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Optimization and Selection of ASF1-Binding Peptides with optimized quantitative bacterial two-hybrid Using qB2H

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ASF1-binding peptides: a model system for qB2H development and binder optimization

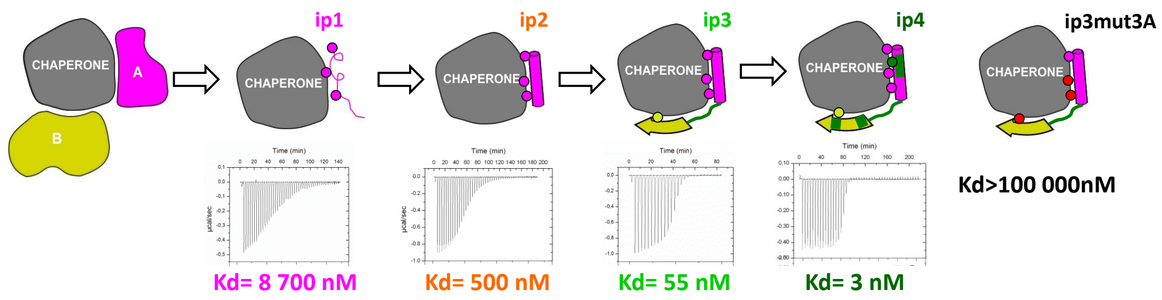
Peptides are powerful tools to perturb protein–protein interactions (PPIs), but their optimization requires detailed knowledge of interaction interfaces and efficient strategies to generate high-affinity binders.

We previously developed synthetic peptides targeting the histone chaperone ASF1 with a broad range of binding affinities.[1] This well-characterized peptide series provided a model system to develop and optimize a quantitative bacterial two-hybrid (qB2H) assay. We then used qB2H to map interaction interfaces and to identify improved ASF1-binding peptides by screening a peptide library. [2]

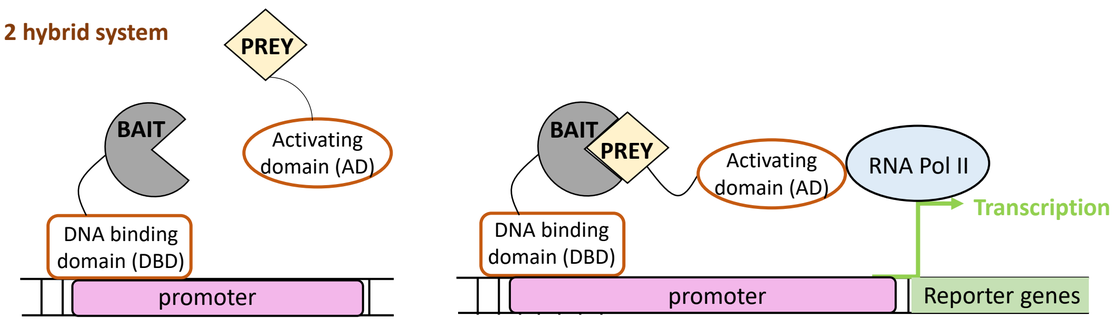
Optimized peptides can further provide molecular templates for the development of foldamer-based binders with enhanced drug-like properties, as illustrated for ASF1-targeting ligands in previous studies[3-5] and **Posters 106 and 76**.

[1] M. Bakail, et al. *Cell Chem Biol* **2019**, 26, 1573–1585 e1510.
[2] A. Guyot, et al. Comput Struct Biotechnol J. 2026 May 25;35(1):0098.
[3] M. Bakail, et al. *Chembiochem* **2019**, 20, 891–895.
[4] J. Mbianda, et al. *Sci Adv* **2021**, 7, eabd9153.
[5] M. E. Perrin, et al. *Chem Commun (Camb)* **2023**, 59, 8696–8699

