PAUL SCHERRER INSTITUT

Distinguishing biologically relevant interfaces from lattice contacts in protein crystals

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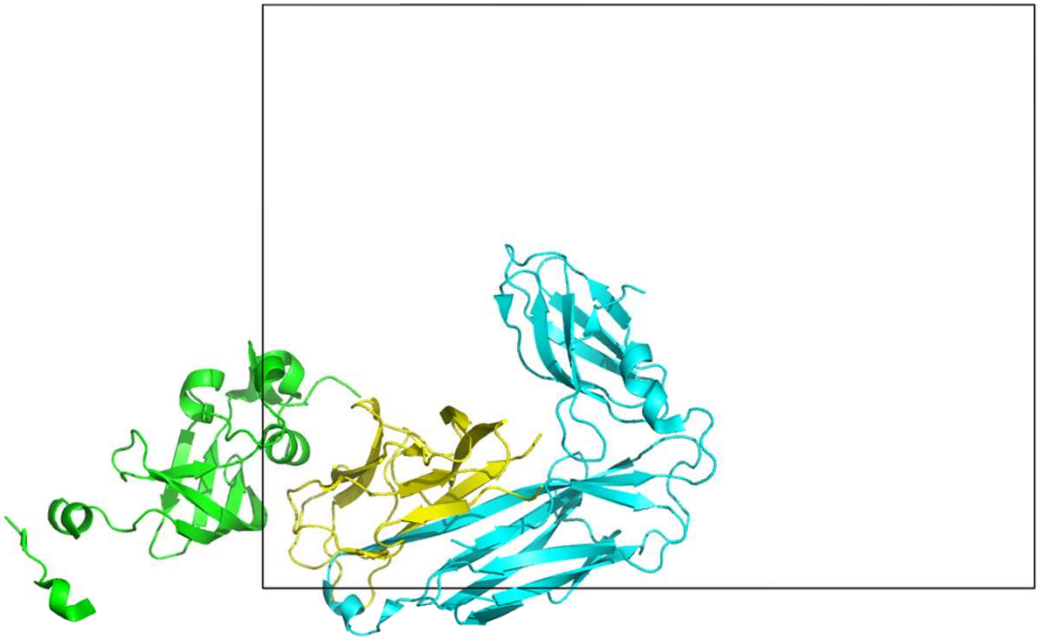
RAMC2013 meeting, Le Bischenberg, 10 September 2013

Crystal packing and biologically relevant interfaces



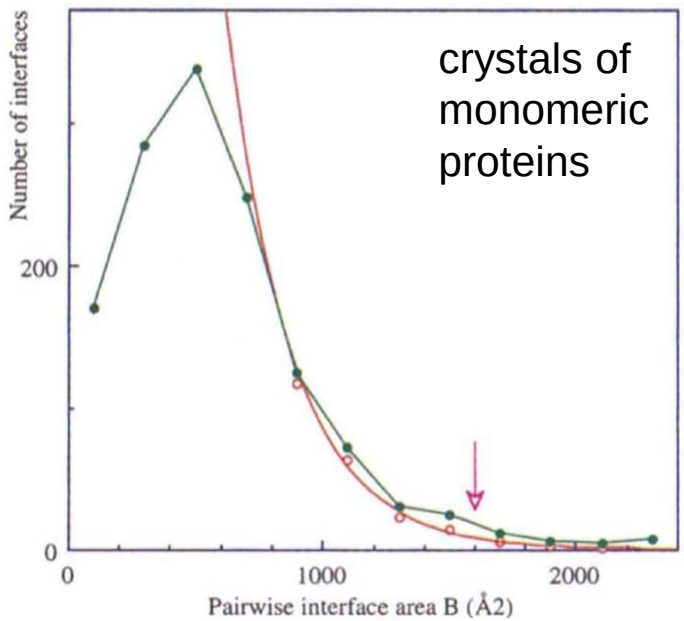
Nishiyama et al., *EMBO J.*, 2005

The first approach: interface area

Interface area (*B*) statistics

For $B > 700$ Å^2, ( )

probability to find a non-specific interface (crystal contact) burying more than B Å^2



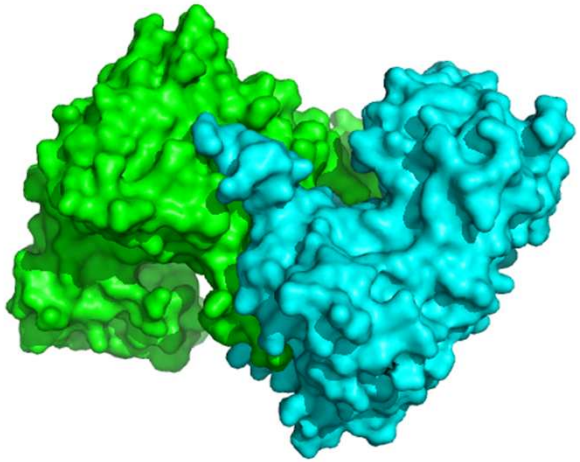
Example: for $B = 1000$ Å^2 $p \approx 9\%$

