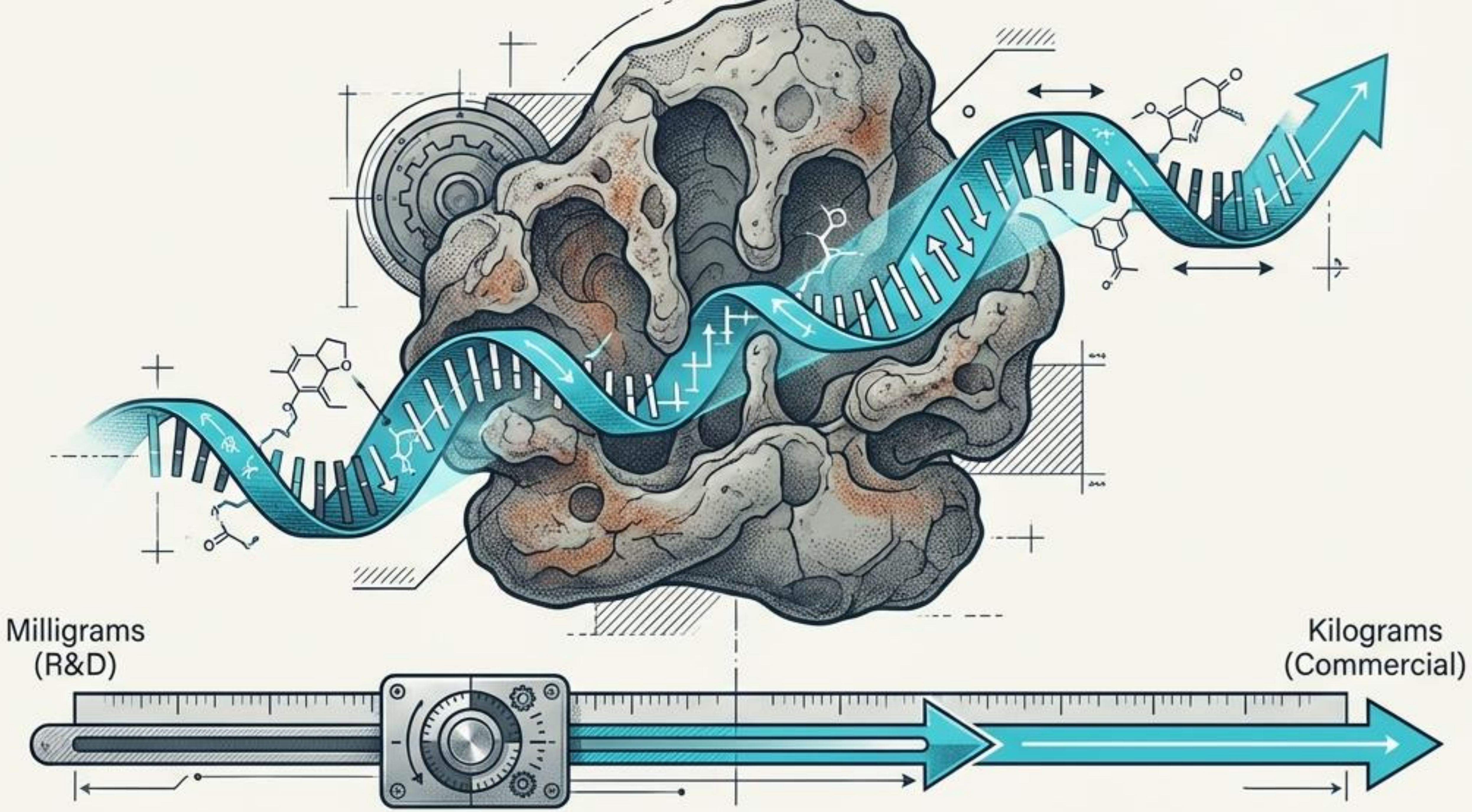
The RNA Engineering Revolution

Adventures in Making Extreme mRNA Molecules

Aaron Larsen, CASSS, 2026



Crystal NAX
A Member of Crystal Pharmatech

Your Trusted Partner
for
Nucleic Acid eXcellence

About Crystal Corporation

Crystal Pharmatech

Crystal NAX
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Crystal Bio Solutions
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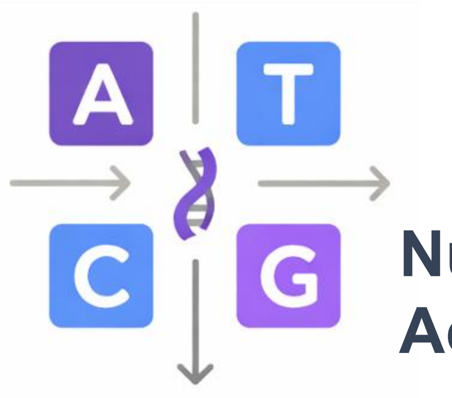
CD CANDOO PHARMATECH

CPT, Candoo, CFS: Small molecule solid-state research | CMC formulation development | cGMP manufacturing
NAX: Nucleic Acid and non-vial delivery research, development and manufacturing
CBS: Biologics Characterization & CMC analytical | bioanalysis & Biomarker | quantitative clinical pharmacology

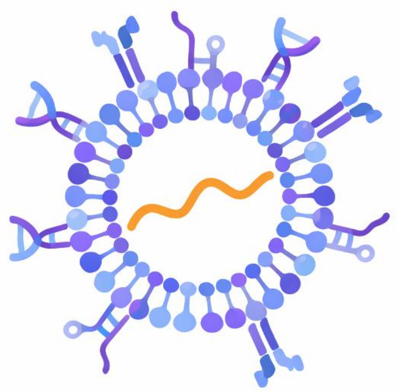
15	3	1000+	2000+	500,000+
Years	Countries	Global Clients	Supported Drugs	Sqft of facilities

About Crystal NAX

3 Key Tech Platforms



Nucleic Acid



Non-viral Delivery

Analytical Development

6 Service Categories

- Catalog Products
- Custom Synthesis
- LNP Formulation
- Analytical Development
- Process Development
- Scale-up Manufacturing

Mission

Unlocking every step of nucleic acid therapy
Providing end-to-end solutions from early R&D to clinical development

NAX End-to-End Services

Early R&D

- Catalog RNA products
- Sequence design & optimization
- DNA construct development
- Strain feasibility studies
- mRNA/saRNA/circRNA/taRNA/siRNA/gRNA synthesis
- LNP formulation synthesis and study
- *In vitro/In vivo* study

Analytical Development

- Customized analytical methods & standards synthesis
- CQA assessment (ID, integrity, purity)
- Method transfer, verification & validation
- Stability studies
- Impurity characterization

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Process Development

- pDNA and linearization process
- mRNA/saRNA/circRNA process optimization
- LNP & tLNP process development
- Process characterization, optimization and transfer

End-to-End Manufacturing

- Scale-up batch for tox studies
- GMP-like & cGMP manufacturing
- Sterile fill and finish
- QC release & IIT/IND support

Executive Summary: Hacking Ancient Biology for Industrial Scale

The transition from acute vaccines to chronic RNA therapeutics requires a fundamental overhaul of both sequence architecture and bioprocessing physics.

Phase 1: The Biological Constraint

Understanding the ribosome's mechanical biases and the limits of natural selection.

Phase 2: Linear Optimization Limits

Navigating the multi-dimensional paradox of codon stability, expression, and manufacturability.

Phase 3: Architectural Shifts

Overcoming the severe CMC and structural barriers of circRNA and saRNA.

Phase 4: Scaling to Kilograms

Abandoning legacy batch reactors for continuous perfusion biomanufacturing.

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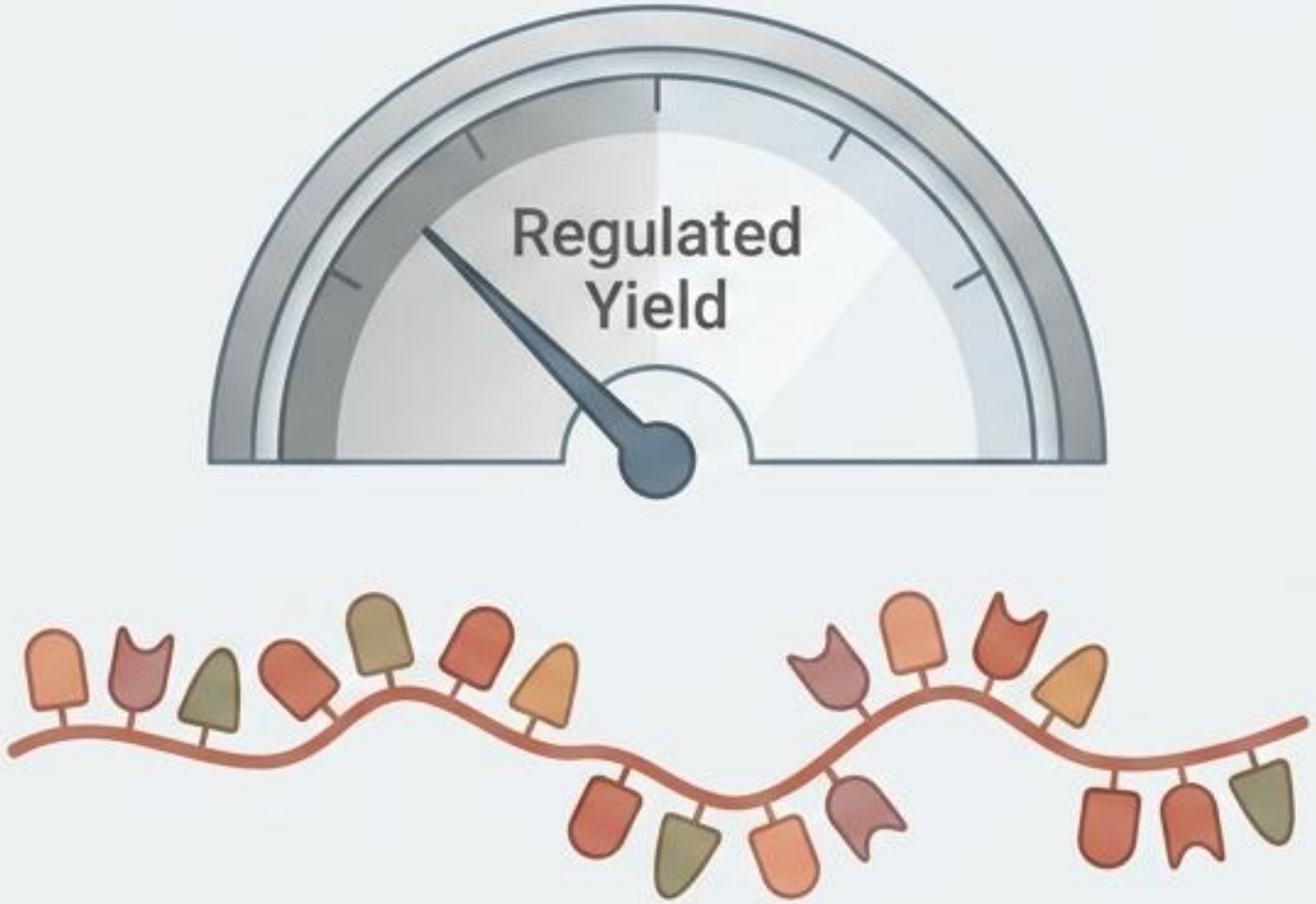
The Fallacy of Natural Selection in Biomanufacturing

The human genome provides a regulatory code, not an industrial production blueprint.

