



Illuminating the invisible – visualizing biomolecular interactions with magnetic force spectroscopy

PRESENTED BY:

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**Eindhoven University
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Too often biology is described through averages, but average does not exist

- Life is **complex** & highly **heterogeneous** at all levels – from **organisms**, to **cells**, to individual **molecules**.
- Understanding this complexity at a **molecular level** is key to **understanding disease** & the **development of new medicines**.

Tools exist to study biological complexity to the level of single cells, but **not to the resolution of individual molecules & their interactions**.



Current analytical solutions don't provide the full picture

For example:

- Isothermal titration calorimetry (ITC)
- Surface plasmon resonance (SPR)
- Microscale thermophoresis (MST)
- Atomic force microscopy (AFM)
- Optical tweezers (OT)
- Nuclear magnetic resonance (NMR)
- Cryo-electron microscopy (Cryo-EM)
- X-ray diffraction (XRD)
- Liquid chromatography mass spectrometry (LC-MS)



Static

Snapshots of bound molecules with no information about dynamic binding kinetics

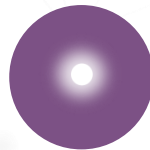
and/or



Indirect

Inferred or surrogate readouts rather than direct measurement of molecular interactions

and/or



Unrepresentative

Data averaged across the whole sample or only able to measure one individual interaction at a time



Depixus' MAGNA One technology – unique capabilities for analysis of biomolecular interactions



Dynamic

Vivid, real-time views of binding events



Direct

Reveal forces & kinetics of molecular interactions & conformations



Detailed

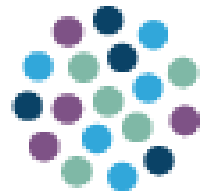
Individual data streams from tens of thousands of molecules reveal the totality, not the average



MAGNA™



Analysis of individual biomolecular interactions opens the door to powerful new biological insights



Molecular heterogeneity



Molecular dynamics

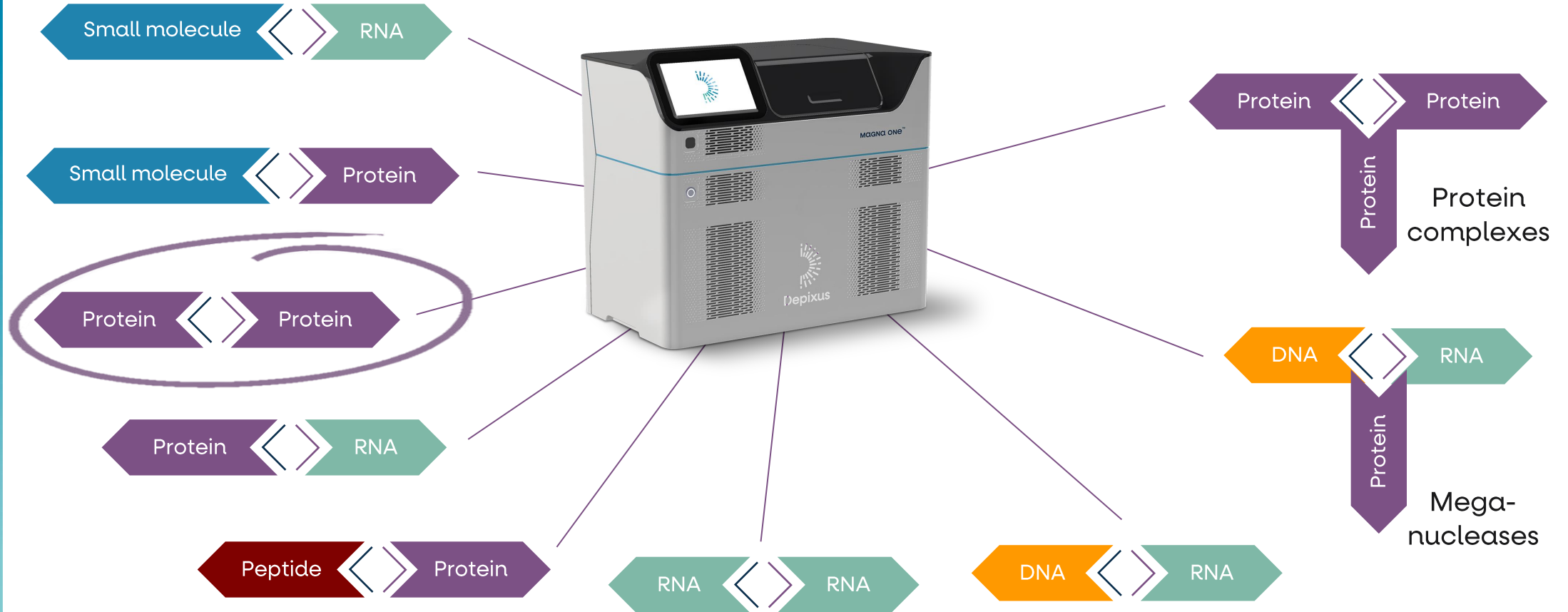


Rare molecular events

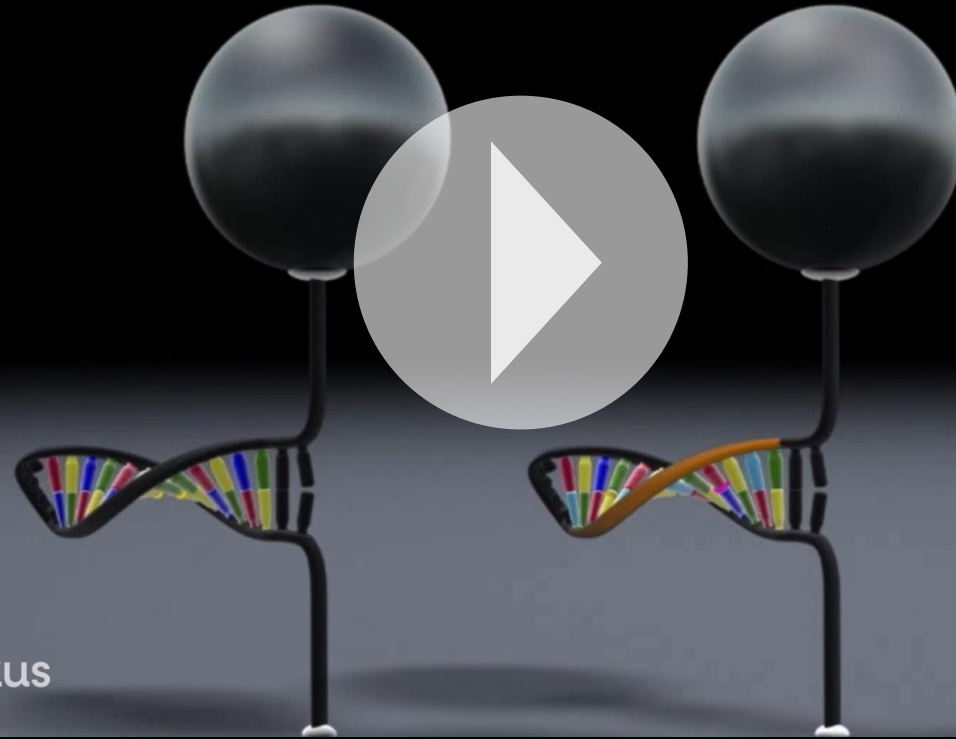



MAGNA One can be used to analyse a wide range of molecular interactions types

From 'simple' to complex...



