V9 – Protein structures

Program for today:

- Structures from protein X-ray crystallography
- Statistics of protein structures
- Statistical potentials

PDB files

X-ray structure 1atp of the cAMP-dependent protein kinase

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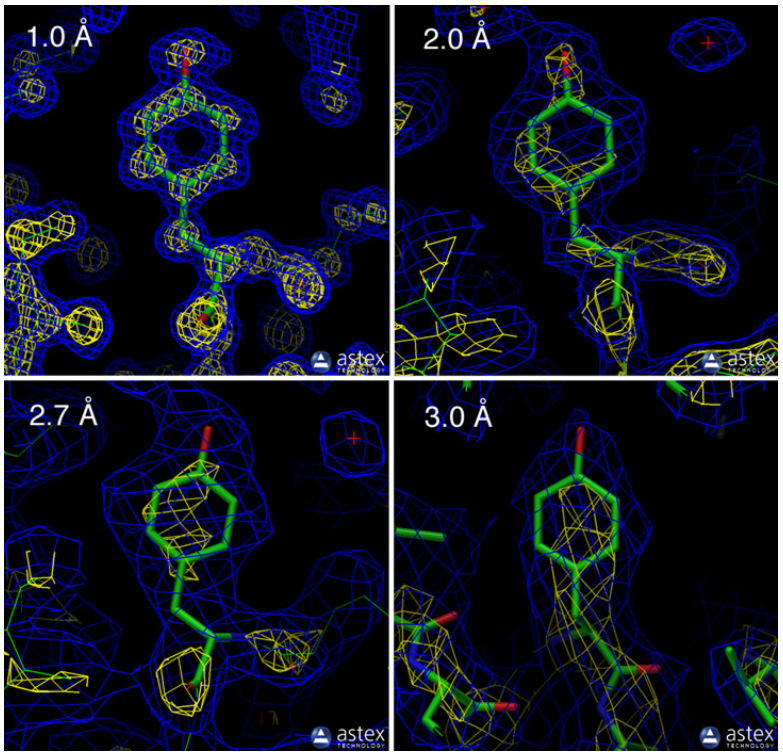
	Atom-number	Atom type	Residue type	Chain ID	Residue number	X-coordinae	Y-coordinate	Z-coordinate	Occupancy	B-factor
ATOM	1	N	VAL	E	15	-6.512	-12.177	-13.595	1.00	64.39
ATOM	2	CA	VAL	E	15	-5.276	-11.431	-13.476	1.00	47.83
ATOM	3	C	VAL	E	15	-4.815	-10.815	-14.785	1.00	35.56
ATOM	4	O	VAL	E	15	-4.806	-9.592	-14.904	1.00	99.02
ATOM	5	CB	VAL	E	15	-4.193	-12.092	-12.629	1.00	100.00
ATOM	6	CG1	VAL	E	15	-2.823	-11.529	-12.987	1.00	50.97
ATOM	7	CG2	VAL	E	15	-4.494	-11.830	-11.149	1.00	35.72
ATOM	8	N	LYS	E	16	-4.475	-11.641	-15.778	1.00	35.94
ATOM	9	CA	LYS	E	16	-4.060	-11.108	-17.074	1.00	55.13
ATOM	10	C	LYS	E	16	-5.100	-10.105	-17.531	1.00	59.23
ATOM	11	O	LYS	E	16	-4.877	-9.036	-18.103	1.00	35.80
ATOM	12	CB	LYS	E	16	-3.916	-12.209	-18.110	1.00	47.57
ATOM	13	CG	LYS	E	16	-2.850	-11.886	-19.158	1.00	100.00
ATOM	14	CD	LYS	E	16	-1.491	-12.525	-18.888	1.00	94.01
ATOM	15	CE	LYS	E	16	-0.665	-11.794	-17.836	1.00	100.00
ATOM	16	NZ	LYS	E	16	-0.505	-12.557	-16.586	1.00	89.11

In high-resolution X-ray structures, one can sometimes resolve different side chain orientations („occupancies“)

Resolution

Resolution : measure of the quality of the data that has been collected on the crystal containing the protein or nucleic acid. If all of the proteins in the crystal are aligned in an identical way, forming a very perfect crystal, then all of the proteins will scatter X-rays the same way, and the diffraction pattern will show the fine details of crystal. On the other hand, if the proteins in the crystal are all slightly different, due to local flexibility or motion, the diffraction pattern will not contain as much fine information.



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Electron density maps for structures with different resolutions. The first three show tyrosine 103 from myoglobin, from entries
1a6m (1.0 Å resolution),
106m (2.0 Å resolution), and
108m (2.7 Å resolution).
The final example shows tyrosine 130 from hemoglobin, from entry 1s0h (3.0 Å resolution).
Blue and yellow contours surround regions of high electron density.
The atomic model is shown with sticks.

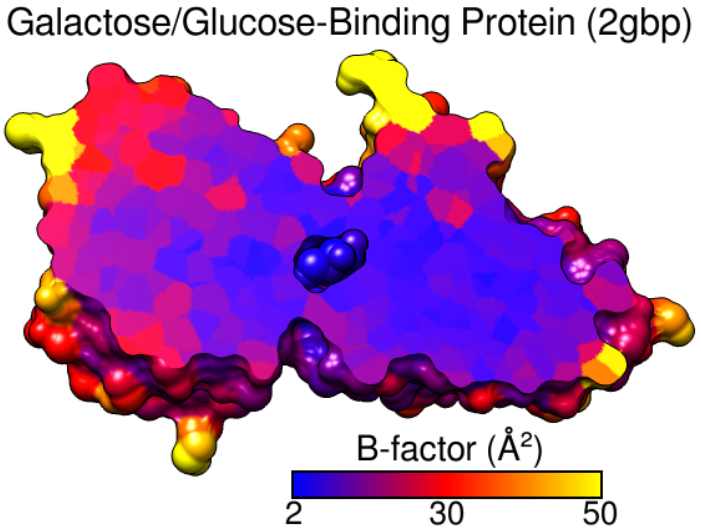
B-factor

The "temperature-factor" or "Debye-Waller factor“ describes the degree to which the electron density of an atom is spread out.
In theory, the B-factor indicates the true static or dynamic mobility of an atom.
However, it can also indicate where there are errors in model building.

