RNA Therapeutics

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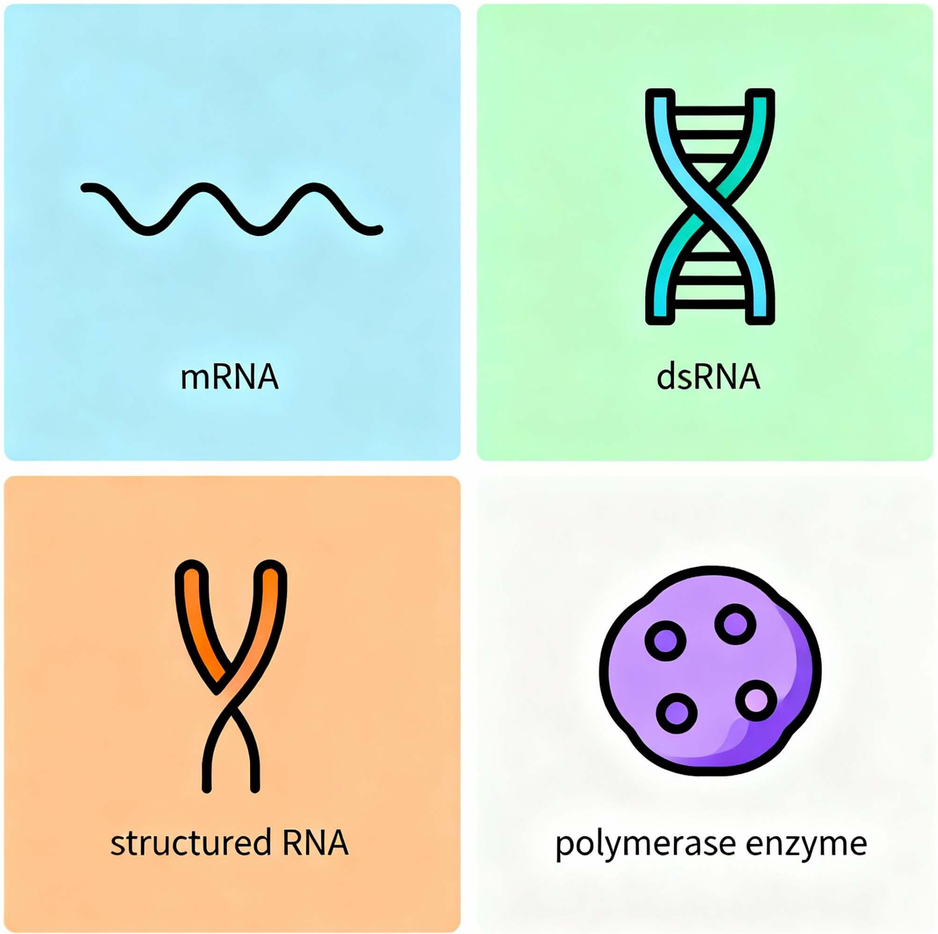
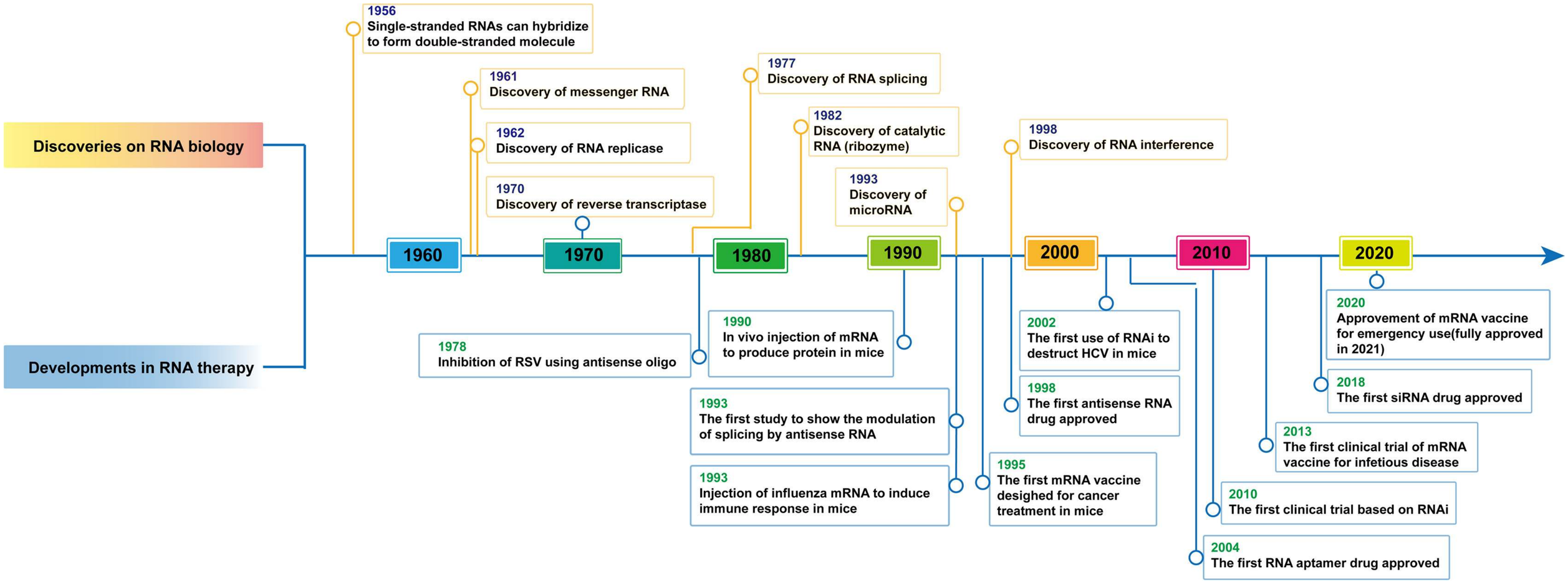
RNA Forecast

Why RNA?

- central to gene expression and regulation
- accessible at multiple interventions points
- can act as **drug**, **drug target**, or **delivery vehicle**
- combines information, structure, and function
- potential to address **undruggable** genes or proteins



RNA therapeutics today

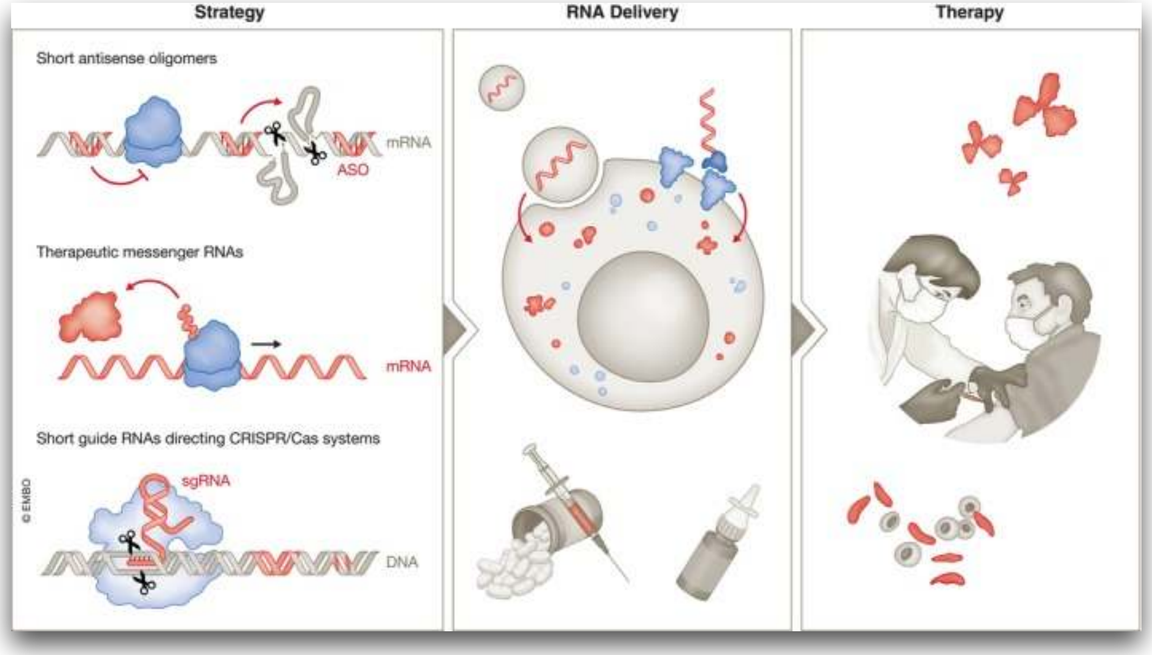


Cell Reports Medicine, 5(5). 2024

RNA as drug vs. RNA as drug target

RNA as drug

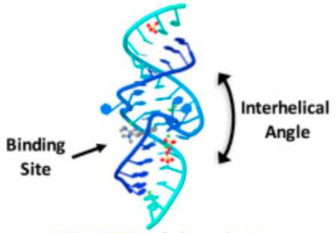
- deliver functional RNAs into cells
- produce a therapeutic effect
- mRNA vaccines, siRNA, ASO, CRISPR



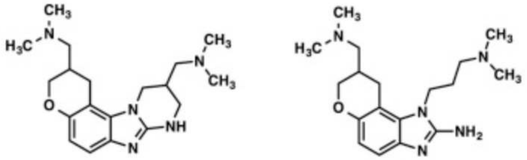
EMBO J 42(21):e114760. 2023

RNA as drug target

- use drugs to bind and modify endogenous RNA



HCV IRES subdomain IIa



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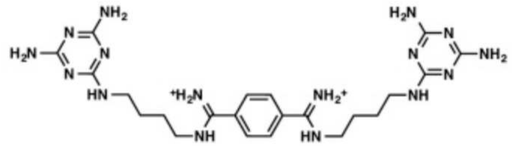
DM1-associated r(CUG)$_6$



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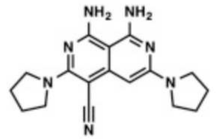

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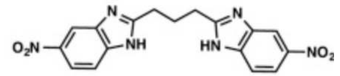

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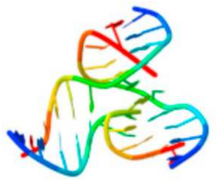
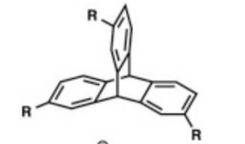
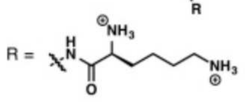


Three-way junction





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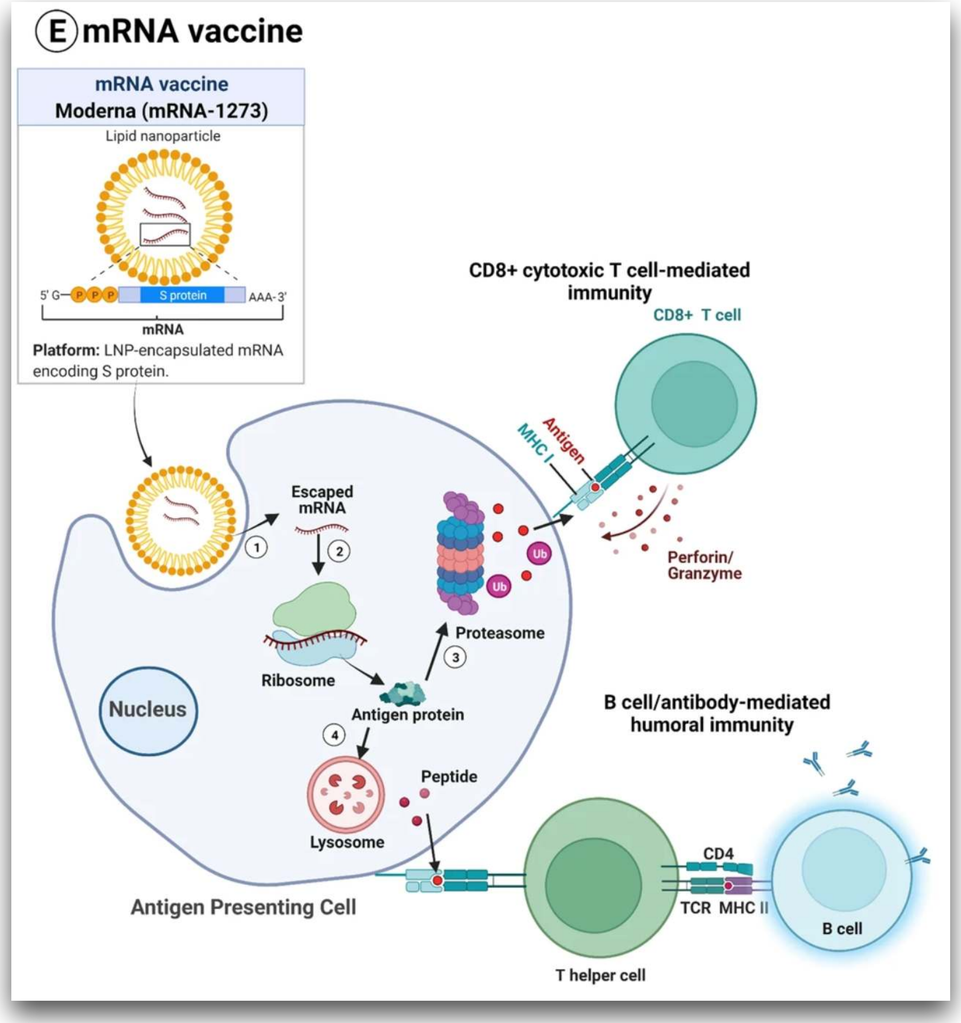
Cell Chem Biol. 23(9):1077–1090. 2016

Classes of RNA therapeutics

- **messenger RNA (mRNA)**
- small interfering RNA (siRNA/miRNA)
- antisense oligonucleotides (ASOs)
- CRISPR/Cas genome editing
- RNA aptamers
- RNA-targeting small molecules

messenger RNA (mRNA)

- deliver genetic instructions for protein production
- transient, non-integratable, programmable
- includes vaccines and experimental protein-replacement concepts



Cell Death Dis. 13(7), 644. 2022

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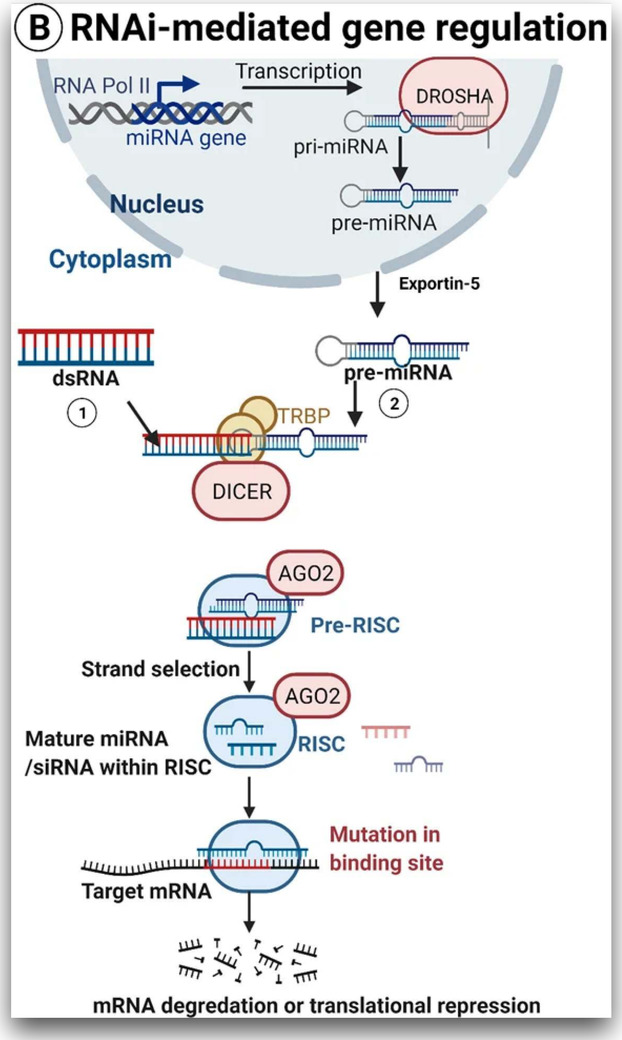
Classes of RNA therapeutics

small interfering RNA (siRNA)

- short ds RNA guide RISC complex -> mRNA degradation
- highly sequence-specific post-transcriptional silencing
- strong clinical development in liver-targeted therapies

microRNA Therapeutics

- modulate endogenous regulatory networks
- therapy either restores a missing regulatory miRNA
- or inhibits a pathogenic miRNA
- main challenge: broad regulatory footprint -> off targets



