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Customized Versus Universal Scoring Functions: Application to Class I MHC–Peptide Binding Free Energy Predictions

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Abstract—A tailor-made free energy scoring method (Fresno) has been compared to six universal scoring functions (Chemscore, Dock, FlexX, Gold, Pmf, Score) for predicting the binding affinity of 26 peptides to the class I human major histocompatibility protein HLA-B*2705. Fresno clearly outperforms all six universal scoring functions.

Predicting absolute binding free energies from three-dimensional coordinates of protein–ligand complexes is one the most challenging issue in current computational chemistry. It is particularly of utmost importance in virtual screening of large databases for finding new hits.[1] Most of the fast empirical scoring functions[2–7] have been parameterized from high-resolution protein–ligand X-ray structures and perform rather well (standard error of about 1 to 1.5 pK unit) in cases where the protein–ligand interface is polar and of reduced dimensions (e.g., proteases). For large contact interfaces, tailoring a target-specific scoring function might be a better approach.[8]

We recently described a new empirical scoring method[9] (Fresno) particularly adapted to such difficult cases because of specific terms accounting for ligand desolvation and protein–ligand repulsion. Fresno was shown to predict with a good accuracy (0.75 pK unit) binding affinity of antigenic nonapeptides to class I MHC proteins from three-dimensional molecular models.[9] One of its advantages is that it can be easily tailored to propose a specific scoring equation for any target. MHC–peptide complexes represent such a difficult case for free energy predictions for several reasons: (i) the MHC binding cleft is huge (about 3000 Å^3); (ii) the ligands are highly flexible; (iii) the polarity of the peptide ligands varies with the MHC-restriction protein. Several approaches for ranking potentially interesting T cell epitopes have been described in the literature.[10] On the one hand, artificial neural networks[11,12] and experimentally-derived statistical position-dependent matrices[13–15] can rank antigenic peptides on the basis of known peptide binding motifs or experimental binding data. However, they require a lot of experimental data in a first training phase, assume independent binding contributions for peptide side chains[16] and cannot predict absolute binding free energies. On the other hand, free energy scoring functions have shown some promise.[3,17–19] Notably, Fresno is the only predictive method that has been extensively validated and for which high-quality quantitative predictions have been reached.[9]

Thus, we decided to compare our method with the state-of-the-art free energy scoring functions[2–7] with the perspective of finding out which one would be the most suitable for virtual screening of the HLA-B*2705 protein. Hence, small-molecular weight nonpeptide inhibitors, which are still lacking for this autoimmune disease-associated protein, might lead to a new series of immunosuppressants.

Predicting Binding Free Energies from Five MHC–Peptide X-ray Structures

We first applied the above-described seven scoring functions to a test set consisting of five MHC–peptide X-ray structures for which experimental binding free energies were available (Table 1). After addition of all

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hydrogen atoms and 100 steps steepest-descent AMBER5[20] energy minimization, the five peptide ligands were scored with SYBYL6.62 (TRIPOS Assoc., Inc.) using either the CScore™ (Chemscore,[2] Dock,[3] Gold,[4] FlexX[5] and Pmf[6] scores) or in-house SPL macros (Score,[7] Fresno[9]). Out of the seven functions tested, Fresno gives by far the best predictive model with a standard error of 3.44 kJ/mol (about 0.6 log unit) in predicting absolute binding free energies. For three universal scoring functions (Chemscore, Dock, Gold), significant correlations could still be found but with lower predictability (predictive error about 4–5 kJ/mol). The remaining three scoring functions (FlexX, Pmf, Score) were not able to find any predictive model.

Predicting Binding Free Energies from 21 MHC–Peptide Models

We next asked whether our scoring method would also outperform universal functions in predicting binding free energies from homology models. Twenty-one nonapeptides (Table 2) were then modeled in the binding groove of our protein of interest, HLA-B*2705 whose X-ray structure in complex with a model peptide (PDB entry 1hsa) had previously been solved at a resolution of 2.1 Å.[22] Peptides were built in the protein binding site (Fig. 1) as previously described.[9]

Table 1. Performance of seven scoring functions on a test set of five HLA-A*0201 peptide X-ray structures (PDB code: 1hhg, 1hhh, 1hhi, 1hhj, 1hhk)[21]

Method	r^{2a}	s_{pred} (kJ/mol)[b]
Chemscore	0.521	4.24
Dock	0.346	4.95
FlexX	0.186	5.53
Gold	0.481	4.42
Pmf	0.003	6.12
Score	0.228	5.38
Fresno	0.895	3.44

[a]Correlation coefficient.
[b]Standard error of prediction.

