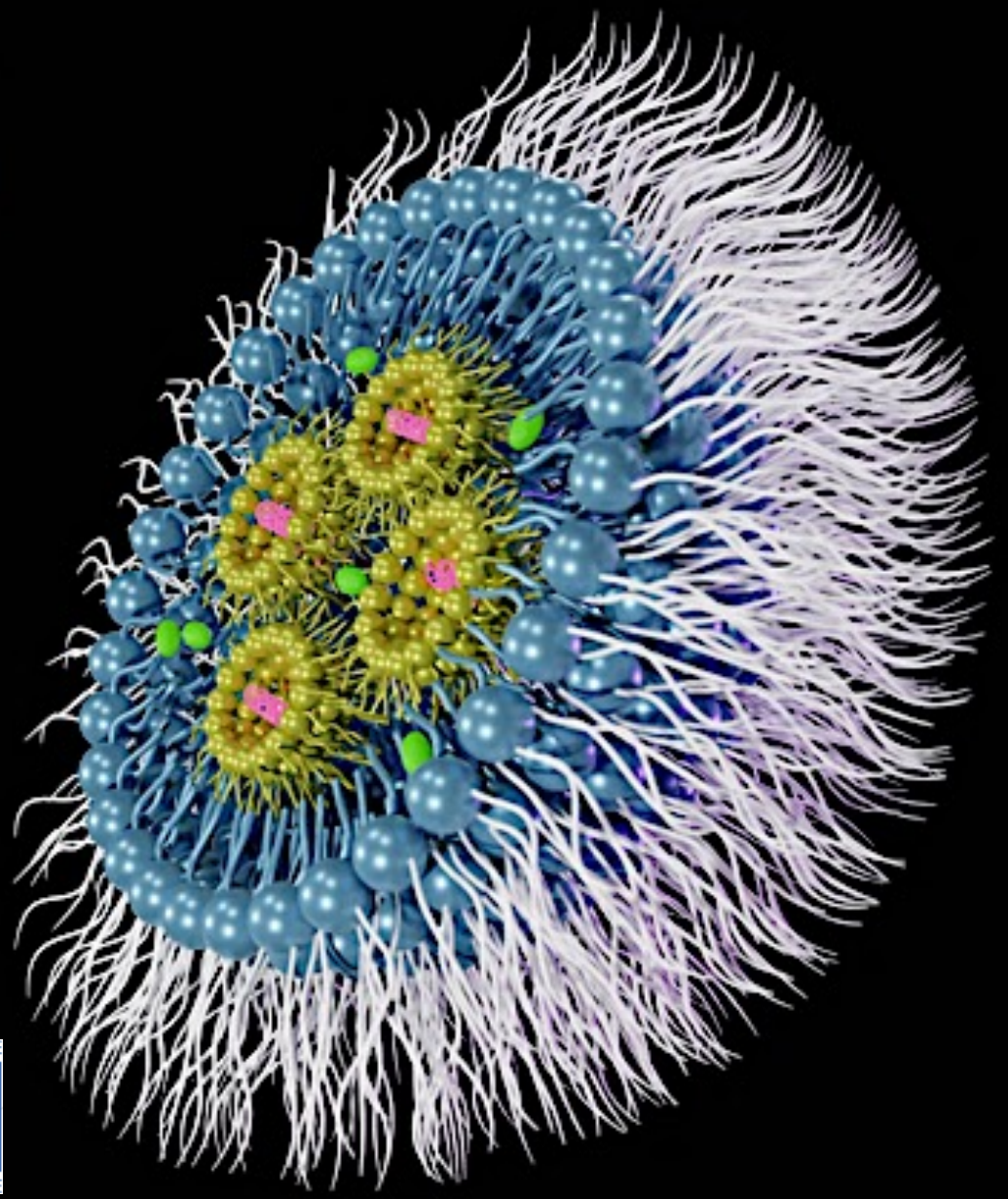
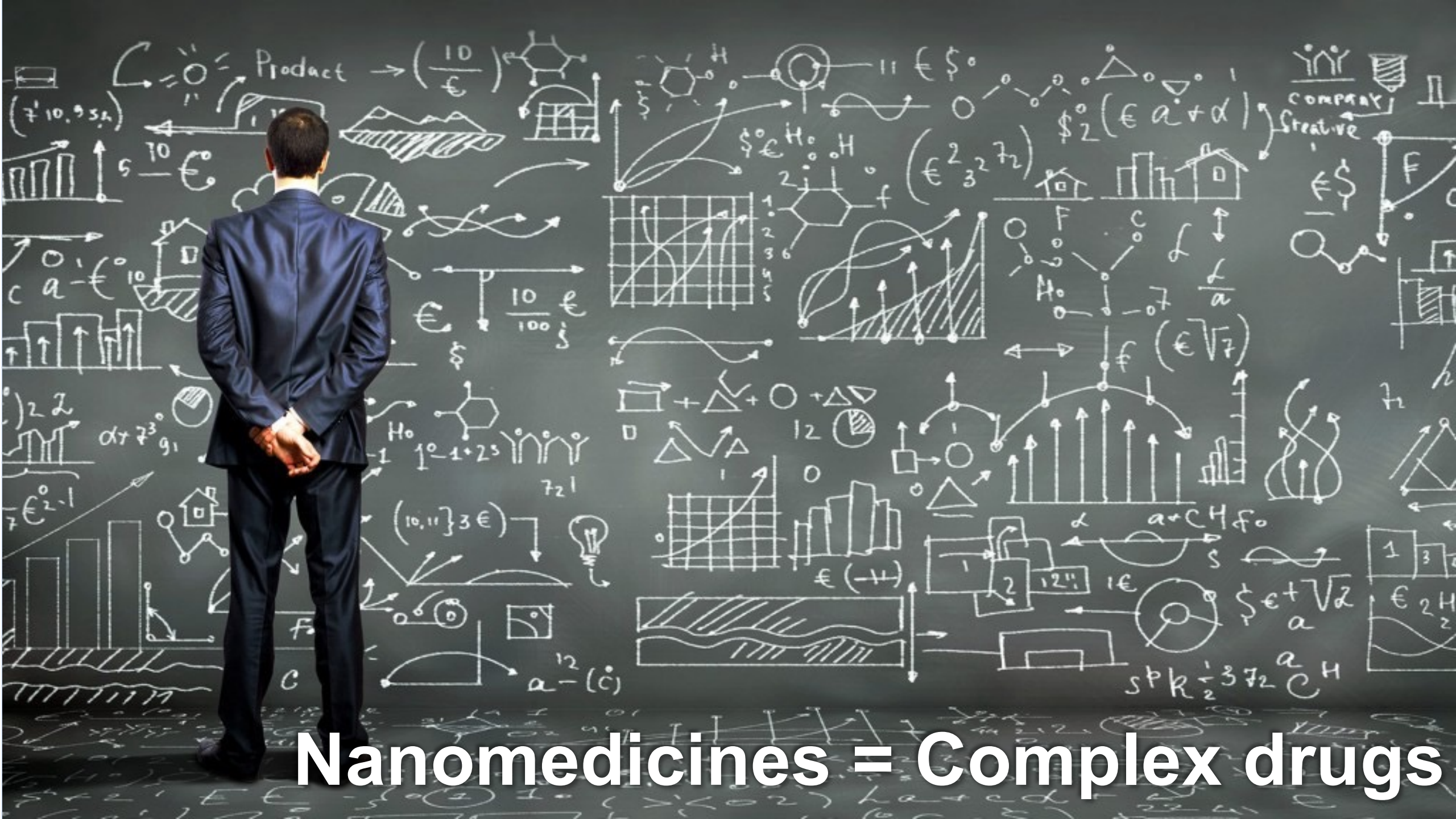
Assurance of the quality of mRNA vaccines for human use: the role of the European Pharmacopoeia

Gerrit Borchard, PharmD, PhD

School of Pharmaceutical Sciences,
University of Geneva, Switzerland

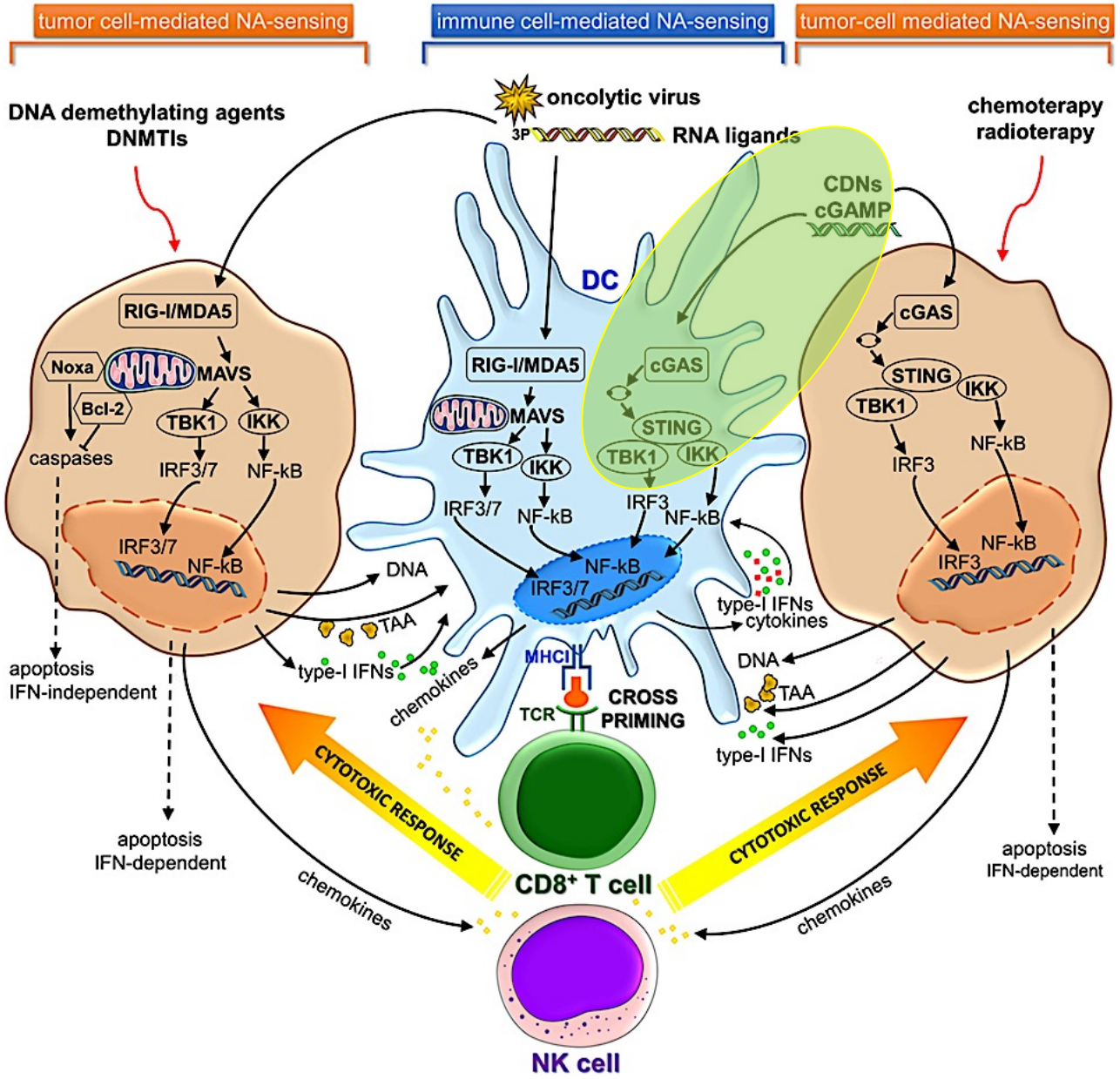




Nanomedicines = Complex drugs

NATURE IS COMPLEX.

