PISA, or a story about macromolecular complexes in bioinformatics and crystallography

Eugene Krissinel

Macromolecular Structure Database
European Bioinformatics Institute, Genome Campus, Hinxton
Cambridge CB10 1SD UK

http://www.ebi.ac.uk/msd
keb@ebi.ac.uk

2nd Annual CCP4 USA Summer School and Workshop
Macromolecular Assemblies

- Complexes of protein, DNA/RNA chains and ligands, stable in native environment
- The way the chains assemble represents the [Protein] Quaternary Structure (PQS)
- Macromolecular assemblies are often the Biological Units, performing certain biochemical functions
- Biological significance of macromolecular assemblies is truly immense

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
PQS is a difficult object for experimental studies

- *Light / Neutron / X-ray / Small angle scatterings*: mainly composition and multimeric state may be found. 3D shape may be guessed from mobility measurements.

- *Electron microscopy*: not a fantastic resolution and not applicable to all objects

- *NMR* is not good for big chains, even less so for protein assemblies.

Very few quaternary structures have been identified experimentally.
PISA, or a story about macromolecular complexes in bioinformatics and crystallography

Macromolecular Structure Database

**PQS are difficult to calculate**

*If we know the sequence ...*

1

VRKERTFLAVEPDGVARGILVGGETIARKYEEKGFVLVGEQLVPEKOLAESHYAEHHKERPEF

*then we can calculate ...*

2

50 - 90% Secondary Structure (CASP 5), depending on method

3

10 - 90% Tertiary Structure (CASP 5), depending on method and target

4

Probably 0%
Quaternary Structure.
Docking of given number of given structures: 5 - 20% success (CAPRI 5)

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
But PQS are assigned to many entries in PDB!

Most of those are PROBABLE Quaternary Structures.

The PDB “rules” are:

1. Depositor’s say prevails.
2. Accept everything which passes formal validation checks.
3. No experimental evidence for PQS is required.
4. If a depositor does not know or does not care (60-80% of instances for PQS), the curator is to decide.
5. The curator may (EBI-MSD) or may not (Rutgers-RCSB, PDBj) use computing/modeling tools to assist the PQS annotation.

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Crystallography is special

Because: A) crystal is made of assemblies

\[ K_d = \frac{\prod_i^n [A_i]}{[A]} \]

\[ \frac{[A]}{[A_i]} = \frac{[A_i]^{n-1}}{K_d} \]

\[ \tan \alpha = n - 1 \]
Crystallography is special

Because: B) there is no need to dock subunits
– the docking is given by crystal structure

Macromolecular interfaces should be viewed as an additional important product of protein crystallography

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
PDB contains a wealth of experimental data on PQS

More than 80% of macromolecular structures are solved by means of X-ray diffraction on crystals.

It is reasonable to expect that PQS make construction blocks for the crystal.

An X-ray diffraction experiment produces atomic coordinates of the Asymmetric Unit (ASU), which is stored as a PDB file.

In general, neither ASU nor Unit Cell has any direct relation to PQS. The PQS may be made of

- a single ASU
- part of ASU
- several ASU
- several ASU parts

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
PDB entries and Biological Units

PDB entry 1P30
A monomer?

Biological unit 1P30
Homotrimer!

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
PDB entries and Biological Units

PDB entry 2TBV
A trimer?

Biological Unit 2TBV
180-mer!

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
PDB entries and Biological Units

PDB entry 1E94

2 Biological Units in 1E94:
A dodecamer and a hexamer!

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
In (very) simple words …

in vivo → crystallization → in crystal

1  2  3

no image or bad image
good image but no associations

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
A simple thing to do

PQS server @ EBI  (Kim Henrick) *Trends in Biochem. Sci.* (1998) **23**, 358
PITA server @ EBI  (Hannes Ponstingl) *J. Appl. Cryst.* (2003) **36**, 1116

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
What is a significant interface?

Depends on the problem.

**Protein functionality:** the interface should be engaged in *any* sort of interaction, including transient short-living protein-ligand and protein-protein etc. associations. Obviously important properties:

- Affinity (comes from area, hydrophobicity, electrostatics, H-bond density etc.)

and properties that may be important for *reaction pathway and dynamics*:

- Aminoacid composition
- Geometrical complementarity
- Overall shape, compactness
- Charge distribution
- etc.

