Fragment Screening

*The *Frag Xtal Screen* for crystallographers*

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Murnau, September 28th 2018
Agenda

1. Frag Xtal Screen

2. XP Screen
Fragments are small but very efficient binders to cover a large chemical space.

<table>
<thead>
<tr>
<th></th>
<th>Fragments</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight</td>
<td>100-250 Da</td>
<td>300-500 Da</td>
</tr>
<tr>
<td>Binding Affinity</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

“Six biophysical screening methods miss a large proportion of crystallographically discovered fragment hits”[1]

Endothiapepsin was screened against 361 fragments using 6 methods in parallel[1]

Which fragments to choose for further investigation?

Crystallographic screening achieved 20% hit rate[1]

Several methods for fragment screening available: **X-ray crystallography** is one of them

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Fragment screening by X-ray crystallography gives direct answers

- Can cover hits not identified by other methods\textsuperscript{[1-3]}
- Identifies several binding sites on the target (protein)
- Yields binding information at atomic level
- Allows structure-guided fragment evolution towards high (nM) affinity binders

Figure adapted from Rees \textit{et al.} (2004) Fragment-Based Lead Discovery \textit{Nat. Rev. Drug Discov.} 3:660.

Crystallographic fragment screening is now a straightforward & user-friendly experiment

For an easy start: The Frag Xtal Screen as 96 well plate

96 fragments produced 145 in-house hits + 8 pdb hits (light blue)
Frag Xtal Screen: A validated library (chemically diverse, highly soluble) for improved hit rates

96 dried fragments (2 x 50 nmol each) are provided in a crystallization plate

Fragment Screening Plate from Jena Bioscience

- Fragment solubilization in crystallization buffer
- Crystal soaking
- Cryocooling
- Automated data collection

For an easy entry into crystallographic fragment screening
High-quality crystals are grown with the XP Screen

1. Frag Xtal Screen

2. XP Screen
The Anderson-Evans Polyoxotungstate $[\text{TeW}_6\text{O}_{24}]^{6-}$ (TEW) is a universal and flexible crystallization additive

- Centrosymmetric, inorganic cluster
- Disk-shaped ion $[\text{TeW}_6\text{O}_{24}]^{6-}$
- Dimensions: 9 x 9 x 3 Å³
- MW: 1615 g/mol (ion)
- Highly soluble in aqueous solutions (100 mM) and stable over a wide pH range
- Provides a valuable anomalous signal for phasing due to 6 tungsten atoms

Size and planar structure are advantageous during crystallization

[1] Mac Sweeney et al. (2018) The crystallization additive hexatungstotellurate promotes the crystallization of the HSP70 nucleotide binding domain into two different crystal forms. PLOS one 13(6): e0199639.
TEW has a high negative charge distributed over a large size.

Steric interference and electrostatic repulsion of three protein patches

TEW links positively charged protein surface regions and provides a certain distance between crosslinked monomers.

TEW prevents steric interference of the molecules

Figure from [1], used courtesy of Prof. Annette Rompel, University of Vienna, Austria

The disk-shaped TEW can act as linker in various orientations

![Diagram showing protein-protein distances](image)

Small protein-protein distance in mushroom tyrosinase PPO4 from *Agaricus bisporus* (PDB code: 4OUA, new structure)

Large protein-protein distance in lysozyme (PDB code: 4PHI, new crystal form)

... and create either smaller or larger protein-protein-distances

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Figures from [1], used courtesy of Prof. Annette Rompel, University of Vienna, Austria

TEW can covalently bind and structurally adapt to fit into the protein molecule

Covalent binding of W to carboxylic oxygen atoms of glutamic acid in aurone synthase from *Coreopsis grandiflora* (PDB code: 4Z12, 4Z13, new structure)[2]

- Increase in crystal quality and resolution
- Can lead to stabilization of flexible protein regions

... without affecting the protein structure or integrity

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TEW mediates and stabilizes crystal contacts

- Get more specific crystal contacts
- Improve crystal quality and resolution
- Induce new crystal forms (e.g. to provide access to binding pockets)
- Solve the phasing problem

Figure from [1], used courtesy of Prof. Annette Rompel, University of Vienna, Austria

XP Screen (CS-350) is a TEW-compatible screen for improved crystal quality and phasing
Thank you!

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