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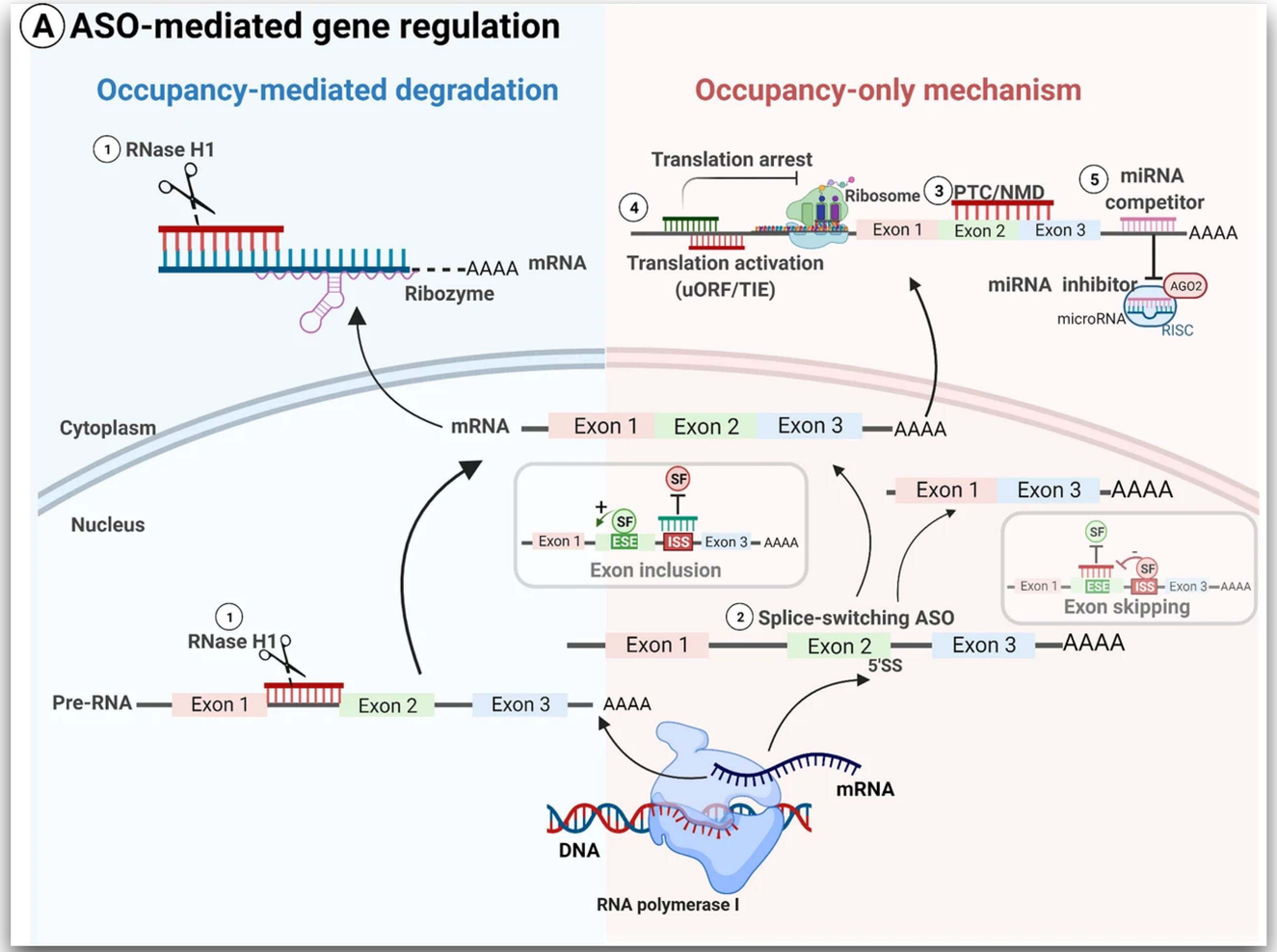
antisense oligonucleotides (ASOs)

- single-stranded NAs binding mRNA targets
- modulate splicing or trigger RNAse H
- first approved RNA therapeutic class



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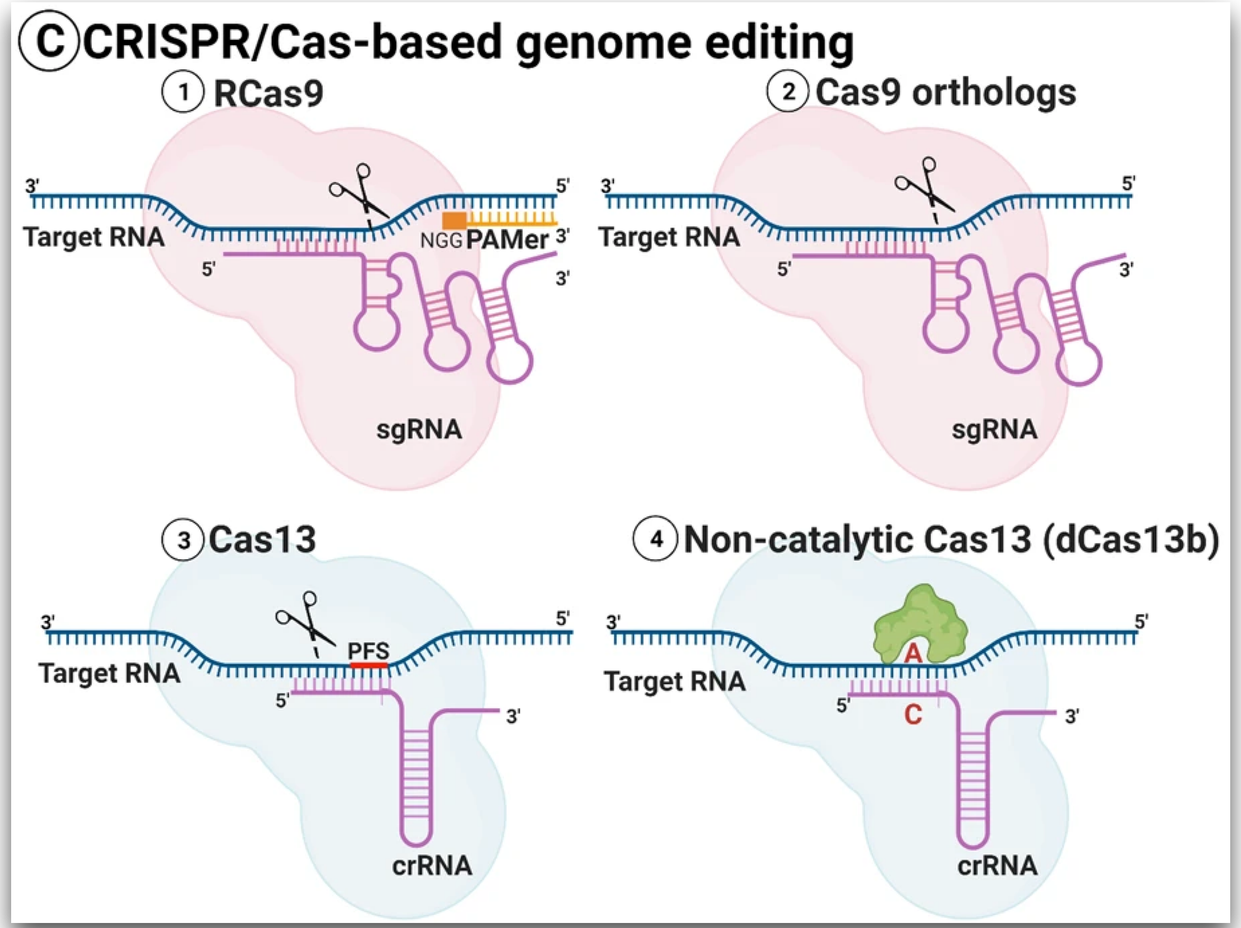
CRISPR/Cas based genome editing

- programmable RNA-guided genome or transcript editing
- uses short guide RNAs to target specific sequences
- correct mutations or modulate gene expression
- delivery typically via mRNA, RNPs, or viral vectors



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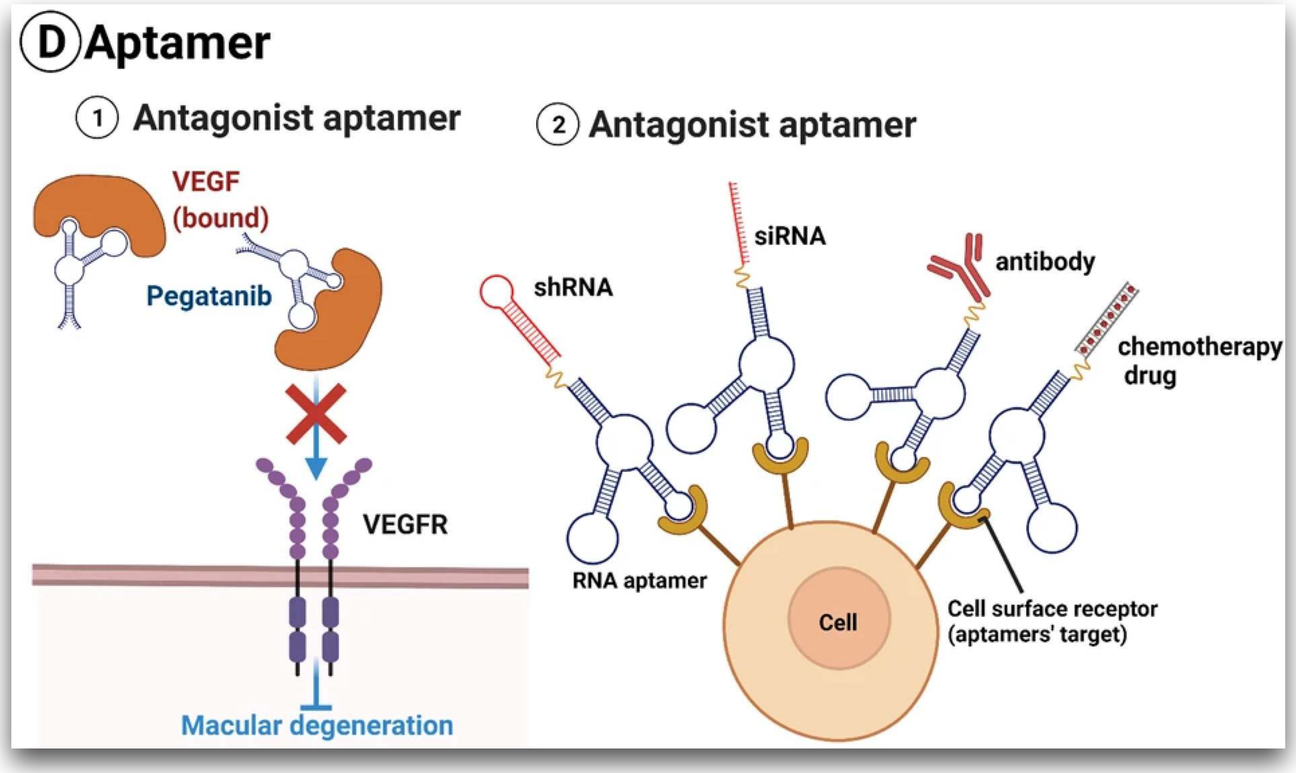
RNA aptamers

- structured RNAs selected for high-affinity ligand binding
- conceptually like chemical antibodies
- fully synthetic and sequence-programmable



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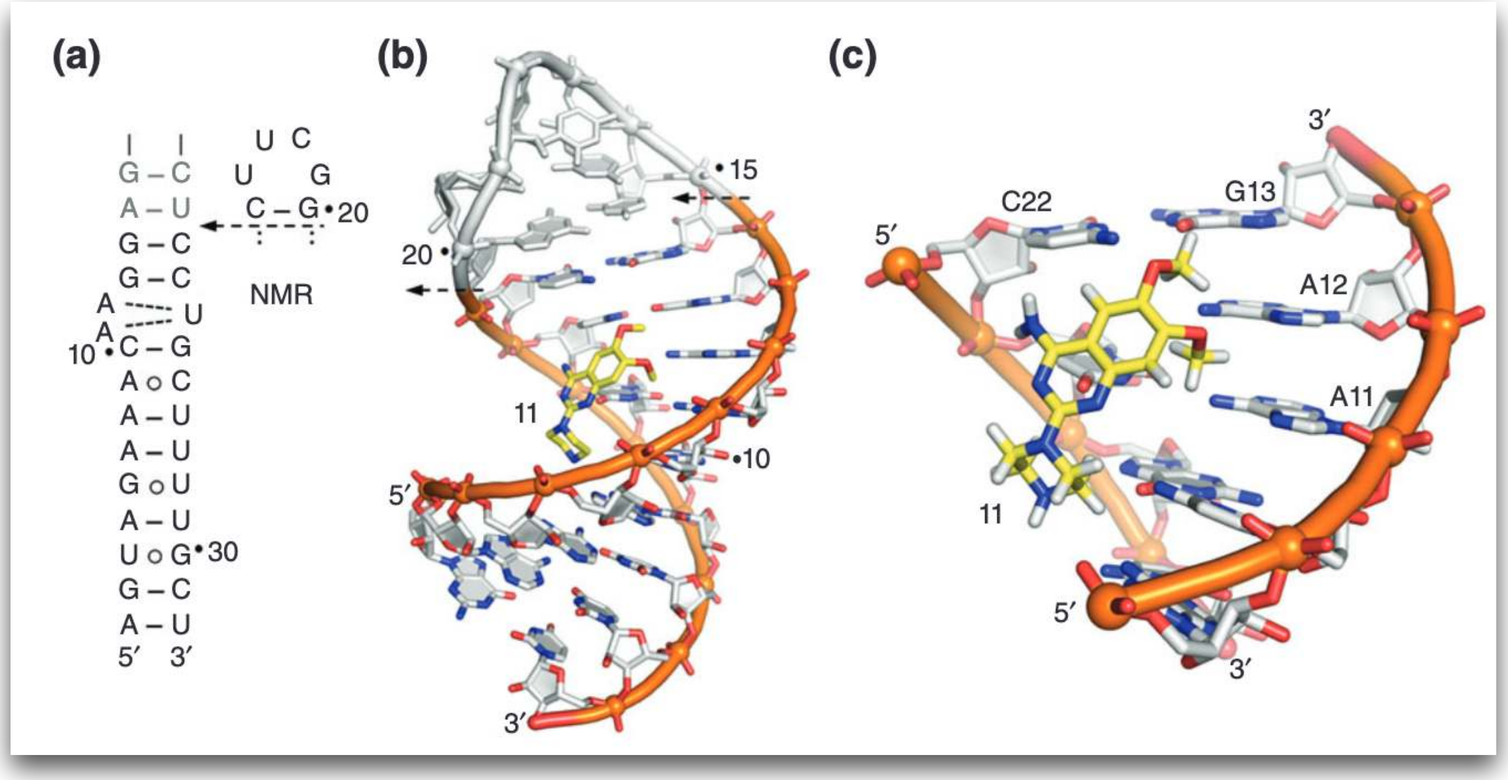
RNA-targeting small molecules

- classical drugs interacting with structured RNA
- antibiotics, splice modulators, viral polymerase inhibitors
- includes nucleoside analogues used in antiviral therapy



