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A potential smoothing algorithm accurately predicts transmembrane helix packing

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Potential smoothing, a deterministic analog of stochastic simulated annealing, is a powerful paradigm for the solution of conformational search problems that require extensive sampling, and should be a useful tool in computational approaches to structure prediction and refinement. A novel potential smoothing and search (PSS) algorithm has been developed and applied to predict the packing of transmembrane helices. The highlight of this method is the efficient manner in which it circumvents the combinatorial explosion associated with the large number of minima on multidimensional potential energy surfaces in order to converge to the global energy minimum. Here we show how our potential smoothing and search method succeeds in finding the global minimum energy structure for the glycophorin A (GpA) transmembrane helix dimer by optimizing interhelical van der Waals interactions over rigid and semi-rigid helices. Structures obtained from our *ab initio* predictions are in close agreement with recent experimental data.

Understanding the nature of interactions between transmembrane helices requires a knowledge of high resolution structures of membrane proteins, which are difficult to obtain using conventional spectroscopic methods such as X-ray crystallography or solution NMR. This necessitates the need for reliable computational methods to characterize the structure of membrane proteins. The 'two-stage' model for membrane protein folding[1] suggests the problem of structure prediction can be divided into two parts: (i) prediction of the membrane spanning helical sequences, and (ii) determination of the optimal packed conformations of transmembrane helices.

We assume that the thermodynamic ground state — that is, the conformation of lowest free energy — is also the one of lowest potential energy. Our primary objective is to find the global energy minimum on a potential energy surface (PES). Novel potential smoothing methods[2–5] for global optimization can be adapted to

study the helix packing problem for transmembrane helices. The potential smoothing algorithm used in this work is an adaptation of the diffusion equation method proposed by Scheraga and coworkers[6,7]. A typical smoothing algorithm for global optimization proceeds as follows. (i) The original potential function $U(\mathbf{r})$ is replaced by a transformed function $U(\mathbf{r},t)$, where t is a control parameter that determines the extent of smoothing. The parameter t is initially set to a large value at which level minimizations from random starting conformations converge to a unique minimum on the deformed convex PES. (ii) The smoothing parameter is slowly reduced according to a chosen reversing schedule followed by minimizations until $t = 0$ at which point the original PES is reached.

The physical interpretation of smoothing is that pairwise interactions between point atoms are altered to be interactions between spacially delocalized atoms where the delocalization is represented by Gaussian probability distributions. On a highly
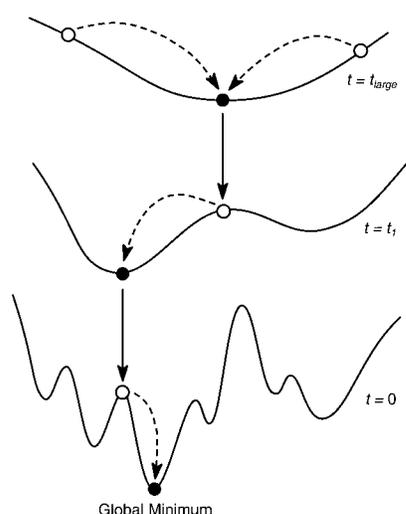

Fig. 1 One-dimensional schematic of the effect of a smoothing protocol on a potential energy surface. The original PES is transformed by successive application of a smoothing operator, where the extent of smoothing is dictated by a control parameter t. The undeformed original surface ($t = 0$), the surface at an intermediate level of smoothing ($t = t_1$) and a highly smoothed surface ($t = t_{large}$) are shown. As the surface is transformed, higher lying minima merge into catchment regions of low lying minima and barriers between minima are progressively lowered. Open circles are starting or intermediate points on each surface. Solid circles are local minima. Dashed arrows show the result of local optimization ending at a local minimum. Solid arrows represent adiabatic movement from a local minimum on one surface to the corresponding starting point on a rougher surface. A simple smoothing protocol consists of repeated cycles of local optimization followed by adiabatic transfer to the next surface. This figure shows an idealized smoothing protocol wherein the unique minimum that remains on the $t = t_{large}$ surface is directly related to the global minimum on the original PES. A series of optimizations followed by a gradual reduction in the level of smoothing will therefore lead back to the global minimum. Note that the reversing protocol depends on a set of discrete Δt steps between surfaces. For small Δt, vertical transfer to a less smooth surface will result in a point close to a transition state whenever a bifurcation has been introduced, as at $t = t_1$ in the figure. The simple protocol will only succeed if there is a consistent bias toward the minimum on the broader, deeper side of the bifurcation.