**Stable macromolecular complexes, PQS:** the interface should make a sound binding. Important properties:

- Sufficient free energy of binding
- something else?

[http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html](http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html)
A Common Identification Problem

This is useless...

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Real and superficial protein interfaces


http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Real and superficial protein interfaces

Most often used discrimination criteria - interface area.

A cut-off at 900 Å² gives about 80% success rate of discrimination between monomers and dimers.

Big proteins will be always sticky if this criteria is true …
Real and superficial protein interfaces

Free energy gain of interface formation.

A cut-off at $-8 \text{ kcal/M}$ gives about 82% success rate of discrimination between monomers and dimers.

Can energy measure be uniform for all weights and shapes?

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Is there a (good) measure of interface significance at all?

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Real and superficial protein interfaces

“No single parameter absolutely differentiates the interfaces from all other surface patches”


Formation of N>2 -meric complexes is most probably a corporate process involving a set of interfaces. Therefore significance of an interface should not be detached from the context of macromolecular complex

“…the type of complexes need to be taken into account when characterizing interfaces between them.”

Chemical stability of macromolecular complexes

- It is not properties of individual interfaces but rather chemical stability of complexes in general that really matters.

- *Macromolecular units will most likely associate into largest complexes that are still stable.*

- A complex is stable if its free energy of dissociation is positive:

\[
\Delta G_{diss}^0 = -\Delta G_{int} - T\Delta S > 0
\]
Chemical stability of macromolecular complexes

\[ \Delta G_{\text{diss}} = -\Delta G_{\text{int}} - T\Delta S > 0 \]
Binding energy

\[ \Delta G_{\text{int}} = \Delta G_{\text{sol}}(A_1, A_2 \ldots A_n) - \sum_{i=1}^{n} \Delta G_{\text{sol}}(A_i) \]

Solvation energy of protein complex
Solvation energies of dissociated subunits

Free energy of H-bond formation
Free energy of salt bridge formation

\[ -E_{\text{hb}} N_{\text{hb}} - E_{\text{sb}} N_{\text{sb}} \]

Choice of dissociation subunits:

Dissociation into stable subunits with minimum \( \Delta G_{\text{diss}} \)

Number of H-bonds between dissociated subunits
Number of salt bridges between dissociated subunits

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
**Solvation free energy**

In this model, binding energy is function of individual interfaces.

\[
\Delta G_{sol} (A) = \sum_k \Delta \sigma_k (a_k - a_k^r)
\]


*Atomic solvation parameters*

*Atom’s accessible surface area*

*Atom’s accessible surface area in the reference (unfolded) state*
Entropy of macromolecules in solutions

\[ S = S_{\text{trans}}(m) + S_{\text{rot}}(\hat{I}, \sigma_S) + S_{\text{surf}}(a) \]

- Translational entropy
  \[ S_{\text{trans}}(m) \approx c_t + \frac{3R}{2} \log(m) \]
- Rotational entropy
  \[ S_{\text{rot}}(\hat{I}, \sigma_S) \approx c_r + \frac{R}{2} \log\left(\frac{I_1I_2I_3}{\sigma_S^2}\right) \]
- Sidechain entropy
  \[ S_{\text{surf}}(a) \approx Fa \]

*\( c_t, c_r \) and \( F \) are semiempirical parameters*


http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Entropy of dissociation

\[
\Delta S = \sum_{i=1}^{n} S(A_i) - S(A_1, A_2 \ldots A_n)
\]

\[
= (n - 1)C + \frac{3R}{2} \log \left( \frac{\prod_i m_i}{\sum_i m_i} \right) + \frac{R}{2} \log \left( \frac{\prod_i \prod_k I_k(A_i)}{\prod_k I_k(A_1 \ldots A_n)} \right) + Fa_{\text{buried}}
\]

By its very nature, entropy of dissociation is function of protein complex rather than that of individual interfaces.
Entropy of dissociation

- Drives thermodynamic systems towards most disordered (dissolved) state.

- Makes bias towards less symmetric states. However, in practice, this is overweighed by binding energy, which is normally maximal in most symmetric states.

