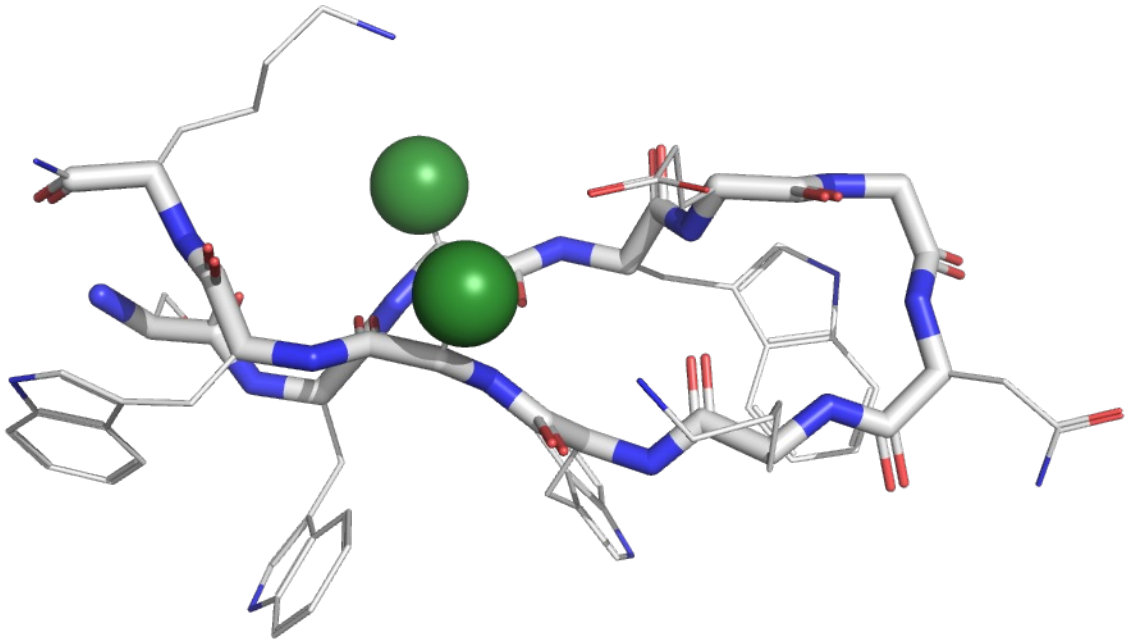
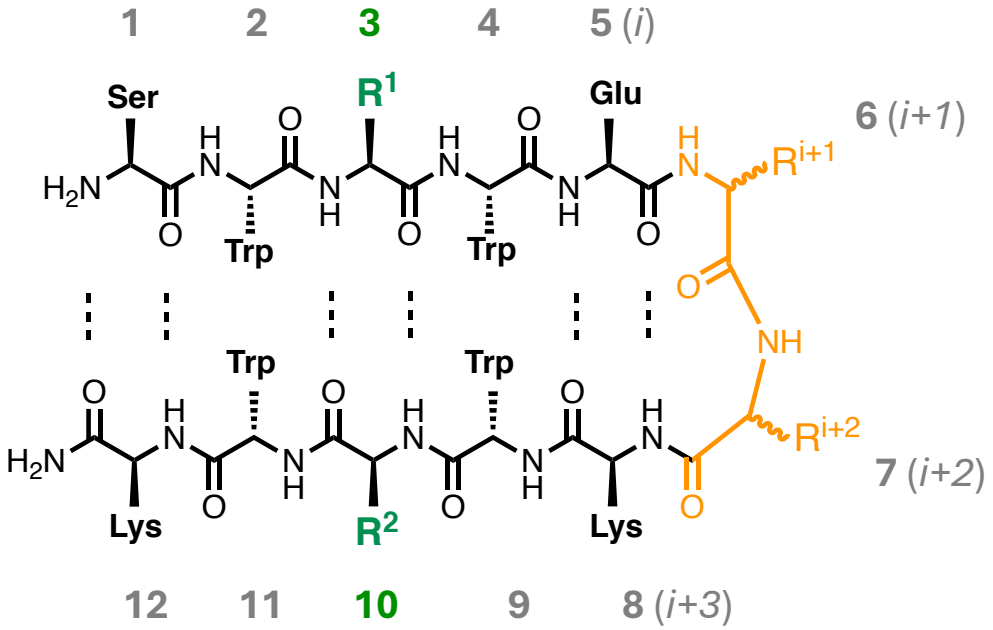
Background Material for Initial Meeting

TrpZip: A stable β-hairpin





1LE0 from Cochran, A. G.; Skelton, N. J.; Starovasnik, M. A. Tryptophan Zippers: Stable, Monomeric β-Hairpins. *PNAS* **2001**, *98*, 5578.

