# Molecular Modelling
# Applied to NMR Structure Determination

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http://aria.pasteur.fr/documentation/courses/saclay-november-2011/saclay_nmr_school.pdf

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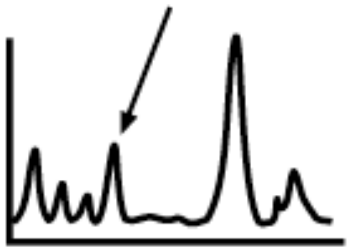
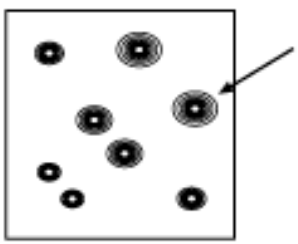
# Overview

- Introduction: the hybrid energy function
- NMR data: distances, angles, orientations, and noise
- Minimization algorithms
- Relation to probability theory

- The hybrid energy function concept
- NMR data: distances, angles, orientation
- Minimization algorithms
- Relation to probability theory

# NMR structure determination steps



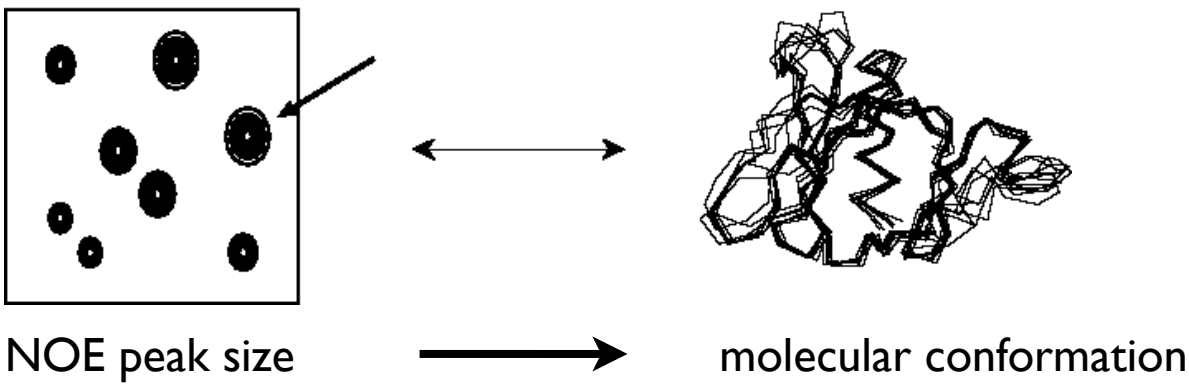


- NMR experiment
- Resonance assignment
- Structural restraints
  - distances
  - NOE assignment
  - torsion angles, orientation
- Structure calculation
- Structure validation

# Structure calculation

- Have molecule and some data on conformation...

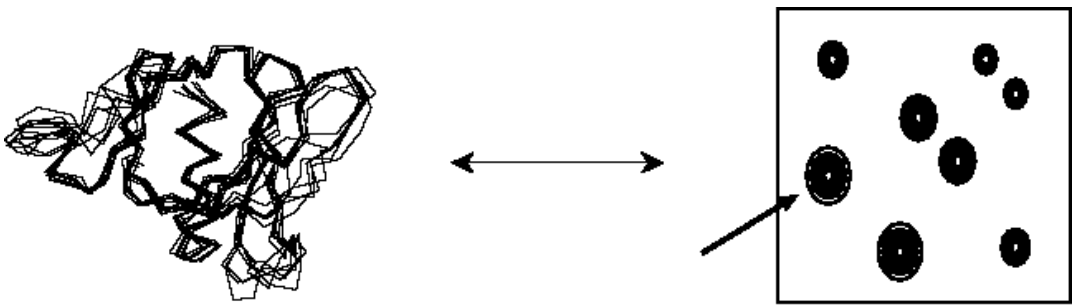
- Objectives:
- find conformation(s) satisfying experimental data
- maintain likely (local) conformation




$$NOE_{ij} \propto r_{ij}^{-6}$$

# "Theory" ;"Forward model"

- Basis: *model* to calculate data from structure
  - model (e.g.): Isolated Spin Pair Approximation for NOE
    - calculate measurement (NOE) from structure (a distance)



molecular conformation → NOE peak size

$$NOE_{ij} \propto r_{ij}^{-6}$$

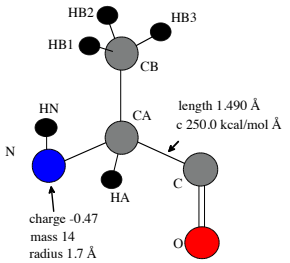
# Hybrid energy function

- weighted sum of data and force field contributions
- each data term carries its own weight
- weights determined by
  - empirical means (trial and error)
  - statistical means (cross-validation)
  - Bayesian probability

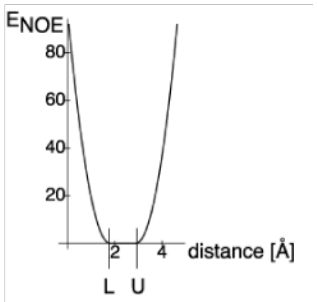
$$E_{hybrid} = E_{phys} + w_{data} E_{data}$$

# Role of molecular modelling

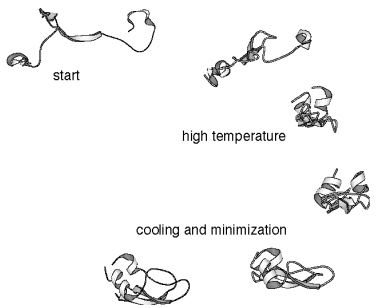
- force field: supplements experimental data by previously known information




- penalty function: provides means to restrain / constrain molecular model to data (e.g., flat-bottom potential)



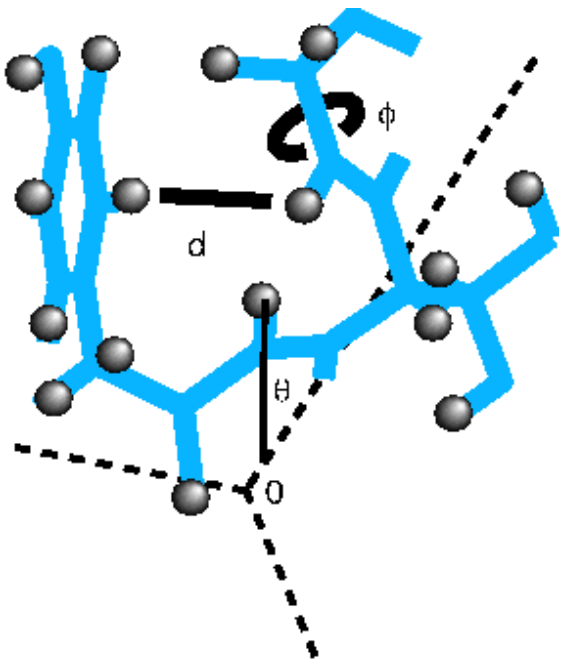

- minimization algorithm: move structure to minimize energy and satisfy data



- Introduction: the hybrid energy function
- NMR data: distances, angles, orientation, noise
- Minimization algorithms
- Relation to probability theory

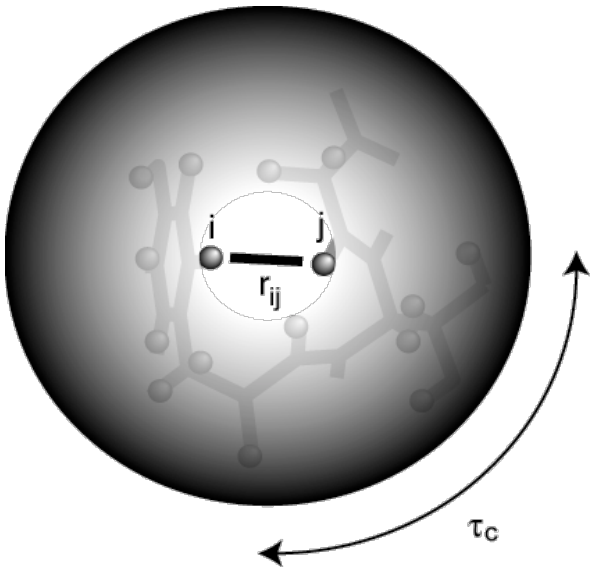
# Experimental data from liquid state NMR

- chemical shift
  - local electronic environment; distances
  - torsion angles
- scalar coupling constants
  - torsion angles
  - distances (hydrogen bonds)
- NOE, ROE
  - interproton distances
- paramagnetic atoms
  - distances, orientation
- residual dipolar couplings etc.
  - bond orientation



# Measurement of distances: NOE

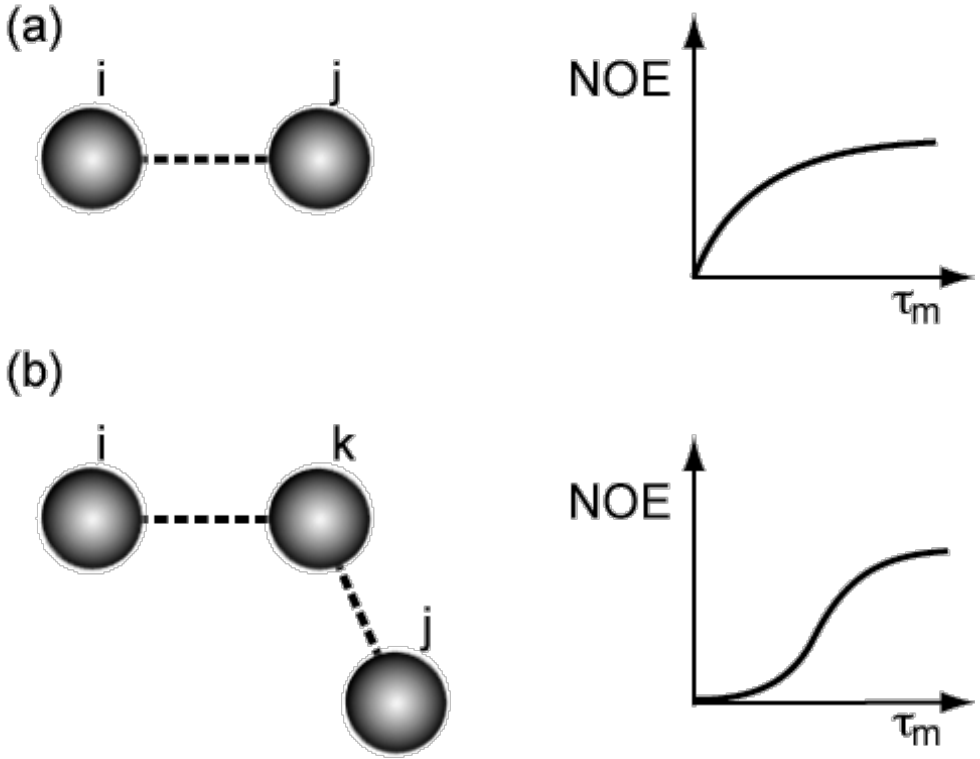
- size of NOESY peak depends on
- crossrelaxation rate
  - the distance between the two protons
  - overall rotational motion
  - internal motion
- mixing time
- ... presence of other protons




$$\sigma_{ij} \propto r_{ij}^{-6} f(\tau_c)$$

# Buildup curves to measure σ

- Crossrelaxation rate from slope of buildup curve
- measure several NOESY at different mixing time
- distinguishes between direct (a) and indirect (b) NOEs



# Distance from crossrelaxation rate/ NOE

- Interproton distance from
  - crossrelaxation rate (neglects internal dynamics)

$$r_{ij} = \left(\sigma_{ij} f\left(\tau_c\right)\right)^{-\frac{1}{6}}$$

- Crossrelaxation rate from:
  - NOE measurements at several mixing times (buildup curve)
  - NOE measurement and relaxation matrix calculation
  - Approximately: use NOE volume/ intensity (neglects spin diffusion)

$$r_{ij} \approx \left(C_{cal} V_{ij}\right)^{-\frac{1}{6}}$$

# Typical interproton distances in proteins

| | | | |
|---|---|---|---|
| methylene group | dHβ2-Hβ3 | 1.8 Å | very strong NOE |
| aromatic | dHδ-Hε | 2.4 Å | strong NOE |
| antiparallel β sheet | dHα-Hα | 2.2 Å | strong NOE |
| parallel β sheet | dHα-Hα | 2.7 Å | strong NOE |
| α helix | dHN-HN(i,i+1) | 2.7 Å | strong NOE |
| α helix | dHα-HN(i,i+3) | 3.3 Å | medium NOE |

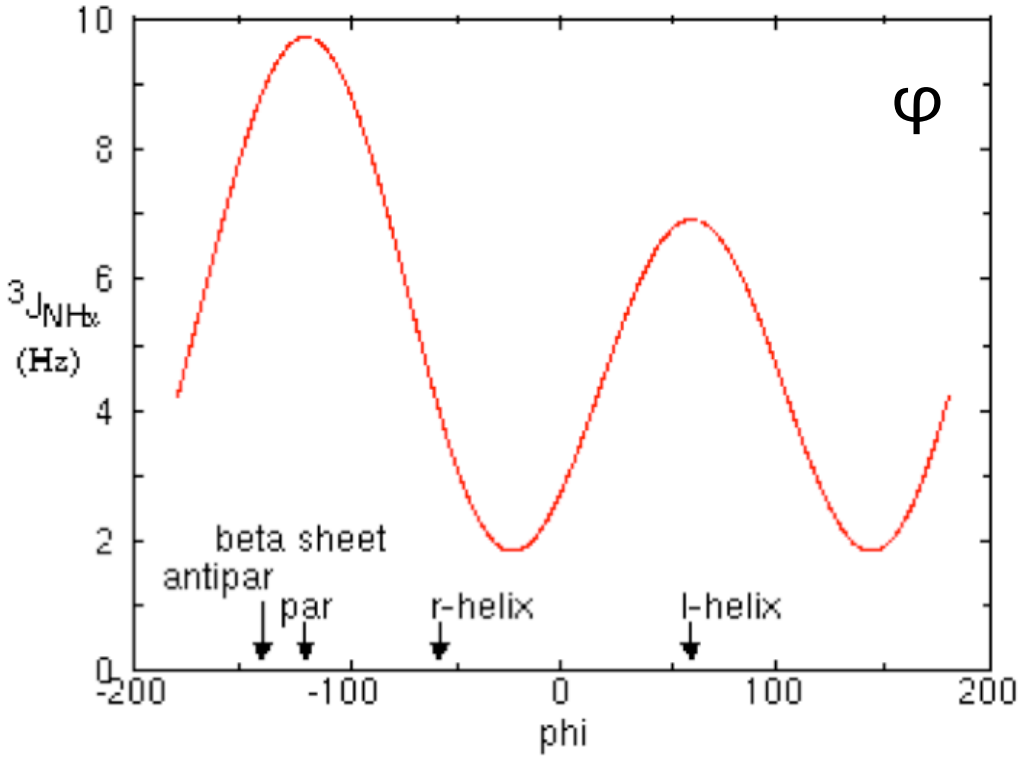
1 Å = 0.1 nm

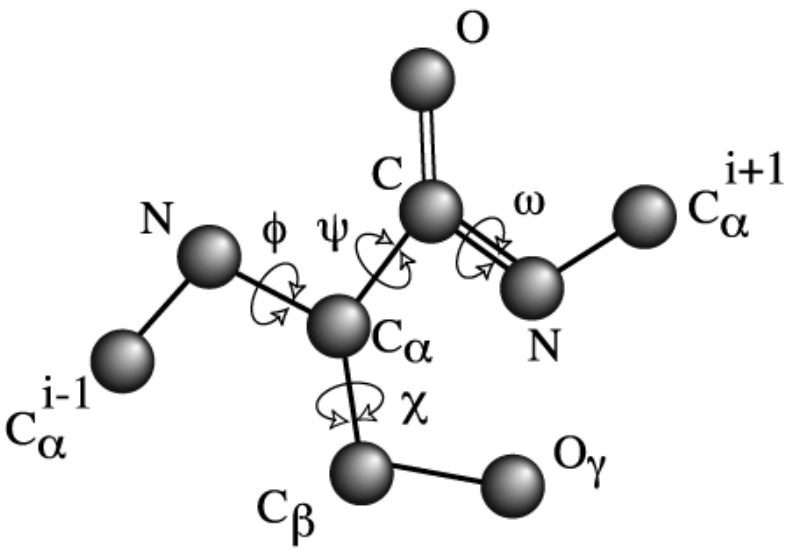
- Known distances can be used to approximately convert NOE peak volumes into distance ranges