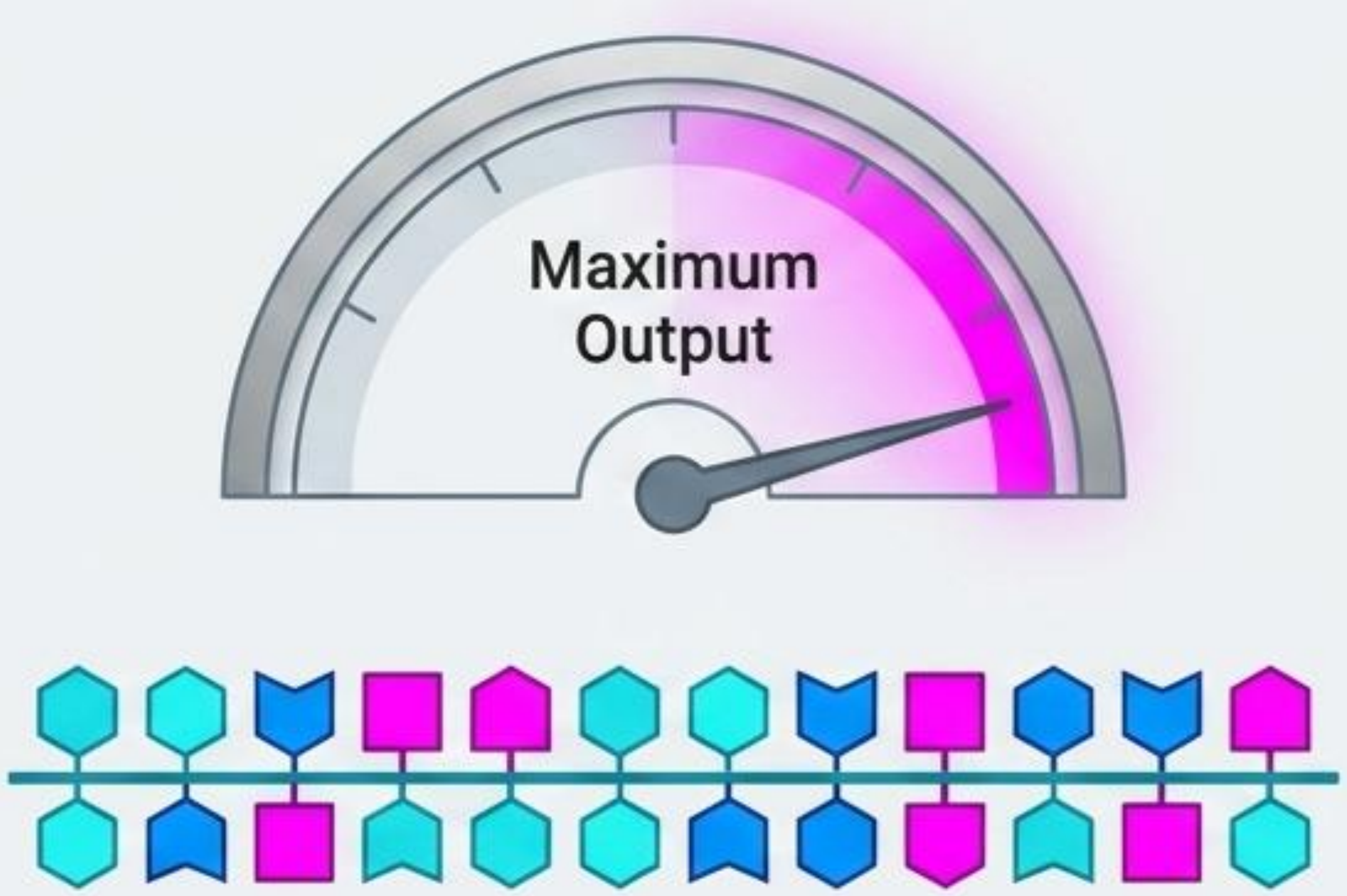
The Wild Type Fallacy

Nature uses sequences to regulate and restrict protein expression, conserving energy and preventing toxicity.



The Engineered Ideal

Therapeutics require maximum output. Relying on genomic wild-type sequences clinically guarantees sub-optimal protein yield, unwanted immune reactivation, and unstable secondary structures.



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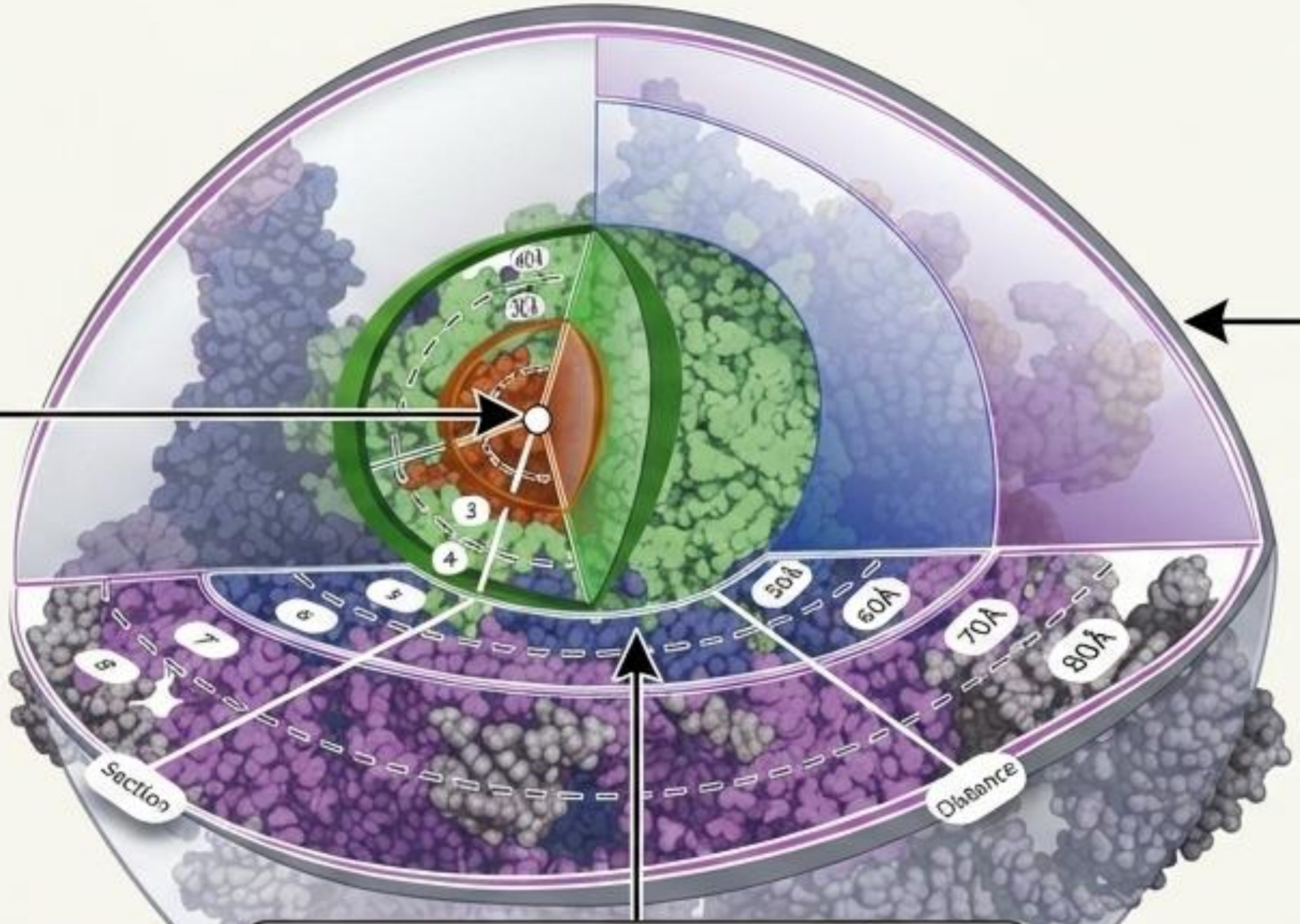
Peeling the Ribosomal Onion

The translation machinery is an ancient molecular fossil that evolved in layers over billions of years. The ancient core was never designed for modern, high-throughput translation.

PT-Origin (0Å)
The ancient catalytic core. Rigid, unmodified, and primarily stabilized by ancient RNA interactions.



