FIDA FOR THE EVALUATION OF DE NOVO DESIGNED BINDERS

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INTRODUCTION

Binder generation algorithms

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Sequence generation algorithms

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Structure prediction algorithms

Revolution in de novo protein binder design

De novo designed binders must be **experimentally validated**

HOWEVER

Binder design campaigns still require screening **tens to hundreds** of designs

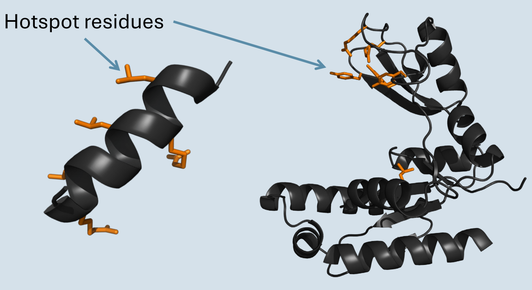
! There is a need for **rapid** and **cost-effective** methods for binder screening

Exploring the potential of FIDA as a tool for screening de novo designed protein binders

METHODS

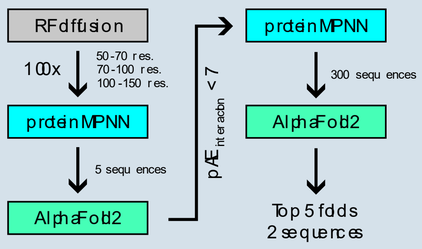
1. Protein design

Protein targets

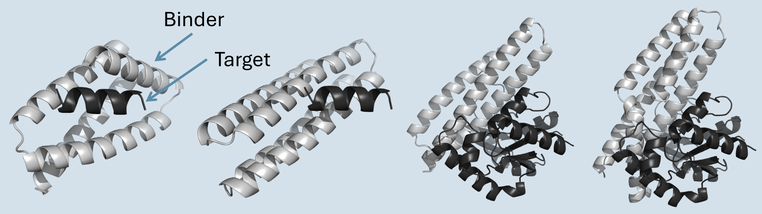


ALFA-tag PSD-95 GK-domain

Workflow

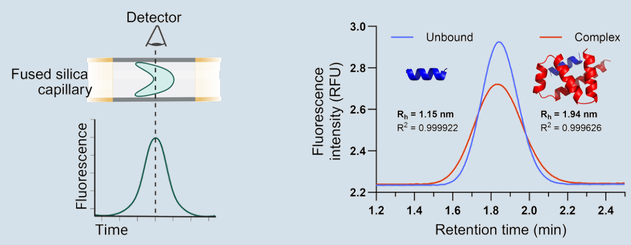


- **10 binders** against ALFA-tag[1] and **12 binders** against the GK-domain[2] were selected for experimental testing.
- Binders A and B represent two **different sequences** for the **same backbone**.



ALFAbinder1A ALFAbinder4A GKbinder1A GKbinder3A

2. Flow-induced dispersion analysis (FIDA)

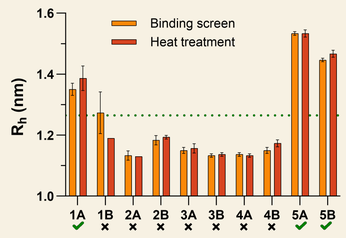


FIDA uses Taylor dispersion analysis to determine the **hydrodynamic radius (Rh)** of fluorescently labeled biomolecules and their complexes[3].

RESULTS

1. Characterization of ALFA-tag binders

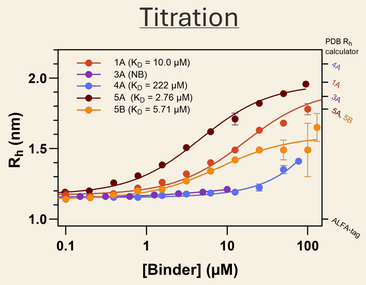
Screening



Indicator: FITC-ALFA Analyte: Purified binders

- Binders **1A**, **1B, 5A and 5B** (10 µM) induced a **10% increase in R_H** (green line) relative to FITC-ALFA (1.15 nm).
- Binders **1A**, **5A and 5B** retained their binding after incubation at **95°C for 15 min**.

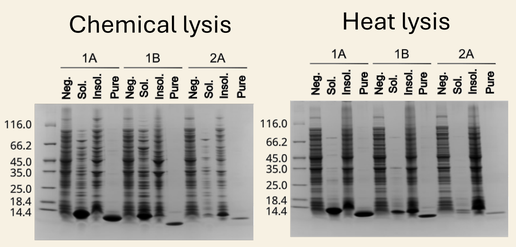
Titration



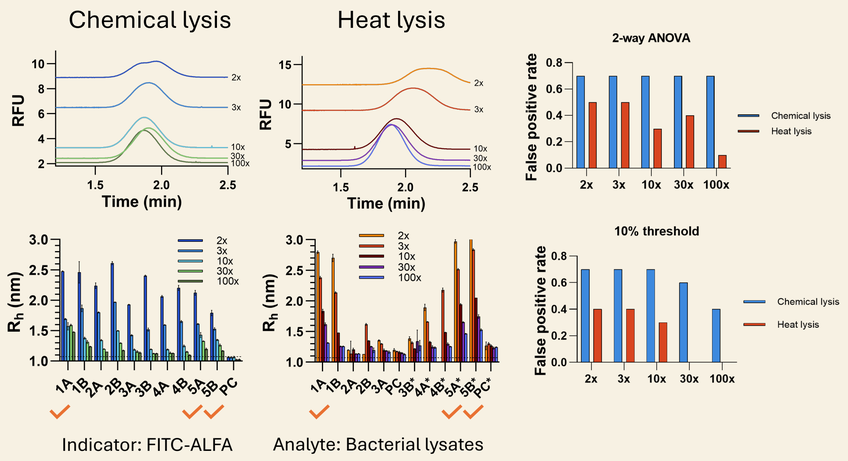
Indicator: FITC-ALFA
Analyte: Purified binders

- **Titration** of all three positive hits produced classical isotherms that could be fitted to a **1:1 binding model**.
- Negative hits (3A and 4A) resulted in **high or no measurable K_D**.