# NOE summary

- richest source of structural information; most important data for structure determination by NMR
- very powerful for qualitative analysis of structures (assignment of secondary structure)
- interactions between residues far apart in sequence
- potentially large errors due to approximate theory to convert NOEs to distances: approximate distance ranges

# Angular information from coupling constants




- 3-bond coupling depends on torsion angle

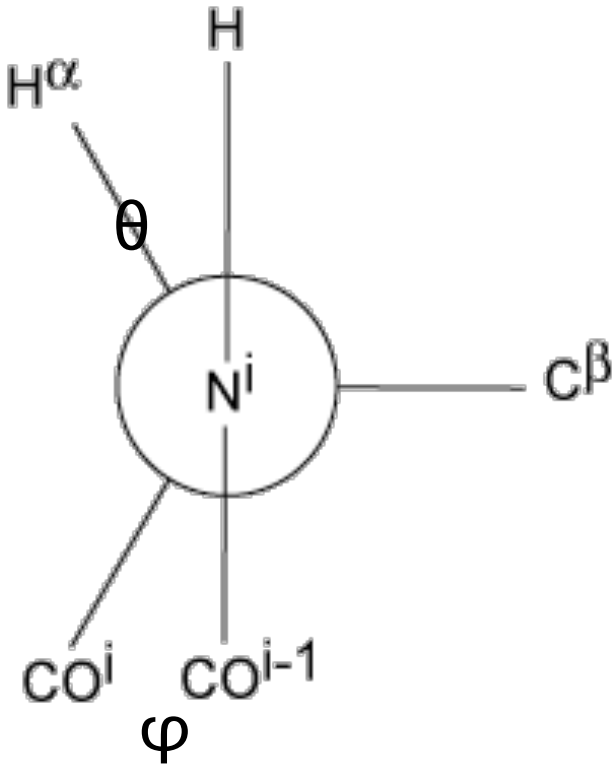
# Coupling constant and angle φ

- General dependency of J on angle:
- Karplus-relationship

$$J = A + Bcos(\theta) + Ccos(2\theta)$$

$$\phi = \theta - 60$$

- The parameters A, B, C need to be parametrized with known (X-ray) structures




| A | B | C |
|---|---|---|
| 6.51 | -1.76 | 1.60 |
| 6.41 | -1.46 | 1.90 |
| 6.98 | -1.38 | 1.72 |

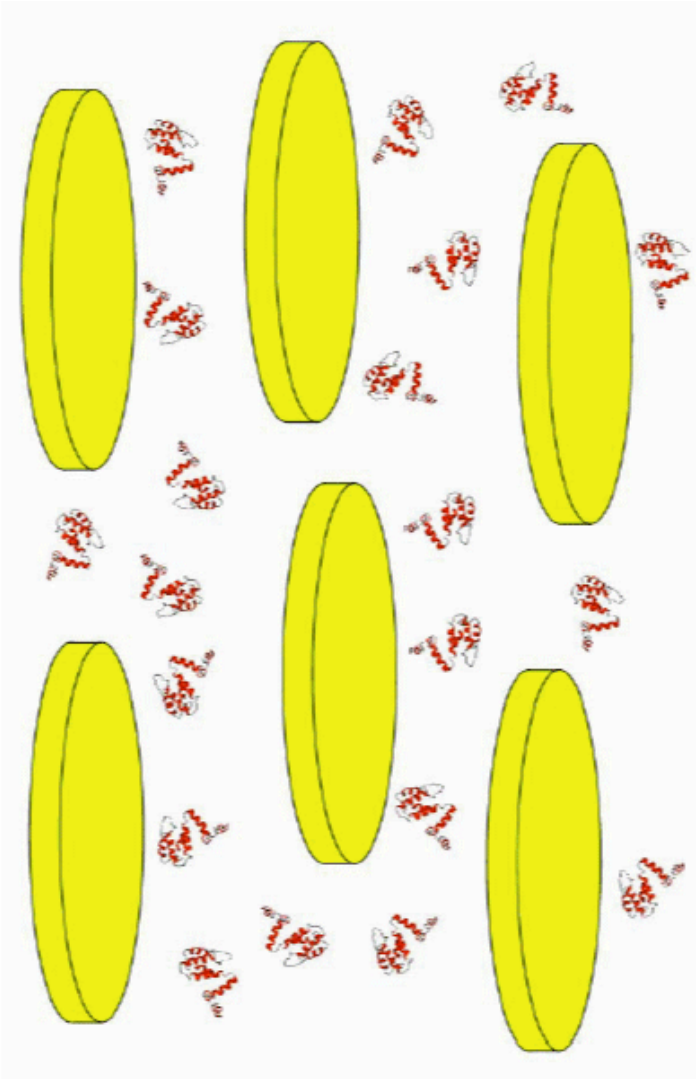
# Residual dipolar couplings from partial alignment

- Partial alignment due to:

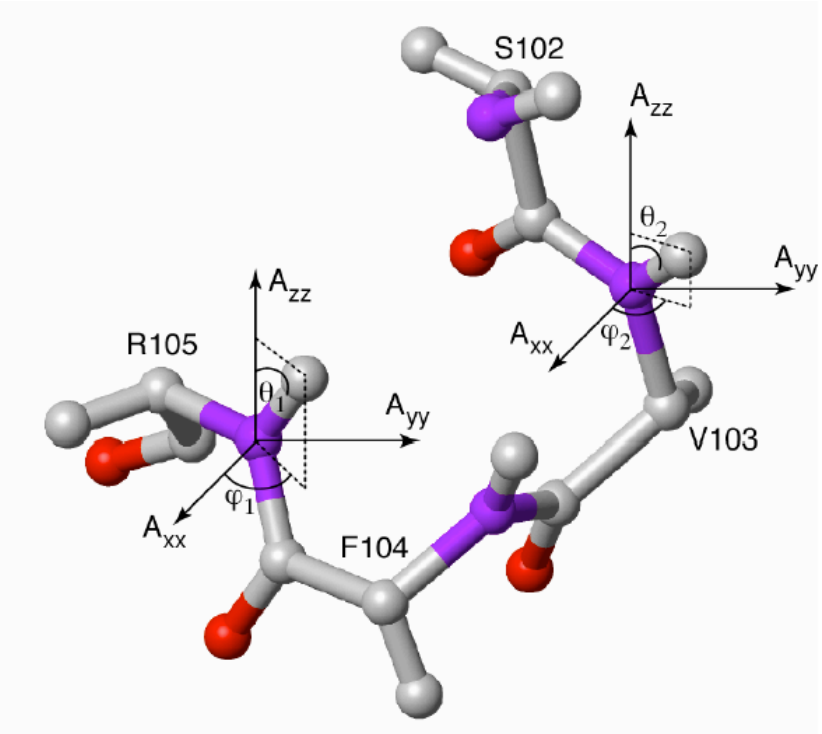
- bicelles
- purple membranes
- phages...

- magnetic interactions (DNA)

- paramagnetic tags...



# Residual dipolar couplings




- Proteins:
  - direction of bond vectors (e.g., N-H) can be determined
  - relative to coordinate system attached to molecule

$$D^{res} \propto \frac{\gamma_i \gamma_j}{r_{ij}^3} \left[ D_{ax}(3\cos^2(\theta) - 1) + \frac{3}{2} D_{rh} \sin^2(\theta) \cos(2\phi) \right]$$

# Noise in Data

- All data contain errors (experimental noise)
- All forward models contain approximations
- No ideal agreement between calculated and measured data possible
- Penalty function for data needs to contain way to include noise
- Automated methods to detect "noise peaks" (violation analysis, network anchoring)

# Distance measurements contain errors

- NOEs only give approximate measure of distance
- measurement errors
  - evaluation of peak volumes
  - experimental parameters
- errors in conversion to distance
  - how to measure crossrelaxation rate?
  - spin diffusion
  - internal dynamics
  - peak broadeing

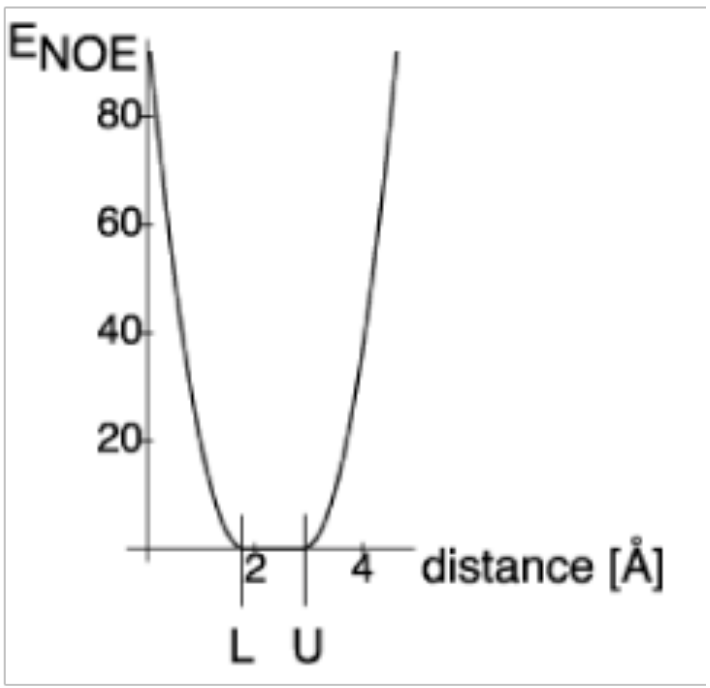
# Distance ranges

| NOE | lower bound | upper bound |
|---|---|---|
| very strong | 1.8 Å | 2.5 Å |
| strong | 1.8 Å | 2.8 Å |
| medium | 1.8 Å | 3.6 Å |
| weak | 1.8 Å | 5.0 Å |
| very weak | 1.8 Å | 6.0 Å |

- Error in measurement:
- derive consistent bounds on distance
- set bounds based on statistical analysis of known structures

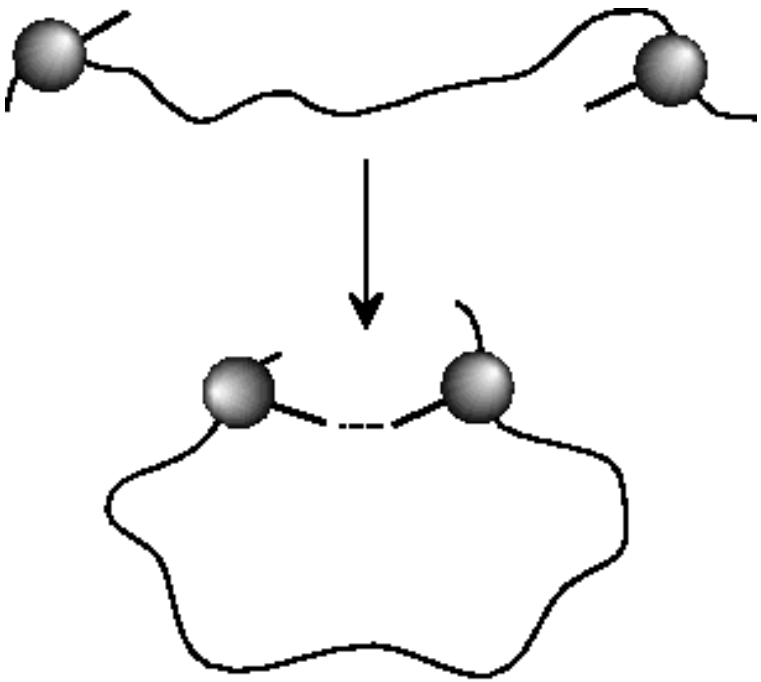
# Standard NOE distance restraint potential

$$E_{data} \propto \sum_{i}^{N_{noe}} \begin{cases} (r_i(\mathbf{X}) - L_i)^2 & \text{if} \quad r(\mathbf{X}) < L_i \\ 0 & \text{if} \quad L_i \leq r(\mathbf{X}) \leq U_i \\ (r_i(\mathbf{X}) - U_i)^2 & \text{if} \quad r(\mathbf{X}) > U_i \end{cases}$$




- sources of error
  - measurement, spin diffusion, internal dynamics
- loose upper and lower bounds
- FBWH potential
  - flat bottom harmonic walls
  - no force between L and U

# Consequence of bounds



- Bounds have to be large enough for cumulative error
- Precise value not (too) important:
- even loose bounds restrict conformational space

- May affect
  - precision of structure
  - validation
  - noise peak recognition (see below)

# Other ways to treat noise

- potential form
- weight in hybrid energy function

$$E_{hybrid} = E_{phys} + w_{data} E_{data}$$

# Data summary

- Forward models contain non-measurable parameters that are necessary for the modelling
  - calibration factor
  - Karplus parameters for scalar couplings
  - tensor parameters
- Data potential needs to include parameter to treat (unknown) "noise"
- The weight in the hybrid energy needs to be set by empirical means

# ...

- influenced by internal dynamics:

- relaxation times
- NOE, ROE

- most data describe
  - the local environment of the protons
  - ...relative to each other
  - not the global conformation of the molecule

- Introduction: the hybrid energy function
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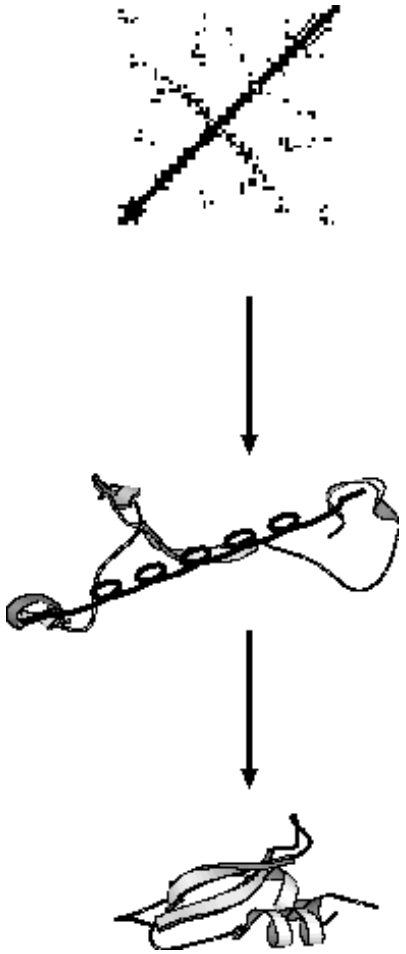
# 3D structure calculation

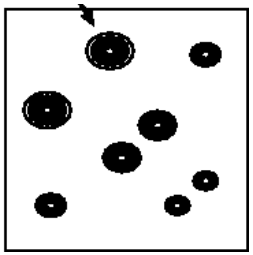
- convert data (1D, 2D)
- + forcefield
- into 3D model



# Structure calculation methods: minimize hybrid energy

- Metric matrix distance geometry (DISGEO, DG2)
- (Energy) minimization ("buildup method", DIANA)
- Simulated annealing (molecular dynamics) from random structures (X-PLOR, CNS)
- Simulated annealing (torsion angle dynamics) from random structures (X-PLOR, CNS, DYANA)

# Data from structure



- Basis of structure calculation: calculate data
  - NOE
    - approximate: distances
    - NOE from relaxation matrix calculations
  - Coupling constants
    - approximate: torsion angles/ Karplus relations;
    - QM calculations
  - RDCs
    - bond orientations in alignment tensor
  - Chemical shifts
    - empirical relations; QM calculations

- include error due to measurement/ approximations

# Structure from data

- Two principles:

- Minimization
  - make random proposal for structure
  - calculate data from structure
  - compare with experiment
  - modify structure to improve agreement

- Sampling
  - make random proposal for structure
  - calculate data from structure
  - make new random proposal

