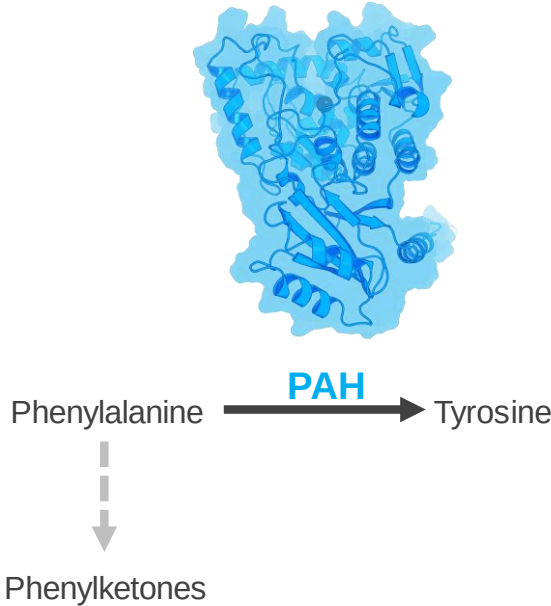
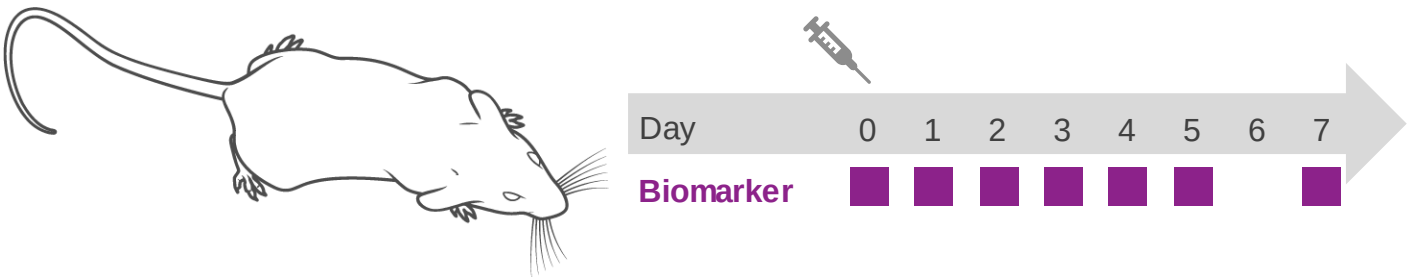
At 72 h post-dose, mouse T cells, B cells, macrophages, and dendritic cells show increased mOX40L from mRNA with 3' idT relative to unmodified mRNA

Phenylketonuria (PKU)

- Deficiency in phenylalanine hydroxylase (**PAH**) → Patients fail to process the amino acid phenylalanine taken up from diet; as a result, phenylalanine can build up to toxic levels

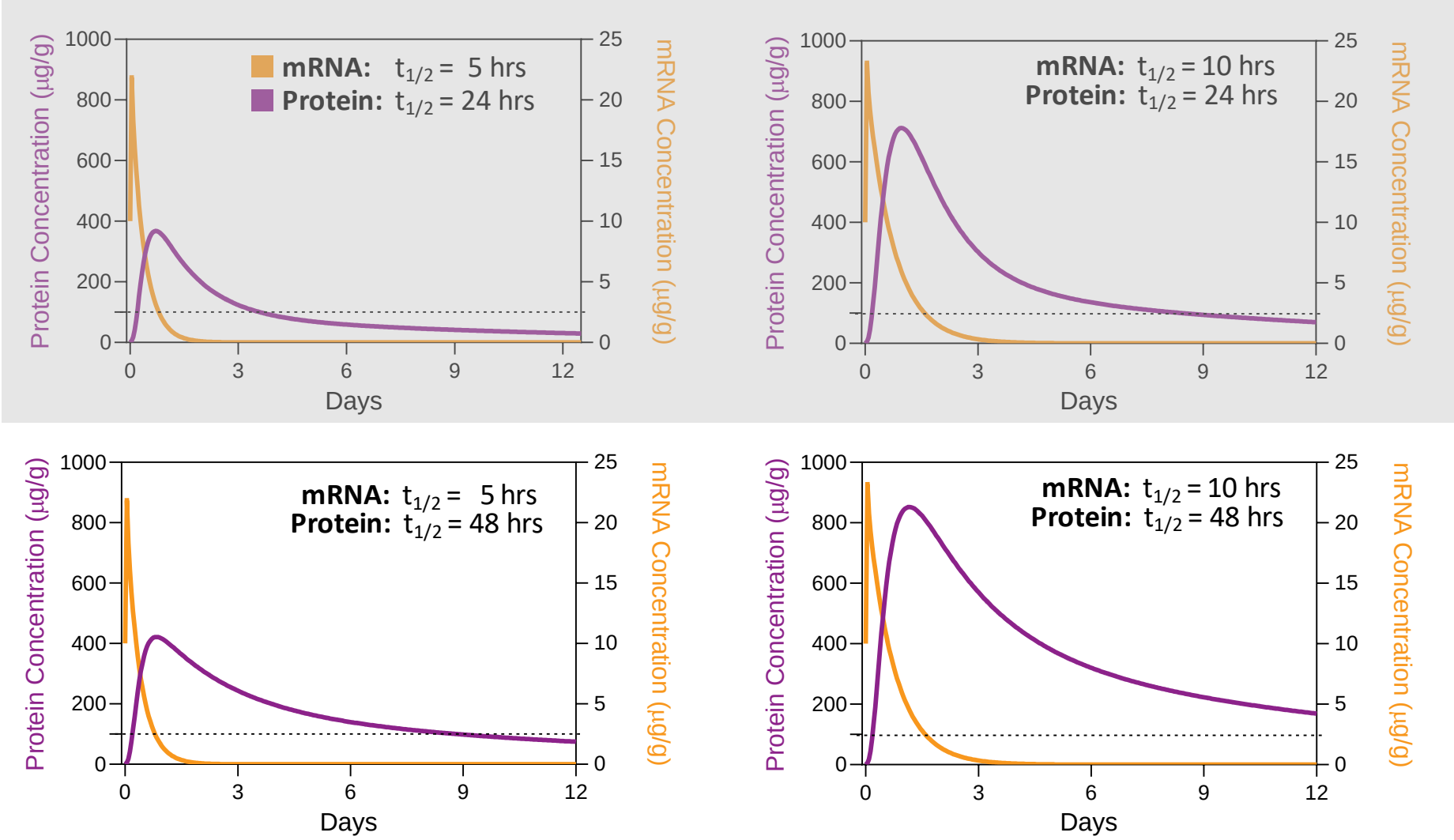
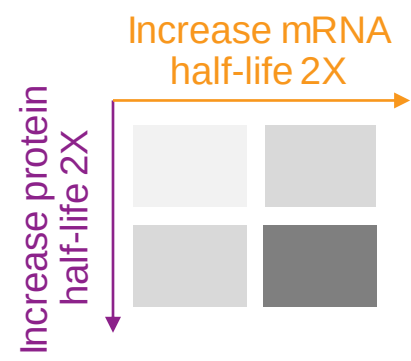


Stabilized PAH mRNA in PKU mouse model

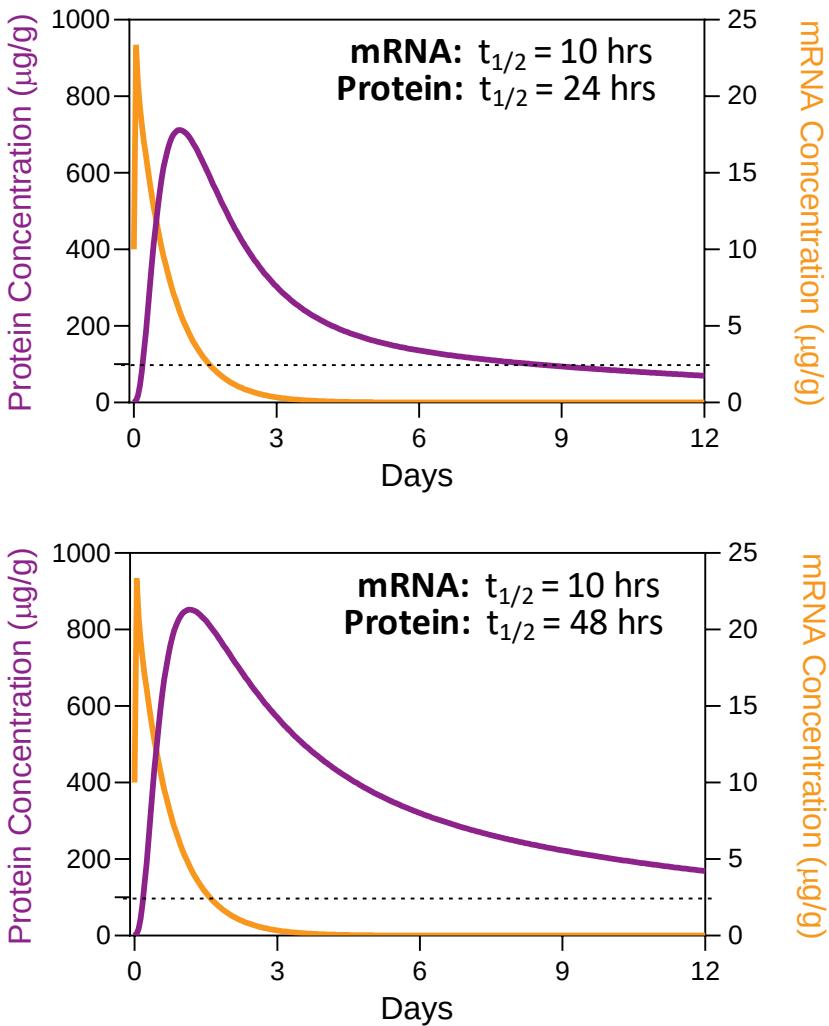
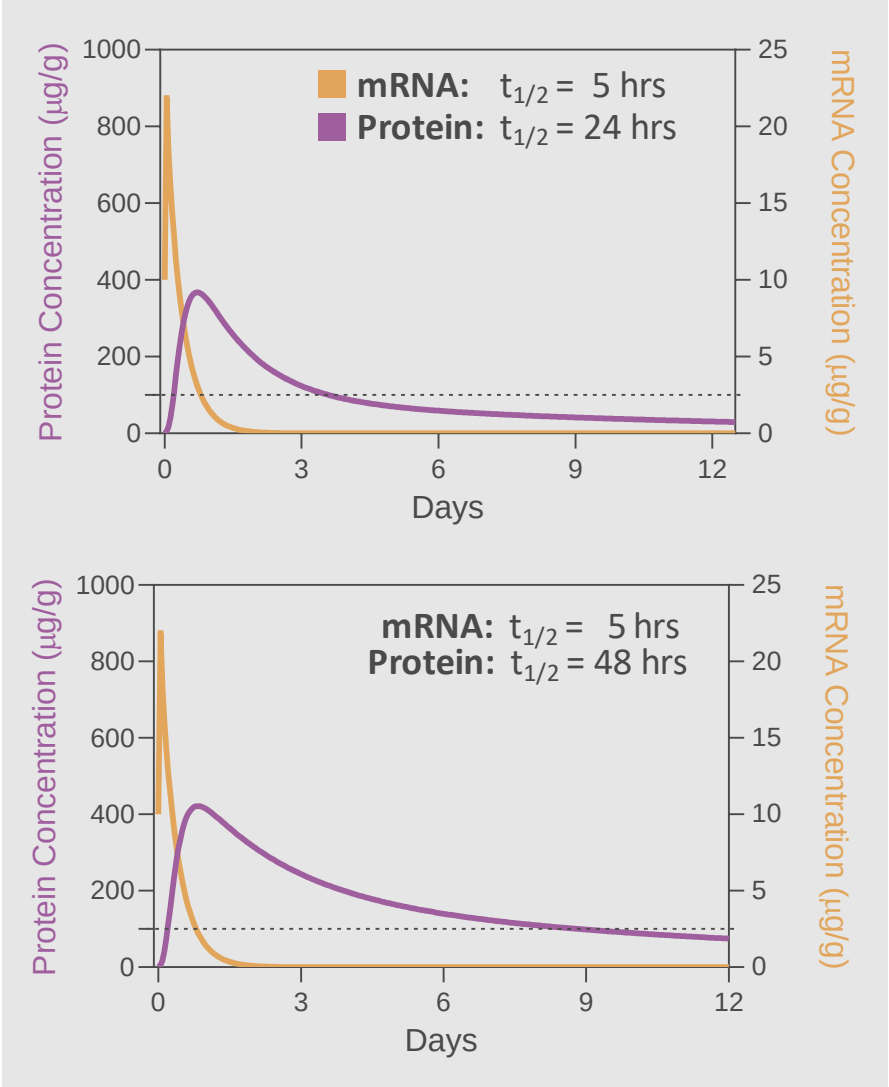
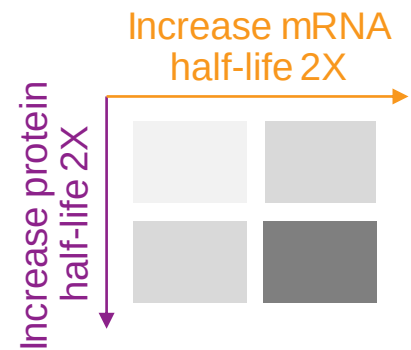


WT PAH expressed from a 3' idT-stabilized mRNA supports sustained reduction of serum phenylalanine levels

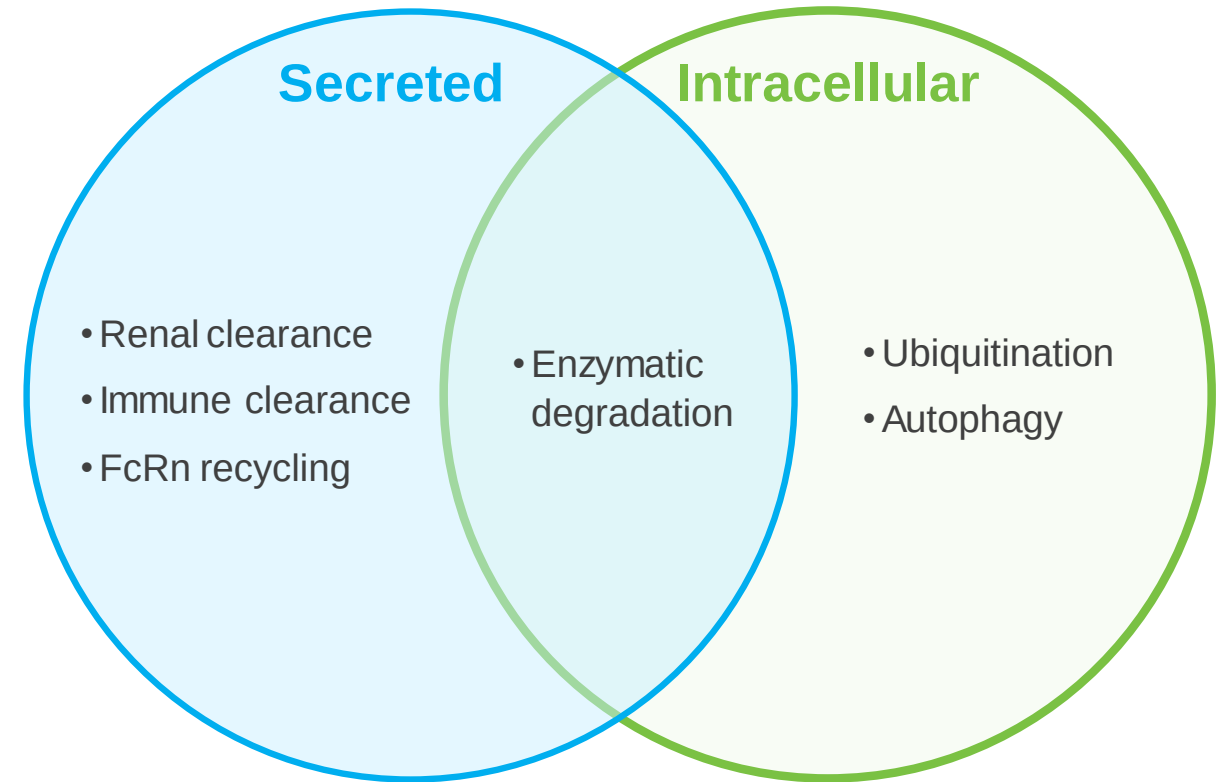
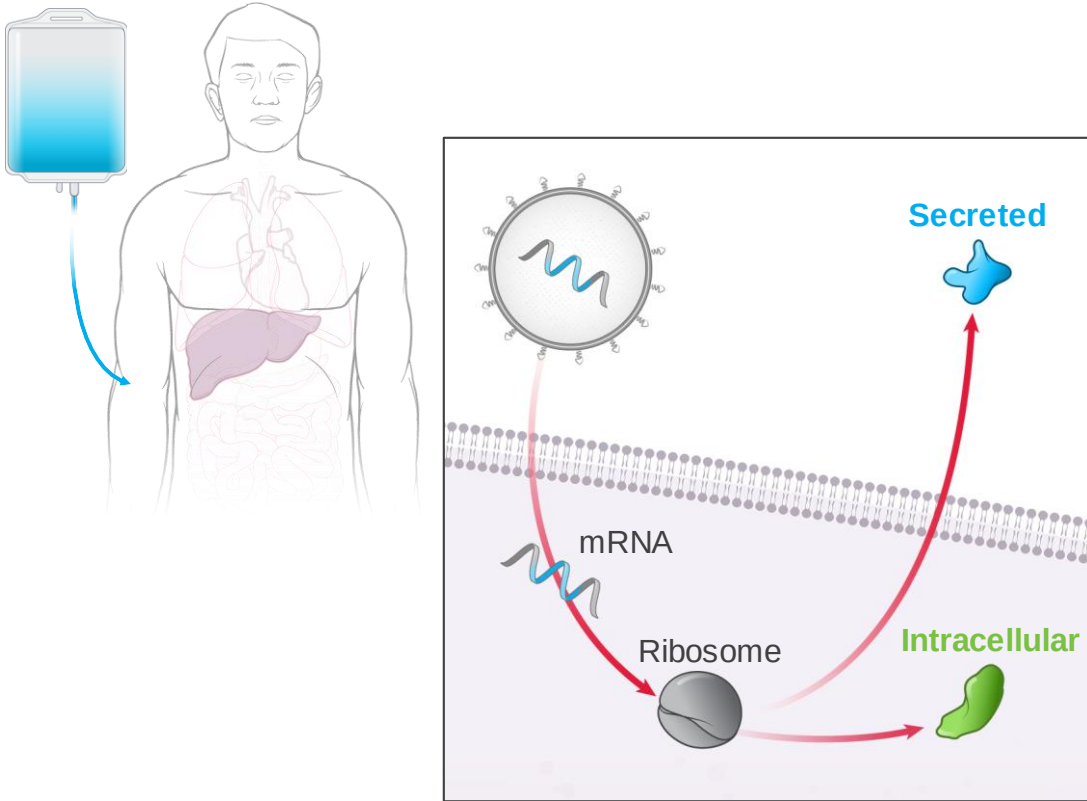
How can we extend mRNA half-life?



How can we extend protein half-life?



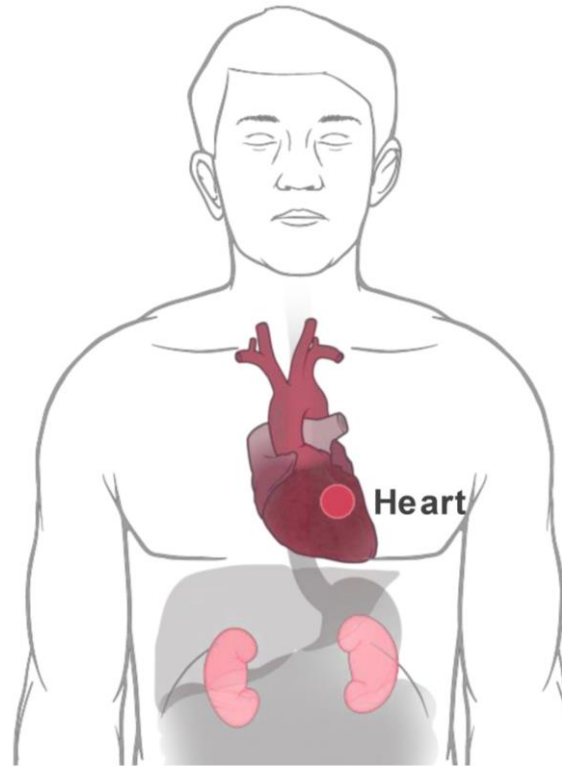
Drivers of *in vivo* protein half-life



Relaxin

Endogenous protein associated with cardiovascular re-modeling

- A naturally occurring hormone (human relaxin-2), present in both men and women, that becomes elevated in pregnant women
- Protects from vascular overwork, increases renal function, promotes cell growth and survival, and maintains vessel structure

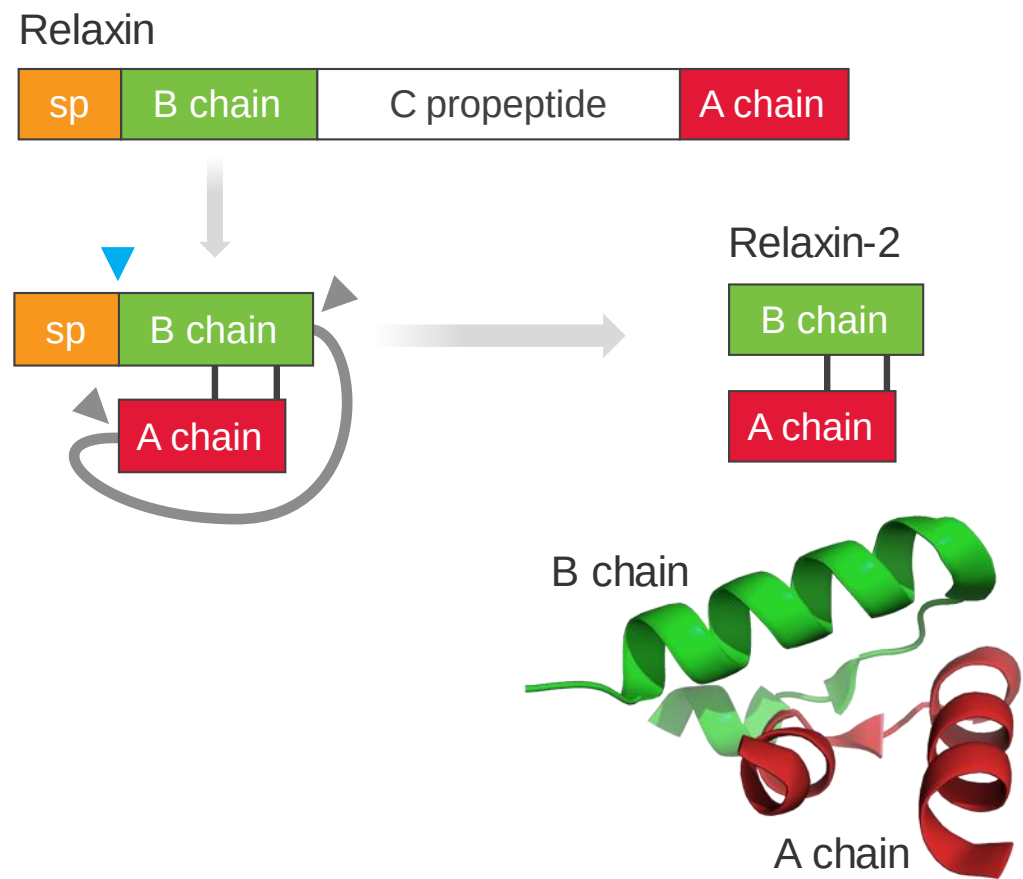


Relaxin is a vasoactive peptide associated with cardiovascular remodeling

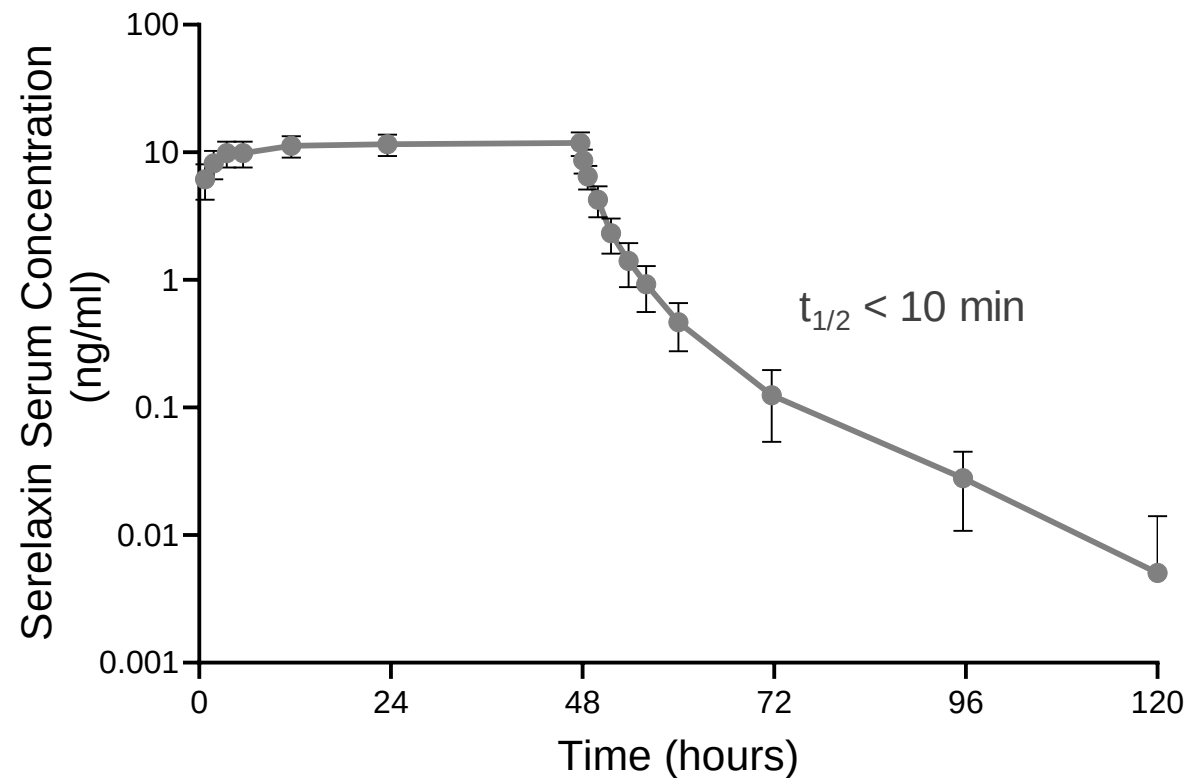
Concept:

IV-administered mRNA encoding relaxin peptide hormone with longer serum half-life to address heart failure

Relaxin-2 and its Achilles' heel

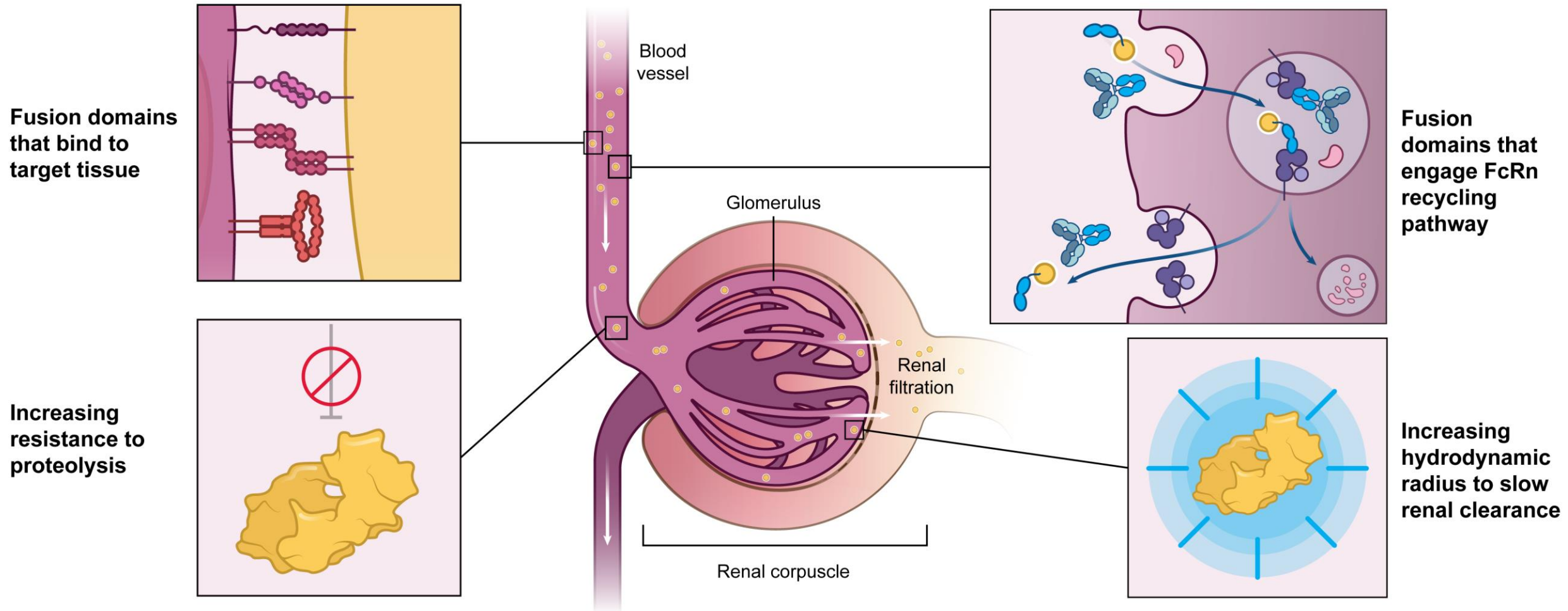


Serelaxin: human recombinant Relaxin-2 protein
Requires continuous infusion over 48 hours

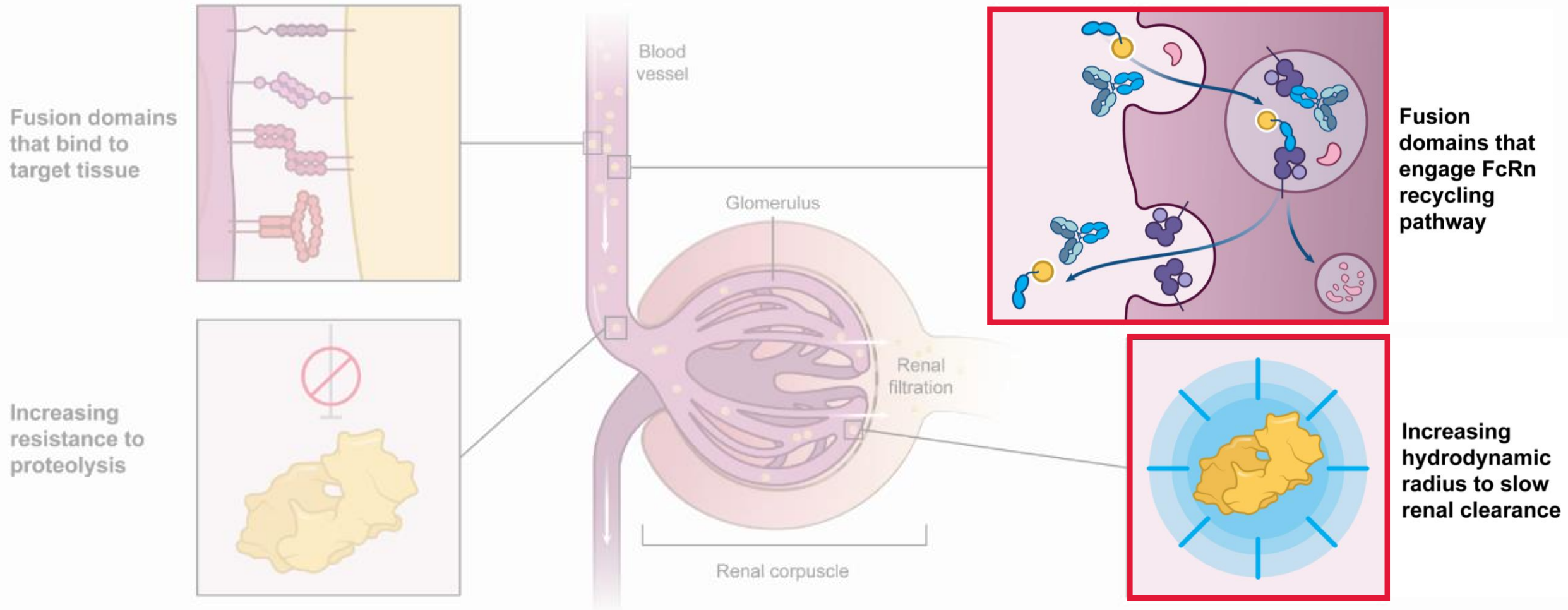


Modified from: Dahlke *et al.* (2015) *J Clin Pharm* doi: 10.1002/jcph.433

Techniques to increase half-lives of serum biologics



Techniques to increase half-lives of serum biologics

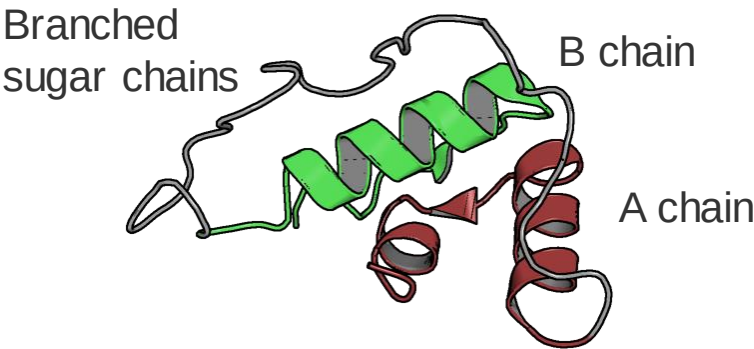
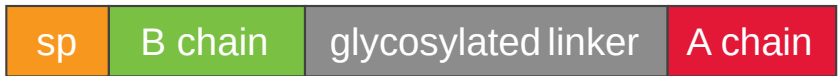


Relaxin-2 constructs

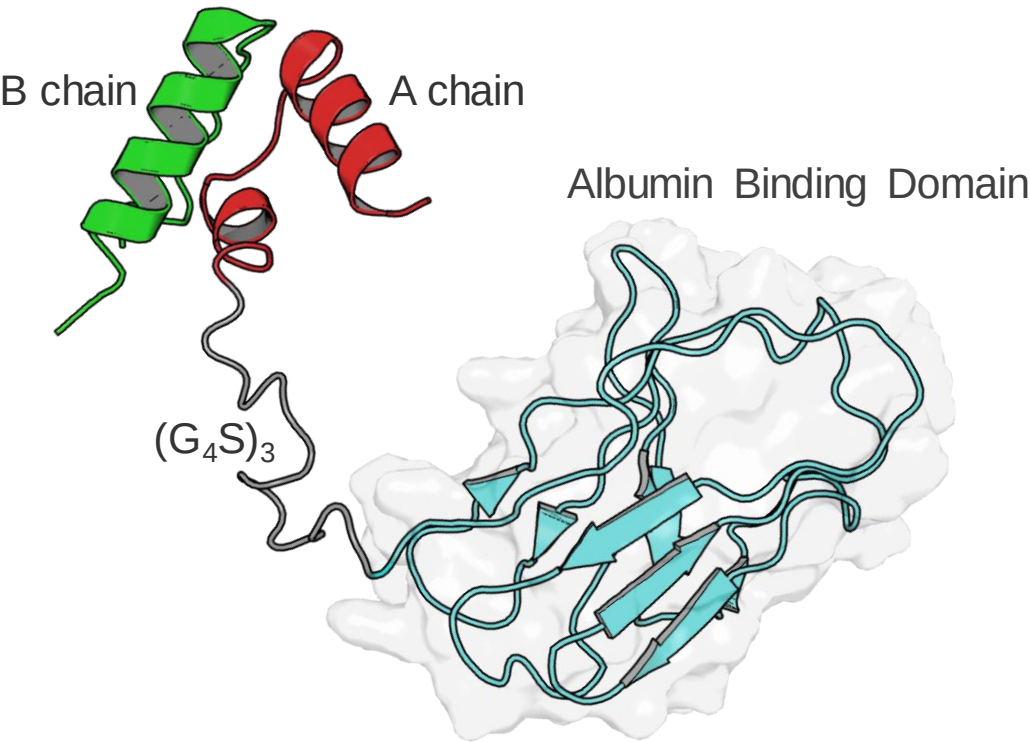
Relaxin



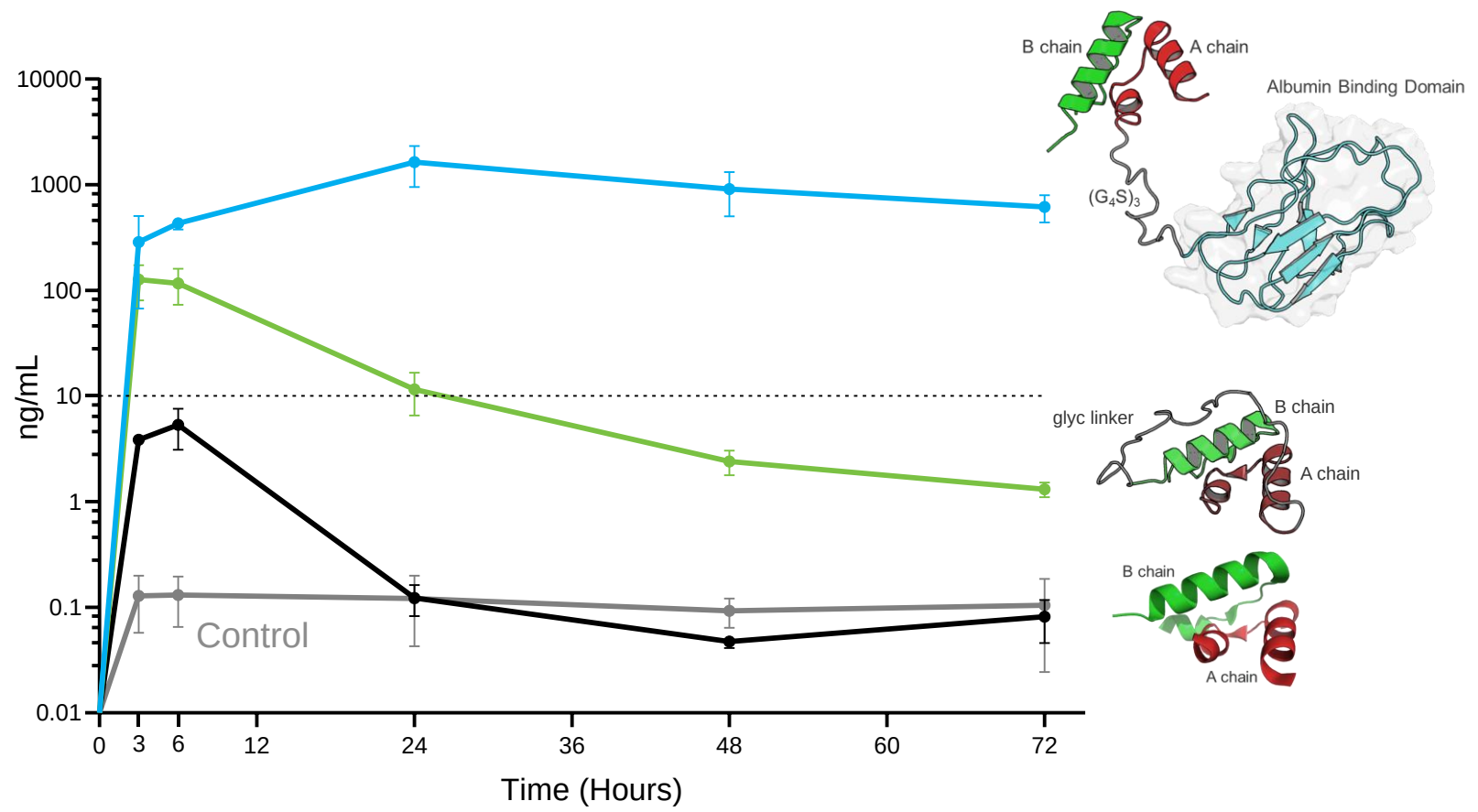
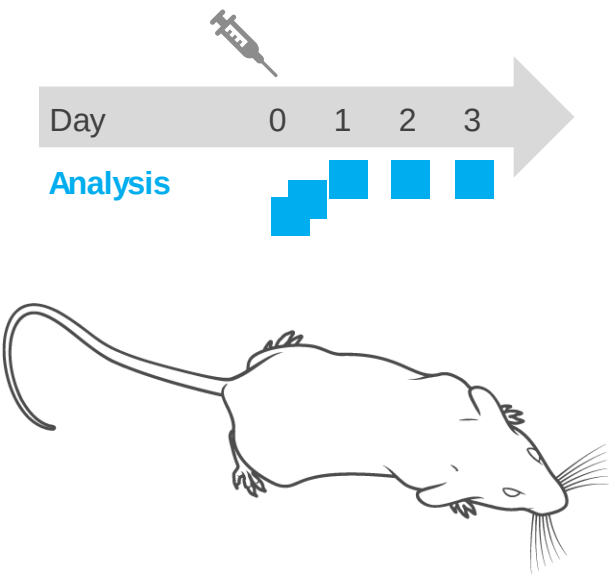
Propeptide replaced with glycosylated linker



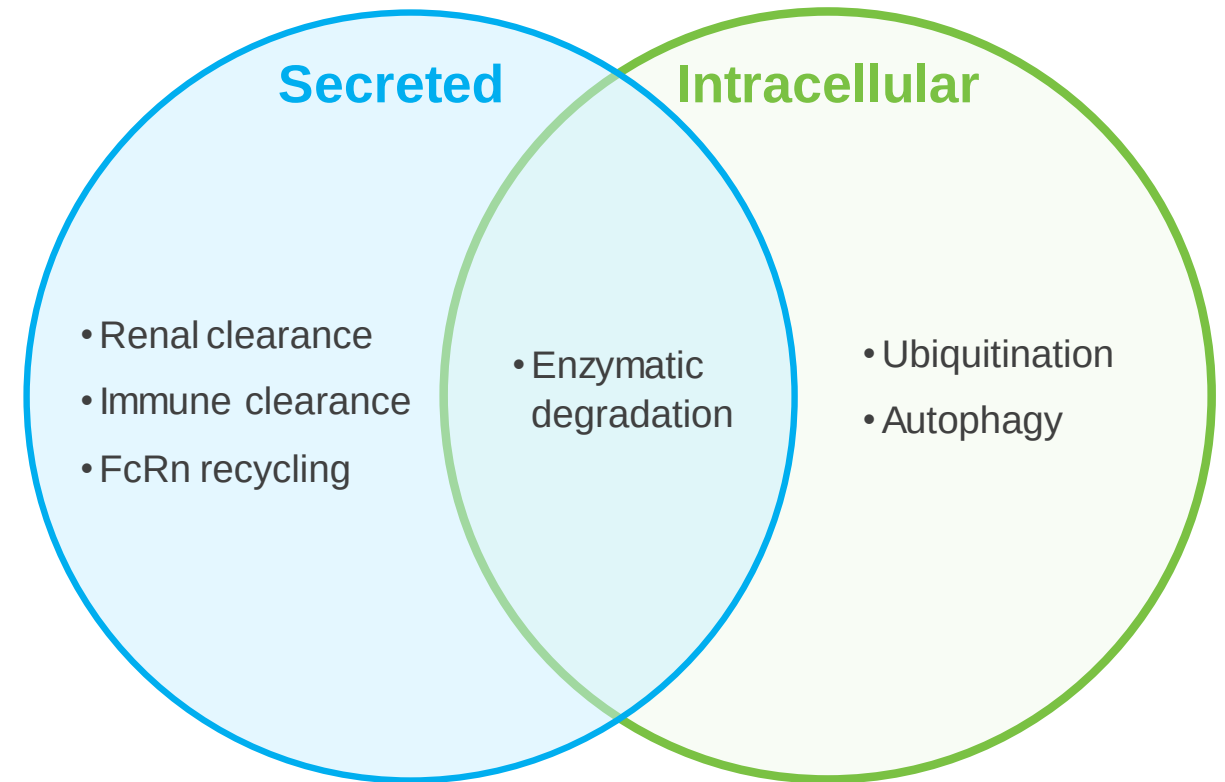
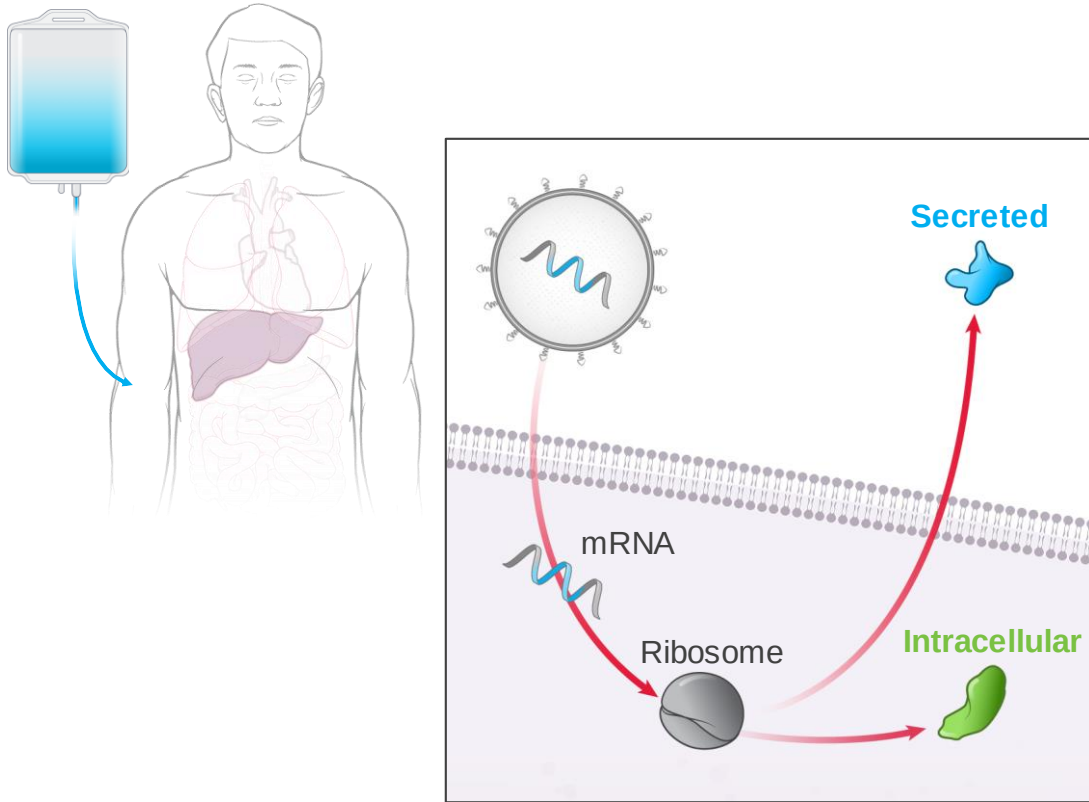
Relaxin fused to albumin-binding domain



Fusion and linker variants improve Relaxin-2 C_{max} and extend half-life

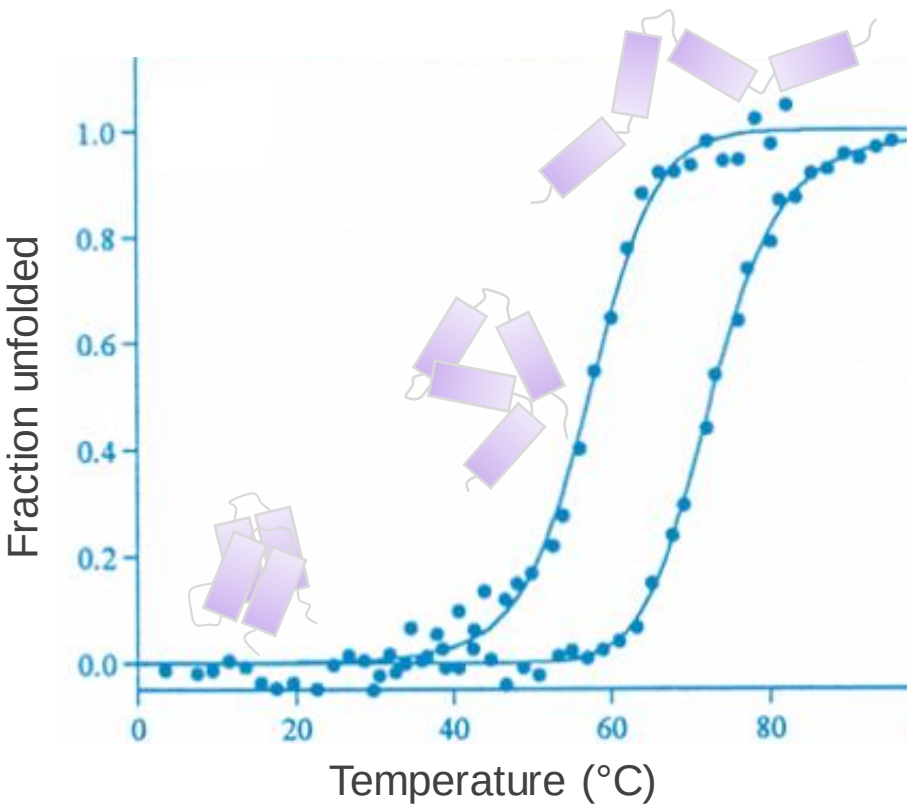


Drivers of *in vivo* protein half-life

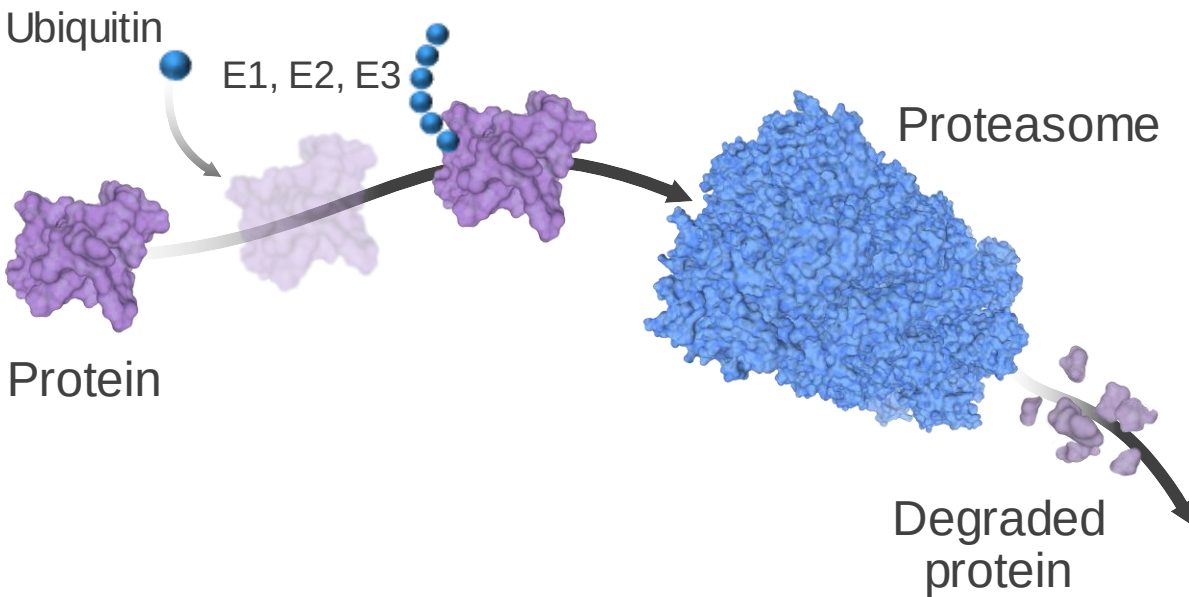


Intracellular protein degradation

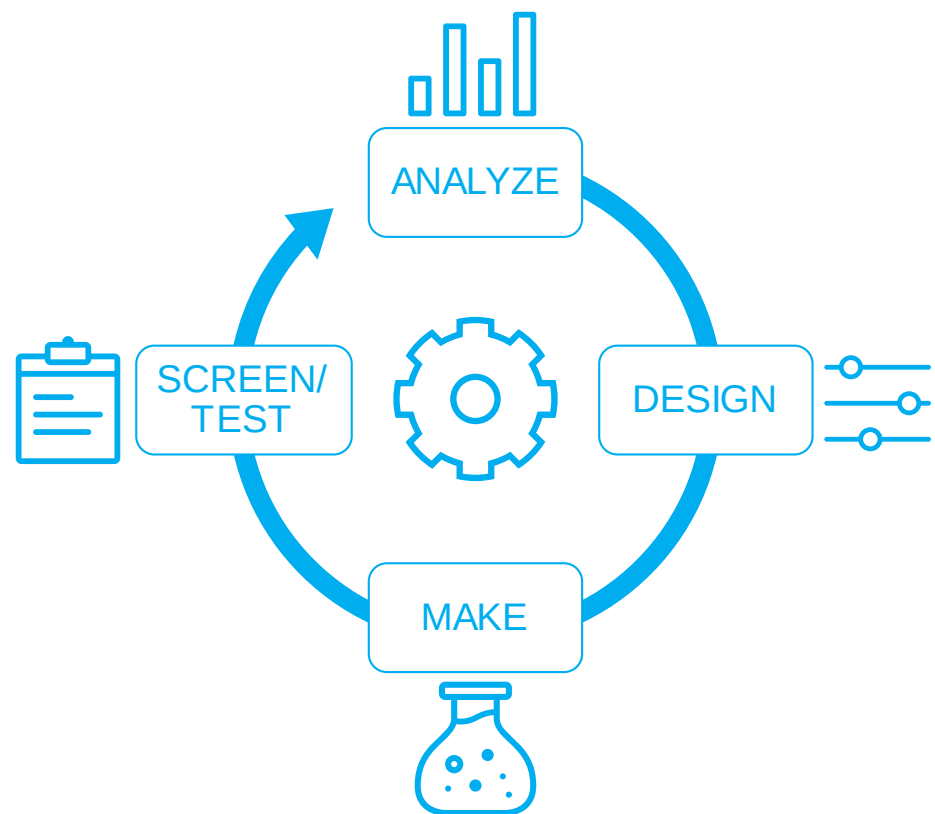
Protein unfolding/denaturation



Ubiquitin-mediated protein degradation

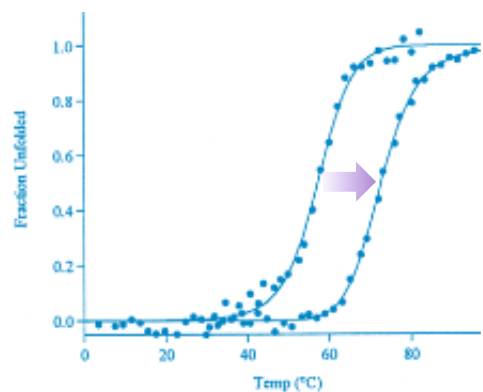


Protein engineering to extend intracellular half-life

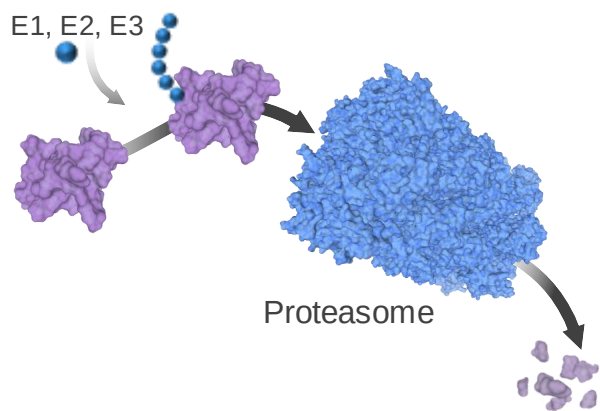


Objectives

Stability

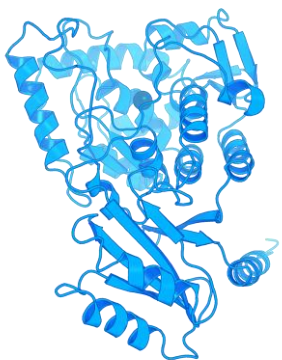


De-ubiquitination

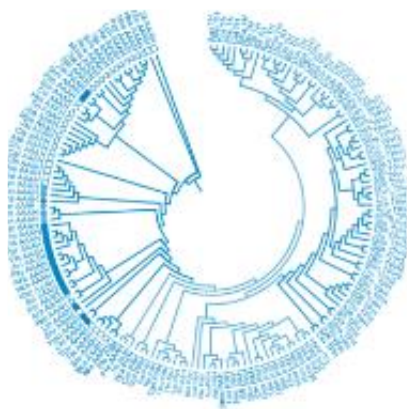


Considerations

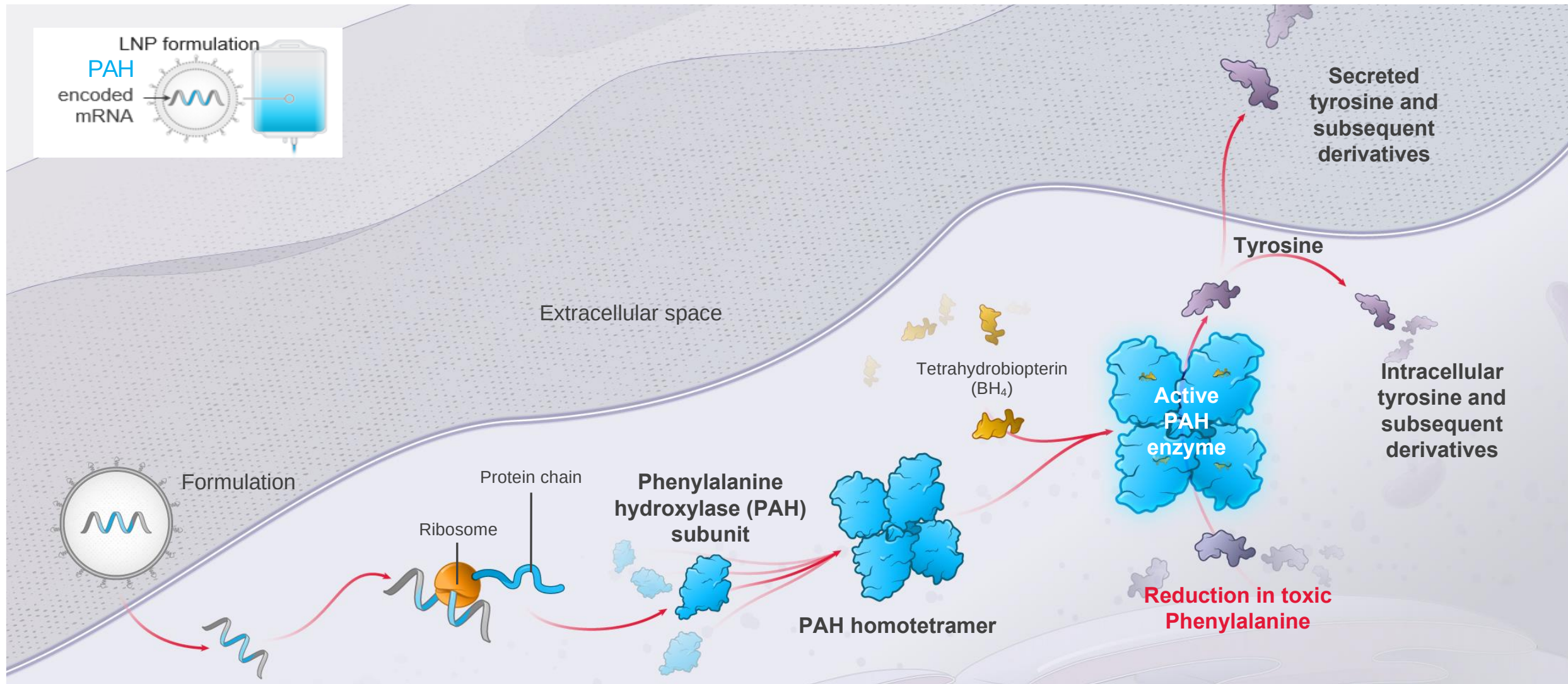
Structural models



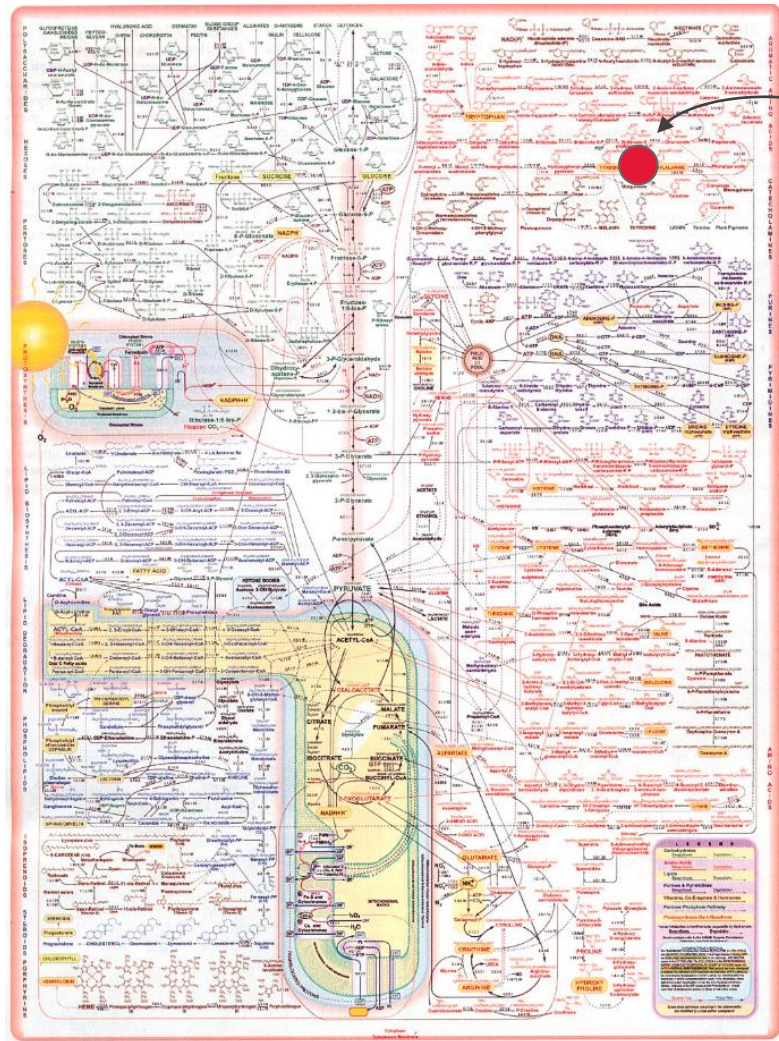
Evolutionary diversity



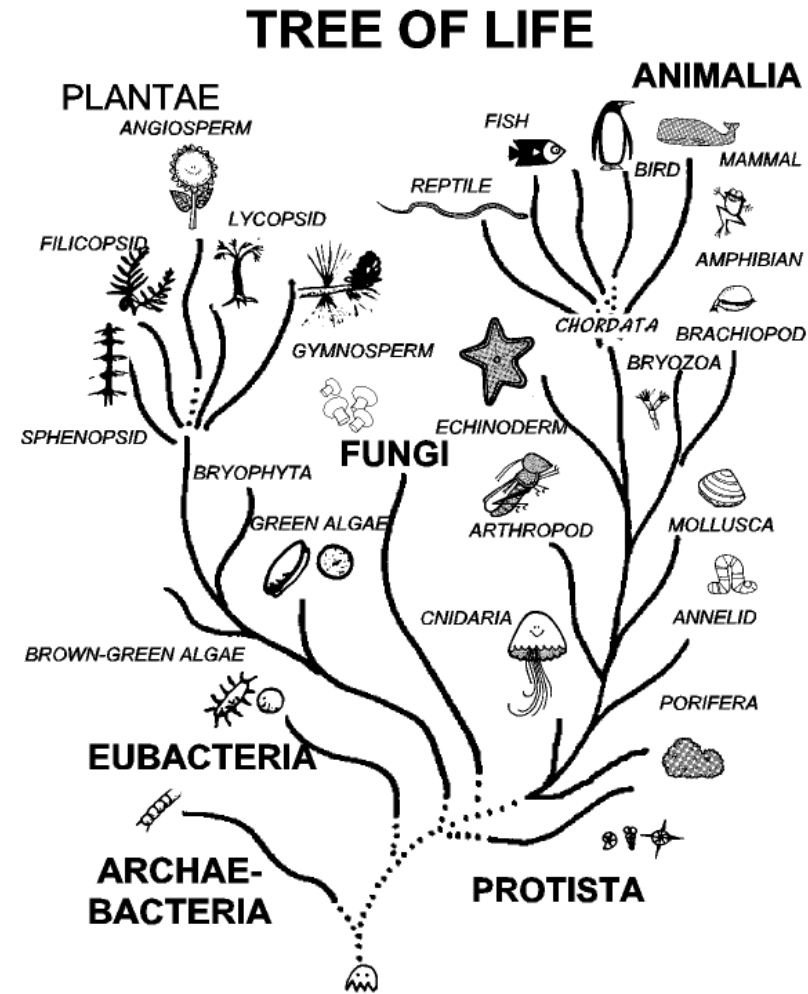
To stabilize phenylalanine hydroxylase (PAH), we needed to understand what amino acids were amenable to change



Since PAH is a central metabolic enzyme, it's present in all branches of life



PAH



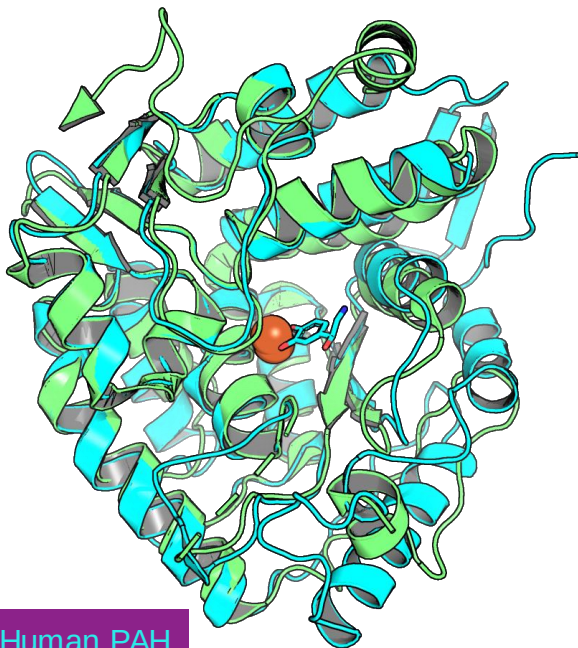
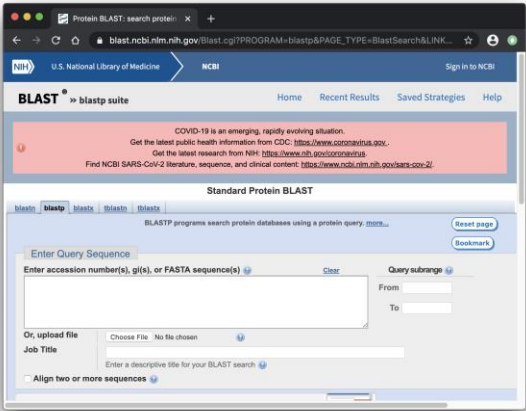
Homology searches can identify related sequences from other species

query sequence: human PAH

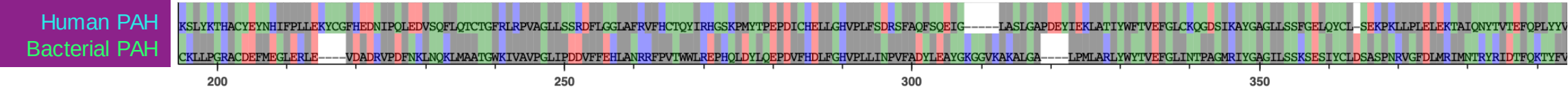
MSTAVLENPGLGRKLSDFGQETSYIEDNCNQNGAISLIFSLKEEVGALAKVLRLEENDV
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FPTIQELDRFANQILSYGAELDADHPGFKDPVYRARRKQFADIAYNRYHGQPI PRVEYM
EEEKKTWGTVFKTLSLYKTHACYEYNHIFP LLEKYCGFHEDNIPQLEDVSQFLQTCTGF
RLRPVAGLLSSRDFLGGLAFRVFHCTQYIRHGSKPMYTPEDICHELLGHVPLFSDRSFA
QFSQEIGLASLAPDEYIEKLATIWFTVEFGLCKQGDSIKAYGAGLLSSFGELOQCLSE
KPKLLPLELEKTAIQNYTVTEFQPLYVAESFNDAKEKVRNFAATIPRPF SVRYDPYTQR
IEVL DNTQQ LKILADSINSEIGILCSALQKIK



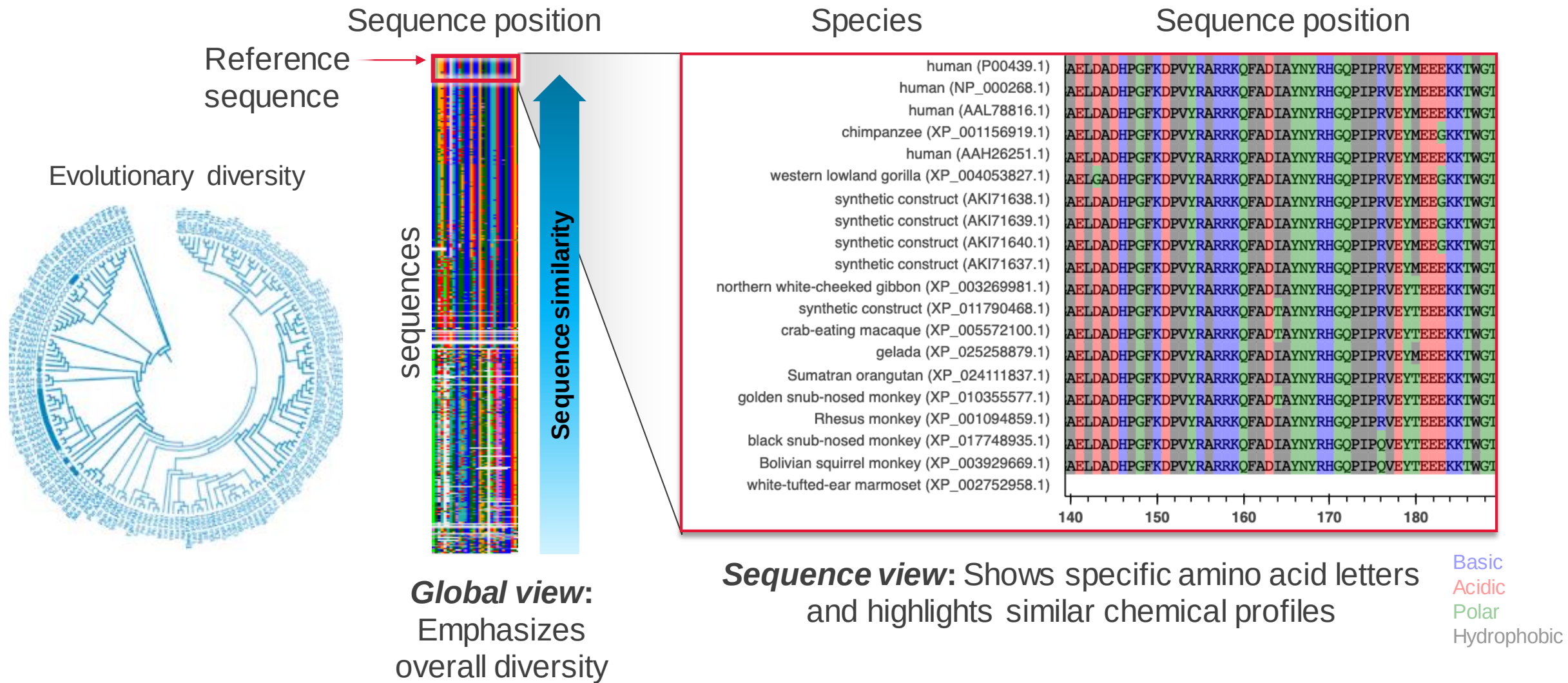
NCBI (NIH)
BLAST database



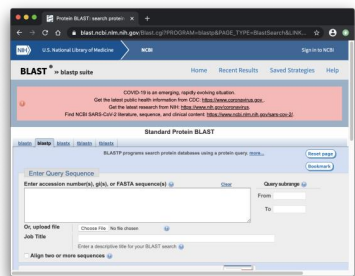
Human PAH
Bacterial PAH



Sequence alignment representations

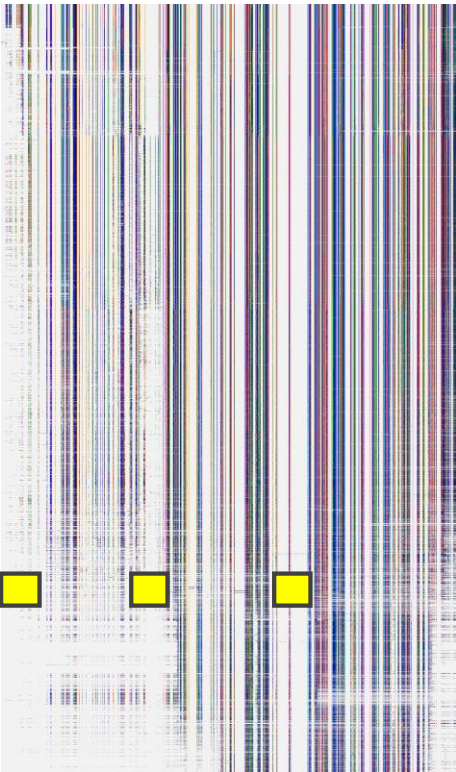


Mining useful substitutions from nature



BLAST homology search

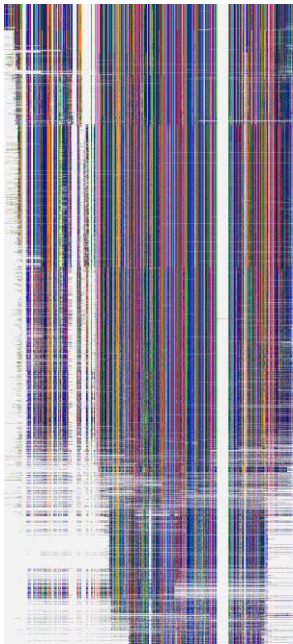
4975 hPAH-like seqs



Large gaps suggest topological differences from human

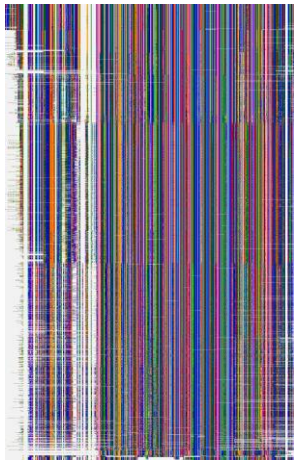
Remove “gappy” sequences

3760 seqs



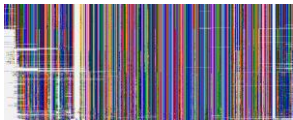
Remove short sequences

2700 seqs



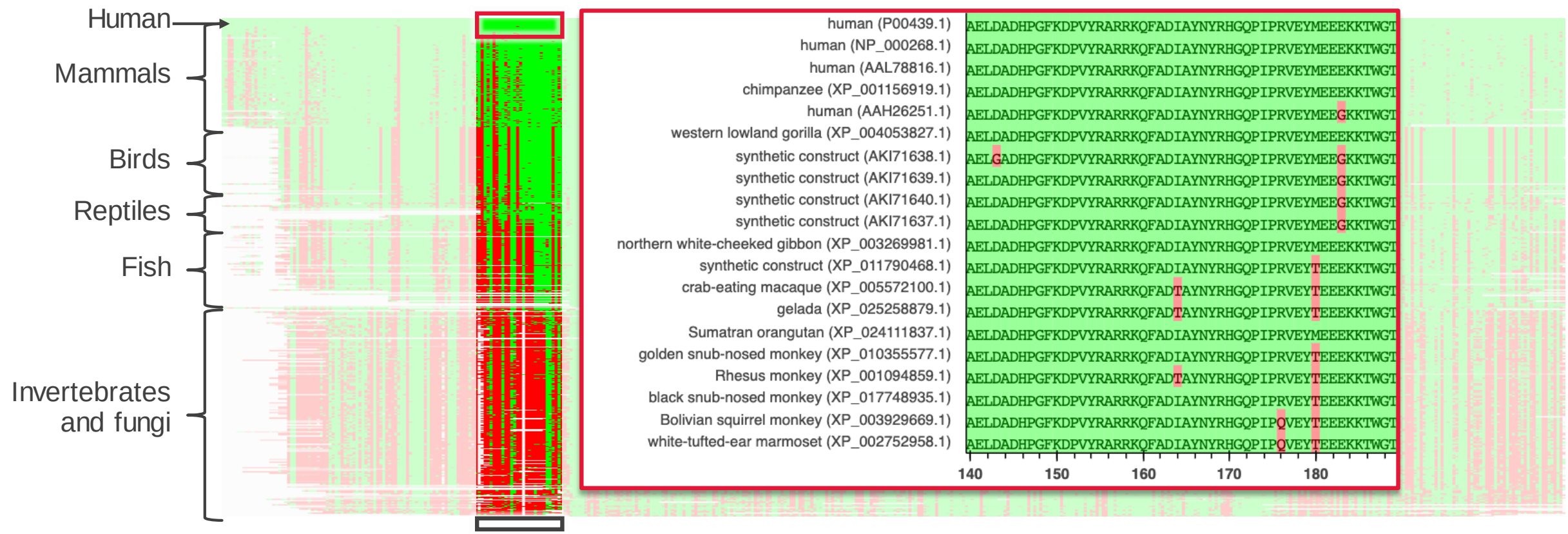
Remove sequences without Phe in description

690 seqs

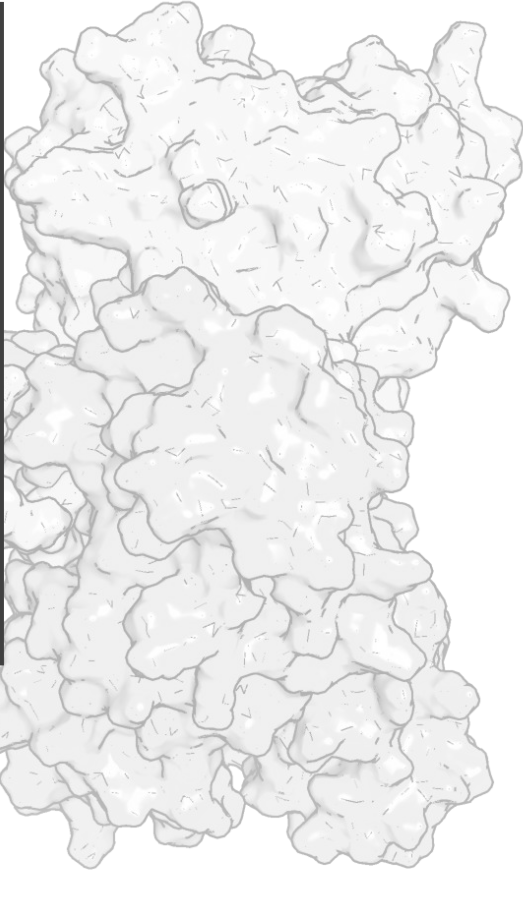
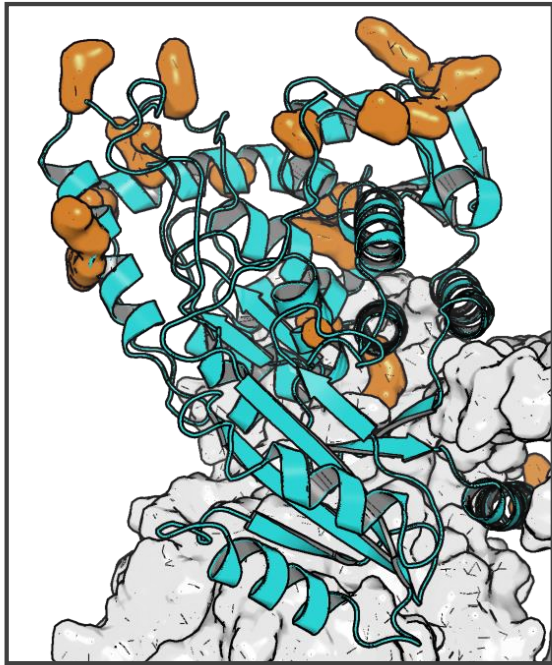


Phenylalanine hydroxylase is evolutionarily related to Tyrosine hydroxylase and Tryptophan hydroxylase

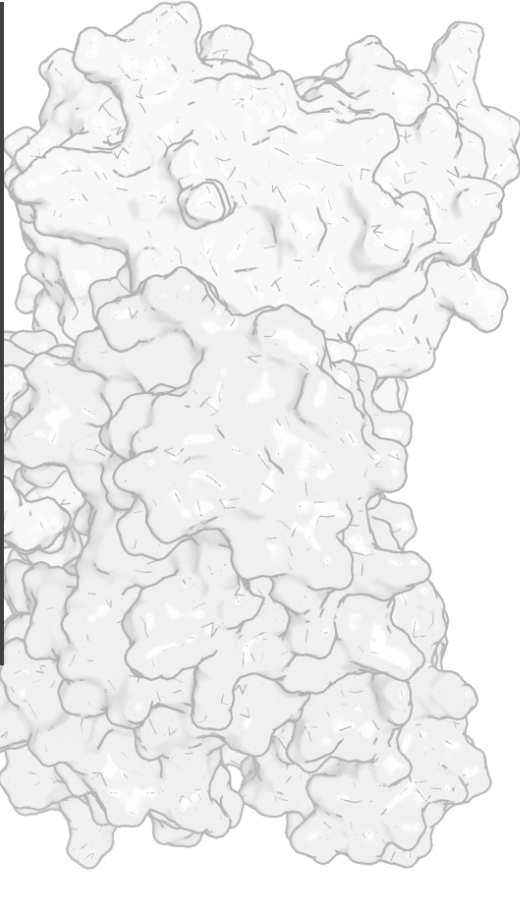
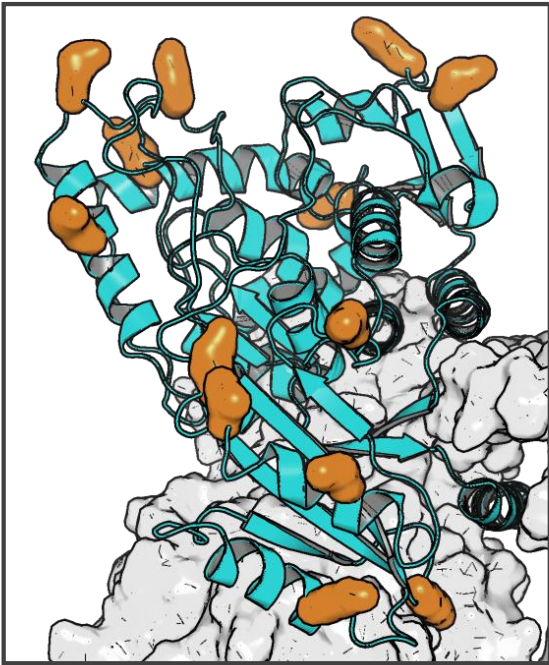
Alignment of PAH sequences



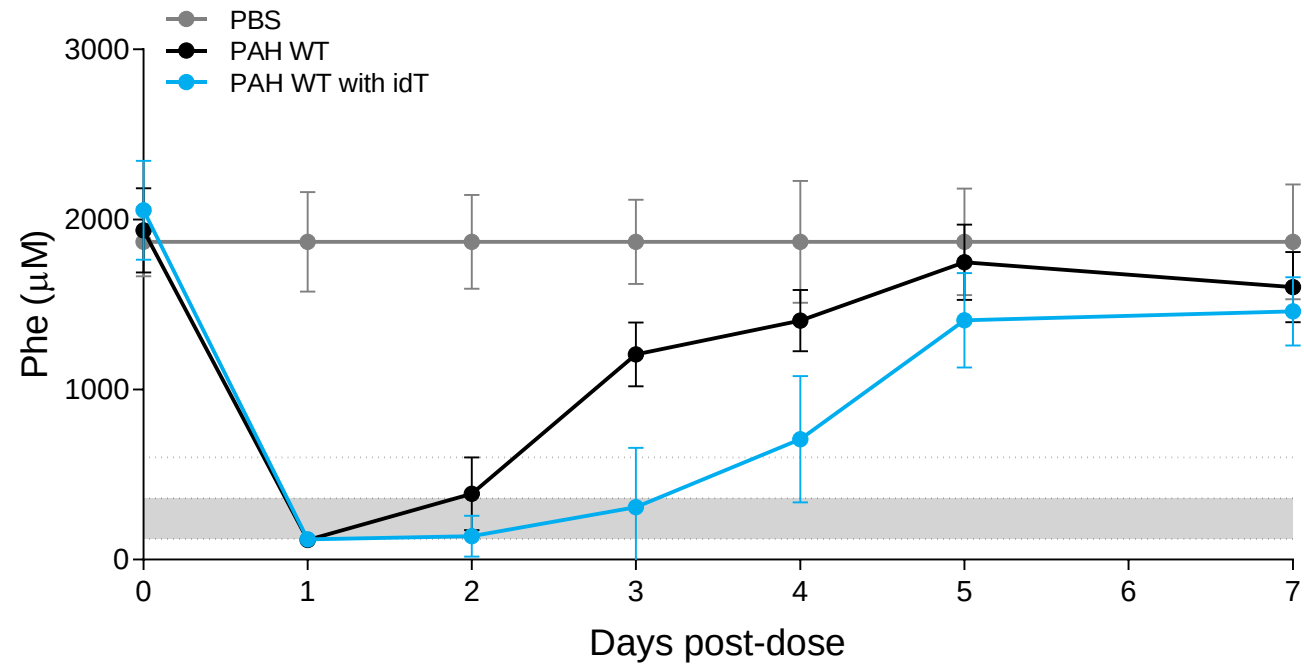
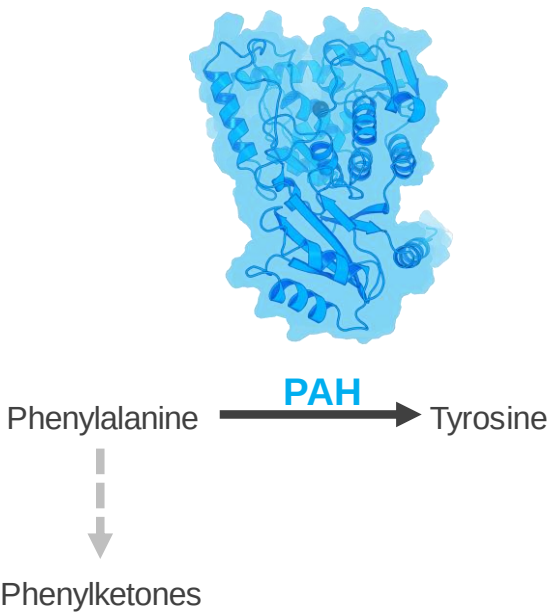
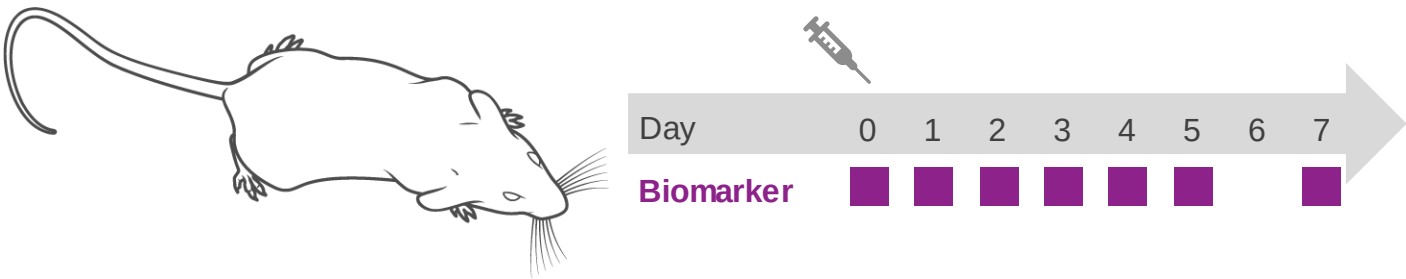
Amino acid changes expected stabilize hPAH folding