Table 2. Sequence and experimental binding free energies of 21 peptides to the HLA-B*2705 protein

Peptide	Sequence	ΔG_{exp}, kJ/mol[a]
1	FRFNGYIHR	−48.51
2	FRFNGLYHR	−48.36
3	RRIKYITLK	−48.28
4	ARLFGIRAK	−47.12
5	FRYNGLIHR	−46.67
6	RRISGVDRY	−46.46
7	ARLYGIRAK	−46.08
8	SRYWAIRTR	−45.86
9	RRYQKSTEL	−45.03
10	KRFEGLTQR	−44.67
11	GRAFVTFGK	−42.65
12	RVMAPRALL	−42.65
13	RRVKEVVKK	−42.57
14	KRYEGLTQR	−42.31
15	YQFTGIKKF	−40.40
16	YQFTGIKKY	−38.03
17	ALFAAAAAK	−37.53
18	YRHDGGNVL	−37.32
19	VADLVGFLL	−27.27
20	RMGAVTTEV	−27.27
21	IMPKTGFLI	−26.56

[a]Calculated from experimental binding data assuming that $\Delta G_{exp} \approx RTln(IC_{50})$.

Briefly, the peptide was built in three steps: (1) backbone coordinates of positions 1, 2, 3, 8 and 9 as well as both charged termini were kept constant and identical to that of the 1hsa crystal structure; (2) rotameric states of side chains at the above-described positions were assigned by searching an in-house three-dimensional database of 37 X-ray structures of class I MHC-bound peptides; (3) the central loop (P4–P7, Pn standing for position n) bulging out of the binding groove was constructed using a knowledge-based loop search procedure.[9] After adding all hydrogen atoms and up to 1000 steepest descent AMBER5 minimization steps of the whole complex, the loop was annealed for 25 ps at 1000 K and cooled down to 50 K for another 25 ps. The last simulated annealing conformer was relaxed again by 100 steps conjugate gradients minimization and used as such for scoring.

Out of the six functions tested, two (FlexX, Score) were unable to find any correlation between computed scores and experimental binding free energies (Table 3).

In both cases, the predicted binding free energy was overestimated by at least two orders of magnitude, mainly because of the overestimation of H-bonding scores that predominates in the latter two functions. Very similar results have been previously described for another MHC–peptide series[9] where binding free energies were scored using the Ludi function,[23] from which FlexX is derived. Several HLA–peptide H-bonds[24] are weaker than expected when looking at their nearly perfect geometry (distances, angles). Hence, they are partially buried and thus accessible to competition with water molecules. Scoring functions with a better balance of polar and apolar interactions, at least for the current set of highly flexible polar ligands, perform better. Chemscore[2] has a rotational entropy that differs from that of Ludi/FlexX and better counterbalances the H-bonding score. The Dock score[3] used in the present study is a molecular mechanics non-bonded term (10–12 Lennard–Jones potential) very similar to that used by AMBER5 for minimizing all complexes. The Gold score[4] is also very dependent of H-bond interactions but is the only one that explicitly describes ligand strain energy. Last, Pmf[5] utilizes a very promising knowledge-based potential of mean forces derived from the statistical distribution of non-bonded contacts in 697 protein–ligand crystal structures and thus is applicable to any receptor–ligand series. Therefore, it is no surprise that it gave a robust model in the present study.

Using the same 21 complexes, we next computed the five different scores of the Fresno function (eq F1, Table 4) to which a new one (buried polar surface area of the ligand) calculated using the SAVOL3 program[25] was added as a possible alternative to the total ligand desolvation energy. All possible linear regressions with at