The B-factor of atom *i* is related to its mean square displacement U_i:

$$B = 8 \pi^2 \ U_i^2$$

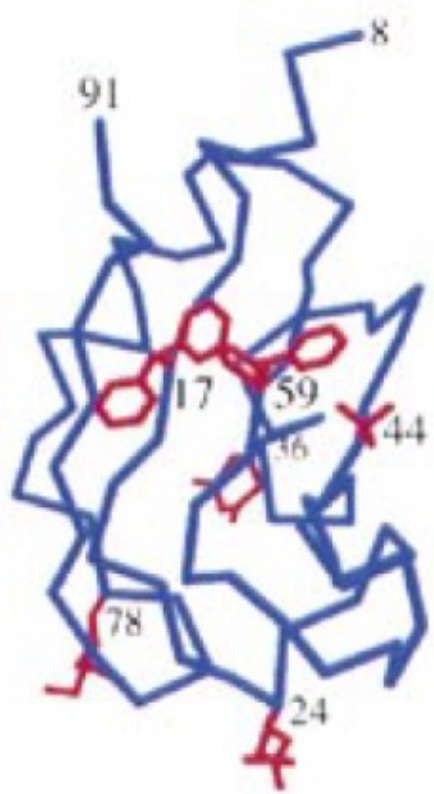
In general, protein structures (should) have larger B-factors in loop regions and on the protein surface and low B-factors in the protein core.



http://pldserver1.biochem.queensu.ca/~rlc/work/teaching/definitions.shtml
https://www.cgl.ucsf.edu/chimera/docs/UsersGuide/tutorials/bfactor.html

Occupancy

1.1 Å structure of heterogeneous nuclear ribonucleoprotein A1:
6 amino acids have alternative side chain conformations
- 3 residues are located in loop regions and are exposed to the solvent: Glu24, Gln36, and Lys78.
- 3 residues are located on the RNA-binding surface: Phe17 on β1, Val44 on β2 and Phe59 on β3.
Phe17 side chain occupancies: 0.65 and 0.35.
Phe59 side chain occupancies: 0.57 and 0.43



Not all of these conformations can be independently adopted by these residues because of potential steric clashes.
Permissible combinations are:
(i) Phe17A/Phe59A/Val44A,B,C;
(ii) Phe17A/Phe59B/Val44A;
(iii) Phe17B/Phe59B/Val44A. These 3 combinations have occupancies of 0.57, 0.08 and 0.35.

Vitali et al. *Nucl Ac Res (2002) 30, 1531–1538*

Head of PDB file 1L3K

REMARK 3 OTHER REFINEMENT REMARKS: RESIDUES PHE 17, VAL 44 AND PHE 59

REMARK 3 SHOW CORRELATED DISORDER IN THE SIDE CHAIN CONFORMATIONS AND

REMARK 3 THIS BEHAVIOR WAS TAKEN INTO CONSIDERATION IN REFINEMENT. THE

REMARK 3 RESIDUES WERE SPLIT IN FIVE PARTS -- B, C, D, K, L,

REMARK 3 CORRESPONDING TO THE FIVE PERMISSIBLE COMBINATIONS OF

REMARK 3 CONFORMATIONS OF PHE 17, PHE 59, AND VAL 44 …

Alternative conformations are only detected in high-resolution data.

Vitali et al. *Nucl Ac Res (2002) 30, 1531–1538*

PDB file 1L3K

ATOM 338 CB	BVAL A 44	-23.016 -1.594 -1.744	0.19	17.60 C
ATOM 339 CB	CVAL A 44	-23.016 -1.594 -1.744	0.20	17.60 C
ATOM 340 CB	DVAL A 44	-23.016 -1.594 -1.744	0.18	17.60 C
ATOM 341 CB	KVAL A 44	-23.016 -1.594 -1.744	0.35	17.60 C
ATOM 342 CB	LVAL A 44	-23.016 -1.594 -1.744	0.08	17.60 C

CB has the same position in the 5 conformers

ATOM 343 CG1	BVAL A 44	-22.101 -2.293 -0.750	0.19	21.01 C
ATOM 344 CG1	CVAL A 44	-22.465 -1.845 -3.138	0.20	21.66 C
ATOM 345 CG1	DVAL A 44	-24.405 -2.206 -1.621	0.18	25.18 C
ATOM 346 CG1	KVAL A 44	-24.405 -2.206 -1.621	0.35	25.18 C
ATOM 347 CG1	LVAL A 44	-24.405 -2.206 -1.621	0.08	25.18 C

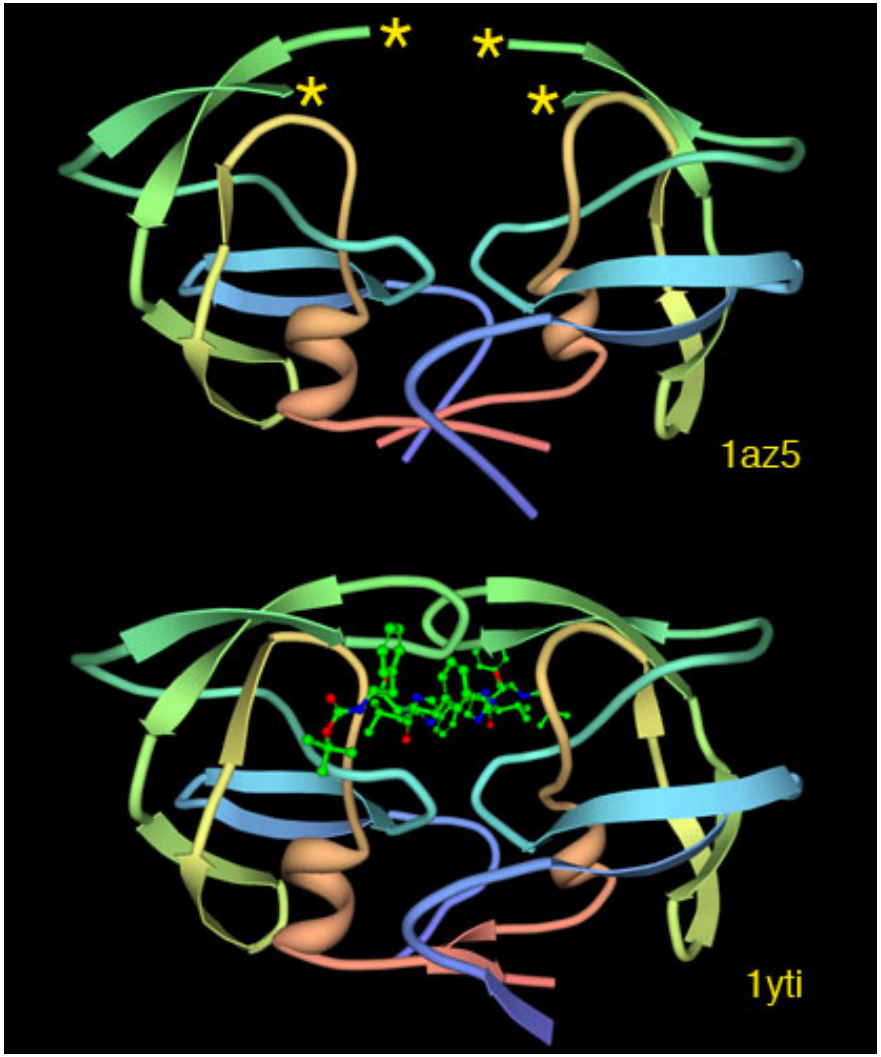
3 alternative conformations: B, C, D/K/L
D, K, L conformers have the same position, but different occupancies

ATOM 348 CG2	BVAL A 44	-24.405 -2.206 -1.621	0.19	25.18 C
ATOM 349 CG2	CVAL A 44	-22.101 -2.293 -0.750	0.20	21.01 C
ATOM 350 CG2	DVAL A 44	-22.465 -1.845 -3.138	0.18	21.66 C
ATOM 351 CG2	KVAL A 44	-22.465 -1.845 -3.138	0.35	21.66 C
ATOM 352 CG2	LVAL A 44	-22.465 -1.845 -3.138	0.08	21.66 C

3 alternative conformations: B, C, D/K/L
D, K, L conformers have the same position

Vitali et al. *Nucl Ac Res (2002) 30, 1531–1538*

Missing loops and tails



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X-ray structure of SIV protease solved without its active site (PDB entry 1az5).
The protein contains 2 loops ("flaps") that were too flexible to be detected in the experiment (shown with stars in the upper picture).