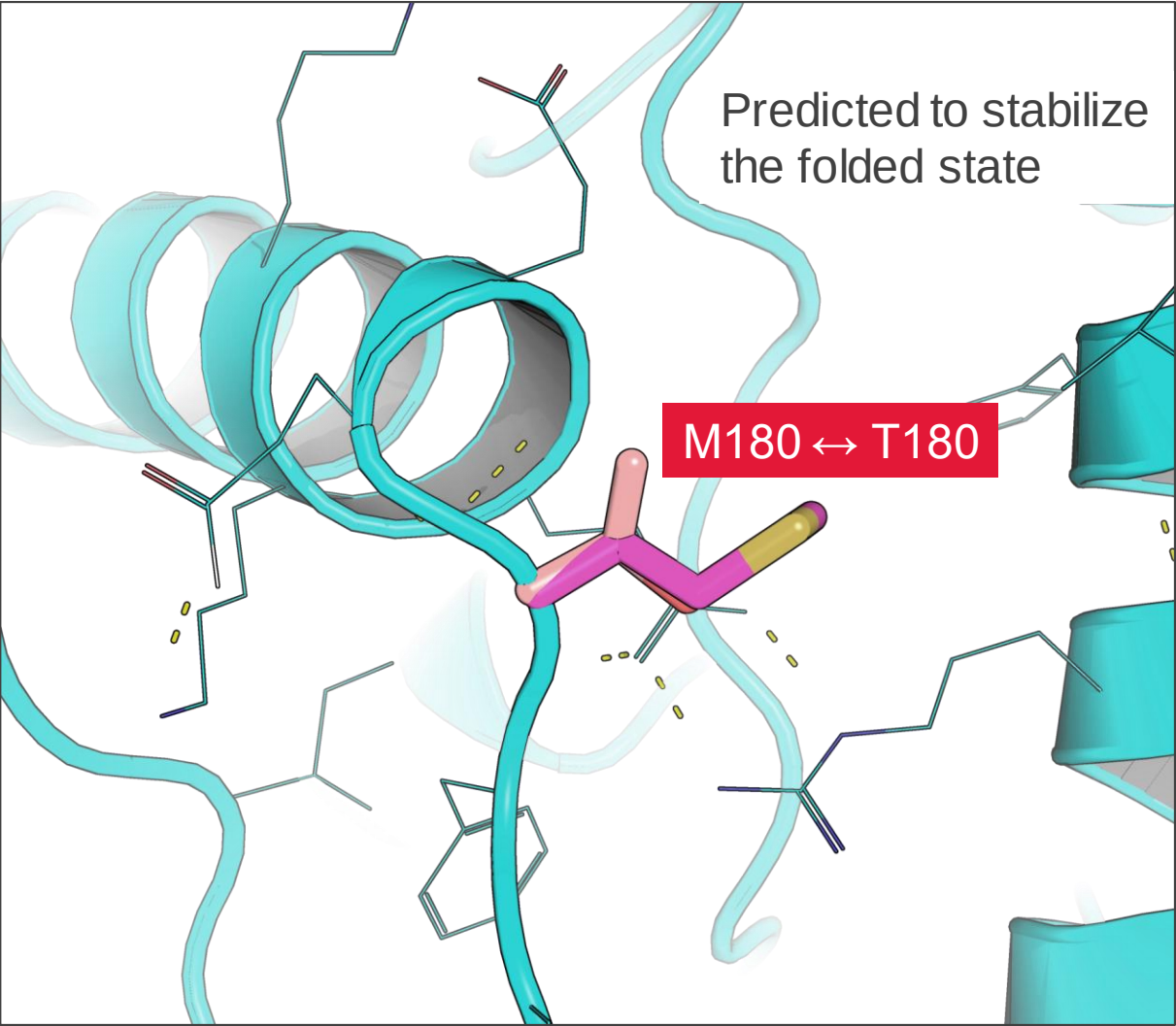
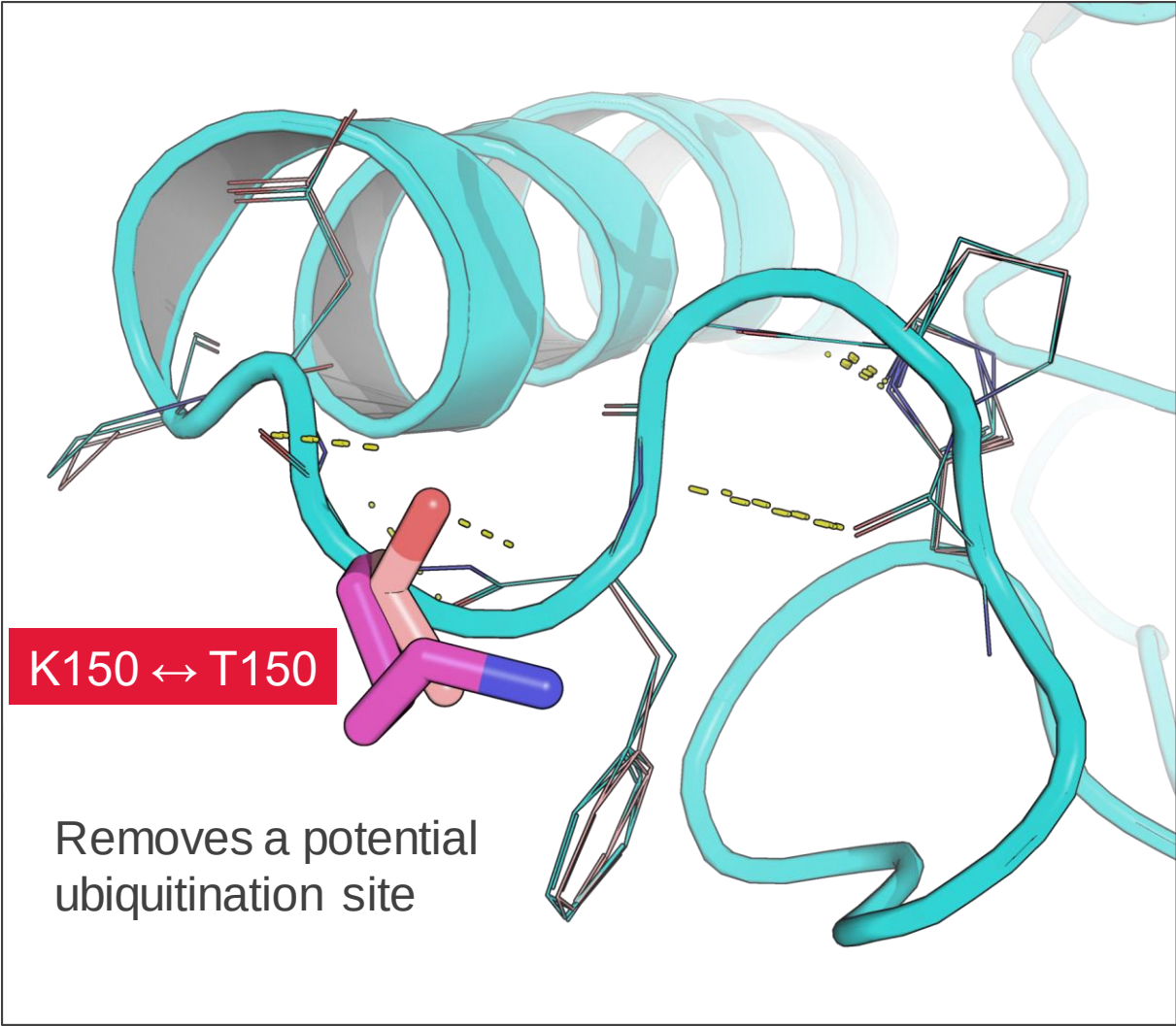
Remove hPAH ubiquitination sites



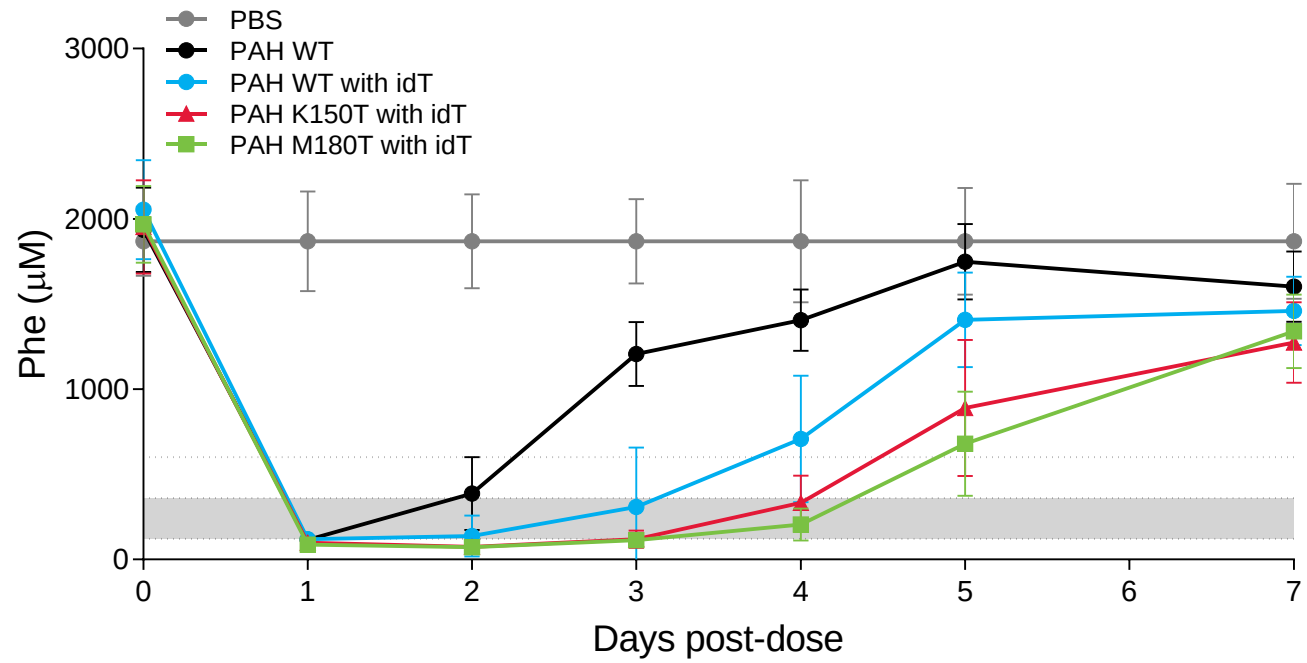
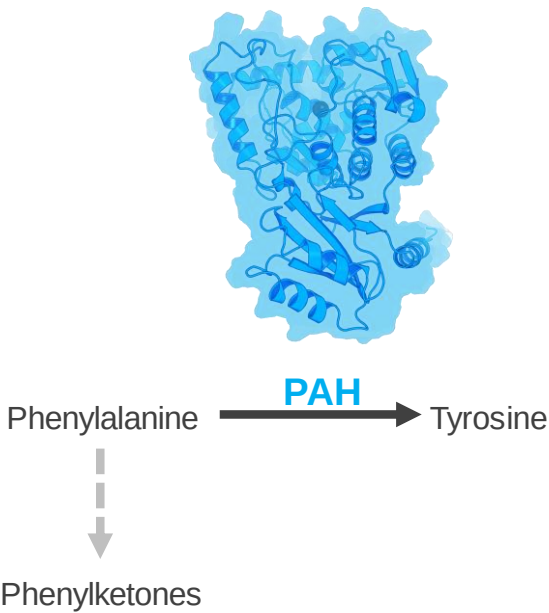
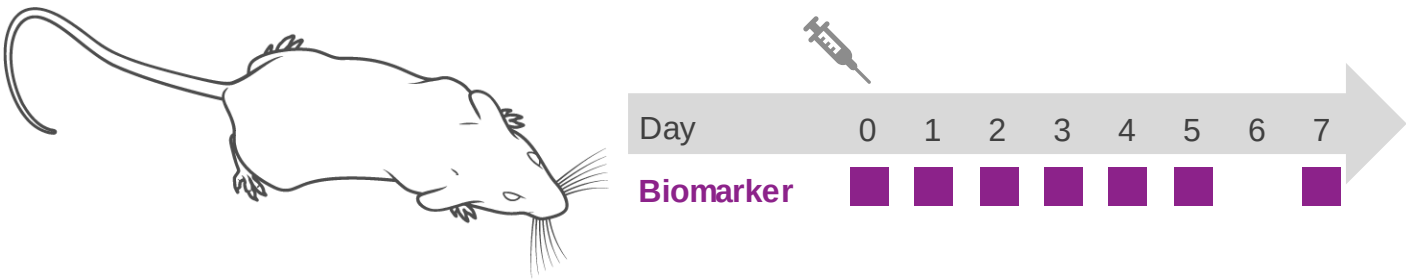
PAH protein variants expressed from idT-protected mRNAs in PKU mouse model



Human PAH engineering



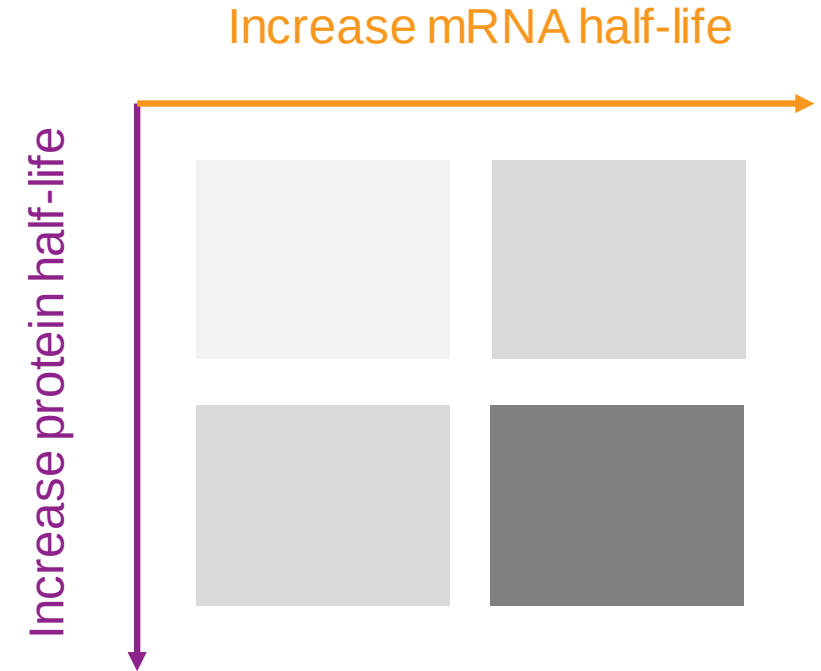
PAH protein variants expressed from idT-protected mRNAs in PKU mouse model



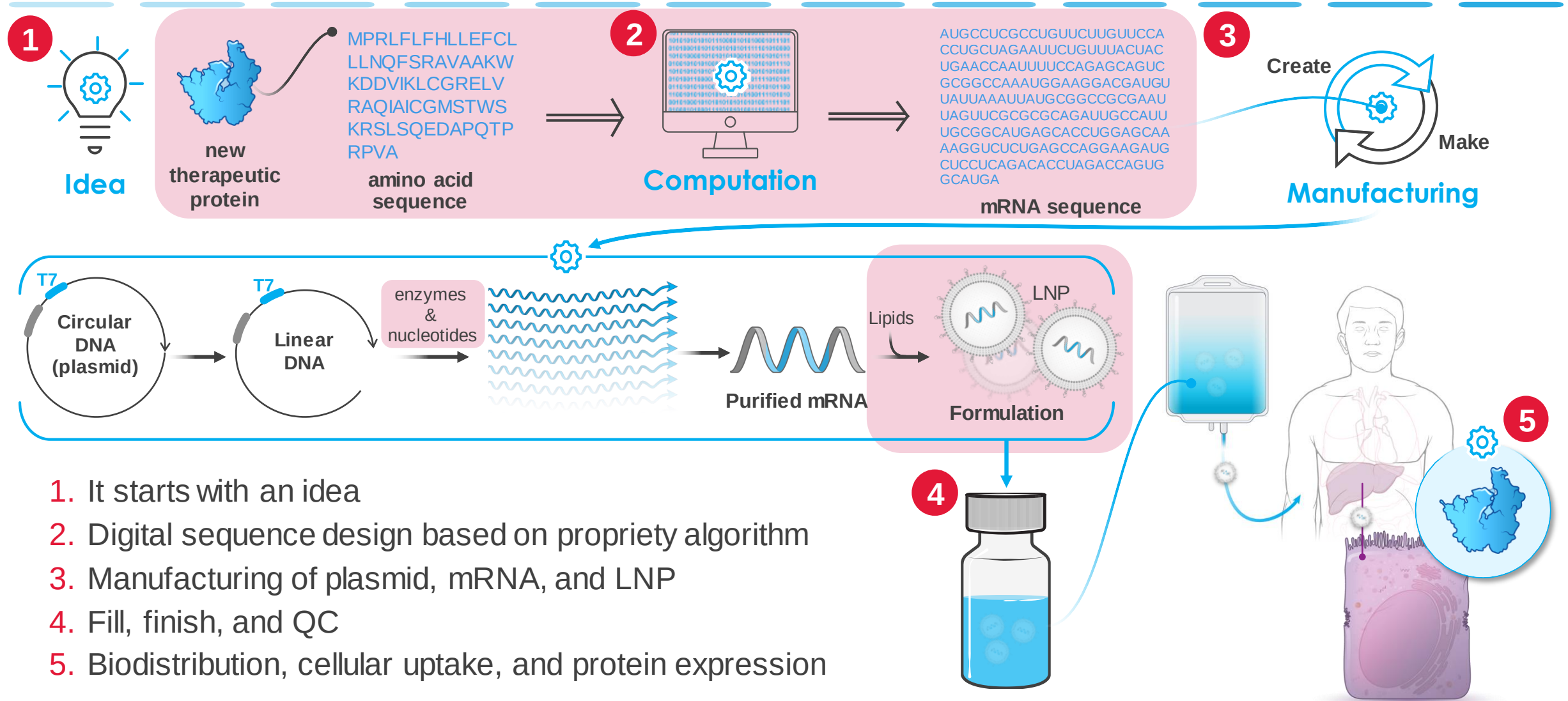
Low serum phenylalanine levels are maintained even further by combining PAH mRNA stabilization and protein engineering

Conclusions

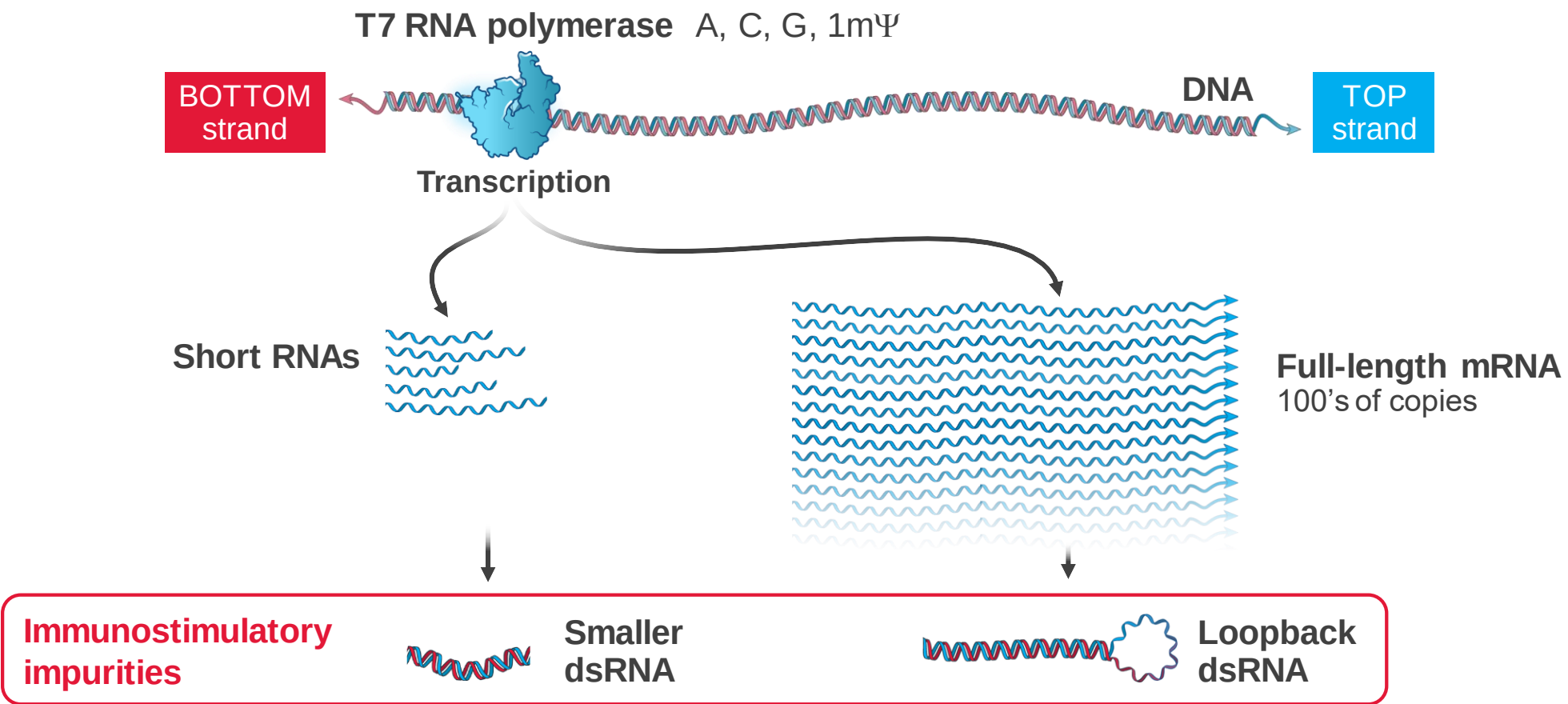
- The duration of pharmacological effect for mRNA medicines is a function of both mRNA and protein half-life
- Understanding the biological rules governing these half-lives gives us multiple levers to engineer the desired pharmacology
- As with other mRNA engineering principles, the effects are additive



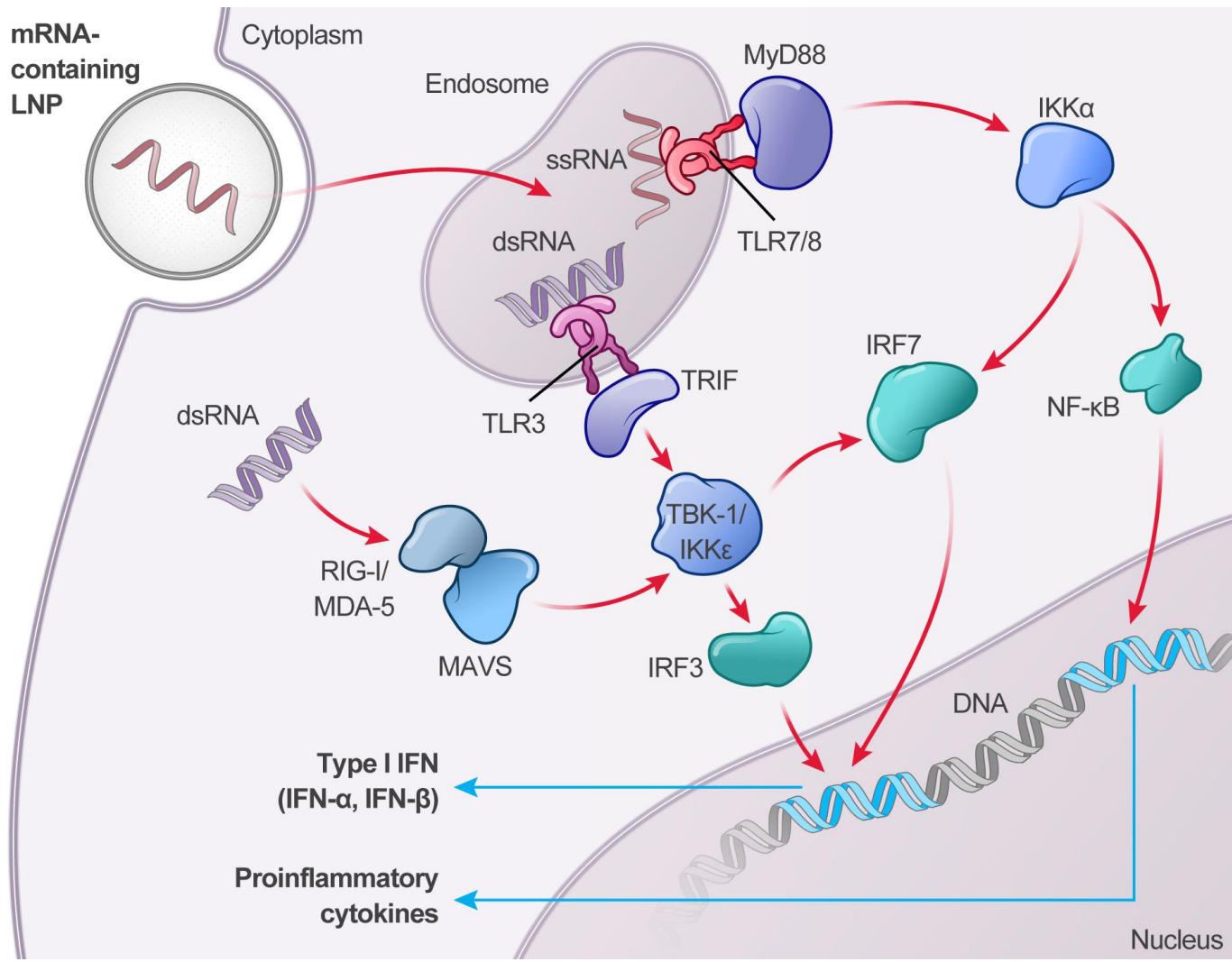
Where does Platform Research fit in?



Wild type T7 RNA polymerase makes both full-length mRNA and dsRNA impurities



dsRNA triggers innate immune responses



Nelson ... Joyal (2020) Science
Advances in press

HPLC purification is commonly used to reduce dsRNA content

Published online 2 September 2011

Nucleic Acids Research, 2011, Vol. 39, No. 21 e142
doi:10.1093/nar/gkr695

Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA

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Received May 26, 2011; Revised August 9, 2011; Accepted August 10, 2011

ABSTRACT

In vitro-transcribed mRNA has great therapeutic potential to transiently express the encoded protein without the adverse effects of viral and DNA-based constructs. Mammalian cells, however, contain RNA sensors of the innate immune system that must be considered in the generation of therapeutic RNA. Incorporation of modified nucleosides both reduces innate immune activation and increases translation of mRNA, but residual induction of type I interferons (IFNs) and proinflammatory cytokines remains. We identify that contaminants, including double-stranded RNA, in nucleoside-modified *in vitro*-transcribed RNA are responsible for innate immune activation and their removal by high performance liquid chromatography (HPLC) results in mRNA that does not induce IFNs and inflammatory cytokines and is translated at 10- to 1000-fold greater levels in primary cells. Although unmodified mRNAs were translated significantly better following purification, they still induced high levels of cytokine secretion. HPLC purified nucleoside-modified mRNA is a powerful vector for applications ranging from *ex vivo* stem cell generation to *in vivo* gene therapy.

INTRODUCTION

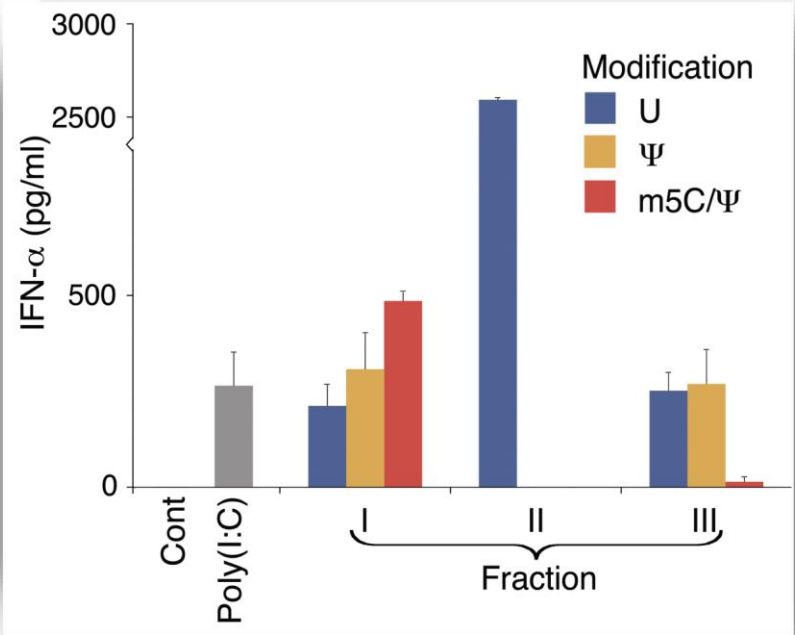
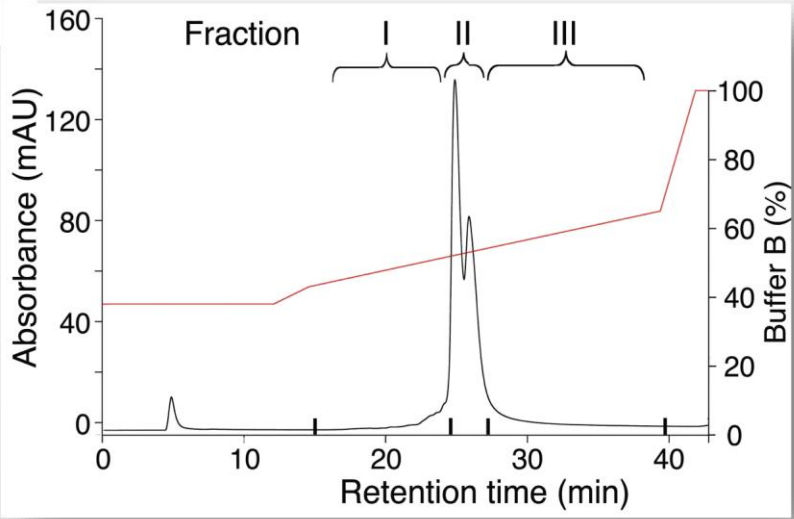
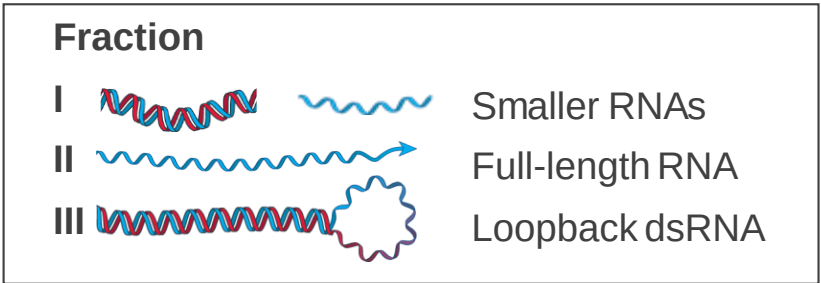
Our understanding of the importance of RNA in biological processes and the therapeutic potential has substantially increased with the discovery of non-coding regulatory RNAs. The use of mRNA has also expanded, including the delivery of mRNA to generate induced pluripotent stem (iPS) cells (1-3) and *in vivo* administration to

express therapeutic proteins (4). The recognition that the immunogenicity of RNA could be reduced by the incorporation of modified nucleosides with a concomitant increase in translation (5), potentially allows efficient expression of intra and extracellular proteins *in vivo* and *ex vivo* without activation of innate immune pathways. Unfortunately, modified nucleoside-containing RNA transcribed by phage RNA polymerase transcription still retains a low level of activation of such pathways (3,5-7). The remaining activation of RNA sensors by nucleoside modified RNA could be because the modifications do not completely suppress the RNAs ability to activate sensors or due to contaminants with structures that activate in the presence of nucleoside modification. It is well established that RNA transcribed *in vitro* by phage polymerase contains multiple contaminants, including short RNAs produced by abortive initiation events (8) and double-stranded (ds)RNAs generated by self-complementary 3' extension (9). RNA-primed transcription from RNA templates (10) and RNA-dependent RNA polymerase activity (11).

Large quantities of RNA can be easily prepared by *in vitro* transcription from DNA templates using phage RNA polymerase or solid-phase chemical synthesis. For uses that require further purification, such as NMR (12), crystallography (13) and therapeutic applications (14), a number of techniques have been developed. Preparative denaturing polyacrylamide gel electrophoresis is commonly used to purify *in vitro*-transcribed RNA, however, this method is suitable only for short RNAs [reviewed in (15)]. Long RNAs can be separated on denaturing agarose gels, but they are not translatable due to covalent modifications introduced by the denaturants glyoxal and formaldehyde (16). Chromatography based on size exclusion can efficiently remove unincorporated nucleoside triphosphates, small abortive transcripts and plasmid template from the desired RNA product

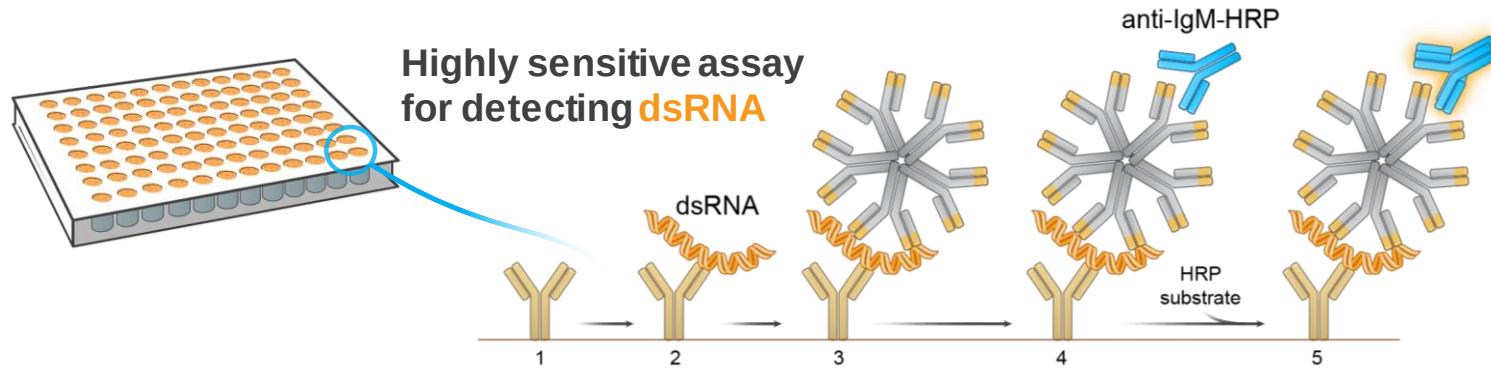
*To whom correspondence should be addressed. Tel: +1 215 573 8491; Fax: +215 349 5111; Email: dreww@mail.med.upenn.edu

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Karikó ... Weissmann (2011) Nucleic Acids Research

By engineering process conditions, we can minimize dsRNA formation by wild type T7 RNA polymerase





Engineering T7 RNA Polymerase to further reduce dsRNA formation

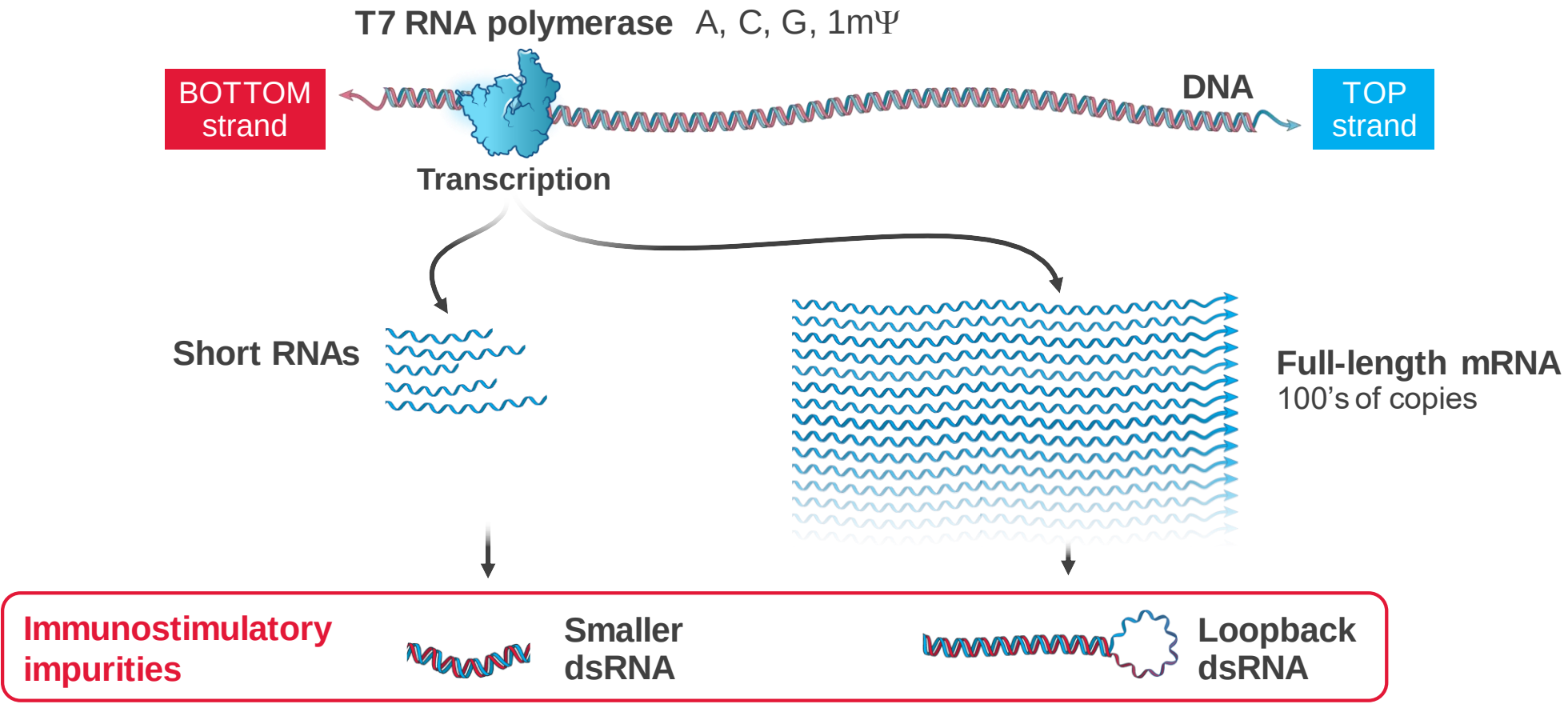
Amy Rabideau, Ph.D.



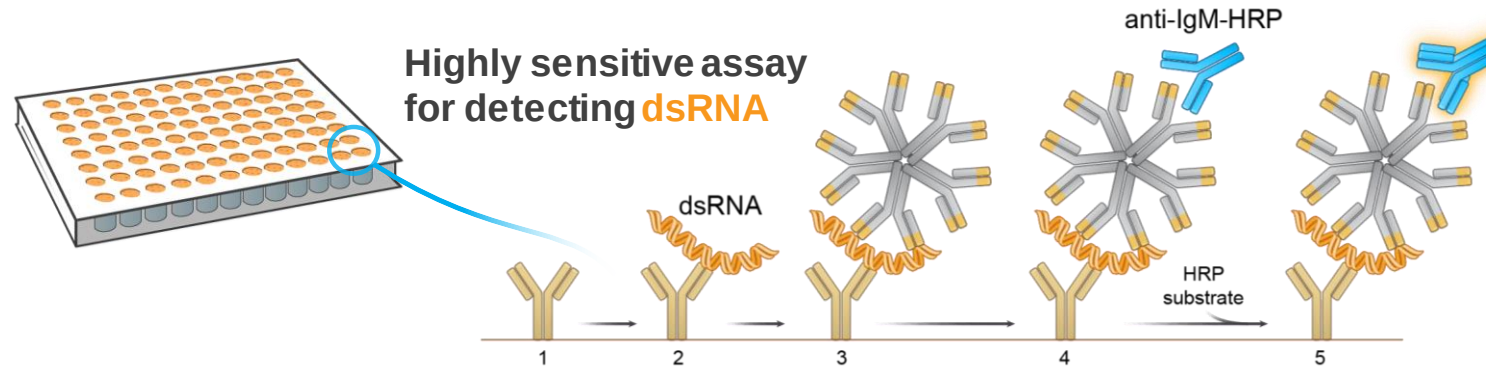
Amy Rabideau, Ph.D.

Principal Scientist, Process Development

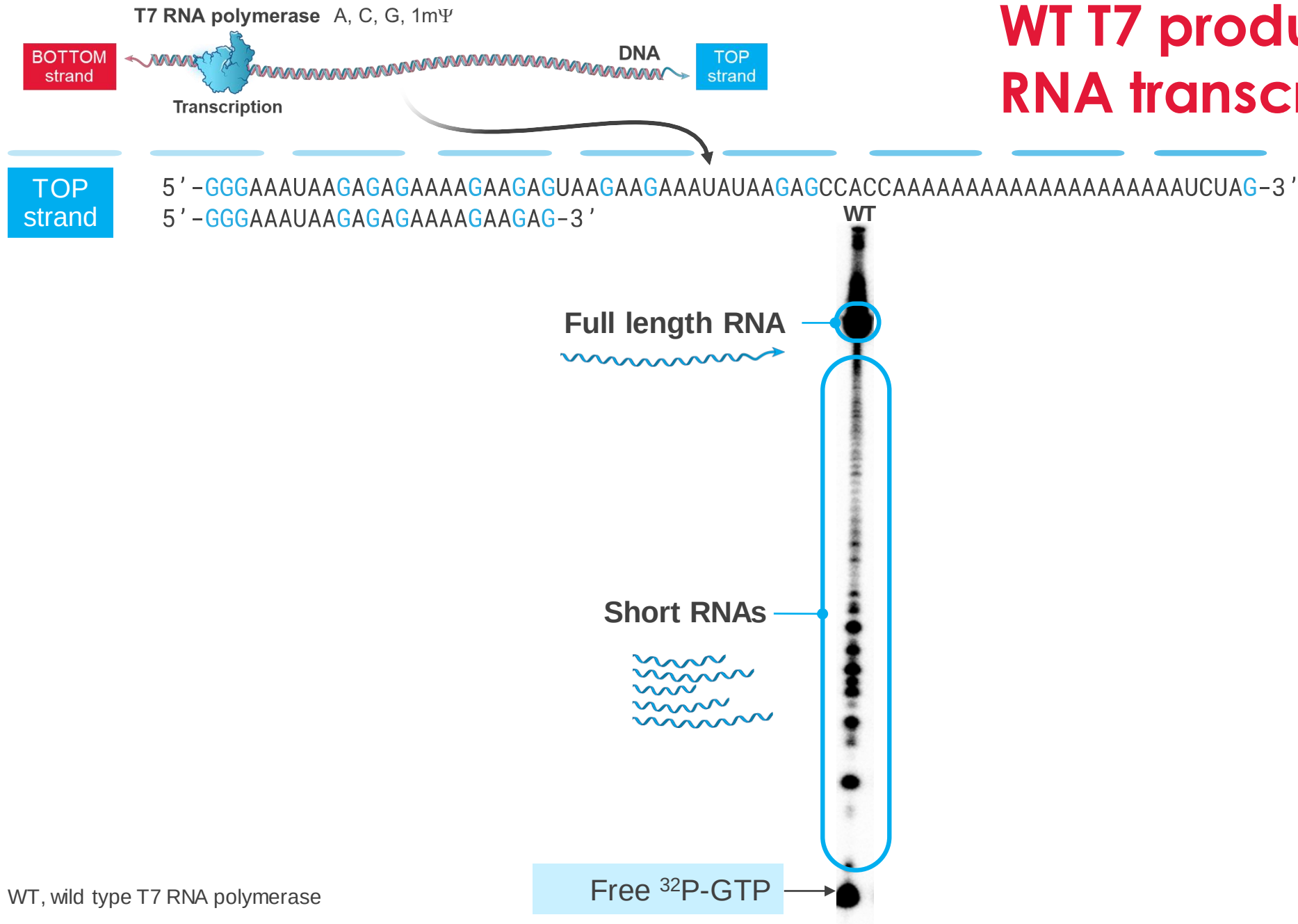
Wild type T7 RNA polymerase makes both full-length mRNA and dsRNA impurities



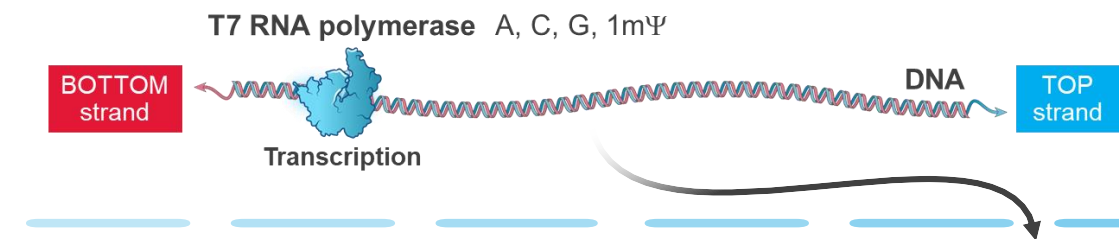
By engineering process conditions, we can minimize dsRNA formation by wild type T7 RNA polymerase



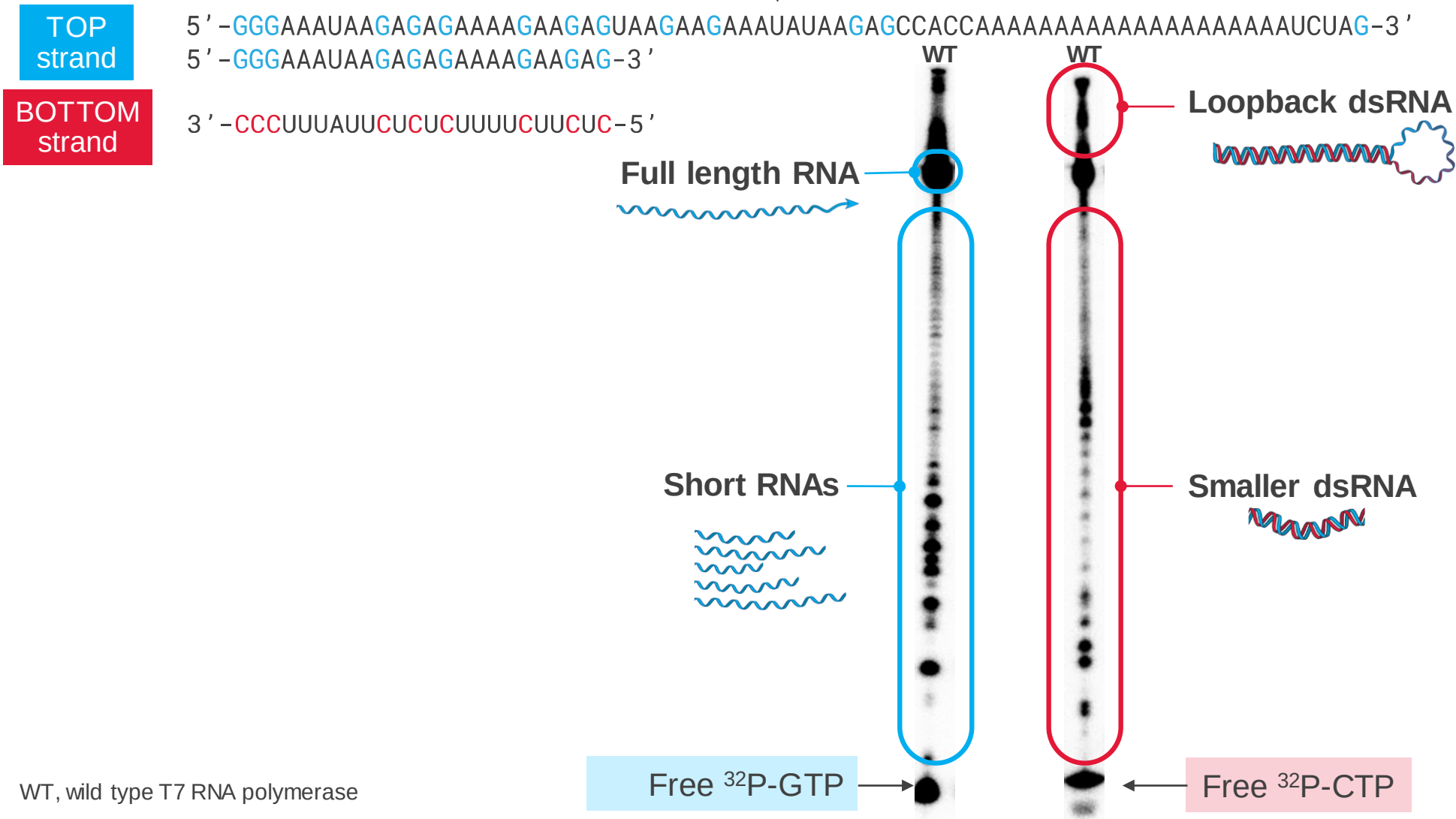
WT T7 produces short RNA transcripts



WT, wild type T7 RNA polymerase



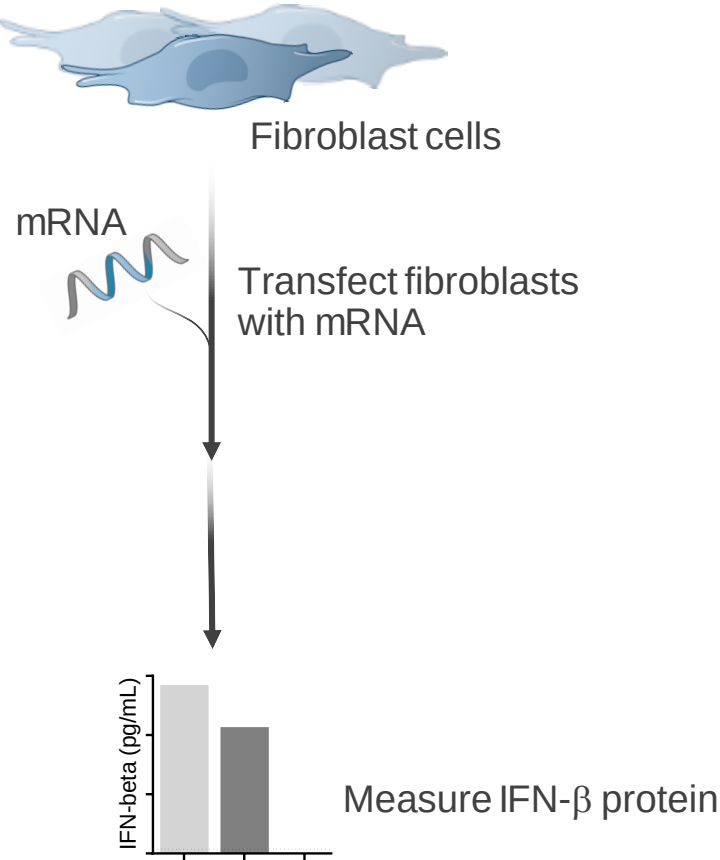
WT T7 produces small and loopback dsRNA



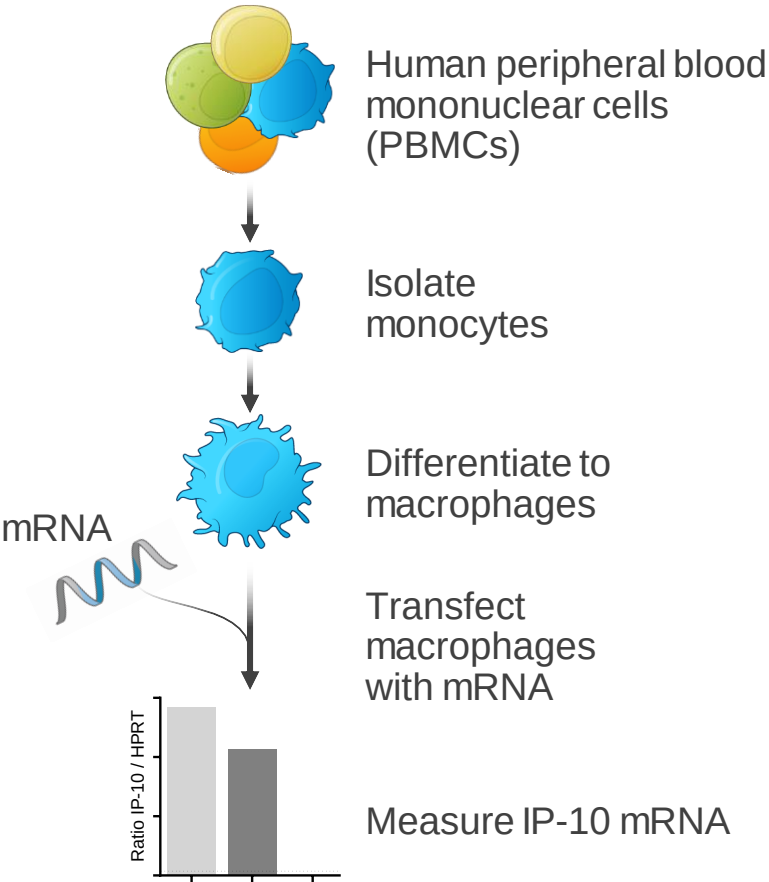
WT, wild type T7 RNA polymerase

Cell-based assays for measuring innate immune responses to dsRNA

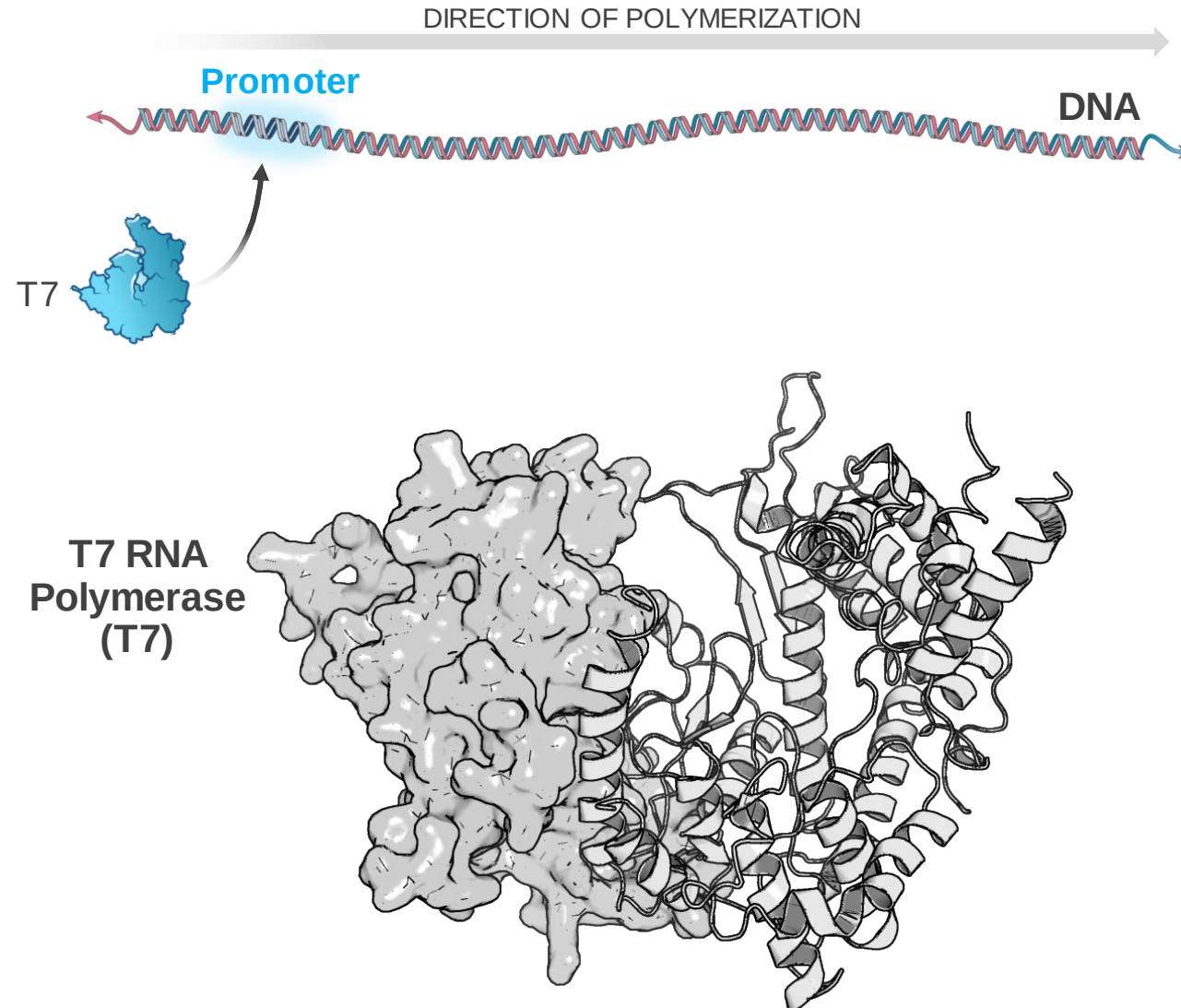
Immune response in fibroblasts



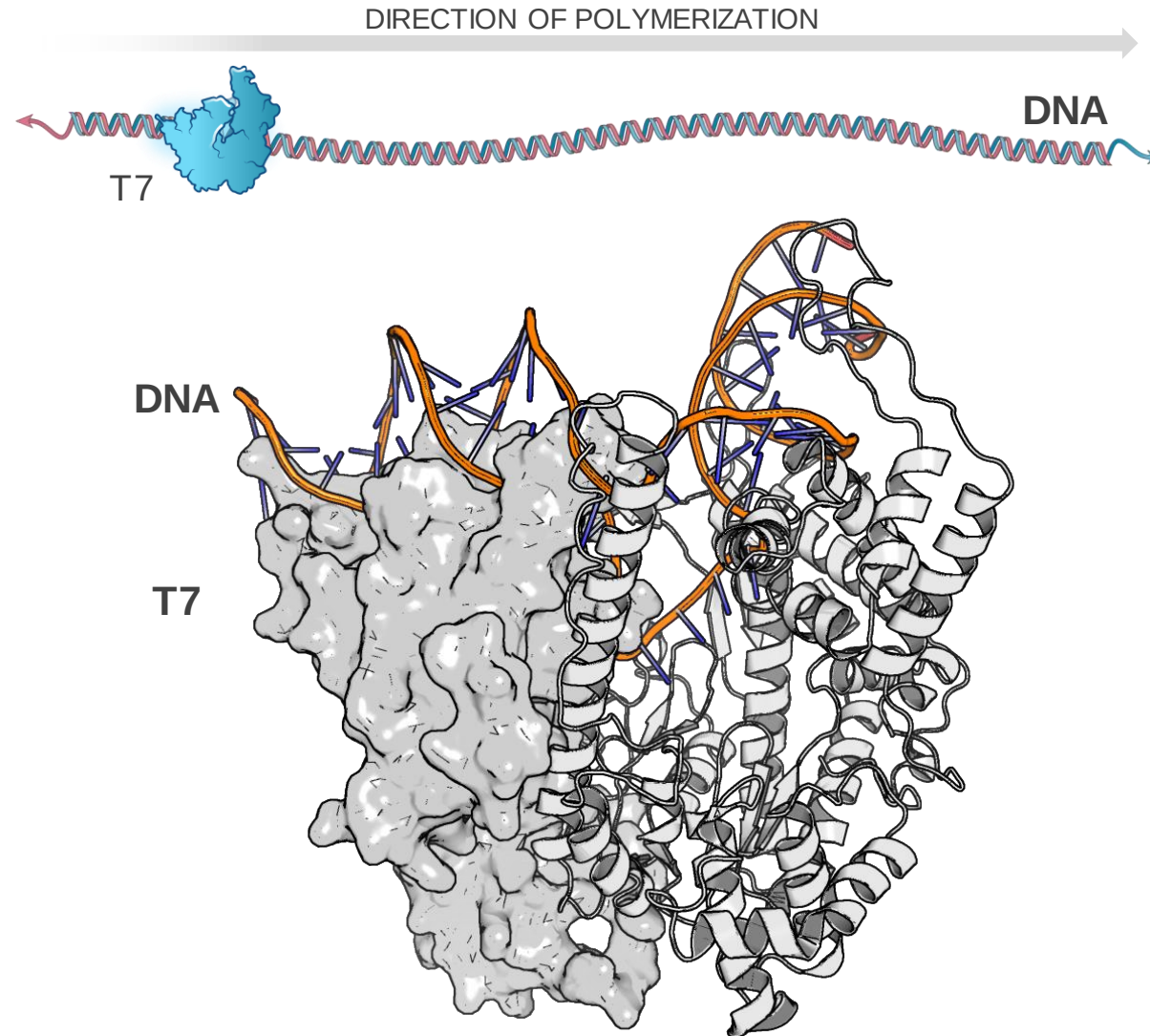
Immune response in macrophages



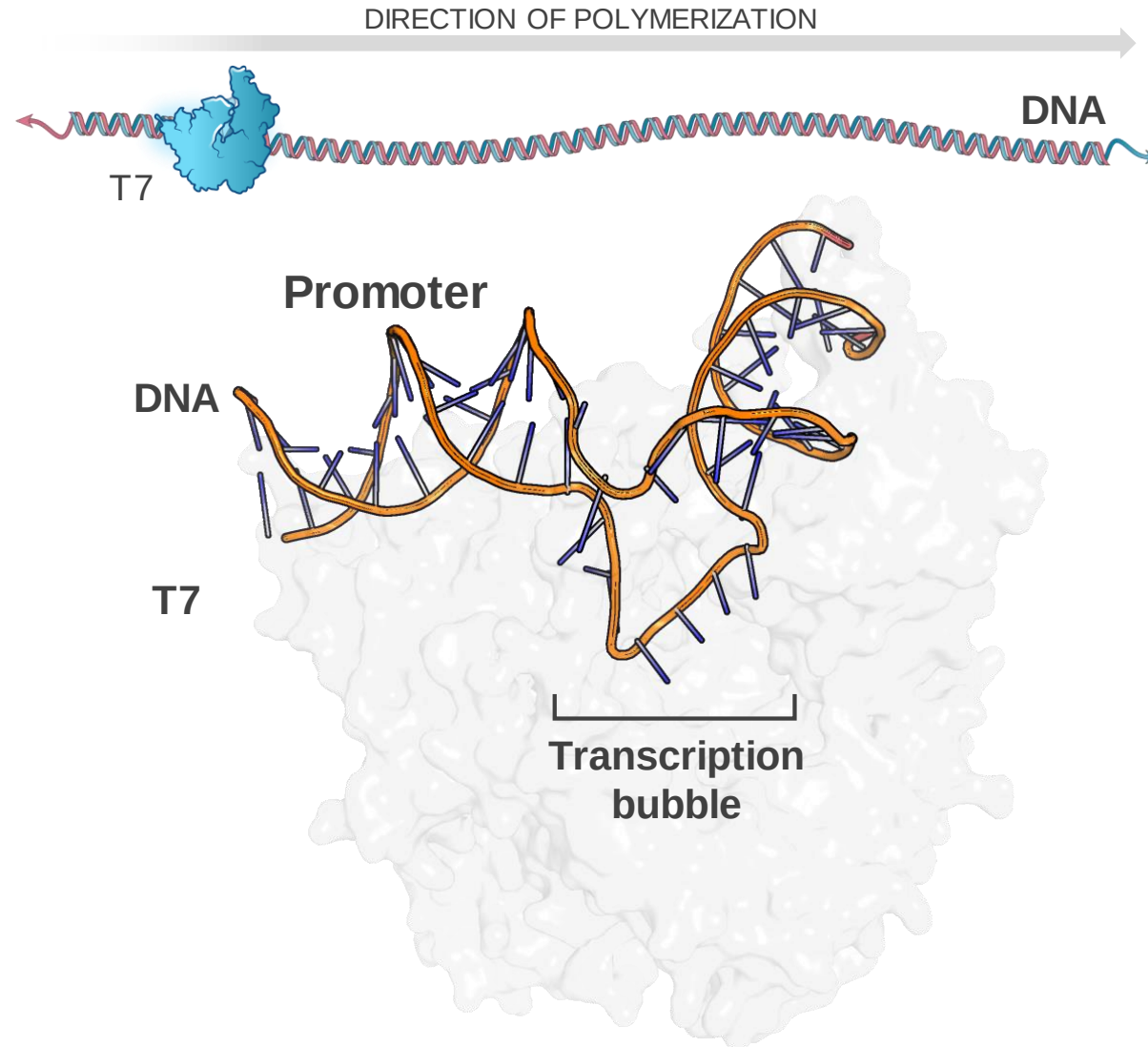
Transcription cycle: Unbound T7 RNA polymerase



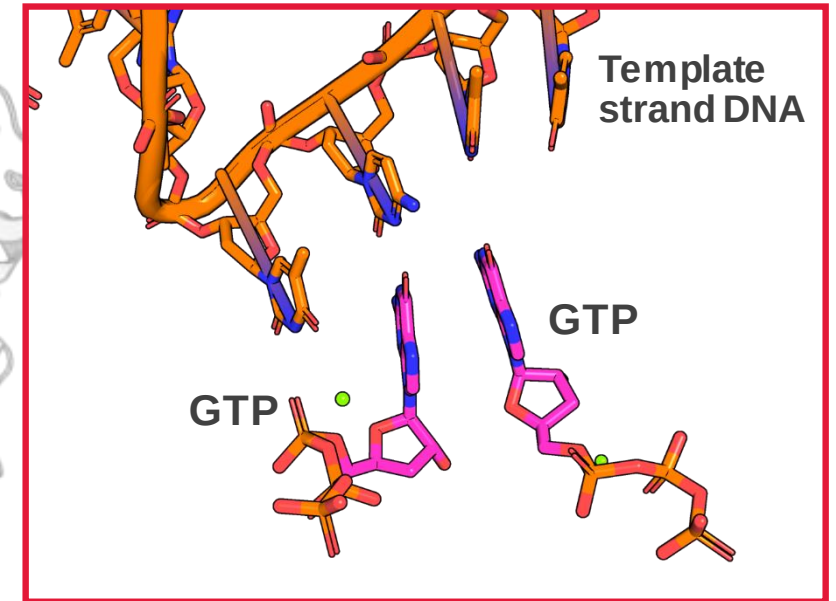
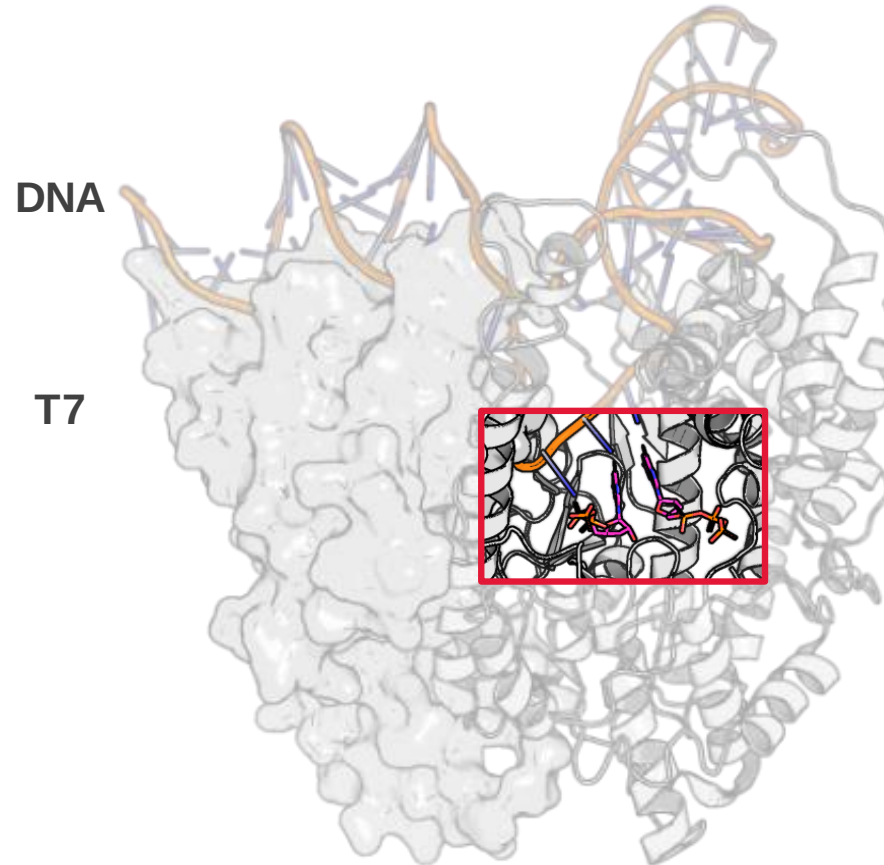
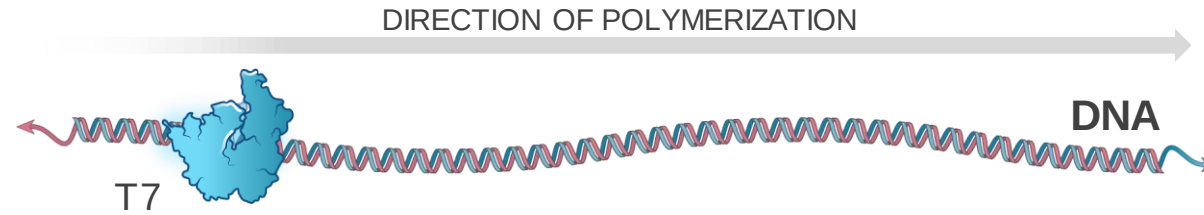
Transcription cycle: Binding promoter in DNA template



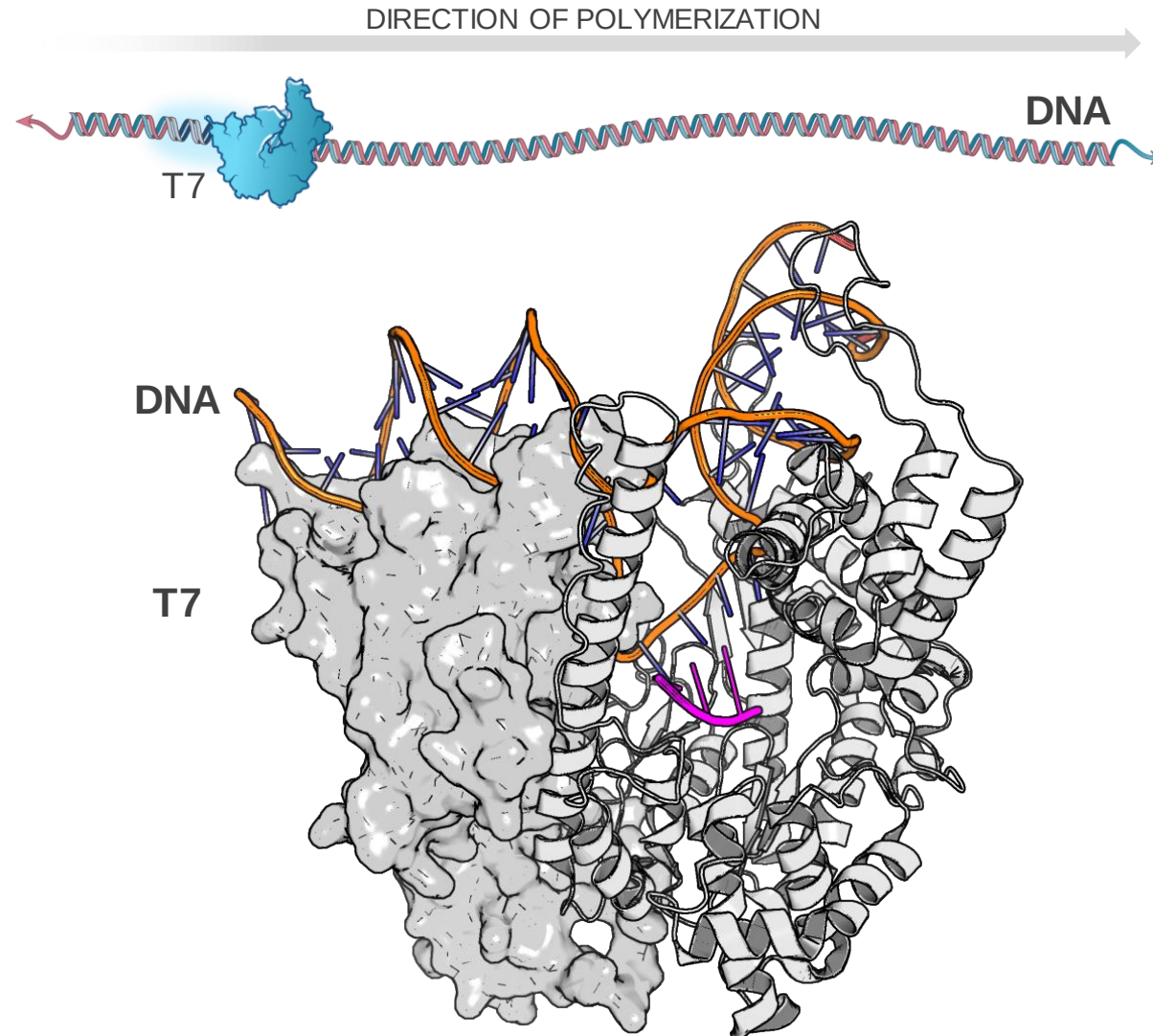
Transcription cycle: Melting DNA to form transcription bubble



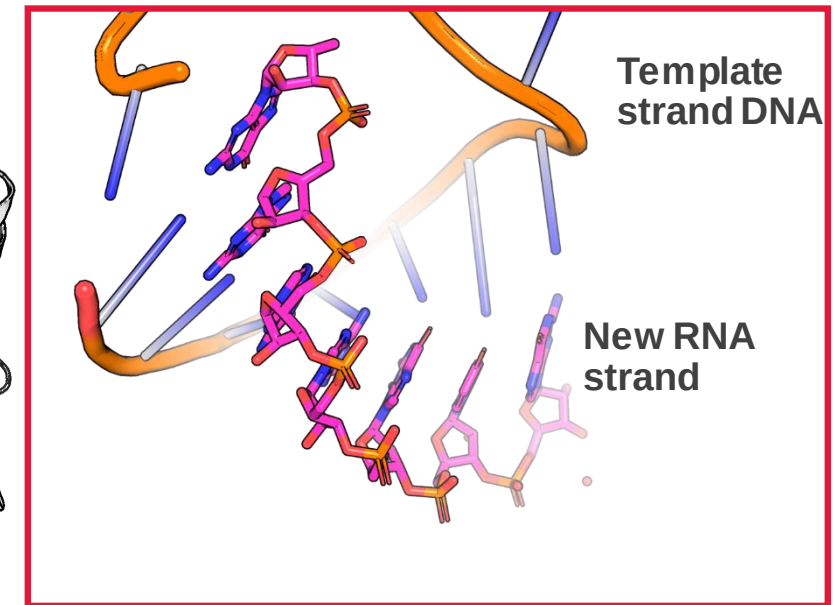
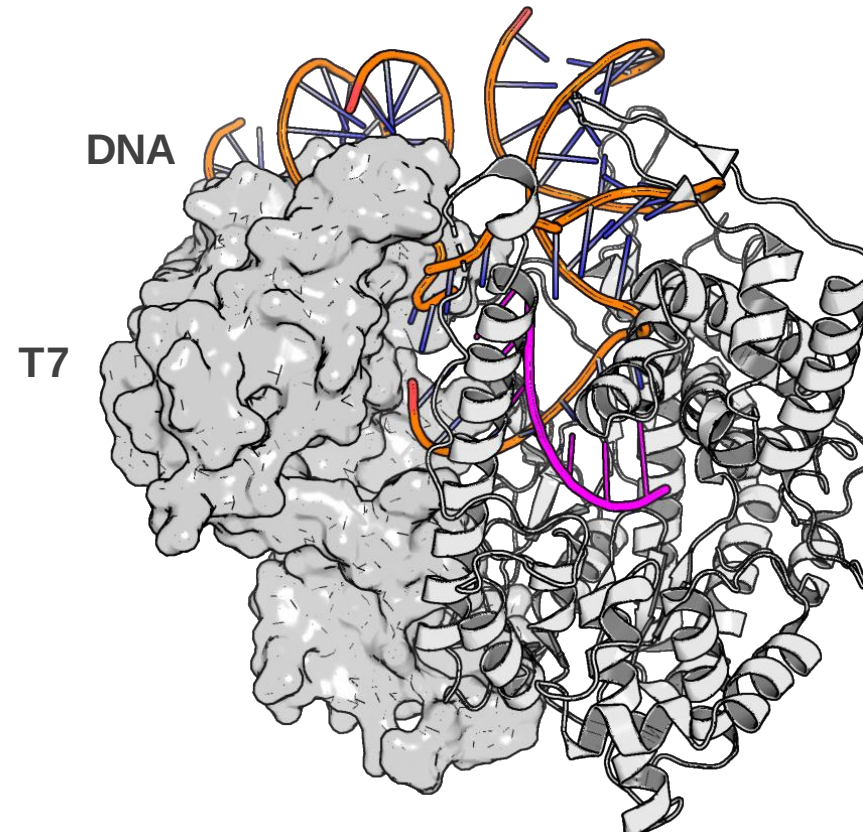
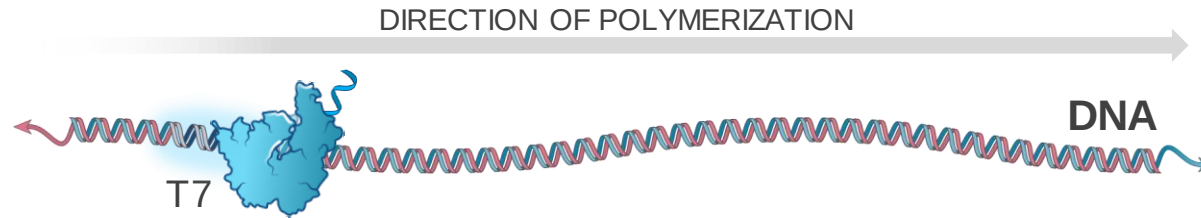
Transcription cycle: *De novo* initiation with 2 GTPs



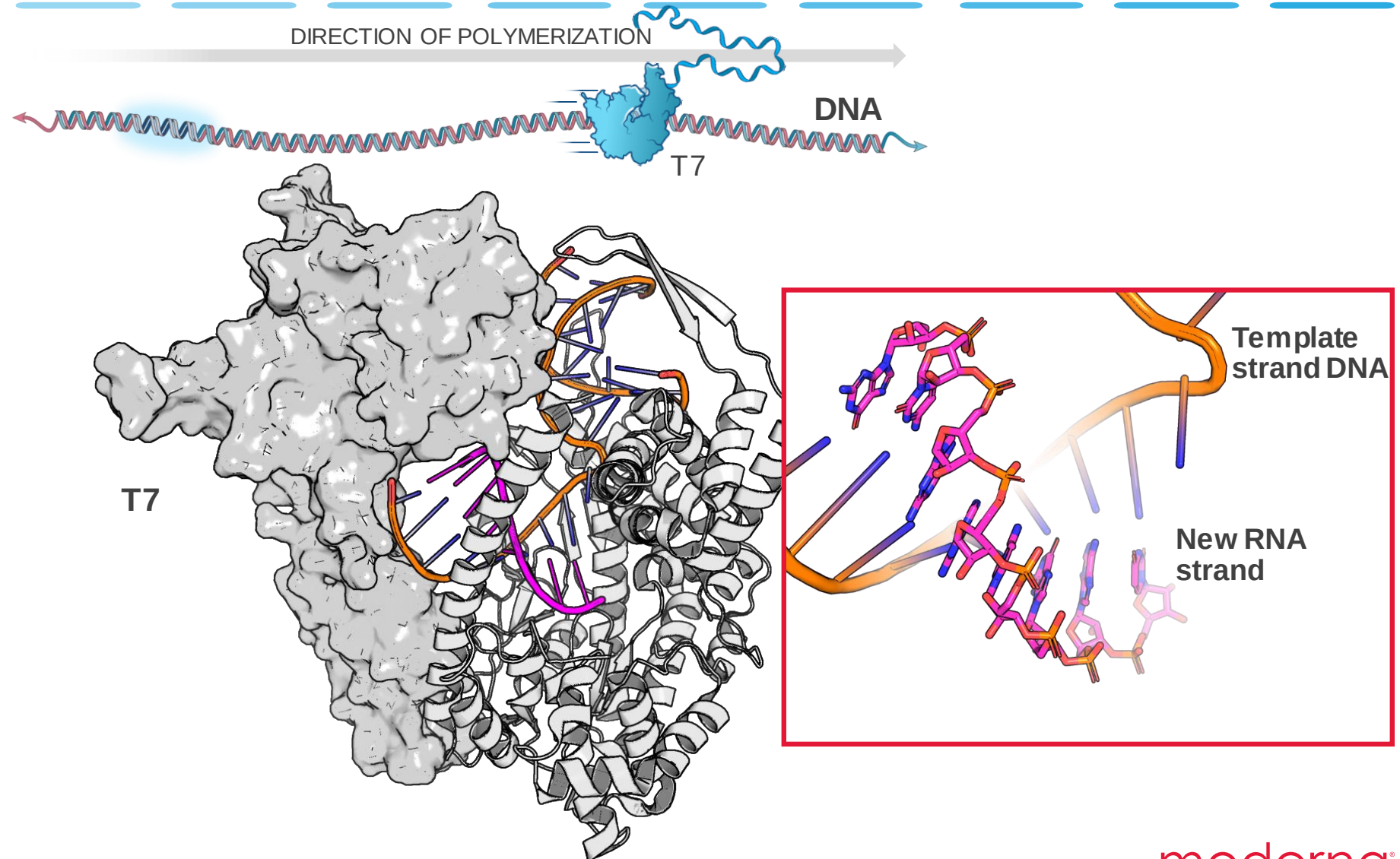
Transcription cycle: Initiating short RNA transcript



Transcription cycle: Transitioning to elongation



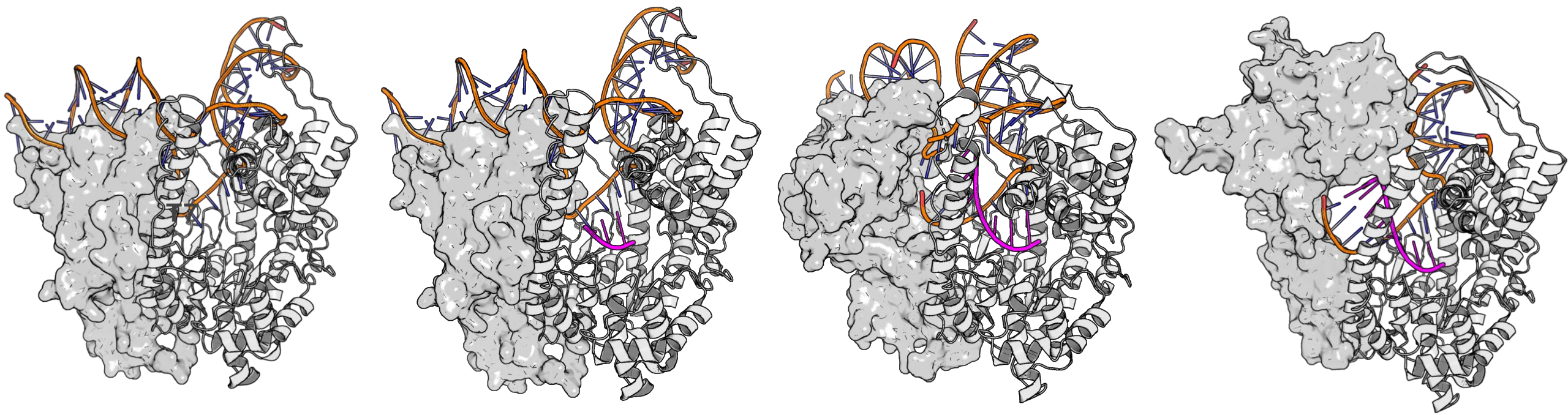
Transcription cycle: Rapidly transcribes RNA



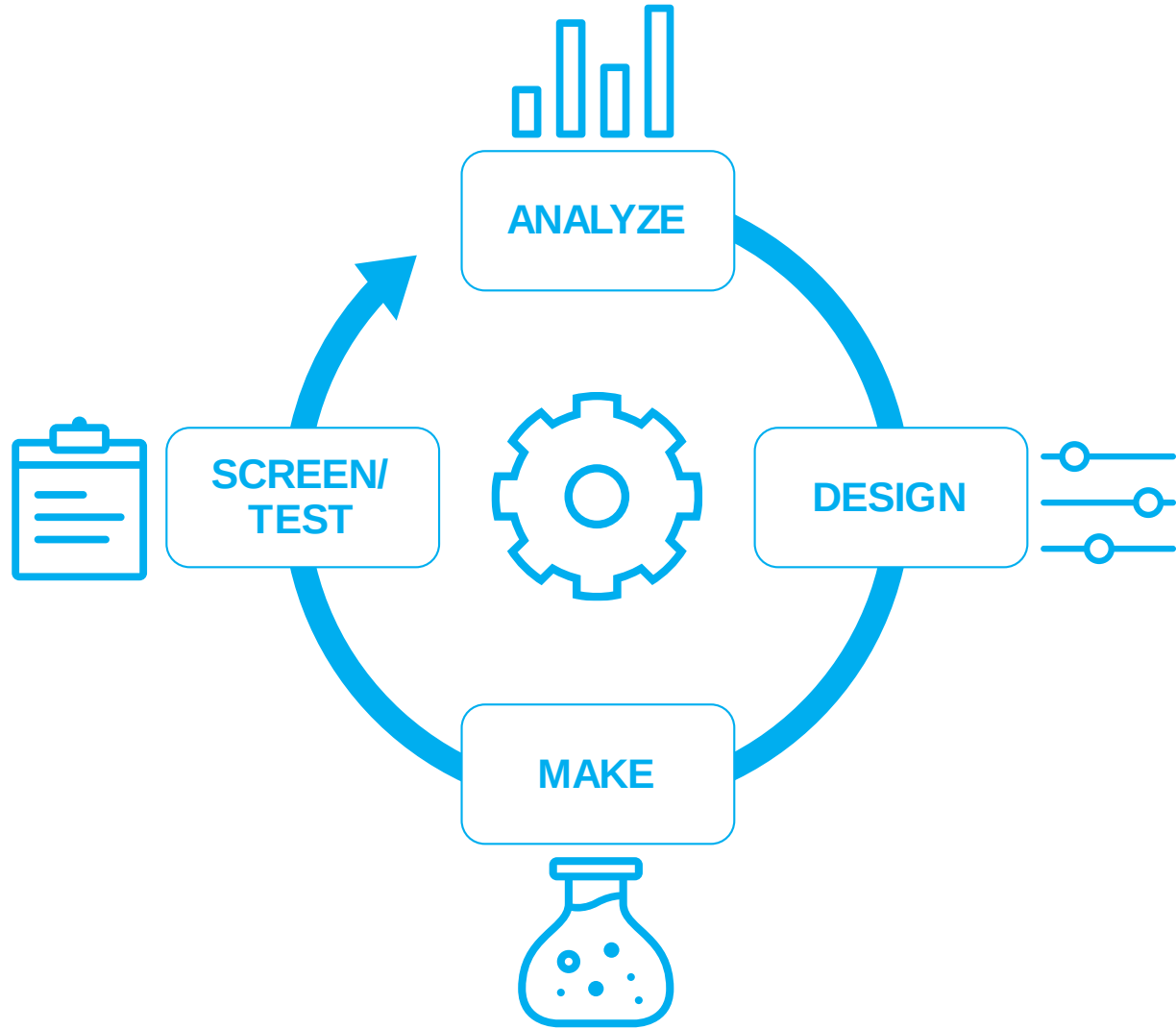
Can we engineer T7 to favor the fast processive state?

