Biomolekulare Strukturvorhersage mit stochastischen Optimierungsverfahren: von der Sequenz zum Medikament

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Nanobiotechnology

Biological macromolecules play an increasing role as functional units in nano-devices.

Urgent need to understand and predict their structural properties and stability.
Computational Nanophysics

DFT, MRCI, DMRG

Landauer Theory, Rate Equations

Kinetic Theory, Molec. Dynamics

Stochastic Optimization

Single Molecule Transport
Hettler, Weber, Schoeller (Aachen), Cuevas (KA)
DFG

DNA Based Molecular Wires
Carell(München), Simon (Aachen)
VW Stiftung

Materials Modelling of Nanotubes and Nanofilms
Krupke, Balaban, Wahlheim, Carrell(München)
VW Stiftung, KIST

Protein Folding & Drug Development
Scheraga (Cornell), Lee (KIST), 4SC AG (München)
DFG, KIST
Biomolecular Structure Prediction

**Protein Folding:** from sequence to structure
- *All-atom free-energy forcefield* that can fold a family of helical proteins
- *Stochastic Optimization Methods* to reproducibly fold proteins with up to 60 amino acids

**Drug Development:** from structure to drug
- *FlexScreen* for in-silico high-throughput screening with flexible protein receptors and ligands for up to 250,000 compounds
- *IntelliScore:* adaptive scoring functions


Herges et al., Nanotechnology 14, 1 (2003)
Protein Structure Prediction

- Proteins are the building blocks and machinery of life
- Sequential molecules assembled from 20 aminoacid building blocks
- Efficient methods to determine the sequence are available
- But, the knowledge of the sequence is insufficient to understand the biological functions
- Structure determination is much more expensive than sequencing

From sequence:
VAL LEU SER PRO ALA ASP LYS THR ASN VAL GLY ....

To structure:
Representation of the Hemoglobin Protein

Structure resolution permits the analysis of biological function
Hemoglobin: Control of biological function

Structure analysis permits control of biological function.

There are 10,000,000 sequences available, but only 25,000 structures.

Movies instead of snapshots, design of inhibitors or enzymes
Prediction Methods

**Homology Models**

Transfer structural information from databases of resolved proteins on the basis of partial sequence similarity.

**Advantage:** Fast, wins present day prediction competitions (CASP)

**Problem:** can reproduce only what is in the database, requires large degree of similarity for successful prediction, need to rank different propositions

**Prediction by Folding**

Solve protein equations of motion: Protein folding occurs on the millisecond time-scale, while molecular dynamics time steps are on the femtosecond timescale

\[ m \ddot{x}_i = - \frac{\partial V(x)}{\partial x_i} \]
Folding Pathway with Molecular Dynamics

Reproducible folding / unfolding has been observed for peptides in helices/bends/beta-sheets for up to 20 amino acids in direct simulation.

For larger proteins, such as the villin headpiece (36 amino acids), one has to rely on rare events (Folding@Home).

256 nodes CRAY T3E

= 85 CPU Years
Protein Structure Prediction by Free Energy Optimization

- **Thermodynamics hypothesis (Anfinsen, 1972):**
  - Proteins are in thermodynamic equilibrium with their environment!
  - Native conformation is the global optimum of the free energy
  - Replace internal energy in the simulation by \textit{effective free energy}
  - Simulation problem is replaced by \textit{structure optimization} problem
  - Structure optimum can be found \textit{without recourse to the folding dynamics}
  - Enormous gain in efficiency, because optimization methods can visit unphysical intermediates
Protein Forcefield PFF01/PFF02

- All atom forcefield (except CH$_n$)
- Bond distances and angles are fixed
- Dihedral angles of backbone and sidechains are free
- Lennard Jones parameterized to experimental structures of 137 proteins
- Electrostatic interaction
group specific dielectric constants (Avbelj, Moult 1992)
correction for main-chain dipole-dipole interaction
- Solvent Model
SASA model based on Eisenberg/McLachlan parameters
- Hydrogen Bonding
parameterized to a set of helical fragments and bends
- Torsional Potential for Backbone dihedral angles

Verma, et.al. (in prep)
Decoy-Generation for Protein A

Generate 10,000 decoys from random and NMR starting configurations, improve the best through repeated optimization (cost 2 CPU years).

Optimization by Stochastic Tunneling

- at any point in the simulation, the detailed structure of the potential above the present best energy $E(R)$ is irrelevant, while the details of the potential below the best energy found are very important.
- compress the potential above $E(R)$ to a fixed interval and stretch the potential below.
- preserve the location and relative order of the minima.

$$f_{\text{eff}}(x) = 1 - e^{-\gamma \otimes [f(x) - f(x_0)]}$$

original potential
red line = present position
green line = present optimum

transformed potential
Reproducible and Predictive Folding of the Trp-Protein with the Stochastic Tunneling Method

The energetically lowest 8 of 25 simulations converged to structures within 1kcal/mol and 2-3 Å RMSB to the native conformation.

Schug; et. al PRL 2003
Basin Hopping Technique

Map the original potential energy surface to a simplified potential by associating each conformation with the conformation of an associated local minimum, optimize on this potential.

