Calculating Protein Structures from Chemical Shifts & Vice Versa

9th CCPN Meeting, July 24, 2009
University of Cumbria, Ambleside UK

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University of Alberta
david.wishart@ualberta.ca
NOE-based NMR

3-6 months

Chemical Shift Assignments

NOE Intensities

J-Couplings

Distance Geometry
Simulated Annealing

active site

N-terminal

C-terminal

8-14

71-73

56-62
Conventional NMR

Advantages
• Robust and well tested
• Yields structure almost every time
• Large body of software available
• Structure reflects dynamics of system

Disadvantages
• NOE measurement is tedious & error prone
• Limited to smaller (<30 kD) proteins
• Time consuming and slow
• Ignores other expt. information
• Lower structure quality than X-ray
Unconventional NMR

- Conventional NMR is still slow and very manually intensive
- Structural proteomics initiatives are looking for better/faster/cheaper routes to protein structure determination
- Use of chemical shifts would skip the NOE assignment problem and allow larger structures to be determined, to higher accuracy and with greater speed
Featured Tools

SHIFTX
http://redpoll.pharmacy.ualberta.ca/shiftx/

CS23D
http://www.cs23d.ca

GeNMR
http://www.genmr.ca
Why Shifts from Structure?

• Can be used for chemical shift refinement
• Can be used for structure generation, fold identification or structure evaluation
• Allows identification of possible assignment errors or spectral folding problems
• Allows correction of chemical shift mis-referencing
• Useful for guiding assignments (if X-ray structure known)
• Useful for ligand or interaction mapping
Shift Calculation Methods

• **SHIFTS (D. Case)**
  - QM calculated hypersurfaces + ring current effects

• **SPARTA (A. Bax)**
  - Matching triplets for sequence + phi/psi/chi1

• **PROSHIFT (J. Meiler)**
  - ANNs from 3D coords + torsion angles

• **SHIFTX (D. Wishart)**
  - ~10 statistically derived hypersurfaces + RC effects

• **SHIFTX 2.0 (D. Wishart)**
  - Machine learning + hypersurfaces + RC effects
Rapid and accurate calculation of protein $^1\text{H}$, $^{13}\text{C}$ and $^{15}\text{N}$ chemical shifts

Stephen Neal, Alex M. Nip, Haiyan Zhang & David S. Wishart

Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, AB, T6G 2N8, Canada

Received 22 November 2002; Accepted 6 March 2003
Comparing δ Predictors

<table>
<thead>
<tr>
<th>Test set of 44 proteins</th>
<th>HA</th>
<th>CA</th>
<th>CB</th>
<th>C</th>
<th>N</th>
<th>HN</th>
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<tbody>
<tr>
<td>ShiftX</td>
<td>0.8687</td>
<td>0.9608</td>
<td>0.9937</td>
<td>0.7724</td>
<td>0.8548</td>
<td>0.6937</td>
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<tr>
<td>Sparta</td>
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<td>0.9692</td>
<td>0.9949</td>
<td>0.8204</td>
<td>0.8845</td>
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<td>ShiftS</td>
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<td>0.9508</td>
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<td>0.6589</td>
<td>0.7239</td>
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<td>ProShift</td>
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<td>0.9431</td>
<td>0.9905</td>
<td>0.7520</td>
<td>0.8025</td>
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<td>ShiftX-2.0</td>
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<td>0.9681</td>
<td>0.9942</td>
<td>0.8099</td>
<td>0.8680</td>
<td>0.7455</td>
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<tr>
<td>10X Cross.Val</td>
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<td>0.9772</td>
<td>0.9963</td>
<td>0.8658</td>
<td>0.9132</td>
<td>0.8124</td>
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</tbody>
</table>
Average Execution Time per 100 Residues

Need to be accurate AND fast
Some Comments

• Shift calculators/predictors are now approaching a level of accuracy that will allow robust chemical shift refinement and robust ID of similar structural folds
• Shift assessment may be the best way to determine the quality of NMR structures
• Shift calculations must be fast to be useful for structure determination & refinement
• Secondary structure is largely defined by C\(_\alpha\), C\(_\beta\), C’ and N shifts
• Tertiary structure info is embedded in N, HN, H\(_\alpha\) and side chain H shifts
Chemical Shifts & Structure