Initiation complex

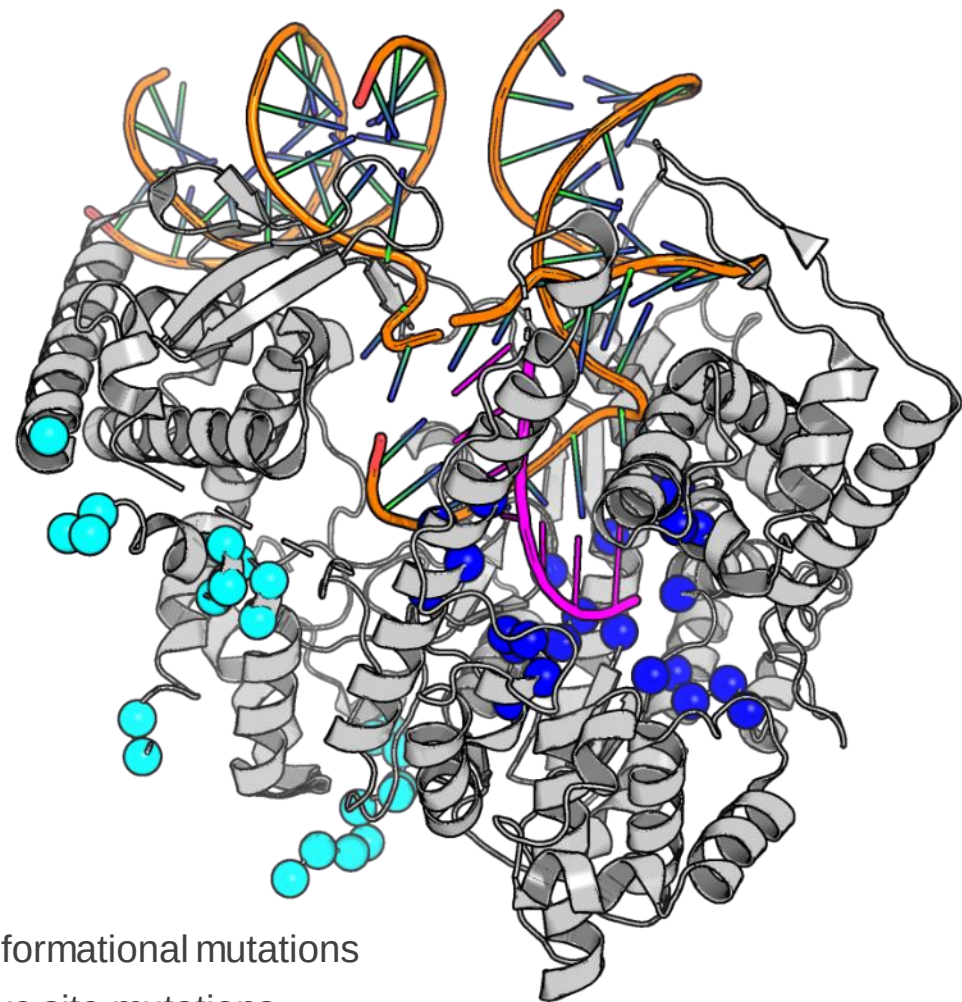
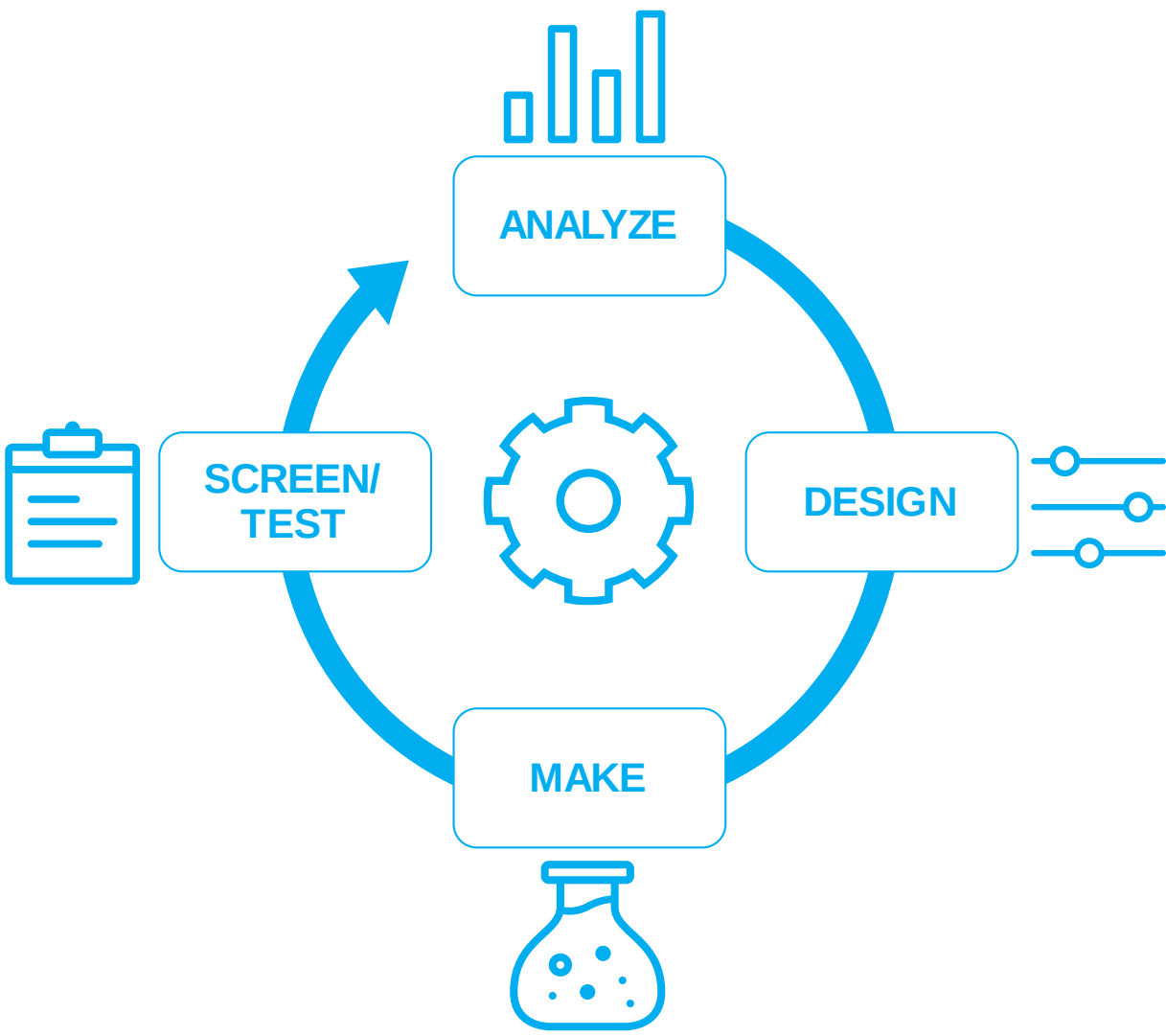
Elongation complex



Moderna computer-aided protein engineering workflow

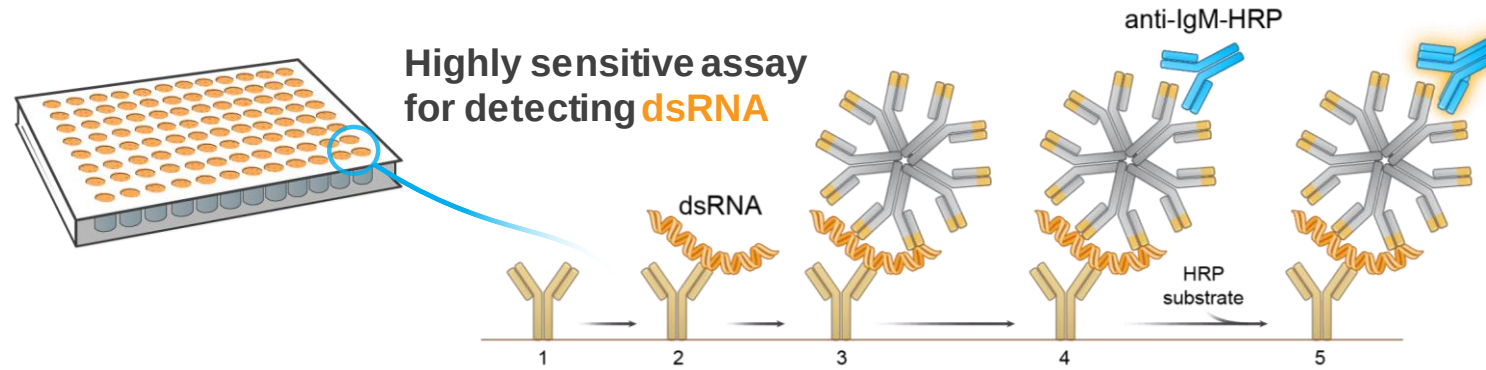


Analyze & Design: Mutation positions in structure

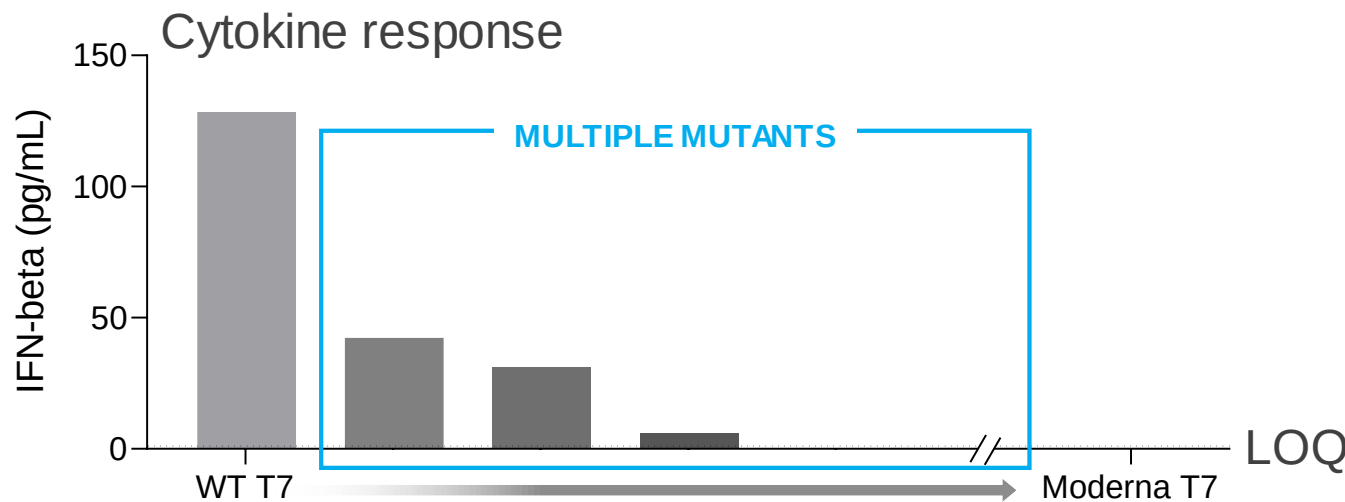
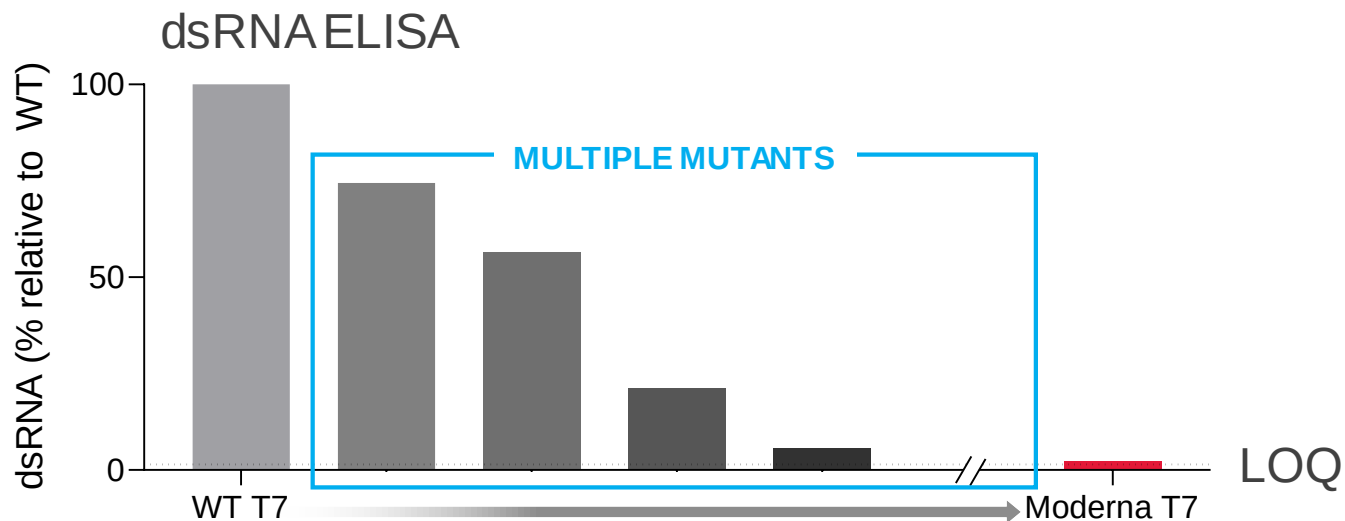
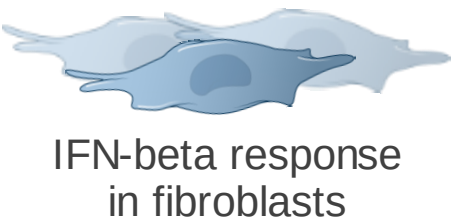
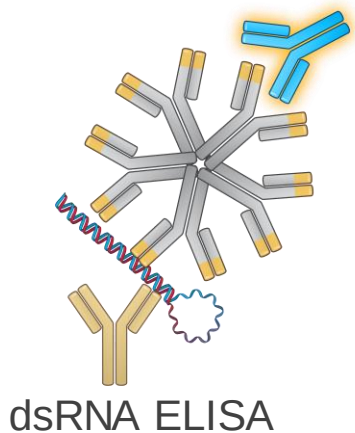


-  Conformational mutations
-  Active site mutations

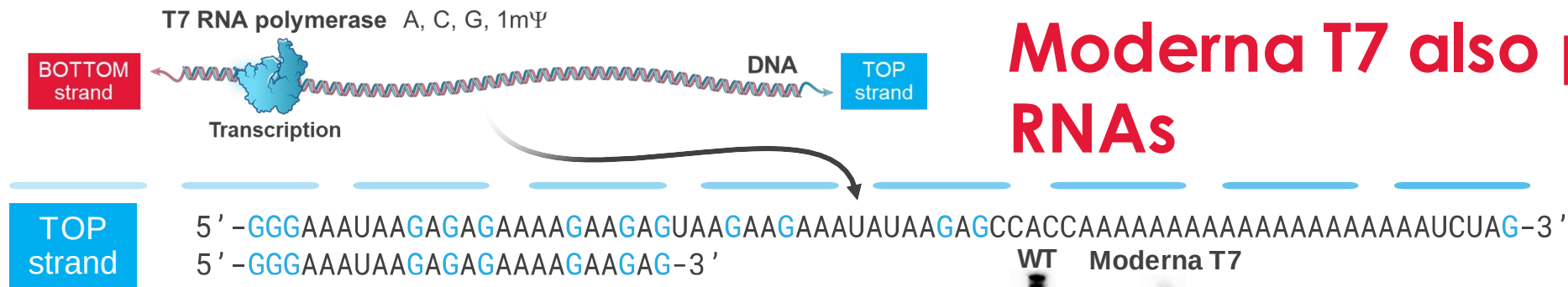
By engineering process conditions, we can minimize dsRNA formation by wild type T7 RNA polymerase



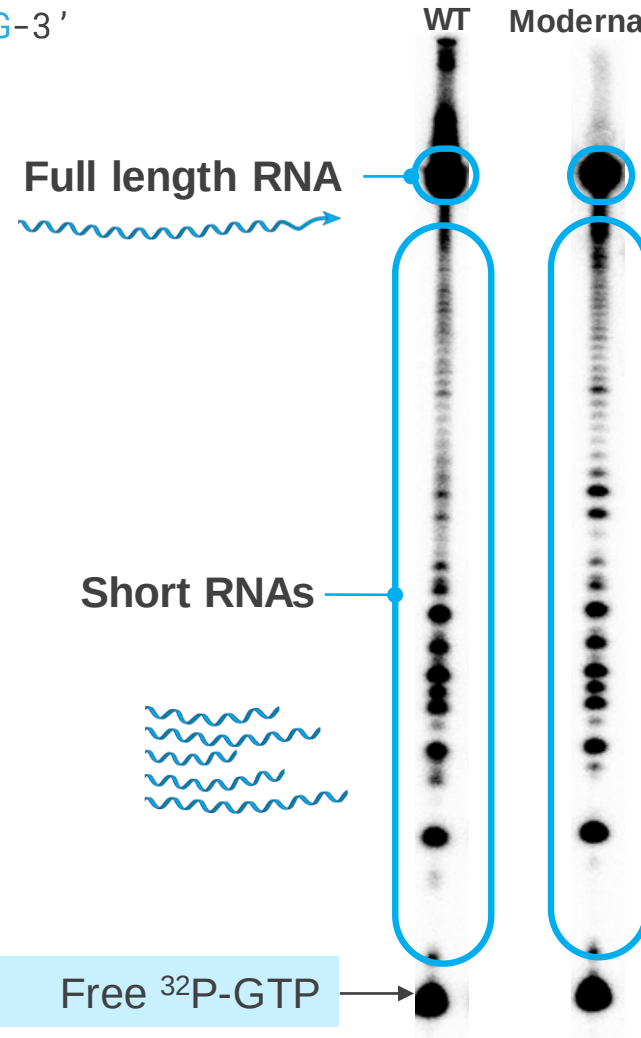
Screen/test: dsRNA content and immune response



LOQ, level of quantitation;
WT T7, wild type T7 RNA polymerase

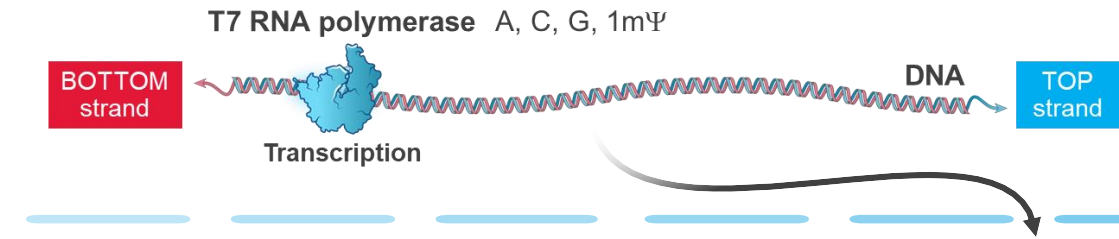


Moderna T7 also produces short RNAs



WT, wild type T7 RNA polymerase

Moderna T7 produces very little dsRNA



5' - GGG AAA UAA GAG AGA AAG AAG UAA GAA GAAA UAU AAG AGC CAC CAAAA AAAAAAAAAAAAAAAAAA UCU AG - 3'

5'-GGGAAAUAAAGAGAGAAAAGAAGAG-3'

3' - CCCUUUAUUCUCUUUUCUUCUC - 5'

Full length RNA

Short RNAs

Loopback dsRNA

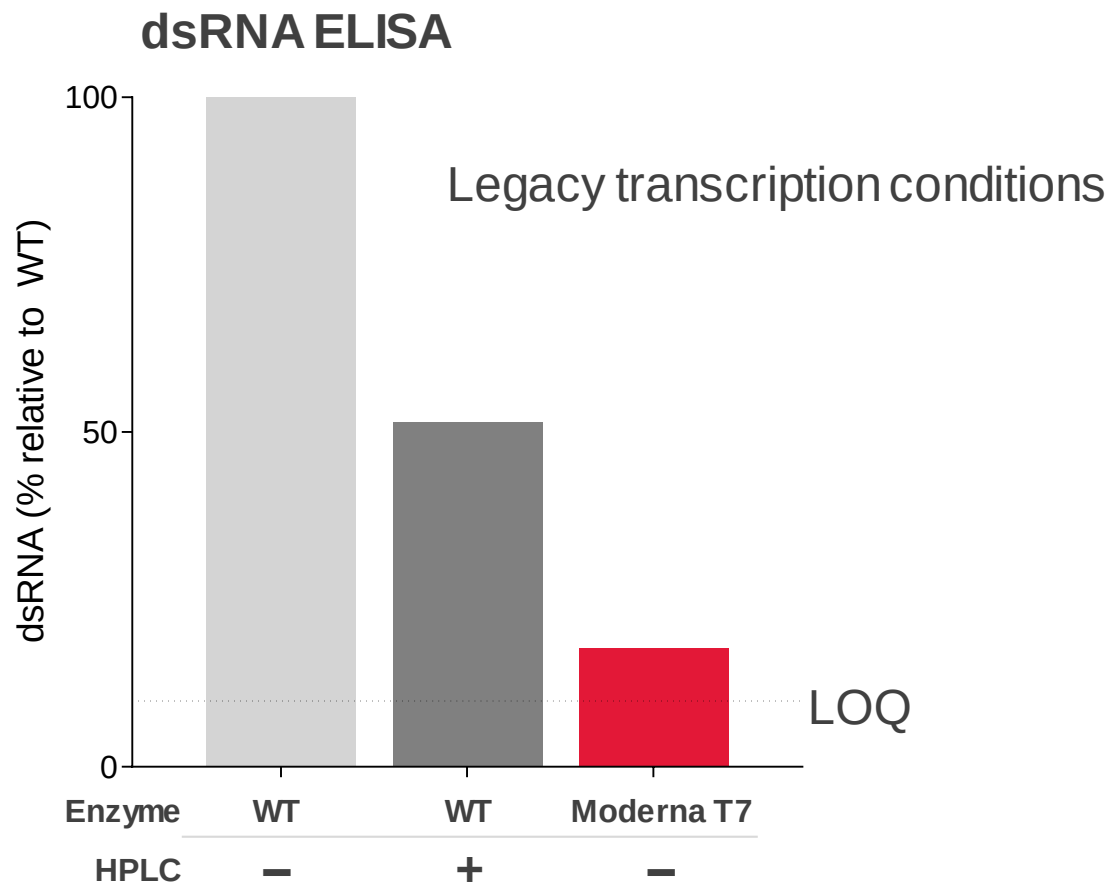
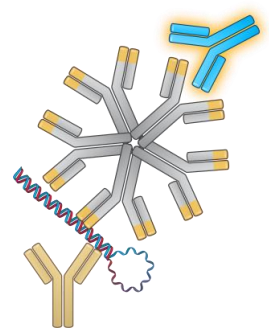
Smaller dsRNA

Free ^{32}P -GTP

Free ^{32}P -CTP

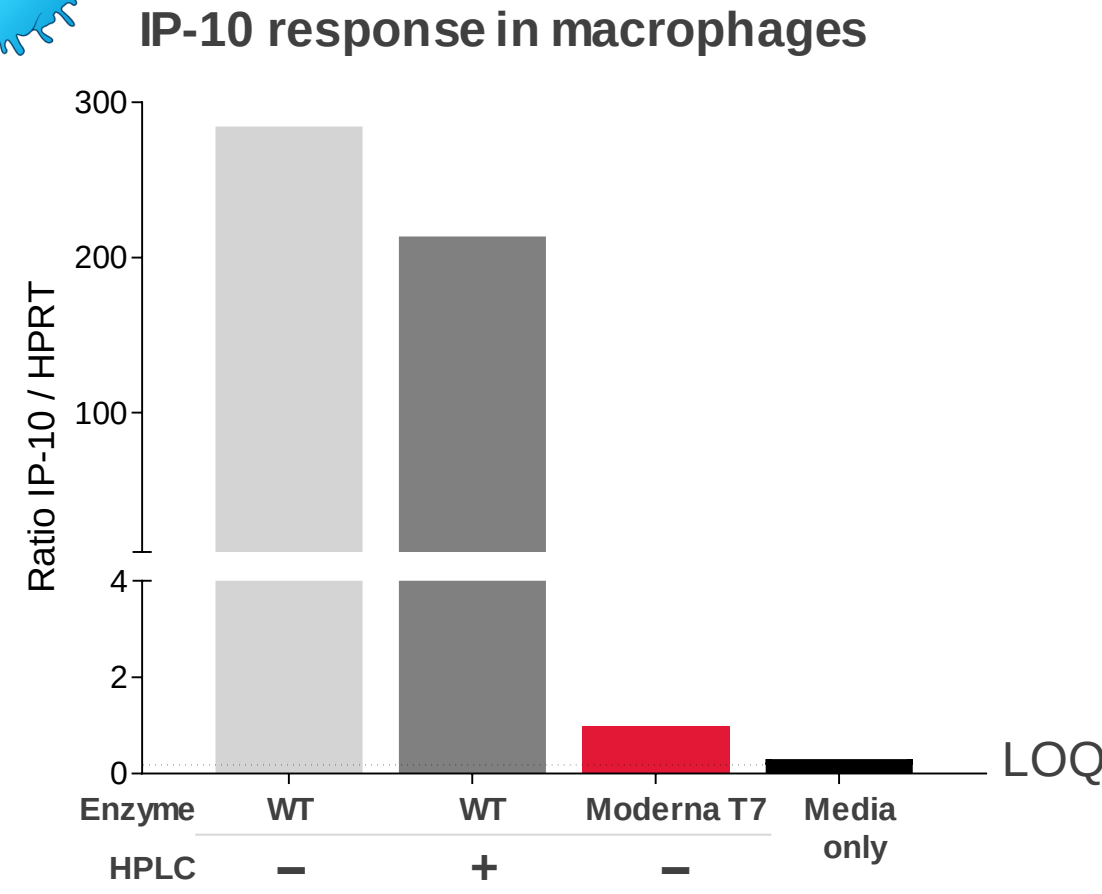
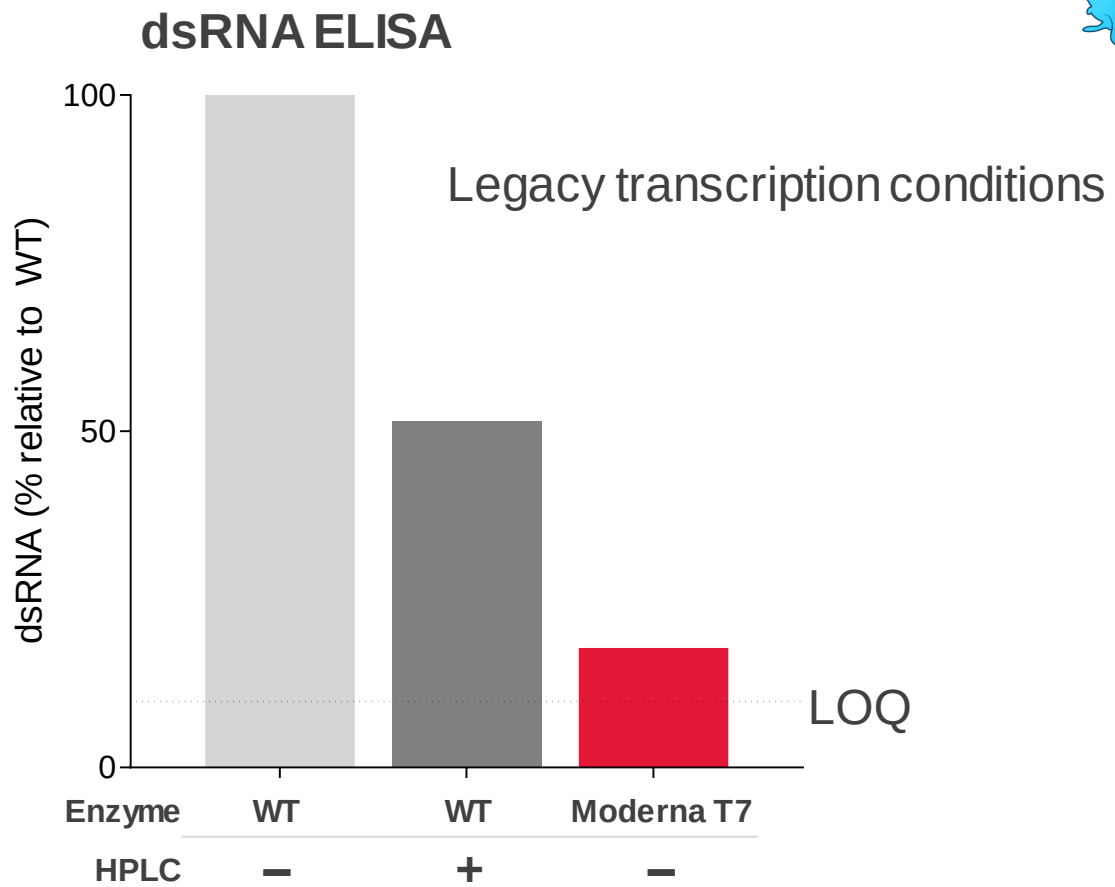
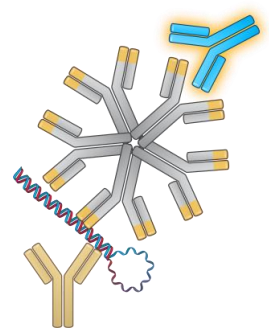
WT, wild type T7 RNA polymerase

Moderna T7 produces mRNA with low dsRNA without HPLC



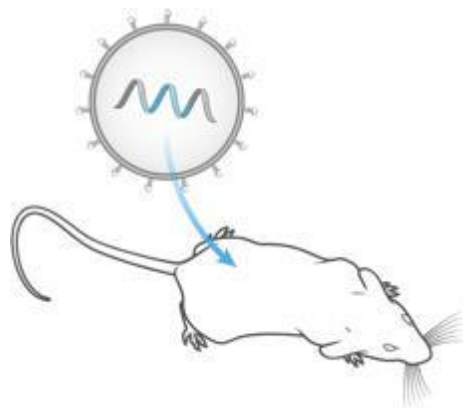
LOQ, limit of quantitation;
WT, wild type T7 RNA polymerase

Moderna T7 produces mRNA with low dsRNA without HPLC

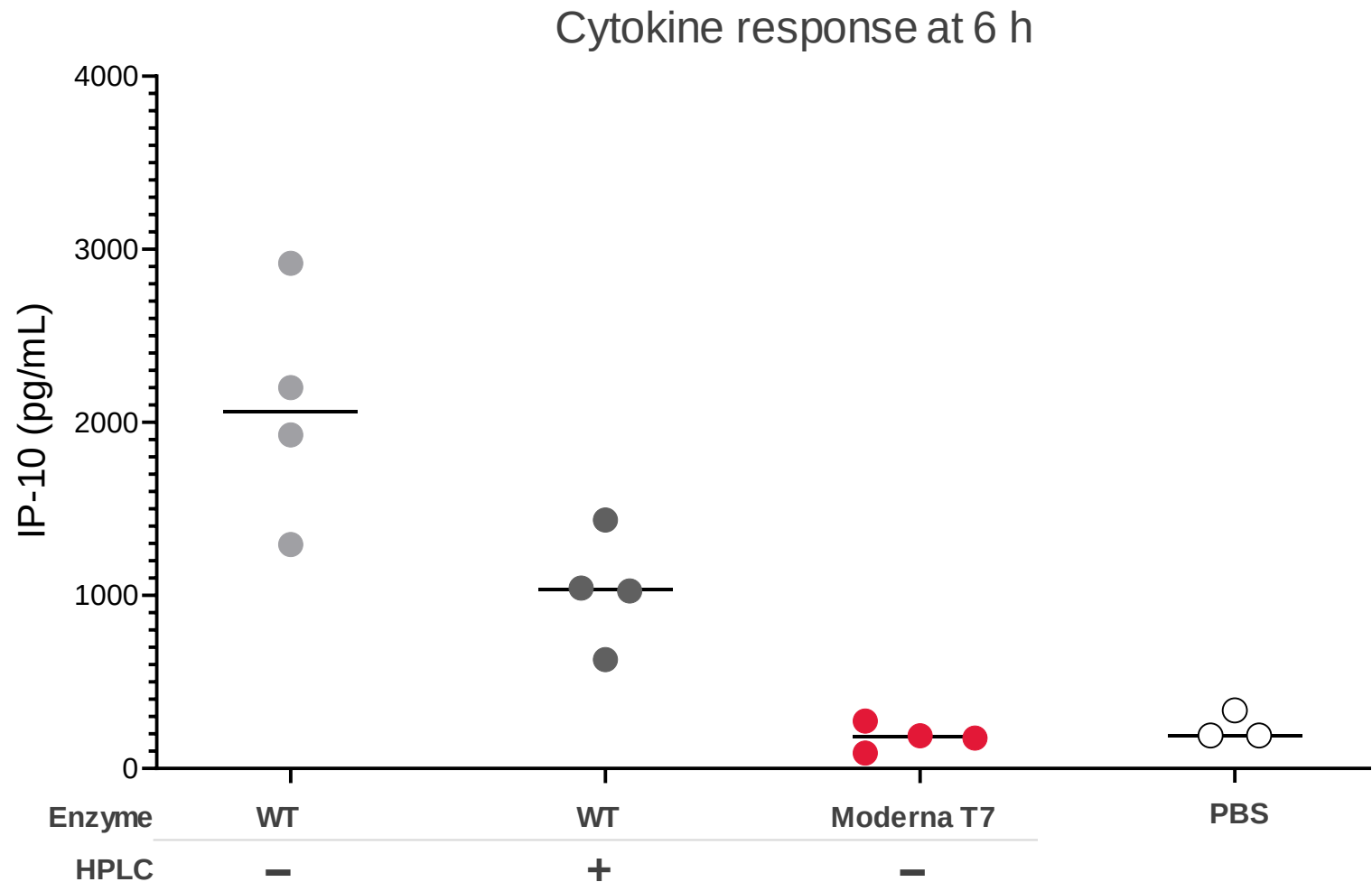


LOQ, limit of quantitation;
WT, wild type T7 RNA polymerase

Moderna T7 produces mRNA with low immune response without HPLC in vivo



C57BL/6 mice
IV administration
0.5 mg/kg mRNA
Analyzed at 6 h post-dose

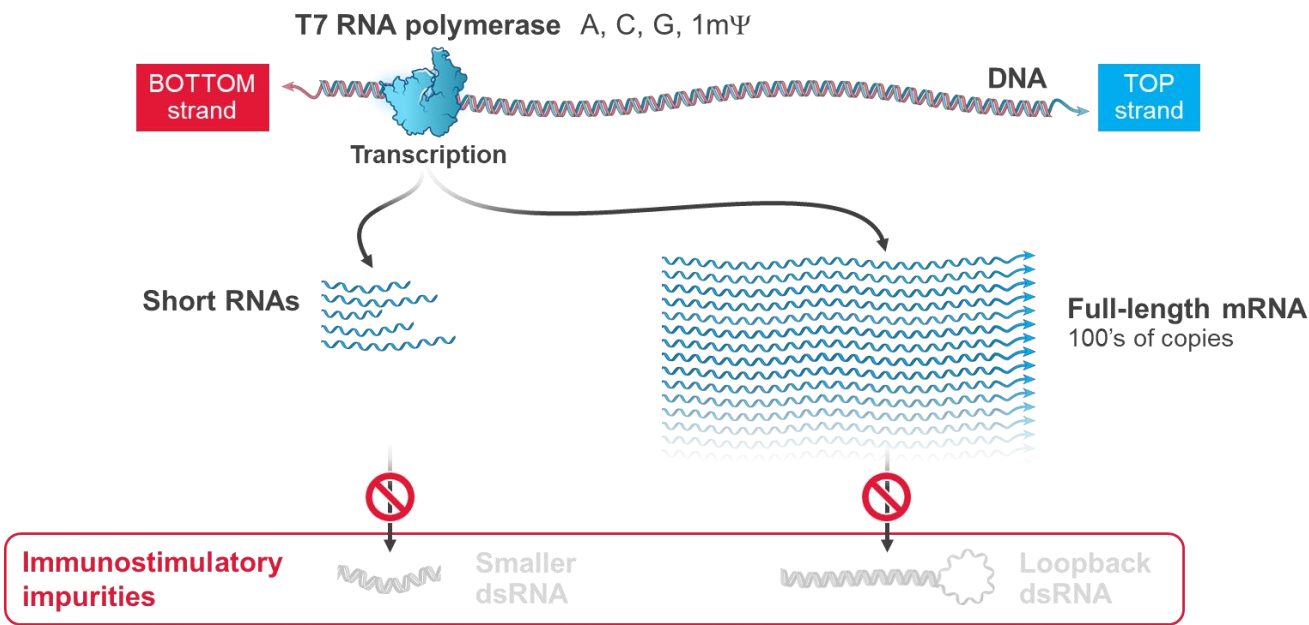


Moderna now has two ways to reduce dsRNA

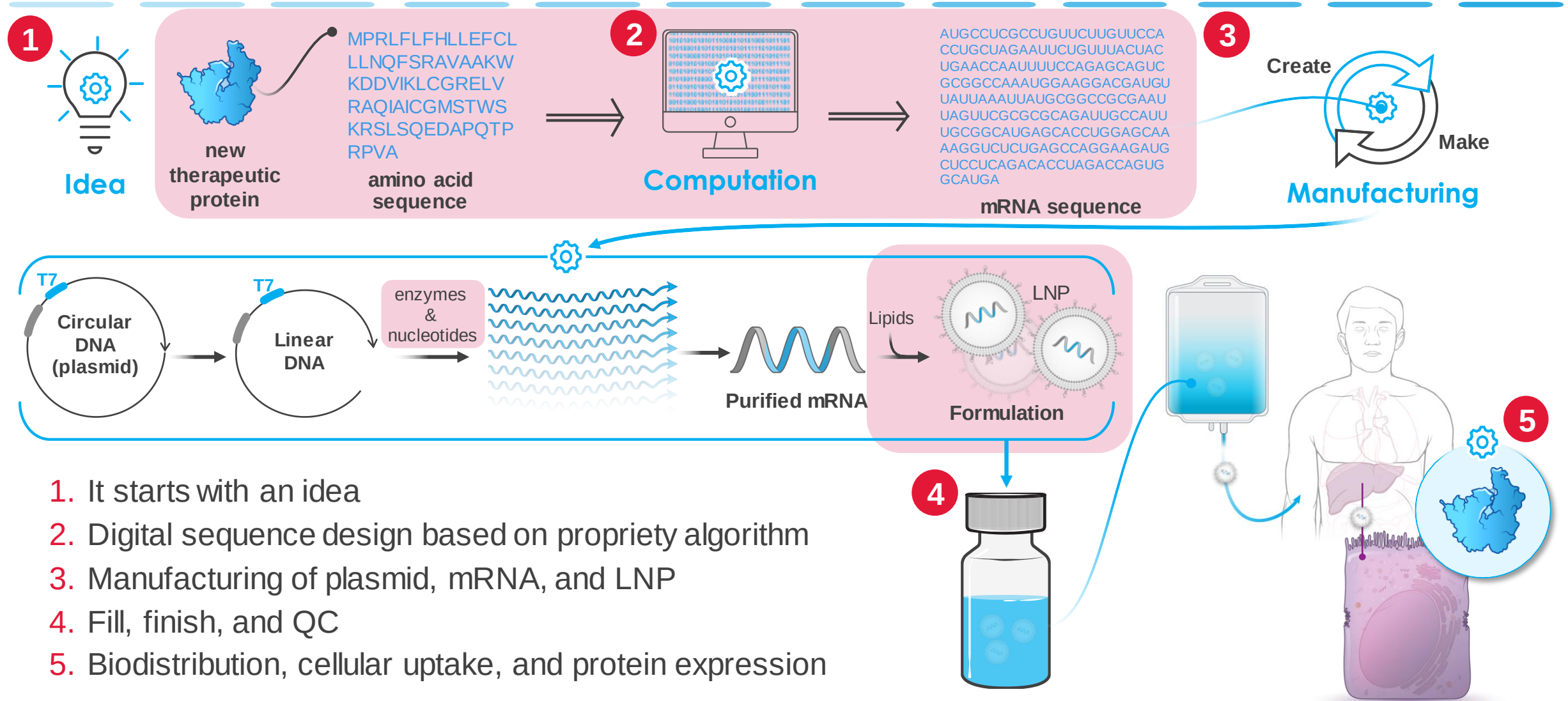
1. Process Optimization



2. Moderna T7

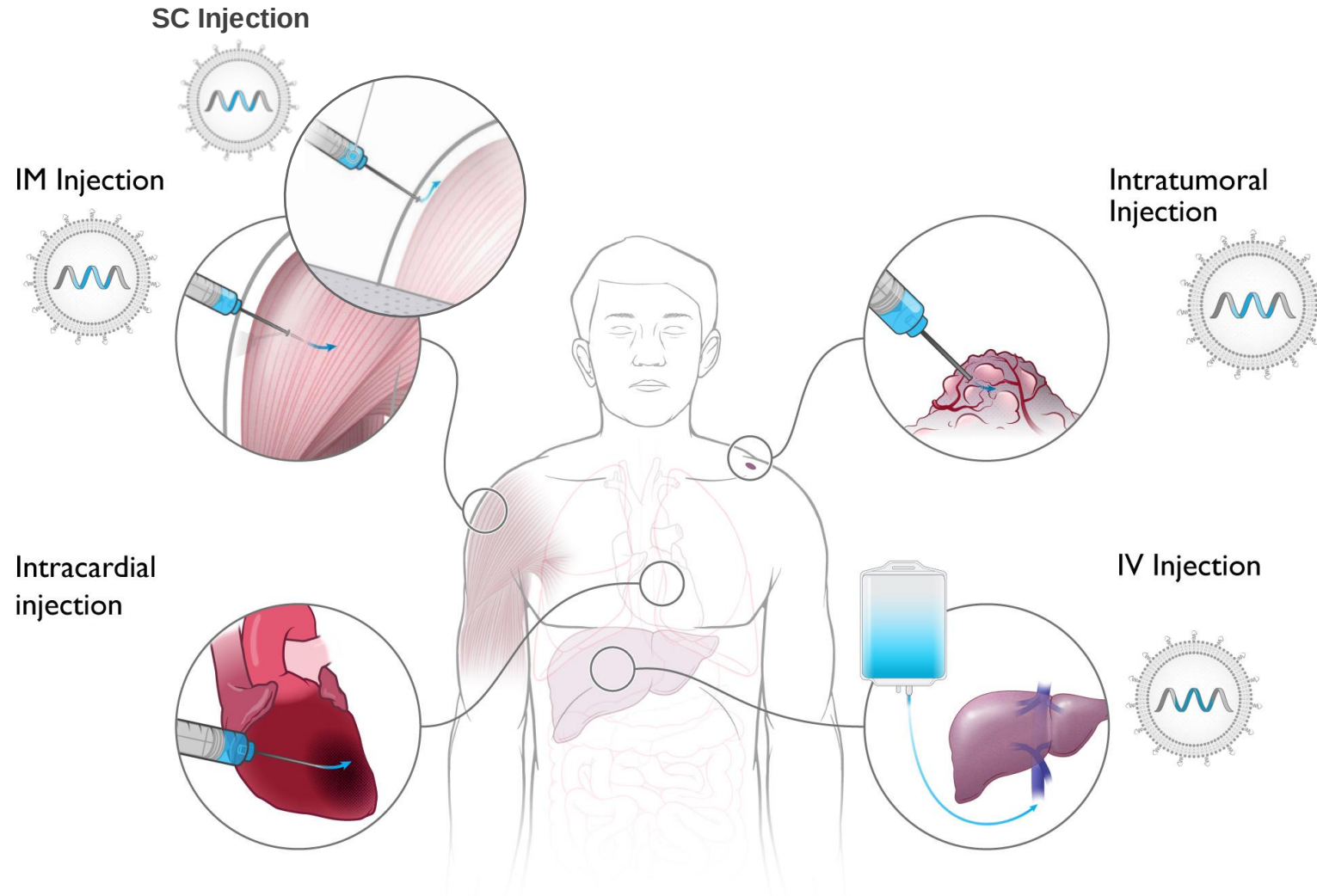


Where does Platform Research fit in?

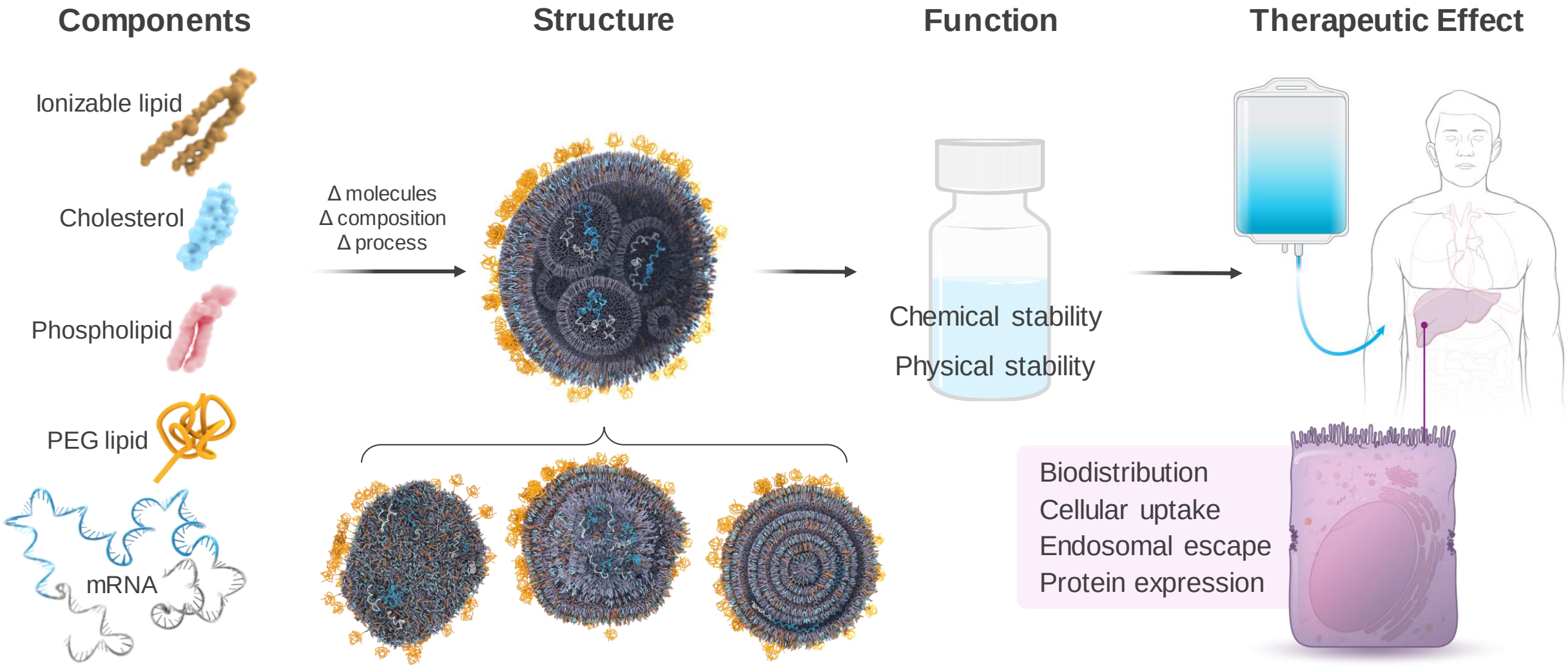


1. It starts with an idea
2. Digital sequence design based on propriety algorithm
3. Manufacturing of plasmid, mRNA, and LNP
4. Fill, finish, and QC
5. Biodistribution, cellular uptake, and protein expression

We target different tissues via multiple routes of administration (ROAs)



Rational structure-based design of LNPs for mRNA starts with understanding the components and their molecular interactions





New LNPs for Liver Delivery

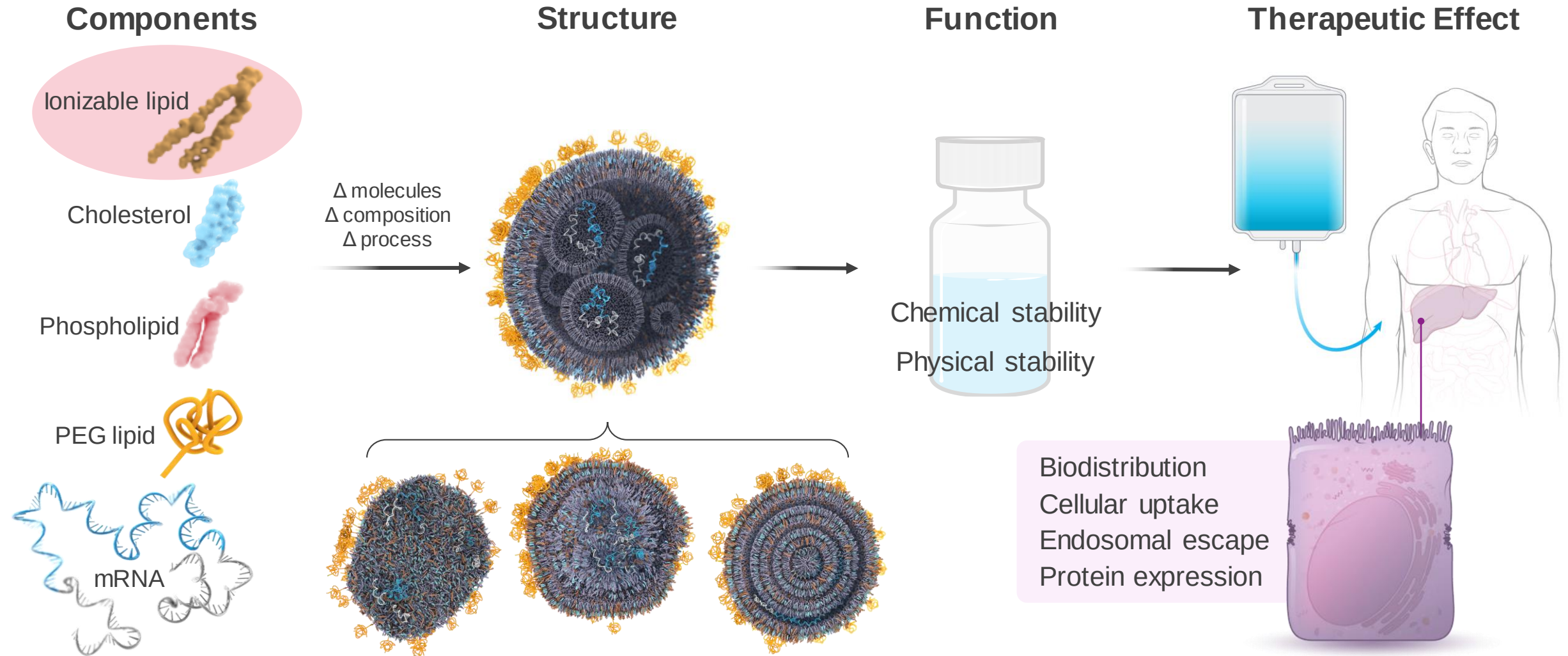
Kerry Benenato, Ph.D.



Kerry Benenato, Ph.D.

Senior Director, Platform Chemistry

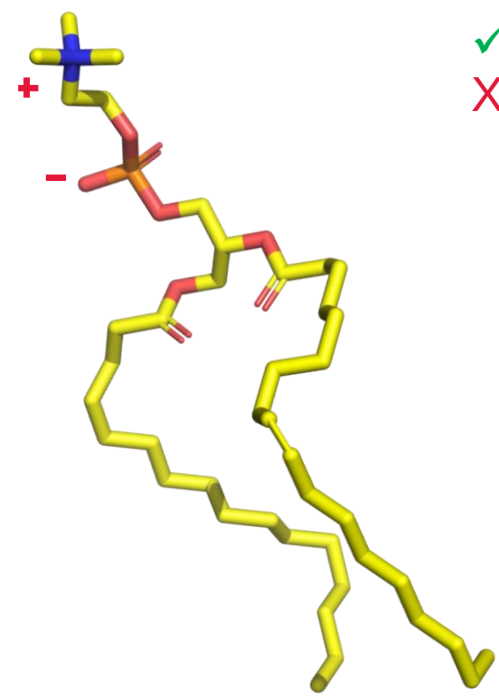
Rational structure-based design of LNPs for mRNA starts with understanding the components and their molecular interactions



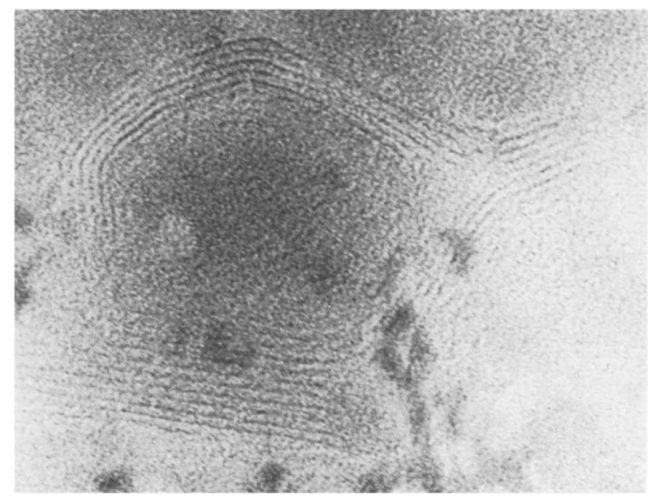
Evolution to the modern day lipid nanoparticle identified the importance of ionizable lipids



Neutral lipids
FEBS lett., 1971, 14, 95

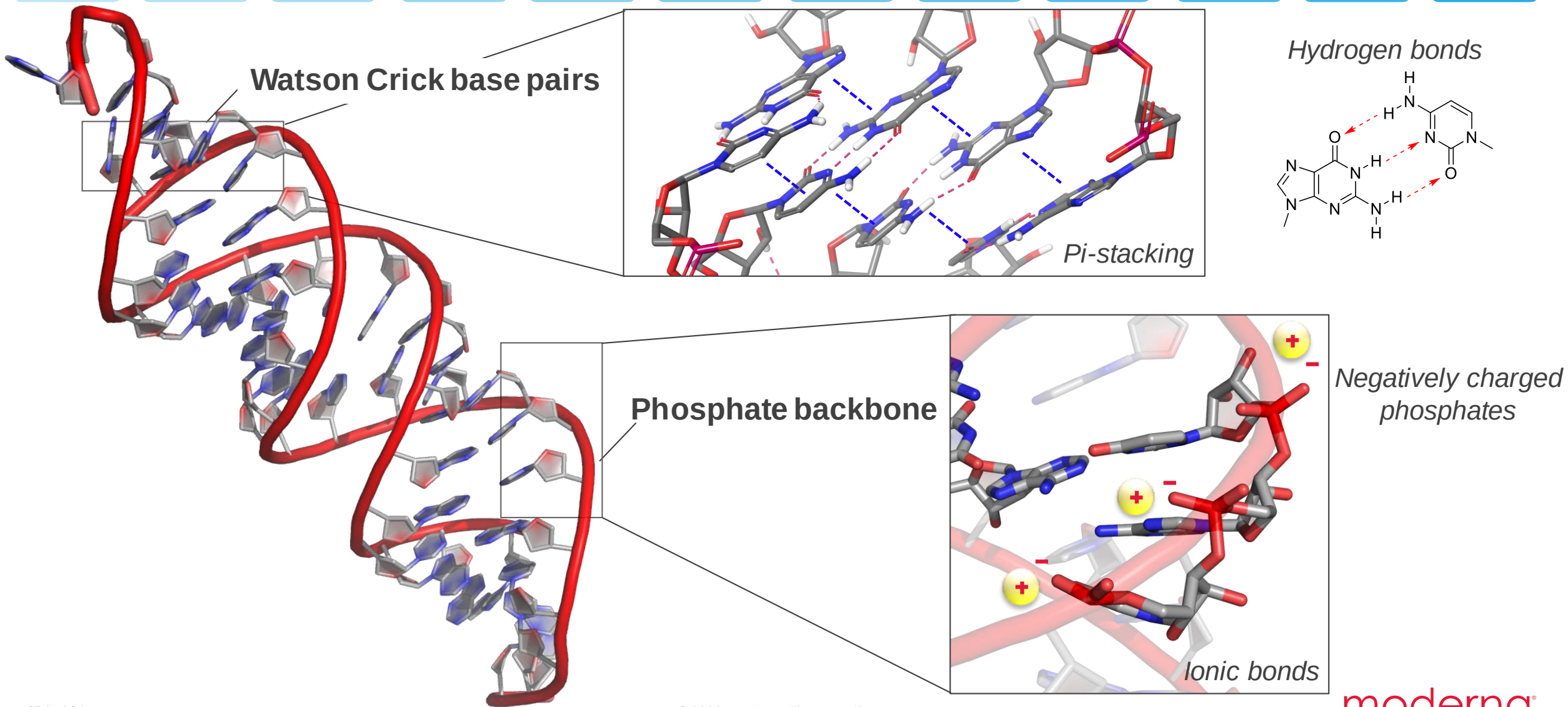


- ✓ Good encapsulation of proteins
- ✗ Poor encapsulation of DNA



Electron micrograph of protein containing liposome

Double-stranded oligonucleotides have a negatively charged backbone



Evolution to the modern day lipid nanoparticle identified the importance of ionizable lipids

Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 7413-7417, November 1987
Biochemistry

Lipofection: A highly efficient, lipid-mediated DNA-transfection procedure

(liposomes/cationic lipid vesicles/gene transfer)

PHILIP L. FELGNER*†, THOMAS R. GADEK*, MARILYN HOLM*, RICHARD ROMAN*, HARDY W. CHAN*,
MICHAEL WENZ*§, JEFFREY P. NORTHROP§, GORDON M. RINGOLD§, AND MARK DANIELSEN†

*Institute of Bio-Organic Chemistry and †Cancer and Developmental Biology, Syntex Research, 3401 Hillview Avenue, Palo Alto, CA 94303; and ‡Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305

Communicated by Avram Goldstein, June 24, 1987 (received for review April 23, 1987)

ABSTRACT A DNA-transfection protocol has been developed that makes use of a synthetic cationic lipid, *N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium chloride (DOTMA). Small unilamellar liposomes containing DOTMA interact spontaneously with DNA to form lipid-DNA complexes with 100% entrapment of the DNA. DOTMA facilitates fusion of the complex with the plasma membrane of tissue culture cells, resulting in both uptake and expression of the DNA. The technique is simple, highly reproducible, and effective for both transient and stable expression of transfected DNA. Depending upon the cell line, lipofection is from 5- to >100-fold more effective than either the calcium phosphate or the DEAE-dextran transfection technique.

Under appropriate conditions, eukaryotic cells can take up exogenous DNA, and a portion of this DNA becomes localized in the nucleus. The phenomenon has been exploited to obtain both transient and stable expression of various genes (1-11). However, due in part to the size and charge of DNA and to the multitude of enzymatic and membrane barriers imposed by the cell, the spontaneous expression in the DNA into the cell and its subsequent expression in the nucleus is a very inefficient process. For this reason, a wide variety of methods have been developed to facilitate this process. These methods include the use of polycations (1-3), calcium phosphate (4, 5), liposome fusion (6), retroviruses (7), microinjection (8), electroporation (9), and protoplast fusion (10). However, all of these methods suffer from one or more problems related to either cellular toxicity, poor reproducibility, inconvenience, or inefficiency of DNA delivery. We have synthesized a cationic lipid that forms liposomes with DNA and here that these liposomes interact with DNA

L-M(TK⁻) cells (ATCC CCL 1.3) are derived from murine L cells and are thymidine kinase-deficient (16). The TA1 cell line is derived from the murine fibroblast cell line 10T½ (17). All cells were grown on plastic tissue culture plates in Dulbecco's modified Eagle's medium with 10% fetal bovine serum in a 10% CO₂, 37°C incubator, except TA1 cells, which were grown in basal minimal essential medium with 10% fetal bovine serum in a 5% CO₂, 37°C incubator.

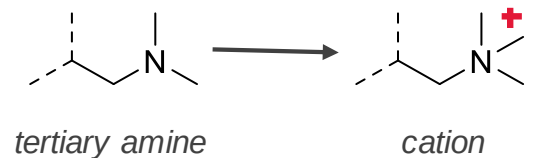
DOTMA Synthesis and Liposome Preparation. DOTMA {*N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium chloride} was prepared as outlined in Fig. 1. A mixture of 3-(dimethylamino)-1,2-propanediol (Aldrich; 1.19 g, 10 mmol), potassium *tert*-butoxide (3.36 g, 30 mmol), and oleyl *p*-toluenesulfonate (12.7 g, 30 mmol) in xylenes (50 ml; 1 torr = 133 Pa) for 30 min and then was heated to 50°C with stirring at room temperature and reduced pressure (30 torr) was stirred for an additional 15 min. The reaction mixture was purged with nitrogen, and the mixture was heated to reflux (~140°C) for 3 hr. After cooling, the resulting solution was diluted with hexane (100 ml), and the organic layer extracted with water (twice, 50 ml each). The organic layer was concentrated, and the residue was chromatographed over silica gel by elution with a mixture of hexanes and diethyl ether (1:2) to afford the intermediate 2,3-dioleoyloxy-1-(dimethylamino)propane as a colorless oil. Quaternization was carried out by condensation of methyl chloride (50 ml) into a Parr pressure apparatus cooled to -78°C and containing this compound (10 g). The sealed vessel was heated behind a safety shield at 70°C for 48 hr. After cooling to 0°C, the reaction vessel was opened, and the excess nitrogen was allowed to evaporate under a stream of nitrogen. The crude residue was recrystallized in a well-ventilated hood. The crude residue was recrystallized from acetonitrile to afford DOTMA as an off-white solid, mp 35-38°C. Full details of the synthesis of DOTMA and analogs will be published elsewhere (T.R.G. and P.L.F.).

Cationic lipids

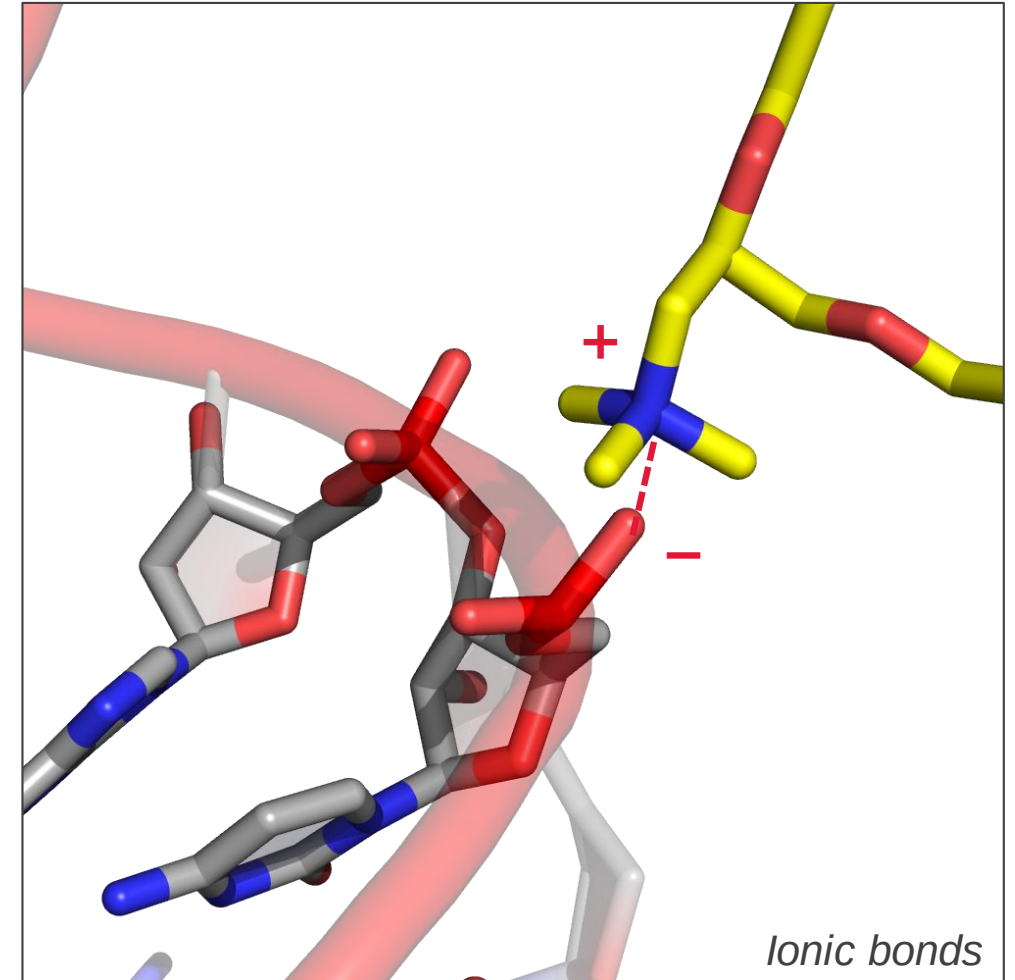
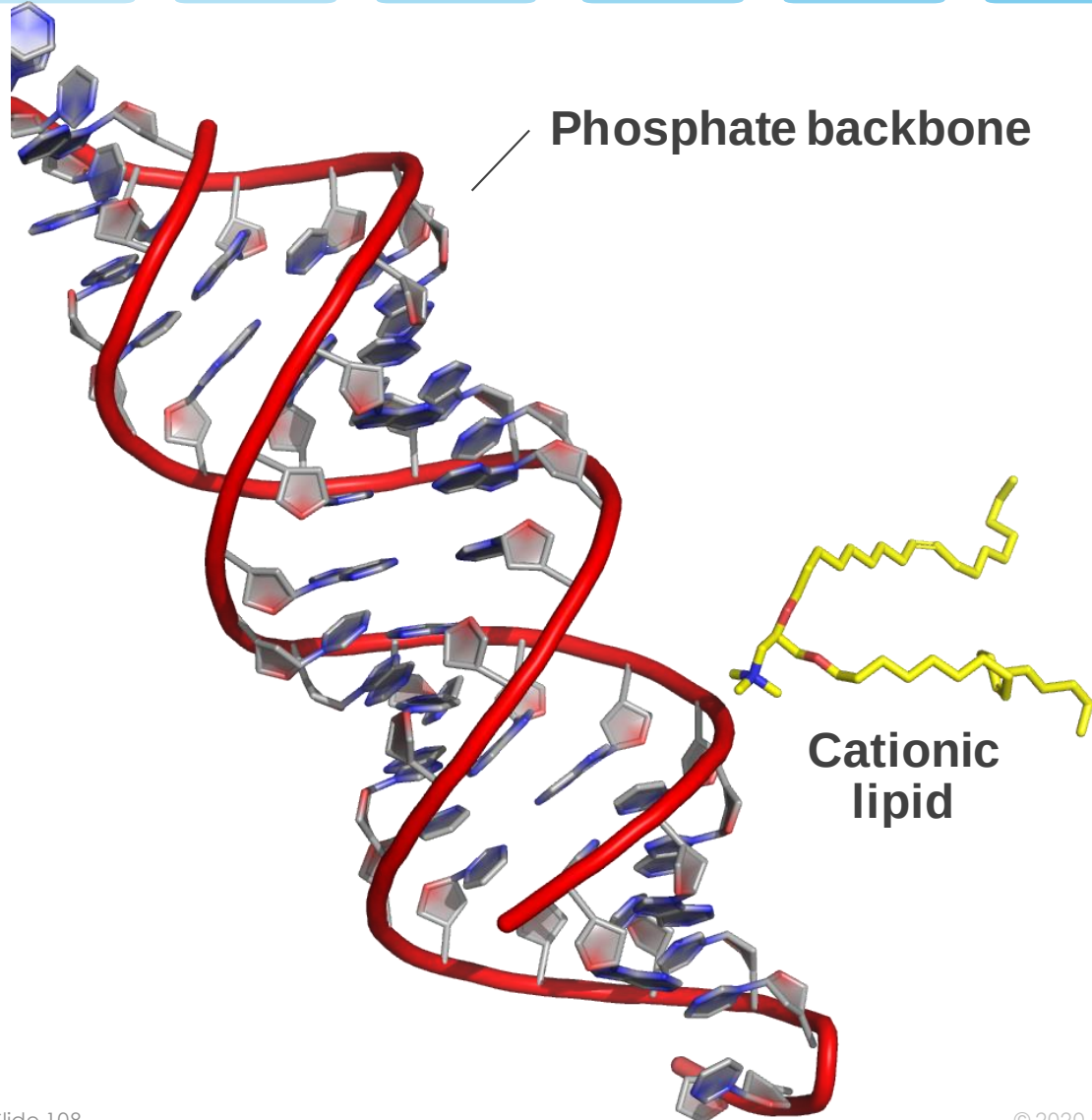
PNAS, 1987, 84, 7413



✓ Good encapsulation of DNA



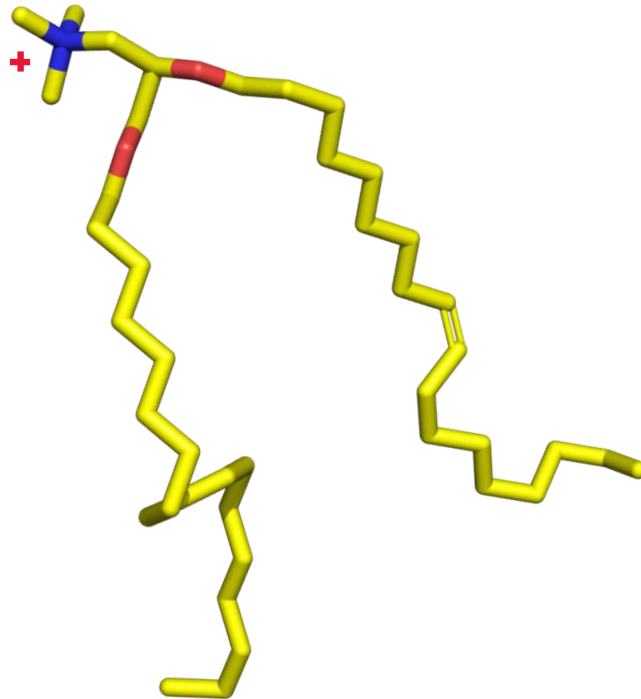
Cationic lipids interact with the negatively charged backbone of double stranded oligonucleotides



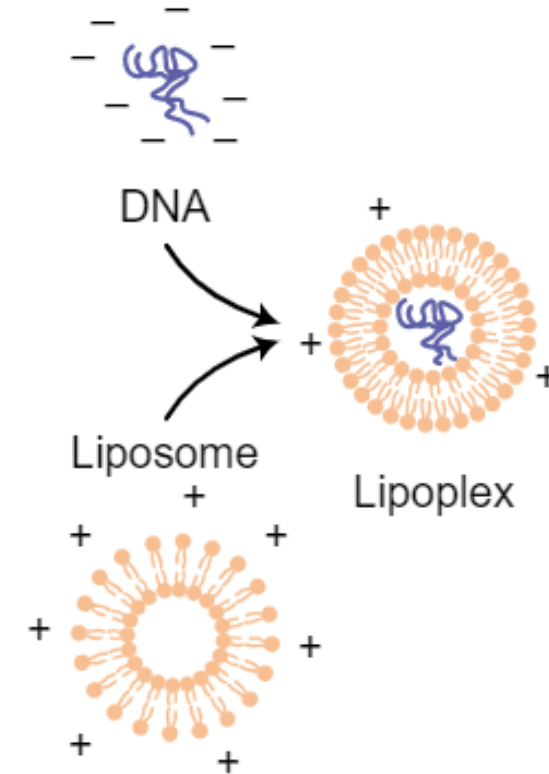
Evolution to the modern day lipid nanoparticle identified the importance of ionizable lipids

Cationic lipids

PNAS, 1987, 84, 7413



- ✓ Good encapsulation of DNA
- ✓ Good *in vitro* delivery efficiency
- ✓ Poor *in vivo* systemic delivery



Evolution to the modern day lipid nanoparticle identified the importance of ionizable lipids

Pharmaceutical Research, Vol. 22, No. 3, March 2005 (© 2005)
DOI: 10.1007/s11095-004-1873-z

Research Paper

A Scalable, Extrusion-Free Method for Efficient Liposomal Encapsulation of Plasmid DNA

Lloyd B. Jeffs,¹ Lorne R. Palmer,¹ Ellen G. Ambegia,¹ Cory Giesbrecht,¹ Shannon Ewanick,¹ and Ian MacLachlan^{1,2}

Received September 17, 2004; accepted December 6, 2004

Purpose. A fully scalable and extrusion-free method was developed to prepare rapidly and reproducibly stabilized plasmid lipid particles (SPLP) for nonviral, systemic gene therapy.
Methods. Liposomes encapsulating plasmid DNA were formed instantaneously by mixing lipids dissolved in ethanol with an aqueous solution of DNA in a controlled, stepwise manner. Combining DNA-buffer and lipid-ethanol flow streams in a T-shaped mixing chamber resulted in instantaneous dilution of ethanol below the concentration required to support lipid solubility. The resulting DNA-containing liposomes were further stabilized by a second stepwise dilution.
Results. Using this method, monodisperse vesicles were prepared with particle sizes less than 200 nm and DNA encapsulation efficiencies greater than 80%. In mice possessing Neuro 2a tumors, SPLP demonstrated a 13 h circulation half-life *in vivo*, good tumor accumulation and gene expression profiles similar to SPLP previously prepared by detergent dialysis. Cryo transmission electron microscopy analysis showed that SPLP prepared by stepwise ethanol dilution were a mixed population of unilamellar, bilamellar, and oligolamellar vesicles. Vesicles of similar lipid composition, prepared without DNA, were also <200 nm but were predominantly bilamellar with unusual elongated morphologies, suggesting that the plasmid particle affects the morphology of the encapsulating liposome. A similar approach was used to prepare neutral egg phosphatidylcholine:cholesterol (EPC:Chol) liposomes possessing a pH gradient, which was confirmed by the uptake of the lipophilic cation safranin O.
Conclusions. This new method will enable the scale-up and manufacture of SPLP required for pre-clinical and clinical studies. Additionally, this method now allows for the acceleration of SPLP formulation development, enabling the rapid development and evaluation of novel carrier systems.

KEY WORDS: DNA; liposome; plasmid; systemic gene delivery.

INTRODUCTION

Systemic delivery of therapeutic genes to disease sites requires a vector with suitable properties. An ideal systemic vector should have the appropriate physical attributes to ensure favorable pharmacokinetics and delivery to disease sites.

Following delivery to the target tissue, the vector must then be taken up by the tissue and express its DNA payload at sufficient levels to give a therapeutic effect. Importantly, a systemic vector must also be safe, well tolerated upon administration, and be non-immunogenic. The utility of viral vectors, cationic lipid-containing DNA complexes (lipoplexes), and polycationic polymer nucleic acid complexes (polyplexes) is limited in a systemic context (1).

Liposomes encapsulating small-molecule drugs such as doxorubicin (2) and vincristine (3) have been shown to be effective in humans. Liposomes can increase drug accumulation at the tumor site by 50 to 100 times compared to the administration of free drug (4,5). This is the result of the

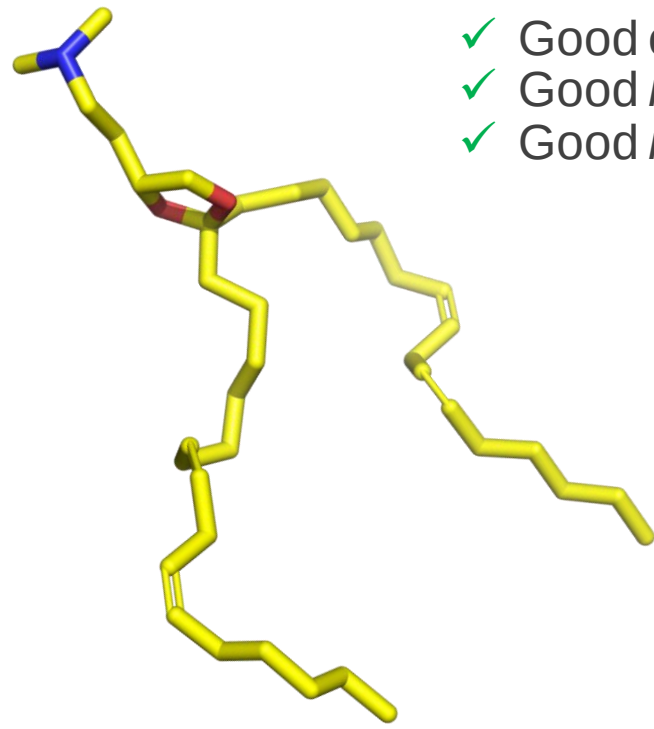
¹ Protiva Biotherapeutics Inc., Burnaby, British Columbia, Canada V5G 4Y1.

² To whom correspondence should be addressed. (e-mail: ian@protivabio.com)

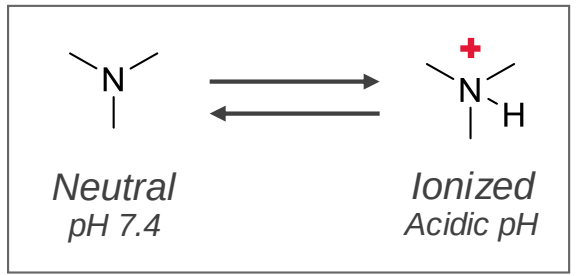
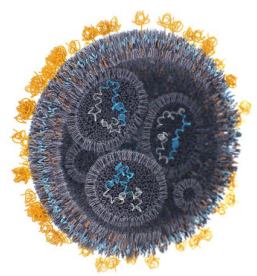
ABBREVIATIONS: Chol, cholesterol; DODAC, dioleoyldimethylammonium chloride; DOPAC, 1,3-bis(sn-3'-phosphatidyl)-2-sn-glycero-3-phosphocholine; EPC, egg phosphatidylcholine; EPC:Chol, egg phosphatidylcholine:cholesterol; SPLP, stabilized plasmid lipid particles.

