in hepatocytes by 50% (CD_{50}) increases from a value of 0.84 μM for CLA[27] to a CD_{50} = 30 μM for CLAIB (Ziegler, K., private communication). It seems likely that flexibility plays a role in the explication of the bioactive function of cyclic peptides of this class. This is not the first example of activity diminishing in a peptide agonist as a consequence of the introduction of conformational constraints. In conformation activity relationship studies aiming to determine the bioactive conformation of peptides, the synthesis of conformationally restricted analogues has become a common practice. Successful examples of this practice are, inter alia, somatostatin[28,29] and enkephalin analogues.[30,31] Negative examples are the cyclic analogues of thymopentin,[32,33] where the conformation restriction introduced in order to reproduce the most probable conformation of thymopentin, as derived by NMR and energy minimization studies, led to a completely inactive analog.

Registry No. Cyclo(Pro-Pro-Phe-Phe-Aib-Aib-Ile-D-Ala-Val), 141666-94-6; cyclo(Pro-Pro-Phe-Phe-Aib-Aib-Ile-D-Ala-Val)·2MeOH·$2H_2O$, 141781-15-9; Z-D-Ala-Val-Pro-Pro-Phe-Phe-OH, 141666-96-8; H-Aib-Aib-Ile-O*t*Bu, 141666-95-7; Z-D-Ala-Val-Pro-Pro-Phe-Phe-Aib-Aib-Ile-O*t*Bu, 141666-97-9; H-D-Ala-Val-Pro-Pro-Phe-Phe-Aib-Aib-Ile-OH·TFA, 141666-99-1.

Communications to the Editor

Use of One-Bond C^α–H^α Coupling Constants as Restraints in MD Simulations

Dale F. Mierke, Simona Golic Grdadolnik,† and Horst Kessler*,‡

Organisch Chemisches Institut
Technische Universität München
Lichtenbergstrasse 4, 8046 Garching, Germany
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Restraints within molecular dynamics (MD) simulations derived from coupling constants using the homonuclear $^3J_{HN-H^\alpha}$[1] coupling and numerous homo- and heteronuclear couplings about a single torsion[2] have been proposed and applied to peptides and proteins. This idea shows promise as these couplings have become more facile to measure, even in natural abundance, with increasing accuracy.[3-5] However, most of these couplings supply information about the ϕ or side chain angles (i.e., χ_1, χ_2); there are no ^3J couplings about the ψ angle which are easily obtained. This led us to explore the use of one-bond couplings as a source of conformational information, namely, the $^1J_{C^\alpha-H^\alpha}$ coupling, which depends on both the ϕ and ψ torsions and is easy to measure (HMQC[6] without proton decoupling). Here we describe the implementation of a penalty function in MD simulations based on the $^1J_{C^\alpha-H^\alpha}$ coupling.

The penalty function is similar to that commonly used for NOE restraints and recently utilized for three-bond couplings:[1,2]

$$E_J = \tfrac{1}{2}K_J(^1J - {}^1J_{exp})^2 \qquad (1)$$

where E_J is the potential energy of the penalty function, K_J is the force constant, ^1J is the coupling constant calculated from the dihedral angles, and J_{exp} is the experimental value. The coupling constant is calculated from the ϕ and ψ dihedral angles using the equation developed for L-amino acids by Egli and von Philipsborn:[7]

$$^1J = A + B\cos^2(\phi + 30°) + C\cos^2(\psi - 30°) \qquad (2)$$

with A, B, and C coefficients of 136.2, 14.0, and −4.9. From an extensive study of a series of peptides and proteins, we have obtained a better fit for alanine using slightly different values: 138.4, 13.7, and −5.2, for A, B, and C, respectively.[8]