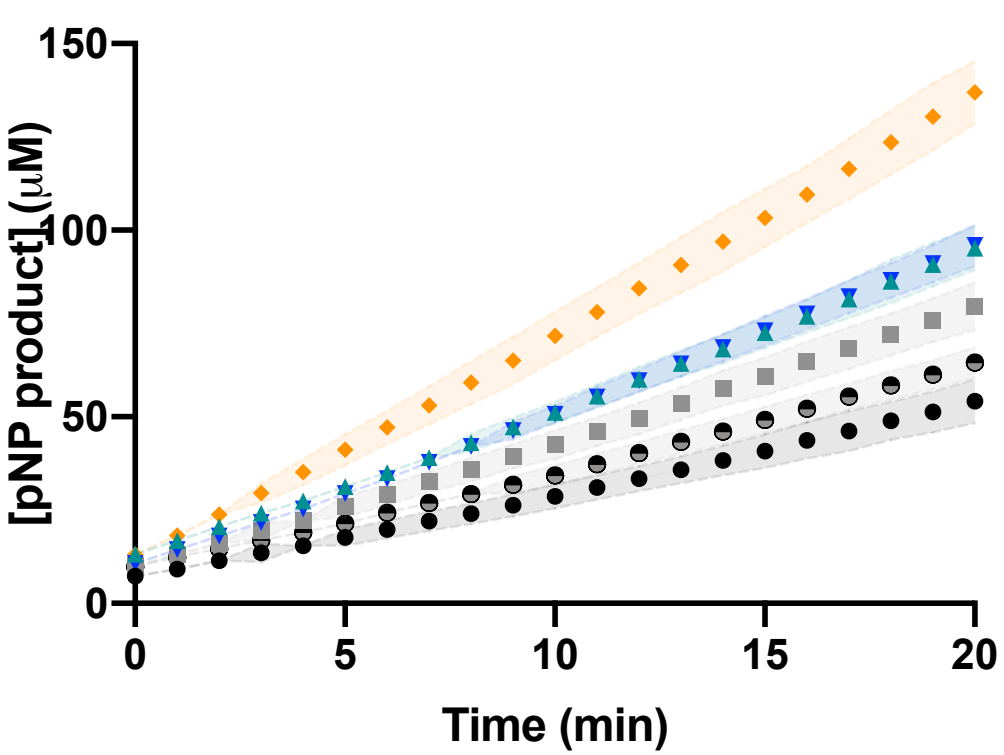
- *Note: the original catalyst designed and tested turned out to not have indication of β-hairpin conformation from CSD analysis of the NMR, so based catalytic sequences on known TrpZip sequence*

- Based on TrpZip type II' β-hairpin
- Install reactive dyads in hydrogen bonding positions 3 & 10
- Explore reactive dyad identity
- Vary the residues in the i+1 and i+2 turn positions to explore the role of β-hairpin conformation
- Eventually investigate effect of dyad positioning in nonHb positions

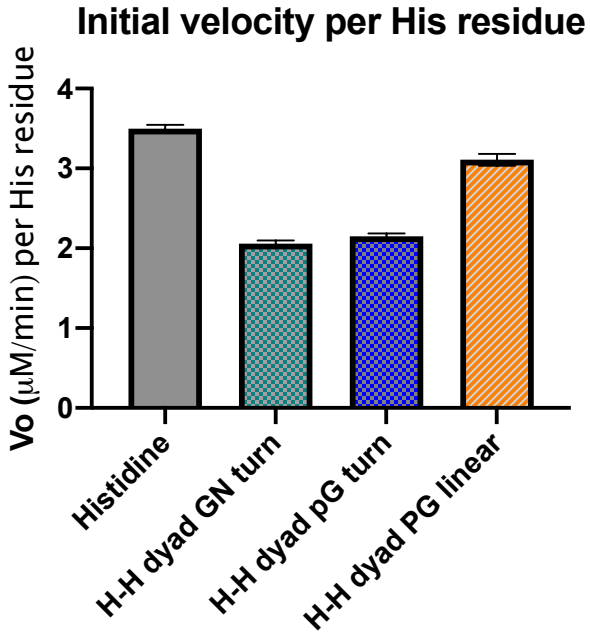
Peptide sequences probing role of β-hairpin