❖ **Synthetic peptides targeting ASF1**

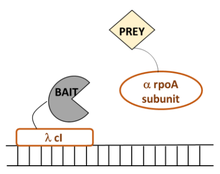


❖ **2 hybrid system**



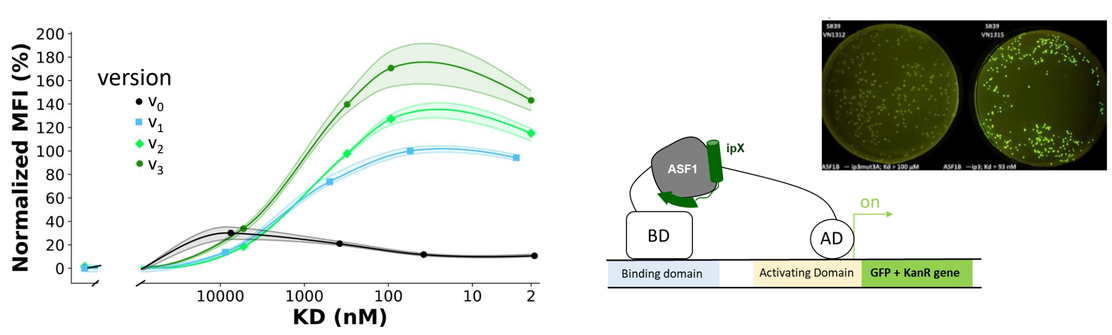
1. qB2H ingeneering

Bacterial two hybrid : Easy manipulation, fast screening, resilient to false results, good for large librairies



Is the fluorescence linear with the affinity between the bait and the prey ?

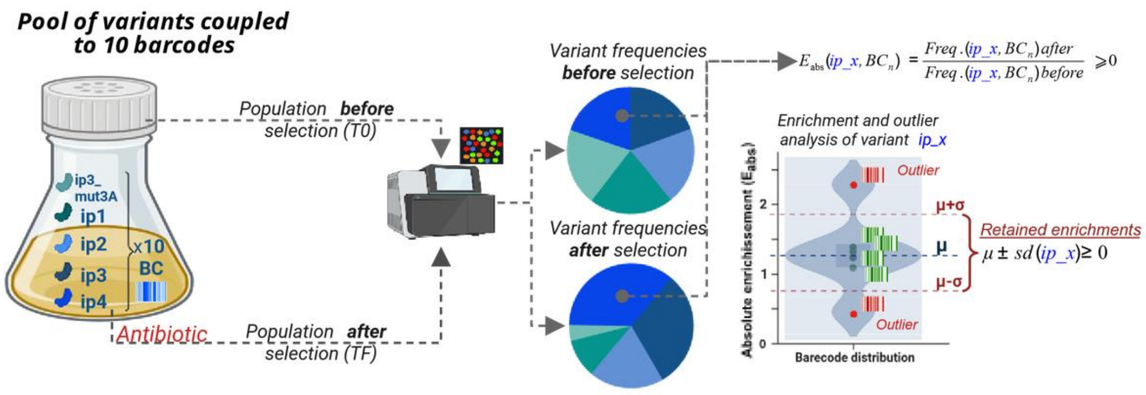
➔ **Test and optimization of reproducibility, signal-to-noise ratio, and correlation between qB2H signal and binding affinity using our series of ASF1-binding peptides spanning a wide range of affinities**