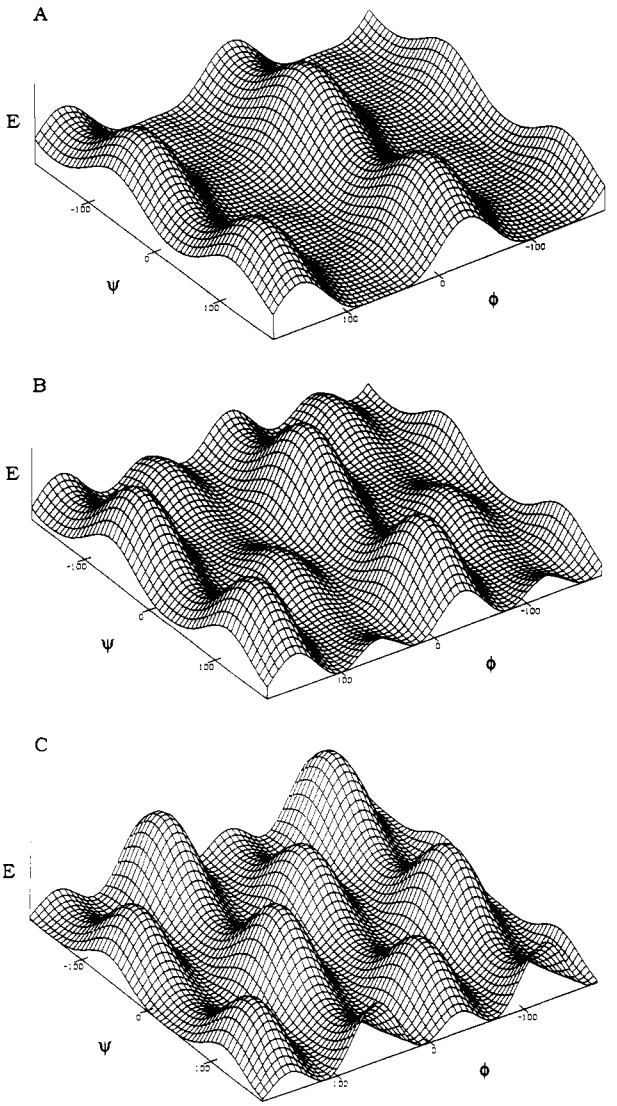

Figure 1. Plot of potential energy as a function of the ϕ and ψ dihedral angles. The energy was calculated using eqs 1 and 2 with K_J = 1.0 kJ mol^{-1} Hz^{-2} for (A) Ala^3 ($^1J_{C^\alpha-H^\alpha}$ = 135.8 Hz), (B) Ala^4 ($^1J_{C^\alpha-H^\alpha}$ = 140.4 Hz), and (C) Ala^5 ($^1J_{C^\alpha-H^\alpha}$ = 143.2 Hz).

The coupling constant restraint function was tested on a model peptide, cyclo[Pro^1-Pro^2-Ala^3-Ala^4-Ala^5], which has been extensively examined in DMSO by NMR.[9] The ^1J couplings (Table

† Permanent address: Institute of Chemistry, Hajdrihova 19, 61000 Ljubljana, Slovenia.
‡ Phone, (89)32093300; FAX, (89)32093210.

(1) Kim, Y.; Prestegard, J. H. *Proteins: Struct. Funct., Genet.* **1990**, *8*, 377–382.
(2) Mierke, D. F.; Kessler, H. *Biopolymers*, in press.
(3) Titman, J. T.; Keeler, J. *J. Magn. Reson.* **1990**, *89*, 640–646.
(4) Kurz, M.; Schmieder, P.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1329–1330.
(5) Schmieder, P.; Kessler, H. *Biopolymers* **1992**, *32*, 435–440.
(6) Müller, L. *J. Am. Chem. Soc.* **1979**, *101*, 4481–4484.
(7) Egli, H.; von Philipsborn, W. *Helv. Chim. Acta* **1981**, *64*, 976–988.
(8) Golic Grdadolnik, S.; Kessler, H. Manuscript in preparation.

