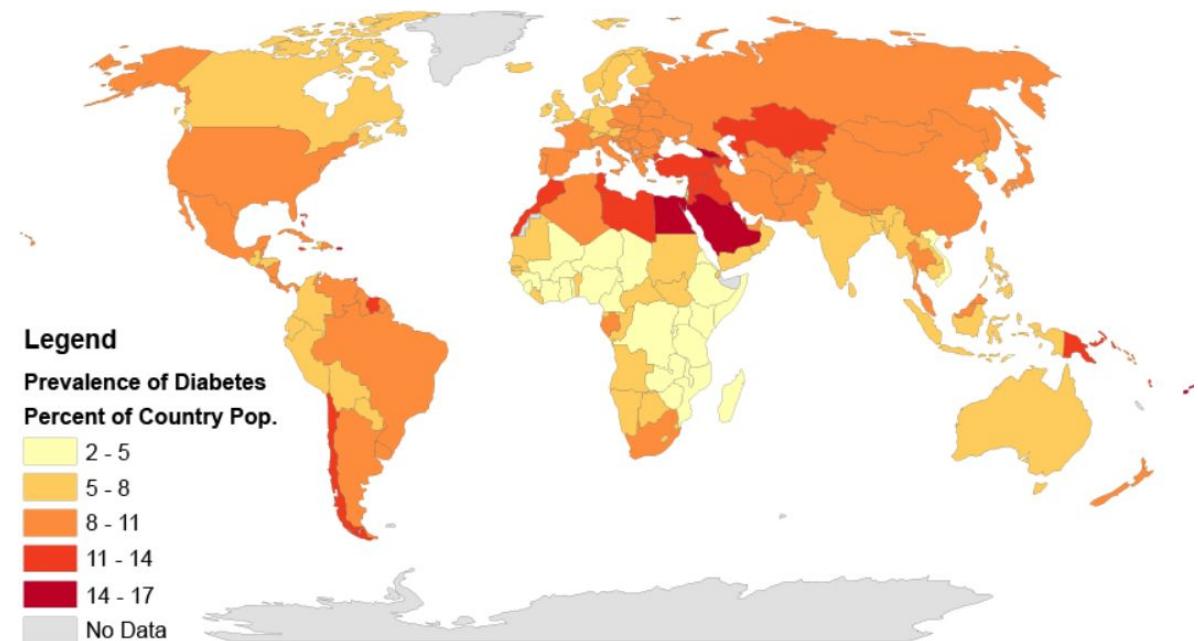


AMIDD 2024 Lecture 3: Key questions in drug discovery

We divided the classroom into five personas:

1. Patients of Type 2 Diabetes
2. Medical doctors
3. Drug discovery company
4. Insurance company
5. The regulatory agency

Questions: (1) What are your main interests and concerns? (2) With which groups do you wish to collaborate? Why? Rank the partners by the priority. (3) What are the ideal and worse scenarios for you?

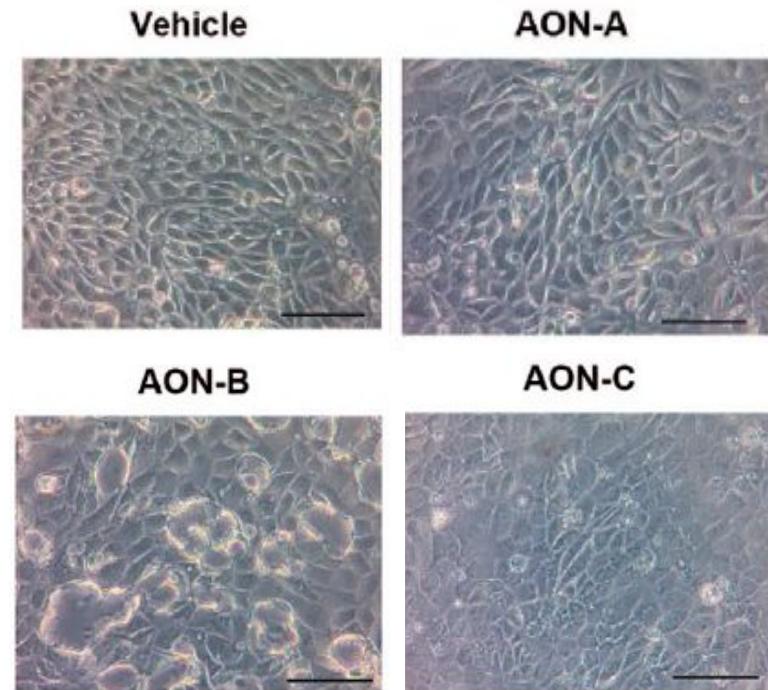
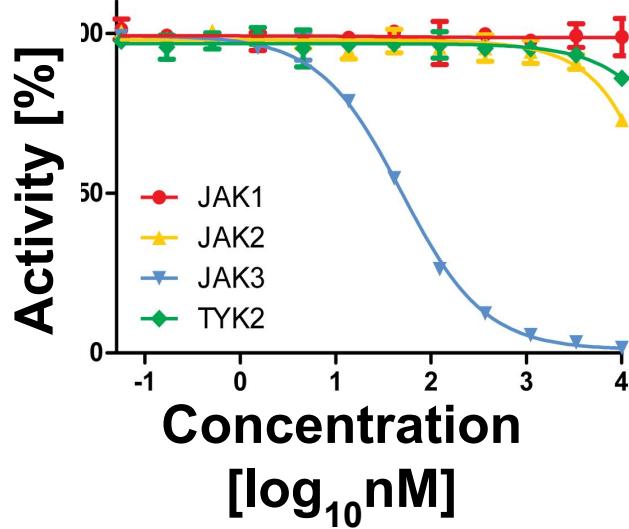


Dr. Jitao David Zhang, Computational Biologist

¹ Pharmaceutical Sciences, Pharma Research and Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche

² Department of Mathematics and Informatics, University of Basel

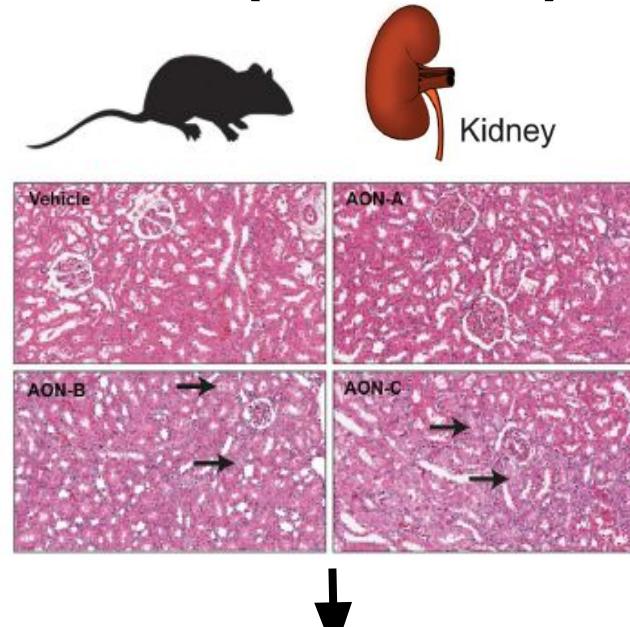
Classical workflow of drug discovery from models' perspective



Cellular assays
(in vitro)

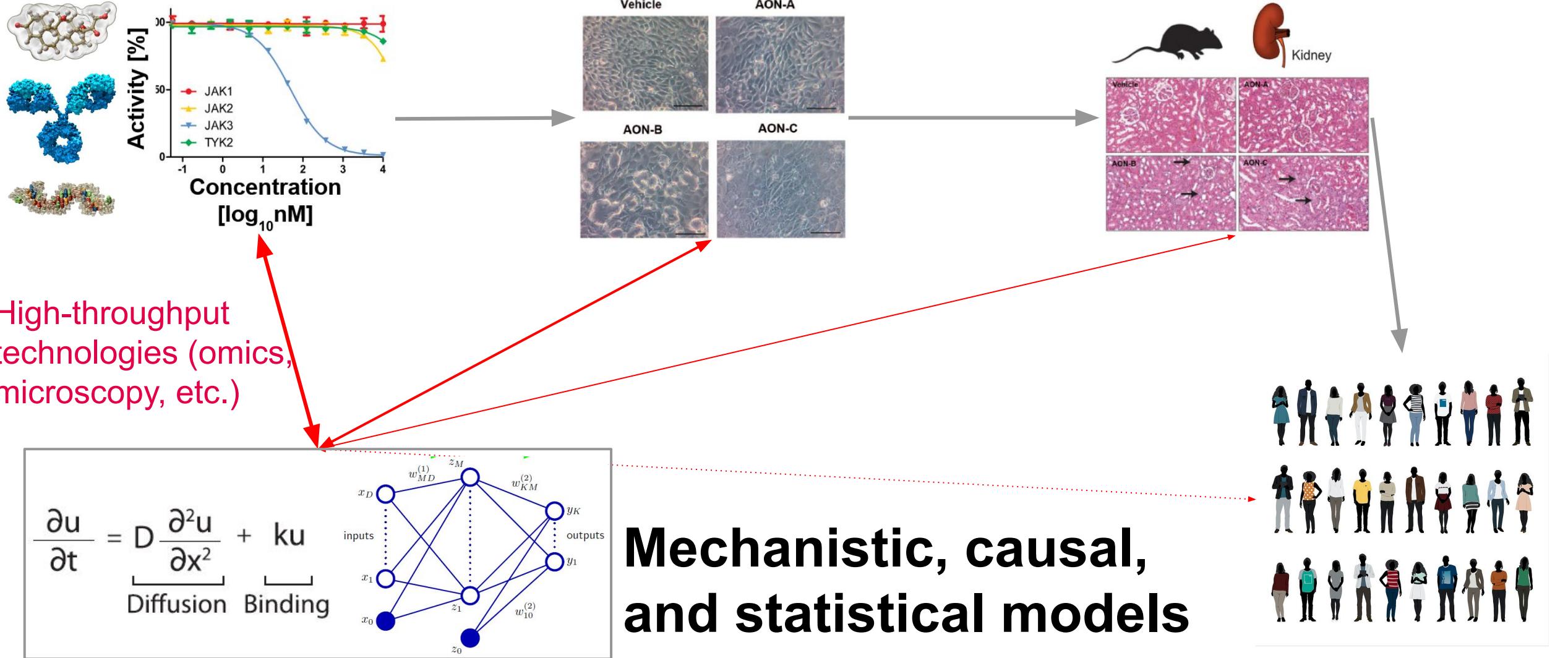
Biochemical or biophysical assays

Animal experiments (*in vivo*)



Clinical trials

Mathematical and computational models integrate data across scales



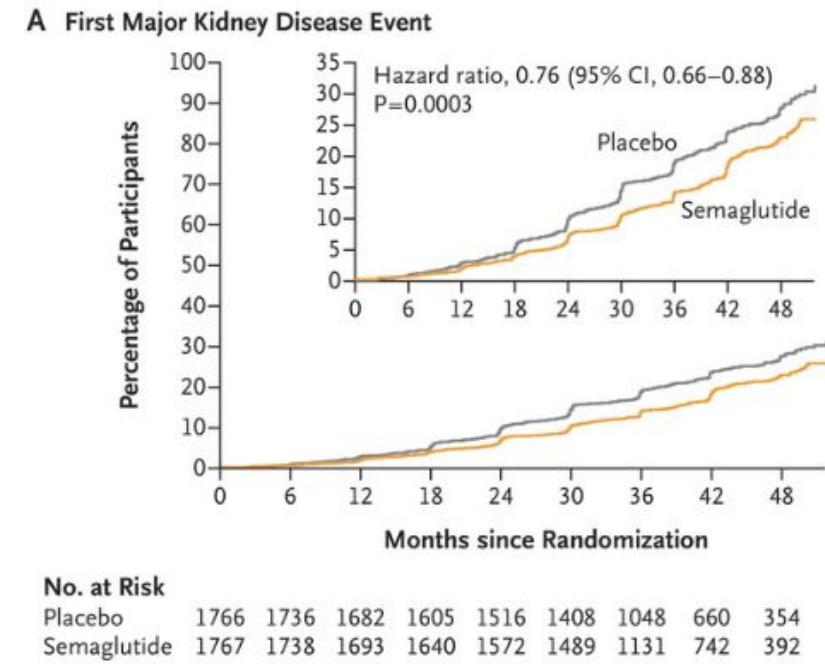
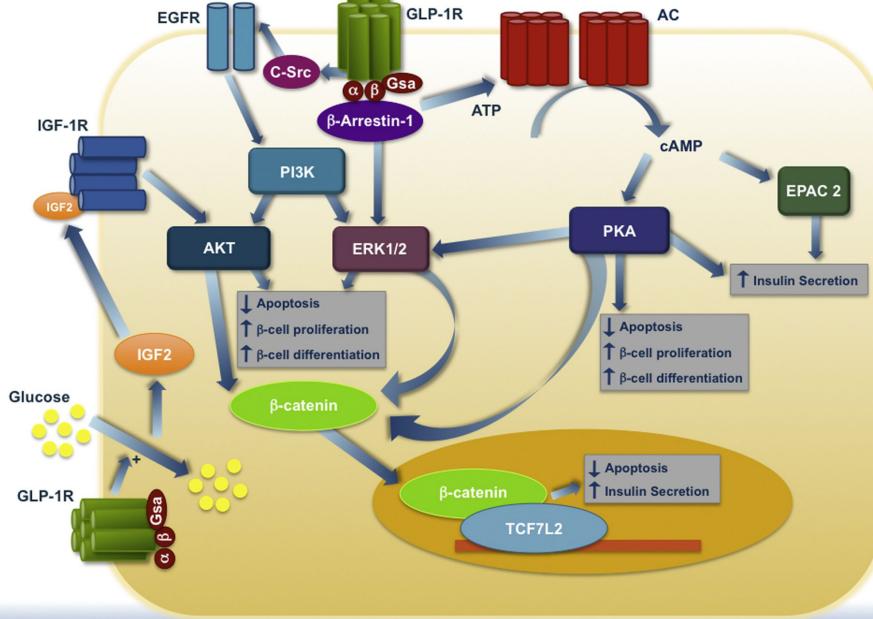
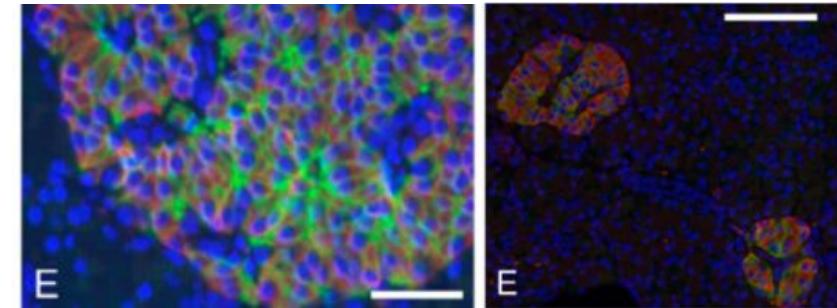
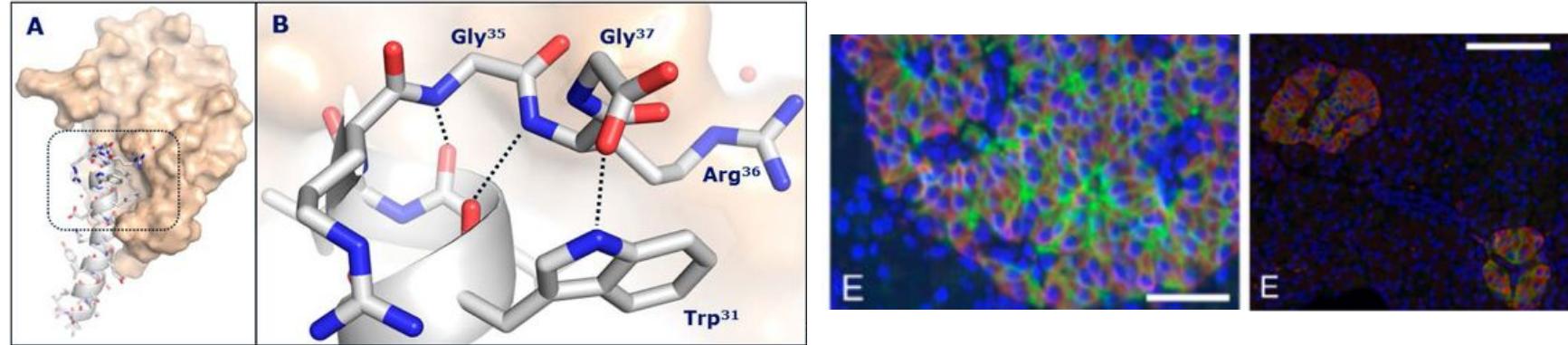
An example of multiscale understanding with semaglutide

Top left: crystal structure of the semaglutide peptide backbone (gray) in complex with its target, GLP-1 receptor (golden surfaces).