More information → Easy(ier) → Less information

Less information → Hard → More information
CS23D (Chemical Shift to 3D Structure)

CS23D is a web server for rapidly generating accurate 3D protein structures using only assigned NMR chemical shifts as input. Unlike conventional NMR methods which require NOE and/or J-coupling data, CS23D uses only chemical shift information to generate a 3D structure of the protein of interest. CS23D accepts chemical shift files in either SHIFTY or BMRB formats and produces a set of PDB coordinates for the protein in about 10-15 minutes. CS23D uses a combination of maximal subfragment assembly, chemical shift threading, shift-based torsion angle prediction and chemical shift refinement to generate and refine the protein coordinates. Tests indicate that CS23D converges (i.e. finds a solution) for about 90% of protein queries. The performance is dependent on the completeness of the chemical shift assignments and the similarity of the query protein to known 3D templates.

To operate this server:
1) Select or Paste to a file (BMRB or SHIFTY format) containing the sequence and chemical shifts of the protein of interest.
2) Type in a VALID email address. The results will be emailed to you.
3) Press the submit button.

For more information on running CS23D:
- Example 2 (BMRB format)
- Example 3 (SHIFTY format)

www.cs23d.ca
CS23D Components

- **RefCor** - corrects chemical shift mis-referencing
- **CSI** - identifies and deliniates secondary structure from chemical shifts
- **PREDITOR** - calculates torsion ($\psi/\phi/\chi/\omega$) angles from chemical shifts
- **PepMake** - generates protein coords from $\psi/\phi$ angles
- **THRIFTY** - performs chemical shift threading to ID similar folds or subfragments
- **SFAssembler** - assembles fragments found via THRIFTY, Homodeller and PREDITOR
- **GAFolder** - refines and minimizes structure using shifts and knowledge-based potentials
THRIFTY & Homodelller

**PREDITOR**
- torsion angles from chemical shifts

**Sequence**
- BLAST against PDB

**Chemical Shifts**
- BLAST against THRIFTY database

**THRIFTY’s Torsion Angle Alphabet**
- I, L, V, P, S, E, Q, G

**Generate Structures via Homodelller**
- Structure Templates
Fragment Assembly

Maximum subfragment assembly (SFAssembler)

Rosetta
Refining with GAFolder

Initial structure

- Call ‘reduce’ to add/adjust hydrogens
- Repair torsion angles with CCD

50 structures

- "Mutate" geometry
- Evaluate, sort 50 evaluations

Best

- 15 evaluations: randomly duplicate, "mutate" and mate structures
- Evaluate, sort ~25 evaluations

Best 10 duplicated

Worst 5 replaced by seed

50 evaluations + (300 iterations * 40 evaluations) = ~12,000 structure evaluations
GAFolder’s Knowledge-based Potentials

- Hydrophobic pairwise potential
- Hydrogen bond geometry
- Number of hydrogen bonds
- Optimized torsion angles
- Bump check (atomic overlaps)
- Charge-charge interactions
- Secondary structure content
- Secondary structure location
- Radius of gyration
- Backbone CS correlations (observed vs. calculated)
3 Different Scenarios

• Input sequence is homologous to known structure or parts of known structure (90% of queries)
  – Use maximal subfragment assembly, homology modeling and chemical shift refinement

• Input shifts are homologous to known structure or parts of known structure (5% of queries)
  – Use chemical shift threading (SimshiftDB/Thrifty), fragment assembly and chemical shift refinement

• No sequence or shift similarity (5% of queries)
  – Use Rosetta with shift restraints and chemical shift refinement
Running CS23D

CS23D is a web server for rapidly generating accurate 3D protein structures using only assigned NMR chemical shifts as input. Unlike conventional NMR methods, which require NOE and/or J-coupling data, CS23D uses only chemical shift information to generate a 3D structure of the protein of interest. CS23D accepts chemical shift files in either SHIFTY or BMRB formats and produces a set of PDB coordinates for the protein in about 10-15 minutes. CS23D uses a combination of maximal subfragment assembly, chemical shift threading, shift-based torsion angle prediction and chemical shift refinement to generate and refine the protein coordinates. Tests indicate that CS23D converges (i.e. finds a solution) for about 90% of protein queries. The performance is dependent on the completeness of the chemical shift assignments and the similarity of the query protein to known 3D folds. If you use the results of the CS23D server in a publication, please cite the following paper:


To operate this server:

1) Select or Paste to a file (BMRB or SHIFTY format) containing the sequence and chemical shifts of the protein of interest.
2) Type in a VALID email address. The results will be emailed to you.
3) Press the submit button.
Running CS23D

1) Select or Paste to a file (BMRB or SHIFTY format) containing the sequence and chemical shifts of the protein of interest.
2) Type in a VALID email address. The results will be emailed to you.
3) Press the submit button.

1) Email Address: david.walker@ualberta.ca
2) Select desired file: Choose File no file selected

OR type (paste) the chemical shift file into the space below (see examples here):

| 4 2 2SER H | β 0.70 0.3000 1 |
| 5 8 4SER H | β 1.31 0.4000 1 |
| 6 4 4MET δα | β 3.40 0.3000 2 |
| 7 4 4MET δα | β 2.11 0.3000 2 |
| 9 4 4MET δα | β 1.94 0.3000 2 |
| 10 4 4MET δα | β 2.30 0.3000 2 |
| 11 4 4MET δα | β 2.30 0.4000 1 |
| 12 4 4MET δα | β 172.22 0.4000 1 |
| 13 4 4MET δα | β 29.90 0.4000 1 |

Number of GAfolder Iterations: 100 (Default)

Number of Ensemble Structures Generated: 10

☐ Ignore exact Matching Structures in Calculation

Submit Reset

Below shows the current status of the CS23D server, indicating which jobs have been set to process.
Running CS23D

Query submitted (GMT)
Your query is being processed (usually takes 10-15 min)...
This page will automatically refresh until the structure prediction is complete.
An email with the results will also be sent to david.wishart@ualberta.ca.
Running CS23D

<table>
<thead>
<tr>
<th>CS23D energy</th>
<th>Before_optimization</th>
<th>After_optimization</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean chemical shift correlation</td>
<td>5.77</td>
<td>-2.95</td>
<td></td>
</tr>
<tr>
<td>Torsion angles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#res in phi/gsi core</td>
<td>68</td>
<td>68</td>
<td>69 (99%)</td>
</tr>
<tr>
<td>#res in phi/gsi allowed</td>
<td>6</td>
<td>6</td>
<td>5 (7%)</td>
</tr>
<tr>
<td>#res in phi/gsi disallowed</td>
<td>2</td>
<td>2</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>#res in phi/psi disallowed</td>
<td>1</td>
<td>1</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>#res in omega allowed</td>
<td>78</td>
<td>70</td>
<td>77 (99%)</td>
</tr>
<tr>
<td>#res in omega disallowed</td>
<td>0</td>
<td>0</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

Final structure reliability: Good

Mean chemical shift correlation
0.75 - 1.00 = High
0.65 - 0.75 = Good
0.55 - 0.65 = Moderate
0.00 - 0.55 = Poor

Download PDB file
Download Ensemble PDB file
View Structure

Thank you for using CS23D!
Return to main page
Why Use CS23D?

10-12 minutes

Chemical Shift Assignments
Chemical Shift Refinement with CS23D

2.45 Å

1.05 Å
Comparison Between CS23D & Conventional Structures

- Calibindin
- GB3
- CspA
- FattBP
- Ubiquitin
- DinI
Comparison Between CS23D, CS-Rosetta & Cheshire