Wiley Int Rev RNA 7.6:726-743. 2016

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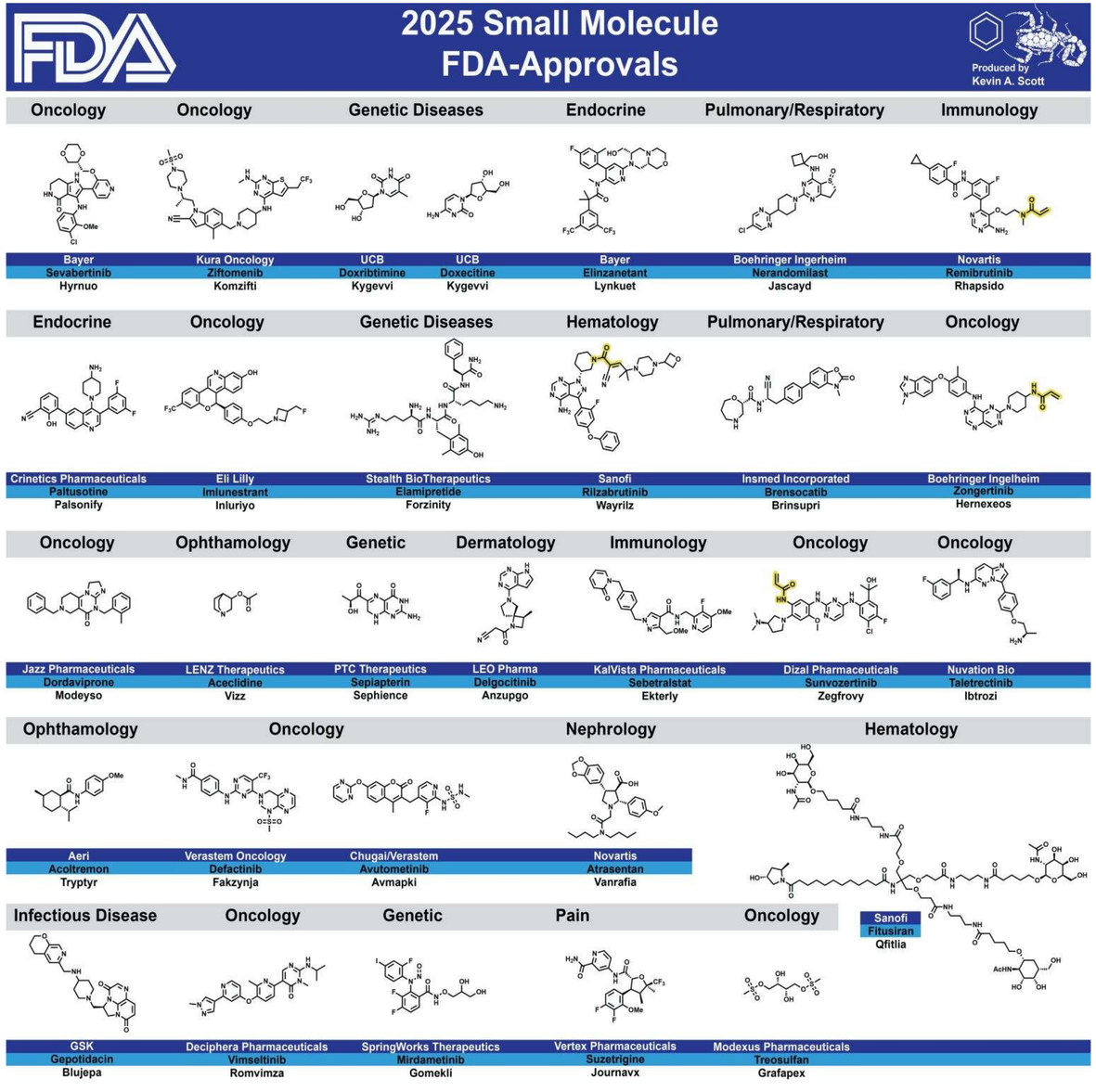

Common challenges in RNA therapeutics

- intrinsic instability and nuclease degradation
- delivery to appropriate tissue/cell type
- avoid unintended innate immune activation
- need for chemical stabilisation and formulation strategies

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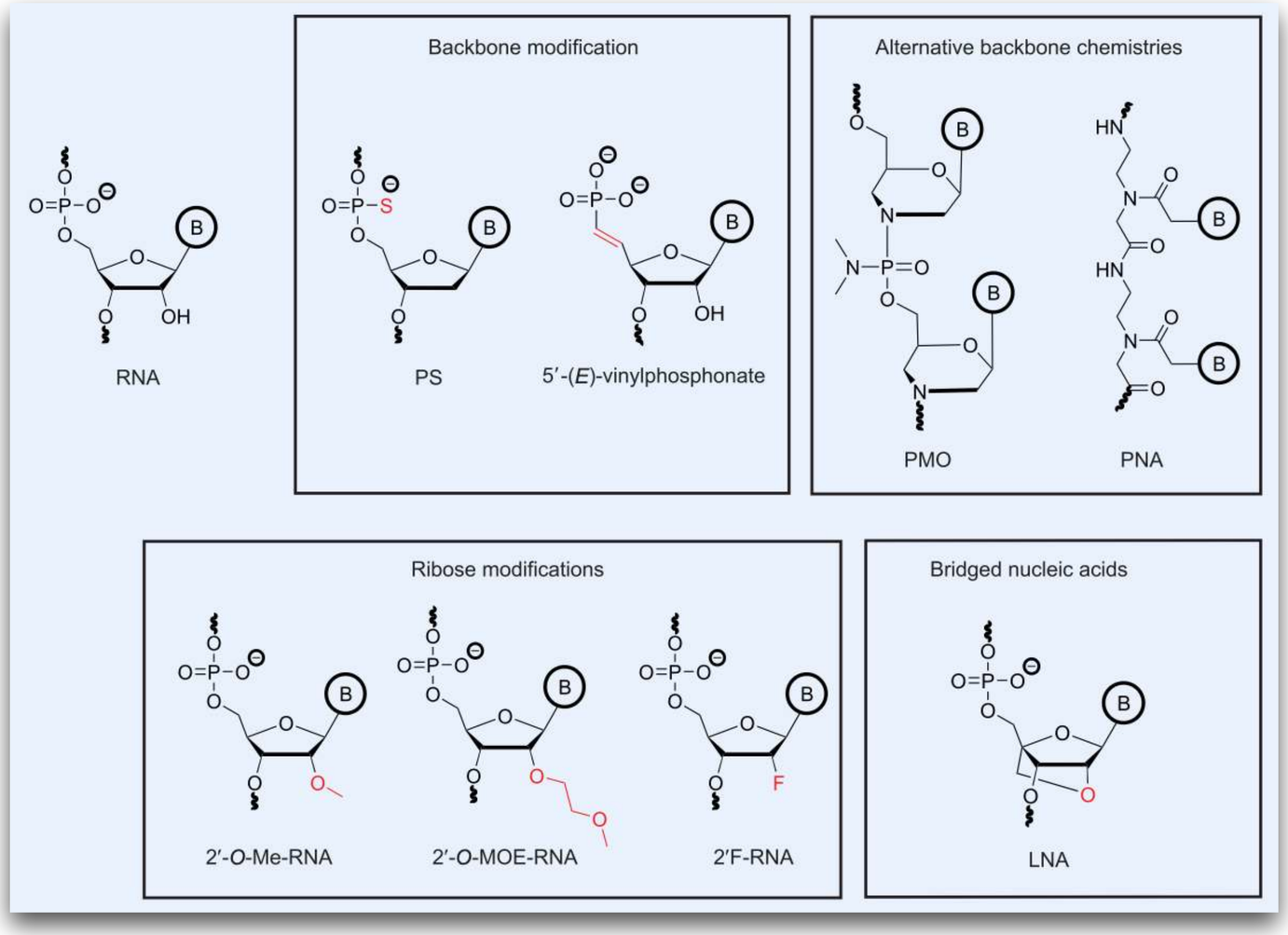

EMBO J 42(21):e114760. 2023

Focus of today's lecture

Nucleoside analogues

which target RNA replication fidelity

RNA aptamers

where RNA itself becomes a therapeutic agent

Focus of today's lecture

Nucleoside analogues

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RNA as target

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RNA as drug

Focus of today's lecture

Nucleoside analogues

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where RNA itself becomes a therapeutic agent

RNA as drug

RNA strand replication

- wildtype often has a higher fitness than mutants
- **selection** counter-acts on copy-errors
- **information** is limited by error rate of polymerase
- all sequence of equal length: sequences space
- neighbours in sequence space differ by one position

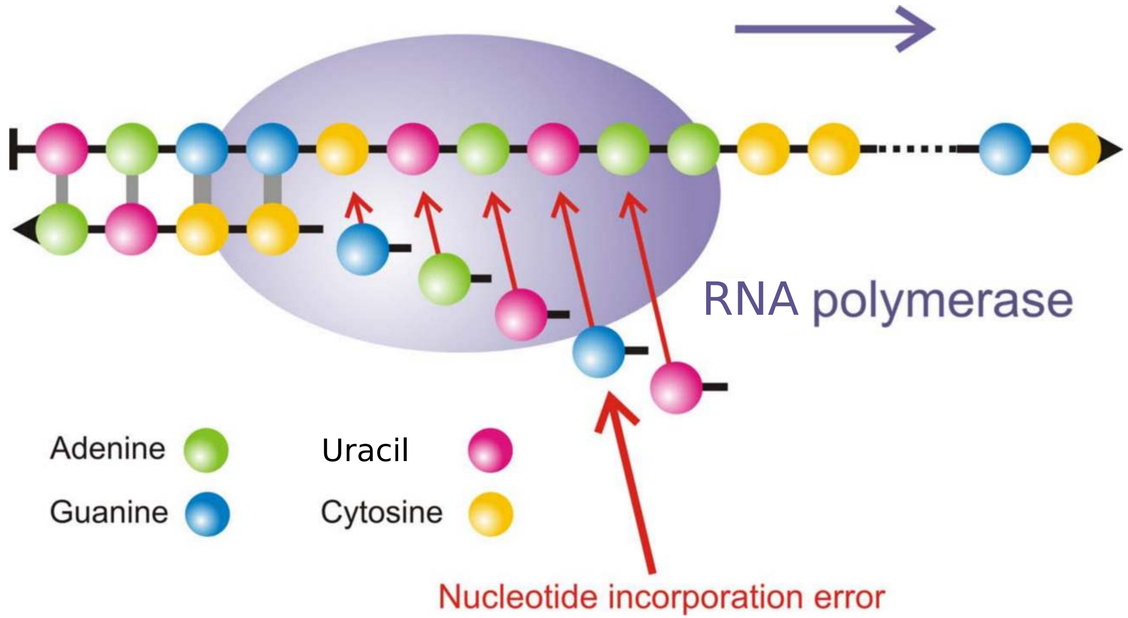
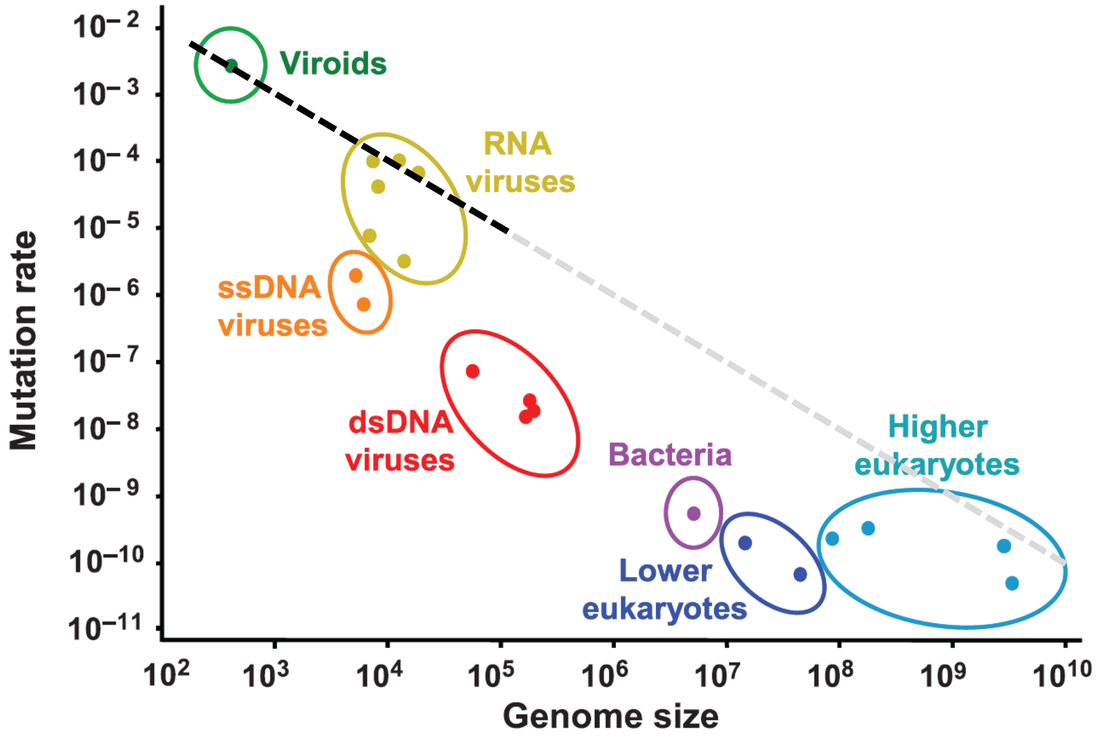


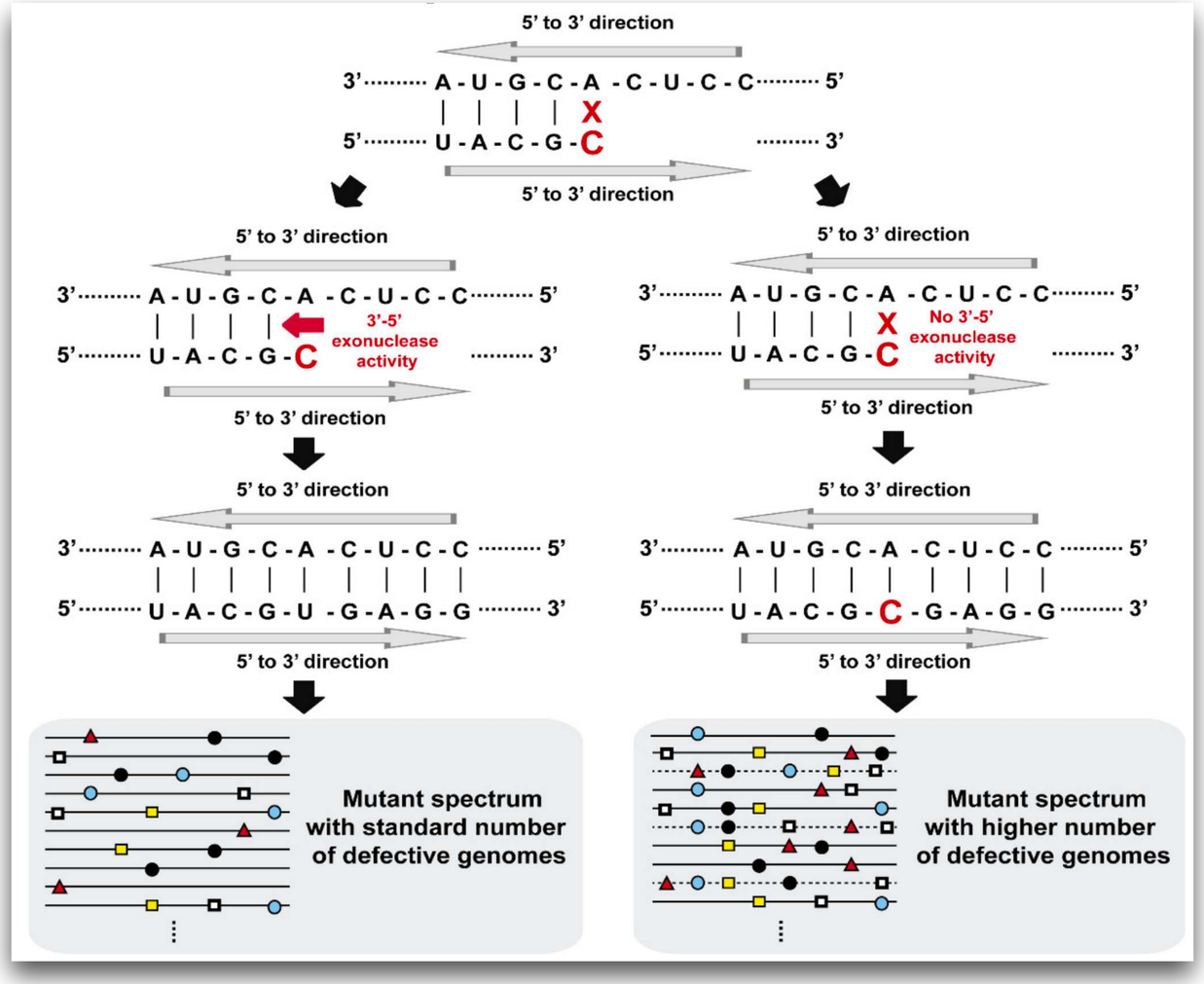
Figure by Peter Schuster

Mutation rates in RNA viruses

- typical error rates 10^{-3} - 10^{-5} per nt
- high variability drives adaptation
- too many errors -> loss of viable genomes



Science. 323, 5919. 2009



Viruses. 13, 1882. 2022

Replicator-mutator dynamics

Manfred Eigen
1927-2019

Peter Schuster
1941-

$$\frac{dx_j}{dt} = \sum_{i=1}^{n} W_{ji} \cdot x_i - x_j \cdot \Phi$$

$$W_{ji} = Q_{ji} \cdot f_i$$

mutation matrix

fitness

$$\Phi = \sum_{i=1}^{n} f_i \cdot x_i$$

$$\sum_{i=1}^{n} x_i = 1$$

- mutation and replication are considered parallel chemical processes

Eigen M 1971, Selforganization of matter and the evolution of biological macromolecules, Naturwissenschaften 58:456 | doi:10.1007/BF00623322