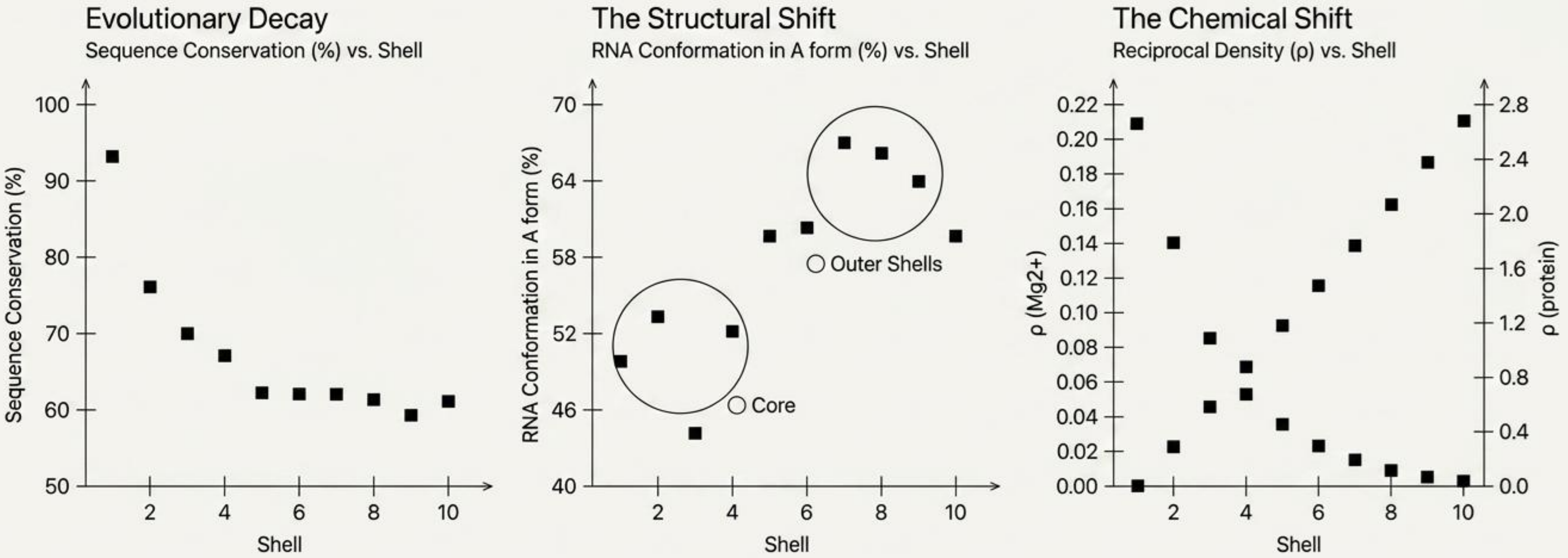
Outer Shells (50Å+)
Late-stage evolutionary accretions dominated by modern protein scaffolding.

Inner Shells (10-40Å)
Fossilized biological architecture with increasing structural complexity.

Hsiao and Williams, Mol Bio and Evol, 2009

Anatomy of a Molecular Fossil

Sequence conservation and structural stability decay outward from the Peptidyl Transferase origin.



Conclusion: RNA sequence engineering is the science of mathematically navigating this shifting, uneven mechanical landscape.

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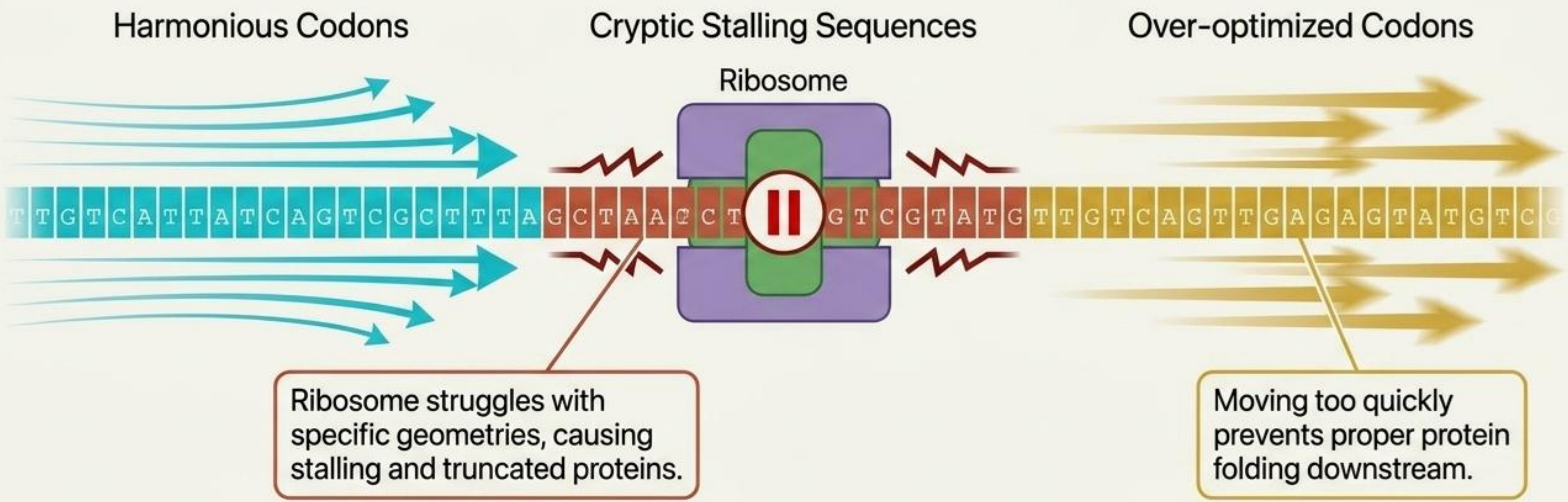
The Ramifications for Translation

The layered evolution of the ribosome creates severe mechanical biases.



Because of its ancient, rigid accretion, the ribosome cannot process all sequences equally.
Sequence engineering forces a negotiated pace.

Linear RNA: The Three Pillars of Optimization

Modern engineering attempts to force wild-type sequences to perform therapeutically by targeting three distinct metrics.

1. Stability

Tweaking G/C content to strengthen the RNA's secondary structure, drastically extending its half-life and preventing rapid degradation inside the cell.

2. Expression

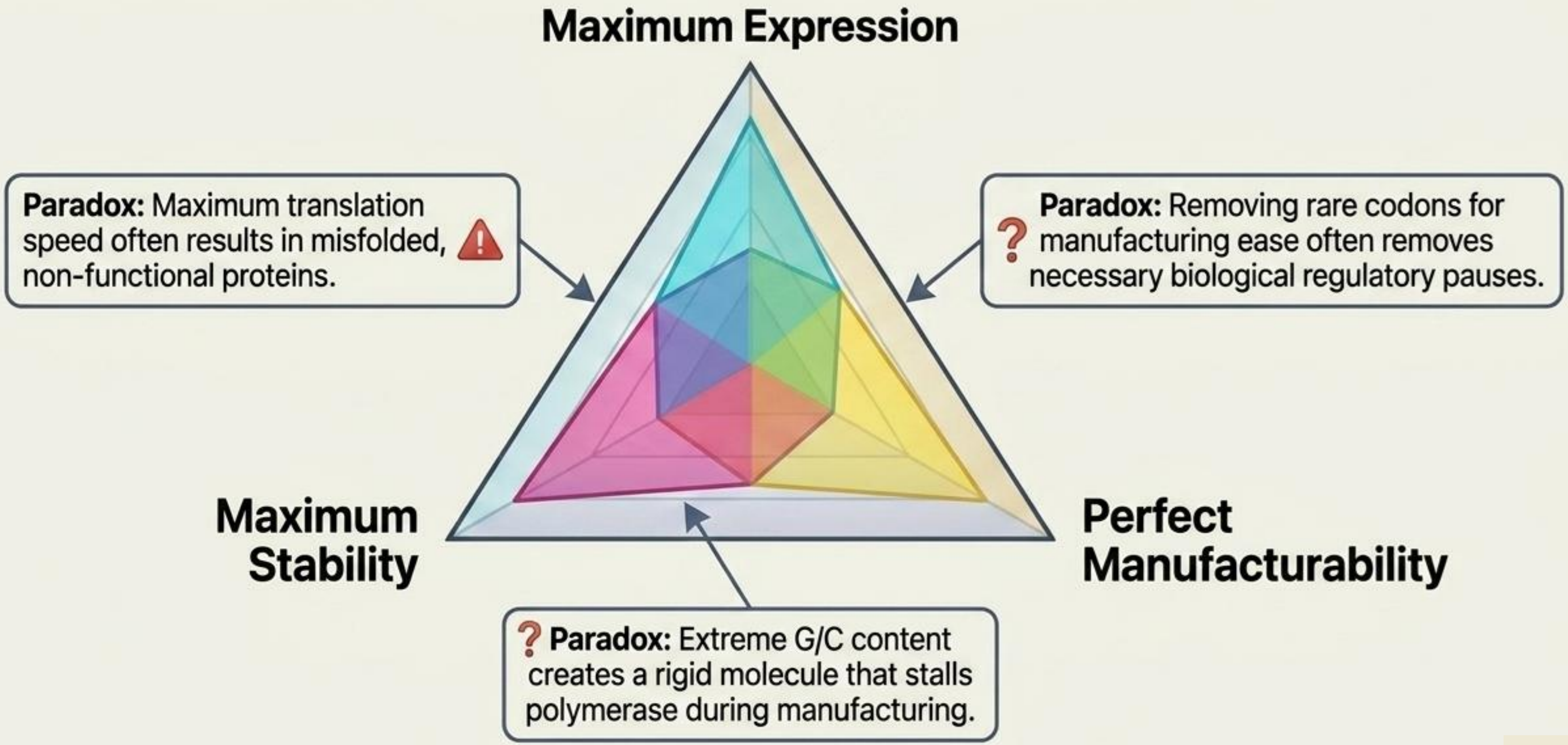
Matching codons to the most abundant tRNAs in the specific target tissue. This minimizes ribosome stalling and maximizes the yield of therapeutic protein.

3. Manufacturability

Identifying and removing repeating loops, extreme secondary structures, and cryptic sequences that cause polymerase enzymes to stutter or detach during In Vitro Transcription (IVT).

Linear RNA and the Optimization Paradox

Codon optimization is a multi-dimensional balancing act, forcing a hard ceiling on linear mRNA capabilities.



