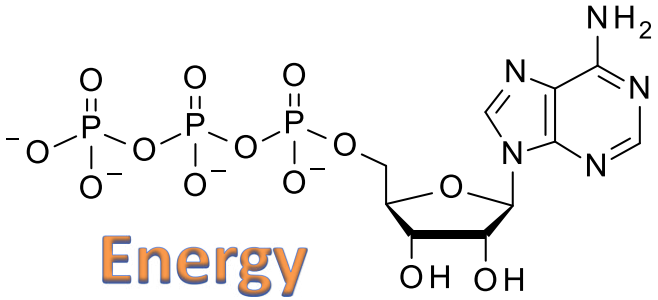
UNIWERSYTET WARSZAWSKI | CeNT CENTRUM NOWYCH TECHNOLOGII

Jacek Jemielity

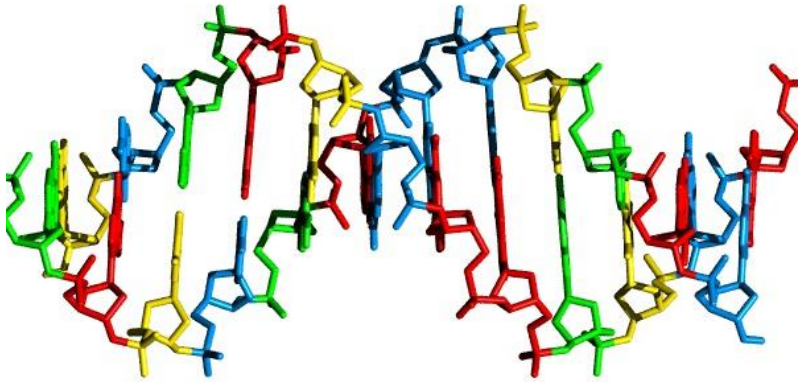
Lab. Chemical Biology

Chemically modified mRNA for therapeutic applications

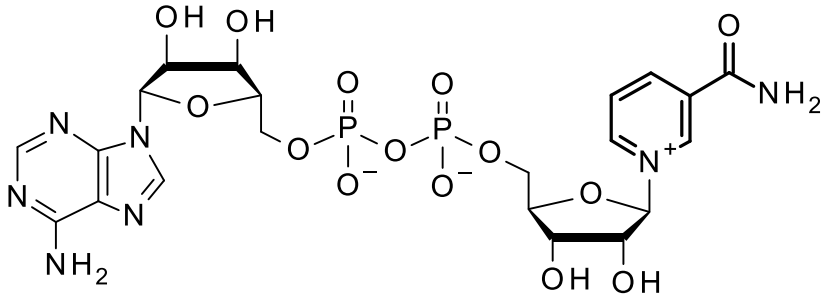
We are focused on modifications of nucleotides



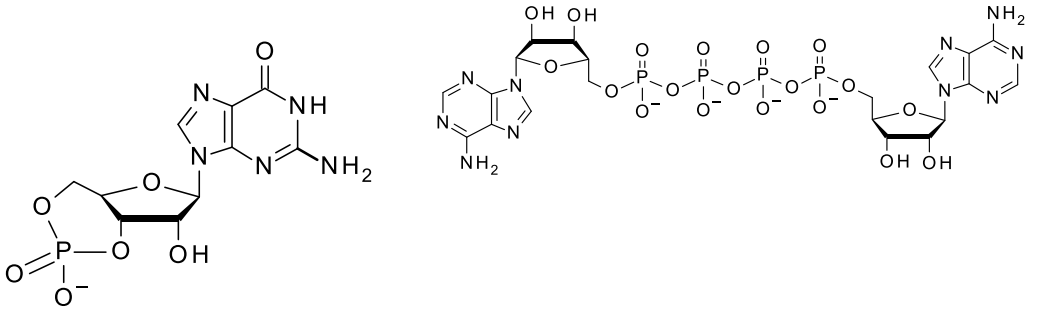
Energy



Genetic material
and gene expression



Co-factors

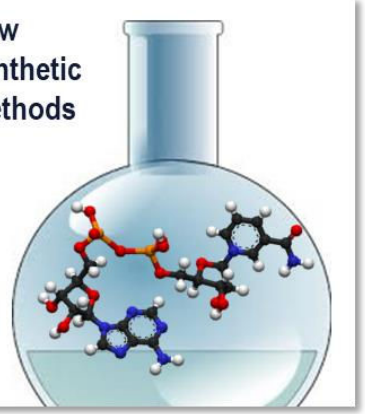


Inter- and intracellular signaling

Plenty of functions → Plenty of applications

Laboratory of Chemical Biology - overview

New synthetic methods



Zang et al. Nature Chemistry (2023)
Chrominski et al. Org. Lett. (2022)
Ziemkiewicz et al. J. Org. Chem (2022)
Chrominski et al. J. Org. Chem (2020)
Warminski et al. Org. Lett. (2017)
Wanat et al. Org. Lett. (2015)
Strenkowska et al. *Org. Lett.* (2012)

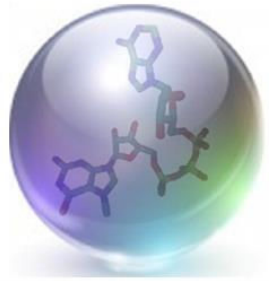
Development of reagents for nucleic acids modifications

Rydzik et al. *Nucl. Acids Res.* (2017)
Mlynarska-Cieslak et al. Org. Lett. (2018)
Warminski et al. Nucl. Acids Res. (2024)
Wojtczak et al. JACS. (2018)
Warminski et al. Top. Curr. Chem. (2017)
6 PCT Patents

Improvment of mRNA properties for gene therapy

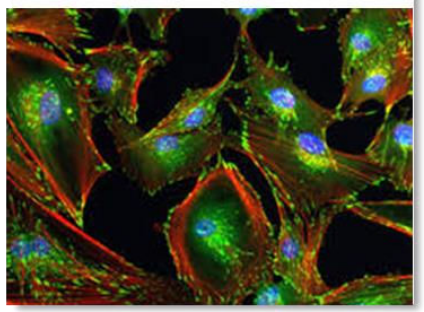
Warminski et al. JACS (2024)
Sikorski et al. *Nucl. Acids Res.* (2020)
Strzelecka et al. RNA (2020)
Perzanowska et al. Chem Eur. J.(2022)
Walczak et al. Chemical Science (2017)
Strenkowska et al. *Nucl. Acids Res.* (2016)
Kowalska et al. *Nucl. Acids Res.* (2014)

Bioconjugation, nucleotide delivery



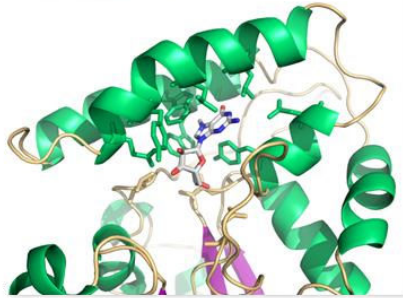
Warminski et al. Adv. Sci. (2024)
Kleczewska et al. Chem. Sci. (2021)
Perzanowska et al. Sci. Rep. (2021)
Zochowska et al. *Nanomedicine: NBM, (2015)*
Kijewska et al. *Biomacromol. (2013)*

Labelled nucleotides, molecular probes



Mamot et al. Nucl. Acids Res. (2022)
Tibble et al. Nature Chem. Biol (2021)
Wanat et al. Chem. Comm. (2018)
Baranowski et al. Nucl. Acids Res. (2020)
Mamot et al. Angewandte Chem. (2017)

