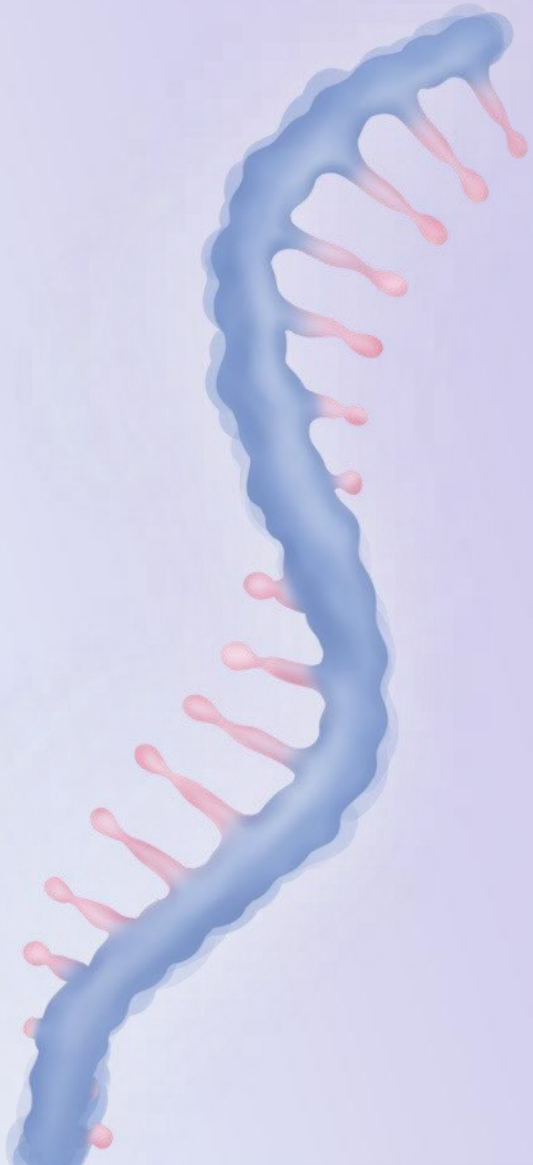

Designing and Modeling Regulatory Mechanisms for Structurally Interacting RNA (sxRNA)

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Professor Daniel Aalberts

01

Motivation

Creating mechanisms for specificity in RNA-based therapeutics

What are RNA-based therapeutics?

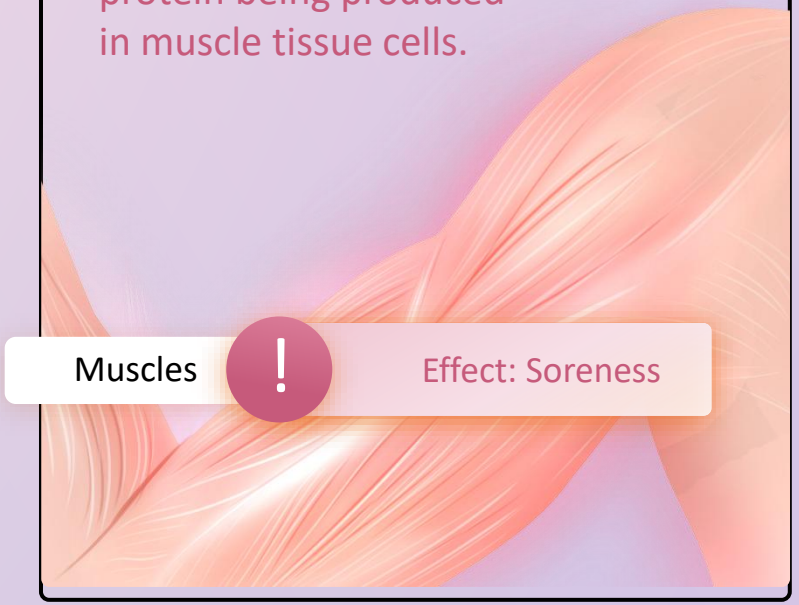
- RNA-based therapeutics act as blueprints, directing cells to produce specific proteins that function as drugs.
- These therapeutics are typically aimed at specific cell types, such as cancer cells in the lung or liver. Expression in non-target regions can have adverse side effects.

Example:

Covid Vaccine

The primary target for mRNA-based COVID vaccines are specific immune cells, called dendritic cells, which cannot be specifically targeted. Much of the Spike protein is therefore expressed in muscle and connective tissue, reducing effectiveness.