Nucleic acid sensing



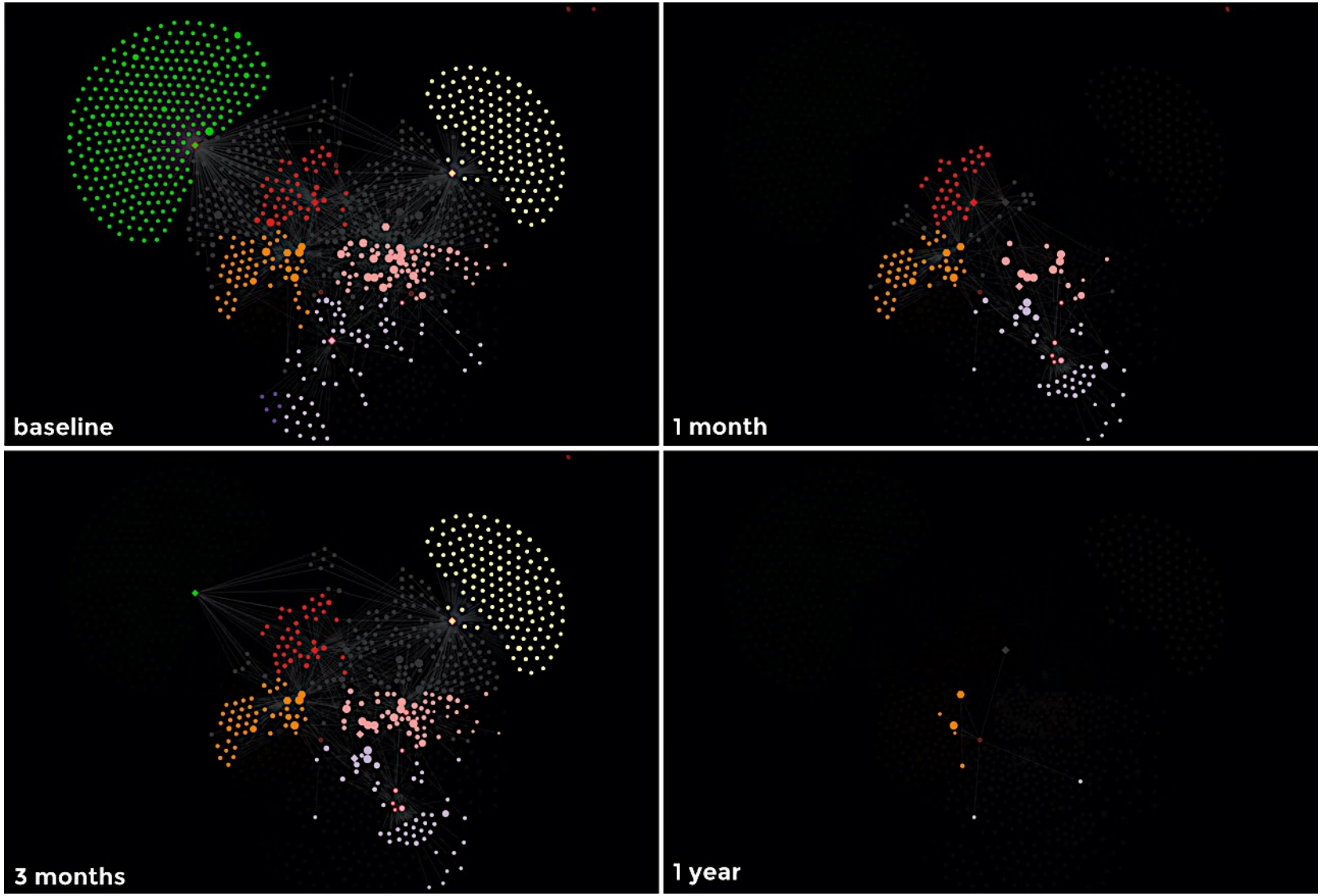
https://doi.org/10.3389/fimmu.2018.00711

SO WHAT?

Diseases are complex and dynamic, as well...

Figure 1: An example of the outcome of a bioinformatics analysis combining patient data with the network analysis platform.

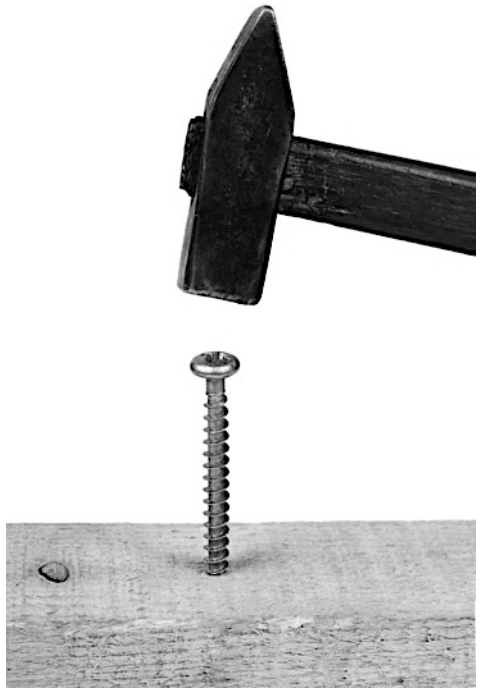
A network model reveals different molecules (nodes, scaled by centrality) and mechanisms (colored network clusters), relevant at different time points after a cardiac event.



...and precisely rather personal.

YES?

TOUS LES PROBLÈMES NE SONT PAS DES CLOUS.



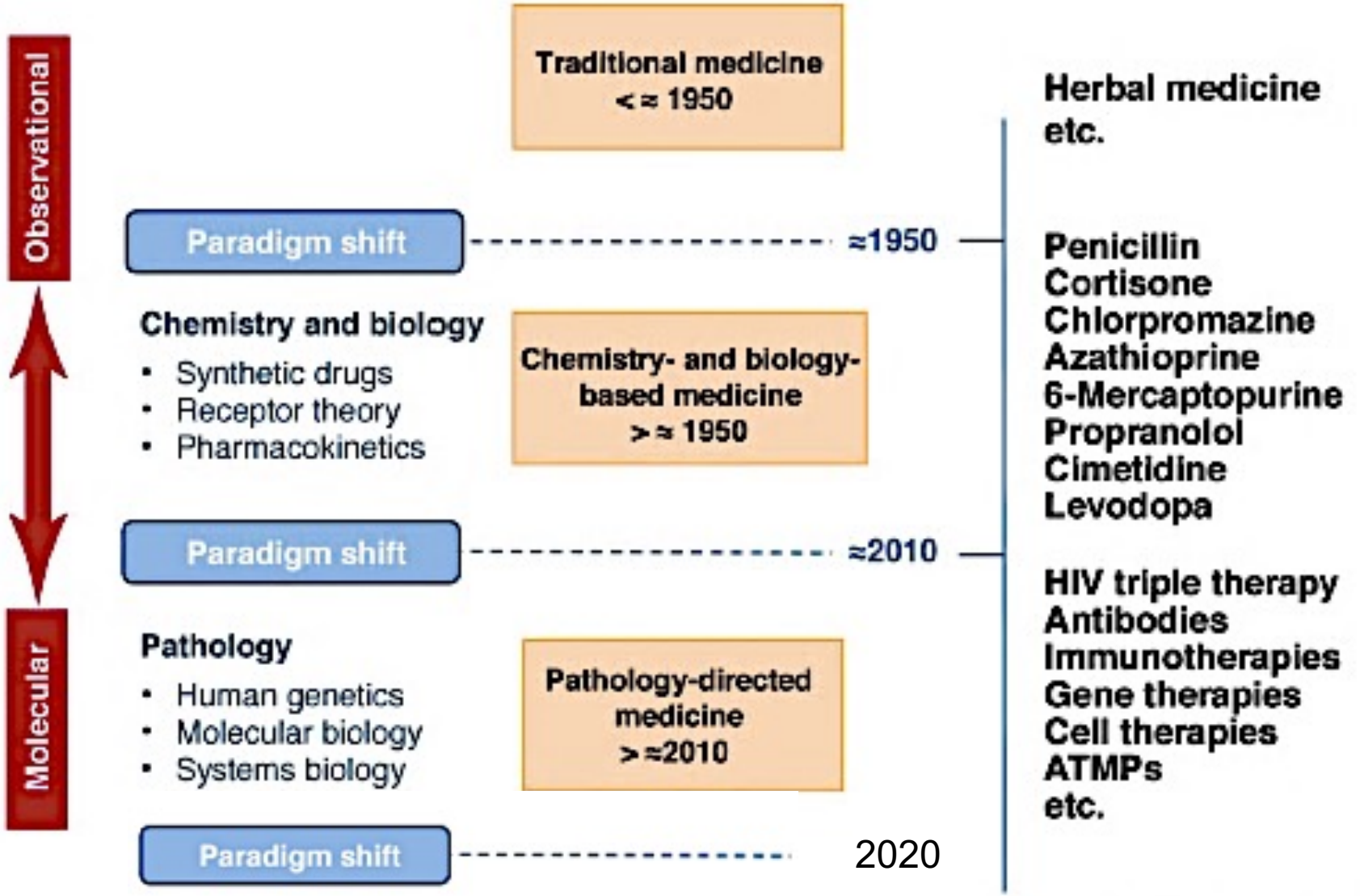
You try to screw a screw with a hammer – you’re screwed...

AN APPROACH THAT TAKES INTO ACCOUNT DYNAMIC COMPLEXITY IS NEEDED.

HOW?

We are in need of more complex tools.

Nanomedicine is part of a pathology-based approach



Next: Information medicine?

Wer hat’s erfunden?

Journal of Pharmaceutical Sciences

Volume 65, Issue 11, 1976, Pages 1624-1627

In vitro studies of poly(methyl methacrylate) adjuvants (Article)

Kreuter, J., Speiser, P.P.

Sch. Pharm., Fed. Inst. Technol., Zurich, Switzerland

Prof. Peter Paul Speiser, 1921-2013

Infection and Immunity

Volume 13, Issue 1, 1976, Pages 204-210

New adjuvants on a polymethylmethacrylate base (Article)

Kreuter, J., Speiser, P.P.

Sch. Pharm., Fed. Inst. Technol., Zurich, Switzerland

Journal of Pharmaceutical Sciences

Volume 62, Issue 9, 1973, Pages 1444-1448

Preparation and in vitro evaluation of cellulose acetate phthalate coacervate microcapsules (Article)

Merkle, H.P., Speiser, P.

Coll. Pharm., Swiss Fed. Inst. Technol., Zurich, Switzerland

FEBS Lett. 1977 Dec 15;84(2):323-6.

Nanocapsules: a new type of lysosomotropic carrier.

Couvreur P, Tulkens P, Roland M, Trouet A, Speiser P.

Pharmazeutische Industrie

Volume 37, Issue 7, 1975, Pages 555-560

Microencapsulation by spray condensation. Polycondensation of aminoplast precondensates in spray coating of disperse systems

[ZUR MIKROVERKAPSELUNG DURCH SPRUHKONDENSATION: DIE POLYKONDENSATION VON AMINOPLAST VORKONDENSATEN BEI DER SPRUHUMHULLUNG DISPERSER SYSTEME]

Merkle, H.P., Speiser, P.