Ionizable lipids

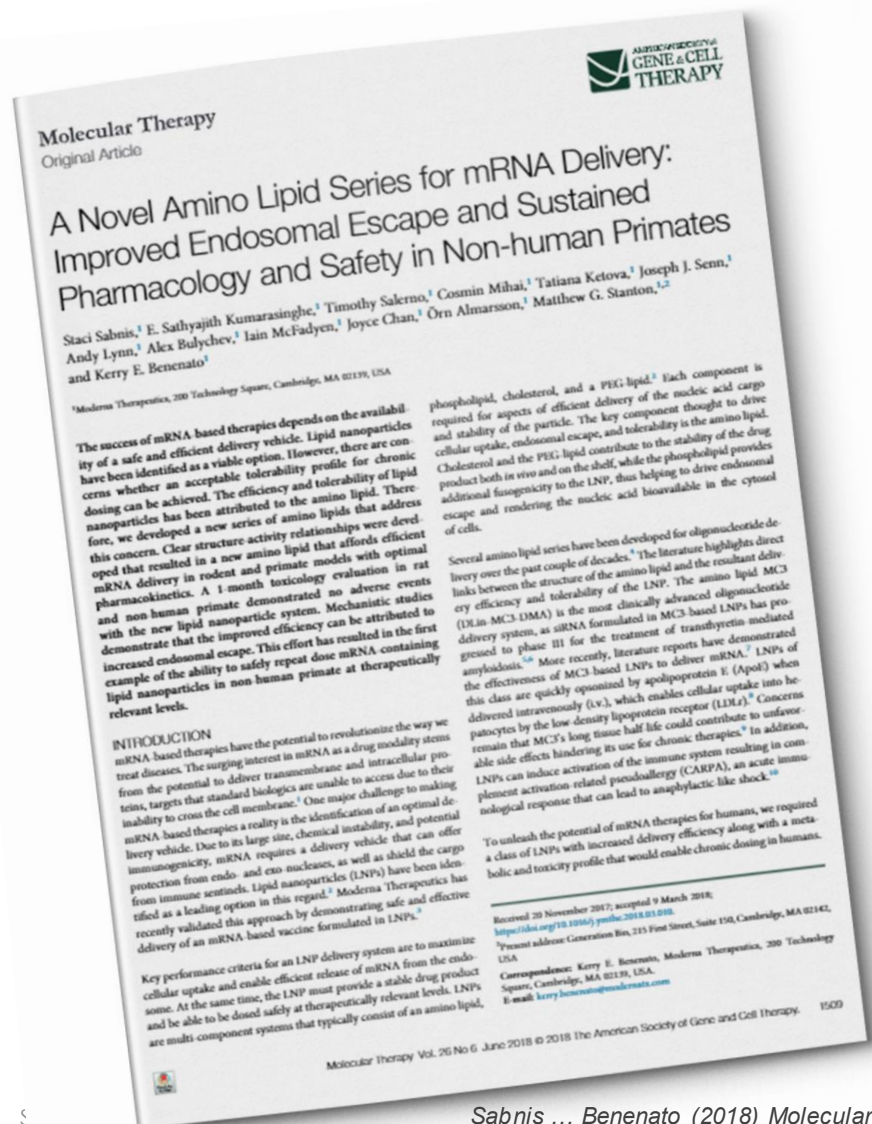
Pharmaceutical Res., 2005, 22, 362



- ✓ Good encapsulation of DNA
- ✓ Good *in vitro* delivery efficiency
- ✓ Good *in vivo* systemic delivery

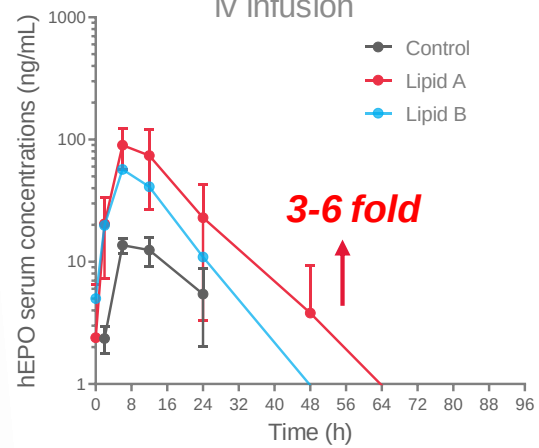


Moderna has invested in development of ionizable lipids for delivery of mRNA



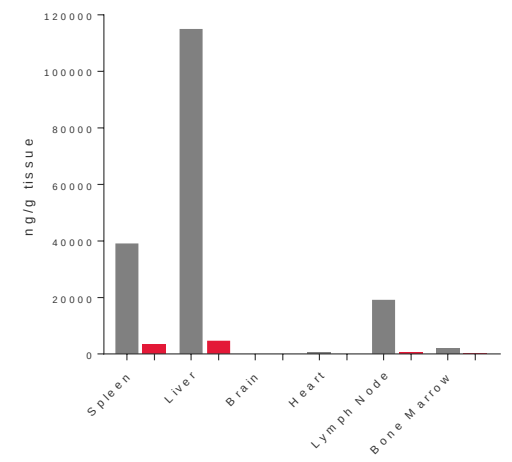
efficiency

hEPO mRNA, 0.01 mg/kg
iv infusion

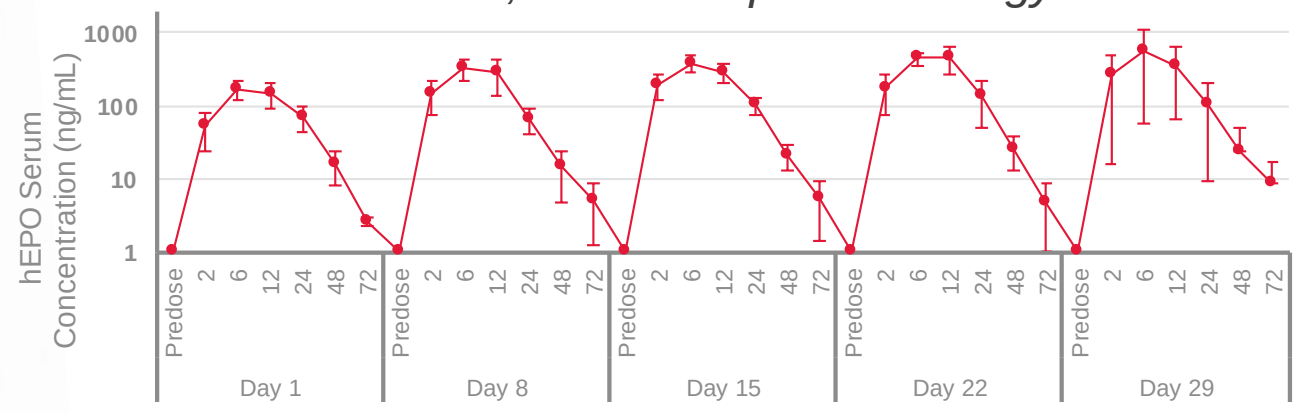


lipid clearance

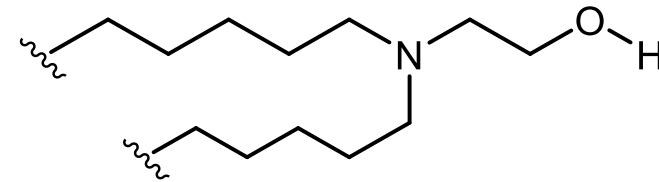
Tissue lipid concentrations, 12 h



safe, sustained pharmacology

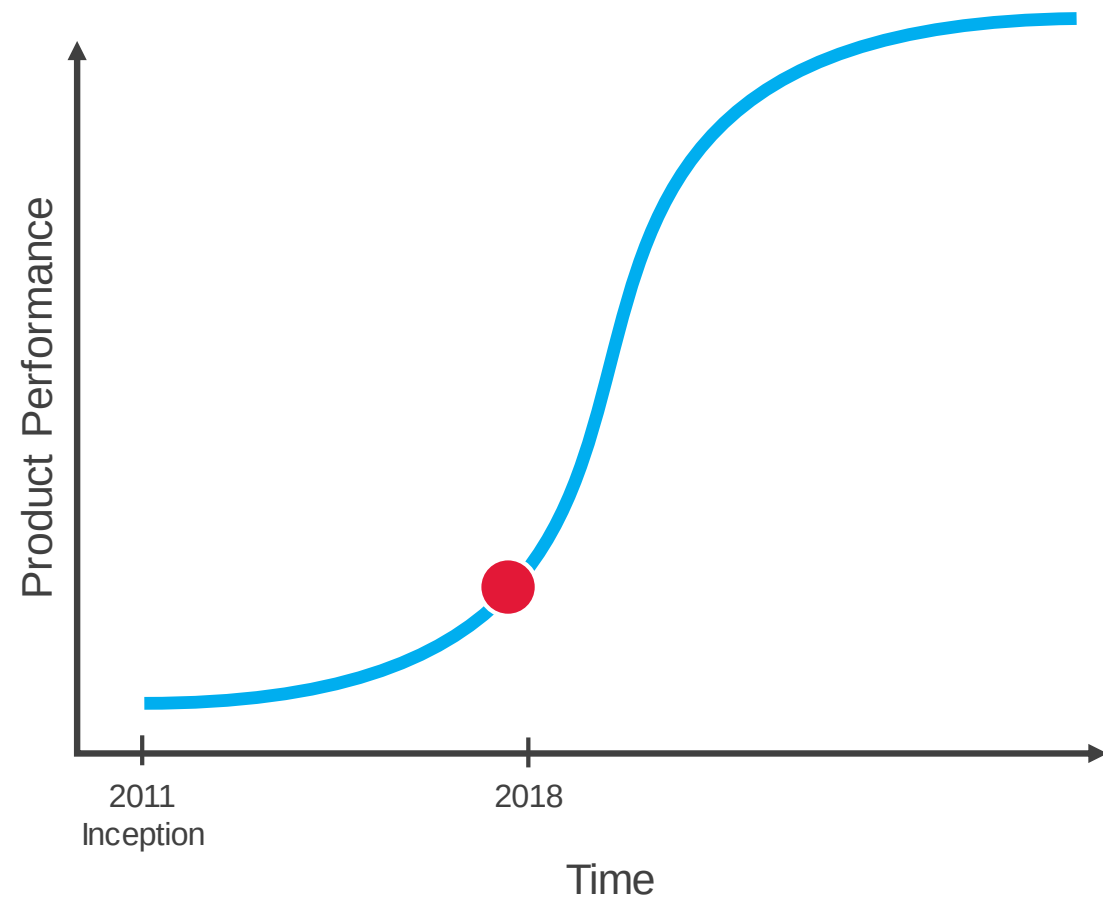


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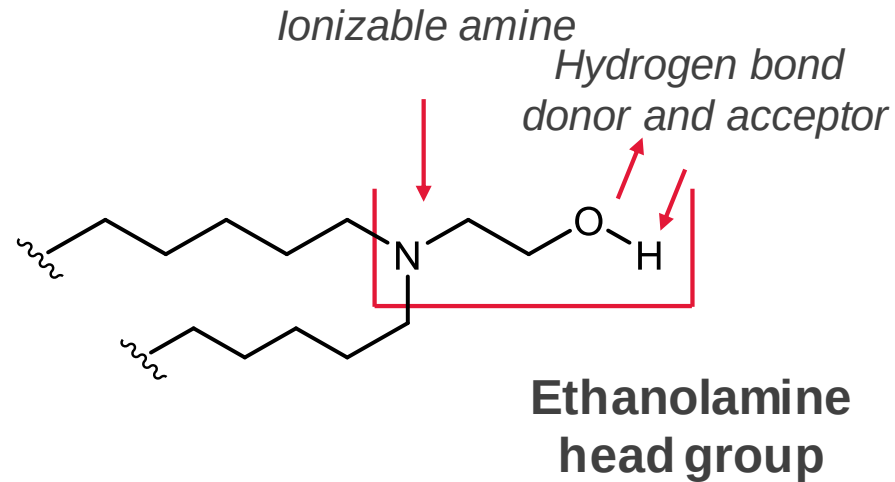


Moderna proprietary ionizable amino lipid

Our commitment to be the best at mRNA science is core to who we are: A 20-year journey

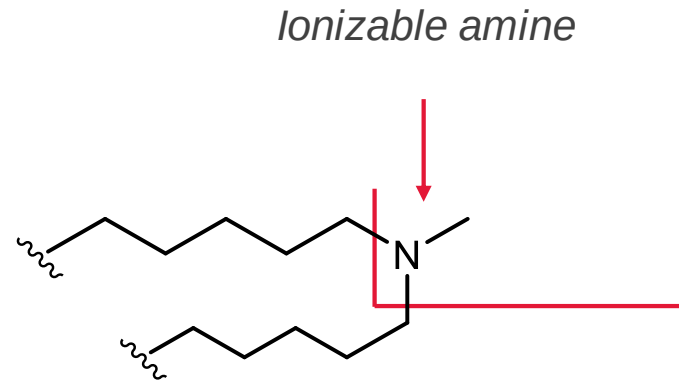


Moderna has invested in development of ionizable lipids for delivery of mRNA

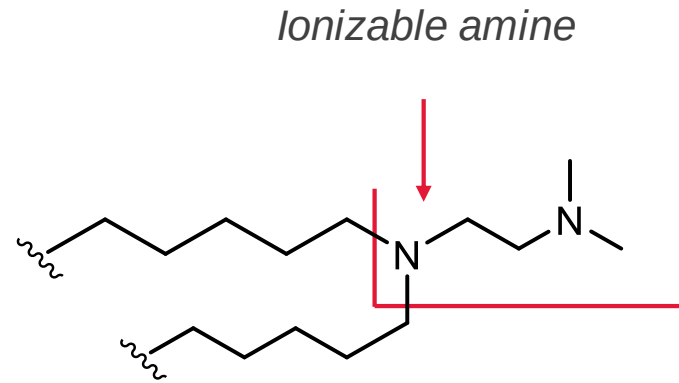


Moderna proprietary ionizable amino lipid

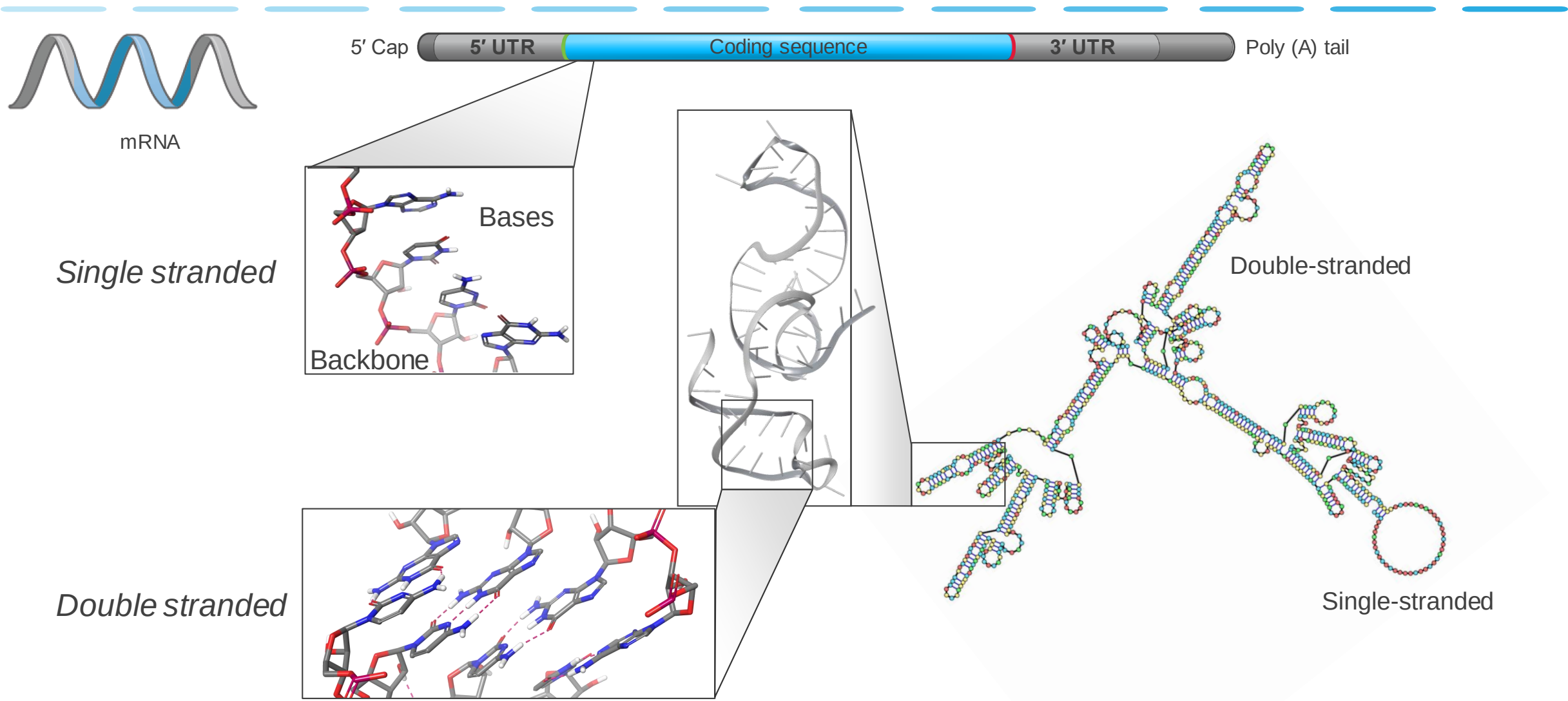
Moderna has invested in development of ionizable lipids for delivery of mRNA



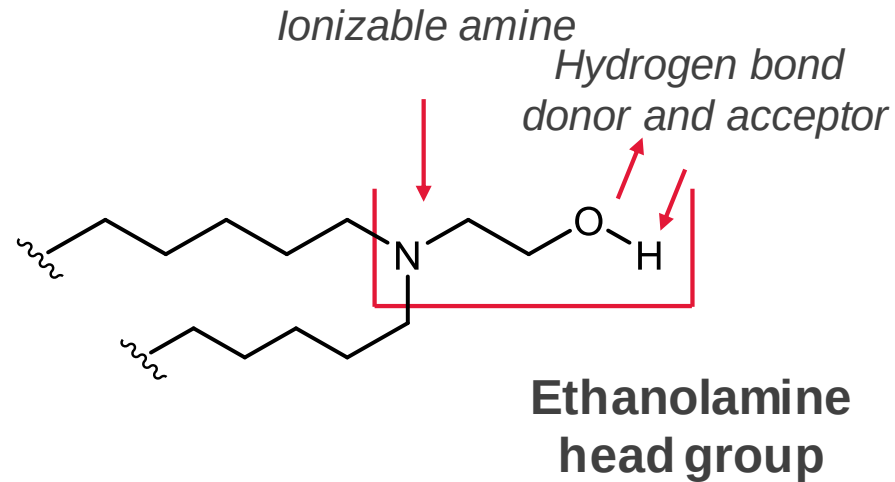
Moderna has invested in development of ionizable lipids for delivery of mRNA



But mRNAs are different: They have both single- and double-stranded regions



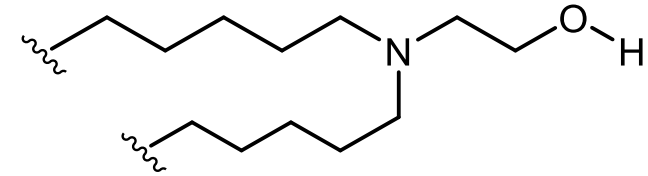
Moderna has invested in development of ionizable lipids for delivery of mRNA



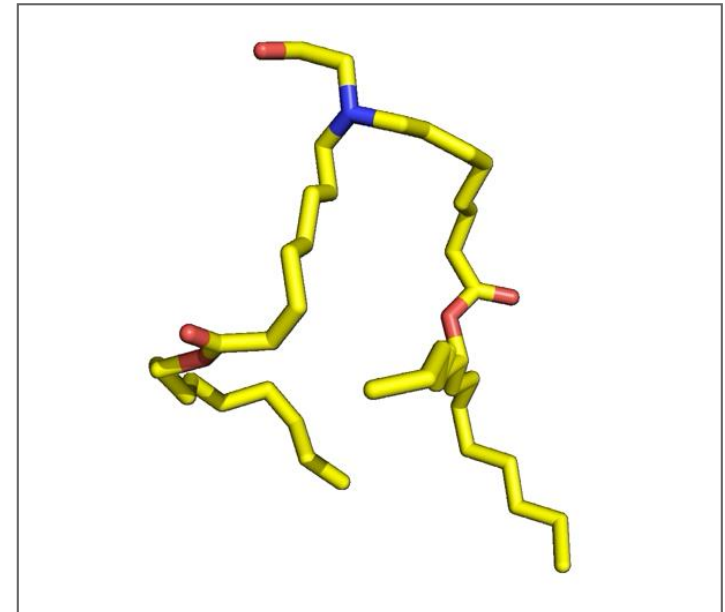
Molecular dynamic simulations allow us to gain insight into how our molecules behave



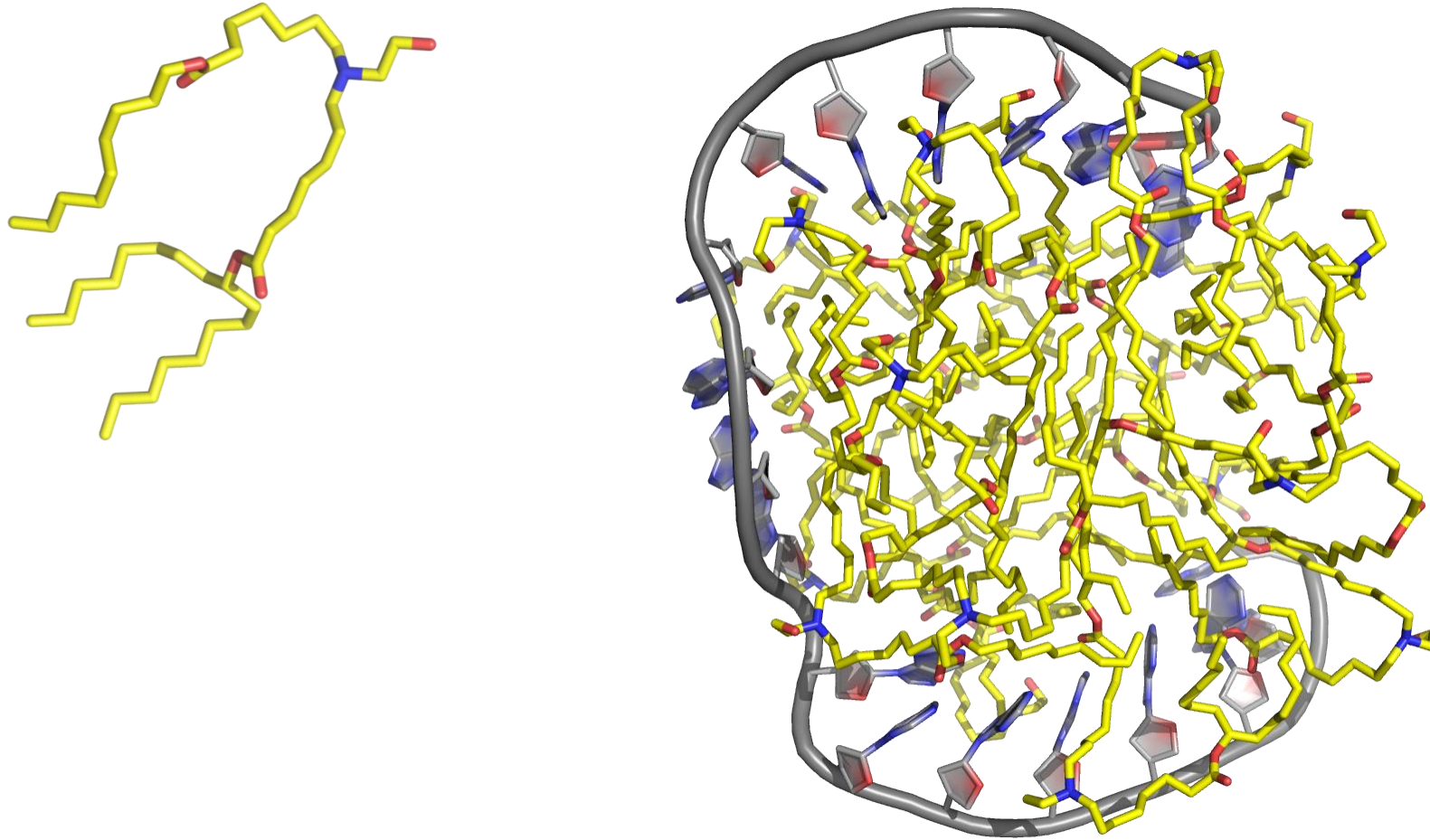
Chemical
Synthesis



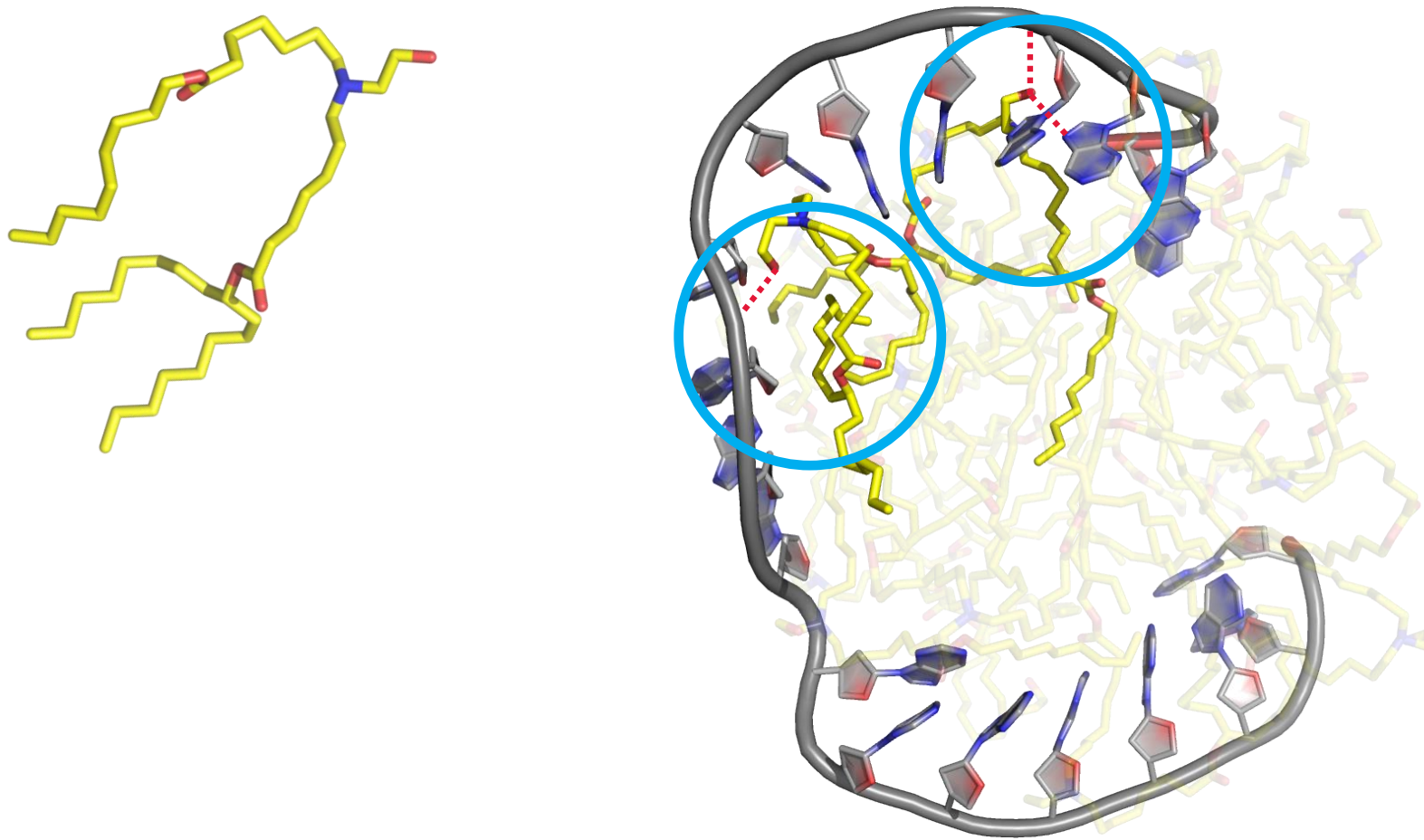
Molecular
Dynamics



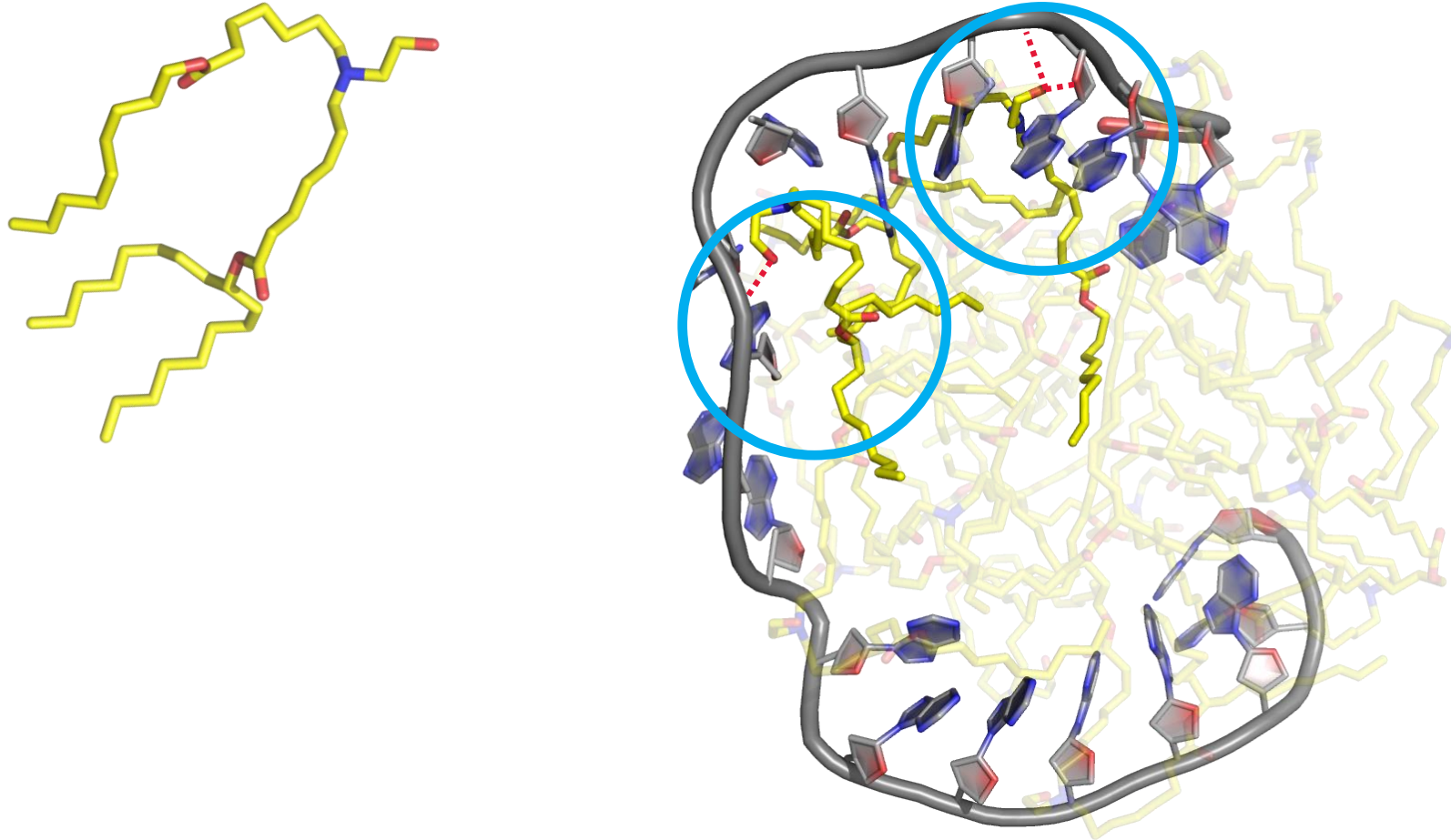
Molecular dynamics simulations indicate the ethanolamine engages in transient hydrogen bonds with the mRNA



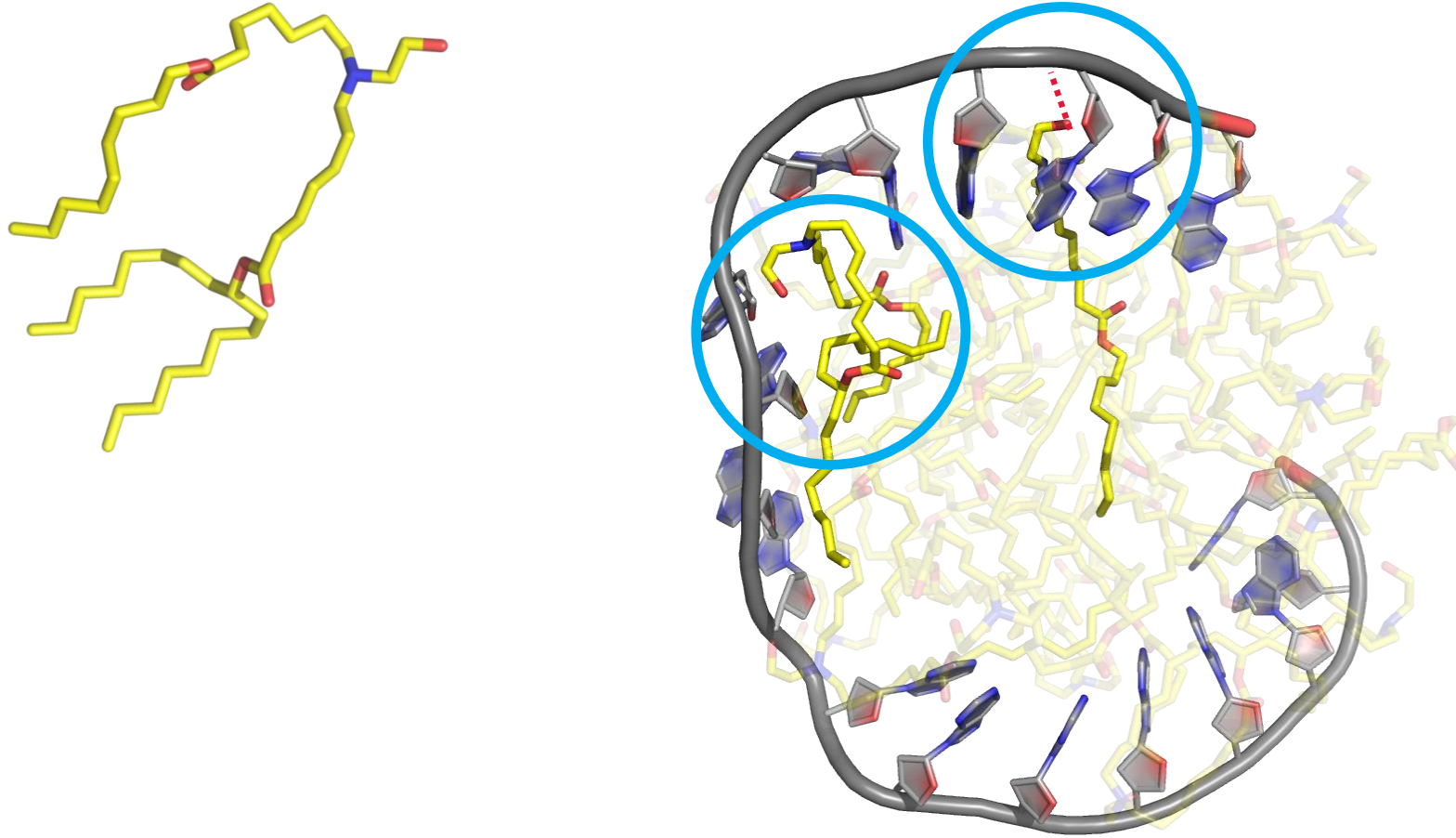
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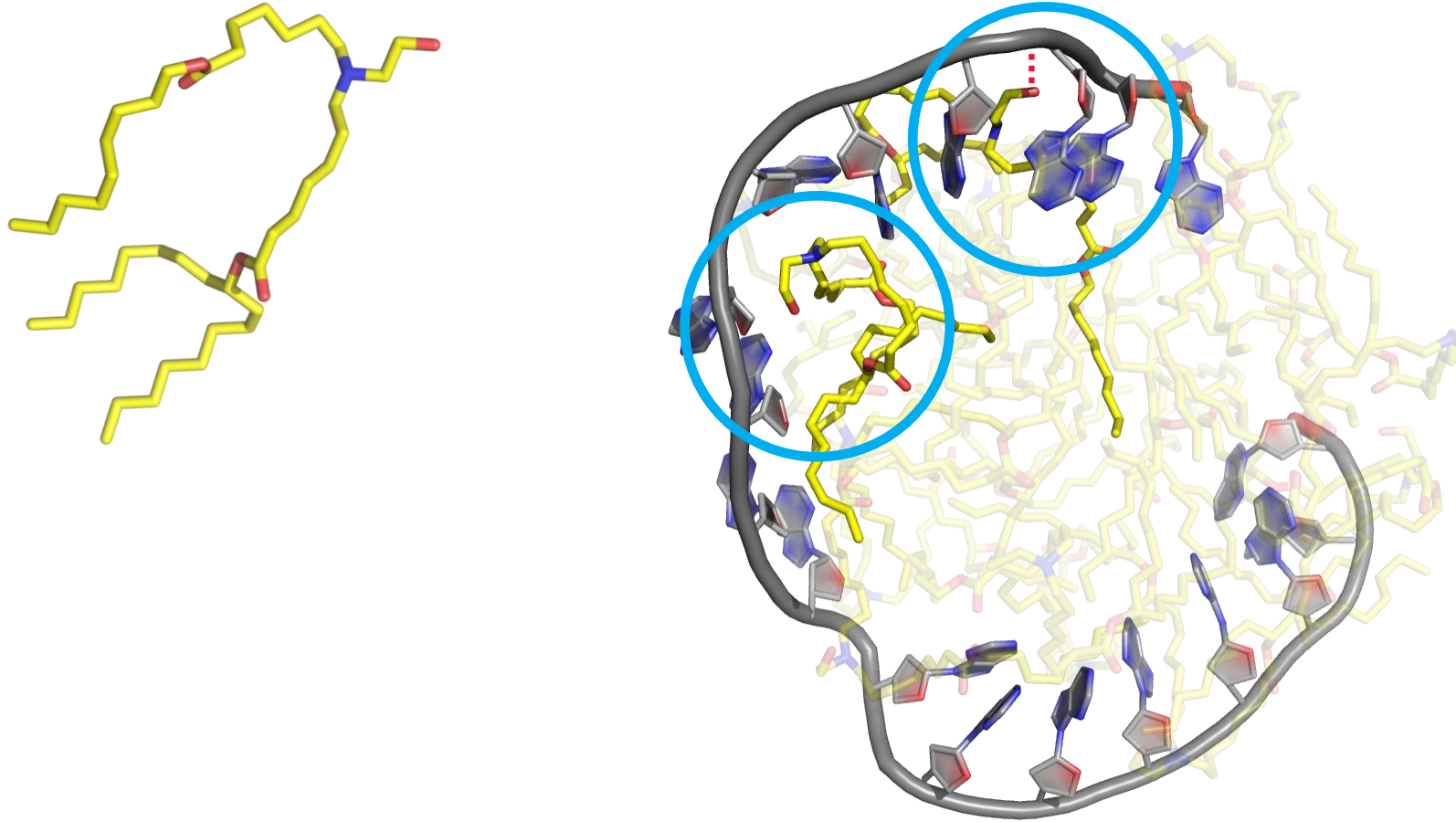
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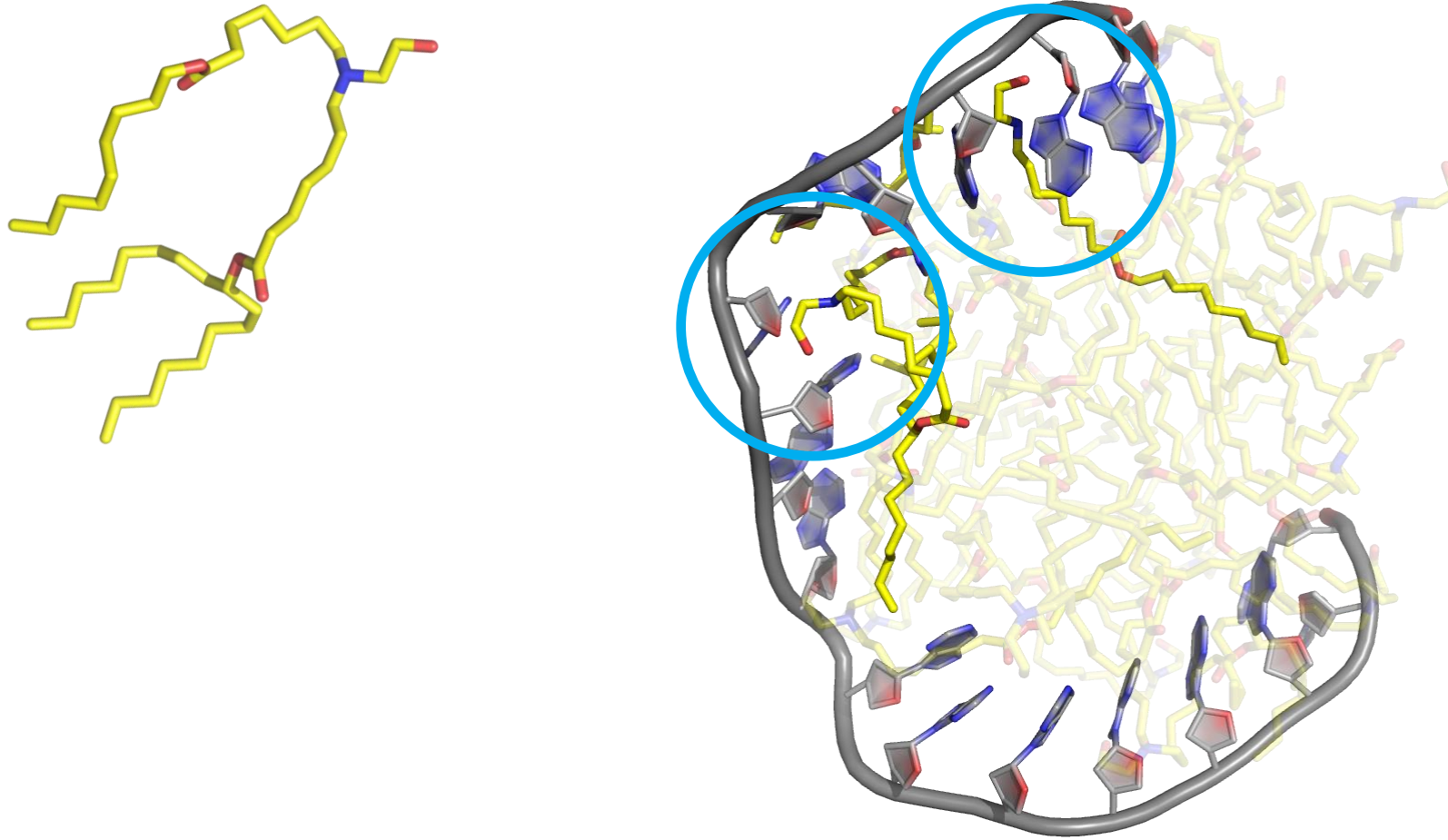
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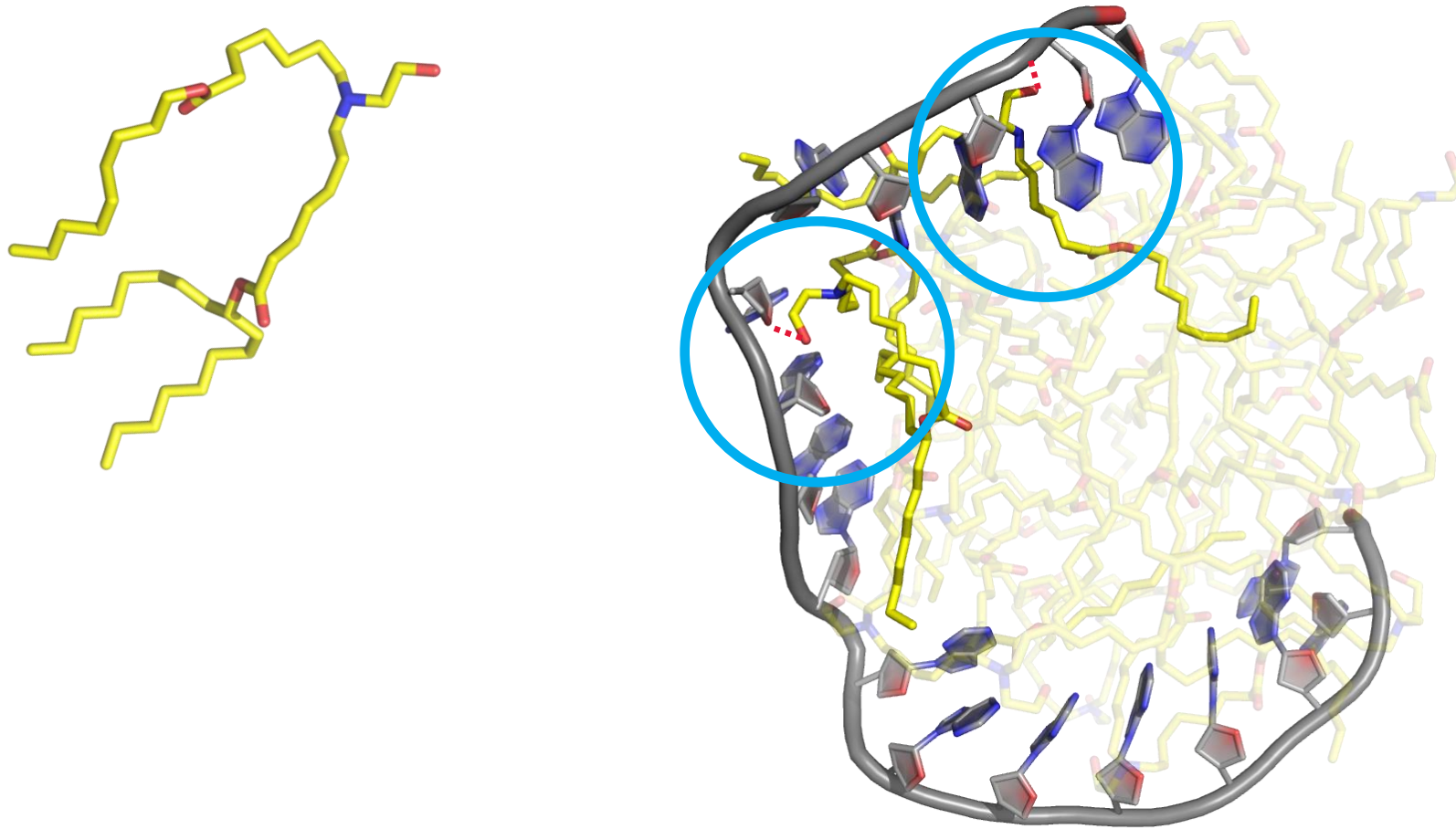
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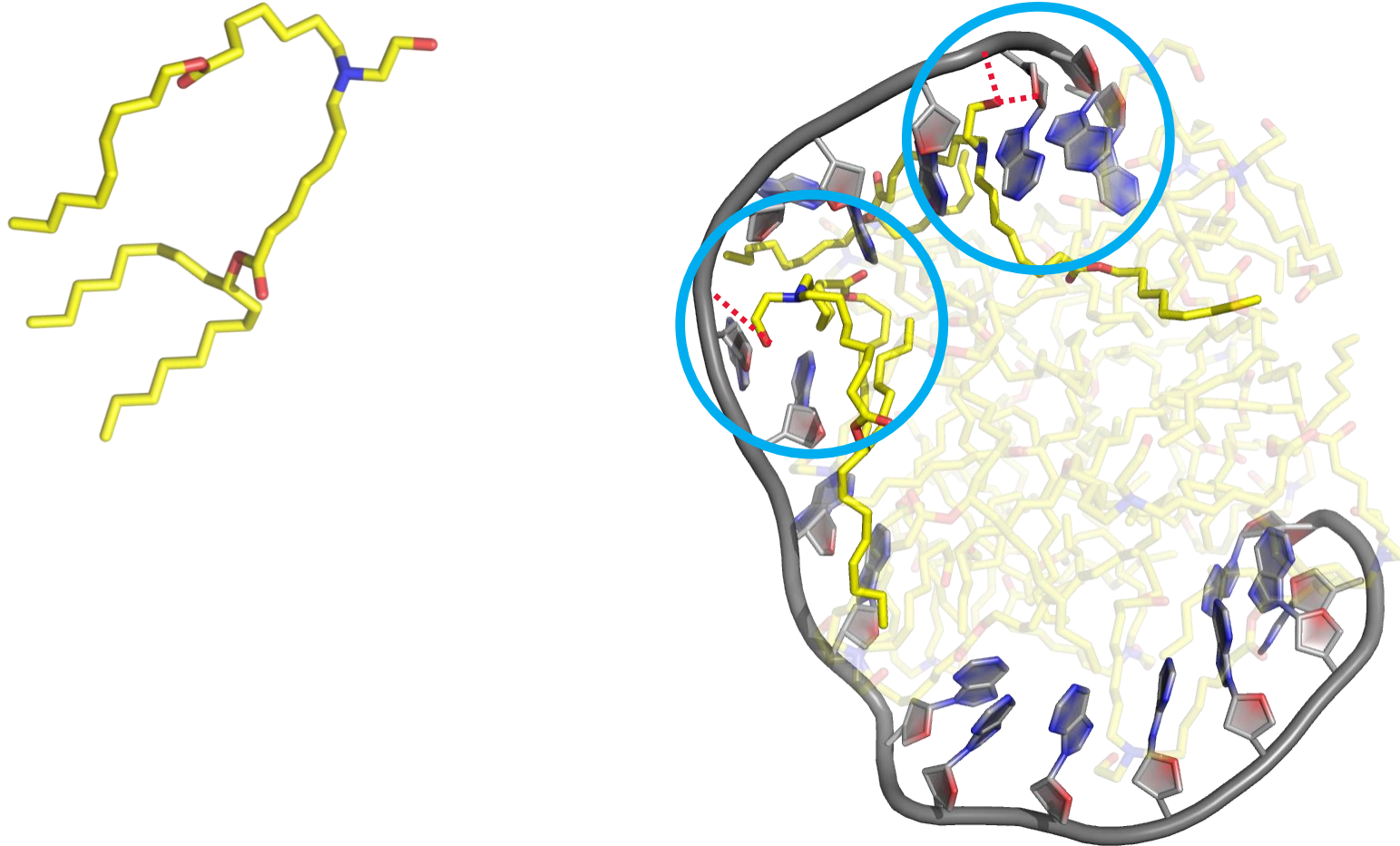
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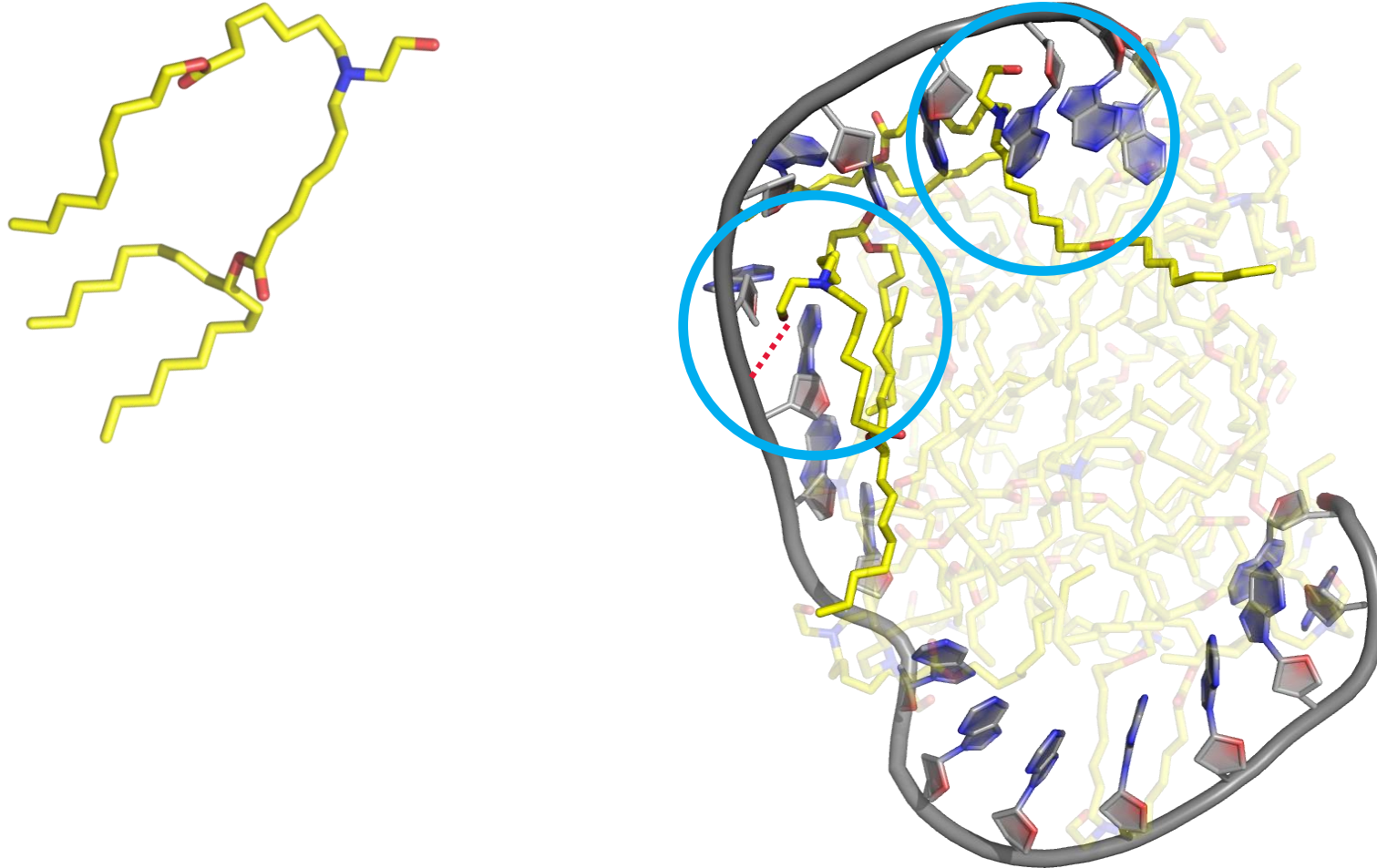
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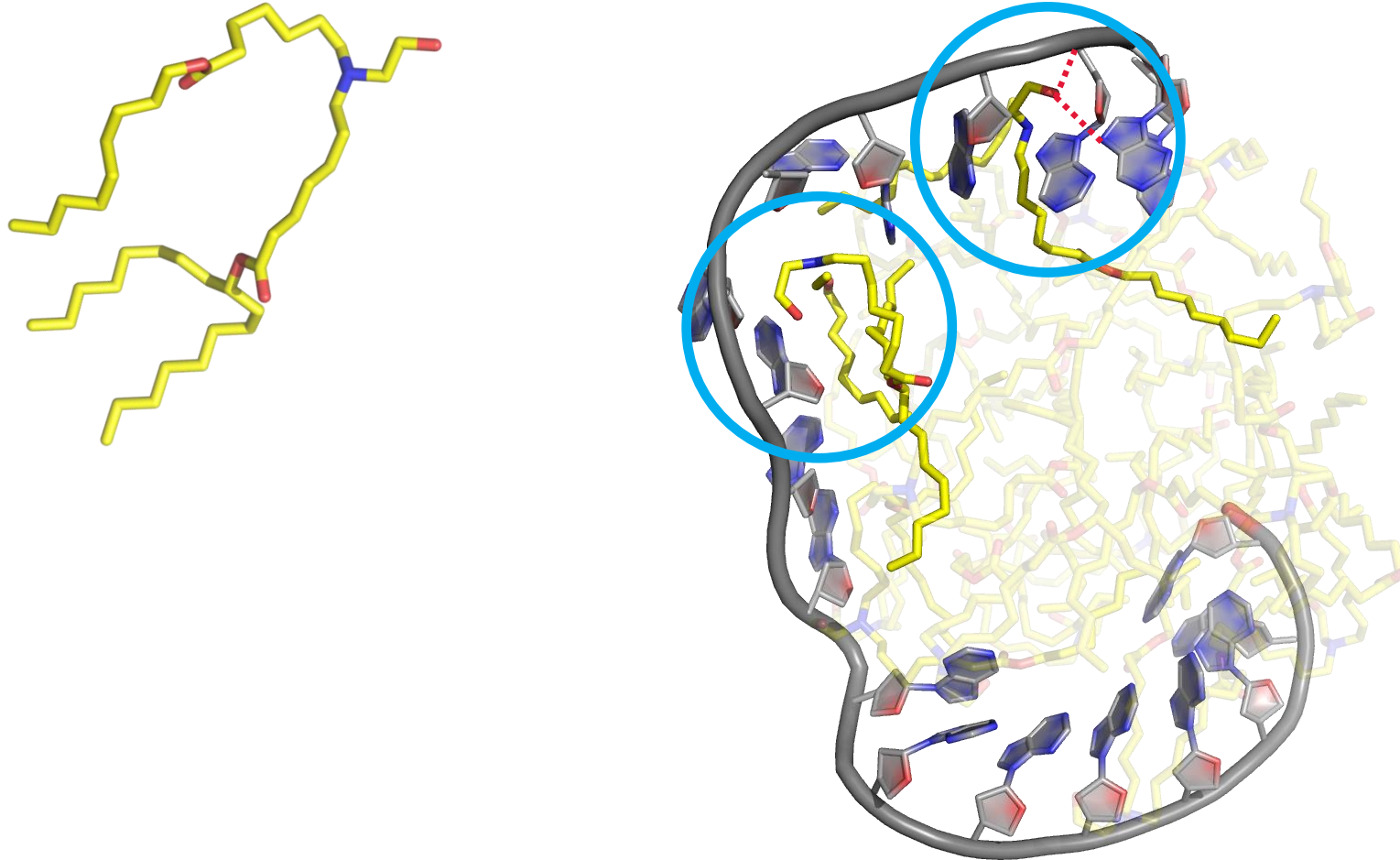
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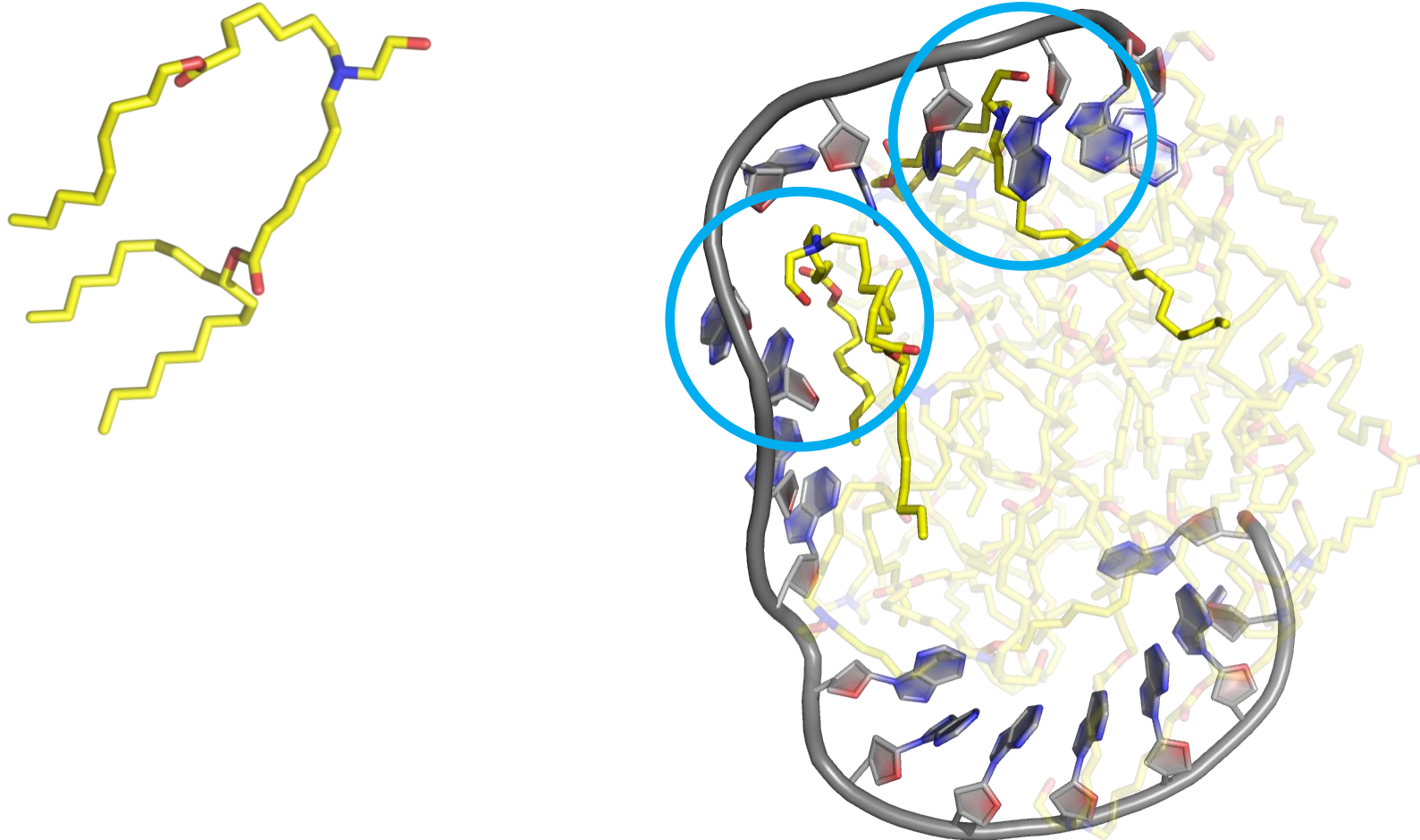
Molecular dynamics simulations indicate the ethanolamine engages in transient hydrogen bonds with the mRNA



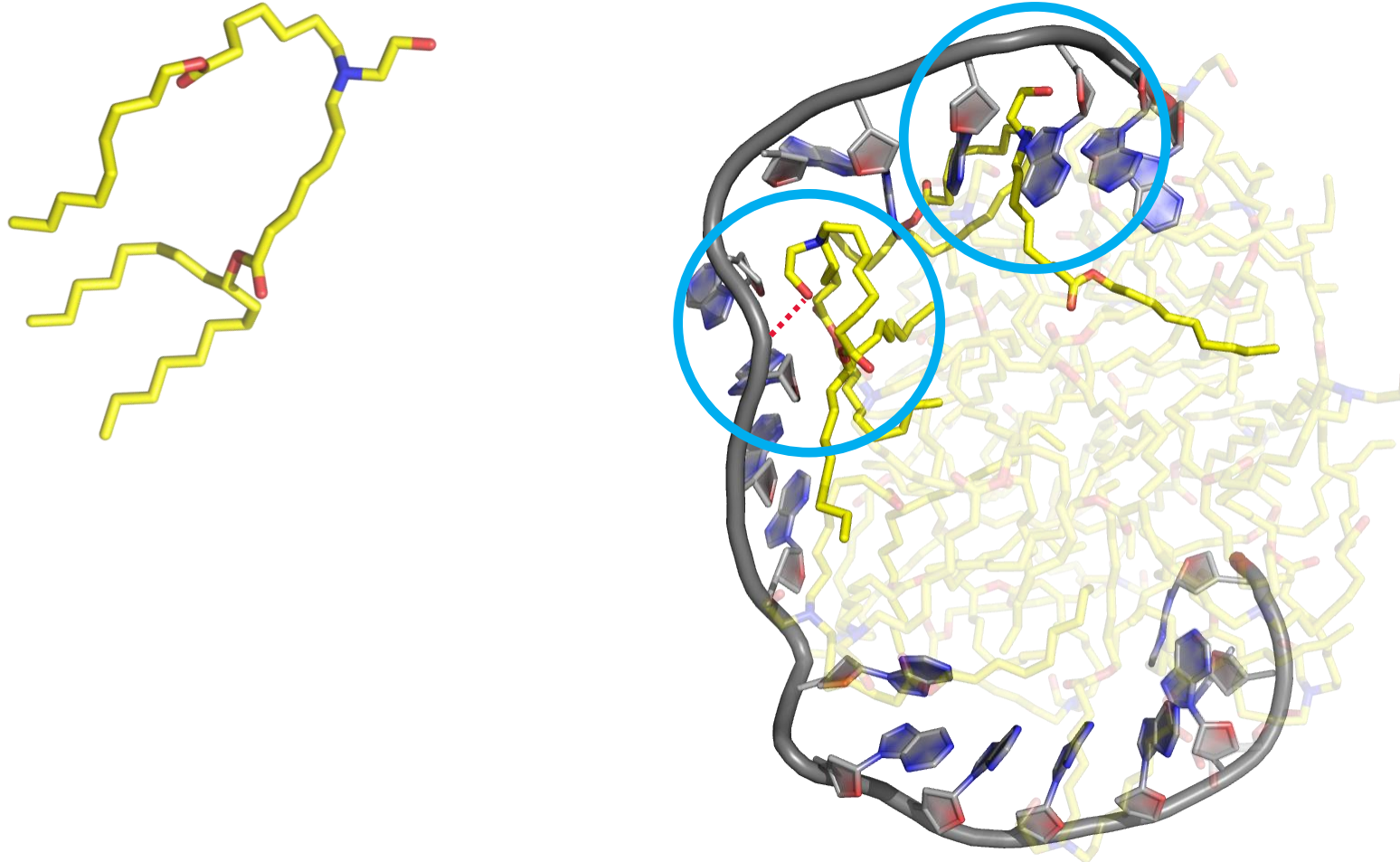
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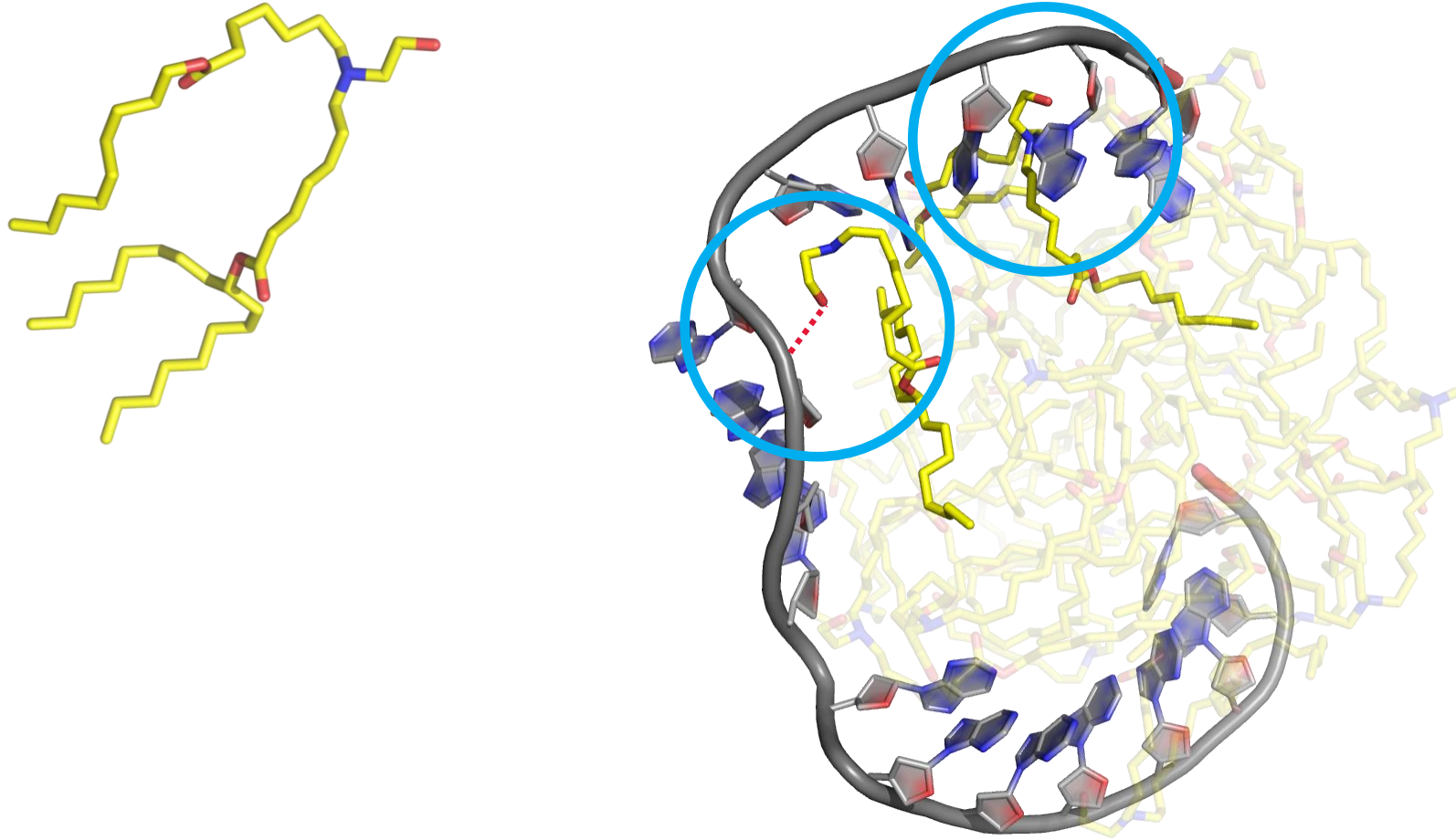
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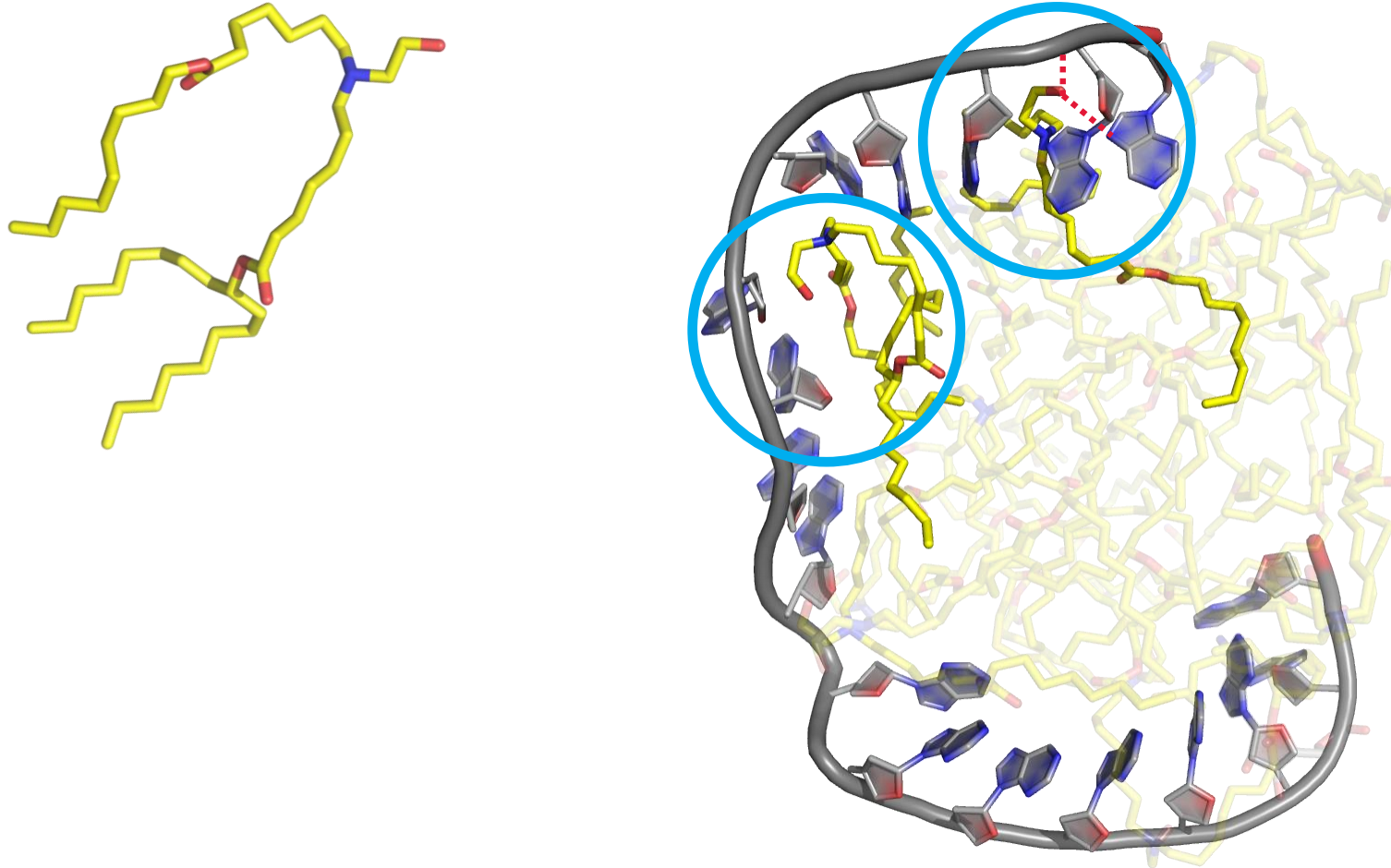
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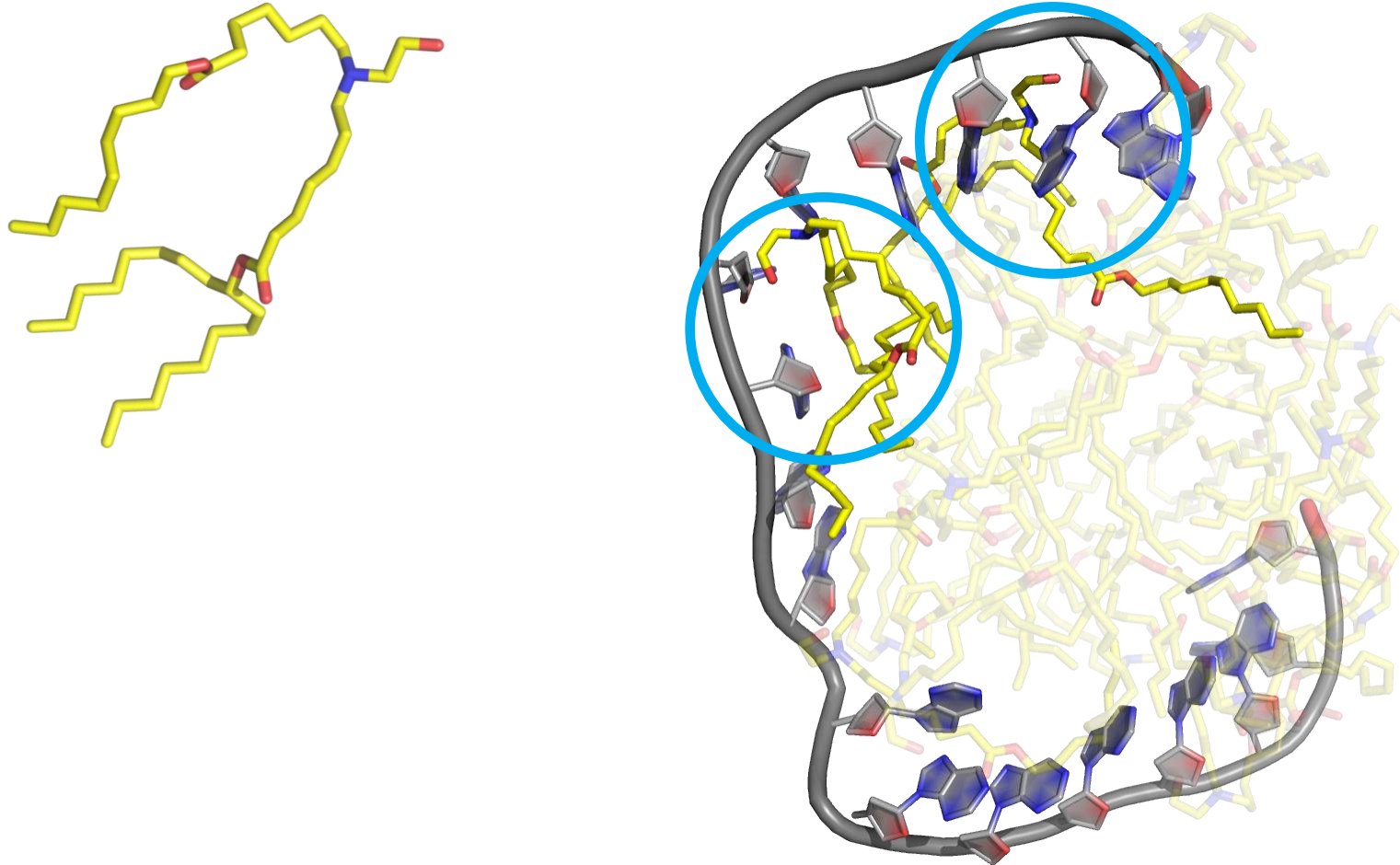
Molecular dynamics simulations indicate the ethanolamine engages in transient hydrogen bonds with the mRNA



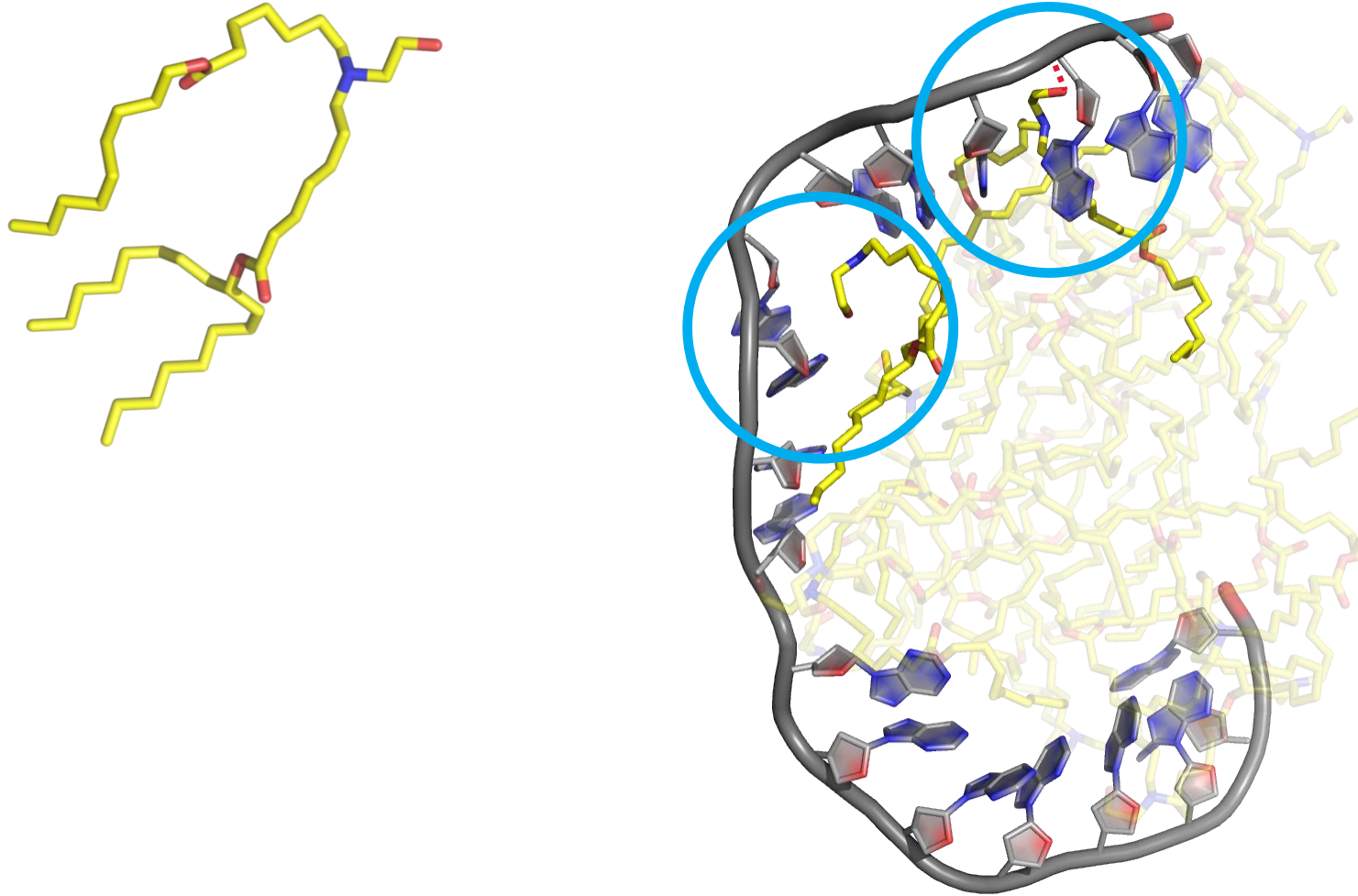
Molecular dynamics simulations indicate the ethanolamine engages in transient hydrogen bonds with the mRNA



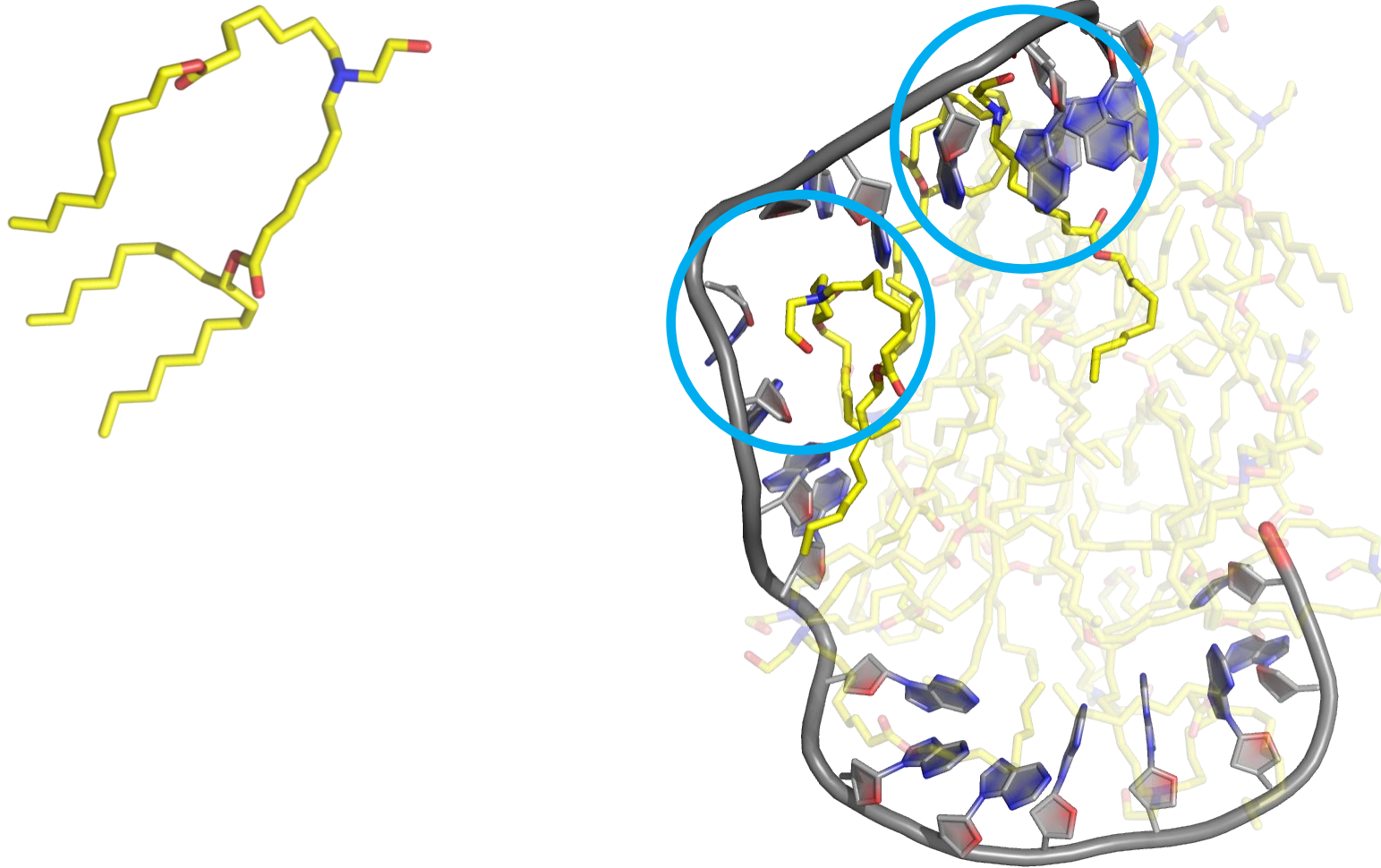
Molecular dynamics simulations indicate the ethanolamine engages in transient hydrogen bonds with the mRNA



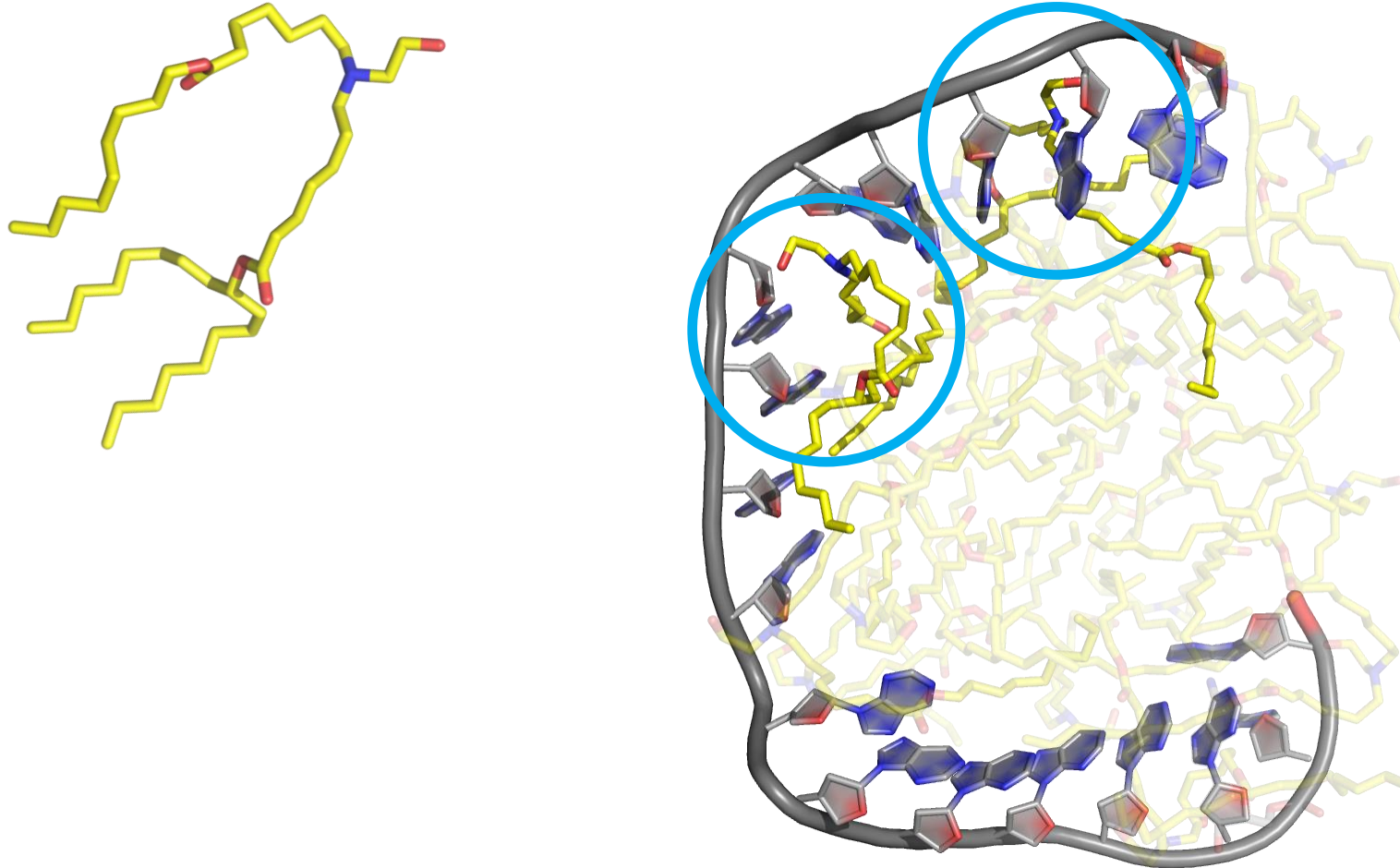
Molecular dynamics simulations indicate the ethanolamine engages in transient hydrogen bonds with the mRNA