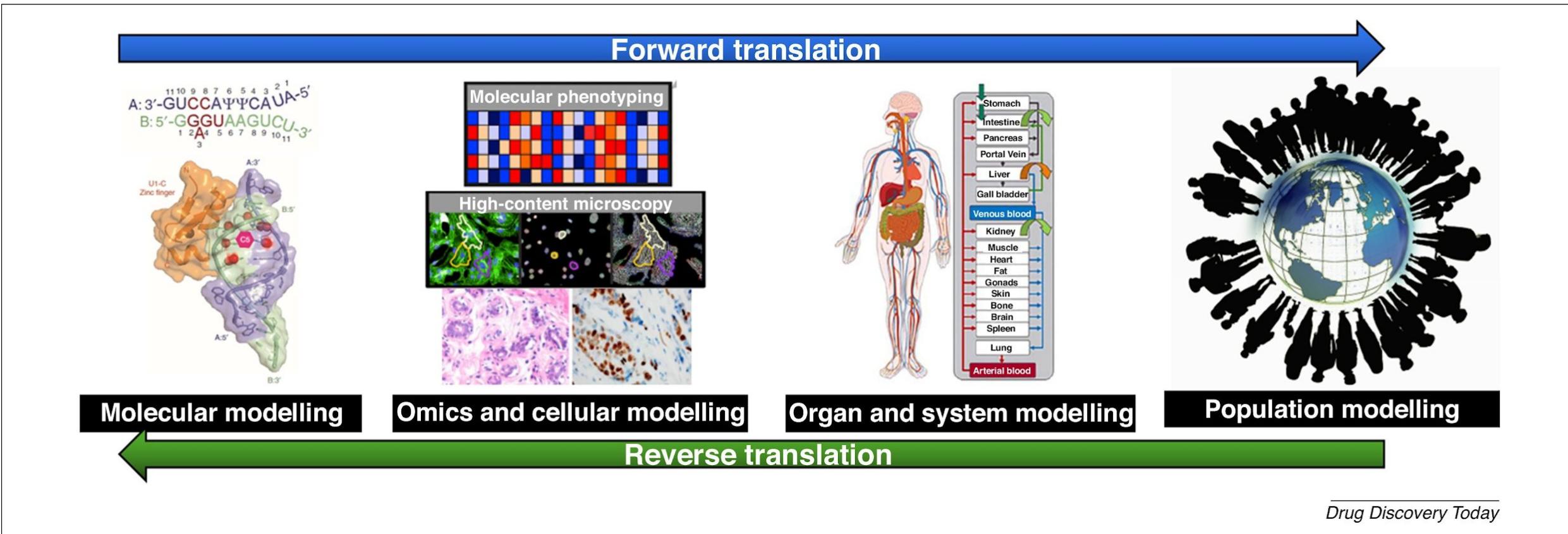
Bottom left: GLP-1 signaling pathway in pancreas beta-cells ([Campbell & Drucker, 2013](#))

Top right: immunostaining of monkey (left) and human (right) pancreas.

Bottom right: the primary outcome of [the FLOW trial](#).



The multiscale modelling view of drug discovery



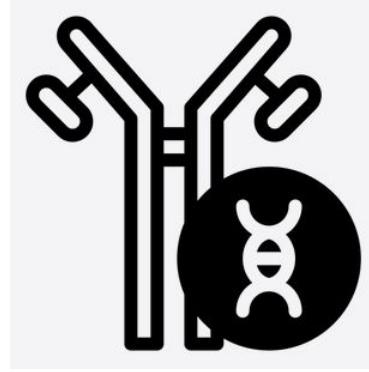
Zhang, Jitao David, Lisa Sach-Peltason, Christian Kramer, Ken Wang, and Martin Ebeling. 2020. "Multiscale Modelling of Drug Mechanism and Safety." Drug Discovery Today 25 (3): 519–34. <https://doi.org/10.1016/j.drudis.2019.12.009>.

Five key questions in drug discovery



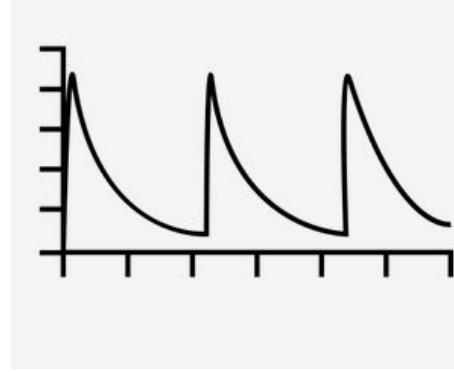
Medical Need

What is the unmet medical need to be addressed?



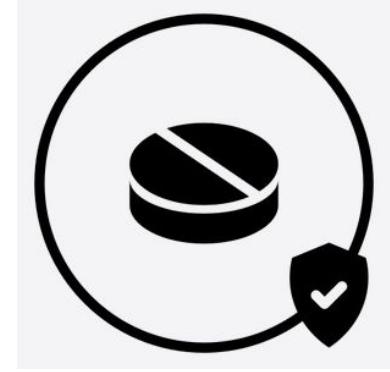
Target & modality

What is the target?
What is the modality?



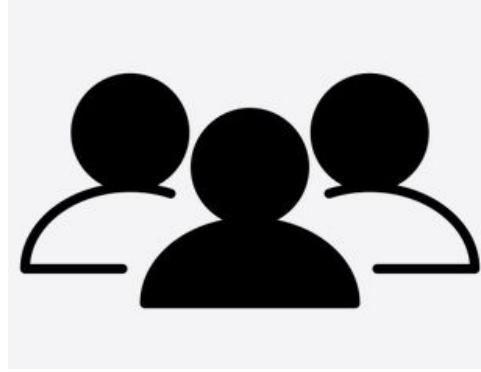
PK/PD

How much drug reach which body part? What does body do to the drug (PK)? What does the drug do to the body (PD)?



Benefit/risk

What is the toxicity of the drug? Is it justifiable given the benefits?



Patient stratification

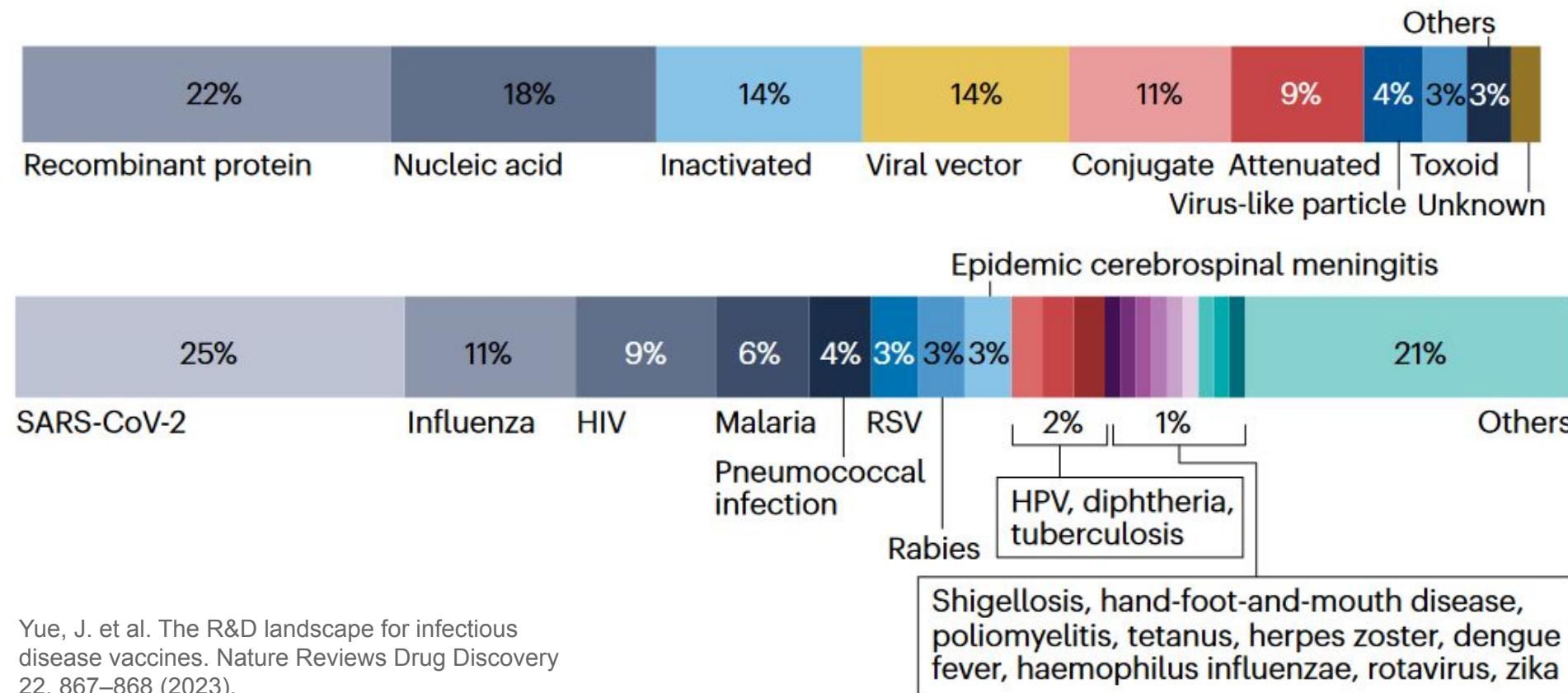
Who are responsive to the drug? Who are susceptible to adverse events?

Nobel Prize in Physiology or Medicine 2023 was awarded to Katalin Karikó and Drew Weissman for “their discoveries concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19”



Main methods for vaccine production before the COVID-19 pandemic:

- **Recombinant protein** (e.g. HBV)
- **Inactivated viruses** (e.g. Influenza and Polio)
- **viral vectors** (e.g. HIV) are.



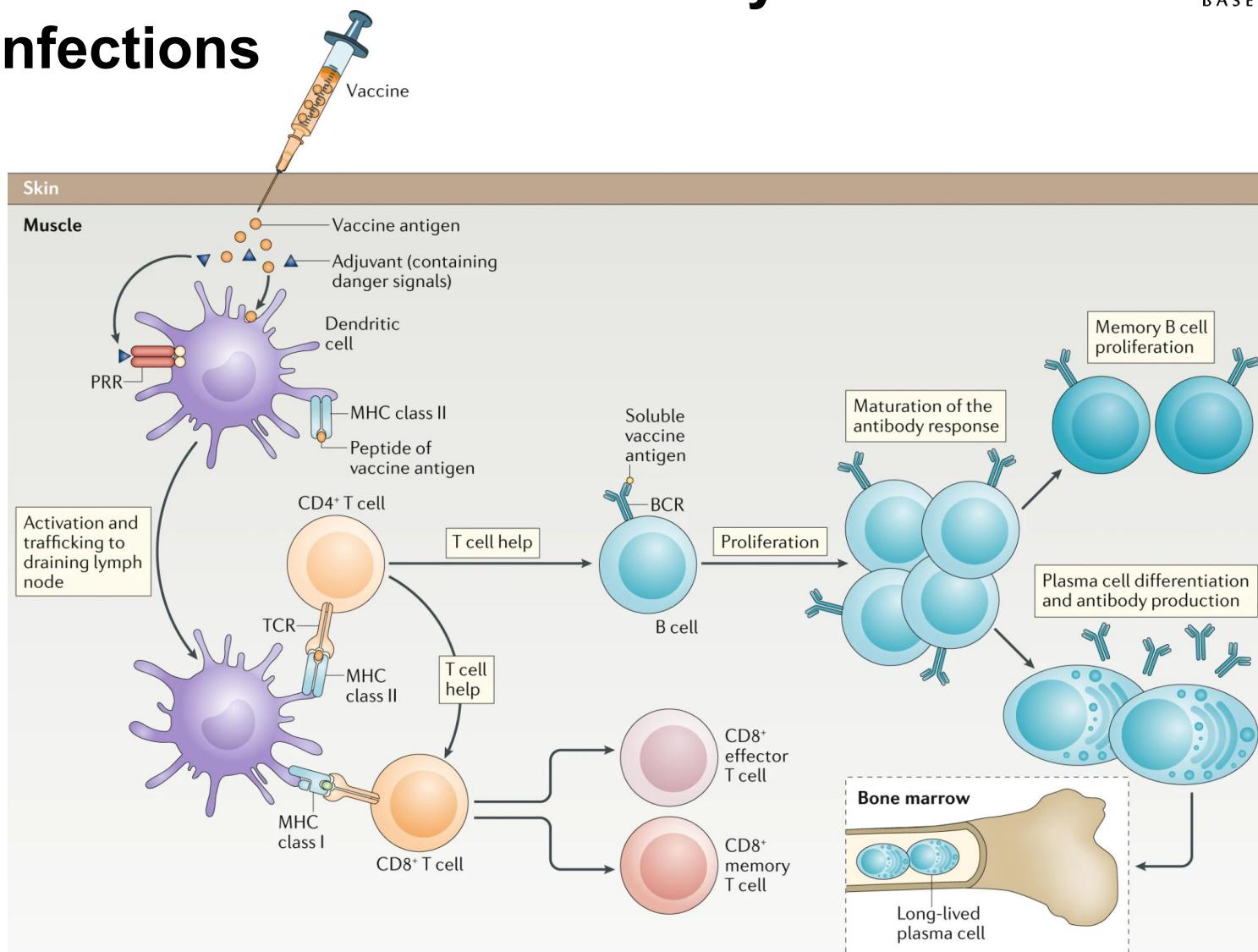
Issues: large-scale cell culture is required, which limits the possibilities for rapid production in response to pandemics.

Vaccine mimics viral infection to activate the immune system to protect body from future infections

Vaccine mimics a viral infection to activate innate and adaptive immune system, while minimizing the pathogenic effects.

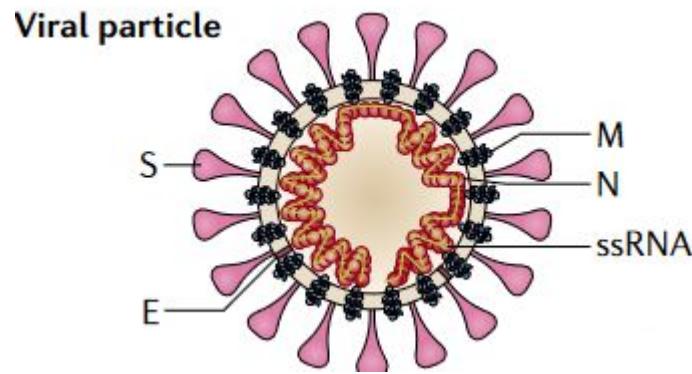
Key players in the game:

1. Viral proteins as *antigens*
2. Antigen-presenting cells (e.g. dendritic cells)
3. T cells (T comes from Thymus, because they mature there)
4. B cells (B comes from bone marrow).