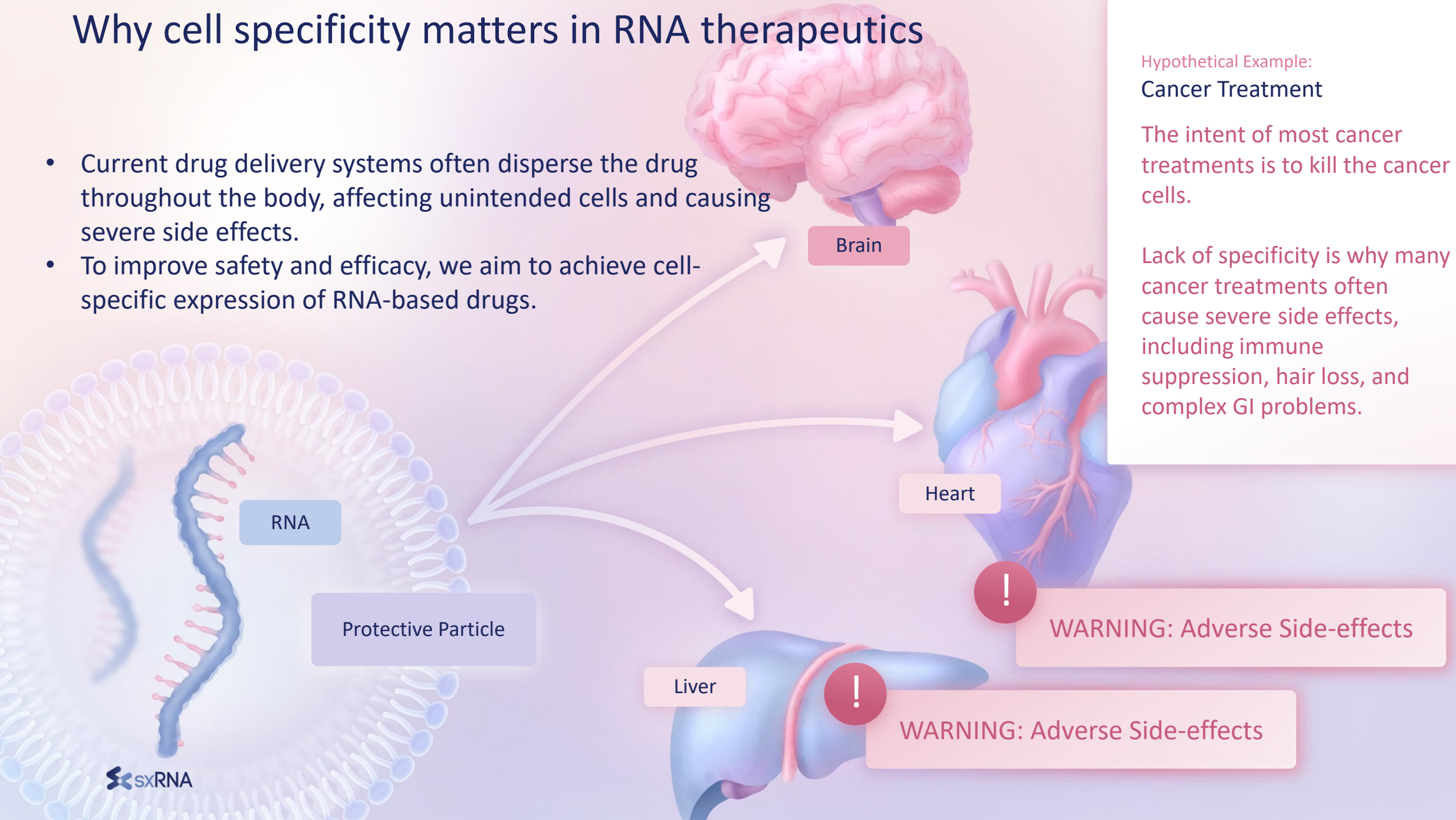
The muscle soreness many experienced results from the Spike protein being produced in muscle tissue cells.



sxRNA

Why cell specificity matters in RNA therapeutics

- Current drug delivery systems often disperse the drug throughout the body, affecting unintended cells and causing severe side effects.
- To improve safety and efficacy, we aim to achieve cell-specific expression of RNA-based drugs.



Hypothetical Example:

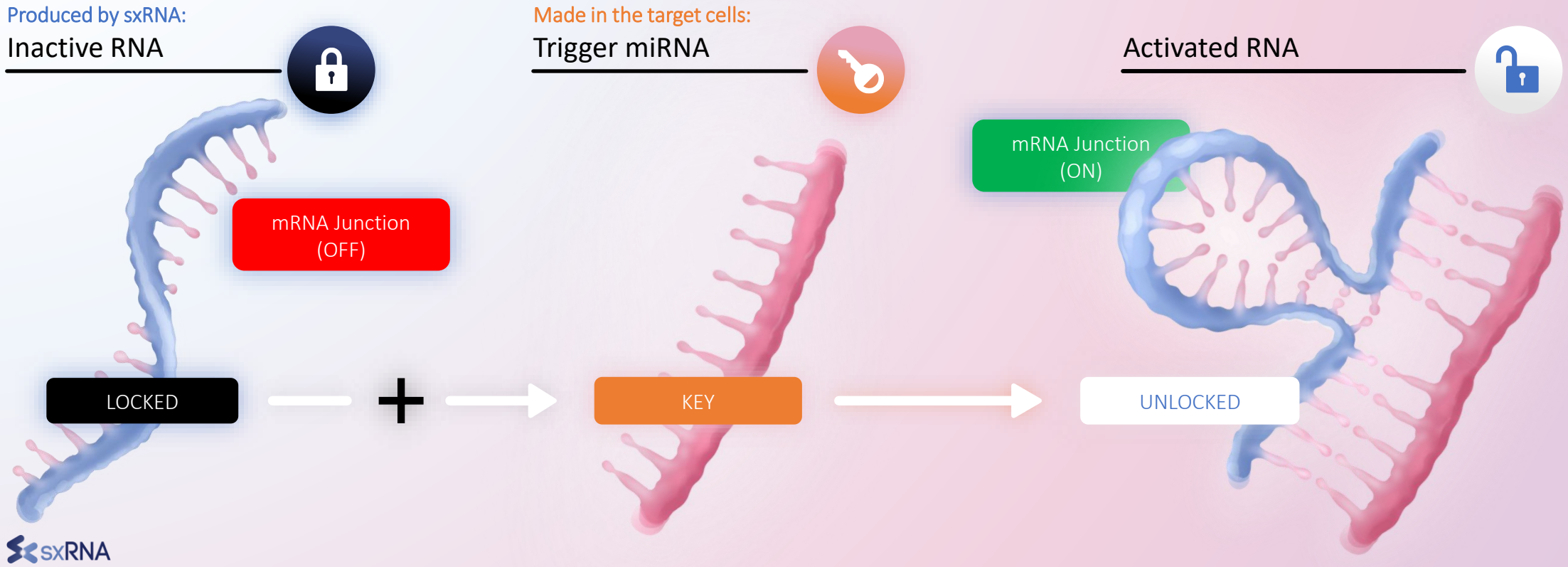
Cancer Treatment

The intent of most cancer treatments is to kill the cancer cells.