Bottom line: the **larger** the interface the more likely to be **bio**

Janin, *Nat Struct Biol*, 1997

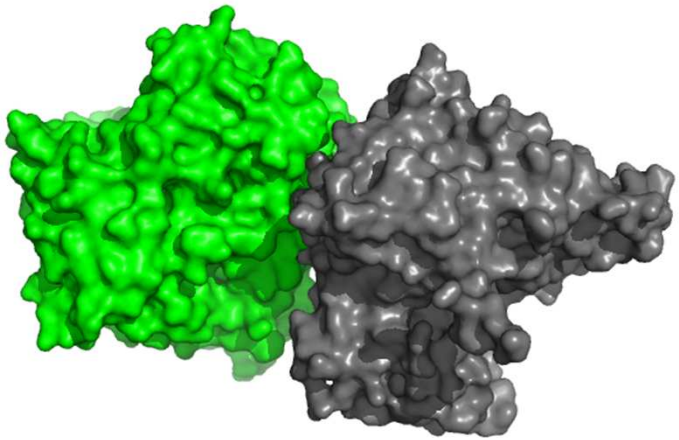
Easy cases

2780 Å^2



large → Biological interface

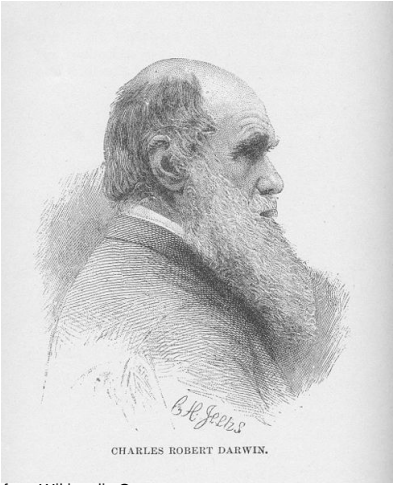
370 Å^2



small → Crystal contact

Evolutionary approach to the problem

Biologically relevant interfaces are the result of **evolution**

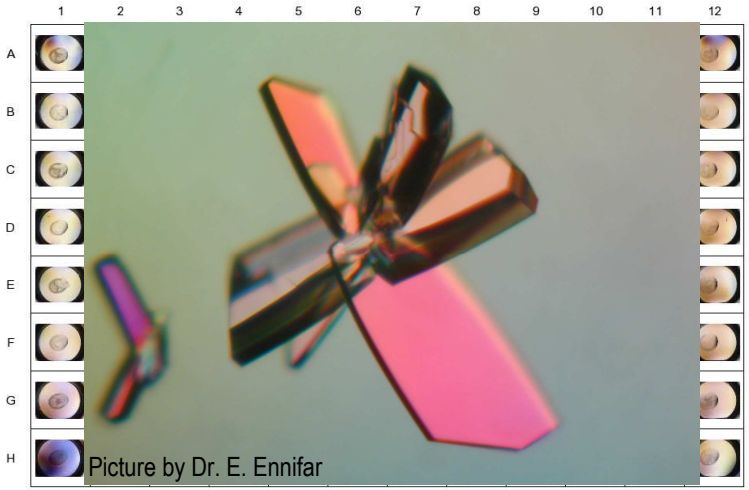


from Wikimedia Commons

Detectable signature of selection pressure

Crystal contacts are not

from Wikimedia Commons

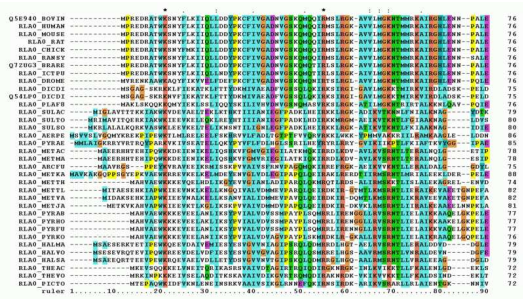


No detectable signature of selection pressure

Entropy ratios for interface classification

Valdar & Thornton[1]: comparison of selection pressure on **interface** versus **surface** residues. Similar approach at the same time by Elcock & McCammon[2]

Sequence entropy of MSA of homologues as metrics of selection pressure[2]:

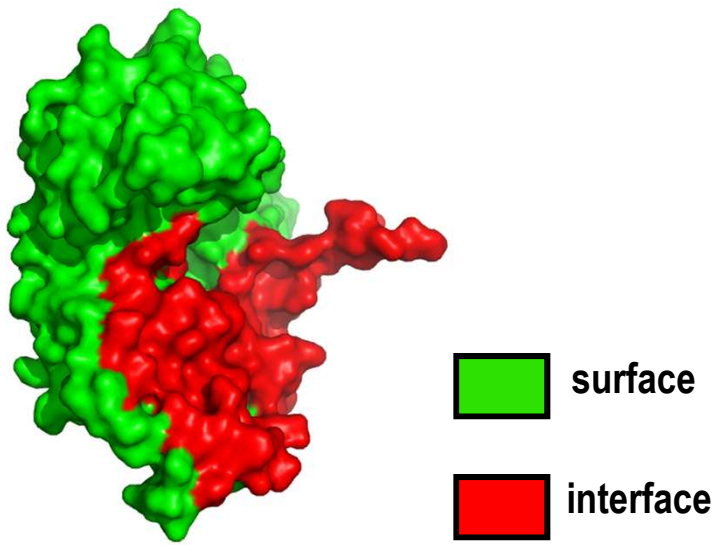


$$s(i) = -\sum_k p_i(k)\log(p_i(k))$$

$p_i(k) \rightarrow$ probability of residue of type *k* at pos. *i*

For **biological** interface: $\frac{<s>_{interface}}{<s>_{surface}} < 1$

For **crystal** contact: $\frac{<s>_{interface}}{<s>_{surface}} > 1$

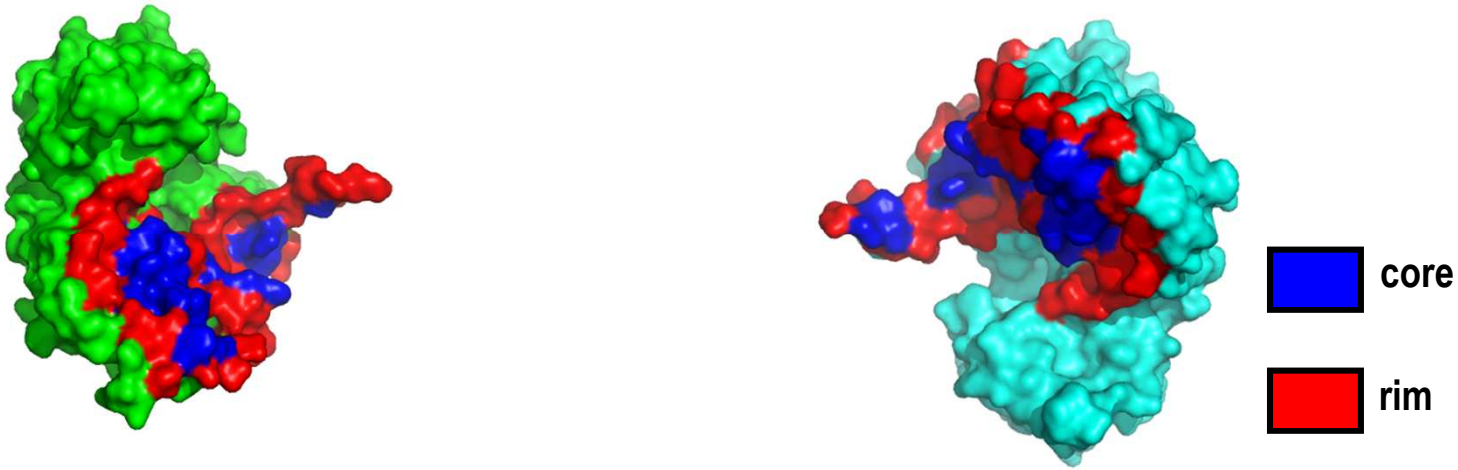


[1]Valdar & Thornton, *J Mol Biol*, 2001

[2]Elcock & McCammon, *PNAS*, 2001

Entropy ratio of interface core and rim

Guharoy & Chakrabarti[1,2]: Core: interface residues with at least one fully buried atom
Rim: the other interface residues



