From de novo roots to biophysical fruits

Building a Taylor-made screening and characterization pipeline for de novo designed proteins

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Background

Recent advances in AI have accelerated de novo protein design.

Increased output makes experimental validation a bottleneck.

Flow-induced dispersion analysis (FIDA) rapidly screens binders.

Synthetic proteins beg for novel characterization approaches.

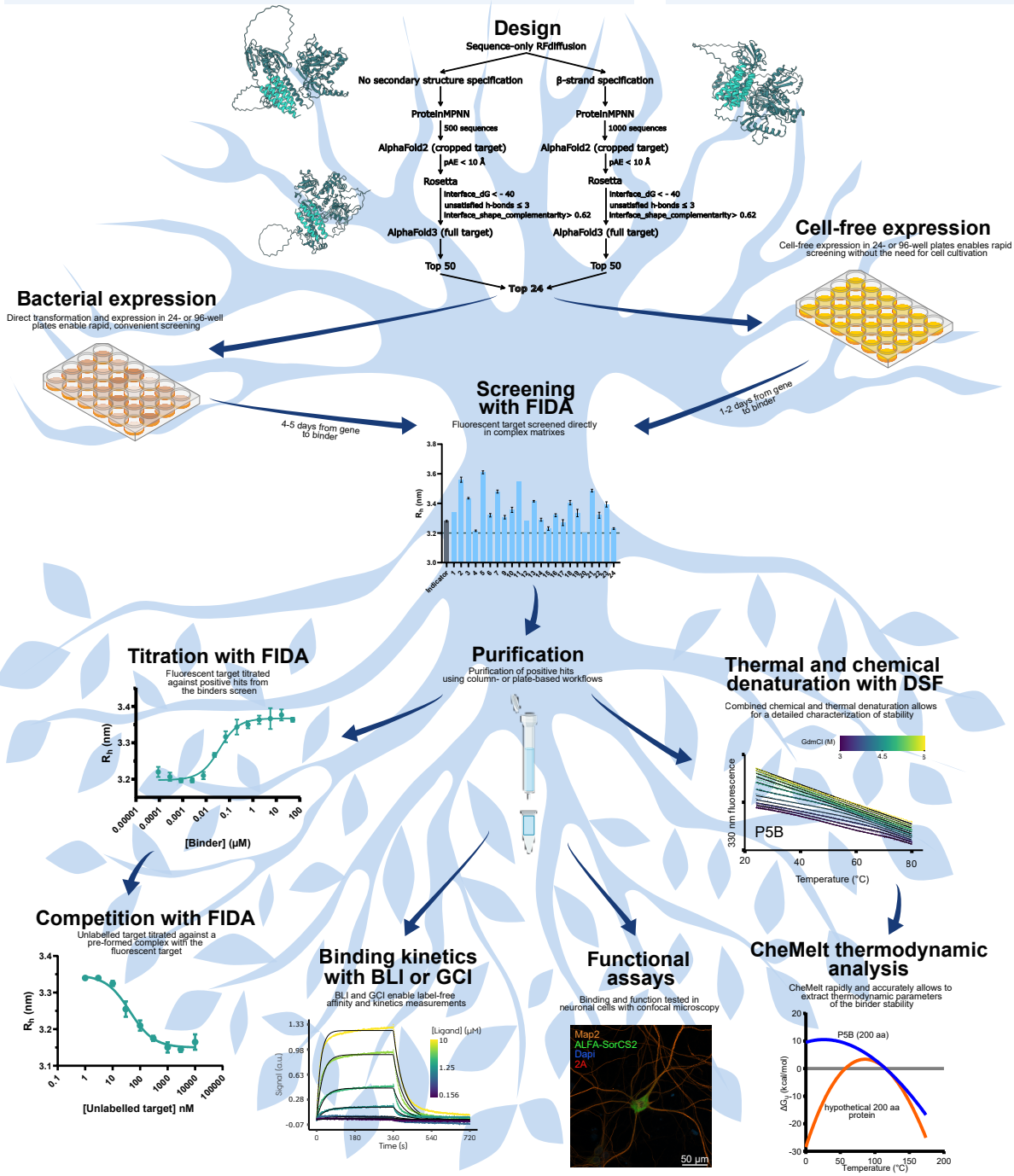
Objectives

Can we establish a solid pipeline for screening of de novo binders.

Can we in few steps screenifor stability, folding and binding?

Can we screen directly in bacterial lysates or cell-free systems?

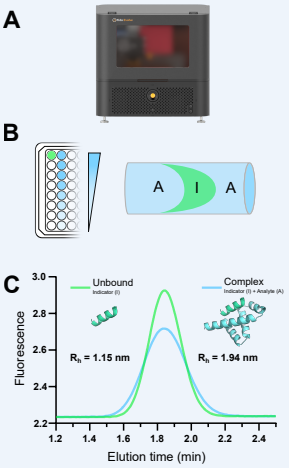
How can we efficiently characterize novel synthetic proteins?



Highlighted methods

Flow Induced Dispersion Analysis (FIDA)

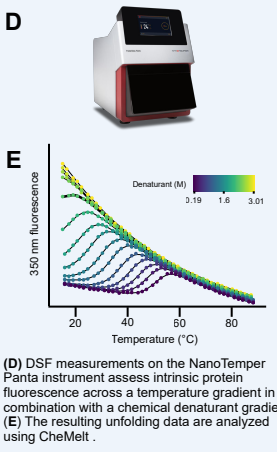
Screening and characterization



(**A**) The FIDA Evolve measures the hydrodynamic radius (Rh) of fluorescent molecules based on Taylor dispersion in a fused-silica capillary, either alone or in complex with a binding partner. (**B**) Complex formation results in a change in R_h. (**C**) A cap-mix approach enables simple assay setup by mixing the fluorescent molecule (indicator, I) with its binding partner (analyte, A).

Differential Scanning Fluorimetry (DSF)

Global analysis of thermal and chemical denaturation using CheMelt



(**D**) DSF measurements on the NanoTemper Panta instrument assess intrinsic protein fluorescence across a temperature gradient in combination with a chemical denaturant gradient. (**E**) The resulting unfolding data are analyzed using CheMelt .

References

Pinheiro F, Nowak JS, Zueva E, Pheasant EC, Grene IK, Lampinen V, Kjaergaard M. Screening de novo designed protein binders in unpurified lysate using flow induced dispersion analysis. Protein Science. 2025;34(10):e70286. DOI: 10.1002/pro.70286.

Lampinen V, Burastero O, Guazzelli IP, Vögele F, Pinheiro F, Nowak JS, García Alai MM, Kjaergaard M. Global analysis of thermal and chemical denaturation using CheMelt: Thermodynamic dissection of highly thermostable de novo designed proteins. Protein Science. 2026. DOI: 10.1002/pro.70800.

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