When the protein was crystallized with inhibitors, however, the loops adopted a stable structure that may be detected (PDB entry 1yti).

R-value and R-free

R-value is the measure of the quality of the atomic model obtained from the crystallographic data.

When solving the structure of a protein, the researcher first builds an atomic model and then back-calculates a simulated diffraction pattern based on that model.

The R-value measures how well the simulated diffraction pattern matches the experimentally-observed diffraction pattern.

A totally random set of atoms will give an R-value of about 0.63,
whereas a perfect fit would have a value of 0.
Typical values of “well refined” protein structures are about 0.20.

Alternative conformations compatible with data

Are X-ray structures of proteins uniquely defined by the data?

Answer: only in the case of ultra-high-resolution data.

As a test, 10 and 20 independent conformers of 3 proteins were generated with a discrete restraint-based modeling algorithm, called RAPPER, based on propensity-weighted φ/ψ and χ angle sampling of the protein backbone.

The PDB structure was used to restrain conformational sampling to only conformations whose C coordinates were within 2 Å of the Cα atoms of the original PDB structures.

Further, all atoms were restrained to lie in regions of positive electron density in a $2F_{obs}$- F_{calc} map phased with the PDB structure.

De Pristo, de Bakker, Blundell, Structure 12 (2004) 831–838

Quality of alternative conformations

Table 1. Crystallographic Quality Metrics

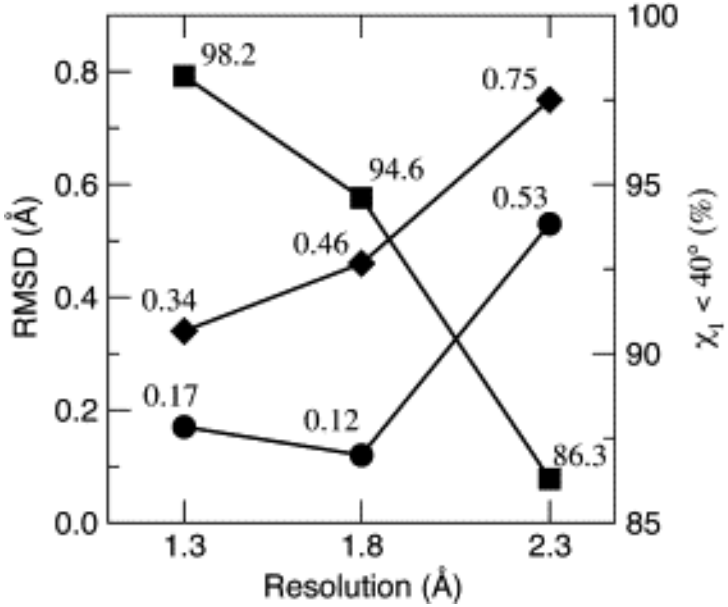
	Amicyanin		HIV Protease		Interleukin-1β	
	PDB	Models[a]	PDB	Models[a]	PDB	Models[a]
Resolution (Å)	8–1.3	20–1.3	50–1.8		15–2.3	55–2.3
R_{work} (%)						
Published[b]	15.5		19.5		15.7	
Refined[c,d]	15.0	14.3–14.8	19.4	17.5–18.3	16.0	15.7–16.2
R_{free} (%)						
Published[b]			23.0		21.0	
Refined[c,d]		16.8–17.1	22.6	20.9–21.9		20.3–21.7
Real-space R	0.981	0.980	0.964	0.967	0.850	0.846
Rms bond[e] (Å)	0.014	0.012	0.014	0.011	0.018	0.017
Rms angles[e] (°)	2.36	1.48	1.84	1.51	2.36	1.70
Allowed ϕ/ψ (%)	100.0	99.6	100.0	99.8	98.0	98.6
Bad rotamers[f]	0/85	0.2/85	2/166	3.3/166	12/129	12.8/140
Esu from R factor[g] (Å)	0.05		0.15		0.23	

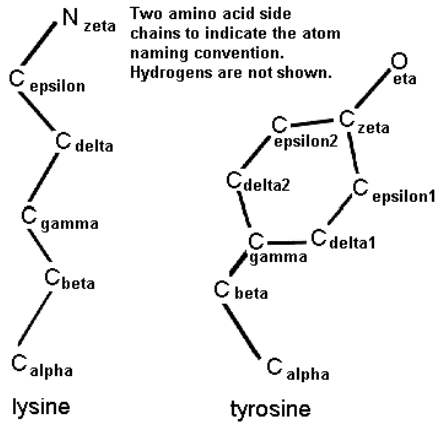
Alternative conformations have equal or better R_{free} values than PDB structure and lower RMS deviations of bond lengths and bond angles from the ideal values.