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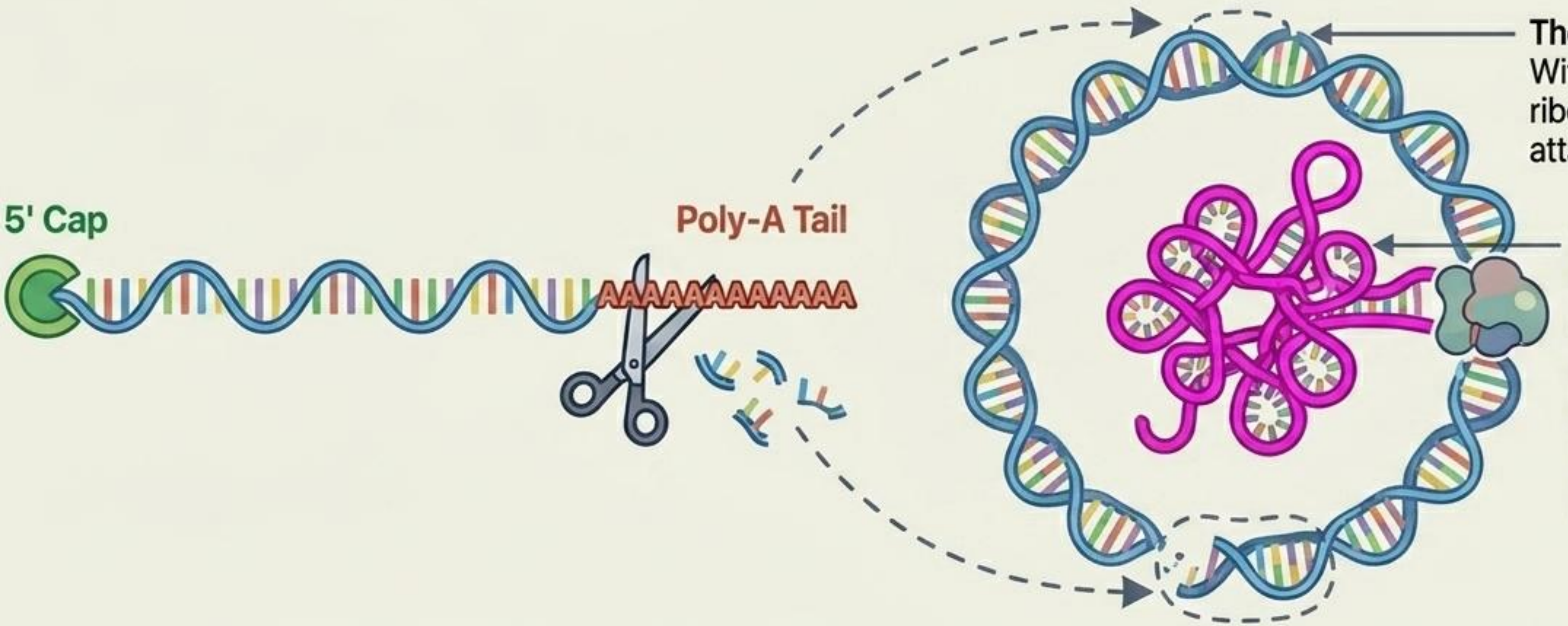
Architectural Shifts: The Circular RNA (circRNA) Paradigm

When linear optimization hits physical limits, engineers must fundamentally change the shape of the molecule.

The Linear Vulnerability

The Circular Solution



The Translation Barrier: Without a 5' cap, the ribosome cannot attach.

The Engineering Fix: Insertion of a highly complex, synthetic Internal Ribosome Entry Site (IRES) to trick the ribosome into binding directly to the circle.

Natural degradation pathways attack the exposed ends, rapidly breaking down the molecule.

By removing the ends, the molecule bypasses natural degradation, granting immense longevity.

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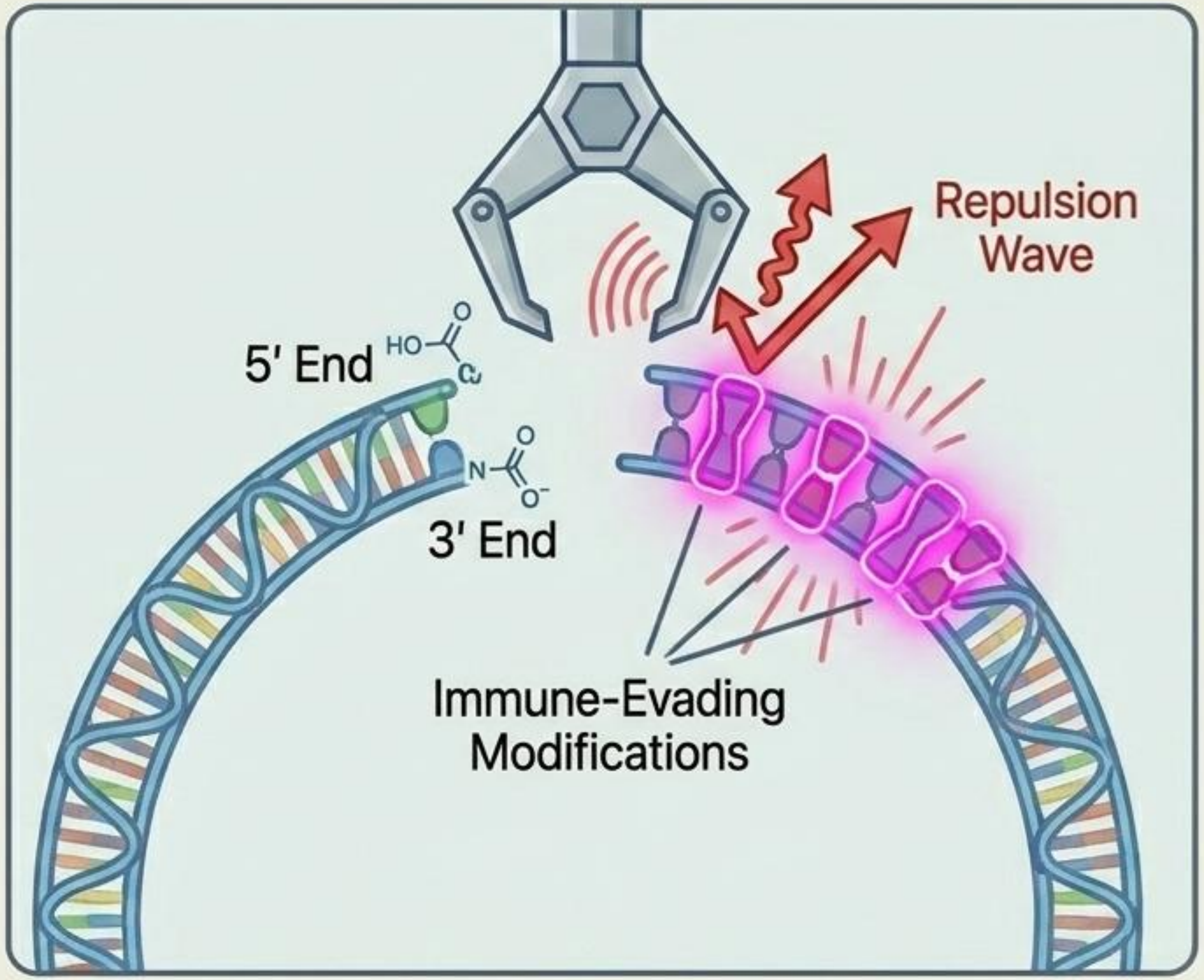
Circular RNA: The Upstream CMC Barrier

The Modification Tolerance Gap forces a choice between manufacturing efficiency and therapeutic stealth.

Manufacturing requires joining linear ends using ligase enzymes. However, these enzymes possess incredibly low tolerance for the modified nucleotides required to hide RNA from the patient's immune system.

Result: Developers must sacrifice immune stealth to achieve functional manufacturing yields.



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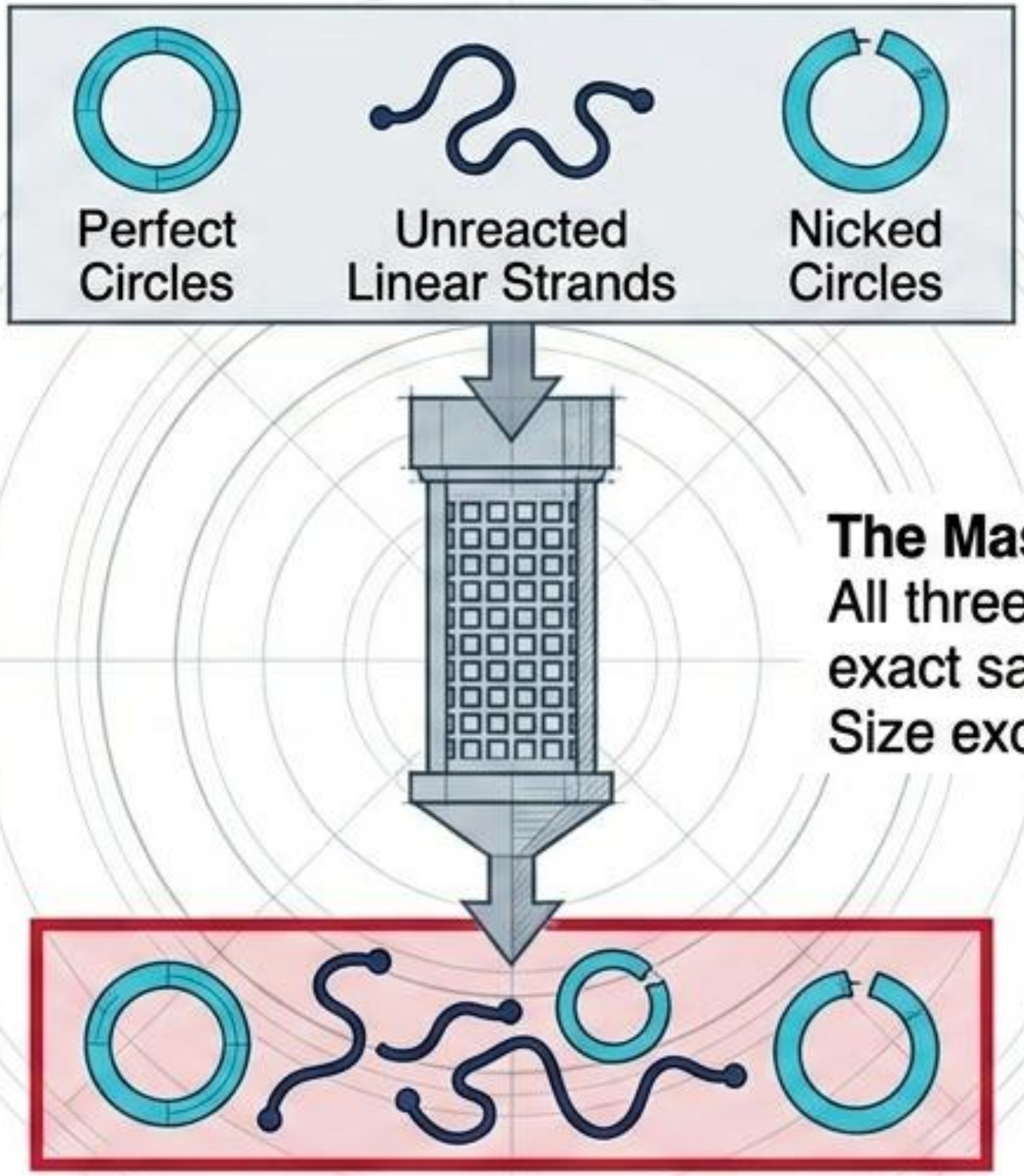
The Downstream Separation Nightmare

Input Mix



The Mass Paradox:
All three molecules share the exact same molecular weight. Size exclusion completely fails.