Eigen M & Schuster 1977, The Hypercycle. A Principle of Natural Self-Organisation. Part A: Emergence of the Hypercycle, Naturwissenschaften 64:541 | doi:10.1007/BF00450633

Eigen M & Schuster 1978, Part B: The abstract hypercycle, Naturwissenschaften 65:7 (1978)

Eigen M & Schuster 1978, Part C: The realistic hypercycle, Naturwissenschaften 65:341 (1978)

Viruses form a quasispecies

perfect replication

- only the master sequence is present in the sequence space

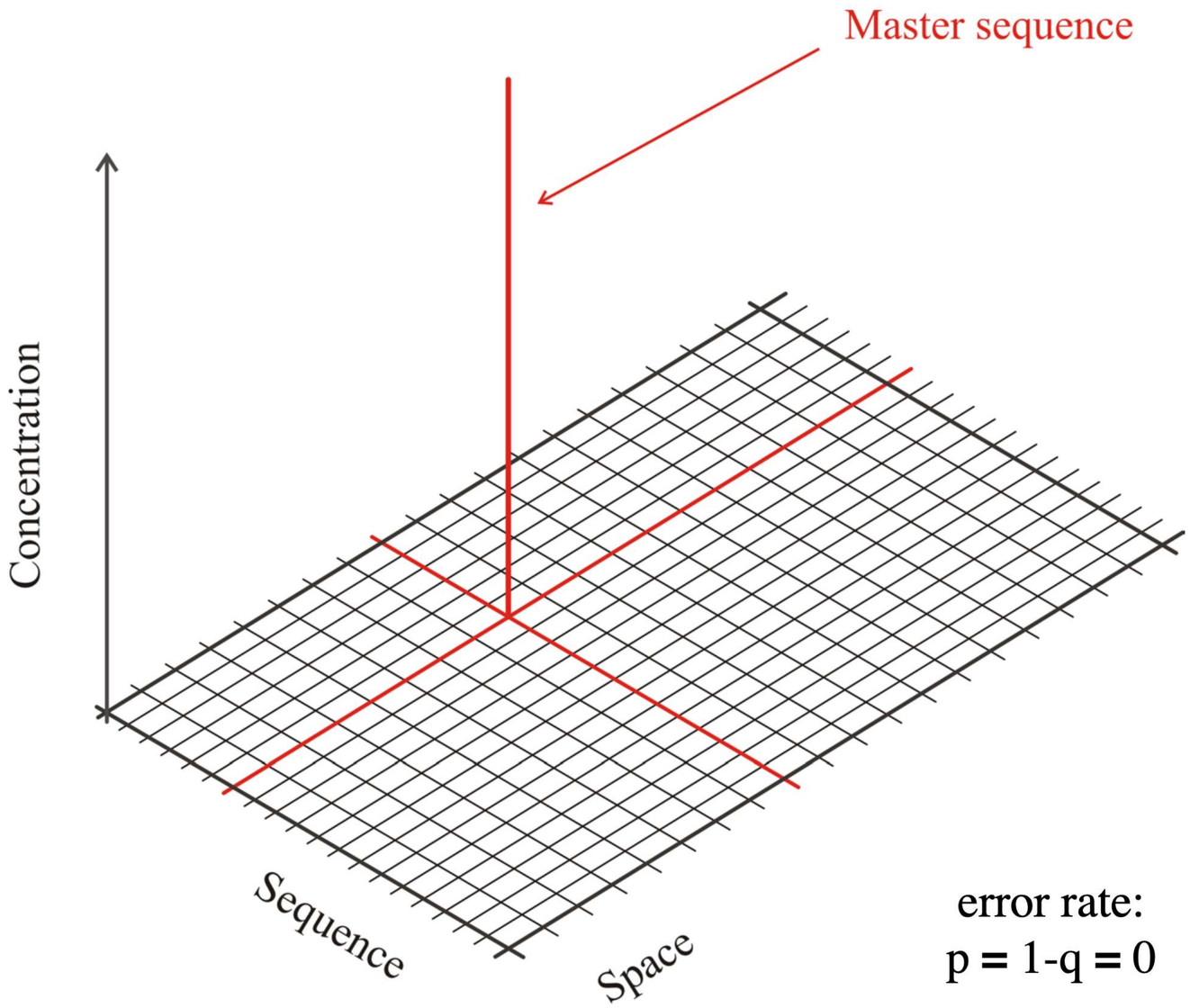


Figure by Peter Schuster

Viruses form a quasispecies

error-prone replication

- a mutant cloud exists around the master sequence space

quasispecies

- virus population is ensemble of mutants
- is a repertoire of variants that allows the virus to overcome selection pressure
- selection acts on the population, not individual genomes

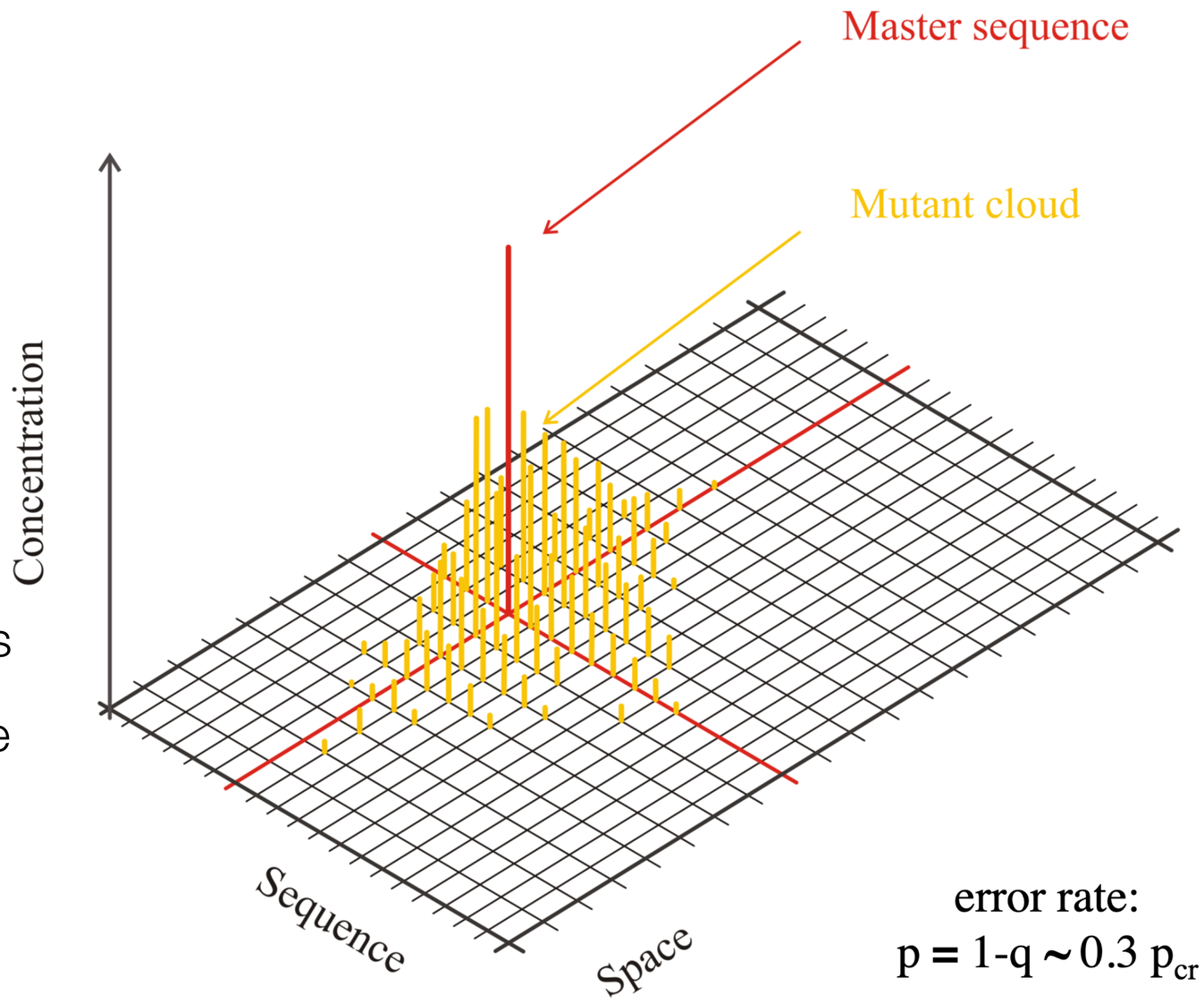


Figure by Peter Schuster

Viruses form a quasispecies

higher error rate

- increases the mutant cloud at the cost of the master sequence
- leads to expansion of the mutant cloud in sequence space

quasispecies

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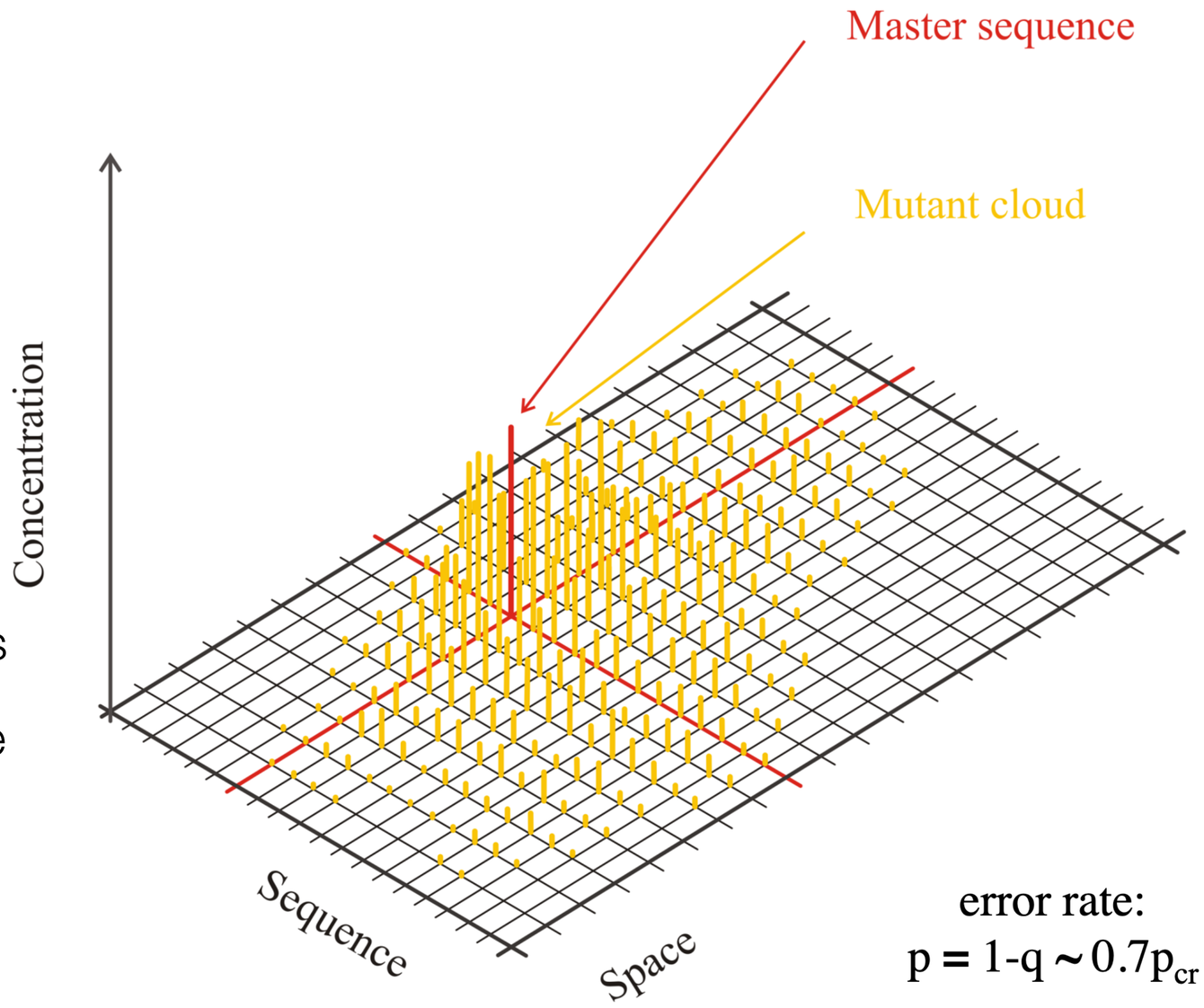

Figure by Peter Schuster

Viruses form a quasispecies

a the critical error rate per is reached

- mutant cloud is equally distributed across sequence space
- genetic information is lost

quasispecies

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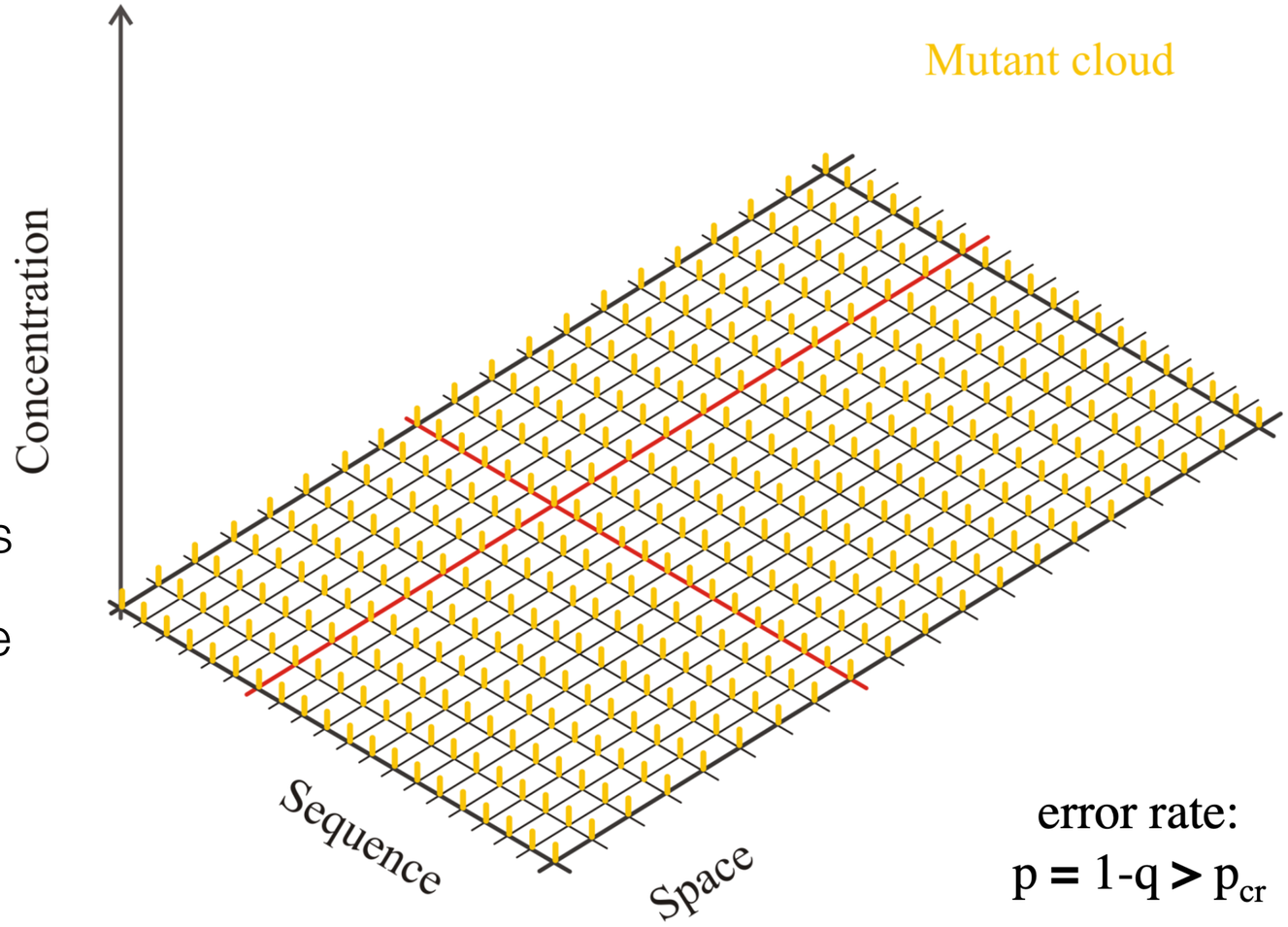


Figure by Peter Schuster

The error threshold (ET)

critical mutation rate beyond which a population's genetic information is destroyed

- below ET, master sequence and its variants are maintained through selection and replication
- above ET, rate of errors overwhelms selection's ability to reproduce an intact genome