Proteins involved in nucleotides and RNA metabolism

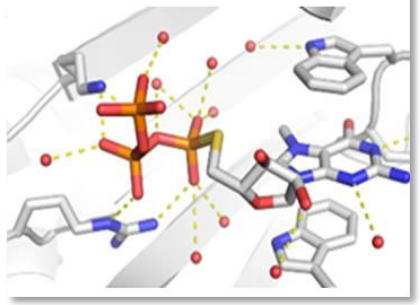


Warminski et al. ACS Chem Biol (2021)
Mugridge et al. Nature Struct. Mol. Biol. (2016)
Mugridge et al. Nature Comm. (2018)
Peters et al. Structure (2022)

High-throughput screening methods

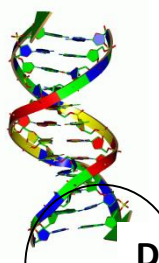
Kasprzyk et al. Antiviral Res. (2021)
Bednarczyk et al. ACS Chem. Biol. (2022)
Kasprzyk et al. Chemistry – Eur. J. (2020)
Kasprzyk at al. Chemistry – Eur. J. (2019)
Strzelecka et al. Sci. Reports (2017)

Inhibitors – design and optimization



Wojtczak et al. JACS (2018)
Mlynarska-Cieslak et al. ACS Chem. Biol.(2022)
Kubacka et al. Pharmaceuticals (2022)
Ziemniak et al RNA (2016)
Kozarski et al. BMC (2018)

mRNA in gene therapy

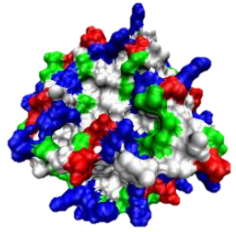


DNA

transcription, export

mRNA

translation



Protein

replication

Genetic information

„recipe" for protein

Body functions: building blocks, catalysis, transport, signaling and much more

GENE (cDNA)

GENE (mRNA)

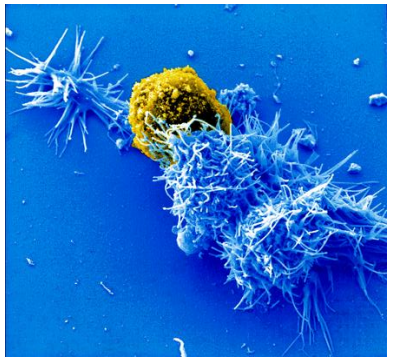
GENE (cDNA)

nucleus

cytoplasm

mRNA Therapy – more than vaccines

Antigens delivery



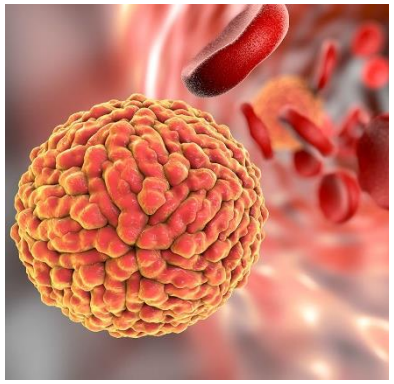
Cancer: immunotherapies based on dendritic cells

Protein suplementation

Genetic diseases
Metabolic diseases

Nuclease delivery

Gene editing by CRISPR/Cas9



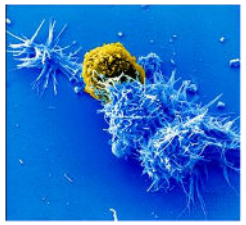
Vaccines against infecious diseases

Regenerative medicine and cellular therapies

Growth factor delivery
(e.g. in cardiovascular diseases)
Generation and modification of stem cells or T cells

mRNA therapy

Antigens delivery



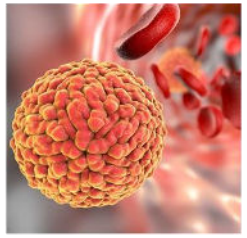
Cancer: immunotherapies based on dendritic cells

Protein suplementation

Genetic diseases
Metabolic diseases

Nuclease delivery

Gene editing by CRISPR/Cas9



Vaccines against infecious diseases

Regenerative medicine and cellular therapies

Growth factor delivery
(e.g. in cardiovascular diseases)
Generation and modification of stem cells or T cells

- *mRNA-based therapies can be developed and tested in just a few weeks, allowing the process to be accelerated from idea to first human trial in less than a year.*
- *All messenger RNA therapies can be manufactured using the same reagents in the same cell-free manufacturing process, enabling rapid and cost-effective GMP production.*
- *This is possible due to the chemical similarity of all messenger RNAs, which differ only in RNA sequence.*

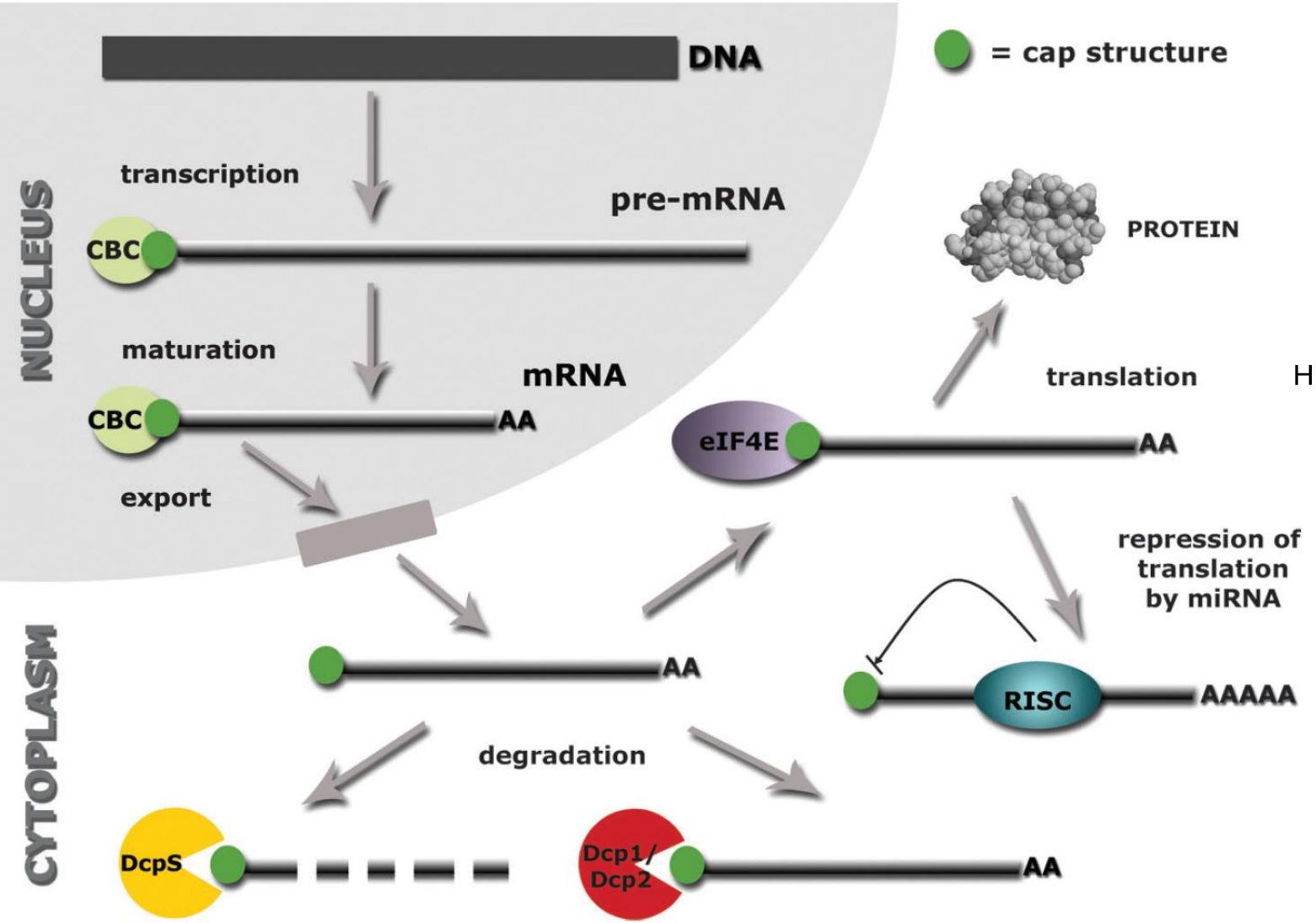
What is important in the context of therapeutic mRNA?