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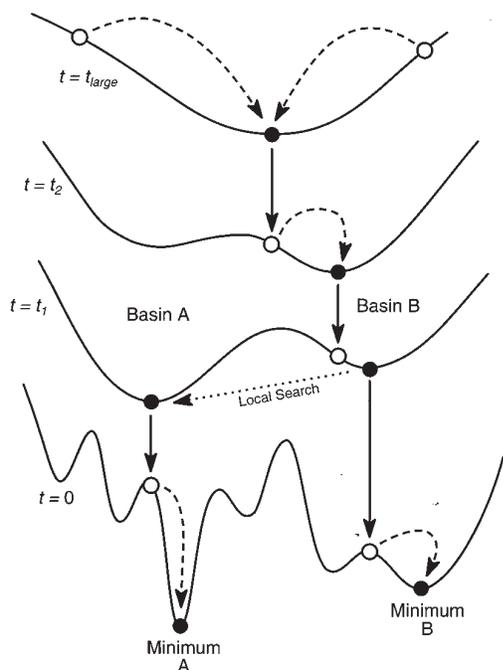
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Fig. 2 Schematic of a more realistic potential smoothing protocol for molecular search problems. This figure shows a crossing between the two surviving minima on the $t = t_2$ surface. A reversing schedule encounters the first bifurcation at $t = t_2$. At this level of smoothing the protocol favors basin B over basin A due to a crossing of relative energies, which is an artifact of the averaging process. The reversing protocol from Fig. 1 follows a path it chooses at the first bifurcation. If bifurcations are sampled where the relative energies of the alternative basins are inverted from the $t = 0$ surface, then the simple method will not converge to the global minimum. Between $t = t_2$ and $t = 0$ there exist values of t for which the energy ordering resembles that of the original PES. A local search process coupled to the smoothing schedule can potentially recognize errors due to earlier energy crossings. For example, a local search represented by the dotted arrow on the $t = t_1$ surface would correctly decide that basin A should be favored over basin B. Local searches are especially efficient when carried out on smoother surfaces since the extent of conformational space sampled is larger than for the original PES. If the global minimum is a very narrow and deep well on the PES then crossings can occur for very small values of the smoothing parameter t. For such problems, smoothing coupled to local search may fail to converge to locate the global minimum due to inadequate local sampling.

deformed PES only a single minimum or a set of nearly degenerate minima remain. Obtaining a single structure on smoothed surfaces implies the reversing protocol is completely independent of starting structures.

A schematic of an idealized smoothing protocol for global optimization is shown in Fig. 1. However, in most conformational problems the smoothing protocol is beset with the type of problem illustrated in Fig. 2. Consider two unique minima A and B from the original PES with conformational energies V_A and V_B such that $V_A < V_B$. For some level of smoothing a rearrangement of minima can result in a crossing of relative energies, that is, $V_B < V_A$ at $t = t_2$. Such crossings are directly related to the spatial volumes of the corresponding minimum energy regions on the original PES[6]. The effect of crossings on a reversing protocol is shown in Fig. 2. If at a bifurcation encountered at some t between t_{large} and t_2 the local minimum chosen is an artifact of a crossing at some smaller t, the reversing protocol will not converge to the global minimum on the original PES.

It is possible to correct for crossings by searching the vicinity of a local minimum on a smooth surface. For example a local search applied at some t near t_1 where $V_A < V_B$ would move the search back into the smoothed basin corresponding to the global minimum A (Fig. 2). Local search corrections have been used successfully to find the global minimum for a cross-section of problems[4,5]. Local searches on smooth surfaces sample larger regions of conformational space due to lower barriers and a reduction in the number of minima.

In this work we describe the application of a potential smoothing and search (PSS) procedure to obtain the global energy minimum for the transmembrane helix dimer of glycophorin A (GpA). The structure of a peptide corresponding to the transmembrane portion of the GpA dimer has been solved by solution NMR spectroscopy[8]. This structure shows an average crossing angle of -40° between the two helices and a dimeric interface with no interhelical hydrogen bonds. The sequence [-TLIIFGV-MAGVIGTILLI-] of each 18-residue helix monomer is hydrophobic except for two threonine residues. In our applica-

tion of the PSS algorithm to calculate the structure of the GpA dimer, we ignored all electrostatic interactions. All computations were performed using the TINKER modeling package[9]. For the energy functions and parameters we use the united-atom OPLS[10] force field suitably modified for potential smoothing[5]. Three different predictions of GpA helix packing were performed using PSS to locate the global minimum. These were (i) packing of rigid helices obtained from the NMR structure, (ii) packing of idealized rigid helices with backbone (ϕ, ψ) angles set to canonical values of (-60°,-45°) and side chain torsion angles (χ) set to values chosen from a backbone dependent rotamer library[11] and (iii) packing of idealized helices with flexible side chains.

