A Computational Tool for Characterizing Solvent Channels in Macromolecular Crystals

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Purpose

To characterize the geometry of solvent channels of macromolecular crystals, aimed at:

1. Choosing a set of crystals for cryocooling experiments that spans a range of solvent channel geometries.
2. Modeling measurements of the diffusion of molecules through the channels.

Methods

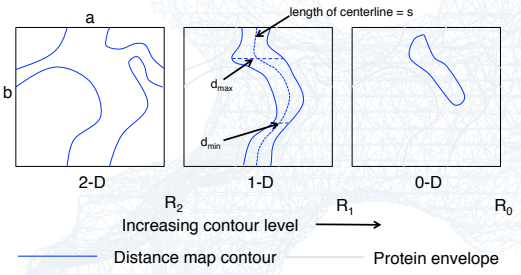
A fortran program, map_channels, was written for analysis of solvent channels using Protein Data Bank coordinates in two steps: (1) Distance map generation and (2) Channel characterization

Step 1. Distance map generation. The unit cell is broken into a grid. At each gridpoint the distance to the closest protein atom is calculated, generating a scalar field which is written as a map (ccp4 format) that can be read by standard crystallographic packages. Visualization of the map gives an intuitive sense of the geometry of the solvent channels.

Step 2. Channel characterization. The distance map is analyzed to determine geometric descriptors of the solvent channel system:

- Solvent content
- Channel diameter in each unit cell direction
- Channel topology
  - 0D – pockets
  - 1D – 1 dimensional pores
  - 2D/3D – 2/3 dimensional pore networks
- For 1D pores:
  - Direction check (along or oblique to cell directions)
  - Tortuosity and bottleneck factors

Sketch of Toy 2D Channel System Characteristics



R_2 = contour level for 2D->1D
R_1 = contour level for 1D -> 0D
R_0 = largest channel-protein distance in cell
Anisotropy = R_1/R_2
Tortuosity = s/b @ R_1
Bottleneck = d_{max}/d_{min} @ R_2

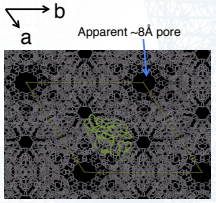
Abstract

Within a macromolecular crystal are interstices commonly referred to as solvent channels, which permit diffusion of small molecules into the crystals, including inhibitors, ligands and cryoprotective agents. Transport of small molecules is useful not only for structural biologists, but also holds potential for applications with crystals acting as microporous materials. Solvent channel geometry is also important in cryogenic cooling processes, but currently determining information about and visualizing solvent channels is cumbersome. Here we describe a computational tool designed specifically to aid in the visualization and characterization of the solvent channels in macromolecular crystals. The channels are defined in terms of a scalar field - the distance to the nearest protein atom – which is written as a map that can be visualized using standard macromolecular crystallography packages. Geometric descriptors of the channels are calculated, including channel radii, anisotropy and tortuosity. A survey of the structures in the protein data bank shows a wide range of channel geometries, and yields a direct relationship between channel diameter and solvent content of the crystal, with some spread in diameter for a given solvent content. Experimental measurements show that under osmotic stress, crystals with a relatively high degree of channel diameter anisotropy tend to crack perpendicular to the direction of the channel with the largest diameter.

Results

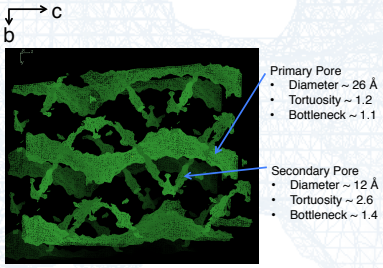
Channel Structure for Hexagonal Thermolysin (PDB code 8TLN – P6$_1$22)

Projection View



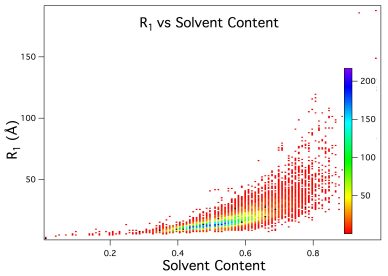
Typical view of crystal packing. The protein is shown as wireframe, yielding a projection view of the solvent channels along the c direction. Here two pores are suggested, of diameters ~3 and ~8 Å. Figure made with Coot.

Distance Map View



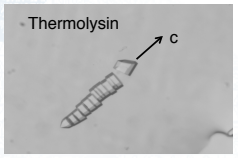
Visualization of the same pore structure using the distance map, contoured at ~6 Å, meaning a spherical object of radius 6 Å could be placed anywhere on the map without a steric clash with the protein. The apparent ~8Å pore on the left is actually helical with diameter of 25 Å. Wrapping around this large pore is another helical shaped pore of diameter ~ 12 Å. Figure made with Coot.

Relationship between Pore Diameter and Solvent Content

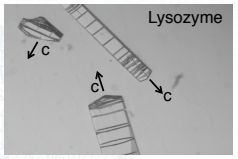


2D histogram of R_1 & solvent content for ~60,000 structures in the protein data bank as of Jan 2011. The structures were analyzed with map_channels using about 20 desktop computers and Xgrid distributed computing. Structures plotted in black are being used for cryo-cooling experiments.

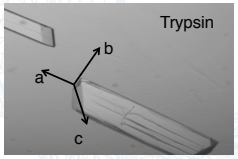
Pore Anisotopy and Crystal Cracking



P6$_1$22 a = b 131 Å, c=93 Å
Solvent content = 0.48
Well: 30% sat'd AmSO4
Dilution for cracking: 33%well (v/v)
Pore diameters:
R_a=R_b=5.9 Å, R_c = 12.9 Å
Pore Anisotropy = 2.2



P4$_3$2$_1$2 a = b= 77 Å, c = 35 Å
Solvent content = 0.34
Well: 1 M NaCl, 0.04 M NaOAc
Dilution for cracking: 50% well (v/v)
Pore diameters:
R_a=R_b=3.2 Å, R_c = 5.7 Å
Pore Anisotropy = 1.8



P2$_1$2$_1$2$_1$ a = 55 Å, b=59 Å, c = 68 Å
Solvent content = 0.47
Well: 25% P8K, 0.2 M AmSO4,
0.1 M benzHCl, 0.1 M Tris 8.0
Dilution for cracking : 25%well (v/v)
Pore diameters:
R_a = 4.7 Å, R_b = 8.7, R_c = 4.7 Å
Pore Anisotropy = 1.9

~ 500 μm

Crystals were first equilibrated to well solution and then placed in a dilution of the well solution using a 0.2 mm cryoloop. Cracks appear perpendicular to the direction of the largest diameter pore.

Acknowledgements

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Background image shows solvent distance map for channels from Tetragonal thermolysin contoured at 15 Å