Lack of specificity is why many cancer treatments often cause severe side effects, including immune suppression, hair loss, and complex GI problems.

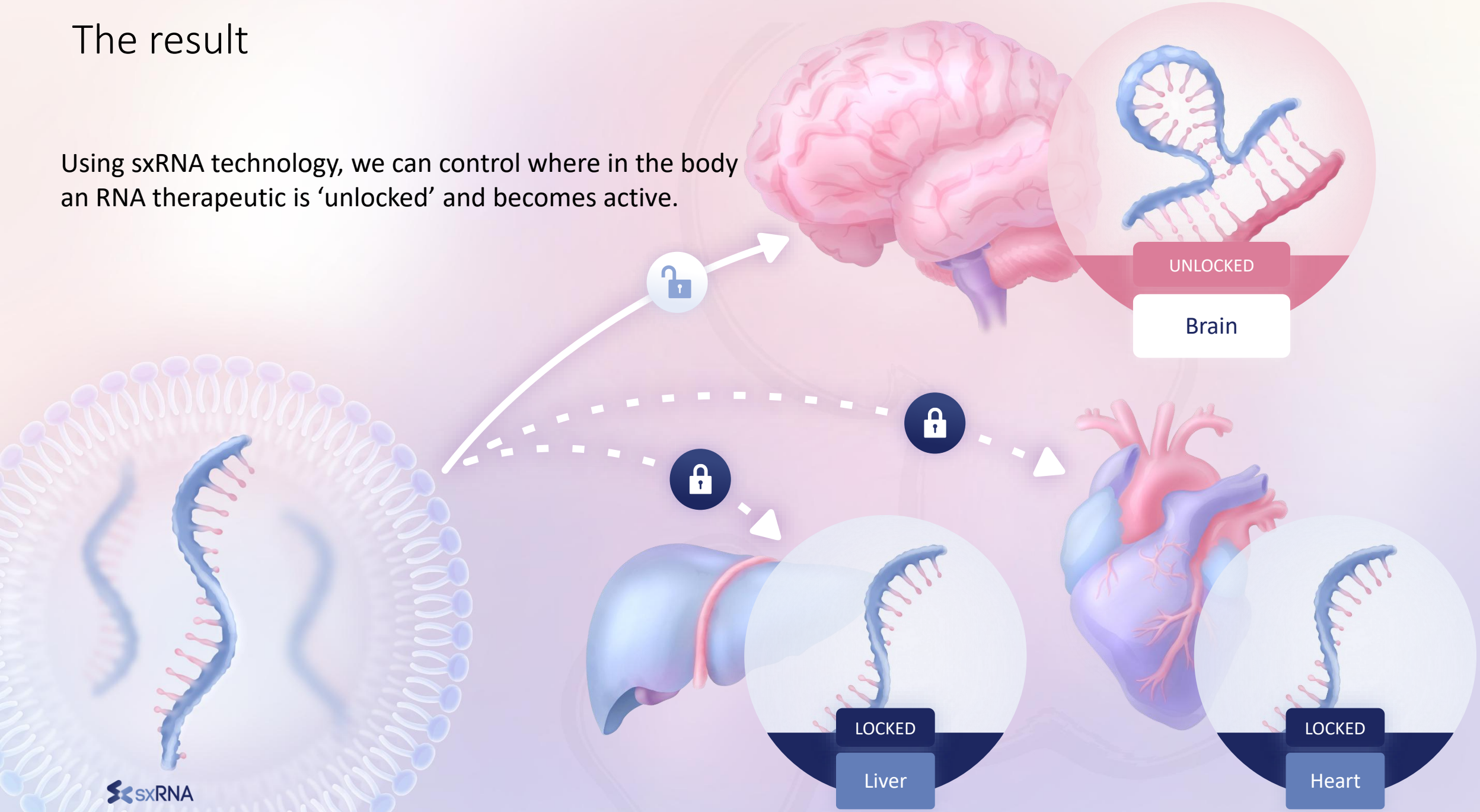
sxRNA – a key and lock mechanism for cell-specificity

- sxRNA is a novel technology developed by our experimental partners, **sxRNA Technologies Inc.**, to LOCK and UNLOCK RNA therapeutics for cell-specific activity.
- The technology uses two RNA components: an **mRNA junction** or "Lock“ embedded in the therapeutic and a **miRNA** "Key" naturally present in the target cells.
- The drug becomes active only when the mRNA Junction and miRNA interact, ensuring precise activation in specific cells.



The result

Using sxRNA technology, we can control where in the body an RNA therapeutic is ‘unlocked’ and becomes active.



Potential applications

- Because there are unique RNA signatures (keys) found in every cell type, there exists an opportunity to utilize sxRNA as a platform for a multitude of diseases.

- The 2024 Nobel Prize in Medicine went to Victor Ambros and Gary Ruvkun for the discovery of miRNA and their role in post-transcriptional gene regulation!

Tissues:

- Brain: miR-9
- Thyroid: miR-135a
- Esophagus: miR-203
- Heart: miR-208
- Kidney: miR-204
- Stomach: miR-188
- Liver: miR-122
- Pancreas: miR-375, 148a
- Colon: miR-192, 194, 215
- Muscle: miR-1, 133a/b
- Epithelial: miR-200c
- Lymph node: miR-150, 142-3p

Cancers:

- Esophagus: miR-373
- Breast: miR-373, 520c
- Lung: miR-183
- Pancreatic: miR-301, 376a
- Testicular: miR-372, 373
- B-cell lymphomas: miR-17
- Neuroblastomas: miR-380

sxRNA

02

Where we come in

Theoretical considerations for engineering sxRNA

Our goals

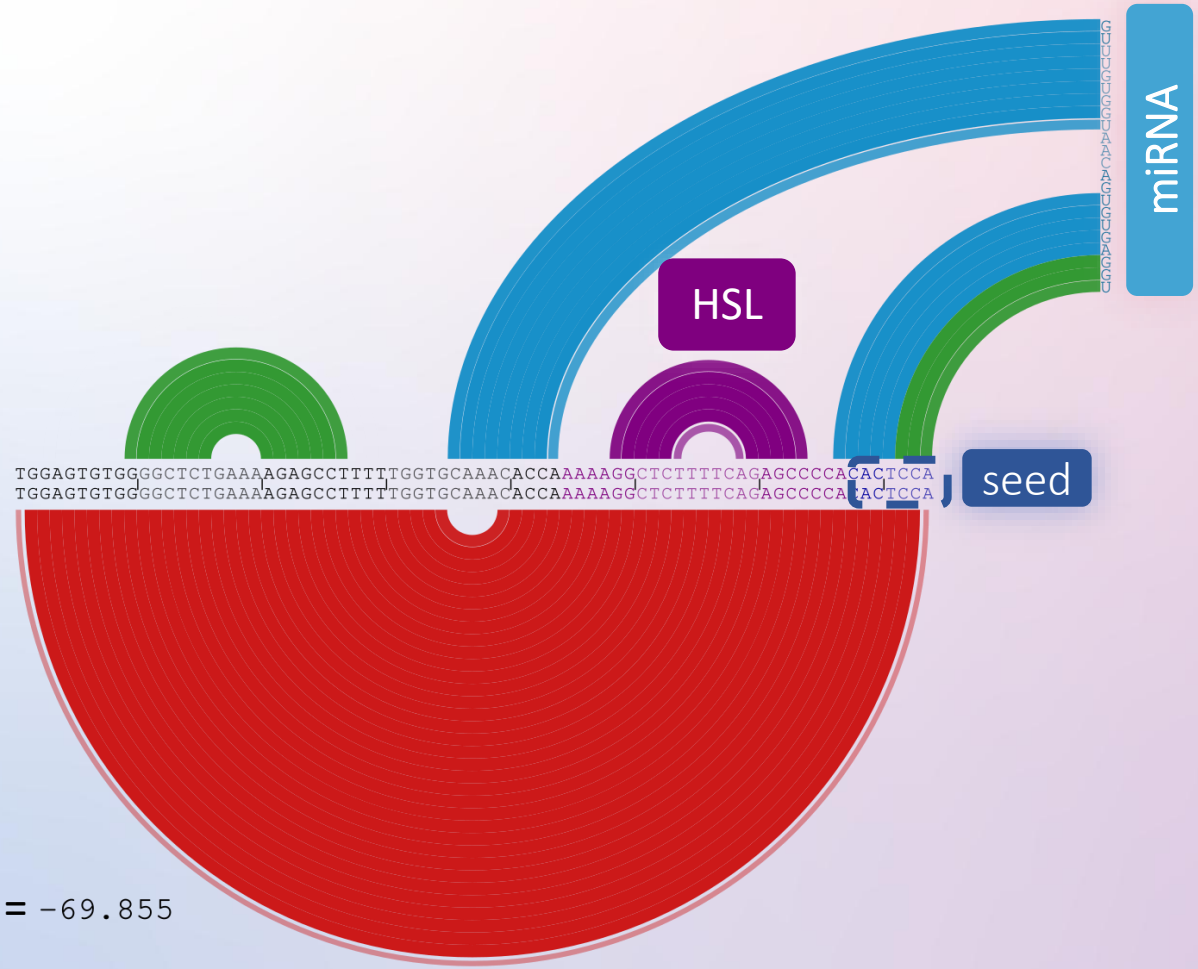
Designing a ‘lock and key’ mechanism that

- Maximizes the free energy difference between the OFF and ON states to ensure smooth transition.
- Ensures that “leaky” intermediate states (where the mRNA junction is active without the **key** miRNA) are as energy unfavorable as possible.