Characterizing predicted structures

Our definitions for the helix packing parameters Ω and d are similar to those of Chothia, et al.[12] Ω is the angle between the two helix axes when projected onto a contact plane. The two helices are exactly parallel if $\Omega = 0$ and perfectly antiparallel for $\Omega = \pm 180°$. d is the closest distance of approach between two helices. We define three additional parameters, two angles, α and β, and a scalar parameter s to measure the packing interface of the GpA helices as compared to the NMR structure.

The angles α and β estimate the rotation of each of the helices in a calculated structure with respect to the corresponding helices from a selected NMR structure. The torsion angle α is defined by four points: the $C\alpha$ of Phe 78, two points on the axis of helix A, and a point along the line of contact. A similar definition of β uses the corresponding set of points for helix B. The NMR structure is the 0° reference state for the angles α and β, where $\alpha = \alpha_{NMR} - \alpha_{predict}$ and $\beta = \beta_{NMR} - \beta_{predict}$. A negative value for either angle implies that the calculated structure is rotated counterclockwise about the helix axis with respect to the NMR structure when looking down from the C-terminus. Small absolute values for the α and β angles imply that the helix faces in contact in the calculated structure are similar those packed in the NMR structure. A translation parameter s measures the relative shift of each helix perpendicular to the line of contact. s is defined as $s = |T_1 - T_2|$, where T_i ($i = 1,2$)

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denotes the distance between the center of mass cm_i and the point on the line of contact cp_i for helix i — that is, $T_i = |cm_i - cp_i|$. Calculation of the five helix parameters Ω, d, α, β and s is summarized in Fig. 3.

Result I. Packing rigid helices from the NMR structure

Coordinates for the individual helices were obtained from the NMR structure[8]. Of the 20 structures in the Protein Data Bank (PDB) file 1AFO, we selected helices from model structure 13 as our reference since it shows the smallest deviations from the 19 other structures in the PDB set. Values for the crossing angle Ω and the contact distance d in the consensus model 13 NMR structure are -44.1° and 6.22 Å, respectively. Starting from the two NMR helices in any arbitrary initial relative orientation, potential smoothing and search (PSS) finds a minimum energy conformation with an interhelical van der Waals energy of -29.56 kcal mol^{-1} on the undeformed PES. The crossing angle is $\Omega = -52.2°$ and the closest distance of approach between the two helices is $d = 6.36$ Å. This is also the structure obtained from a rigid body local energy minimization of the NMR dimer structure using the undeformed OPLS force field.

The rotation angles about the helical axes are $\alpha = -0.4°$ and $\beta = 6.2°$ indicating the contact interface between the two helices is very similar to the NMR structure. We computed the root mean square (r.m.s.) deviation for a Cα superposition of the predicted structure and each of the 20 NMR structures. The smallest value for the r.m.s. deviation is 0.64 Å.

We verified that the predicted structure is in fact the global minimum by characterizing the undeformed OPLS PES through a series of systematic two-body grid searches over all the rigid body degrees of freedom. This extensive search generates a total of 5,834 conformationally distinct minima. The distribution of interhelical energies for the 5,834 minima on the undeformed OPLS PES is shown in Fig. 4a. The global minimum from the grid search is identical to the structure predicted by the PSS calculation. The PSS calculation is completely independent of starting orientation of the two individual helices and is considerably more efficient and general than any systematic or random search procedure.

Result II. *Ab initio* prediction using rigid idealized helices

The calculation described above uses helix monomer conformations from the NMR structure and the result could be biased by the choice of NMR internal coordinates for the helices. We removed this possible bias by using idealized helices and side chain conformations obtained from a backbone dependent rotamer library[11]. Such a library may or may not reflect the rotamer preferences for side chains in a membrane environment[13]. The only information derived from the NMR structure is the sequence of the individual helices.

A two-body grid search identified 4,105 conformationally distinct local minima for the packed idealized helices with a distribution of energies as shown in Fig. 4b. The global minimum has an interhelical van der Waals energy of -31.84 kcal mol^{-1}, $\Omega = -52.8°$, $d = 6.58$ Å, $\alpha = -2.7°$ and $\beta = -3.5°$. The smallest value for the r.m.s. deviation from a Cα superposition of the global minimum on each of the 20 NMR structures is 0.73 Å. The undeformed PES defined by the modified OPLS force field is dotted by a large number of distinct minima, many of which are energetically close to the global minimum. Table 1 lists the structural parameters for the ten lowest energy structures obtained from the grid search. Comparison of the appropriate structural parameters indicates that the lowest energy con-
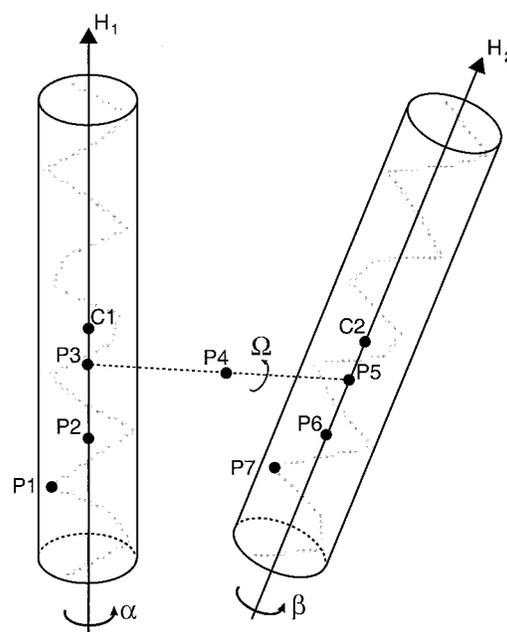