Name	Sequence												
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	predicted conformation
TrpZip control	Ser	Trp	Thr	Trp	Glu	Gly	Asn	Lys	Trp	Thr	Trp	Lys	β-hairpin
H-H dyad GN turn	Ser	Trp	**His**	Trp	Glu	Gly	Asn	Lys	Trp	**His**	Trp	Lys	β-hairpin
H-H dyad pG turn	Ser	Trp	**His**	Trp	Glu	dPro	Gly	Lys	Trp	**His**	Trp	Lys	β-hairpin
H-H dyad PG linear	Ser	Trp	**His**	Trp	Glu	Pro	Gly	Lys	Trp	**His**	Trp	Lys	linear

Catalysis results



- H-H dyad GN turn
- H-H dyad pG turn
- H-H dyad PG linear
- Histidine
- TrpZip control
- No catalyst



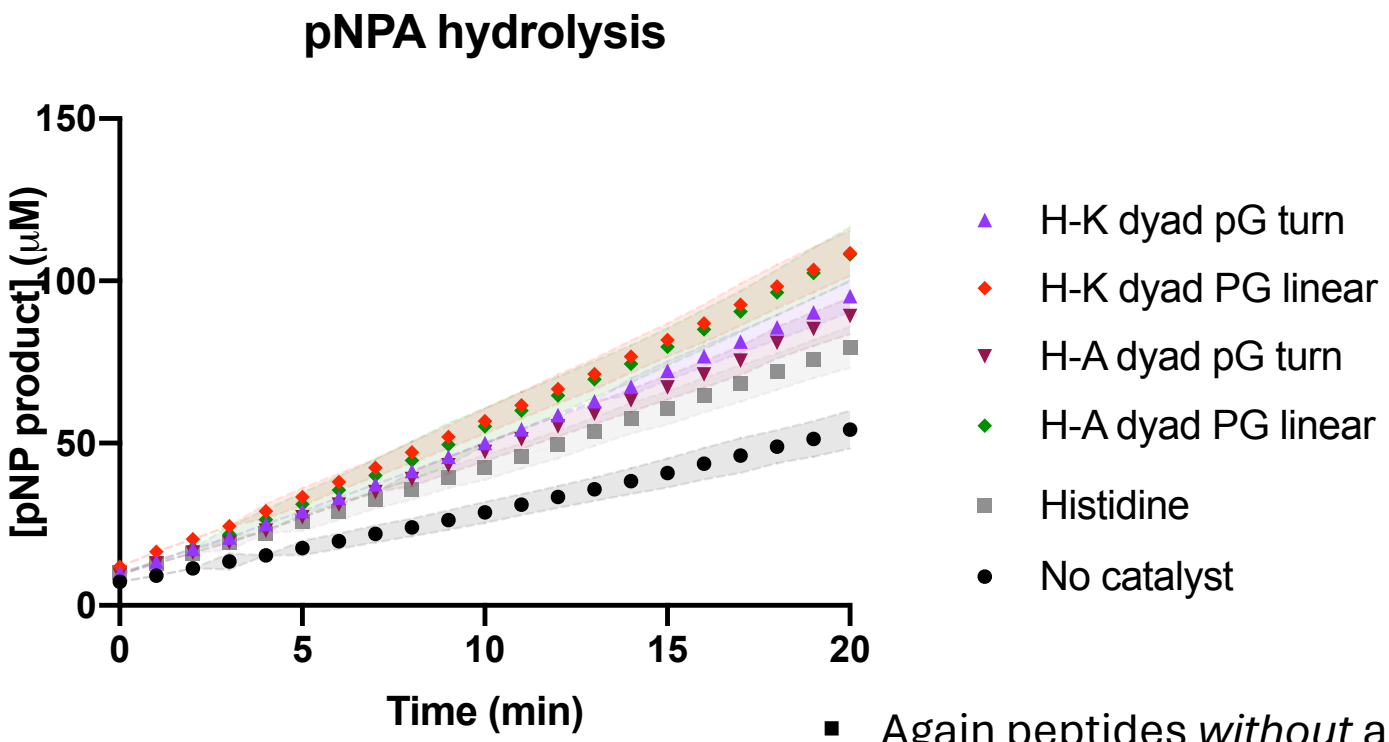
- For these peptides, β-hairpin conformation is detrimental to catalytic activity
- The peptide context does not enhance reactivity compared to histidine, the initial velocity *per histidine equivalent* is lower for the peptide catalysts regardless of conformation