→ they look like “better” structures

De Pristo, de Bakker, Blundell, Structure 12 (2004) 831–838

Difference between models and PDB structure





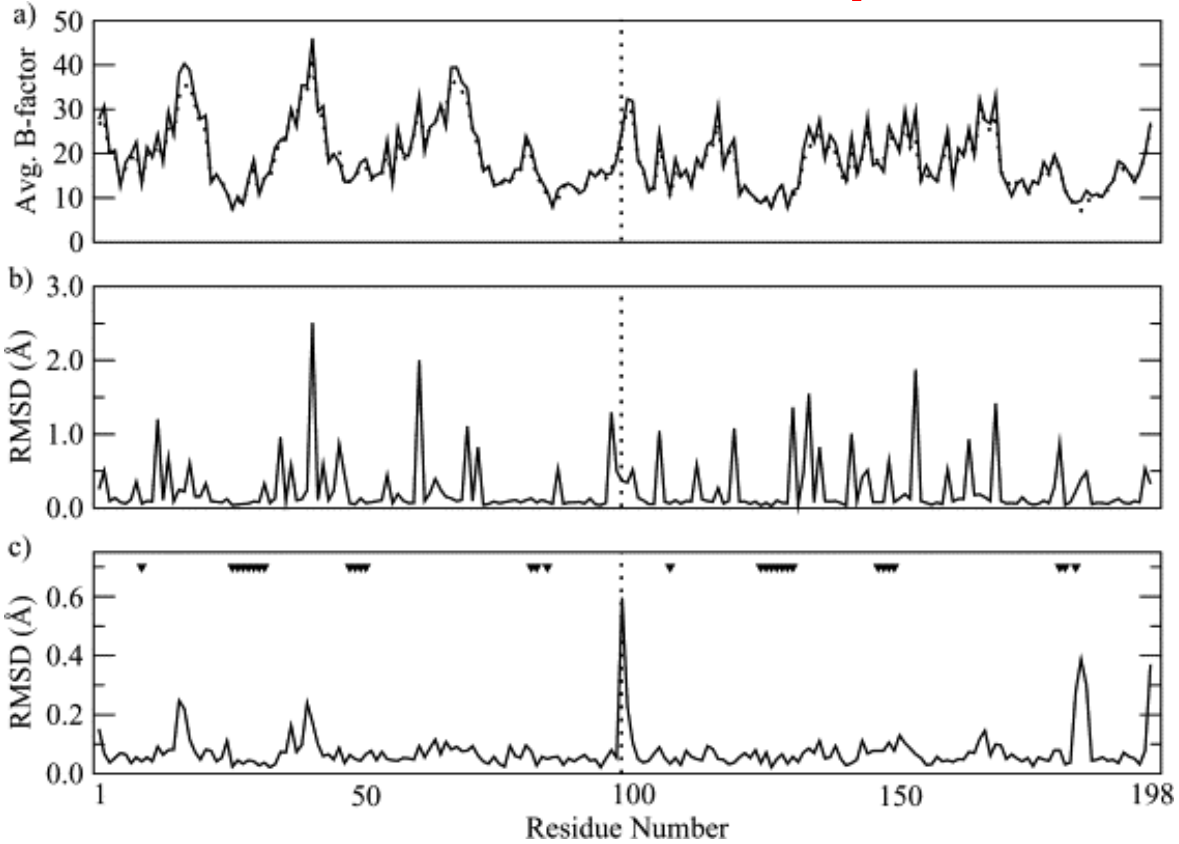
De Pristo, de Bakker, Blundell, Structure 12 (2004) 831–838

Amicyanin (1.3 Å resolution),
HIV protease (1.8 Å)
h-IL1β (2.3 Å).

Pairwise differences among the PDB and alternate models increase with lowered resolution

Circles: main chain RMSD
Diamonds: all-atom RMSD
Squares: rotamer state conservation : fraction of residues with side chain χ_1 angle within 40° of the PDB structure.

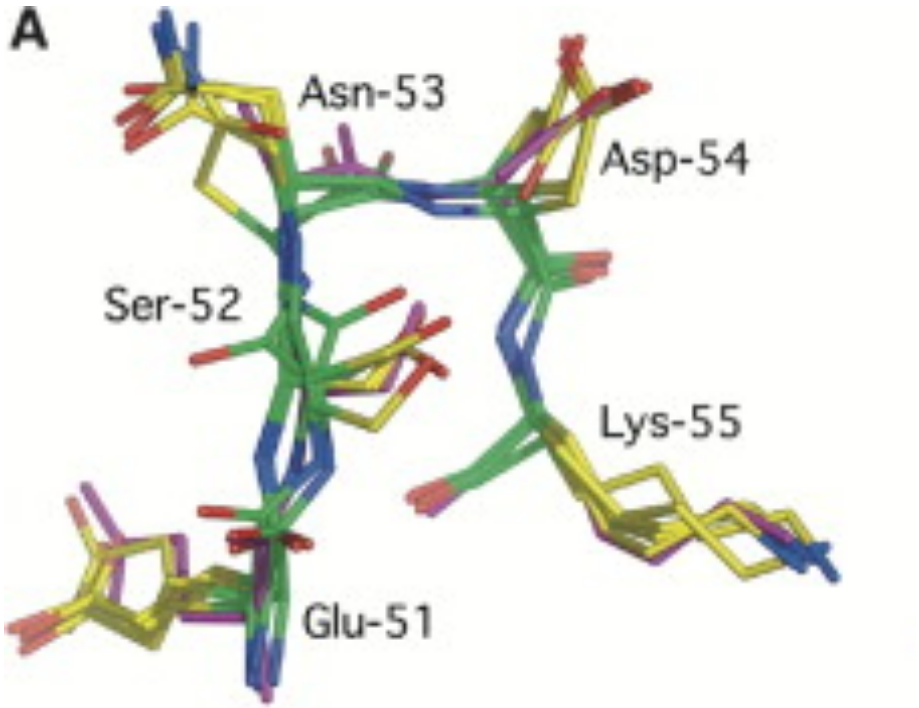
B-factors and RMSD per Residue for HIV Protease



De Pristo, de Bakker, Blundell, Structure 12 (2004) 831–838

Averaged B factor (A) of the PDB structure (dots) and the five alternate models (line). Note the similarity of the average B factors between the PDB and RAPPER models. All-atom (B) and main chain (C) rmsd for each residue of the alternate models compared to the PDB structure.
Triangles indicate residues in contact with the inhibitor molecule. The vertical dotted line denotes the break between the two chains of the protease dimer.

Main Chain and Side Chain Heterogeneity in Human Interleukin-1β (2.3 Å)

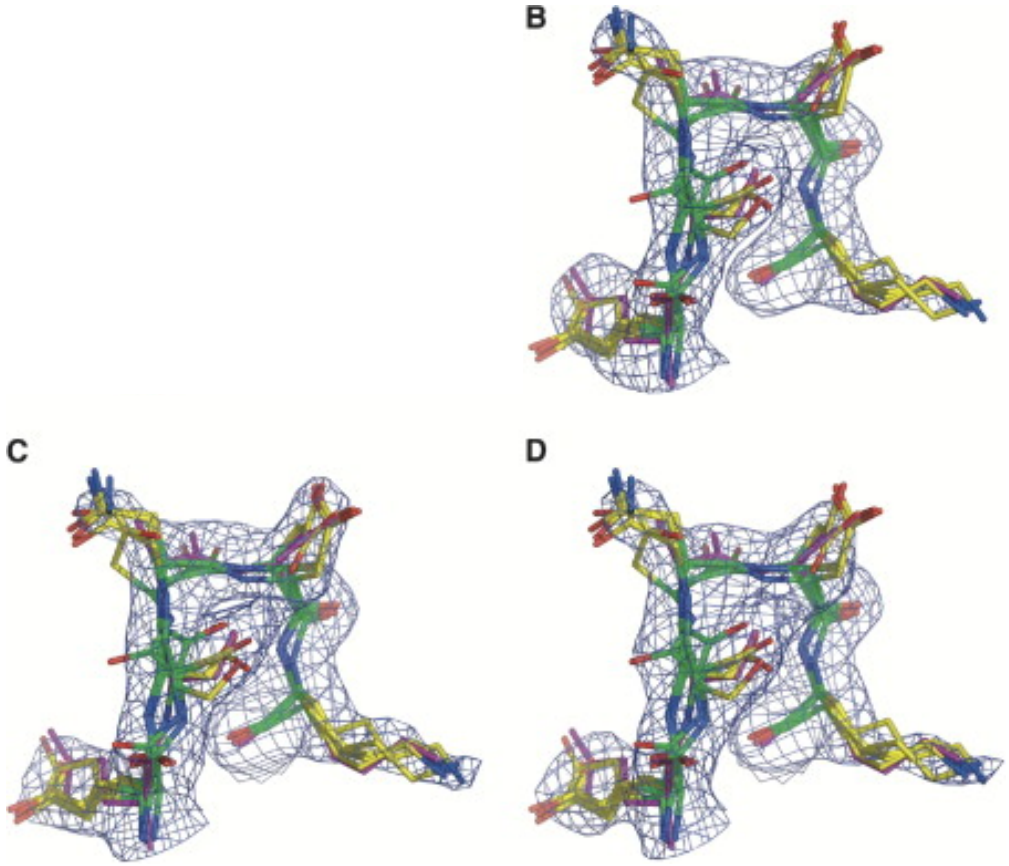


Shown are residues 51–55 from h-IL1β. The PDB structure is in magenta and the five alternate models are colored according to: nitrogen, blue; oxygen, red; main chain carbon, green; side chain carbon, yellow.