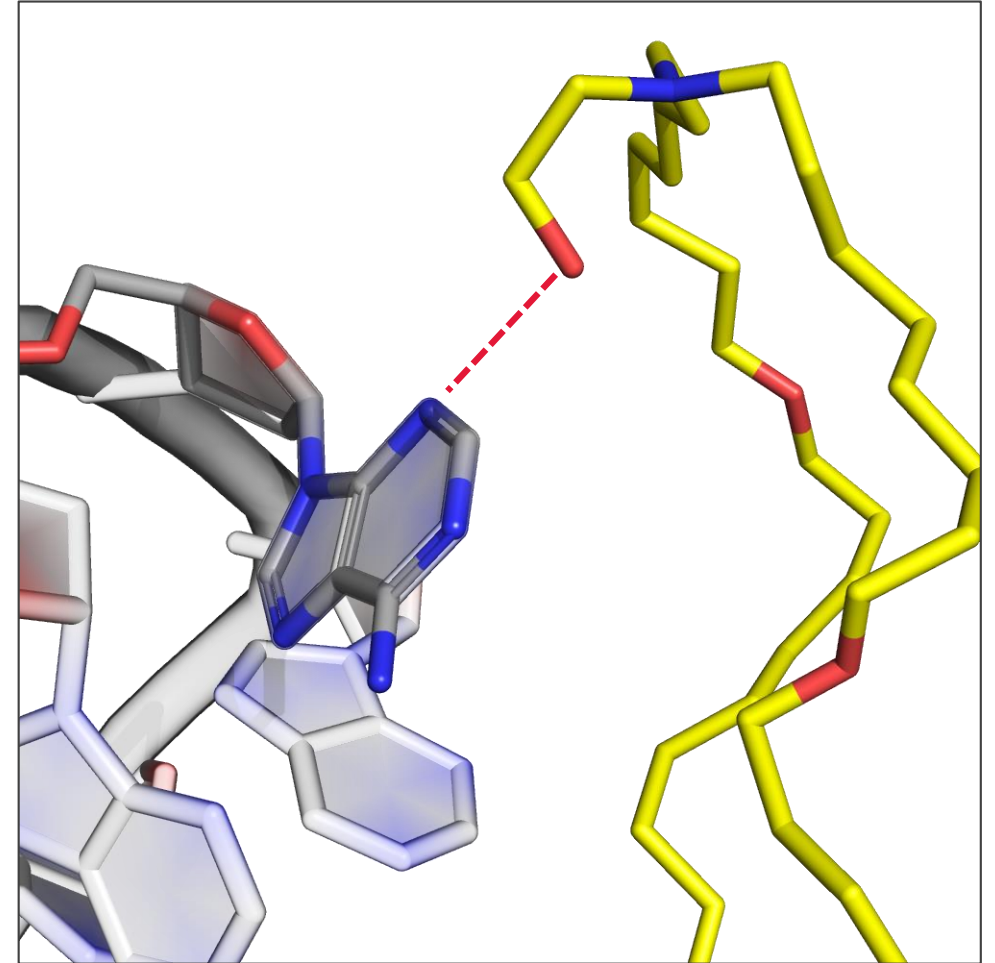
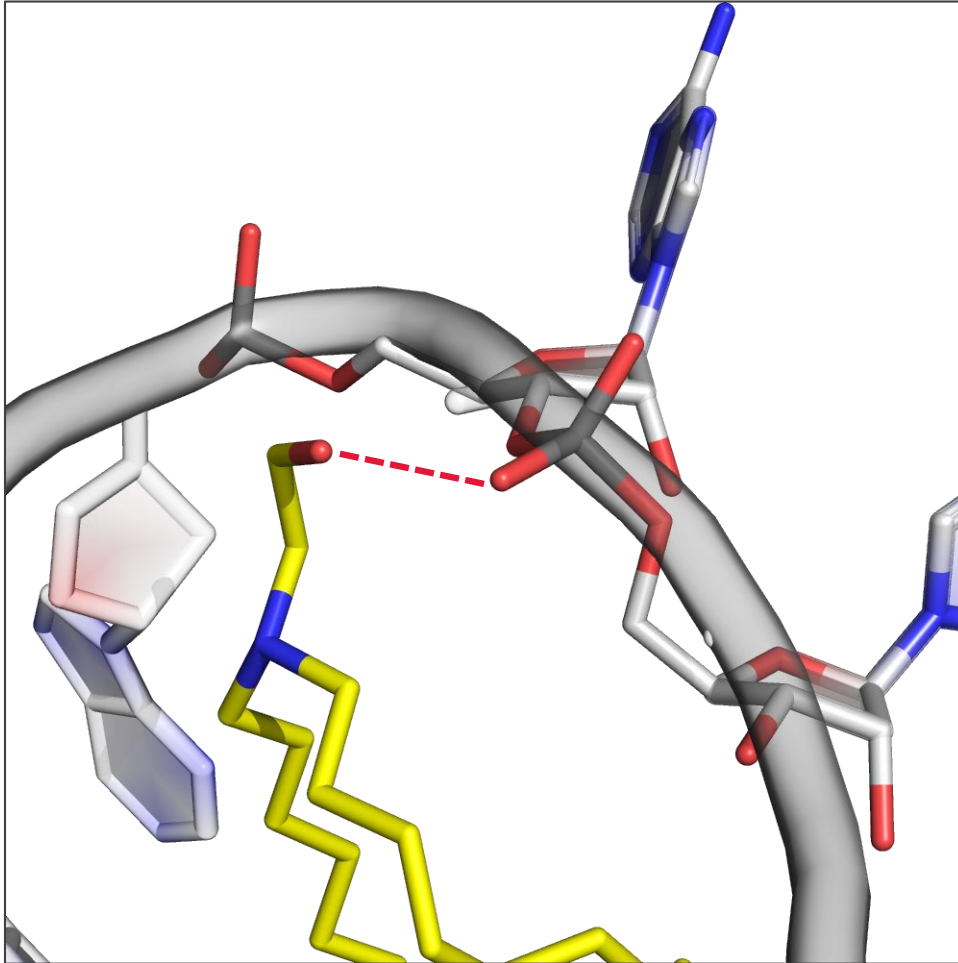
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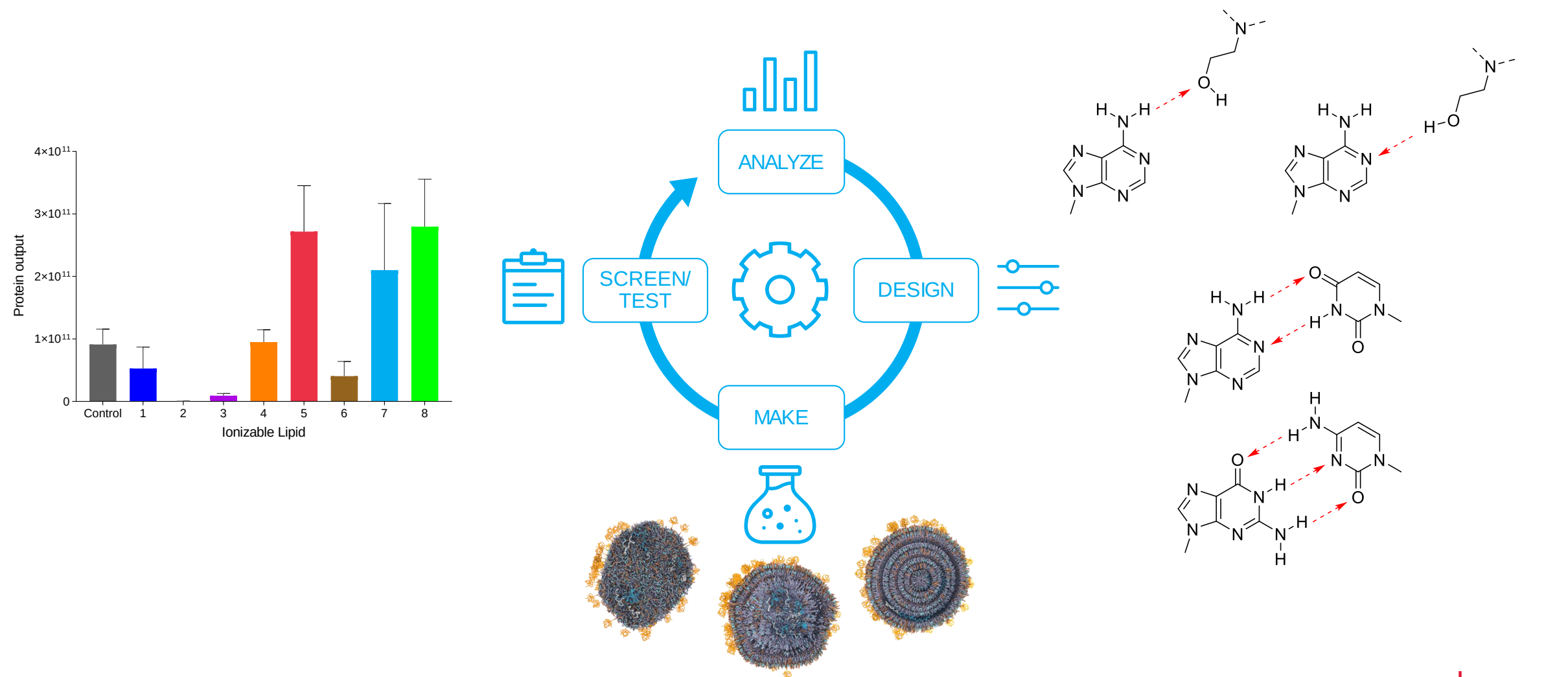
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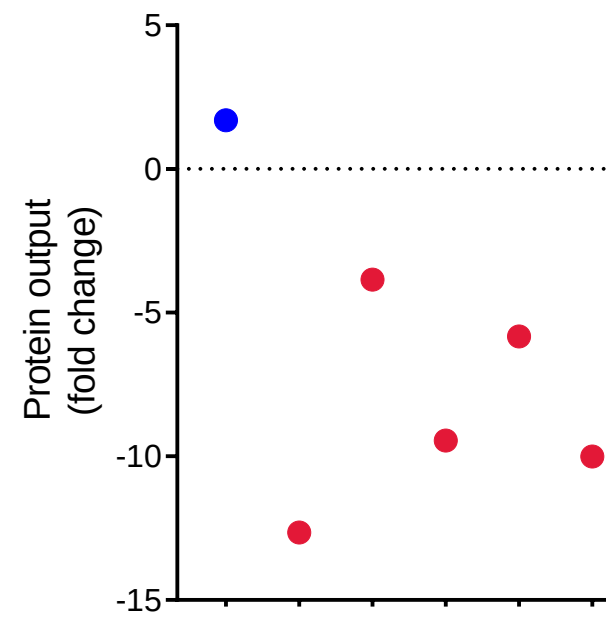
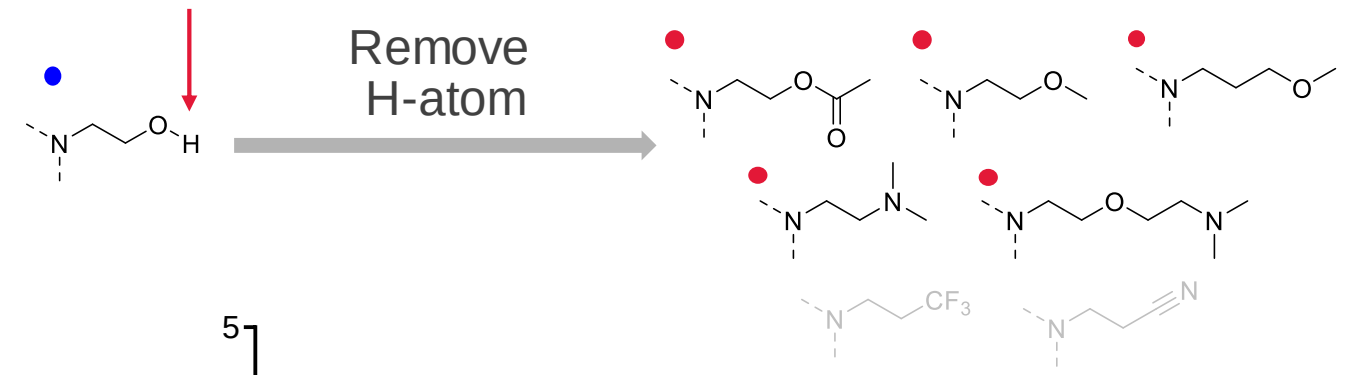
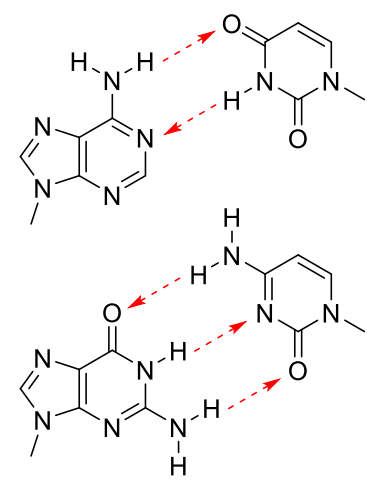
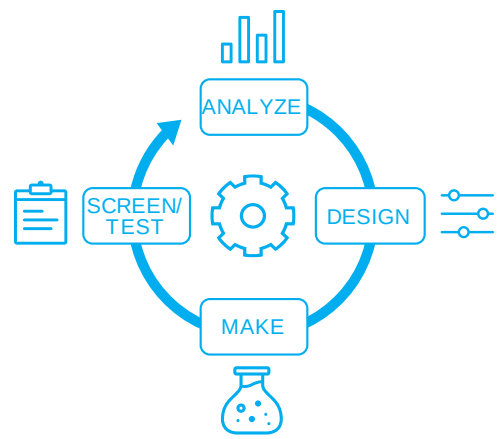
The ethanolamine hydrogen bonds to the phosphate backbone and the nucleobases



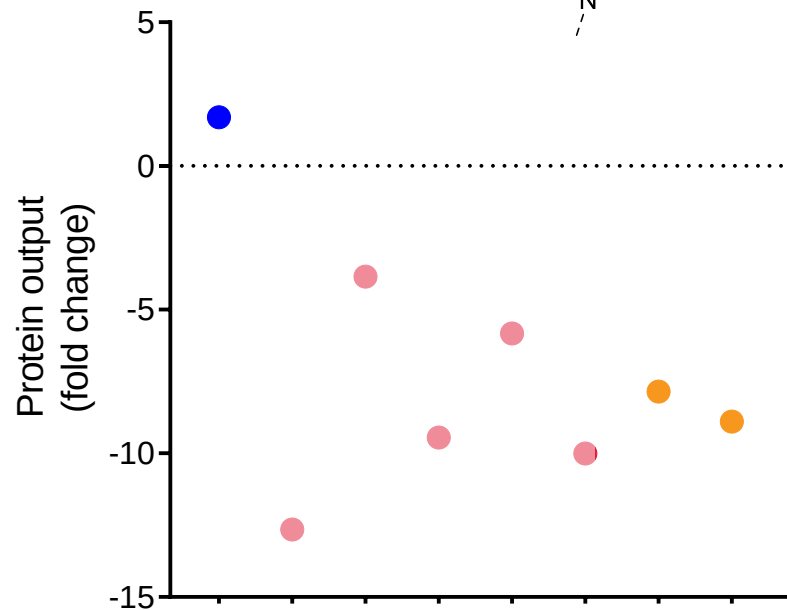
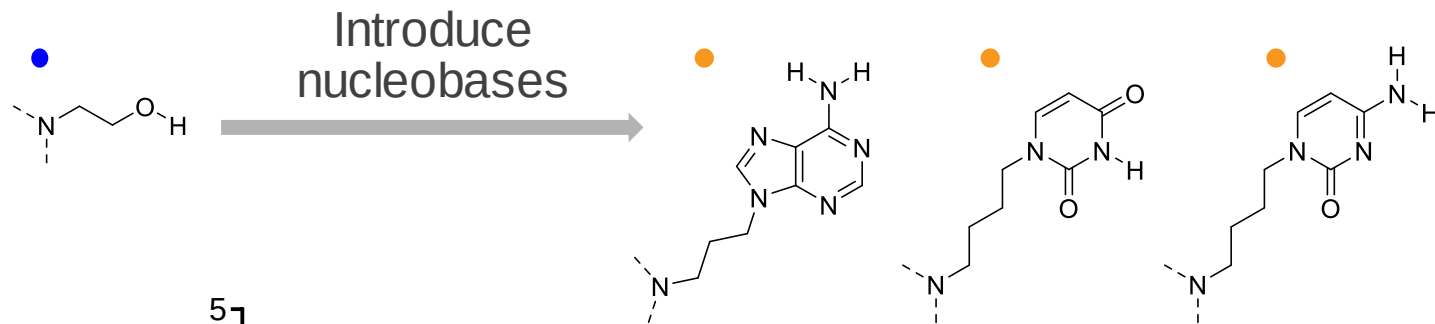
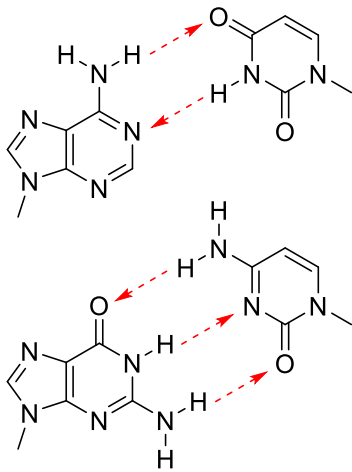
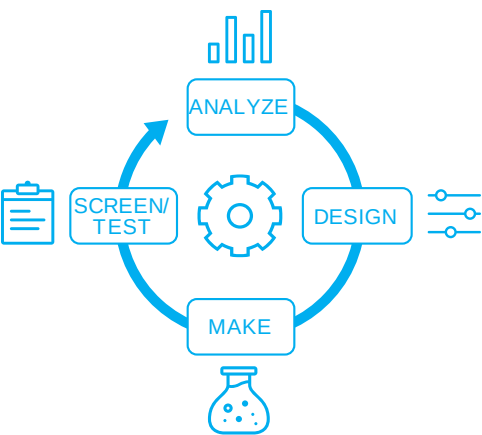
Could we design lipids with even stronger interactions with the nucleobases?



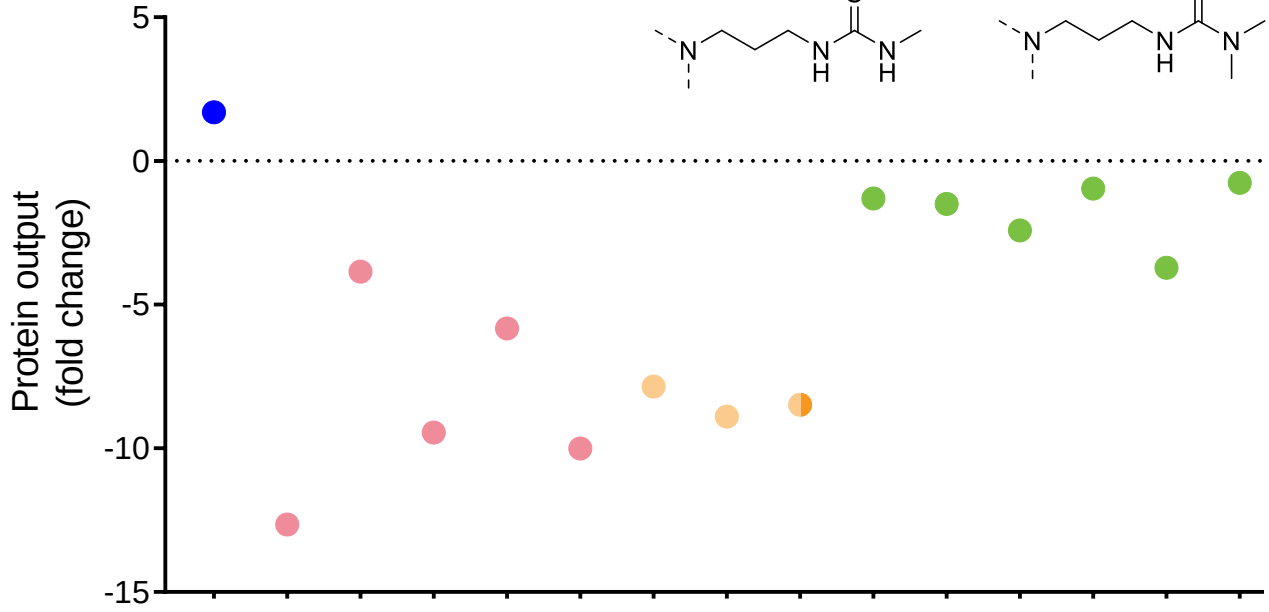
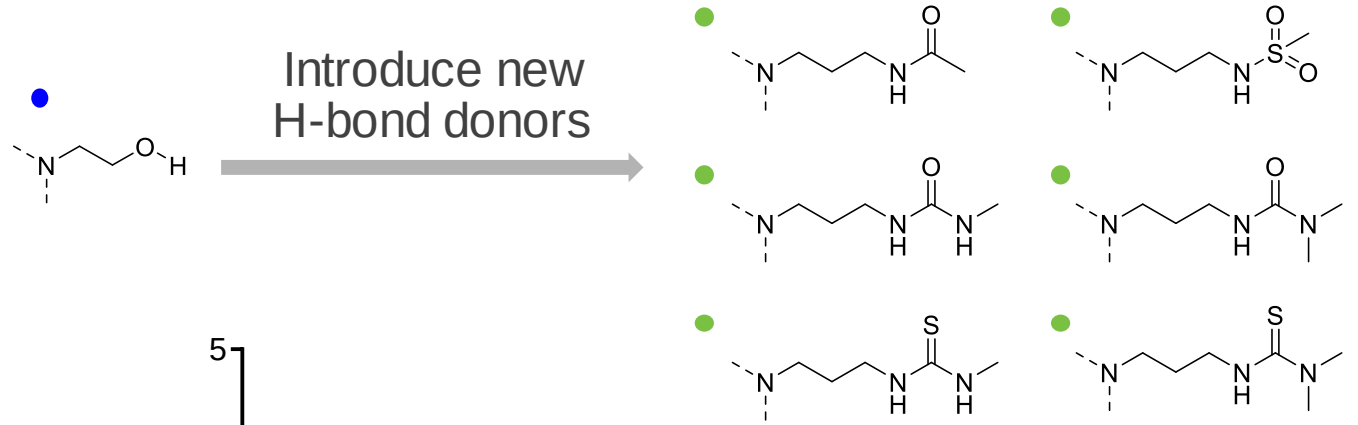
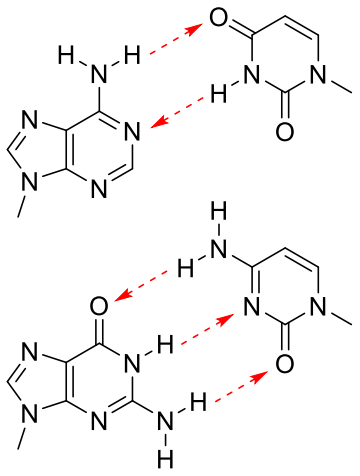
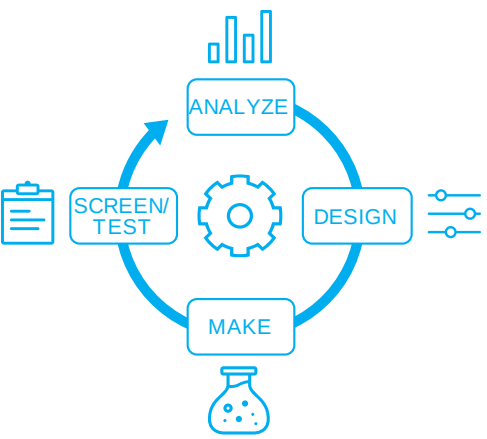
Hydrogen atom is critical for activity



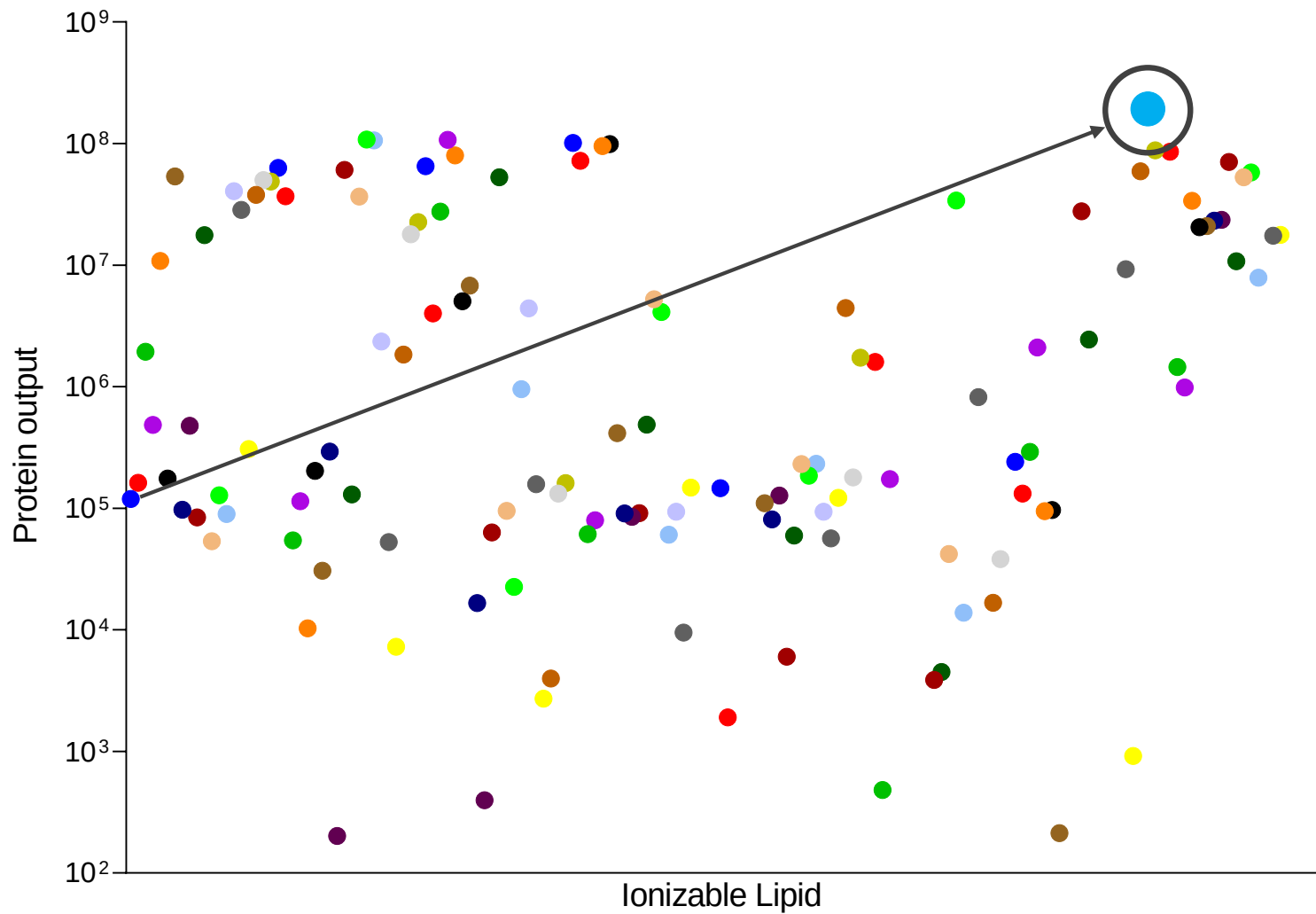
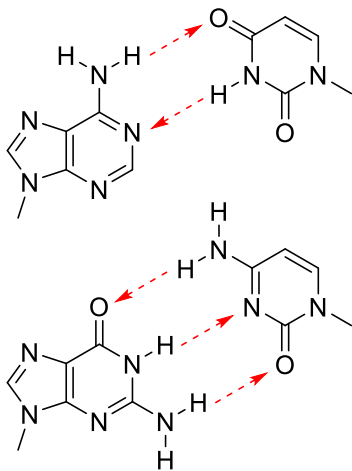
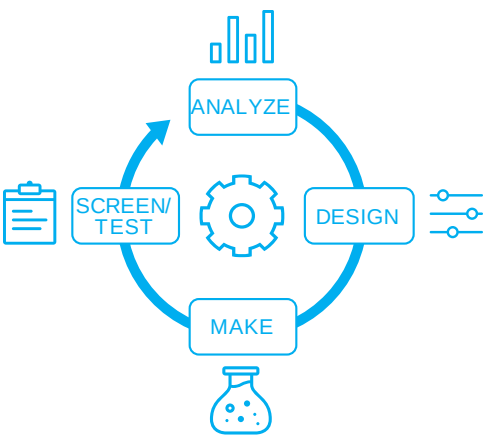
Nucleobases are too specific



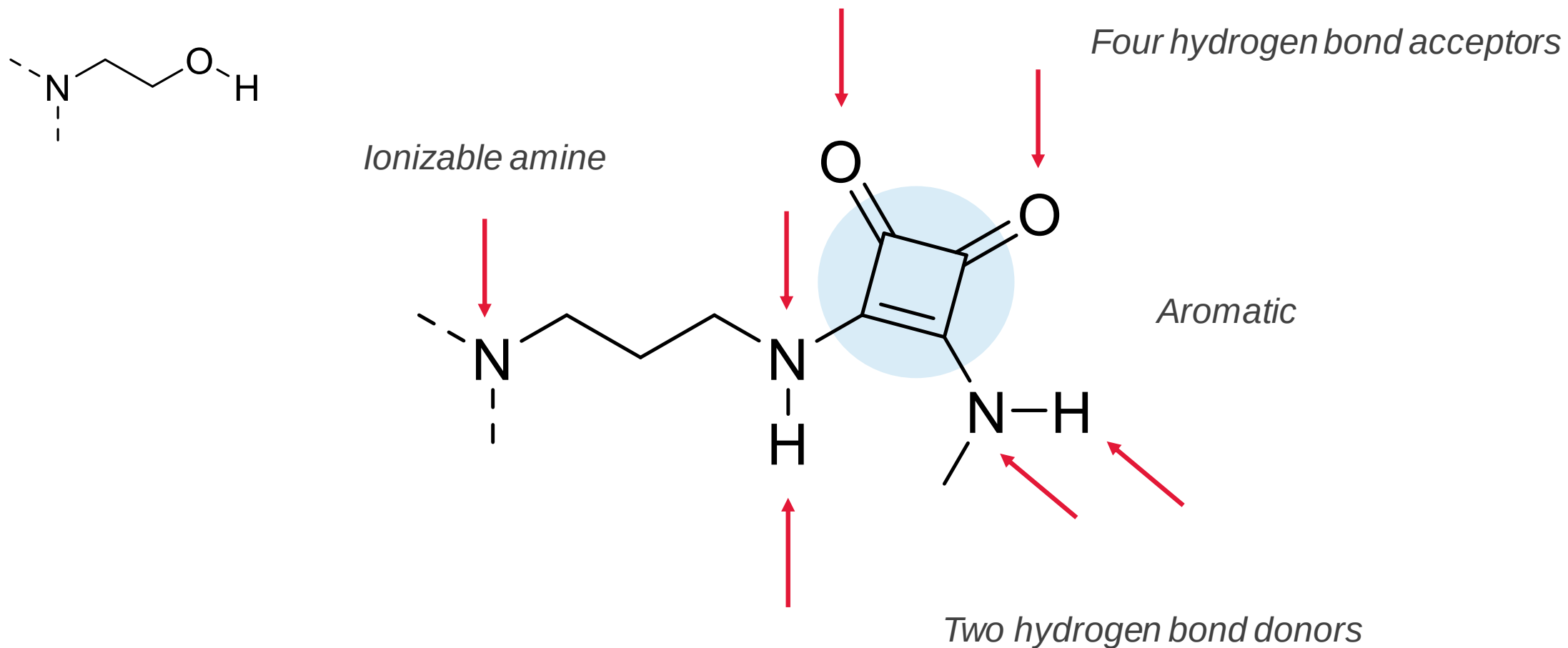
Hydrogen bond donors and acceptors are critical for activity



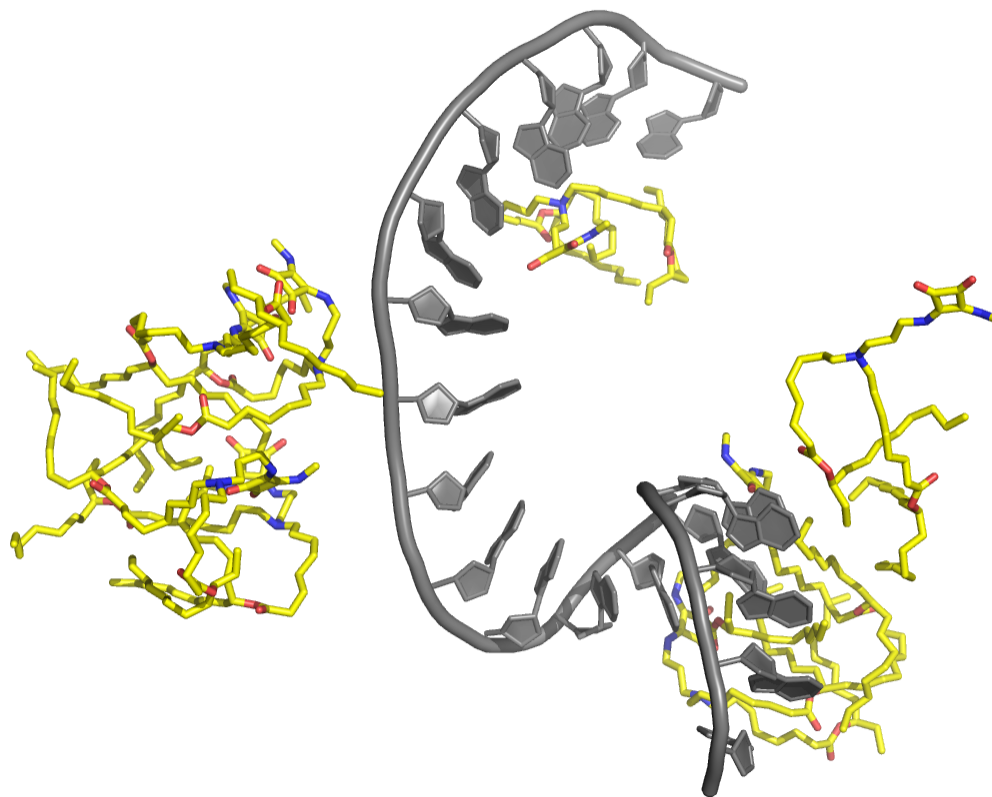
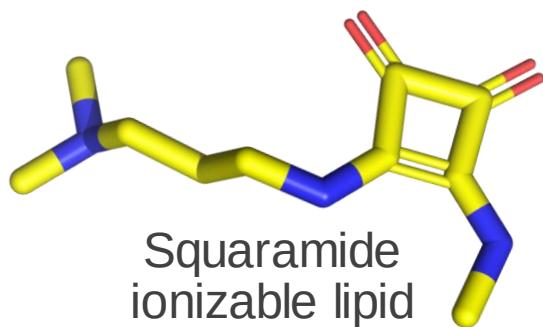
Further development of structure activity relationships identified a new lead structure



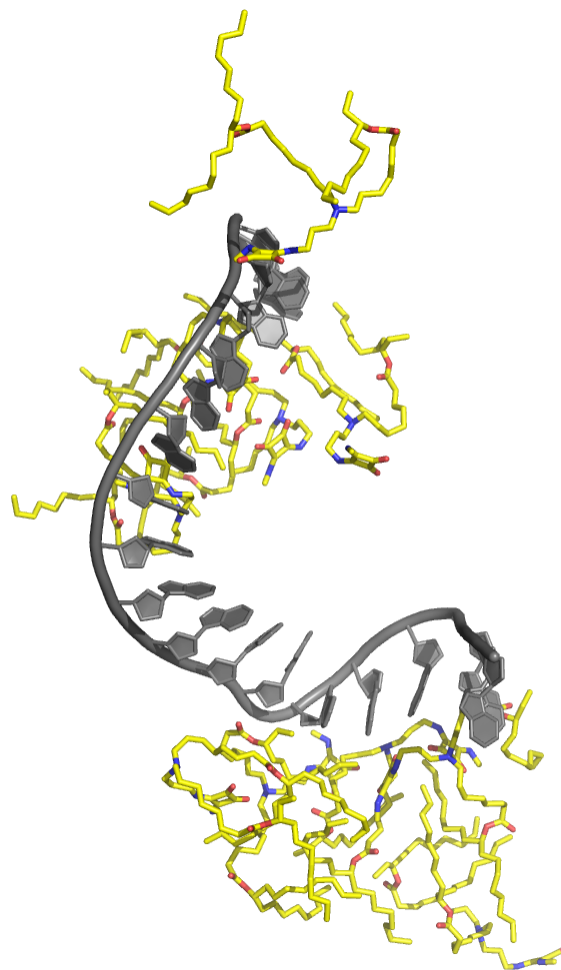
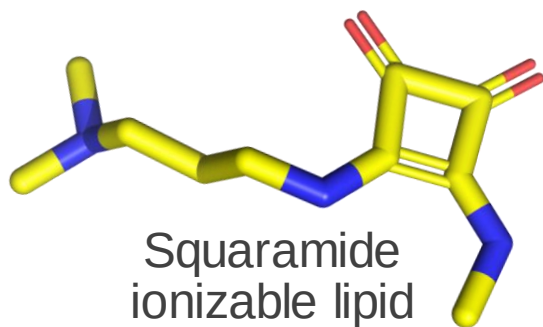
New lead structure is a squaramide ionizable amino lipid



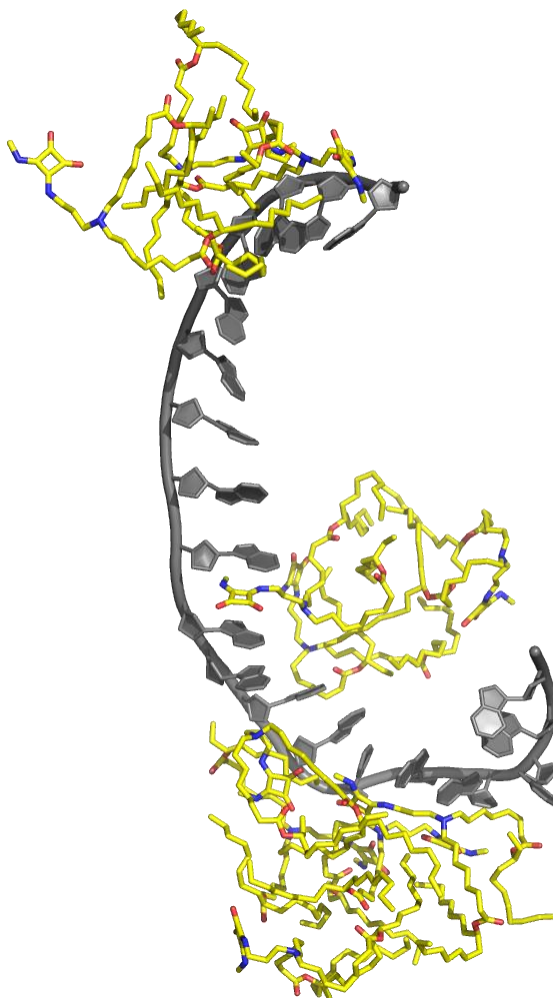
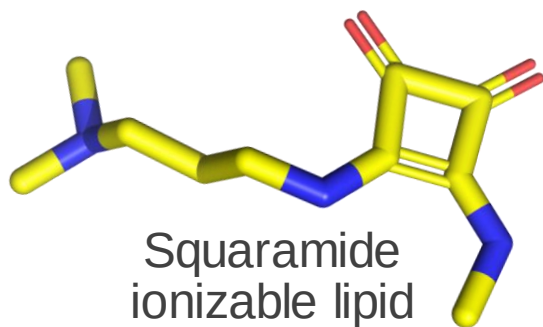
Simulations of squaramide ionizable lipids interacting with mRNA demonstrate preferential interactions with the nucleobases



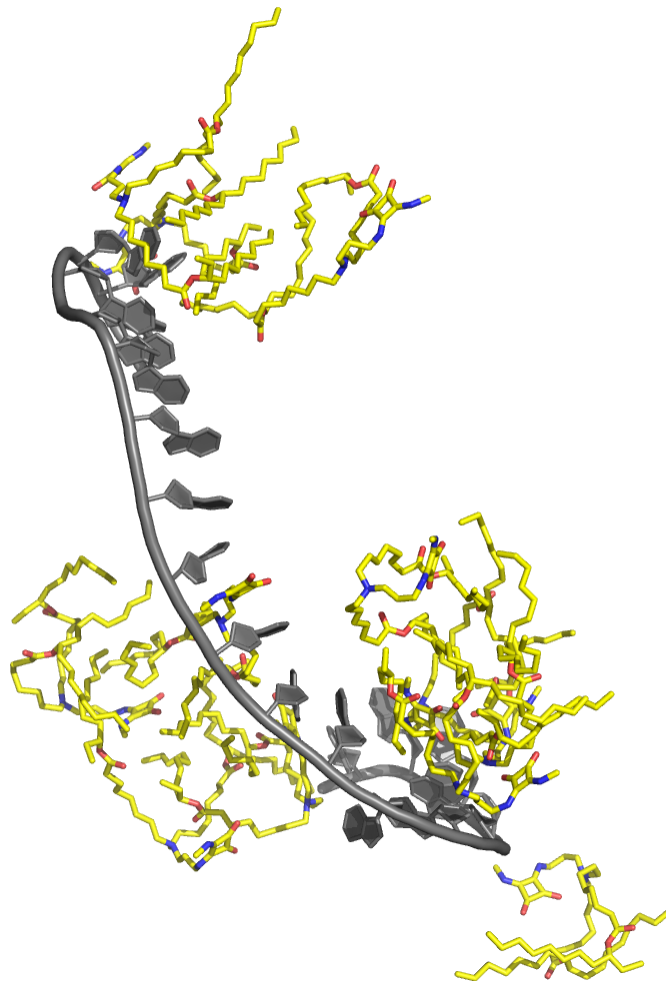
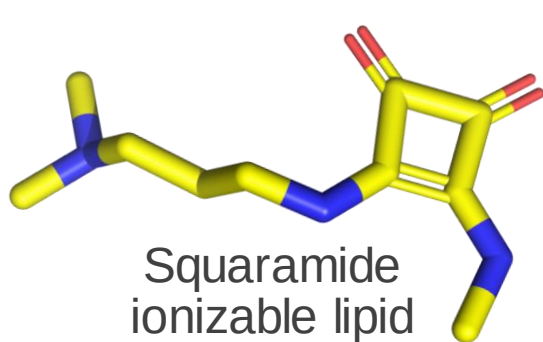
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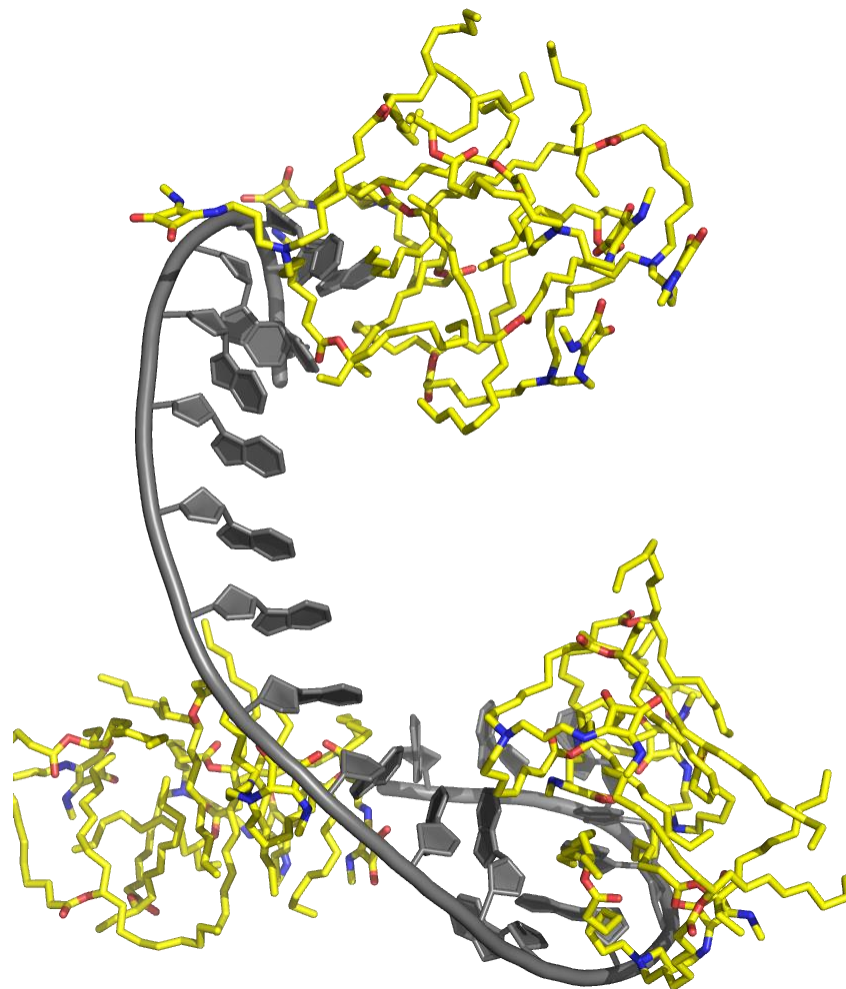
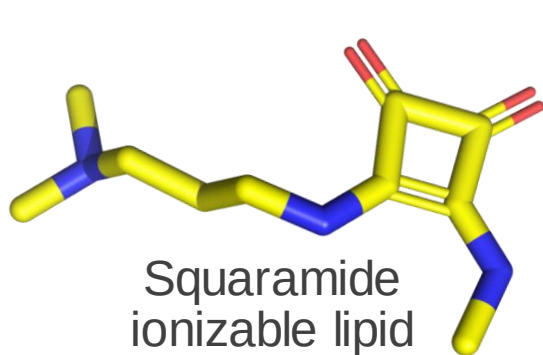
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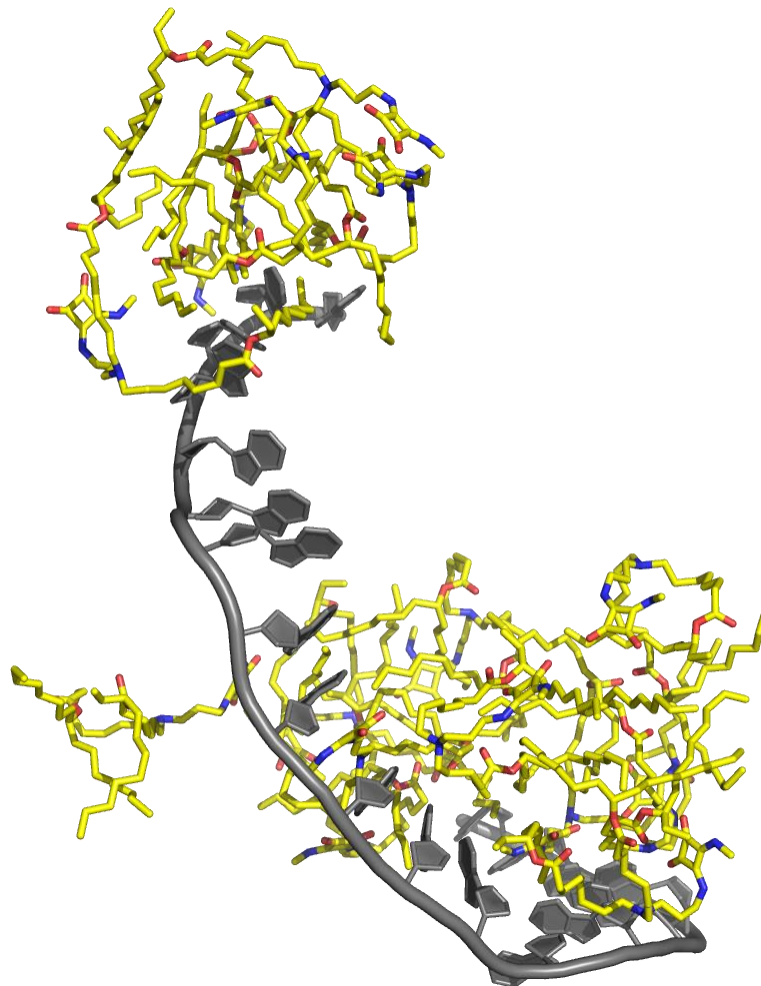
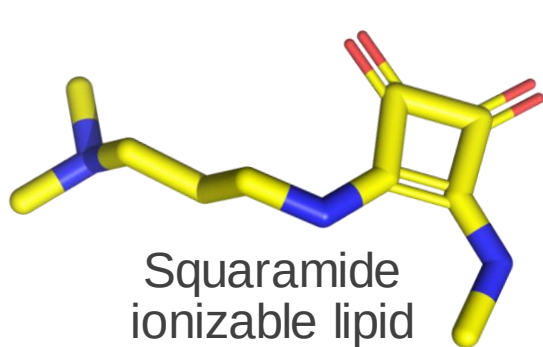
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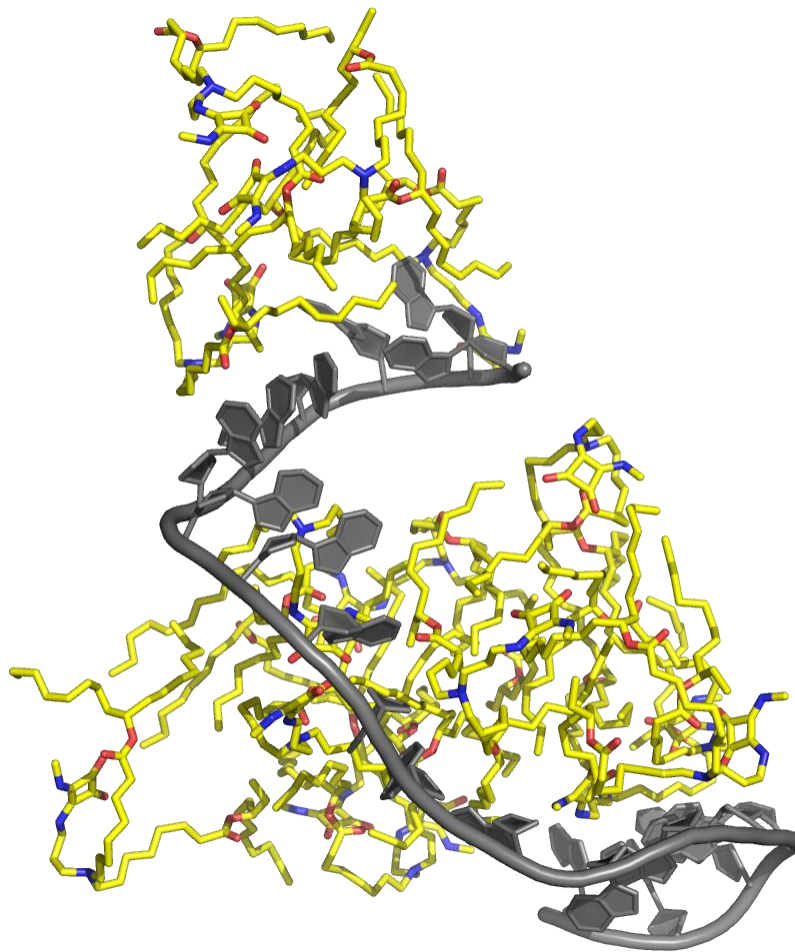
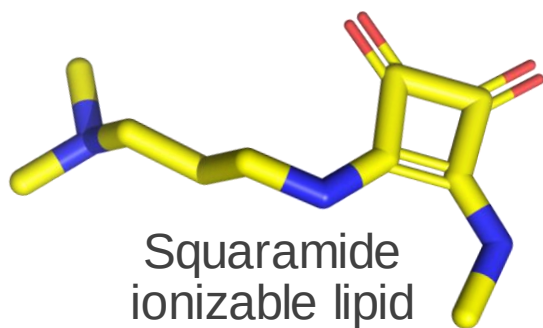
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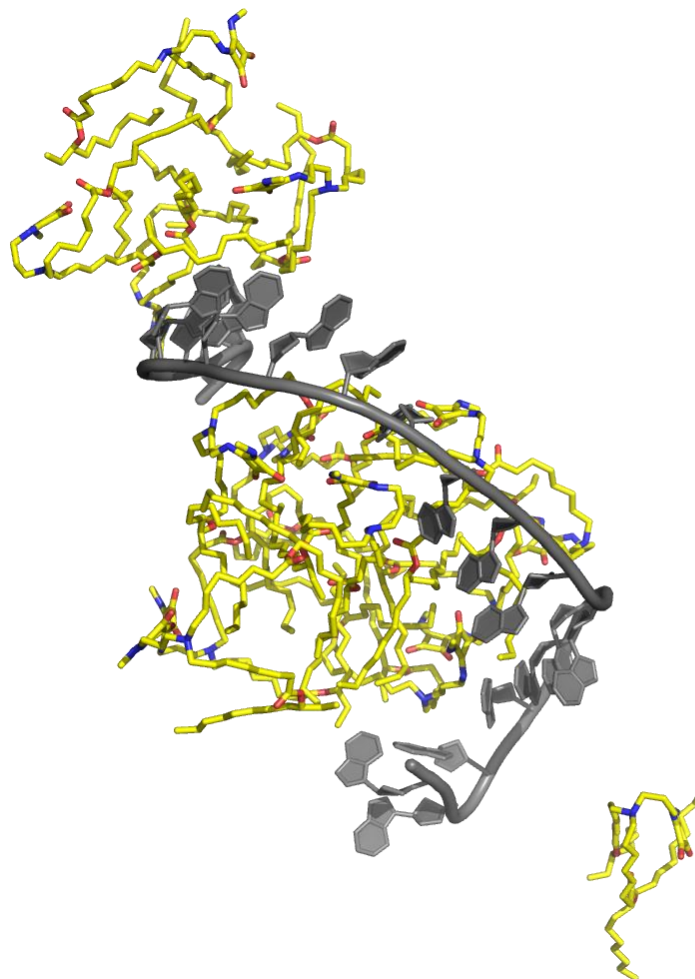
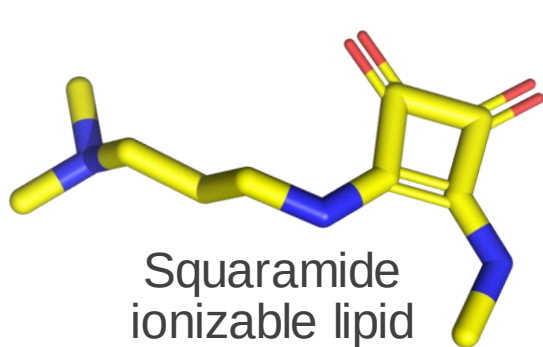
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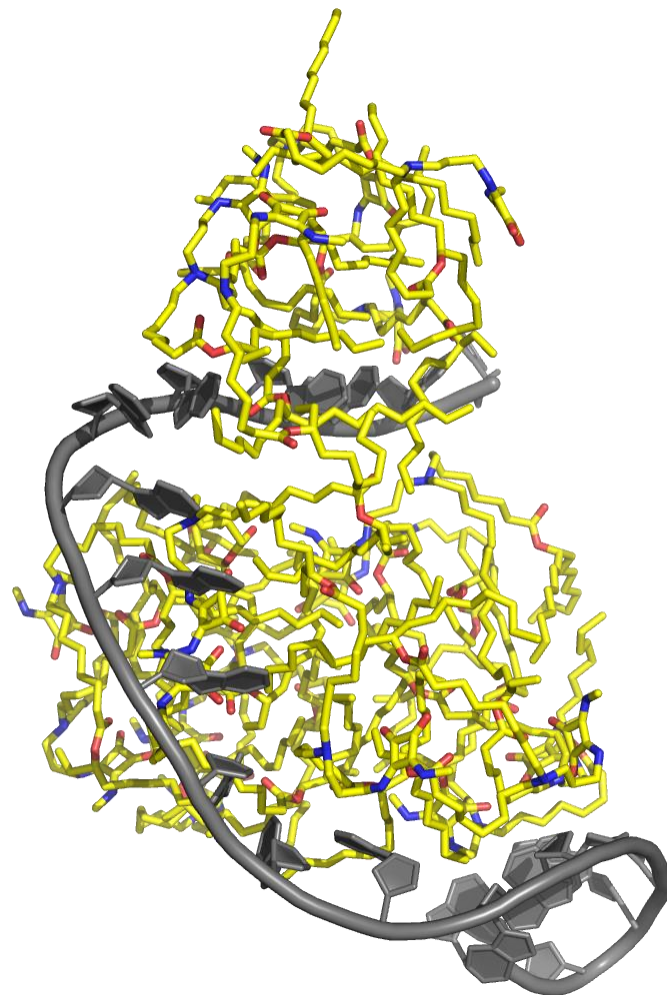
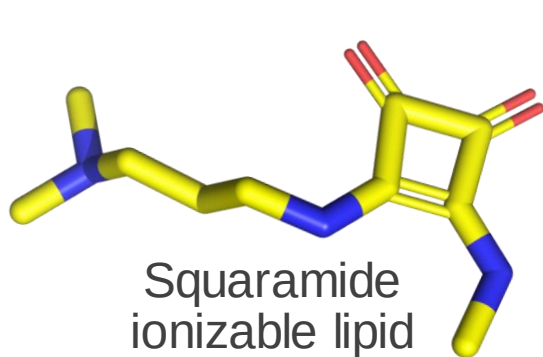
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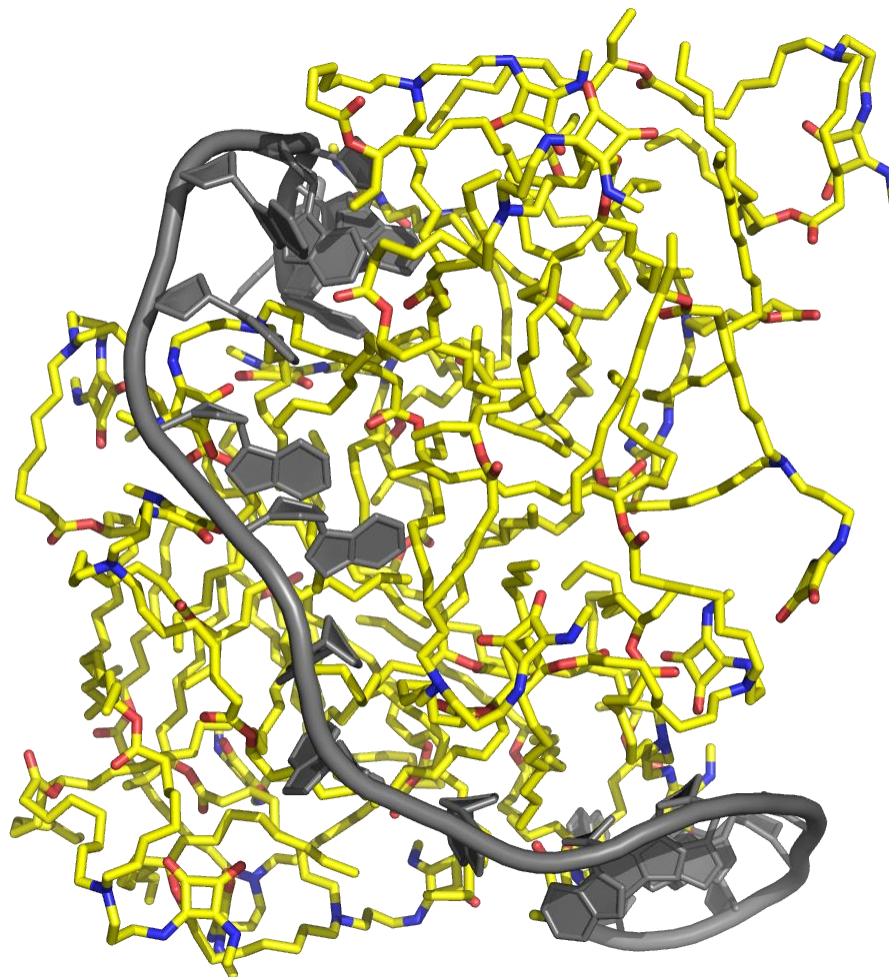
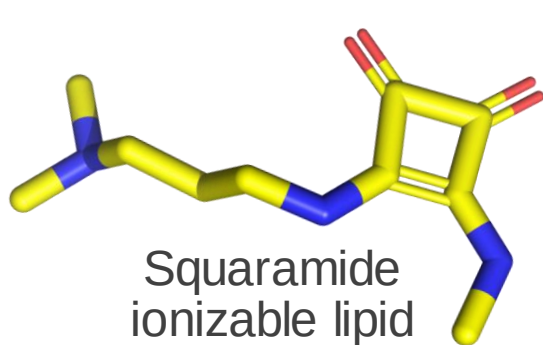
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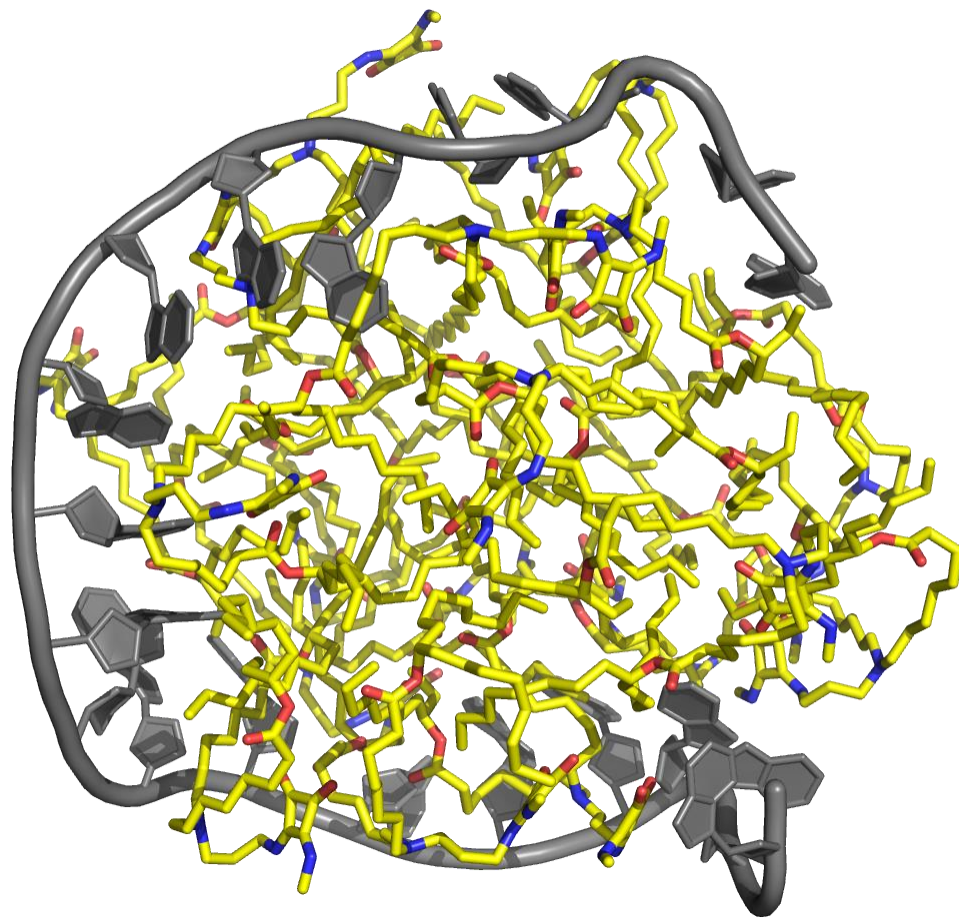
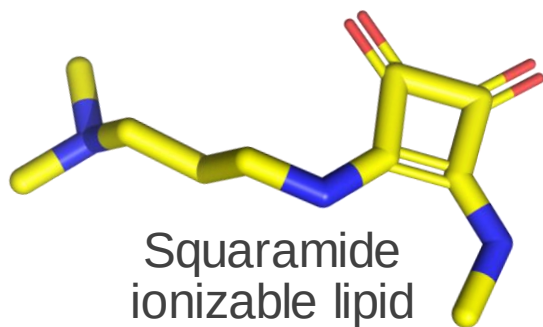
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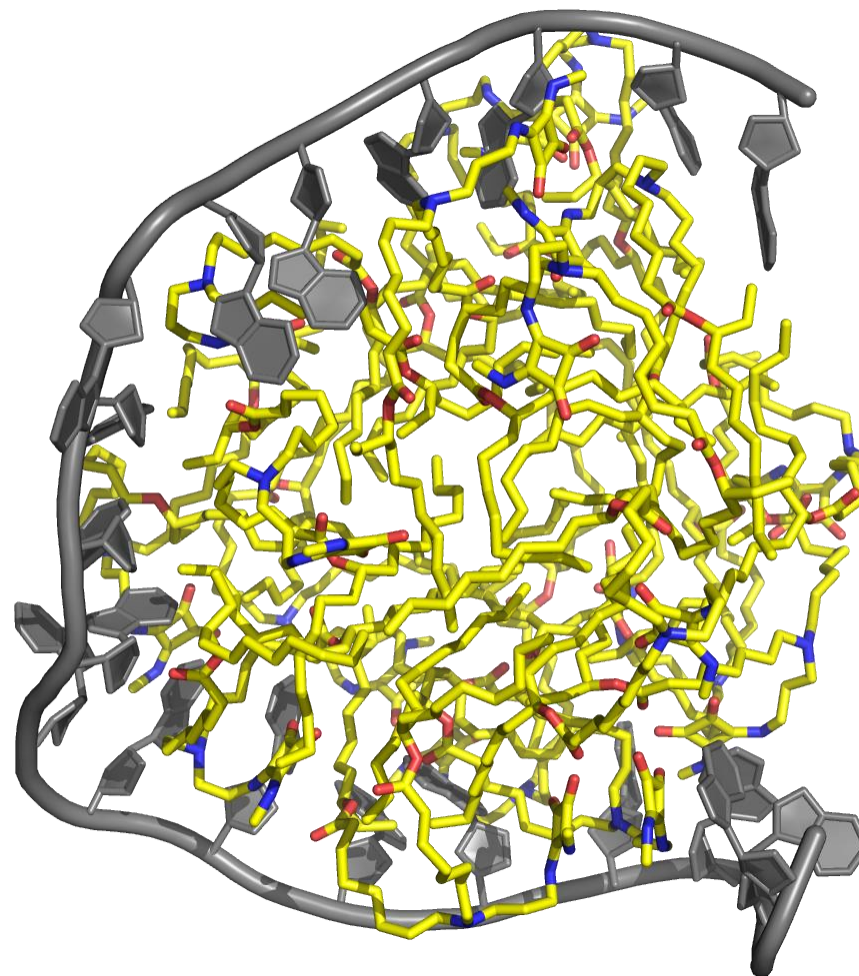
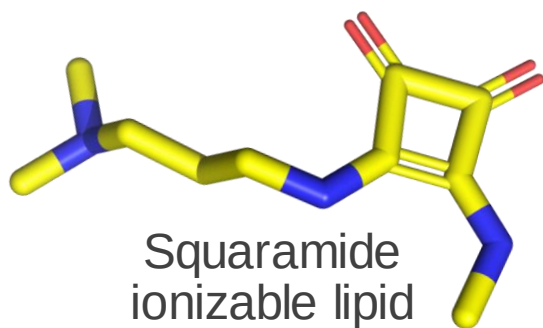
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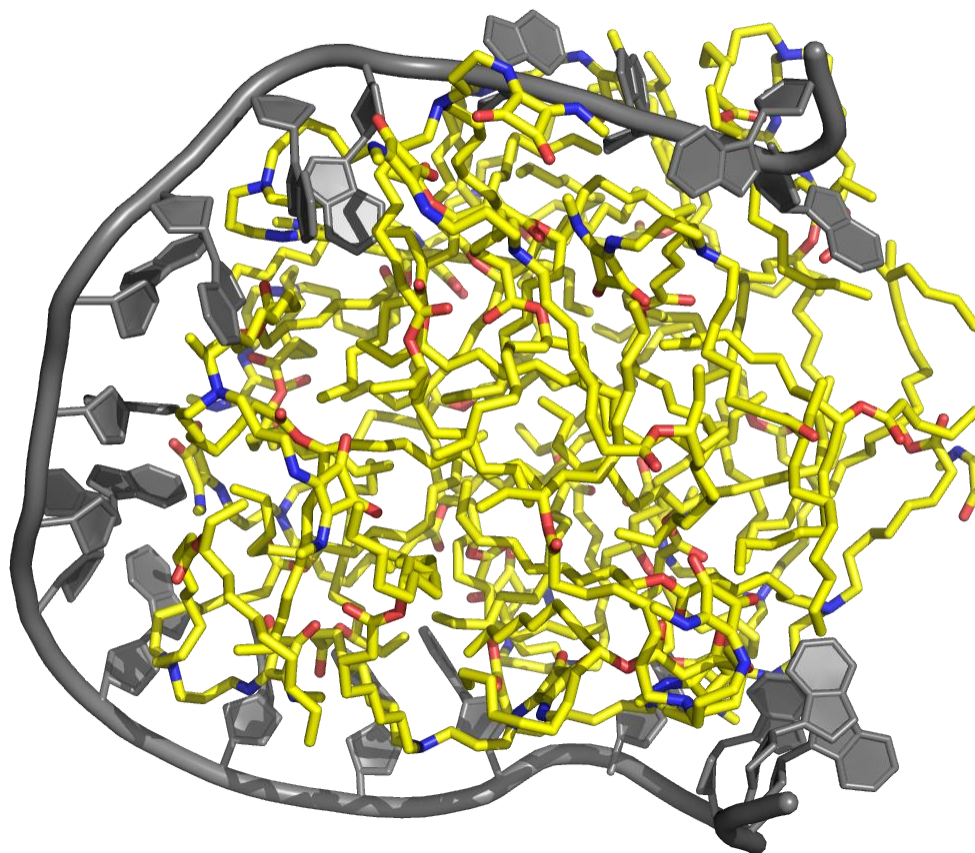
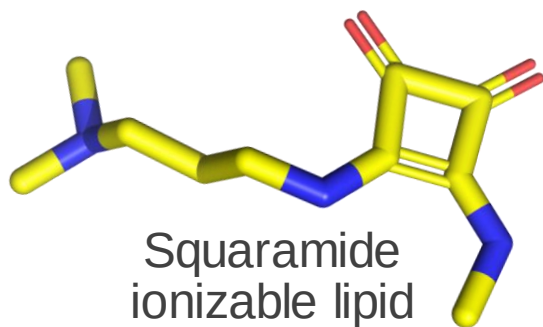
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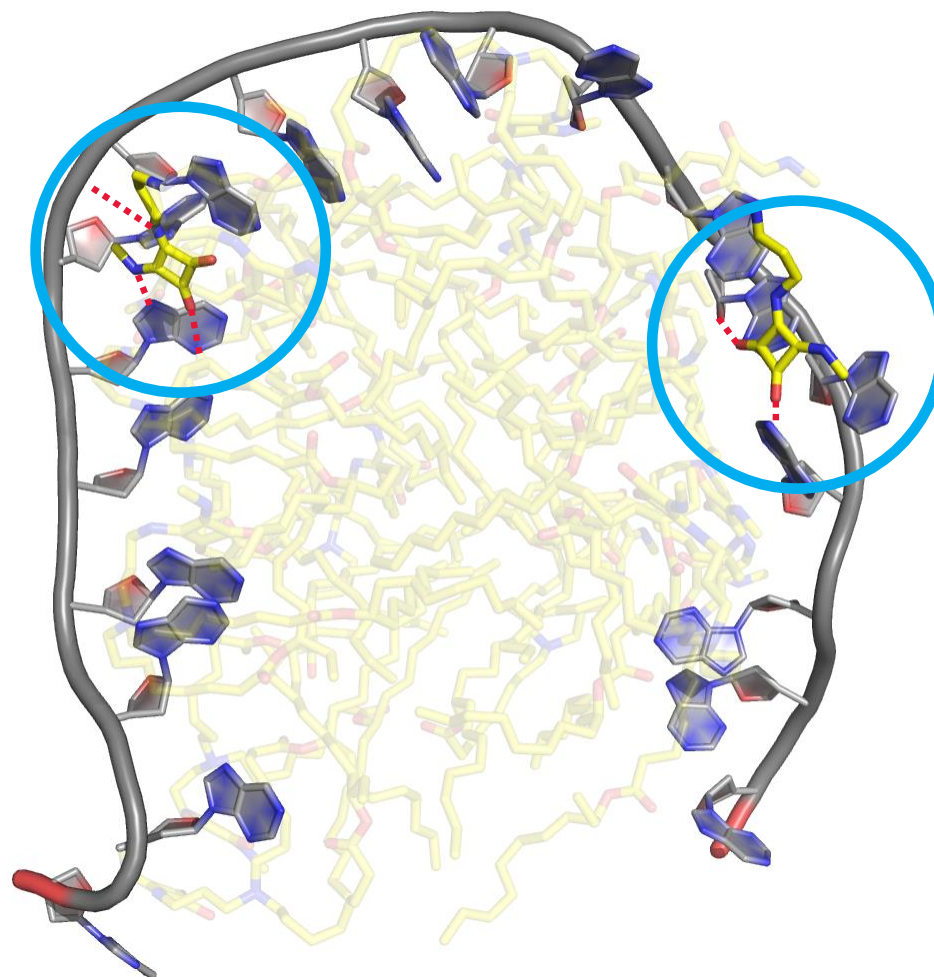
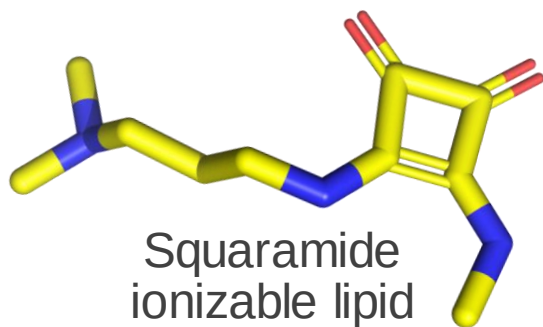
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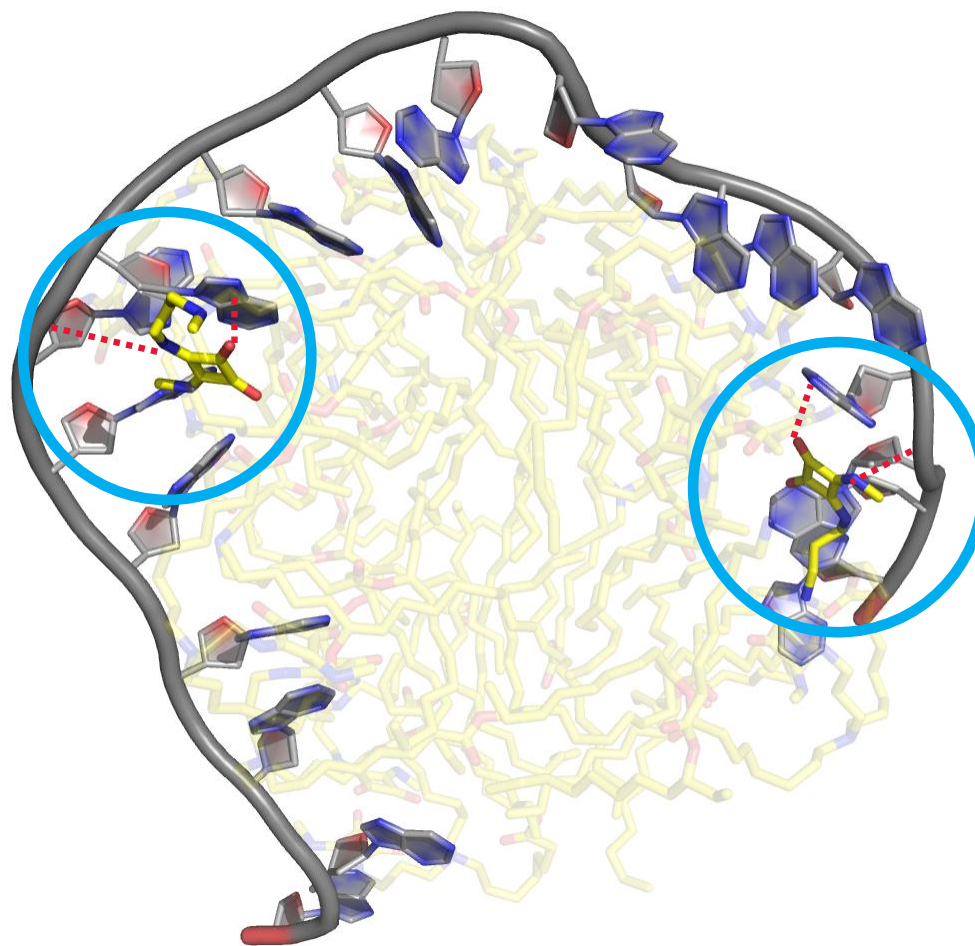
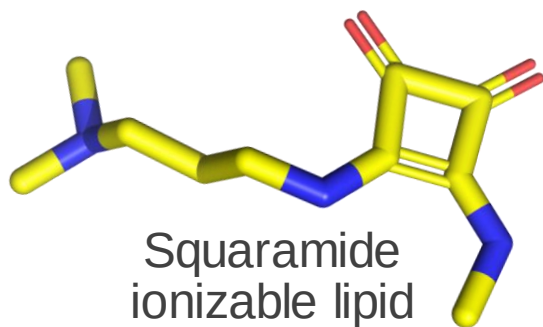
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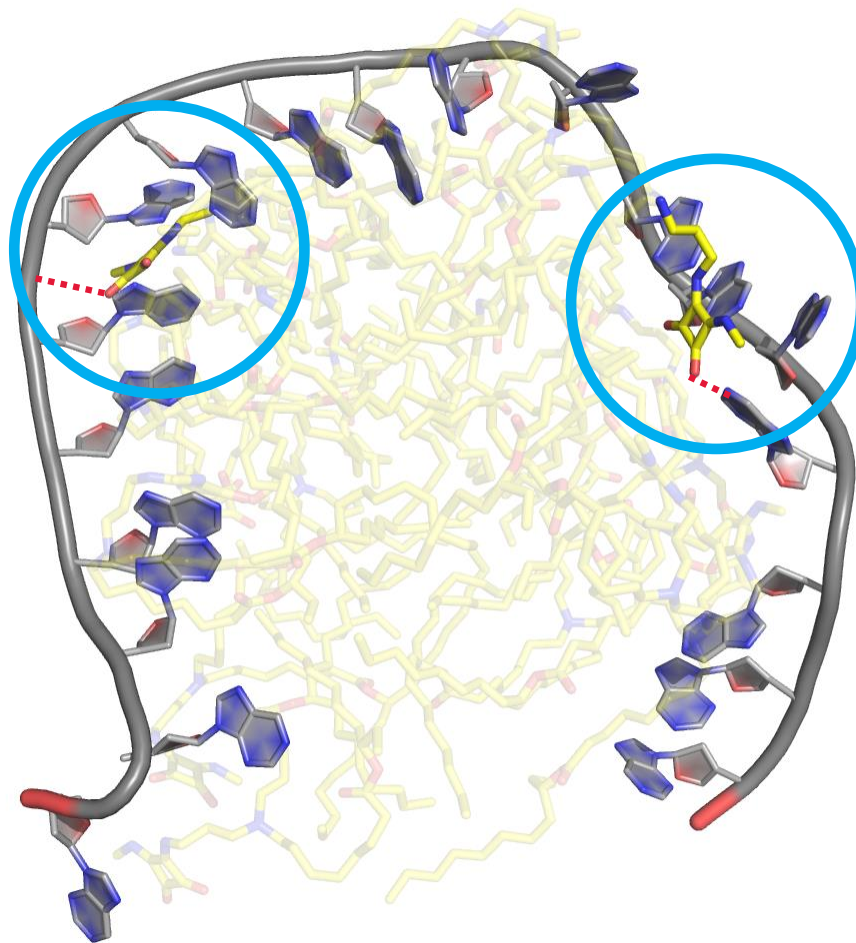
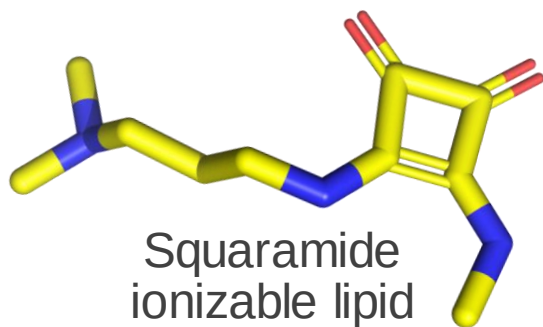
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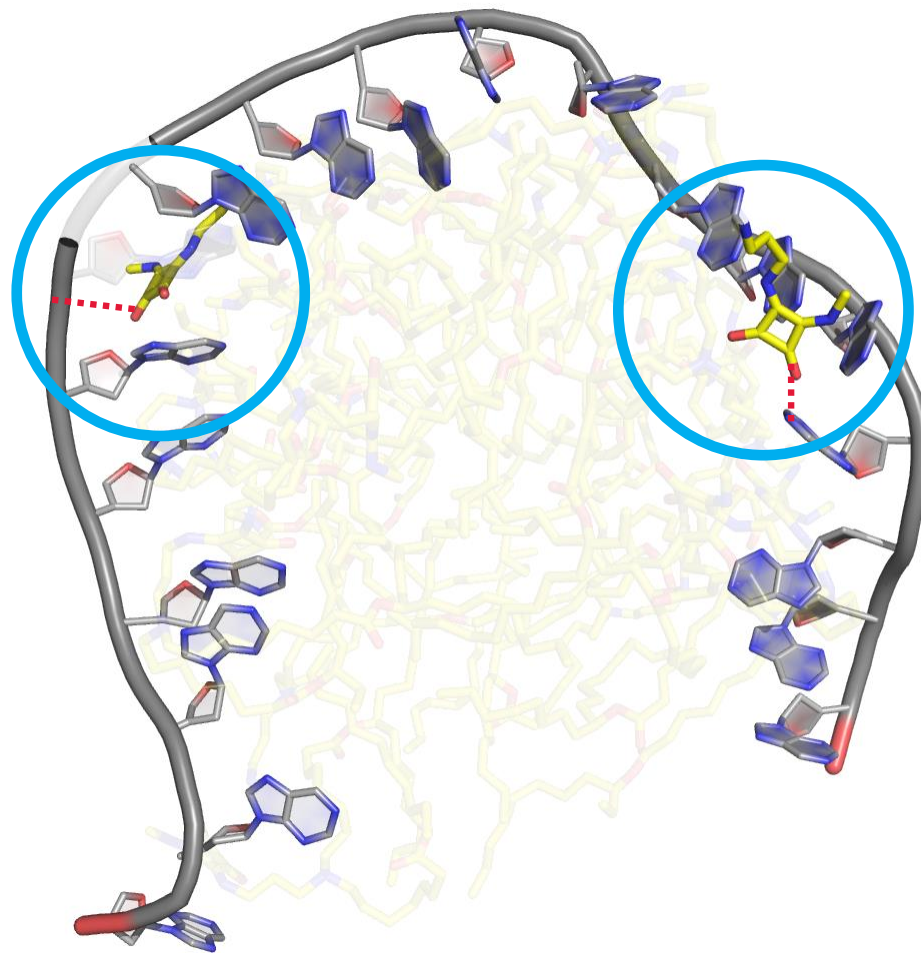
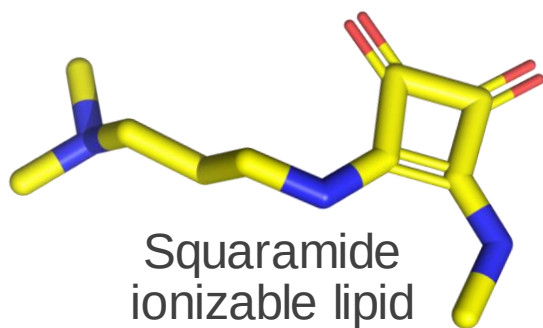
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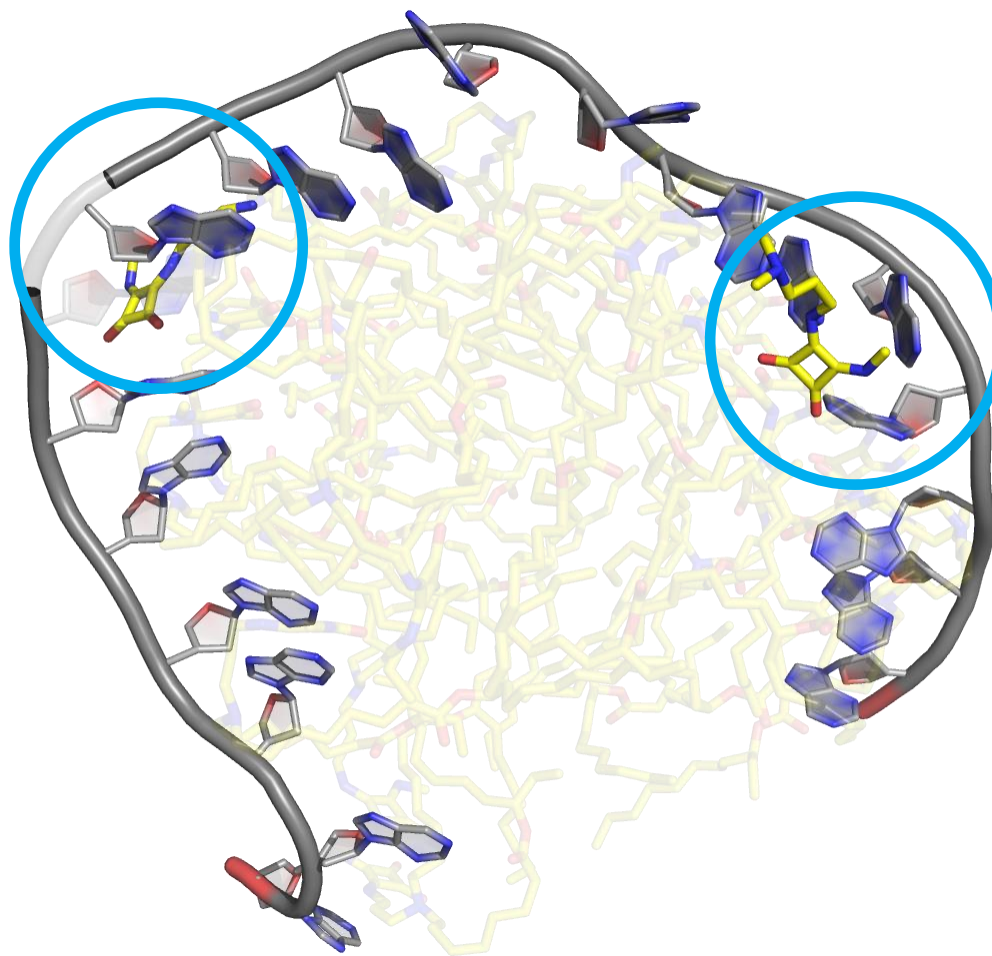
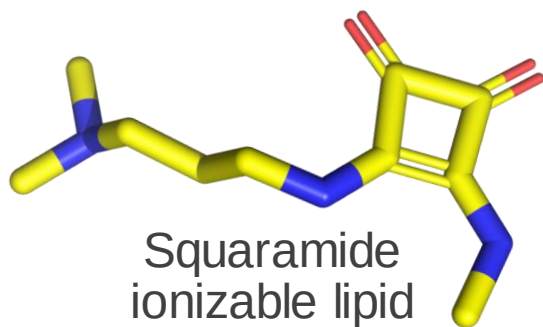
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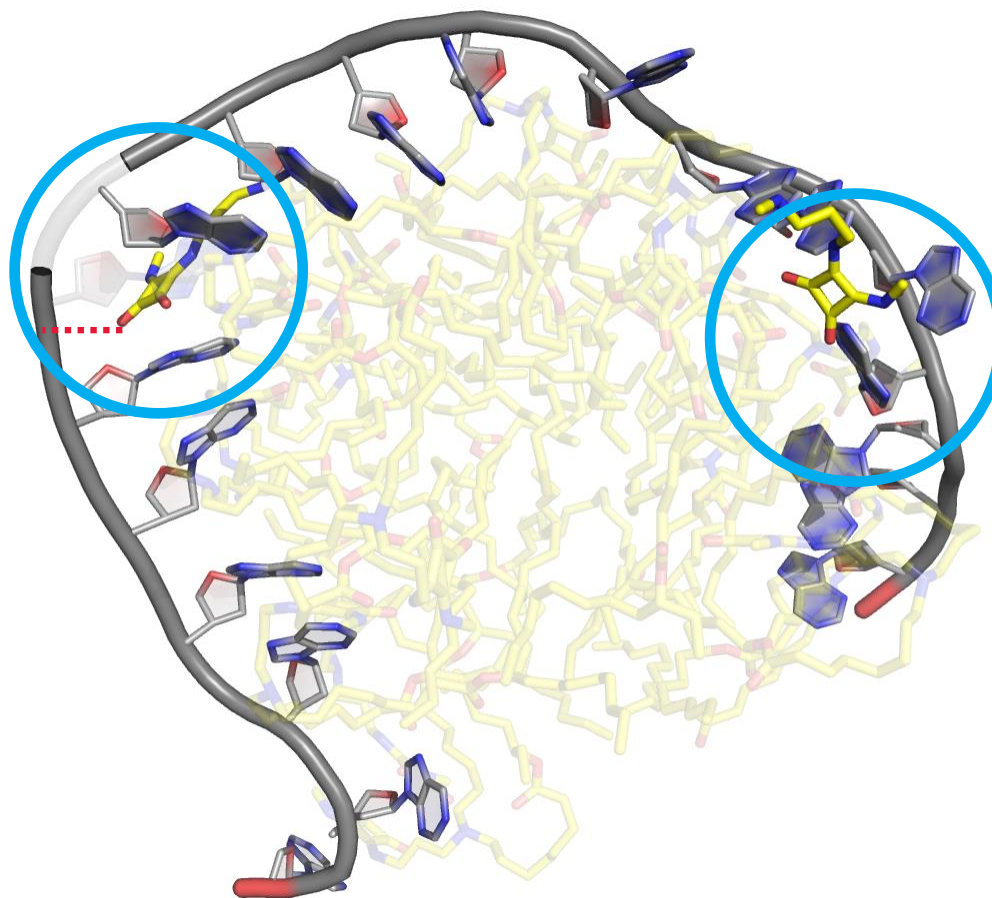
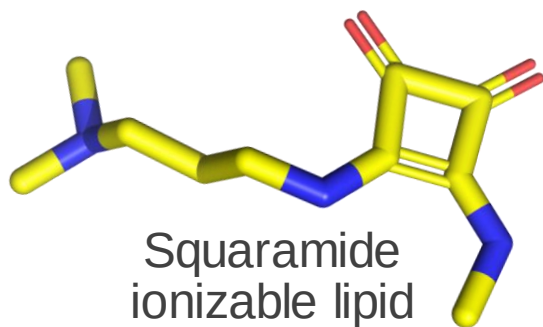
Simulations of squaramide ionizable lipids interacting with mRNA demonstrate preferential interactions with the nucleobases