High levels of exogenous mRNA expression

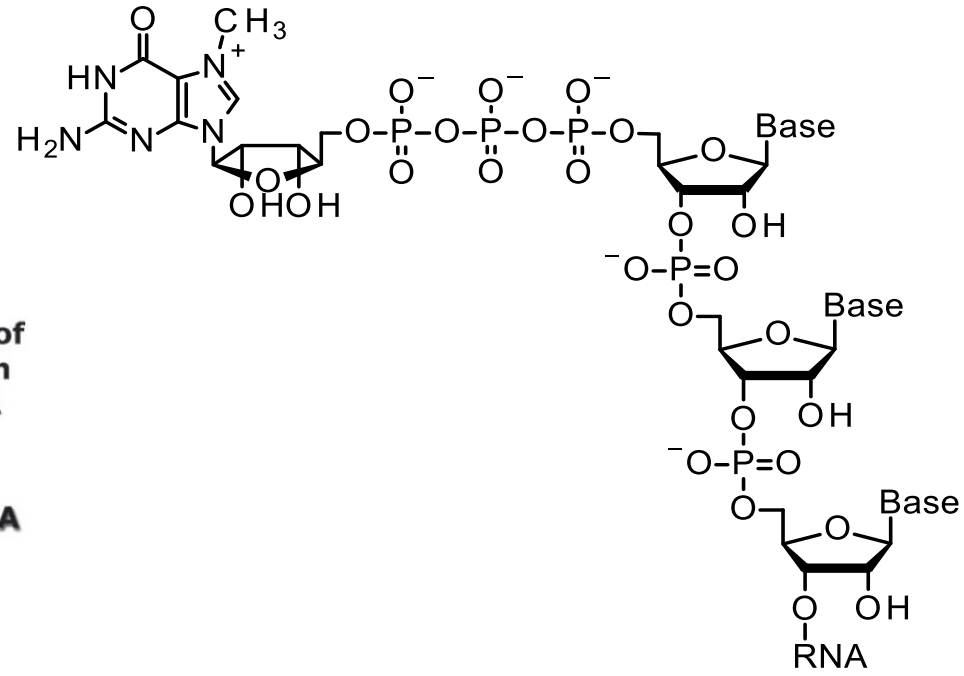
Achieve the highest possible biological response with the lowest possible dose

- Lack of reactogenicity (non-specific immune system response to mRNA)
- Efficient delivery to target cells
- Efficient release of mRNA from the formulation
- Affinity for the translational machinery
- Stability of the mRNA
- Purity of the synthetic mRNA
- Other cellular mechanisms?

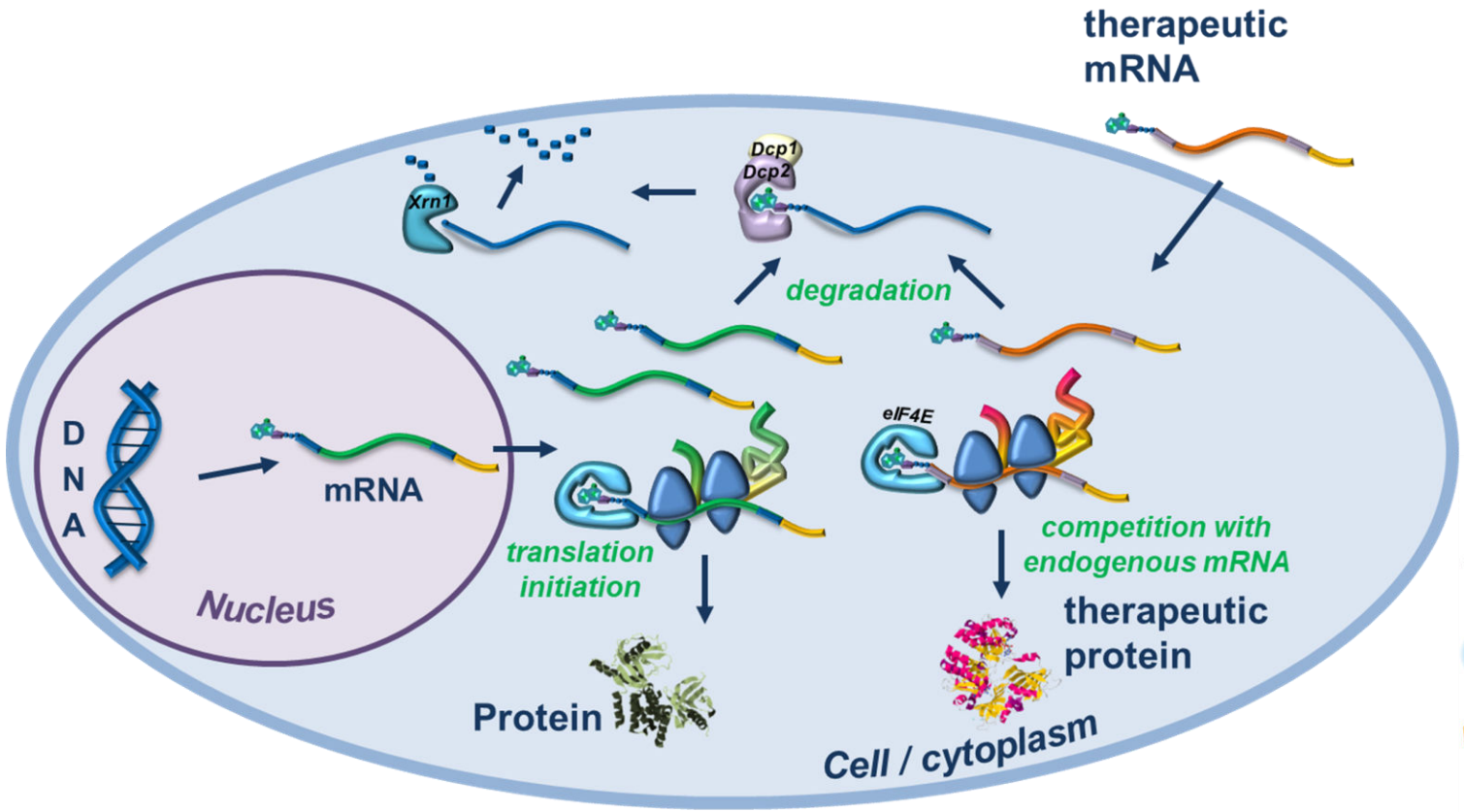
The 5' end of mRNA (cap) - why is it important

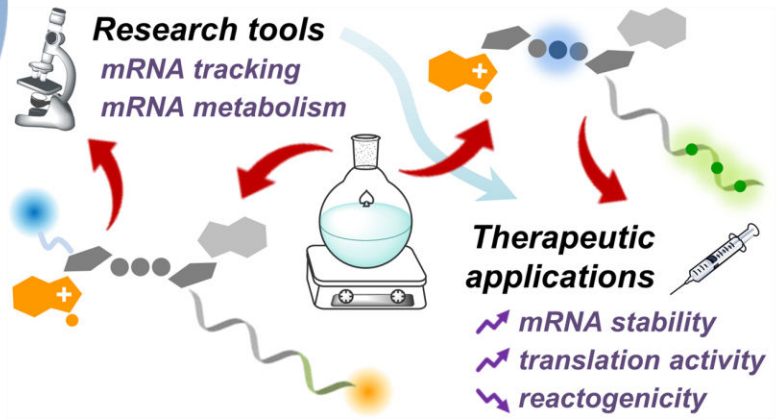


mRNA
500-6000 nucleotides



How to change mRNA properties?