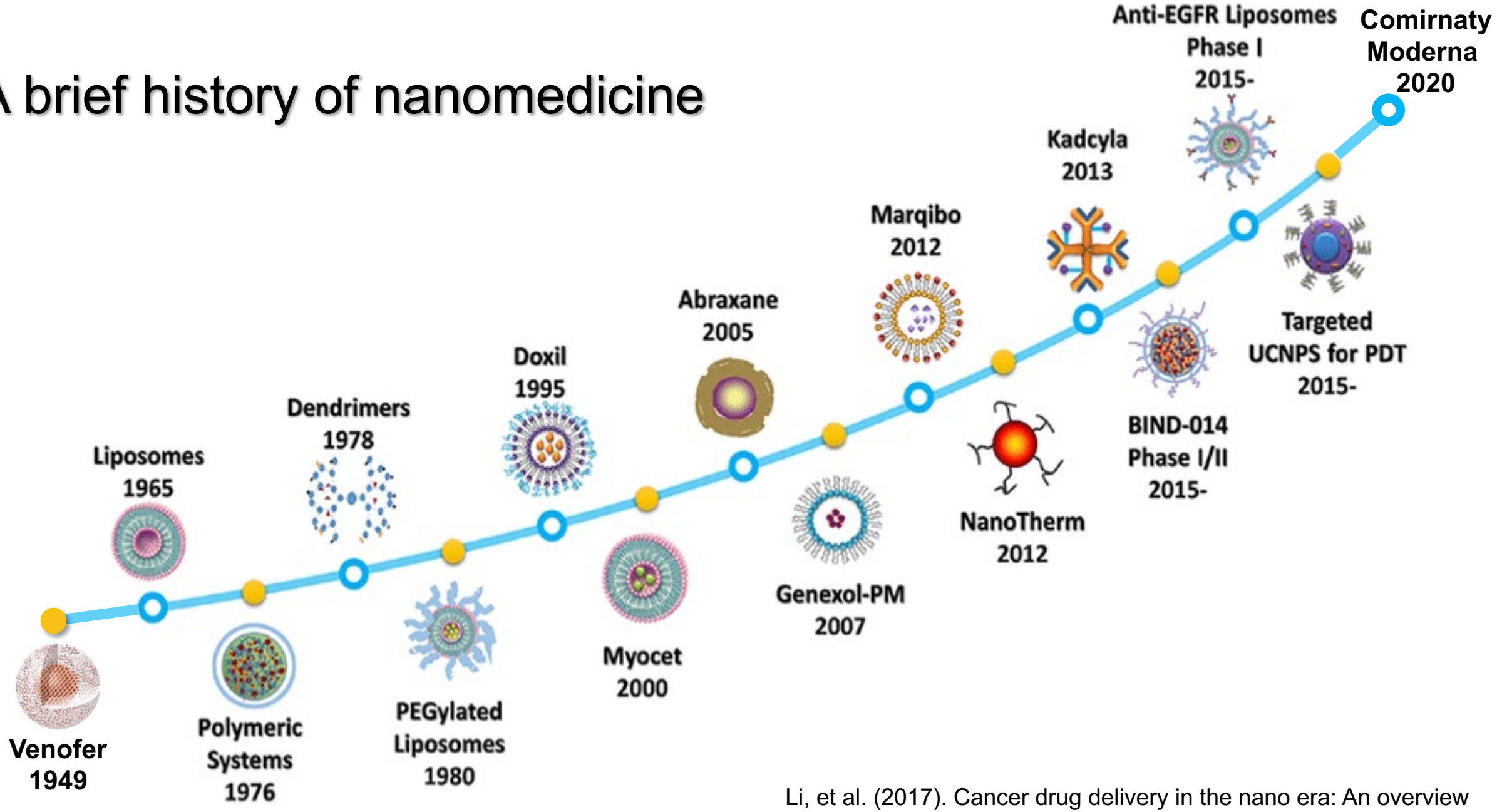
Galen Abt., Pharmazeut. Inst., ETH, Zurich, Switzerland

Paul Ehrlich et "*Der Freischütz*"

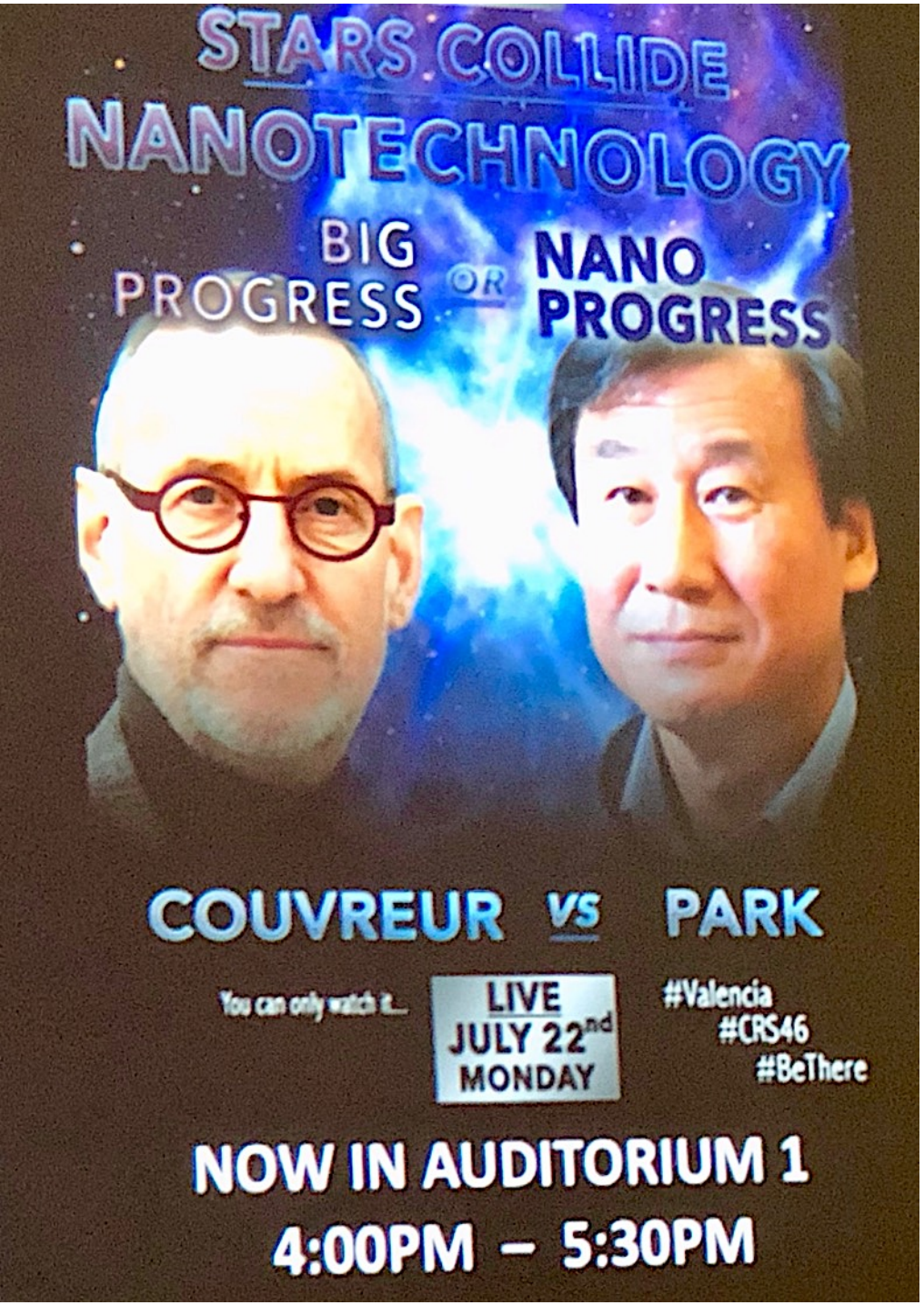
- *Der Freischütz* est un opéra allemande de Carl Maria von Weber.
- *Freikugeln* ("balles magiques") sont fourni par le diable en échange de l'âme de Max, le garde-chasse du Prince.
- Ces balles qui atteindraient leur cible sont utilisées lors d'un concours de tir dont l'enjeu est la nomination du nouveau garde-chasse et obtenir ainsi la main d'Agathe, la fille du garde forestier.
- Paul Ehrlich (1854-1915) a assisté à cet opéra à Francfort, et lui a donné le concept du "drug targeting" (ciblage des médicaments).
- Salvarsan: premier médicament contre la syphilis et premier agent chimiothérapeutique, considéré comme balle magique, a été développé par Ehrlich et Sahachiro Hata (1910).

Honestly Paul, where are we?

A brief history of nanomedicine

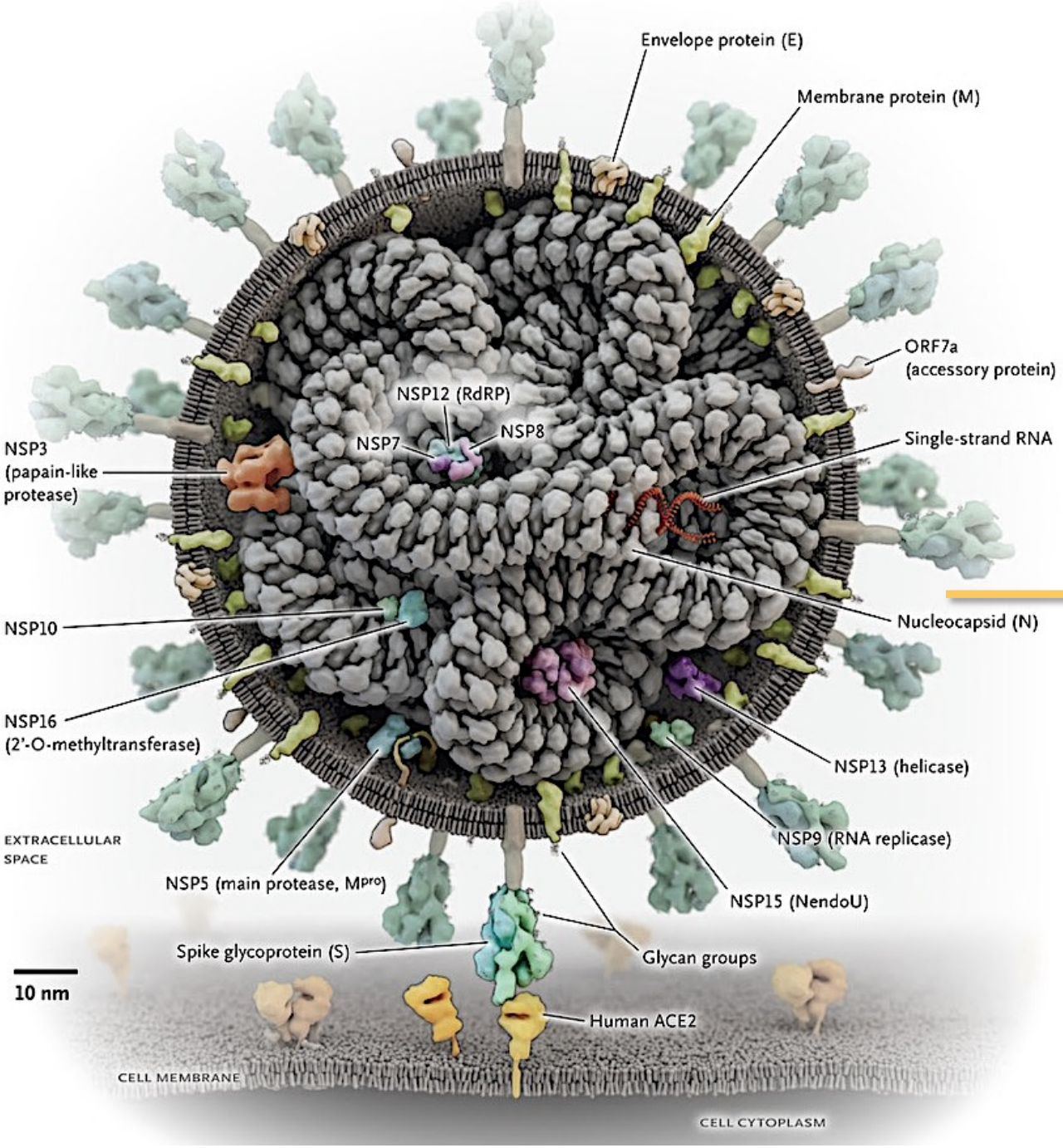


Li, et al. (2017). Cancer drug delivery in the nano era: An overview and perspectives. Oncology Reports. 38. doi: 10.3892/or.2017.5718.