Bow Diagrams – A Method to Visualize Base Pairings

Free energy: $G_{A\mu} = -50.0431$

ON: $G_{A\mu}$



≈

OFF: G_B

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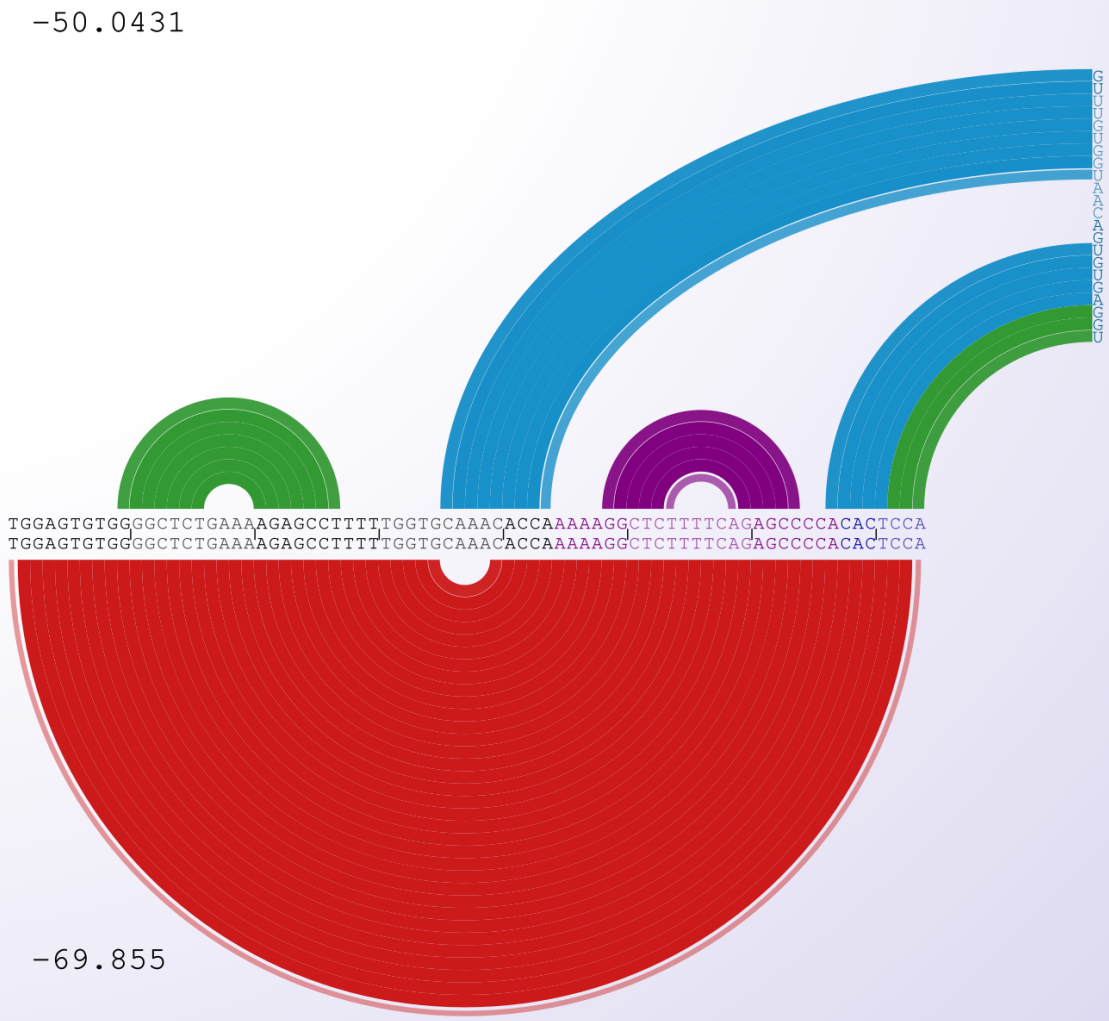
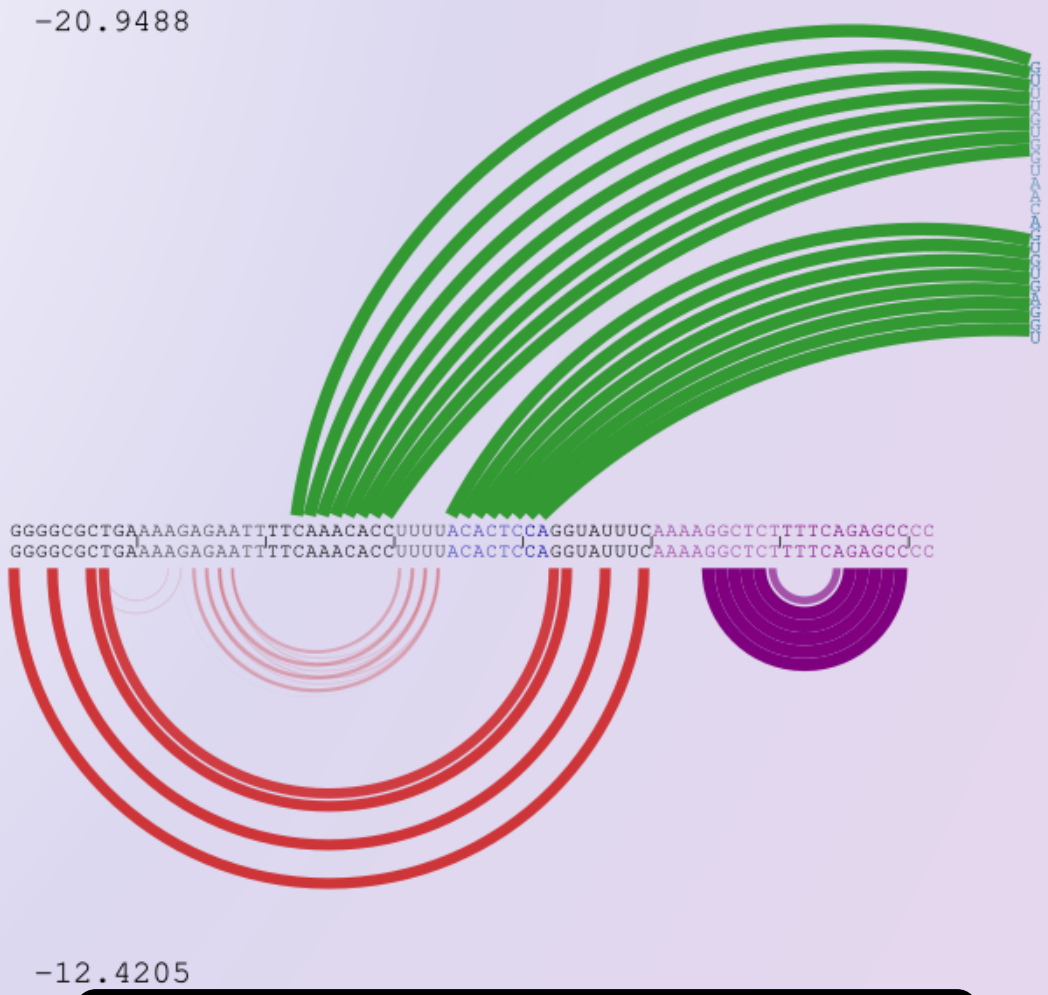
Hairpin loop

Free energy: $G_B = -69.855$

Work started by Elena Deegan-Krause (‘26)