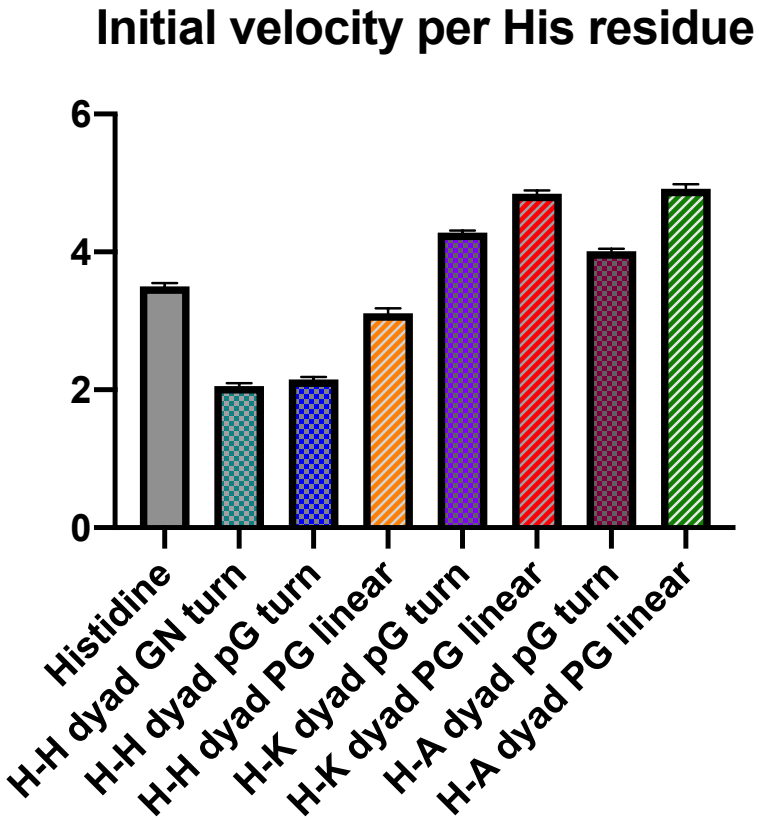
Sequences probing role of dyad identity

Name	Sequence												
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	predicted conformation
H-K dyad pG turn	Ser	Trp	**His**	Trp	Glu	DPro	Gly	Lys	Trp	**Lys**	Trp	Lys	β-hairpin
H-K dyad PG linear	Ser	Trp	**His**	Trp	Glu	Pro	Gly	Lys	Trp	**Lys**	Trp	Lys	linear
H-A dyad pG turn	Ser	Trp	**His**	Trp	Glu	DPro	Gly	Lys	Trp	**Ala**	Trp	Lys	β-hairpin
H-A dyad PG linear	Ser	Trp	**His**	Trp	Glu	Pro	Gly	Lys	Trp	**Ala**	Trp	Lys	linear

*Did not do NMR analysis on these analogues, so assuming the conformations are similar to the H-H dyads,
i.e. that the **pG**-containing sequences have some β-hairpin character
and that the **PG**-containing sequences do not