- Responsible for complex “instability”.

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
PISA, or a story about macromolecular complexes in bioinformatics and crystallography

We know how it looks, can we see it?

We now know (or we think that we know) how to evaluate chemical stability of protein complexes:

\[ \Delta G^0_{diss} = -\Delta G_{\text{int}} - T\Delta S > 0 \]

which depends on interface properties as well as on the geometry of protein complex and dissociated subunits.

How to find stable complexes in a crystal?

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Enumerating assemblies in crystal

- crystal is represented as a periodic graph with monomeric chains as vertices and interfaces as edges
- each set of assemblies is identified by engaged interface types
- all assemblies may be enumerated by a backtracking scheme engaging all possible combinations of different interface types

Example: crystal with 3 interface types

<table>
<thead>
<tr>
<th>Assembly set</th>
<th>Engaged interface types</th>
<th>Assembly set</th>
<th>Engaged interface types</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>000 - only monomers</td>
<td>5</td>
<td>100 - dimer N3</td>
</tr>
<tr>
<td>2</td>
<td>001 - dimer N1</td>
<td>6</td>
<td>101</td>
</tr>
<tr>
<td>3</td>
<td>010 - dimer N2</td>
<td>7</td>
<td>110</td>
</tr>
<tr>
<td>4</td>
<td>011</td>
<td>8</td>
<td>111 - all crystal</td>
</tr>
</tbody>
</table>

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Detection of Biological Units in Crystals: Method Summary

1. Build periodic graph of the crystal
2. Enumerate all possibly stable assemblies
3. Evaluate assemblies for chemical stability
4. Leave only sets of stable assemblies in the list and range them by chances to be a biological unit:
   - Larger assemblies take preference
   - Single-assembly solutions take preference
   - Otherwise, assemblies with higher $\Delta G_{\text{diss}}$ take preference

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Classification of protein assemblies

Assembly classification on the benchmark set of 218 protein structures published in


<table>
<thead>
<tr>
<th></th>
<th>1mer</th>
<th>2mer</th>
<th>3mer</th>
<th>4mer</th>
<th>6mer</th>
<th>Other</th>
<th>Sum</th>
<th>Correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>1mer</td>
<td>49</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>55</td>
<td>89%</td>
</tr>
<tr>
<td>2mer</td>
<td>3</td>
<td>71+11</td>
<td>0</td>
<td>2+1</td>
<td>0</td>
<td>0</td>
<td>76+12</td>
<td>93%</td>
</tr>
<tr>
<td>3mer</td>
<td>1</td>
<td>0</td>
<td>22</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>24</td>
<td>92%</td>
</tr>
<tr>
<td>4mer</td>
<td>2</td>
<td>2+1</td>
<td>0</td>
<td>26+6</td>
<td>0</td>
<td>1</td>
<td>31+7</td>
<td>84%</td>
</tr>
<tr>
<td>6mer</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0+1</td>
<td>10+2</td>
<td>0</td>
<td>10+3</td>
<td>92%</td>
</tr>
</tbody>
</table>

196+22 <= 196 homomers and 22 heteromers

Total: 196+22 90%

Fitted parameters:
1. Free energy of a H-bond: $E_{hh} = 0.51$ kcal/mol
2. Free energy of a salt bridge: $E_{sb} = 0.21$ kcal/mol
3. Constant entropy term: $T \cdot C = 11.7$ kcal/mol
4. Surface entropy factor: $T \cdot F = 0.57 \cdot 10^{-3}$ kcal/(mol*Å²)

Classification error in $\Delta G_{diss}^0 : \pm 5$ kcal/mol
Classification of protein-DNA complexes

Assembly classification on the benchmark set of 212 protein – DNA complexes published in


<table>
<thead>
<tr>
<th></th>
<th>2mer</th>
<th>3mer</th>
<th>4mer</th>
<th>5mer</th>
<th>6mer</th>
<th>10mer</th>
<th>Other</th>
<th>Sum</th>
<th>Correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>2mer</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>3mer</td>
<td>6</td>
<td>96</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>105</td>
<td>91%</td>
</tr>
<tr>
<td>4mer</td>
<td>0</td>
<td>2</td>
<td>83</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>85</td>
<td>98%</td>
</tr>
<tr>
<td>5mer</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>60%</td>
</tr>
<tr>
<td>6mer</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>1</td>
<td>15</td>
<td>87%</td>
</tr>
<tr>
<td>10mer</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>Total:</td>
<td>212</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>93%</td>
<td></td>
</tr>
</tbody>
</table>