For **biological** interface: $\frac{<s>_{core}}{<s>_{rim}} < 1$

For **crystal** contact: $\frac{<s>_{core}}{<s>_{rim}} > 1$

[1]Guharoy & Chakrabarti, *PNAS*, 2005 [2]Chakrabarti & Janin, *Proteins*, 2002

New datasets: DCxtal and DCbio

Need for new datasets for method development

"Classical" datasets contain

- Too many "easy-to-predict" (obvious by area) interfaces
- Many old structures with no R-free statistics and no deposited structure factors

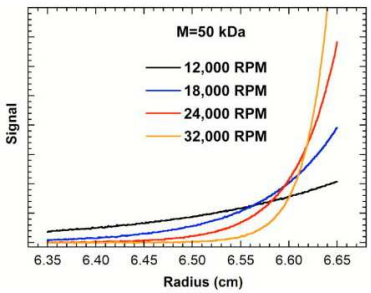
Two new datasets[1]:

- **DC xtal**: large crystal contacts, with BSA 1000 Å^2 and above
- **DC bio**: small biological interfaces

Criteria:

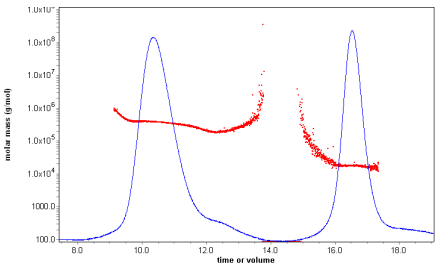
- Good quality structures by strict filtering (resolution, R-free, data deposition)
- Clear **biophysical evidence** for oligomeric state in solution from literature
- No domain swaps or other ambiguous cases