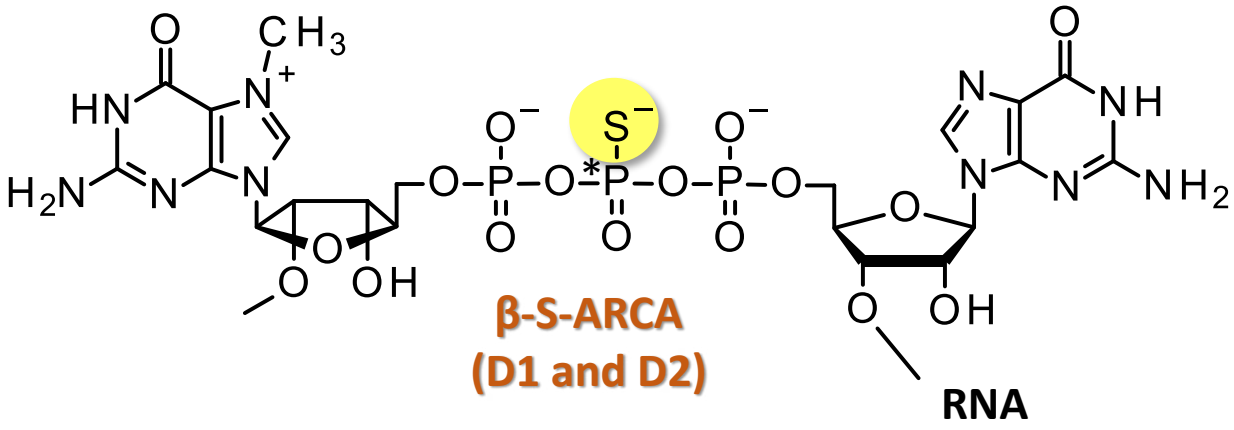




Chemical Modifications of mRNA Ends for Therapeutic Applications
M. Warminski, A. Mamot, A. Depaix, J. Kowalska, J. Jemielity
Accounts of Chemical Research **56**, 2814-2826 (2023)

How it started: beta-S- ARCA for mRNA modification

- our first invention used in 12 clinical trials



Beneficial mRNA properties:

Increased affinity for translational machinery, eIF4E

Resistance to mRNA degradation (Dcp2)

Increased mRNA half-life in vivo (3x)

Increased efficiency of protein biosynthesis (5x)

= More protein from the same amount of mRNA

Kowalska J., et al. *RNA* **14**, 1119-1131 (2008)

Grudzien-Nogalska E, et al. RNA 13, 1745-1755 (2007)

Jemielity J et al. U.S. Patent No 8,153,773, 10th April 2012

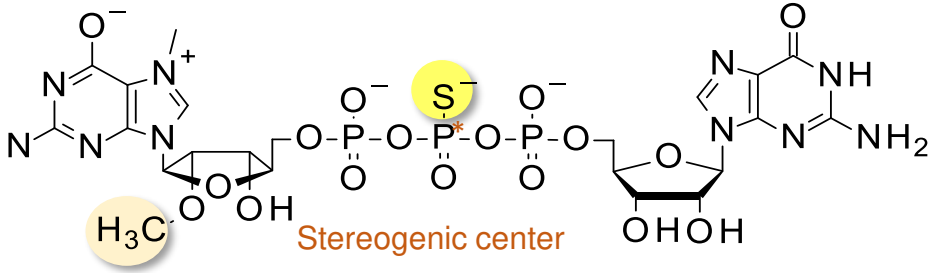
Licensed for BionNTech

Sublicensed for Sanofi, Genentech, Pfizer

2012-2025: 11 clinical trials on cancer vaccines (BioNTech, Mainz, Germany)