Fig. 3 Schematic of a helix dimer illustrating the method used to compute helix packing parameters. H_1 and H_2 denote the helix axes for helix 1 and helix 2 respectively. P4 is a point along the line of contact connecting the two helices between points P3 and P5. P1 is the Cα position of Phe 78 for helix 1, P2 is a point on the helix axis H_1 and C1 is the location of the center of mass of helix 1. Similarly P7 is the Cα position of Phe 78 for helix 2, P6 is a point on the helix axis H_2, and C2 is the location of the center of mass for helix 2. The crossing angle Ω is the torsion angle defined by the points P2, P3, P5 and P6. The distance of closest contact d is the distance between the points P3 and P5. The angle α that measures the rotation of helix 1 about its axis H_1 is the torsion angle defined by the points P1, P2, P3 and P4. Similarly, the angle β that measures the rotation of helix 2 about its axis H_2 is the torsion angle defined by the points P7, P6, P5 and P4. The scalar shift parameter s is defined as $|T_1-T_2|$, where T_1 is the distance between points P3 and C1 and T_2 is the distance between points P5 and C2.

former is in fact closest to the NMR structure. Results from Table 1 establish the complex nature of a typical PES; in other words, dissimilar structures can have similar conformational energies.

As with the NMR-derived helices, the PSS method successfully locates the global minimum irrespective of the starting orientation of the idealized helices. The local search component of PSS is crucial to finding the global minimum. For instance a potential smoothing calculation without local search finds a conformation with an interhelical energy of -25.80 kcal mol^{-1}, $\Omega = -33.08°$, $d = 8.88$ Å, $\alpha = 3.16°$, $\beta = 199.76°$ and $s = 4.07$ Å. A Cα superposition of this structure on the NMR structure gives an r.m.s. deviation of 3.99 Å.

Result III. *Ab initio* prediction using flexible side chains

Of the 18 residues in the helix monomer sequence, only leucine, phenylalanine and methionine do not exhibit a clear preference for a single side chain rotamer in a helical environment[11]. The most general calculation would be to allow complete flexibility of χ-angles for residues that do not show a clear preference for a single rotamer. Side chain torsions based on local motions can be coupled to the 'rigid body' degrees of freedom to find the global minimum. We chose the six 'rigid body' degrees of free-
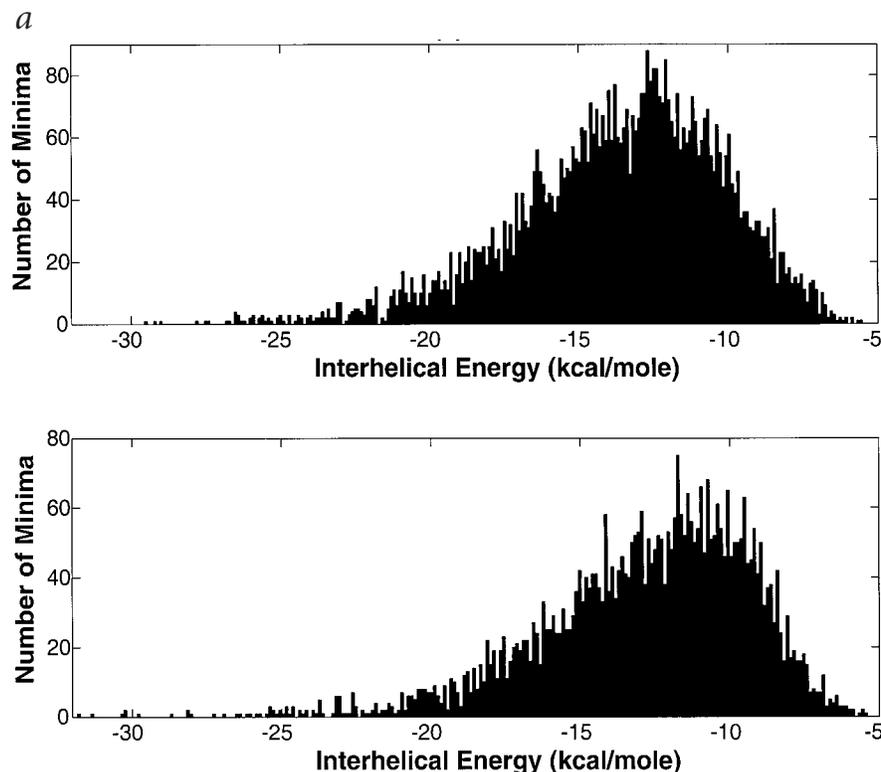
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Fig. 4 *a*, Distribution of interhelical energies for the 5,834 local minima found from a two-body grid search for helices from the consensus NMR structure for the GpA helix dimer[8]. The global minimum on this grid has an energy of -29.56 kcal mol⁻¹, and is also located by PSS. The low energy conformers obtained from this grid search show dissimilarities from the NMR structure akin to the results for the idealized helices described in Table 1. *b*, Distribution of interhelical energies for the 4,105 local minima found from a two-body grid search for idealized helices built using (ϕ,ψ) angles for a canonical α-helix and χ-angles from a rotamer library[11]. The global minimum on this grid has an energy of -31.84 kcal mol⁻¹. This is also the structure found using the PSS algorithm. In both panels the minima have been grouped into 0.1 kcal mol⁻¹ bins.

