

# Depixus

## See biology as it happens

- **Reveal biomolecular interactions**
- **Decode disease mechanisms**
- **Unlock more effective therapies**

## Single molecule real-time analysis of dynamic nucleic acid interactions

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### INTRODUCTION

Despite the enormous technological advances made in genomics over recent years, the latest tools still fall short in their ability to fully capture the dynamic molecular interactions within cells that are essential for understanding the underlying biology of health and disease. Based on established force spectroscopy technology,<sup>1</sup> MAGNA™ is a novel platform for analyzing the complex interactions between different biomolecules, with potential application across a broad range of areas within life science research. We have already generated rich data sets revealing how nucleic acids, DNA- & RNA binding proteins, antibodies, and small-molecule compounds bind to their targets. As an example of this, we demonstrate how MAGNA™ can be used to explore the binding events between nucleic acids (in this case RNA), and ligands such as proteins and small molecule compounds, to inform the development of RNA-targeted therapeutics.<sup>2</sup>

Nucleic acids are captured within a flow cell, anchored to a planar glass surface at one end and bound to a micron-scale paramagnetic bead at the other. The flow cell is inserted into the MAGNA™ instrument. Molecules are unfolded using a movable magnet above the flow-cell, with the position of each magnetic bead tracked by an optical system. Multiple cycles of unfolding and refolding are performed using an increase then a reduction in force (figure 1), either in the presence or absence of ligand such as a small molecule or binding protein. Thousands of individual molecules are available for repeated, non-destructive interrogation, enabling detailed characterization of binding kinetics.

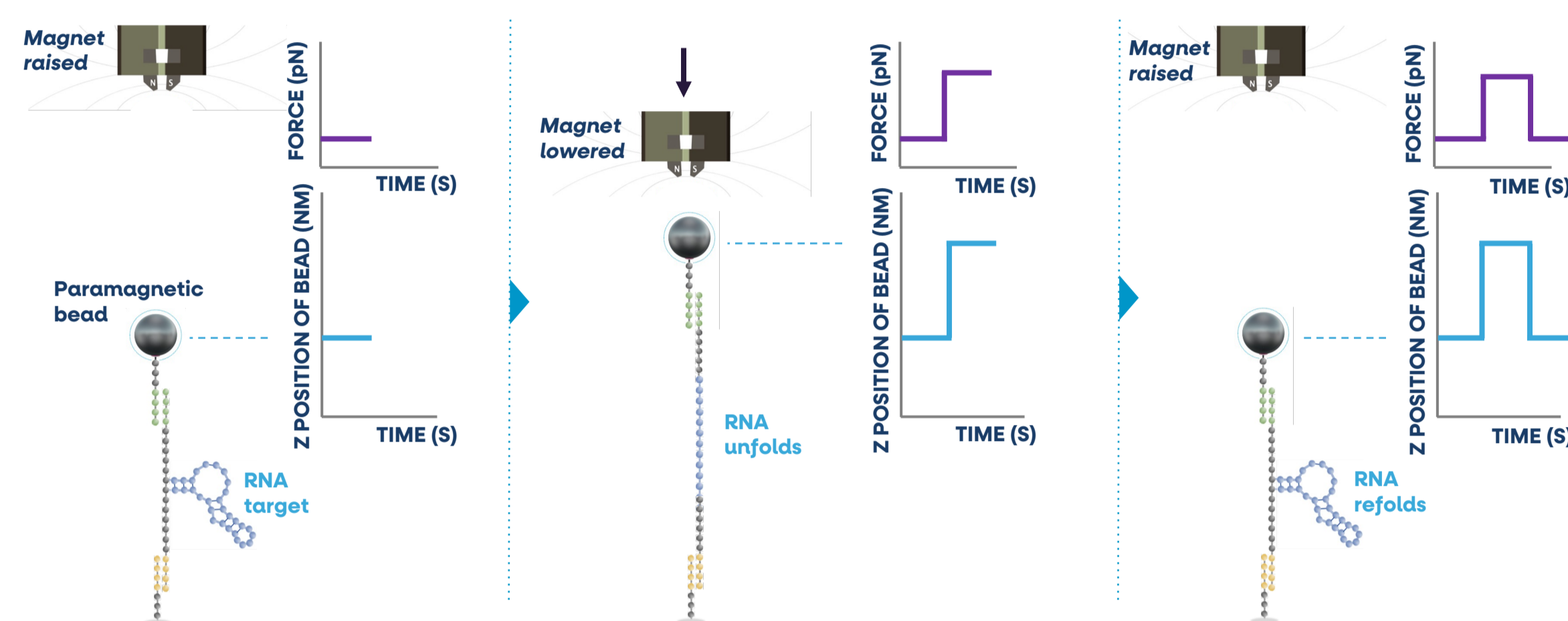
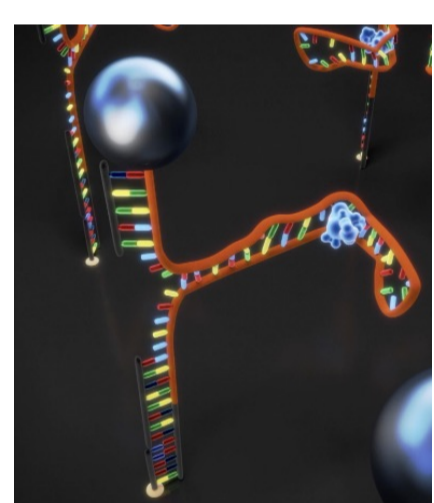