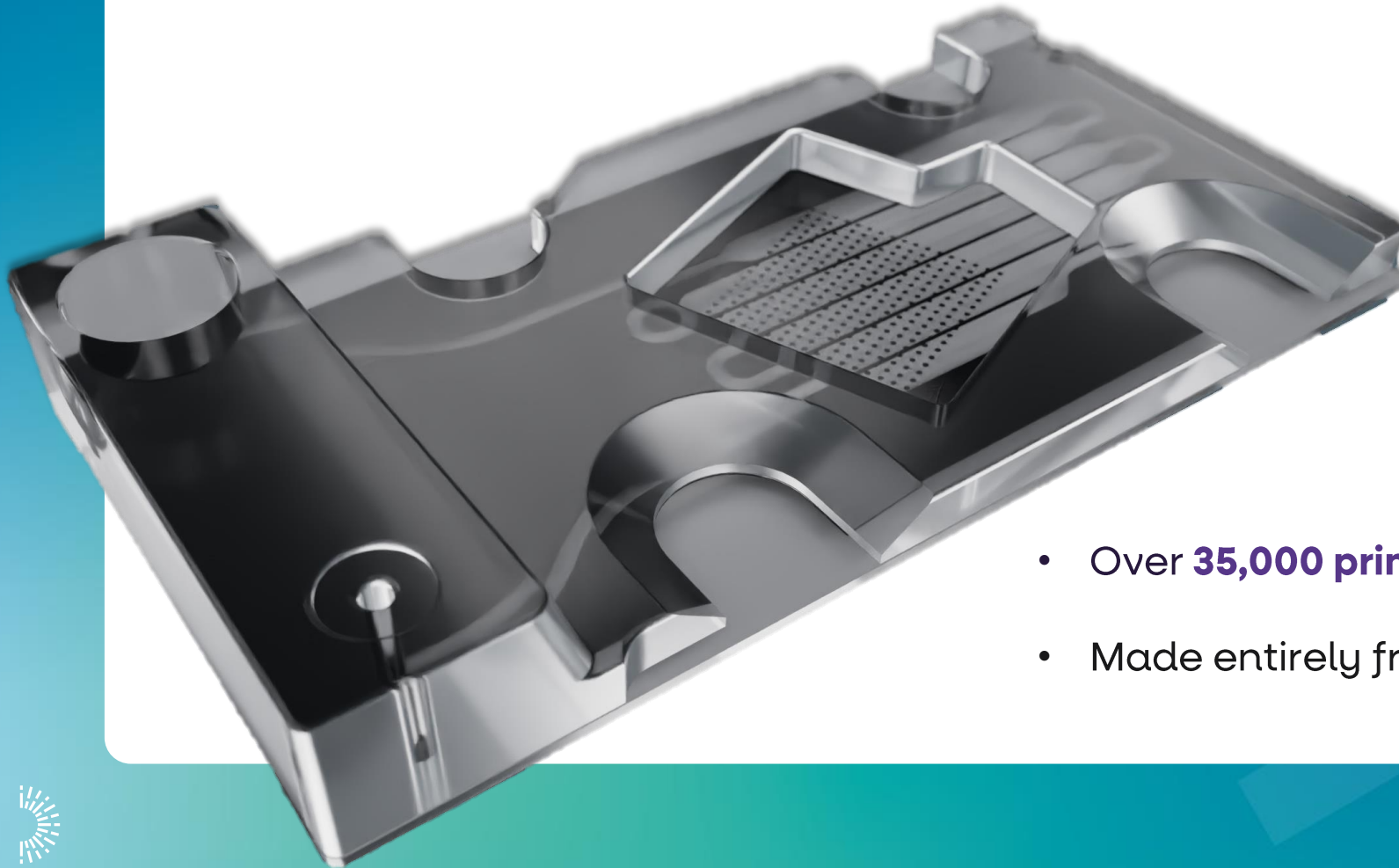
How MAGNA™ works – the principles



 Depixus

 Simple DNA hairpins – a model system to explain the basics

At the heart of MAGNA One is a multi-use cartridge for simultaneous analysis of 1,000's of individual molecular interactions



- Over **35,000 printed spots**
- Made entirely from a **biocompatible plastic**

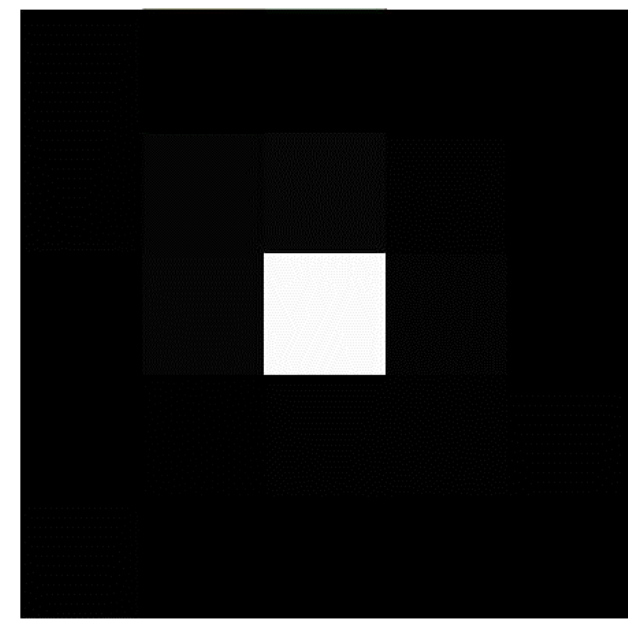
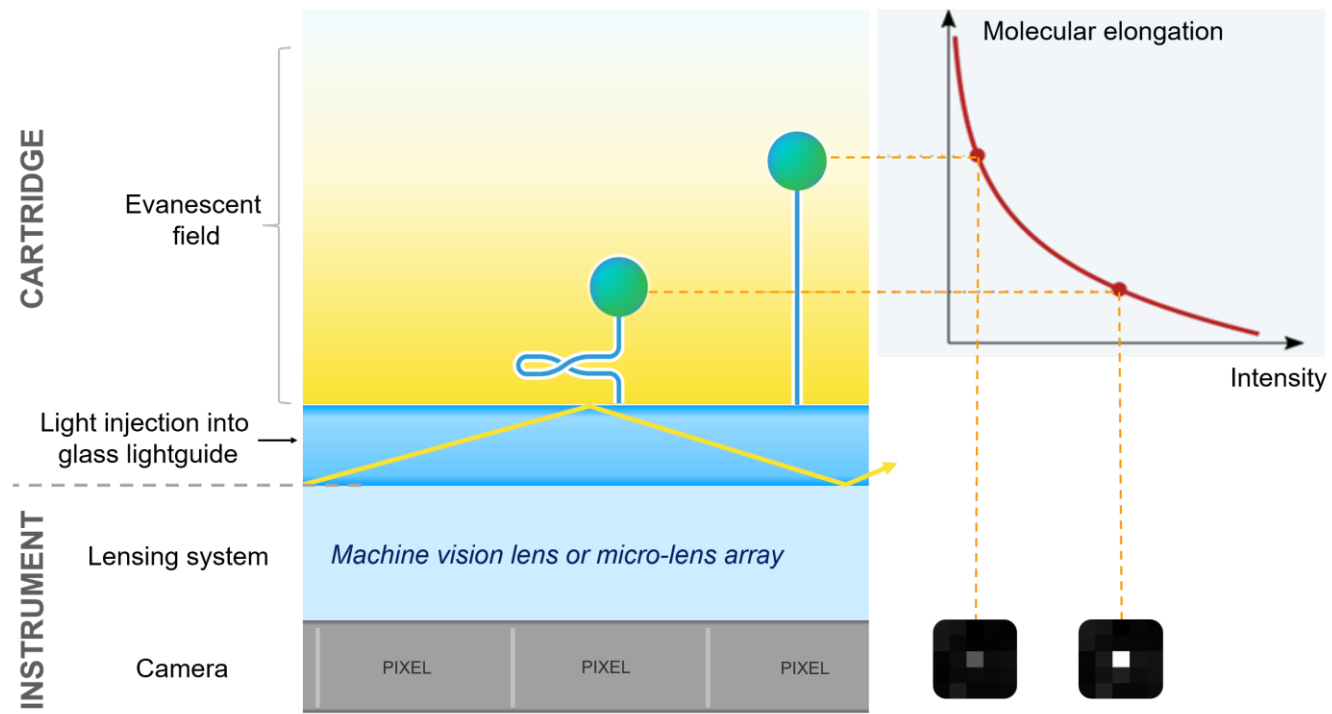