dom for each helix as well as the (χ_1,χ_2) angles of Leu 75, Leu 89, Leu 90, Phe 78 and the (χ_1,χ_2,χ_3) angles of Met 81 as optimization parameters. The PSS calculation optimizes the energy defined as a sum of the side chain torsional potentials and the intra- and interhelical van der Waals interactions.

The GpA helices are known from previous experiments to adopt a topologically parallel orientation[8,14]. For the flexible side chain calculation, the system was restricted to the parallel regime by harmonic springs that attach the ends of each helix to mobile positions on planes representing the membrane boundaries. The restraint uses a very small force constant of 0.01 kcal mol⁻¹ Å⁻¹ so that the difference in restraint energy between $\Omega = 0°$ and $\Omega = \pm50°$ orientations is less than 0.05 kcal mol⁻¹, while an orientation of $\Omega = 180°$ results in a penalty of 20 kcal mol⁻¹. The restraints do not hinder or significantly bias the complete sampling of approximately parallel helix packings.

A PSS optimization over the 34 'rigid body' and torsional degrees of freedom finds a structure with an interhelical van der Waals energy of -31.04 kcal mol⁻¹. The interhelical energy for this structure is slightly higher than the idealized rigid helix result, but the total energy including torsional and intrahelical terms is several kcal mol⁻¹ lower. The helix parameters and rotation angles are $\Omega = -47.87°$, $d = 6.78$ Å, $\alpha = -6.2°$ and $\beta = -7.0°$. The smallest r.m.s. deviation of this structure from a Cα superposition on the set of NMR structures is 0.59 Å. Fig. 5 shows the transmembrane helices from the experimental NMR structure[8] and the global minimum from the OPLS potential energy surface found using a PSS calculation. The difference in crossing angle between this particular NMR model structure and the global minimum is very small, and in all other respects the two structures are quite similar. It should be

stressed here that this represents a completely general calculation that does not use any information derived from the experimental structure determination.

Result IV. Analysis of related homodimer sequences
In Table 2 we compare the side chain interfaces for GpA helix dimer conformations from the consensus NMR model and the three structures found on the modified OPLS potential energy surfaces by using PSS. The side chain interface is quantified as the loss in accessible surface area for a given side chain upon packing. The results in Table 2 suggest that the -GVxAG- sequence motif is the major determinant of the overall helix dimer structure.

Brosig and Langosch[15] have studied the importance of the -GxxxG- sequence motif in inducing GpA-like dimerization of transmembrane helices using 13-residue poly-methionine and poly-valine chains as host sequences in a natural membrane environment. Interaction between transmembrane segments is measured using an assay based on transcriptional activation by the ToxR protein. They studied a chimeric construct where activation is only possible when the GpA-like membrane spanning helix anchor dimerizes. Thus, transcriptional activity serves as an indirect measure of transmembrane helix dimerization. The results of Brosig and Langosch[15] suggest that the sequence [-VVVVGVVVGVVVV-] shows considerably reduced transcription activation when compared to the native GpA dimer sequence. We determined the lowest energy conformation for the mutant sequence using the PSS algorithm and found a structure with helix parameters of $\Omega = 127.92°$ and $d = 5.84$ Å. The packing pattern for this structure is very different from the GpA dimer and does not involve the -GVVVG- motif. The

