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FIDA-Based Screening of De Novo Binders in Bacterial Lysates and Purified Protein Titration

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Jan Nowak¹, Francisca Pinheiro^{1,2,3}, Elena Zueva¹, Magnus Kjaergaard^{1,2,3}

¹Department of Molecular Biology and Genetics, Aarhus University, Universitetsbyen 81, 8000 Aarhus C, Denmark;

²Center for Proteins in Memory – PROMEMO, Danish National Research Foundation, 8000 Aarhus C, Denmark;

³The Danish Research Institute for Translational Neuroscience (DANDRITE), Aarhus University, Universitetsbyen 81, 8000 Aarhus C, Denmark



Jan Nowak

Aarhus University

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Francisca Pinheiro, Jan S. Nowak, Elena Zueva, Emily C. Pheasant, Ida Kjærsgaard Grene, Vili Lampinen, Magnus Kjaergaard

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Protocol status: Working**We use this protocol and it's working****Created:** August 14, 2025**Last Modified:** August 29, 2025**Protocol Integer ID:** 224673

Keywords: characterizing biomolecular binder, biomolecular binder, screening of de novo binder, de novo binder, quantitative affinity determination through titration, quantitative affinity determination, purified protein titration flow, bacterial lysate, labelled molecule, complex biological sample, induced dispersion analysis, treated cell lysate, fida, dispersion analysis, events in complex biological sample, cell lysate

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Abstract

Flow-induced dispersion analysis (FIDA) offers a rapid and cost-effective approach for screening and characterizing biomolecular binders. By measuring changes in the hydrodynamic radius of fluorescently labelled molecules and their complexes, FIDA enables direct detection of binding events in complex biological samples, such as heat-treated cell lysates, without immobilization. This allows identification of candidates with high affinity and stability in a single measurement, followed by quantitative affinity determination through titration. The low sample requirements and compatibility with crude preparations make FIDA a versatile tool for evaluating large sets of computational or experimentally generated binder designs.

Guidelines

Fida 1 instrument (Fidabio, Søborg, Denmark) with a 480 nm detector has been used to develop this protocol, however, it is also applicable to other detector types and newer iterations of the instrument.

Materials

Experimental buffer: neutral pH buffer with salt and either 0.05% v/v Tween 20 or 0.03% v/v Pluronic f127. E.g: 20 mM phosphate 300 mM NaCl pH 7.4 0.05% Tween 20.

Fidabio consumables <https://shop.fidabio.com/>

96-well pressure-plateFidabioCatalog #220-010

Permanent Coated CapillaryFidabioCatalog #100-002

Standard capillaryFidabioCatalog #100-001

Standard capillaryFidabioCatalog #100-001

Pressure-vialsFidabioCatalog #201-200

Protocol materials

 Standard Fida Capillary **Fidabio Catalog #100-001**

 Permanent Coated Fida Capillary **Fidabio Catalog #100-002**

 1 M NaOH

 Acetic acid **Fisher Scientific**

 Pressure-vials **Fidabio Catalog #201-200**

 96-well pressure-plate w. plate seal **Fidabio Catalog #220-002 or 220-010**

Safety warnings

 Use protective glasses when installing or removing the Fida capillary.

Use gloves when handling 1 M NaOH and any other toxic materials during the experiments.

Before start

The indicator (the reporter molecule in the Fida method) should be labelled with a fluorophore compatible with the detector; FITC and Alexa 488 are effective for the 480 nm detector. Unreacted dye should preferably be removed, for example by size-exclusion chromatography or RP-HPLC. The sample should be as monodisperse as possible and remain stable under the assay buffer conditions.



Installing a capillary

1h 7m

1 Turn on the instrument and detector.

1m

2 Move the autosampler to the capillary position.

1m

Note

The autosampler position should appear as "Capilla" in the position dashboard.



3 Install the capillary (Standard Fida Capillary **Fidabio Catalog #100-001** or Permanent Coated Fida Capillary **Fidabio Catalog #100-002**) according to the manufacturer's instructions (Fidabio).

5m

4 Wait until the baseline is stabilized (approx. 01:00:00).

1h



Determining optimal indicator concentration

1h 15m

5 Make 5 20 μL dilutions of indicator in a range of concentrations between 200 nanomolar (nM) to 20 nanomolar (nM) using assay buffer.

10m

6 Transfer the indicator solutions to a 96-well pressure-plate (96-well pressure-plate w. plate seal **Fidabio Catalog #220-002 or 220-010**).

5m

7 Fill one well with 100 μL assay buffer.

8 Prepare vials (Pressure-vials **Fidabio Catalog #201-200**) with 1.5 mL 1 M NaOH and 1.5 mL assay buffer.



If using a Permanently Coated Capillary, avoid using NaOH entirely or replace it with 0.5 % volume (approx. 80 millimolar (mM))

Acetic acid **Fisher Scientific** .