Classification error in $\Delta G_{diss}^0 : \pm 5$ kcal/mol
Free energy distribution of misclassifications

Number of misclassifications vs. $|\Delta G_{diss}^0|$ [kcal/mol]

Misclassifications:
- 1hcn (4:2)
- 1h3u (8:4)
- 1cx (12:6)
- 1bn (2:1)
- 1cg2 (4:2)
- 2hex (10:1)
- 3g (15:1)

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Example of misclassification: 1QEX

BACTERIOPHAGE T4 GENE PRODUCT 9 (GP9), THE TRIGGER OF TAIL CONTRACTION AND THE LONG TAIL FIBERS CONNECTOR

Predicted: homohexamer
Dissociates into 2 trimers
\[ \Delta G^0_{diss} \approx 106 \text{ kcal/mol} \]

Biological unit: homotrimer
Dissociates into 3 monomers
\[ \Delta G^0_{diss} \approx 90 \text{ kcal/mol} \]

http://www.ebi.ac.uk//msd-srv/prot_int/pistart.html
Example of misclassification: 1QEX

BACTERIOPHAGE T4 GENE PRODUCT 9 (GP9), THE TRIGGER OF TAIL CONTRACTION AND THE LONG TAIL FIBERS CONNECTOR


http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Example of misclassification: 1QEX

BACTERIOPHAGE T4 GENE PRODUCT 9 (GP9), THE TRIGGER OF TAIL CONTRACTION AND THE LONG TAIL FIBERS CONNECTOR

1QEX trimer

Wrong mainchain tracing!

1S2E trimer
Correct mainchain tracing
Classed correctly

1QEX hexamer

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Example of misclassification: 1D3U

TATA-BINDING PROTEIN / TRANSCRIPTION FACTOR

Predicted: octamer

Dissociates into 2 tetramers

\[ \Delta G_{diss}^0 \approx 20 \text{ kcal/mol} \]

Functional unit: tetramer

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Example of misclassification: 1CRX
CRE RECOMBINASE / DNA COMPLEX REACTION INTERMEDIATE

Predicted: dodecamer
Dissociates into 2 hexamers
\[
\Delta G_{diss}^0 \approx 28 \text{ kcal/mol}
\]

Functional unit: trimer

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Example of misclassification: 1CRX
CRE RECOMBINASE / DNA COMPLEX REACTION INTERMEDIATE

Structure of Cre recombinase complexed with DNA in a site-specific recombination synapse.

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Example of misclassification: 1TON

Predicted: dimer

Dissociates at
\[ \Delta G_{diss}^0 \approx 37 \text{ kcal/mol} \]

Biological unit: monomer

Apparent dimerization is an artefact due to the presence of Zn\(^{2+}\) ions added to the buffer to aid crystallization. Removal Zn from the file results in \[ \Delta G_{diss}^0 \approx 3 \text{ kcal/mol} \]

Example of misclassification: 1YWK

Predicted: homohexameric
\[ \Delta G_{\text{diss}} \approx 4.4 \text{ kcal/mol} \]
dissociating into 3 dimers

Believed to be: monomeric
6 units in ASU

Structural homologue
1XRU:
RMSD \approx 0.9 \text{ Å}
Seq.Id \approx 50%
Homohexameric with
\[ \Delta G_{\text{diss}} \approx 9.3 \text{ kcal/mol} \]
Choice of ASU
Example of misclassification: 1YWK

Predicted: homohexameric

$\Delta G_{\text{diss}} \approx 4.4 \text{ kcal/mol}$
dissociating into 3 dimers

Believed to be: monomeric

6 units in ASU

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Reasons for the misclassification of Biological Units

- Crystal packing may introduce interactions that are not engaged in native environment
- Definition of Biological Unit is based on physiological function and may be to a certain degree subjective
- Interaction artifacts are often due to the addition of binding agents in order to aid crystallization