# Structure from data

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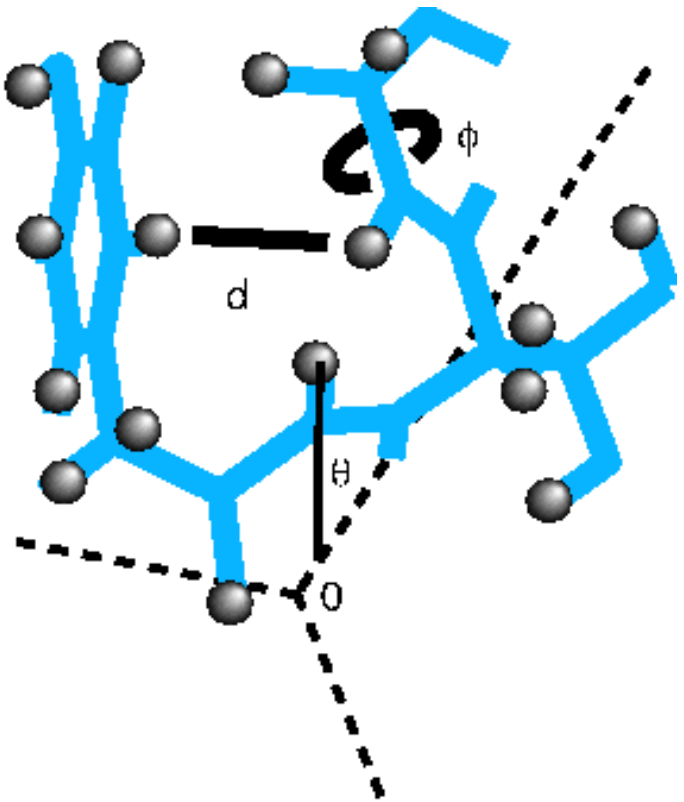
  - calculate data from structure
  - make new random proposal

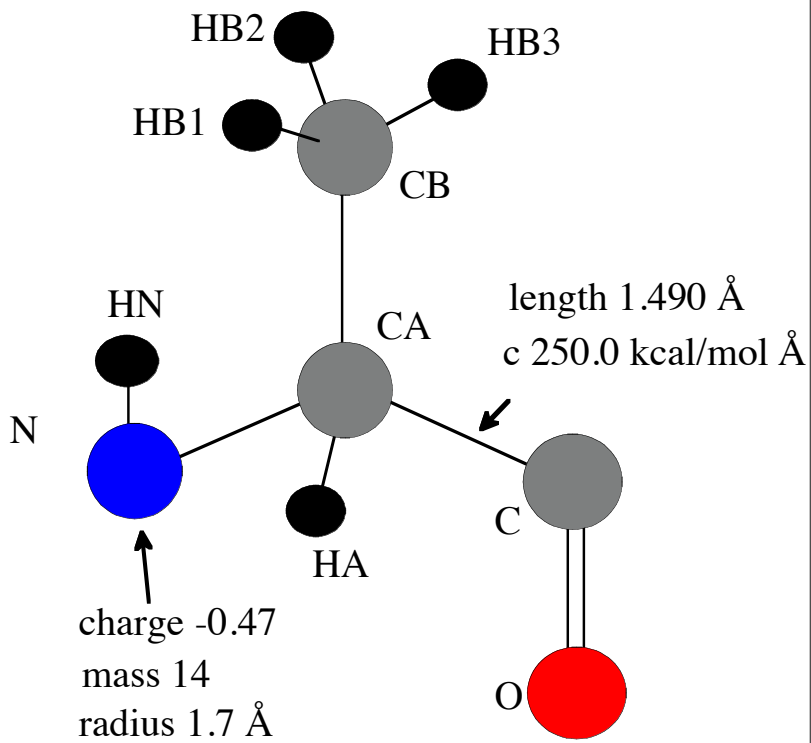
# Experimental distances insufficient

- Data are incomplete:
  - NOE distance ranges only for protons
  - torsion angle ranges for some atoms
  - (orientation for some bonds)
- for most atoms no direct experimental observation



# Additional data: prior information

- Need prior information for building blocks: amino acids or nucleic acids
- topology (which atoms are connected)
- parameters
  - bond lengths
  - bond angles
  - planarity
  - chirality
  - atomic radii



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# Structure calculation: Simulated Annealing

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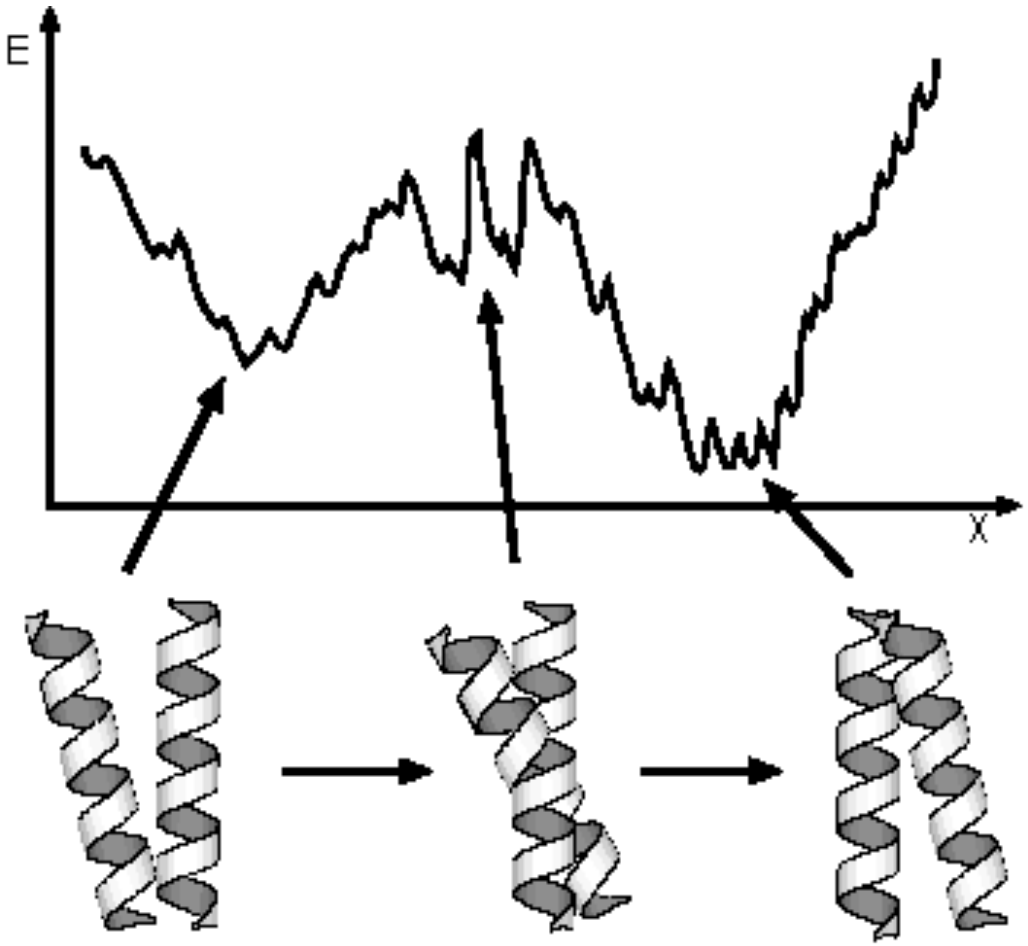
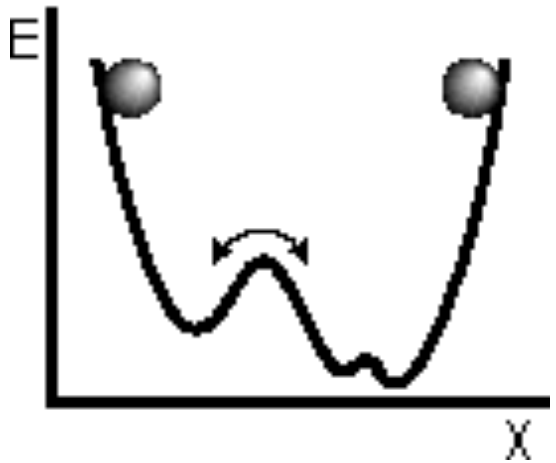
# Multiple minimum problem

High energy barriers to fold protein

Standard minimization only "downhill"



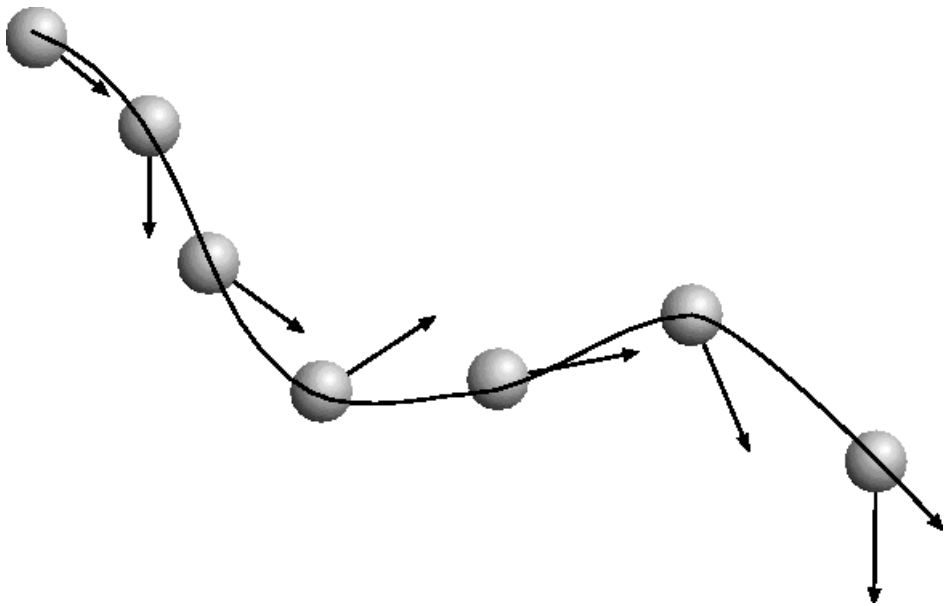
# Minimization by molecular dynamics




$$\frac{d^2 \vec{r}_i}{dt^2} = -\frac{c}{m_i} \frac{\partial}{\partial \vec{r}_i} E_{hybrid}$$

- Molecular dynamics solves Newton's equations of motion
- Molecular dynamics can overcome local energy barriers

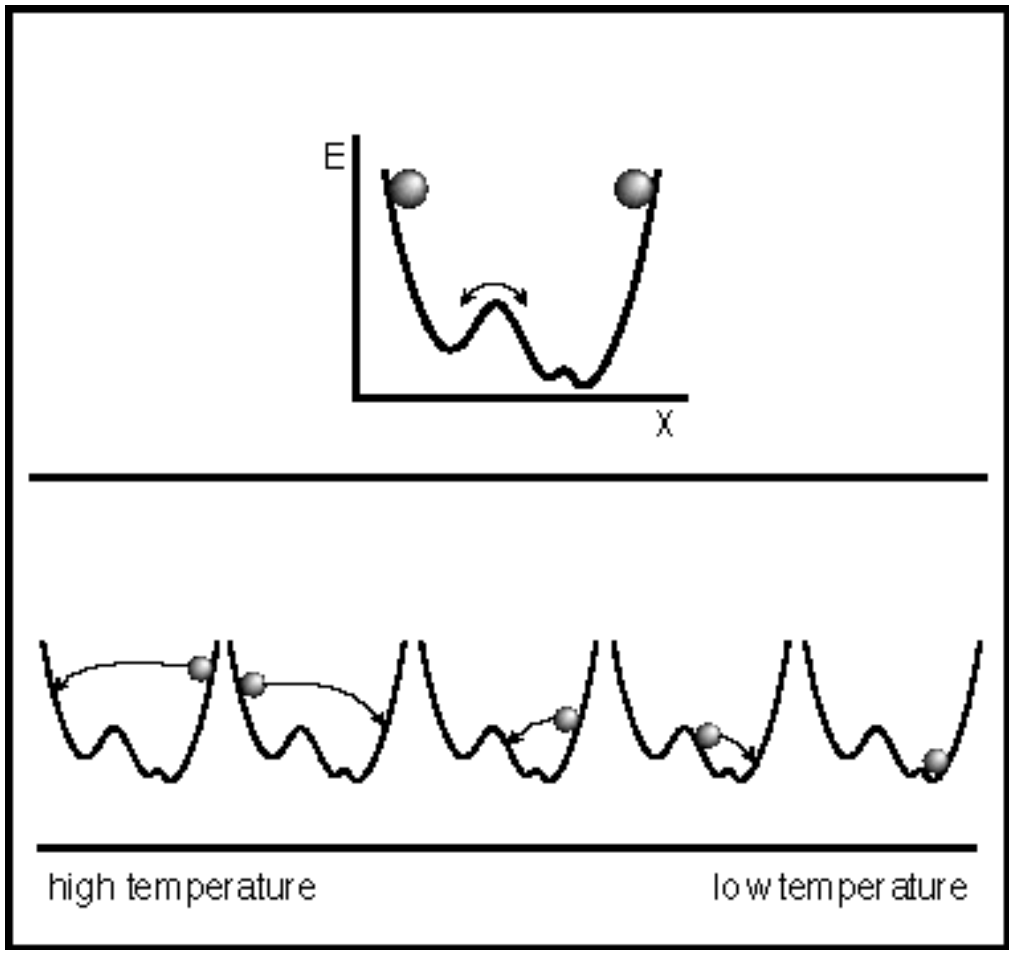
# Newton dynamics



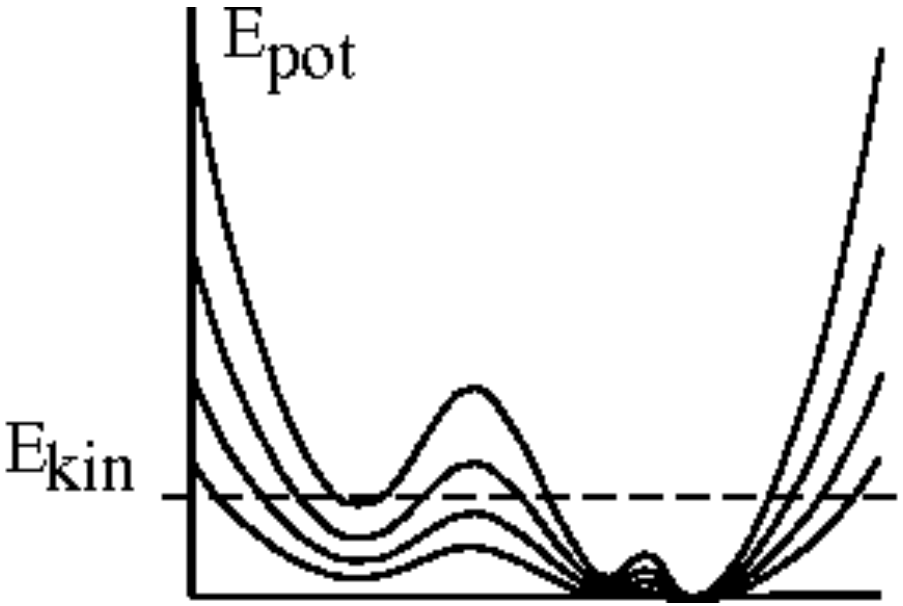
- Direction of motion depends on
- force (derived from force field and experimental restraints)
- momentum

