

COMPUTATIONAL DESIGN OF HYPER-STABLE ANTI-GNRH PROTEINS

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GnRH is a well-validated target for anti-reproductive technologies in male and female cats and dogs of all ages. Effective neutralization of GnRH will be a surgical procedure free alternative to sterilization of both female and male cats and dogs.

We are designing anti-GnRH proteins (AGPs) using computational protein structure prediction and de novo protein design tools (Rosetta software) developed in the Baker lab. We aim to identify hyper-stable immune silenced proteins capable of binding GnRH with high affinity, effectively neutralizing it before it can bind GnRH receptors in the pituitary gland.

We have built a library of helical protein binder topologies that can engulf as much of GnRH as possible. We docked the GnRH peptide and optimized the binding cleft to ensure all hydrogen bonds were satisfied using an improved version of our Hydrogen Bond Network (HBnet) algorithm. We have also eliminated the designs in which single unpaired charged groups are buried in the core of the protein. This removes the burden on the folding free energy caused by the single buried charged group. We ordered the genes to encode the twenty best designs selected after computational screening. We have characterized those designs after expressing in *E. Coli* as thermostable and monomeric species. We have also done yeast surface display (YSD) to express those proteins on the surface of the yeast and followed by flow cytometry and fluorescently-labeled GnRH variants to test for GnRH binding. YSD will enable us to test designs in a high-throughput fashion. Based on the results we have had in the first round of designs, we have improved our design protocol and we are in the process of ordering 100 designs to be co-transformed in yeast to be tested for display and binding.

Even weak binders will be taken into consideration for potential candidates since binding affinity can be improved by affinity maturation protocol. Best binding candidates (with nm binding affinity) will then be selected for future collaborative work with the Foundation's partners to produce recombinant adeno-associated virus (rAAV) gene transfer vectors encoding the AGPs (rAAV-AGPs), which would then be evaluated for their safety and efficacy as single dose sterilization agents in rodent studies conducted at the University of Washington.