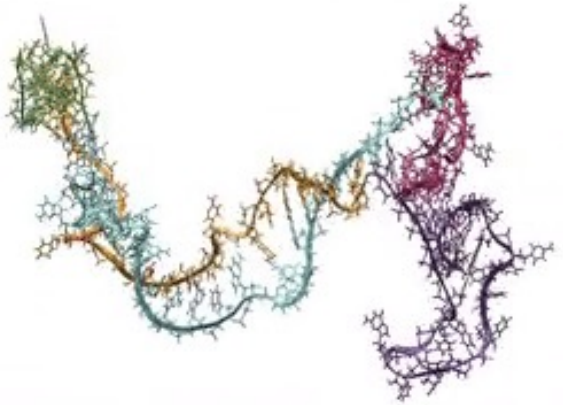


*"**Disappointing outcomes** of nano-sized formulations (nanoformulations) in clinical studies indicate that our overall approach of nanomedicine needs serious reevaluation. (…) we all have to find the reality by absorbing the truth and fight our way out of the egg to **break the ill-conceived illusion of the nanomedicine.**"*

K. Park, J. Control. Rel. 267 (2017) 2-14

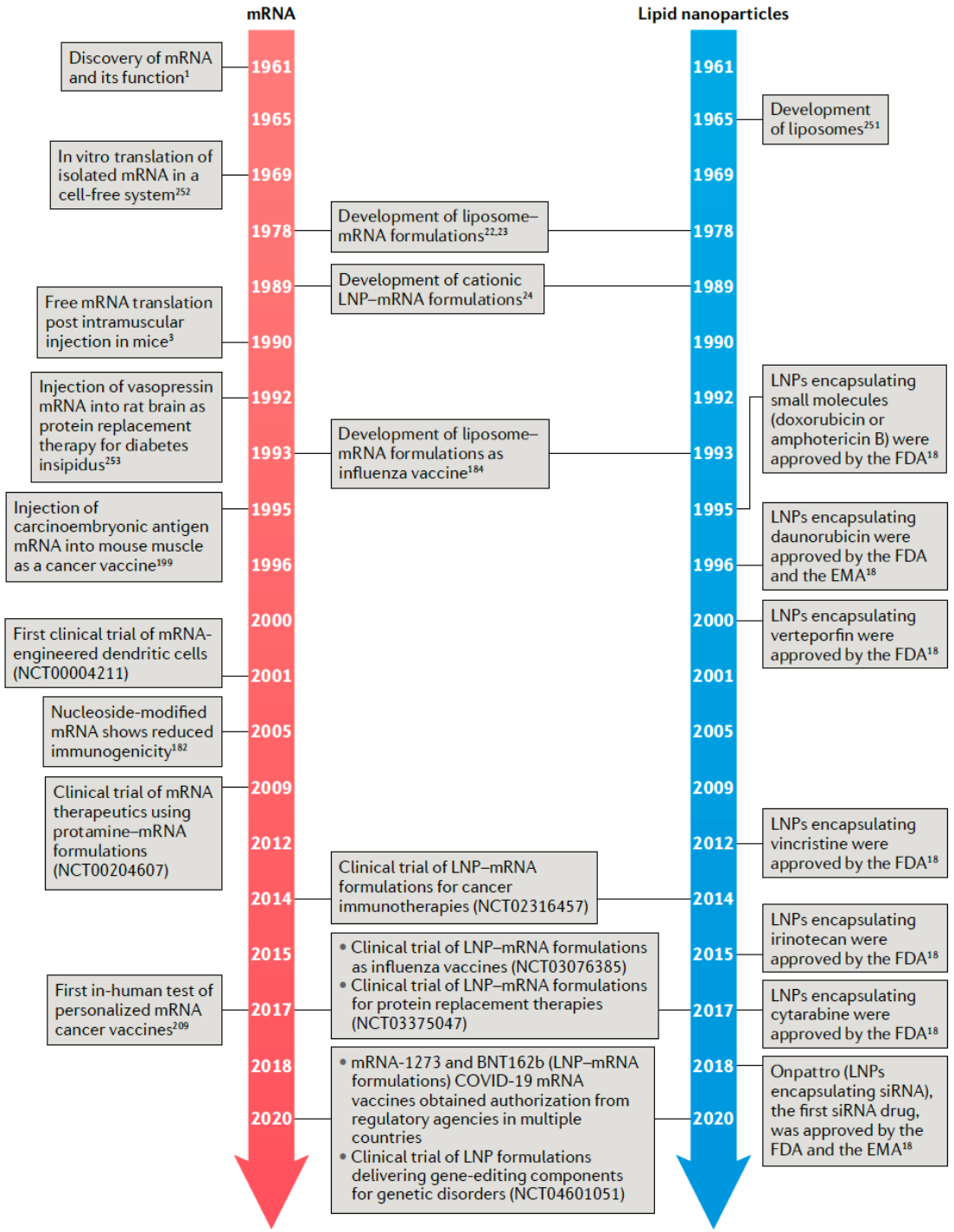


Pfizer/BioNTech's S-protein mRNA



4,284 nucleotides

1388 kDa molecular weight

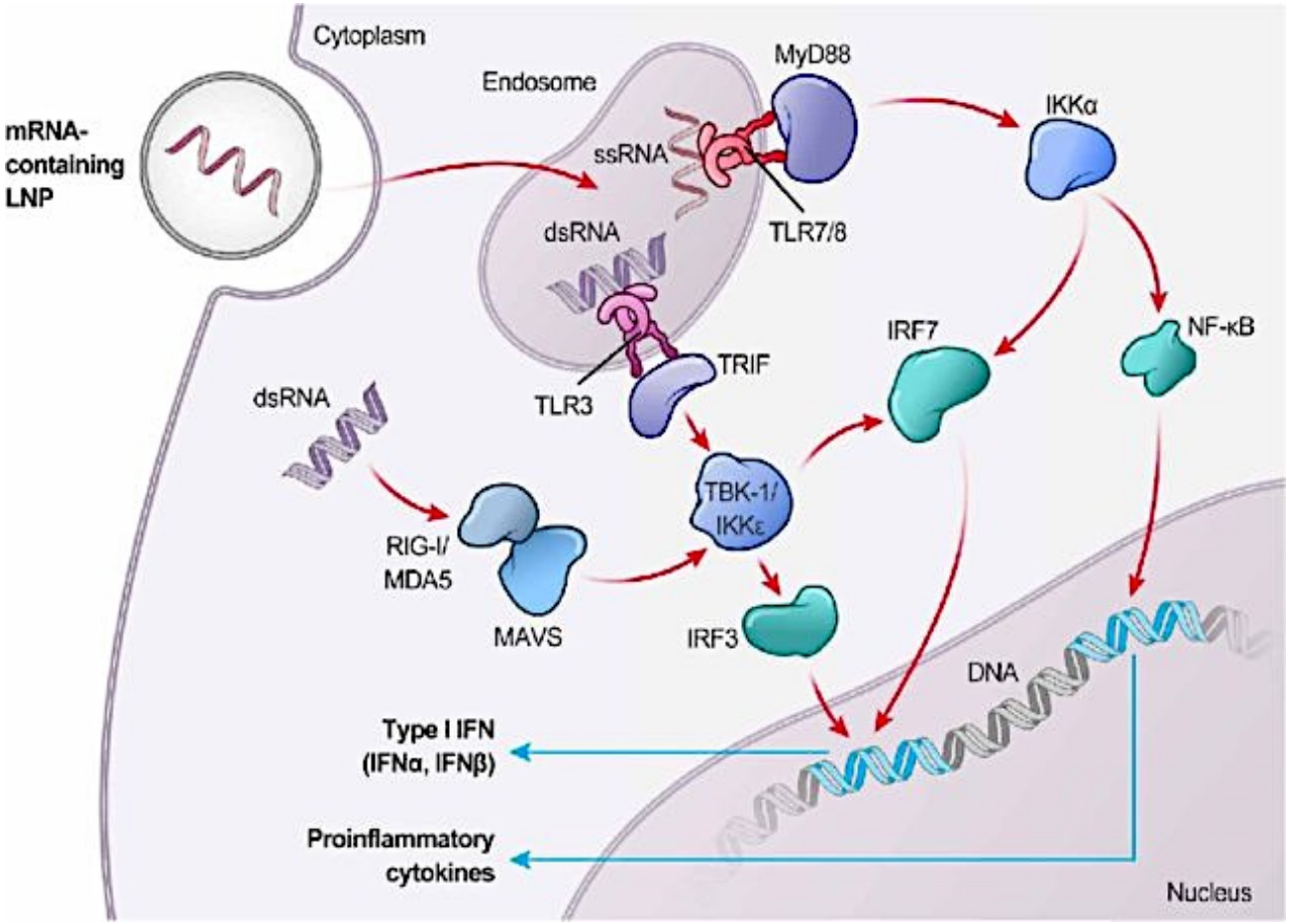


60 years of mRNA...

...and its formulation, the “lesser known sister”.