Genentech/BioNTech: Personalized cancer vaccines, 10 different solid tumors

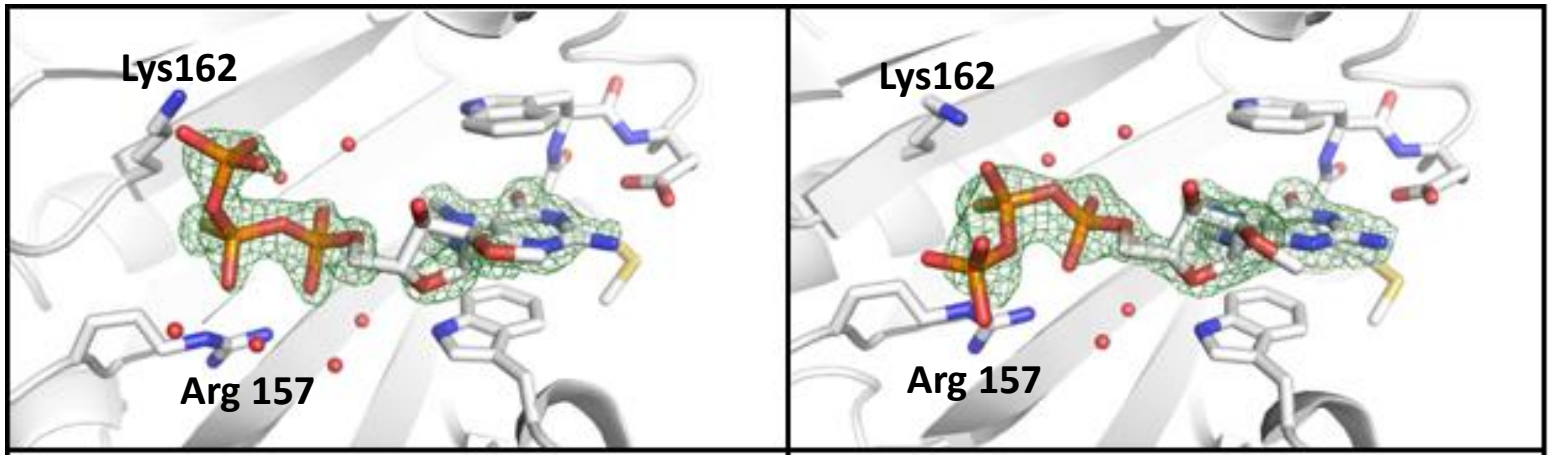
Thio-effect in translation

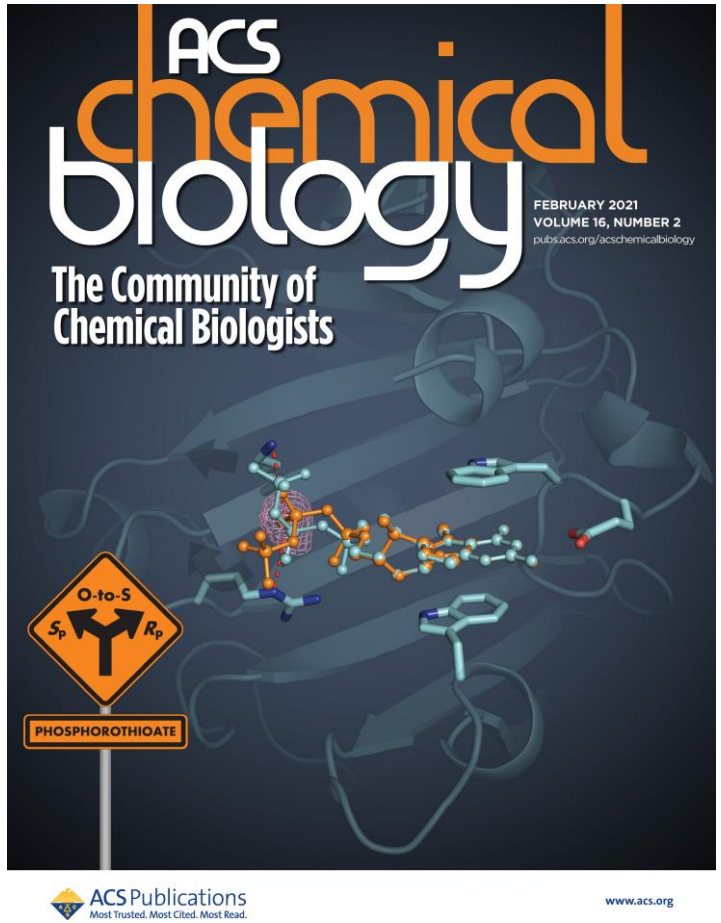


(R)-β-S-ARCA D1

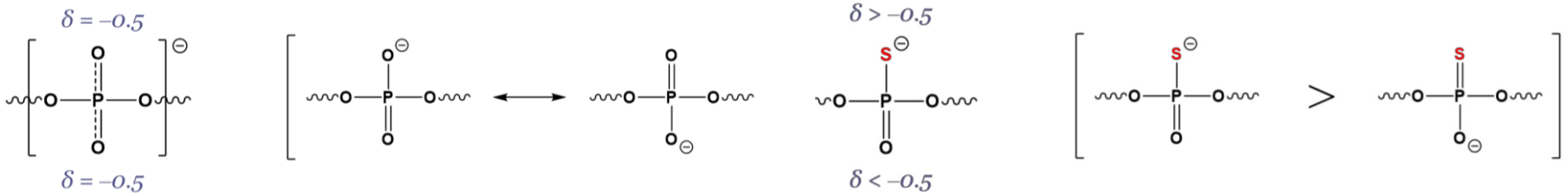
(S)-β-S-ARCA D2

	K_{AS} [μM^{-1}]
D1	43.1 ± 1.4
D2	19.3 ± 2.2
m^7GpppG	9.4 ± 0.4



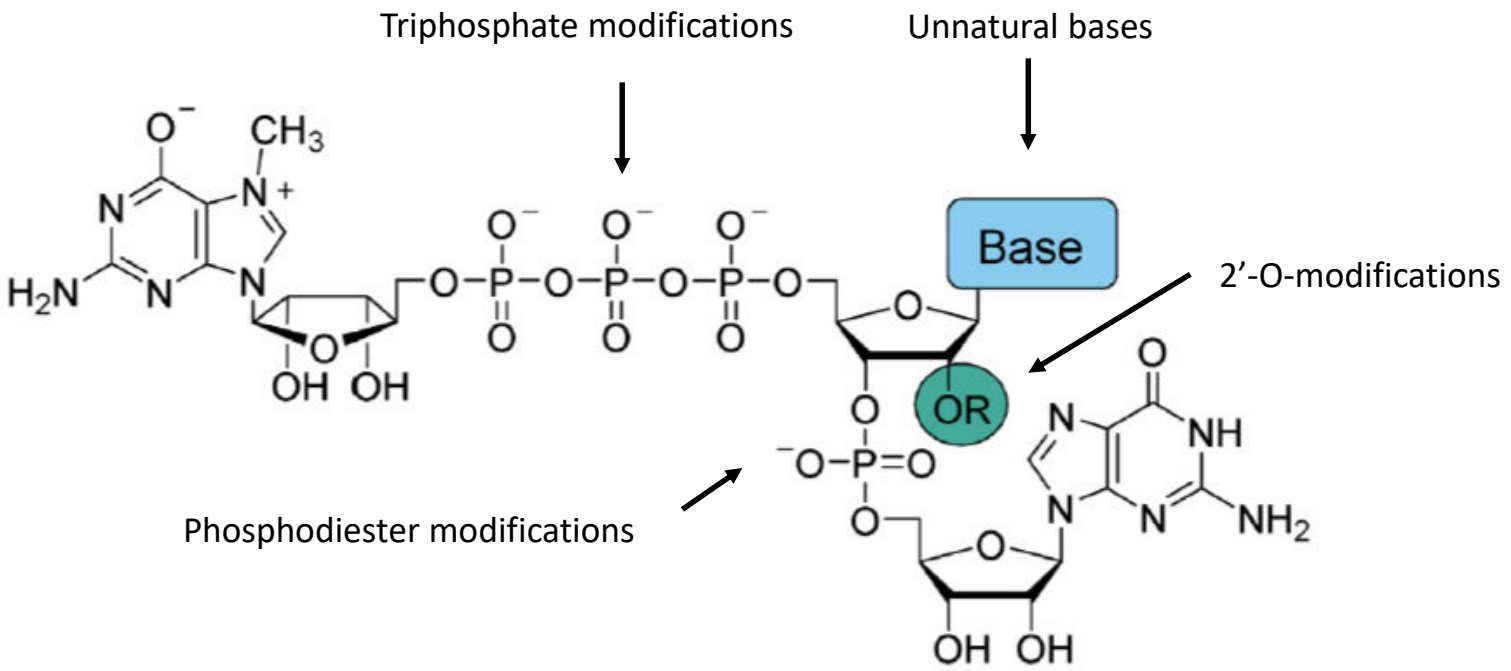


What is the stabilization of the eIF4E - β-S-ARCA complex due to?



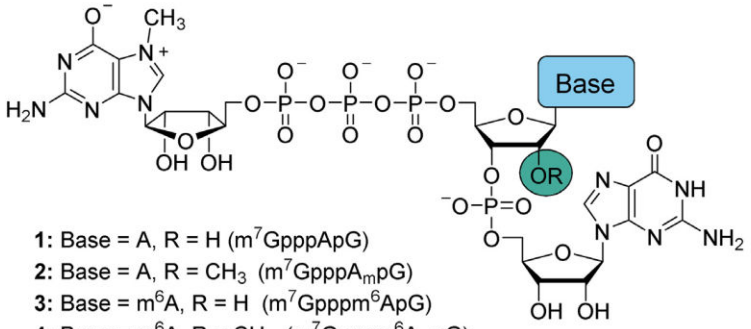
Warmiński et al. ACS Chem. Biol. 2021

Trinucleotide analogues of cap - new opportunities in the design of therapeutic mRNAs



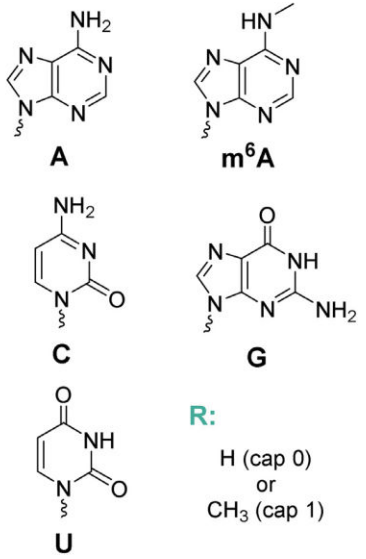
Sikorski et al. *Nucleic Acids Research*, 2020, 48, 1609 – 1626, *NAR Breakthrough Article*

Does the first transcribed nucleotide matter?