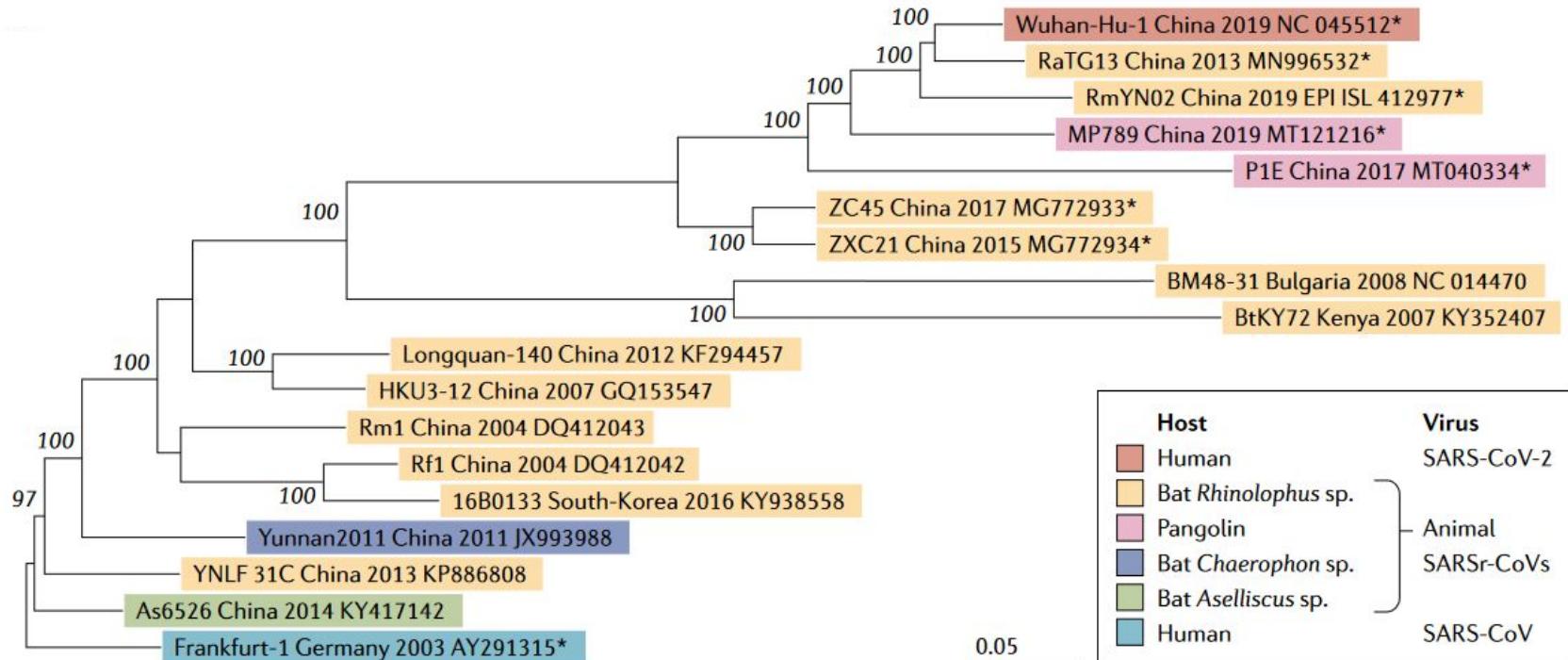


Pollard, A. J. & Bijker, E. M. A guide to vaccinology: from basic principles to new developments. Nature Reviews Immunology 21, 83–100 (2021).

Coronavirus is a RNA virus infecting human and other species



The coronavirus virion consists of structural proteins, namely spike (S), envelope (E), membrane (M), nucleocapsid (N)

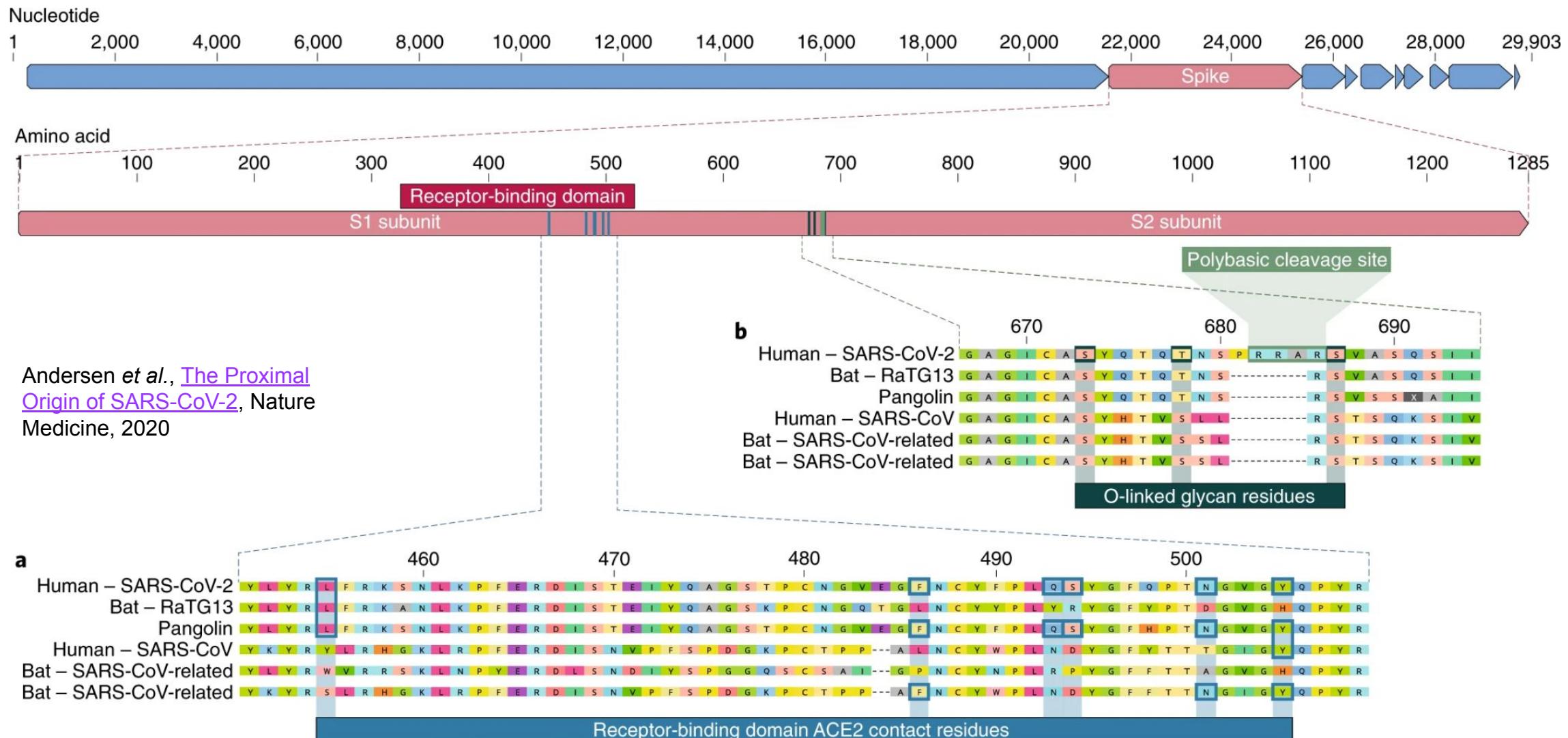


Host	Virus
Human	SARS-CoV-2
Bat Rhinolophus sp.	SARS-CoVs
Pangolin	Animal
Bat Chaerophon sp.	SARS-CoVs
Bat Aselliscus sp.	
Human	SARS-CoV

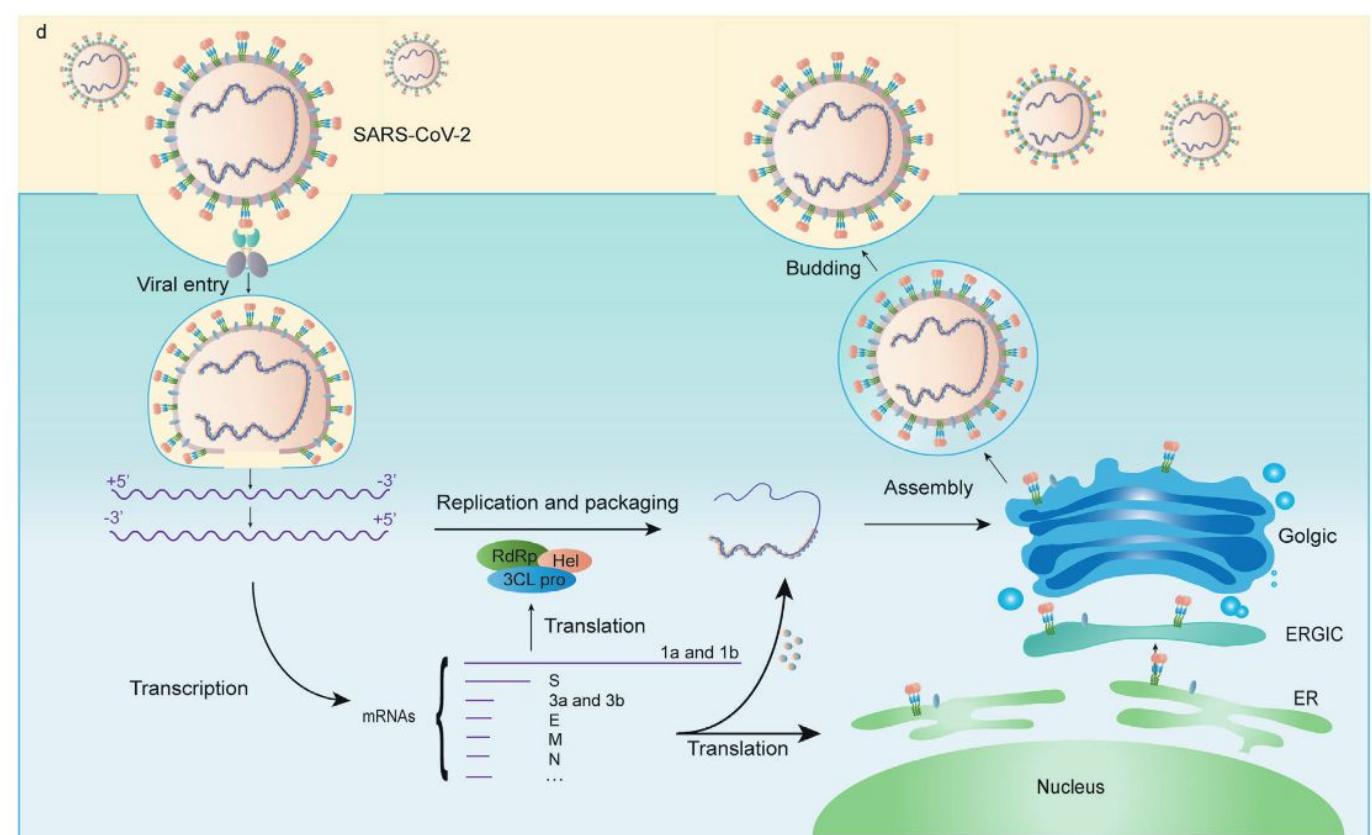
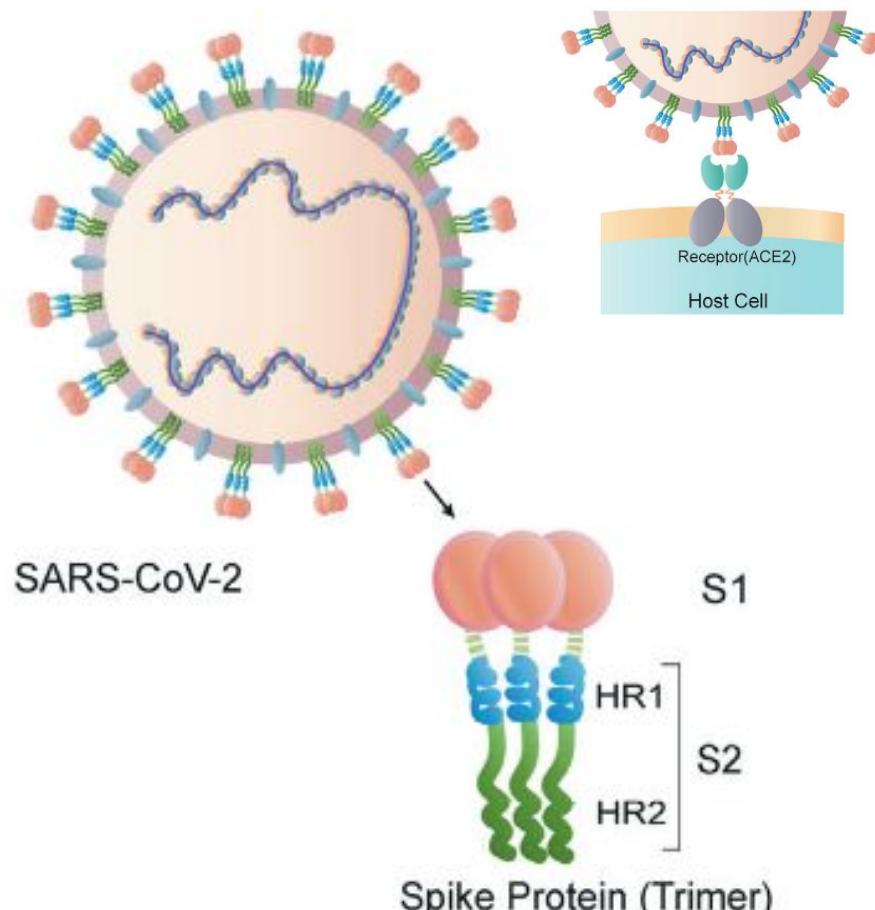
Phylogenetic relationships of representative members of the species Severe Acute Respiratory Syndrome (SARS)-related coronavirus

V'kovski, P., Kratzel, A., Steiner, S., Stalder, H. & Thiel, V. Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol 19, 155–170 (2021).

Sequence of the spike protein is largely conserved between corona and related viruses



Spike protein of coronavirus is responsible for viral entry into human cells



Huang, Y., Yang, C., Xu, X., Xu, W. & Liu, S. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. *Acta Pharmacol Sin* 41, 1141–1149 (2020).