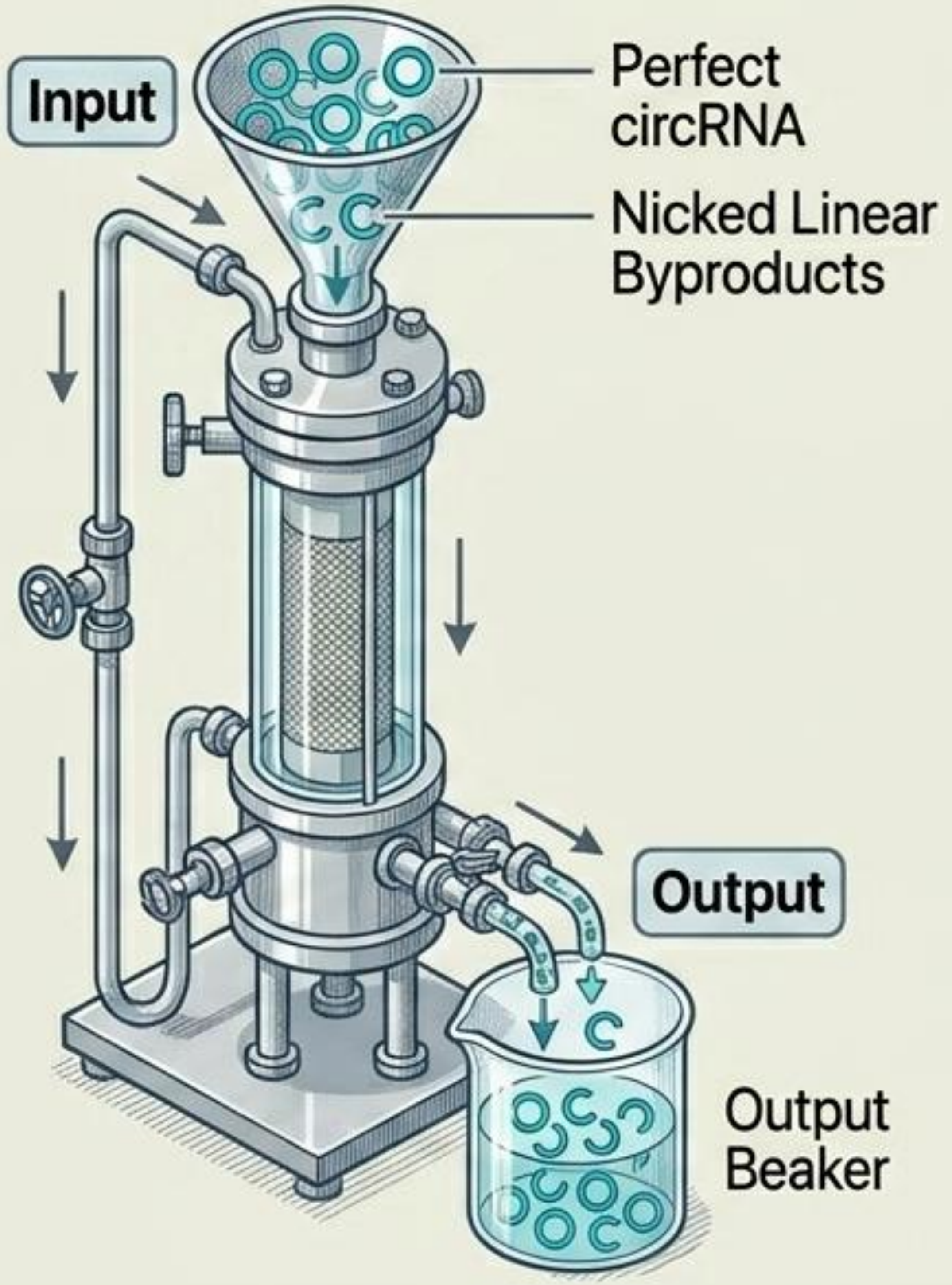
The immune system is hyper-sensitive to linear byproducts, requiring near-perfect purity. Because size exclusion is impossible, separating identical-mass molecules relies on subtle topological differences via slow, expensive chromatography and enzymatic polishing.

Leads to massive yield losses of 30%+ from downstream separation alone.

Circular RNA: The Downstream Separation Nightmare

Industrial purification is the primary bottleneck for circular constructs.

- Circularization reactions are never 100% efficient.

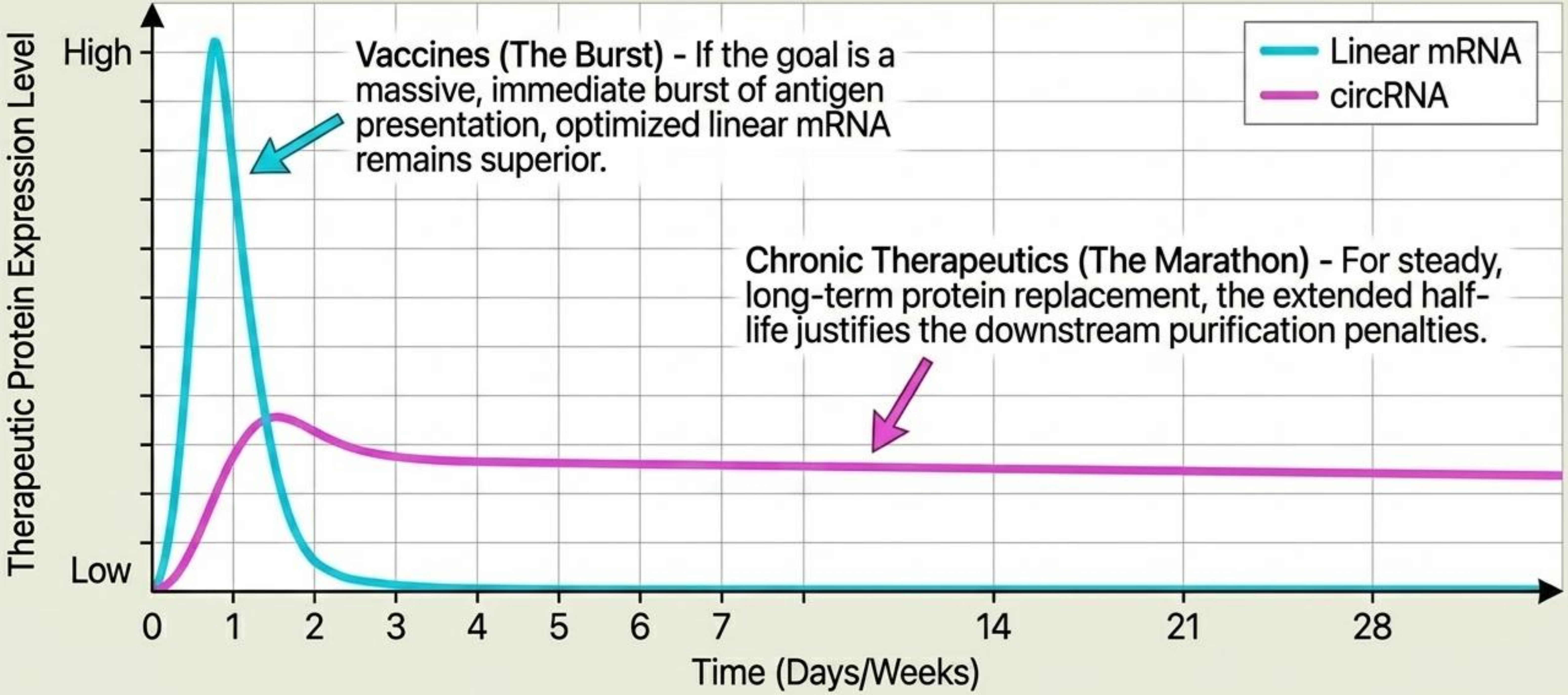


The immune system is hyper-sensitive to linear byproducts. However, separating a completed circle from a 'nicked' linear strand of the exact same molecular weight is mathematically and physically excruciating.

- **Result:** Requires extremely slow, costly chromatography and enzymatic polishing, routinely driving yield losses above 30%.

Assessing Modalities: Does Circularity Matter?

Balancing the manufacturing penalty against the clinical objective.



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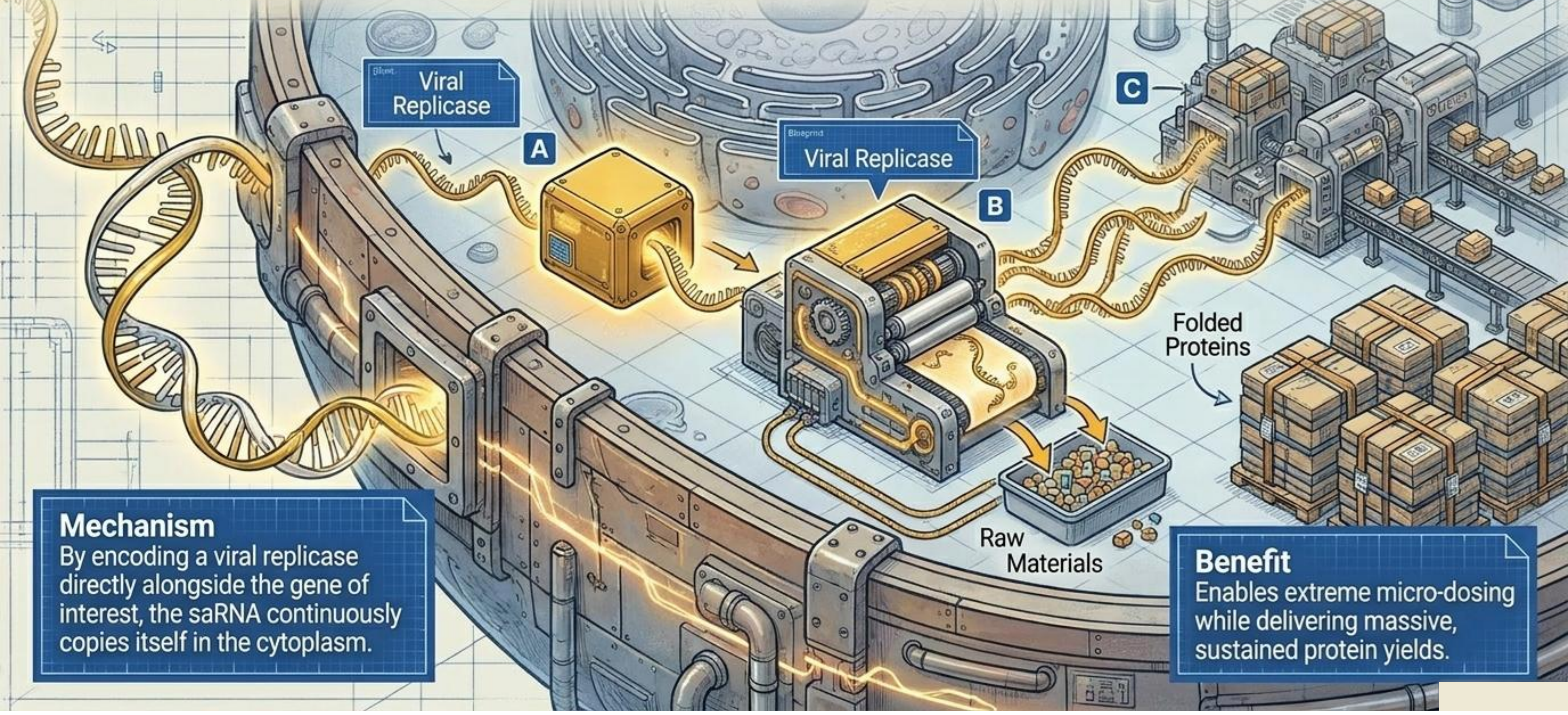
Self-Amplifying RNA (saRNA): The Cellular Factory

Turning the patient's own cell into an autonomous biomanufacturing plant.