AUC



from Cole, *MethodsCell Biol*, 2008

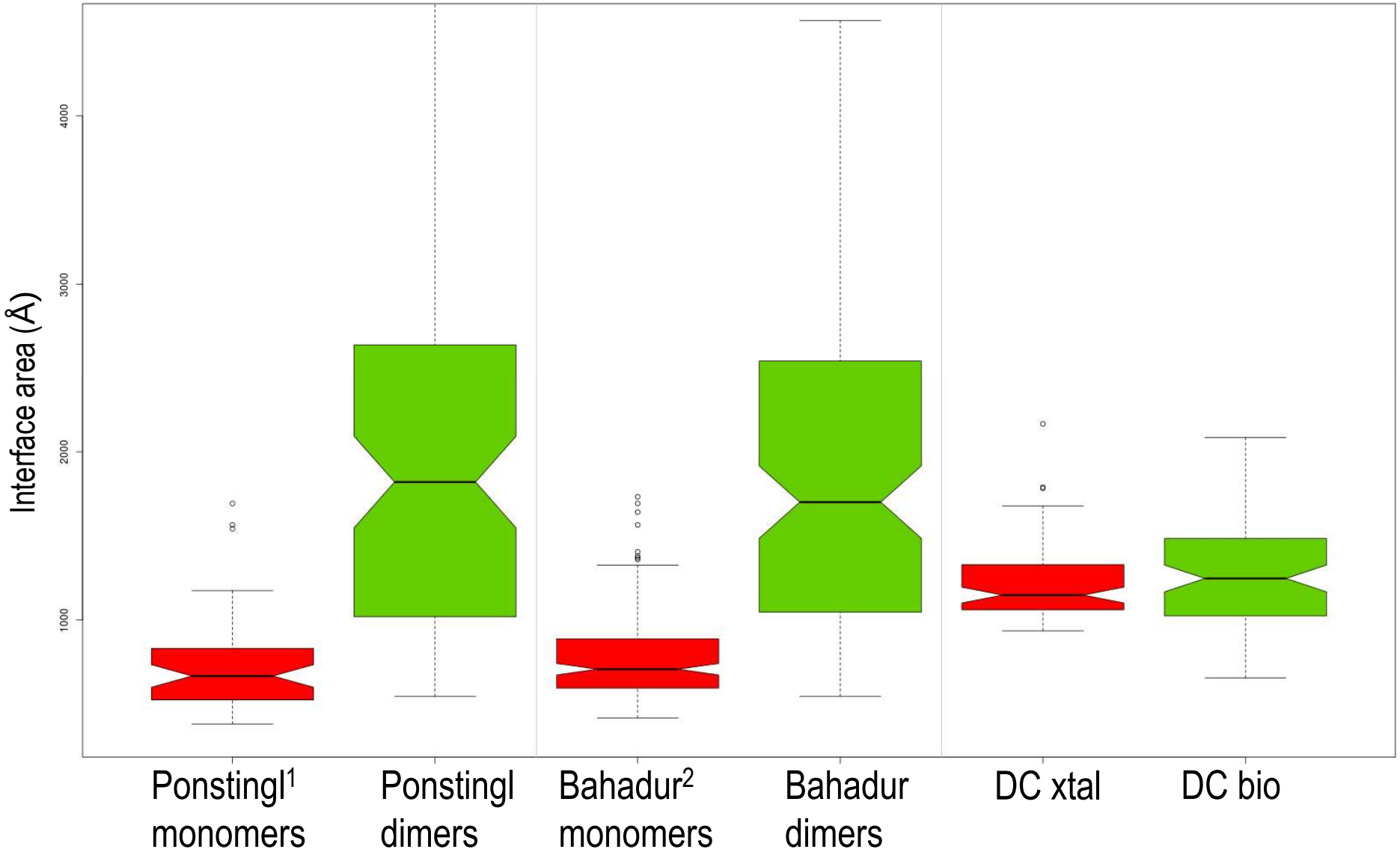
Light scattering



from Gut et al, *JMB*, 2009

[1]Duarte et al., *BMC Bioinformatics*, 2012

Area distributions of different interface datasets



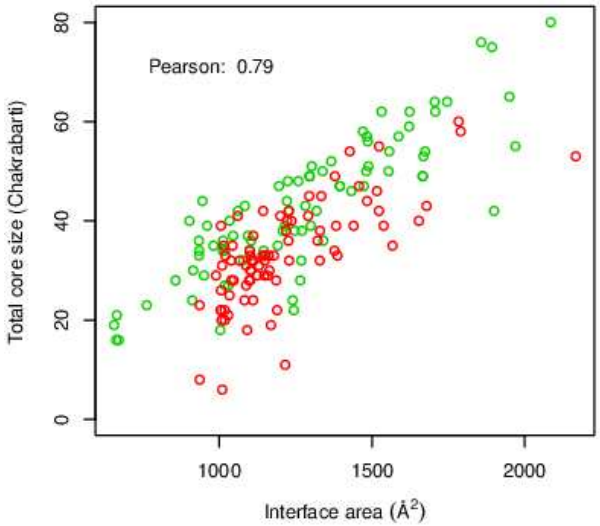
[1]Ponstingl et al., *J Appl Cryst,* 2003 [2]Bahadur et al., *J Mol Biol,* 2004

Core size: a geometric predictor

Core size: # of core residues

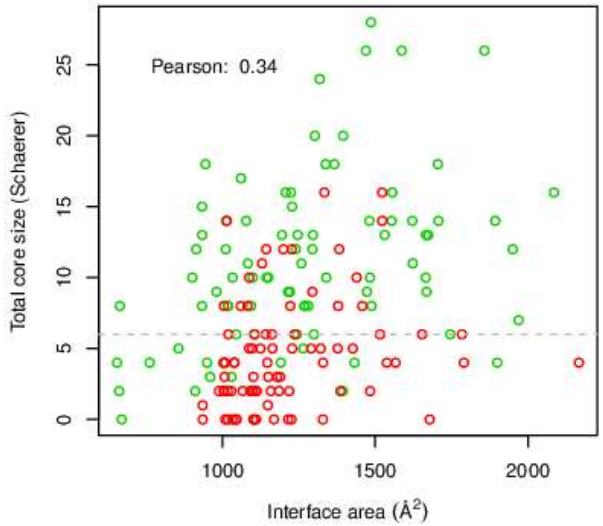
DC xtal set
DC bio set

Our definition



Chakrabarti & Janin, *Proteins*, 2002

Core residues: those with >1 fully buried atom



Schärer et al, *Proteins*, 2010

Core residues: **the fully buried ones**

Our entropy-based interface scoring indicators

Core vs rim:

Introduced by Guharoy and Chakrabarti (2005)

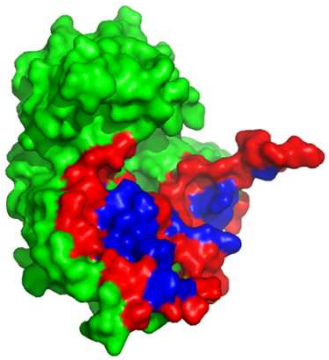
Our core definition (2010)

- Measure: ratio

$$\frac{\langle s_{core} \rangle}{\langle s_{rim} \rangle}$$

< threshold: bio

> threshold: xtal



Interface vs surface:

Introduced by Valdar/Thornton, Elcock/McCammon (2001)

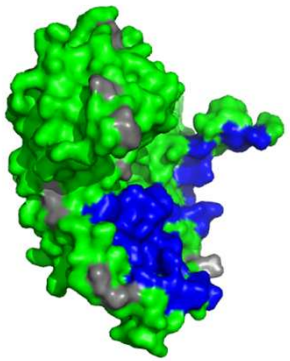
Our modifications:

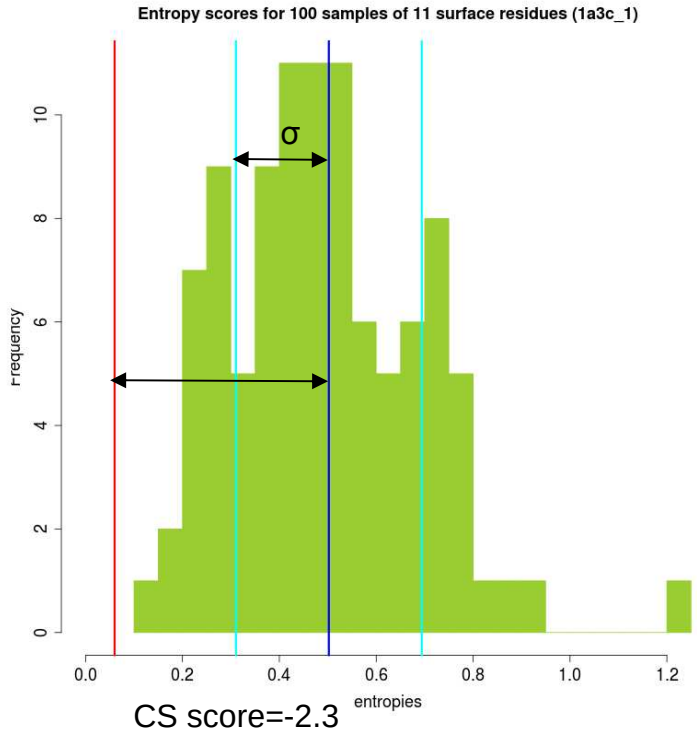
- **core** vs. surface (CS score)
- our core definition
- measure:

$$\frac{\langle s_{core} \rangle - \mu_{surface}}{\sigma_{surface}}$$

< threshold: bio

> threshold: xtal





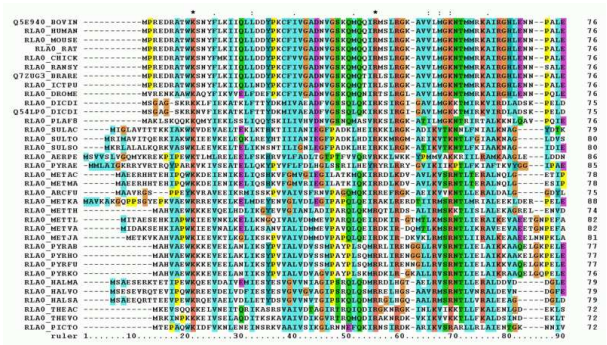
CS score=-2.3

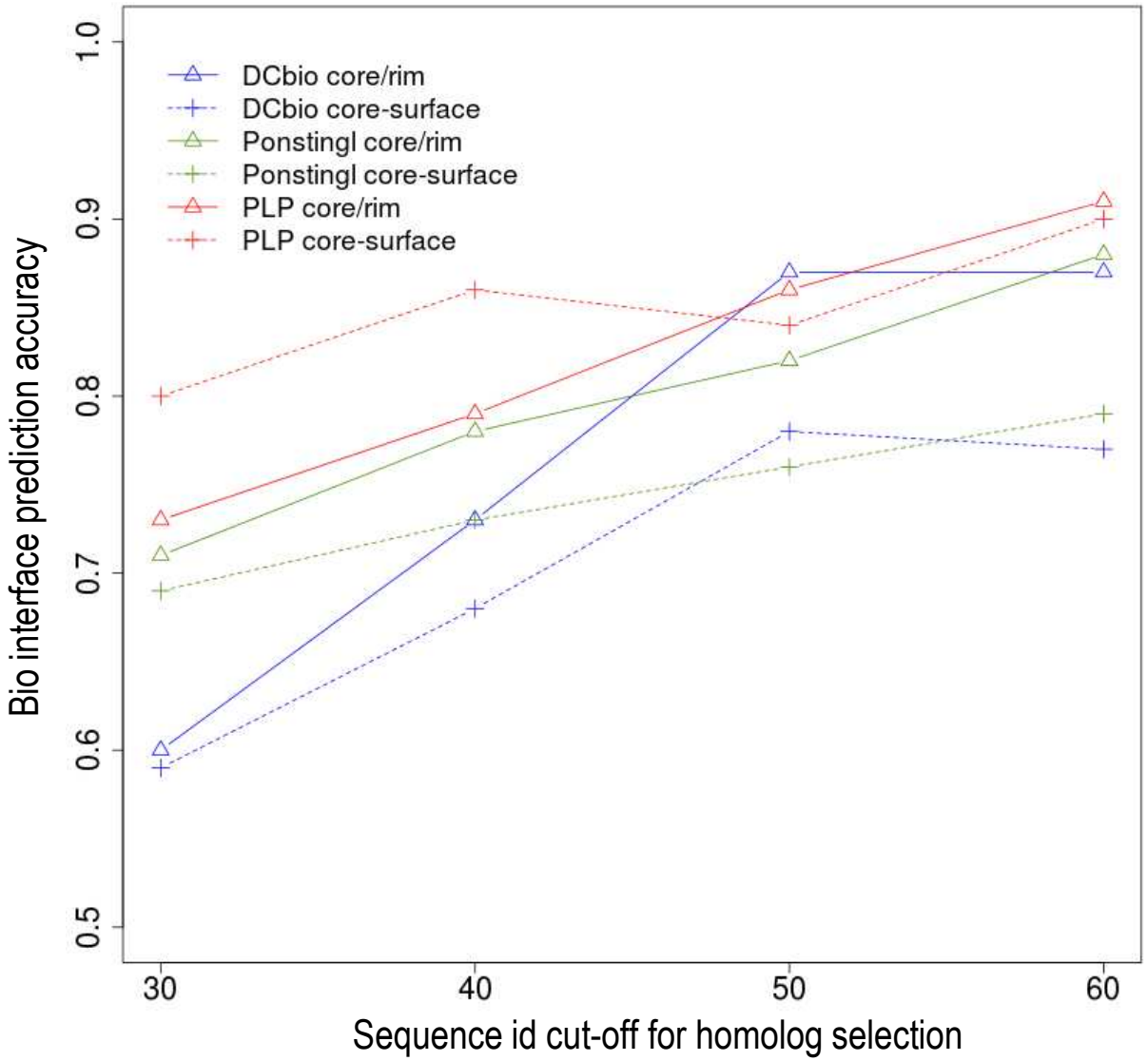
Homolog selection for MSA

Most approaches until now try to pool in as much data as possible: alignments include low sequence identity homologs

We take a conservative approach (now feasible): less data to get better accuracy

Redundancy reduction: UniRef100 and sequence clustering per alignment

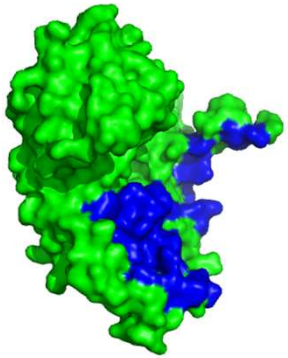




Combining geometry and evolutionary information

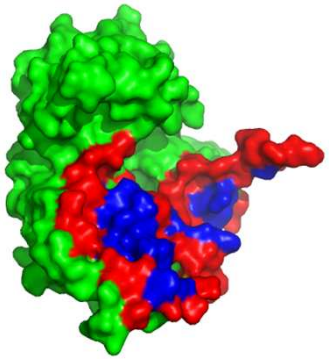
1) Core size:

# of core residues < threshold1: xtal
> threshold1: bio



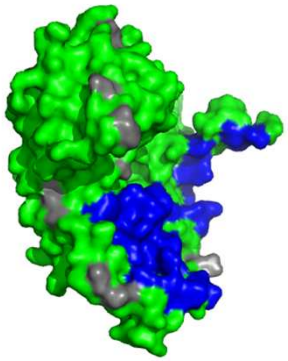
2) Core vs. rim:

$$\frac{\langle s_{core} \rangle}{\langle s_{rim} \rangle}$$ < threshold2: bio
> threshold2: xtal



3) Core vs. surface:

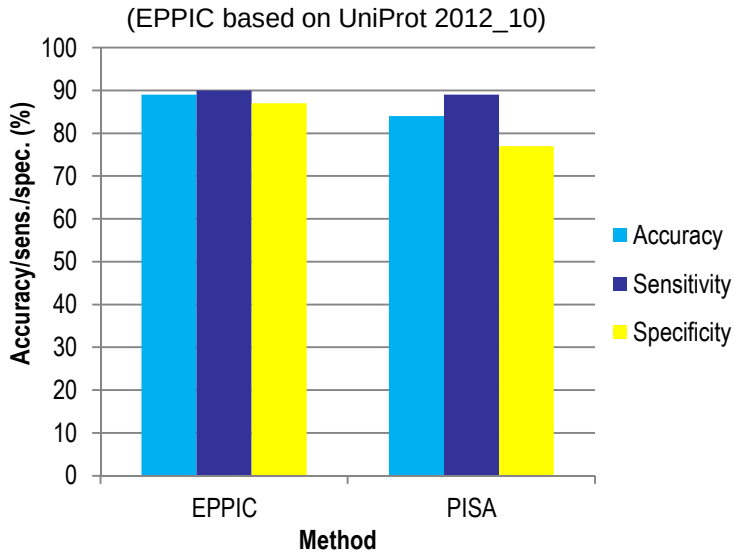
$$\frac{\langle s_{core} \rangle - \mu_{surface}}{\sigma_{surface}}$$ < threshold3: bio
> threshold3: xtal



Final call:
by majority of the three criteria

Thresholds chosen by systematic runs vs DCbio and DCxtal

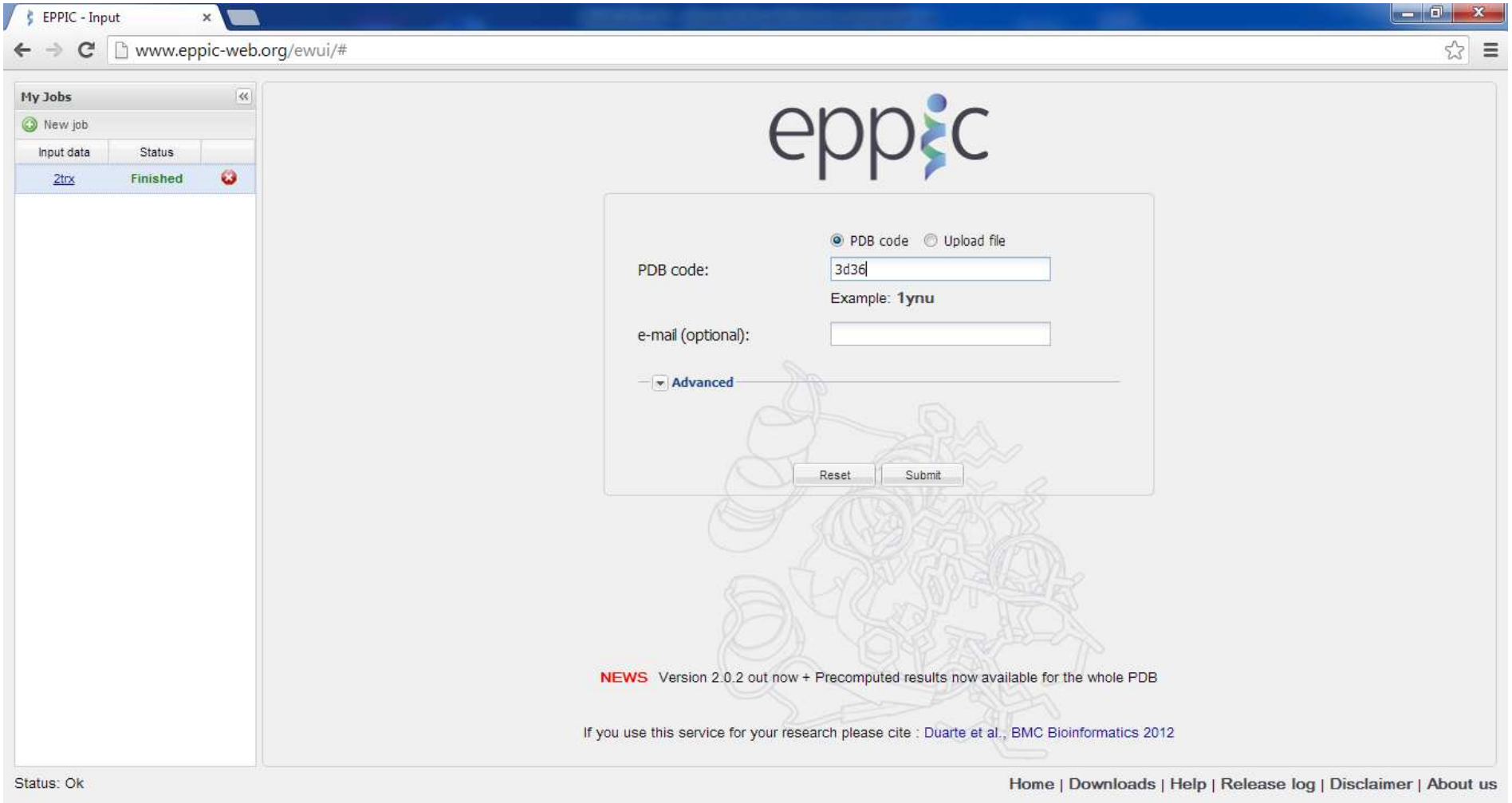
Benchmarking:



Dataset: Ponstingl

Web server: www.eppic-web.org

- Input: PDB entry code or PDB file uploaded by the user
- Rich web application (GWT/ext-js)