0002-7863/92/1514-8283$03.00/0

Table I. $^1J_{C_\alpha-H_\alpha}$, $^3J_{HN-H_\alpha}$, and $^3J_{HN-C_\beta}$ Coupling Constants Measured for Cyclo[Pro[1]-Pro[2]-Ala[3]-Ala[4]-Ala[5]] in DMSO

residue	$^1J_{C_\alpha-H_\alpha}$ (Hz)	ϕ^a (deg)	ψ^a (deg)	$^3J_{HN-H_\alpha}$ (Hz)	ϕ^b (deg)	$^3J_{HN-C_\beta}$ (Hz)	ϕ^c (deg)
Ala[3]	135.8	−120 ± 30	−140 ± 40	8.0	−153	2.0	−90
		60 ± 30	40 ± 40		−87		−30
					44		63
					78		177
Ala[4]	140.4	−150 ± 10	−60 ± 30	6.7	−160	3.0	−60
		−90 ± 10	120 ± 30		−80		73
		90 ± 10			32		167
		90 ± 10			88		
Ala[5]	143.2	−170 ± 10	−60 ± 20	6.6	−161	2.5	−81
		−70 ± 10	120 ± 20		−79		−39
		10 ± 10			31		68
		110 ± 10			89		172

[a] Values and ranges estimated from energy profiles following eqs 1 and 2. Each of the ϕ values can be paired with each of the ψ values. [b] Values of 9.4, −1.1, and 0.4 were used for *A*, *B*, and *C* following the standard Karplus equation.[16] [c] Values of 4.7, −1.5, and −0.2 were used for *A*, *B*, and *C* following the standard Karplus equation.[16]

I) were obtained with an accuracy of 0.3 Hz by HMQC without decoupling. The slices containing the coupling were removed from the 2D data set and processed as 1D spectra, allowing for greater zero filling. The best way to visualize the restraint from the C^α–H^α coupling is by a plot of the energy as determined by eqs 1 and 2 as a function of ϕ and ψ, shown in Figure 1. The ϕ and ψ torsions of the minima obtained from examination of the energy profiles are included in Table I. The figure indicates that, with larger coupling constants, the minima are more clearly defined. The previously determined $^3J_{HN-H^\alpha}$ and $^3J_{HN-C^\beta}$ values,[4,9] both about the ϕ dihedral, are included in Table I for comparison.

The MD simulations were carried out in vacuo and in DMSO[10] using the GROMOS program[11] following a protocol previously described.[12] Starting structures were created by application of dihedral angle restraints to the ϕ,ψ of the three alanines to 180°; the cyclic compound cannot obtain these constraints and therefore is of high energy and is removed from the structures found in solution, which suits our purposes.

Starting from this structure, the *J* restraints were applied following different procedures: (1) application of both the ^1J and ^3J couplings, with equal force constants (separate simulations using force constants of 0.25, 0.5, 1.0, and 5.0 kJ mol^{-1} Hz^{-2}); (2) application of only the ^1J for 20 ps and then slowly increasing the force constant of the ^3J couplings; and (3) similar to procedure 2 but starting with the ^3J couplings. These numerous simulations each in DMSO and in vacuo resulted in only two conformations, each of which are minima in regard to the coupling constants. The first conformation has ϕ and ψ values of (−67°,155°), (−68°,151°), (61°,−104°), (−83°,−15°), and (−81°,−45°) for Pro[1] to Ala[5], respectively, and is close to the conformation observed from NOE-restrained MD [corresponding ϕ and ψ values of (−73°,155°), (−65°,148°), (74°,−83°), (−115°,−20°), and (−63°,−42°)]. The distance restraint violation (using the 18 NOEs measured for this compound) is 19 pm. The second conformation with ϕ and ψ values of (−72°,160°), (−66°,−51°), (−99°,−103°), (−83°,78°), and (−159°,−62°) contains a γ-turn about Ala[4] and is well removed from the NOE structure (distance restraint violation of 50 pm). MD simulations starting with either of these two structures and applying the NOEs quickly produce the conformation previously reported, in agreement with NOEs and couplings (distance restraint violation of 8 pm).

