De Novo Design of Hyper-Stable Anti-GnRH Proteins

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AIM:
We will target GnRH using computational design tools to create anti-GnRH mini-proteins (AGPs) that efficiently bind and sequester GnRH preventing its ability to bind to GnRH receptors. A single injection of a recombinant adeno-associated virus (rAAV) gene therapy will endow muscle or liver cells of cats and dogs to continuously secrete the AGPs into the blood stream causing lifelong sterility.

GnRH:

To design de novo GnRH binders, we used various techniques and utilized our in-house built ROSETTA software.

Different GnRH conformations were targeted
1YY1   4D5M   Gierarch’92

Larger designs

Smaller Designs

Designs with water at the interface

Computational Screening of Designs:
Forward folding to ensure we have the lowest energy structures

Experimental Screening of Designs:
No Display = No Target Binding
Display with Target Binding
Yeast that are not displaying the Aga2P target
Yeast that are displaying the Aga2P target

Level of binding

Level of expression

Yeast Surface Display (YSD) / Fluorescence Activated Cell Sorting (FACS)

We have filtered designs via the following metrics
• forward fold metric<=20.0
• ddG of binding <=-25
• Shape complementarity >=0.55
• Buried polar groups == 0
• Number of hydrogen bonds >= 4
• Alanine count <= 10

Overview of the Multiplex Gene Assembly

Multiplex Assembly Protocol:
• PCR1 using primers for the pool-specific adaptors will amplify the subpool of genes in each length-range.
• PCR2 using pETCON3 primers will assemble the genes.
• Please note that chimeras do occur due to assembly of oligos from different gene.
• This will enable testing of thousands of designs simultaneously.

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