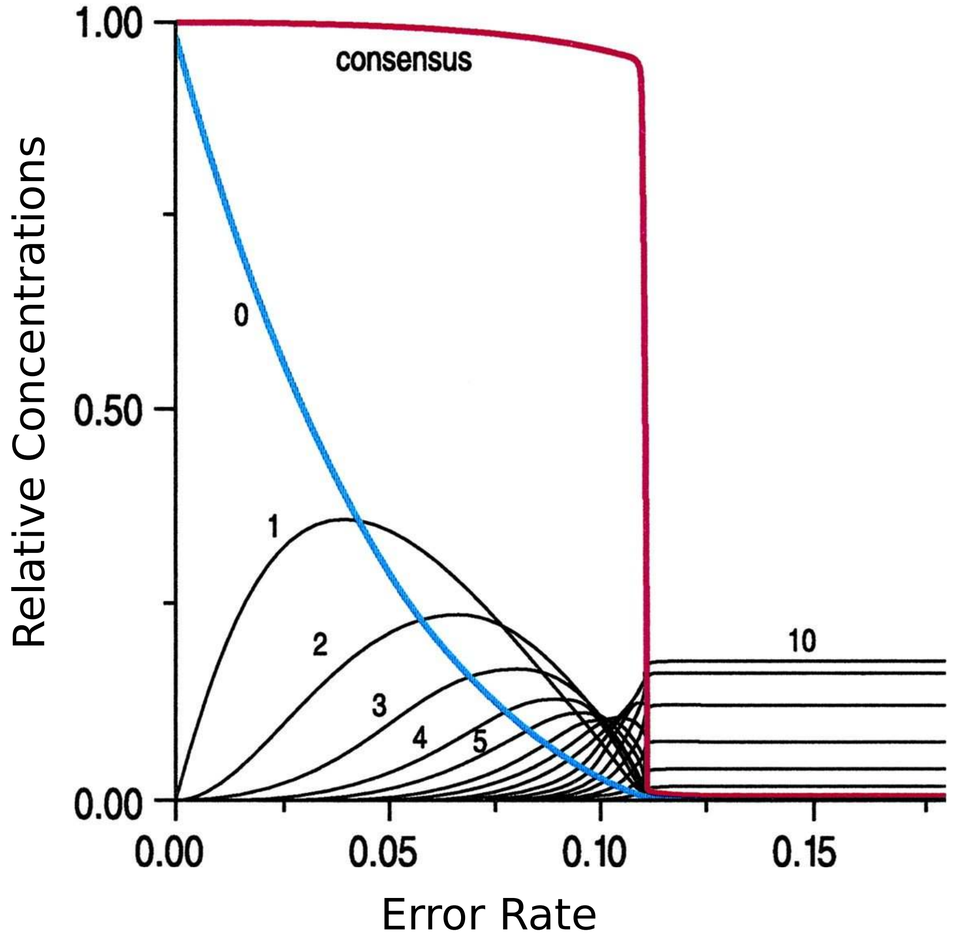
Applications & Implications

- **Origin of life:** how life could have originated from self-replicating molecules w/o error-correction
- **Viral evolution:** Many RNA viruses operate close to their ET, making them vulnerable to drugs
- **Antiviral therapy:** Increasing mutation rate of a virus beyond ET, leading to population collapse

Eigen's error catastrophe

increasing the error rate:

- relative concentration of **master sequence** decreases
- relative concentrations of mutants increase
- **consensus sequence** shows a phase transition
- here the critical error rate is at ~0.11



modified from PNAS 99(21):13374-76. 2002

Eigen's error catastrophe

increasing the error rate:

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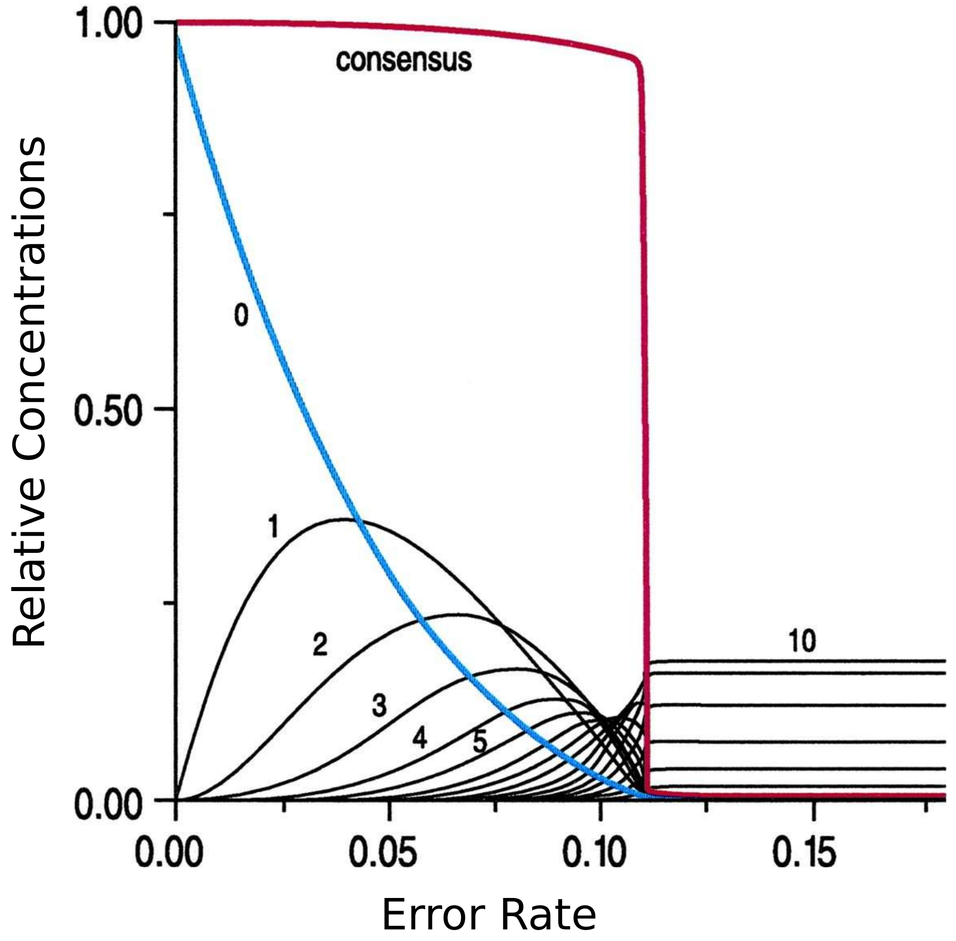
take-home message:

push the virus beyond the critical error rate, so it cannot maintain essential functions



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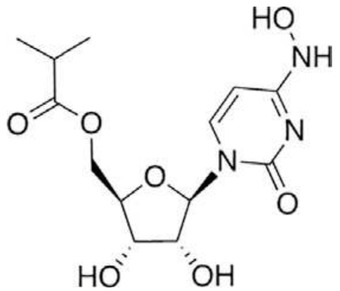
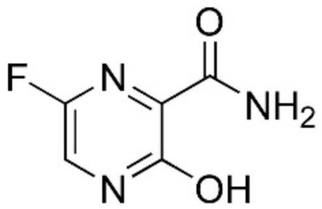
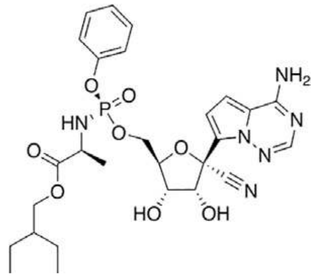
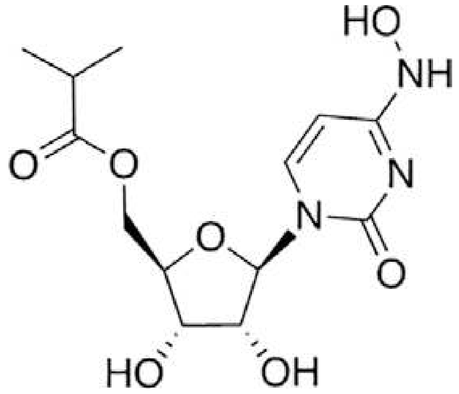
Nucleoside analogues

- small molecules that mimic natural nucleosides
- incorporated by viral polymerases
- alter replication outcomes (termination or mispairing)

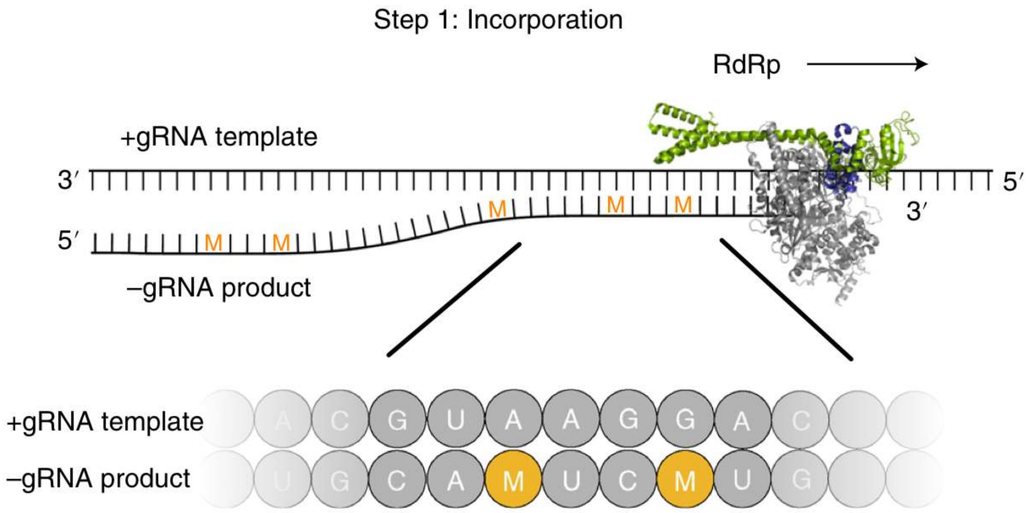
Molnupiravir	**Favipavir**	**Remdesevir**
		
• lethal mutagenesis	• lethal mutagenesis	• directly inhibits viral RdRP
• mimics nucleotides C and U	• affects G → A and C → U transition rate	• mimics nucleotides A
• prodrug is converted to ribonucleoside	• broad-spectrum antiviral effect	• initially developed as antiviral agent against Ebolaviruses

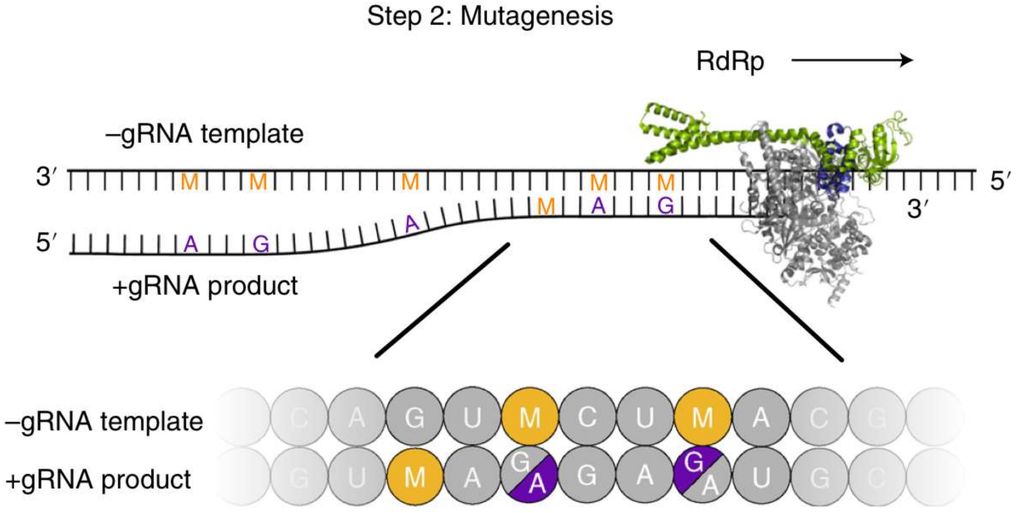
Molnupiravir



Molnupiravir

- prodrug of N4-hydroxycytidine (NHC)
- converted to its active form molnupiravir-triphoshpate (MTP) in the cell
- competes most effectively with CTP for incorporation into the product RNA
- once incorporated (as monophosphorylated MNP), RNA synthesis proceeds without stalling
- a templating MNP can form base pairs with GTP and ATP into the new product strand

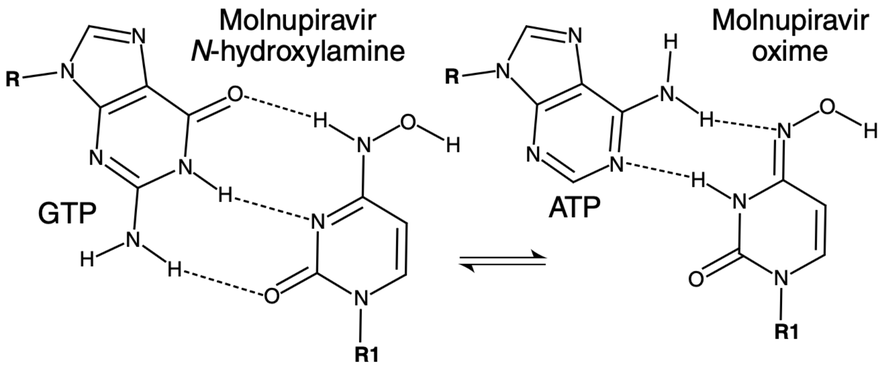




Nat Struc Mol Biol 28:740-46. 2021

Nat Struc Mol Biol 28:706-11. 2021

Molnupiravir



G-to-A transitions

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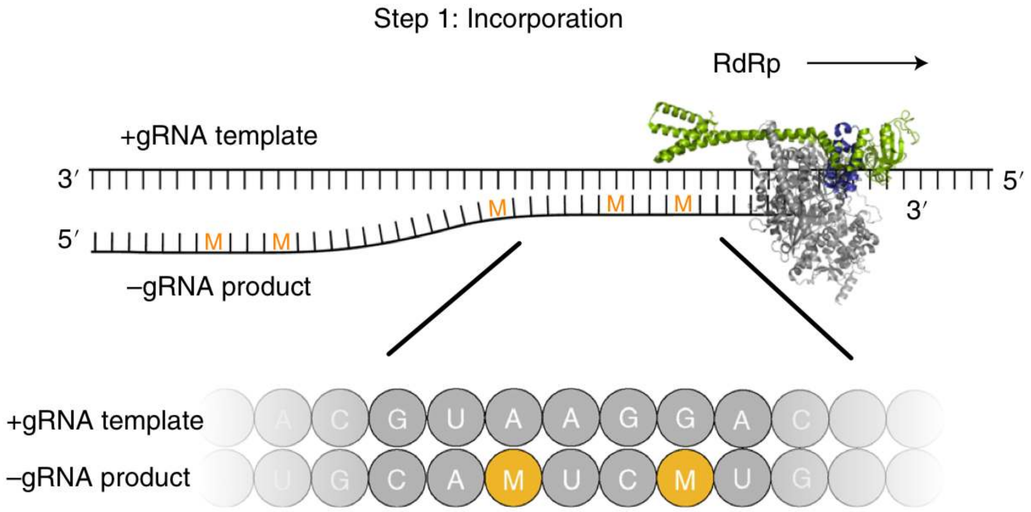

