Background:

Ovastacin is an oocyte-specific oolemmal receptor involved in sperm and egg adhesion and fertilization. Plays a role in the polyspermy inhibition. Research into the function of ovastacin suggest it acts as a protease for the post-fertilization cleavage of ZP2. After fertilization and cortical granule exocytosis, ovastacin cleaves the sperm-binding ZP2 at the surface of the zona pellucida. Without correct function of ZP2, the zona pellucida is unable to support further sperm binding.

Zona pellucida regulation is critical for correct fertilization; potentially providing an avenue for non-hormonal contraception. Non-hormonal contraception has the potential to provide excellent contraception options without the problems and side-effects associated with current pharmaceutical options. Ovastacin, a protease, is expressed in mature oocytes, and when active, cleaves ZP2 found in the extracellular matrix of the zona pelucida. Once ZP2 is cleaved, the zona pellucida no longer allows for secondary fertilization.

Fetuin-B has been identified as an inhibitor of ovastacin. ZP2 cleavage by ovastacin seems to be closely linked to zona pellucida hardening, since the knockout of fetuin-B results in ZP hardening before fertilization and causes female infertility in mice.

Human Ovastacin sequence and domains:

Length: 431 aa
Mass: 45.936 kDa

```
        10        20        30        40        50        60        70        80
MEGVGGLWPWVLGLLSLPGVILGAPLASSCAGACGTSFPDGLTPEGTQASGDKDIPAINQGLILEETPESSFLIEGDIIR
PSPFRLLSATSNKWPMGGSGVVEVPFLLSSKYDEPSRQVILEALAEFERSTCIRFVTYQDQRDFISIIPMYGCFSSVGRS
GGMQVVSLAPTCLQKGRGIVLHELMHVLGFWHEHTRADRDRYIRVNWNEILPGFEINFIKSQSSNMLTPYDYSSVMHYGR
LAFSRRGLPTITPLWAPSVHIGQRWNLSASDITRVLKLYGCSPSGPRPRGRGSHAHSTGRSPAPASLSLQRLLEALSAES
RSPDPSGSSAGGQPVPAGPGESPHGWESPALKKLSAEASARQPQTLASSPRSRPGAGAPGVAQEQSWLAGVSTKPTVPSS
EAGIQPVPVQGSPALPGGCVPRNHFKGMSED
```

Signal sequence

Propeptide

Catalytic domain

C-terminal peptide

Project Plan:

Recombinant protein expression of human ovastacin (of usable yields) has not been yet published to the knowledge of the SGC. A reliable and repeatable protein expression protocol will be established by investigating a number of cloning experiments in Sf9 insect cells. Co-expression of ovastacin and fetuin-B may be required to stabilize ovastacin during these experiments.

It is possible that expression of human ovastacin will not be successful and as such a known expression of mouse ovastacin will (provided by Hagen Körschgen, Universitätsmedizin der Johannes Gutenberg-Universität, Mainz) also be used in parallel.

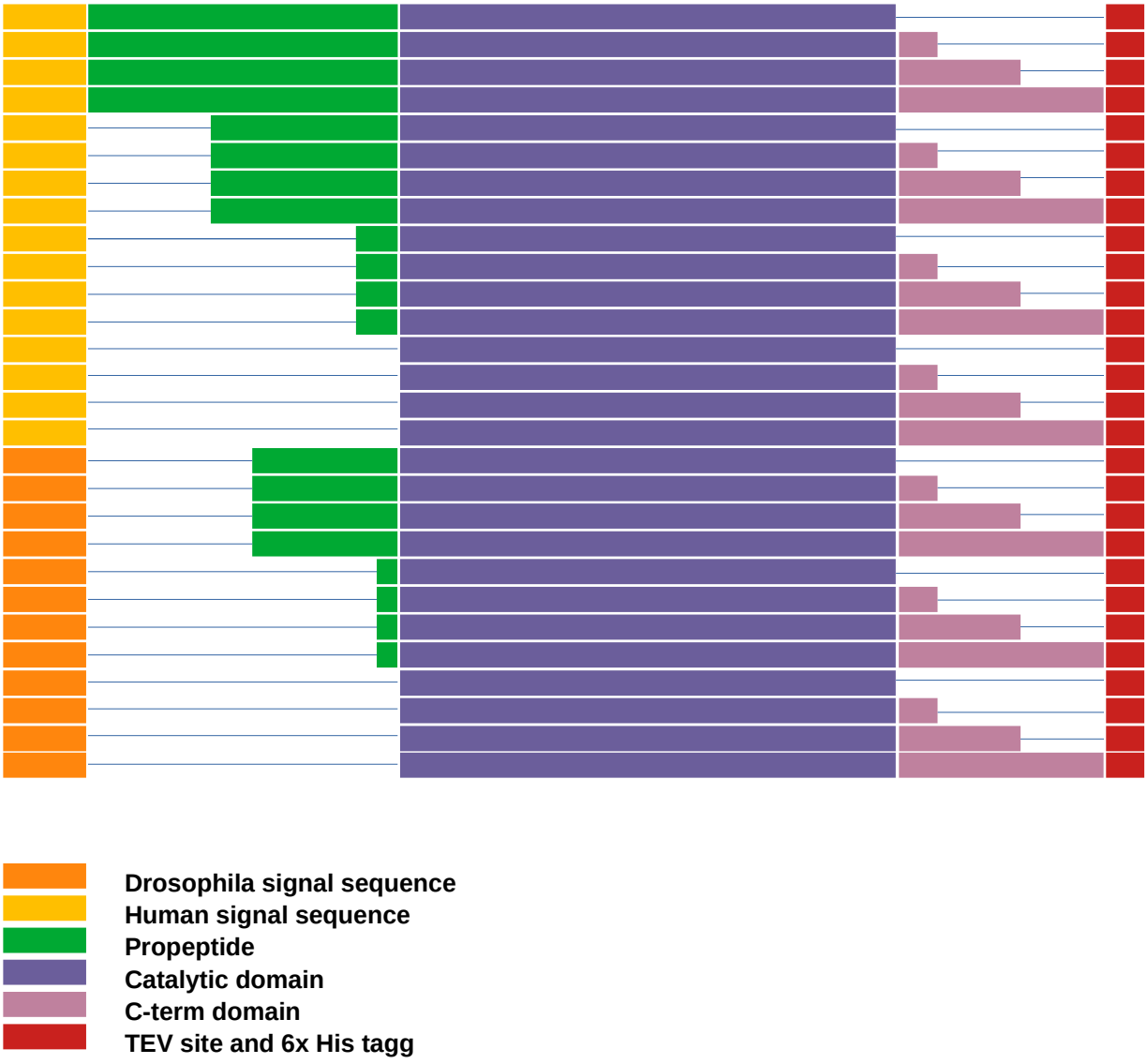
Obtaining usable quantities of ovastacin will allow for crystallization screening and optimisation experiments with the goal of structural determination of human ovastacin to below 2 Å; it is