Note the pronounced backbone variability and side chains with anisotropic motion (Ser52, Asn53, Lys55) and multiple discrete conformations (Glu51, Asp54, Lys55).

De Pristo, de Bakker, Blundell, Structure 12 (2004) 831–838

Main Chain and Side Chain Heterogeneity in Human Interleukin-1β

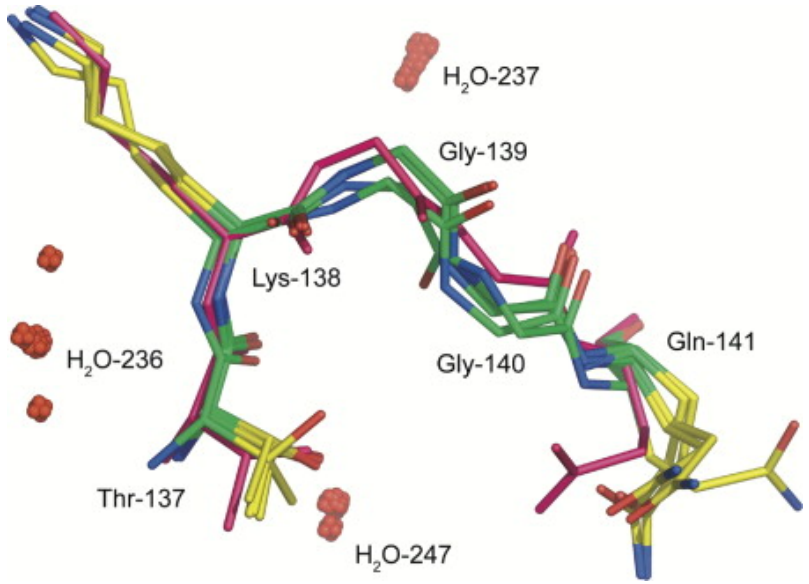


(B)–(D) show simulated-annealing omit maps contoured at 1 σ, for the original PDB structure (B) and alternate models 2 (C) and 3 (D).

→ Maps are practically indistinguishable.

De Pristo, de Bakker, Blundell, Structure 12 (2004) 831–838

Main Chain and Water Heterogeneity in Human IL-1β



Residues 137–141 from h-IL1β are shown, highlighting backbone variability and disordered side chains and waters.

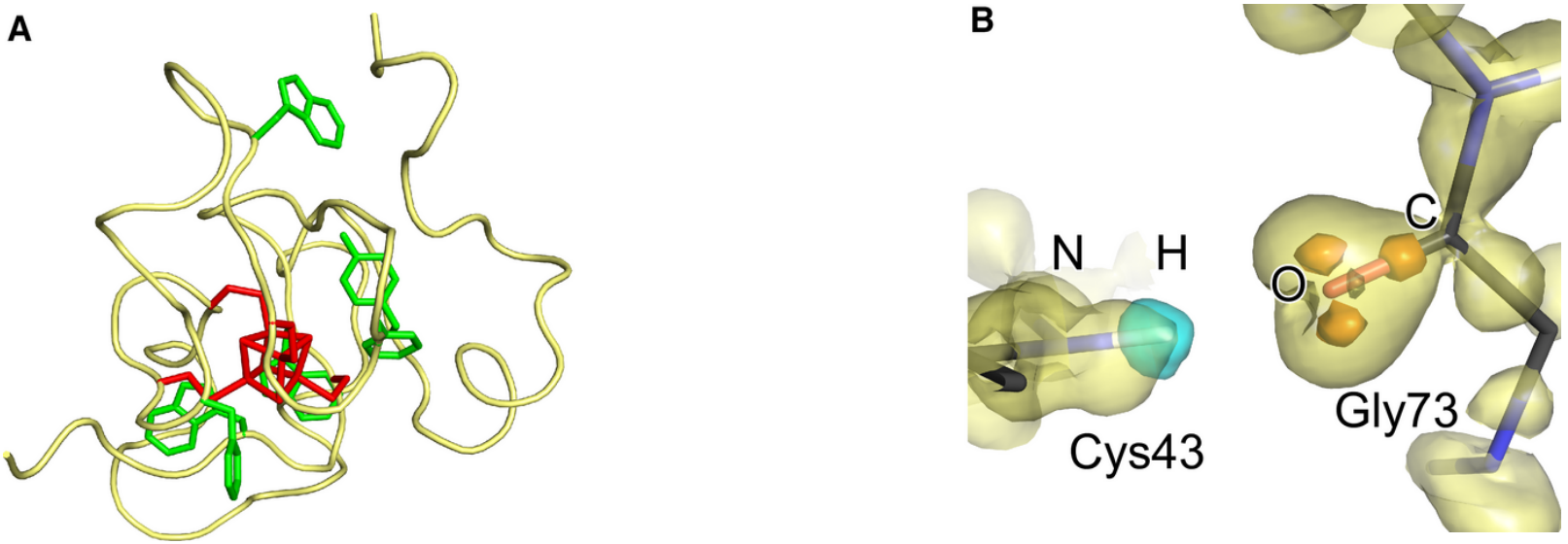
Note the significant variability in the main chain (Gly139 and Gly140) and side chain (Thr137 and Lys138) conformations, while Gln141 appears to be total disordered.

Waters H_2O-237 and H_2O-247 are well ordered, whereas H_2O-236 has a mean square displacement of 3.5 Å.

Mid-range resolution structures do **not** provide unique information about atomic positions and relative orientations.

De Pristo, de Bakker, Blundell, Structure 12 (2004) 831–838

Ultra high resolution structure (0.48 Å) of HiPIP



R_{free} = 0.078! At this resolution, enormous levels of detail can be detected.

(Left) The overall structure of HiPIP is shown as a tube model, where aromatic residues and the iron–sulfur cluster are represented as green and red sticks.

(Right) Hydrogen bonding formed between lone pair electrons of the carbonyl O of Gly73 and the amide H atoms of Cys43.

Takeda, Miki, FEBS J. (2017)

Statistics on protein structures: derive understanding from statistical enrichment

Idea: some positions in/on protein structures are energetically more favorable for certain amino acids → these amino acids should be enriched there

The energetics is difficult to estimate.

