FLOW INDUCED DISPERSION ANALYSIS (FIDA) TO MEASURE gRNA-NUCLEASE INTERACTIONS FOR CRISPR-CAS DEVELOPMENT

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Introduction

FIDA is a microfluidic technology which utilizes the phenomenon of Taylor's dispersion to obtain absolute in solution hydrodynamic radius (R_h) of biomolecules.

Here we demonstrate how Fida 1 can be employed as an effective tool for characterization of guide RNA (gRNA)-Cas nuclease interactions and as an automated gRNA screening method.

The interaction analysis and the screening assay requires only a single, fluorescently labelled RNA strand and consumes only 40 nL of it for each measurement.

Method

In Fida 1, the sample flows from the autosampler, through the capillary and towards the detector, which measures the fluorescence of a labelled molecule, called "indicator".

Here FAM labelled synthetic gRNA was used as indicator for characterization of gRNA-Cas interaction, as well as for gRNA screening.

Experiments were performed on a Fida 1 instrument employed with 480 nm LED detection using a high-sensitivity coated capillary (Fida Biosystems). Working assay buffer was 50 mM HEPES, 700 mM NaCl, 0.5 mM TCEP, 0.05% Pluronic-F-127, pH 7.5. Flow Induced Dispersion Analysis was performed by flushing the capillary with 4 µl of analyte sample (the unlabelled titrant), followed by 40 nL indicator plug injection and finally mobilizing the plug towards detection window by analyte flushing.

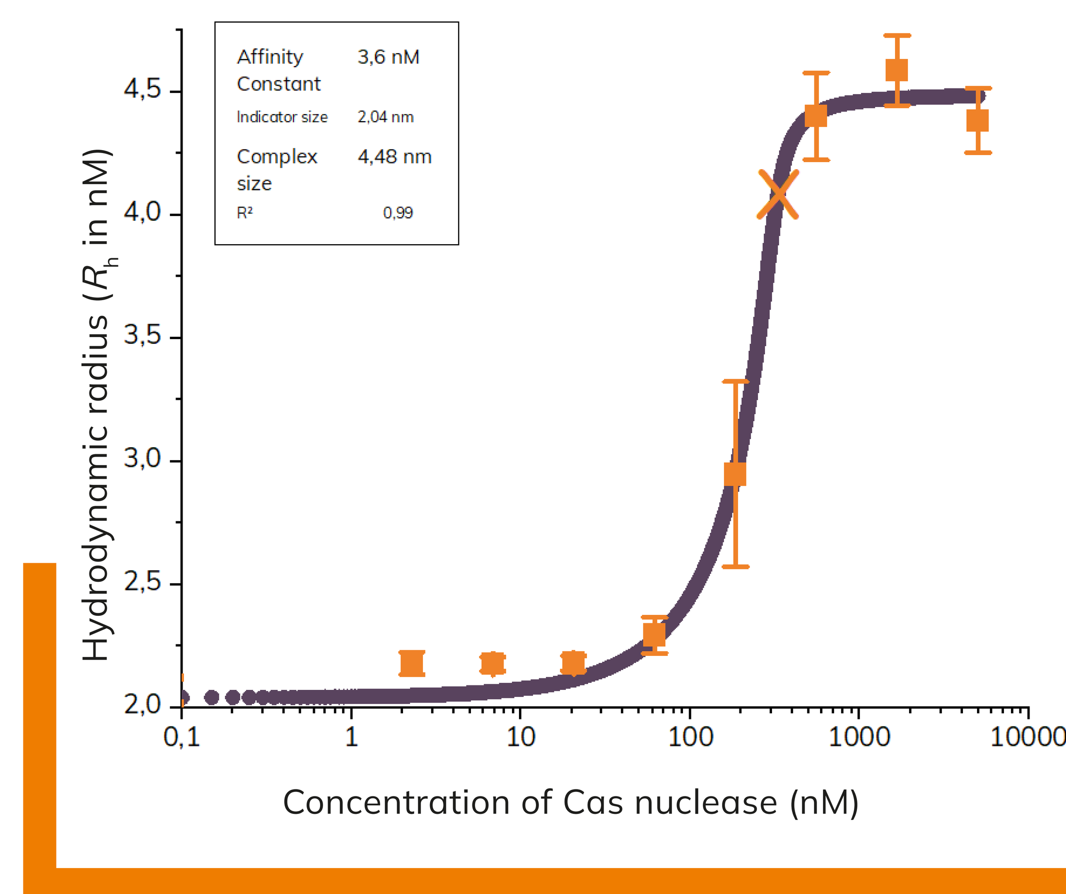
I. Conditions for gRNA Cas interaction assay:
Indicator: 10 nM FAM labelled RNA + Cas nuclease
Analyte: Cas nuclease

II. Conditions for gRNA screening for relative binding to Cas nuclease:
Indicator: 10 nM FAM labelled RNA + 300 nM Cas nuclease + cold RNA
Analyte: 300 nM Cas nuclease + cold RNA

Fida 1

Results

1 Characterization of RNA Cas nuclease interaction



The hydrodynamic radius R_h of unbound gRNA was determined to be 2.2828 ± 0.04 nm