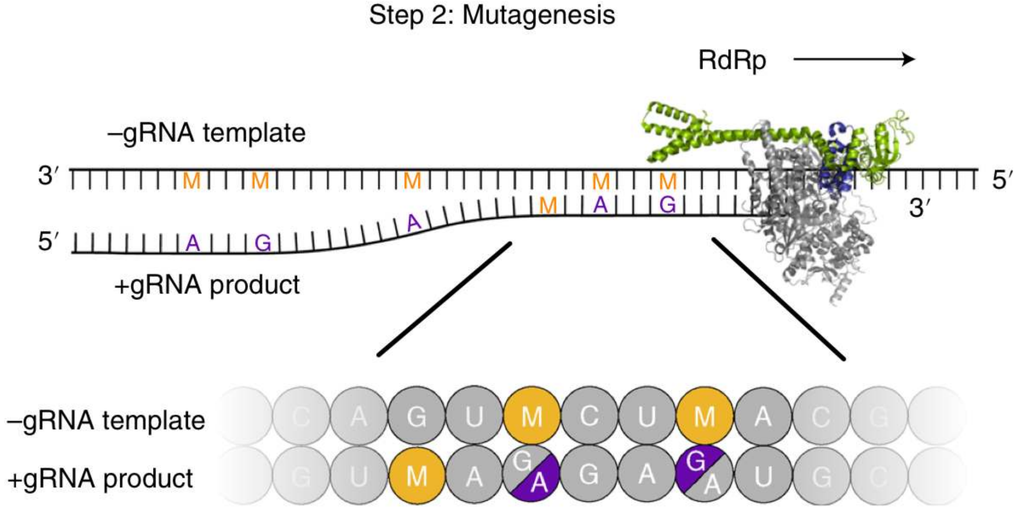

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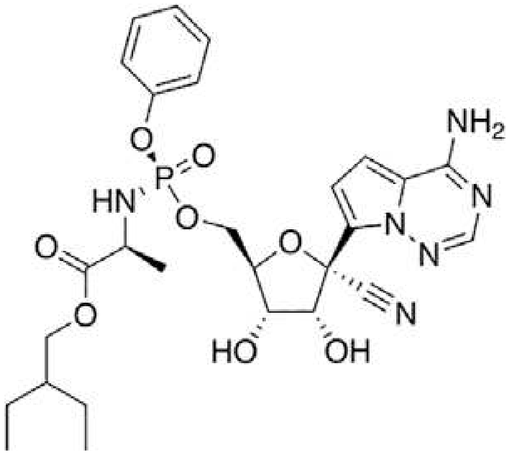
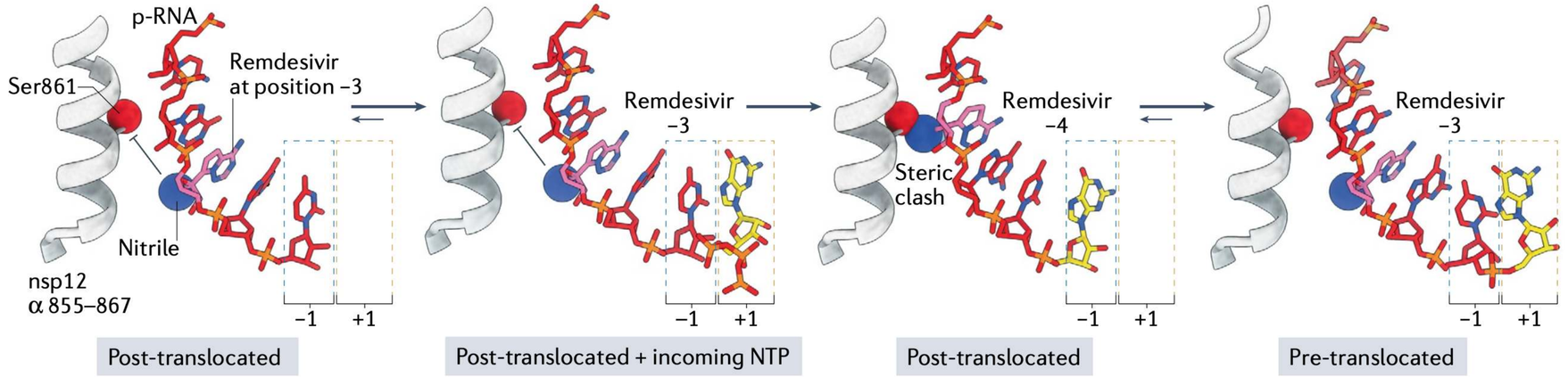
Remdesevir



Remdesevir

- prodrug metabolized into remdesevir-teriphosphate (RTP), an ATP analogue
- RdRP incorporates RTP into nascent viral RNA instead of ATP
- once RTP is incorporated, RdRP adds three more nucleotides
- at position i+3, the 1'-nitrile group induces a steric clash with RdRP (S861 in SARS-CoV-2)
- this leads to RdRP stalling, and a delayed chain termination ('i+3 mechanism')
- inhibition of viral RNA synthetis & evasion of proofreading



Nat Rev Mol Biol 23:21-39. 2022

Other nucleoside analogues

Drug	Viral target	Mechanism
Sofosbuvir	HCV	cytidine analogue → lethal mutagenesis
Ribavirin	multiple RNA viruses	guanosine analogue → mutagenesis + GTP depletion
Acyclovir	HSV/VZV	deoxyguanosine analogue → obligate chain termination
Tenofovir	HIV/HBV	AMP analogue → chain termination

From nucleoside analogues to RNA aptamers

Nucleoside analogues

which target RNA replication fidelity

RNA as target

RNA aptamers

where RNA itself becomes a therapeutic agent

RNA as drug

From nucleoside analogues to RNA aptamers

Nucleoside analogues

which target RNA replication fidelity

RNA as target

small molecules that modulate errors

RNA aptamers

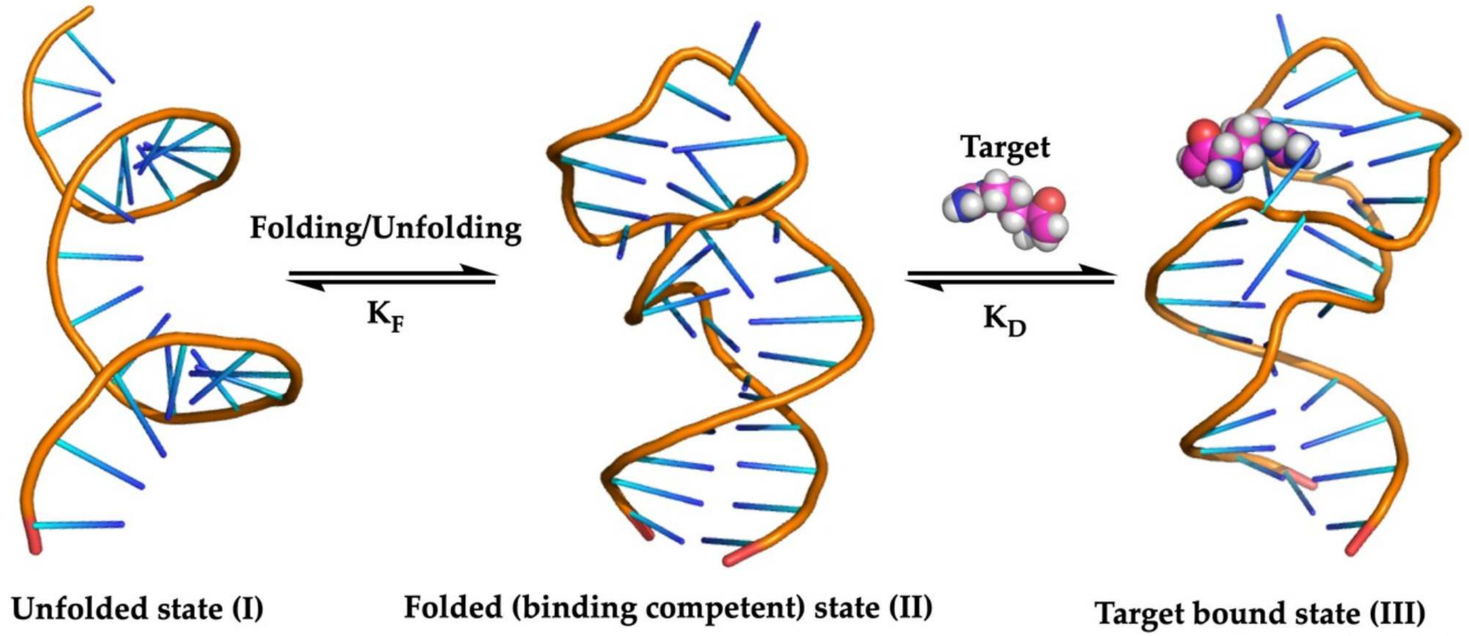
where RNA itself becomes a therapeutic agent

RNA as drug

structured RNAs performing molecular recognition

What are RNA aptamers?

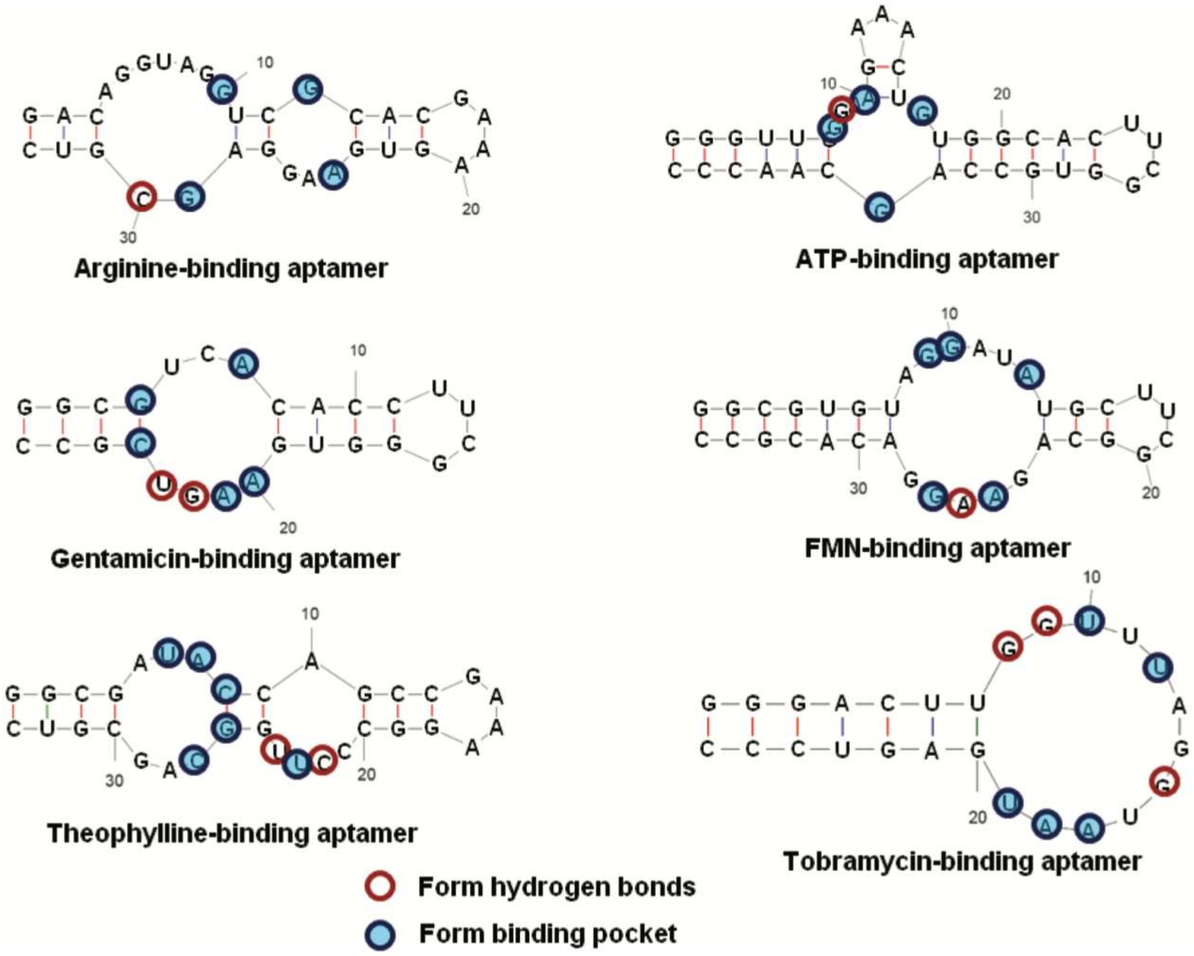
- short, structured RNAs with defined 3D fold
- bind atoms / small molecules / peptides / proteins with high affinity
- fully synthetic and sequence-programmable
- conceptually similar to antibodies (“chemical antibodies”)



IJMS 21(12):4522. 2020

Therapeutic advantages of RNA aptamers

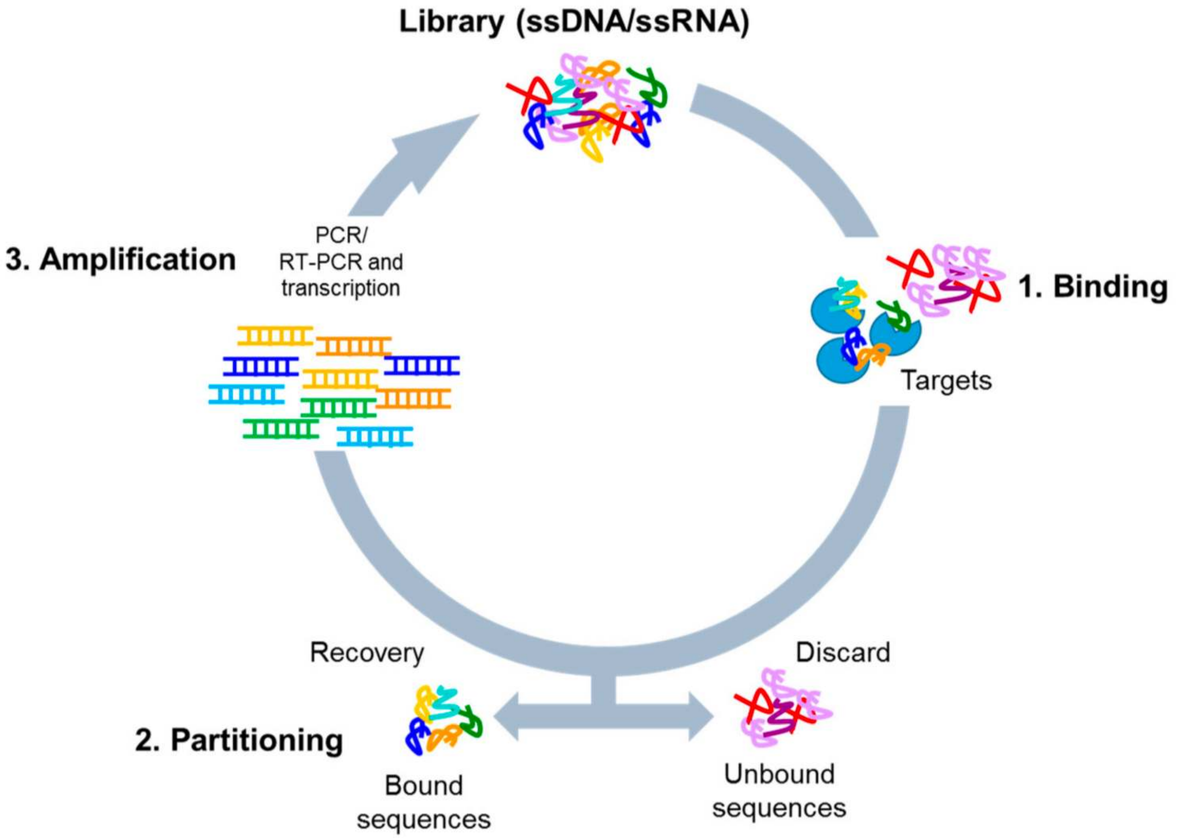
- high specificity through structural comple-mentarity
- small size -> rapid tissue penetration
- no immunogenicity protein scaffolds
- chemical synthesis-> high reproducibility
- can target ‘undruggable’ proteins that lack small-molecule pockets



NAR 37(12):e87. 2009

The SELEX process

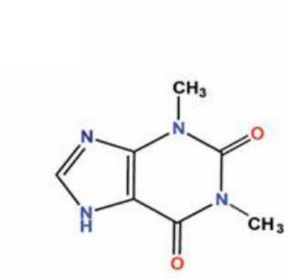
- SELEX: iterative in vitro selection
- start from a large RNA library (~10^{13} variants)
- cycles of binding -> partition -> amplification
- enrichment of high-affinity binders