DNA or RNA encodes genetic information of all life forms that we know, including viruses

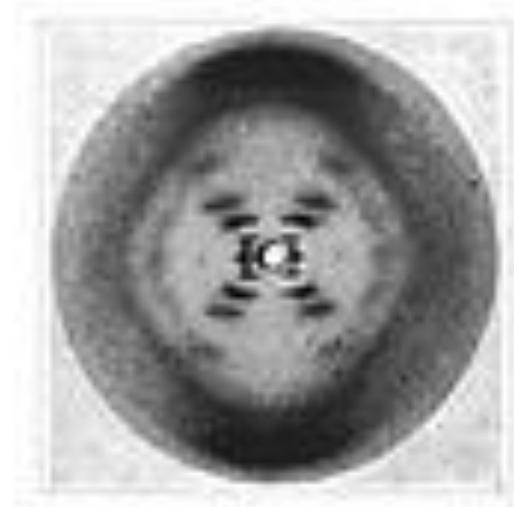
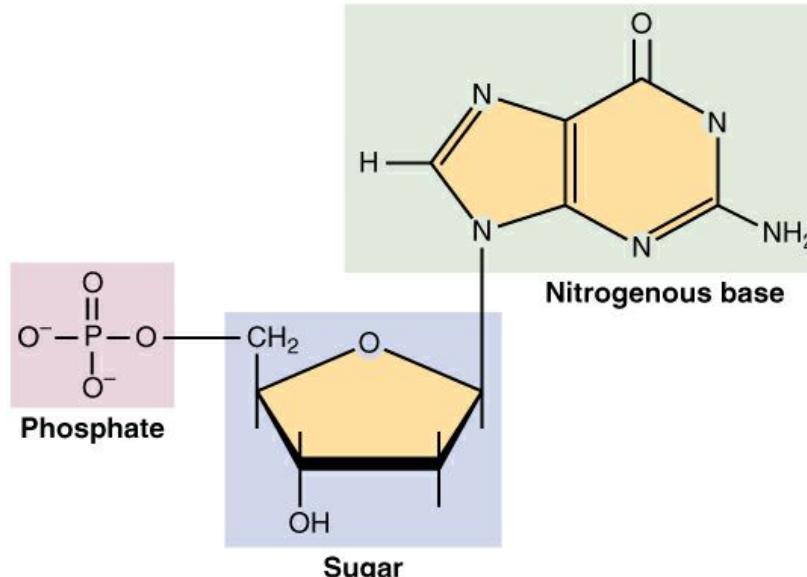
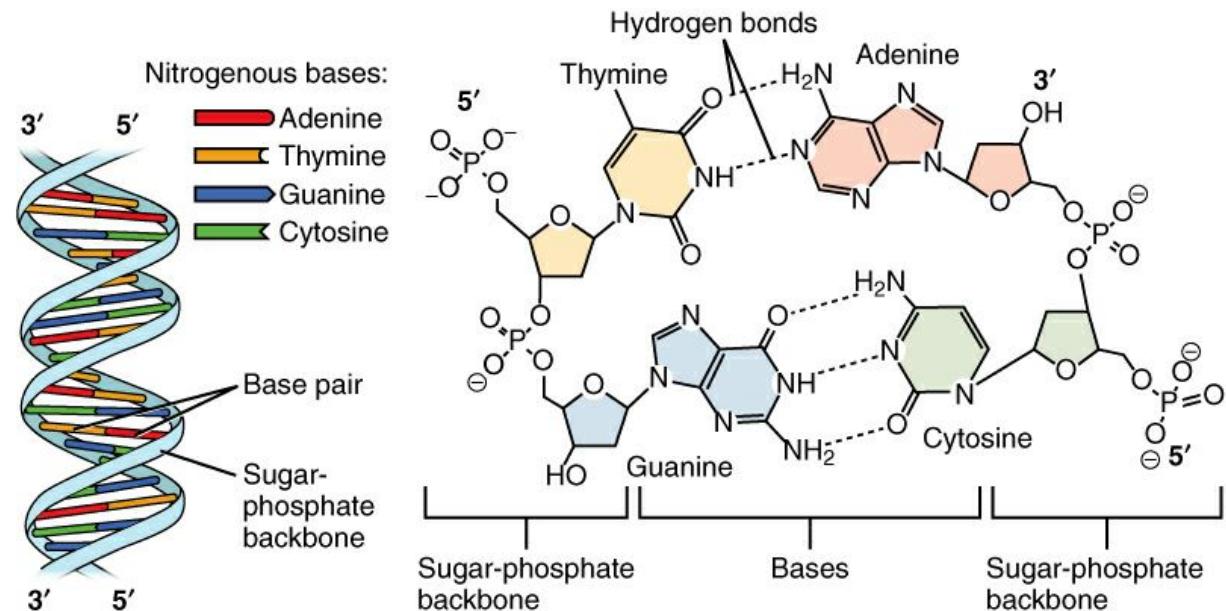


Photo 51, X-ray diffraction image of DNA

Franklin R, Gosling RG (1953)
 "Molecular Configuration in Sodium Thymonucleate". *Nature* 171: 740–741.



From the textbook OpenStax Anatomy and Physiology, discovered through Wikimedia, reused under the CC license.

RNA is transcribed from DNA and translated into protein

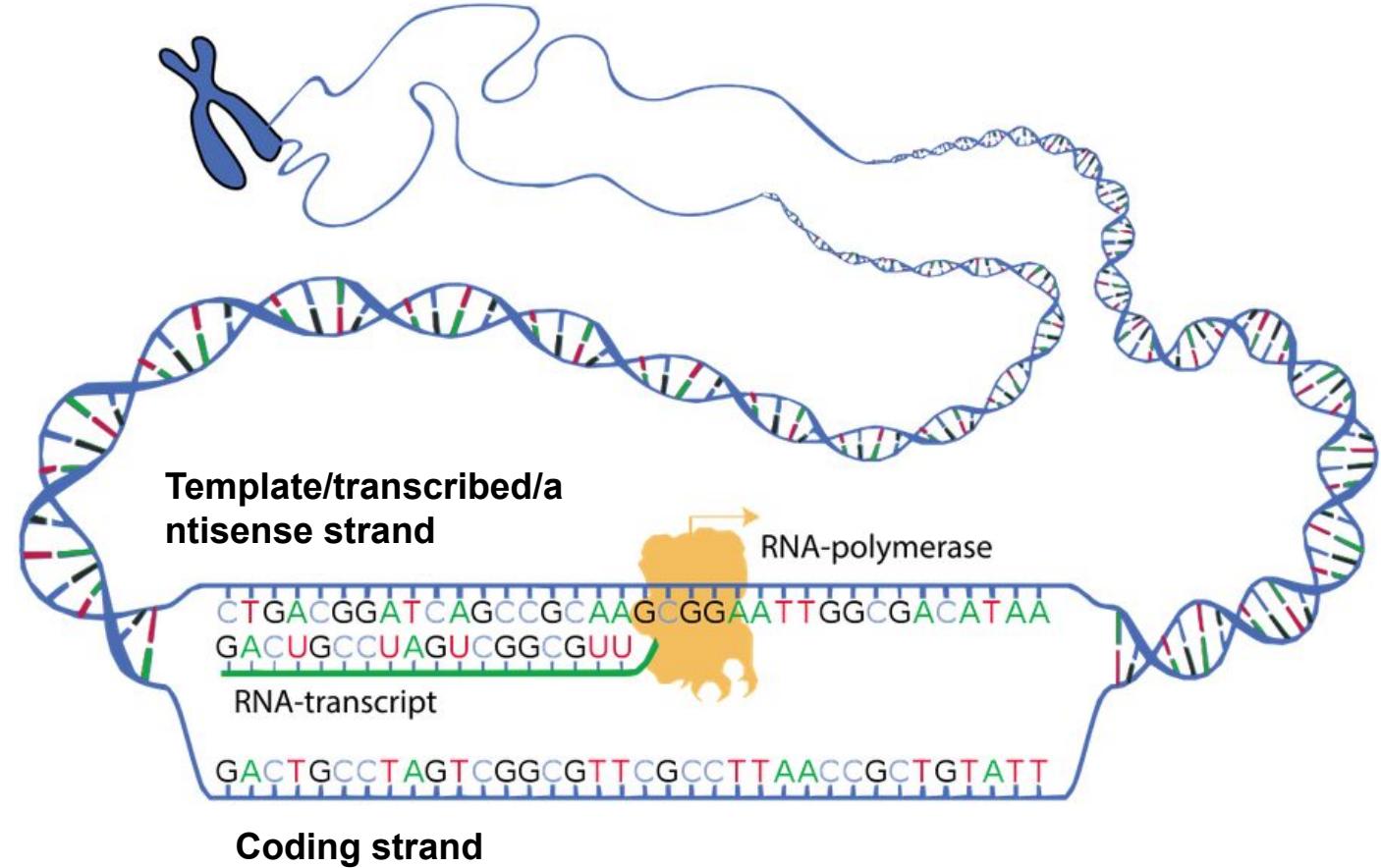
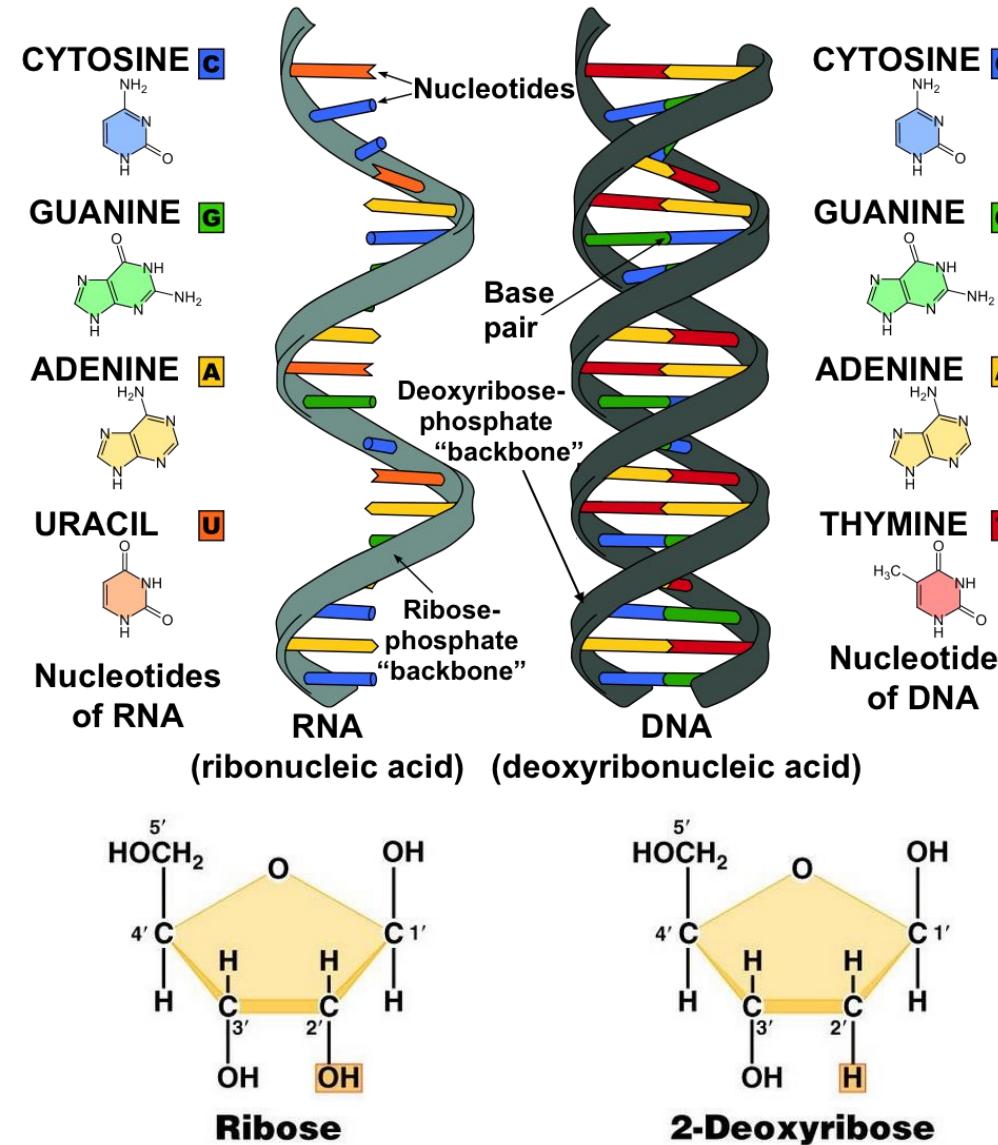
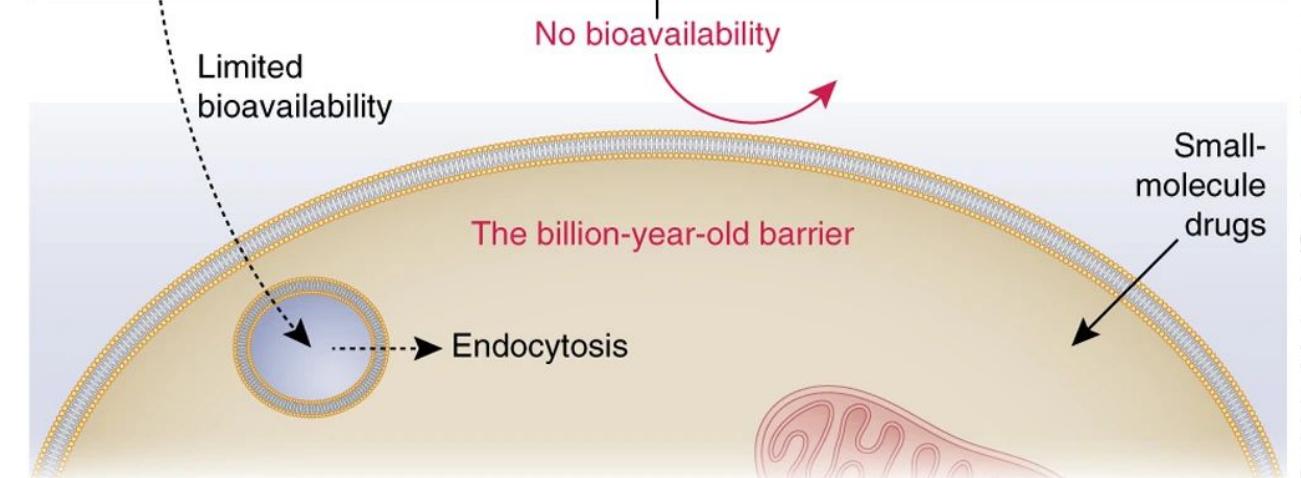
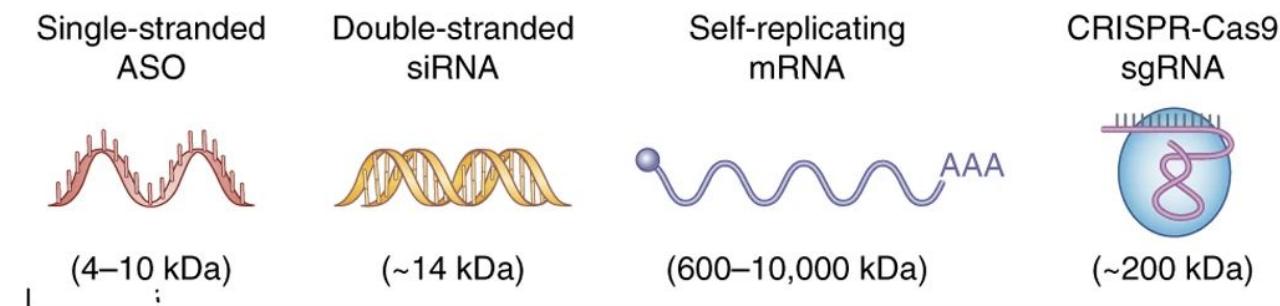
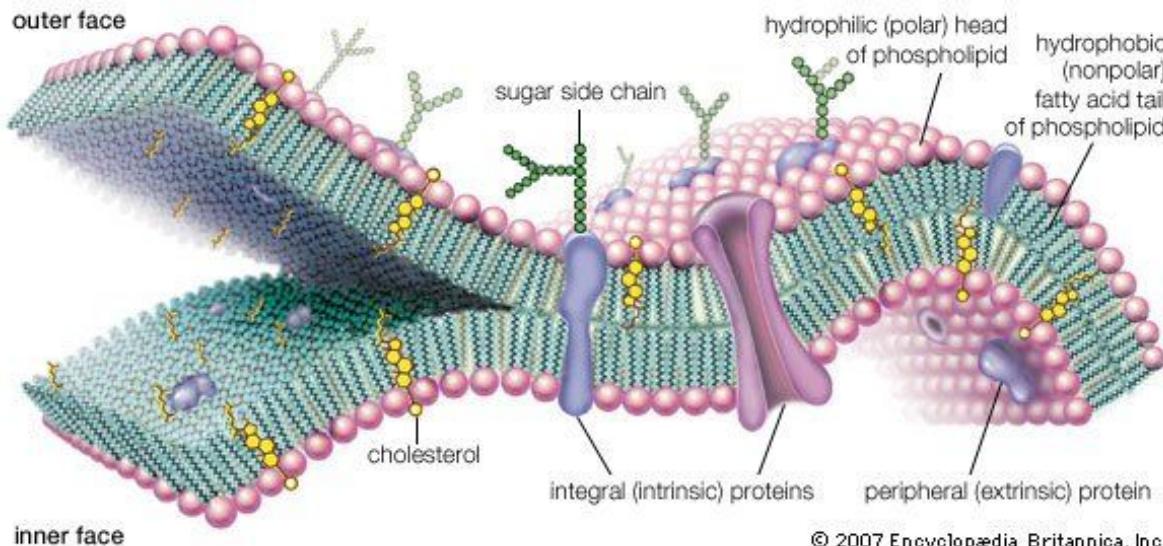


Figure: https://commons.wikimedia.org/wiki/File:DNA_transcription.svg and https://en.m.wikipedia.org/wiki/File%3AHAR1F_RF00635_rna_secondary_structure.jpg. Original work by wikipedia user: OrgreBot and user:Ppgardne. Used under CC-SA 3.0 license.