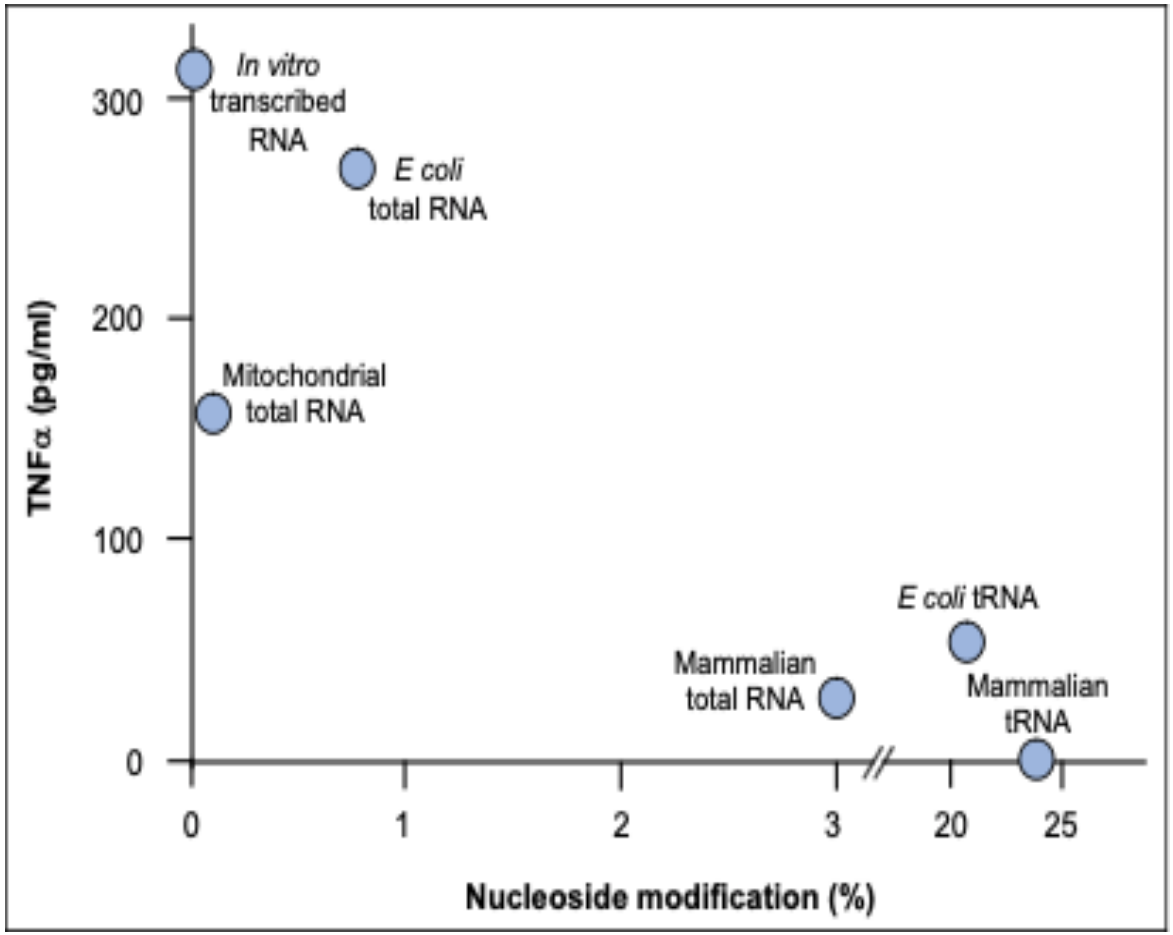
Hou, X., Zaks, T., Langer, R., Dong, Y.
Lipid nanoparticles for mRNA delivery. *Nat Rev Mater* (2021).
https://doi.org/10.1038/s41578-021-00358-0

L'ARNm interagit avec les récepteurs de l'immunité innée, provoquant une inflammation.



Nelson et al., Sci. Adv. 2020, 6, DOI: 10.1126/sciadv.aaz6893

Les modifications nucléosidiques naturelles suppriment l'activité immunostimulante de l'ARN.



K. Karikó & D. Weissman, Curr. Opin. Drug Discov. Devel. 2007, 10:523-32.

Incorporation of Pseudouridine Into mRNA Yields Superior Nonimmunogenic Vector With Increased Translational Capacity and Biological Stability

Katalin Karikó[1], Hiromi Muramatsu[1], Frank A Welsh[1], János Ludwig[2], Hiroki Kato[3], Shizuo Akira[3] and Drew Weissman[4]

[1]*Department of Neurosurgery, University of Pennsylvania, Philadelphia, Pennsylvania, USA;* [2]*Laboratory of RNA Molecular Biology, The Rockefeller University, New York, New York, USA;* [3]*Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan;* [4]*Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA*

«I felt like a God!»

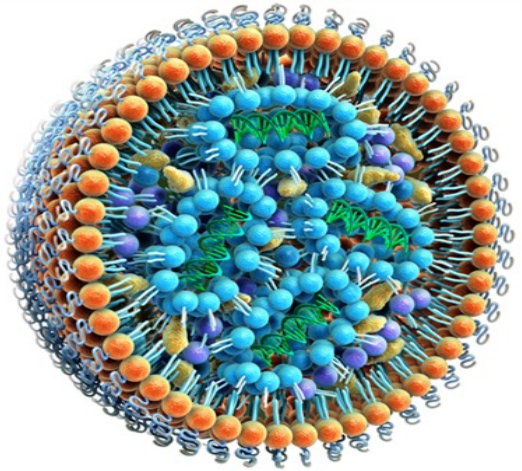
Mol. Therap. 16, 2008, 1833-1840, doi: 10.1038/mt.2008.200

2023

2021

SAPhS
Swiss Academy
of Pharmaceutical
Sciences
www.saphw.ch

What are we talking about?



Step 1: DNA template

- prepare DNA (*E. coli*, cell-free)
- purify linear DNA template
- Freeze product

Step 2: mRNA

- prepare mRNA (cell-free)
- purify mRNA
- Freeze product

Step 3: Drug product

- formulate LNP
- buffer exchange by TFF
- filter-sterilize
- fill & finish, freeze

At the onset of the pandemic, very few companies were able to manufacture GMP grade DP!

Storage ?

ISPSO
INSTITUT DES SCIENCES PHARMACEUTIQUES DE SUISSE OCCIDENTALE

UNIVERSITÉ DE GENÈVE

Chula VRC
Chula Vaccine Research Center
Faculty of Medicine, Chulalongkorn University

Freeze-drying a monovalent mRNA-LNP dengue serotype 1 vaccine

A. Ramos Barros[1], Aya Halmi[1], C. Khawsang[2], E. Prompetchara[2],C. Ketloy[2], G. Borchard[1]

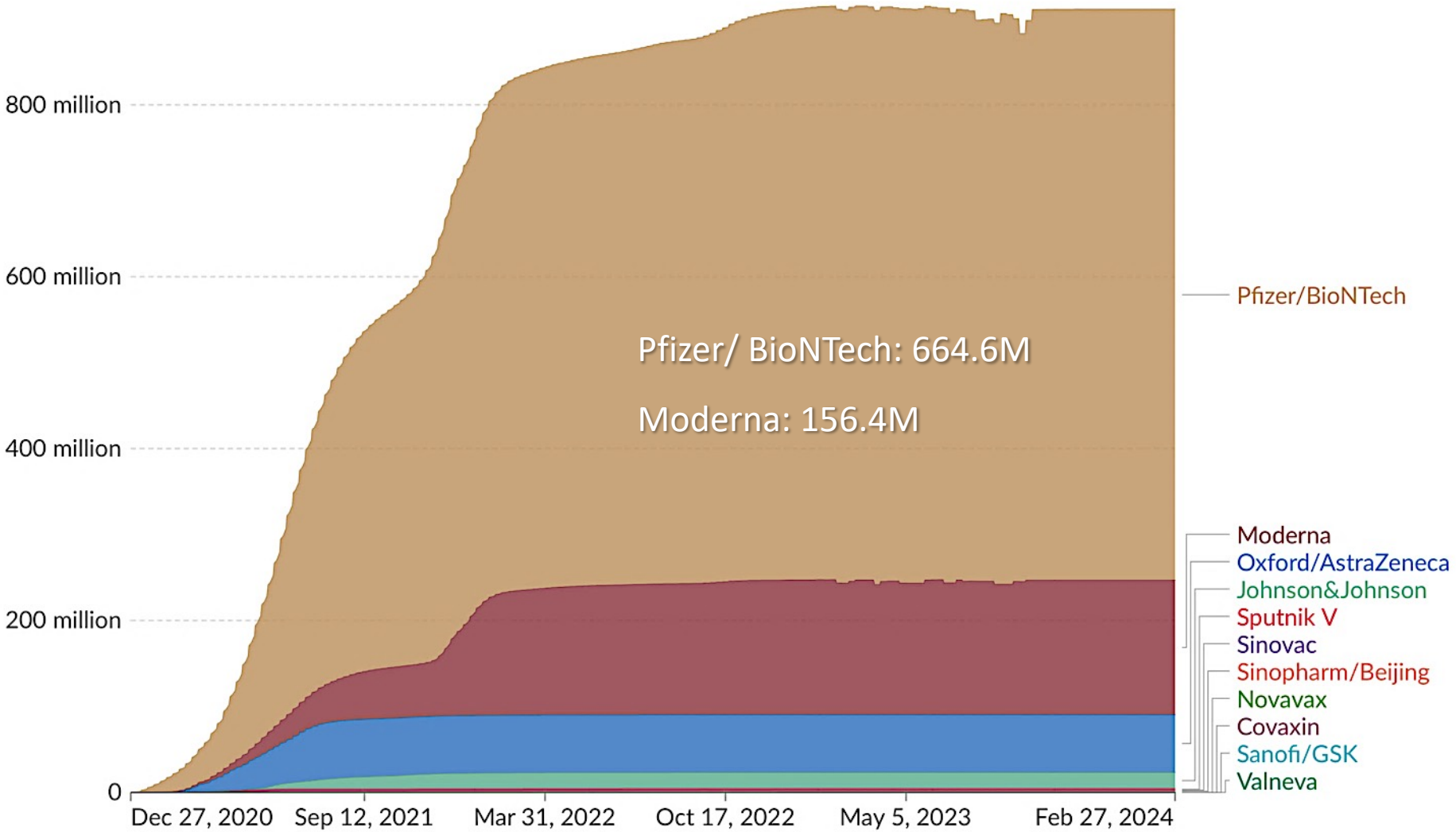
[1]Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), University of Geneva, Rue Michel-Servet 1, Geneva, Switzerland

[2]Chula Vaccine Research Center (VRC), Faculty of Medicine, Chulalongkorn University,1873 RamaIV Rd., PathumWan, Bangkok, 10330, Thailand

COVID-19 vaccine doses administered by manufacturer, European Union

Our World in Data

All doses, including boosters, are counted individually.



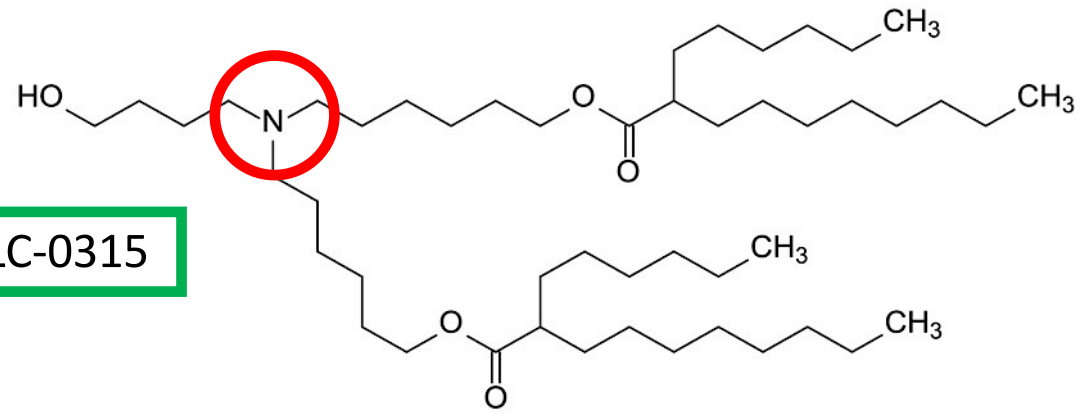
Pfizer/ BioNTech: 664.6M

Moderna: 156.4M

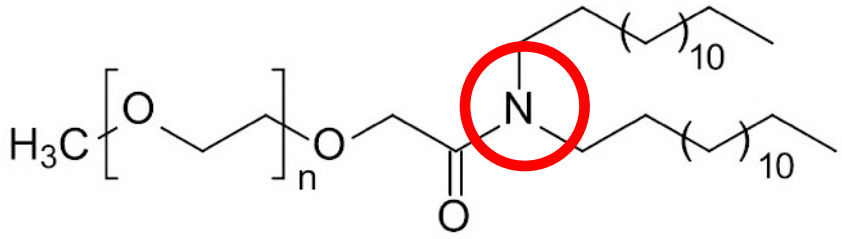
Data source: Official data collated by Our World in Data