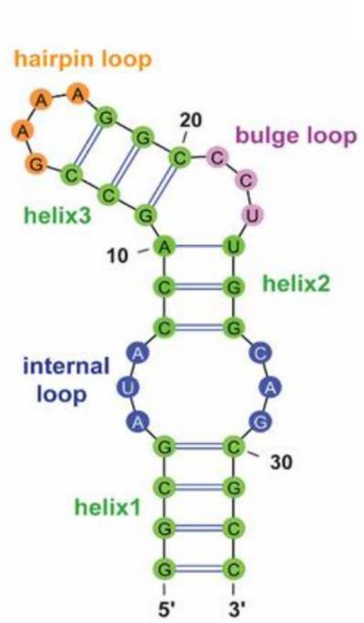


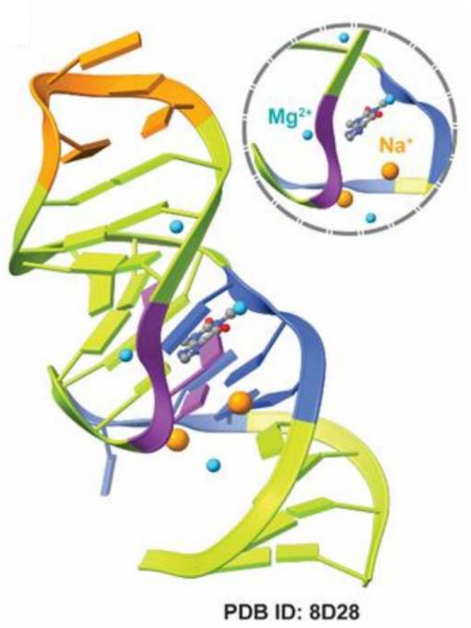
Biomedicines 5(3):39. 2017

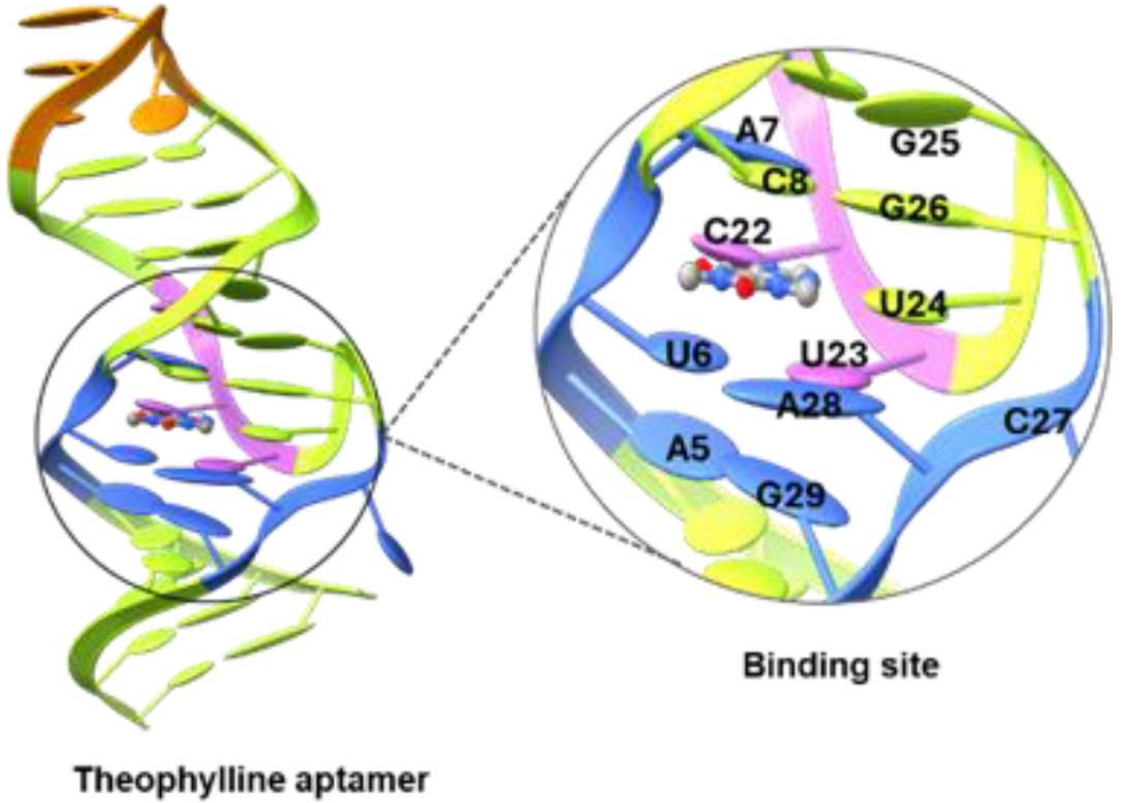
RNA structure as the basis for recognition

- RNA fold determines function
- binding pockets determined by stems, loops, junctions
- non-canonical basepairs stabilize 3D structure
- precise tertiary contacts enable picomolar affinities









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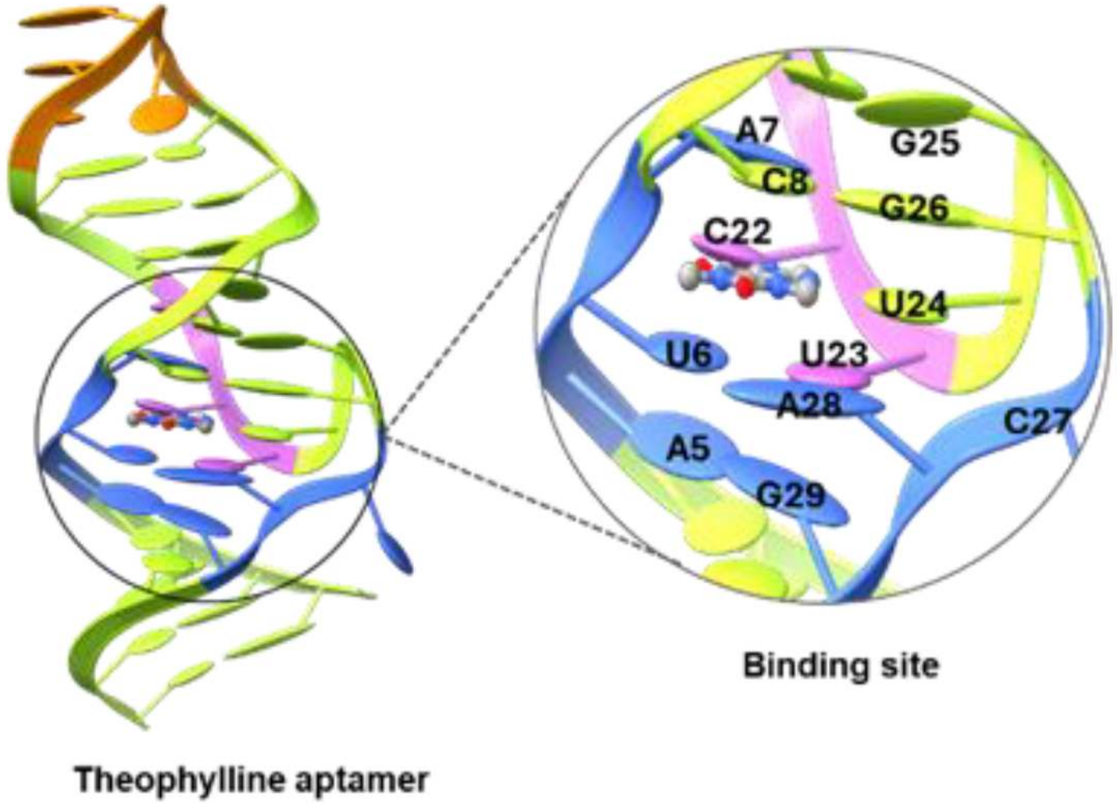
Binding modes

- different binding modalities, depending on target (protein / small molecules
- shape complementarity
- electrostatics
- VdW interactions
- hydrogen bonding network



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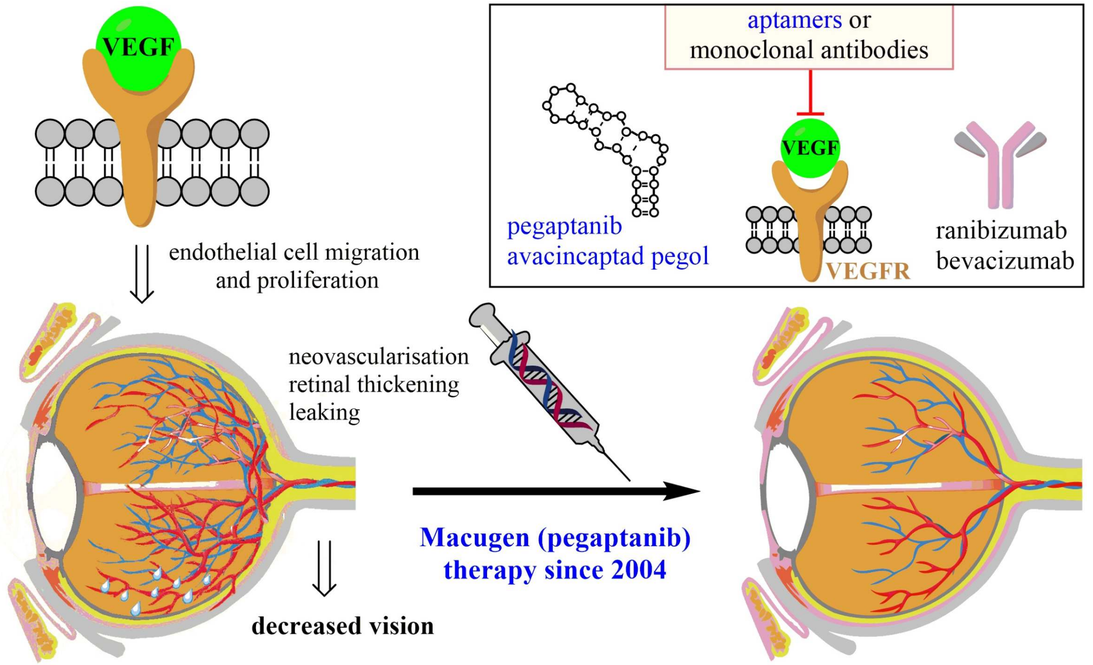
Therapeutic mechanisms of aptamers

- block protein-protein interactions
- inhibit receptor activation
- prevent enzyme access to substrates
- targeting ligands for drug delivery
- act as logic modules in synthetic circuits



Pharmaceutics 17(3):394. 2025

Clinical landscape

- Pegaptanib (VEGF targeting aptamer) - first FDA approved aptamer
- aptamers in development: oncology, coagulation, immunomodulation
- increasing interest in aptamer diagnostics

Aptamers as engineering modules

- modular design enables synthetic control
- aptamer domains can be fused to sensors or effectors
- works as on/off switches in complex RNA folds
- basis for **artificial riboswitches** and **programmable RNA devices**

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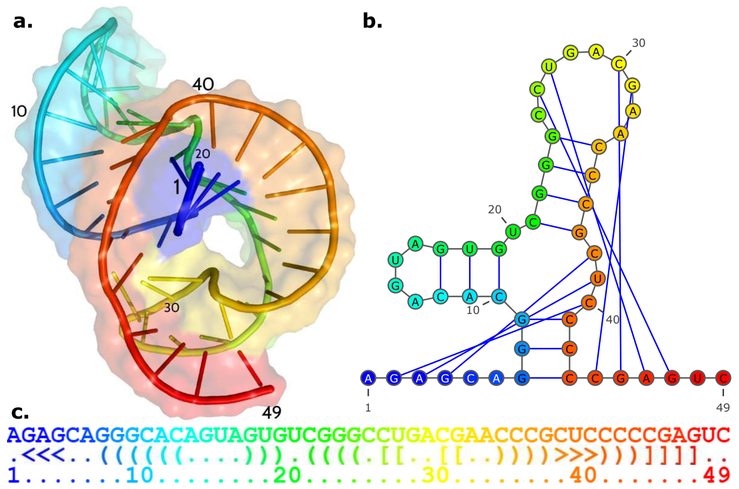
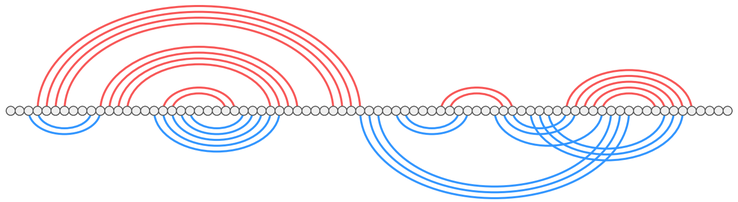


AeFV xrRNA

+

theophylline aptamer

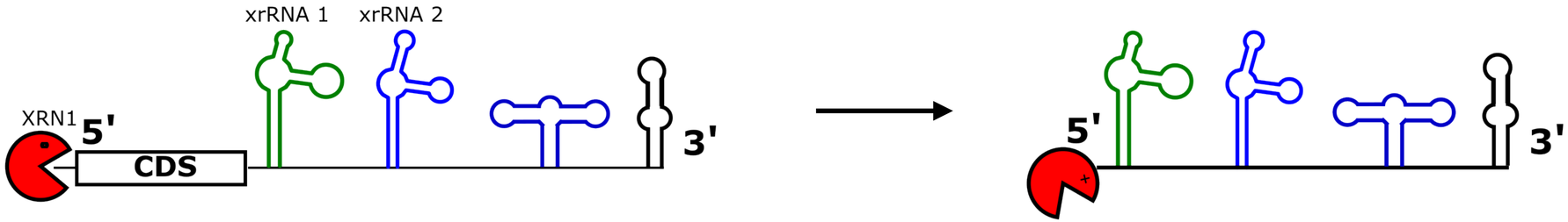
=

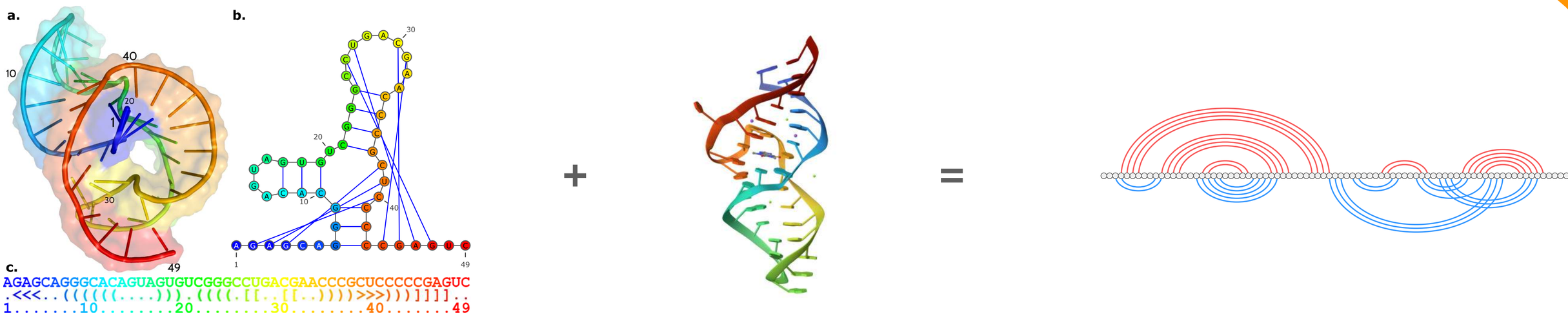


xrRNA riboswitch

Aptamers as engineering modules

- Flaviviruses hijack the host mRNA degradation machinery by stalling XRN1
- 5’-> 3’ exoribonuclease XRN1 degrades RNA





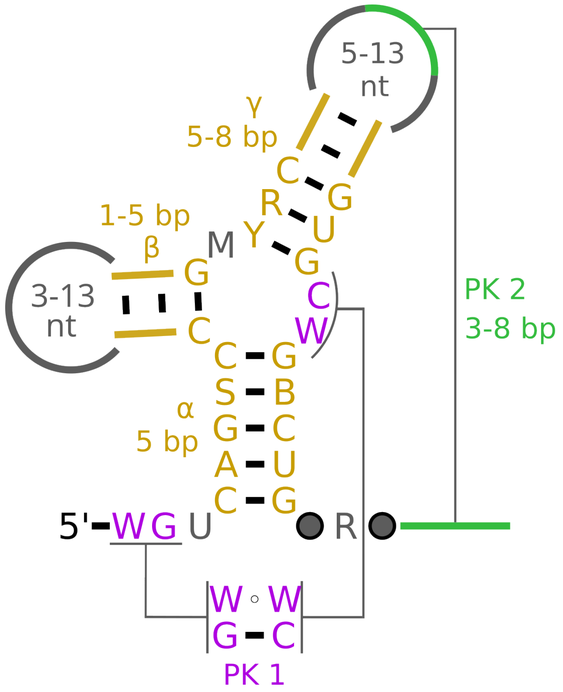
AeFV xrRNA

theophylline aptamer

xrRNA riboswitch

Designing artificial xrRNAs

- Infrared is a framework for solving declaratively modelled problems
- Sample sequences using a set of constraints and functions
  - Bases that should form basepairs need to be complementary
  - Positions with conserved bases are fixed
  - Length of individual features and total length
- Sampling from model will likely not achieve target structure
- Use Monte Carlo approach to
  - **minimize Ensemble Defect**
    average number of incorrectly folded nucleotides in the thermodynamic ensemble
  - **maximize Equilibrium Probability**
    probability of target structure in the thermodynamic ensemble