A summary of what we have learned so far in the context of coronavirus

1. What is the unmet medical need to be addressed? We need a vaccine to prevent a large population of individuals from being infected by coronavirus, which have severe consequences.
2. What are the target(s) of our drug? Spike protein is conserved: immune reaction is desired.
3. Where should the drug go in patient's body, what does body do to the drug, and what does the drug do to the body? Due to time constraints, classical vaccine may not meet the need. How about mRNA vaccines?
4. What is the safety profile of the drug in light of its benefits? To be investigated.
5. Who are responsive to the drug, or susceptible to adverse events? To be investigated.

Three essential challenges for mRNA-based therapies: delivery, stability, and *unwanted immune responses*



Key challenges:

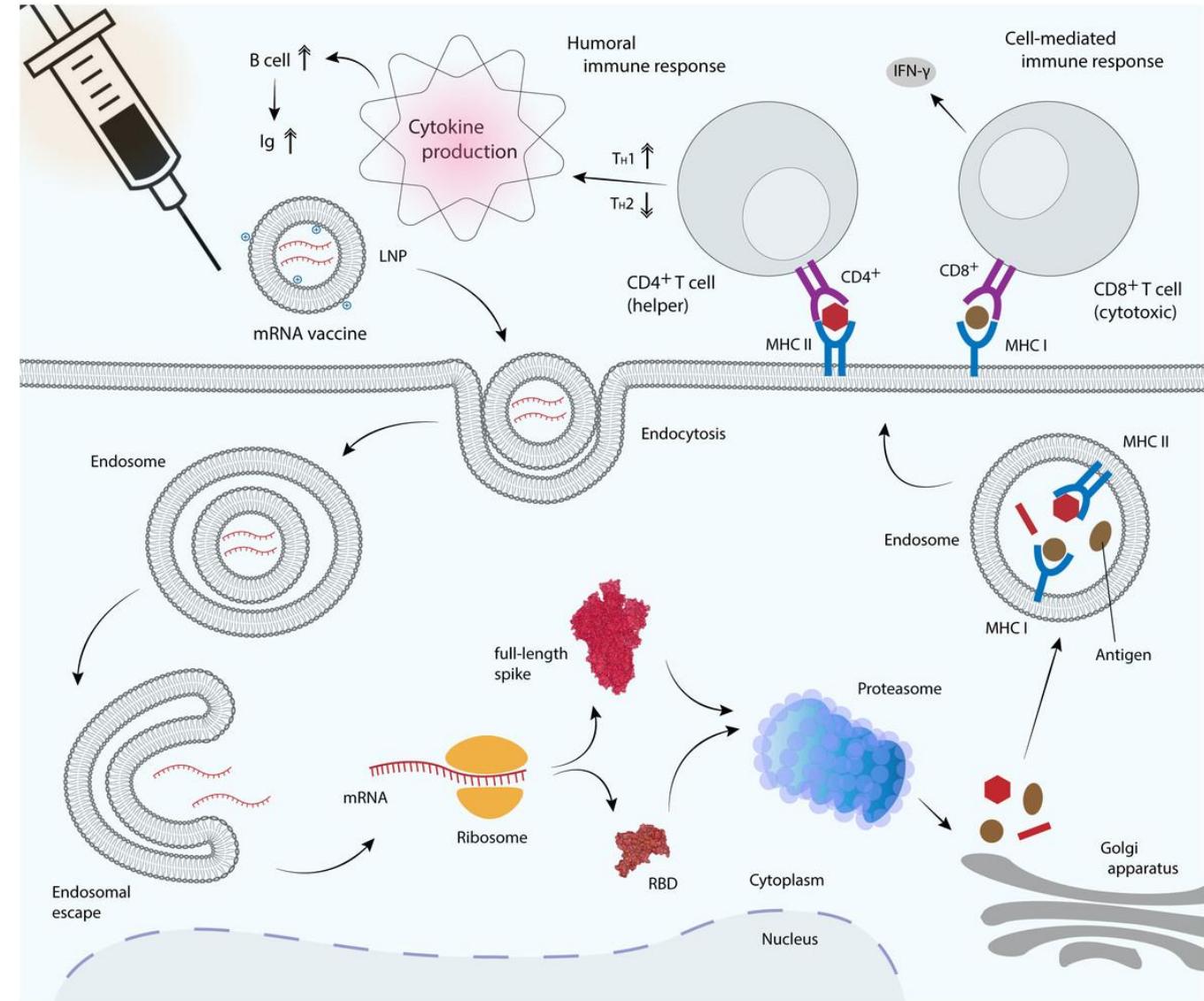
- mRNAs are too large and charged to pass lipid bilayers.
- mRNAs are readily degraded by ribonucleases.
- Exogenous mRNAs cause immunogenicity.*

Left: Cell membrane, copyright of Encyclopedia Britannica, Inc. Right: The four-billion-year-old barrier to RNA therapeutic

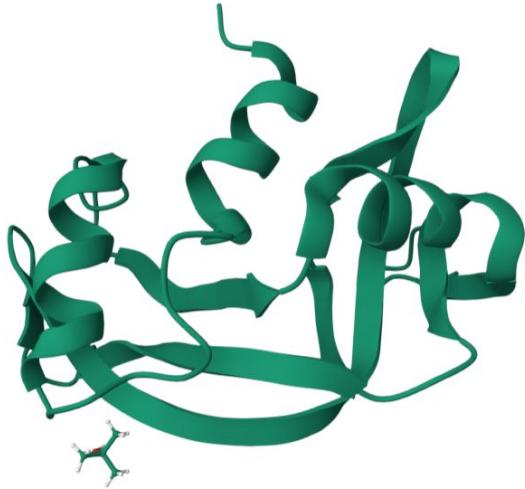
Lipid NanoParticles (LNP) helps delivering RNAs into cells

- Lipid nanoparticles can take mRNA vaccines as cargos, and deliver them into human cells.
- In the cell, mRNA encoding the part of the spike protein sequence is translated into proteins with the human protein translation mechanism.
- Synthesized proteins will be degraded and exposed on cell surface, which will be recognized by antigen presenting cells.

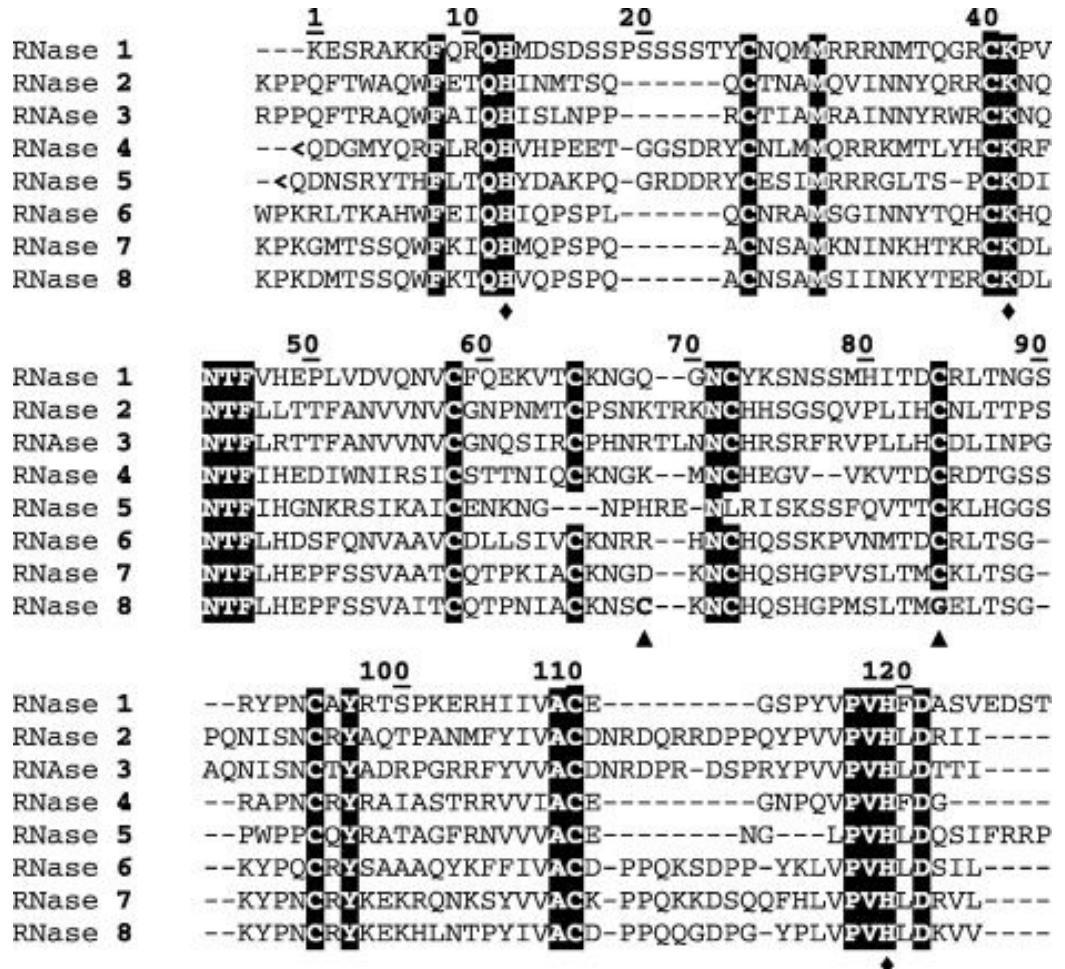
Salleh, Mohd Zulkifli et al. "[Immunogenicity Mechanism of mRNA Vaccines and Their Limitations in Promoting Adaptive Protection against SARS-CoV-2](#)." PeerJ 10 (March 9, 2022)



RNAs are degraded by proteins known as ribonucleases (RNAases)



- mRNAs are too large and charged to pass lipid bilayers.
- mRNAs are degradable by ribonucleases (RNases).** RNases belong to *enzymes*, a class of proteins that catalyse chemical reactions.
- Exogenous mRNAs induce immunogenicity.



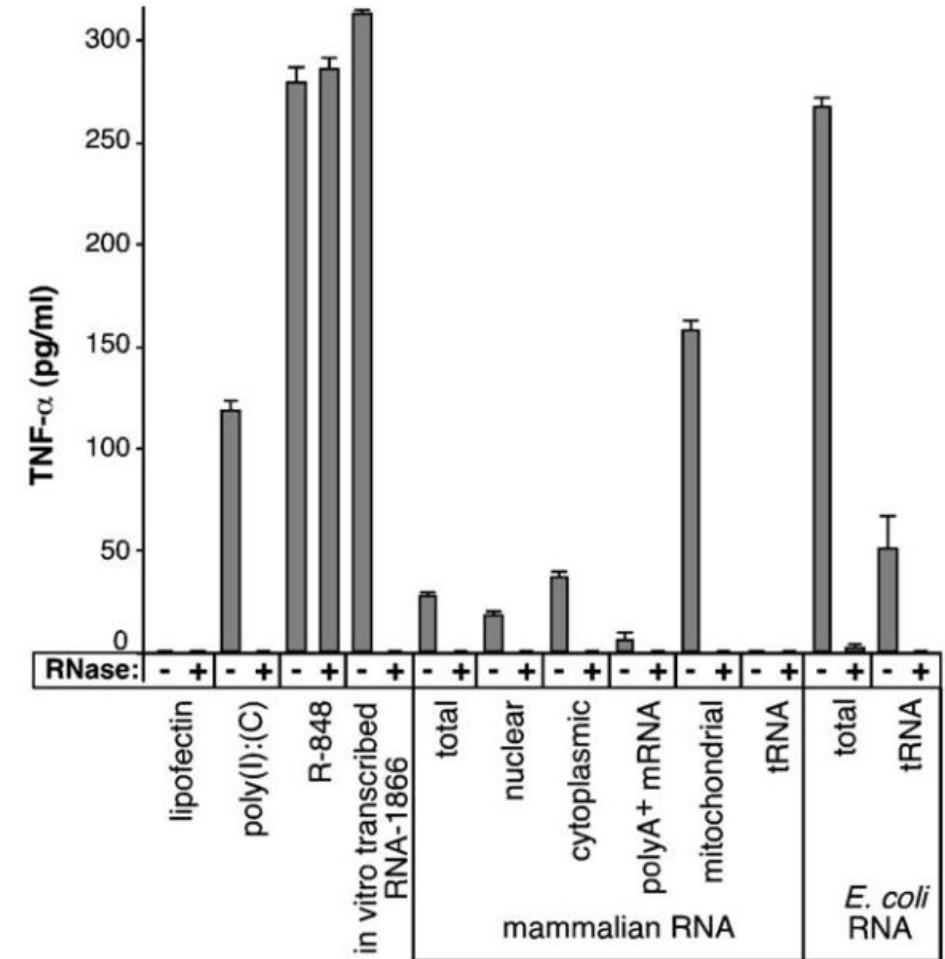
Left: Structure of PDB [7RSA](#). Right: alignment of protein sequences of 8 canonical human RNases (ribonuclease A family). [Sorrentino FEBS Letters, 2010](#).

Unmodified RNA induces unwanted immune reactions: modifying RNA can reduce or remove them

Exogenous RNAs induce immunogenicity. RNAs are synthesized from four ribonucleotides: ATP (adenosine triphosphate), CTP (cytidine triphosphate), UTP (uridine triphosphate), and GTP (guanosine triphosphate).