# Temperature control and variation: "MD-simulated annealing"





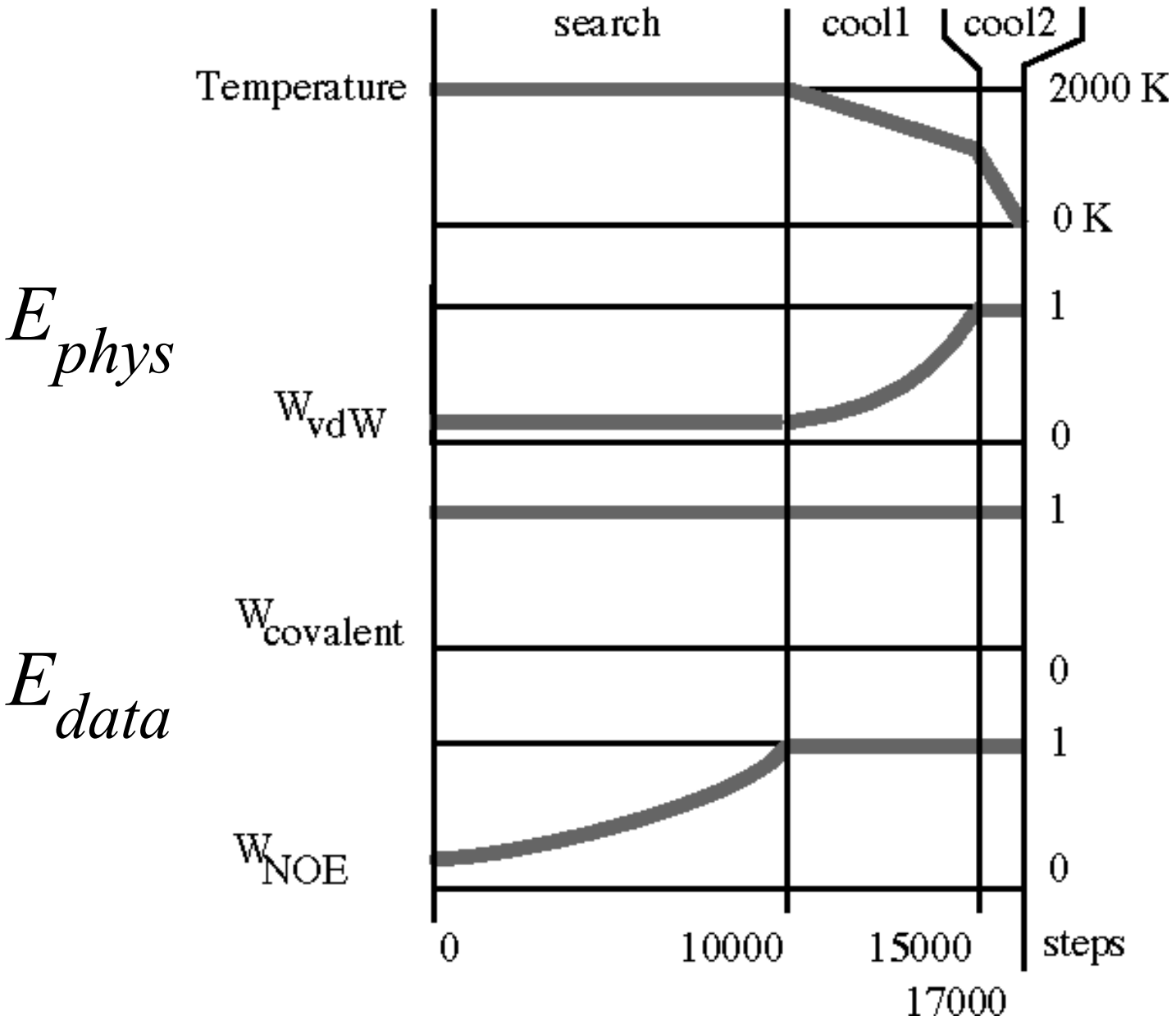
# Energy scaling



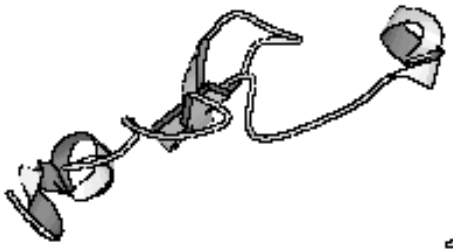

- more flexible annealing schemes
- different variation of different energy terms

- e.g.:
- $E_{chem}$ / $E_{exp}$
- $E_{covalent}$ / $E_{exp}$ / $E_{nonbond}$

- equivalence:
- mass/ energy/ temperature scaling


search
cool1
cool2
Temperature
2000 K
0 K
$E_{phys}$
1
$W_{vdW}$
0
1
$W_{covalent}$
$E_{data}$
0
1
$W_{NOE}$
0
0
10000
15000
steps
17000

start

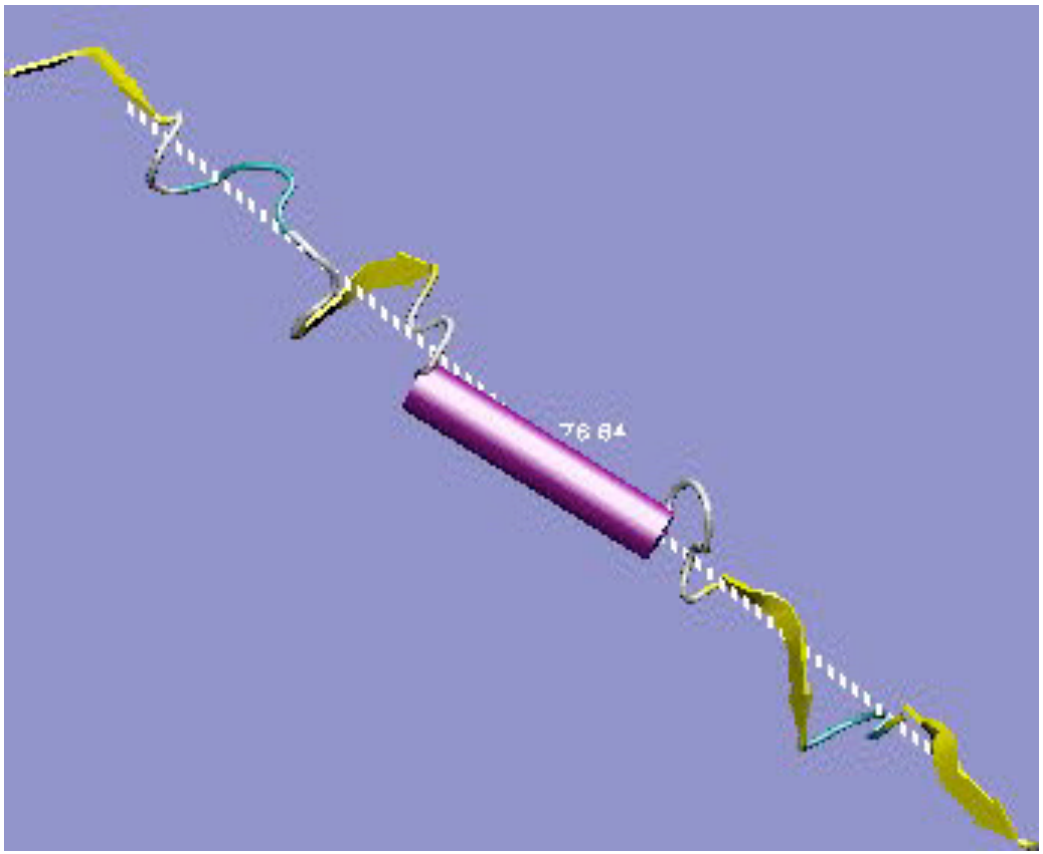


high temperature

cooling and minimization

# Structure calculation with MD

- NMR data: distances
- Start: random structure
- Difficult search problem: many degrees of freedom



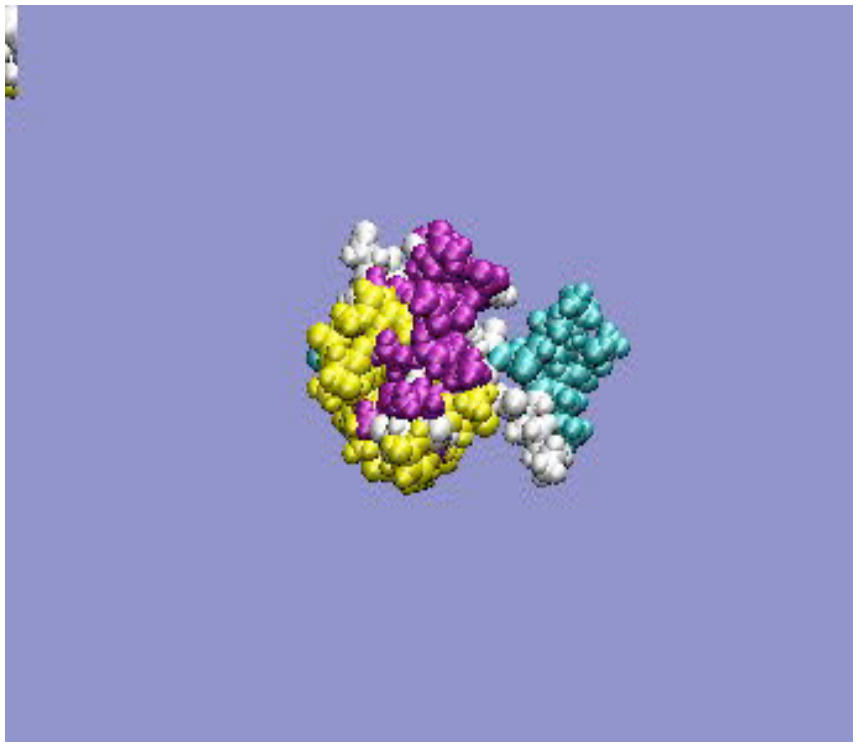
# Structure calculation with MD

1988:

48 hours per structure on mainframe (DISGEO, Havel)

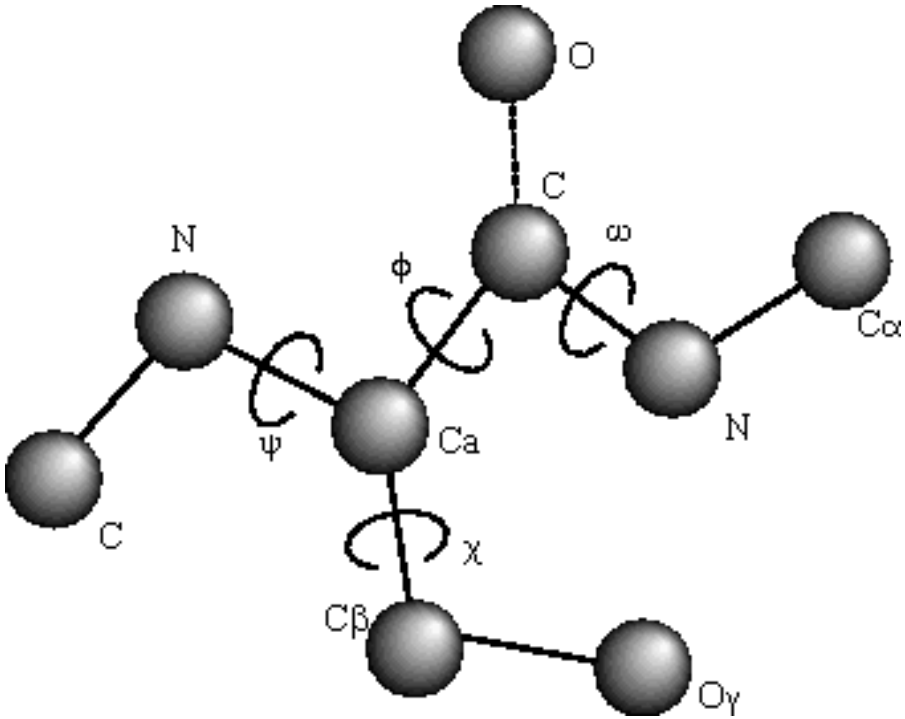
2001:

seconds per structure on PC



# Torsion angle dynamics

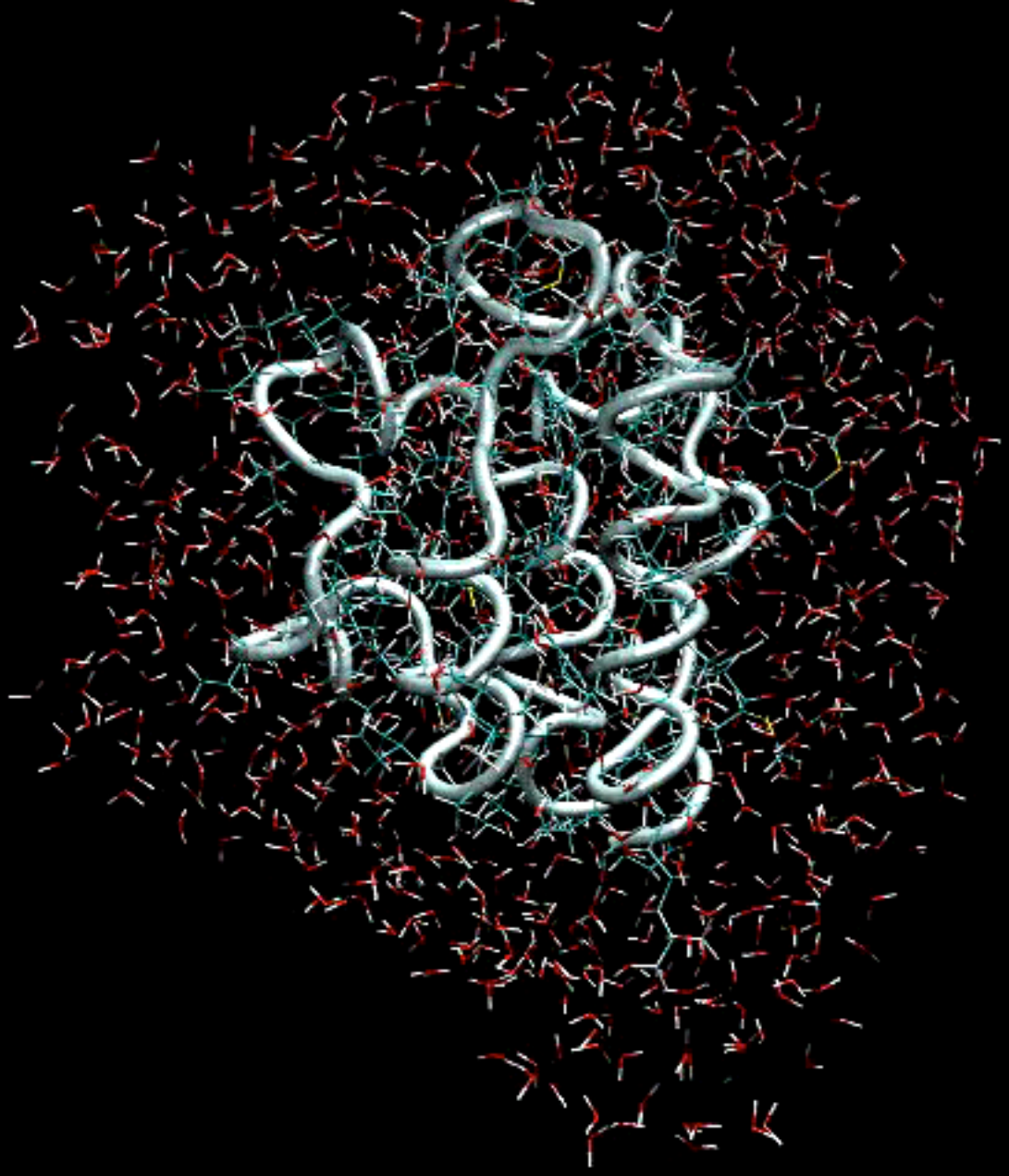
- dynamics time step dictated by bond stretching: waste of CPU time
- important motions are around torsions
- ~ 3 degrees of freedom per AA
- (cf 3Natom for Newton dynamics)
- Available in DYANA, X-PLOR, CNS, X-PLOR-NIH



# Typical protocol

- calculation with simplified force field, torsion angle dynamics
  - no electrostatics, simplified van der Waals
- refinement with Cartesian dynamics
- very short final refinement with "full" force field in water

# Final refinement in H2O



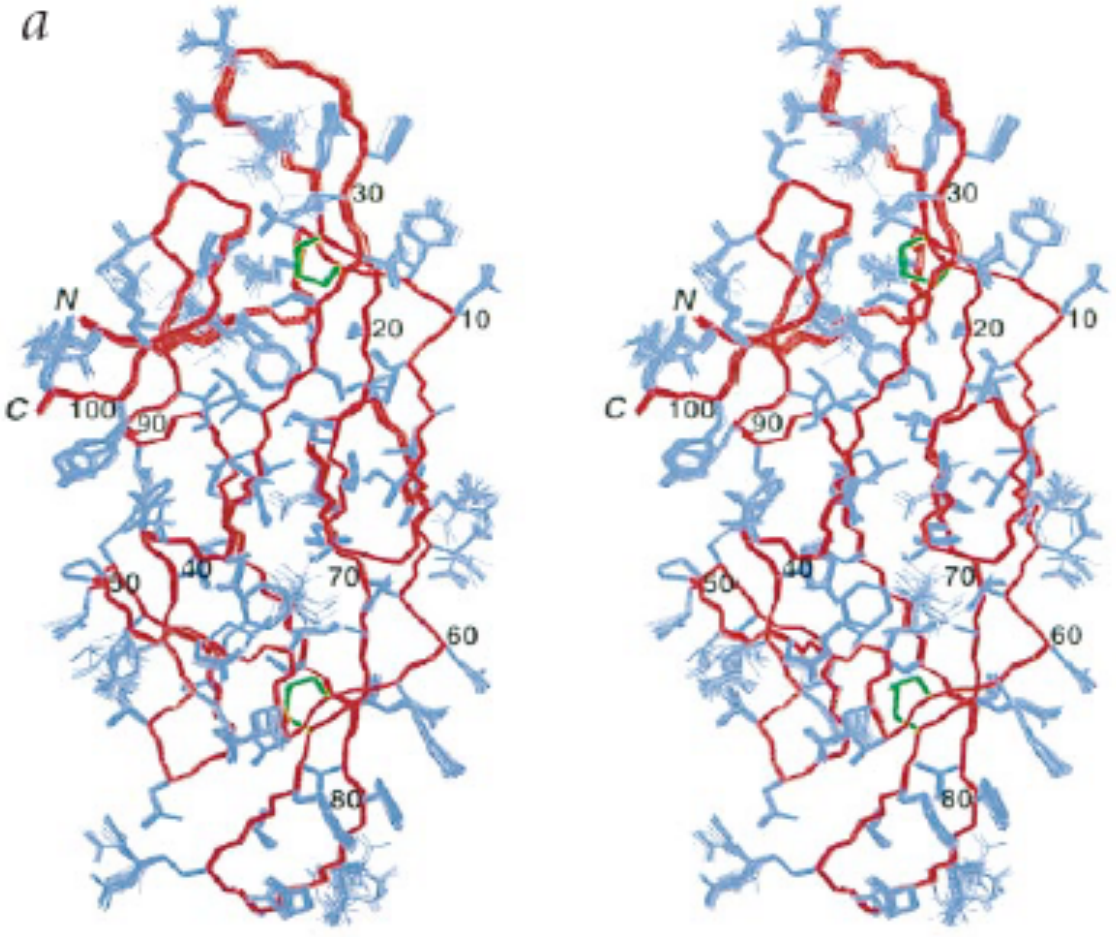
# Calculation of structure ensembles

- with identical data/ restraints:

- repeat calculation (20-100 times)
- random variation of initial conditions (starting structure/ velocities)

- obtain information on
  - uniqueness / different folds
  - "dynamics"

# High quality structure ensembles



# Meaning of structure ensembles

- Simple way to assess uniqueness of solution
- This has very little to do with dynamics
- Distribution depends on
  - data
  - data representation
  - algorithm
  - forcefield
  - algorithm parameters
  - ...