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Fig. 5 a, Ribbon drawing derived from the transmembrane helix portion of the experimental NMR structure (PDB file 1AFO, Model 13). **b**, The corresponding helix backbone and side chains from the global minimum determined by the PSS algorithm. Regions involved in interhelical packing are very similar in the two structures, and the r.m.s deviation for superposition over all Cα atoms is 0.59 Å. Drawings were generated using MOLSCRIPT[18].
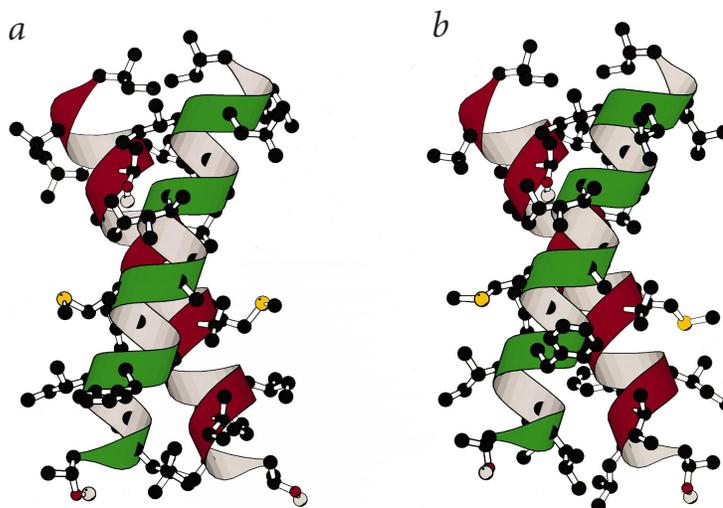
results of Brosig and Langosch[15] also suggest that the alternative sequence [-LIVVGAVVGAVVT-] has dimerization characteristics similar to that of GpA. For this sequence, the lowest energy dimer structure generated by our PSS algorithm is highly symmetric with an interhelical interface defined by the -GAVVGA- sequence motif. The overall structure is very GpA-like with helix parameters of Ω = -47.92° and d = 5.48 Å.

Computational aspects

We have demonstrated that the PSS method finds the lowest energy structure of the GpA helix dimer, which for the OPLS force field minus electrostatic interactions agrees with the experimental result[8]. A very important aspect of our algorithm is its computational efficiency. A method based on molecular dynamics simulated annealing (MDSA) searches has also been used to generate a calculated structure that is in good agreement with the experimental data[16]. The model structure of Adams *et al.*[16] and the NMR structure[8] show an r.m.s. deviation of 0.8 Å for the backbone atoms of residues 74–91. Their method is based on an exhaustive two-body search implemented by a series of 512 MDSA runs from a grid of starting conformations. Molecular dynamics simulations were performed at 600 K and 300 K, with 5,000 steps at each temperature for each configuration, followed by energy minimization. An important feature of these MDSA simulations is the large scale sampling of conformational space achieved through global search methods. The PSS method is different from an MDSA-based protocol in that it obviates the need for multiple simulations from different starting conformations. This is because the results of a PSS calculation are, by construction, independent of the starting conformation.

A useful measure of the efficiency of a simulation protocol is to estimate the total number of potential energy evaluations required. A single function call implies the calculation of both the energy and gradient. For the helix dimer studied here a typical MDSA simulation, as implemented by Adams *et al.*[16], would require upwards of 10,000 function calls for each run. The global search component of their calculation will require approximately 500 independent MDSA runs, for a total of at least $500 \times 10,000 = 5 \times 10^6$ function calls. The PSS method used in this work is based on a local search protocol that searches along all the eigenvectors of a rigid body or flexible helix Hessian matrix. The PSS method with flexible side chains

on average requires 50–100 thousand function calls when exhaustive local searches are coupled to the smoothing protocol. Thus a 'brute force' PSS is 50–100 times faster than a simulated annealing global search for locating the global minimum of the GpA helix dimer. If only a few local search directions are chosen, by means of a heuristic selection procedure, a significant further reduction in computational effort is feasible.

Potential smoothing can be thought of as a 'projection method'; that is, the important catchment region is projected out by reducing barriers between minima. In direct contrast, barriers are always present during simulated annealing and the global minimum is located by generating a trajectory that follows a reaction path by thermal activation over barriers. Because the number of barriers to be negotiated by MDSA grows exponentially with the size of the system, projection methods become relatively more efficient for larger problems. The term 'projection' as used here refers to the relation of the deepest, broadest catchment region on the undeformed surface to the single minimum remaining on a highly deformed surface.