Figure 1. Tethered nucleic acids are subjected to multiple cycles of unfolding and refolding using an increase then a reduction in force.

### ASSAY MODES

MAGNA™ can be configured in three distinct assay modes:

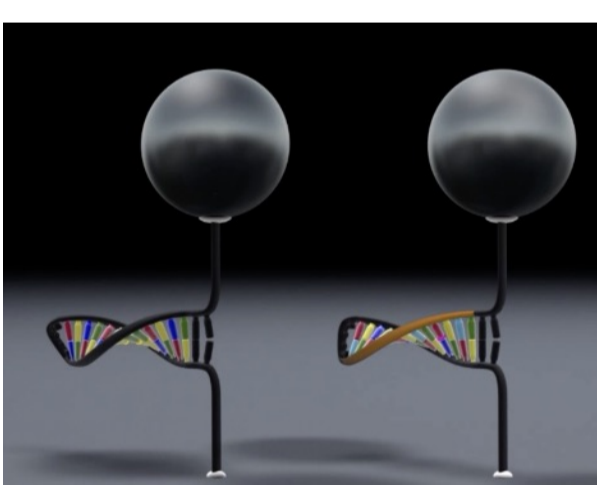


**Binding structure mode** is used to test whether the three-dimensional structure of a target RNA is stabilized or destabilized by a binding partner such as a protein or small molecule, as shown in figure 1. If a ligand stabilizes the structure, more force will be required to unfold it, while destabilizing molecules have the opposite effect.

- Applications include RNA-targeted drug discovery, RNA binding protein research

**Binding strength mode** tests molecular interactions. Pairs of target molecules are tethered at a fixed distance apart along oligonucleotide strands. When no magnetic force is applied, the oligo is relaxed so that the tethered molecules can interact. As the magnetic force is applied, they are pulled apart and their binding force is measured.

- Applications include protein-protein interactions, small molecule, protein and DNA-binding protein interactions



**Binding location mode** is used to determine the precise location of a ligand binding site within an RNA/DNA hairpin. As the magnetic force is applied, the hairpin unzips and can be interrogated with ligands such as antibodies or oligos. The target molecules refold as the force is released, pausing when a ligand is encountered and enabling the exact binding site to be determined.

- Applications include nucleic acid QC, epigenetic biomarker discovery

### STUDYING RNA-LIGAND BINDING

In Interaction mode, MAGNA™ can be used to characterize interactions between RNA and small molecule compounds, as well as with RNA binding proteins. A slow ramping up of force enables the calculation of binding kinetics by observing how a ligand that binds to an RNA, such as a small molecule compound

alters the force at which the structure changes from a folded to an unfolded state (figure 2A). This reveals whether the ligand has a stabilizing or destabilizing effect on the target RNA (2B), and the concentration dependence of the interaction (2C).