For proteins: local minimization by simulated annealing

Herges, Wenzel, PRL (2005)
Proteins Folded with Basin Hopping

The energetically lowest six of 20 independent simulations converged to 2-3 Å RMSB to the native conformation.
Visualization of the Folding Landscape

- generate decoys that explore the entire low energy surface
- start with the lowest energy decoy
- Associate all decoys in the next higher energy window with existing families, when they are structurally similar, otherwise create a new family
- Family membership is associative: if A is in the same family as B, and B in the same family as C, A and C are also in the same family.
- As the energy increases, families unite
- Generates inverted tree-structure

Complete topological characterization of the low energy part of the free energy surface

Herges, et. al. Structure (2005)
Energy Landscape of the Villin Headpiece

Herges, Structure (2005)
1VII Decoys

NMR

N

M

A

B

C
PFF02 stabilizes small beta peptides, reproducible folding. Up to 24 amino acids, no mixed systems to date. Decoy studies show that the helical proteins are not destabilized in the new forcefield!
Folding the trp.

30% of the simulations converge to the native conformation within exp.
Resolution, speedup: $10^5$

Cochran, PNAS (02), Snow, PNAS (04), Yang, JMB (04)
Internal Free Energy Surface
Adaptive Parallel Tempering

Run a number of parallel simulations at different temperatures and exchange their conformations according to:

$p = \max\left(1, e^{-\Delta \beta \Delta E}\right)$

(preserve thermodynamic equilibrium)

Better: adjust temperatures to control exchange rates

Even better: duplicate the best conformation to highest temperature

Reproducible Folding of the Bacterial Ribosomal Protein L20 using distributed optimization

In a population of 2000/200/50 structures in a distributed optimization approach the native state occupies the three lowest conformations and occurs 4 additional times.

Decoy set from Rosetta, 43 Proteins, (Tsai et al. Proteins 2003), over 1800 decoys for each protein

- PFF01 stabilizes all helical proteins except one.
- For the helical proteins, where near-native decoys are in the set, PFF01 selects a near-native decoy in 9 of 21 cases, but always in the top ten.
- For the one exceptional case, the experimental structure has since been replaced in the PDB
- Significant enrichment even for mixed and beta-sheet systems, but no predictive selection
- average Z-score < -3
Protein structure prediction by decoy refinement

Pdb: 1afi, 72 amino acids, 2.2 A bRMSD
Protein Structure Prediction

1A32, 65 AA, 1.01 Å bRMSD
Protein Structure Prediction

1POU, 70 AA, 2.71 Å bRMSD
Protein Structure Prediction

1VIF, 48 AA, 1.45 Å bRMSD
Conclusions

- We have developed and validated all-atom free-energy forcefields that stabilize the native conformation of many proteins as their global optimum.
- We have developed and adapted efficient optimization methods that find the global optimum of the protein free-energy surface.
- Based on the thermodynamic hypothesis we have predictively folded several proteins with both alpha-helix and beta-sheet secondary structure.
- We can characterize the low-energy structure of the protein free energy surface (and possibly reconstruct the folding dynamics).
- Using decoy sets generated from heuristic methods we can predict the structure of proteins from many distinct structure classes.
Computational Drug Discovery

Selection of ligands as molecular switches for structurally characterized protein receptors.

Old approach: QSAR, fast but unspecific

New approach: Atomistic simulation of the docking process

In 2002: 18 drugs in clinical trials worldwide
In silico Lead Screening

- Choice of possible ligands from the database
- Synthesis and test of the selected ligands (expensive !!!)
- Improvement through combinatorial chemistry and high throughput screening
- Data base size: 10,000,000, i.e. approx 50 ms / molecule
- High specificity of the receptor-ligand pair (key-lock principle) requires atomistic simulations
- Affinity depends on intermolecular interactions
Screening of dihydrofolate reductase

- Receptor for methotrexate (MTX, pdb-entry 4dfr)
- 10000 chemical compounds from nciopen3D database
- MTX was scoring best
- Other top ranking leads display specific binding pattern

Docking to thymidine kinase

Ranking of 10 substrates against 10000 database ligands for different receptor X-ray conformations

**FlexScreen: Receptor Flexibility**

- The consideration of side-chain mobility is a significant improvement in model.
- The price is a dramatic increase in the number of variables in the optimization problem.
- While energy evaluations are more expensive, the optimization method is unaffected.

Merlitz, et.al. : J. Comp. Chem. (submitted)
Database screen with receptor flexibility

Left: Screen to rigid receptor conformation (1ki2, gcv). Docked: 8 of 10 substrates.
Right: 15 flexible bonds enabled. Docked: All 10 of 10 substrates.
IntelliScore: Adaptable Scoring Functions

Rational development of scoring functions for particular receptors and databases

(1) Perform a screen using FlexScreen to obtain ranking of ligands
(2) Synthesis and Affinity measurement
(3) Rationally adjust the Parameterization of the Scoring Function to improve the correlation between the measured and predicted affinities

Repeat steps (2)-(4) until a suitable ligand has been found
FlexScreen / IntelliScore

- The stochastic tunneling method provides an efficient docking algorithm for flexible ligand / flexible receptor screens in FlexScreen.
- FlexScreen screens the NCIopen database (ca. 250,000 ligands) in about 1 week turnaround time.
- FlexScreen is able to identify known ligands in the top of the database using an atomistic representation of receptor and ligand (industrial test with 4SC AG, München).
- The IntelliScore approach permits a rational evolution of existing all-atom scoring functions for specific receptors and databases.
Group Members, Collaborators and Funding

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