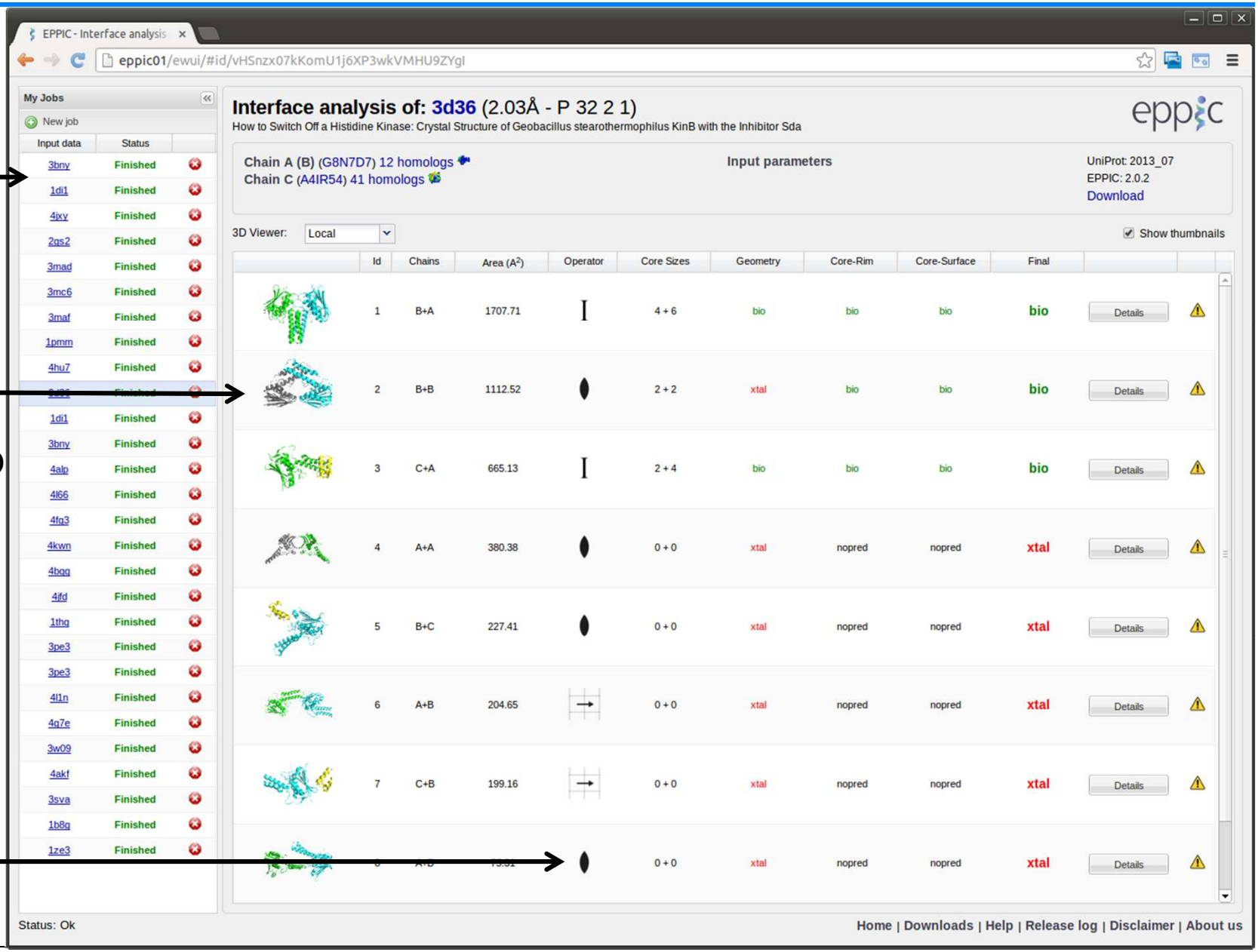
- Output: lists all interfaces with main features and predictions (bio or xtal)