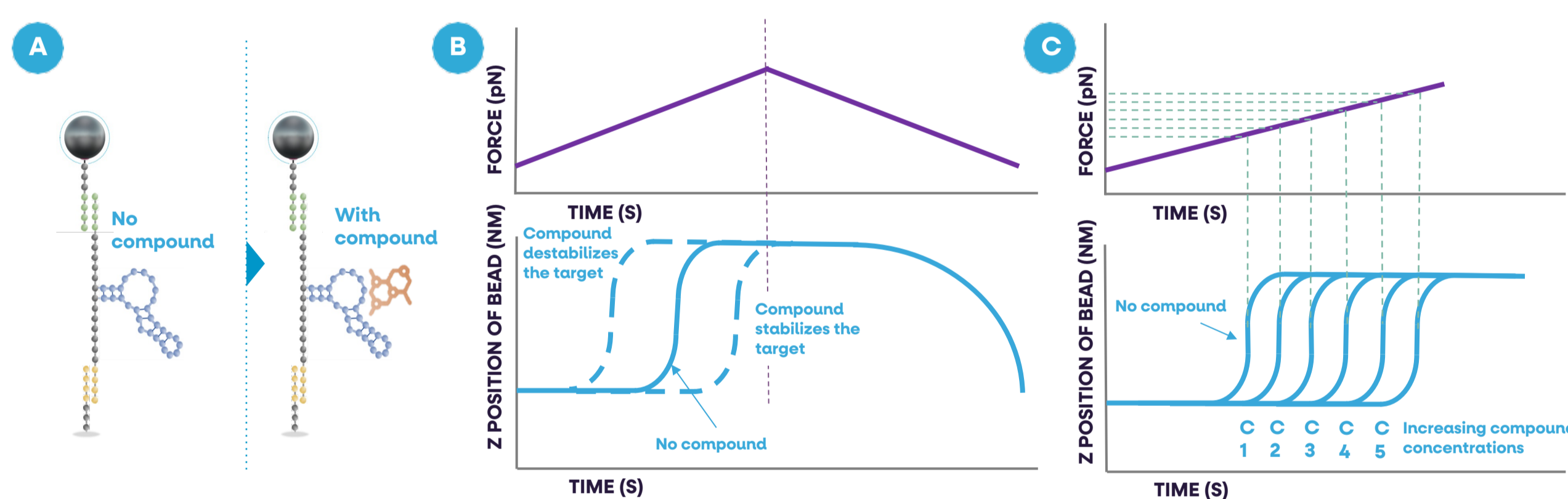


Figure 2. A) MAGNA™ can be used to study the dynamics of interactions between nucleic acids and ligands. B) Force ramp experiments show whether a ligand stabilizes or destabilizes an RNA target and C) the concentration dependency of the interaction.

### CHARACTERIZING SMALL MOLECULE-RNA INTERACTIONS

MAGNA™ was used to investigate the properties of four compounds that have been shown to bind to the HIV trans-activation response element (HIV-TAR)<sup>3</sup> – a conserved RNA structure known to be critical for viral replication.

MAGNA™ reveals the binding affinities of the four compounds and the concentration dependency of their interactions with target RNA (figure 3A-D).

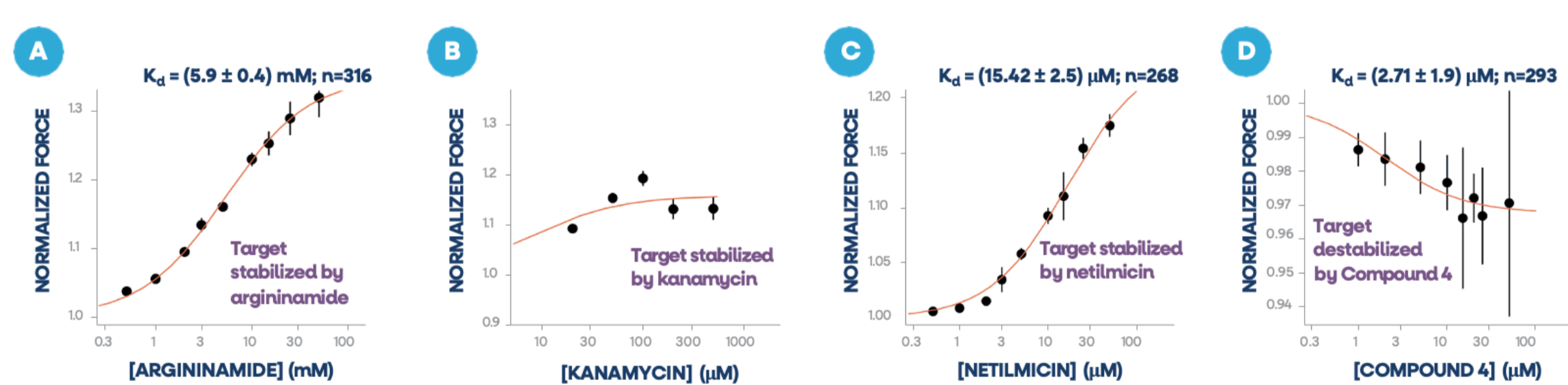


Figure 3. Force ramp data for HIV-TAR unfolding in the presence of four compounds: A) Argininamide B) Kanamycin C) Netilmicin and D) Proprietary compound 4. The number of HIV-TAR molecules (n) analyzed in each condition is shown, and the unfolding force at each cycle is normalized to the median force of each compound in the control condition. The force at the maximum distribution of each bead is taken and the median force of all beads is shown for each condition, with calculated  $K_d$  shown for each compound.

### CHARACTERIZING PROTEIN-RNA INTERACTIONS

MAGNA™ can be used to characterize the interactions of RNA binding proteins with RNA, by revealing how they stabilize or destabilize their target RNA structures. Furthermore, MAGNA™ can be used to study the interplay between nucleic acids, proteins and small molecules – for example, to explore whether a small molecule disrupts the binding of a protein to its RNA target.

