# A designed Franken protein that specifically targets a large DNA site

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## Background

Synthetic biology: Introduce heterologous biological elements to biological systems for a desired outcome[1]

- Detection of stimulus e.g., HIV[2]
- Compound production e.g., artemisinin[3]

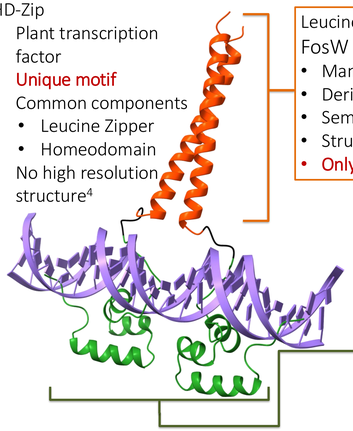
Need heterologous elements orthogonal to the host → avoid unwanted interactions[1]

↳ Orthogonal DNA binder: HinZip

## Frankenprotein = Hin DBD + Leucine Zipper

HD-Zip

- Plant transcription factor
- Unique motif
- Common components
  - Leucine Zipper
  - Homeodomain
- No high resolution structure[4]



Leucine Zipper (LZ) Dimerization Element FosW

- Mammalian
- Derived from basic zipper LZ
- Semi rationally designed[5]
- Structural basis for dimerization[6]
- Only homodimerizes

DNA Binding Domain (DBD)

→Homeodomain (HD)

- Helix-turn-helix motif
- Recognize 7-8 bp DNA site[7]

Hin DBD (Hin)

- HD-Like domain
  - Helix-turn-helix motif [8]
- From *Salmonella typhimurium*
- Recognizes 12 bp DNA site
  - Hix Sites[9]

**Figure 1.** Model HinZip dimer bound to 2 sp invHixC. Made using ChimeraX and 1HCR and 5FV8 structures

## DNA Target Site Design

- Wild type Hin DNA targets: HixL and HixR
  - Each target has two half-sites
- HixC: inverted repeat of half site
  - Half-site identical in HixL and HixR[9]
- InvHixC
  - Inverted half sites in HixC
  - Varied spacer between half sites

  → accommodate structure, binding and dimerization of HinZip

**Table 1.** Hix sites DNA sequences

| Site | Sequence |
|---|---|
| HixL | TTCTTGAAAACCAAGGTTTTTGATAA |
| HixR | TTTTCCTTTTGGAAGGTTTTTGATAA |
| HixC | TTATCAAAAACATGTTTTTGATAA |
| invHixC | TGTTTTTGATAAXTTATCAAAAACA |

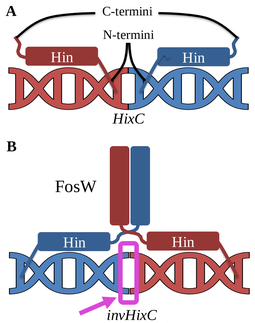



**Figure 2.** *A.* Hin bound to HixC. *B.* HinZip bound to invHixC

## Protein Sequences: Hin DBD + FosW LZ

[redacted]

## Bacterial-one-Hybrid Assays (B1H)

- Semi-quantitative
  - More growth to the right = stronger binding[10]
- Binding of constructs to various invHixC spacers

**Table 2.** Sequences of the DNA sites used

| Target site | Sequence |
|---|---|
| 0 sp invHixC | TGTTTTTGATAA---------TTATCAAAAACA |
| 2 sp invHixC | TGTTTTTGATAAGA-------TTATCAAAAACA |
| 5 sp invHixC | TGTTTTTGATAAGAGAG----TTATCAAAAACA |
| 7 sp invHixC | TGTTTTTGATAAGAGAGAG--TTATCAAAAACA |
| 9 sp invHixC | TGTTTTTGATAAGAGAGAGAGTTATCAAAAACA |
| Half invHixC | ACCGTGCGTGGT---------TTATCAAAAACA |
| Non-specific | GCTGCAGGAATGCCACGTGGCCCA |

**Table 3.** Summary of B1H data. The number of + indicates the strength of binding. – indicates no binding

| Target site | HinZip | Hin | HinZip/LA |
|---|---|---|---|
| 0 sp invHixC | ++ | + | ++++ |
| 2 sp invHixC | +++ | + | ++ |
| 5 sp invHixC | ++ | + | ++++ |
| 7 sp invHixC | ++ | + | +++ |
| 9 sp invHixC | +++ | + | +++ |
| Half invHixC | + | + | +++ |
| Non-specific | - | - | - |