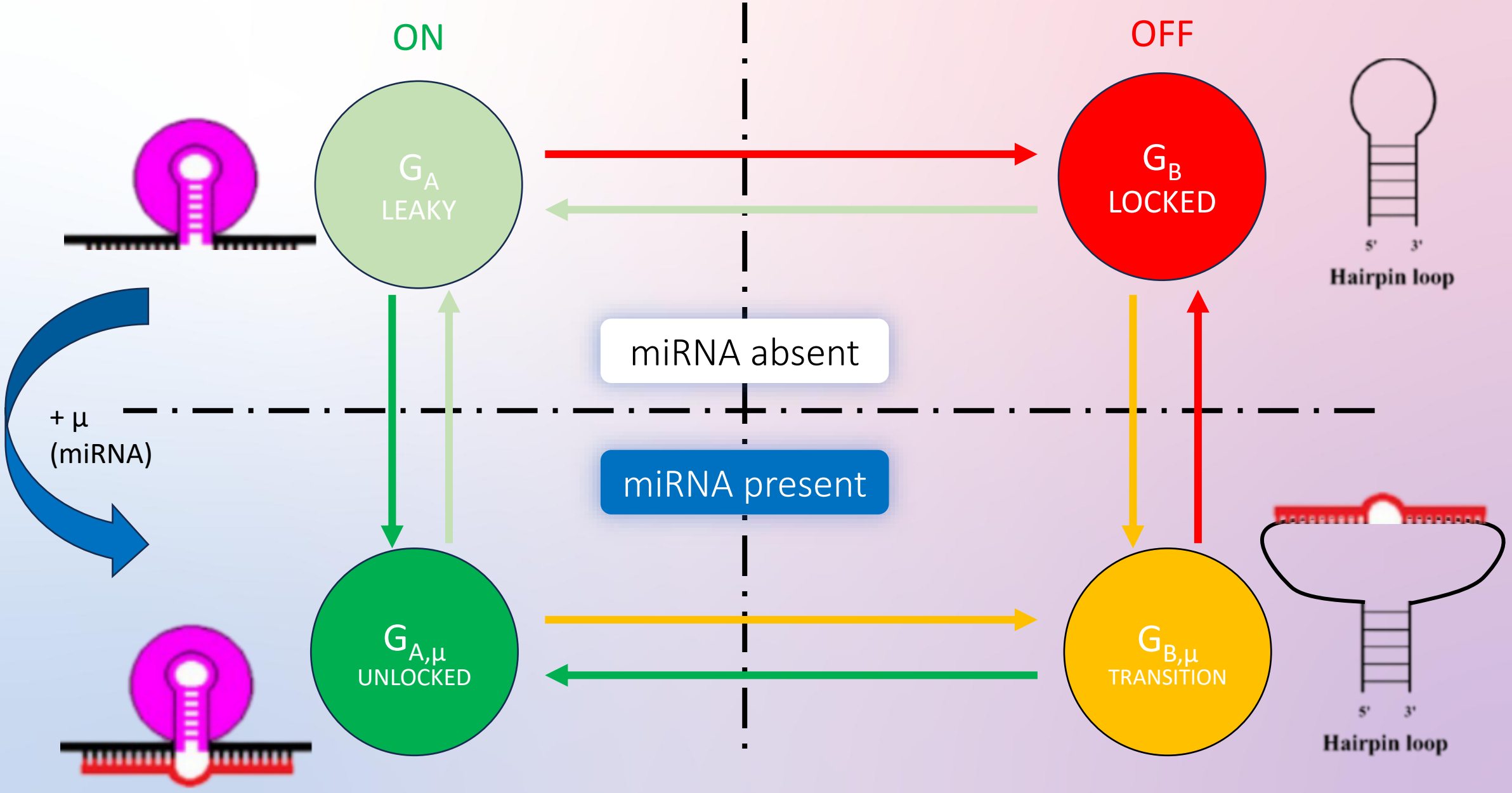


3WJ – first design for sxRNA

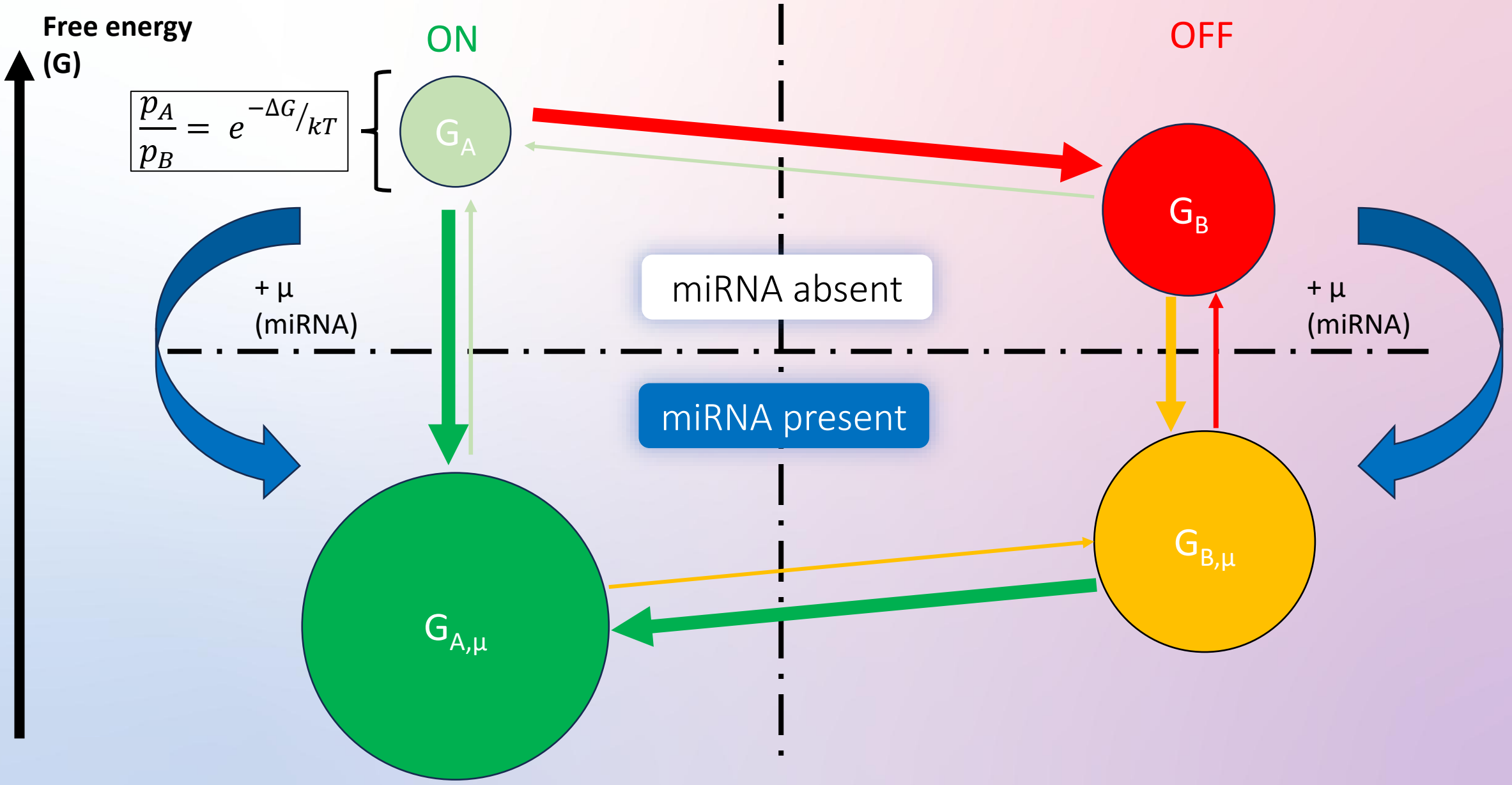


Design 2 – seed region placed in the hairpin of mRNA loop

Competing States in Thermodynamic Equilibrium



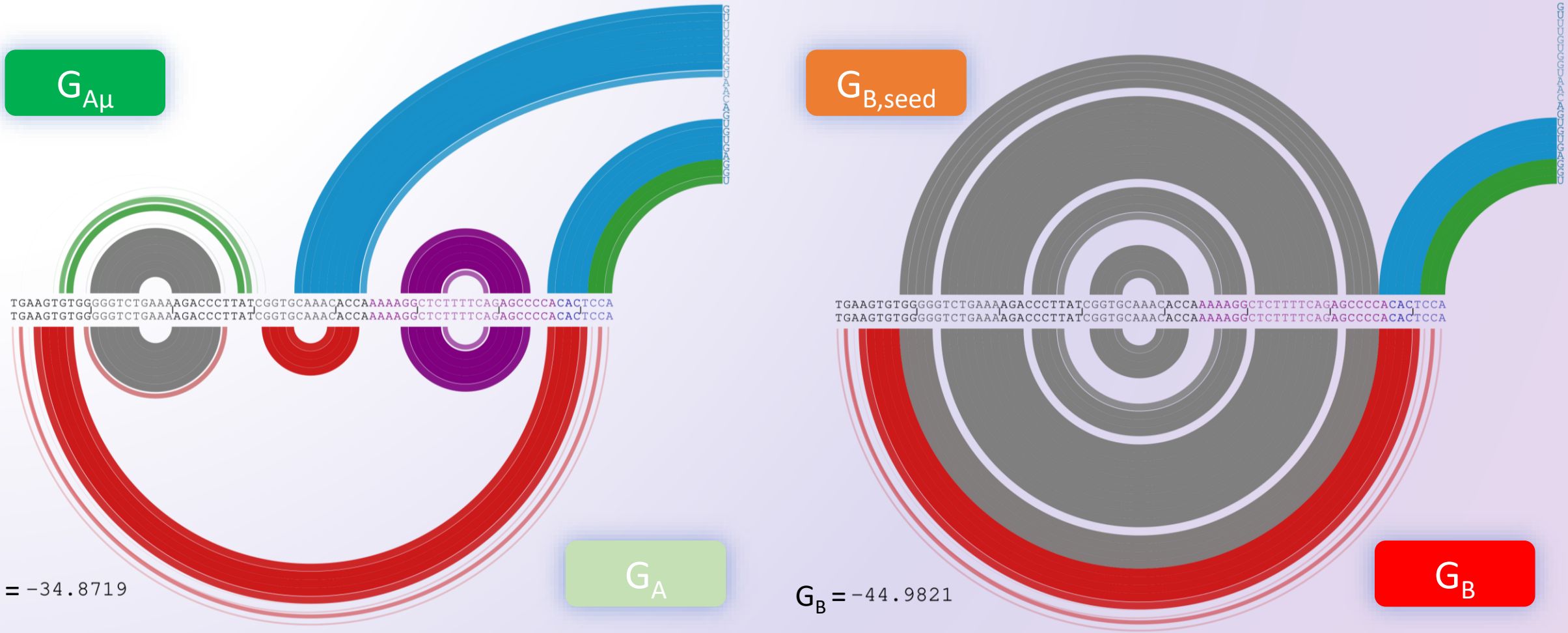
The Energy Landscape We Want



Results for 3WJ Design

$G_{A\mu} = -51.4079$

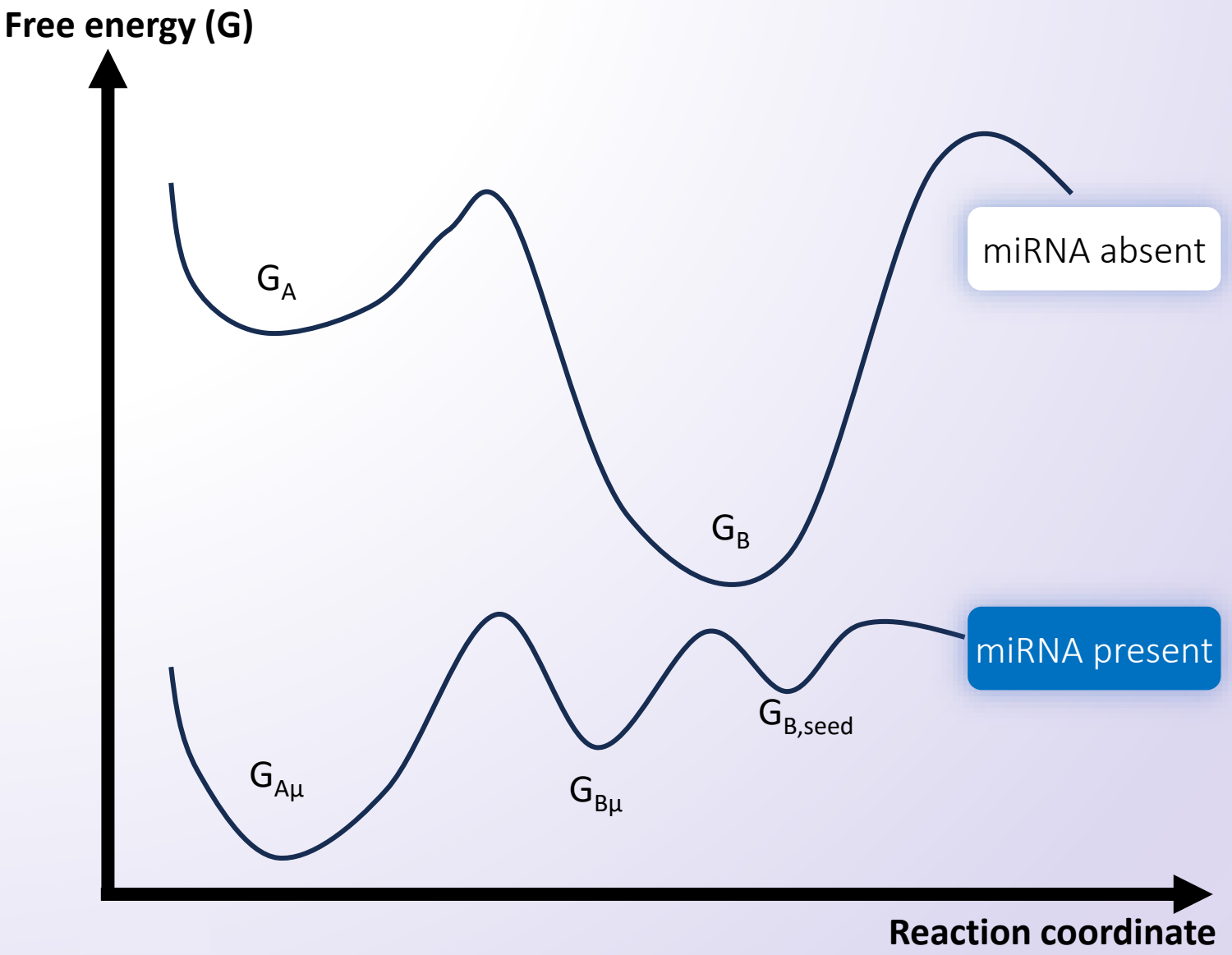
$G_{B,seed} = -45.7504$



$G_A = -34.8719$

$G_B = -44.9821$

Numerical Results



G_A = -34.88 kcal/mol