The accuracy of the results predicted by our simple potential function and the tunable computational efficiency of PSS makes the current protocol attractive for global optimization and conformational searching to solve typical problems such as X-ray and NMR structure refinement, docking of ligands to active sites and prediction of antibody loop conformation. We are currently pursuing further improvements to the smoothing algorithms and generalizations to include electrostatics and continuum solvation models in molecular docking methods.

Methods

Force field and parameters. The force field is a modification of the original united atom OPLS[5,10]. It is parameterized to replace each of the pairwise Lennard-Jones 12-6 van der Waals terms as a sum of two Gaussians:

$$U_{LJ} \approx U_{gauss} = \sum_{i=1}^{2} a_i \exp\left(-b_i r^2\right)$$

Parameters for the two Gaussian approximation are chosen to fit a canonical Lennard-Jones 12-6 function with a hard sphere radius (σ) and well depth (ϵ) of one. The magnitude of the repulsive Gaussian, a_1, determines the height of the excluded volume energy barrier. We use values of (a_1,b_1) = (14,487.1 kcal mol^{-1}, 9.05148 Å) and (a_2,b_2) = (-5.55338 kcal mol^{-1}, 1.22536 Å) for σ = 1 Å and ϵ = 1.0 kcal mol^{-1} (ref. 17). These reference coefficients are scaled according to the σ and ϵ values for each pairwise interaction using the values prescribed by

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the OPLS force field. Relative energies of all conformations obtained using the Gaussian approximation show extremely good correlation with corresponding values obtained using standard OPLS.

Potential function smoothing. A potential function $U(\mathbf{r})$ is transformed to $U_d(\mathbf{r},t)$ such that $\partial U_d / \partial t = \Lambda\{U_d\}$ where Λ is a multidimensional diffusion operator. The deformed Gaussian van der Waals function that is an analytical solution to the diffusion equation is of the form:

$$U(r,t) = \sum_{i=1}^{2} \frac{a_i}{(1+4t)^{3/2}} \exp\left(\frac{-b_i r^2}{1+4t}\right)$$

where t is the deformation parameter that controls the extent of potential smoothing. For the torsional potential term, the smoothed functional form becomes:

$$\frac{1}{2}\sum_j V_j\left(1+\cos(j\omega+\phi)\exp(-j^2 t)\right)$$

where ω is the torsional angle value, j is the periodicity, Vj is the half-amplitude, ϕ is a phase factor and t is the deformation parameter.

Potential smoothing and search (PSS) algorithm. Our method for local searches is derived from an algorithm proposed by Nakamura, et al.[4]. At some chosen value of t along the reversal protocol the level of smoothing is reduced followed by local minimization. The system is moved out of this local minimum along a set of search directions corresponding to the eigenvectors of the Hessian matrix at the current local minimum.

The system is moved along a search direction i and the conformational energy is computed at each equidistant point k along the search direction. If the energy at point k satisfies the two inequalities $V_{i,k-1} > V_{i,k}$ and $V_{i,k-1} > V_{i,k+1}$ it is chosen to be a new point from which to start a minimization. This pair of conditions suggests apparent downhill progress on a PES. If the energy of the alternate minimum is lower than the energy of the original minimum, the system is moved to the alternate location and the search process is iterated until no new minima of lower energy can be found at the current level of smoothing.

For purely rigid helices the degrees of freedom are the three translations and three rotations for each rigid body and the only term in the potential function is the van der Waals potential. For the docking of helix backbones with flexible side chains the degrees of freedom are translations and rotations to describe global motions for the helices and torsional angles for the side chains. The potential function in this case includes both van der Waals and torsional terms. In terms of rotational coordinates each step along a search direction represents a 5° displacement. Similarly for translational coordinates the step size is approximately 0.5 Å.

The initial value of the smoothing parameter in all calculations was set to $t = 4.25$. Local searches along Hessian eigenvector directions are performed for all values of $t < 4.0$ during the reversing schedule. A typical reversing schedule includes fifty to a hundred values of t between $t = 4.25$ and $t = 0$. For the PSS calculation based on the individual NMR helices, the local search finds alternate low energy conformers on the $t = 1.8$, $t = 0.54$, $t = 0.23$ and $t = 0.18$ surfaces. In the corresponding calculation for idealized helices, local search finds alternate low energy conformers on the $t = 4.25$, $t = 3.44$, $t = 0.67$ and $t = 0.35$ surfaces.

Generating idealized helix conformations. An idealized helix structure for the capped monomer (Acetyl-TLIIFGVMAGVIGTILLI-NHCH$_3$) was generated using idealized bond lengths and bond angles that are part of the TINKER program[9]. The backbone dihedral angles were set to $(\phi,\psi) = (-60°,-45°)$ for a canonical α-helix and the peptide bond was set *trans*. Values for the side chain torsional angles (χ) were chosen from a backbone dependent rotamer library[11]. Each helix was then minimized in torsional space using the full undeformed OPLS force field *in vacuo* to a gradient convergence criterion of 0.0001 kcal mol^{-1} radian^{-1}. The individual helices have fully opti-

mized intrahelical hydrogen bonds and show an average r.m.s. deviation of 0.4 Å for a superposition of corresponding backbone Cα atoms from the NMR structures.