BUT the frequency of amino acids can be easily computed as a statistical average over all known protein structures.

Hayat et al. Comput Biol Chem (2011) 35, 96–107

Statistics on protein structures

Q: how does the amino acid composition of trans-membrane barrels (TMB) differ in the membrane from that in the cytosol?

Split the membrane into the non-polar membrane-core (aliphatic lipid tails) and the medium-polarity membrane interface region (phospholipid head-groups).

We compiled a non-redundant data set of known TMB structures by removing those protein sequences for which less than 20 homologous sequences were found or where the pair-wise sequence identity of the aligned retrieved sequences was greater than 80%.

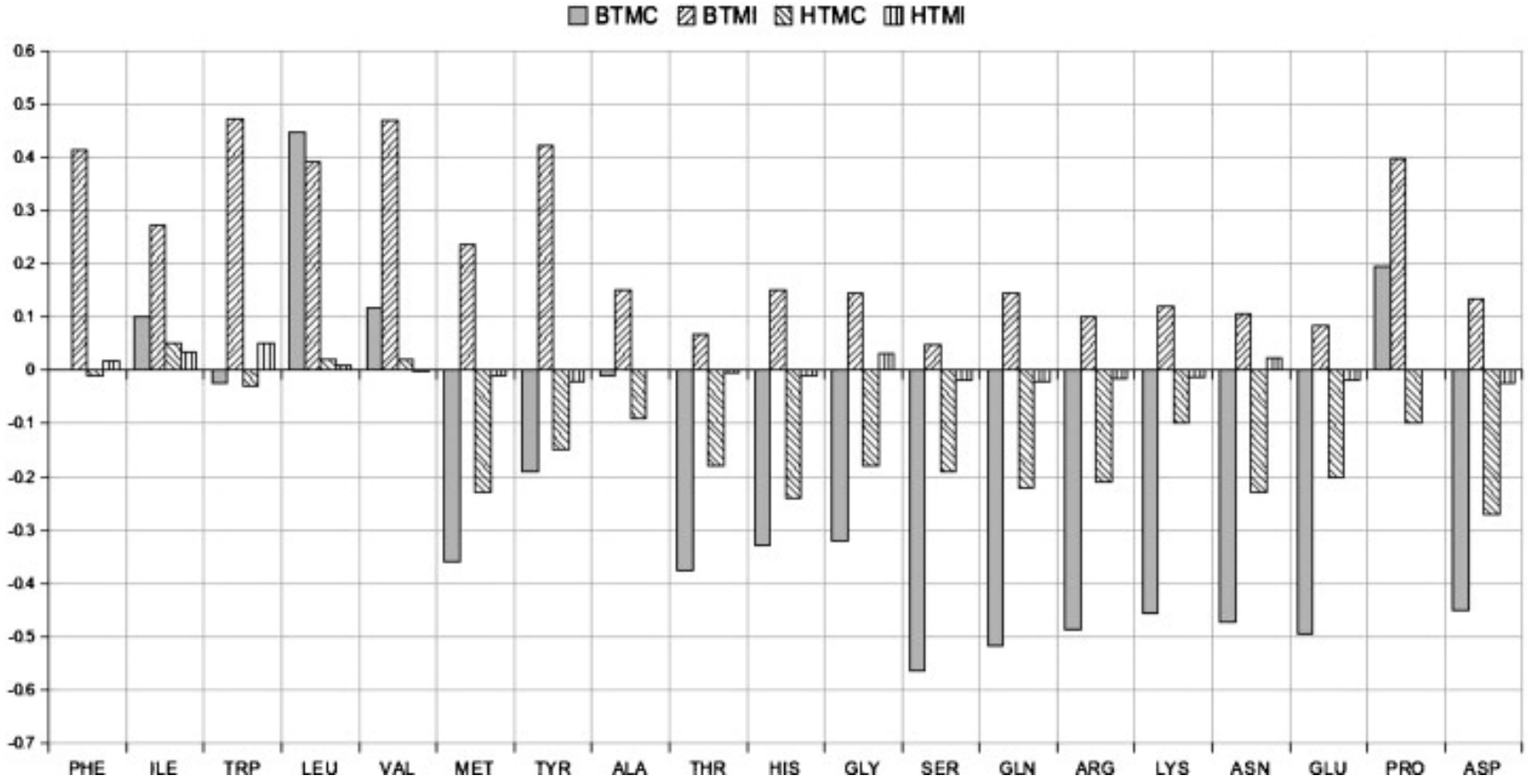
The final data set for TMBs comprises of 20 protein chains with 1725 and 572 TM residues in the hydrophobic core and interface regions, respectively

Hayat et al. Comput Biol Chem (2011) 35, 96–107

Propensity scale: over / under-representation

Logarithmic ratio of presence in membrane core for beta-barrels (BTMC) and helical membrane proteins (HTMC)

or in interface region of membrane (BTMI and HTMI) vs. full sequence.



Hayat et al. Comput Biol Chem (2011) 35, 96–107

Composition of protein interfaces

Q: Are protein-protein interfaces comparable to protein-ligand interfaces?

Dataset : 174 protein-protein complexes and 161 protein-ligand complexes.

These complementary PP and PL datasets fulfill the following criteria:
(i) PP: PL pairs represent pairs of complexes, where one protein may bind either a second protein or a small molecule ligand at the same interface,

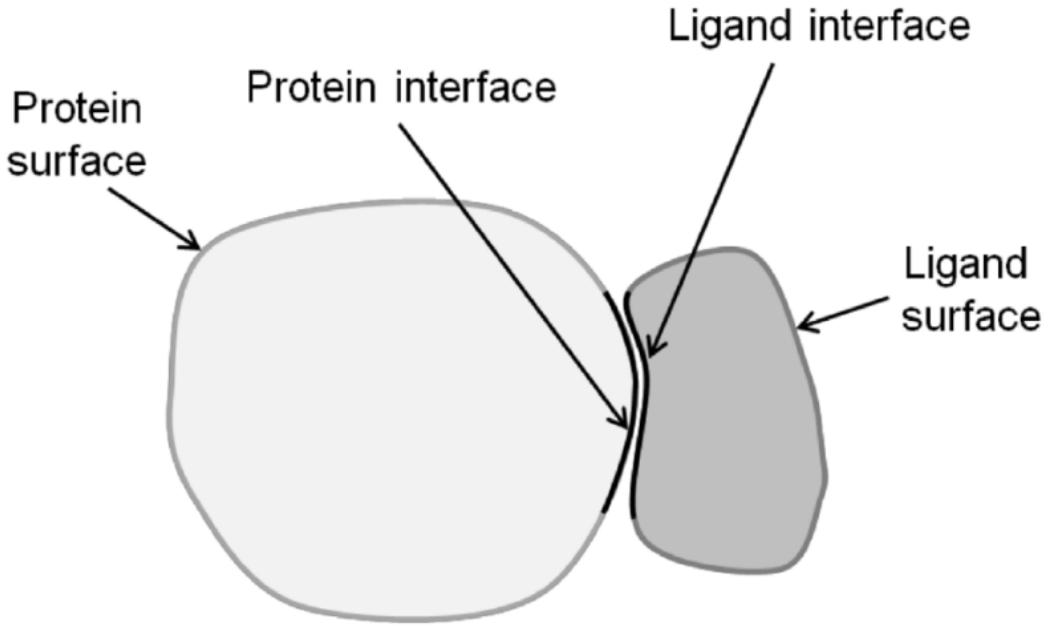
(ii) every pair of the dataset is represented as (P_{i1}, P_{i2}): (P_{i3}, L_j), where P_{i1}, P_{i2} and P_{i3} are three proteins and L_j is a small molecule ligand,

(iii) P_{i1} and P_{i3} share at least 40% sequence identity, and

(iv) the aligned positions in the binding interfaces of P_{i1}–P_{i2} and P_{i3} –L_j have at least 2 residues in common.