OurWorldInData.org/covid-vaccinations | CC BY

Comirnaty's formulation

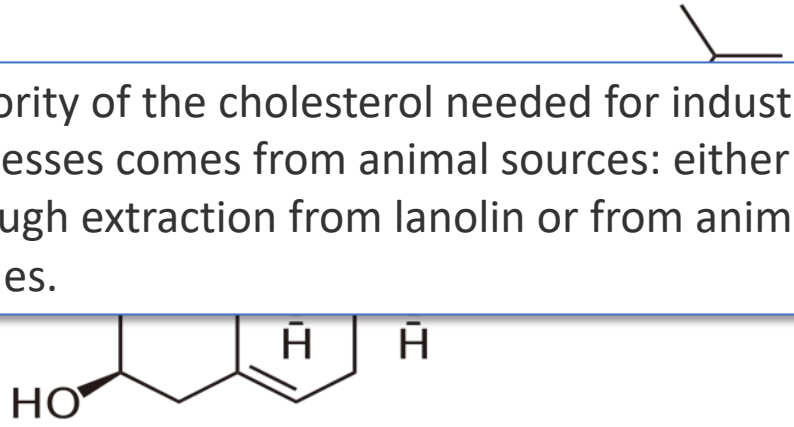


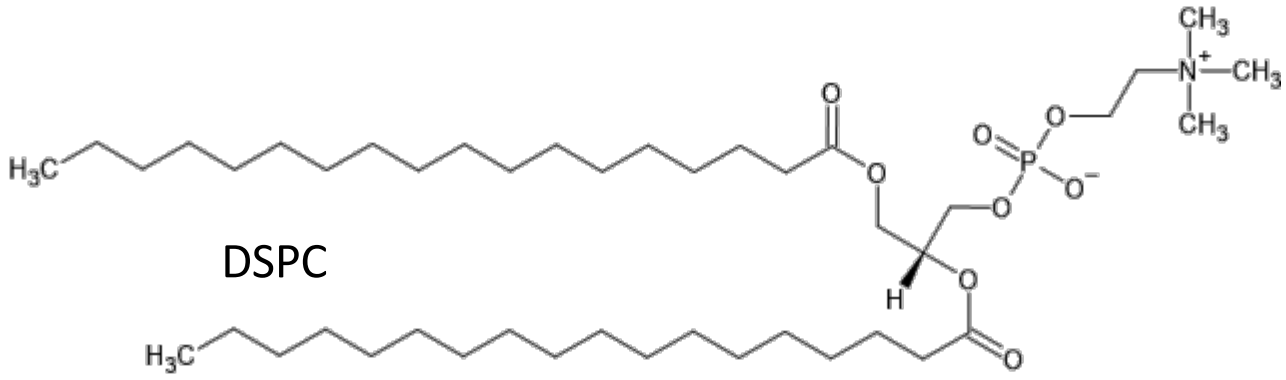
ALC-0315



ALC-0159

Majority of the cholesterol needed for industrial processes comes from animal sources: either through extraction from lanolin or from animal tissues.





DSPC

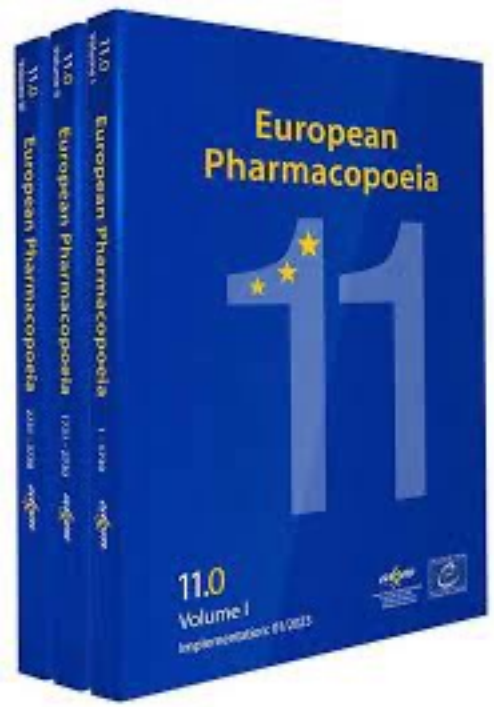
0.2 mg/dose = 130 tons in EU doses alone

How do you source and assure quality of these compounds for billions of doses?

KCl, KH_2PO_4, NaCl, Na_2HPO_4, sucrose, aq. ad inj.

“...mRNA vaccines are nanomedicines...”

CASSS CMC Strategy Forum Europe 2021, October 2021

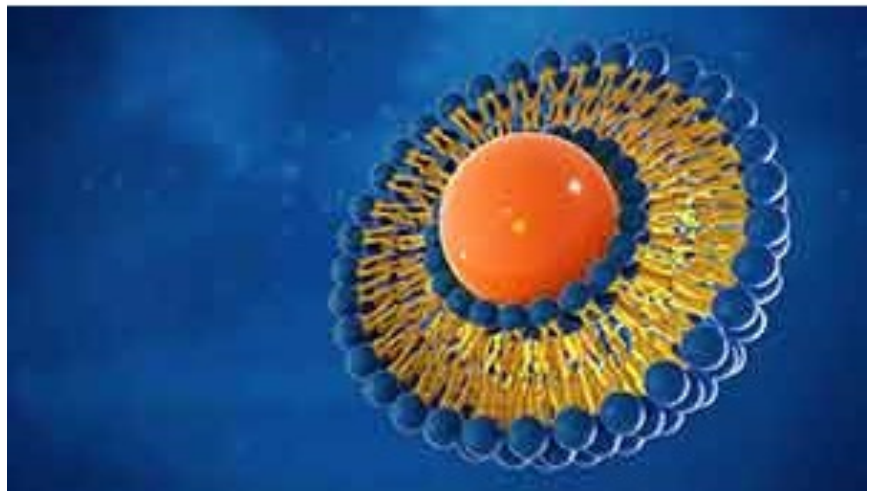


European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

COUNCIL OF EUROPE
CONSEIL DE L'EUROPE

?

Quality requirements for nanomedicines: which role for the European Pharmacopoeia?



7-8 June 2022

Council of Europe premises

40 registrants (67 including speakers & EDQM staff) from **15** countries: 5 academia, 15 authorities, 16 industry

Outcomes

- Creation of a Working Party on mRNA vaccines (mRNAVAC)
- Appointment at November 2022 session of the Ph. Eur. Commission
- Develop a consolidated strategy for future standards addressing these vaccines and their components
- The ideas and proposals put forward on this topic during the recent EDQM Symposium on Nanomedicines will be taken into account

https://www.edqm.eu/en/-/quality-requirements-for-nanomedicines-what-role-should-the-european-pharmacopoeia-play-

mRNAVAC Working Party – terms of reference

mRNAVAC Working Party (mRNA Vaccines for human use)

Terms of reference

- Drafting and revision of texts in the field of mRNA vaccines for human use

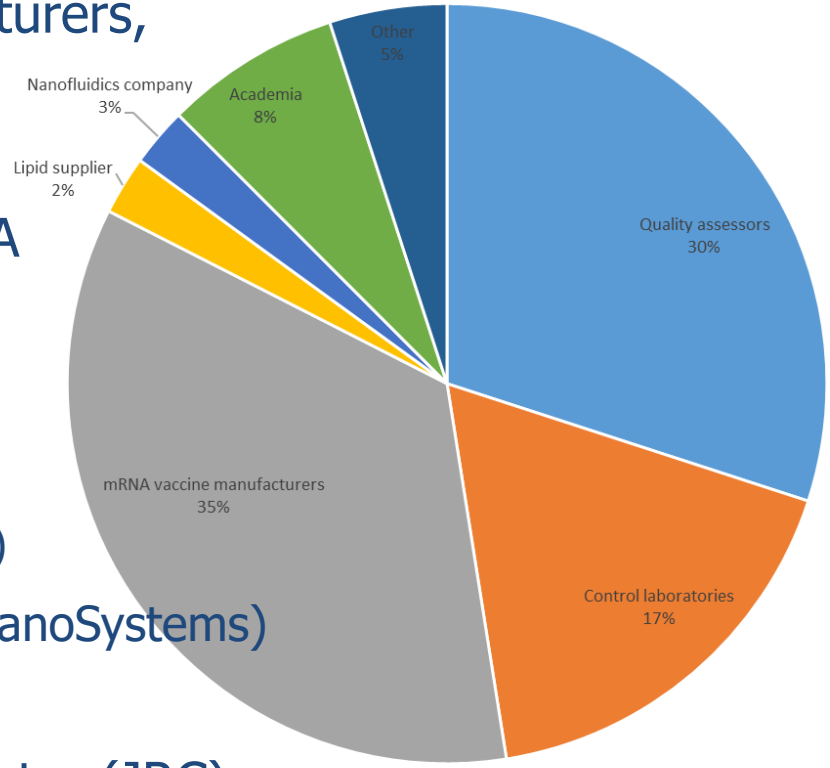
Profile for experts

- Current expertise in analytical procedures related to the quality control of mRNA vaccines for human use, their components and their formulation
- Significant experience in one or more of the following fields:
  - Quality control of mRNA vaccines for human use and their components in a pharmaceutical manufacturing setting
  - Quality control/batch release/market surveillance of mRNA vaccines for human use and their components in an independent testing laboratory (e.g. OMCL)
  - Pharmaceutical development related to the formulation of mRNA vaccines for human use
  - Analytical development related to mRNA vaccines for human use and their components
  - Assessment of the relevant parts of applications for marketing authorisation within a medicines agency

mRNAVAC Working Party – composition

- Experts appointed at COM 174
- **43 Members** from various areas of activities: vaccines, mRNA, nanomedines / nano-formulation
- Regulatory authorities, national control labs, mRNA vaccine manufacturers, lipid supplier, nanofluidics company, academia
- Regulators/NCLs:
  - European regulators but also US FDA, Health Canada, TGA, TFDA

    → **Global effort!**
- Industry:
  - 14 experts from 6 mRNA vaccine manufacturers (Moderna, Pfizer / BioNTech, eTheRNA, GSK, Sanofi-Pasteur, Novartis)
  - 1 lipid supplier (Lipoid GmbH), 1 nanofluidics company (Precision NanoSystems)
- 6 Group 15 experts including its Chair (S. Andersen)
- 1 representative from the European Commission's Joint Research Centre (JRC)
- Chair: G. Borchard