# Evaluation of structures

- Energetic criteria
  - $E_{chem}$
  - RMS from ideal values for covalent interactions
  - number of large deviations
- Comparison to other structures, "knowledge-based"
  - e.g., WhatIf
- Satisfaction of experimental data
  - restraint violations
  - $E_{data}$
  - RMS from data / bounds
- Statistical criteria for data
  - crossvalidation
- `http://proteins.dyndns.org/cing`

- Introduction: the hybrid energy function
- NMR data: distances, angles, orientation
- Minimization algorithms
- Relation to probability theory

# Minimisation and probability

- Where do potential forms come from
- Where do all the parameters come from
  - bounds
  - weights
  - any parameter required by theory

# Probability and energy

$$E_{hybrid} = E_{phys}(\mathbf{X}) + w_{data} E_{data}(D, \mathbf{X})$$

# Probability and energy

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- force field: background information I

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$$E_{hybrid} = E_{phys}(\mathbf{X}) + w_{data} E_{data}(D, \mathbf{X})$$

- force field $E_{phys}$ ⇔ probability (Boltzmann)
- probability of distortion of molecule
- force field: background information I
- prior probability

$$P(\mathbf{X}|I) = \exp\left[-\frac{E_{phys}(\mathbf{X})}{kT}\right]$$

# Probability and energy

$$E_{hybrid} = E_{phys}(\mathbf{X}) + w_{data} E_{data}(D, \mathbf{X})$$

# Probability and energy

$$E_{hybrid} = E_{phys}(\mathbf{X}) + w_{data} E_{data}(D, \mathbf{X})$$

- similar: $E_{data}$ ⇔ probability

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- probability that data is correct, given structure X:

# Probability and energy

$$E_{hybrid} = E_{phys}(\mathbf{X}) + w_{data} E_{data}(D, \mathbf{X})$$

- similar: $E_{data}$ ⇔ probability
- probability that data is correct, given structure X:
- "likelihood"

# Likelihood

- Example:
- Gaussian distribution of error for r,
- standard deviation σ,
- ⇒ probability is

$$P(D|\mathbf{X},\sigma) \propto \exp\left[\frac{-(r-r(\mathbf{X}))^2}{2\sigma^2}\right]$$

# Likelihood and restraint potential

- Inversely, if we know probability distribution, we can derive potential

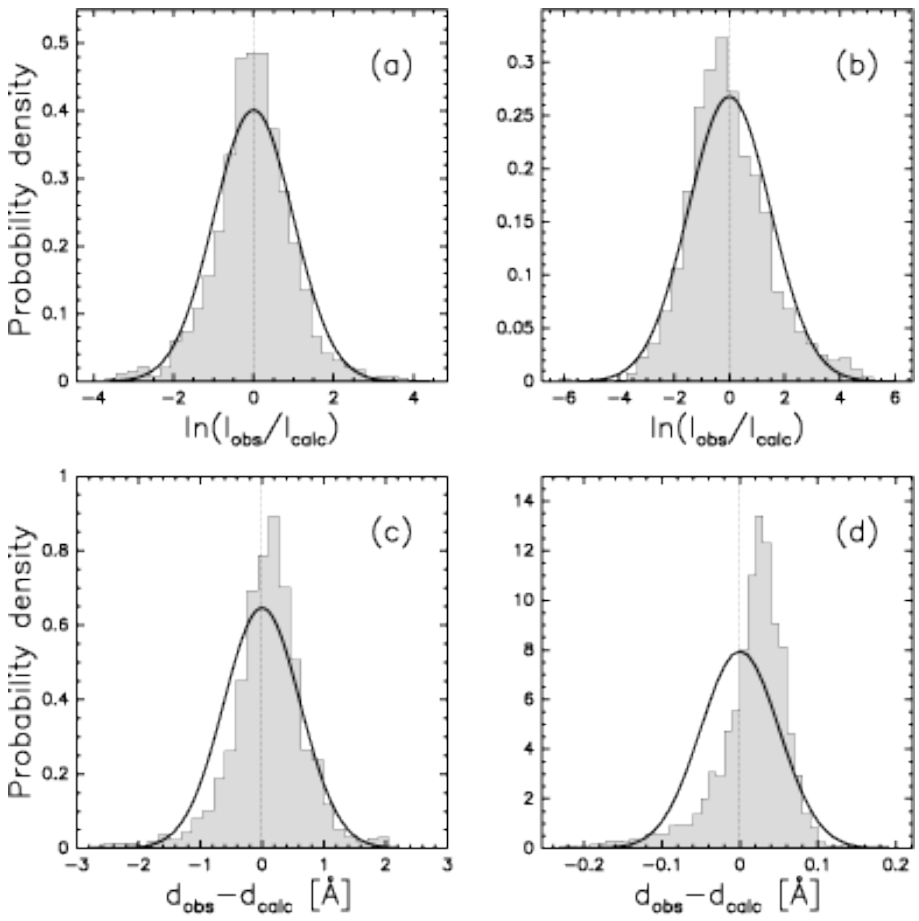
$$E_{data} \propto -log\left[P(D|\mathbf{X},\sigma)\right]$$

- For Gaussian error, harmonic potential ("least squares")

$$E_{data} \propto \frac{1}{2\sigma^2}\left(r - r(\mathbf{X})\right)^2$$

- The weight is related to the error in the data

# Distances (NOEs) do not follow Gaussian



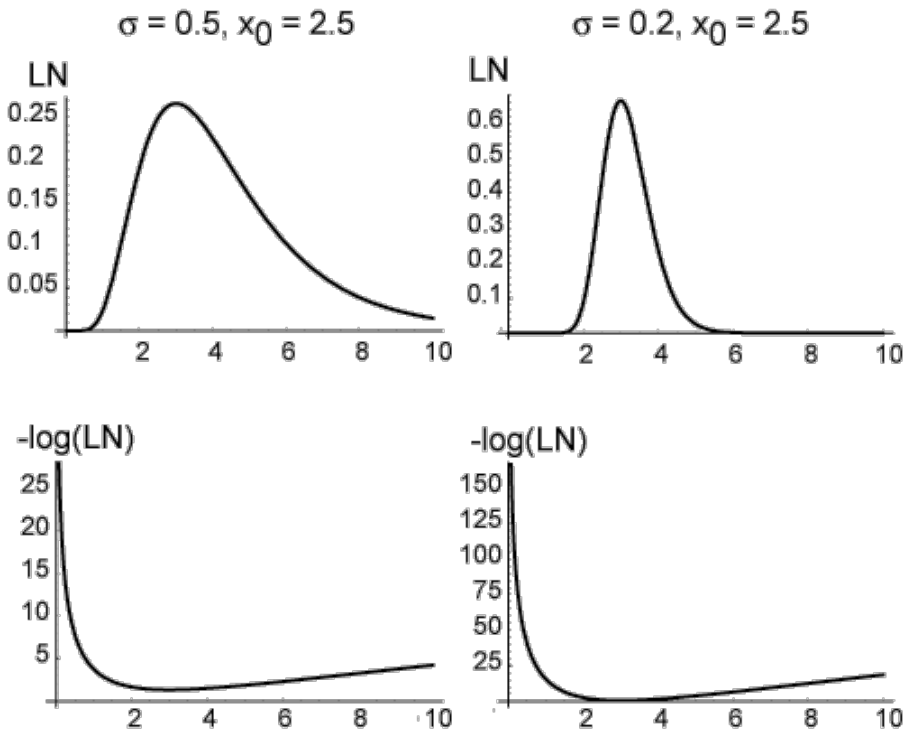

Gaussian distribution of logarithms

Gaussian distribution

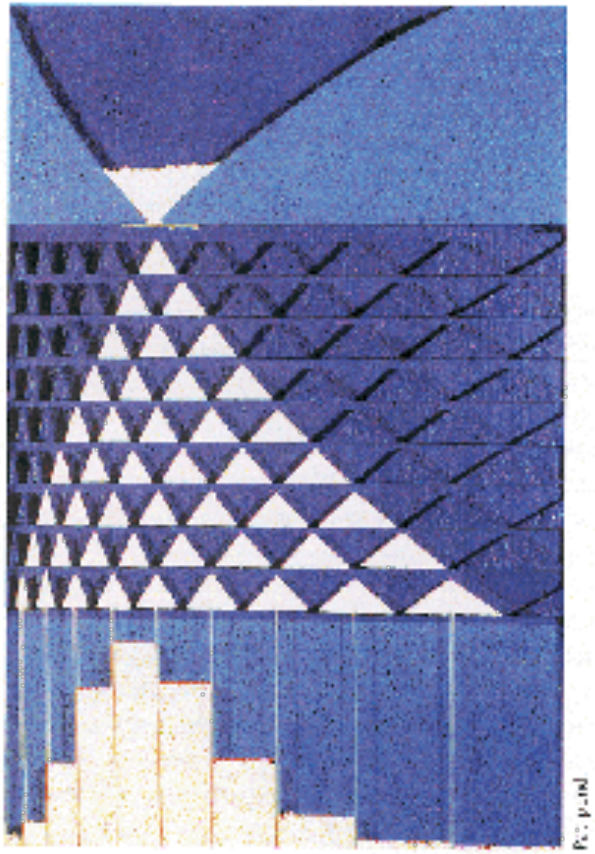
Rieping, Habeck, Nilges, JACS 2005

# Log-normal distribution

- Log-normal distributions
- and derived potentials




$$\mathrm{LN}(x_0, x, \sigma) \equiv \frac{1}{\sqrt{2\pi\sigma^2}x_0} \exp[-\frac{1}{2\sigma^2}(\log[x_0] - \log[x])^2]$$



# ...Life is LogNormal

- ... there are a lot of data with only positive values
- examples on
- http://stat.ethz.ch/~stahel/lognormal/
  - no theoretical derivation

| Disciplines | | mu* | sigma* |
|---|---|---|---|
| Medicine | Onset of Alzheimer disease | ~ 60 years | 1.2 |
| | Latent periods of infectious diseases | Hours to months | 1.5 |
| | Survival time after diagnosis of cancer | Months to years | 3 |
| Environment | Air pollution in the U.S.A. | 40-110 PSI | 1.5-1.9 |
| | Rainfall | 80-200 m3 (x103) | 4-5 |
| | Species abundance in ecology | - | 6-30 |
| Social sciences and linguistics | Income of employed persons | 6.700sFr | 1.5 |
| | Lengths of spoken words | 3-5 letters | 1.5 |

# Joint probability from prior and likelihood



- To calculate joint probability from single probabilities, multiply:

Probability of a structure:
Posterior Probability

likelihood

$$P(\mathbf{X}|D,I) \propto P(\mathbf{X}|I)P(D|\mathbf{X},\sigma,I)$$

prior distribution

# Hybrid energy revisited



$$E_{hybrid} = E_{phys}(\mathbf{X}) + w_{data} E_{data}(D, \mathbf{X})$$

- The hybrid energy function is negative logarithm of joint probability
- Minimum energy corresponds to maximum probability
- Relative weight "should" depend on data quality
- story is incomplete (what about $w_{data}$?)

Probability of a structure

likelihood

$$P(\mathbf{X}|D, I) \propto P(\mathbf{X}|I) P(D|\mathbf{X}, \sigma, I) \bullet \bullet \bullet$$

prior distribution

# Bayesian determination of data weight

$$E_{hybrid} = E_{phys} + w_{data} E_{data}$$

- Data weight has influence on structure quality
- Bayesian analysis:

$$w_{data} = \frac{k_B T}{2RMS^2}$$

- Update iteratively during structure calculation
- weight ⇔ overall data quality
- only possible for "least squares"-type potential

Habeck M, Rieping W, Nilges M (2006). PNAS 103:1756

# Summary

- Minimizing hybrid energy corresponds to maximizing the probability of a structure, given data and force field
- ...if one knows the data quality, scale factors, ...
- Relative weights
  - usually set empirically (trial and error, experience, cross validation)
  - Bayesian determination of weight possible
- Relationship of error distribution and restraint potentials

1. Introduction: relating data to structure
2. Hybrid energy and treatment of errors
3. Minimisation of hybrid energy
4. Relation to probability theory

# Problems inherent in minimisation

data are incomplete: solution is degenerate
data are inconsistent: strictly speaking, no solution exists
many unknown parameters are necessary ("nuisance parameters")
no objective figures of merit for structures
no consistent concepts of data quality evaluation

# Example NOE

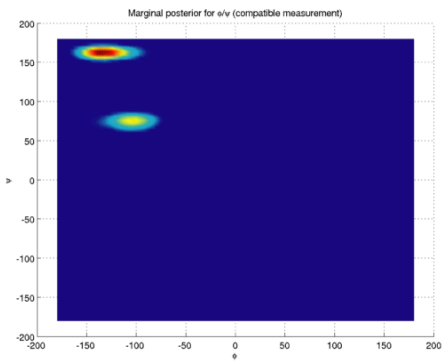
- incompleteness: assignments, NMR-"visibility"
- inconsistency: approximate theory, noise
- unknown quantities: calibration, data consistency

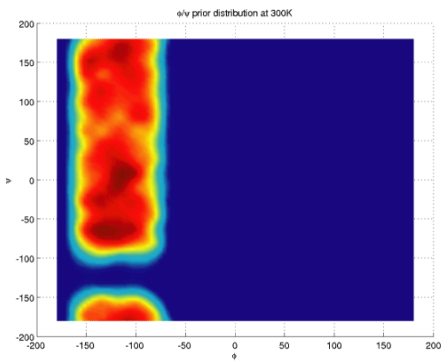
- basic question: how well do my data determine the structure remains unanswered, need of heuristics:
  - cross validation
  - independent validation

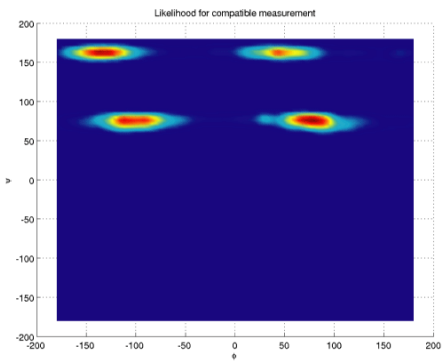
- standard approach works in practice with sufficient data of good quality
- sparse data:
  - problems with determining auxiliary parameters
  - structure calculation difficult
- no estimation of uncertainties in coordinates or data
  - RMSDs and R-factors depend on all auxiliary parameters
  - few restraints can change result drastically
  - no concept to evaluate data quality (”don’t overfit”...“use data not used in structure calculation”...)