Mohamed et al.
PLoS ONE (2015)
10, e0140965

Protein:ligand interface

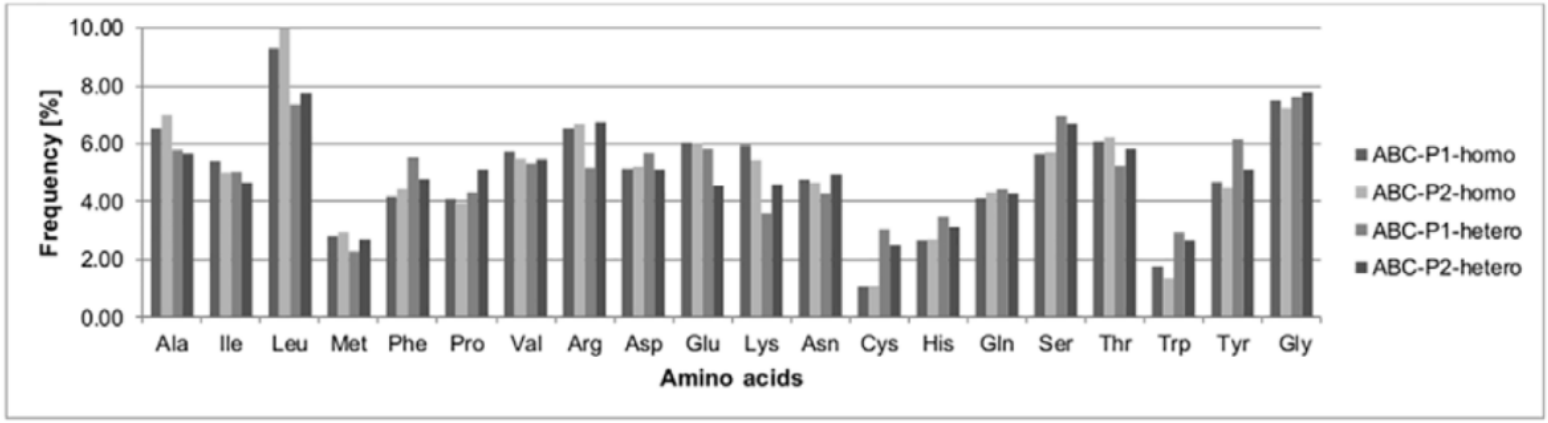


$$Interface\ residue\ propensity\ AAj = \left(\frac{\sum interface\ residues\ of\ type\ j}{\sum all\ interface\ residues}\right) / \left(\frac{\sum surface\ residues\ of\ type\ j}{\sum all\ surface\ residues}\right)$$

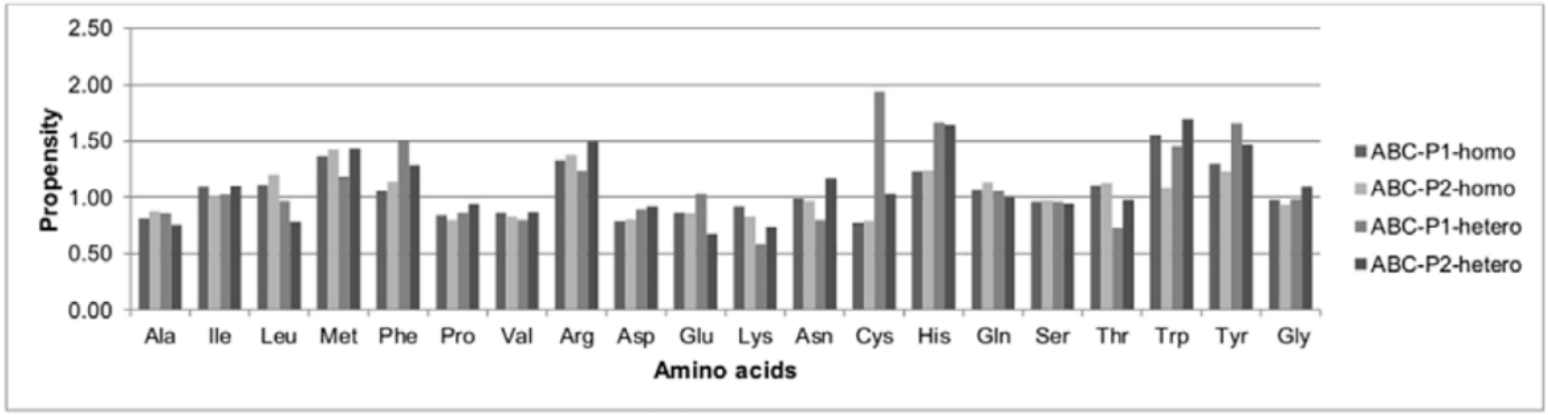
An interface residue propensity of > 1.0 indicates that a residue type occurs more frequently in interfaces than on the protein surface in general.