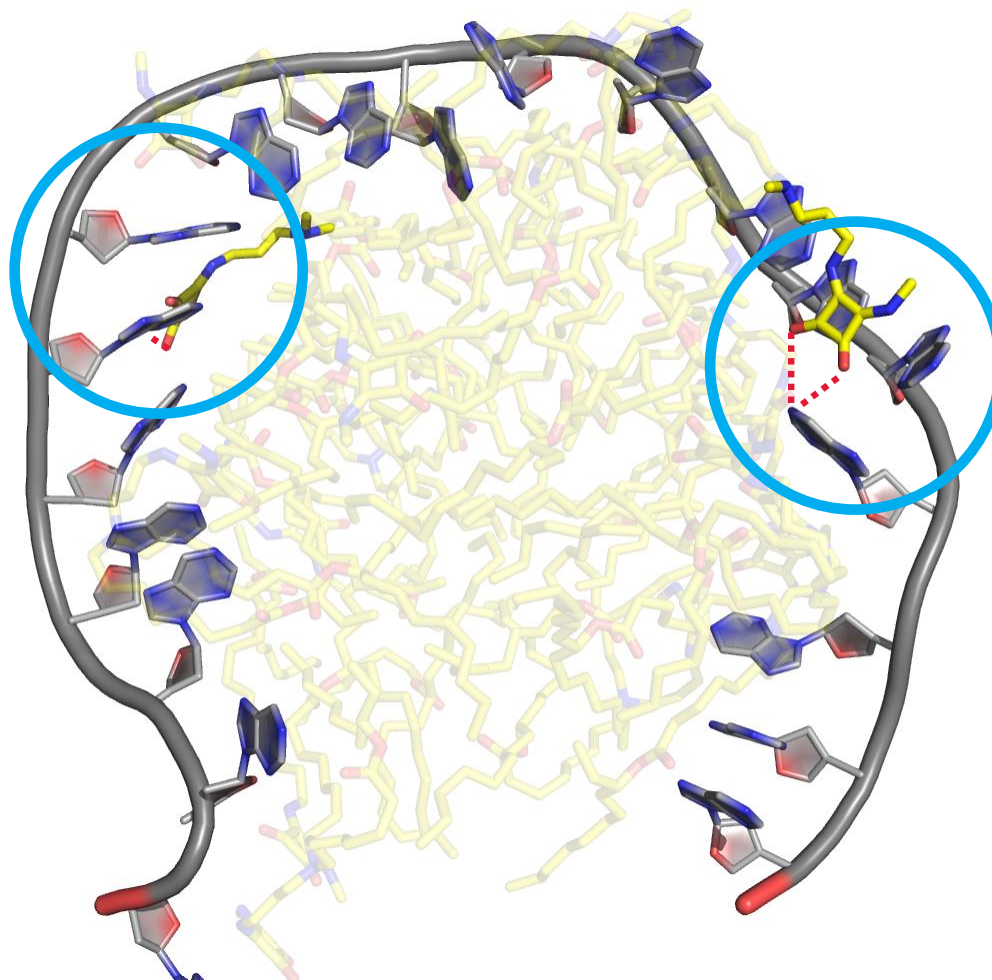
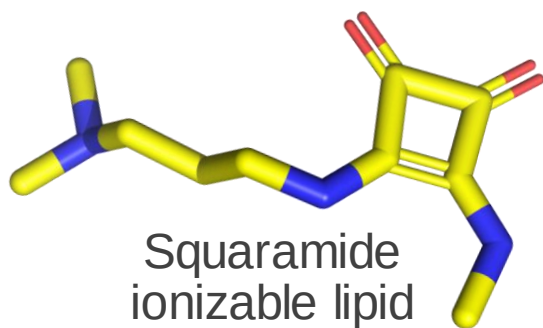
Simulations of squaramide ionizable lipids interacting with mRNA demonstrate preferential interactions with the nucleobases



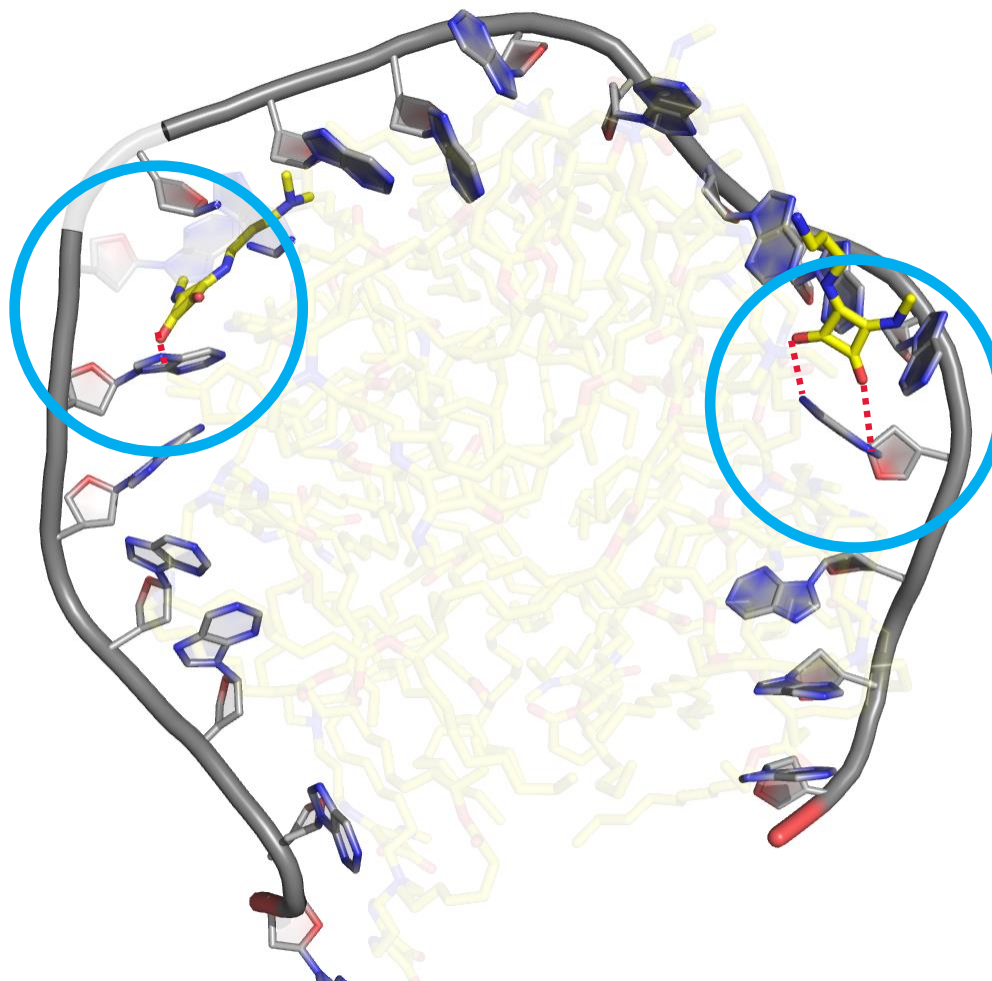
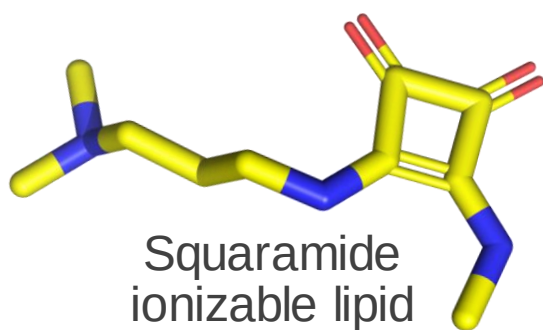
Simulations of squaramide ionizable lipids interacting with mRNA demonstrate preferential interactions with the nucleobases



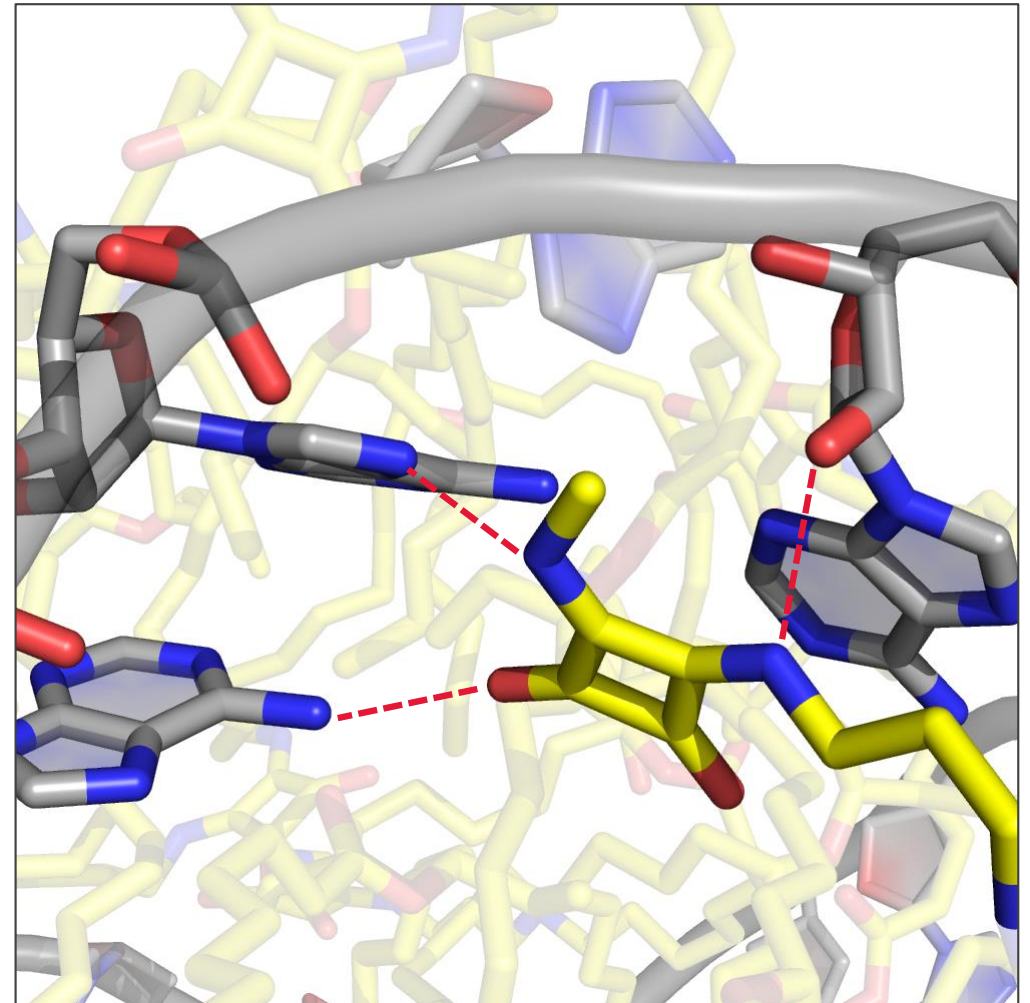
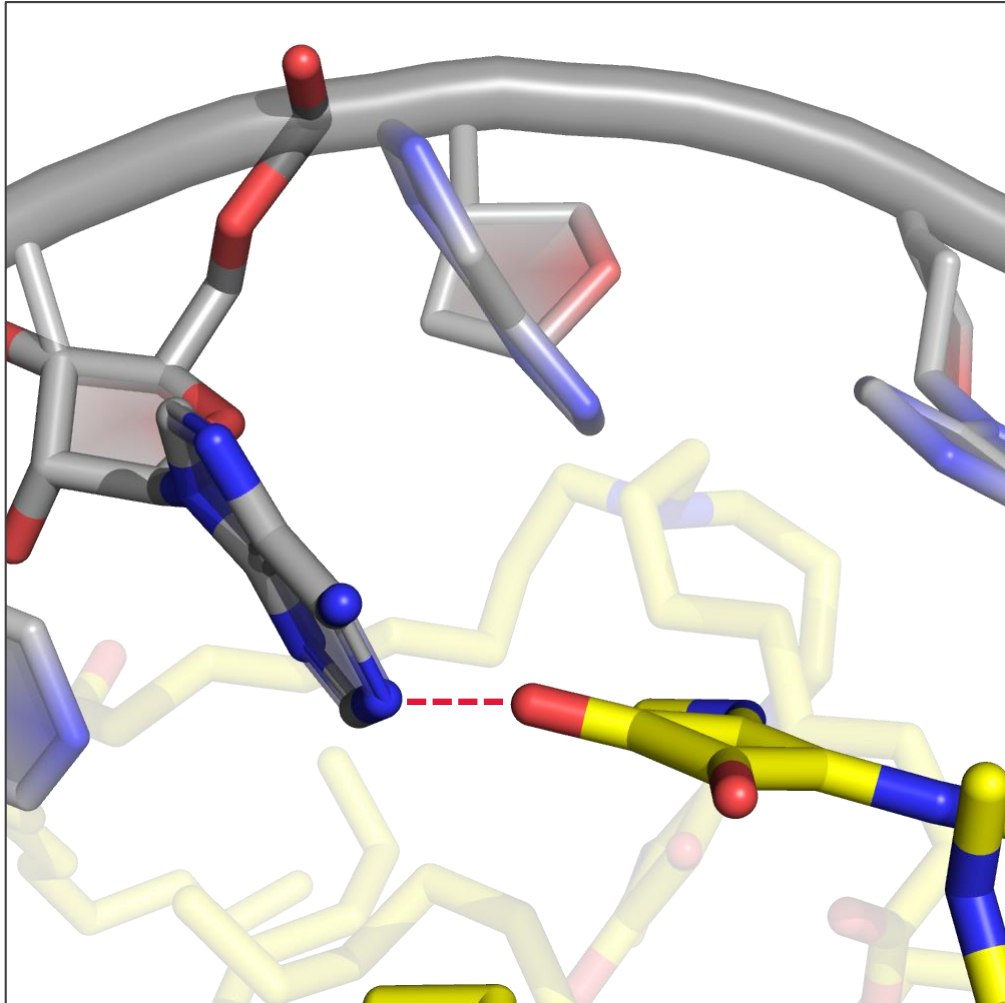
Simulations of squaramide ionizable lipids interacting with mRNA demonstrate preferential interactions with the nucleobases



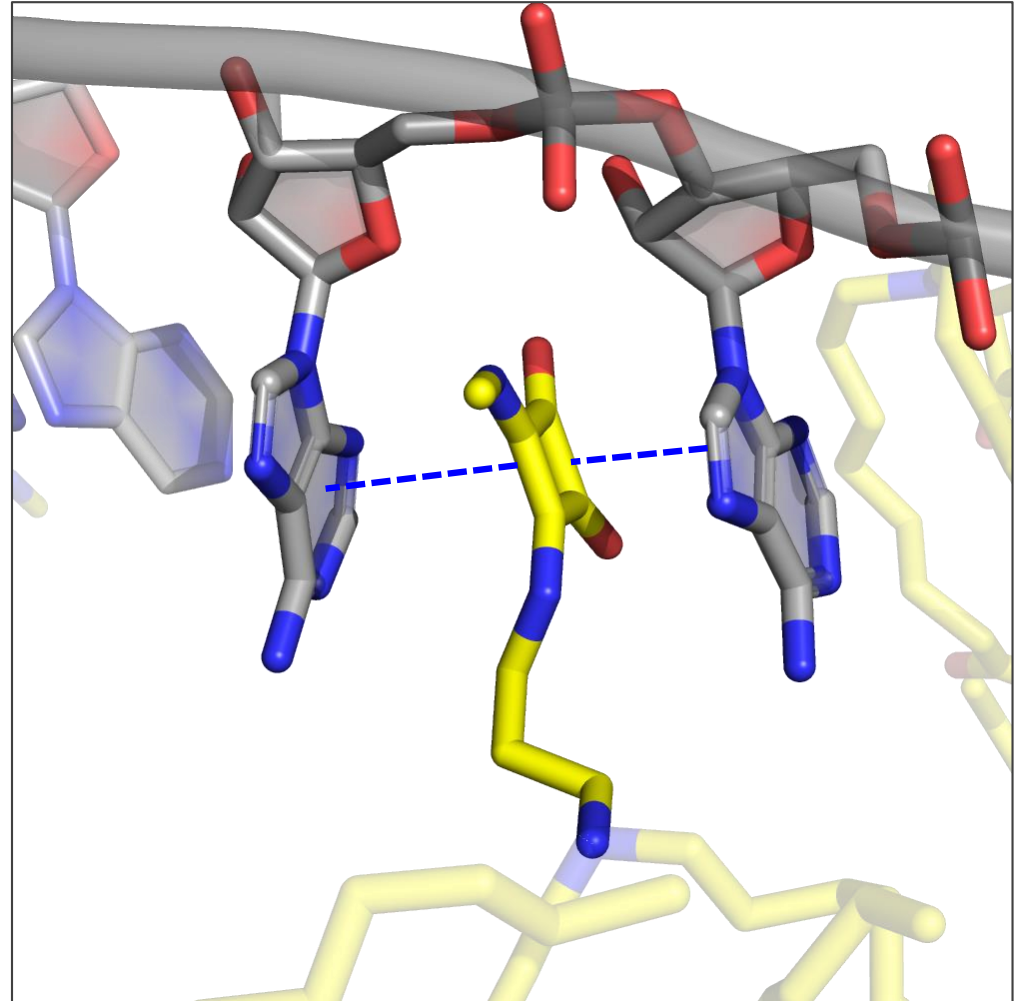
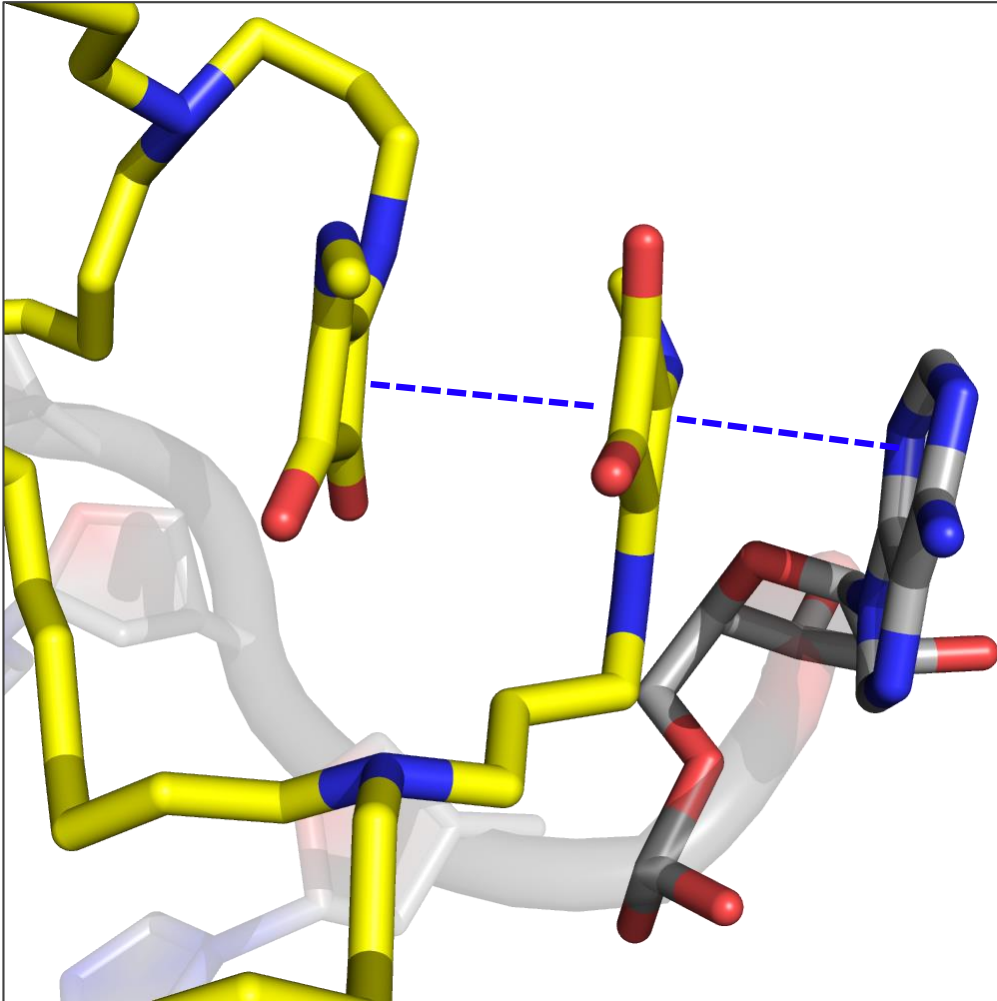
Simulations of squaramide ionizable lipids interacting with mRNA demonstrate preferential interactions with the nucleobases



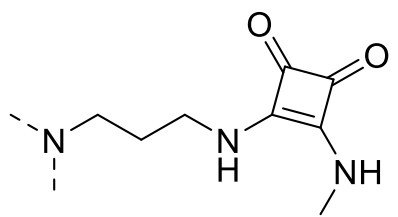
Squaramide ionizable lipids bind mRNA via hydrogen bonds



Squaramide ionizable lipids bind mRNA via pi-stacking

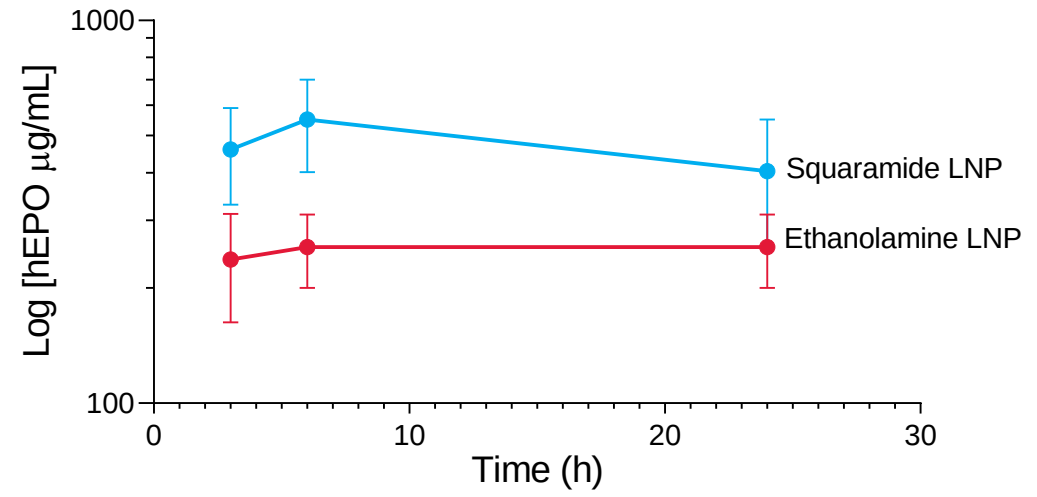


Squaramide based ionizable lipids afford improved protein expression after iv administration

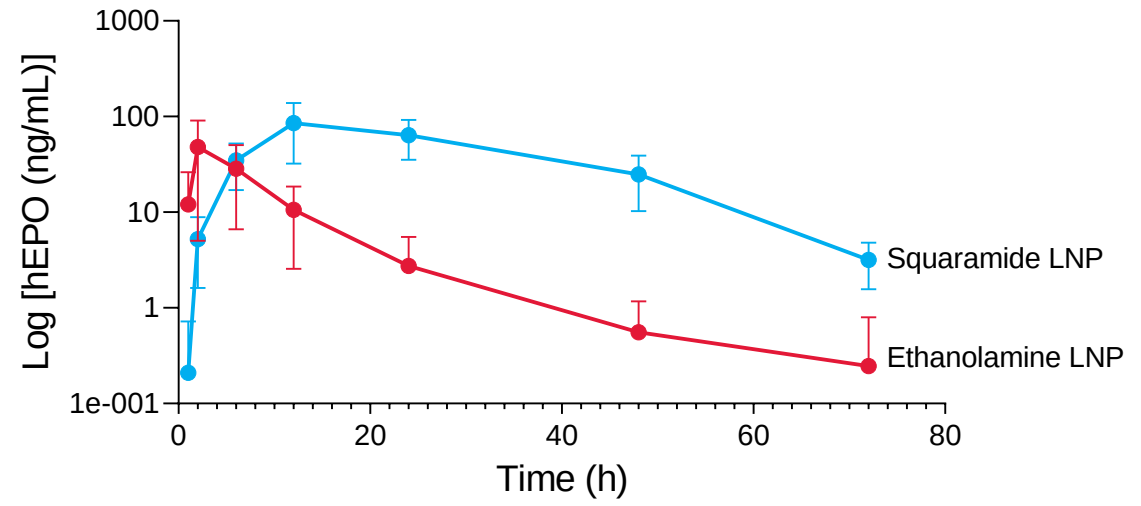


Squaramide ionizable lipid

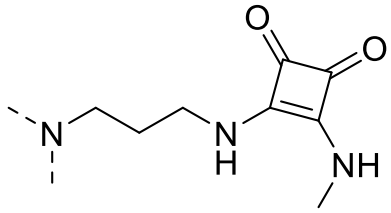
Mouse
IV infusion
0.5 mg/kg
hEPO mRNA



NHP
IV infusion
0.1 mg/kg
hEPO mRNA

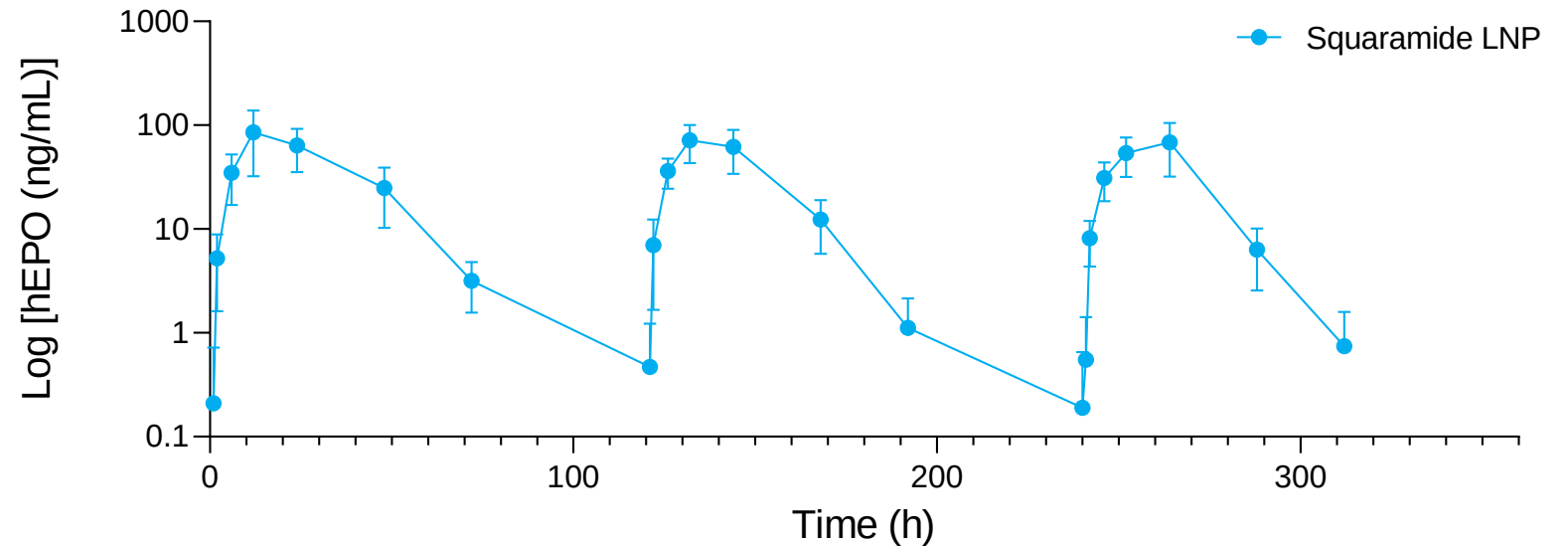


Squaramide based ionizable lipids afford sustained pharmacology



Squaramide
ionizable lipid

NHP
IV infusion
0.1 mg/kg
hEPO mRNA
Biweekly dosing



Squaramide based ionizable lipids efficiently deliver to hepatocytes in cyno liver

NHP

IV infusion

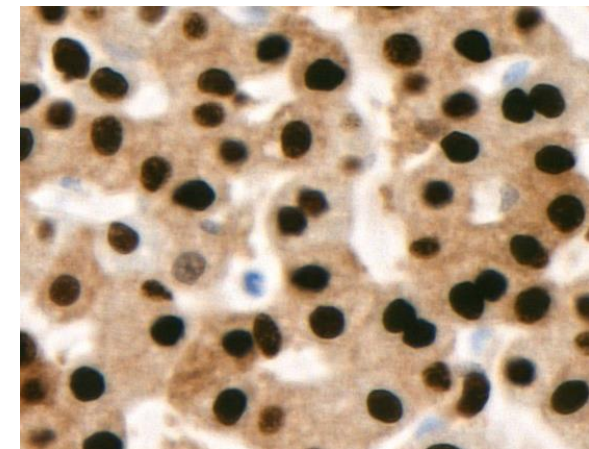
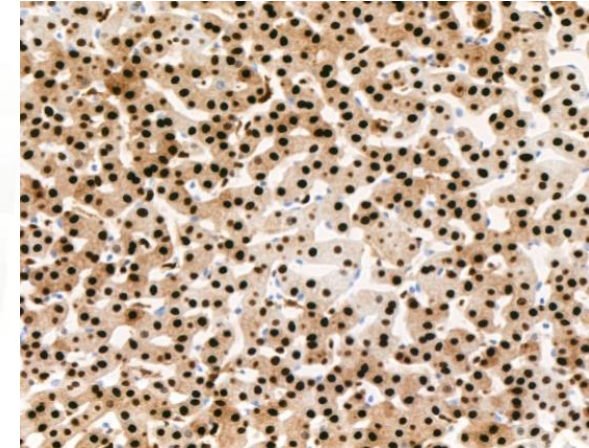
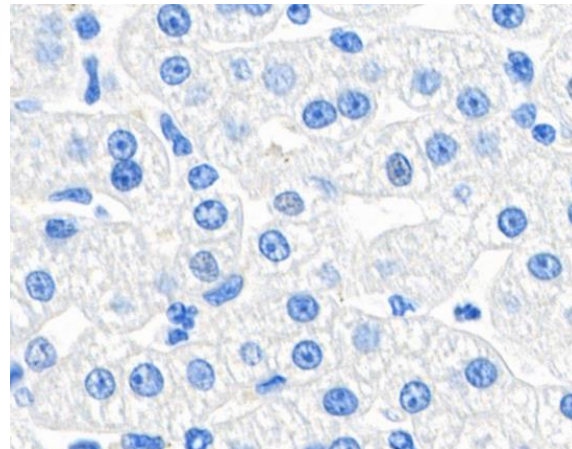
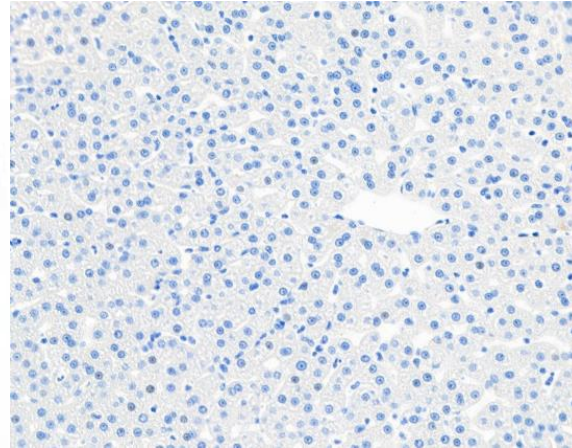
2 mg/kg

NPI-Luc mRNA

6 h immunohistochemistry

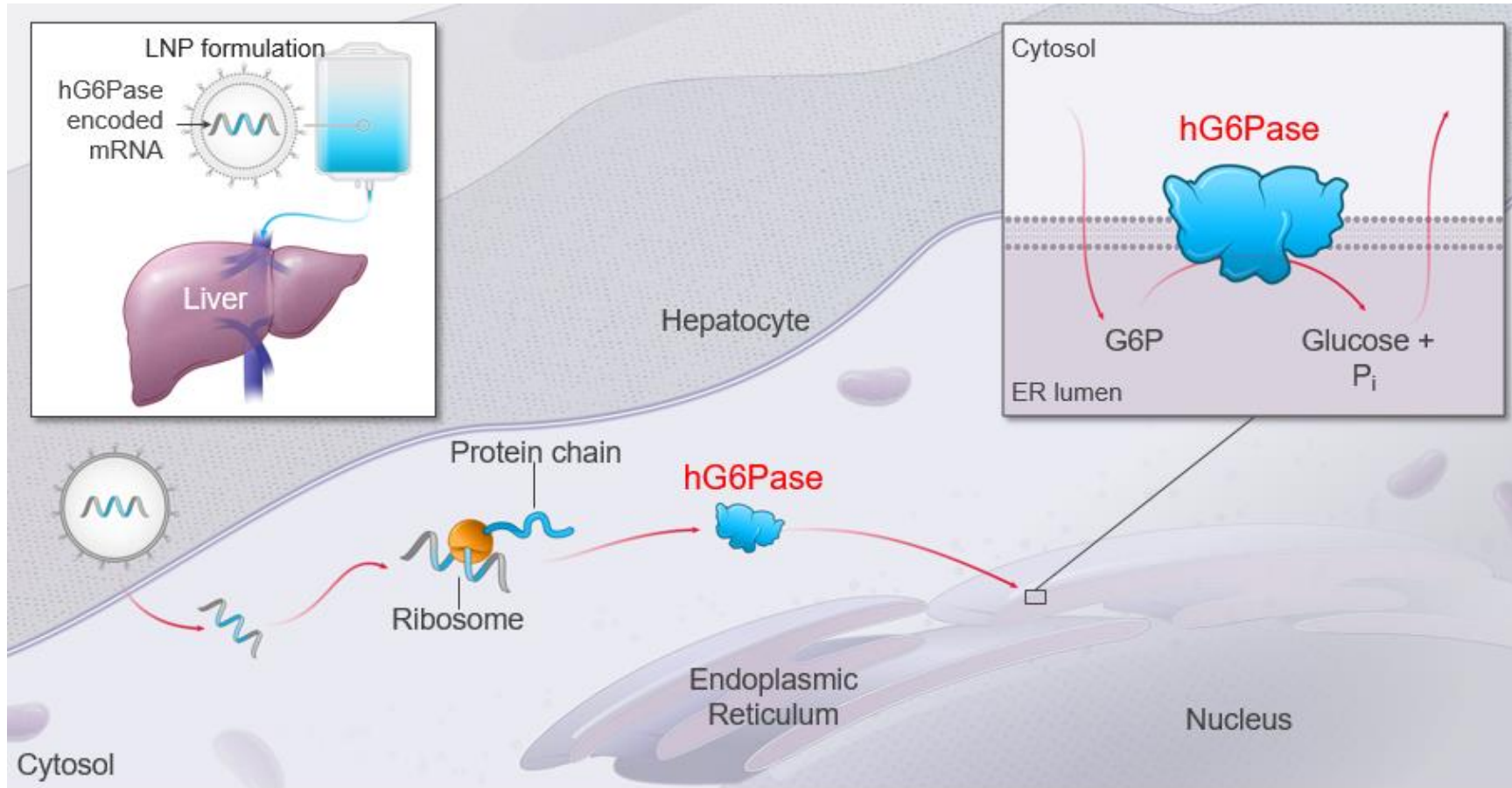
untreated control liver

treated liver



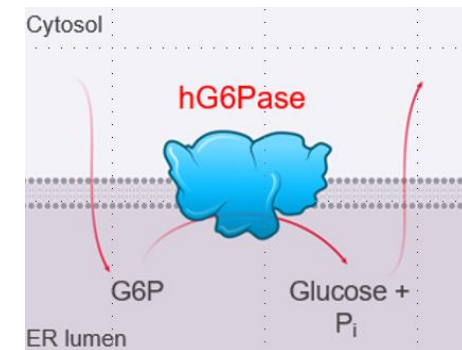
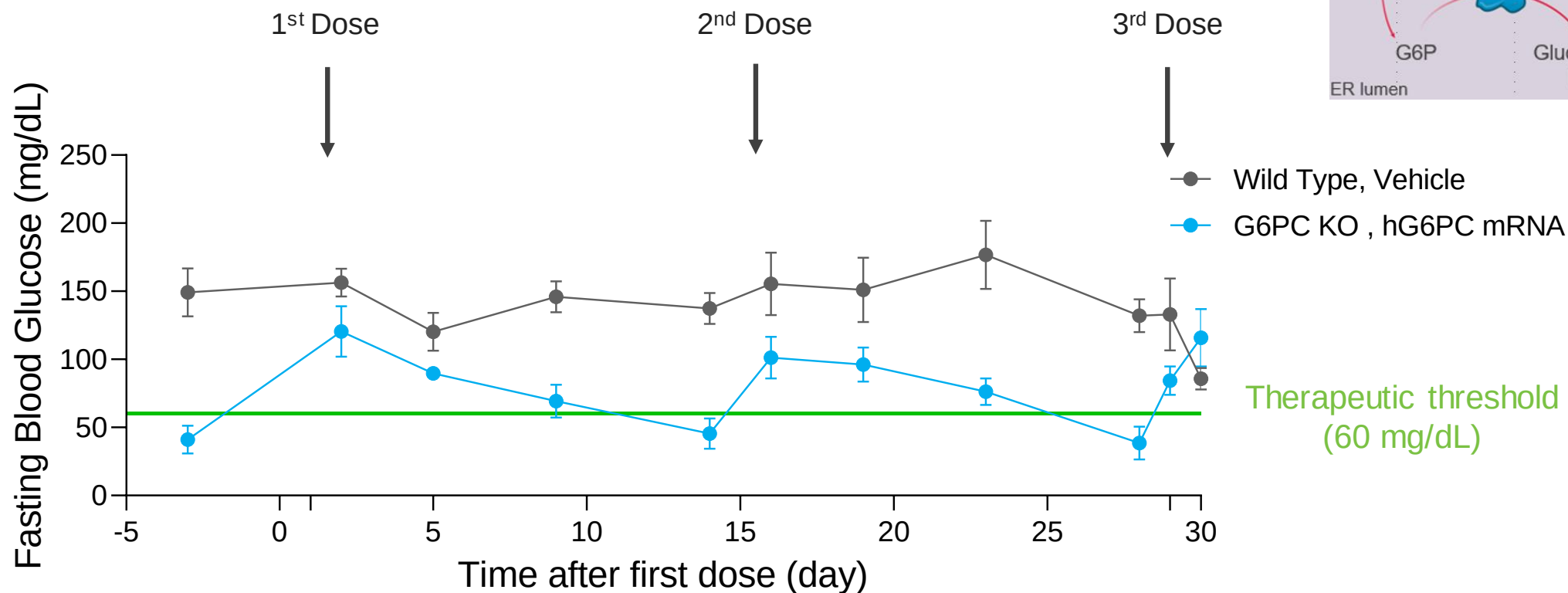
Glycogen Storage Disease Type 1a (GSD1a)

- Deficiency in glucose-6-phosphatase (**G6Pase**), encoded by G6PC gene
→ Patients fail to maintain blood glucose level and suffer hypoglycemia during fasting

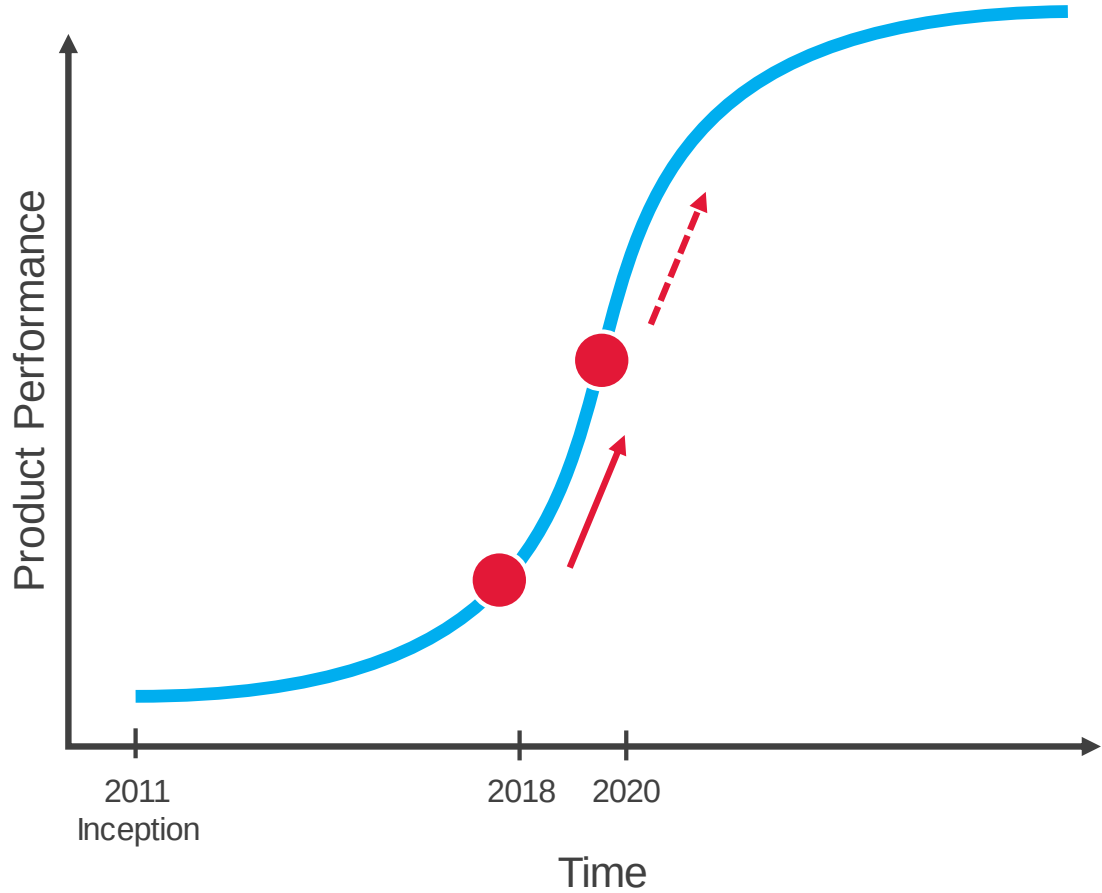
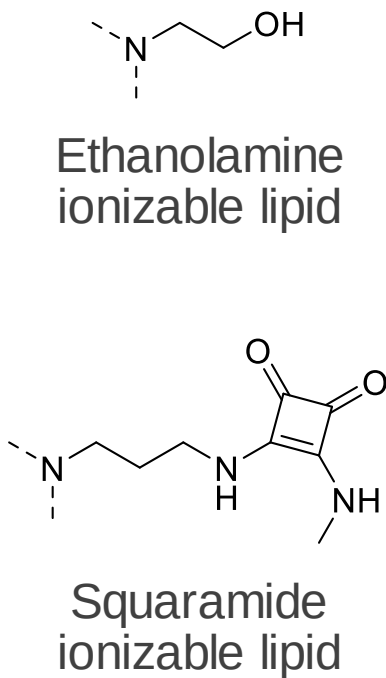


Sustained improvement in fasting blood glucose with the squaramide LNP

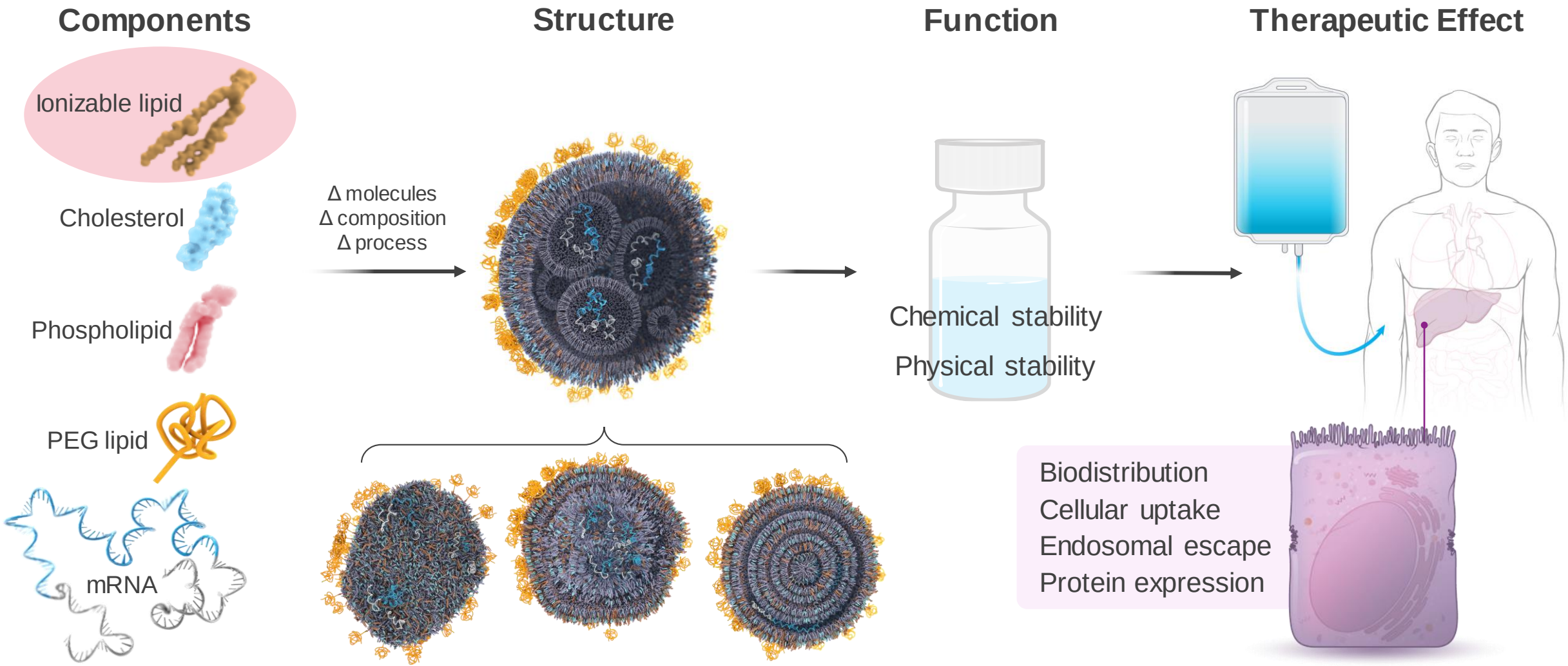
G6PC KO mouse
IV bolus
0.2 mg/kg
G6Pase mRNA



Our commitment to be the best at mRNA science is core to who we are: A 20-year journey



Rational structure-based design of LNPs for mRNA starts with understanding the components and their molecular interactions





Impact of LNP Size on Vaccine Immunogenicity

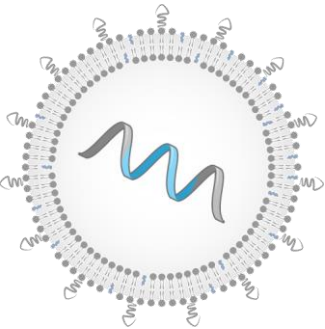
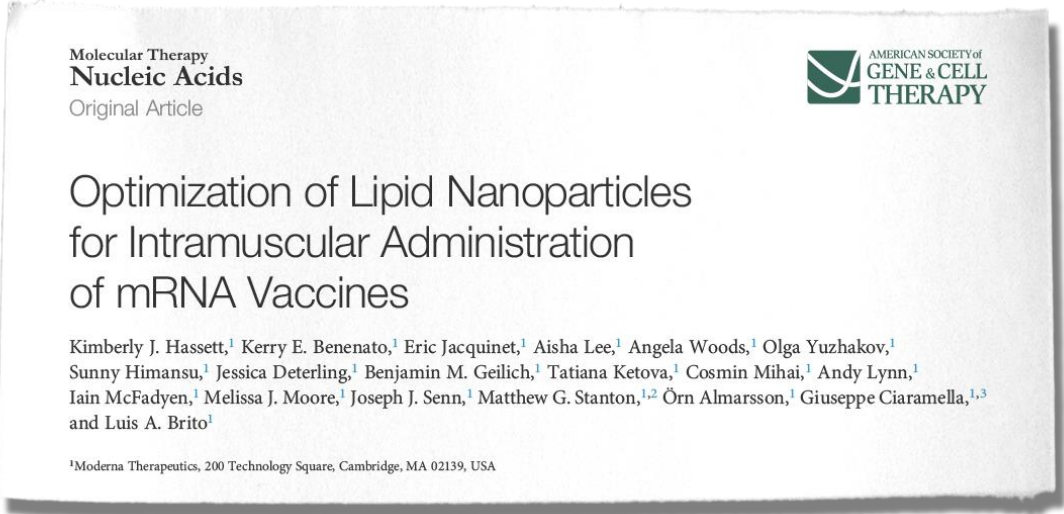
Kimberly Hassett, Ph.D.



Kimberly Hassett, Ph.D.

Senior Scientist, Formulation Science

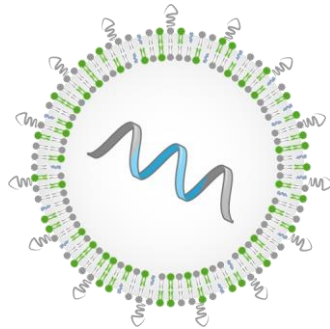
Moderna's proprietary vaccine ionizable lipid improves local expression and tolerability without impacting immunogenicity



Generic
LNP



Improvement of
reactogenicity



Vaccine-optimized
LNP



Expression



Tolerability

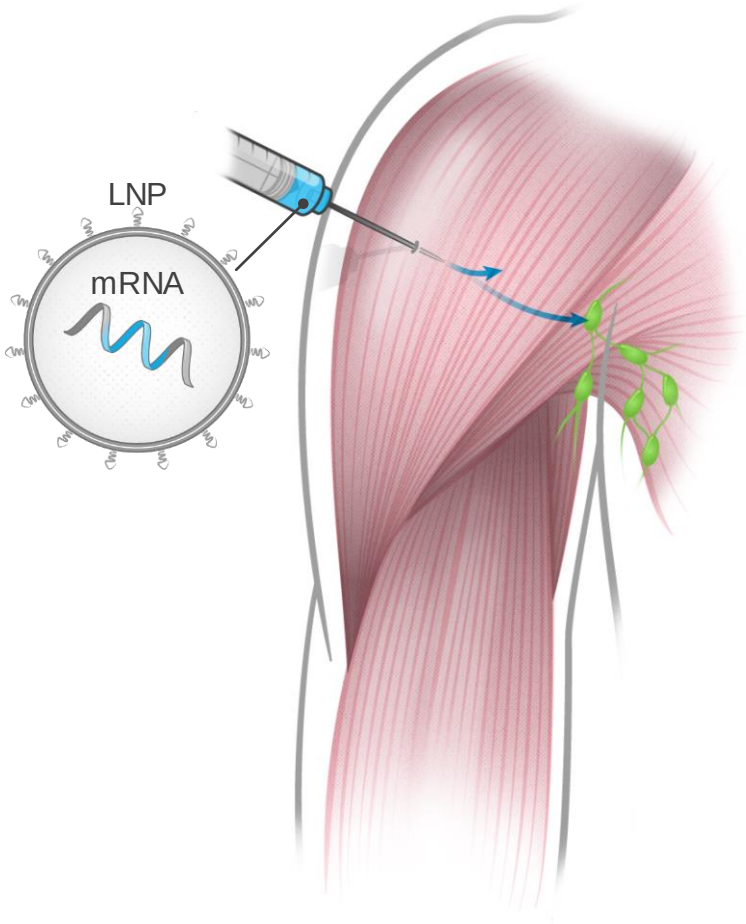


Immunogenicity

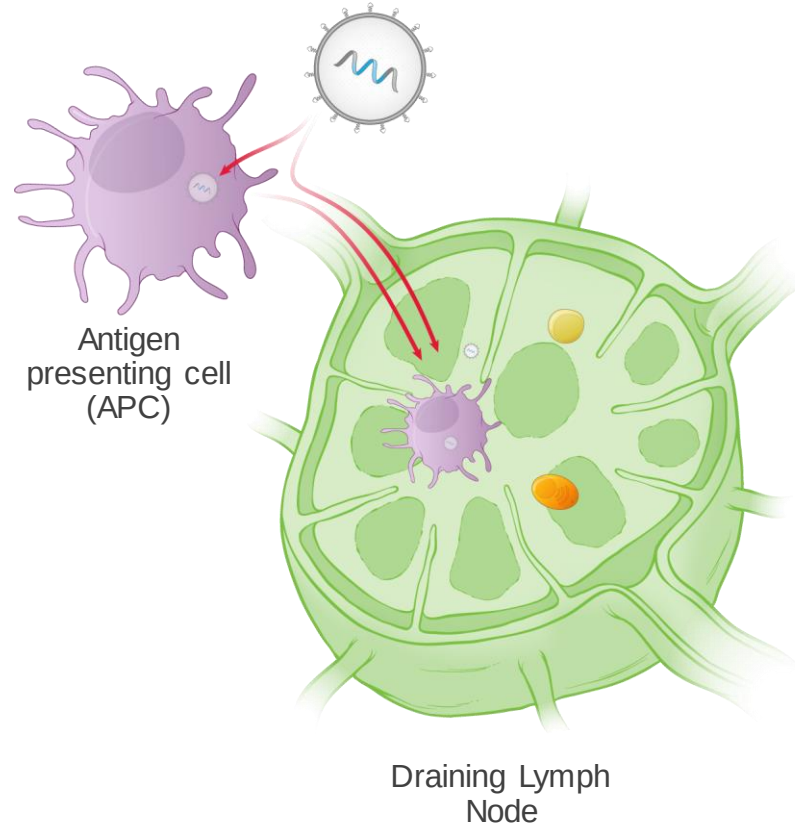


Generating an immune response with mRNA vaccines

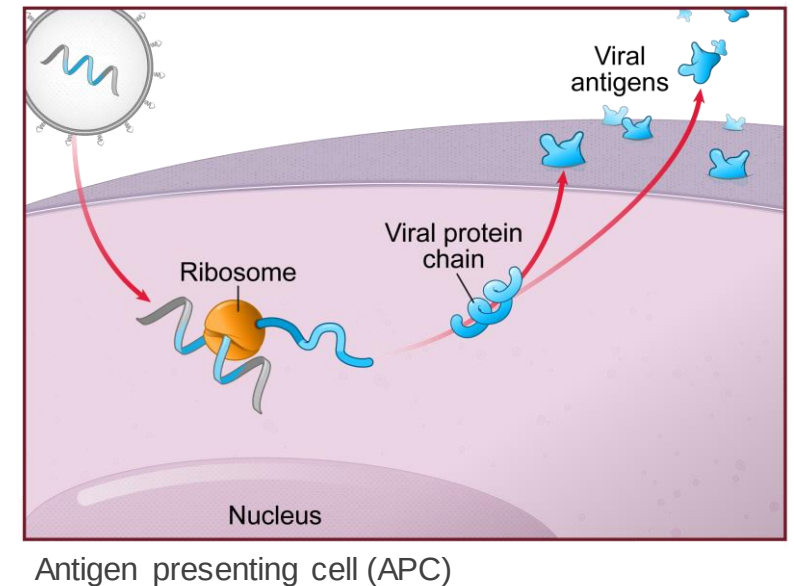
- 1 Recruitment of immune cells to the site of administration



- 2 Migration of LNPs and APC to the draining lymph node



- 3 LNP uptake and antigen expression in cells at the injection site and in draining lymph nodes



Literature suggests particle size can impact immune response



Brewer . . . Russell (2004)

Vesicle Size Influences the Trafficking, Processing, and Presentation of Antigens in Lipid Vesicles¹



Howard . . . Mao (2019)

Critical size limit of biodegradable nanoparticles for enhanced lymph node trafficking and paracortex penetration



Prabha . . . Labhasetwar (2002)

Size-dependency of nanoparticle-mediated gene transfection: studies with fractionated nanoparticles



Manolova . . . Bachmann (2008)

Nanoparticles target distinct dendritic cell populations according to their size



Carstens . . . Jiskoot (2011)

Effect of vesicle size on tissue localization and immunogenicity of liposomal DNA vaccines



Bachmann . . . Jennings (2010)

Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns



Benne . . . Slütter (2016)

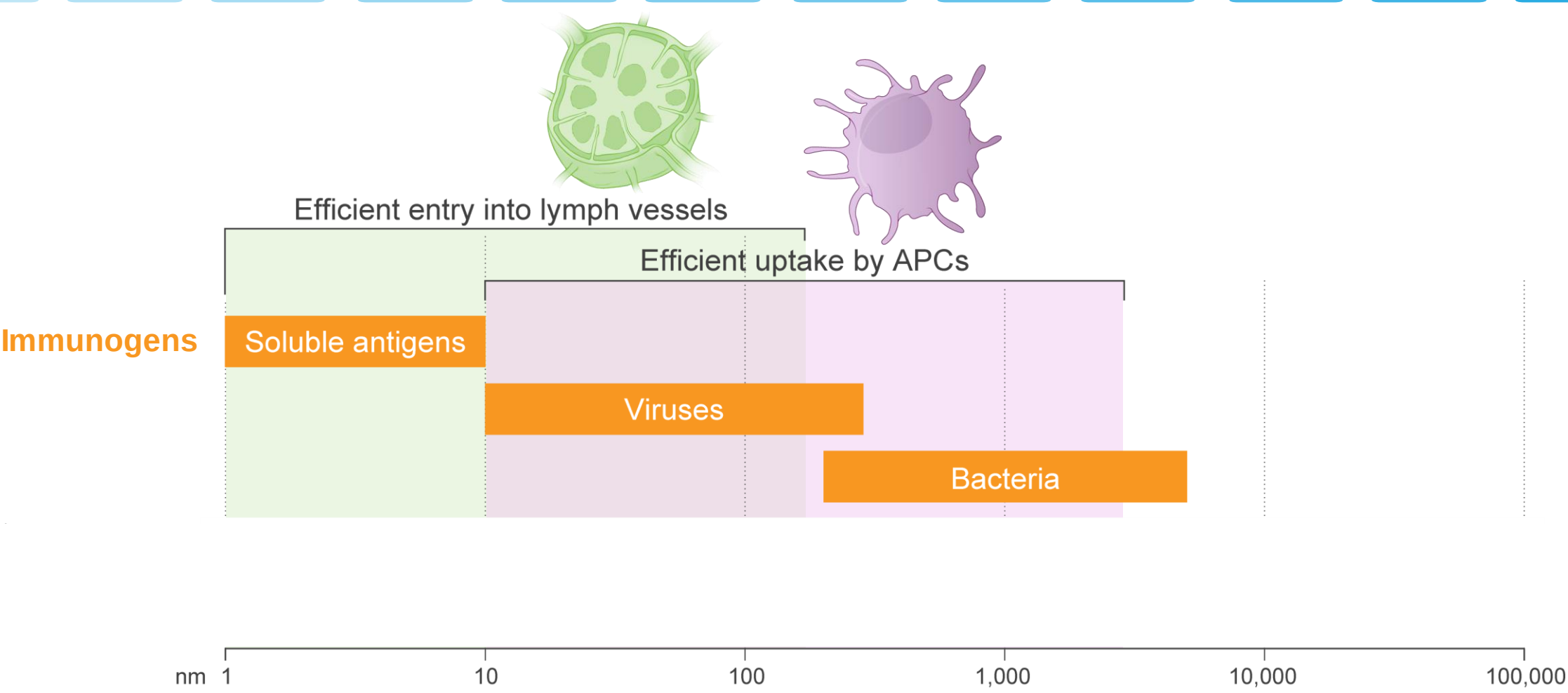
Orchestrating immune responses: How size, shape and rigidity affect the immunogenicity of particulate vaccines



Shang . . . Nienhaus (2016)

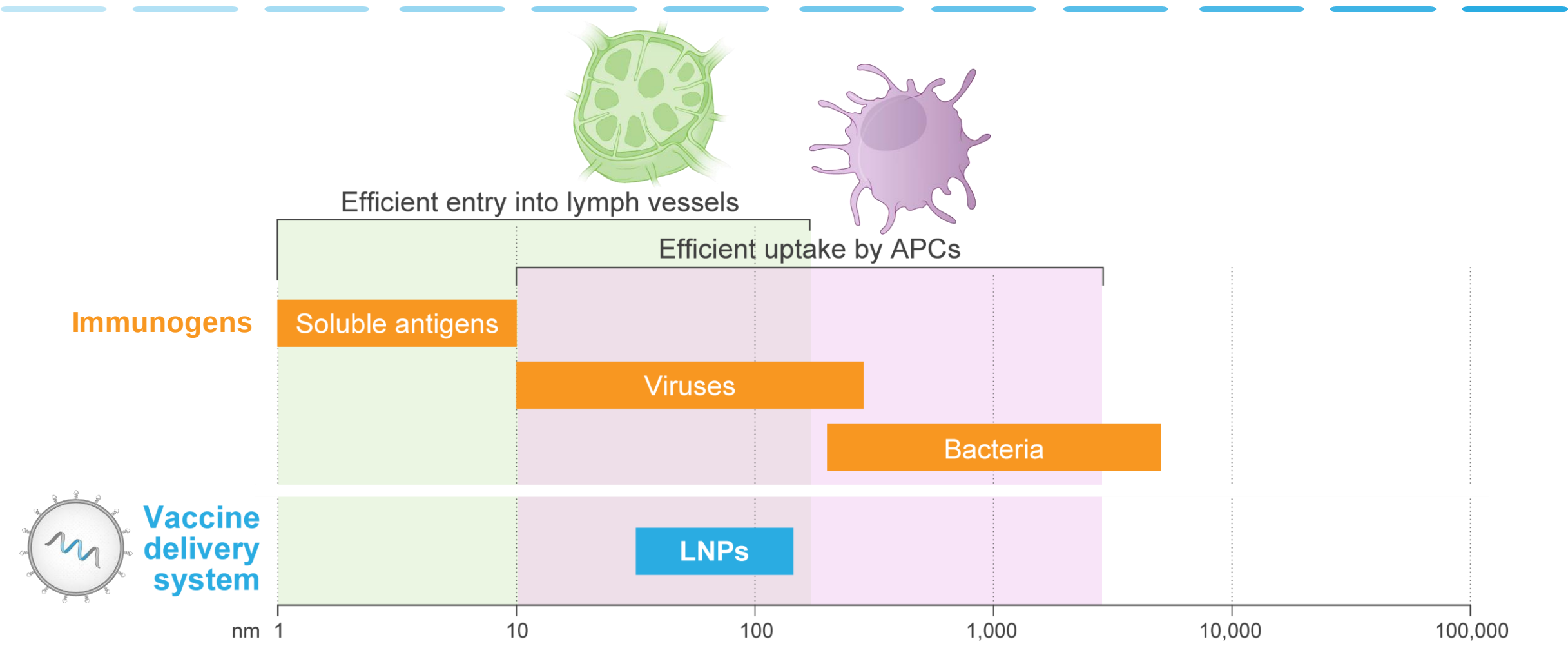
Engineered nanoparticles interacting with cells: size matters

Particle size affects lymphatic vessel entry and APC uptake



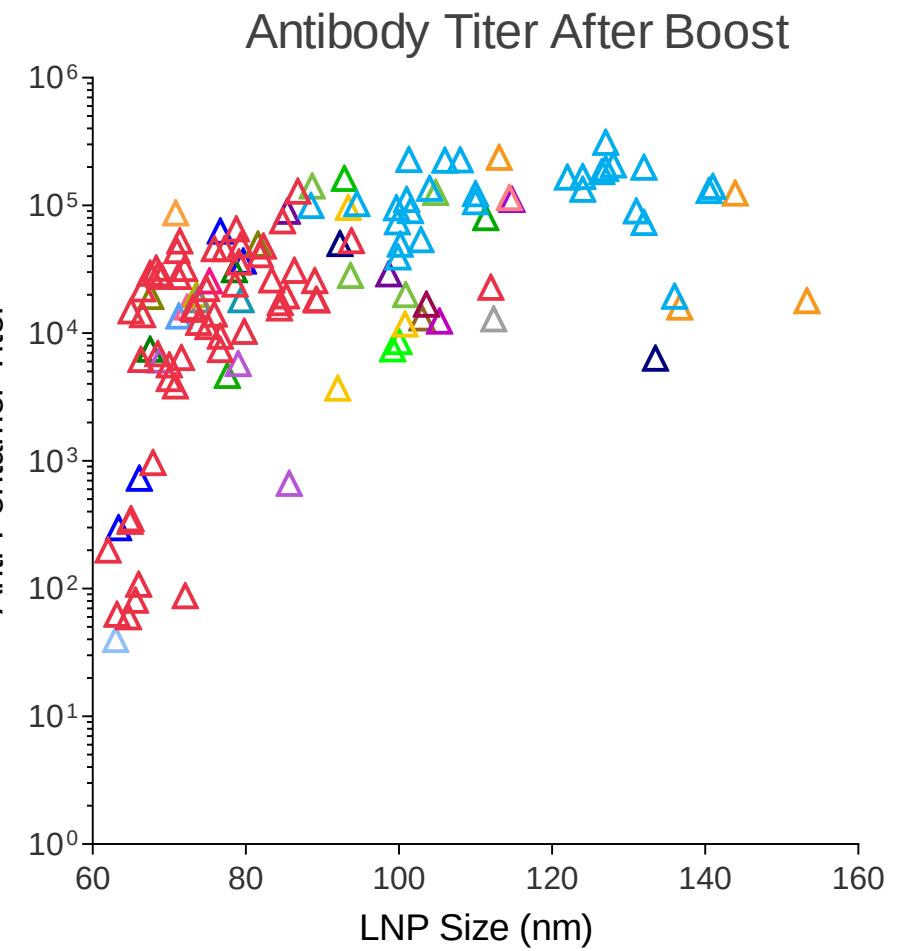
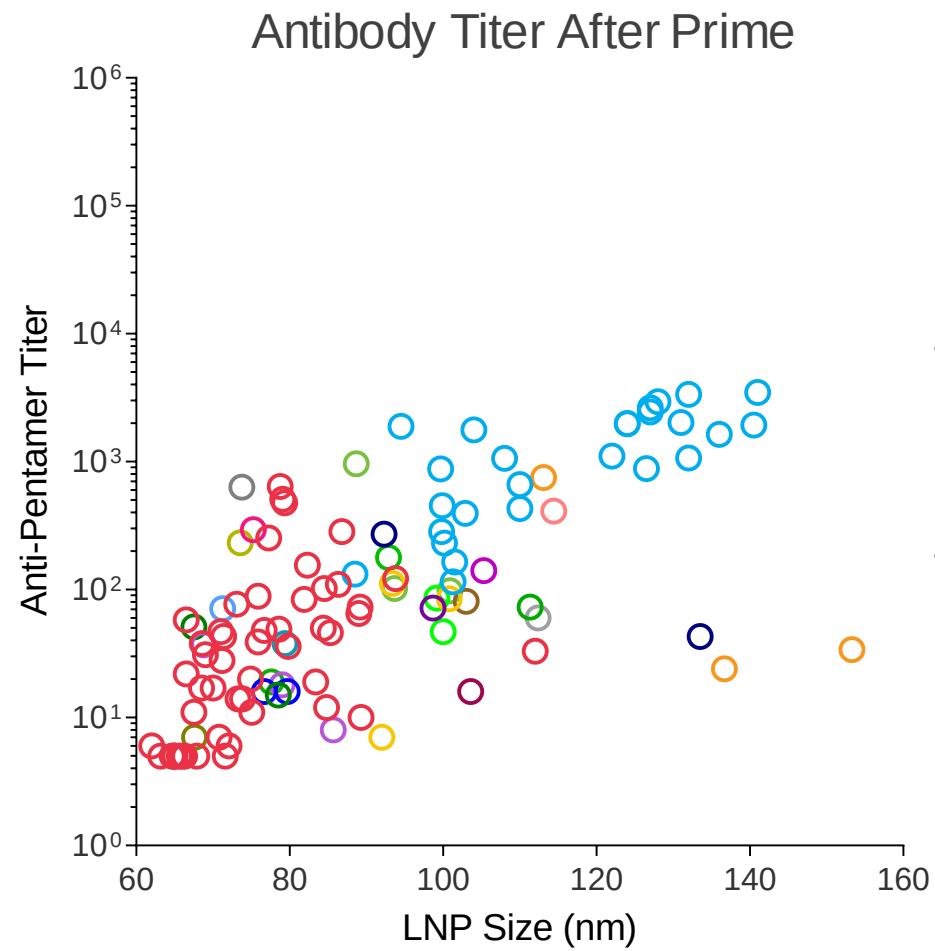
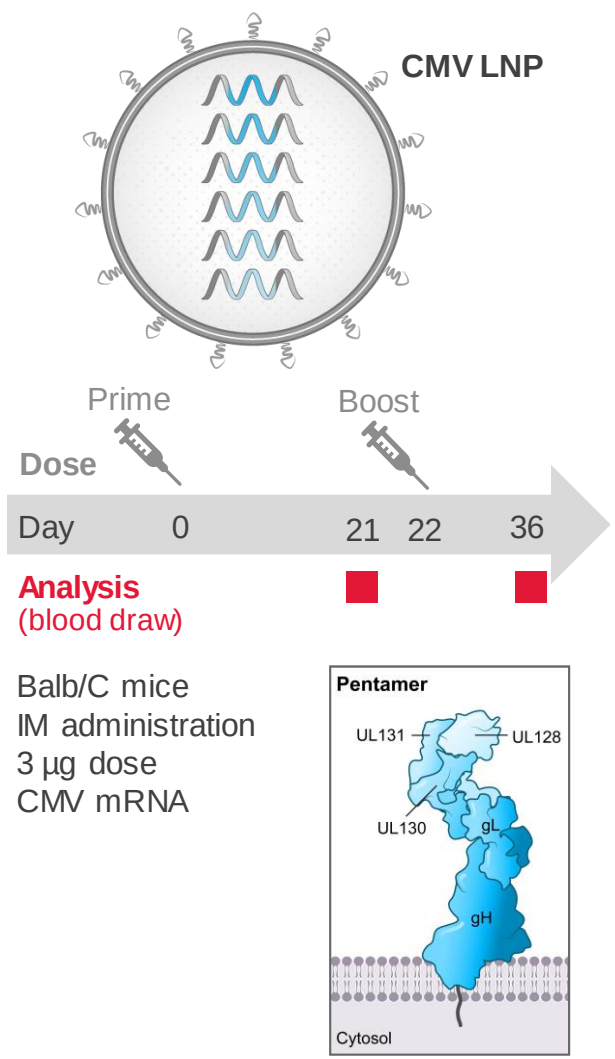
Modified from Bachmann MF and GT Jennings. "Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns" *Nature Reviews Immunology* (2010).

Particle size affects lymphatic vessel entry and APC uptake



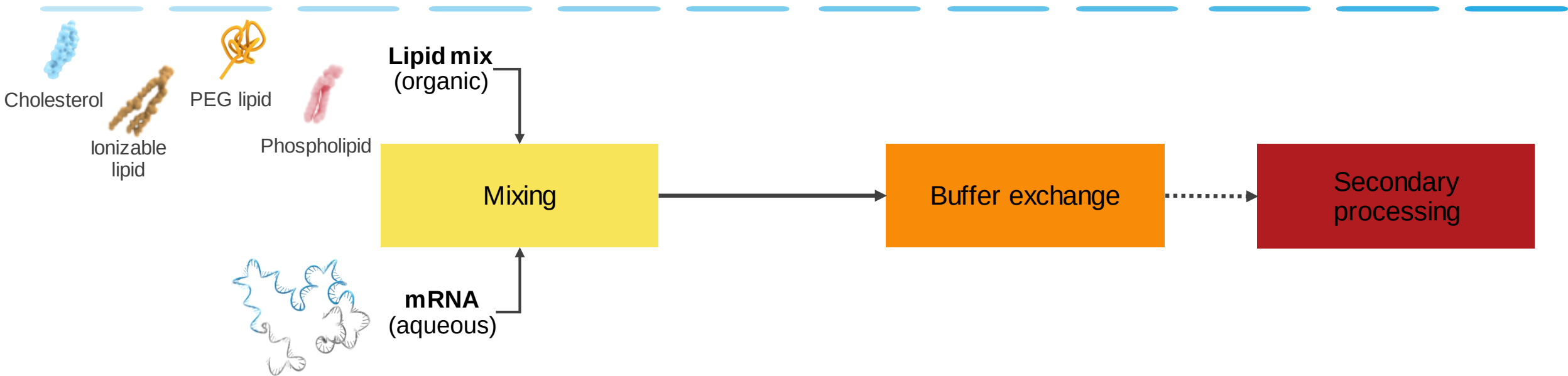
Modified from Bachmann MF and GT Jennings. "Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns" *Nature Reviews Immunology* (2010).