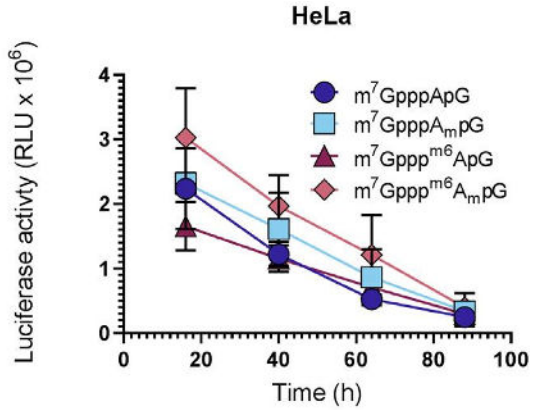
1: Base = A, R = H ($m^7GpppApG$)
2: Base = A, R = CH_3 (m^7GpppA_mpG)
3: Base = m^6A, R = H (m^7Gpppm^6ApG)
4: Base = m^6A, R = CH_3 ($m^7Gpppm^6A_mpG$)
5: Base = C, R = H ($m^7GpppCpG$)
6: Base = C, R = CH_3 (m^7GpppC_mpG)
7: Base = G, R = H ($m^7GpppGpG$)
8: Base = G, R = CH_3 (m^7GpppG_mpG)
9: Base = U, R = H ($m^7GpppUpG$)
10: Base = U, R = CH_3 (m^7GpppU_mpG)

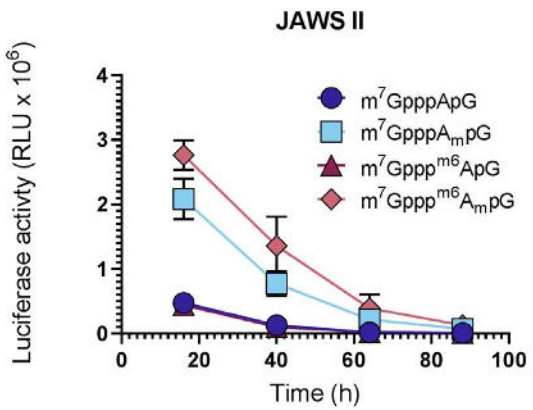
Bases:

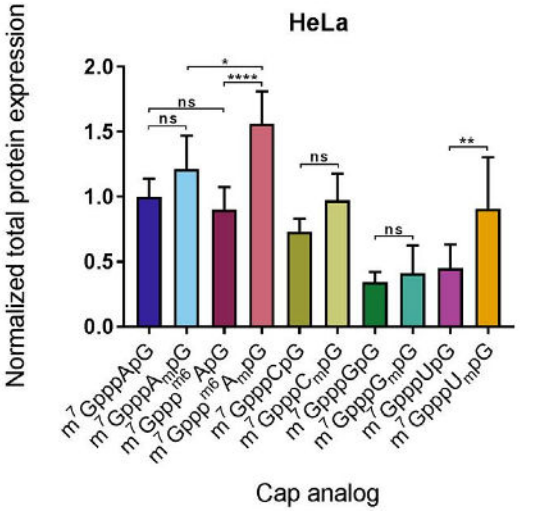


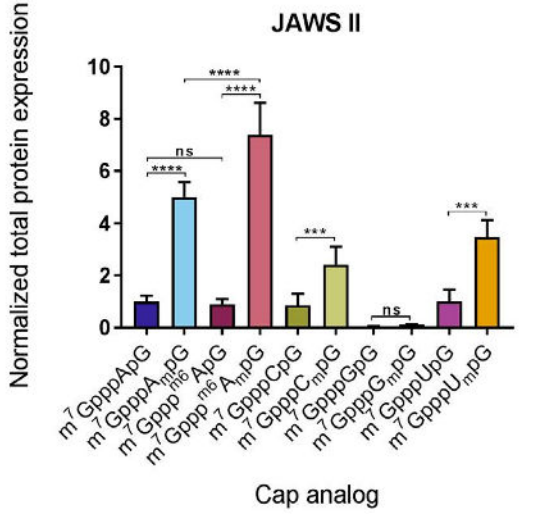
R:

H (cap 0)
or
CH_3 (cap 1)









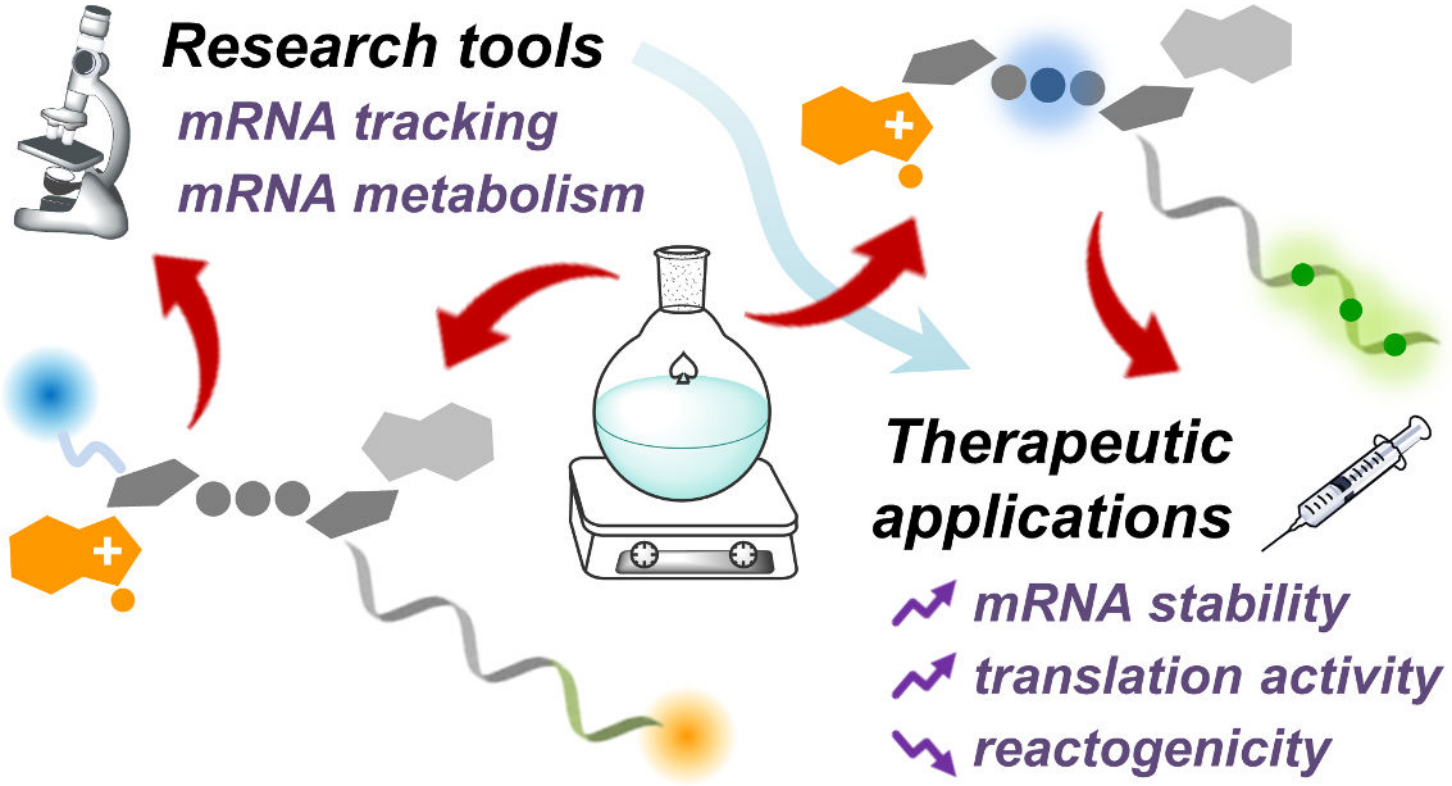
Answer: Yes, but why?