The utilization of one-bond $^1J_{C^\alpha-H^\alpha}$ couplings as conformational restraints in MD simulations has been illustrated for a model cyclic pentapeptide. The results indicate that coupling constants, especially when more than one coupling about a torsion is available, are a valuable source of conformational restraints. Dynamics, either free rotation of a side chain or multiple backbone conformations,[13] has been purposely avoided in this simple example since the constant restraints may not be appropriate. The approach of time-dependent restraints,[14] as has been utilized for NOEs,[15] seems to be a viable alternative for cases involving dynamics.

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Registry No. Cyclo[Pro-Pro-Ala-Ala-Ala], 135866-42-1.

(9) Kurz, M. Ph.D. Thesis, Technical University of Munich, Munich, Germany, 1991.
(10) Mierke, D. F.; Kessler, H. *J. Am. Chem. Soc.* **1991**, *113*, 7466-7470.
(11) van Gunsteren, W. F.; Berendsen, H. J. C. Groningen molecular simulation library manual (GROMOS), Biomos B. V., Groningen, 1987.
(12) Kurz, M.; Mierke, D. F.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 210-212.
(13) Kessler, H.; Griesinger, C.; Müller, A.; Lautz, J.; van Gunsteren, W. F.; Berendsen, H. J. C. *J. Am. Chem. Soc.* **1988**, *110*, 3393-3398.
(14) Torda, A. E.; Brunne, R. M.; Huber, T.; Kessler, H.; van Gunsteren, W. F. *J. Biomol. NMR*, submitted for publication.
(15) Torda, A. E.; Scheek, R. M.; van Gunsteren, W. F. *Chem. Phys. Lett.* **1989**, *157*, 289-294.
(16) (a) Karplus, M. *J. Chem. Phys.* **1959**, *30*, 11-15. (b) Karplus, M. *J. Am. Chem. Soc.* **1963**, *85*, 2870-2871.

Prediction of Water Binding Sites on Proteins by Neural Networks

Rebecca C. Wade,*,† Henrik Bohr,‡ and Peter G. Wolynes‡

European Molecular Biology Laboratory
Meyerhofstrasse 1, 6900 Heidelberg, Germany
Noyes Laboratory, School of Chemical Sciences
University of Illinois, 505 South Mathews Avenue
Urbana, Illinois 61801
Received May 18, 1992

The ability to predict ligand binding sites on biological macromolecules is an important goal in biotechnology. Because water plays a crucial role in the binding of ligands to proteins, we focus here on the prediction of water binding sites on proteins. We describe neural networks trained using crystallographic data to predict water sites on the basis of amino acid sequence and secondary structure. These networks make predictions on the atomic scale and surprisingly produce results comparable to those from other known methods of predicting water sites, even though the latter use tertiary structure information. The networks may be used to analyze relationships between the positions of water sites and protein sequence and secondary structure.

Feed-forward networks with one hidden layer were employed. These are known to have the ability to generalize molecular biology data.[1-6] Two different networks were used to determine (1)

* Author to whom correspondence should be addressed.
† European Molecular Biology Laboratory.
‡ University of Illinois.

(1) Qian, N.; Sejnowski, T. J. *J. Mol. Biol.* **1988**, *202*, 865-884.
(2) Bohr, H.; Bohr, J.; Brunak, S.; Cotterill, R. M. J.; Lautrup, B.; Nørskov, L.; Olsen, O. H.; Petersen, S. B. *FEBS Lett.* **1988**, *241*, 223-228.
(3) Holley, L. H.; Karplus, M. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 152-156.
(4) Bohr, H.; Bohr, J.; Brunak, S.; Cotterill, R. M. J.; Fredholm, H.; Lautrup, B.; Petersen, S. B. *FEBS Lett.* **1990**, *261*, 43-46.

0002-7863/92/1514-8284$03.00/0