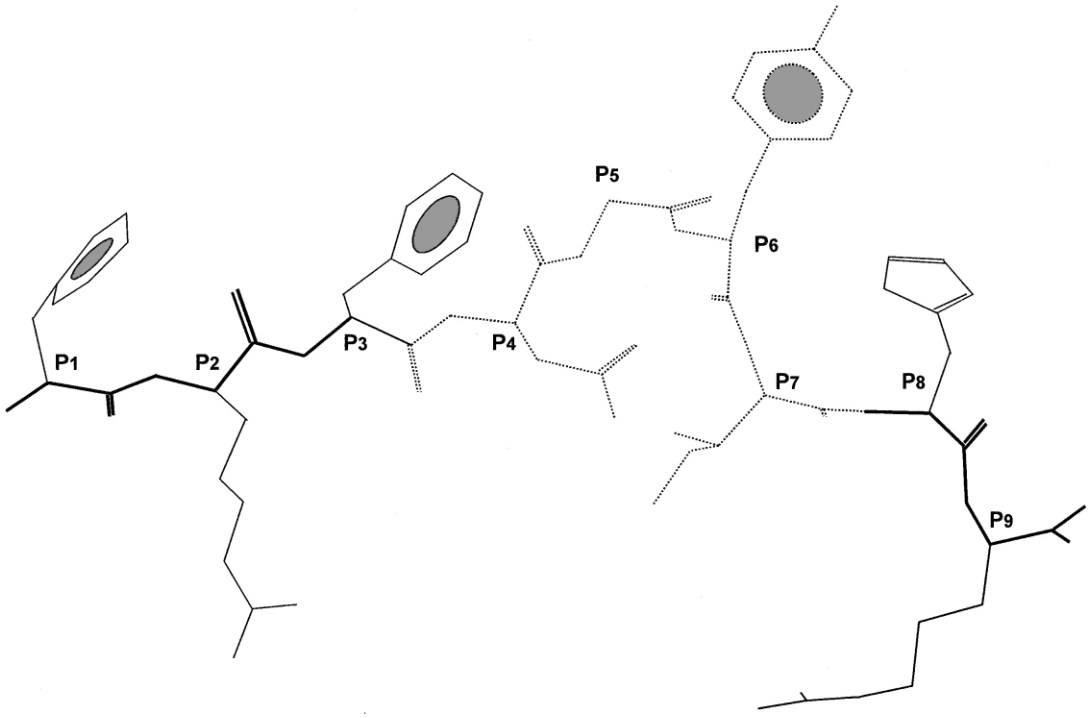

Figure 1. Building a nonapeptide (e.g., peptide **1**) in the binding groove of HLA-B*2705. Conserved backbone atoms (P1–P3, P8–P9) have fixed coordinates and are shown as bold lines. Corresponding side chains, built by homology modelling,[9] are displayed as thin lines. The rest of the peptide structure, displayed as dotted lines, is obtained by a knowledge-based loop search procedure.

least three parameters were obtained and analyzed for their statistical and physicochemical values. Using five or four terms (eqs F1–F5) led to models with good correlations but meaningless equations (positive coefficients for lipophilic interactions, negative coefficients for BP and DESOLV or BPSA scores). Two models (F6, F7) with good crossvalidated coefficients and relevant coefficient signs were further selected. The first one comprises only three terms, as in the Chemscore function, taking into account H-bonding, lipophilic interactions and rotational entropy of the ligand. As expected from the highly polar nature of the ligands, the H-bond term largely predominates in the equation and provides about 2/3 of the total binding free energy. The minor role of the lipophilic contribution (about 10 kJ/mol) explains why more complex equations (with four or five terms) including the LIPO term were unable to find a meaningful regression, as the LIPO scores are relatively small and with a limited variance. Omitting the lipophilic score (eqs F7–F10), a weak but negative contribution of the desolvation energy could be found (eq 7) at the condition that the repulsive BP parameter was also discarded. DESOLV scores are very high for all 21 peptides with again a too small variance for real statistical interpretation. The BP parameter was not required in any of the final two selected regressions, probably because all complexes have been thoroughly energy-minimized before scoring. It might however be useful for virtual screening purpose.

Table 3. Performance of six universal scoring functions for the test set of 21 HLA-B*2705/peptide complexes

Scoring method	Q^2_{LOO}[a]	s_{press}[b]	r^{2c}	s^d
Chemscore	0.465	5.21	0.569	4.68
Dock	0.503	5.03	0.582	4.60
FlexX	−0.020	7.20	0.181	6.45
Gold	0.508	5.00	0.613	4.44
PMF	0.524	4.92	0.601	4.50
Score	−0.153	7.65	0.043	6.97

[a]Crossvalidated correlation (leave-one-out PLS analysis).
[b]Standard error of prediction (kJ/mol).
[c]Fit to experimental data (linear regression).
[d]Standard deviation of the fit (kJ/mol).