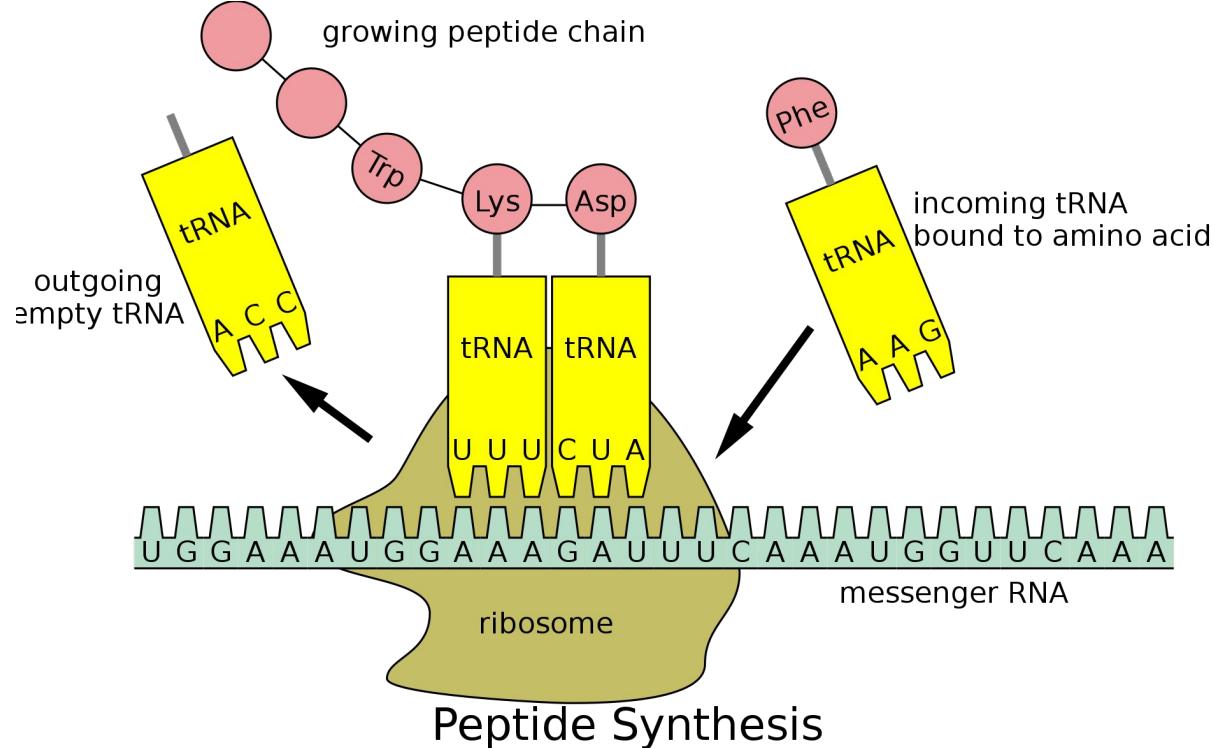
When unmodified RNAs are delivered into cells, they induce unwanted immune reaction. They activate the surface proteins known as Toll-like receptors (TLRs), which leads to the release of cytokines including the tumor necrosis factor alpha (TNF-alpha). TLRs and TNF-alpha are also activated by bacterial and viral infections and mediate their killing.

Some type of RNA, however, does not induce immunogenicity, for instance human *tRNA*. This finding by Karikó and Drewman made major contributions to the successful development of SARS-CoV-2 mRNA vaccines.

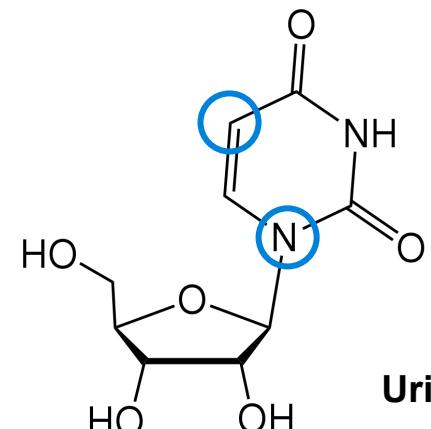


Karikó, K., Buckstein, M., Ni, H. & Weissman, D. Suppression of RNA Recognition by Toll-like Receptors: The Impact of Nucleoside Modification and the Evolutionary Origin of RNA. *Immunity* 23, 165–175 (2005).

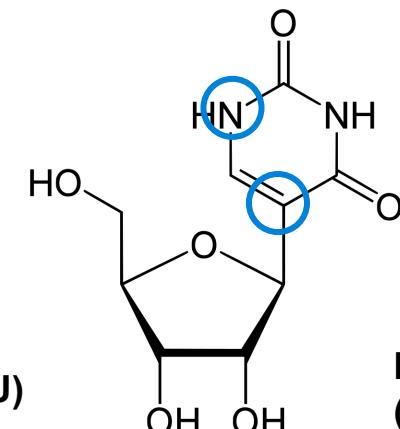
Human tRNA contains *pseudouridine*, a modified uridine, which does not induce immunogenicity



Left: tRNAs transfer amino acids to ribosome for protein translation. Top right: tRNA structure, with the TΨC loop highlighted in the blue ellipse. Below: structures of uridine and pseudouridine.

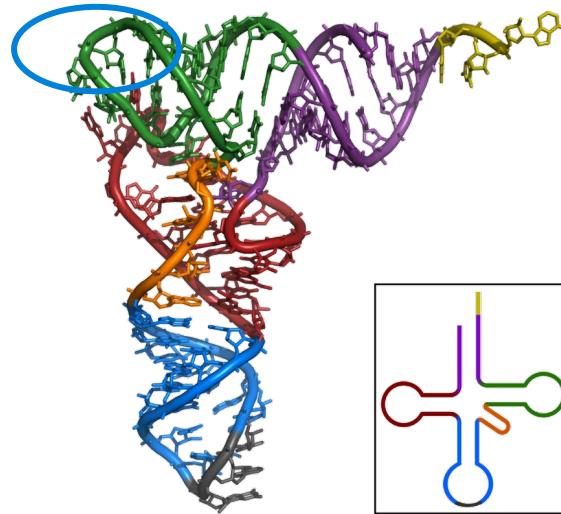


Uridine (U)

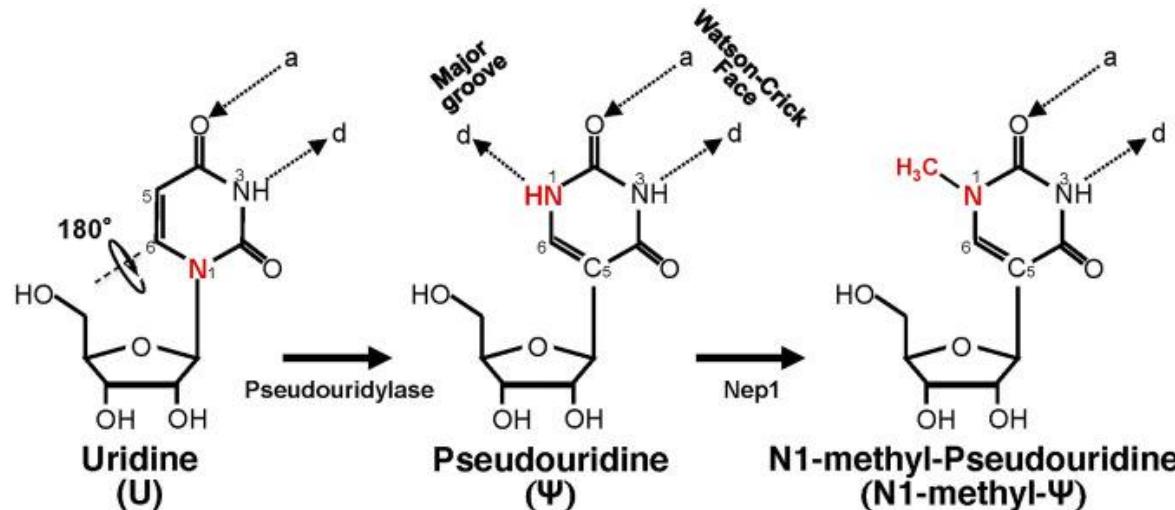


Pseudouridine
(psi, Ψ)

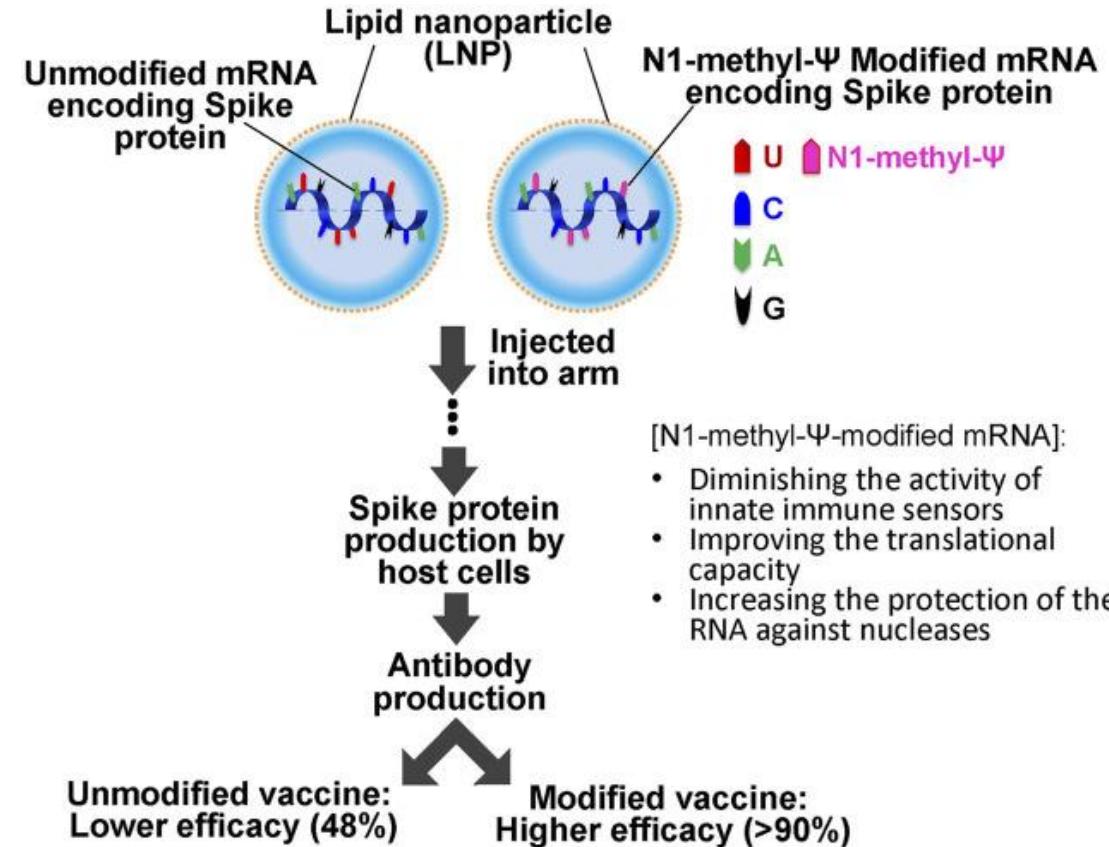
By Boumphreyfr vector conversion by Glrx - File:Peptide syn.png, CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=101457889>. By Yikrauul, CC BY-SA 3.0, via Wikimedia Commons.



Further modification (N1-methyl-Ψ) and LNP delivery are critical for the success of mRNA vaccines

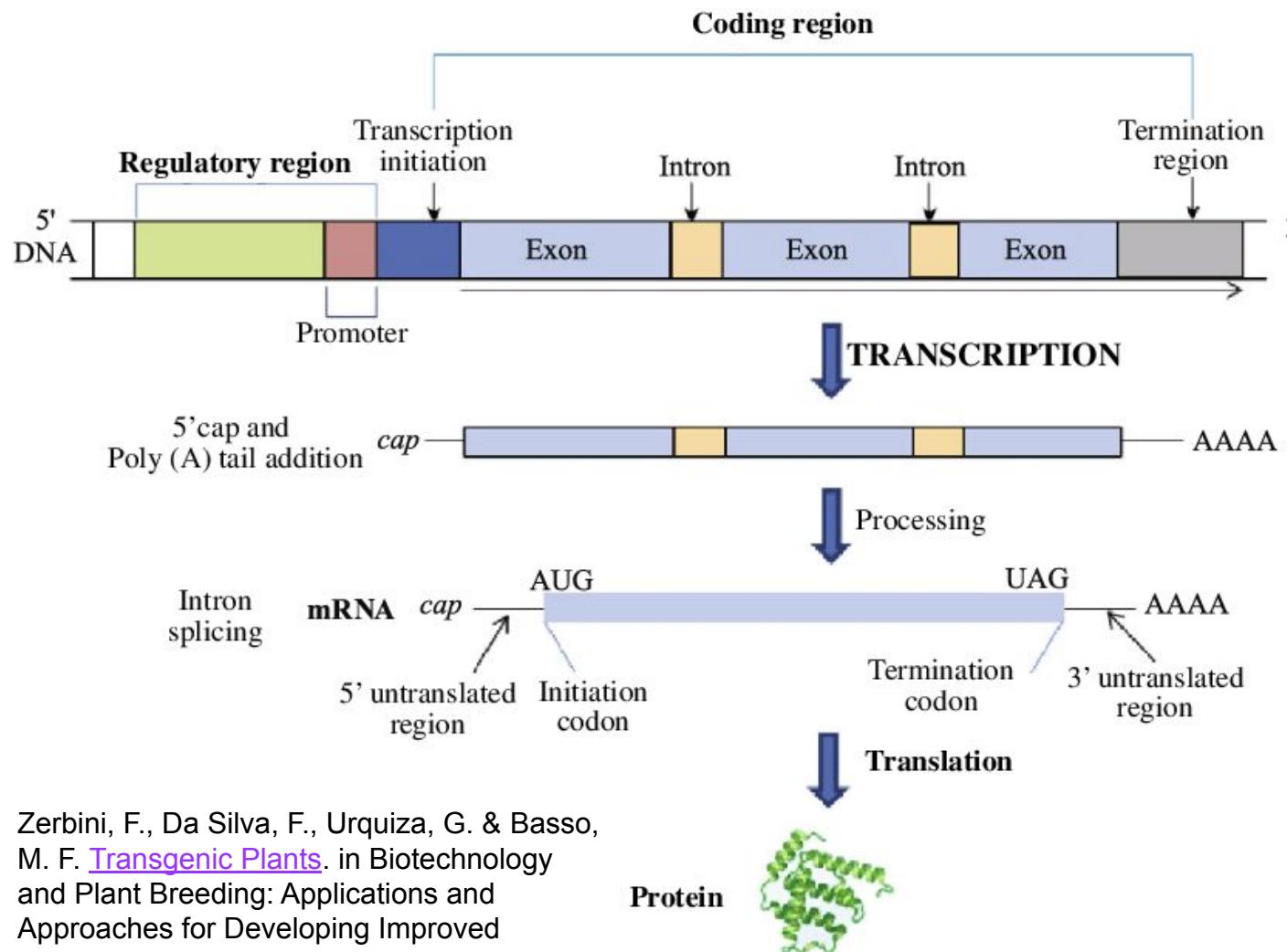


mRNA vaccines against human SARS-CoV-2 viruses, developed in 2020 by Pfizer-BioNTech and Moderna Therapeutics (comirnaty® and spikevax®, respectively), reached clinical efficacies higher than 90%. Both benefited from modified RNA and LNP. Curevac mRNA vaccine (CVnCoV), which used LNP but not modified RNA, reached an efficacy of 48%.



Morais, P., Adachi, H. & Yu, Y.-T. The Critical Contribution of Pseudouridine to mRNA COVID-19 Vaccines. *Front Cell Dev Biol* 9, 789427 (2021).

