A case study of the effects of metal ions and TCEP on the crystallization of a cysteine protease.

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Introduction

- There are multiple factors that can influence your crystallization experiment.
- Simple observations of your drop can sometimes bring you useful informations that could explain why your protein does or does not crystallize, e.g. gooeyness of the drop.
- Increased thermal stability has been reported as useful for crystallization, but our results show this is not always the case.
- Even destabilizing ions can have a positive influence on crystallization.

Experiment, what we observed

- Poor quality crystals were obtained with type I inhibitors. Matrix seeding helped to improve the crystal quality and diffraction.
- TCEP was essential to prevent gooeyness of the crystallization droplet and allows crystallization for a longer period.
- Magnesium at high concentration appears to thermally destabilize the protein but allows crystallization of type II inhibitors.
- Compound characterization/selection criteria is of a high importance for binding of the inhibitor in the co-crystallization:
  - IC50 in low salt: < 1uM
  - Solubility at pH7.4: > 100uM

Seeding is essential to get co-crystals

With a standard concentration of TCEP (~ 1mM), we observed that the drops become “gooey”. We found that this disturbed the crystallization and decided to test different concentrations of TCEP. We finally obtained crystals after 5 days by adding 10mM TCEP to the protein prior to drop set-up. It appears that high concentration is needed for late nucleation. We suspect oxidation or crosslinking.

Influence of Magnesium Formate in crystallization of type II inhibitor, DSF and NMR data:

<table>
<thead>
<tr>
<th>TCEP (mM)</th>
<th>MgFormate (mM)</th>
<th>DSF and NMR data</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>180mM MgFormate (final 30mM)</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>30mM MgFormate (final 5mM)</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>300mM MgFormate (final 50mM)</td>
</tr>
<tr>
<td>0</td>
<td>20</td>
<td>600mM MgFormate (final 100mM)</td>
</tr>
<tr>
<td>0</td>
<td>50</td>
<td>600mM MgFormate (final 100mM)</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>120mM MgFormate (final 20mM)</td>
</tr>
<tr>
<td>0</td>
<td>200</td>
<td>1M MgFormate (final 133mM)</td>
</tr>
</tbody>
</table>

DSF shows that high concentrations of magnesium decrease the Tm of the protein and seems to destabilize it. In our case this also corresponds to a positive effect on crystallization. The higher the magnesium concentration, the more better the crystals. The low resolution of the dataset (2.3Å) did not allow us to demonstrate binding of magnesium. Further experiments are planned with manganese.

Additional information, apo crystals

Addition of Mg also favors apo crystallization:
- no hits without Magnesium
- 17 hits with 100mM Magnesium

Conclusion:

- We suspect that the 0.3M magnesium formate contained in the seeds solution contributes to crystallization.
- Apo crystallization favored in the presence of high concentration of magnesium.
- It seems that magnesium affects the crystallization of the protein by destabilizing it or changing the conformation.
- That is why we believe that magnesium has a strong influence on the crystallization of our protein.

Follow-up experiments:
- Test of high concentrations of ions with other proteases by DSF.
- Replace magnesium by other ions and see if we observe the same effects.
- Try to understand why high concentrations of TCEP decreases gooeyness of drops.