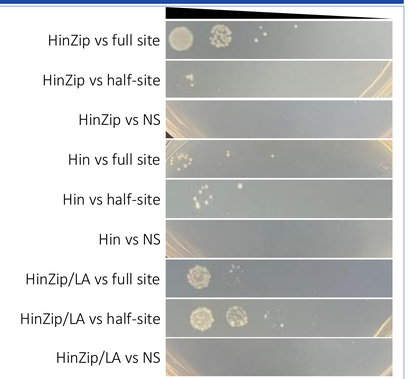



**Figure 3.** Sample B1H data, 5 mM 3-AT plate. Full site is 2 sp invHixC. Half-site is half invHixC.

- No binding to non-specific site → Specific binding
- HinZip binds better than Hin → LZ improves binding
- Spacer differentiation: only HinZip → Dimerization plays a role
- HinZip binding to half invHixC = Hin binding to half invHixC → HinZip cooperativity
- HinZip/LA shows the most growth
  - Alanine : helix stabilizer [11]

## Electrophoretic Mobility Shift Assay (EMSA)

- Quantitative binding assay
- Obtain binding constant and Hill coefficient (cooperativity)[12]
- Hin and HinZip/LA have monomers on gel. HinZip only Dimer → LZ induces dimerization

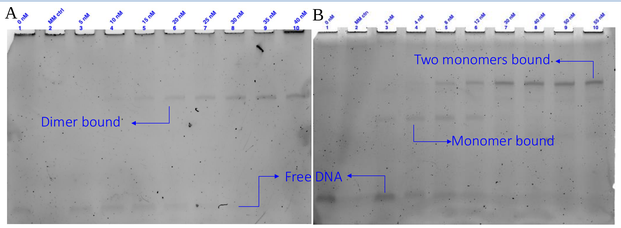



**Figure 4.** EMSA gels of *A.* HinZip binding to 0 sp invHixC *B.* HinZip/LA binding to 0 sp

## Biophysical Data

**Table 4.** Summary of $K_d$ data acquired from EMSA in nM (unless otherwise stated)

| | 0 sp invHixC | 2 sp invHixC | 5 sp invHixC | 9 sp invHixC | Half-site | NS |
|---|---|---|---|---|---|---|
| **HinZip** | 18.7±3.4 | 23.2±0.3 | 17.2±0.6 | 24.5±4.6 | **> 2 µM** | > 2 µM |
| **HinZip/LA** | 117.1±48.7 | 729.3±150.3 | — | 203.8±54.4 | **300±201** | > 2 µM |
| **Hin** | – | 78.1±20.7 | — | 126.9±75.0 | – | >2 µM |

- HinZip higher specificity compared to HinZip/LA → dimerization = more specificity

Circular Dichroism (CD):

Insights into secondary structure

- **HinZip: Highest % Helicity**
  - HZ 222:208 ~1=coiled-coil [13]

**Table 5.** Summary of CD data

| Protein | HinZip | Hin | HinZip/LA |
|---|---|---|---|
| **% Helicity** | **28%** | 21% | 27% |
| **222:208** | **1.02** | - | **0.83** |

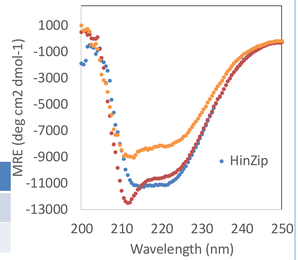



**Figure 5.** Representative CD

Dynamic Light Scattering (DLS):

Insights into size and oligomerization

**Table 6.** Summary of DLS data

| Protein | HinZip | Hin | HinZip/LA |
|---|---|---|---|
| **Oligomer Observed** | 900 nM | 150 nM | - |

- HinZip shows oligomers at 900 nM
- HinZip/LA no oligomers up to 2 µM

→ Dimerization capabilities of HinZip

## Conclusion and Future Directions

- Created an orthogonal, specific DNA binder. Large, unique DNA target site (24-35 bp).
- HinZip would be used to modulate synthetic biological circuits efficiently and effectively
- Alanines stabilize the protein
  - Introduce Ala mutations → stabilize 2° α-helical structure.

## References

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