- 9 Put the silicone plate-seal on the 96-well plate and place it in Tray 1, the vials from previous step in Tray 2, and an empty vial in the Waste tray.
- 10 Define the sequence of steps in the experiment in the Fida Instrument software under the "Sequence" tab using the following steps:

10.1 Define indicator positions according to the indicator positions in the 96-well plate.

10.2 Define analyte position as the well with  100 μ L assay buffer.

10.3 Choose waste position according to placement of vial in Tray waste.

10.4 Select a single replicate.

10.5 Choose the *Standard Capillary* method if using Standard Capillary (or Permanently Coated Capillary with acetic acid) or *Coated Capillary* method if using a Permanently Coated Capillary with buffer only. Open both methods for clarification.

Make sure that the vials in Tray 2 match the position in the chosen method. You can see and edit method under "Method" tab.
- 11 Run the sequence.
- 12 Choose the lowest indicator concentration that still provides a satisfactory S/N of >50 after smoothing in the Fida analysis software.

Also, assess the "stickiness" of the indicator by examining the symmetry of the Taylogram; right-side asymmetry suggests interactions between the indicator and the capillary. This issue can be mitigated by optimizing the buffer (e.g., increasing salt concentration or changing detergent) or by using a permanently coated capillary. Additionally, evaluate the monodispersity of the indicator by checking the polydispersity index (PDI), where low PDI values indicate a more uniform sample.



1h

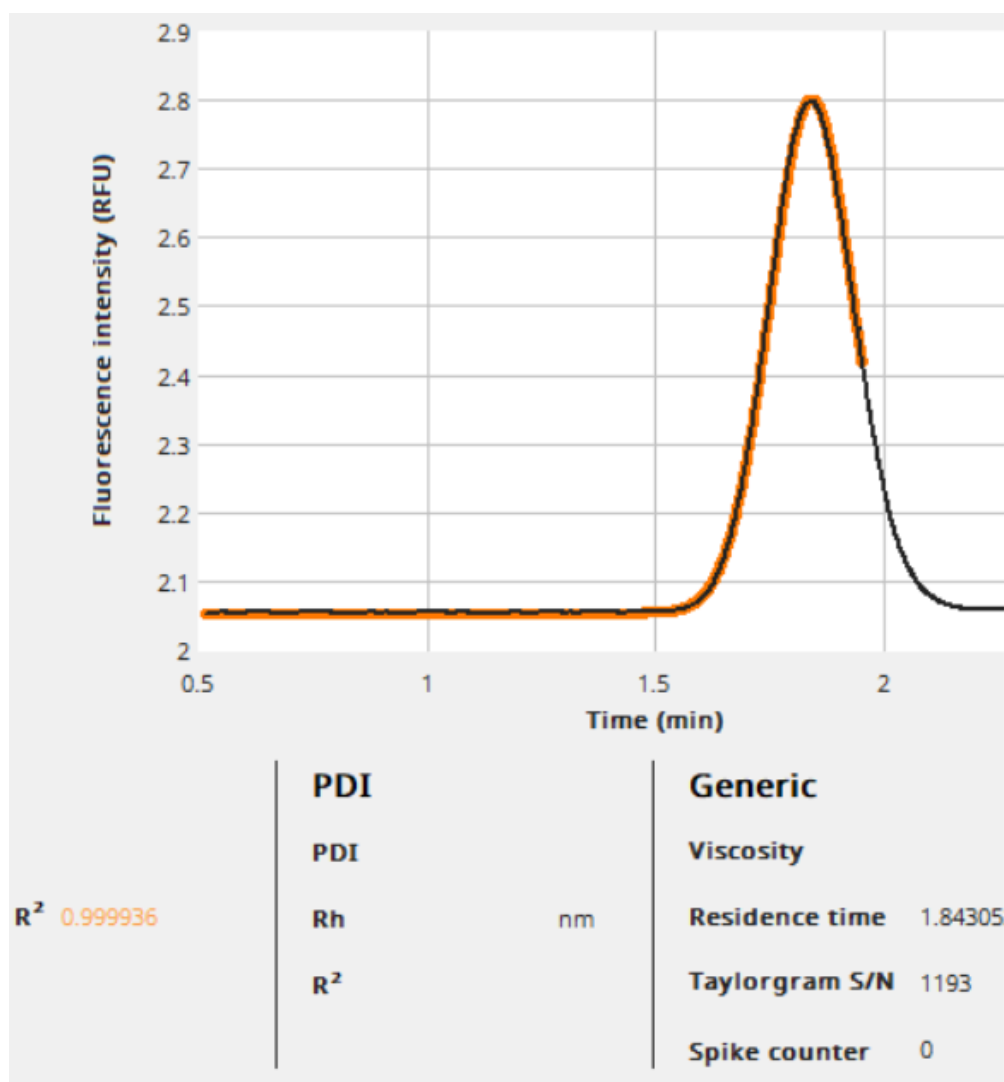


Note

In case of proteins labeled with kits, some unreacted dye may be present. Solve this, using a multi-species fit with a species locked to a ~0.7 nm size.