A retrospective analysis shows a trend between CMV LNP size and immunogenicity

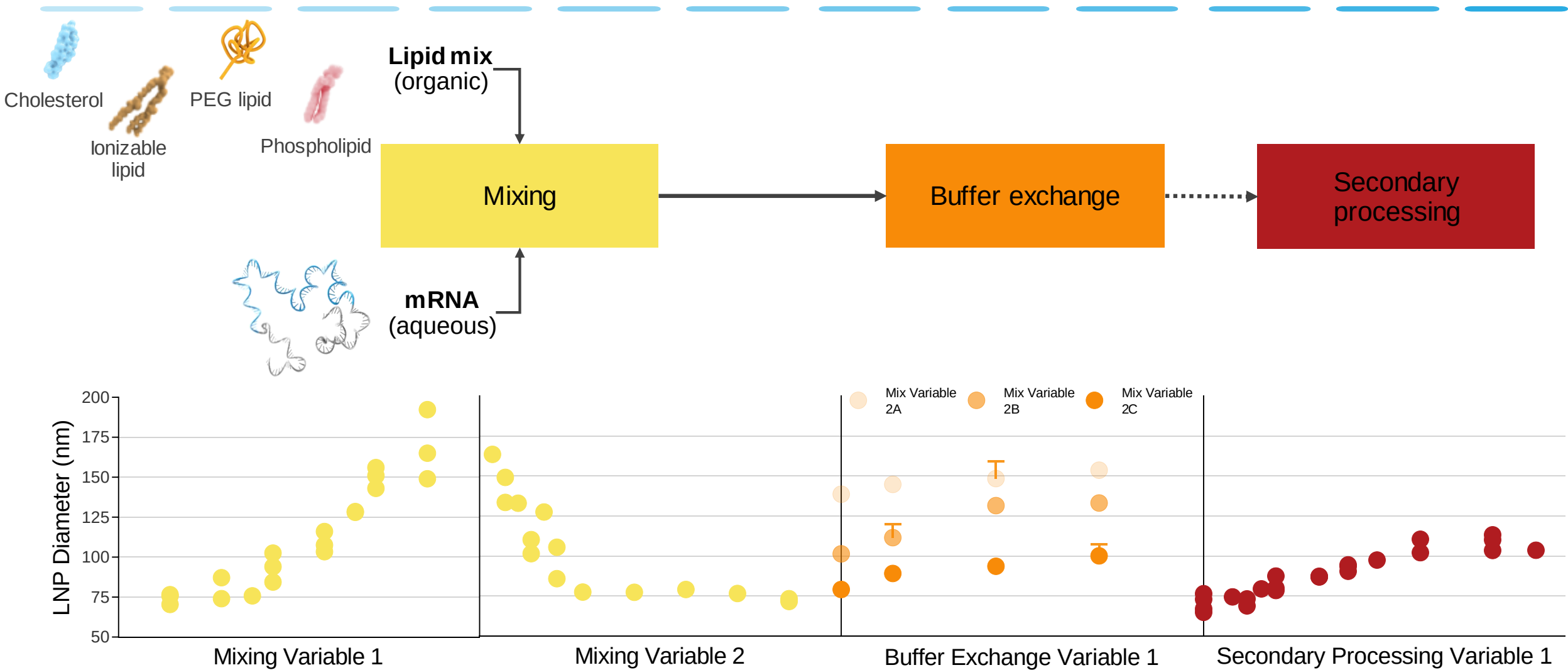


The retrospective analysis contained 23 CMV immunogenicity studies evaluating 129 LNP formulations

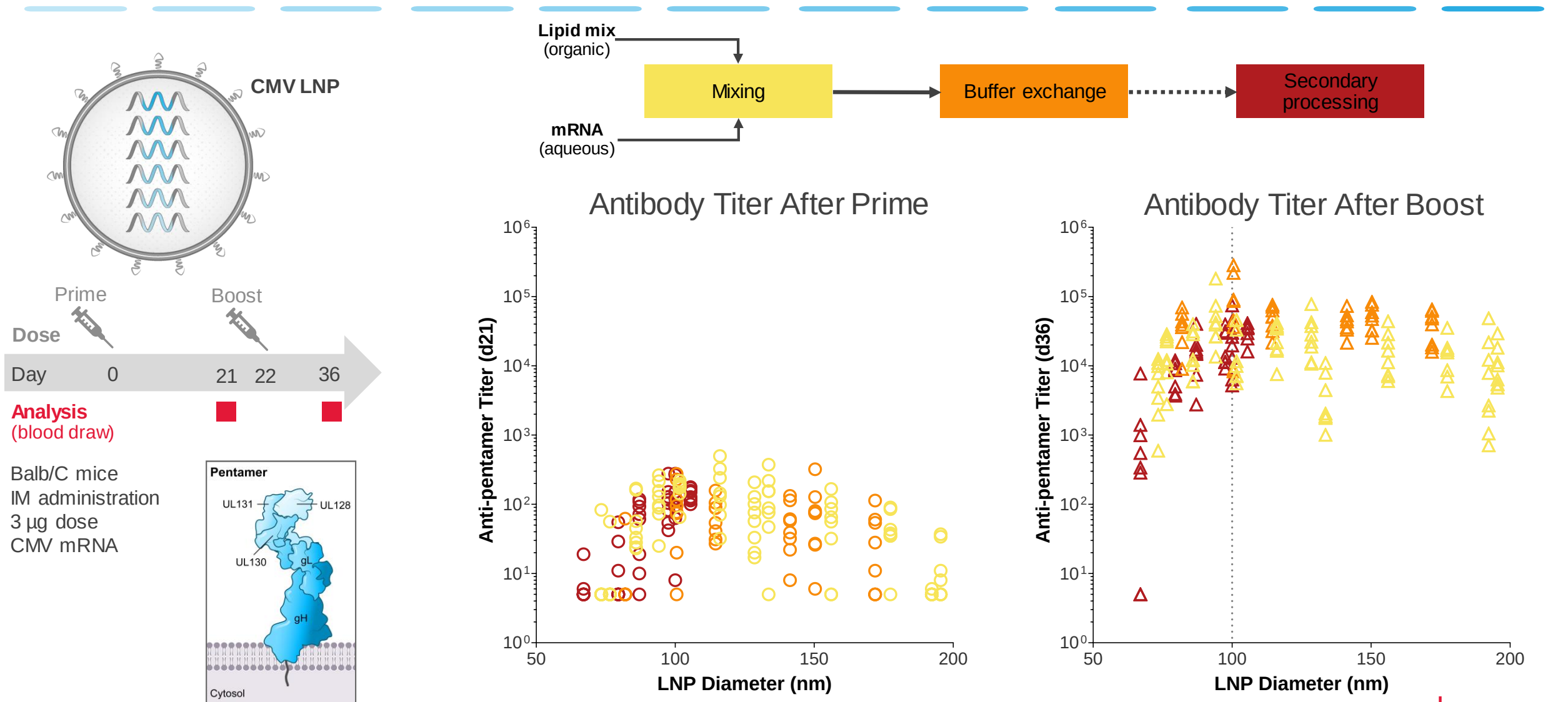
LNP size can be varied by controlling process parameters



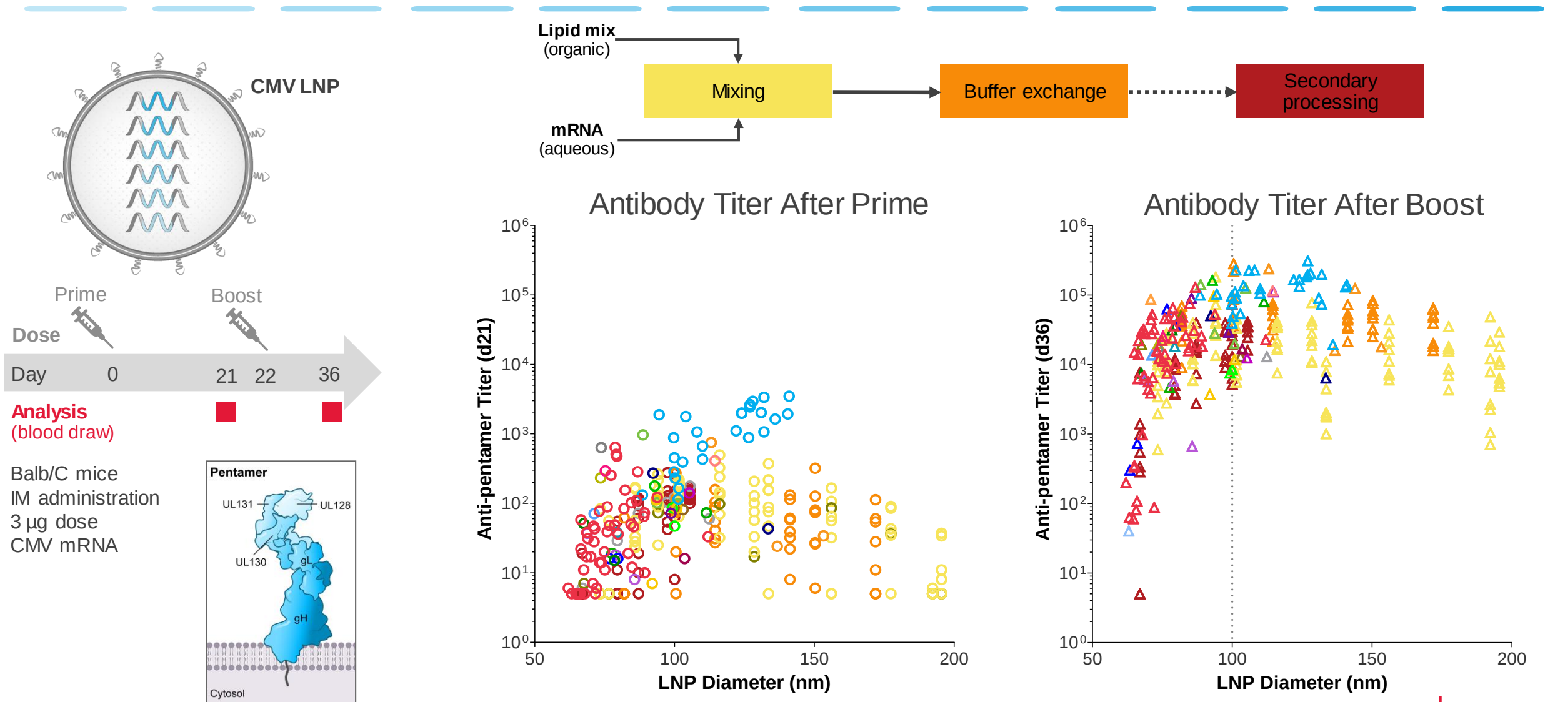
LNP size can be varied by controlling process parameters



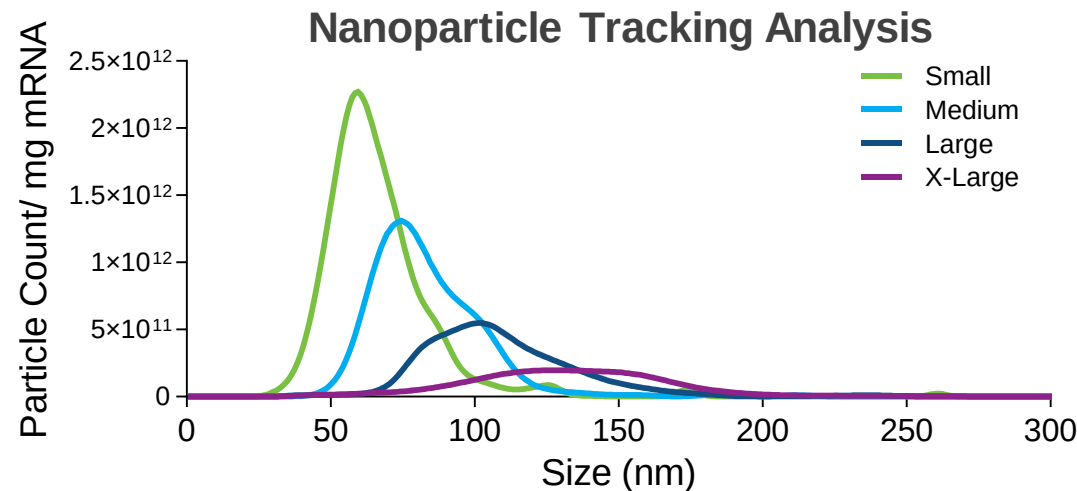
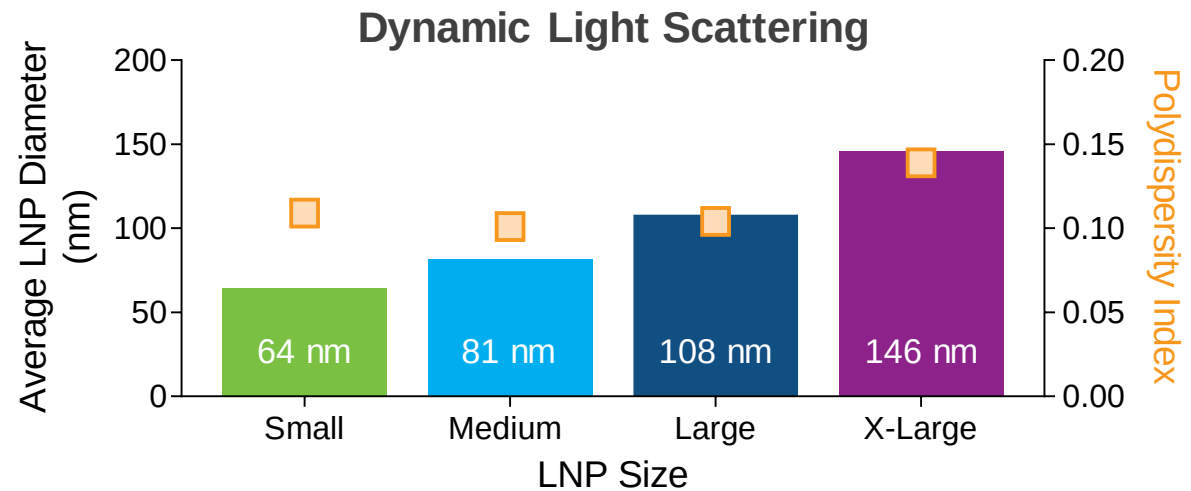
Optimal CMV LNP size is ~100 nm in mice



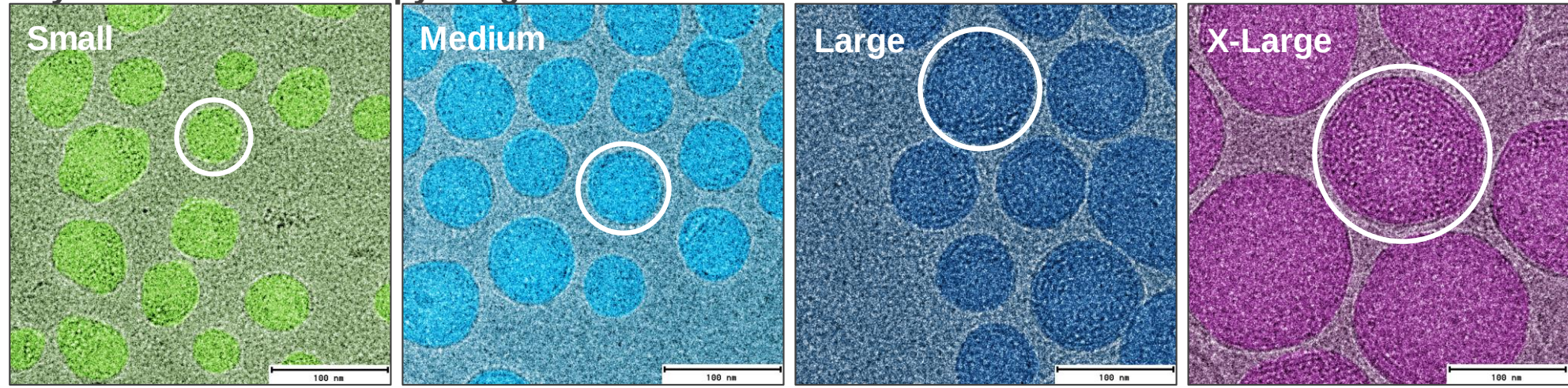
Optimal CMV LNP size is ~100 nm in mice



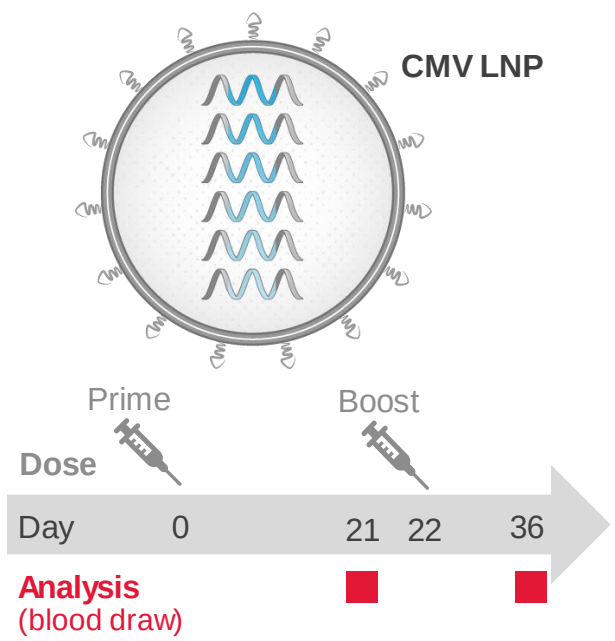
Different sized LNPs were selected for further evaluation



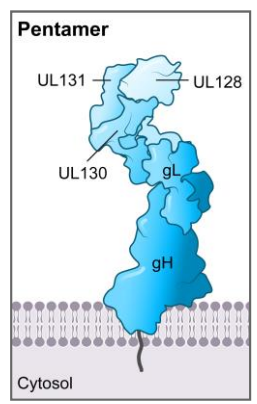
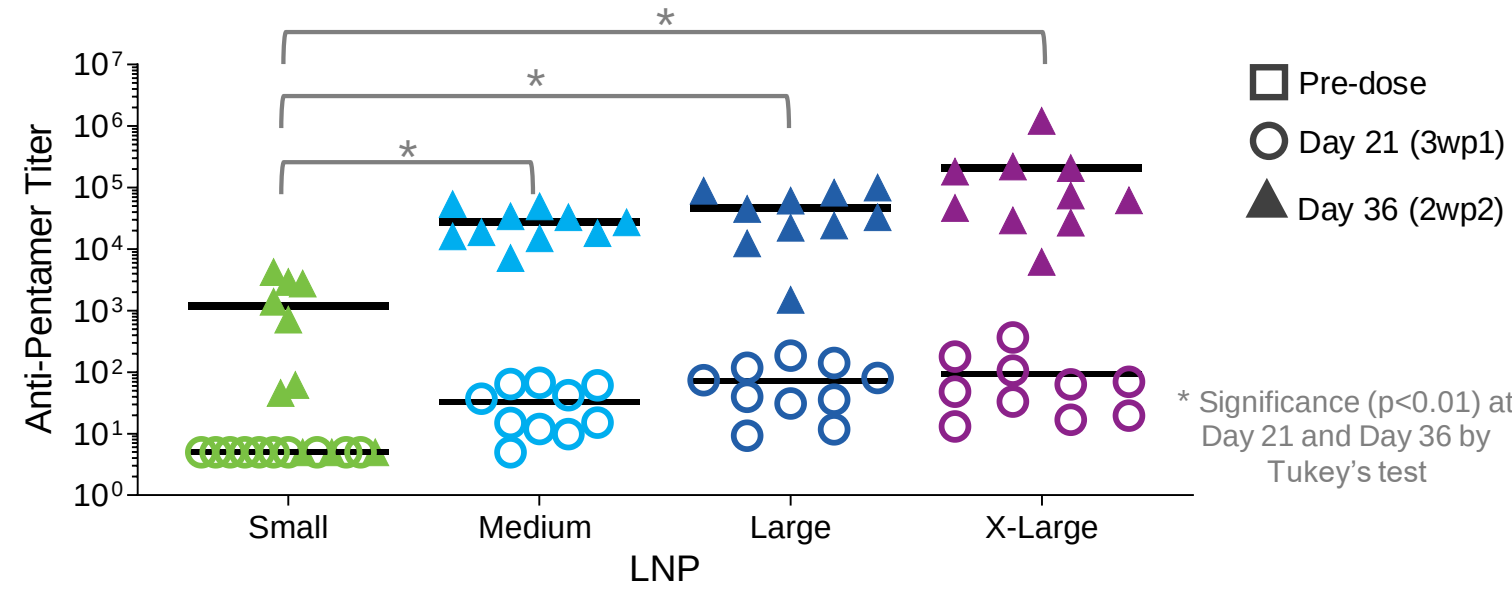
Cryo-Electron Microscopy Images



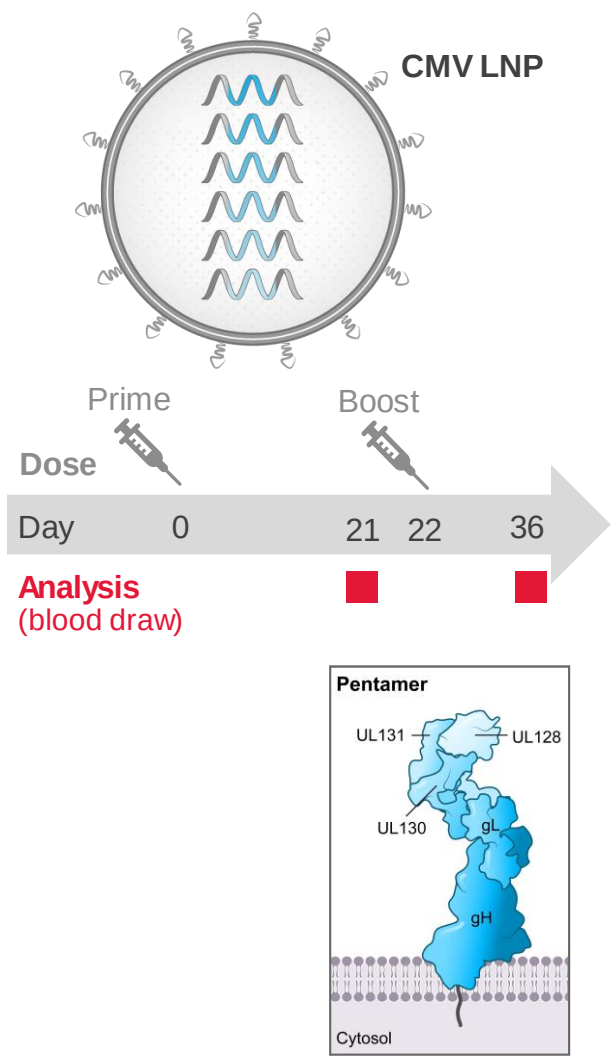
CMV LNP vaccine size evaluated in mice



Mouse
Balb/C mice
IM administration
3 µg dose
CMV mRNA

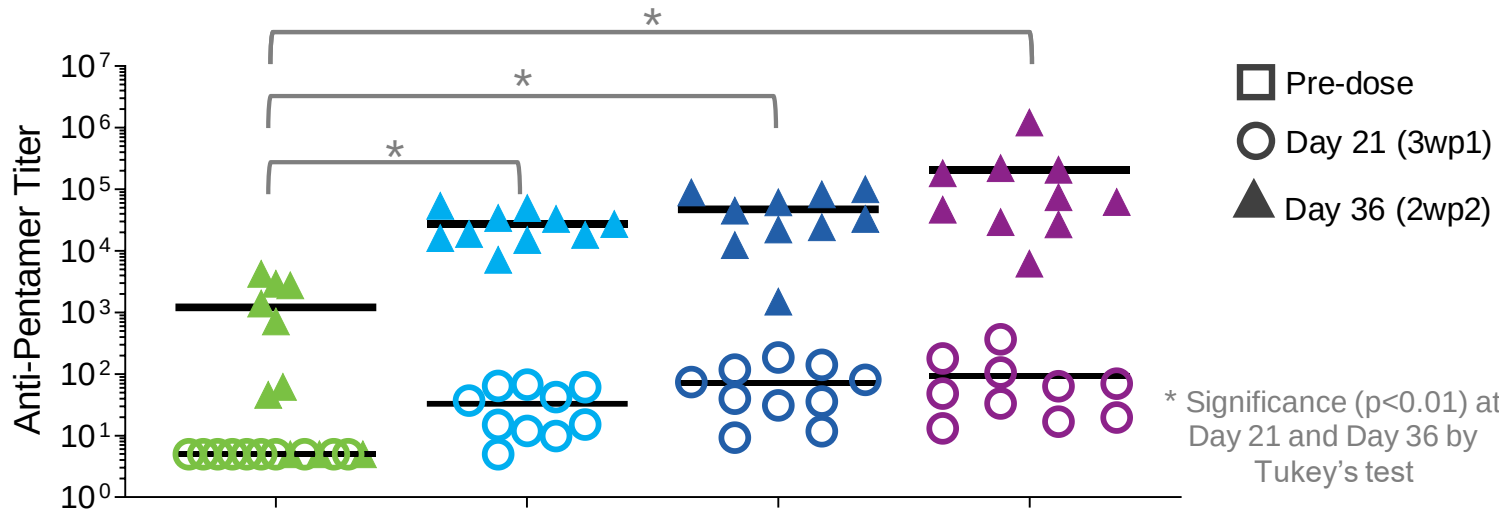


CMV LNP vaccine size evaluated in mice and NHP models



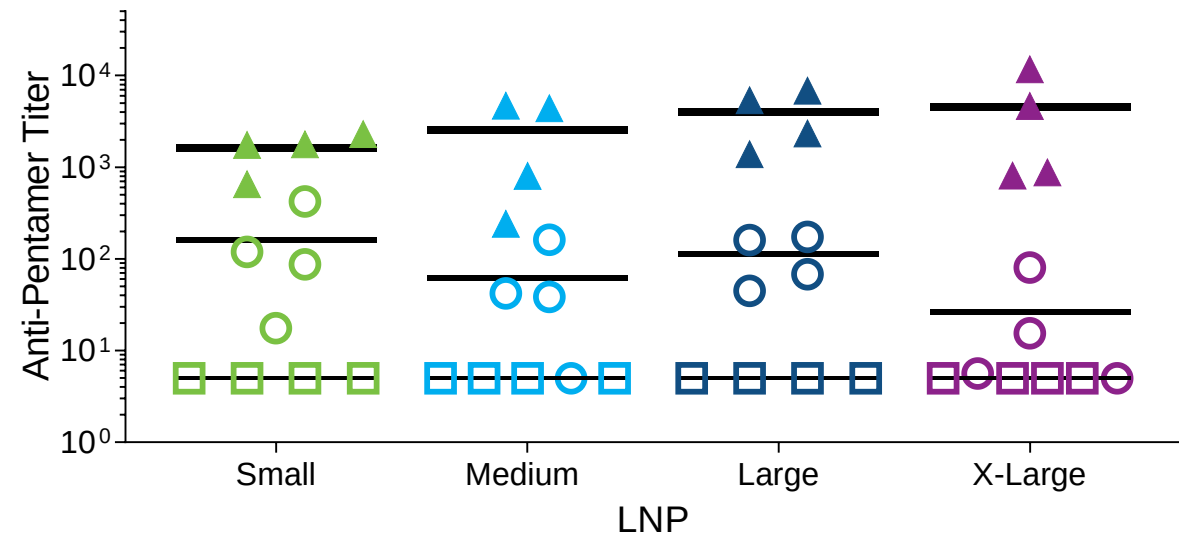
Mouse

Balb/C mice
IM administration
3 µg dose
CMV mRNA



NHP

Cynomolgus monkey
IM administration
30 µg dose
CMV mRNA



Conclusions

- LNP size can be controlled while maintaining lipid composition by modulating formulation process parameters
- In mice, LNP sizes below 80nm induce antibody titers dependent on particle size
- In mice, LNP sizes above 100nm consistently generate high antibody titers independent of particle size
- NHPs are more tolerant to changes in LNP particle size
- Current size range of Moderna's mRNA vaccine LNPs is appropriate for optimal immune responses in primates

2020 Science Day agenda

Introduction	<i>Stéphane Bancel, CEO</i>	8:00-8:10 AM	10 min
Chapter 1 Extending Pharmacology - Controlling the duration of our pharmacologic effect - Enhancing mRNA and protein half-lives	<i>Melissa Moore, Ph.D., Chief Scientific Officer, Platform Research</i>	8:10-8:45 AM	35 min
Chapter 2 T7 Enzyme Engineering to Reduce dsRNA Production	<i>Amy Rabideau, Ph.D., Principal Scientist, Process Development</i>	8:45-9:05 AM	20 min
Chapter 3 New LNPs for Liver Delivery	<i>Kerry Benenato, Ph.D., Senior Director, Platform Chemistry</i>	9:05-9:25 AM	20 min
Chapter 4 Impact of LNP Size on Immunogenicity	<i>Kimberly Hassett, Ph.D., Senior Scientist, Formulation Science</i>	9:25-9:40 AM	15 min
Break		9:40-9:50 AM	10 min
Chapter 5 Our Progress Toward Creating a mRNA Vaccine for HIV - Vaccine design to elicit broadly neutralizing antibodies against HIV - NIH-Moderna HIV-1 vaccine	<i>Andrea Carfi, Head of Research, Infectious Disease</i> <i>William Schief, Ph.D., Scipps, International Aids Vaccine Initiative (IAVI)</i> <i>Paolo Lusso, M.D., Ph.D., National Institute of Allergy and Infectious Diseases (NIAID)</i>	9:50-10:50 AM	60 min
Conclusion	<i>Stephen Hoge, M.D., President</i>	10:50-11:00 AM	10 min
Q&A		11:00-11:30 AM	30 min

Andrea Carfi



Andrea Carfi

Head, Infectious Disease Research

mRNA vaccines overview

Key characteristics and differentiation

1. Large product opportunity

- ✓ Ability to do complex antigens
- ✓ Ability to do combination vaccines

2. Higher probability of technical success

- ✓ Vaccine mechanism of action (MOA)
- ✓ B-cell and T-cell response

3. Accelerated research and development timelines

- ✓ Time to clinical study/market
- ✓ Uniform process allows for fast scale up

4. Greater capital efficiency over time vs. recombinant technology

- ✓ Lower capex
- ✓ Greater flexibility

Moderna's vaccine franchise:

- **7 vaccines** for major unmet needs
- **>1,500 healthy volunteers** enrolled in vaccine clinical trials
- Observed neutralizing antibodies to viral antigens **against all eight viruses** for which we have had clinical trial readouts

HIV collaborations with BMGF, IAVI and NIH

Important part of Moderna's commitment to public health

Commitment to public health

- ✓ Long standing collaborations with BMGF, IAVI and NIH
- ✓ HIV remains a high unmet need with 2.1 million new annual infections¹
- ✓ In ~30 years, only 6 HIV-1 efficacy vaccine trials

BILL & MELINDA
GATES *foundation*

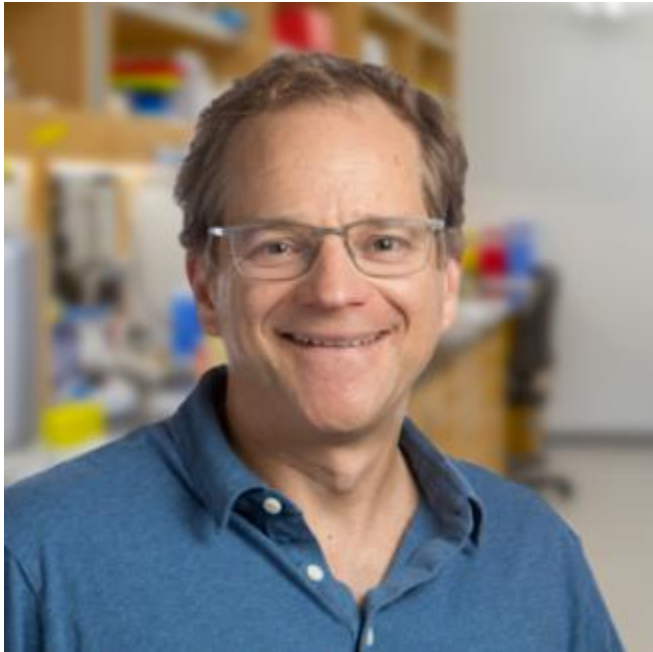
Applying mRNA to challenging biological and technological problems

- ✓ Moderna's mRNA technology to accelerate HIV vaccine discovery by rapid production and clinical testing for novel vaccine candidates
- ✓ mRNA vaccine platform allows for rapid iterative cycles of design and human testing enabling novel vaccination strategies
- ✓ mRNA technology expands the antigen design space enabling the generation of vaccine candidates that can not be easily manufactured otherwise



William Schief, Ph.D.

*Professor, Immunology and Microbiology, Scripps
Executive Director, Vaccine Design, IAVI*



William Schief is a Professor in the Immunology and Microbial Science Department at The Scripps Research Institute in La Jolla, CA, Director for Vaccine Design at the International AIDS Vaccine Initiative (IAVI), and an Associate Member of the Ragon Institute of MGH, MIT and Harvard. He is also a founder and board member of CompuVax, Inc., a startup biotech company focused on vaccine design.

William received a B.S. in Applied Mathematics from Yale University and a Ph.D. in Physics from the University of Washington. Dr. Schief's work focuses on computation-guided and structure-based design of immunogens and immunization regimens, with the goal of inducing broadly neutralizing antibodies against HIV and other pathogens that have frustrated traditional vaccine design strategies.

Paolo Lusso, M.D., Ph.D.

Chief, Viral Pathogenesis, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases (NIAID)



Dr. Lusso was born in Torino, Italy. He received his M.D. from the University of Torino and his Ph.D. from the University of Bologna. He is board certified in Internal Medicine and Infectious Diseases. From 1986 to 1994 he worked as a Visiting Scientist in the Laboratory of Tumor Cell Biology, NCI, Bethesda, MD.

From 1994 to 2007, he directed the AIDS Research Laboratory at the San Raffaele Institute in Milan, Italy. In 2007, he returned to the US where he became the Chief of the Viral Pathogenesis Section in the Laboratory of Immunoregulation, NIAID, Bethesda.

He is an elected Member of the *European Molecular Biology Organization (EMBO)* and a Fellow of the *American Academy of Microbiology*. His research is focused on the mechanisms of HIV/AIDS pathogenesis and the development of an HIV vaccine. His 1995 discovery of the HIV-suppressive chemokines was nominated “Breakthrough of the Year” by *Science* magazine.

Vaccine Design to Elicit Broadly Neutralizing Antibodies Against HIV

William Schief
Professor, Immunology and Microbiology, Scripps
Executive Director, Vaccine Design, IAVI

Moderna Science Day

June 2, 2020

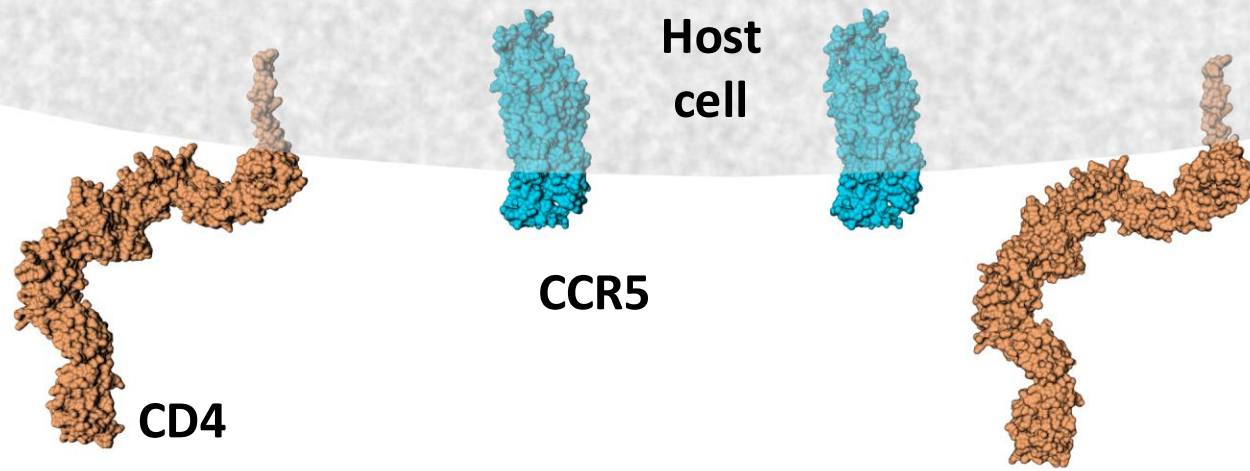
Information in following slides are from a third-party source. While we believe such information is reliable, we have not independently verified any third-party information, and make no guarantee, express or implied, as to the accuracy and completeness of it

HIV/AIDS Continues to Devastate

- **1.8 million new infections per year (nearly 5,000 per day)**
- **37 million people living with HIV – 40% not receiving ART**
- **1 million deaths per year due to HIV**
- **35 million people have died due to HIV**

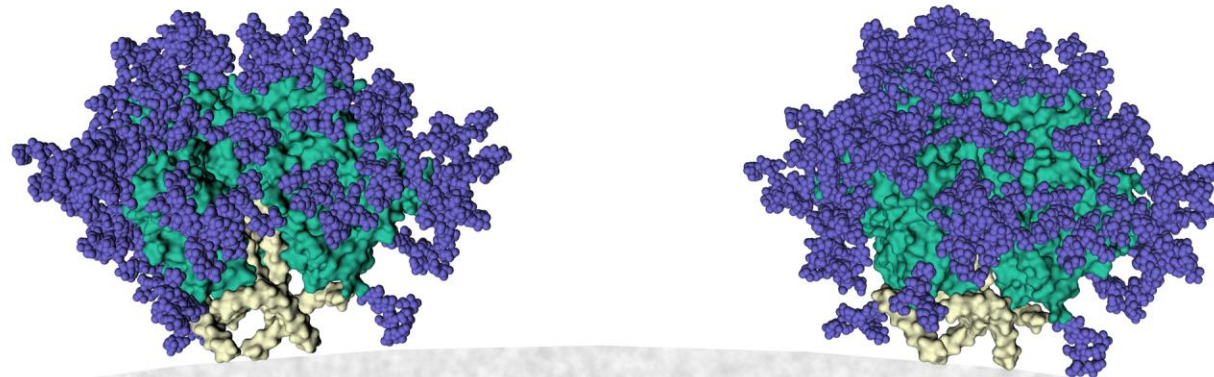
We need a vaccine.

Source: UNAIDS Fact Sheet 2018

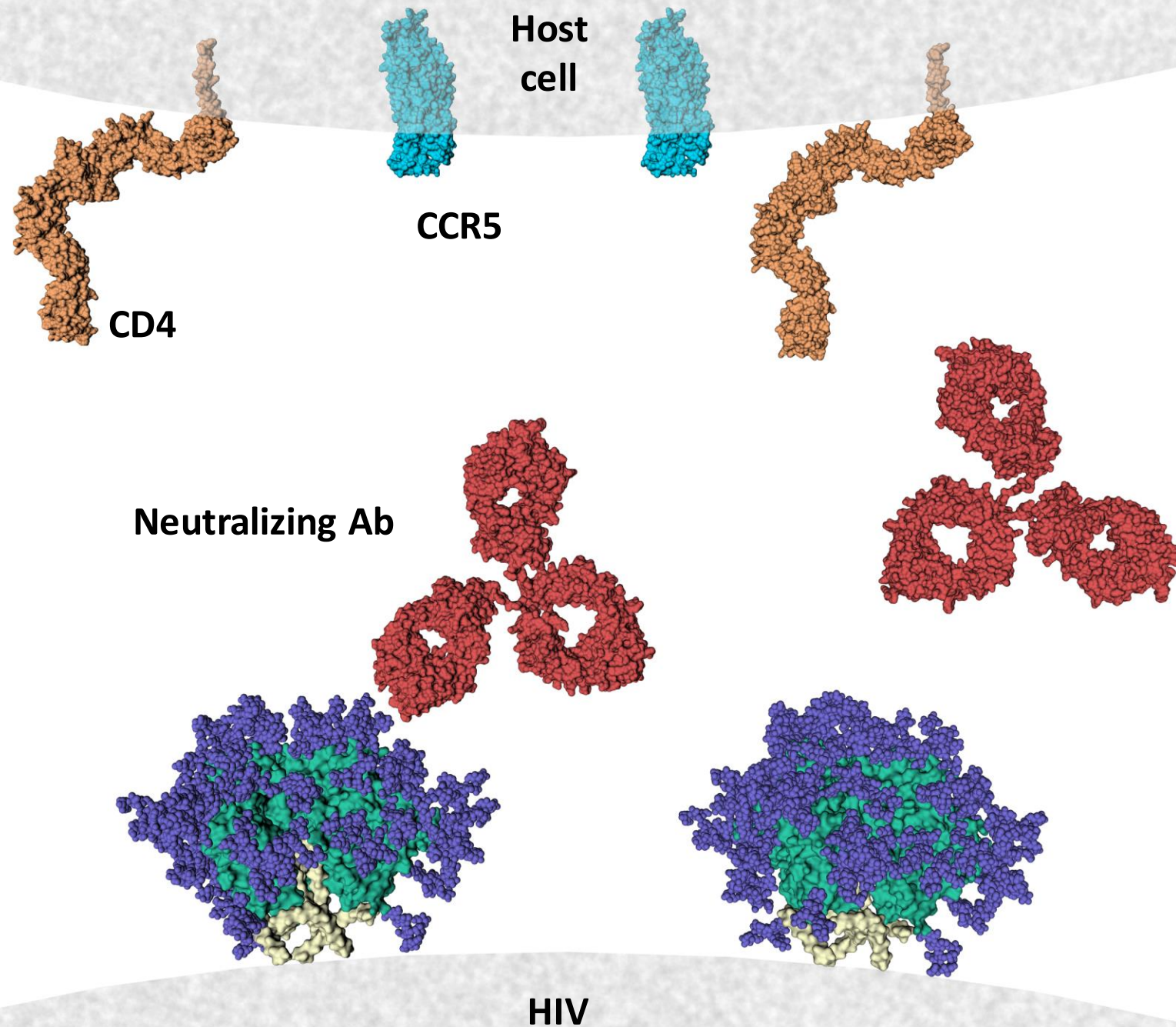


HIV entry

Env spike = $(gp120)_3 (gp41)_3$



- Neutralizing Abs block HIV entry
- Successful vaccines induce neutralizing Abs
- For HIV, need to induce broadly neutralizing Abs (bnAbs)



Sequence Diversity of HIV ENV Dwarfs that of Influenza HA

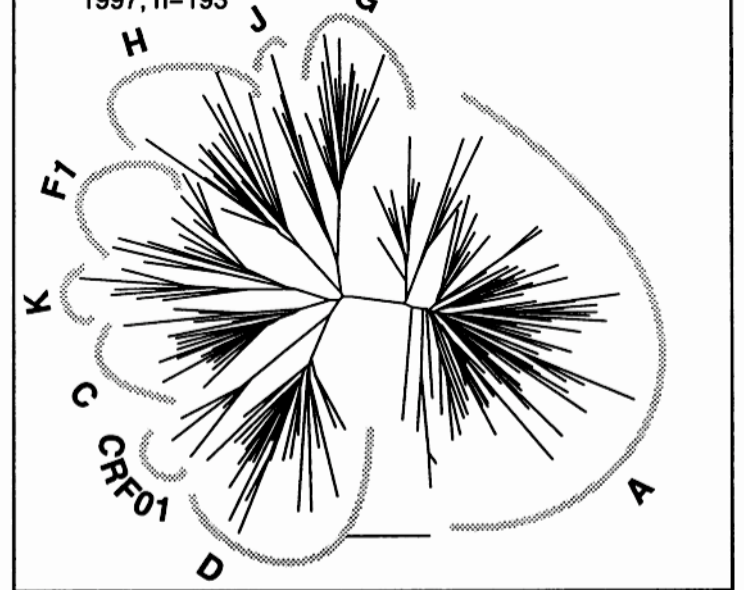
b) 1996 Influenza Sequences
Hemagglutinin (H3)
n=96



c) HIV-1 Single Individual (v2-C5)
Subtype B
Asymptomatic phase
Year 6 post sero-conversion
n=9

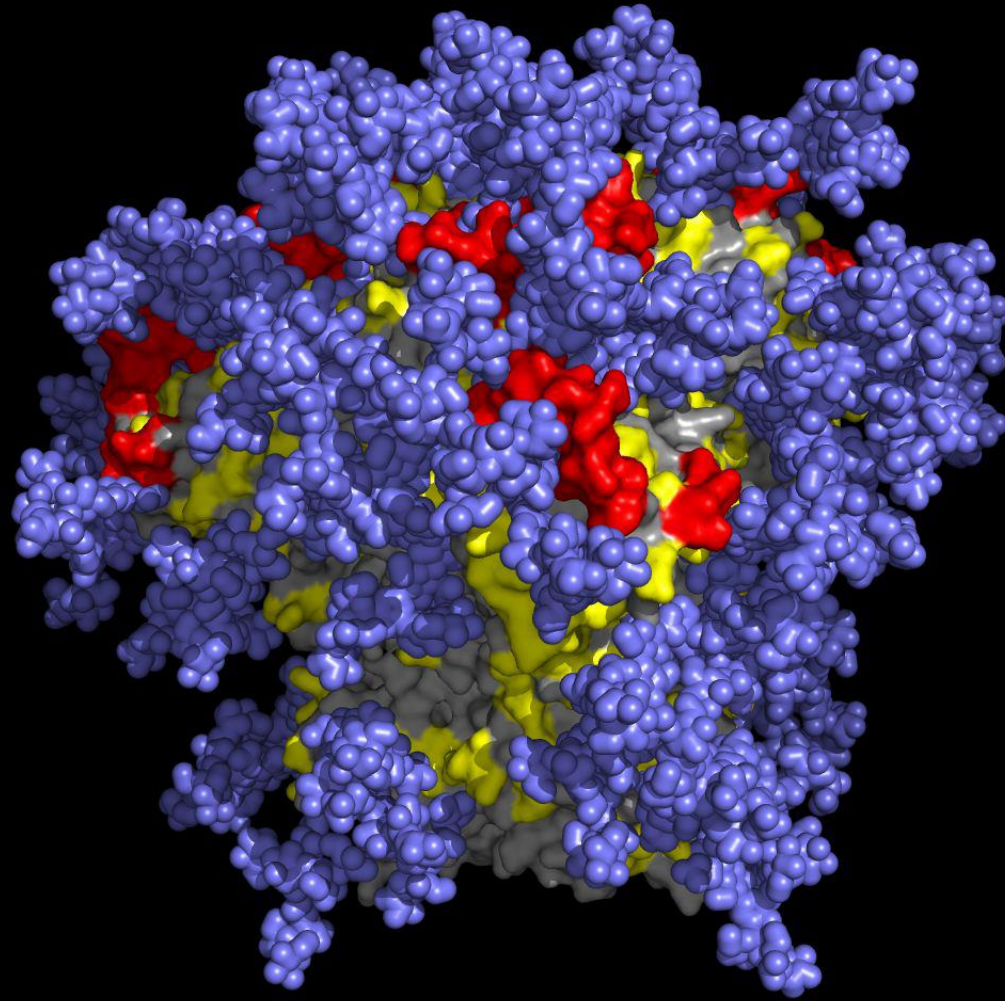


f) Democratic Republic of the Congo
1997, n=193



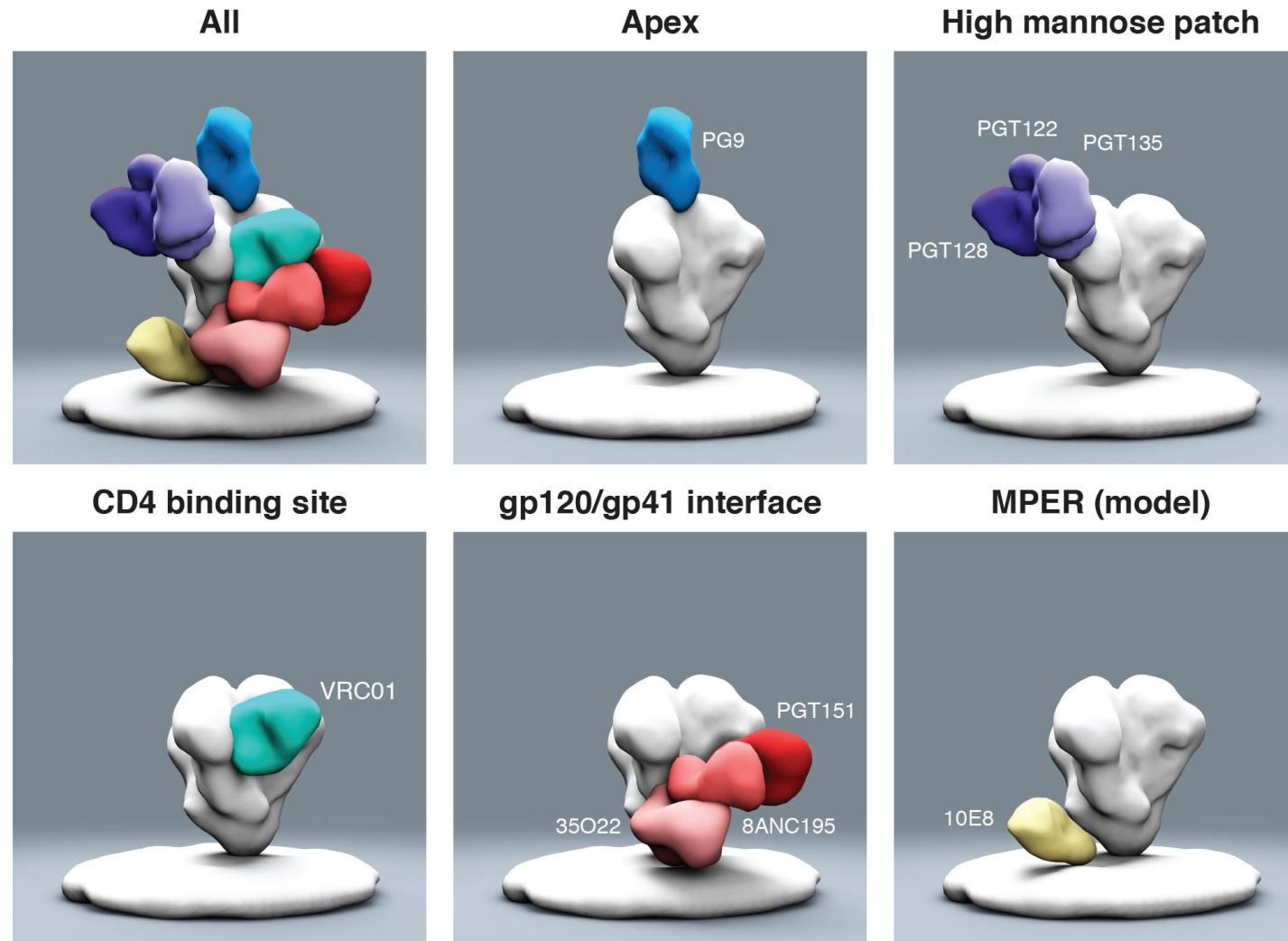
Korber et al., (2001) Brit. Med. Bull.

HIV Envelope Trimer: the Target of Neutralizing Antibodies



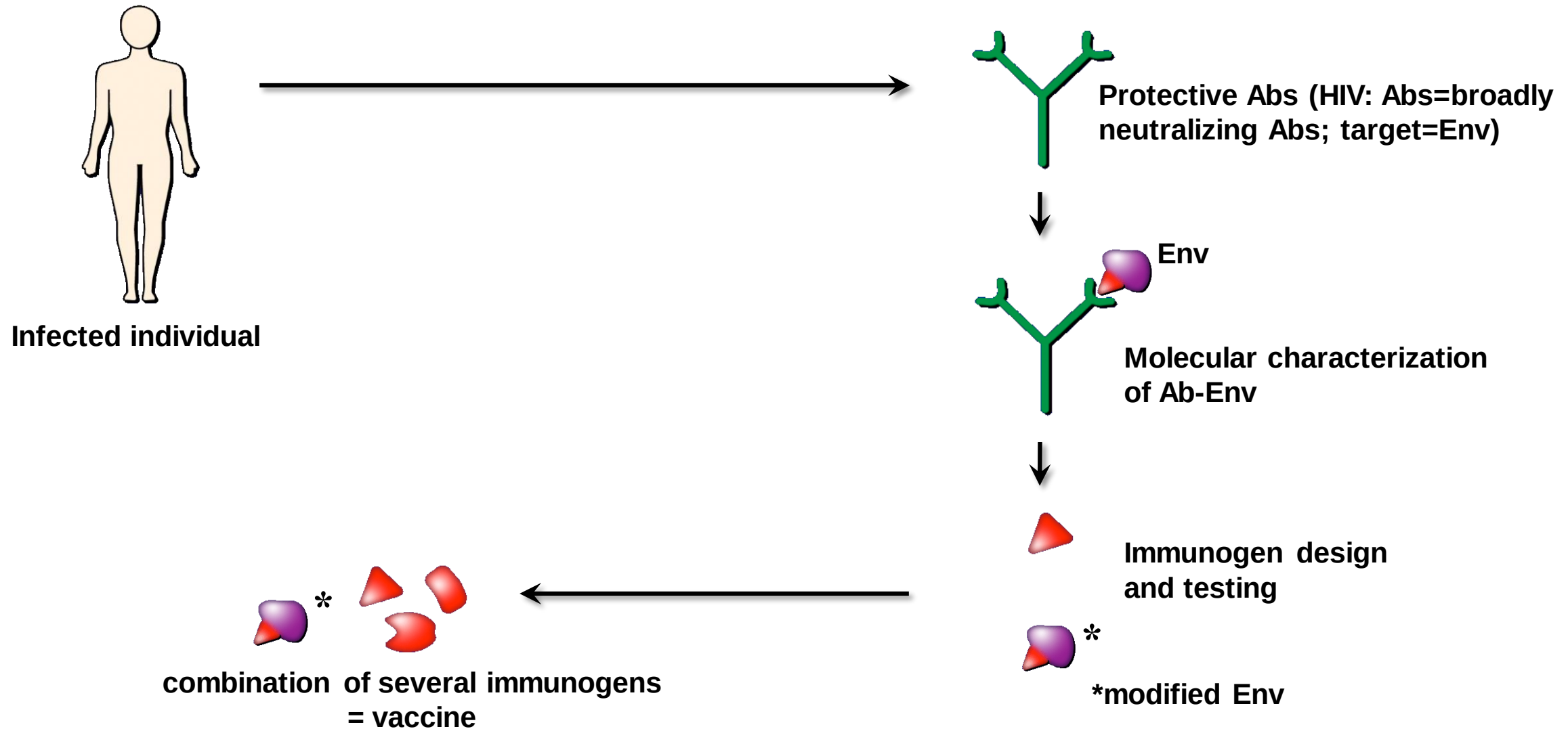
Structure, Glycosylation, and Sequence Variation Pose Major Challenges to the Development of Broadly Neutralizing Antibodies

Prototype HIV Broadly Neutralizing Antibodies (bnAbs)



- bnAbs neutralize diverse isolates, some up to 99% of all isolates.
- bnAbs provide sterilizing immunity in NHP models
- If vaccine can elicit bnAbs, could prevent HIV infection

Reverse Vaccinology 2.0



(adapted from Burton, Nat. Rev. Immunol., 2:706, 2002; Rappuoli et al, J Exp Med, 2016; Burton, CSHL Perspectives, 2017).

HIV Vaccine Goal

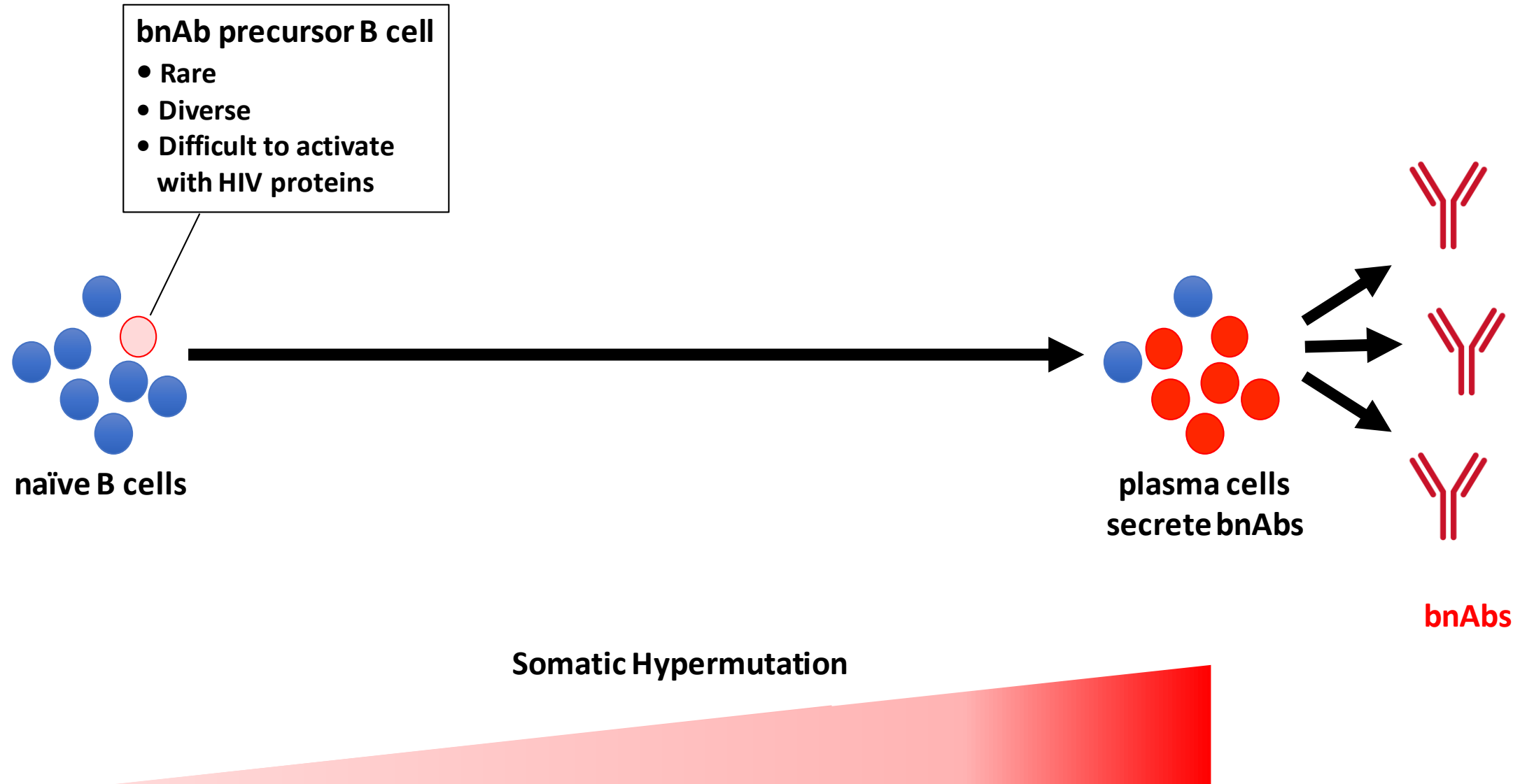
Develop a vaccine that elicits sustained protective levels of broadly neutralizing antibodies (bnAbs) in humans

Hypothesis: An effective HIV vaccine will need to consistently induce bnAbs against 2-3 different sites on the HIV Env in most vaccine recipients.

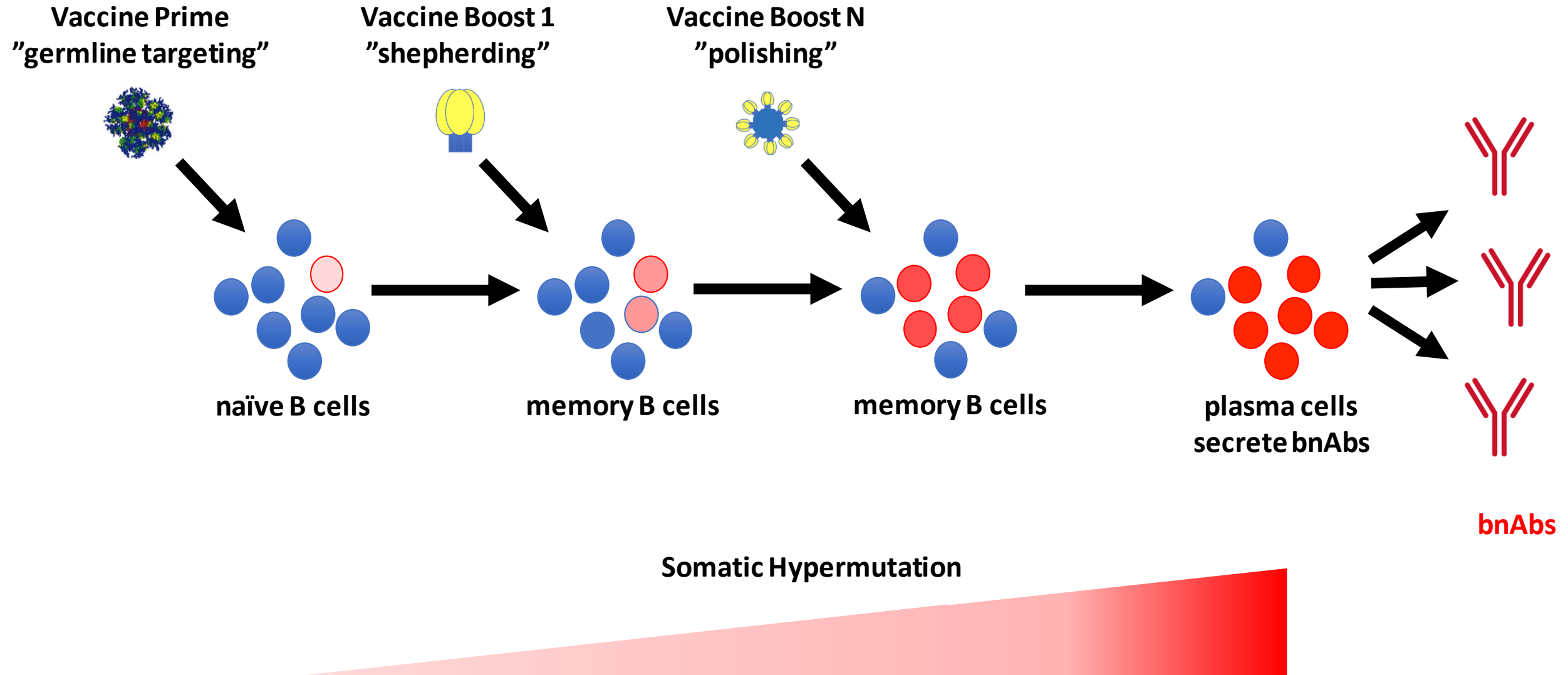
Targeting multiple sites is necessary to provide adequate coverage against the huge diversity of global isolates.



How will an HIV Vaccine Induce Broadly Neutralizing Antibodies (bnAbs)?



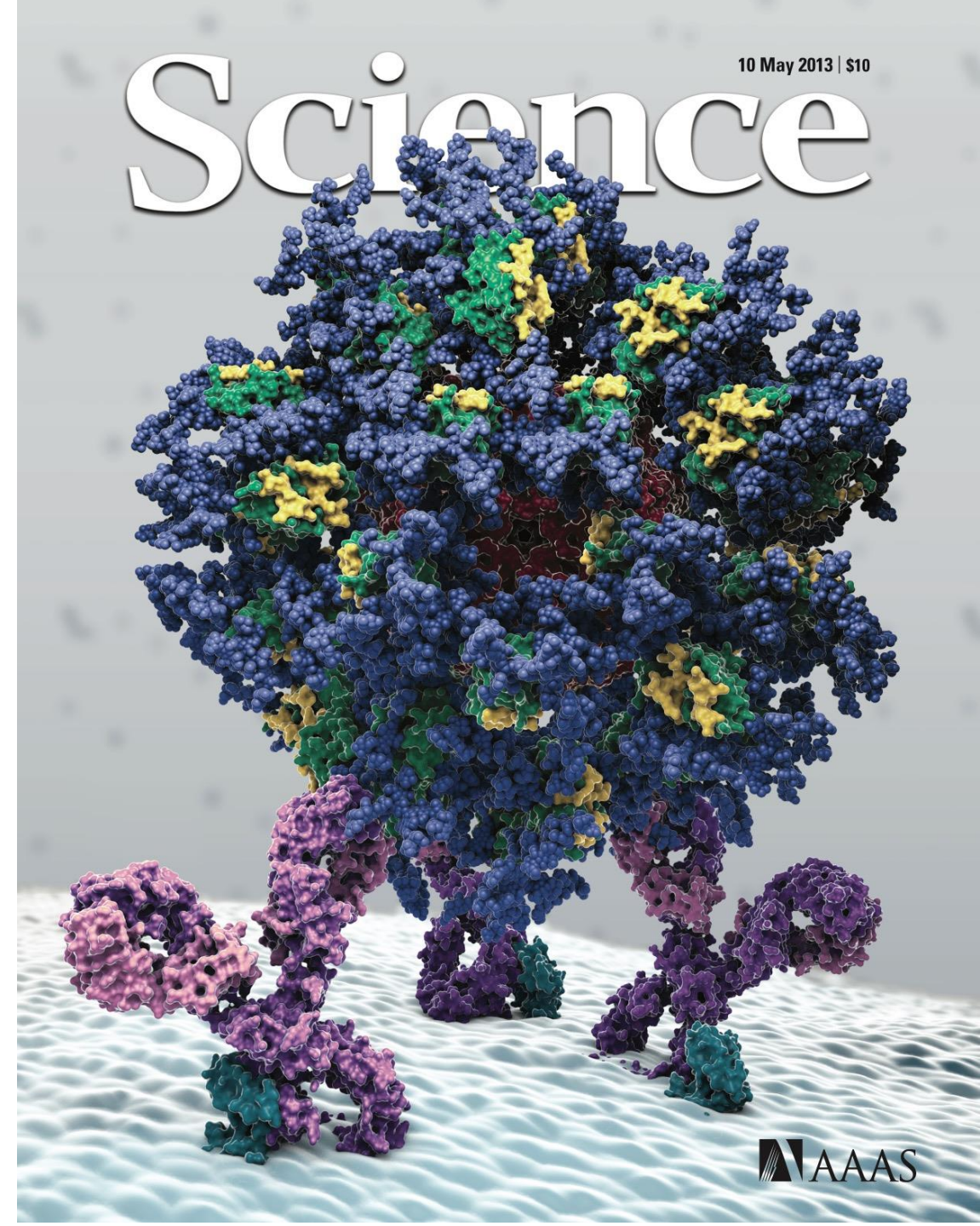
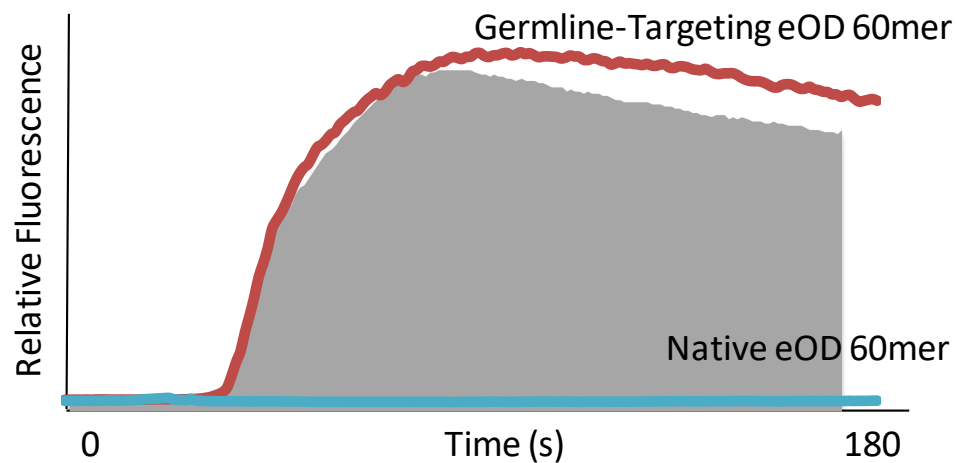
Strategy to induce bnAbs: Germline-Targeting Vaccine Design



Rational HIV Immunogen Design to Target Specific Germline B Cell Receptors

Jardine, Julien, Menis et al., Science, 2013

Germline-targeting nanoparticle activates bnAb germline precursor B cells, but similar nanoparticle lacking germline affinity fails to activate.

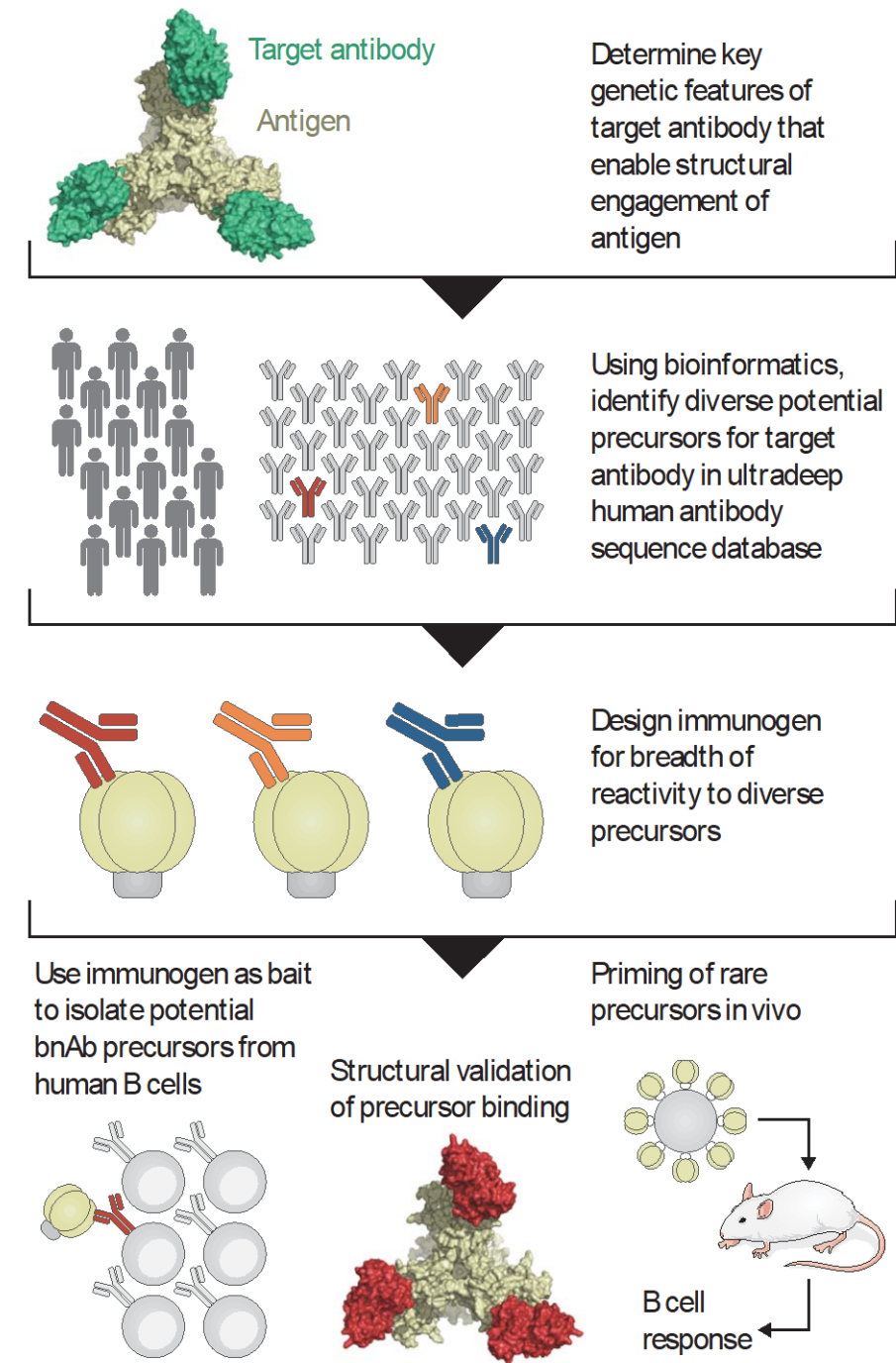


A generalized HIV vaccine design strategy for priming of broadly neutralizing antibody responses

Jon M. Steichen*, Ying-Qing Lin*, Colin Havenar-Daughton*, Simone Pecetta*, Gabriel Ozorowski*, Jordan R. Willis*, Laura Toy, Devin Sok, Alessia Liguori, Sven Kratochvil, Jonathan L. Torres, Aleksandr Kalyuzhnyi, Eleonora Melzi, Daniel W. Kulp, Sebastian Raemisch, Xiaozhen Hu, Steffen M. Bernard, Erik Georgeson, Nicole Phelps, Yumiko Adachi, Michael Kubitz, Elise Landais, Jeffrey Umotoy, Amanda Robinson, Bryan Briney, Ian A. Wilson, Dennis R. Burton, Andrew B. Ward, Shane Crotty†, Facundo D. Batista†, William R. Schief†

“Our repertoire-guided germline-targeting approach provides a framework for priming the induction of many HIV bnAbs and could be applied to most HCDR3-dominant antibodies from other pathogens.”

Steichen, et al. Science, 2019



Proof of principle for germline-targeting vaccine design: bnAbs can be elicited starting from human GL B cells

Immunity

HIV Vaccine Design to Target Germline Precursors of Glycan-Dependent Broadly Neutralizing Antibodies

Article

Authors

Jon M. Steichen, Daniel W. Kulp,
Talar Tokatlian, ..., Darrell J. Irvine,
Michel C. Nussenzweig,
William R. Schief

Correspondence

nussen@mail.rockefeller.edu (M.C.N.),
schief@scripps.edu (W.R.S.)

In Brief

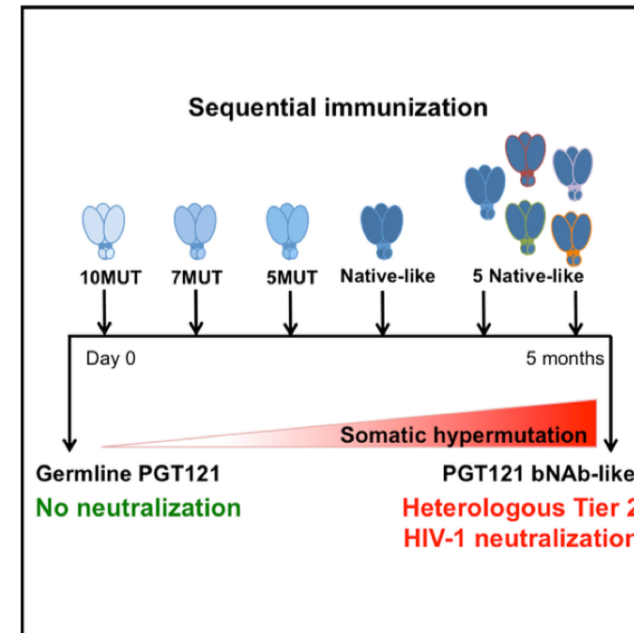
Elicitation of broadly neutralizing antibodies (bnAbs) is a critical HIV vaccine goal. Steichen et al. have developed immunogens that prime germline-precursor B cells for the bnAb PGT121 and can therefore initiate bnAb induction. The authors have also designed boosting immunogens to shepherd the antibody maturation to develop bnAbs.

Cell

Sequential Immunization Elicits Broadly Neutralizing Anti-HIV-1 Antibodies in Ig Knockin Mice

Article

Graphical Abstract



Authors

Amelia Escolano, Jon M. Steichen,
Pia Dosenovic, ..., Dennis R. Burton,
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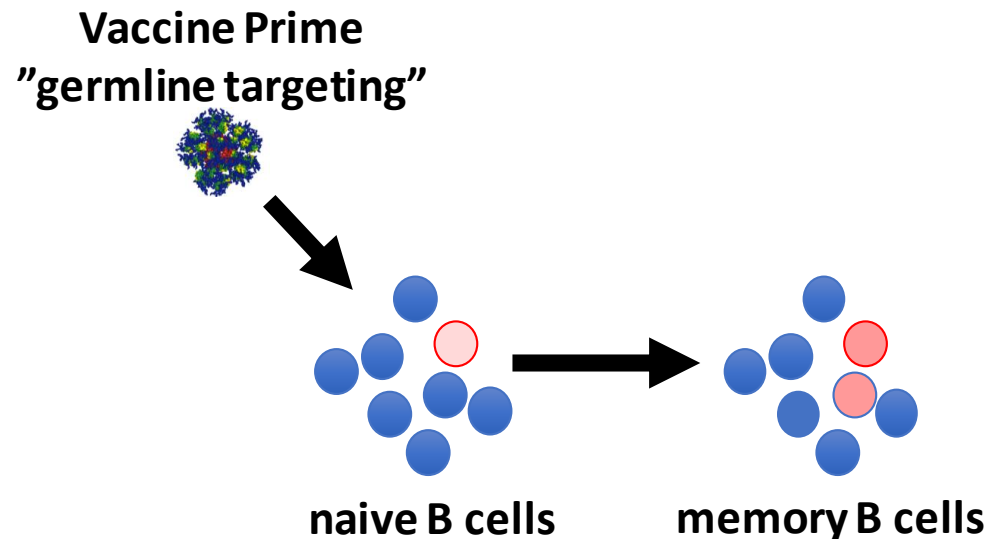
In Brief

Generation of HIV broadly neutralizing antibodies—a major goal in vaccine research—can be achieved through sequential immunization with HIV envelope glycoproteins designed to engage PGT121 antibody precursors and their intermediates.

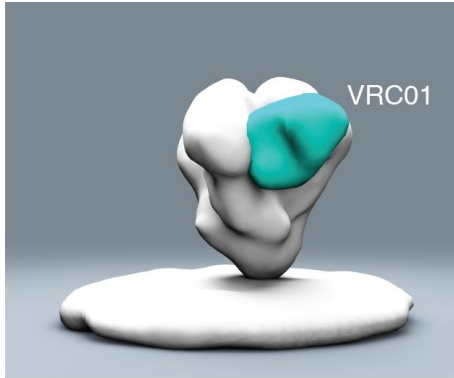
- Caveats:**
- BnAb precursor frequency was ~100% in this mouse model → Too easy, no competition from other B cells
 - Priming immunogen had only modest affinity for bnAb precursors
 - Not ready for human testing – priming immunogen needs higher affinity, broader specificity

Eliciting bnAbs in humans – importance of consistent priming

- Consistent priming of bnAb precursors is a vaccine requirement
- Consistent priming will likely require a priming immunogen that can target a diverse precursor pool for any one class of bnAb (due to human genetic diversity and antibody recombinational and junctional diversity)
- Priming immunogen needs appreciable affinity and avidity for diverse precursors (diverse human naïve B cells)

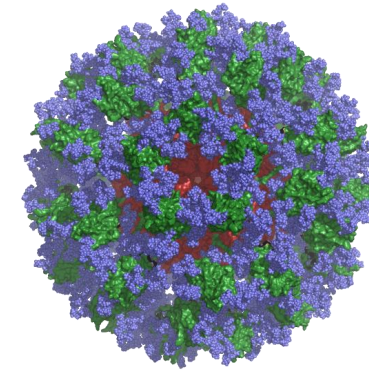


Lead Project for Germline-Targeting Vaccine Design: VRC01



VRC01-class bnAbs

- bind the gp120 CD4bs
 - require VH1-2 and 5AA L-CDR3 to engage CD4bs
 - have diverse H-CDR3s and light chains
- Need priming immunogen with appreciable affinity and avidity for diverse VRC01-class human naïve precursors

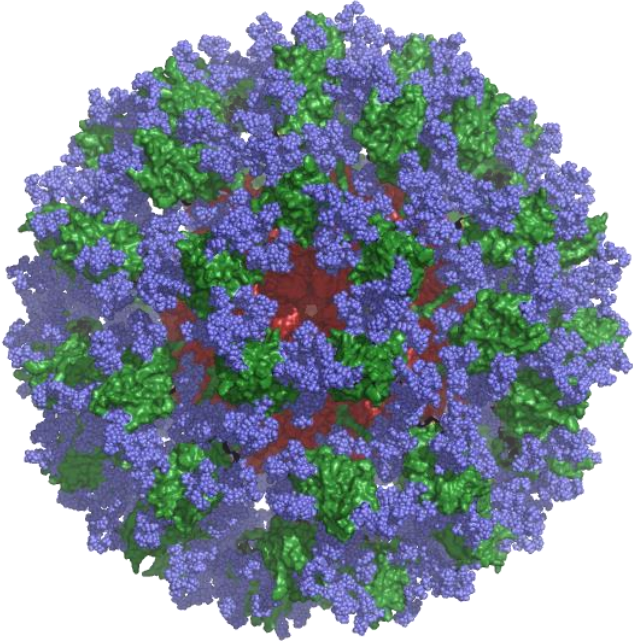


Germline-Targeting Immunogen

- eOD-GT8 60mer
 - Self-assembling nanoparticle presenting 60 copies of an engineered gp120 outer domain
- ✓ Has appreciable affinity and avidity for diverse VRC01-class human naïve precursors
- ✓ Primes VRC01-class responses in stringent mouse models
- ✓ Induces VRC01-class memory responses that can be boosted toward bnAb development in mouse models

Jardine et al. Science 2013, 2015, 2016;
Briney et al. Cell 2016; Tian et al. Cell 2016; Sok et al. Science 2016
Abbott et al. Immunity 2018; Havenar-Daughton et al. Sci. Trans. Med 2018

IAVI G001 Phase I Trial: eOD-GT8 60mer/AS01B



- First-in-human test of germline targeting
- Self-assembling nanoparticle immunogen
- Trial start: Sept 2018 (1st immunization)
- Conducted at FHCRC (Seattle) and GWU (Washington, DC)
- Critical readout by B cell sorting/sequencing at FHCRC (Cohen/McElrath) and NIH/VRC (Koup/McDermott)

Study Group	N	eOD-GT8 60mer dose	Week 0	Week 8
1 (low dose)	18	20 µg	eOD-GT8 60mer/ AS01B	eOD-GT8 60mer/ AS01B
	6	-	placebo	placebo
2 (high dose)	18	100 µg	eOD-GT8 60mer/ AS01B	eOD-GT8 60mer/ AS01B
	6	-	placebo	placebo
Total	48			

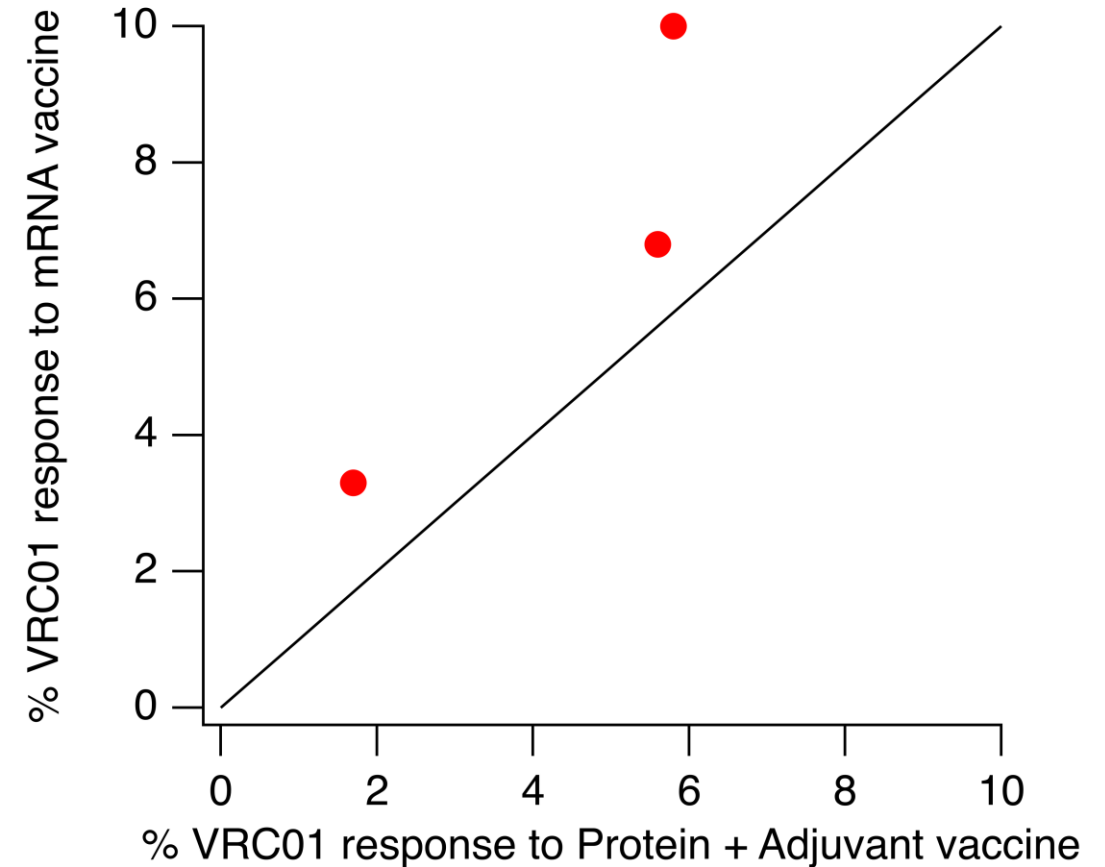
Moderna mRNA vaccine platform in HIV vaccine development

Challenge: To develop a highly effective HIV vaccine, we will need to carry out many iterative human clinical trials. If we rely on GMP protein manufacture, our progress will be limited by the relatively slow pace and high cost of manufacture.

Solution: We are hopeful that Moderna mRNA will provide a rapid, economical, and highly immunogenic vaccine platform to enable expeditious iterative human vaccine optimization.

Moderna mRNA vs protein for priming VRC01-class responses in a stringent mouse model: BCR sequencing indicates that mRNA performs at least as well as protein

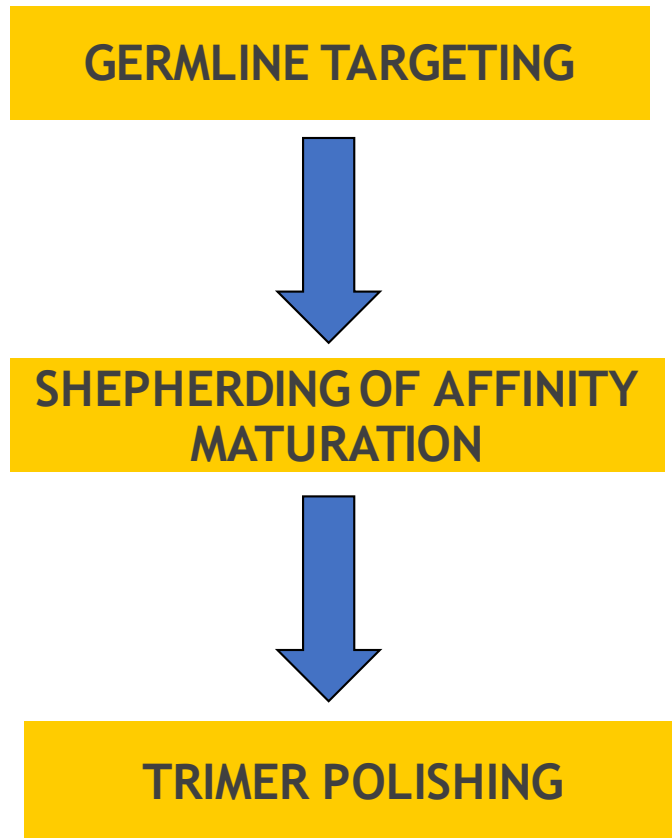
- VH1-2 mouse model (Tian et al Cell 2016)
- Three different immunogens tested as mRNA vs protein + adjuvant
- Immunogens are variants of eOD-GT8 60mer
- Single immunization at day 0
- mRNA (10 µg) via IM route
- protein (20 µg) + SAS adjuvant via IP route
- Readout is epitope-specific B cell sorting/BCR sequencing at d42
- >100 BCR sequences recovered in each of 6 cases
- What % of epitope-specific B cells at d42 are VRC01-class?



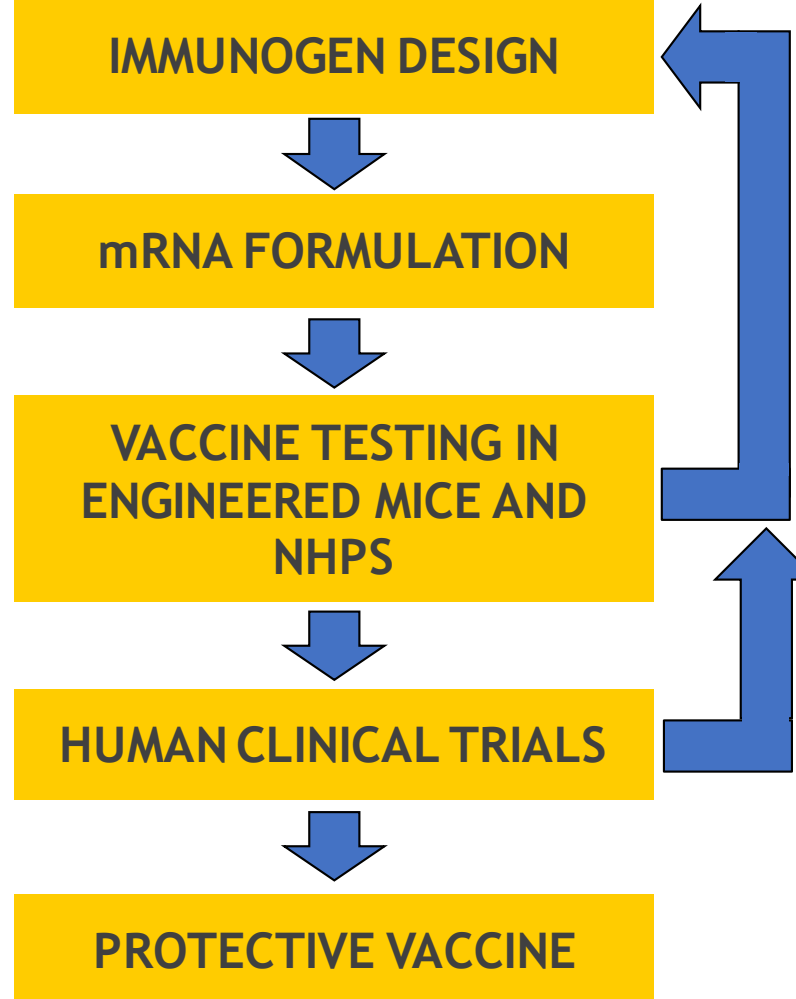
Moderna Collaboration with Schief/Burton/IAVI:

Developing an mRNA-based HIV Vaccine that Induces BnAbs, through Expeditious Iterative Human Vaccine Evaluation

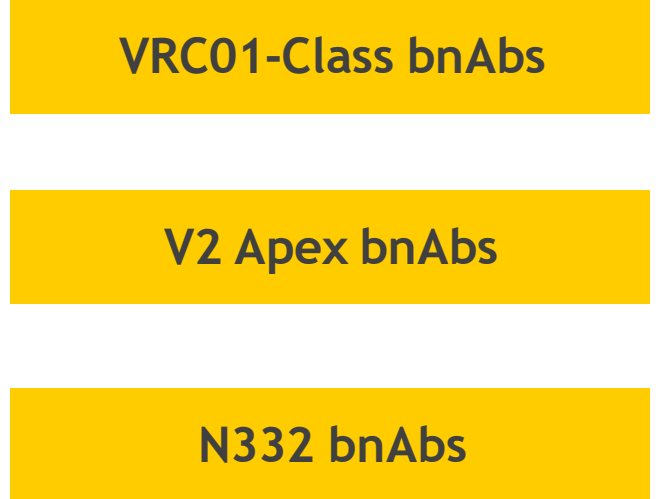
Overall Vaccine Strategy



Iterative Vaccine Design/Test Cycle



Vaccine Targets (bnAbs)



- IAVI, BMGF and NIH funding
- Animal exps with: Alt, Batista, Crotty, Haynes, Nemazee, Silvestri, Verkoczy

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Anita Sarkar

Harvard

Fred Alt
Ming Tian
Sai Luo

Emory

Guido Silvestri
Diane Carnathan

La Jolla Institute

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Colin Havenar-
Daughton
Robert Abbott
JH Lee

Yu (Alex) Kato
Ivy Phung

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Pia Dosenovic
Amelia Escolano
Lotta von Boehmer

Ragon

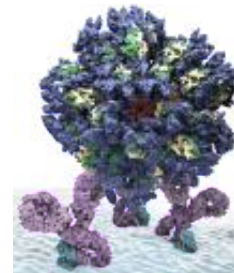
Facundo Batista
Ying-Cing Lin
Simone Pecetta
Sven Kratochvil
Eleonora Melzi
Xuesong Wang

Moderna

Andrea Carfi
Sunny Himansu
Samantha Falcone
Karina Steiger-Gonzalez
Phil White
Sophia Zendzian

IAVI

Mark Feinberg
Labeeb Abboud
Antu Dey
Olayinka Fagbayi
Khoah Le
Dominick Laddy
Dagna Laufer
Karen Tran



National Institute of
Allergy and
Infectious Diseases



BILL & MELINDA
GATES *foundation*



 Ragon Institute
of MGH, MIT and Harvard

NIH-Moderna HIV-1 Vaccine

An mRNA-Based Approach to the Development
of a Protective HIV-1 Vaccine

Paolo Lusso, M.D., Ph.D.