Catalysis results

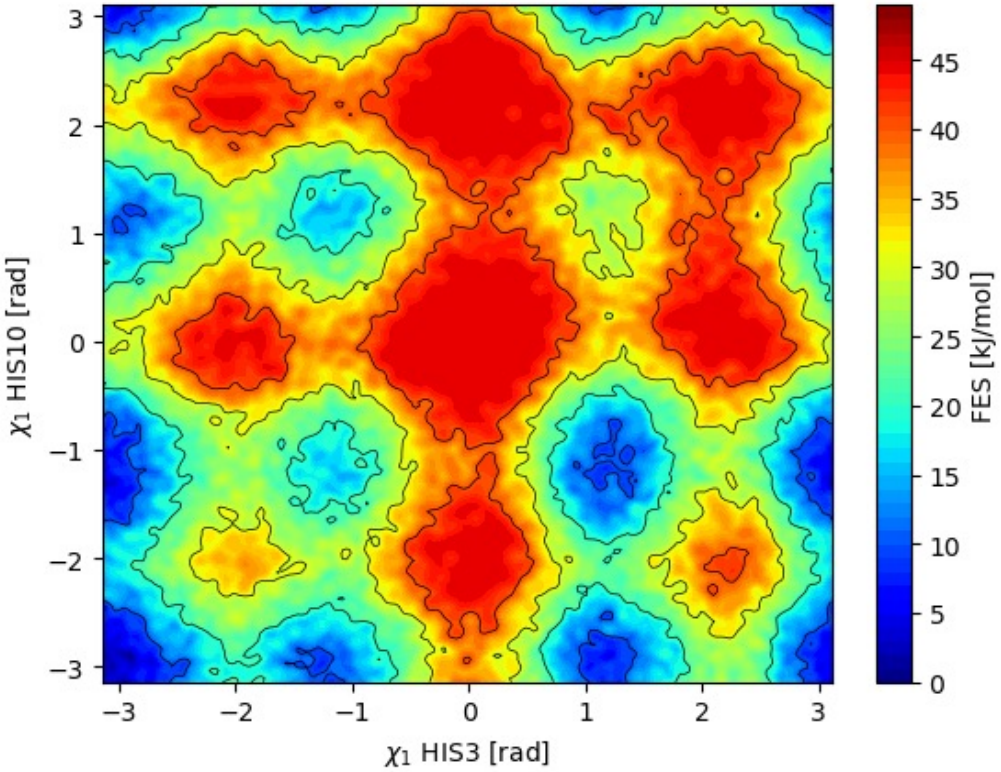


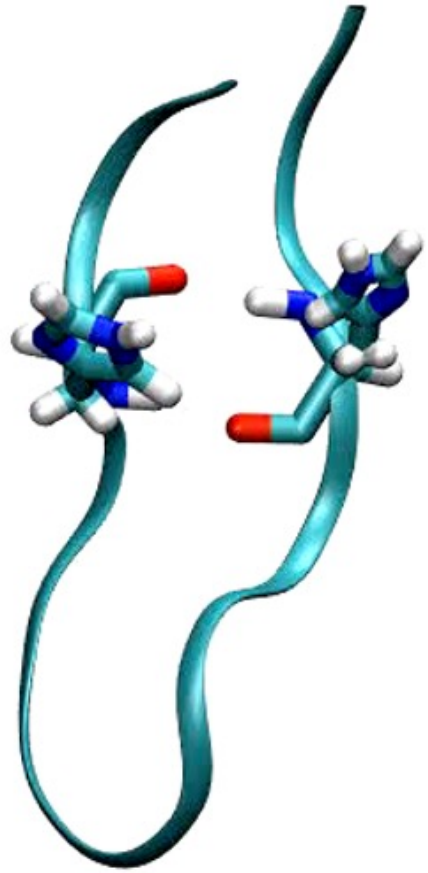


Conditions
25 ºC
5 mM pNPA
0.1 mM catalyst (2 mol %)
100 mM HEPES buffer pH 7.4, 5% MeCN
9 replicates total in 3 separate experiments

- Again peptides *without* a β-hairpin conformation are more active catalysts
- The initial velocity *per histidine equivalent* trends are:
  - Lower activity per His equivalent for the peptide catalysts with β-hairpin conformation regardless of dyad identity
  - His-His dyads are less active catalysts per His equivalent than peptides with a single His residue. Unclear why identity of position 10 should affect position 3 in the 'linear' sequences – may indicate some secondary structure formation
  - The effects observed are not large, but the trends are consistent

Conformational Sampling





Initial Proposed Structures

I: TrpZip4	Gly-Glu-Trp-Thr-Trp-Asp-Asp-Ala-Thr-Lys-Thr-Trp-Thr-Trp-Thr-Glu
II: 4H13H_pG	Gly-Glu-Trp-His-Trp-Asp-Asp-{D-Pro}-Gly-Lys-Thr-Trp-His-Trp-Thr-Glu
III: 4H13H_PG	Gly-Glu-Trp-His-Trp-Asp-Asp-Pro-Gly-Lys-Thr-Trp-His-Trp-Thr-Glu

1. Can we identify the key conformational basins for the three initial peptides?

- Will SPIB likely distinguish between these mutants?
- Can we calculate the histidine dyads in the basins?
- Are there limits (formal or practical) on the numbers and types of OPs to be included?

2. How is the best way to define CVs/OPs for homologous systems?

- Do we build a bespoke one for each mutant?
- Can we take the full set of structures and only include conserved OPs?
- Are there elements that might be conserved but not equivalent?
  - i.e. ϕ/ψ angles for different amino acids (particularly proline)