The three-term regression equation (F6) led to a model with higher predictability (predictive error of 4.37 kJ/mol) than that previously reported for the best universal scoring function (Pmf, press = 4.92 kJ/mol; Table 3). To really check its extrapolation power, six peptides (**1**, **5**, **9**, **13**, **17** and **21**, Table 2) were removed from the training set, and a new equation computed for the remaining 15 complexes. The new equation (eq 1) was relatively similar to that obtained for the whole dataset (eq F6, Table 4).

$$\Delta G_{bind} = -7.830 - 2.274.HB - 0.042.LIPO + 1.096.ROT$$
$$(Q^2_{LOO} = 0.564, s_{press} = 5.03\,kJ/mol, n = 15). \quad (1)$$

When used for predicting the binding free energy of the six peptides in the new test set, a very good correlation ($r^2_{pred} = 0.850$, $s_{pred} = 3.49$ kJ/mol, $n = 6$) was obtained (Fig. 2). Using the same protocol, the universal scoring function shown to perform the best in the current study (Pmf) led to much weaker predictions ($r^2_{pred} = 0.492$, $s_{pred} = 4.99$ kJ/mol, $n = 6$). As the experimental binding free energy was spread over four orders of magnitude in the test set, we here demonstrate the robustness of the

Table 4. Performance of Fresno for the test set of 21 HLA-B*2705/peptide complexes

Eq[a]	K	α	β	γ	δ	ε	ε′[b]	Q^2_{LOO}[c]	s_{press}[d]	r^{2}[e]	s[f]
F1	−7.278	−1.008	0.007	1.367	−0.055	−0.009		0.636	5.167	0.831	3.296
F2	−9.131	−0.914	0.015	1.374	−0.051		−0.013	0.692	4.732	0.826	3.351
F3	−7.197	−1.230	0.011	1.196	−0.051			0.705	4.468	0.821	3.290
F4	−9.125	−1.688	−0.028	0.954			−0.016	0.685	4.667	0.775	3.688
F5	−6.858	−1.966	−0.038	0.830		−0.006		0.620	4.926	0.773	3.703
F6	−6.819	−2.082	−0.033	0.733				0.664	4.370	0.768	3.632
F7	−15.538	−2.190		0.725		0.001		0.577	4.907	0.725	3.953
F8	−17.378	−1.462		1.126			−0.027	0.675	4.471	0.749	3.781
F9	−7.136	−1.117		1.252	−0.041		−0.011	0.734	4.280	0.822	3.277
F10	−6.532	−1.065		1.333	−0.05	−0.009		0.689	4.388	0.831	3.198

[a]Fresno equation: $\Delta G_{bind} = K + \alpha(HB) + \beta(LIPO) + \gamma(ROT) + \delta(BP) + \varepsilon(DESOLV)$; HB: H-bond, LIPO: lipophilic contacts, ROT: rotational entropy term, BP: repulsive term, DESOLV: desolvation term.
[b]ε′ is the regression coefficient of a new property (buried polar surface area, BPSA) replacing the original desolvation (DESOLV) term.
[c]Crossvalidated correlation (leave-one-out PLS analysis). The robustness of each PLS model was checked either by altering the order of the data lines (no influence on Q^2 and s_{press}) or assigning random values to experimental binding free energies (loss of any correlation).
[d]Standard error of prediction (kJ/mol).
[e]Fit to experimental data (linear regression).
[f]Standard deviation (kJ/mol).

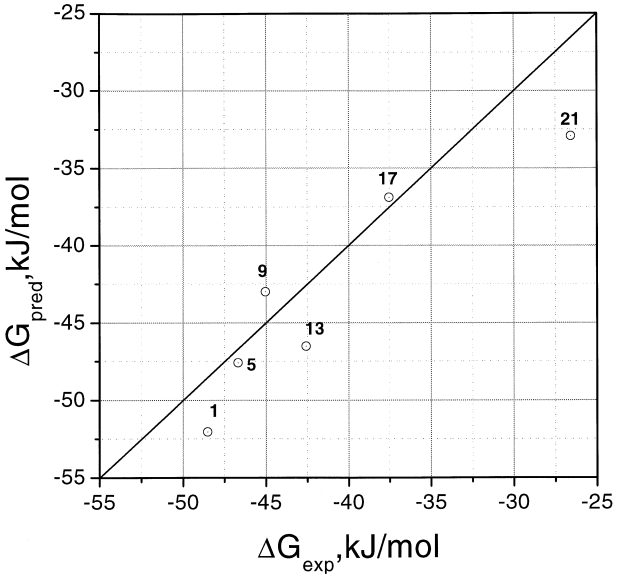

Figure 2. Experimental versus Fresno-predicted binding free energies for a test set of six peptides (**1**, **5**, **9**, **13**, **17**, **21**; see Table 2).

Fresno function for predicting binding free energy of polar, flexible ligands with large intermolecular contacts to their target proteins.