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SEQ WGUCAGGCCXXXXNNNXXXXXXXXGCYACNXXXXXXXXXXXNNNXXXXXNXXXXXXXXNGUGCWGCCUGXXXXXXXXXXXNNN
SS  ...((((((((((.......)))))).(((((((.....................)))))))..)))))..............
PK1 ((.....................................................))...................
PK2 ........................................(((((((.........................)))))))
```

Towards xrRNA riboswitches

- design an inducible molecular device that allows for targeted RNA degradation
- riboswitch comprises **expression platform (E)** and **aptamer (A)**
- we use artificial xrRNAs as expression platform and theophylline/neomycin aptames

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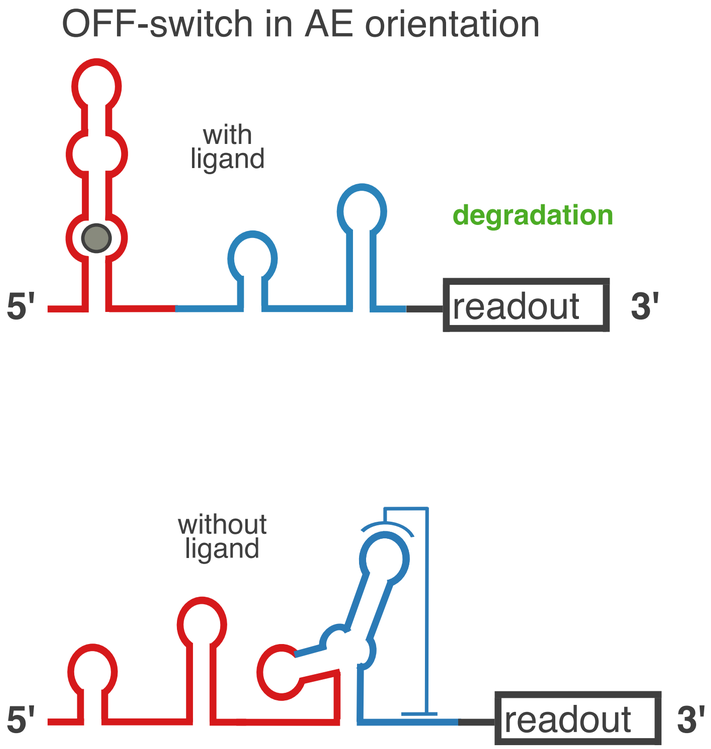
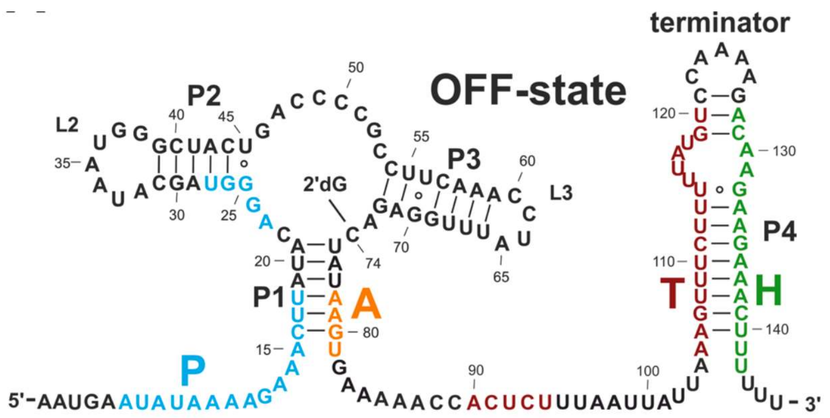


recall Ivo's Monday lecture



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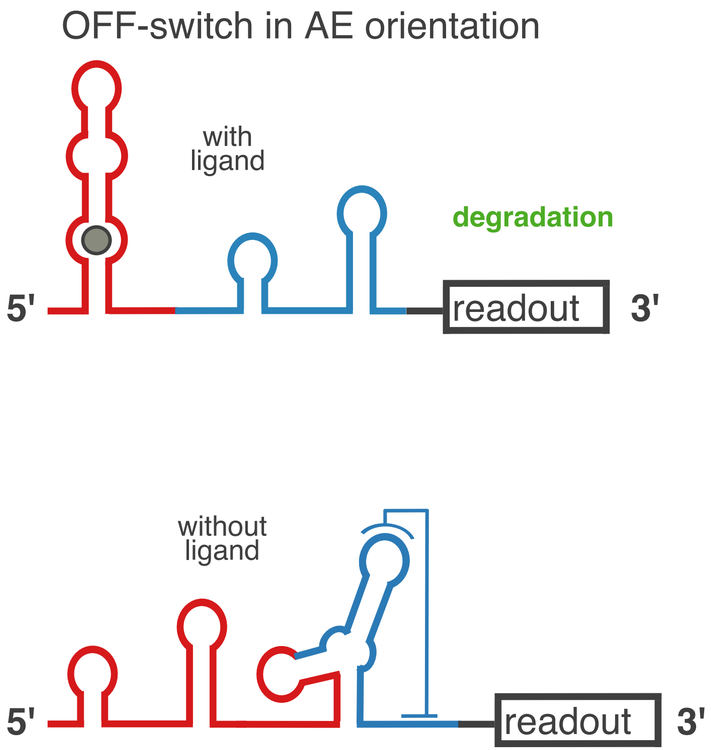

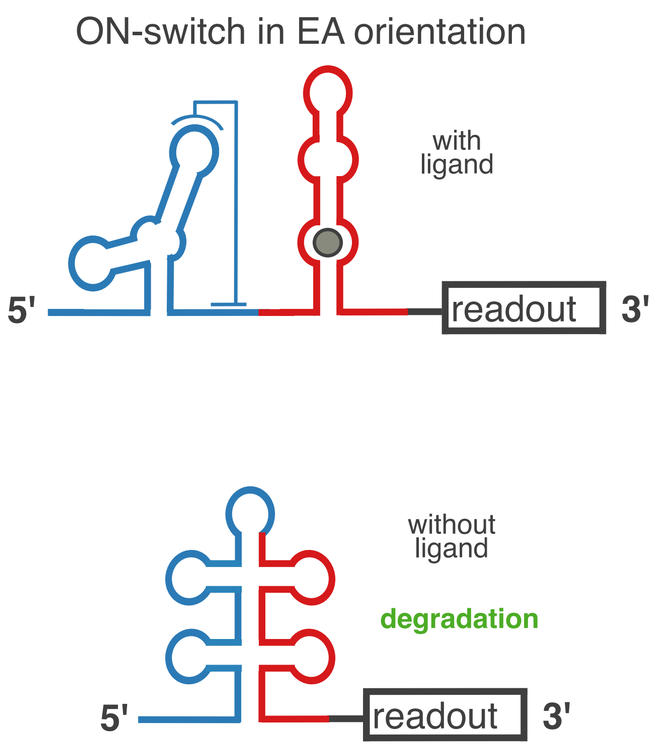

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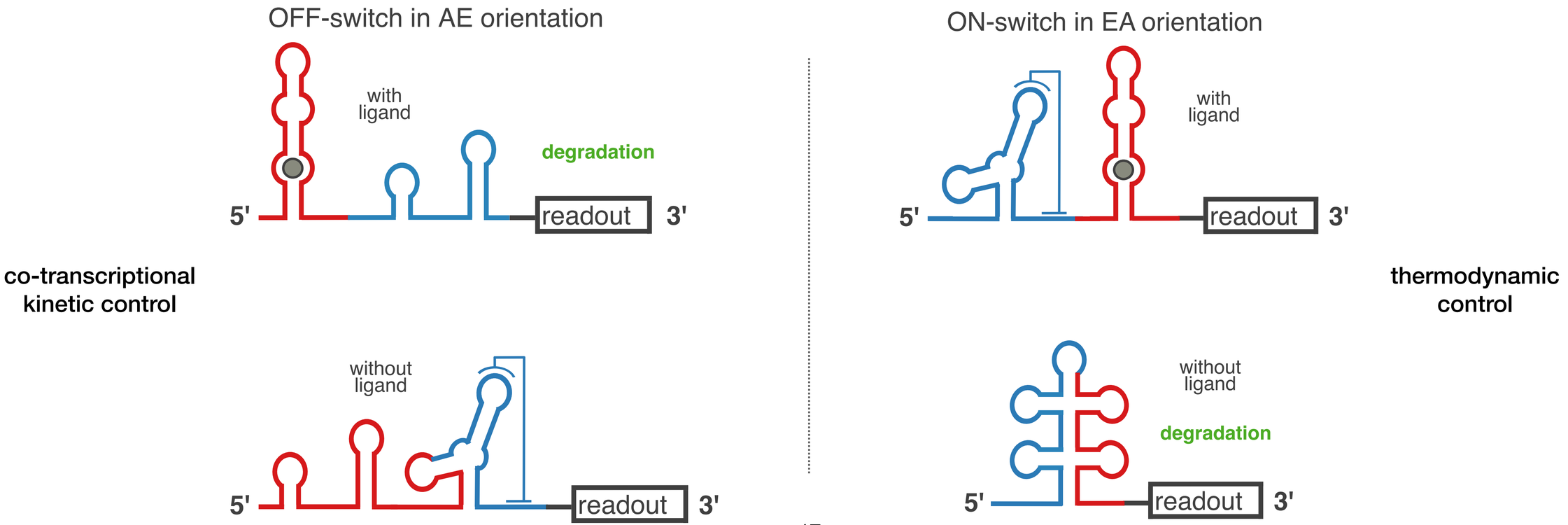

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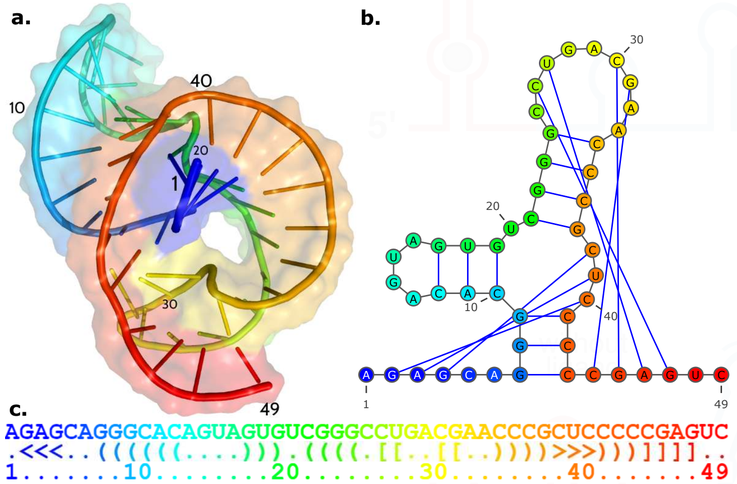
We need a thorough understanding of xrRNAs and aptamers first



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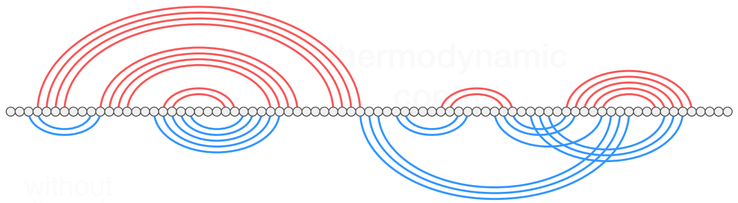
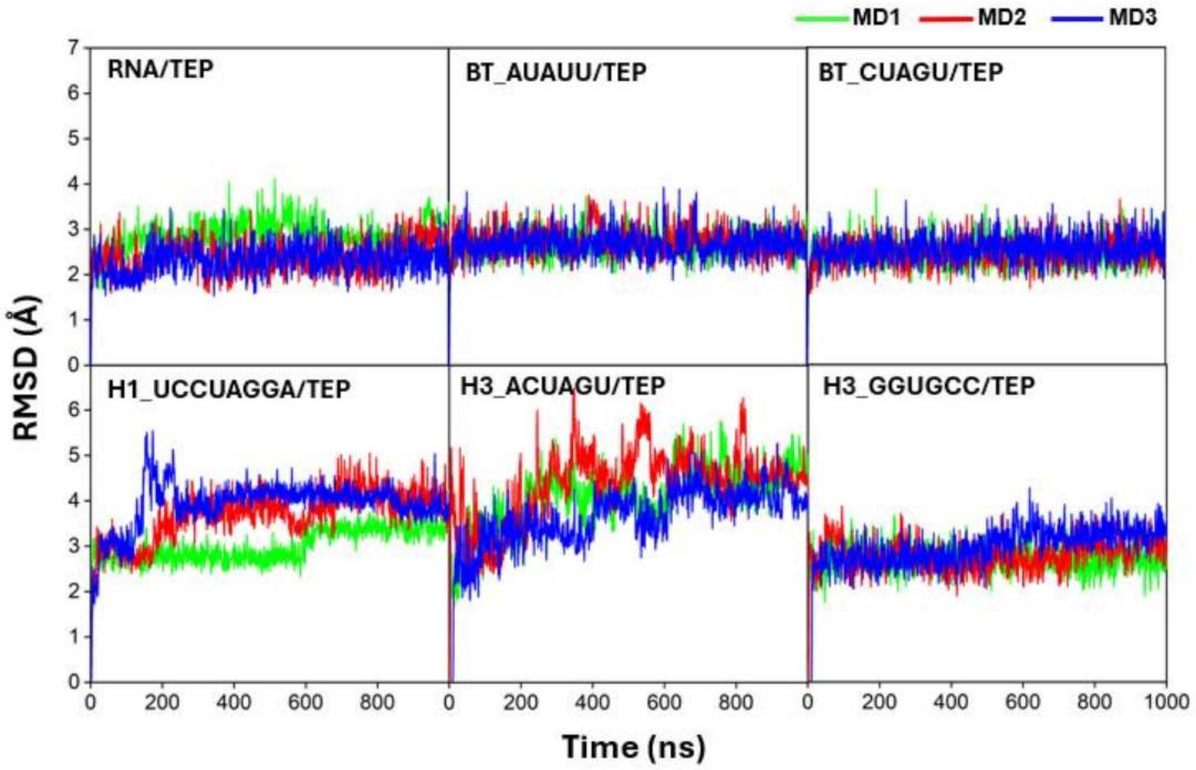
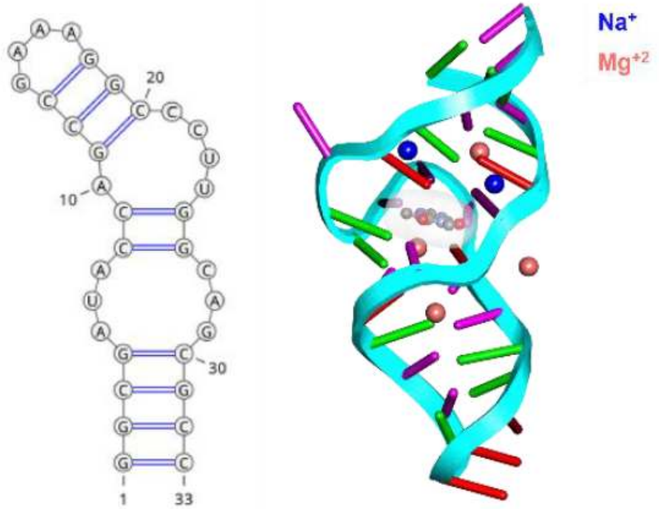


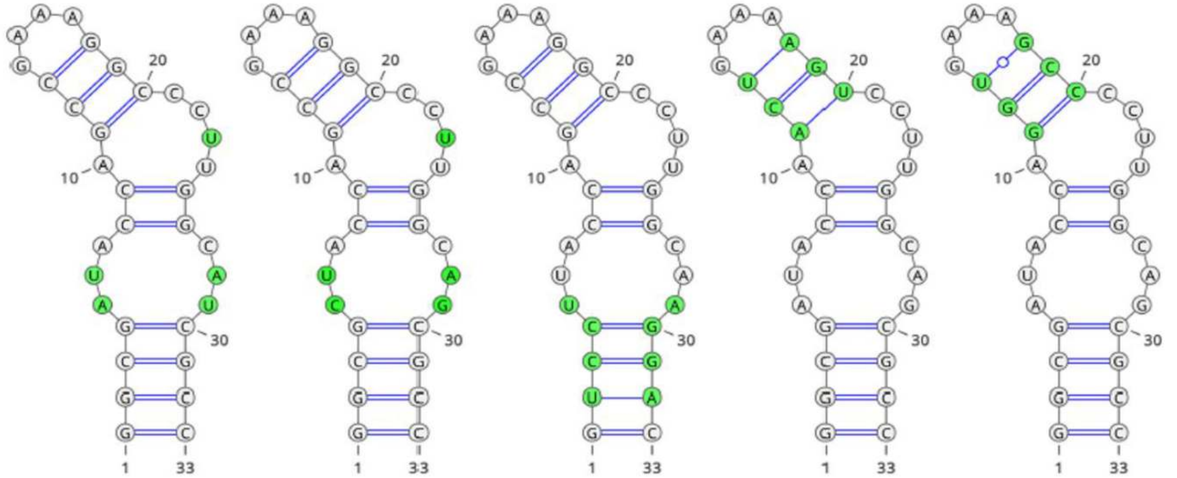
xrRNA riboswitch

Mutating the theophylline aptamer

- mutate different positions of the theophylline aptamer
- perform MD simulations to confirm positions that can be mutated without affecting ligand binding







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