Chief, Viral Pathogenesis

Laboratory of Immunoregulation

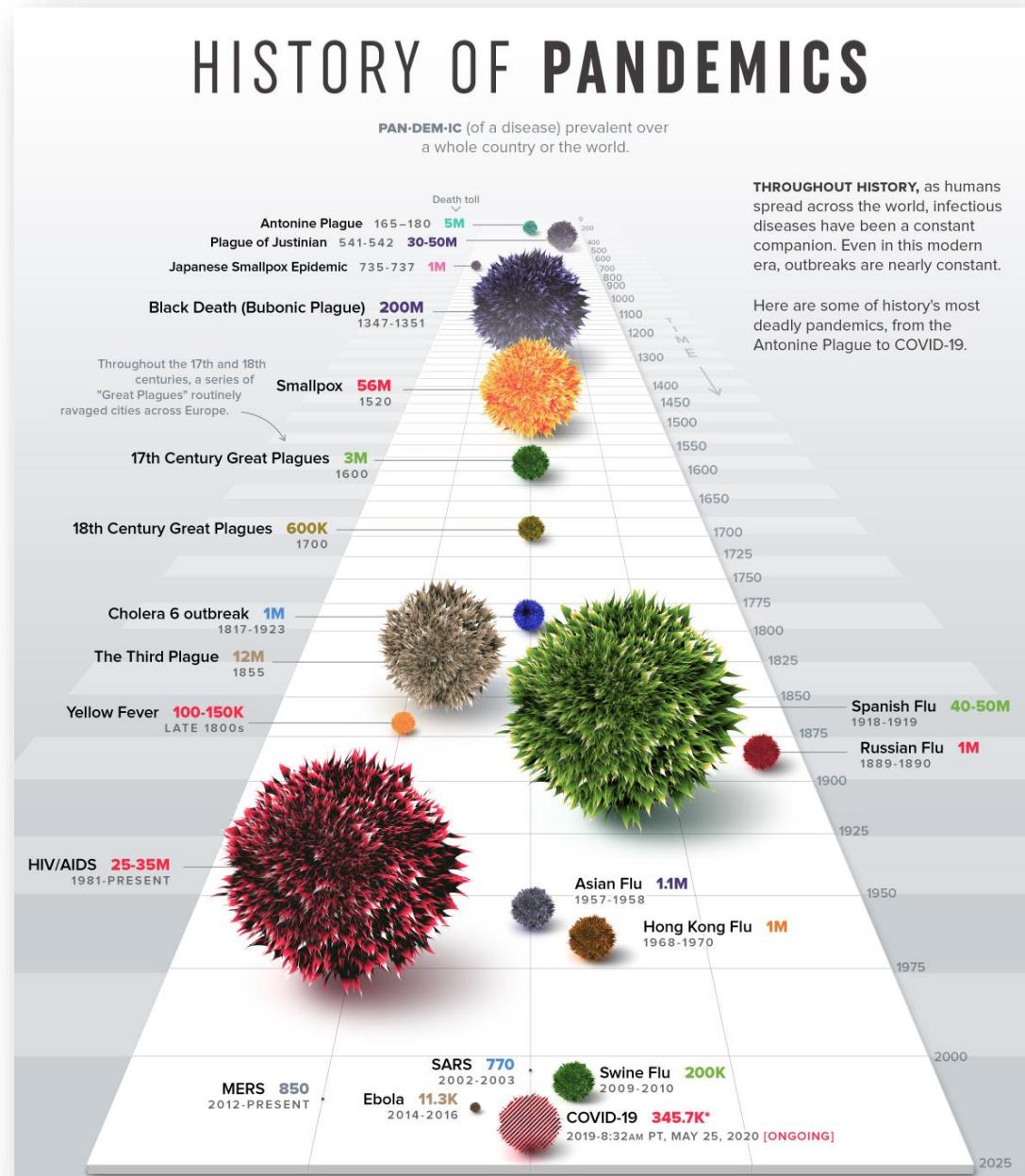
National Institute of Allergy and Infectious Diseases (NIAID)

Supported by:

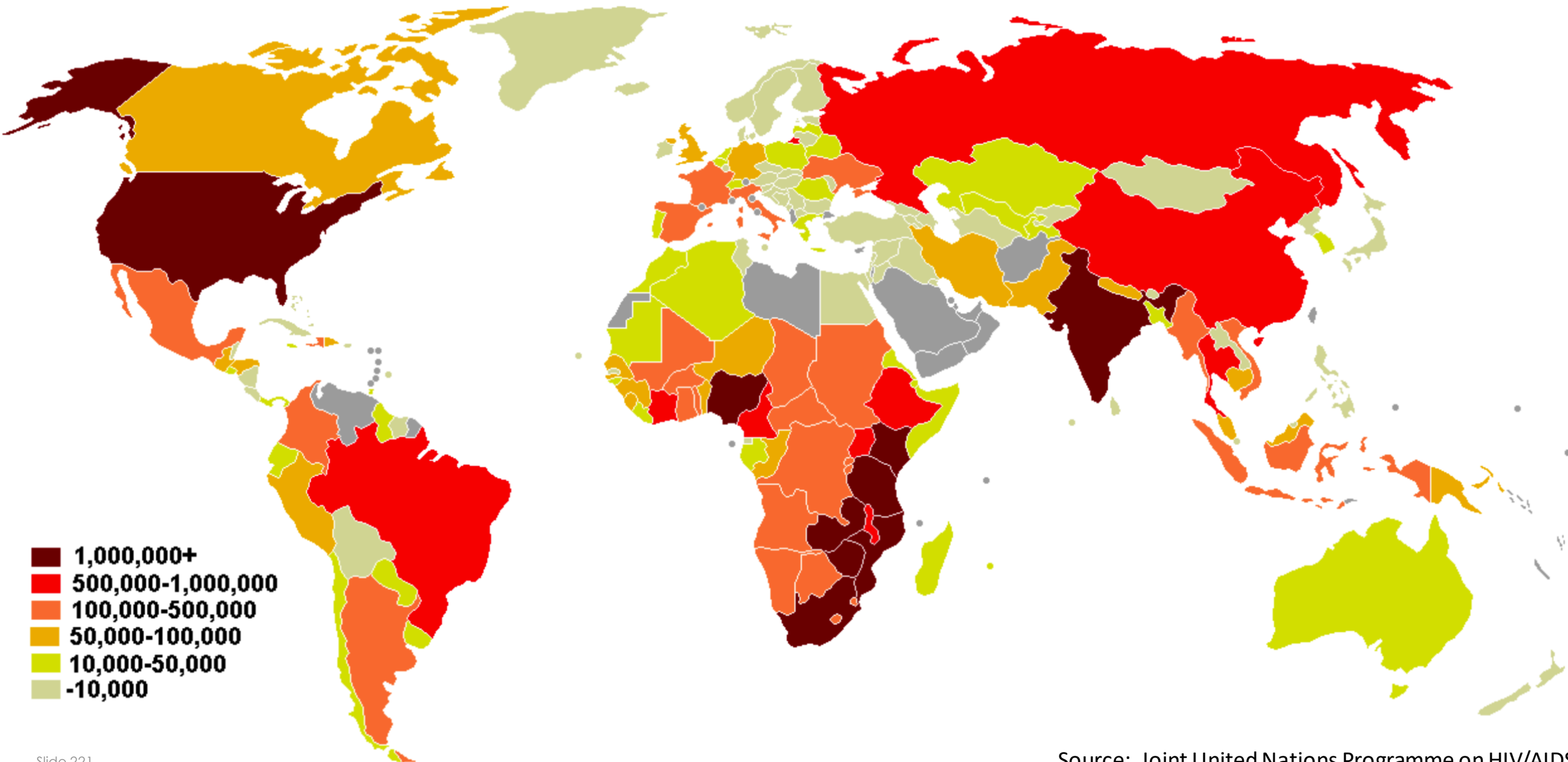


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Greatest Pandemics in History



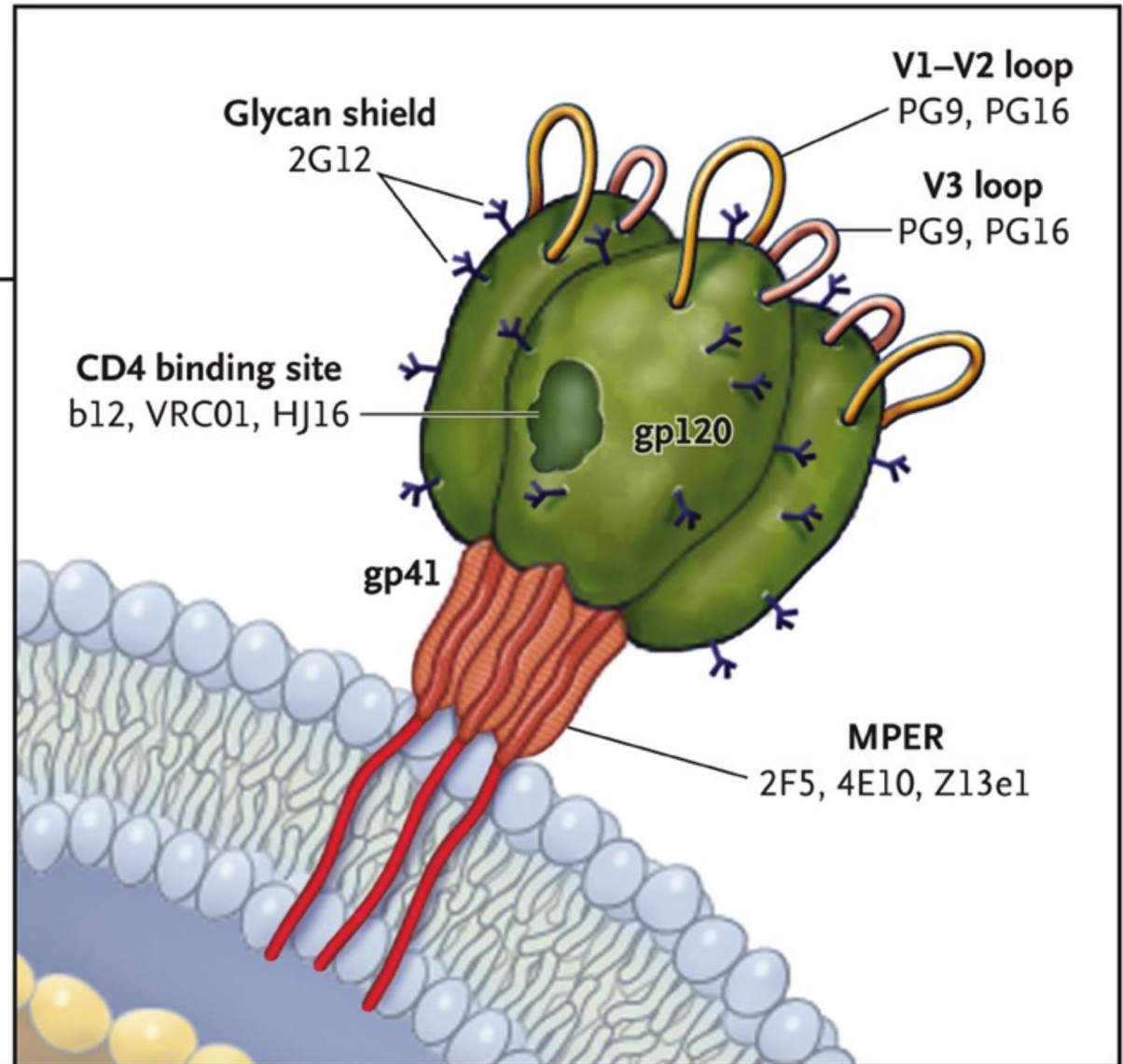
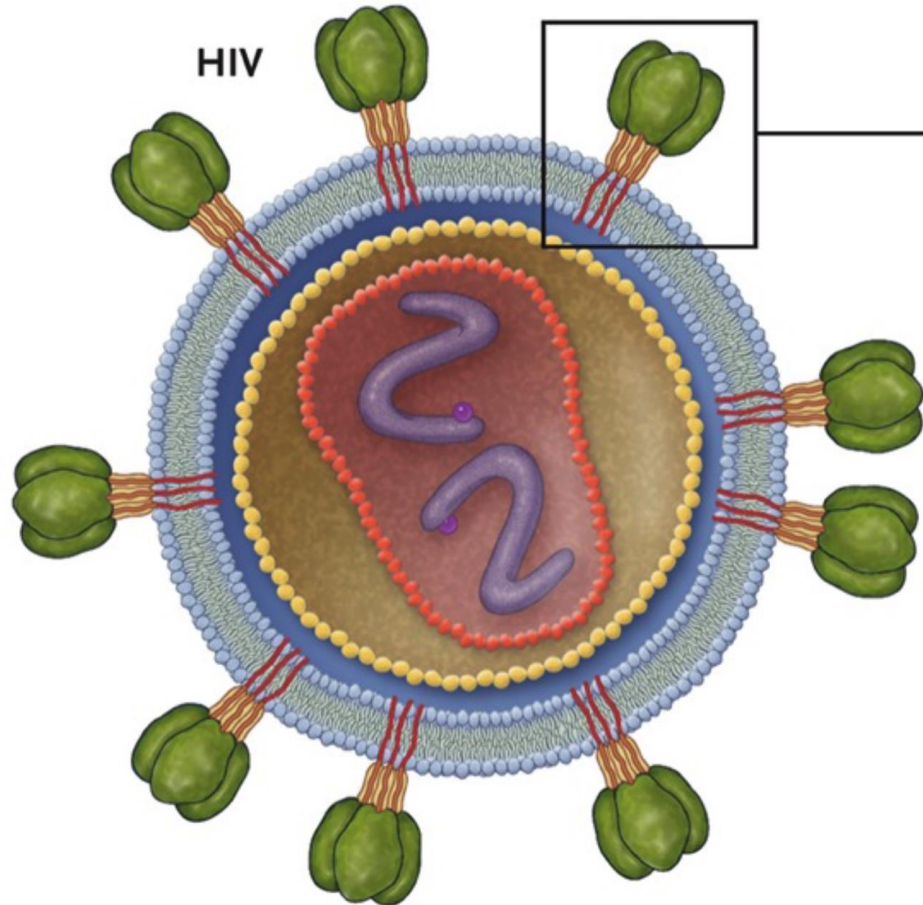
People Living with HIV/AIDS (2019)



Two Key Lessons Learned from Decades of HIV Vaccine Research

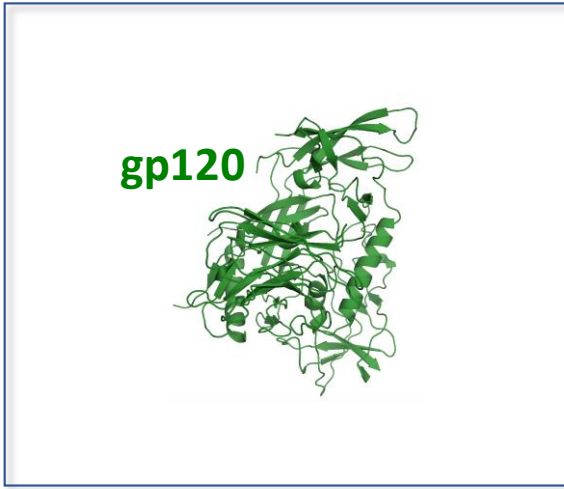
- **A protective vaccine is feasible**
 - **Induction of broadly neutralizing antibodies (bNAbs) is an essential condition for a protective vaccine**
-

The HIV-1 Envelope Is a Membrane-Anchored Trimer



Different Forms of the HIV-1 Envelope Utilized as Vaccines

1. Soluble, monomeric gp120 subunit

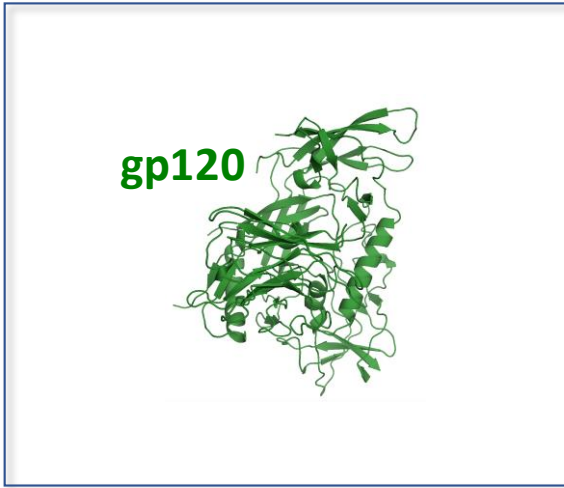


Ineffective as vaccine

- Elicits nonprotective antibodies

Different Forms of the HIV-1 Envelope Utilized as Vaccines

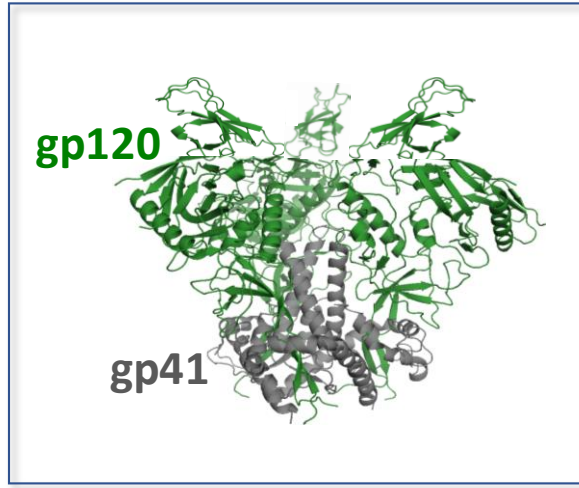
1. Soluble, monomeric gp120 subunit



Ineffective as vaccine

- Elicits nonprotective antibodies

2. Soluble, stabilized (SOSIP) trimer

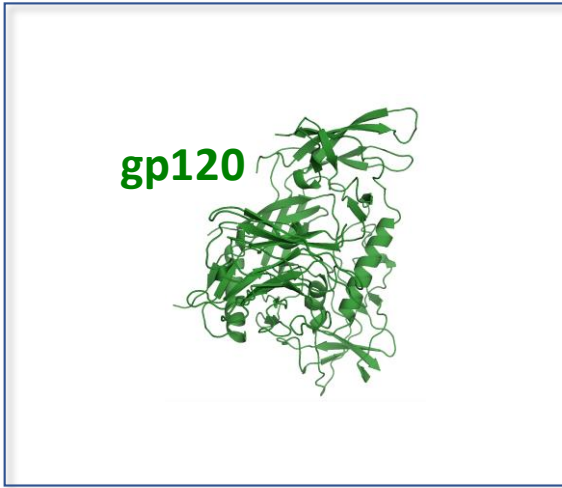


Sub-optimal for vaccine

- Elicits off-target antibodies

Different Forms of the HIV-1 Envelope Utilized as Vaccines

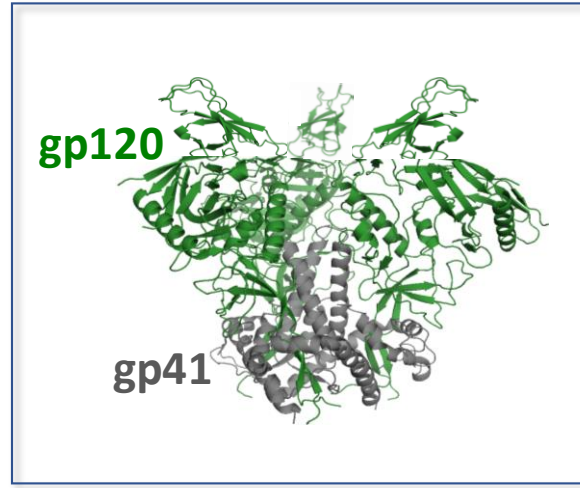
1. Soluble, monomeric gp120 subunit



Ineffective as vaccine

- Elicits nonprotective antibodies

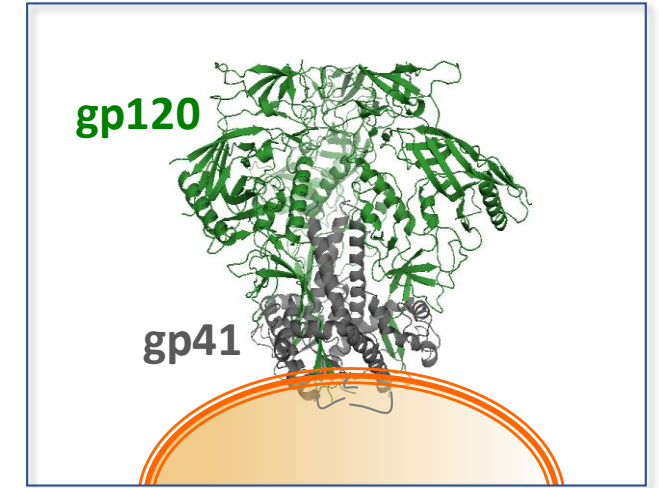
2. Soluble, stabilized (SOSIP) trimer



Sub-optimal for vaccine

- Elicits off-target antibodies

3. Membrane-anchored pre-fusion trimer



Best for vaccine

- Same form as in real-life virus
- Best suited for mRNA expression

The NIH-Moderna HIV-1 Vaccine Approach

1. Use membrane-anchored Env expressed *in vivo* by mRNA
 2. *In vivo* production of virus-like particles (VLP) by co-formulation of Env with Gag
 3. Priming with Ab ancestor-engaging transmitted/founder (T/F) Env
 4. Intensive heterologous boosting with multi-clade, glycan-repaired tier-2 Envs (*i.e.*, real-life HIV-1 strains)
-

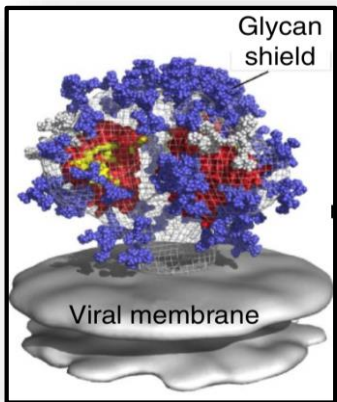
The NIH-Moderna Approach: Key Points

1. Use membrane-anchored Env expressed *in vivo* by mRNA

The NIH-Moderna Approach: Key Points

1. Use membrane-anchored Env expressed *in vivo* by mRNA

Main advantages:



- Native antigenic state (best mimic of virion-associated Env)
- Endogenous protein processing (native glycosylation)
- Lack of “distracting” epitopes (e.g., SOSIP-trimer base)

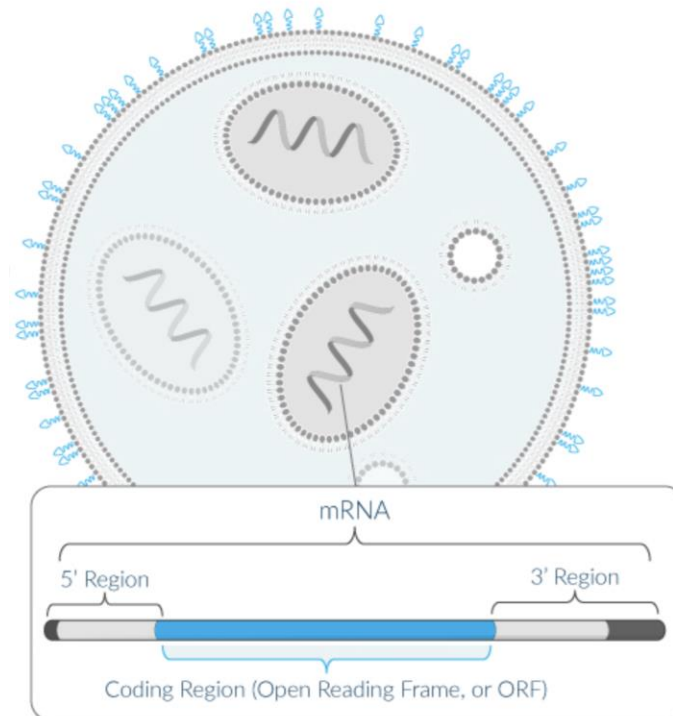
The NIH-Moderna Approach: Key Points

2. *In vivo* production of virus-like particles (VLP) by co-formulation of Env with Gag

The NIH-Moderna Approach: Key Points

2. *In vivo* production of virus-like particles (VLP) by co-formulation of Env with Gag

mRNA formulated in lipid nanoparticles

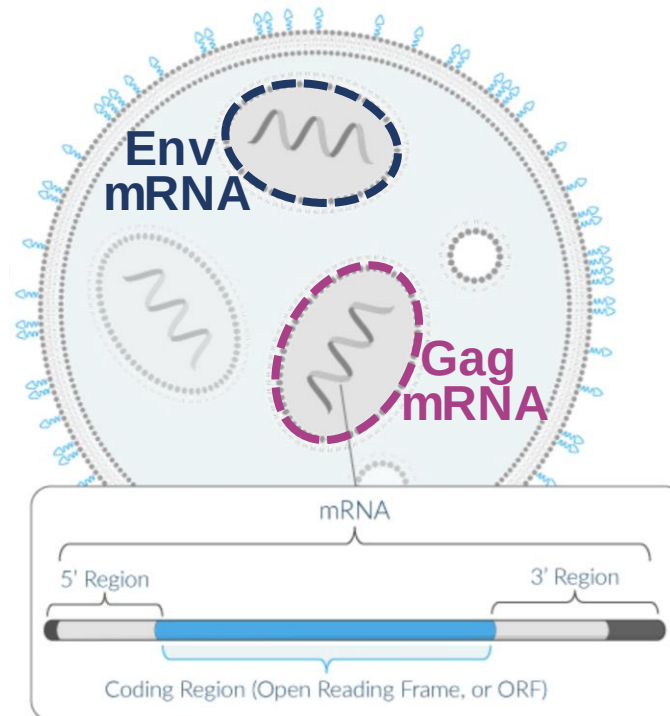


The NIH-Moderna Approach: Key Points

2. *In vivo* production of virus-like particles (VLP) by co-formulation of Env with Gag

mRNA formulated in lipid nanoparticles

Co-formulation of
Gag+Env mRNAs



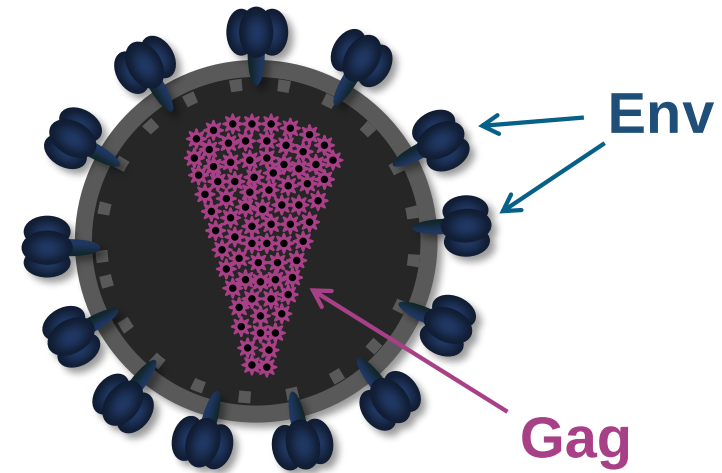
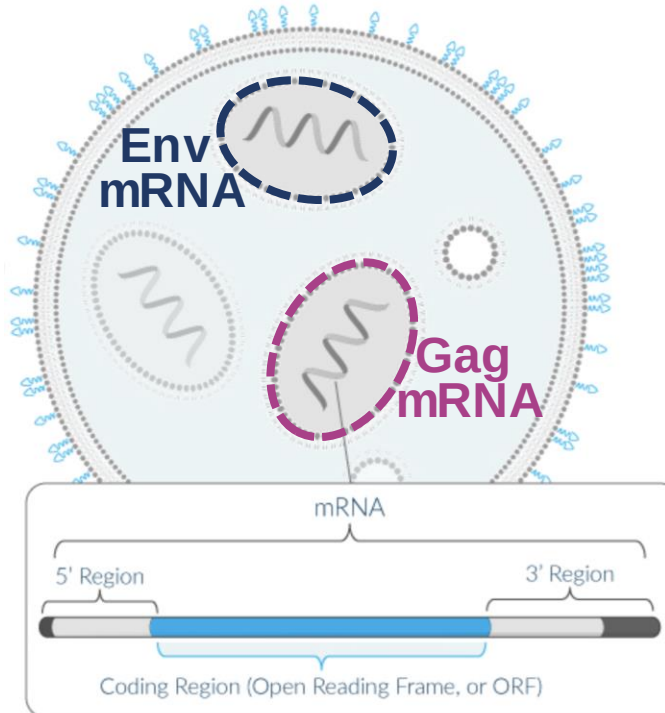
The NIH-Moderna Approach: Key Points

2. *In vivo* production of virus-like particles (VLP) by co-formulation of Env with Gag

mRNA formulated in lipid nanoparticles

In vivo-produced virus-like particle (VLP)

Co-formulation of
Gag+Env mRNAs



The NIH-Moderna Approach: Key Points

2. *In vivo* production of virus-like particles (VLP) by co-formulation of Env with Gag

Main advantages:

- Native antigenic state (closest proxy to HIV virions)
- Life size (optimal APC uptake/processing)
- Extracellular release (travel to afferent lymph nodes)
- Induction of both Env and Gag responses

The NIH-Moderna Approach: Key Points

3. Priming with Ab ancestor-engaging transmitted/founder (T/F) Env

Main advantages:

- *Ab initio* recruitment of rare precursor antibodies for the generation of broadly neutralizing antibodies

The NIH-Moderna Approach: Key Points

4. Intensive heterologous boosting with multi-clade, glycan-repaired tier-2 Envs (*i.e.*, real-life HIV-1 strains)

Main advantages:

- Selective immune focusing on “shared” epitopes across different HIV-1 isolates and clades, *i.e.*, bNAb epitopes
- Exclusion of distracting, non-neutralizing epitopes, *i.e.*, glycan holes, V3 loop, bridging sheet, etc.
- Mimicking of sustained antigenic stimulation that occurs in patients who eventually develop bNAbs

VPS-M1 Study: Design and Rationale

Prime

(1 dose)

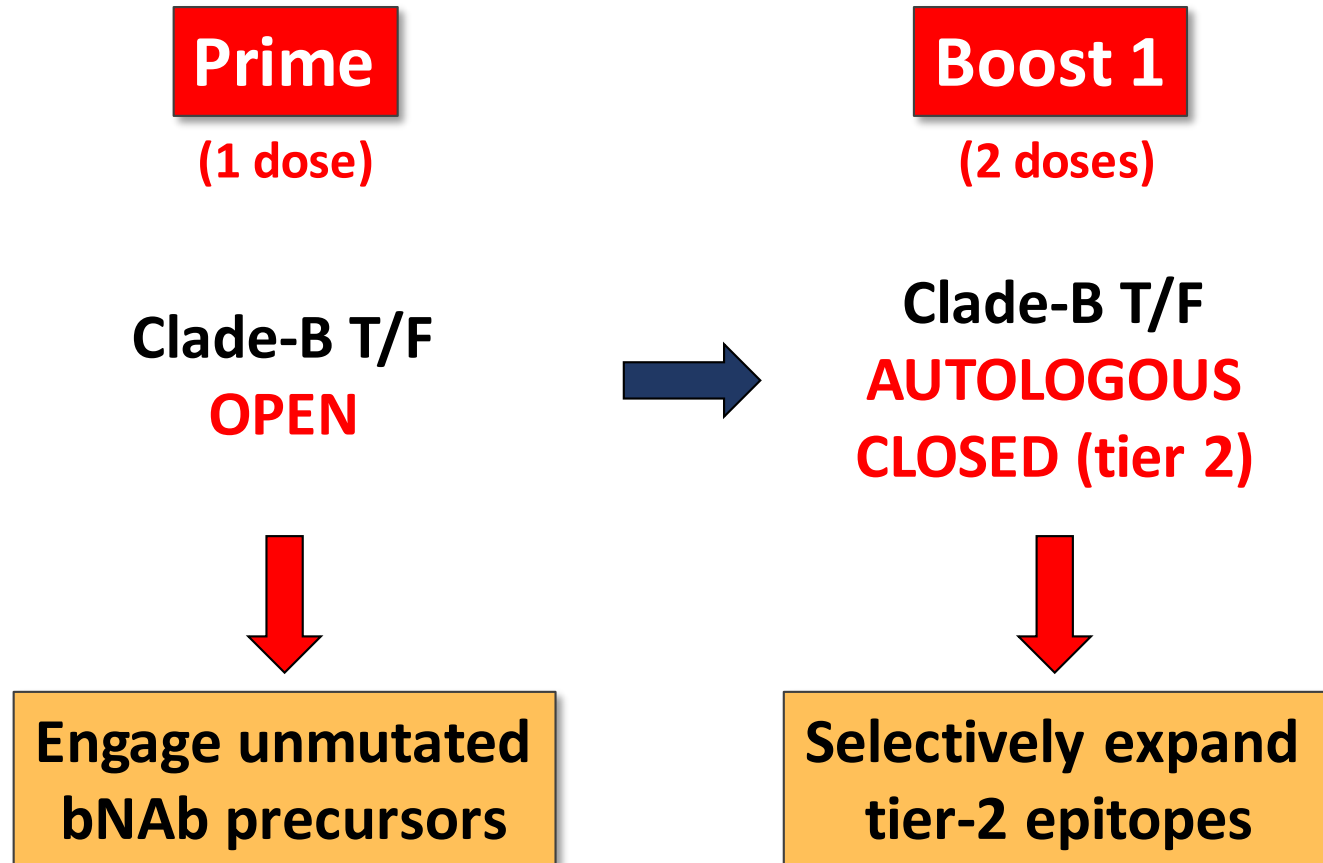
Clade-B T/F

OPEN

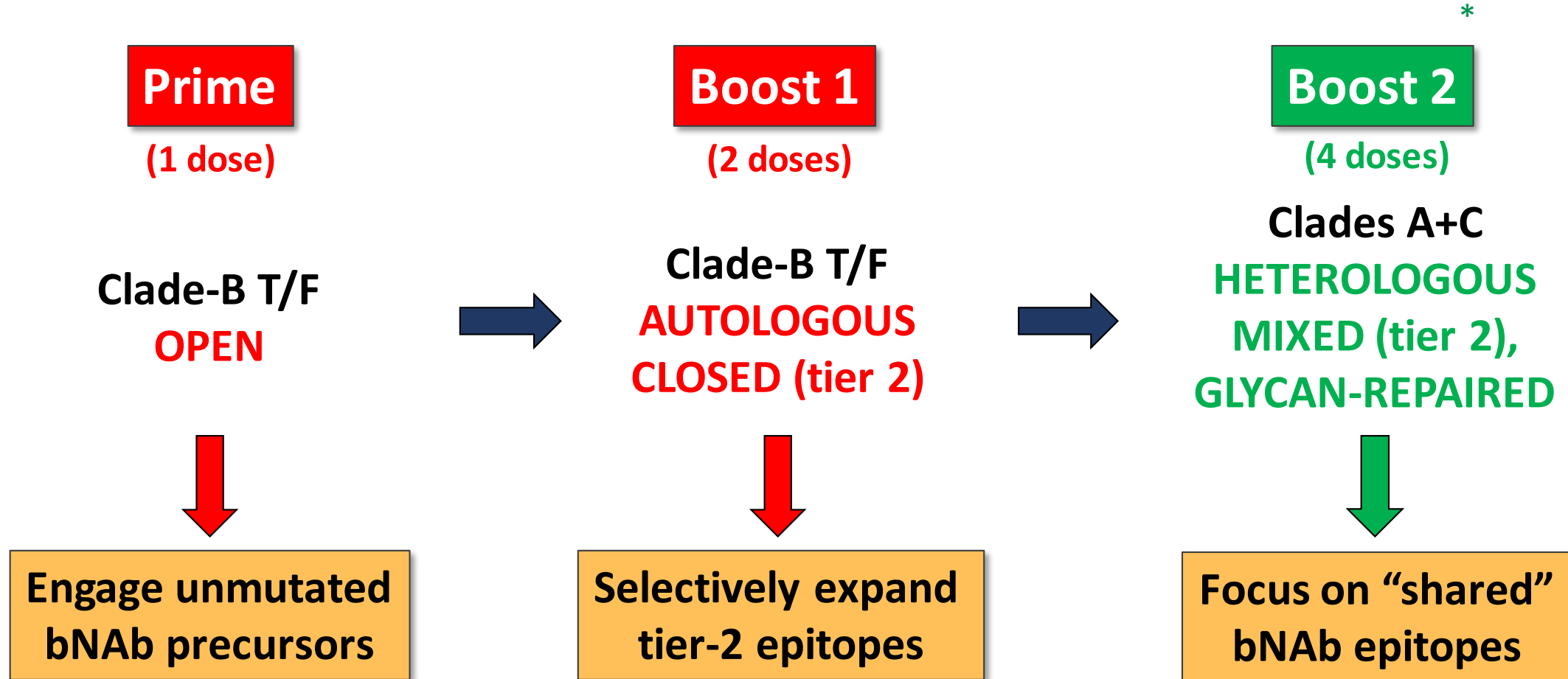


**Engage unmutated
bNAb precursors**

VPS-M1 Study: Design and Rationale



VPS-M1 Study: Design and Rationale



VPS-M1: Study Design

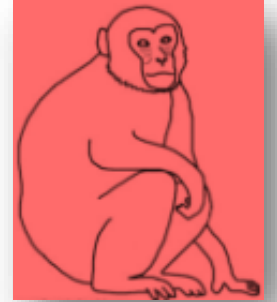
Species:

Rhesus macaques (*M. mulatta*), juvenile, A01⁻, B08⁻, B17⁻

Study arms:

4 (n = 4 animals each)

- Arm 1: mRNA prime, SOSIP boost, WT Envs
- Arm 2: mRNA prime, SOSIP boost, 113-429 locked Envs
- Arm 3: mRNA only, WT Envs
- Arm 4: mRNA only, 113-429 locked Envs



Inoculation:

Intramuscular

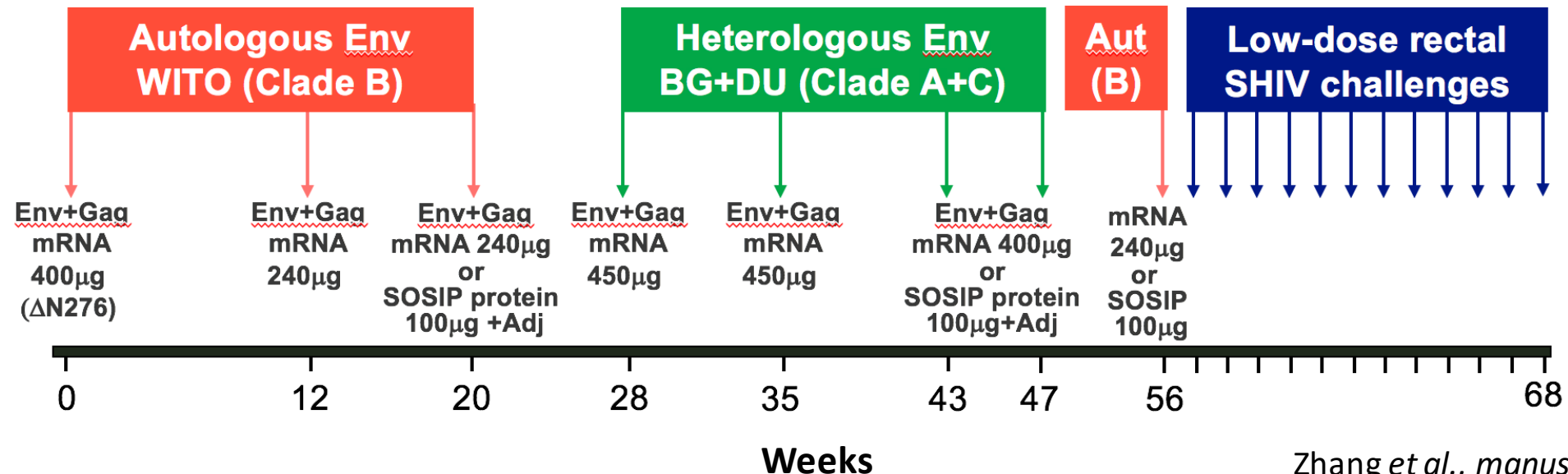
Env mRNA or protein:

WITO (clade B), BG505 (clade A), DU422 (clade C)

all glycan-repaired and 375Y-mutated

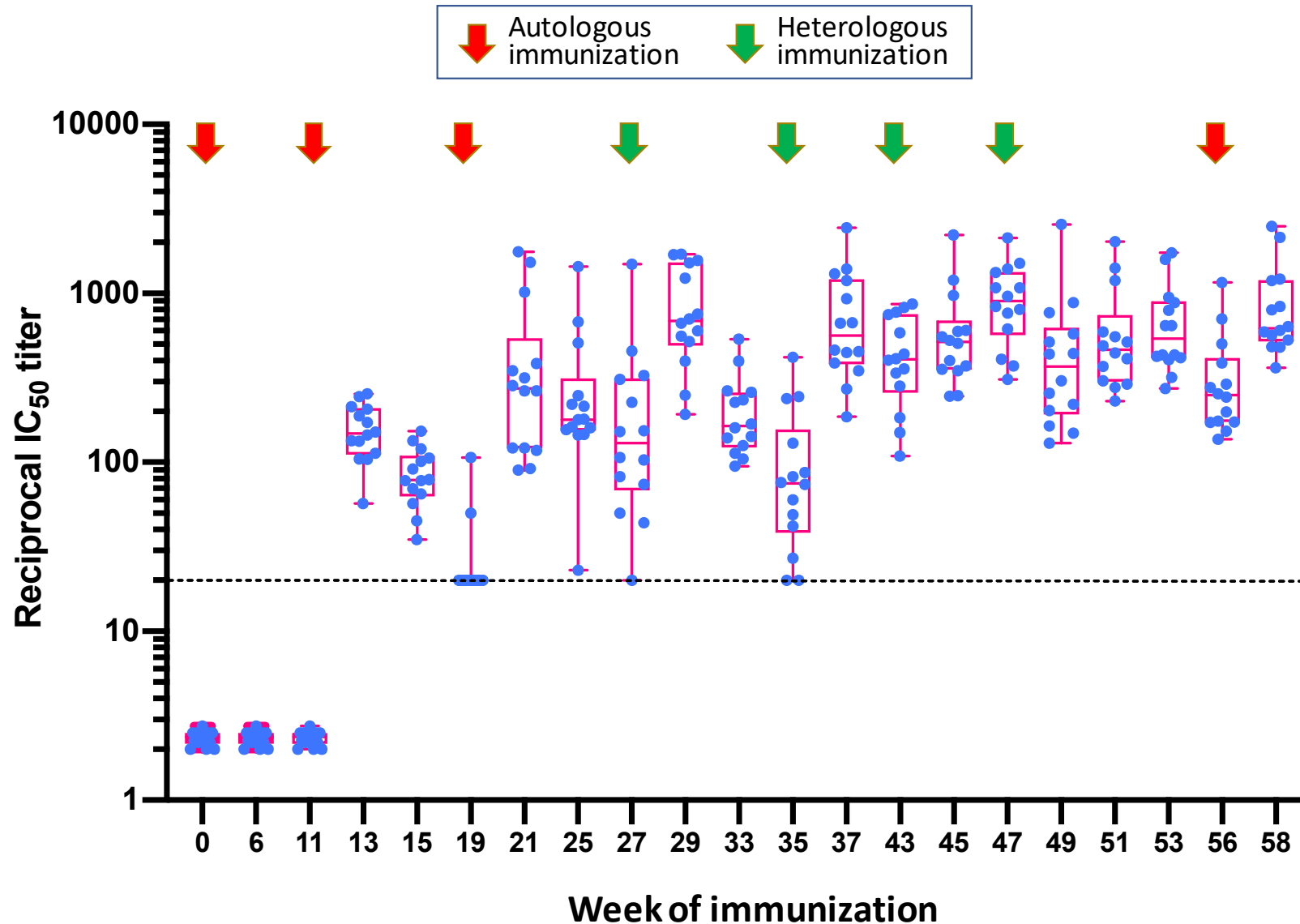
Gag mRNA:

SIVmac239

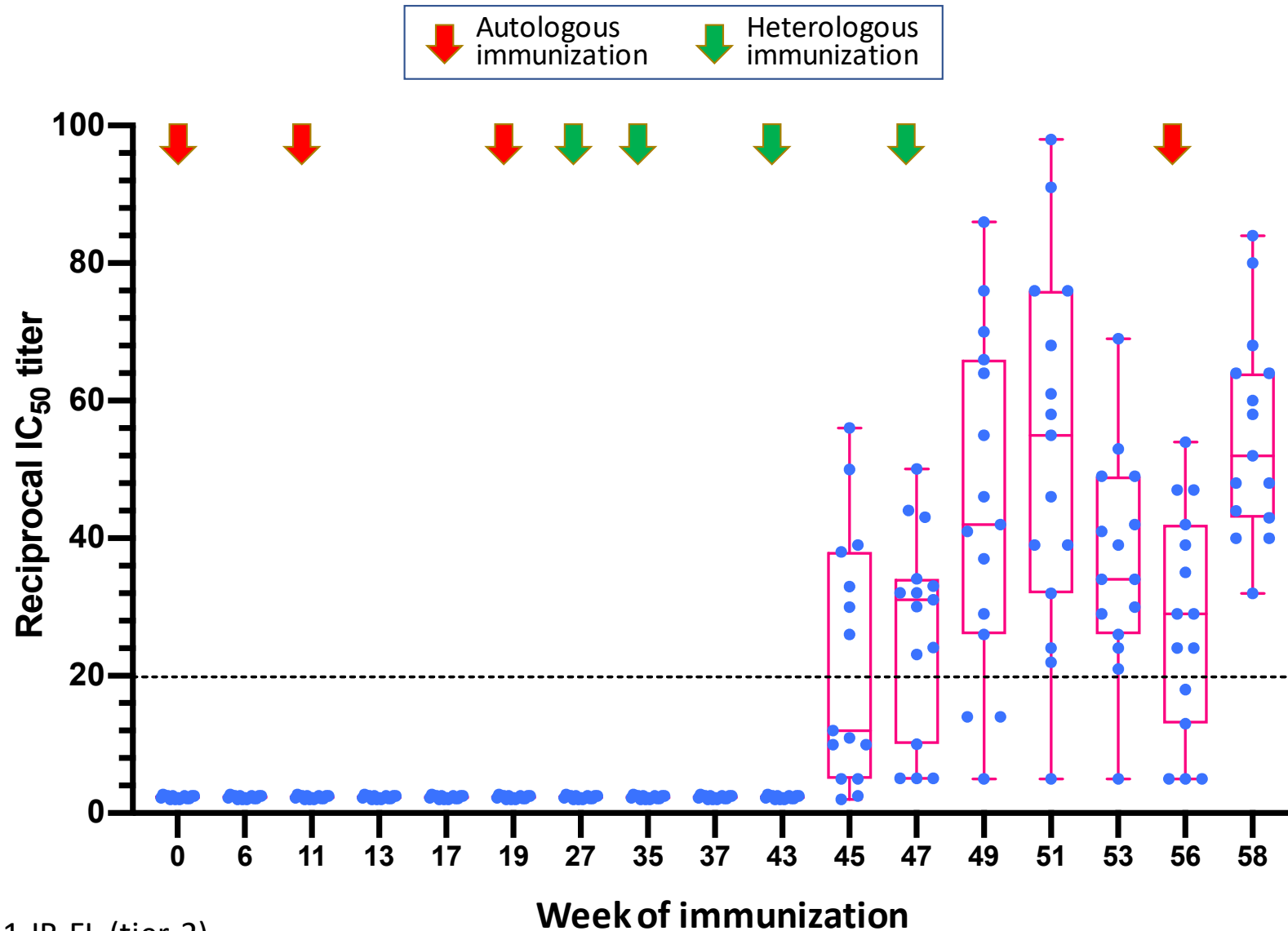


Induction of Broadly Neutralizing Antibodies

Progressive Induction of Autologous HIV-1 Neutralization in Macaques during the Course of the Immunization Phase



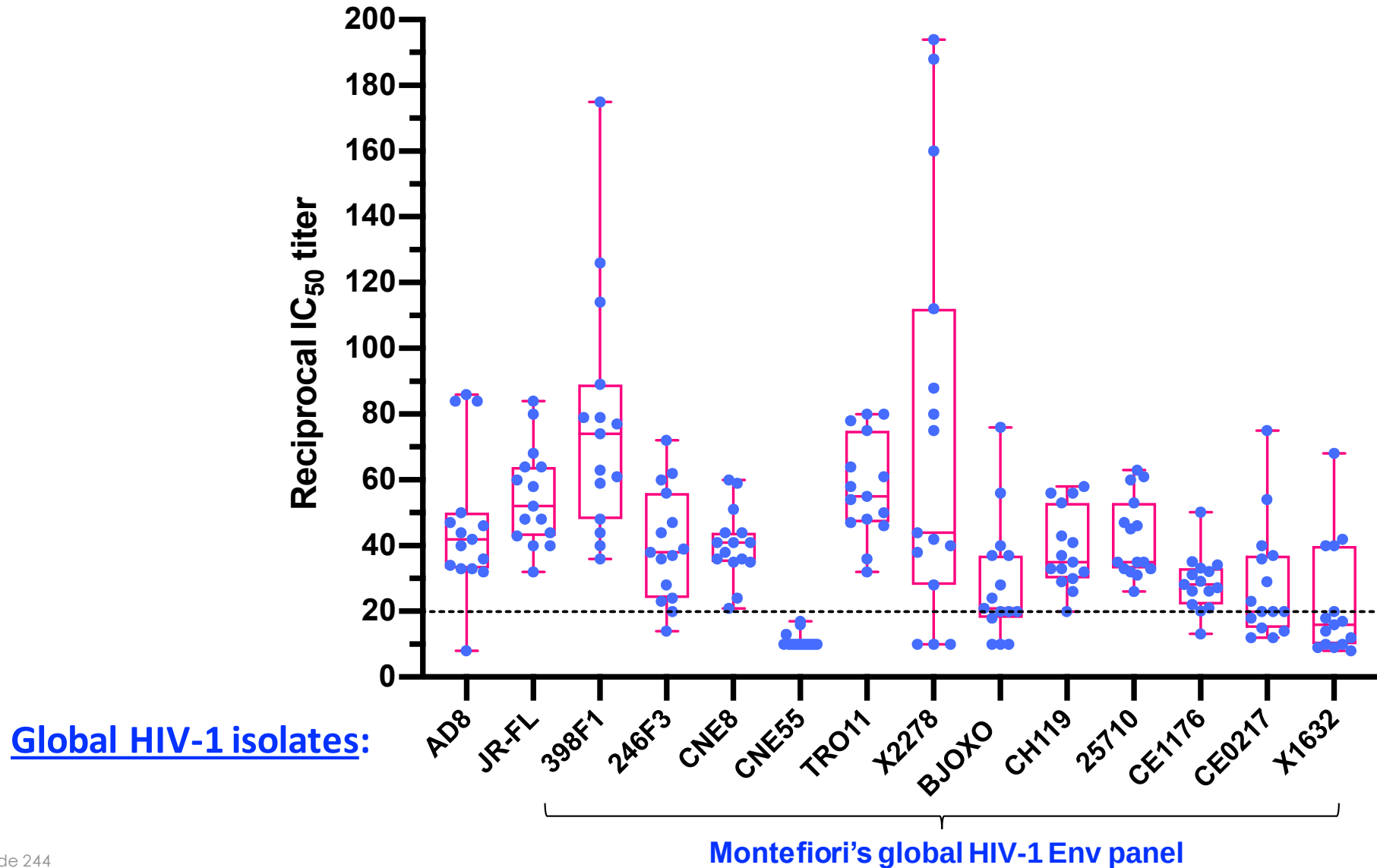
Appearance of Heterologous HIV-1 Neutralization* in Macaques after Repeated Heterologous Immunizations



*Neutralization of HIV-1 JR-FL (tier-2)

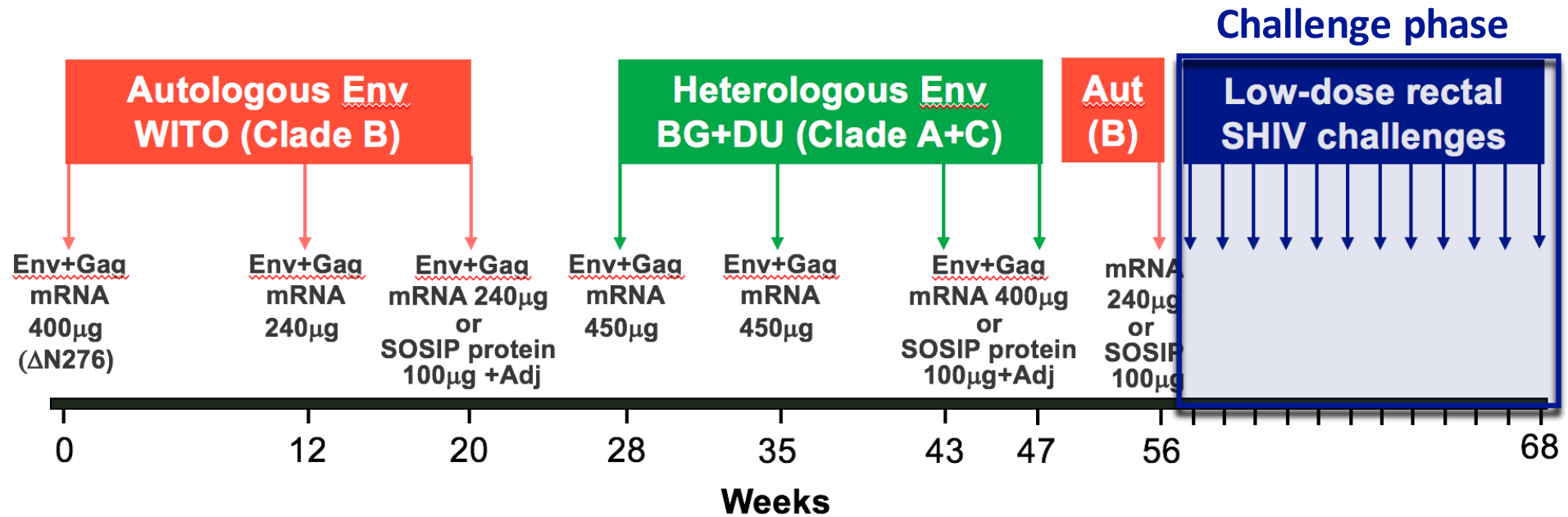
Zhang et al., manuscript in preparation

Broad-Spectrum Heterologous Tier-2 Neutralization in Macaques at the End of the Immunization Period (Wk. 58)

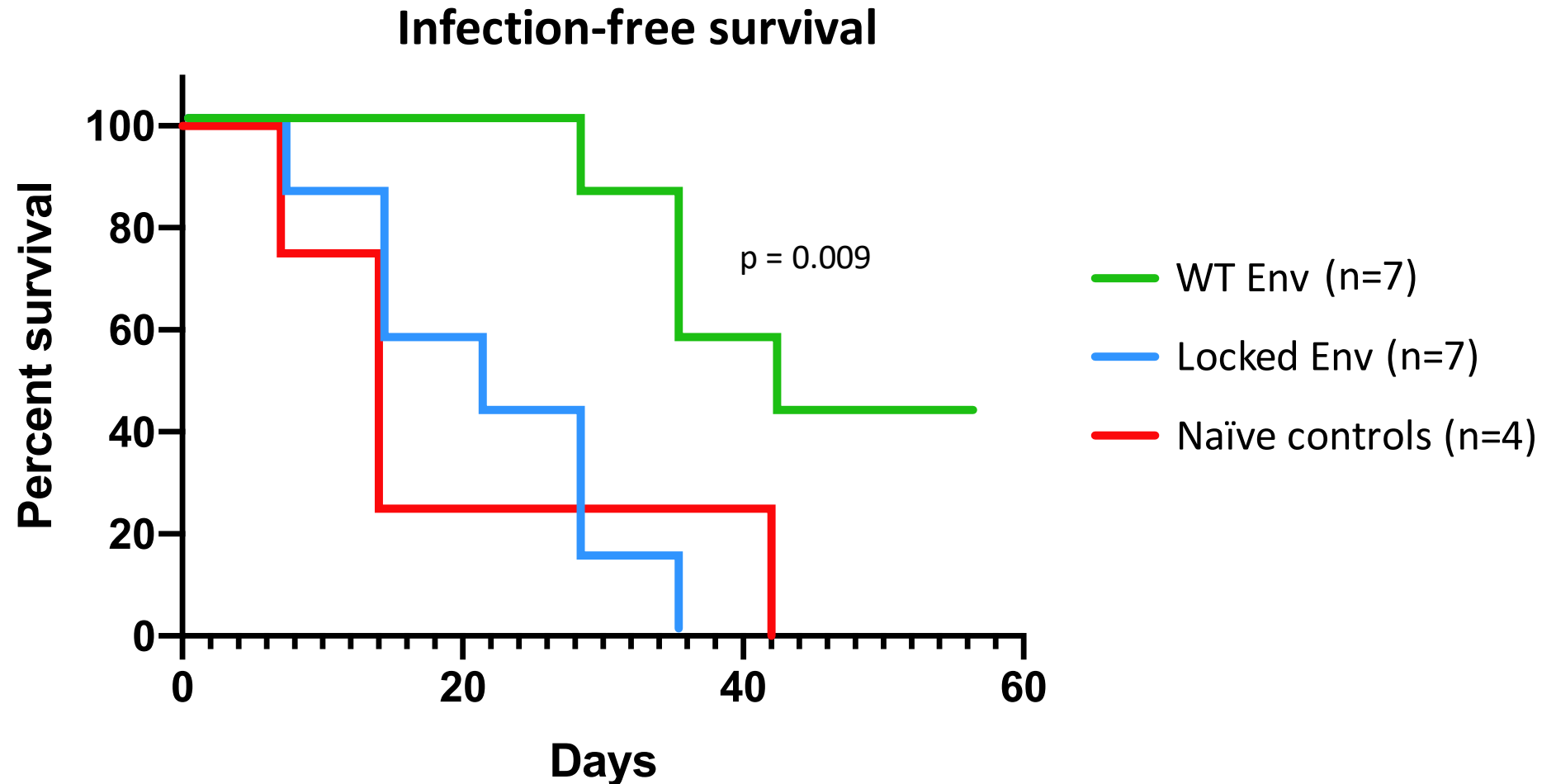


Heterologous Tier-2 SHIV Challenge

VPS-M1: Study Design

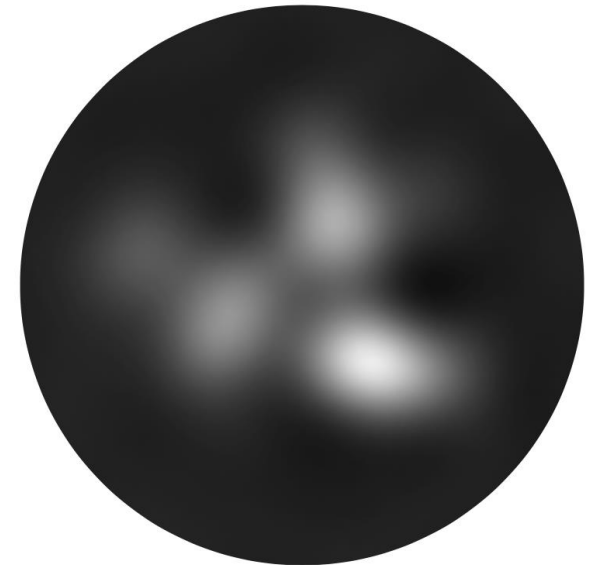
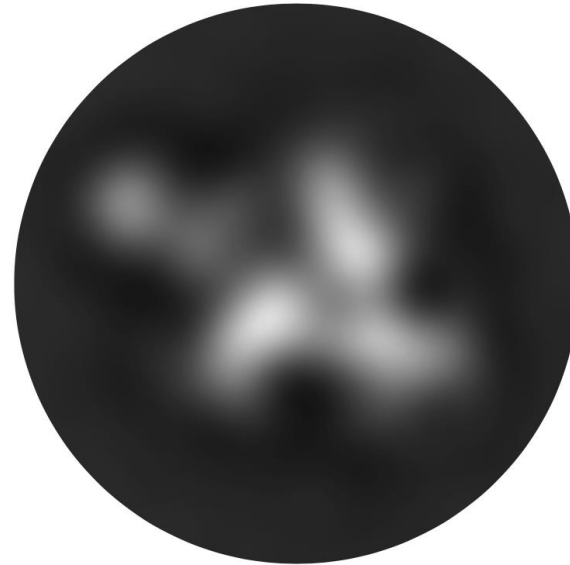
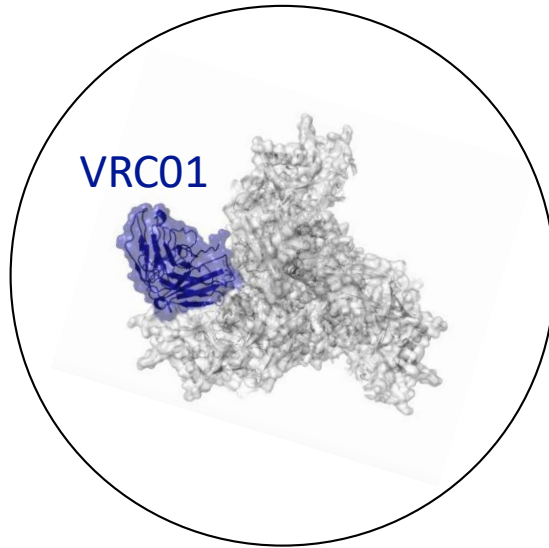


Protection from Pathogenic Tier-2 SHIV (AD8) Challenge in Immunized Macaques



Correlates of Protection

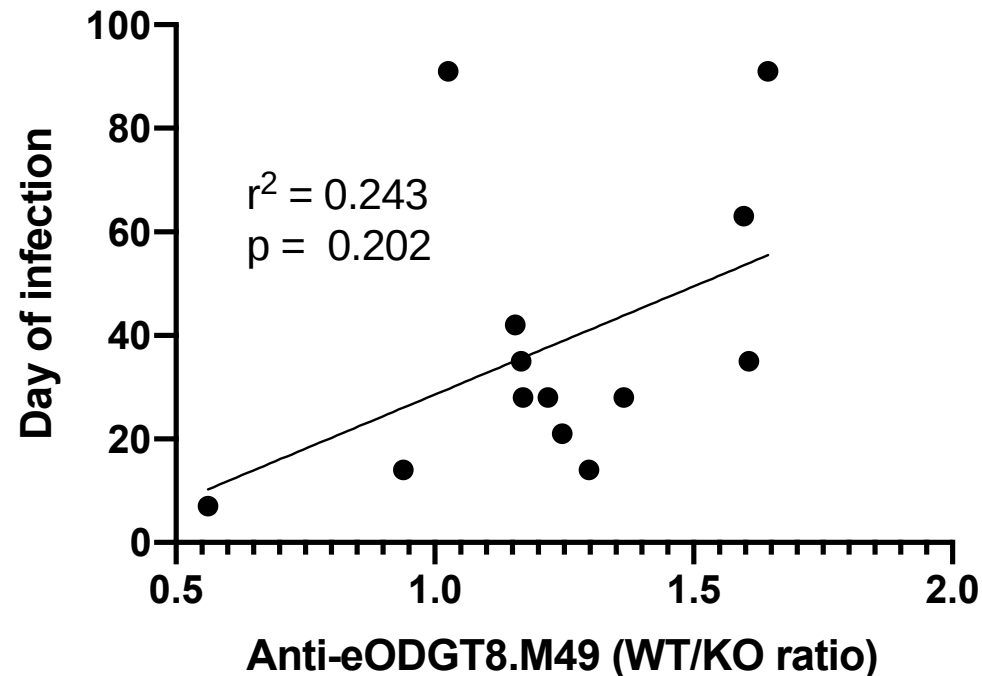
Antibodies to the CD4-Binding Site (VRC01-Like) Detected in Serum from a Protected Animal



Antibodies to the CD4-Binding Site (Probe M49) Correlated with Protection from SHIV-AD8 Infection in Vaccinated Macaques

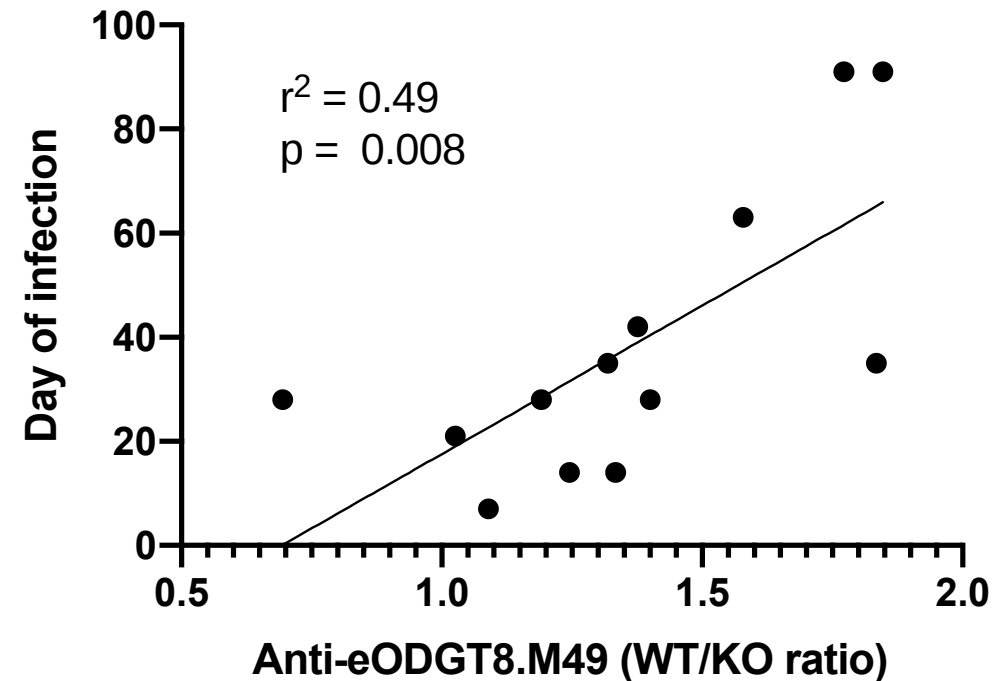
Week 37

eODGT8.M49 antibodies

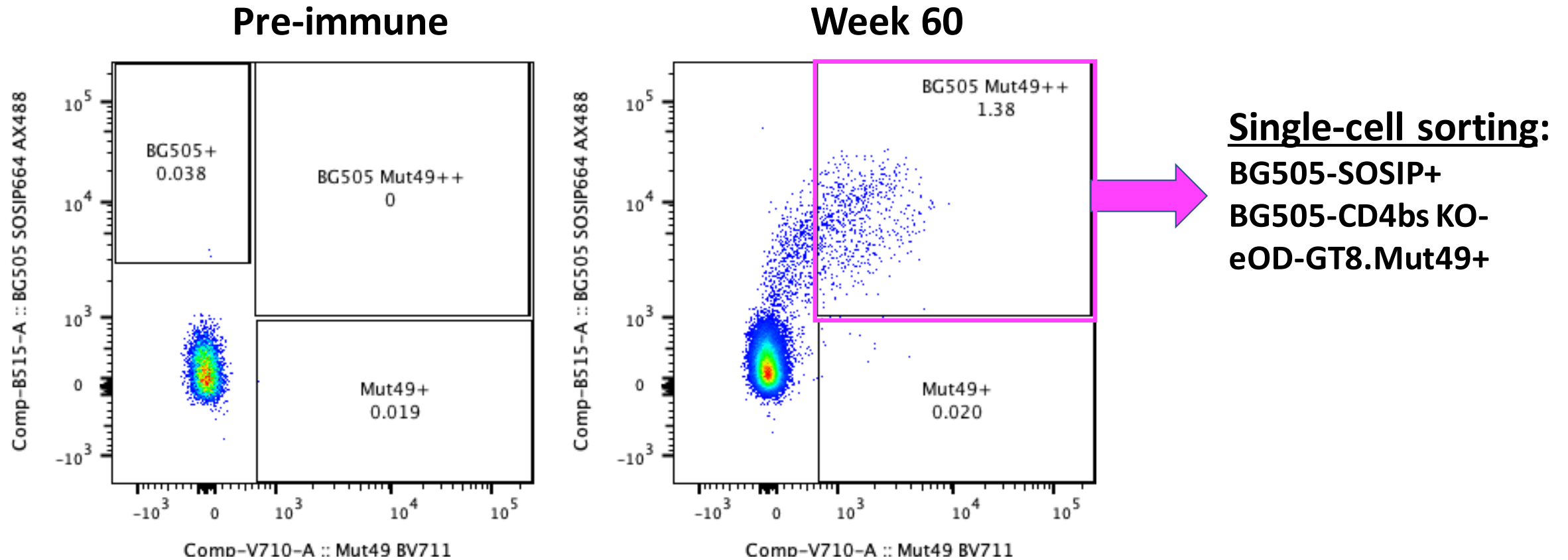


Week 58

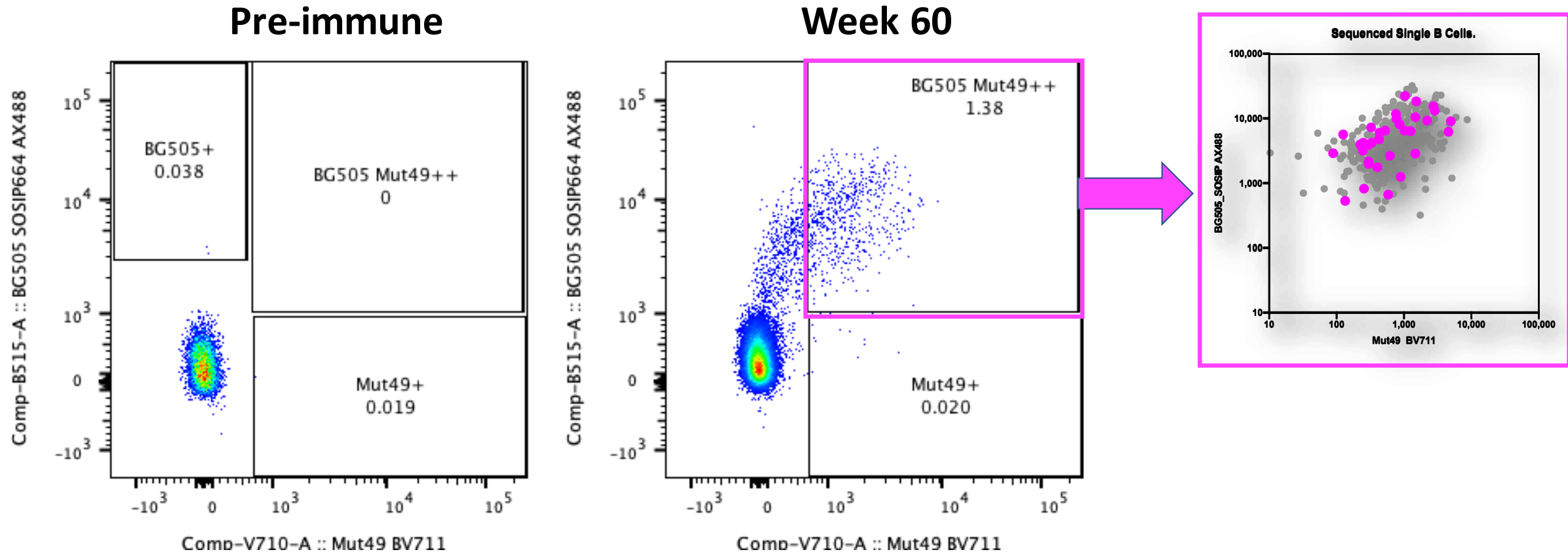
eODGT8.M49 antibodies



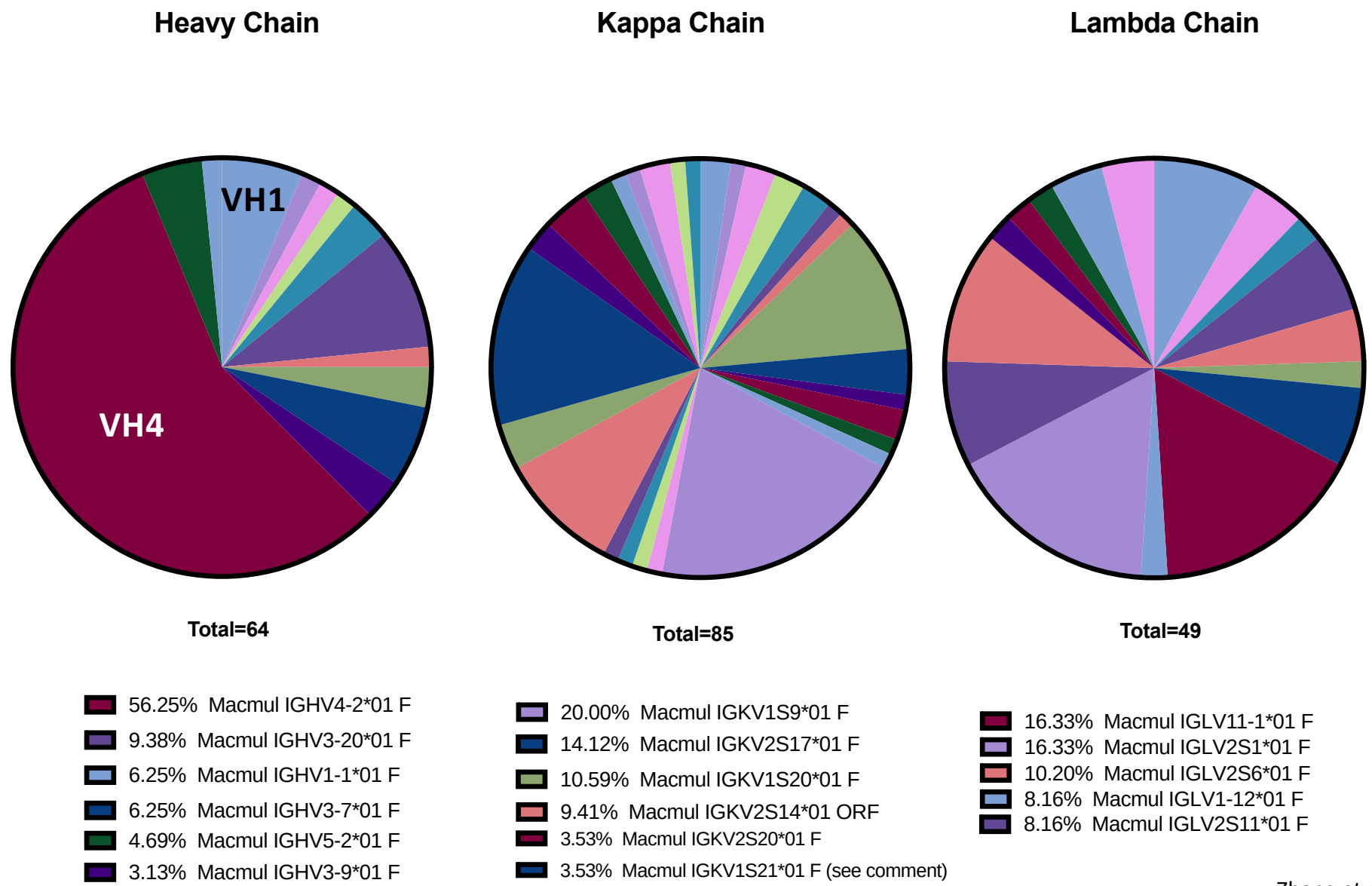
Single-Cell Sorting of eOD-GT8.M49-Binding B Cells from a Protected Macaque (#9)



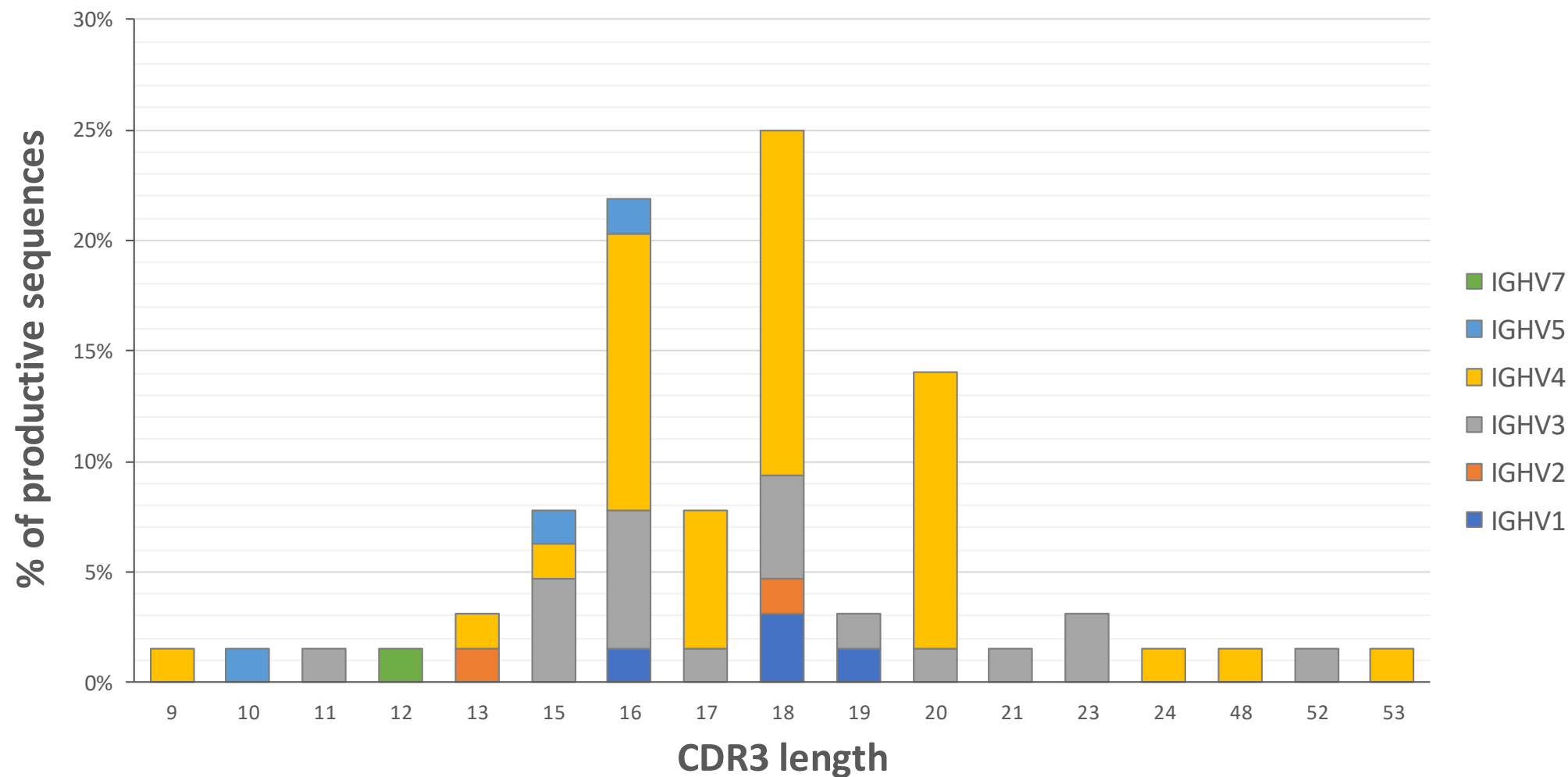
Single-Cell Sorting of eOD-GT8.M49-Binding B Cells from a Protected Macaque (#9)



Prevalence of Heavy Chain Variable Gene (VH) Usage among CD4-BS-Specific Antibodies Cloned from a Protected Macaque (#9, week 60)



CDR3 Length Distribution in Heavy Chain Variable (VH) Genes of CD4-BS-Specific Cloned Antibodies



HCDR3 length: 9-53 aa. (average 18.4)

The NIH-Moderna HIV-1 Vaccine Approach

1. Use membrane-anchored Env expressed *in vivo* by mRNA
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Close mimicry of real-life infection with native virus

NIH-Moderna HIV-1 Vaccine: Next Steps

1. Repeat/expand the first pre-clinical NHP study to:
 - Confirm initial results
 - Further optimize immunogens
 - Streamline protocol
2. Design first-in-human clinical trial with an mRNA-based HIV-1 vaccine

The logo of the Bill & Melinda Gates Foundation, featuring the text "BILL & MELINDA GATES foundation" in a serif font, with "GATES foundation" in a smaller, italicized font.



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Qingbo Liu, Ph.D.

Hana Schmeisser, Ph.D.

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Elisabeth Narayanan, Ph.D.

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Isabella Renzi, Ph.D.

Vlad Presnyak, Ph.D.

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Giuseppe Ciaramella, Ph.D.

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Jason Gorman, Ph.D.

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Yaroslav Tsybovsky, Ph.D.

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Bioqual Inc.

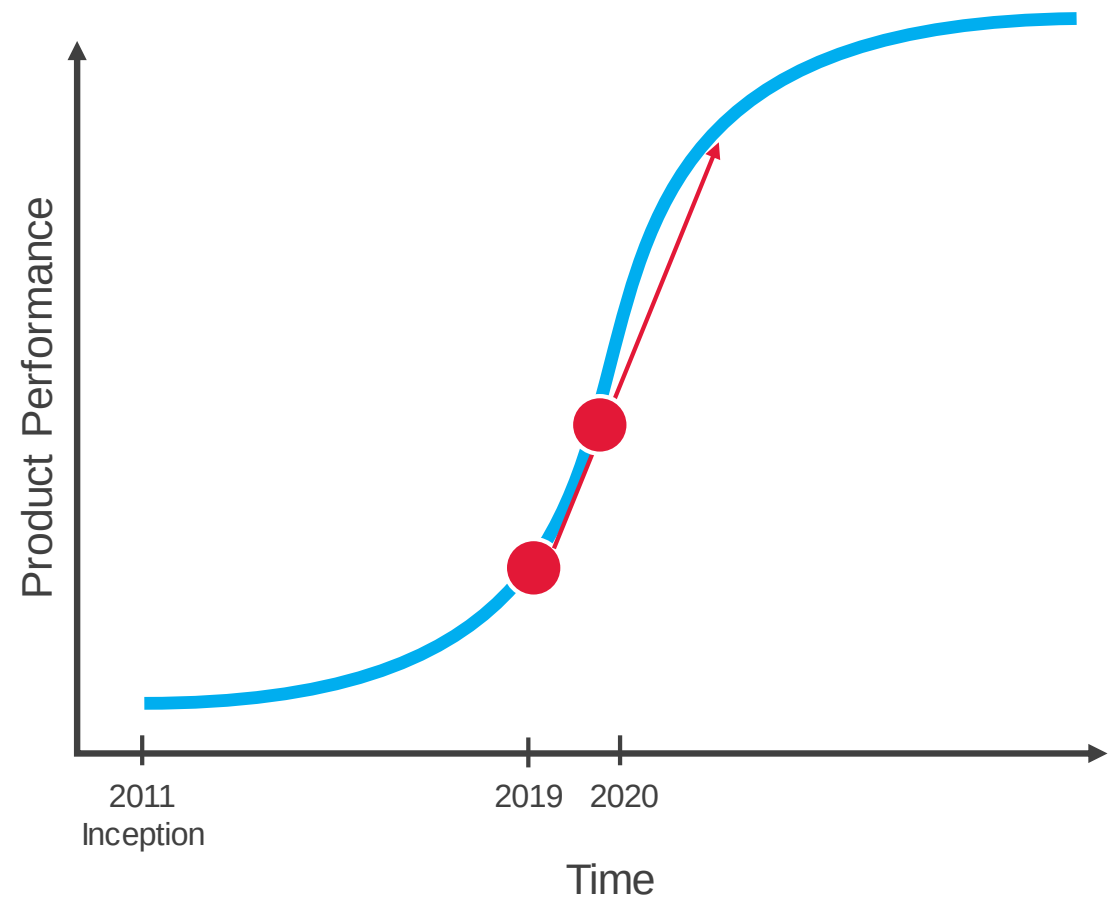
Deborah Weiss, VDM

Jonathan Misamore, VDM



Conclusion

Our commitment to be the best at mRNA science is core to who we are: A 20-year journey



2018 Science Day Themes and Publications

- Development of proprietary ionizable lipids for improved systemic mRNA delivery
- Development of proprietary ionizable lipids with improved tolerability for mRNA vaccines
- Use of miRNA target sites to create "smart" mRNAs
- Translation initiation and "leaky scanning"
- How artificial intelligence can help us design better coding sequences

A Novel Amino Lipid Series for mRNA Delivery: Improved Endosomal Escape and Sustained Pharmacology and Safety in Non-human Primates

Staci Sabnis,¹ E. Sathyajith Kumarasinghe,¹ Timothy Salerno,¹ Cosmin Mihai,¹ Tatiana Ketova,¹ Joseph J. Senn,¹ Andy Lynn,¹ Alex Bulychev,¹ Iain McFadyen,¹ Joyce Chan,¹ Örn Almarsson,¹ Matthew G. Stanton,^{1,2} and Kerry E. Benenato¹

Sabnis ... Benenato (2018) Mol Ther

Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines

Kimberly J. Hassett,¹ Kerry E. Benenato,¹ Eric Jacquinet,¹ Aisha Lee,¹ Angela Woods,¹ Olga Yuzhakov,¹ Sunny Himansu,¹ Jessica Deterling,¹ Benjamin M. Geilich,¹ Tatiana Ketova,¹ Cosmin Mihai,¹ Andy Lynn,¹ Iain McFadyen,¹ Melissa J. Moore,¹ Joseph J. Senn,¹ Matthew G. Stanton,^{1,2} Örn Almarsson,¹ Giuseppe Ciaramella,^{1,3} and Luis A. Brito¹

Hassett ... Brito (2019) Mol Ther Nuc Acids

MicroRNAs Enable mRNA Therapeutics to Selectively Program Cancer Cells to Self-Destruct

Ruchi Jain,¹ Josh P. Frederick,² Eric Y. Huang,³ Kristine E. Burke,⁴ David M. Mauger,⁵ Elizaveta A. Andrianova,¹ Sam J. Farlow,² Summar Siddiqui,⁶ Jeffrey Pimentel,⁶ Kahlin Cheung-Ong,² Kristine M. McKinney,^{3,*} Caroline Köhrer,¹ Melissa J. Moore,¹ and Tirtha Chakraborty^{1,†}

Jain ... Chakraborty (2018) Nuc Acid Ther

2019 Science Day Themes and Publications

- Why we incorporate modified nucleotides into our mRNAs
- Coding sequence design: Codon optimality vs RNA secondary structure
- 5'-UTR design
- The physical and computational methods we employ to understand LNP structure
- An early glimpse into our Immune LNP

Impact of mRNA chemistry and manufacturing process on innate immune activation

Jennifer Nelson^{*†}, Elizabeth W. Sorensen^{*‡}, Shrutika Mintri[§], Amy E. Rabideau, Wei Zheng, Gilles Besin^{||}, Nikhil Khatwani[¶], Stephen V. Su[#], Edward J. Miracco, William J. Issa, Stephen Hoge, Matthew G. Stanton^{**}, John L. Joyal^{††}

Nelson ... Joyal (2020) Science Advances

mRNA structure regulates protein expression through changes in functional half-life

David M. Mauger[¶], B. Joseph Cabral^{¶,1}, Vladimir Presnyak[¶], Stephen V. Su^{¶,2}, David W. Reid[¶], Brooke Goodman[¶], Kristian Link[¶], Nikhil Khatwani^{¶,3}, John Reynders^{¶,4}, Melissa J. Moore^{¶,5}, and Iain J. McFadyen^{¶,6}

[¶]Platform Research, Moderna, Inc., Cambridge, MA 02139

Mauger ... McFadyen (2019) PNAS

Human 5' UTR design and variant effect prediction from a massively parallel translation assay

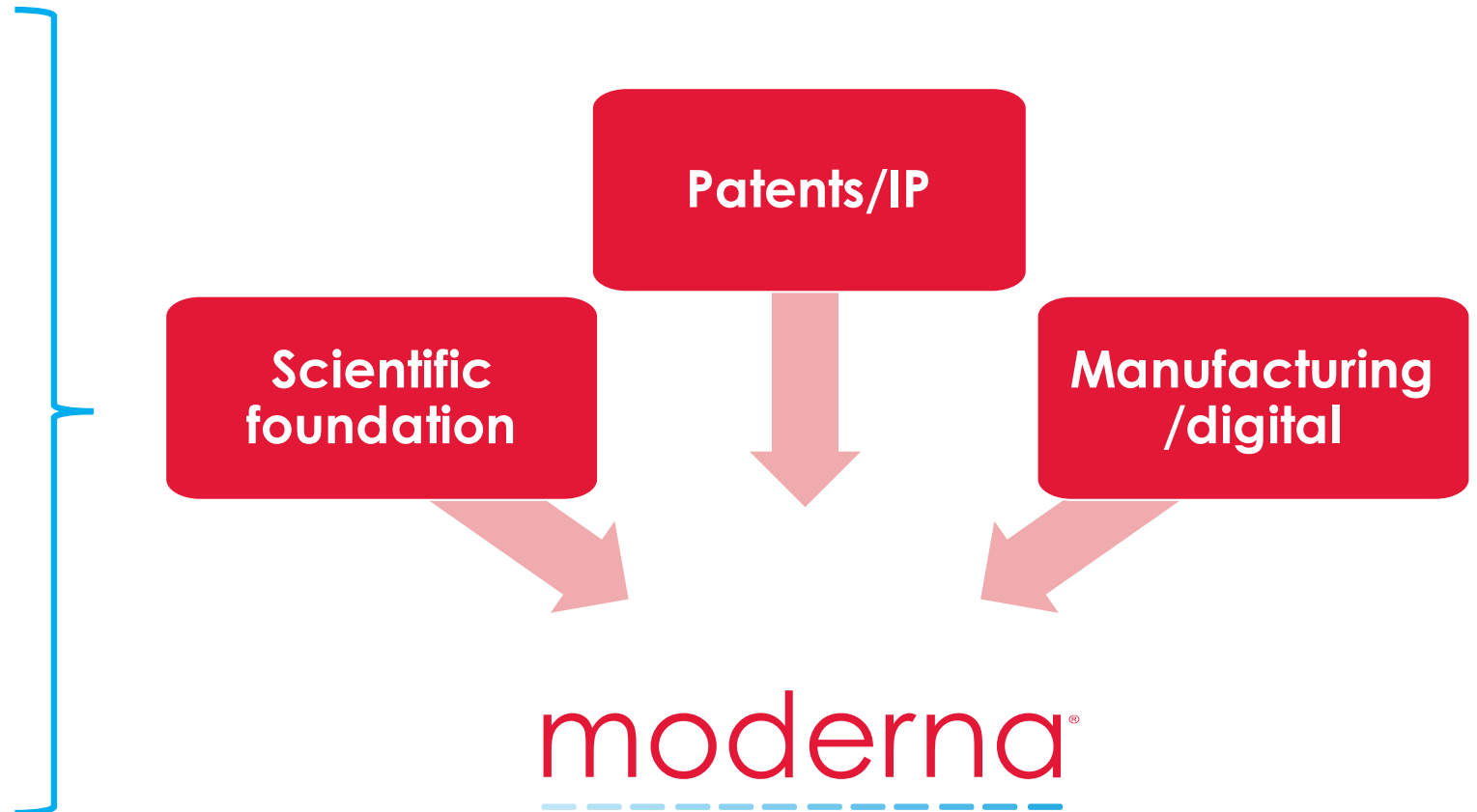
Paul J. Sample^{¶,1,5}, Ban Wang^{¶,1,5}, David W. Reid^{¶,2}, Vlad Presnyak^{¶,2}, Iain J. McFadyen^{¶,2}, David R. Morris[¶] and Georg Seelig^{¶,1,4*}

Sample ... Seelig (2019) Nature Biotech

mRNA platform science builds our foundation

Themes from today's presentations

- How we are extending pharmacology
- T7 enzyme engineering to reduce dsRNA production
- New LNP's for liver delivery
- Impact of LNP size on immunogenicity
- Progress towards creating a mRNA vaccine for HIV





Q&A