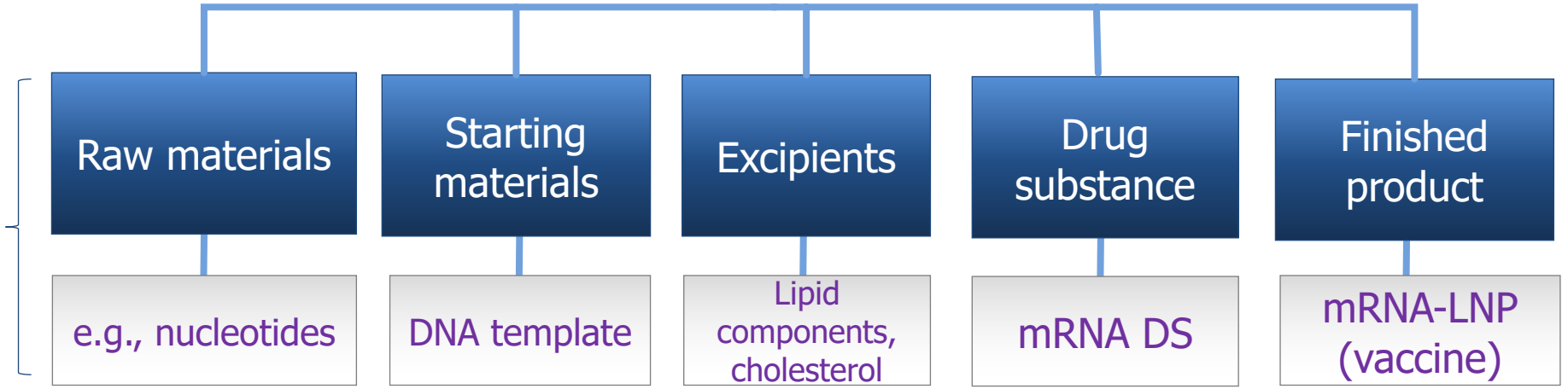
mRNAVAC Working Party – country distribution

Reminder

16 countries, 4 continents

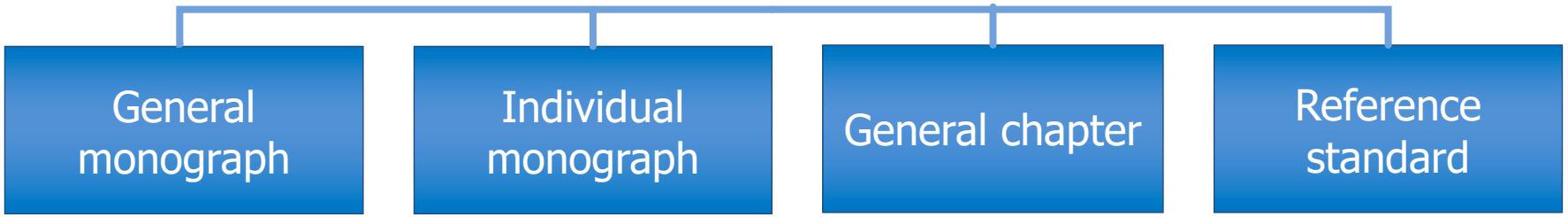
What role can the Ph. Eur. play in setting standards for mRNA vaccines?

1) Areas for standardisation



→ *Additional info: feedback from EDQM Nanomedicines Symposium*

2) Format / Type of Ph. Eur. text



→ *Additional info: induction training*

mRNAVAC Working Party

1) EDQM Nanomedicines Symposium:
- Dedicated session on mRNA vaccines
- Brainstorming session on Role for the Ph. Eur in setting standards for mRNA vaccines and nanomedicines

2) COM 173:
- Outcome of Nano Symposium
- Establishment of the mRNAVAC WP

Newsroom

Ph. Eur. Commission establishes a new working party on mRNA vaccines

EDQM | STRASBOURG, FRANCE | 03/08/2022

At its 173rd session in June 2022, the European Pharmacopoeia (Ph. Eur.) Commission decided to start working on mRNA vaccines by establishing the mRNAVAC Working Party, entrusted with elaborating quality standards supporting this emerging field that will be included in the Ph. Eur.

The newly created Working Party's first task will be to develop a consolidated strategy for future standards addressing these vaccines and their components. The ideas and proposals put forward on this topic during the recent EDQM Symposium on Nanomedicines will be taken into account during the process, as will the experience gained with these vaccines during the pandemic.

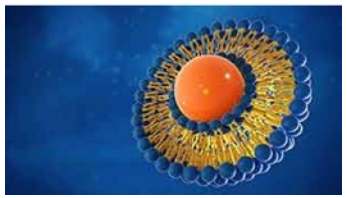
Specialists with experience in the formulation of mRNA vaccines and the analytical procedures used in the quality control of these vaccines and their components (e.g. from licensing authorities, official medicines control laboratories, industry or academia) are invited to apply to join the new Working Party.

More information on how to apply can be found here.

3) COM 174:
- Nomination of Experts and Chair

4) mRNAVAC WP 'Get together' (virtual):
- To set the scene, welcome Members
- To kick off the work (action plan, preparatory work for face-to-face meeting)

Jun 2022



Jun 2022

Nov 2022

Dec 2022

mRNAVAC Working Party

5) mRNAVAC WP Induction training (virtual)

- Many members with no prior experience in Ph. Eur. work
- Need for training on Ph. Eur. & Ph. Eur. processes (how the Ph. Eur. works, elaboration & revision of texts…)
- 2 virtual meetings

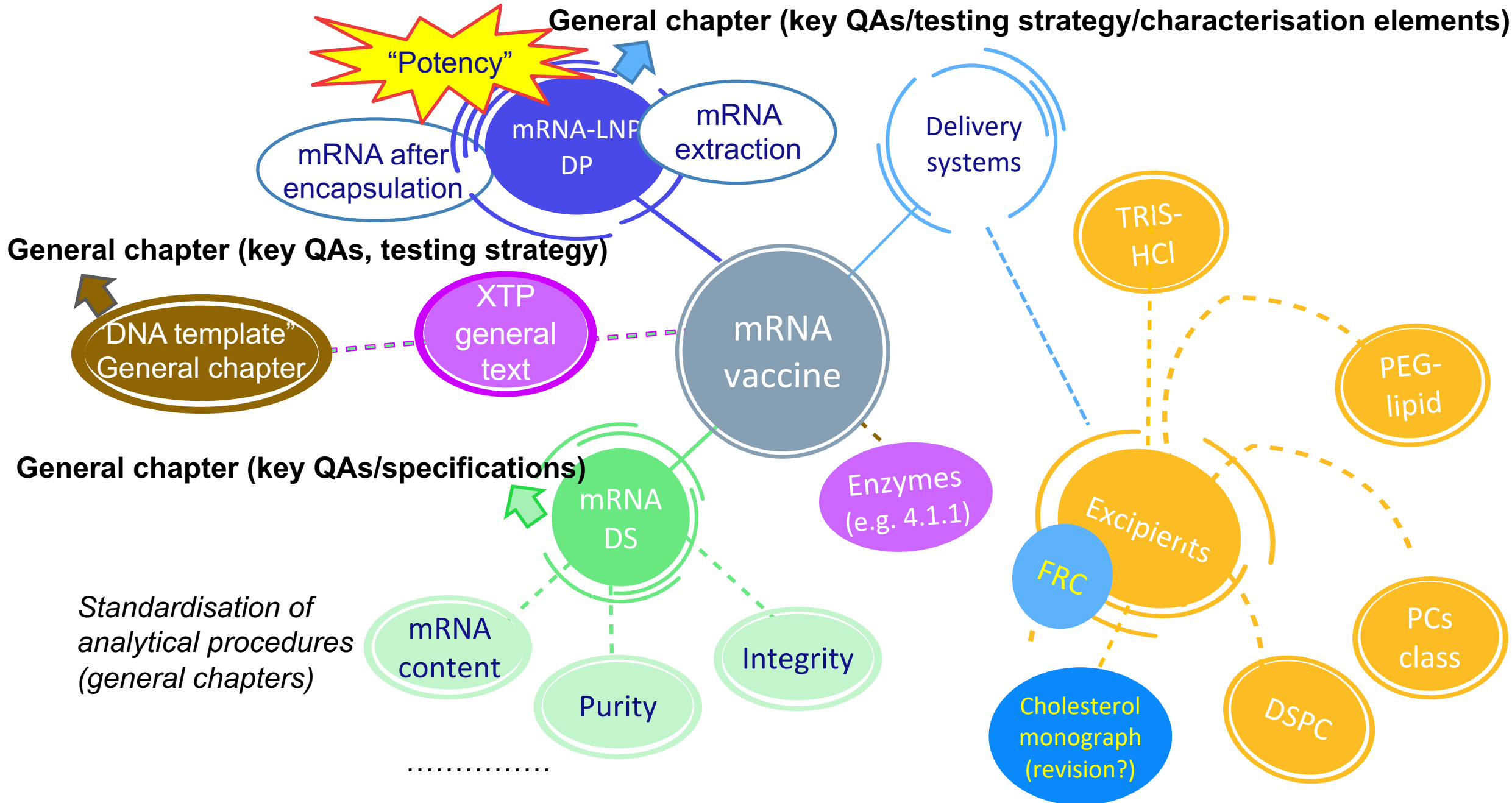
6) 1st mRNAVAC WP meeting (in-person): **kick-off & brainstorming**

- Feedback from the EDQM nanomedicines symposium
- Brainstorming on how to address mRNA vaccines in the Ph. Eur. (future texts & areas for standardisation

Jan 2023

Feb 2023

mRNA Vaccines: Proposed "Roadmap" (from 1st mRNAVAC WP meeting, Feb. 2023)



mRNAVAC Working Party – Activities since EPC175

1) <u>EPC175</u>:
- Feedback from 1st mRNAVAC WP meeting
- **First additions to work programme** (5.36, 5.39 & 5.40)

2) <u>Drafting groups meetings</u> (virtual)
- Kick off drafting of Ph. Eur. **chapters 5.36** (DP), **5.39** (DS) and **5.40** (DNA template)
- Discuss the outline /structure and content
- Appoint Rapporteurs and assign work

3) <u>2nd plenary mRNAVAC WP meeting</u> (virtual)
- Agreement on draft outlines for each text
- Organisation of drafting work

5 May 31 May & 21 June 29-30 June

Mar 2023 May June

Phase 1: Outline /structure and key elements of each chapter

mRNAVAC Working Party – Activities since EPC175 (cont'd)

6) Drafting groups meetings (virtual)

- Review of draft sections prepared by Rapporteurs
- Discussion on Drafting Group comments
- Consolidation of draft texts

15, 22 & 28 Sep

7) 3rd mRNAVAC WP meeting (in-person)

- Detailed review of consolidated texts and outstanding comments
- Agreement on draft chapters 5.36, 5.39 & 5.40

11-13 October

4) Drafting by Rapporteurs

5) Peer review – comments from Drafting Group members

8) Final verification of draft texts

July 2023 August September October November 2023

Phase 2: drafting of individual sections by Rapporteurs (working in "pairs")

Phase 3: initial review & consolidation by Drafting Groups

Phase 4: Final review by mRNAVAC WP

mRNAVAC Working Party

10) 4th Plenary mRNAVAC WP meeting (in-person)
- Continue discussion "other streams"
- Agreement on proposed strategy and actions

9) Drafting groups meetings (virtual)
- Discussion "other streams"
- Prepare proposals for further discussion

EPC 178:
- Update on work programme

EPC 179:
- Potential additions to the work programme

- Continue discussions in drafting groups ("other streams")
- Consolidate strategies
- Prepare proposals for addition to the work programme

Review of stakeholders' comments (5.36, 5.39, 5.40)

11) Plenary mRNAVAC WP meeting (in-person)

25 & 26 Jan | 15-16 Feb.