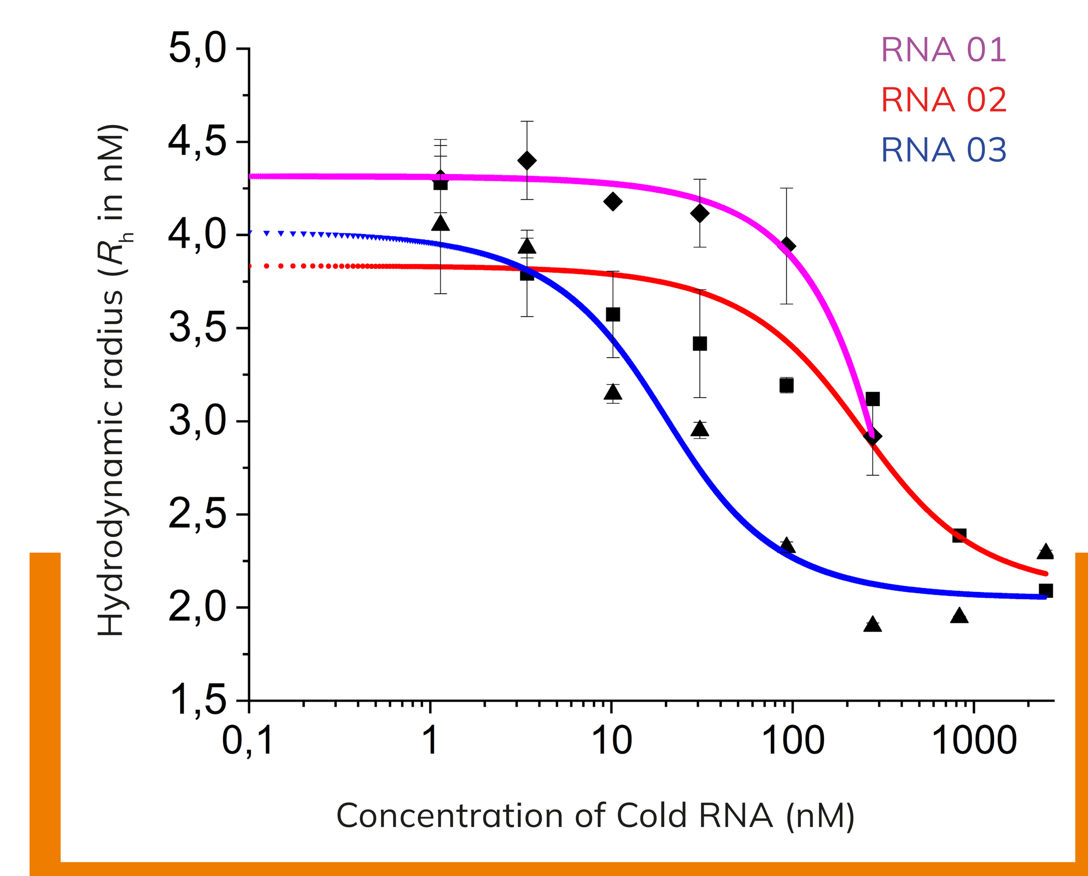
Titration of 10 nM FAM labelled gRNA with increasing concentration of Cas nuclease generated a sigmoidal shaped binding curve, confirming the formation of gRNA-Cas complex with a measured size of 4.48 nm.

The complex saturation was observed at approximately 300 nM Cas concentration, and this condition was used in further experiments.

2 gRNA screening and estimation of relative binding affinities to Cas nuclease

The complex saturation condition from step 1, i.e. 10 nM of FAM gRNA + 300 nM of Cas nuclease were used to perform a competition assay with unlabeled gRNAs. The unlabeled, cold gRNAs were titrated with fixed concentrations of FAM-labeled gRNA and Cas nuclease. Three cold gRNAs were tested, including the unlabelled version of the previous FAM-labelled gRNA, as well as two therapeutic gRNAs.

The complex size of 4.00 ± 0.5 nm was observed to decrease with increasing amount of cold gRNA. At high concentrations of gRNA, the R_h of free FAM labelled gRNA, i.e. 2.28 nm was detected. This indicates favorable competition of cold gRNA against FAM labelled gRNA in complex with Cas nuclease. The competition patterns obtained for each gRNA enables efficient ranking and selection for strong or weak binders.



Outcome of analysis using Fida software:

- gRNA-Cas complex size measurement
- Kd (affinity of gRNA - Cas interaction)
- Relative comparison of several gRNA interaction with the nuclease

Conclusions

In this work, we demonstrated the ability of Fida 1 to perform characterization of RNA-Cas complexes using only few nanogram amount of sample. We showed successful detection of the hydrodynamic radius of free RNA, and the respective increase of this radius in the presence of Cas nuclease, which indicated binding interactions. Additionally, we designed a novel method for obtaining relative binding affinities of Cas nucleases with different gRNAs, using a single labelled RNA variant. The method is easy to setup in any standard laboratory and can be adopted for a high throughput screening (HTP), which enabled mechanistic studies of RNA-nuclease interactions, as well as efficient guide RNA lead discovery.

The method was developed using the Fida 1 instrument which offers straightforward assay development, walk away automation, and absolute (R_h) measurement with ultra low sample consumption.