# Inference instead of deduction

- *inference*:
  - assign a *probability* to *each* molecular conformation
- use probability theory:
  - prior probability from physical model (force field)
  - likelihood from forward model



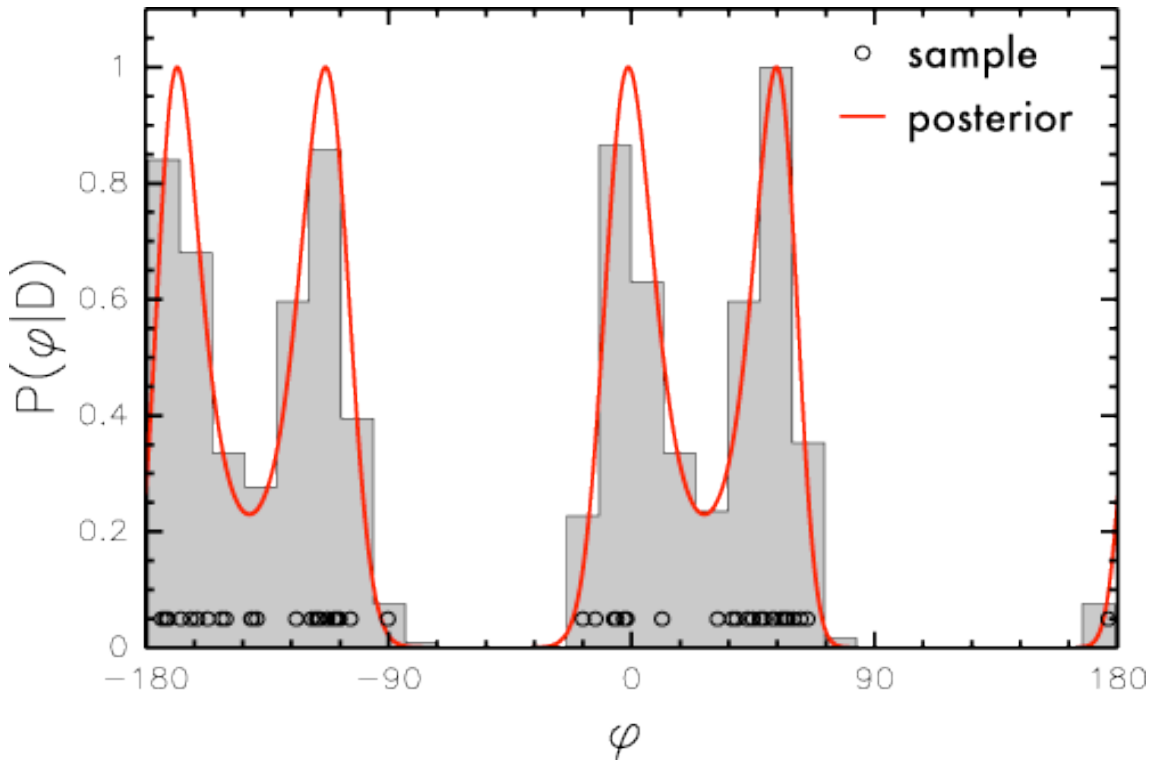




**posterior** **prior** **likelihood**

$$P(\theta, \sigma, \gamma | D, I) \propto P(\theta, \sigma, \gamma | I) P(D | \theta, \sigma, \gamma, I)$$

# Sampling




- Posterior P(X|D) is extremely complex for realistic problem
  - too many degrees of freedom to do "integration"
- Take representative samples (Markov Chain Monte Carlo)

# Sampling probability distributions



- Sampling is computationally much more complex than structure calculation by minimization
- cf calculating partition function in statistical mechanics

- Algorithm uses
  - hybrid Monte Carlo
  - torsion angle dynamics
  - replica exchange
  - Tsallis distribution

# Sampling probability distributions



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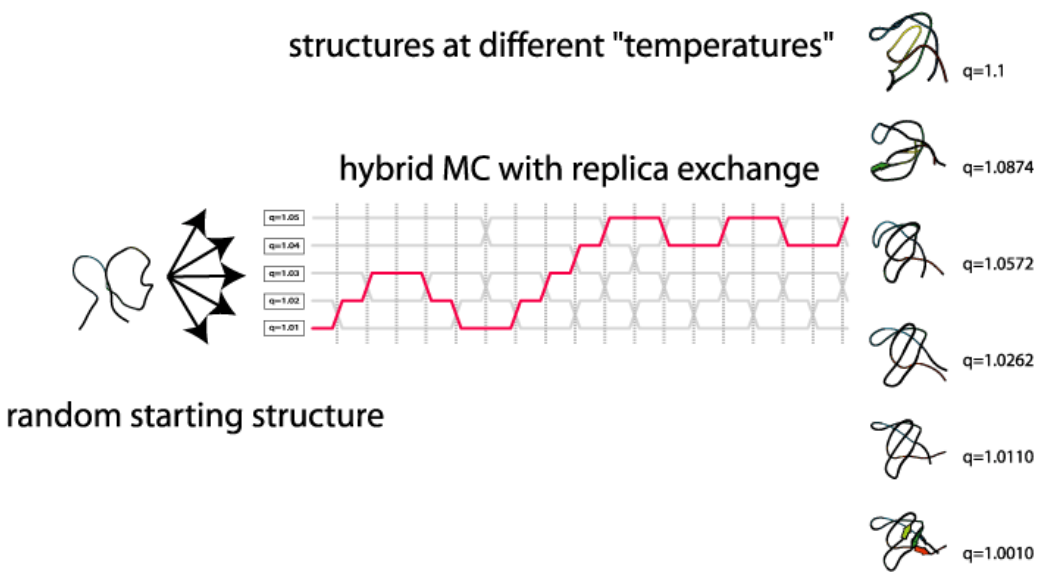


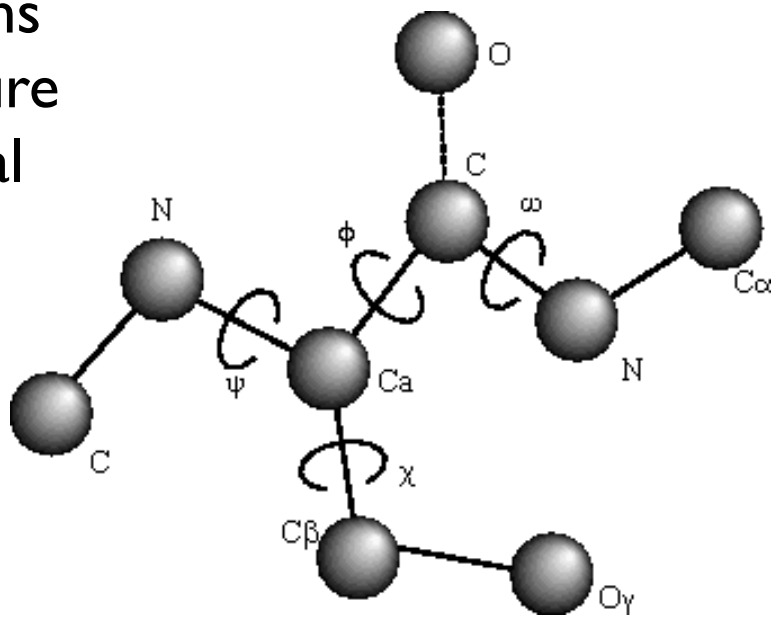
# Hybrid Monte Carlo Algorithm

- Monte Carlo is inefficient for polypeptides (polymers in general):
  - most moves either high non-bonded or covalent energy
  - many correlated degrees of freedom
- Combination of Molecular Dynamics and Monte Carlo:
  - assign random momenta
  - run short NVE MD to get new proposal state (e.g., 200 steps)
  - evaluate with Metropolis criterion on total energy

# Torsion angle dynamics

- Important degrees of freedom: torsion angles
- True torsion angle dynamics: equations of motion with a complicated structure (time-dependent masses, non-diagonal mass matrix)

$$M(\phi)\frac{d^2\phi}{dt^2} + C(\frac{d\phi}{dt}, \phi) = 0$$




- for *sampling* sufficient:

$$m\frac{d^2\phi}{dt^2} = -\frac{\partial}{\partial\vec{r}}E_{hybrid}$$

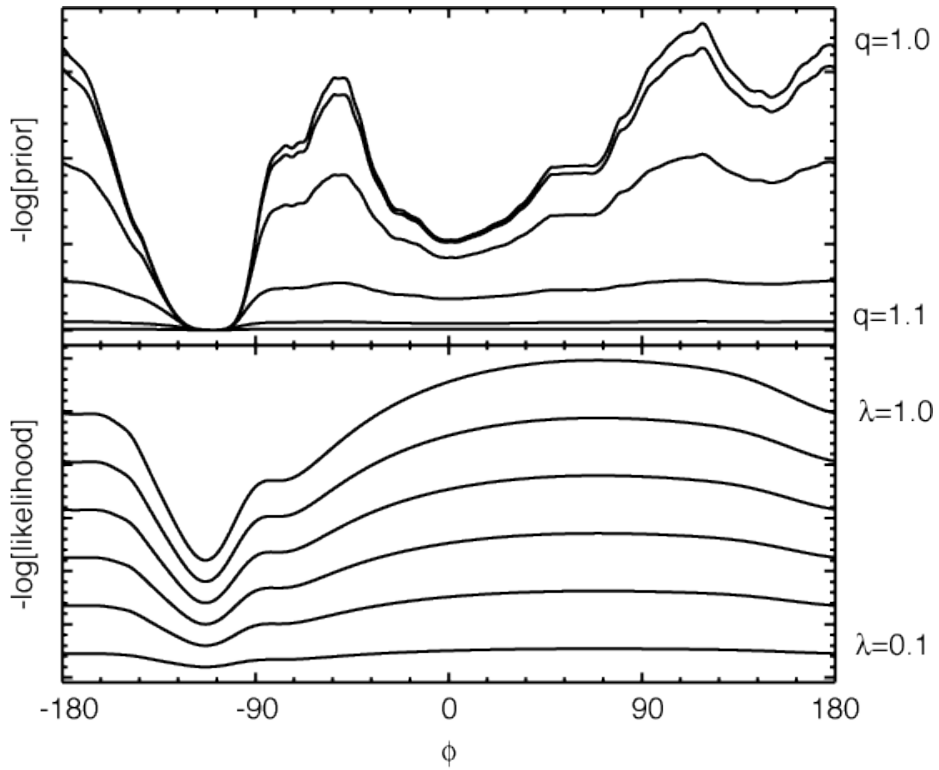
# Replica hybrid MC algorithm



- start 25-50 hybrid Monte Carlo trajectories in parallel: replicae
- replicae run at different constant conditions (temperatures, weights)
- every 50 hybrid Monte Carlo steps:
  exchange conformations between replicae;
- preserve "detailed balance”

# "Temperatures" and Tsallis distribution

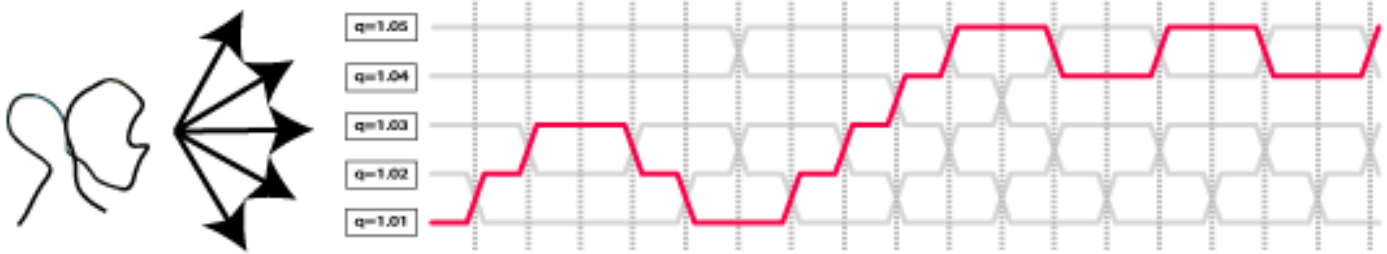
$$E(\theta;q) = \frac{q}{\beta(q-1)} \log[1 + \beta(q-1)[E(\theta) - E_{min}]]$$




- prior (force field): high T -> non-Boltzmann statistics (q; Tsallis)
- likelihood (data): high T -> exponent λ

structures at different "temperatures"

hybrid MC with replica exchange




random starting structure


q=1.1


q=1.0874


q=1.0572

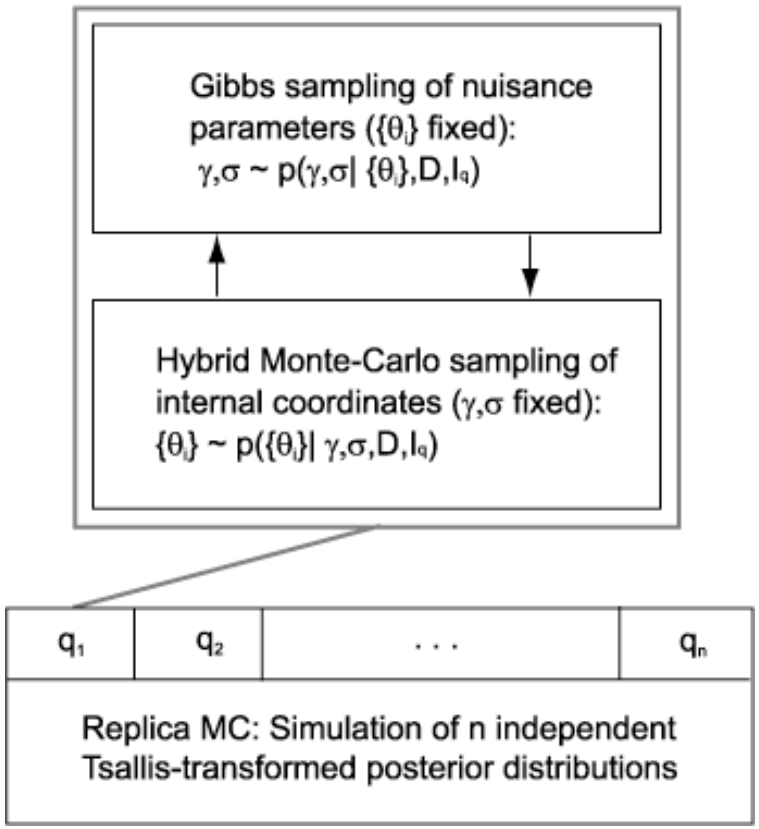

q=1.0262


q=1.0110


q=1.0010

# Sampling over nuisance parameters





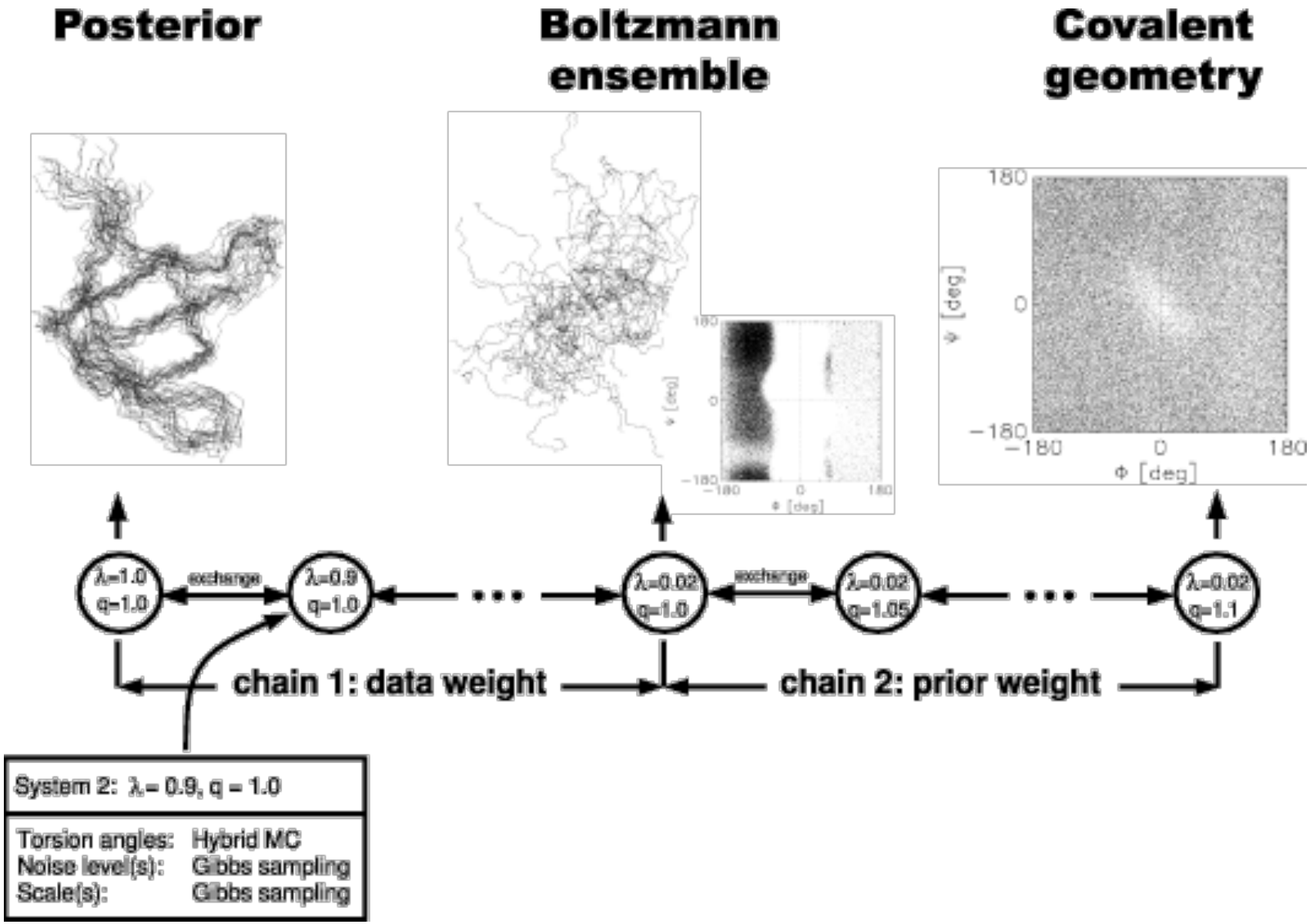

data quality ⇔ weight

scale factor

other parameters

not assumed known

(usually determined by empirical methods: experience, crossvalidation)