January | February | March | April | May | June | July | August | Sep | Oct | November

"**Other streams**":
- Analytical standardisation (stream 5)
- Lipid excipients (stream 6)
- Raw materials (stream 4)

Public consultation Pharmeuropa 36.2 (5.36, 5.39 & 5.40)
(deadline for comments: 31 August 2024)

mRNA vaccines: Elaboration of chapters 5.36, 5.39 & 5.40

	Text	Content	Status
mRNA-LNP	General chapter *mRNA Vaccines for human use (5.36)*	• Scope: Production and control of **mRNA and sa-RNA packaged in lipid nanoparticles** (mRNA-LNP medicinal product i.e. vaccine). Mono- and multivalent vaccines • Quality attributes and testing strategy for the mRNA-LNP medicinal product • Analytical procedures that may be used for analysis, to establish product consistency and for quality control of the mRNA-LNP medicinal product • Formulation (key element of mRNA vaccines)	Draft completed 
mRNA substance	General chapter *mRNA Substances for the production of mRNA vaccines for human use (5.39)*	• Scope: Production and control of **mRNA active substances** that are used in the manufacture of mRNA vaccines • Quality attributes and testing strategy for mRNA substance • Analytical procedures that may be used for mRNA analysis, to assess consistency and for quality control • Manufacture of mRNA active substance	Draft completed 
Starting material	General chapter *DNA Template for the preparation of mRNA substances (5.40)*	• Scope: Production and control of the **DNA template** (**starting material for preparation of the mRNA component)** • Production of the linear DNA template (e.g. linearised plasmid DNA or linear DNA derived enzymatically) • Quality attributes and testing strategies for the linear DNA template	Draft completed 

mRNA Vaccines: Elaboration of chapters 5.36, 5.39 & 5.40

5.40. DNA TEMPLATE FOR THE PREPARATION OF mRNA SUBSTANCES

DNA template for the preparation of mRNA substances (5.40.)

mRNA substances, DNA templates for the preparation of (5.40.)

1. DEFINITION

A DNA template is a linear double-stranded DNA used as a starting material for the manufacture of mRNA substances for the production of mRNA vaccines for human use. The linear DNA template is transcribed *in vitro* using a cell-free enzymatic reaction to produce the corresponding mRNA substance.

The DNA template may be a linearised plasmid DNA that has been produced in bacteria or may be derived enzymatically using a cell-free process. For the latter, different technologies such as PCR or rolling circle amplification can be used.

Regardless of the production method, the linear DNA template contains the promoter sequence for the RNA polymerase used for mRNA transcription, the sequence to be transcribed into the mRNA, which consists of the 5′ and 3′ untranslated regions (UTR), the open reading frame for the encoded antigen and, if appropriate, the poly(dA:dT) tract for the poly(A) tail.

Certain aspects of this general chapter may apply regardless of the intended use
the mRNA that is transcribed from the linear DNA template.

2. PRODUCTION

2.1. *GENERAL PROVISIONS*

Production of plasmid DNA is based on a bacterial cell-bank system. Plasmid DNA is amplified in bacterial cells and then purified as the circular form. In order to be used for *in vitro* transcription, the circular plasmid DNA is then linearised with a suitable restriction endonuclease.

Production of DNA by enzymatic technologies based on cell-free amplification of DNA can also be used. This starts with a small quantity of DNA to be amplified (input DNA). Some technologies give rise to a covalently closed DNA form that then has to be linearised as for plasmid DNA, others produce a linear form with the appropriate 3′ end required for mRNA transcription. To ensure the consistency of the input DNA, a master DNA stock is established.

2.2. *LINEARISED PLASMID DNA*

Plasmid construction. The plasmid is composed of:

– the plasmid backbone that contains multiple restriction endonuclease recognition sites for insertion of the genetic insert and the bacterial elements necessary for plasmid production (such as selectable genetic marker(s) for the selection of cells that carry the recombinant plasmid) and the recognition sequence for the endonuclease used for linearisation;

5.39. mRNA SUBSTANCES FOR THE PRODUCTION OF mRNA VACCINES FOR HUMAN USE

mRNA substances for the production of mRNA vaccines for human use (5.39.)

1. DEFINITION

mRNA substances for the production of mRNA vaccines are single-stranded mRNA molecules encoding one or more target antigens for induction of an immune response against an infectious agent. They are used as active substances for the production of prophylactic vaccines against infectious diseases.

mRNA substances are produced by a cell-free enzymatic process (referred to as *in vitro* transcription) using a suitable DNA template encoding the required antigen sequence.

The sequence of the mRNA may contain one or more open reading frames that encode the target antigen(s), flanking untranslated regions (UTRs), a 5′ cap (or alternative) and a 3′ poly(A) tail. The mRNA may contain naturally occurring nucleosides (modified or unmodified) and synthetic nucleosides. The mRNA backbone may be optimised.

In the case of

2. PRODUCTION

2.1. *GENERAL PROVISIONS*

The production method for a given mRNA substance must have been shown to yield consistently comparable batches. Substance specifications and relevant in-process tests and limits are set.

Process validation.

The production process is validated for the following aspects, including (but not limited to):

– consistency of the production process on an appropriate number of batches;
– adequate removal of product- and process-related impurities (for example, enzymes, DNA template and dsRNA if applicable);
– reusability of purification components (for example, chromatographic resin if applicable or tangential flow filtration membrane lifetime), with limits or acceptance criteria being set as a function of the validation.

Characterisation.

The mRNA substance is characterised in order to determine its structure, physico-chemical properties, purity and ability to be translated into the protein that it encodes.

5.36. mRNA VACCINES FOR HUMAN USE

mRNA Vaccines for human use (5.36.)

1. DEFINITION

mRNA vaccines for human use are preparations containing mRNA molecules compatible with the cellular protein translation machinery encoding for antigens capable of inducing a specific and active immunity in humans against an infecting agent or the toxin or antigen produced by it.

A suitable delivery system is necessary for the effective protection and administration of the mRNA substances. The scope of this general chapter is limited to lipid nanoparticle (LNP)-based delivery systems.

mRNA vaccines using LNPs as delivery system may contain one or more mRNA substances encapsulated in LNPs. LNPs are noncovalent, multicomponent assemblies, heterogeneous in their size, composition, and surface properties of the LNP subpopulations. They are composed of lipid and lipid-like components capable of encapsulating mRNA to ensure the desired product stability. The purpose of the LNPs is to protect the mRNA from enzymatic degradation by nucleases and enable cytosolic delivery of the mRNA.

PRODUCTION

GENERAL PROVISIONS

Production of mRNA vaccines using LNPs as a delivery system is based on self-assembly of the lipid and RNA (see 5.39 *mRNA substances for the production of mRNA vaccines for human use*) components resulting in encapsulation of the mRNA substance. This may be achieved by introducing a solution of the lipid components in a suitable solvent into a solution containing one or more mRNA substances in a suitable buffer, via a mixing system that is capable of controlling the flow rate, and thereby the mixing rate, and the ratio of the components. The resulting mRNA-containing LNP dispersion is further processed through a suitable purification process (e.g. ultrafiltration/diafiltration) to ensure adequate removal of product- and process-related impurities, medium exchange, and concentration adjustment.

Process validation

The production process is validated for the following aspects, including (but not limited to):

– consistency of the production process during mixing of the lipids and mRNA, the formation of RNA-containing LNPs, purification, formulation, final bulk vaccine production, and fill and finish steps;

– acceptable operational range for various processing parameters to ensure consistency in the quality of the product;

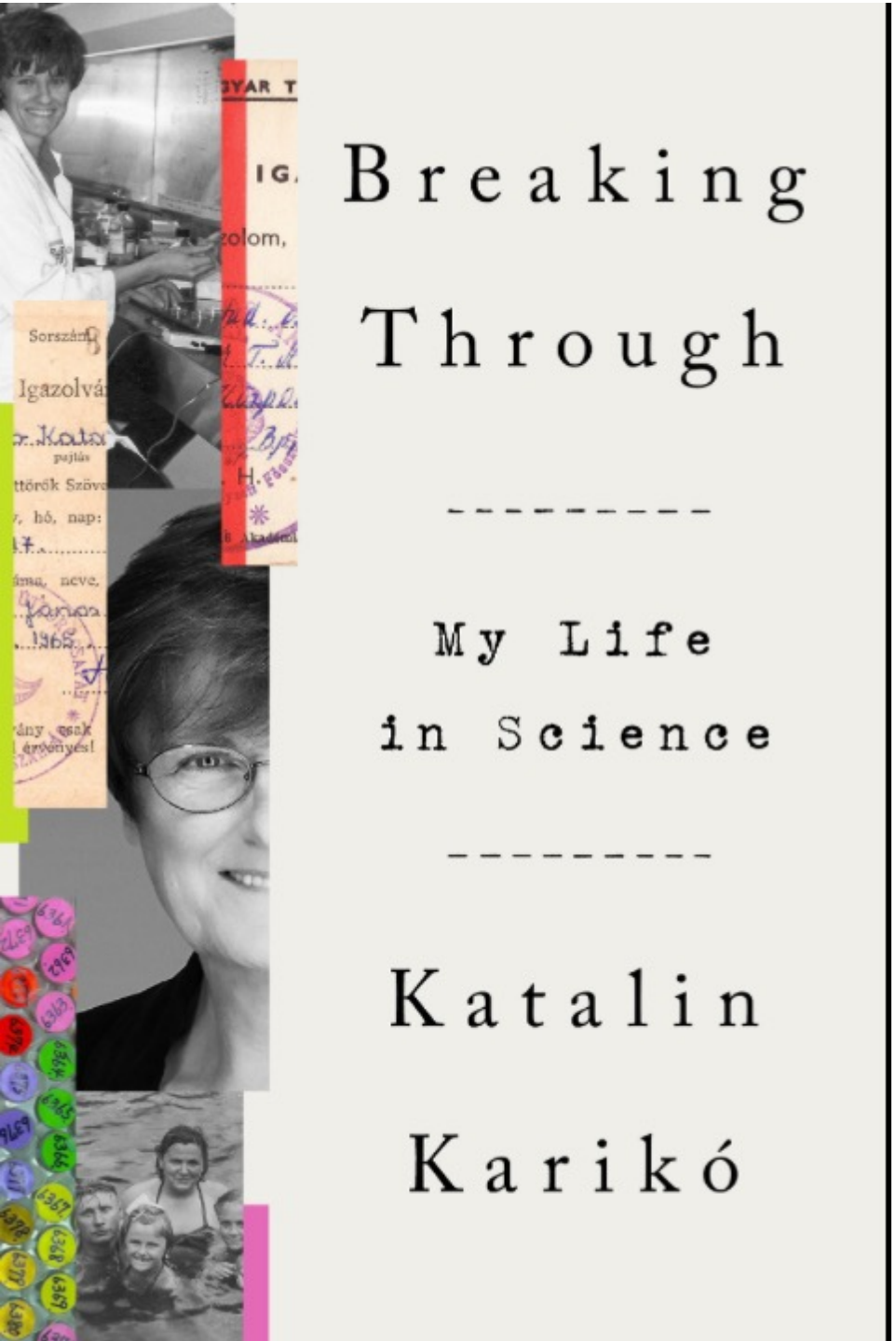
– critical processing steps and their acceptance criteria, including the manufacture of any intermediates;

Texts published in Pharmeuropa!

mRNA vaccines: Current status

- Drafted the 3 proposed general chapters (mRNA-LNP DP, mRNA DS, DNA template) in dedicated sub-teams

- Continue the discussions on other topics
  (incl. excipients, raw materials, standardisation of analytical procedures and reference standards)



“Our home is simple, small. It is constructed, literally, from the earth that surrounds it: clay and straw, pressed into adobe walls, whitewashed, then covered with a thick roof of reeds.

We live in a single room. The house is larger than this one room, but for most of the year, the other rooms are too cold for anything but storage. We live where the heat is.”

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