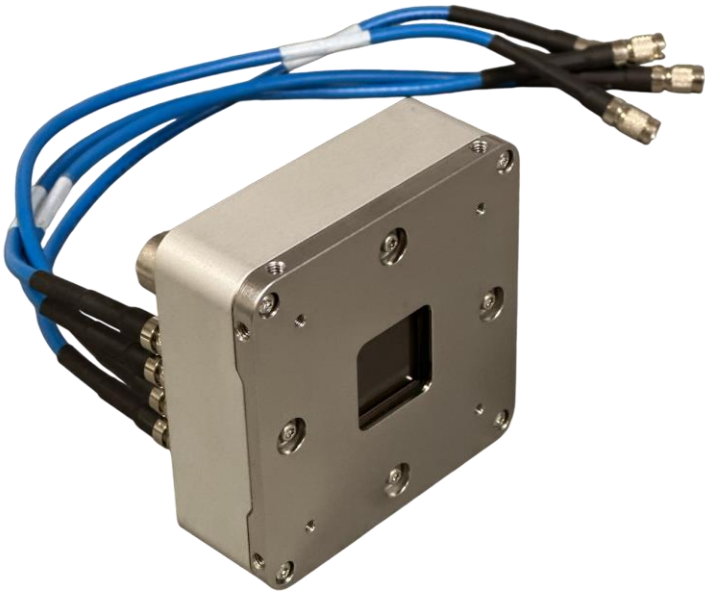
**Micron-scale spots are printed on the floor of flow cell
using a super-resolution inkjet printer**



Bead position is tracked with nanometric precision using evanescent field illumination (EFI), with light from each bead captured by a unique pixel



Measuring very small variations of bright light at 10' s of Hz requires a highly specialized camera



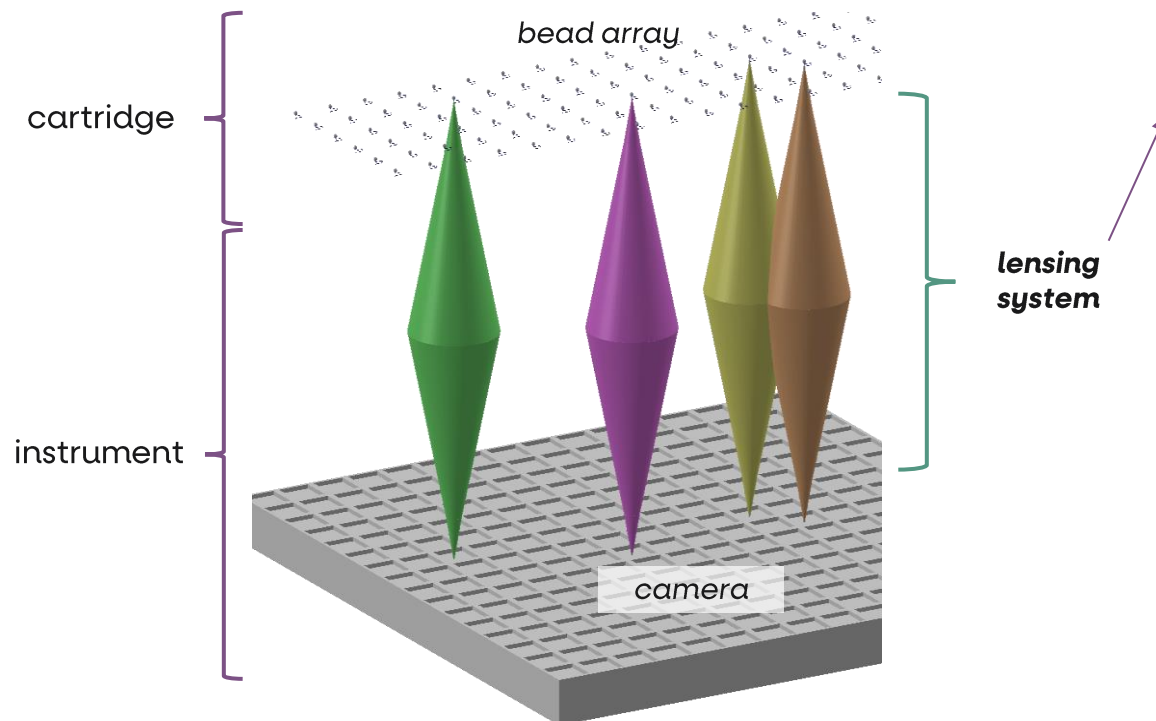
- A very high full-well capacity (**2,000,000 e⁻**)
- A large pixel size (**12 μm x 12 μm**)

→ Detection of a **1 nm bead movement** over a **dynamic range of 1 μm**

Adimec
A TELEDYNE COMPANY



The camera needs to be married with a lensing system with similarly tight specifications to match bead-molecule complexes with camera pixels



Lens requirements

1. Very low-distortion
2. 1:1 magnification
3. Single-pixel confinement of the signal
4. Highest possible aperture to collect maximal amount of light

→ **field lens** (or telecentric machine vision lens)



Followed by (1) nanometric focusing & (2) micron-scale alignment of the bead signals to individual pixels to ensure a high pixel fill factor

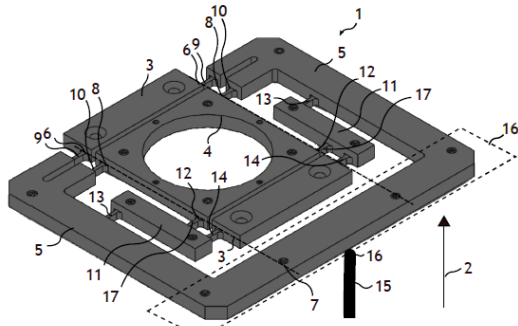


Figure 1

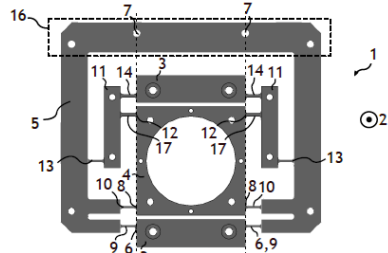


Figure 2

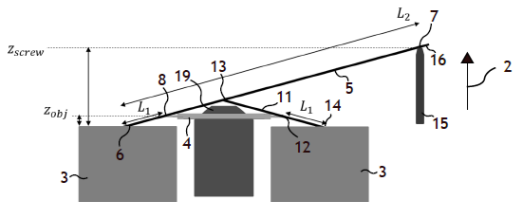


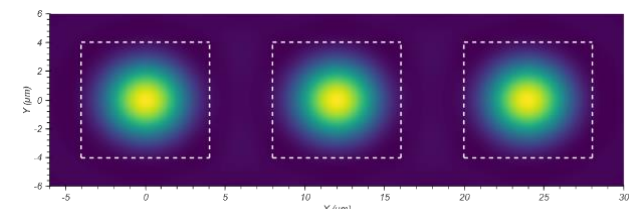
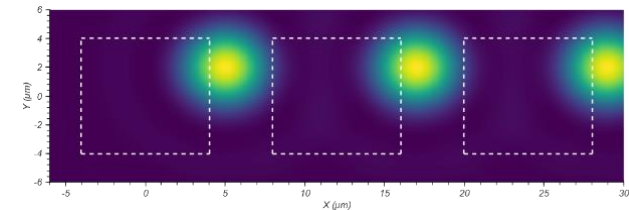
Figure 3