Rieping, W, Habeck M, Nilges, M. Science, 309:303-306

# Typical trace (SH3 domain)

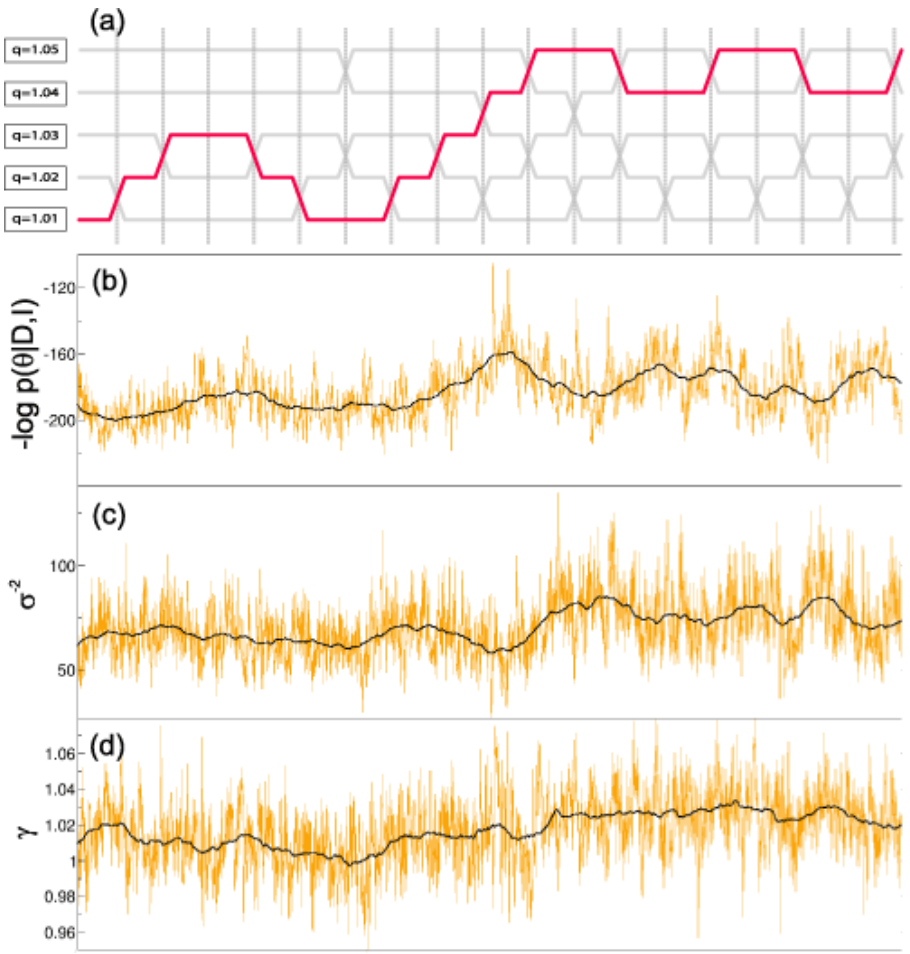


replica exchanges

"energy"

data variance

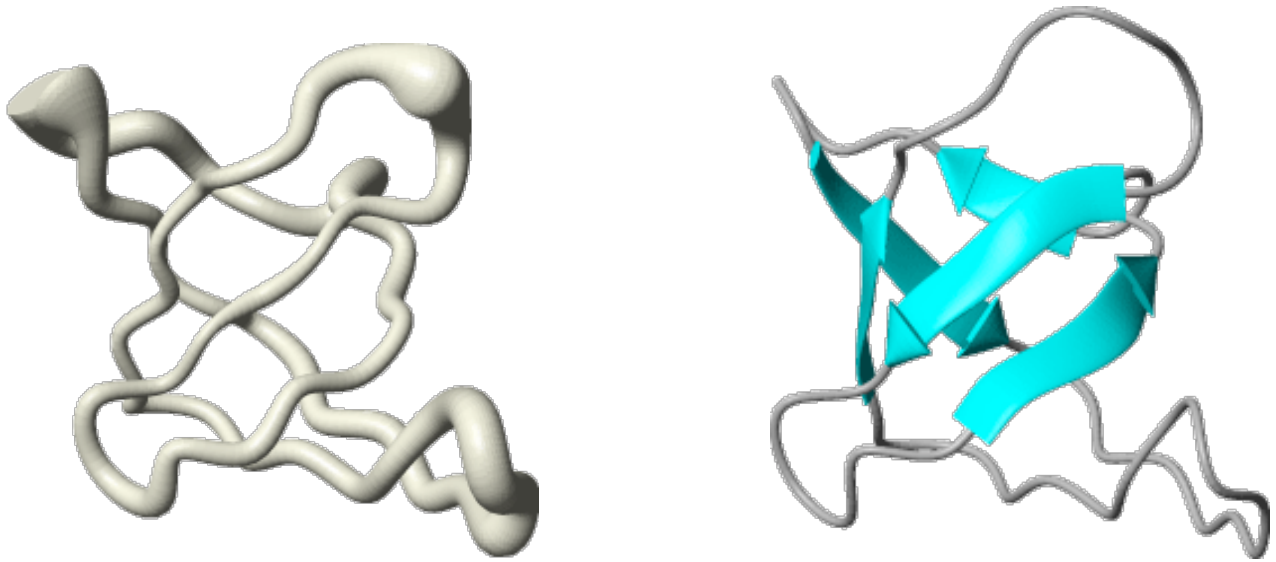
calibration



# Program ISD

SH3 (Campbell):

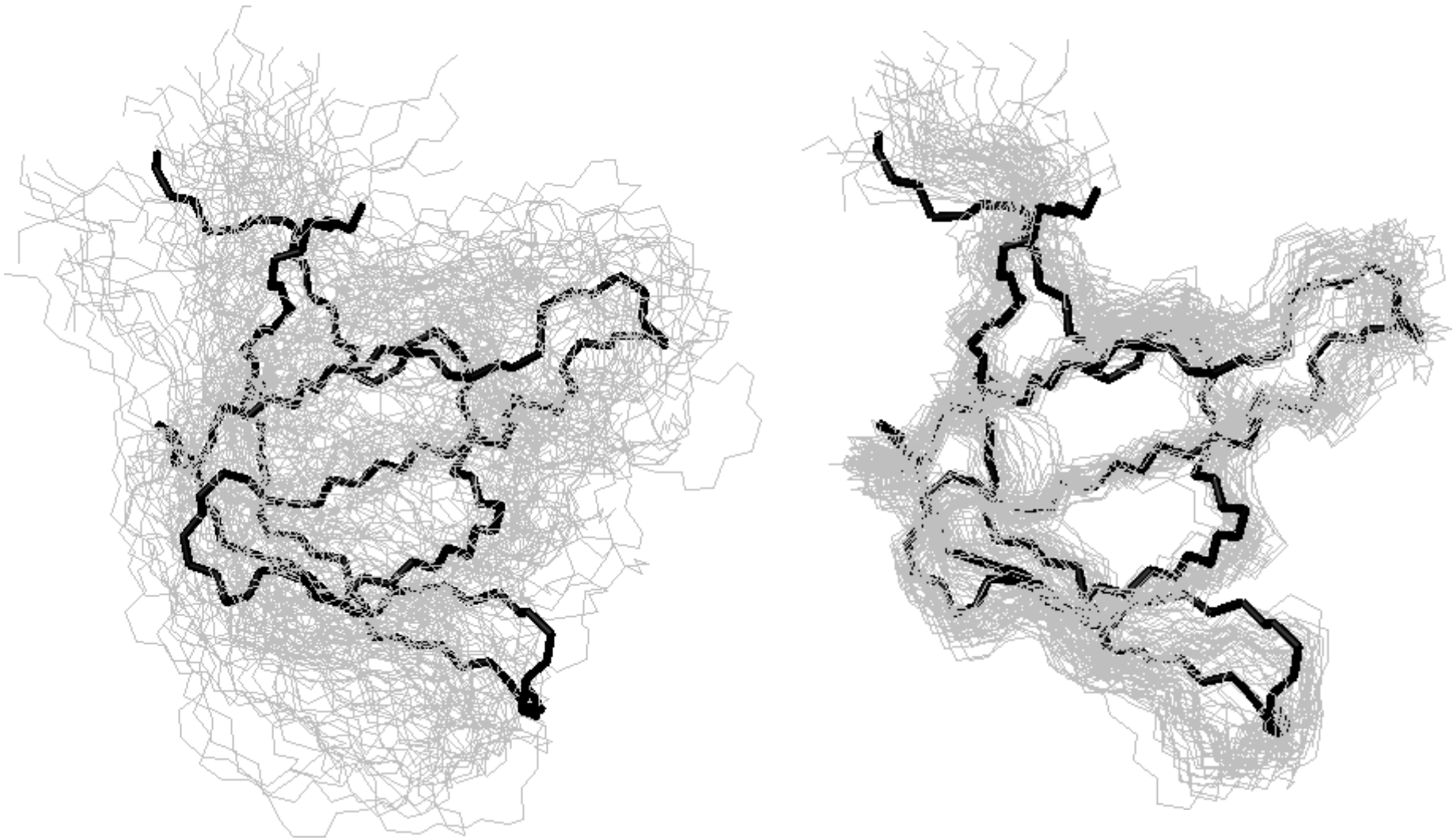
- 150 NOEs from perdeuterated domain
- sparse data set; standard structure calculation does not produce unique fold



Rieping, Habeck, Nilges, Science (2005)

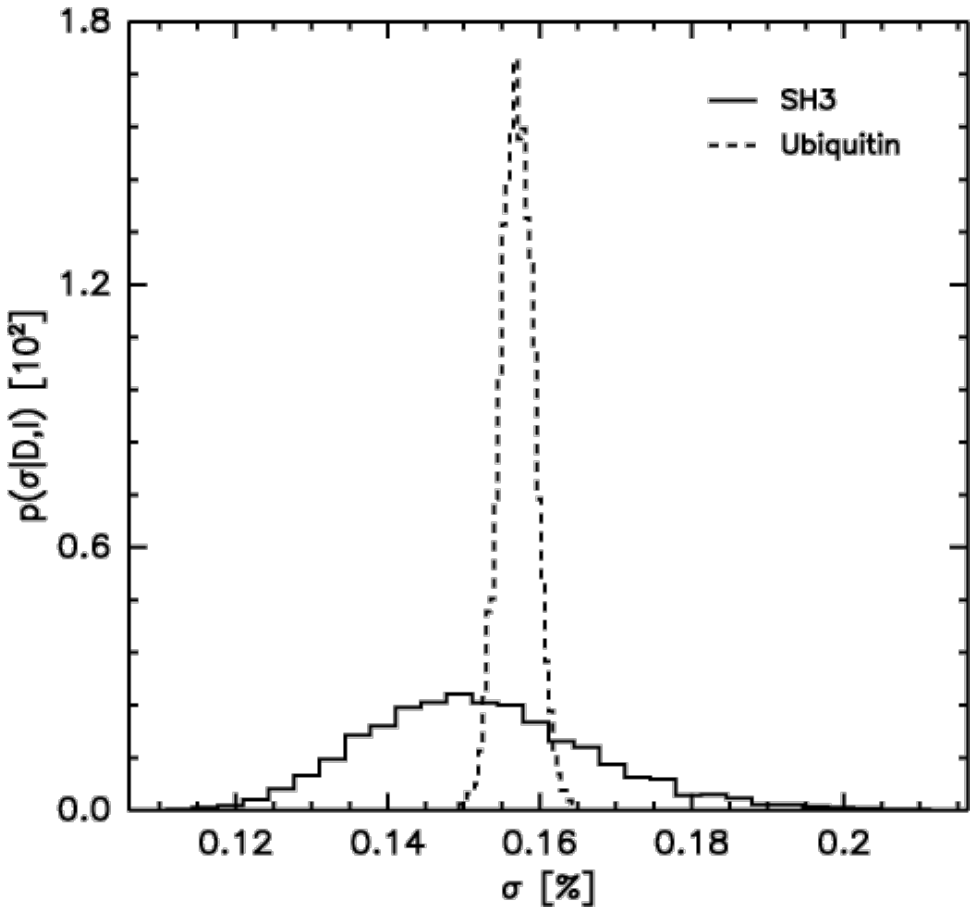
# Comparison to standard result





# Distribution of σ in Ubiquitin and SH3

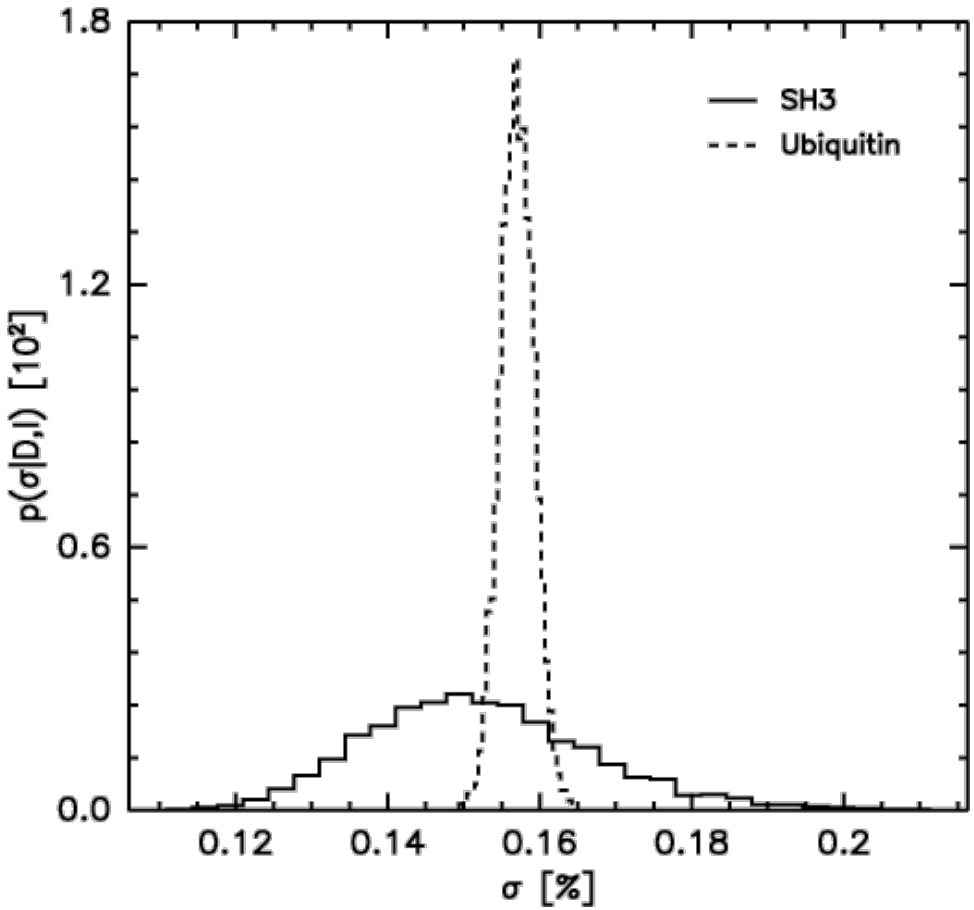





- Distributions for all parametres
- No fixed "weight" but distribution
  - "marginalization": integration over all other parameters
    - coordinates
    - scale factor