Web server: output page for PDB entry 3d36

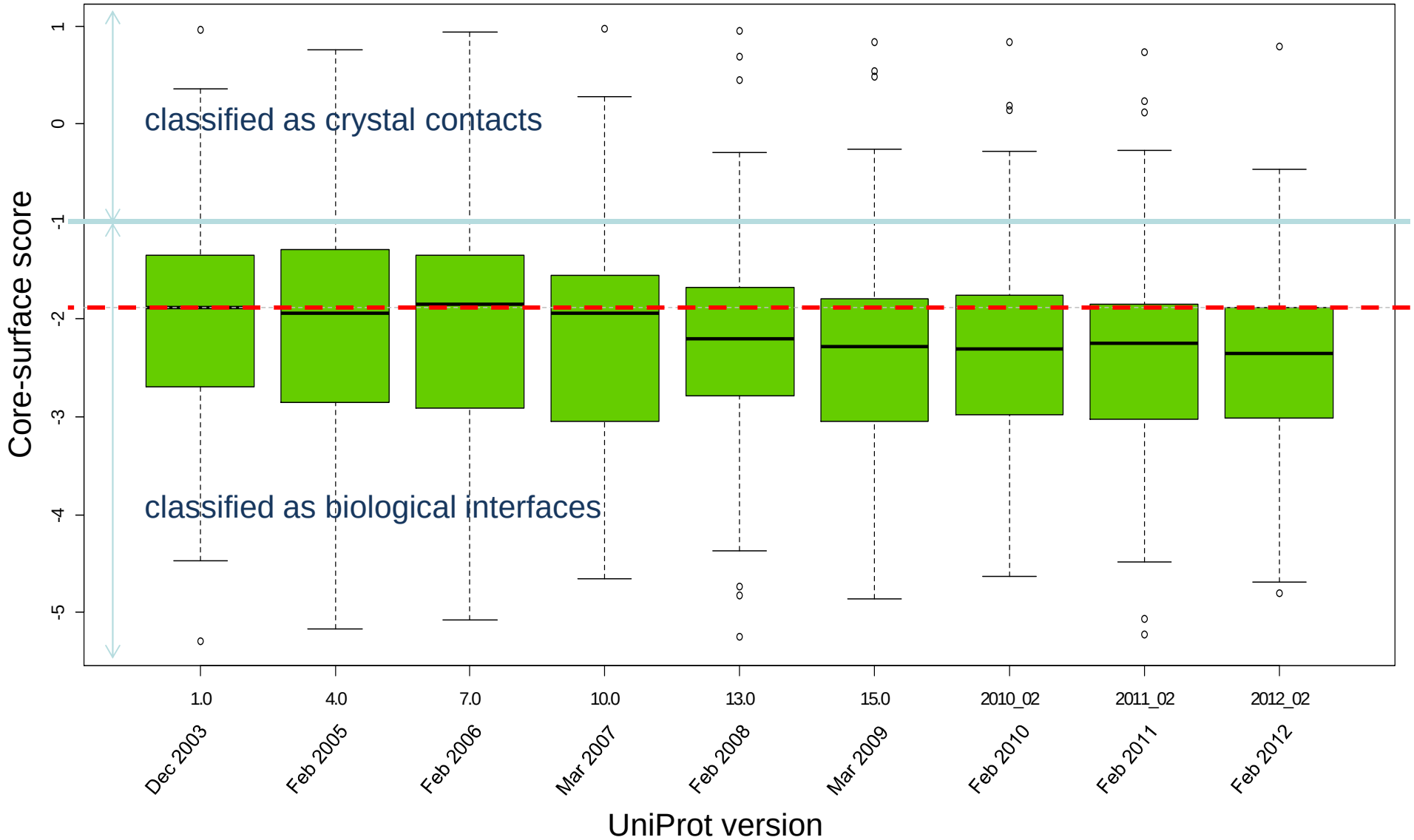
Jobs panel

Interface thumbnail: click to show in 3D (Jmol or PyMOL)

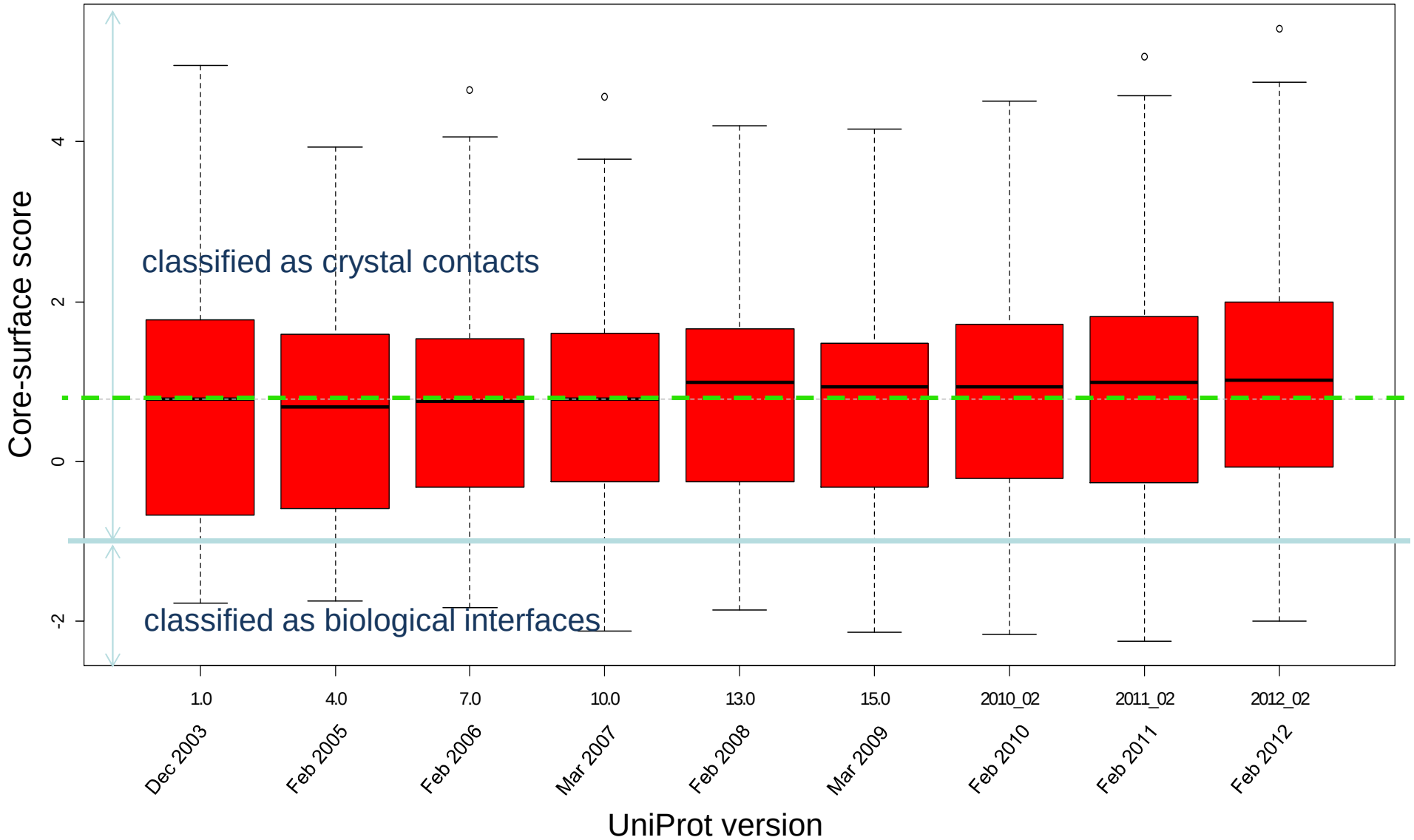
Interface symmetry operator



Bio interfaces: core-surface scores over the years



Xtal interfaces: core-surface scores over the years



Conclusions and outlook

- **Core size** (# of core residues by our definition) is an important geometric determinant of bio interfaces: good interface packing is essential for a bio interface
- **Sequence entropy** can be used with satisfactory performance by combining **core/rim** ratio and **core/surface** score and using close homologs only
- **Evolution-based method**: results can only improve with sequence database growth (numerically shown for 2002-2012)
- Implemented in a robust, open-source **software** package and web server: www.eppic-web.org
- The method works satisfactorily on **membrane proteins** as well (Duarte *et al.*, 2013, under review)
- In the works/outlook:
  - Prediction of **biological assemblies** based on interface calls and symmetry
  - PDB-wide statistical analysis of bio and xtal interfaces

Acknowledgements

Paul Scherrer Institut
(Biology and Chemistry, LBR)
Jose M. Duarte
Kumaran Baskaran
Nikhil Biyani
Martin A. Schärer

Bela Hullar (SyBIT/ETH Zurich)
Adam Srebniak (SyBIT/ETH Zurich)

Derek Feichtinger & Valeri Markushin
(SciComp group, PSI)

University of Zurich

Prof. Amedeo Caflisch
Prof. Andreas Wagner

Funding and support:

Forschungskommission PSI

SyBIT
SystemsX.ch
Biology IT

FNSNF
Swiss National Science Foundation