(1)
**NANOMETRIC
 FOCUSING
 DEVICE**

z-axis

(2)
**ALIGNMENT
 VIA CARTRIDGE
 MARKERS**

x-y axis

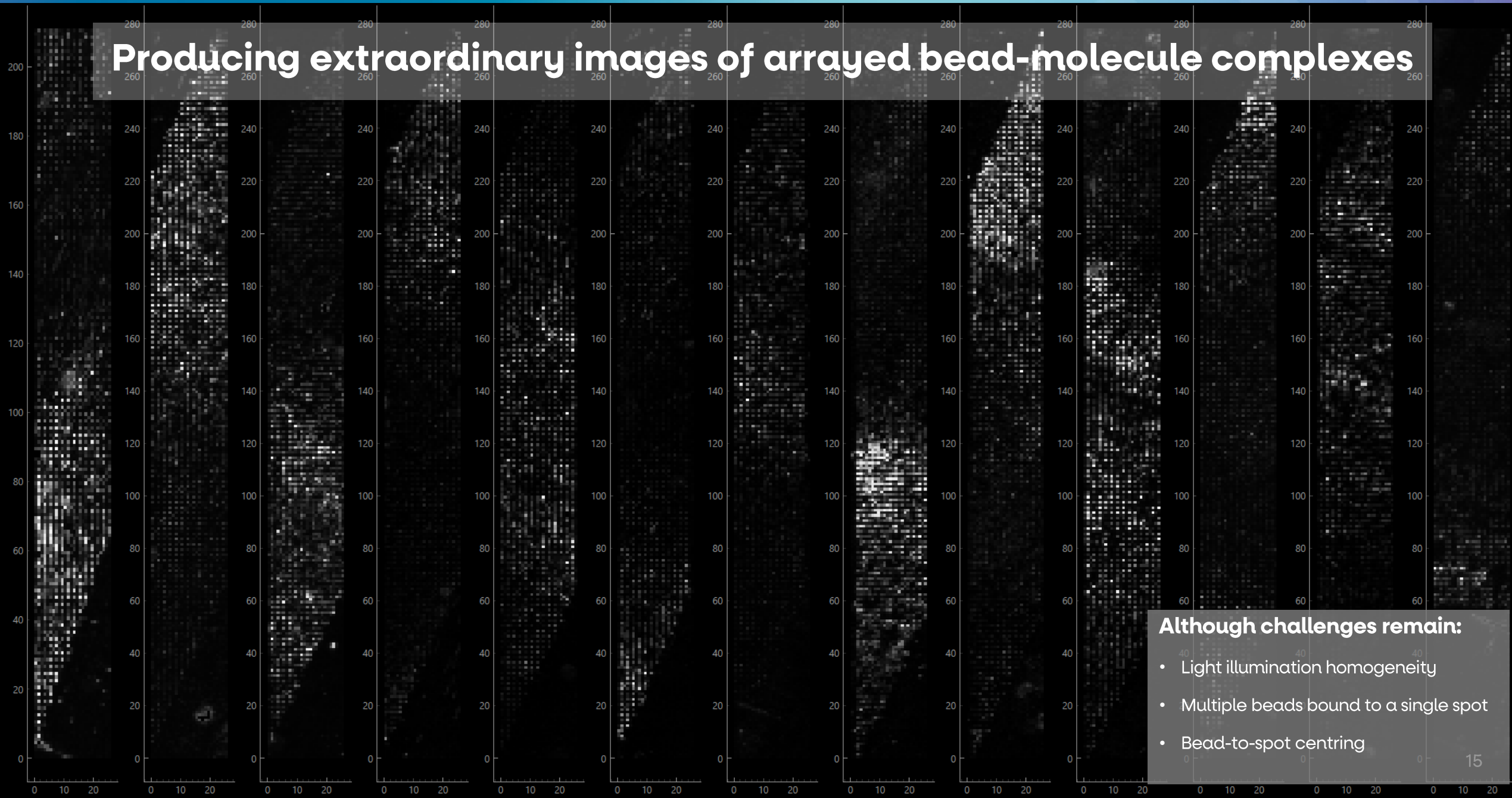


→ **high pixel fill factor**



Real-world data

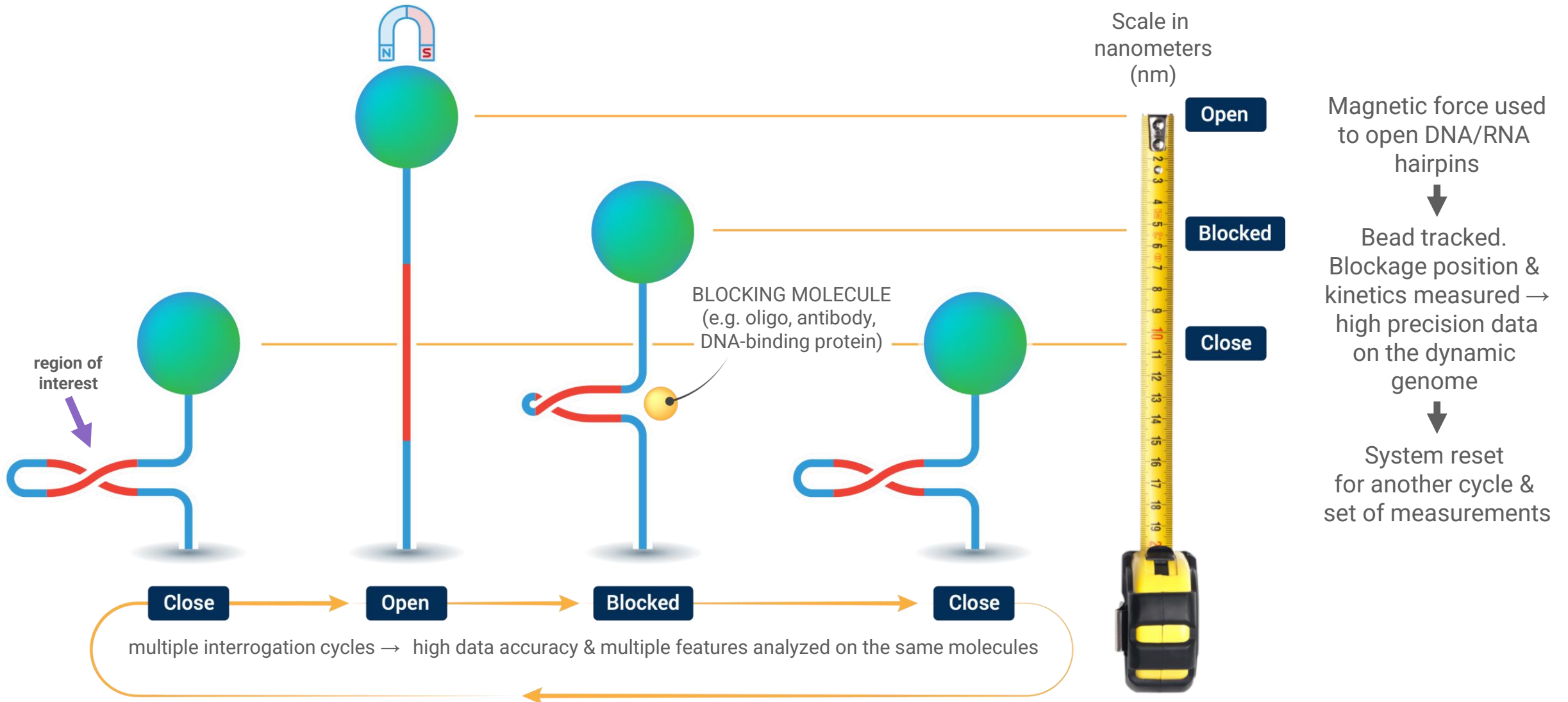
Producing extraordinary images of arrayed bead-molecule complexes



Although challenges remain:

- Light illumination homogeneity
- Multiple beads bound to a single spot
- Bead-to-spot centring

