Protein Crystallography and Fragment-Based Drug Design Approach

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Outline

- Basic principles and methods of protein crystallography
  - Crystallization
  - Data collection
  - From diffraction intensities to a molecular structure
- Practical application
  - Fragment based drug design (principles and examples)
- Summery
- Q&A
Examples of protein crystals. From left to right: β-secretase inhibitor complex; human farnesyl pyrophosphatase in complex with the nitrogen-containing bisphosphonate drug zoledronic acid; Crystals of the Abl kinase domain in complex with imatinib
Source: Novartis Institutes for BioMedical Research;

Crystal packing of a human thrombin complex.
Crystallization of protein–ligand complexes

Principle of the method of vapor diffusion in hanging drops.

- Reagents
- Protein stock [0.1–1 mM]
- Compound stock [25–100 mM] in DMSO
- Crystallization buffer

- Crystallization, standard protocol
  1:1

- Co-crystallization, standard protocol
  [DMSO] < 2–5%
  1:50

- Soaking experiment
  [DMSO] < 2–5%
  1:50

- Co-crystallization, “ligand fishing” protocol
  dilution = 1:300
  incubation concentration = 50:1

Molar excess of ligand
- 450 to 1,500-fold

(Jean-Michel Rondeau, The Practice of Medicinal Chemistry)
Data collection

The setup for an X-ray experiment.

(Jean-Michel Rondeau, The Practice of Medicinal Chemistry)
From diffraction intensities to a molecular structure

Bragg’s equation: \[ n\lambda = 2d \sin \theta \]
From diffraction intensities to a molecular structure

The phase problem

Fourier transform → Electron Density → Phases

Heavy-atom derivatives
Anomalous dispersion
Molecular replacement
PRACTICAL APPLICATIONS

(Jean-Michel Rondeau, The Practice of Medicinal Chemistry)
What is Fragment?

“Rule of Three” (Kjell N, 2003)

- MW <300
- No of H-bond donors ≤ 3
- No. of H-bond acceptors ≤ 3
- ClogP ≤ 3
- NROT* ≤ 3
- PSA ≤ 60 Å

David C and et al. 2004

*The number of rotatable bonds
Low-Quality HTS Hit

David C and et al. 2004
Fragment Based Drug Design Approach

Application of Fragment Growing and Fragment Linking to the Discovery of Inhibitors of Mycobacterium tuberculosis Pantothenate Synthetase

Fragment growing

Fragment linking
Overview of the experiment

*M. tuberculosis* pantothenate synthetase

Fragment library (1300 fragments)

- Fluorescence-based thermal shift
- Ligand-based NMR spectroscopy

Isothermal titration calorimetry

- Validating and quantifying binding
- Obtaining structural information

X-ray crystallography

*In silico* design + Chemical synthesis

Biological assays

Inhibition assays
a) $K_D$ 1100 µm
LE 0.36

1

1a $K_D$ 500 µm
LE 0.32

b) $K_D$ 210 µm
LE 0.28

2

c) $K_D$ 29 µm
LE 0.26

3

d) $K_D$ 1.5 µm
LE 0.26

4
Pymol Models
Summary

- Protein crystallography is a key player in drug discovery, especially fragment based drug design.
- The principles of protein crystallography are crystallization, data collection and interpretation.
- Two major techniques of fragment based drug design are growing and linking approaches.
Recommended References


Kjell Någren. A ‘Rule of Three’ for fragment-based lead discovery? DDT, 8, No. 19 October 2003


Chapter 30: Protein Crystallography and Drug Discovery (Jean-Michel Rondeau and Herman Schreuder)
THANK YOU FOR YOUR ATTENTION