Conclusions

The main advantage of Fresno over universal scoring functions is that it may be easily calibrated for a specific protein–ligands series and further used for predicting the affinity of new compounds. Of course, recalibrating parameters of universal scoring functions for a peculiar target is feasible but requires modifications of the source code. Fresno can be applied to any protein–ligands series that significantly differs from the panel of X-ray structures (proteases, sugar and steroid-binding proteins, immunoglobulins) usually used to calibrate universal scoring functions. As Fresno scores are obtained within a second for any protein–ligand complex, it is particularly adapted to rank hits obtained from virtual screening of chemical databases. In the peculiar case of MHC–peptide complexes, we are currently using Fresno for tailoring free energy scoring functions to the most common HLA alleles. Our scoring function is particularly well suited to the rapid computer prediction of unknown MHC binding motifs. Furthermore, combining Fresno to knowledge-based T-cell epitope prediction algorithms[26–29] should allow the high-throughput binding energy prediction of potential epitopes from the primary structure of any protein.

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References

1. Walters, W. P.; Stahl, M. T.; Murcko, M. A. *Drug Discov. Today* **1998**, *3*, 160.
2. Eldridge, M.; Murray, C. W.; Auton, T. A.; Paolini, G. V.; Lee, R. P. *J. Comput.-Aided Mol. Des.* **1997**, *11*, 425.
3. Ewing, T. J. A.; Kuntz, I. D. *J. Comput. Chem.* **1997**, *18*, 1175.
4. Jones, G.; Wilett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. *J. Mol. Biol.* **1997**, *267*, 727.
5. Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. *J. Mol. Biol.* **1996**, *261*, 470.
6. Muegge, I.; Martin, Y. C. *J. Med. Chem.* **1999**, *42*, 791.
7. Wang, R.; Liu, L.; Lai, L.; Tang, Y. *J. Mol. Model.* **1998**, *4*, 379.
8. Verkhiver, G.; Appelt, K.; Freer, S. T.; Villafranca, J. A. *Protein Eng.* **1995**, *8*, 677.
9. Rognan, D.; Laumoeller, S. L.; Holm, A.; Buus, S.; Tschinke, V. *J. Med. Chem.* **1999**, *42*, 4650.
10. Buus, S. *Curr. Opin. Immunol.* **1999**, *11*, 209.
11. Gulukota, K.; Sidney, J.; Sette, A.; DeLisi, C. *J. Mol. Biol.* **1997**, *267*, 1258.
12. Honeyman, M. C.; Brusic, V.; Stone, N. L.; Harrison, L. C. *Nat. Biotechnol.* **1998**, *16*, 966.

13. Stryhn, A.; Pedersen, L. O.; Romme, T.; Holm, C. B.; Holm, A.; Buus, S. *Eur. J. Immunol.* **1996**, *26*, 1911.
14. Parker, K. C.; Bednarek, M. A.; Coligan, J. E. *J. Immunol.* **1994**, *152*, 163.
15. Lamas, J. R.; Paradela, A.; Roncal, F.; López de Castro, J. A. *Arthritis Rheum.* **1999**, *42*, 1975.
16. Fremont, D. H.; Stura, E. A.; Matsumura, M.; Peterson, P. A.; Wilson, I. A. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2479.
17. Froloff, N.; Windemuth, A.; Honig, B. *Protein Sci.* **1997**, *6*, 1293.
18. Altuvia, Y.; Sette, A.; Sidney, J.; Southwood, S.; Margalit, H. *Hum. Immunol.* **1997**, *58*, 1.
19. Zhang, C.; Cornette, J. L.; DeLisi, C. *Protein Sci.* **1997**, *6*, 1057.
20. Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, J. K. M.; Ferguson, D. M.; Spellmeyer, D. M.; Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179.
21. Madden, D. R.; Garboczi, D. N.; Wiley, D. C. *Cell* **1993**, *75*, 693.
22. Madden, D. R.; Gorga, J. C.; Strominger, J. L.; Wiley, D. C. *Cell* **1992**, *70*, 1035.
23. Böhm, H. J. *J. Comput.-Aided Mol. Des.* **1994**, *8*, 243.
24. Bouvier, M.; Wiley, D. C. *Science* **1994**, *265*, 398.
25. Pearlman, R. S.; Skell, J. M.; Deanda, F. Laboratory for Molecular Graphics and Theoretical Modeling, College of Pharmacy, University of Texas, Austin, TX 78712.
26. http://bimas.dcrt.nih.gov/molbio/hla_bind/
27. http://www.uni-tuebingen.de/uni/kxi
28. http://sdmc.krdl.org.sg/immuno/predintro.html
29. http://www.epimmune.com/start.html