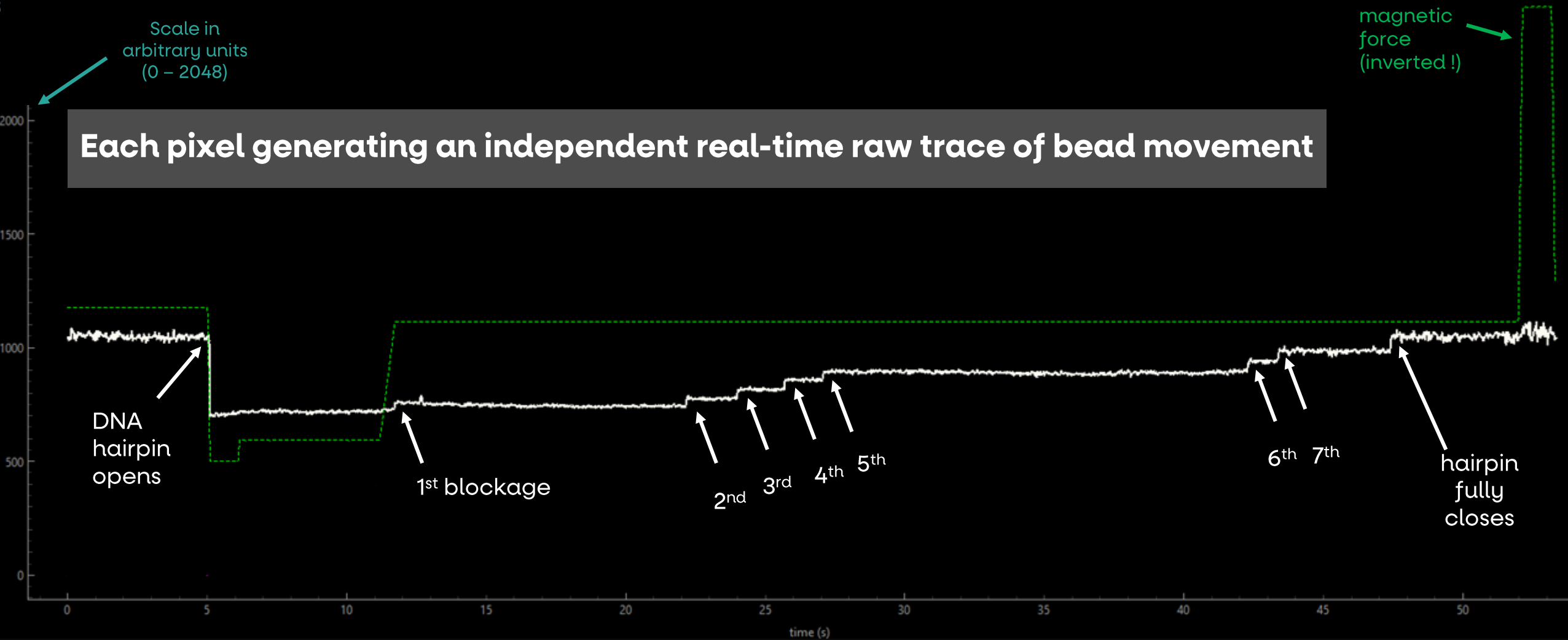
MAGNA is a highly accurate molecular scale 'measuring tape'



Scale in
arbitrary units
(0 - 2048)

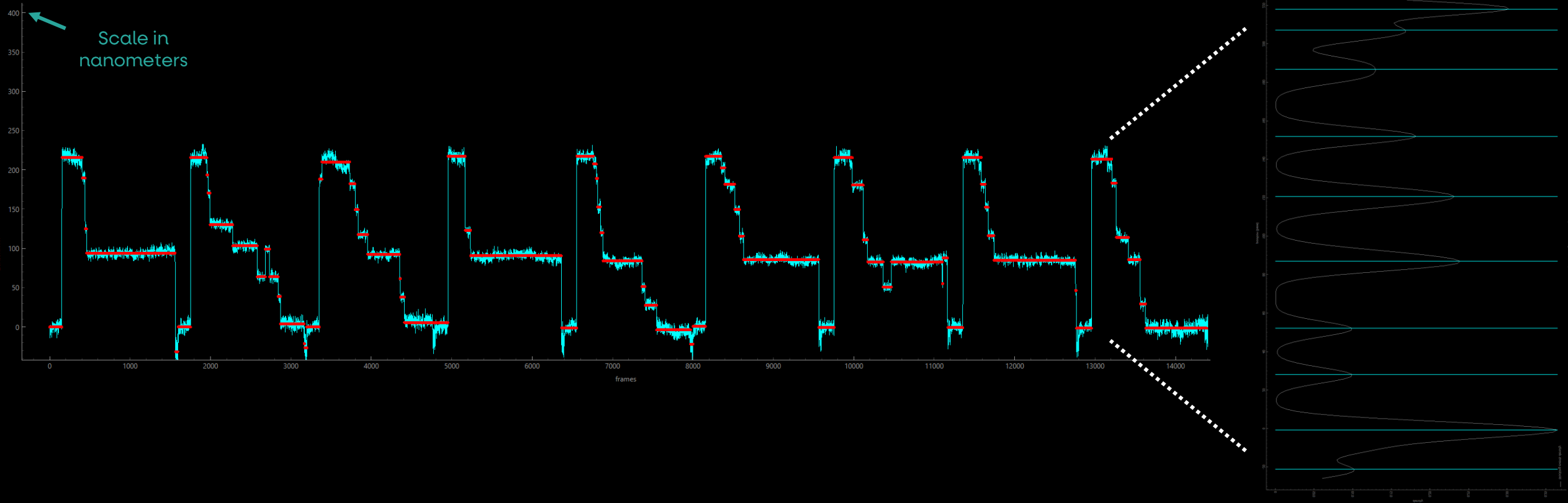
magnetic
force
(inverted !)

Each pixel generating an independent real-time raw trace of bead movement



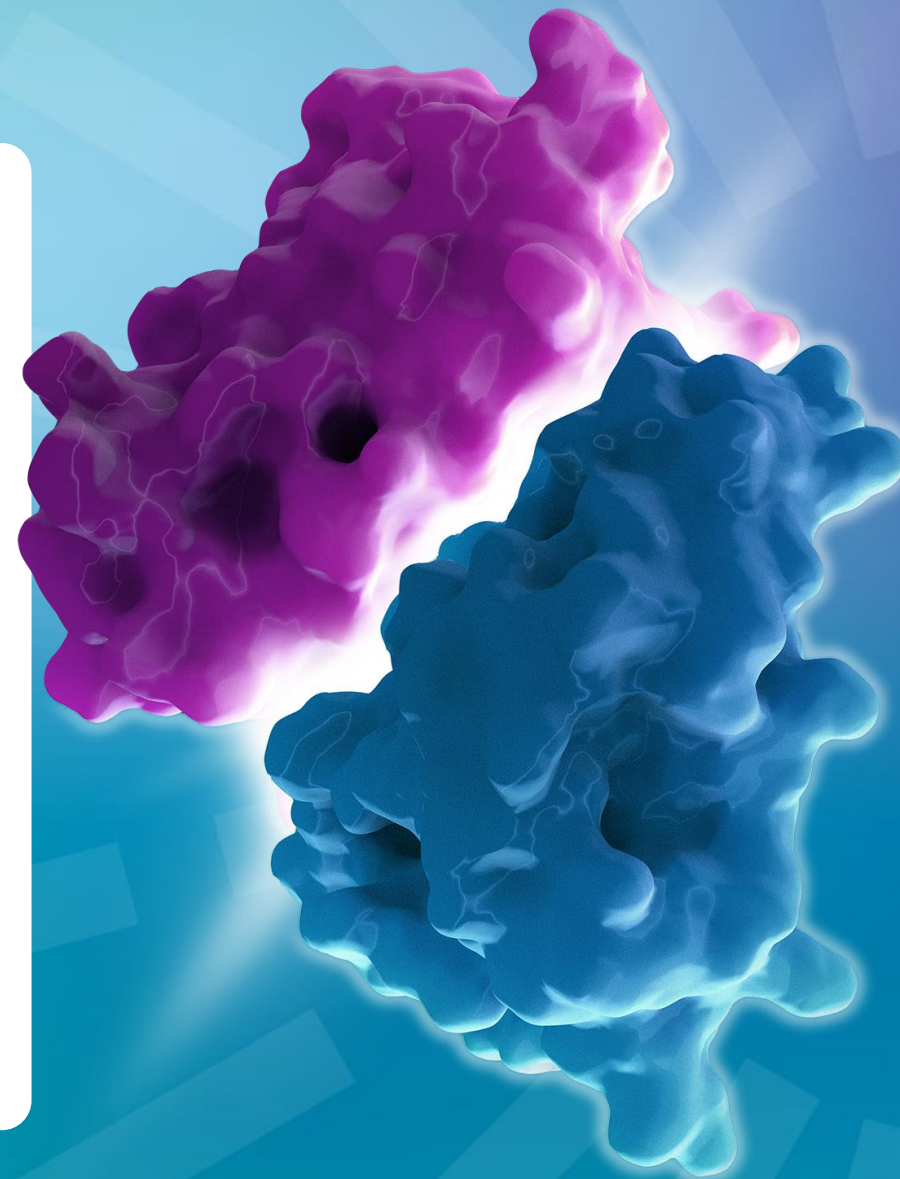
With signal processing tools then used to ...