Mechanism

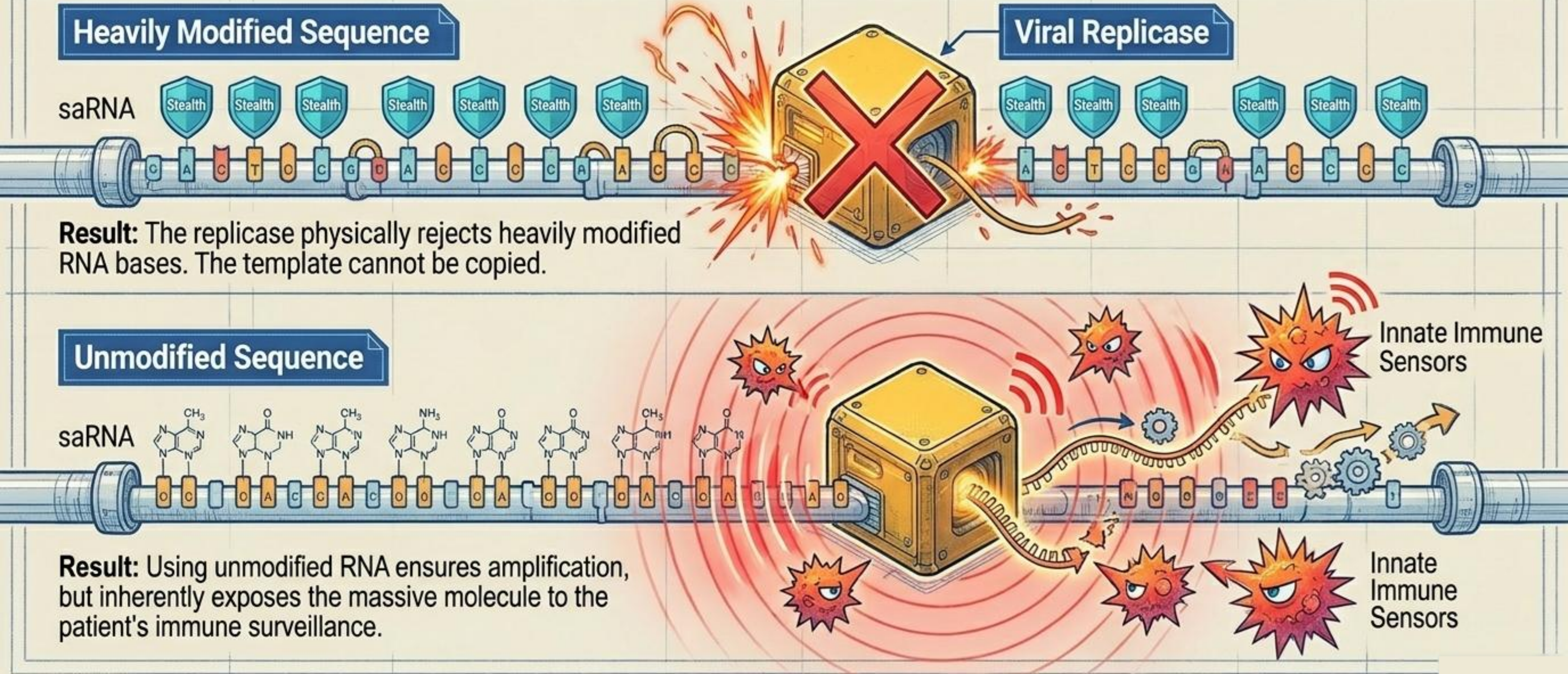
By encoding a viral replicase directly alongside the gene of interest, the saRNA continuously copies itself in the cytoplasm.

Benefit

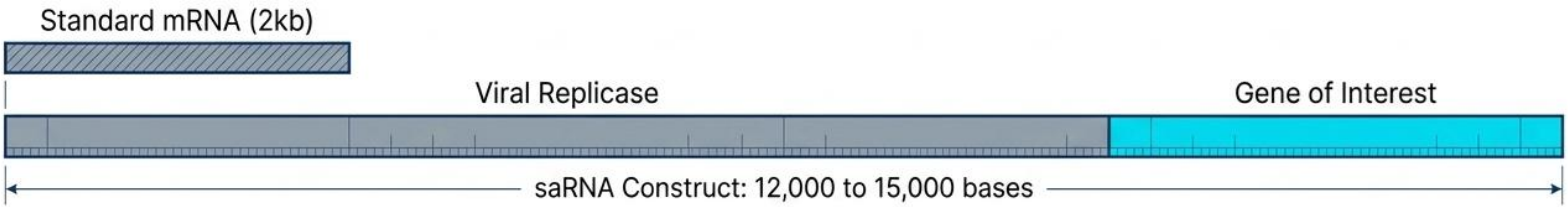
Enables extreme micro-dosing while delivering massive, sustained protein yields.

saRNA Chemical Barrier: The Replicase Tolerance Trap

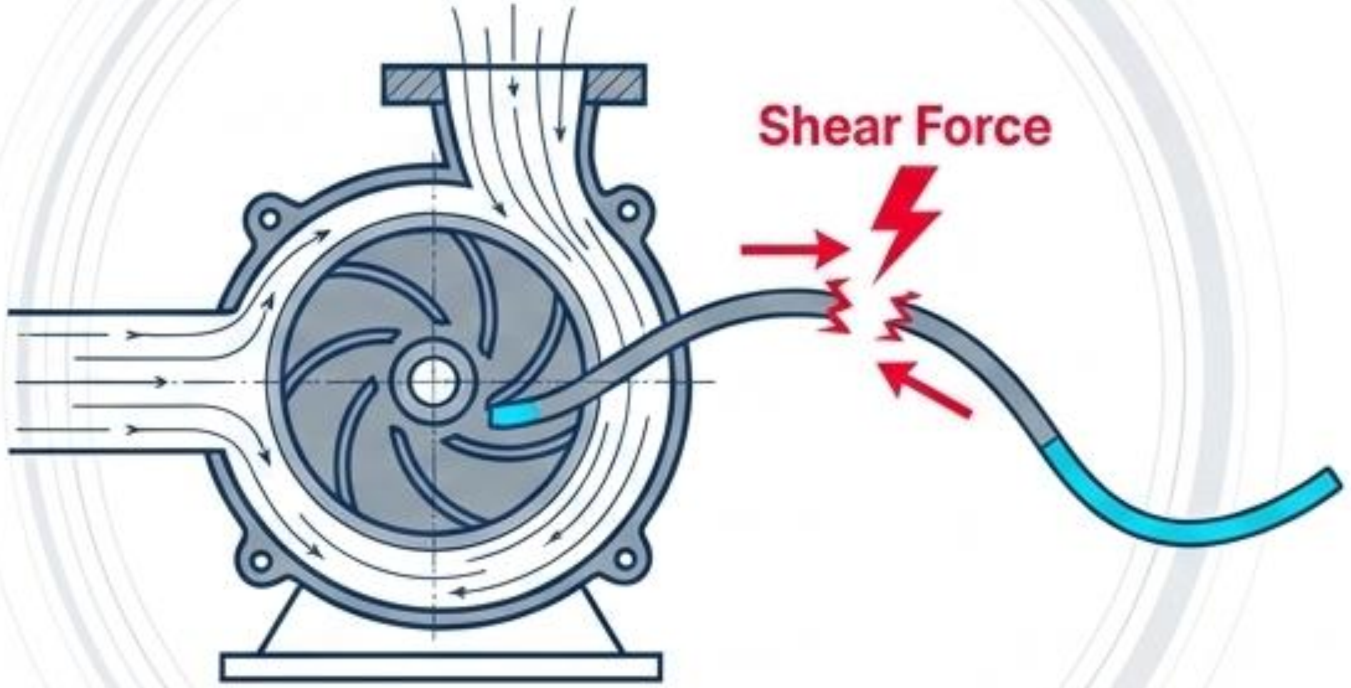
The viral replicase suffers from a modification tolerance gap with immediate immunological consequences.