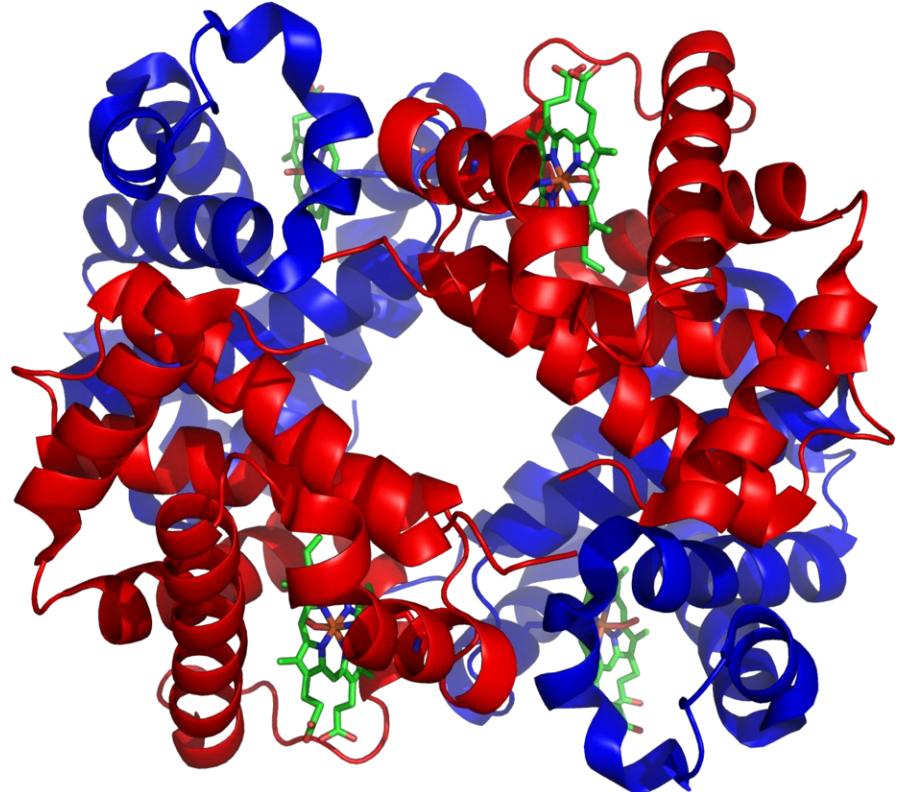
Coding sequence of the spike protein alone is not enough: mRNA transcription depends on 5'-UTR and 3'-UTR, too



The process of gene expression in eukaryotes:

1. RNA polymerase, an enzyme, binds to the promoter region of the gene. It reads the DNA from the 5' untranslated region (UTR) to the 3' UTR to synthesize pre-mRNA.
2. Pre-mRNA receives a modified nucleotide (7-methylguanosine triphosphate) at the 5' end as a cap, and a repeated adenine sequence (poly-A tail) at the 3' end.
3. Pre-mRNA is spliced to remove introns. Mature mRNA contains the 5' cap, 5'-untranslated region (5'-UTR), coding sequence, 3'-untranslated region (3'-UTR), and a poly-A tail.
4. Mature mRNA is transported from the nucleus to the cytoplasm for translation.

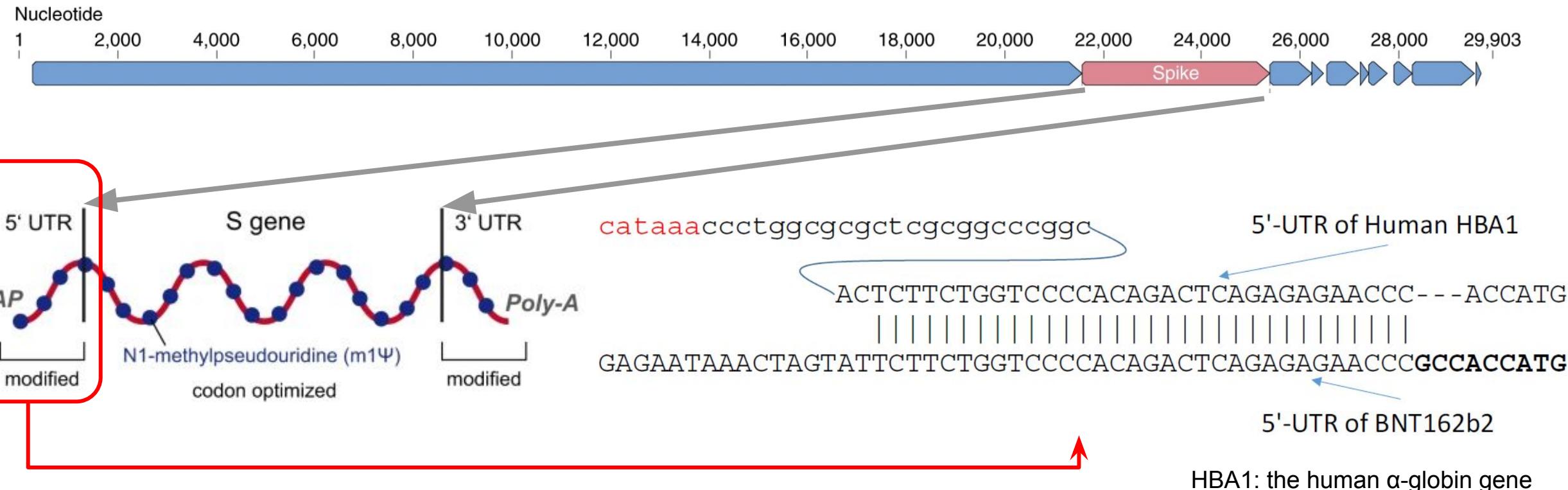
5'-UTR of human hemoglobin is a good choice to make sure that the vaccine sequence is stable and highly translated



1	-MVLSPADKTNVKAANGVGAHAGEYGAEALELMFLSFPITKTYFPHD-----LSHGS	53	P69905	HBA_HUMAN
1	MVHLTPEEKSAVTALNGKVNV--DEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGN	58	P68871	HBB_HUMAN
1	MVHLTPEEKTAVNALNGKVNV--DAVGGEALGRLLVVYPWTQRFFESFGDLSSPDAVMGN	58	P02042	HBD_HUMAN
	: * : * : * : * . * . * * * : * : * : * : * * *			
54	AQVKGHGKKVADALTNAVAHVDDMPNALSALSDLIAHKLRLVDPVNFKLISHCLLVTLAAH	113	P69905	HBA_HUMAN
59	PKVKAHGKKVLGAFSDGLAHLNDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHH	118	P68871	HBB_HUMAN
59	PKVKAHGKKVLGAFSDGLAHLNDNLKGTFSQLSELHCDKLHVDPENFRLLGNVLVCVLAHM	118	P02042	HBD_HUMAN
	: * : * * * * * : * : * : * : * : * : * : * : * : * : * .			
114	LPAEFTPAPVHASLDKFLASVSTVLTSKYR	142	P69905	HBA_HUMAN
119	FGKEFTPPVQAAYQKVVAGVANALAHKYH	147	P68871	HBB_HUMAN
119	FGKEFTPQMQAAYQKVVAGVANALAHKYH	147	P02042	HBD_HUMAN
	: * * * : * : * : * : * : * : * :			

- Hemoglobin (left) is a protein that transports oxygen.
 - Hemoglobin consists of three subunits: alpha, beta, and delta. They are encoded by three highly similar genes known as HBA, HBB, and HBD (above).
 - Hemoglobin is present in erythrocytes (red blood cells) of almost all vertebrates.
 - The protein is essential, therefore the mRNA is relatively stable and highly translated.

LNP, modified RNA, and 5'-UTR of HBA are all essential to make effective *and* safe vaccines against coronavirus



References: Heinz, Franz X., and Karin Stiasny. "Distinguishing Features of Current COVID-19 Vaccines: Knowns and Unknowns of Antigen Presentation and Modes of Action." *Npj Vaccines* 6, no. 1 (August 16, 2021): 1–13. <https://doi.org/10.1038/s41541-021-00369-6>; *Assemblies of putative SARS-CoV2-spike-encoding mRNA sequences for vaccines BNT-162b2 and mRNA-1273* (github.com/NAalytics); Xia, Xuhua. "Detailed Dissection and Critical Evaluation of the Pfizer/BioNTech and Moderna MRNA Vaccines." *Vaccines* 9, no. 7 (July 3, 2021): 734. <https://doi.org/10.3390/vaccines9070734>.

A summary of what we have learned so far in the context of coronavirus

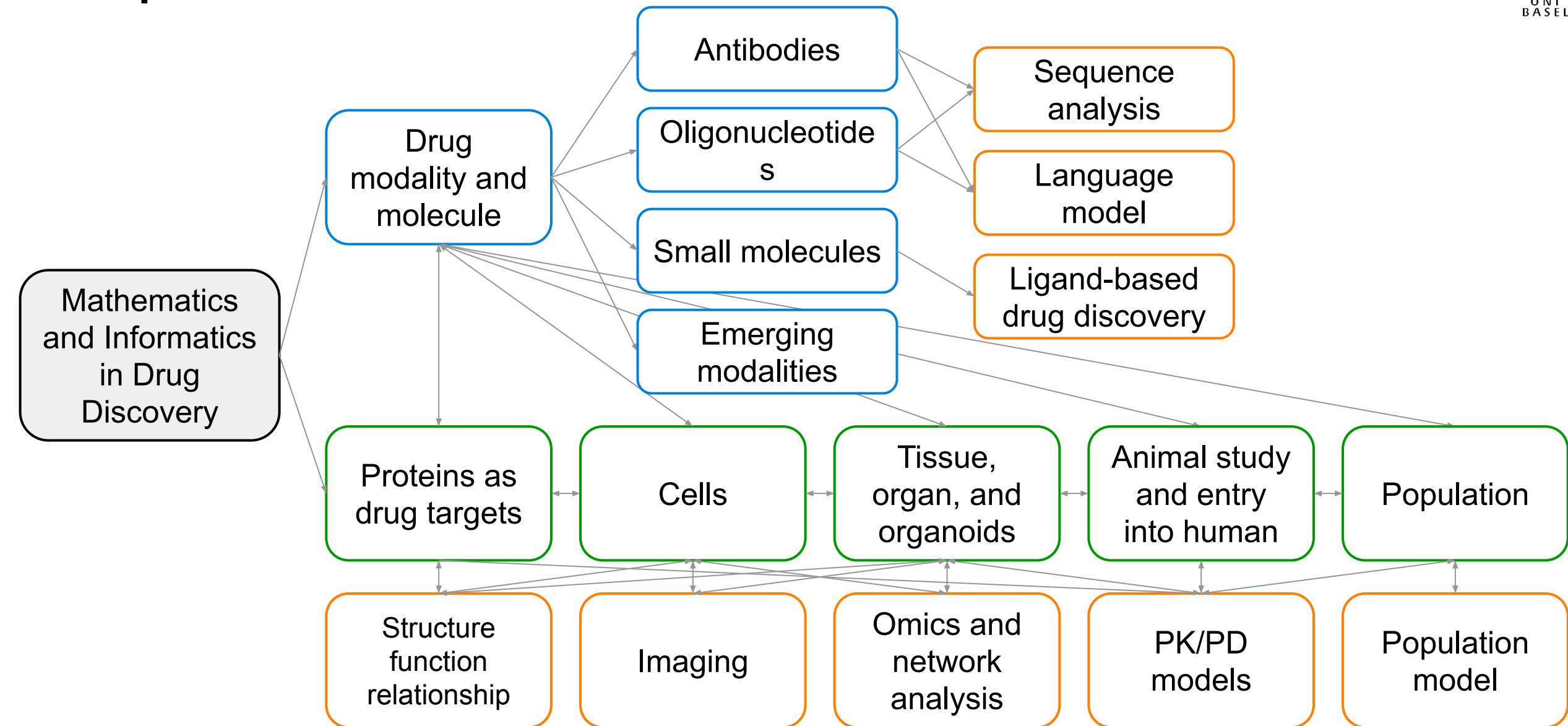
1. What is the unmet medical need to be addressed? [We need a vaccine to prevent a large population of individuals from being infected by coronavirus, which have severe consequences.](#)
2. What are the target(s) of our drug? [Spike protein is conserved: immune reaction is desired.](#)
3. Where should the drug go in patient's body, what does body do to the drug, and what does the drug do to the body? [Thanks to LNP, N1-mythel-Ψ, and 5'-UTR of HBA1, the mRNA vaccination can enter cells with minimal side effects. In cells, spike protein RNA is synthesized into proteins, which are digested, presented, and elicit immune response.](#)
4. What is the safety profile of the drug in light of its benefits? [Initial study: Polack, F. P. et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. New England Journal of Medicine 383, 2603–2615 \(2020\), and watch \[this video\]\(#\).](#)
5. Who are responsive to the drug, or susceptible to adverse events? [Updated regularly by regulatory agencies, for instance \[European Medicines Agency\]\(#\).](#)

Conclusions

1. Drug discovery is an interdisciplinary effort to solve medical and technical challenges.
2. Biological understanding, including sequence analysis, is key for indication and target selection.
3. Modern drug discovery needs to address five key questions:
 - a. **Unmet medical need**
 - b. **Target(s) and modalities**
 - c. **Pharmacokinetics** (what body does to the drug) **and pharmacodynamics** (what the drug does to the body)
 - d. **Safety** (benefit/risk assessment)
 - e. **Patient enrichment/stratification**

Backup slides

The path of the course



Interests and concerns of companies working on drug discovery: summary of our previous discussions

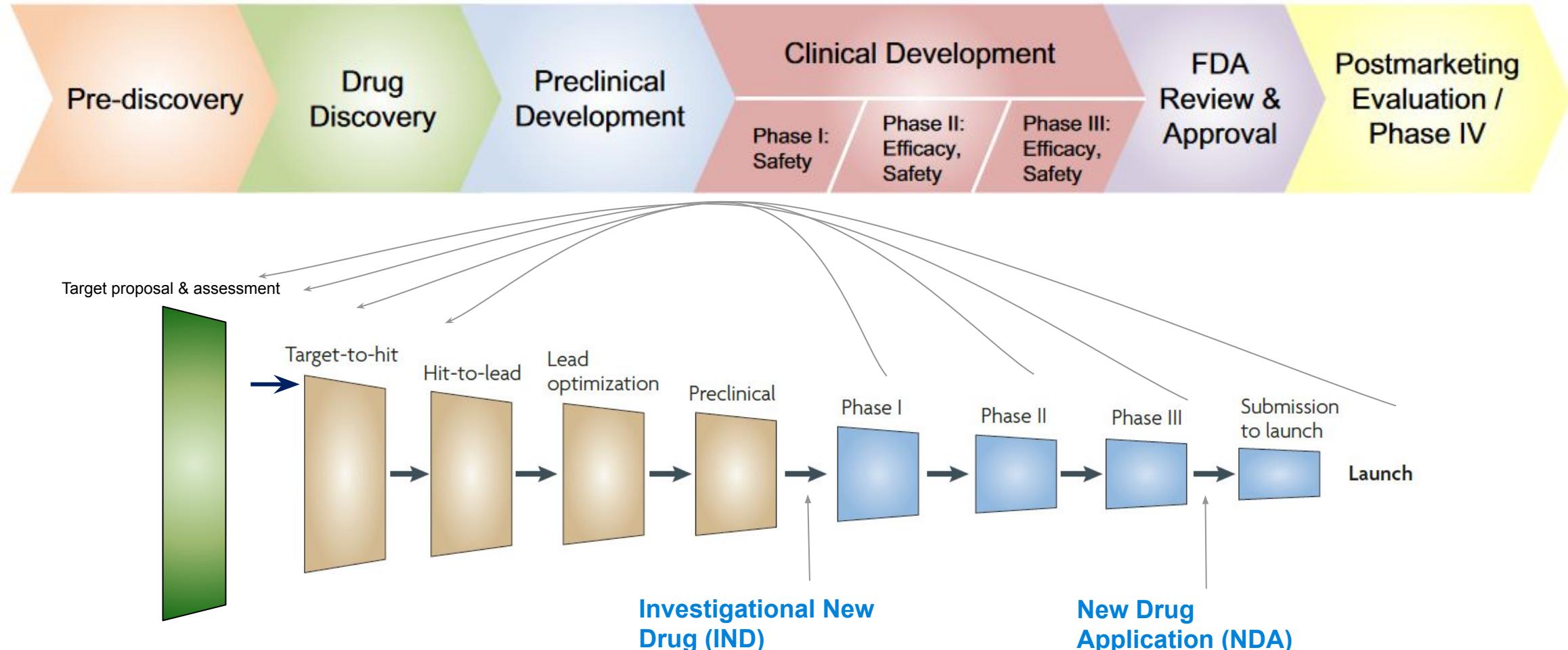
Interests

- Return of Investment
 - Commercial potential
 - Cycle time
- Good reputation
 - Efficacy of the drug
 - Safety of the drug
 - Market access
- Environmental, social, and governance (e.g. fighting internal corruption, diversity of board members).