- Convert the raw pixel signal into bead vertical position
- Detect blocking events
- Map detected events to expected events (if known)

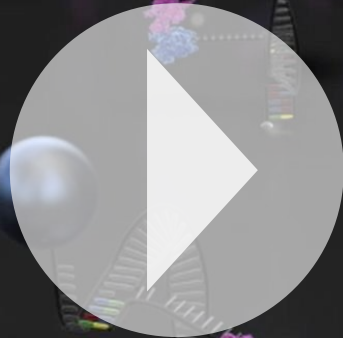



MAGNA for studying protein-protein interactions (PPIs)

- what's real
- what's biologically important
- generate best-in class drugs



MAGNA for the study of PPIs



 Depixus

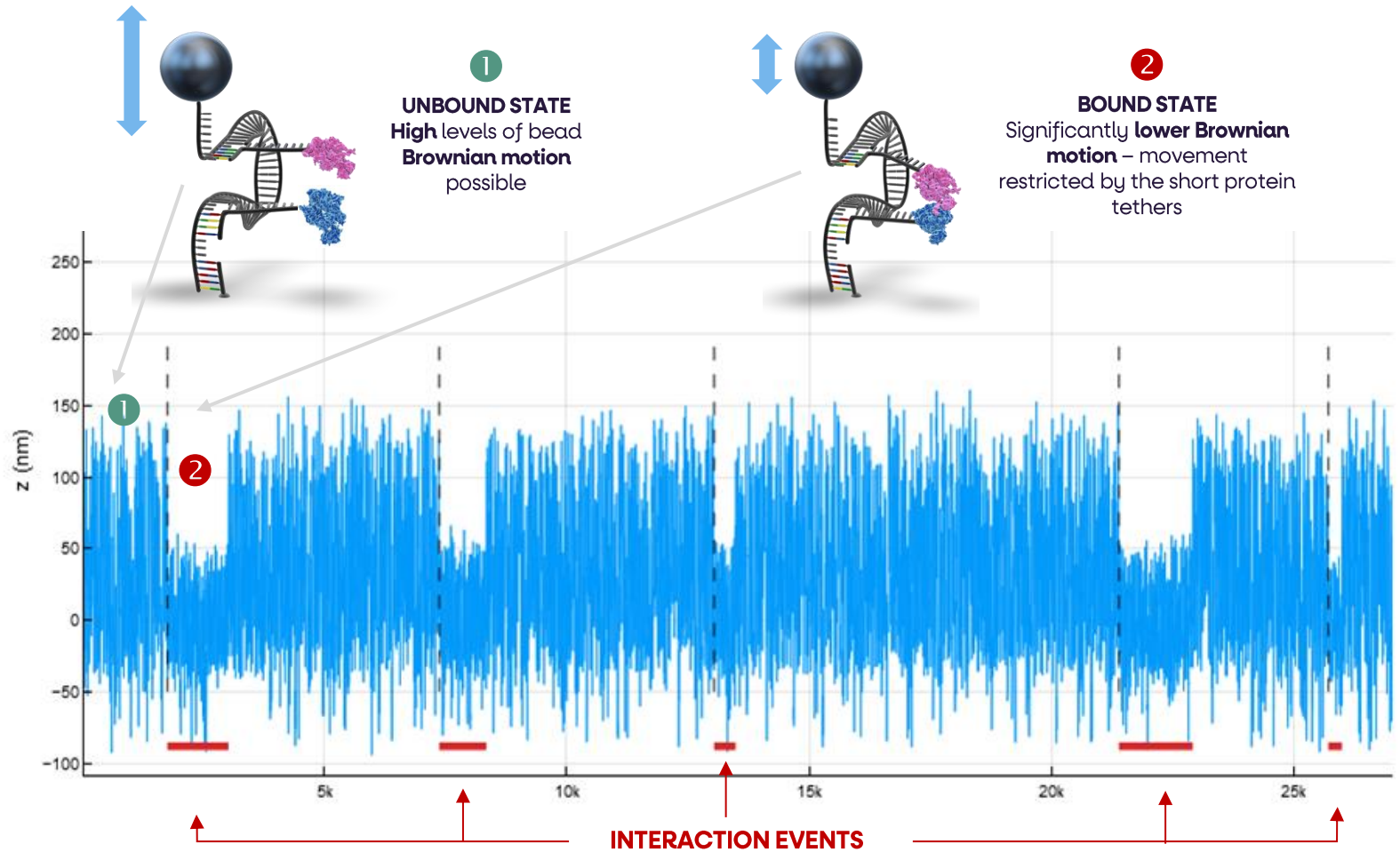
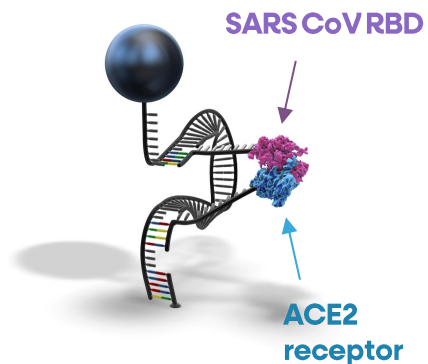


An example of the MAGNA raw signal, with real-time detection of individual protein-protein interaction events

What are we seeing?

SARS-Cov RBD - ACE2 receptor binding

- Real-time, z-axis movement of a single bead *
- No force applied
- Single protein pair
- Duration shown = ca. 16min



* Note: only one trace shown here, but thousands of such interactions can be studied in parallel

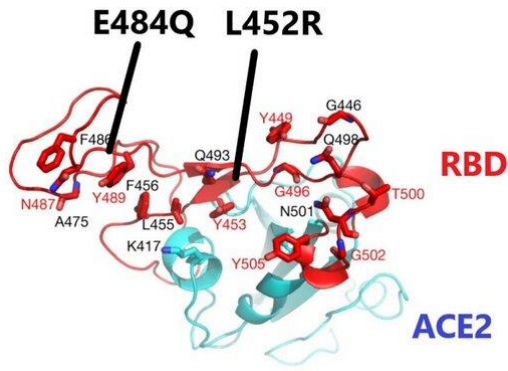


Additional assay capabilities

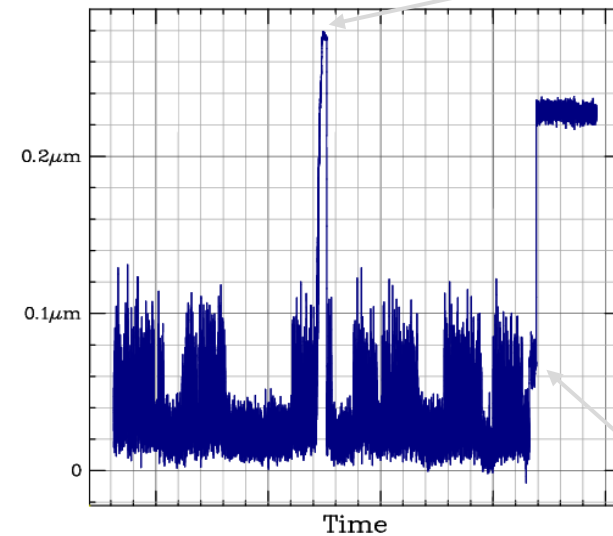
What are we seeing?

SARS-Cov RBD - ACE2 receptor binding

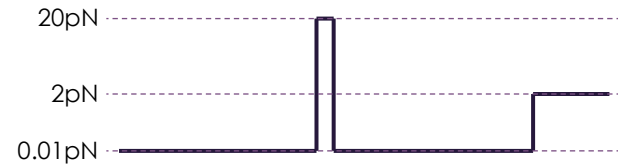
- Real-time, z-axis position of a single bead
- High force spike used to 'reset' assay
- Force used to determine energy required to break an established interaction



PPI ONLY



Force profile →



3

ASSAY RESET
Molecule fully elongated
(first at 20 pN, then at 2 pN)

4

'BREAKING STRAIN'
Force increased to the breaking strain of the interaction
(here ca. 2 pN)



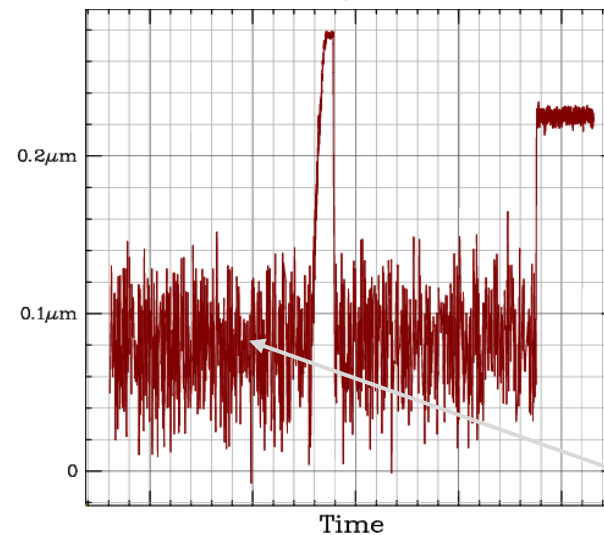
Addition of a third analyte

What are we seeing?