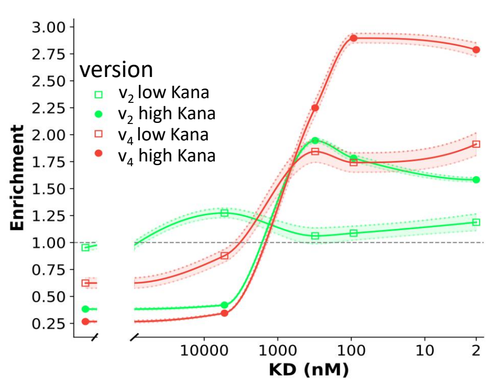


Version	V0 *	V1	V2	V3	V4
Number of Plasmids	3	2	**1**	1	1
cI expression	pZS22	lac_UV5	lac_UV5	**L_tetO**	L_tetO
cI RBS	—	strong	strong	**weaker**	**very weak**
qB2H promoter	Two cI binding sites	Single cI binding site	single cI binding site	Optimized single cI binding site	Optimized single cI binding site
Reporter	eGFP	eGFP + KanR	eGFP + KanR	weak eGFP + KanR	**KanR only**
Main step	**Initial system**	promoter optimization	single plasmid	quantitative optimization	library selection / NGS

** McLaughlin RN et al. Nature. 2012;491:138–142.*

Does fitness correlate linearly with bait–prey affinity?



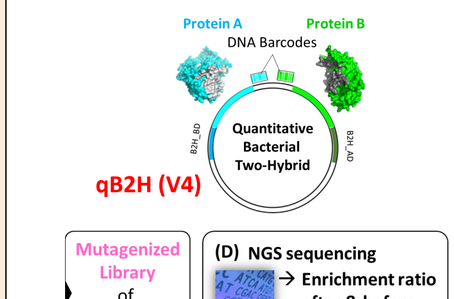


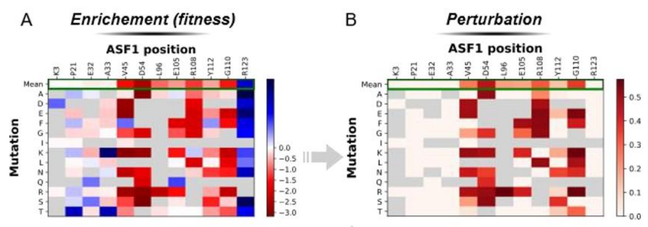
✓ **Optimized version (V4) qB2H response is linear from 5µM to 50nM**

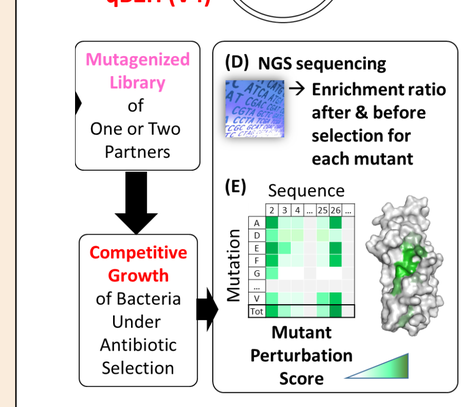
- **Bacterial strain** → **SB39**
  Best Kd- fluorescence correlation, lowest variability
- **Plasmid number** → **1 plasmid**
  Simpler, robust quantitative readout
- **qB2H promoter** → **Single λ cI-binding site**
  Best Kd and signal correlation
- **cI expression** → **L_tetO + weak RBS**
  Tight and reduced expression of the cI fusion ↑signal-to-noise, ↓ stochasticity
- **Reporter** → **Weak KanR only** (v4)
  ↓ metabolic burden, improved quantification

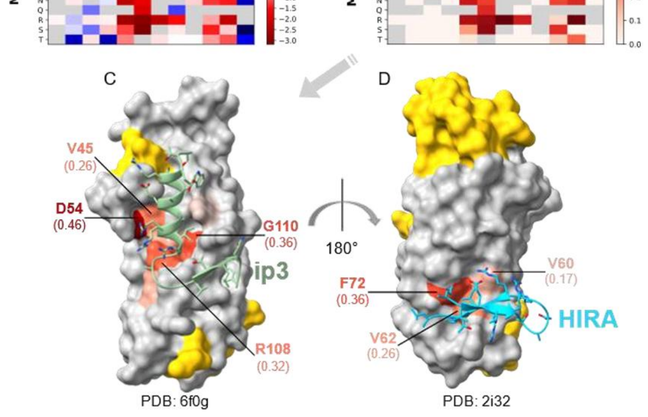
2. interface mapping application

Can qB2H map PPIs using a mutant library?