- **Affinity to translation machinery** X
- **Susceptibility to decapping** X
- **Reactogenicity** X

The identity and methylation status of the first transcribed nucleotide in eukaryotic mRNA 5′ cap modulates protein expression in living cells
Sikorski et al. *Nucleic Acids Research*, 2020, 48, 1609 – 1626,

NAR Breakthrough Article

We study phenomena to understand them and thus better design therapeutics



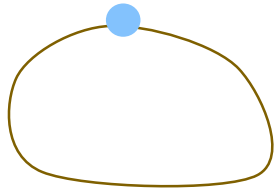
Chemical modifications of mRNA ends for therapeutic applications.
Marcin Warminski, Adam Mamot, Anais Depaix, Joanna Kowalska, Jacek Jemielity.
Accounts of Chemical Research **56** , pp.2814-2826 (2023)

Ongoing projects on mRNA field

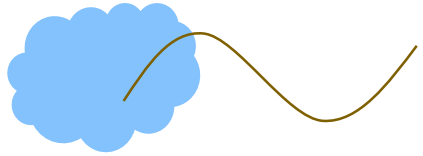
New 5’ cap modifications

PolyA modifications

mRNA fluorescent labelling



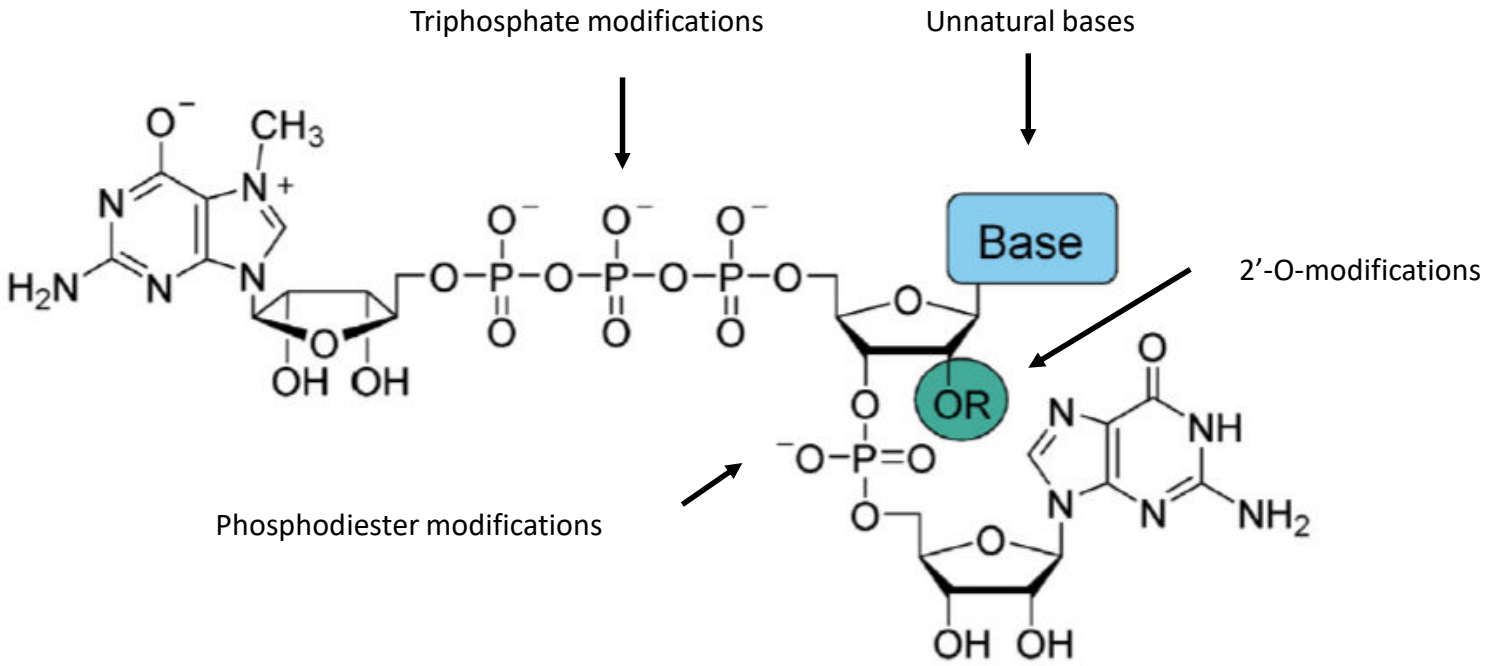
Chemical circularization



RNA - protein interactions

RNA purification methods

Library of trinucleotide cap analogues



90+ Cap library

for for various applications,
4 PCT patent applications

exploRNA THERAPEUTICS

Spin off University of Warsaw

Game-changing mRNA Innovations

Company snap shot

- mRNA innovations company **launched in 2019** with strong IP in hand (**5 patent families**).
- Laboratories and offices in Warsaw & Białystok.
- Library **of 100+ cap analogs**, high quality **mRNA synthesis know-how**, and **own therapeutic programs at pre-clinical stage.**
- Full expertise for mRNA innovation R&D in house.
- Offering 2 products that generated interest resulting in **40+ evaluation & collaboration agreements with partners** in the US, EU and Asia.
- First eastern European company receiving grant from Bill and Melinda Gates Foundation (800+ kUSD).

Team 30

- Biology; 10
- MNG & admin; 7
- mRNA team; 7
- Cap synthesis team; 6

4 Professors **8 PhDs** **4 PhD candidates**

Explorna Team has comprehensive expertise to develop mRNA modifications and mRNA-based therapeutics in house

Prof. Jacek Jemielity
Chief Executive Officer (CEO)

World-recognized and award-winning Nucleic Acids Chemists specilizing in the design of mRNA modifications for 20+ years

Co-inventors of 10+ patents and patent applications and co-authors of 100+ scientific publications

Prof. Joanna Kowalska
Chief Technology Officer (CTO)

Prof. Jakub Gołąb
Chief Scientific Officer (CSO)

World-recognized expert immunologist with strong background in experimental medicine Previously co-founder of OncoArendi Therapeutics (now Molecure), with current market cap of 60+ MUSD

Prof. Dominika Nowis
Director of Biology

Award-winning immunologist who discovered the cardiotoxic effects of proteasome inhibitors and statin-dependent decrease in glucose uptake by cancer cells

Marek Baranowski, PhD
Director of Key Reagent Production

Expert in reagent production and quality control with strong background in chemistry and biophysics and 10+ years of hands-on laboratory experimence

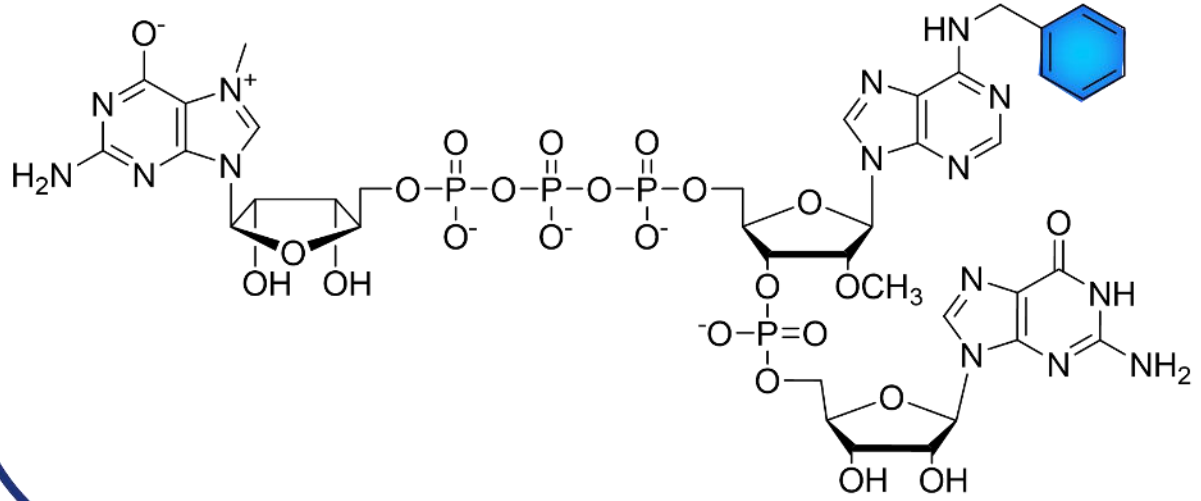
Design, synthesis, and purification of cap and NTP analogs structure verification, stability studies