Characterizing the PES using two-body grid searches. For the two-body grid searches, starting conformations for a pair of helices were generated by rotations about individual helix axes in 40° increments from 0° to 360° coupled to rotations about the line of closest contact in 20° increments from 0° to 360°. For each complete rotational search we obtain $18 \times 81 = 1,458$ unique conformations. We generated 40 independent rotational searches by translating the helices along their respective helix axes. This procedure resulted in a total of 58,320 starting conformations for energy minimizations. Each of the starting conformations were then minimized using a quasi-newton method in rigid body space where the degrees of freedom are the three independent rotations and three translations for each rigid helix. Redundant minima were eliminated from the original set. All minimizations were carried out to a gradient convergence criterion of 0.0001 kcal^{-1} mol^{-1} per rigid degree of freedom on the undeformed PES.

CPU information. All calculations were performed on a Digital 2100 Server with 250 MHz DEC Alpha CPUs running Digital Unix 4.0. Total time for rigid helix PSS calculations was <30 min on a single CPU with the minimum required search directions and up to 2.5 h with exhaustive searching.

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A potential smoothing algorithm accurately predicts transmembrane helix packing

Tables 1 and 2 were inadvertently omitted from the text of the paper. They are printed below in full. We regret this error and any confusion it may have caused.

Table 1 Comparison of conformational energies and structural parameters for the set of ten lowest energy conformers obtained from a two-body grid search using model built idealized helices

Interhelical vdW Energy (kcal mol^{-1})	Crossing angle Ω	Contact distance d (Å)	Helix A rotation angle α	Helix B rotation angle β	Relative shift s (Å)	Smallest r.m.s. Cα superposition on NMR structures
-31.84	-52.81	6.58	-2.75	-3.75	0.00	0.74
-31.31	-159.91	7.49	69.54	68.54	0.00	8.28
-30.37	-165.69	7.33	48.54	47.54	0.00	7.96
-30.23	-135.86	6.62	25.22	24.22	0.00	6.00
-29.75	154.84	7.64	19.14	18.14	0.00	10.02
-28.72	144.14	7.38	3.02	79.97	4.47	9.76
-28.19	-144.63	6.97	57.09	56.09	0.00	6.65
-28.04	-124.14	6.30	46.42	45.42	0.00	7.22
-27.25	-50.82	6.83	103.85	67.89	0.68	4.19
-26.97	159.26	7.70	22.86	21.86	0.00	9.84

Table 2 Comparison of the side chains in the helix dimer interface for the three PSS generated low energy conformers of the GpA helix dimer and the NMR structure[1]

Side chain residue	Δ(%$_{sasa}$) for consensus NMR structure	Δ(%$_{sasa}$) for PSS global minimum using NMR helices	Δ(%$_{sasa}$) for PSS global minimum using idealized helices	Δ(%$_{sasa}$) for PSS flexible minimum using ideal helices and fexible side chains
Ile 76 A	42.3	23.6	31.1	32.9
Gly 79 A	93.2	86.9	81.3	79.1
Val 80 A	41.1	38.3	31.4	33.9
Ala 82 A	7.4	10.7	12.0	8.5
Gly 83 A	86.1	95.5	99.6	95.9
Val 84 A	27.6	27.7	25.9	29.8
Thr 87 A	61.5	58.3	56.9	57.8
Ile 91 A	22.1	16.9	11.3	15.3
Leu 75 B	43.1	44.9	37.4	15.2
Ile 76 B	40.6	16.5	31.1	34.5
Gly 79 B	92.9	93.3	81.3	80.3
Val 80 B	42.8	34.8	31.4	34.0
Ala 82 B	8.2	18.3	11.9	8.5
Gly 83 B	87.3	97.5	99.6	95.9
Val 84 B	27.8	25.3	25.9	29.3
Thr 87 B	58.6	58.6	56.9	57.6
Ile 91 B	22.0	14.0	11.3	15.4

[1]Side chains in the interface are characterized using the change in percentage of solvent accessible surface area upon packing. For a given side chain Δ(%$_{sasa}$) is defined as the difference in percent solvent accessible surface area of the side chain in the isolated helix monomer and the corresponding percentage for the packed dimer structure. Only side chains with a greater than 10% change in their percentage of accessible surface upon packing are reported. A solvent probe radius of 1.4 Å was used.