# Thinking Outside of the Box to Solve a Novel Protein Structure

Kirsten Kahler and Shawn Williams
GlaxoSmithKline, Computational and Structural Chemistry, Research Triangle Park, NC, USA

## Introduction

Computational and Structural Chemistry has a strong commitment to fully support GSK Drug Discovery efforts. As part of this commitment, the crystallography group in RTP provides structural support for previously published and novel targets for multiple therapeutic areas.

Protein X is a novel target from a pathogenic species. This protein is a member of the polymerase family and is potentially a target for anti-infection therapy. Solved structures are available for homologous proteins from other species with 45-95% sequence identity.

This poster will explain how thinking outside of the box was crucial for crystallization and structural determination of Protein X.

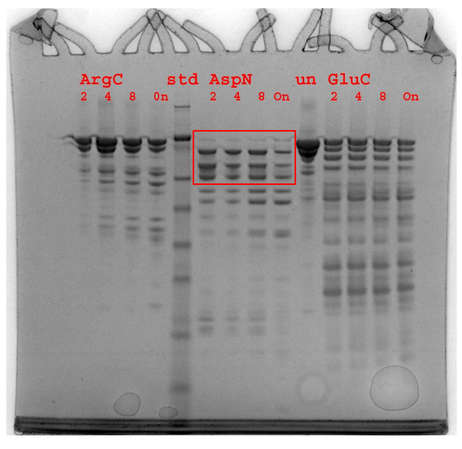
## Identifying a construct for crystallography

### Start with Known Structures

- The structure of Protein X had not been solved, however there were published structures of this class of protein from other species.
- Perform sequence alignment to identify conserved features, domain boundaries, possible construct limits.

### Perform Limited Proteolysis

- Run limited proteolysis experiments with a variety of proteases to identify possible truncation constructs
- The study with Protein X included six different proteases: Trypsin, Chymotrypsin, Carboxypeptidase Y, GluC, ArgC, and AspN.
- Below is an SDS PAGE gel showing several time points from the study. The bands of interest, boxed in red, were identified by peptide sequencing.



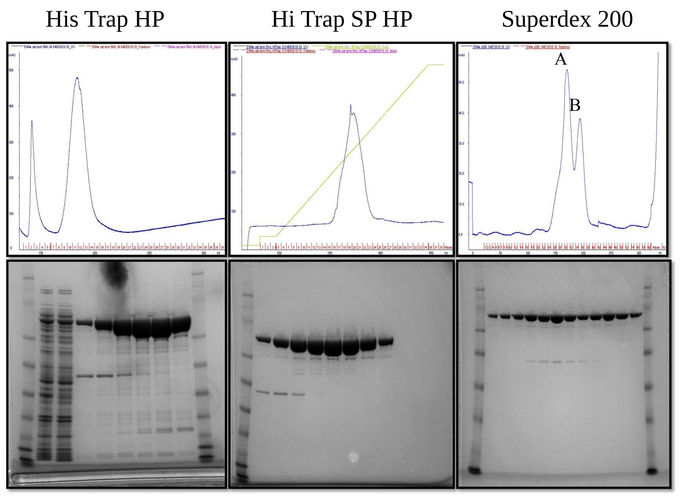

### Key Points

- Protein termini from limited proteolysis correlated well with alignments to constructs of published structures
- Tentative "catalytic domain" chosen as initial construct to pursue for crystallization trials.

## Benzonase: the solution for crystallizable protein

- Protein X is a polymerase, so there was a possibility that DNA, RNA and/or nucleotides were remaining bound during the purification.
- The first few purifications contained standard lysis procedures followed by Ni affinity, SP ion exchange, and S200 size exclusion chromatography. However, these purified protein preparations would not crystallize.

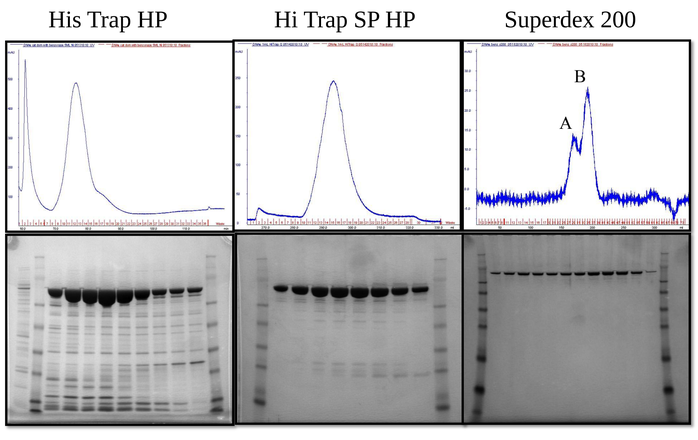
**Without Benzonase**




### Benzonase Changes Protein X's Behavior

- Benzonase, a genetically engineered endonuclease that degrades all forms of DNA and RNA (single stranded, double stranded, linear, circular), was added during cell lysis to reduce DNA/RNA to oligonucleotides.
- Purified using same chromatography scheme (Ni, SP, & S200 columns)
- Protein from two S200 peaks was kept separate (A&B).
- Amount of Protein X in longer retention pool, B, increased after adding Benzonase during lysis
- Both S200 peaks were judged to be identical by bioanalytical techniques, however <u>only longer retention pool, B, crystallized</u>.

**With Benzonase**




### Key Points

- Benzonase treatment changed characteristics of Protein X, detectable by gel filtration
- Benzonase treatment necessary for crystallization.

## Thinking outside of the Box! Cross-seeding with a totally unrelated family member protein

- The first crystals of Protein X were obtained by cross-seeding with crystals of an unrelated polymerase family member, Protein Y, using the crystallization conditions of Protein Y.
- Matrix seeding was done with Protein X crystals to find additional conditions.

**THE DIFFERENCE BETWEEN CRAZY AND BRILLIANT IS SUCCESS!!**

- Try thinking outside of the box when traditional methods are not producing crystals of a novel protein. A crazy idea may turn out to be the start of success!

## Solving a novel structure Using Balbes

- Best initial Protein X diffraction data was 3.4Å, requiring robust structure solution and refinement. Sub 3Å data was later obtained in the presence of an inhibitor.
- Structure solved using Balbes, which cut out homologous domains from deposited structures to perform molecular replacement.
- Refinement included "gelly" constraints and non-crystallographic symmetry restraints using Refmac5.6.