Through the Looking Glass: Functional Ambidexterity in an Ancient Nucleic Acid-Binding Protein

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2. Resurrecting an Ancient dsDNA Binding Motif

The Helix-Hairpin-Helix (HhH) motif is an ancient peptide element that binds to both RNA and DNA. If duplicated and fused, the HhH motif forms an independently folding dsDNA-binding protein domain

3. From Phase Separation to dsDNA Binding

Modern dsDNA binding domains may be descendants of ancient peptides that phase separated with RNA



Flexible Peptide that Forms Coacervates with RNA

dubbed the $(HhH)_{2}$ -Fold.

HhH Nucleic Acid Binding Motif ('peptide')



Duplicated Motif Forming a dsDNA Binding Domain with an (HhH)₂-Fold ('protein')



Longo et. al. PNAS 2020 117(27):14731-15739

4. Phase Separation is Robust to Chiral Inversion

1. Homochirality is at the Heart of Biology





Dimerizing HhH Peptide that Forms Coacervates with RNA



Folded Domain with an (HhH)₂-Fold that Binds dsDNA

When does homochirality matter?

Seal et. al. JACS 2022 144(31):14150-14160

5. dsDNA Binding is Robust to Chiral Inversion

Inverting the chirality of the HhH peptide, either completely or every other amino acid, did not disrupt phase separation with *D*-PolyU (natural chirality)

Sequence shuffling disrupts phase separation.

Coacervation with *D*-polyU (RNA)











D/L-HhH Peptide *L*-HhH Peptide With Shuffled Sequence

6. The Mode of dsDNA Binding is Largely

What if a protein fell "through the looking glass" into the mirror world?

Total chiral inversion of the (HhH)₂-Fold does not abolish *D*-dsDNA (natural chirality) binding, and is less disruptive than entropy mutations within the HhH binding motif (the '5G' construct).



Mirror image circular dichroism spectra confirming total chiral inversion of the $(HhH)_2$ -Fold



Retained Upon Chiral Inversion

The regions of the canonical HhH binding loop (PGIGP) within the (HhH)₂-Fold mediates binding of both the natural and the mirror protein forms.





D-(HhH)₂-Fold (Mirror)

7. Take-Home Messages (and Questions!)

- Both the HhH motif and the (HhH)₂-Fold are 'functionally ambidextrous' – they can bind to either chirality of nucleic acid.
- Is the (HhH)₂-Fold special or is functional ambidexterity a property of the most ancient proteins and peptides?
- The 'veil' that separates mirror worlds may not be as \bullet impenetrable as previously assumed.