Docking of the Dictyostatin, 16-Normethyldictyostatin, and the Discodermolide, 14-Normethyldiscodermolide, to β-Tubulin

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Microtubules – What Do They Do?

- Intracellular transport
- Motility (cilia and flagella)
- Shape (cytoskeleton)
- Cell division (mitosis)
  - Spindle formation

http://www-vis.lbl.gov/Vignettes/KDowning-Microtubules/
So What Are Microtubules?

- Monomers – α and β-tubulin
  - Nucleotide-binding zone (GTP)

- Dimers - α,β heterodimers
  - Interface includes nucleotide of α-tubulin

So What Are Microtubules?

- Protofilaments – straight chain of dimers
  - Interface includes nucleotide (GTP → GDP)

- Lateral Contacts – bind protofilaments to form microtubule wall
  - Homogenous contacts (α,α or β,β)
  - M Loop
So What Are Microtubules?

- Left-handed, 3-start helix

- Minus end (stable)
  - Capped with α-tubulin w/ embedded nucleotide (GTP)
  - Lateral contacts strong, independent of nucleotide state

- Plus end (dynamic)
  - Capped with β-tubulin w/ exposed nucleotide (GTP or GDP)
  - Lateral contacts
    - GTP – strong
    - GDP – weak

http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb2/part1/microtub.htm

Taxanes and Epothilones

- Binds to β-tubulin adjacent to the M loop

- Stabilizes lateral contacts of plus end
  - Overrides hydrolysis-induced conformational change in M loop

- Rests in pocket of β subunit occupied by extra 8 amino acids of S9-S10 loop in α-tubulin
**Taxanes and Epothilones**

- Binding modes experimentally determined
  - Taxol - electron diffraction (1JFF)
  - EpoA - cryoelectron microscopy (1TVK)

- No common pharmacophore
  - Taxol – pairing of aromatic side chains w/ complementary residues
  - EpoA – extensive hydrogen bonding network

**Discodermolides and Dictyostatins**

- Highest known affinity for taxane binding site
- 16-normethyl analogue of dictyostatin
  - Equipotent against wild-type β-tubulin
  - Many fold less potent if Phe270→Val mutation
- 14-normethyl analogue of discodermolide still untested
Goals

- Dock discodermolide and dictyostatin into the taxane/epothilone binding pocket of β-tubulin
- Pay special attention to dictyostatin C16 methyl group and its proximity to Phe270
- See if discodermolide could possibly dock similarly with the analogous C14 methyl group interacting with Phe270

Methods

- CAChe
  - Utilize Dock into ActiveSite feature
  - Active site was varied from 3 to 5Å
    - Any residue within given distance of Taxol or EpoA
  - Rigid and flexible docking was performed (PMF)
    - For Taxol and EpoA, rigid docking was performed with the known conformation (from PDB file)
    - Flexible dockings and DCD/DCT dockings performed on energy minimized conformations (Cerius², MMFF94)
  - Optimize Current Pose feature used to refine results
  - Gives one possible conformation
Methods

- MOE – followed MOE-Dock Tutorial
  - Addition of hydrogens and energy minimization (MMFF94 and implicit solvation)
  - Docking using residues within 7.5Å
  - 25 runs; 6 cycles each
  - Gives 25 results
    - Compared to experimental data (i.e. PDB file or focusing on C16/C14 – Phe270 interaction)
  - Energy minimize feature used to refine results

Taxol – CAChe

- Tubulin/Taxol binding mode determined from electron diffraction (1JFF)
- Docking of flexible Taxol
- Docking of rigid Taxol into 1JFF using 3Å neighbors as active site
- Docking of rigid Taxol into 1TVK using 3Å neighbors as active site
- Docking of rigid Taxol into 1TVK using 5Å neighbors as active site
- Docking of rigid Taxol into 1JFF using 5Å neighbors as active site
**Taxol – MOE**

Most favorable docking of flexible Taxol onto 1JFF

Energy optimization of most favorable conformation

**Epothilone A – CAChe**

Tubulin/EpoA binding determined from cryoelectron crystallography

Docking of flexible EpoA followed by optimization of current position

Docking of rigid EpoA into 1TVK using 3Å as active site

Docking of rigid EpoA into 1JFF using 3Å as active site

Docking of rigid EpoA into 1TVK using 5Å as active site
Epothilone A – MOE

- Tubulin/EpoA binding mode (1TVK) in MOE
- Eleventh most favorable docking of flexible EpoA into 1JFF
- Energy optimization of eleventh most favorable docking of flexible EpoA

16-normethyldictyostatin – CAChe

- Docking of rigid DCT1 into 1TVK with a 3Å active site
- Docking of flexible DCT1 into 1JFF with a 5Å active site
- Docking of rigid DCT1 into 1JFF with a 5Å active site
- Optimization of current pose of flexible docking
16-normethyldictyostatin – MOE

- Relationship of C16 methyl group to Phe272 of second lowest energy confirmation
- Relationship of C16 methyl group to Phe272 after energy minimization
- Binding pocket (3Å neighbors) of second lowest energy confirmation
- Binding pocket (3Å neighbors) after energy minimization

14-normethyldiscodermolide – CAChe

- Docking of rigid DCD into 1JFF with a 3Å binding pocket
- Docking of flexible DCD into 1JFF with a 5Å binding pocket
- Docking of rigid DCD into 1TVK with a 5Å binding pocket
- Optimization of current pose of flexible docking
The image on the right is a possible binding mode for dictyostatin based on MOE modeling.

According to the possible conformations given by MOE, the C14 of DCD, while analogous to the C16 of DCT, probably does not interact with Phe270.
Complications and Shortcomings

- Two different PDB files
  - Different β-tubulin structures
    - Alpha carbons – most divergent next to M loop, which is next to the binding pocket
    - Side chain conformations

- Different pharmacophores
  - Issue for CAChe
  - Taxol and EpoA have different neighbors
    - May help explain why Taxol worked better with larger spheres of influence and EpoA with smaller active sites
  - DCD and DCT may be influenced by residues different than those that affect Taxol and EpoA

- Determining which dockings were “correct”
  - Difficult to simply superimpose images
  - PDB files do not match completely
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