G_B = -44.98 kcal/mol

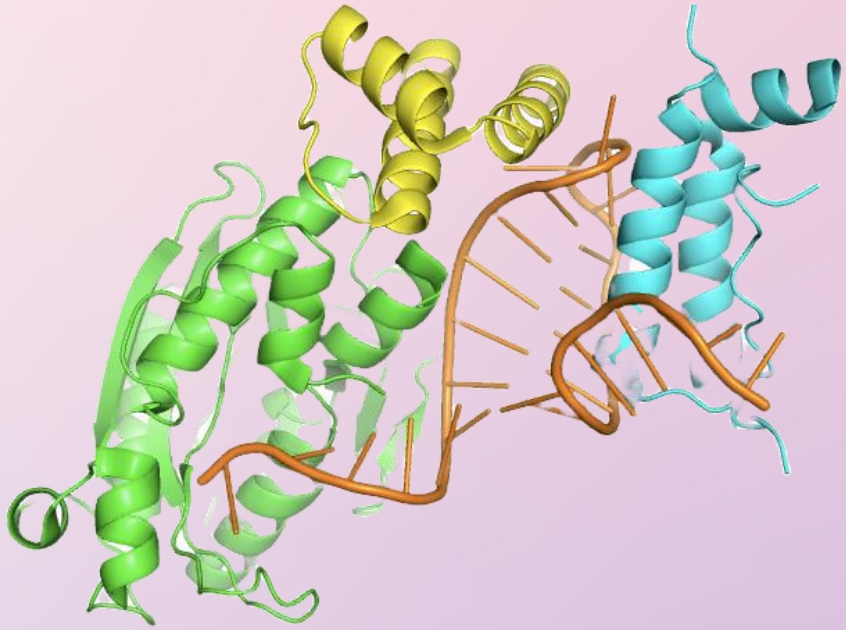
$G_{B,seed}$ = -45.75 kcal/mol

$G_{A\mu}$ = -51.41 kcal/mol

Further considerations to take into account

So far, we have modeled only the free energy landscape of the mRNA-miRNA complex, excluding the effects of protein interactions that may activate or inhibit the complex. Key considerations include:

- Stem-Loop Binding Protein (SLBP): Binds to the HSL loop, activating the mRNA sequence.
- Cutting Protein: Binds to the “CCCC” bases at the 3’ UTR of the HSL loop, potentially inhibiting ribosome activity and preventing mRNA translation.



Acknowledgements

- This research is being conducted in collaboration with **sxRNA Technologies Inc.** and **Professor Scott Tenenbaum**'s research group at the University of Albany.
- I would like to thank **Elena Deegan-Krause** ('26) for her contributions to this research project.
- Moreover, I would like to thank **Professor Daniel Aalberts** for his invaluable guidance throughout the semester.