As an example, we used MAGNA™ to interrogate the real-time binding of an inactive form of the pre-miRNA processing protein Dicer to the precursor form of miR-21 – a microRNA that plays an important role in the pathogenesis of many cancers.<sup>4</sup> Force ramp measurements indicated that more force was required to unfold pre-miR-21 in the presence of Dicer, showing that the protein is binding to and stabilizing the RNA structure (figure 4).

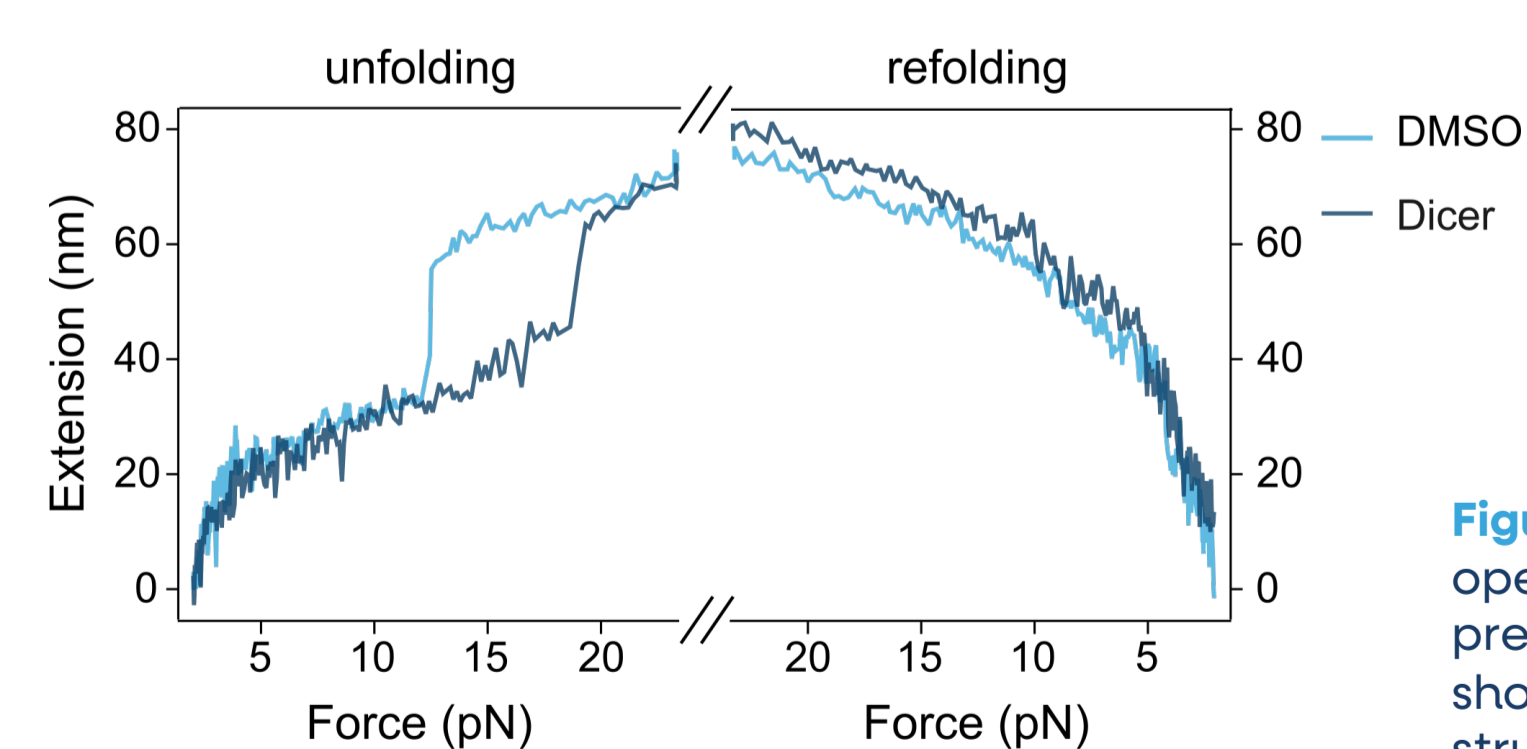


Figure 4. Force ramp data from two opening/closing cycles of pre-miR-21 in the presence of inactivated Dicer or DMSO control, showing that the protein is binding to and stabilizing the RNA structure.

### SUMMARY

**MAGNA™ is the only analytical method that can fully capture the dynamic nature of individual molecular interactions at scale:**

- Direct readout of interactions between 3-D nucleic acid structures and ligand activity without the need for surrogate measurements
- Data from thousands of individual molecules rather than bulk averages
- Measure changes in binding affinity with nucleic acid targets and ligands, including proteins and small molecules
- Explore binding kinetics of nucleic acids target and ligands
- Probe the interplay between a wider range of biomolecules using three different assay modes

### REFERENCES

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4. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. *Science*. 2001;294(5543):853–8.

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