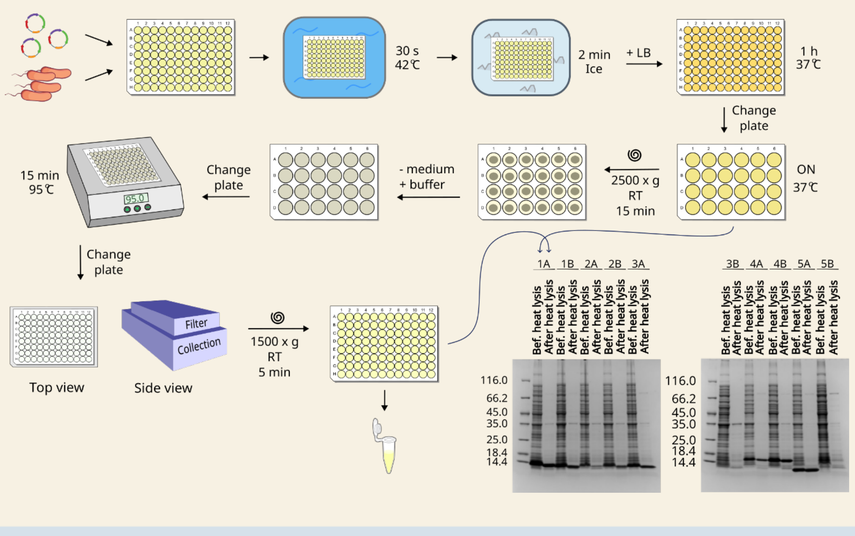
2. Screening directly on bacterial lysates



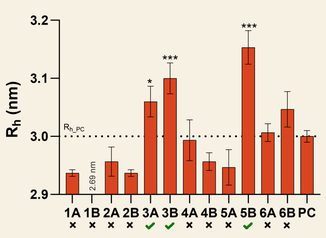
- De novo designed binders are often **highly thermostable**, making it possible to use **heat** to lyse the bacteria.
- **Heat lysis** (95°C, 15 min) resulted in **highly pure lysates** (Sol.), surpassing the purity achieved with chemical lysis.
- Binding can be screened directly **in bacterial lysates**.
- **Diluting** the lysates is a good strategy to **decrease** signal mismatch (at 488).
- Screening for binding with **a 30-fold dilution of heat-treated lysate** excludes all false positives without sacrificing the detection of true positive hits.



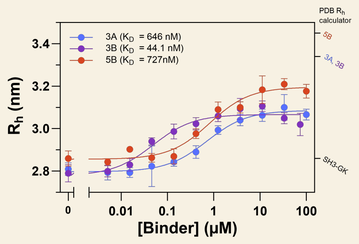
3. Expression and purification of de novo designed binders using heat lysis



4. Evaluation of de novo designed binders against complex targets

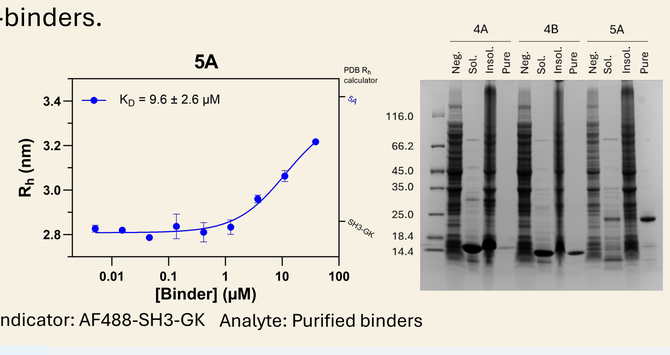


Indicator: AF488-SH3-GK
Analyte: Heat lysates



Indicator: AF488-SH3-GK
Analyte: Purified binders

- Binders **3A, 3B and 5B** induced a **statistically significant increase** in the Rh of SH3-GK compared to the negative control (PC).
- Titration experiments showed **nanomolar affinities** for GK-binders.



Indicator: AF488-SH3-GK Analyte: Purified binders

- Binder 5A, which has a low micromolar affinity, was **not identified in the screening**, probably due to its **low expression**.

DISCUSSION

- Screening in **heat-treated** bacterial lysates allows **rapid** and **efficient** identification of binders with **high affinity** and **heat stability**.
- Binding **can be screened** using chemical lysates, at the expense of purifying and titrating a higher number of proteins.
- The Rh threshold is a **subjective** choice, that should be **optimized** case-by-case.

TO BE CONTINUED...

WANT TO KNOW MORE?

Pinheiro *et al.*, 2025

Grene *et al.*, 2025

Nowak *et al.*, 2025

REFERENCES

1. Götzke, H. *et al*. The ALFA-tag is a highly versatile tool for nanobody-based bioscience applications. Nat Commun. (2019) 10:4403; 2. Li H., *et al*. Characterizing the binding sites for GK domain of DLG1 and DLG4 via molecular dynamics simulation. Front Mol Biosci. (2020) 7:1; 3. Jensen, H. and Østergaard, J. Flow induced dispersion analysis quantifies noncovalent interactions in nanoliter samples. J Am Chem Soc. (2010) 132(12):4070-4071.

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