expected molecular replacement using the previously published homology models should be succesful.

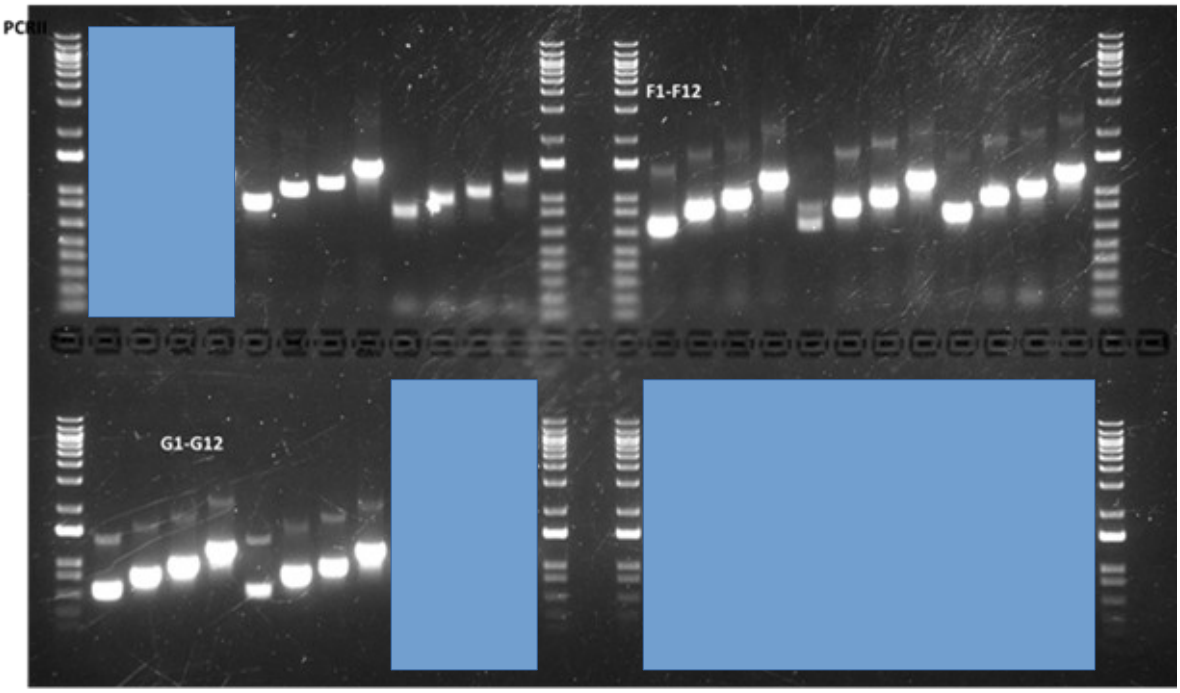
Thermal shift assays will be used to identify possible inhibiting ligands which can then be used for crystal soaking experiments as well as kinetic determinations via ITC.
Further investigations will depend on the success of these main initial goals.

Current status:

A diagram shown below demonstrates the various C- and N-terminal truncations of full length human ovastacin. All constructs contain the complete catalytic domain as well as a 6xHis tag, cleavable with TEV protease.



PCR experiments suggests successful cloning of 28 human ovastacin derivative constructs into pFB-CT6H-LIC was achieved. All constructs are currently stored in purified DNA solutions as well as DH10bac glycerol stocks.



Next steps:

Small scale expression test possibly leading to a large scale expression of the most successful candidate(s).