Concerns

- Low or no return of investment
 - Lack of efficacy of drugs
 - Unfavorable benefit/risk profiles of the drug
 - No approval from agency
 - Cost, time, effectiveness of R&D
 - Competitor
 - Poor targets or disease models due to lack of reproducibility of published data
 - Companion diagnostic
- Intellectual property
- Idea and knowledge management
- Acceptance by doctors and patients
- Legal concerns

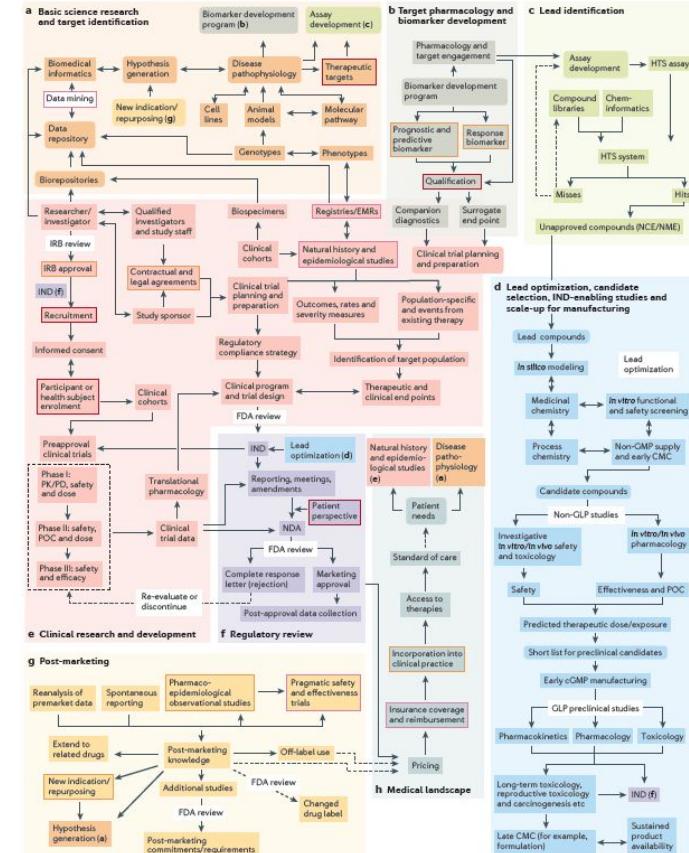
Chevron diagrams as a pipeline view of drug discovery and development



FDA: US Food and Drug Administration. Top: Wagner, J. A. et al. [Application of a Dynamic Map for Learning, Communicating, Navigating, and Improving Therapeutic Development](#). Clinical and Translational Science 11, 166–174 (2018). Bottom: Adapted from Paul et al. [How to Improve R&D Productivity: The Pharmaceutical Industry's Grand Challenge](#). Nature Reviews Drug Discovery, 2010.

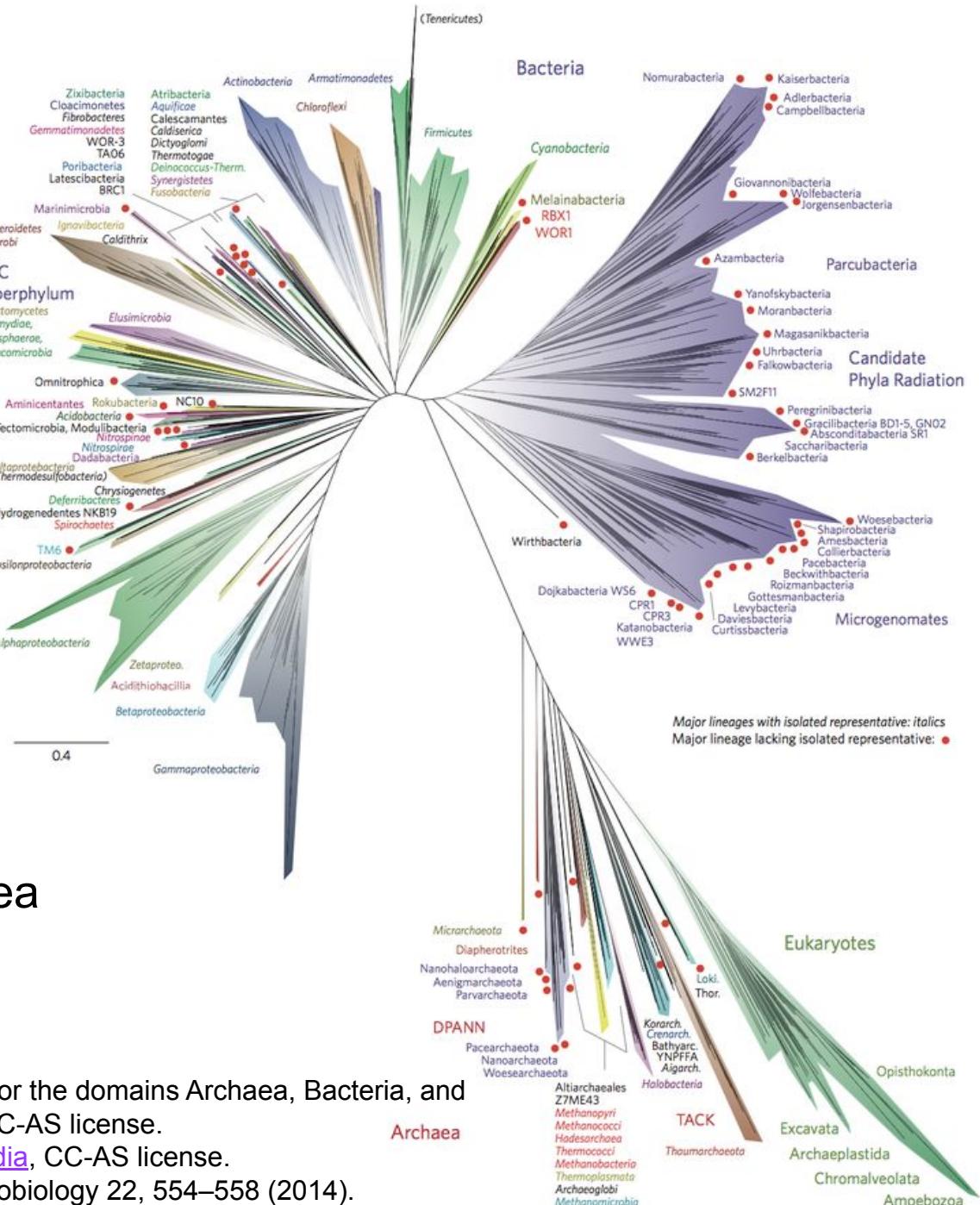
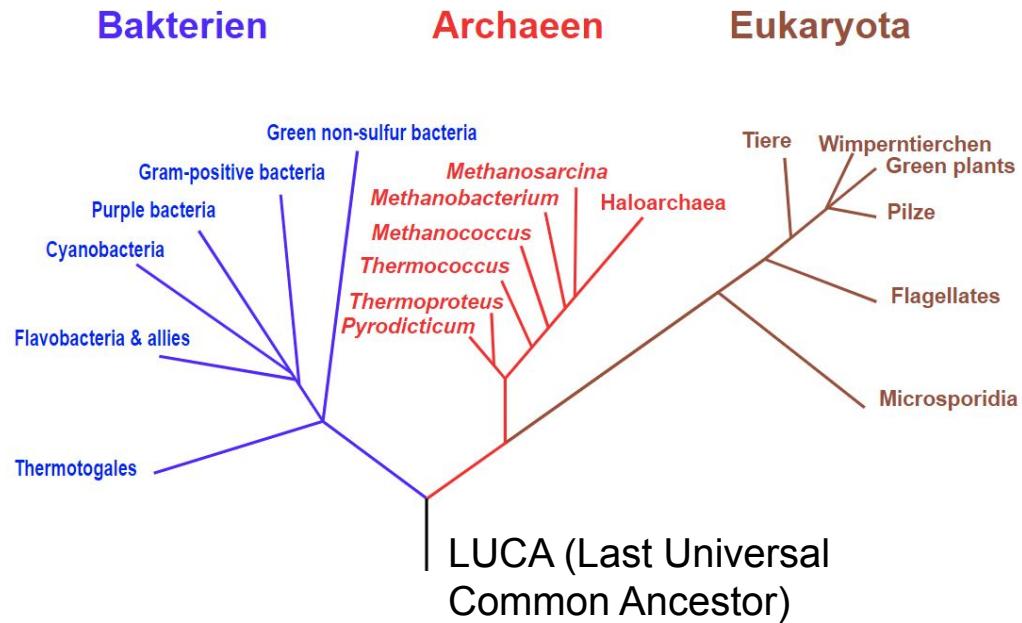
A dynamic map for drug discovery, development, and deployment

1. **Basic science research and target identification.** *What causes the disease? What do we want to achieve? Which protein can I target with which modality?*
 2. **Target pharmacology and biomarker development.** *What is the effect of targeting the protein? What we can measure to confirm that the protein is properly targeted?*
 3. **Lead identification.** *How can we find a starting point of a new drug?*
 4. **Lead optimization and clinical candidate selection.** *What are criteria to define a good drug? How can I improve the starting material?*
 5. **Clinical research and development.** *Does it work in human? How about efficacy and safety profiles?*
 6. **Regulatory review.** *Should we approve the drug?*
 7. **Post marketing.** *How does the drug work in real world?*



Wagner, J. et al. [A dynamic map for learning, communicating, navigating and improving therapeutic development](#). Nat Rev Drug Discov 17, 150–150 (2018).

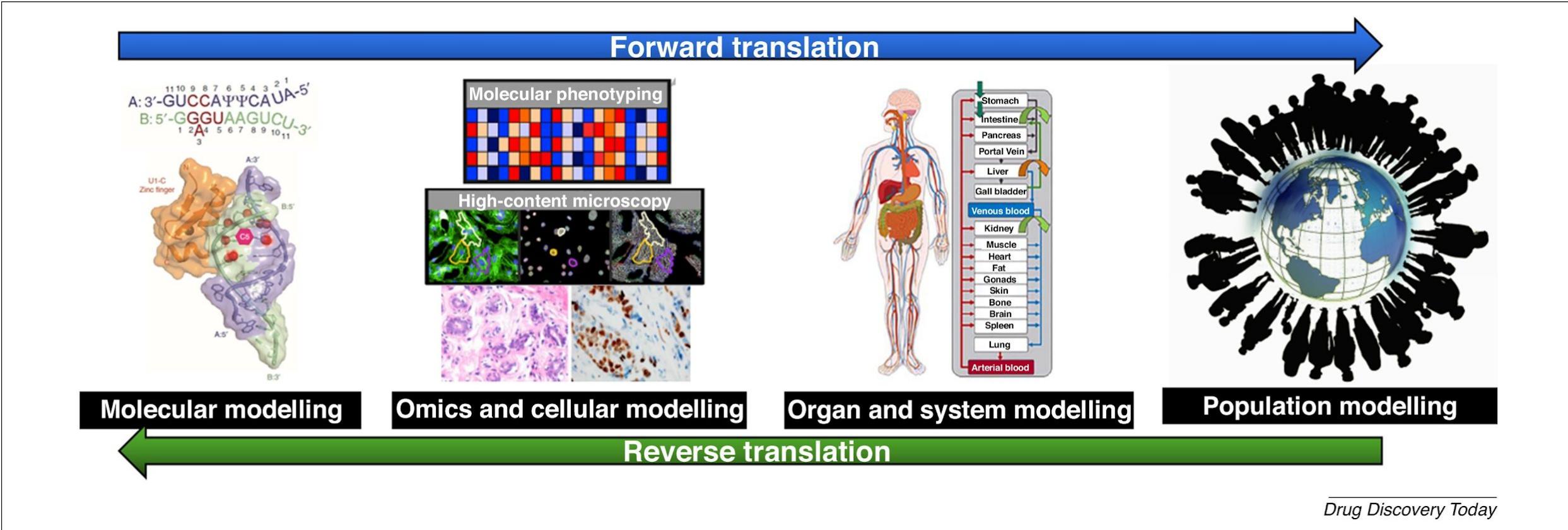
Virus is evolutionarily special



- The three-domain model of *cellular* life: (eu-)bacteria, archaeabacteria, and eukaryotes.
- The two-domain model: bacteria as one branch, archaea and eukaryotes as the other.
- Virus fits in no domain of neither models.

1. Woese, C. R., Kandler, O. & Wheelis, M. L. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc Natl Acad Sci U S A* 87, 4576–4579 (1990). Figure from [Wikimedia](#), reused with CC-AS license.
1. Hug, L. A. et al. A new view of the tree of life. *Nat Microbiol* 1, 1–6 (2016). Figure from [Wikimedia](#), CC-AS license.
1. Forterre, P., Krupovic, M. & Prangishvili, D. Cellular domains and viral lineages. *Trends in Microbiology* 22, 554–558 (2014).

The multiscale modelling view of drug discovery



Zhang, Jitao David, Lisa Sach-Peltason, Christian Kramer, Ken Wang, and Martin Ebeling. 2020. "Multiscale Modelling of Drug Mechanism and Safety." Drug Discovery Today 25 (3): 519–34. <https://doi.org/10.1016/j.drudis.2019.12.009>.

Five key questions in drug discovery

1. What is the unmet medical need to be addressed?
2. What are the target(s) and what is the modality of our drug?
3. Where should the drug go in patient's body, what does body do to the drug, and what does the drug do to the body?
4. What is the safety profile of the drug in light of its benefits?
5. Who are responsive to the drug, or susceptible to adverse events?

The *meta*-question: What knowledge, data, and tools do we have to address these questions?

Right target

- Strong link between target and disease
- Differentiated efficacy
- Available and predictive biomarkers

Right tissue

- Adequate bioavailability and tissue exposure
- Definition of PD biomarkers
- Clear understanding of preclinical and clinical PK/PD
- Understanding of drug–drug interactions

Right safety

- Differentiated and clear safety margins
- Understanding of secondary pharmacology risk
- Understanding of reactive metabolites, genotoxicity and drug–drug interactions
- Understanding of target liability

Right patient

- Identification of the most responsive patient population
- Definition of risk–benefit for a given population

Right commercial potential

- Differentiated value proposition versus future standard of care
- Focus on market access, payer and provider
- Personalized health-care strategy, including diagnostics and biomarkers

Morgan, P. et al. [Impact of a five-dimensional framework on R&D productivity at AstraZeneca](#). *Nature Reviews Drug Discovery* 17, 167–181 (2018).