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>PDB ID</th>
<th>BMRB ID</th>
<th>CS-ROSETTA RMSDbb (Å)</th>
<th>CHESHER RMSDbb (Å)</th>
<th>CS23D RMSDbb (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM1442</td>
<td>BSBO</td>
<td>5921</td>
<td>1.22</td>
<td>1.32</td>
<td>1.84</td>
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<tr>
<td>Calbindin</td>
<td>4ICB</td>
<td>390</td>
<td>1.39</td>
<td>1.47</td>
<td>2.41</td>
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<tr>
<td>Hpr</td>
<td>1HDN</td>
<td>2371</td>
<td>1.99</td>
<td>1.83</td>
<td>0.96</td>
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<tr>
<td>Ubiquitin</td>
<td>1UBQA</td>
<td>5387</td>
<td>0.82</td>
<td>1.33</td>
<td>1.34</td>
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<tr>
<td>FF domain</td>
<td>2UCZ</td>
<td>6711</td>
<td></td>
<td>1.46</td>
<td>1.12</td>
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<tr>
<td>HR2106</td>
<td>2HZ5</td>
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<td>1.85</td>
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<tr>
<td>Spo0F</td>
<td>1SRR</td>
<td>5899</td>
<td>1.67</td>
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<td>1.88</td>
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<tr>
<td>Apo 1fabp</td>
<td>1LFO</td>
<td>4098</td>
<td>1.72</td>
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<td>2.11</td>
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<td>CspA</td>
<td>1MJC</td>
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<td>1.43</td>
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<tr>
<td>XcR50</td>
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<td>2.41</td>
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<tr>
<td>Profilin</td>
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<td>2.26</td>
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<td>1.8</td>
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<tr>
<td>GB3</td>
<td>2OED</td>
<td>15283</td>
<td>0.74</td>
<td></td>
<td>0.98</td>
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</tbody>
</table>
## CS23D Summary

### Advantages
- Simplifies NMR-based structure determination by orders of magnitude
- 1000-10,000X faster than competing methods (Cheshire and CS-Rosetta)
- Not limited by size of protein (generally)
- Only system with robust CS refinement

### Disadvantages
- Can’t solve every protein it is given (~95% effective)
- Doesn’t perform quite as well as CS-Rosetta on totally novel folds
- Doesn’t handle protein complexes
- No other input (NOEs, J-couplings, RDCs) accepted
GeNMR (Improving on CS23D)

www.genmr.ca
<table>
<thead>
<tr>
<th>GeNMR Advantages</th>
<th>CS23D Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can solve almost every protein it is given (99% effective)</td>
<td>Can’t solve every protein it is given (~95% effective)</td>
</tr>
<tr>
<td>Performs better than CS-Rosetta on totally novel folds</td>
<td>Doesn’t perform quite as well as CS-Rosetta on totally novel folds</td>
</tr>
<tr>
<td>Handles protein complexes</td>
<td>Doesn’t handle protein complexes</td>
</tr>
<tr>
<td>Accepts NOEs (J-couplings, RDCs for Sept. 2009)</td>
<td>No other input (NOEs, J-couplings, RDCs) accepted</td>
</tr>
</tbody>
</table>
Running GeNMR
Running GeNMR

Query submitted

Your query is being processed (usually takes 10-15 min)...

This page will automatically refresh until the structure prediction is complete.
An email with the results will also be sent to david.wishart@ualberta.ca.
Running GeNMR

Your query has finished processing.

- [Download coordinates of the best-score model](#)
- [Download coordinates of the NMR ensemble](#)

<table>
<thead>
<tr>
<th></th>
<th>Before_optimisation</th>
<th>After_optimisation</th>
<th>Expected</th>
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</thead>
<tbody>
<tr>
<td>CB23D2.0 energy</td>
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<td>5.20</td>
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<tr>
<td>Mean chemical shift correlation</td>
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<td>NOE Violations</td>
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<tr>
<td>Knowledge-Based Score</td>
<td>-11.25</td>
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<tr>
<td>Chemical Shift Score</td>
<td>11.54</td>
<td>7.28</td>
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</tr>
</tbody>
</table>

**Torsion angles**
- #res in phi/psi core: 72
- #res in phi/psi allowed: 5
- #res in phi/psi generous: 3
- #res in phi/psi disallowed: 3
- #res in omega allowed: 94
- #res in omega disallowed: 6

**Final structure reliability: Good**

- Mean chemical shift correlation
  - 0.75 - 1.00 = High
  - 0.65 - 0.75 = Good
  - 0.55 - 0.65 = Moderate
  - 0.00 - 0.55 = Poor