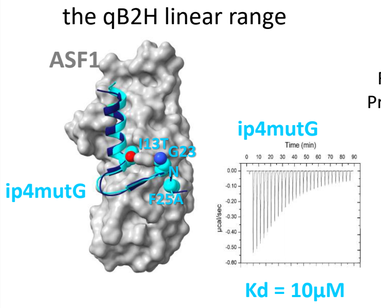


✓ **qB2H (v4) enables PPI interface mapping**

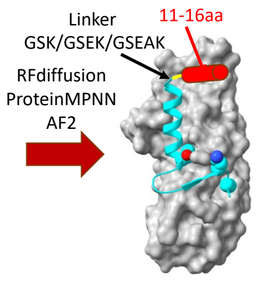
3. Screening for improved variants

Can qB2H identify improved variants from AI-designed libraries?

1) Rational mutant design to start at the lower end of the qB2H linear range



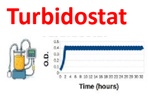
2) Peptide lengthening using generative AI tools



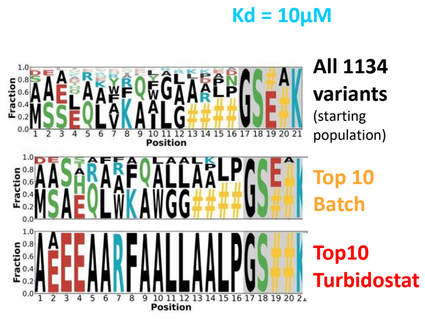
3) Test different selection pressure

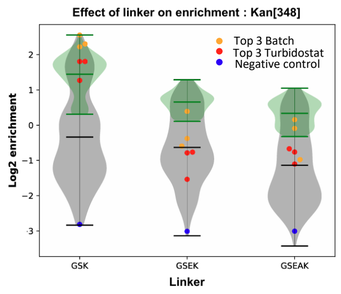


short term (4-5h) batch selection, increasing kanamycin concentration



long term (24-32h) selection using turbidostat, fixed kanamycin concentration

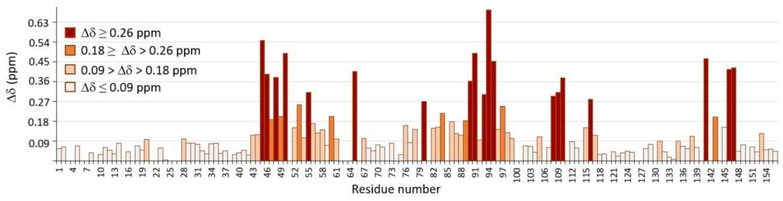


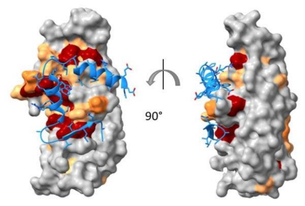


3) Affinity measurement of the two Top1

ip4mutG	10 000nM
Top 1 Batch	209nM
Top 1 Turbidostat	135nM

4) Mapping the interacting surface by NMR of the best binder selected





✓ **qB2H (v4) can identify binders with improved affinity from AI-designed libraries**

Conclusion & Perspectives

Guyot, et al. Comput Struct Biotechnol J. 2026 May 25;35(1):0098

- ASF1-binding peptides enabled the development of an optimized quantitative B2H assay (qB2H v4).
- qB2H v4 enables quantitative PPI analysis, interface mapping, and selection of improved binders from large libraries.
- Coupling qB2H selection with generative AI provides an efficient strategy to identify higher-affinity ASF1-binding peptides.

➔ Selected peptides provide promising templates for the development of foldamer-based ASF1 binders

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