saRNA and the 15kb Physical Barrier

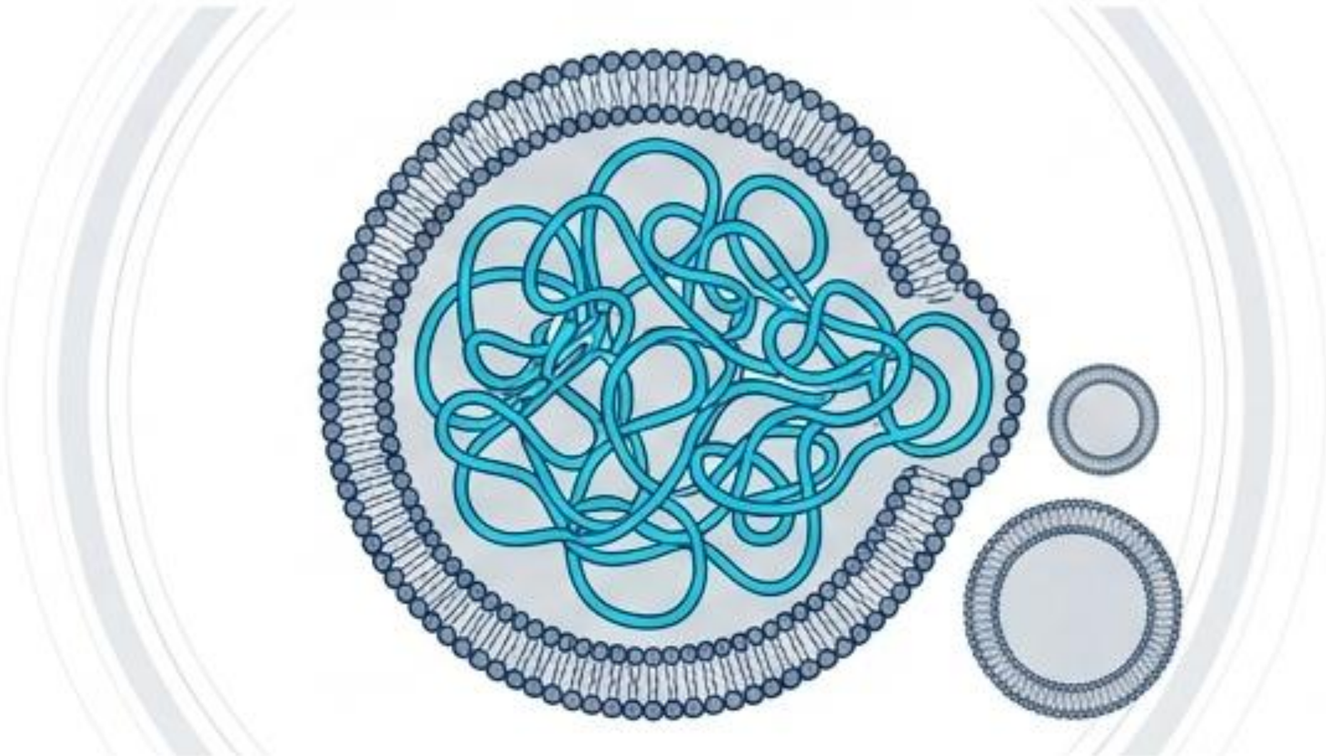


The Manufacturing Breakpoint



Standard mixing and high-pressure pumping literally snap the ultra-fragile 15kb molecule in half.

The Delivery Breakpoint



Inefficient LNP packaging of massive cargo yields aggregated particles or entirely empty lipid shells.

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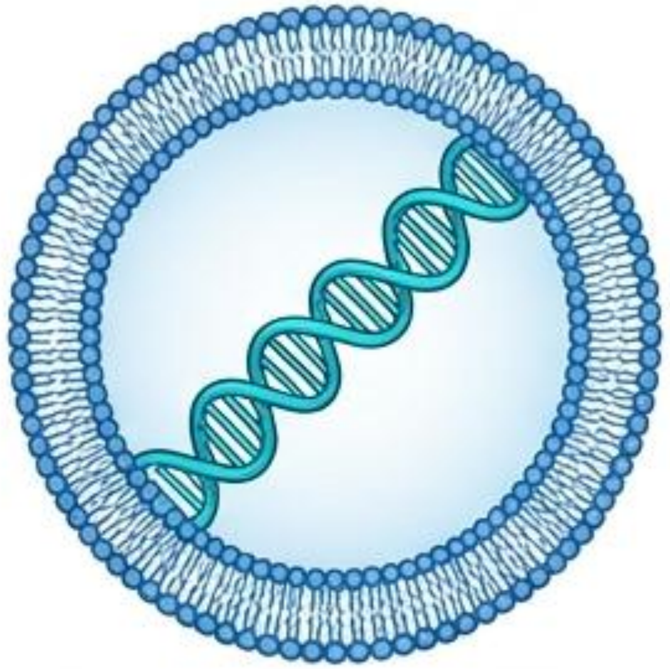
saRNA Physical Barrier: The 15-Kilobase Limit

At extreme lengths, the physical dynamics of the molecule collapse during manufacturing.

Linear mRNA
(2kb Scale)

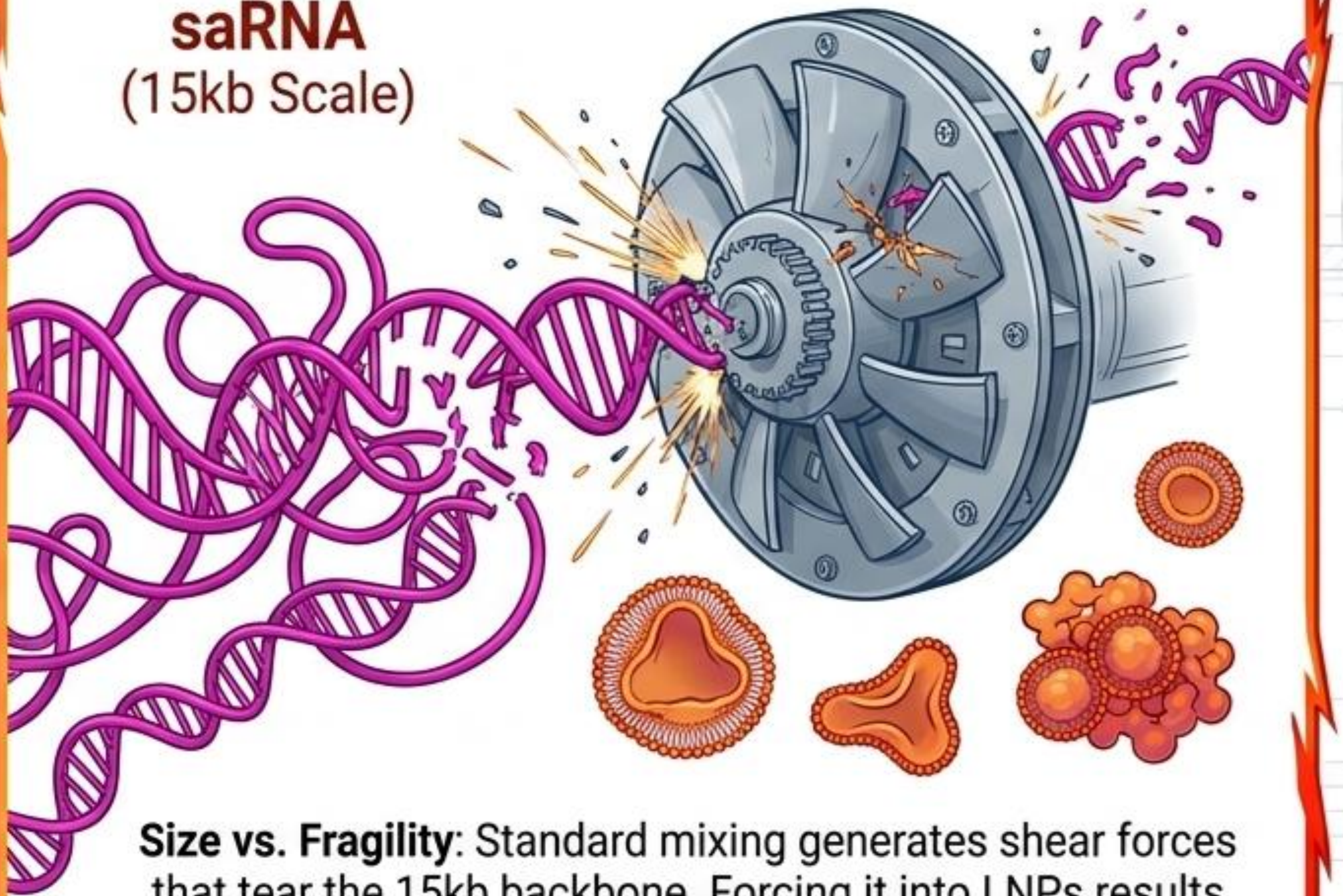


Standard encapsulation thermodynamics.

2kb

15kb
7.5x

saRNA
(15kb Scale)



Size vs. Fragility: Standard mixing generates shear forces that tear the 15kb backbone. Forcing it into LNPs results in empty shells and severe aggregation.

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saRNA: The Cellular Race

Clinical success relies entirely on the engineered RNA outpacing the immune system.

The Viral Illusion

Amplifying saRNA produces double-stranded intermediates. The cell interprets this as an active viral infection and triggers a catastrophic Type I Interferon response.

The Goal

The saRNA must produce enough therapeutic protein before the cell chemically shuts down all local translation to starve the perceived virus.

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Architectural Trade-Offs: The Modality Matrix

Diagnostic comparison of RNA architectures across critical CMC and clinical dimensions.

Dimension	Linear mRNA	Circular RNA (circRNA)	Self-Amplifying (saRNA)
Construct Size	~2-4 kb	~2-4 kb	12-15 kb (Massive)
Expression Profile	Acute, massive burst	Low, extended plateau	High, sustained output
Upstream Bottleneck	Sequence secondary structure stability	Synthetic IRES design & ligation efficiency	Replicase modification intolerance
Downstream / Physical Bottleneck	Poly-A tail integrity	Separating exact-weight linear byproducts	Shear-force degradation & empty LNP shells

