Depixus

MAGNA™ is a powerful tool for visualizing complex biomolecular interactions in real-time and at scale.

Revealing…

- **Detailed binding kinetics**
- **Nucleic acid structures**
- **Protein binding domains**
- **Drug mechanisms of action**

MAGNA™: 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,[1] 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.[2]

HOW MAGNA™ WORKS

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, which is inserted into a MAGNA™ instrument (**figure 1A**). Multiple molecules are then available for repeated, non-destructive interrogation, enabling detailed characterization of interactions with proteins and small molecule compounds. The molecules of interest, in this case RNA, are subjected to multiple cycles of unfolding and refolding using steadily increasing and decreasing forces, either in the presence or absence of ligand such as a small molecule compound or binding protein (**figure 1B**).

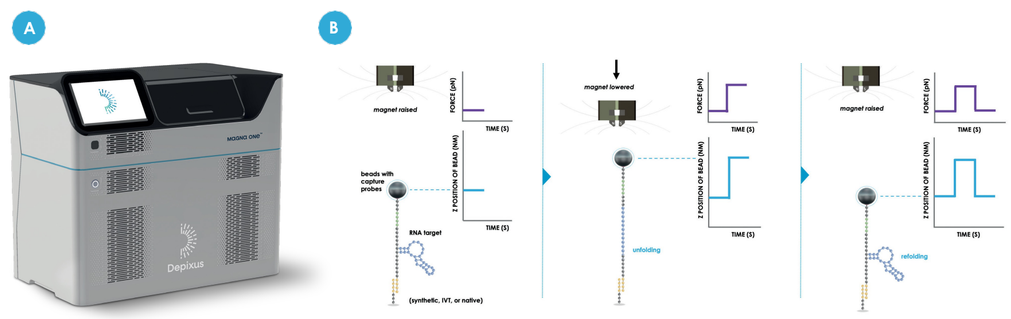

Figure 1. A) The Magna One™ instrument, B) RNA is subjected to multiple cycles of being unfolded and refolded using steadily increasing and decreasing forces.

USING MAGNA™ TO STUDY RNA-LIGAND BINDING

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

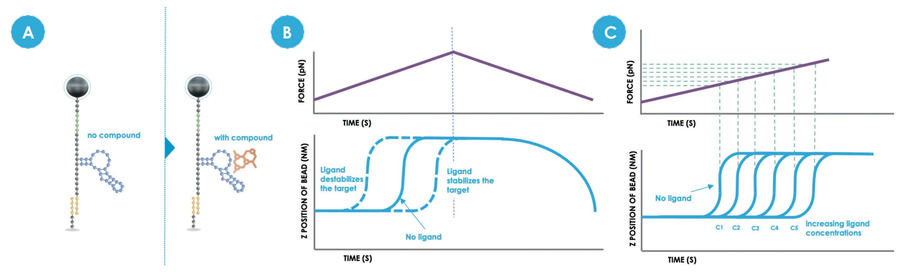

Figure 2. A) MAGNA™ can be used to study the dynamics of interactions between nucleic acids and ligands. B) Force ramp experiments show whether a ligand stabilizes or destabilizes an RNA target and C) the concentration dependency of the interaction.

MAGNA™ can be used to characterize interactions between RNA and small molecule compounds, as well as with RNA binding proteins. It can also be used to study the complex interplay between a wide range of biomolecules, including oligonucleotides and antibodies.

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) [3] - a conserved RNA structure known to be critical for viral replication.

Force ramp experiments reveal the binding affinities of the four compounds and the concentration dependency of their interactions with target RNA (**figure 3A-D**).

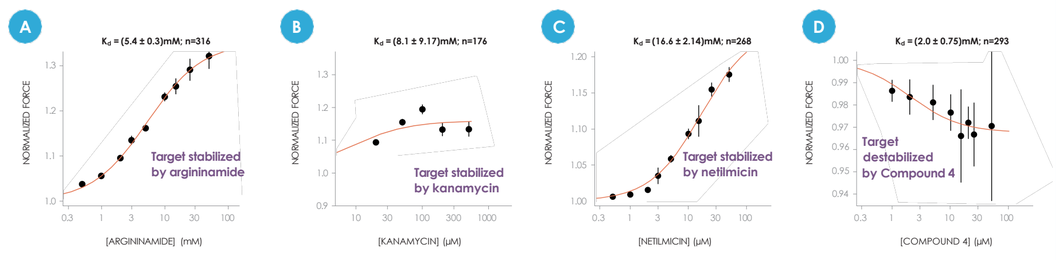

Figure 3. Force ramp data for HIV-TAR unfolding in the presence of four compounds. The number of HIV-TAR molecules (n) analyzed in each condition is shown, and the unfolding force at each cycle is normalized to the median force of each compound in the control condition. The force at the maximum distribution 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.[3] 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**).

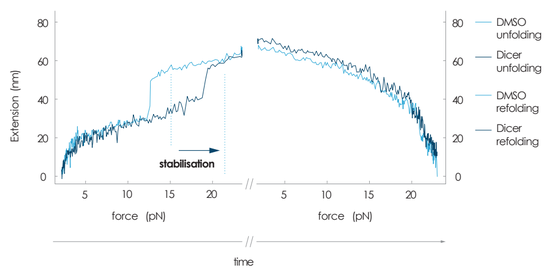

Figure 4. Force ramp data from two opening/closing cycles of pre-miR-21 in the presence of inactivated Dicer or DMSO control, showing that the protein stabilizes the RNA structure.

SUMMARY

MAGNA™ is an exciting novel platform with a number of features and advantages that make it superior to existing methods for capturing the dynamics of biomolecular interactions, including:

- Direct readout of the dynamic relationship between nucleic acid structure and ligand activity across a wide dynamic range (nM to mM)
- Measuring changes in binding affinity with different ligands and nucleic acid substrates including proteins and small molecules
- Exploring binding kinetics of small molecule and protein interactions with target nucleic acids
- Probing the interplay between a wider range of biomolecules, including nucleic acids, proteins, oligonucleotides and chemical compounds

REFERENCES

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