*Make sure that the indicator has the correct size using either the PDB correlator in the Fida analysis software or an **MW to Rh calculator**.*

Expected result





Screening

10h 52m

- 13 Dilute the bacterial lysates with a factor of 30 using the assay buffer to a final volume of  50 μL directly in the 96-well plate. Use a non-expressing clone as control.

10m

Note

This dilution factor strongly depends on the expression method, as the result of this approach will depend both on the affinity of the binder and its expression level. This dilution factor has proven effective for [reference].

- 14 Add  50 μL assay buffer to an empty well of the 96-well plate.

1m

- 15 Refill vials in Tray 2 and empty the Waste vial.

1m

- 16 Put the silicone plate-seal on the 96-well plate and place it in Tray 1.

- 17 Define the Sequence in Fidabio instrument software.

10m



- 17.1 Define indicator position according to the chosen indicator concentration in the previous section.

- 17.2 Define analyte positions as the wells with the diluted lysates and the single well with assay buffer.

- 17.3 Select triplicates.

- 17.4 Choose the same Method as in the previous section. Only use the same Method for 7 samples (21 total replicates) as the buffers in Tray 2 will run out. In case of >7 samples, make a copy of the Method and change the buffer locations.



E.g: Open Standard Capillary Method and change NaOH to position 3 and buffer to position 4, and save it as "Standard Capillary 2". Load that method in the Sequence.

Make sure that the vials in Tray 2 match the position in the chosen Method(s).

17.5 Change the Waste vial position in the Sequence for each time a Method is changed.

18 Run the Sequence.

10h



19 Evaluate the Rh of the samples compared to the control. As a rule of thumb, FIDA can reliably distinguish changes of 5%, however, the cutoff for the screening is highly dependent on the size of the indicator and the complex.

30m

Note

Lysates may induce high viscosity, so viscosity compensation may be necessary. Use the indicator in assay buffer as reference.

Titration of purified positive hits

7h 33m

20 Make a 15-point 2- or 3-fold serial dilution of the purified binders, preferably in a range between at least 100 times lower and at least 100 higher relative to the expected K_D .

20m

Note

E.g.: If the expected K_D is [M] 100 nanomolar (nM) , make a 2-fold serial dilution between [M] 10 micromolar (μ M) and [M] 0.61 nanomolar (nM) .

If expected K_D is unknown, use 3-fold dilution and extend the concentration range.

20.1 For a 2-fold dilution, fill a well with  100 μ L of the binder at its highest concentration.

20.2 Fill subsequent 14 wells with  50 μ L of assay buffer.



- 20.3 Transfer  50 μL of the binder at its highest concentration to the next well and mix by pipetting. Transfer  50 μL of the mixture to the next well and continue until finishing the whole dilution series.

Note

For 3-fold dilution, transfer  25 μL between each well .

- 21 Fill a well with  50 μL of assay buffer as control. 1m
- 22 Refill vials in Tray 2 and empty the Waste vial(s). 1m
- 23 Put the silikon plate-seal on the 96-well plate and place it in Tray 1. 1m
- 24 Define the Sequence in Fidabio instrument software. 1m
- 24.1 Define indicator position according to the chosen indicator concentration in the previous section. 1m
- 24.2 Define analyte positions as the wells with the dilution series and the single well with assay buffer. Fill out the concentration of the dilutions. 1m
- 24.3 Select triplicates. 1m
- 24.4 Choose the same Method as in the previous sections. Only use the same Method for 7 samples (21 total replicates) as the buffers in Tray 2 will run out. In case of >7 samples, make a copy of the Method and change the buffer locations. 5m



E.g: Open Standard Capillary Method and change NaOH to position 3 and buffer to position 4, and save it as "Standard Capillary 2". Load that method in the Sequence.

Make sure that the vials in Tray 2 match the position in the chosen Method(s).



24.5 Change the Waste vial position in the Sequence for each time a Method is changed.

1m

24.6 Run the Sequence.

7h

25 Evaluate the data in the Fida analysis software in case of an isotherm, fit to a suitable binding model.



Note

*Compare the size of the indicator and the complex using either the PDB correlator in the Fida analysis software or an **MW to Rh calculator**.*