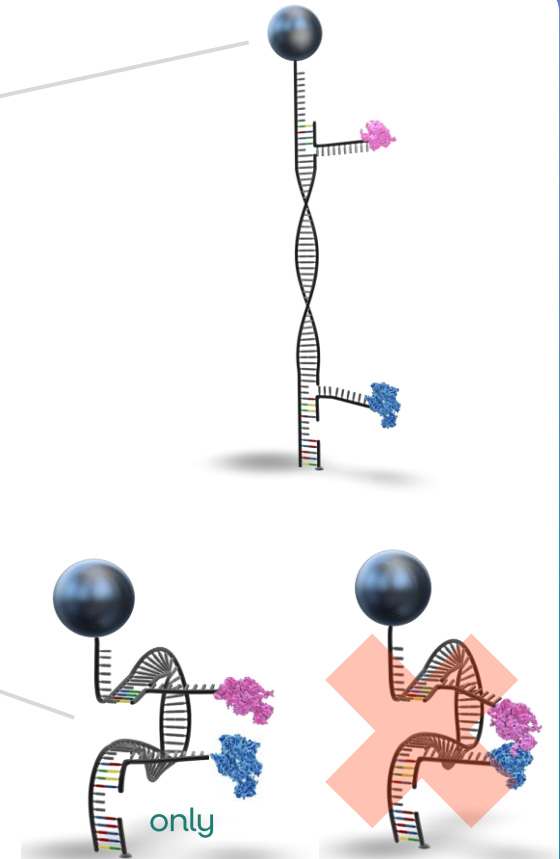
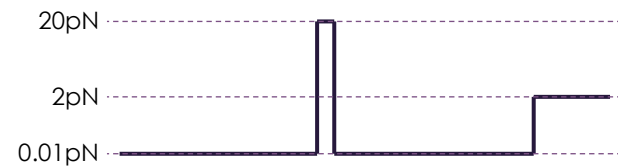
A peptide ('DX600') binds to the ACE2 receptor, blocking its interaction with SARS-CoV 2 RBD.

- No further interactions observed
- A 'permanent' blockage of the interactions between the two proteins is seen.

WITH PEPTIDE 'DX600'



Force profile →

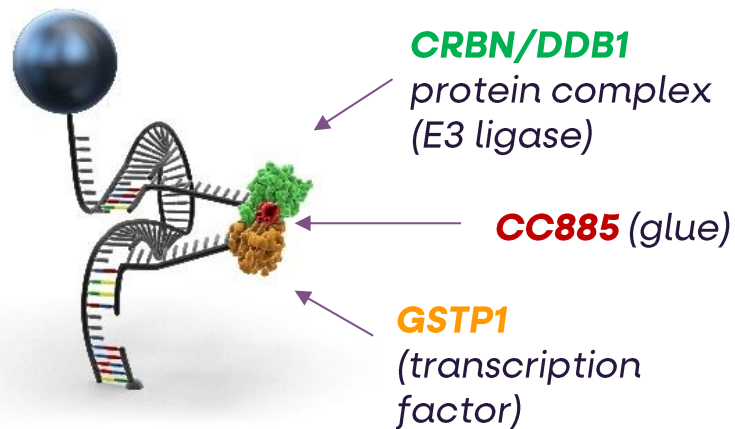


MAGNA for studying molecular glues

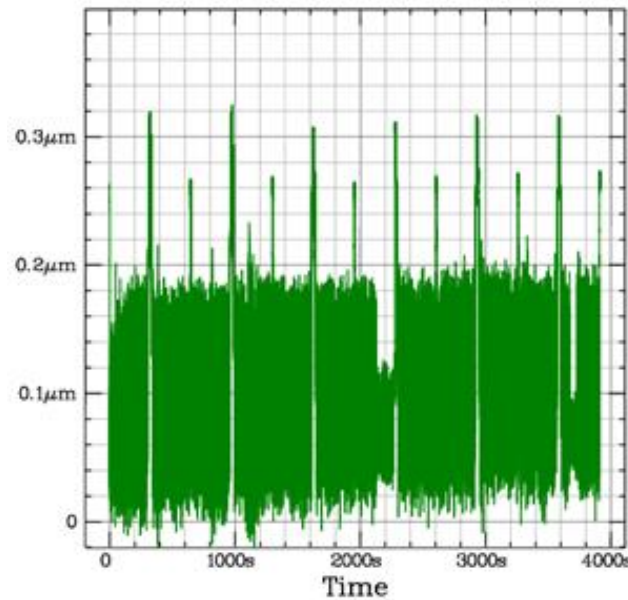
What are we seeing?

Study of a molecular glue modulating an E3 ligase to target protein interaction.

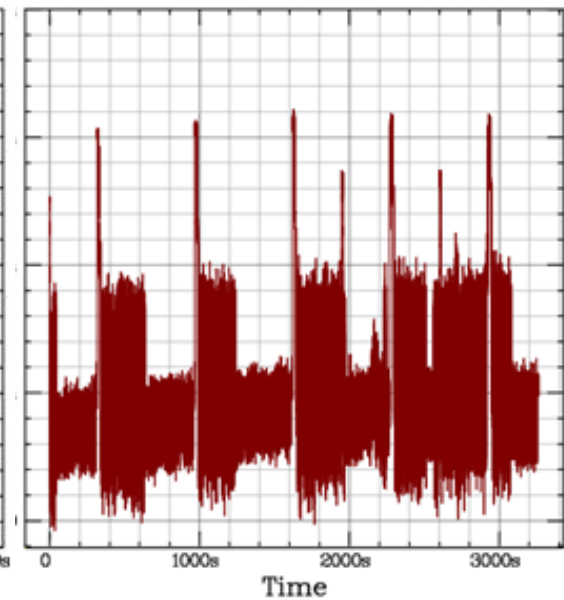
- Real-time, z-axis movement of a single bead
- No force applied
- Duration shown = **2 x 1hr traces**