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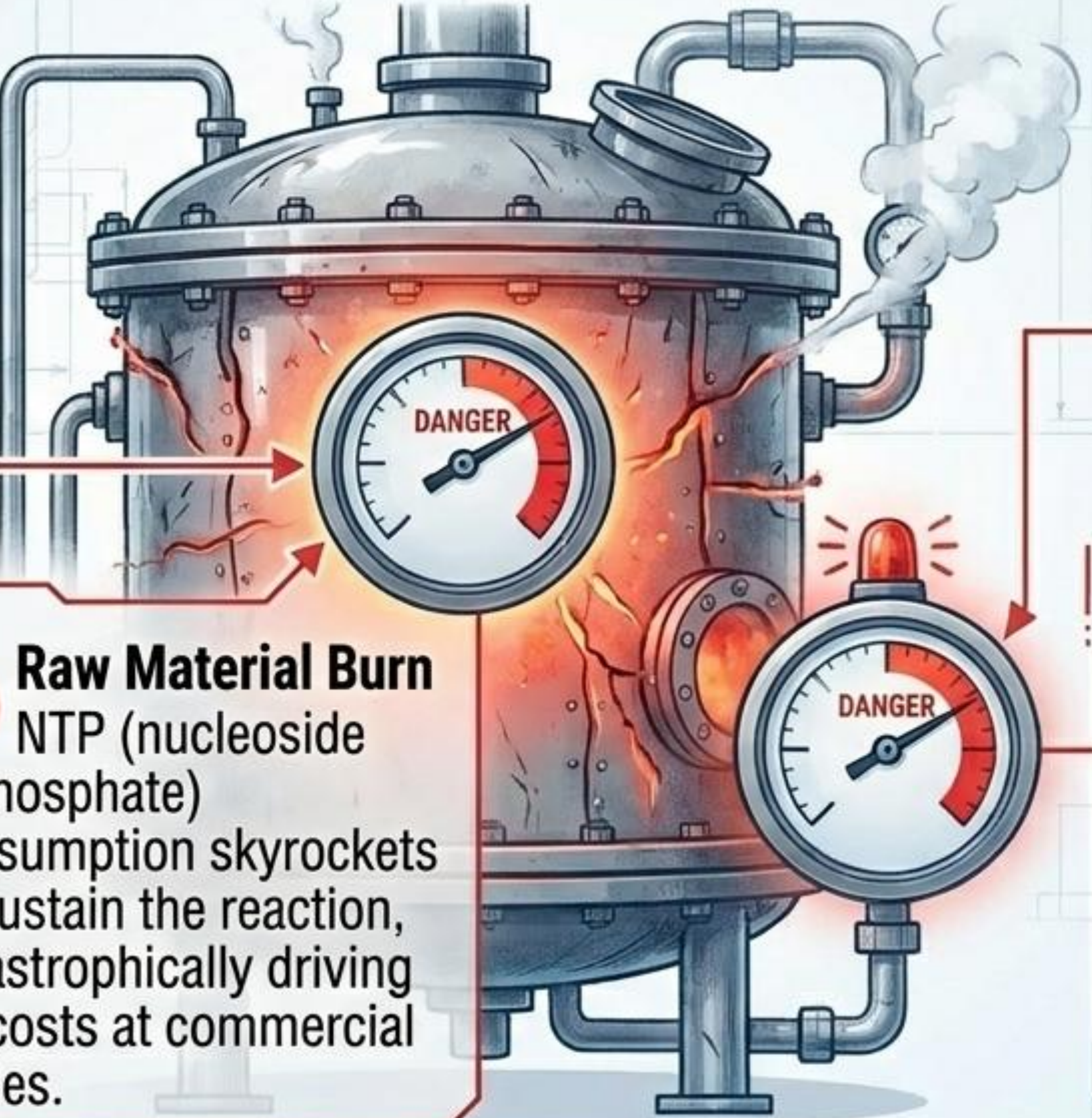
Scaling the Impossible: The Collapse of Batch IVT

Standard legacy bioprocessing is the fundamental bottleneck to commercial scale.



Yield Degradation

Polymerase efficiency drops exponentially on 15kb templates. The enzyme simply falls off the strand before finishing.

Raw Material Burn

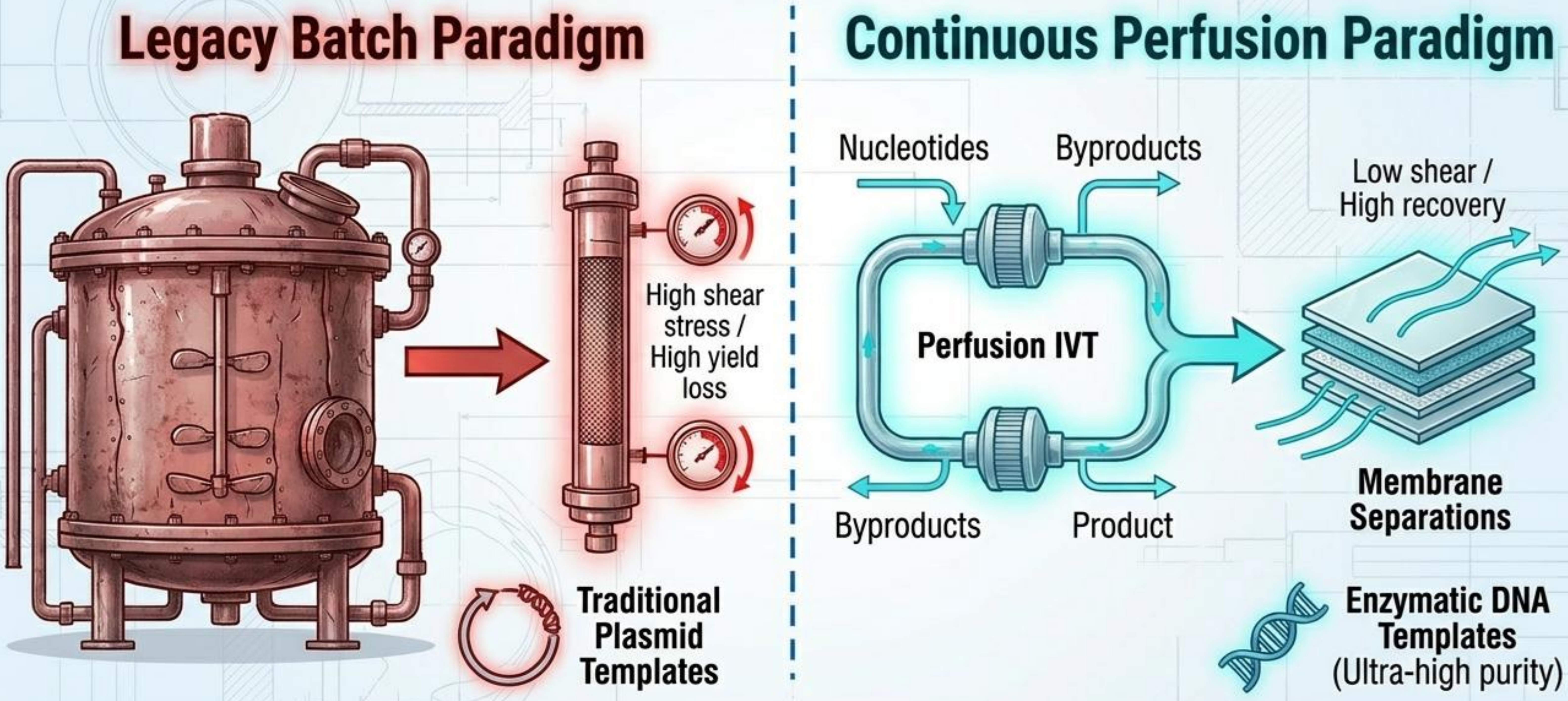
NTP (nucleoside triphosphate) consumption skyrockets to sustain the reaction, catastrophically driving up costs at commercial scales.

Mechanical Limits

The strict requirement
The strict requirement to eliminate shear stress means standard high-pressure pumping and vigorous agitation must be abandoned, suffocating mass transfer in the tank.

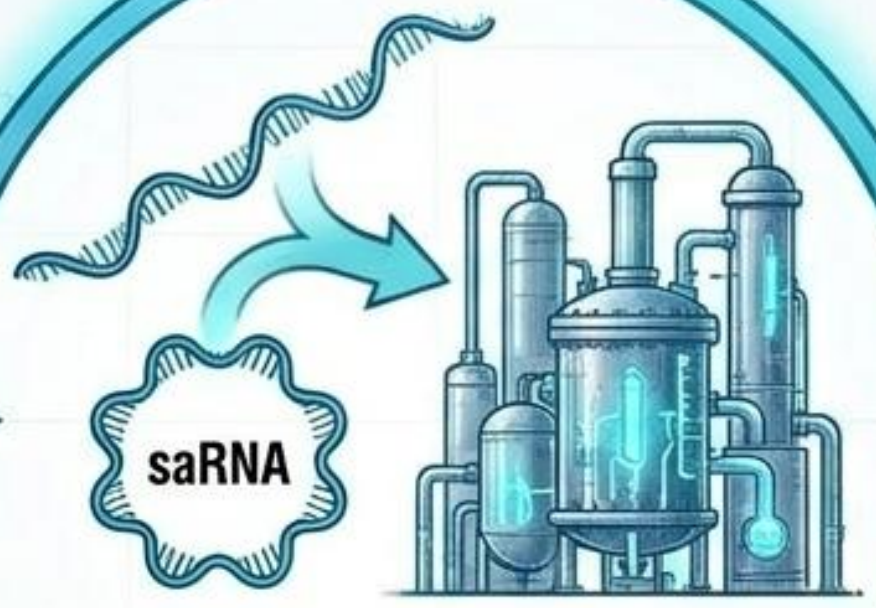
The Future Paradigm: Continuous Bioprocessing

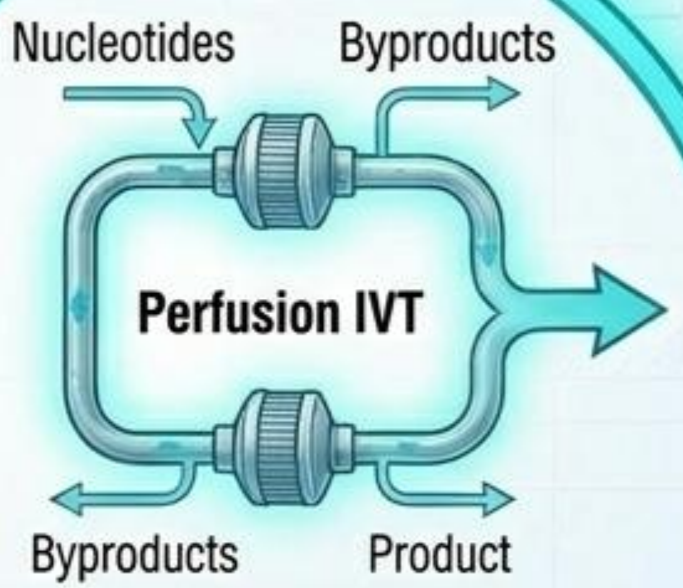
The road to commercial RNA therapeutics demands an entirely new industrial scale.



Synthesizing the RNA Engineering Revolution

From evolutionary constraints to industrial reality.





The Biological Baseline

We must map the mechanical biases of life's ancient molecular fossils to understand the limits of translation.

The Architectural Shift

When sequence optimization hits physical limits, we engineer novel shapes, accepting severe CMC trade-offs for clinical longevity.

The Scale-Up Paradigm

To deliver these fragile, massive molecules at commercial scale, we must abandon legacy batch reactors for continuous manufacturing.

The mg-to-kg journey requires the combined expertise of structural biology, sequence engineering, and advanced bioprocessing.

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THANK YOU

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