Mohamed et al.
PLoS ONE (2015)
10, e0140965

Frequencies vs. propensities



B



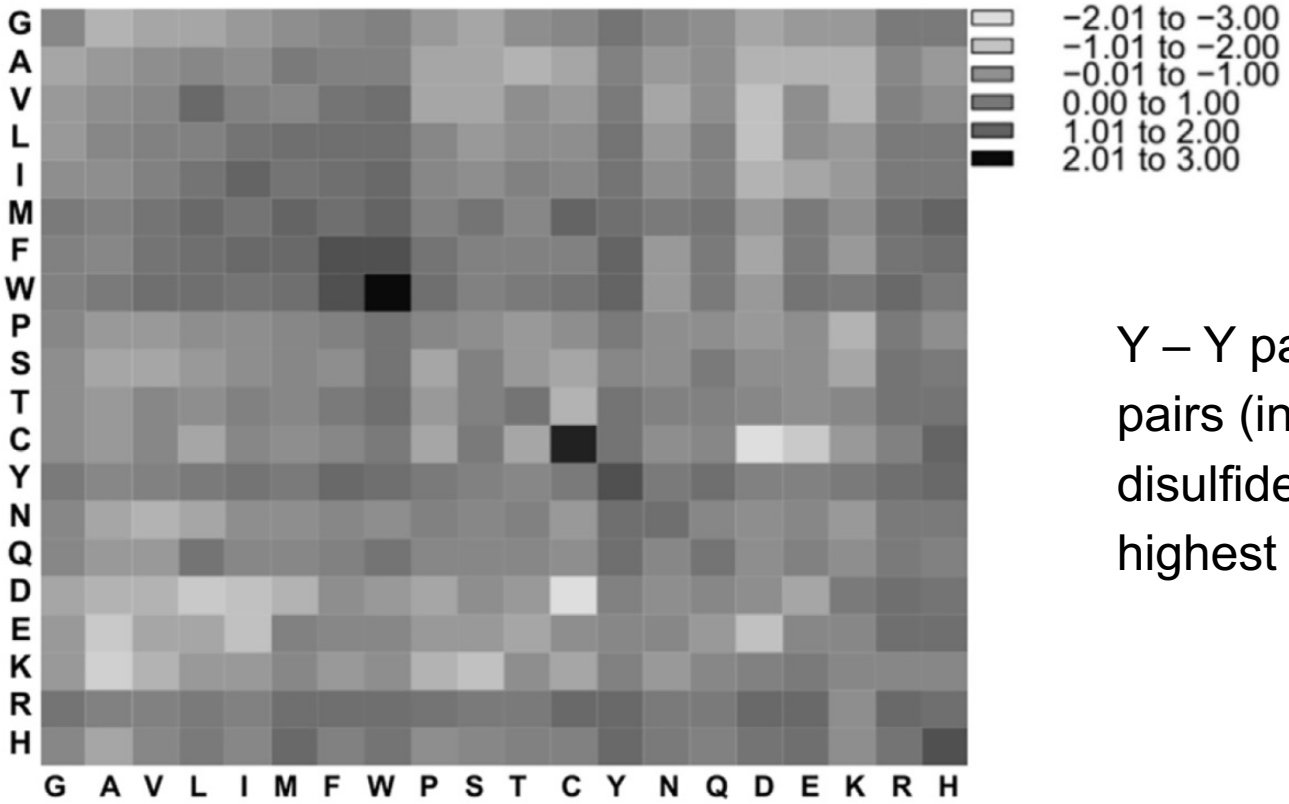
Frequencies are raw counts.

Propensities are normalized by the proportion of the amino acids.

Trp has overall a low frequency, but is frequently found at interfaces.

Mohamed et al.
PLoS ONE (2015)
10, e0140965

Amino acid pairing propensity at interface



Y – Y pairs and C – C pairs (inter-protein disulfide bridges) have highest propensities.

$$Normalization = \frac{\left(\frac{\sum contacts\ of\ residue\ pair\ XY}{\sum all\ residue\ contacts}\right)}{\left(\frac{\sum observed\ X\ on\ surface}{\sum all\ surface\ residues\ of\ first\ protein}\right)\left(\frac{\sum observed\ Y\ on\ surface}{\sum all\ surface\ residues\ of\ second\ protein}\right)}$$

Mohamed et al.
PLoS ONE (2015)
10, e0140965

Statistical potential: Boltzmann inversion

$$P(r) = \frac{1}{Z} e^{-\frac{F(r)}{kT}}$$

Probability *P(r)* at position *r* according to Boltzmann distribution as a function of the free energy *F(r)* at this position.
k is the Boltzmann constant, *T* is the temperature.

This can be re-arranged into

$$F(r) = -kT \ln P(r) - kT \ln Z$$

and taken with respect to a reference state with distribution $Q_R(r)$.

$$\Delta F(r) = -kT \ln \frac{P(r)}{Q_R(r)}$$

This is called a statistical potential,
e.g. from the probability to find two amino acids at a certain distance *r* from eachother one can derive their effective interaction free energy.

Sippl MJ (1990). J Mol Biol. **213**: 859–883.
www.wikipedia.org

Rosetta energy function

David Baker and co-workers justified PMFs from a Bayesian point of view and used these in the construction of the coarse grained ROSETTA energy function.

According to Bayesian probability calculus, the conditional probability $P(X \mid A)$ of a structure X , given the amino acid sequence A , can be written as:

$$P(X \mid A) = \frac{P(A \mid X)\, P(X)}{P(A)} \propto P(A \mid X)\, P(X)$$

$P(X \mid A)$ is proportional to the product of the likelihood $P(A \mid X)$ times the prior $P(X)$

Sippl MJ (1990). J Mol Biol. **213**: 859–883.
www.wikipedia.org

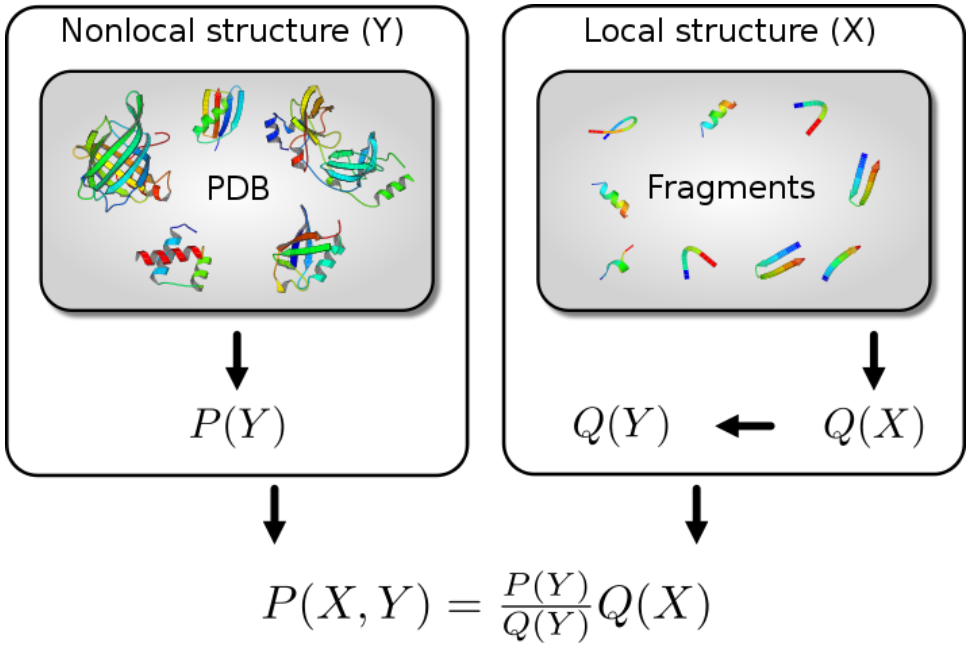
Rosetta energy function

By assuming that the likelihood can be approximated as a product of pairwise probabilities, and applying Bayes' theorem, the likelihood can be written as:

$$P(A \mid X) \approx \prod_{i<j} P(a_i, a_j \mid r_{ij}) \propto \prod_{i<j} \frac{P(r_{ij} \mid a_i, a_j)}{P(r_{ij})}$$

where the product runs over all amino acid pairs a_i, a_j (with $i < j$), and r_{ij} is the distance between amino acids i and j.

The assumption that the likelihood can be expressed as a product of pairwise probabilities is questionable.



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www.wikipedia.org