<table>
<thead>
<tr>
<th>i</th>
<th>1</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
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<tr>
<td>Protein Name (PDB ID)</td>
<td>Sequence ID (%)</td>
<td>RMSD (Å) to reference PDB</td>
<td>Calculation Time (min)</td>
<td># of distance restraints</td>
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<tr>
<td>-----------------------</td>
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<td>------------------------</td>
<td>--------------------------</td>
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<tr>
<td><strong>Scenario (a) – Shift data only -- query has homologue in database</strong></td>
<td></td>
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<tr>
<td>Ubiquitin (1UBQA)</td>
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<td>10</td>
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<td>Ig Domain of Palladin (2DM2A)</td>
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<td>Abl Kinase (2HYYA)</td>
<td>98</td>
<td>1.76</td>
<td>19</td>
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<td>RGD-Hirudin (2JOOA)</td>
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<tr>
<td><strong>Scenario (b) – Shift data only -- query has NO homologue in database</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ubiquitin (1UBQA)</td>
<td>---</td>
<td>2.55</td>
<td>18</td>
<td>---</td>
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<td>4-helix Bundle (2I7UA)</td>
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<td>1.48</td>
<td>22</td>
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<td>Discoidin Domain DDR2 (2Z4FA)</td>
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<td>1.63</td>
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<td>CheW (2HO9A)</td>
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<td>25</td>
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<tr>
<td><strong>Scenario (c) – NOE only -- query has homologue in database</strong></td>
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<td>Cyclophilin (1CWCA)</td>
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<td>4096</td>
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<td>Regulatory Protein E2 (1A7G)</td>
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<td>16</td>
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<td>Serine Protein Inhibitor (3C12)</td>
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<td>DnaB (1JWE)</td>
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<td>1194</td>
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<td></td>
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<tr>
<td>Superoxide Dismutase (2AF2)</td>
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<td>1.27</td>
<td>14</td>
<td>2672</td>
<td></td>
<td></td>
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<tr>
<td>PyJ Protein (1FAF)</td>
<td>95</td>
<td>0.05</td>
<td>10</td>
<td>870</td>
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<td></td>
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<tr>
<td>Neurotoxin II (1NOR)</td>
<td>87</td>
<td>0.92</td>
<td>16</td>
<td>540</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Scenario (d) – NOE data only -- query has NO homologue in database</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ubiquitin (1UBQ)</td>
<td>---</td>
<td>1.38</td>
<td>21</td>
<td>1318</td>
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<tr>
<td>Forkhead FOXO4 (1E17)</td>
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<td>1294</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Profilin (1AWI)</td>
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<td>1.96</td>
<td>46</td>
<td>1794</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mu DNA Binding Protein (2EZI)</td>
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<td>2.43</td>
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<td>1009</td>
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<tr>
<td>SV40 ORI Binding Protein (1TBD)</td>
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<td>46</td>
<td>1709</td>
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</tr>
<tr>
<td><strong>Scenario (e) – NOE + Shift data -- query has homologue in database</strong></td>
<td></td>
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<tr>
<td>Response Regulator SpoOF (1FSP)</td>
<td>96</td>
<td>1.17</td>
<td>16</td>
<td>1835</td>
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<tr>
<td>Profilin (1AWI)</td>
<td>99</td>
<td>0.37</td>
<td>28</td>
<td>1794</td>
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<td>Interleukin 4 (1BBN)</td>
<td>87</td>
<td>1.68</td>
<td>20</td>
<td>917</td>
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<tr>
<td>Metalloproteinase 12 (1YCM)</td>
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<td>1.44</td>
<td>19</td>
<td>3544</td>
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<td>Ubiquitin (1UBQ)</td>
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<td>0.42</td>
<td>25</td>
<td>1318</td>
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Summary

• Chemical shifts are the richest (but most ignored) source of structural information in NMR
• Structure determination by chemical shifts is roughly where conventional NMR was in 1990 (still room for improvement)
• Critical need to include side chain CS’s and to improve chemical shift calculation accuracy & speed
• Critical need to improve conformational sampling and energy minimization for shift-based refinement
• Despite these caveats, it is important for the BioNMR community to test CS23D, CS-Rosetta or Cheshire and provide feedback
Thanks to…

- Scott Montgomerie
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- Ben Zhou
- Jack Liang