- Theoretical models of macromolecular complexation are simplified
- Experimental data are of a limited accuracy
- No explicit account for concentrations, temperature, pH and ionic strength
Comparison with current PDB annotation

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Web-server PISA
http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html

A new MSD-EBI tool for working with **Protein Interfaces**, **Surfaces** and **Assemblies**
PISA, or a story about macromolecular complexes in bioinformatics and crystallography


Session 564-RI-ACR map

Found interfaces

<table>
<thead>
<tr>
<th>#</th>
<th>Structure</th>
<th>Score</th>
<th>NN</th>
<th>Range</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E</td>
<td>96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>D</td>
<td>95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>90</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>C</td>
<td>97</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>D</td>
<td>74</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>B</td>
<td>74</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>C</td>
<td>74</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>B</td>
<td>60</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>D</td>
<td>61</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>C</td>
<td>60</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>E</td>
<td>60</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>B</td>
<td>63</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>E</td>
<td>12</td>
<td>7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ΔG indicates the change in free energy of the interface compared to the total solvation of the interacting proteins. Negative ΔG indicates a positive protease activity, and thus a higher binding affinity of the interface.

Interfaces in PDB 1stm crystal

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
### Probable Quaternary Structures

PISA, or a story about macromolecular complexes in bioinformatics and crystallography

**2nd Annual CCP4 USA Summer School and Workshop, APS at ANL, Illinois, 24 June – 2 July 2009.**

**Macromolecular Structure Database**

#### Session 564-RI-ACR map

- **query**
- **interfaces**
- **structures**
- **assemblies**

PQS sets 1 to 4 of total 4

### Analysis of protein interfaces suggests that the...

<table>
<thead>
<tr>
<th>PQS set</th>
<th>Size</th>
<th>Formula</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2x60</td>
<td>A\textsubscript{60}</td>
<td>A\textsubscript{12}B\textsubscript{12}C\textsubscript{12}D\textsubscript{12}E\textsubscript{12}</td>
</tr>
<tr>
<td>2</td>
<td>24x5</td>
<td>A\textsubscript{5}</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8x3</td>
<td>A\textsubscript{3}</td>
<td>E\textsubscript{9}</td>
</tr>
<tr>
<td>4</td>
<td>24x2</td>
<td>A\textsubscript{2}</td>
<td>A\textsubscript{B}</td>
</tr>
<tr>
<td></td>
<td>12x2</td>
<td>A\textsubscript{2}</td>
<td>DE</td>
</tr>
</tbody>
</table>

**http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html**

[Open Jmol view](http://www.ebi.ac.uk - Assembly 1stm/1:1 - Mozilla Firefox)

- Rendering: Spacefill
- Background: Black
- Zoom: 100%
- Screen: 50%
- Spin

**Close**
Conclusions

• Chemical-thermodynamical models for protein complex stability allow one to recover biological units from protein crystallography data at 80-90% success rate

• Considerable part of misclassifications is due to the difference of experimental and native environments and artificial interactions induced by crystal packing

• Functional significance of protein interfaces cannot be reliably inferred only from their properties. Due to entropy contribution and entangled interactions, interface function is also subject to protein complex composition and geometry.

• Protein interface and assembly analysis software (PISA) is available, please use it

http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html
Acknowledgements

Kim Henrick  
*European Bioinformatics Institute*

Mark Shenderovich  
*Structural Bioinformatics Inc.*

Hannes Ponstingl  
*Sanger Centre*

Sergei Strelkov  
*University of Leuven*

MSD & PDB teams  
*EBI & Rutgers*

CCP4  
*Daresbury-York-Oxford-Cambridge*

~4000 PISA users  
*Worldwide*

Biotechnology and Biological Sciences Research Council (BBSRC) UK

General introduction, job assignment and gentle push

Helpful discussion

Sharing the expertise and benchmark data

“Mystery” of bacteriophage T4

Everyday use of PISA, examples, verification and feedback

Encouragement and publicity

Using PISA and feedback

Research grant No. 721/B19544

http://www.ebi.ac.uk/ MSD-srv/prot_int/pistart.html