Green trace –
protein pair only



Red trace – molecular
glue **CC885** at **10nM**
concentration

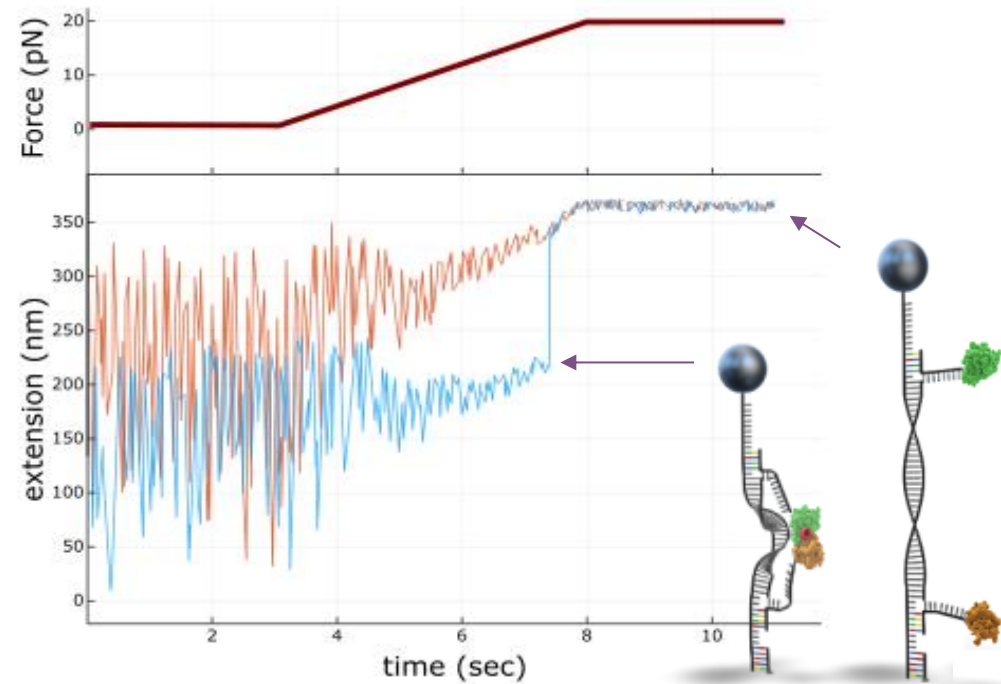
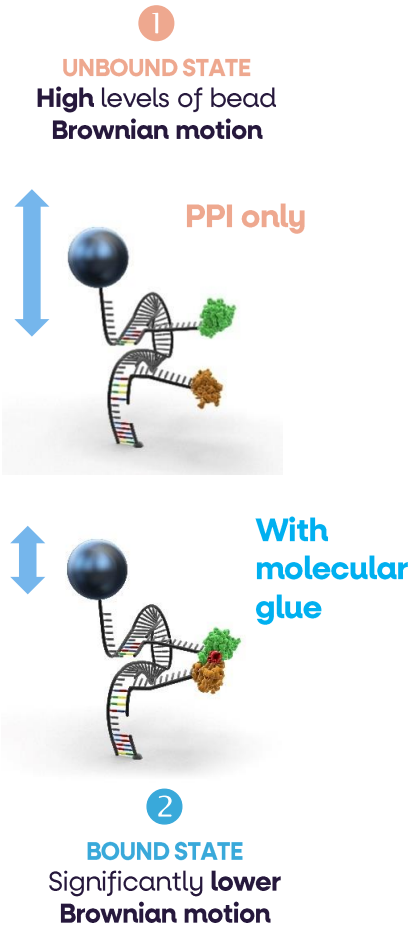


MAGNA for studying molecular glues

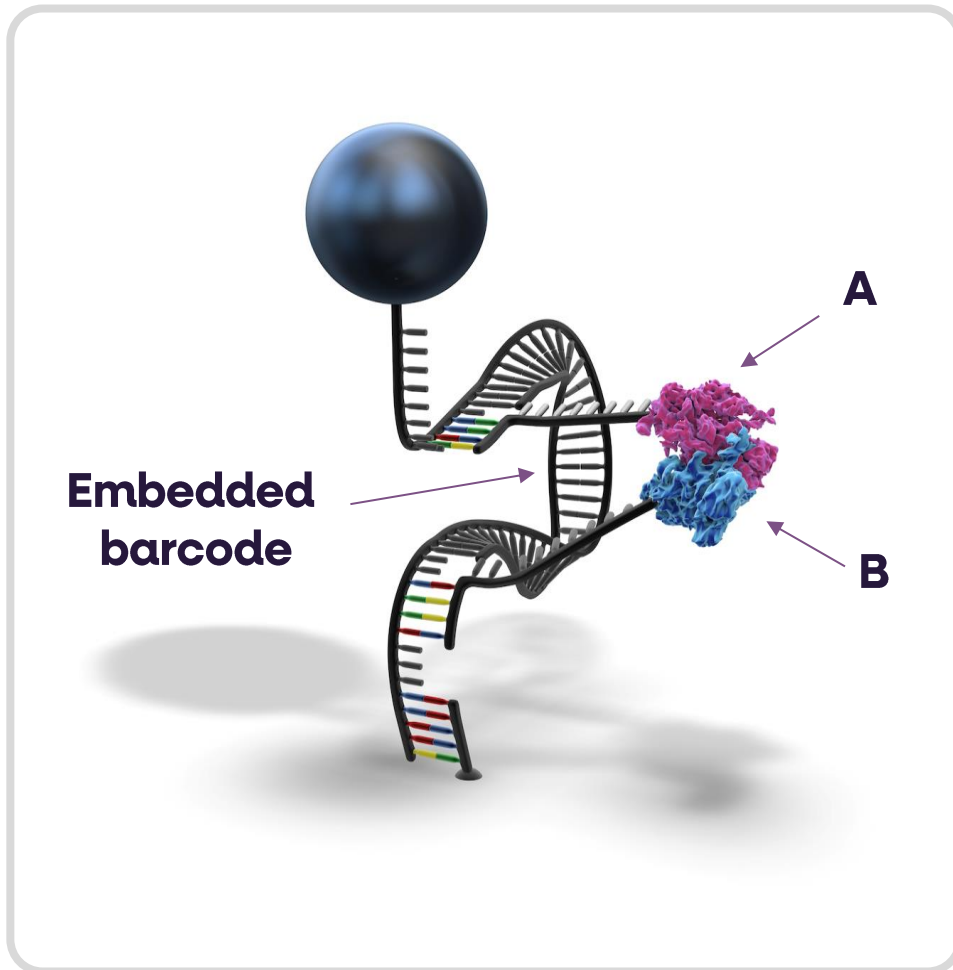
What are we seeing?

E3 ligase CRBN/DDB1 – GSTP1

- Real-time, z-axis movement of a single bead
- Force ramped from 0.01pN to 20pN



This binding strength assay is highly versatile & can be multiplexed



- **A & B** can be many different molecule types
 - **Proteins** – including antibodies
 - **Peptide**
 - **DNA or RNA**
 - **Small molecules**
- Embedded barcode enables **multiplexing**
- 90% of the **assay components are standard**
- Starting to **reveal new binding features**
- Validated across multiple PPIs, including:
 - **EGF : EGFR**
 - **CD40 : CD40L**
 - **PD1 : PD-L1**



MAGNA today & looking to the future

We are weeks away from our launch of MAGNA One



Take off for MAGNA One ...

- Successful **beta testing** program - **launched Dec 2023.**
- **Early-access sales** program - **October 2024.**



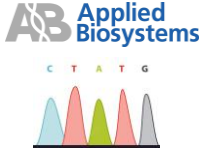



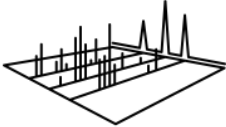






First commercial instruments currently under construction

With a supply of Adimec cameras awaiting future installations



Scalable technologies bring step-changes in value

Tools development is often incremental, but scalable technologies that for the first time bring **large volumes of high-quality** data to a field, can generate enormous value.

	Tools for targeted analysis	reads/sample	High-throughput, high-content tools – opening huge new biological understanding	
Genomics	 <p>Applied Biosystems Sanger sequencing</p>	> 10,000x	 <p>Acquired by Illumina \$600m (2007)</p>	 <p>Market cap. \$22.5bn (2023)</p>
Proteomics	 <p>PAGE gel electrophoresis</p>  <p>Conventional mass spectroscopy</p>	> 1,000x	 <p>Acquired by Danaher \$900m (2010)</p>	 <p>Acquired - Thermo Fisher \$3.1bn (2023)</p>
Interactomics	 <p>BIACORE Acquired by GE \$390m (2003)</p>  <p>Atomic force microscopy</p>  <p>Optical tweezers</p>	> 10,000x		



In conclusion

- **MAGNA One – a powerful new optical approach to real-time analysis of biomolecular interactions at massive scale ...**

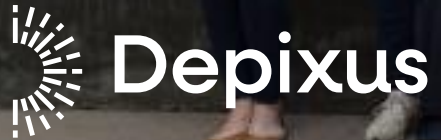
Opens the door to:

- new biological insights
 - functional assays generating unparalleled kinetic data
 - working with low abundance samples
- ... across a **wide range of interaction types & affinities.**



→ **unlocking faster routes to better therapies**

Acknowledgements



THE GRANTS & AWARDS WE'VE RECEIVED FROM...



Innovate UK

OUR INVESTORS



& OUR COLLABORATORS



Thank you!

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See biology as it happens