- DNA template production
- Optimization of in vitro transcription
- mRNA synthesis and purification
- mRNA formulation
- mRNA and LNP Quality control

- Cell culture studies
- Animal studies (mice)
- Development of disease models

AvantCap®: Nature-inspired design for improved biological properties

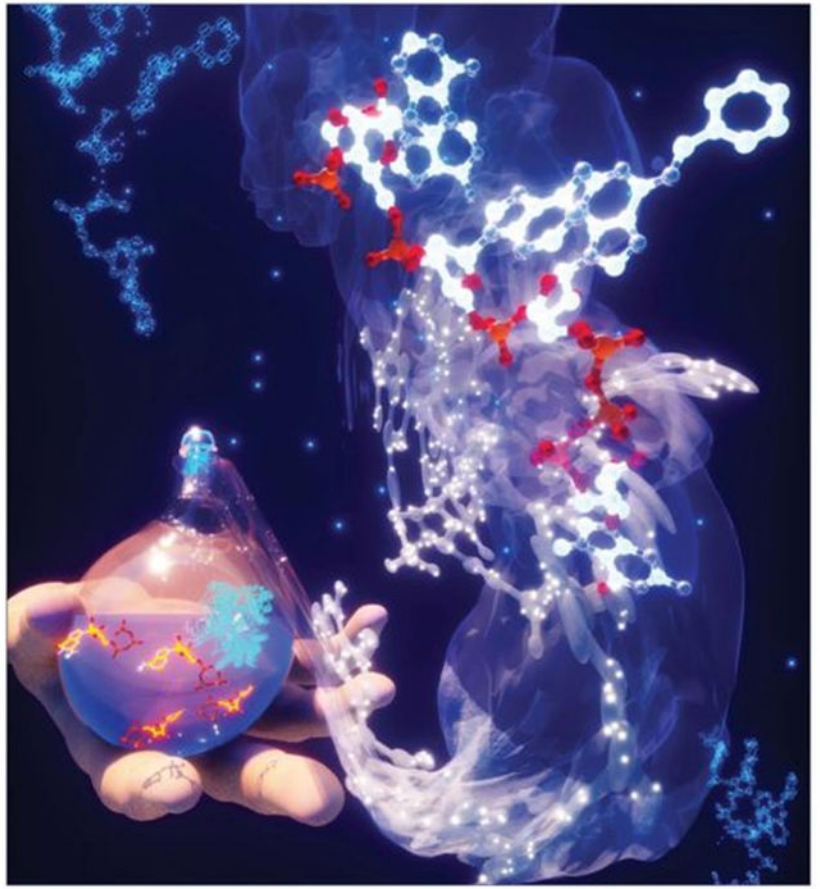
AvantCap®



exploRNA THERAPEUTICS

March 27, 2024
Volume 146
Number 12
pubs.acs.org/JACS

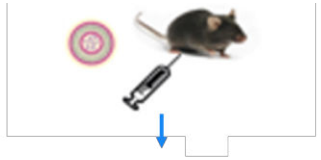
JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

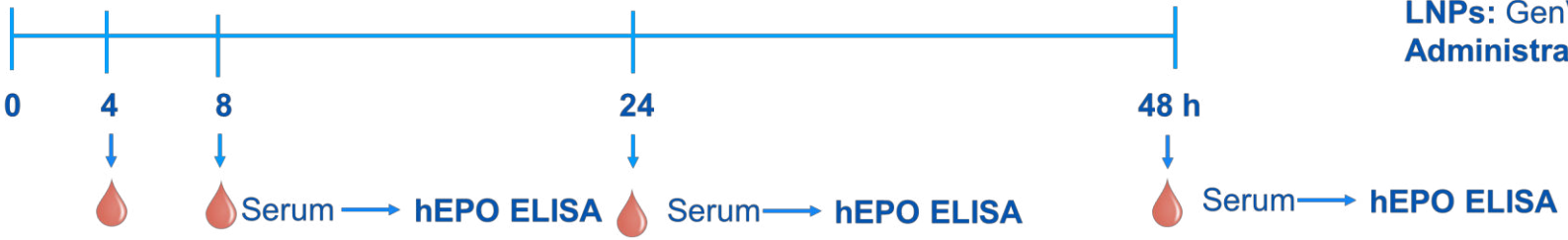


ACS Publications
Most Trusted. Most Cited. Most Read.
www.acs.org

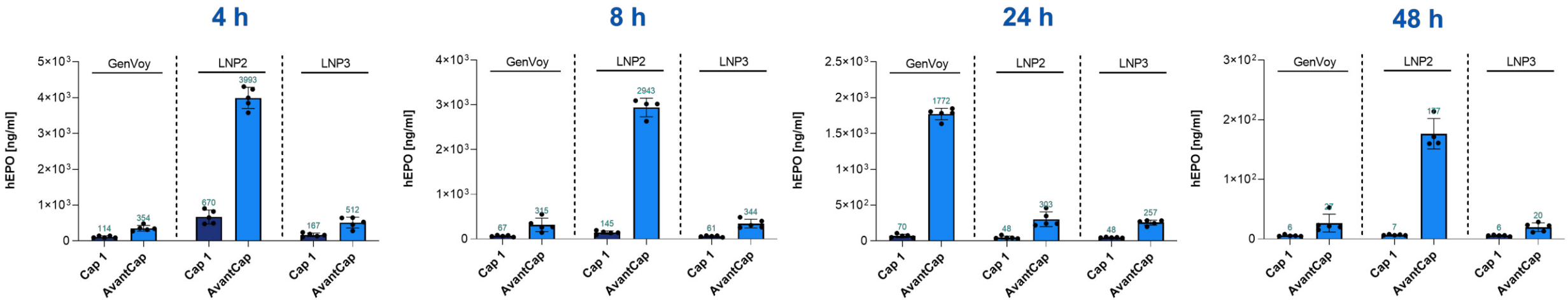
Warminski et al. JACS **146**, 8149–8163 (2024)

AvantCap® augments total protein expression in vivo: hEPO in C57BL/6 mice





mRNA: human EPO, non-modified U
Dose: 1 µg
Caps: Cap 1, AvantCap
LNPs: GenVoy, LNP 2 (clinical grade), LNP3 (clinical grade)
Administration: intravenous



AvantCap® increases EPO production up to 25-fold

Warminski et al. JACS **146**, 8149–8163 (2024)

Traction

MARKETS

USD 5.17B TAM value in 2028

10.4% CAGR 2024-2033

RESULTS

200-600% protein output improvement over current industry leader

PROJECTS

40+ projects started with partners

3 Therapeutics PoC

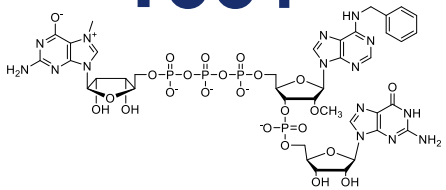
PRODUCTS

ExploCap® AvantCap®

2 products commercialized

CAPs LIBRARY

100+



IP / FTO

2 families with granted patents
3 families with patents pending
2 patents being finalized
FTO available