# Distribution of σ in Ubiquitin and SH3



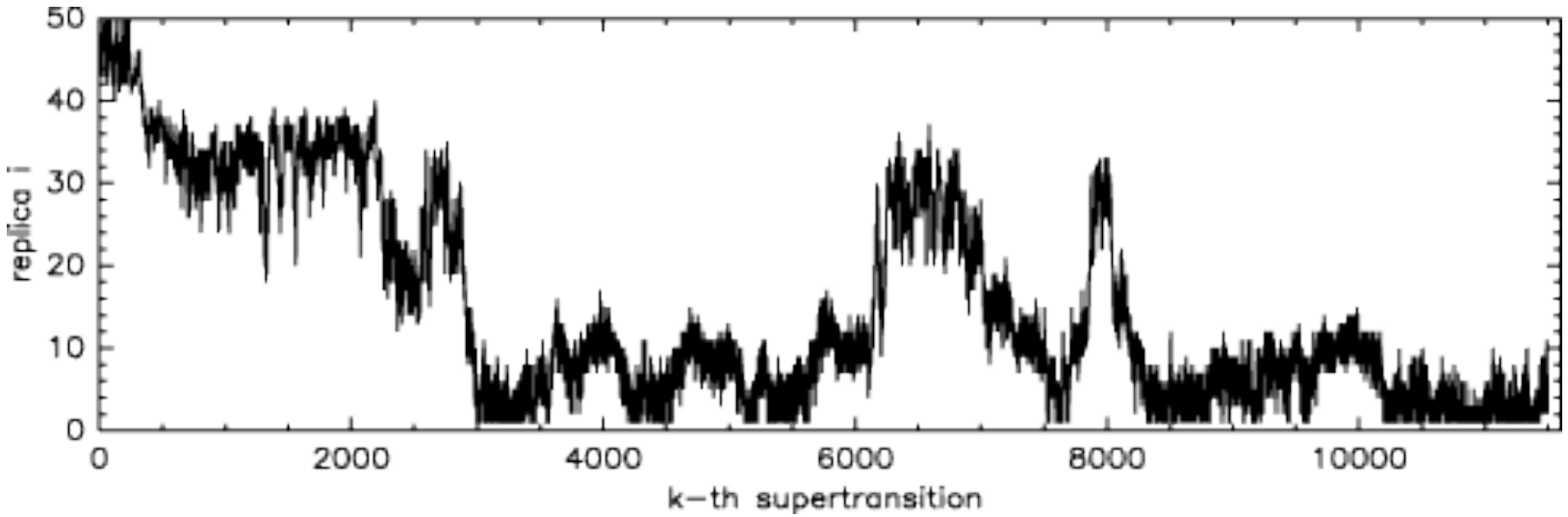



- Distributions for all parametres
- No fixed "weight" but distribution
  - "marginalization": integration over all other parameters
    - coordinates
    - scale factor

$$P(\sigma \mid D,I) = \int d\theta d\gamma P(\sigma \mid \theta,\gamma) P(\theta,\gamma \mid D,I)$$

# Computational requirements






- a few days on 50 Linux PCs
  - every "supertransition" is 50 short dynamics trajectories
  - in total, > 25000000 hybrid Monte Carlo steps
  - convergence of *distribution*, not only structures

# Literature: modelling, x-plor, CNS


- Brooks, B.R., Bruccoleri, R.E., Olafson, B.D., States, D.J., Swaminathan, S. & Karplus, M. (1983). CHARMM: A Program for Macromolecular Energy, Minimization, and Dynamics Calculations. J. Comp. Chem. 4, 187–217.
- Brunger, A.T. (1992). X-PLOR. A System for X-ray Crystallography and NMR. New Haven: Yale University Press.
- Brunger A.T., Clore, G.M., Gronenborn, A.M. & Karplus, M. (1986). Three–Dimensional Structure of Proteins Deter- mined by Molecular Dynamics with Interproton Distance Restraints: Application to Crambin. Proc. Natl. Acad. Sci. U.S.A. 83, 3801–3805.
- Brunger, A.T., Kuriyan, J. & Karplus, M. (1987a). Crys- tallographic R Factor Refinement by Molecular Dynamics. Science 235, 458–460.

# Literature: reviews, NMR calculations


- Braun, W. Distance geometry and related methods for protein structure determination from NMR data. Quart. Rev. BioPhys. 19:115-157, 1987.
- Brunger, A. T. and Nilges, M. Computational challenges for macromolecular structure determination by X-ray crystallography and solution NMR spectroscopy. Quart. Rev. BioPhys. 26:49-125, 1993.
- Güntert, P. Structure calculation of biological macromolecules from NMR data. Quart Rev Biophys 31:145-237, 1998.
- Nilges, M. and O'Donoghue, S. I. Ambiguous noes and automated noe assignment. Progr. Nucl. Magn. Reson. Spectrosc. 32:107-139, 1998.
- Guntert P. Automated NMR structure calculation with CYANA. Methods Mol Biol. 2004;278:353-78

# Literature: Bayesian




- Rieping W, Habeck M, Nilges M. (2005) Inferential structure determination. Science, 309:303-306
- Habeck M, Nilges M, Rieping W. (2005) Bayesian inference applied to macromolecular structure determination. Physical Reviews E
- Habeck M, Rieping W, Nilges M. (2005) Bayesian Estimation of Karplus Parameters and Torsion Angles from Three-bond Scalar Couplings Constants. J Magn Reson
- Habeck M, Nilges M, Rieping W. Replica-exchange Monte Carlo scheme for bayesian data analysis. Phys Rev Lett. 2005 Jan 14;94(1):018105.
- Rieping W, Habeck, M, Nilges, M (2006). Refinement against NOE intensities using a lognormal distribution improves the quality of NMR structures. JACS,
- Nicastro G, Habeck M, Masino L, Svergun, D, Pastore, A. J Biomol NMR 2006, 36, 267–277.
- Bayrhuber M, ..., Habeck M ... et al. (2008). Structure of the human voltage-dependent anion channel. Proc. Natl. Acad. Sci. USA, 105: 15370–5.

# Practicals

- http://aria.pasteur.fr/documentation/courses/saclay-november-2011/unfolding.tar.gz
- http://aria.pasteur.fr/documentation/courses/saclay-november-2011/nmrcalc.tar.gz

# Introduction to CNS

# CNS overview

- X–PLOR minimizes the hybrid energy function

  $E_{hybrid} = E_{phys} + w_{exp}E_{exp}$

- where $E_{phys}$ could be
  - a molecular dynamics force field (CHARMM, AMBER, OPLS/ AMBER)
  - a modified/ geometric force field (Engh/Huber, PROLSQ, PARALLHDG)
  - a distance geometry target function
- and Eexp would be derived from:
  - X-ray data
  - NMR data (distances, NOE volumes, torsion angles)
  - other (e.g. positional restraints)
  - ...

# Minimization Methods

- Minimization:
  Powell's conjugate gradient minimization
- Molecular dynamics:
  (numerical solution of Newton's equations of motion) with temperature variation (simulated annealing)
- Torsion angle dynamics
- Rigid body minimization
  (with Powell's method)
- Grid search
  through command language
- Monte Carlo simulated annealing through command language

# Minimized parameters

- the coordinates
- some other porperties
  - occupancies
  - temperature factors

# Analysis of coordinates

- conformational energy
- deviations from ideal geometry
- deviations from experimental data: R-values
- Crossvalidated R-values (free R-factor)

# Calculations with CNS

- With Echem alone, CNS can be used for
  - Energy minimization
  - MD calculations
  - MD analysis (correlation functions)
  - Free energy calculations
    (perturbation method, thermodynamic integration)

# X–PLOR is an interactive program

- typing "cns" generates the prompt cns>
- there is some online help

  `cns> help`

  produces a list of available commands
- CNS has a powerful interpreted command language
  - variables (real and string)
  - if–statements
  - for– and while–loops
  - "vector" manupulations (e.g. coordinates) – data manipulations
  - mathematical functions
    - example:

```
evaluate ($count = $count + 1)
if ($count > 5) then
     do (x = ran()*y^2)
end if
```

- commands are in general not case sensitive:
  `HELP = hElp`
- commands can go over several lines
- some commands have fixed number of parameters:

```
ASSI (resi 1 and name ha) (resi 1 and name hn) 3.0 1.0 1.0
```

- others end with “end”
  `noe scale dist 50 end`
- usually, 4 characters (sometimes 3 or 5) are sufficient:

```
ASSI = ASSIG = ASSIGN
```

# Comment lines

- 3 types of comment lines:
- exclamation mark !
  the rest of the line is ignored example

```
coor   ! this is a comment...
```

- curly brackets
  the contents of the { } is ignored; example

```
dynamics verlet init{ial}t = 1000 end
```

- REMARK
  the line beginning with REMARK is ignored, but stored and written to the next output file (especially coordinate file)

# Opening Files: @ and @@

- In general, files are opened with @ or @@.
- Both switch the "command stream" to the file.
- @–files are stored on internal command buffer (for loops or if–statements) and are only opened once in a loop

- @@–files are only parsed and cannot contain loops or if-statements

- "@ for command files – @@ for data files"
- Warning: some commands expect only the file name:

```
read trajectory
        input = coords.crd
end
```

# Filenames

- Filenames are case sensitive (UNIX), and can be specified with absolute or relative path:

```
@@../../parallhdg.pro
@@/data4/Users/nilges/toppar2/parallhdg.pro
```

- Environment variables can be used

```
@@TOPPAR:parallhdg.pro
```

- where TOPPAR has been defined by

```
setenv TOPPAR /data4/Users/nilges/toppar2
```

# Topology and the PSF file

- Topology file: residue library that defines standard molecule components (amino acids, nucleotides):
- atom definitions (masses)
  residue definitions (covalent topology) • charges
  patches ("presidue") for modifications:
  - peptide bond
    disulfide bridge
    N– and C–termini
- no coordinates!
  no bond lengths, force constants etc!
- The topology of a specific molecule is stored in the PSF:
  - Sequence + Topology → PSF

# Example of a residue in TOPALLHDG.PRO

```
residue ALA
  group
    atom N   type=NH1 charge=-0.36 end
    atom HN  type=H   charge= 0.26 end
    atom CA  type=CT  charge= 0.00 end
    atom HA  type=HA  charge= 0.10 end
    atom CB  type=CT  charge=-0.30 end
    atom HB1 type=HA  charge= 0.10 end
    atom HB2 type=HA  charge= 0.10 end
    atom HB3 type=HA  charge= 0.10 end
    atom C   type=C   charge= 0.48 end
    atom O   type=O   charge=-0.48 end
   bond N HN bond N CA bond CA HA bond CA CB
   bond CB HB1 bond CB HB2 bond CB HB3 bond CA C bond C O
   improper HA  N   C   CB  !chirality CA
   improper HB1 HB2 CA HB3  !methyl group CB
end
```

# generate.inp

```
topology
    @@TOPPAR:topallhdg.pro
end
segment
    name="    "
    chain
       @@TOPPAR:toph19.pep
       sequence
          Ala Ala end
       end end
REMARK ALA dipeptide
write structure
    output=INPUT:diala.psf
end
stop
```

- "`segment`" defines a new segment of the molecular structure.
- several segments possible (e.g. in complexes or multimers) – protein – DNA – water
- the name of the segment corresponds to the PDB coordinate file (last 4 characters before card number)
- "`chain`" concatenates residues, with definitions in file toph19.pep
- sequence" specifies the sequence
- "`sequence ... end`" can be replaced by "`coor @@example.pdb`" (note: "end" in coor file!)
- the REMARKs will be written to the PSF file
- PSF file is written by `WRITE PSF ... END`

# Example of a patch in TOPALLHDG.PRO

```
presidue PEPT
   add bond -C +N
   add angle -CA -C +N
   add angle -O  -C +N
   add angle -C  +N +CA
   add angle -C  +N +HN
   add improper -O  -C  +N  +CA
   add improper +HN +N  -C  -CA
   add improper -CA -C  +N  +CA
end
```

# Description of PSF file

- Note: usually no need to look at the file – do not modify
- Header: REMARK records
  Filename,date etc are generated by WRITE

```
PSF
         3 !NTITLE
   REMARKS FILENAME="diala.psf"
   REMARKS ALA dipeptide
   REMARKS DATE:07-Sep-95  10:16:16   created by ...
```

- list of all atoms

```
    23 !NATOM
       1       1    ALA  CA   CT      0.220000       1
       2       1    ALA  HA   HA      0.100000       1
...
      22        2     ALA  OT1  OC     -0.570000       1
      23        2     ALA  OT2  OC     -0.570000
```

- list of all bonds

```
22 !NBOND: bonds
9 1 1 2 1 3
3 5 3 6 1 7
...
21 22 21 23
```

- same for
- – bond angles
  – dihedrals
  – impropers
  – hydrogen bond donors and acceptors – non–bonded groups

# A simple energy minimization

- To minimize, we need
- PSF file
- energy parameters
- starting coordinates (X–PLOR PDB format)

```
structure @@diala.psf end
parameter @@TOPPAR:parallhdg.pro end
coor @@diala.pdb end
mini powell nstep= 50 end
REMARK after 50 steps powell
write coor output=diala_min.pdb end
stop
```

# CNS scripting language

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# General features



- variables (symbols)
- if–statements
- loops
- atom selection
- data structure manipulations
- many application statements
- mathematical functions
  for variable and data structure manipulations

# Symbol definitions and the EVALuate statement

- recognized by $ sign
- symbols are defined and manipulated by EVALuate

```
evaluate ($count = 0)
evaluate ($filename = "dg.pdb")
```

- Symbols can be
  - real numbers
  - strings
  - logical
- the type definition is implicit by usage
- type conversion by encode and decode

```
evaluate ($name = encode($count))
evaluate ($number = decode($name))
```

- $? produces list of all defined symbols

# Arithmetic operations

- Standard operations
  + - * / ** ^ ( )

```
evaluate($number = (5*$count)^(3+$count))
```

- mathematical functions

```
cos  sin  ran ...
evaluate($number = sin( $count*ran() ))
```

# Special symbols

- Fundamental constants $pi $kboltz
- Results of certain operations (incomplete list) – PRINt statements define $result

```
print angle
evaluate ($rms_angle = $result)
```

- SHOW statements define $result

```
show average (x) (all)
evaluate ($x_ave = $result)
```

- ENERgy, MINImiz and DYNAmics define energy terms

```
energy end
display $ener $bond $angl
```

# IF statements

- basic structures:
  - IF ( condition ) THEN commands END IF
  - IF ( condition ) THEN commands ELSE commands END IF
  - "case" statement
    - IF ( condition )
    - THEN commands
    - ELSEIF (condition)
    - THEN commands ...
    - END IF
- can be nested
- note: ELSEIF is not ELSE IF

- two “end if” necessary

```
if ( $count eq 1 )
   then
    coor copy end
   else
    if ($count eq 2)
   then
    coor fit end
   end if
end if
```

- one “end if” necessary

```
if ( $count eq 1 )
   then coor copy end
elseif ($count eq 2)
   then coor fit end
end if
```

# Loops

- WHILE loop:
  WHILE (condition) LOOP loop–name
- commands
  END LOOP loop–name

```
evaluate ($count = 1)
while ($count le 10) loop main
      evaluate ($count = $count + 1)
end loop main
```

- FOR loop 1:
  FOR variable IN (set)

```
for $filename in ( "sa_1.pdb" "sa_3.pdb" )
   loop main
   coor @@$filename
end loop main
```

- FOR loop 2:
  FOR variable IN ID (selection)

```
for $loopid in id (all) loop main
   vector show element (x) (id $loopid)
end loop main
```

# Atom selection

- elect atoms for certain operations
- selection by atom name
- wildcards and ranges
- selection by atom property
- different "queries" can be connected by AND / OR
- "queries" can be negated by NOT
- parantheses necessary for combinations of AND, OR, NOT

# Selection by atom name

- The atom name consists of
- – SEGId, segment name defined by SEGMent
- – RESId, residue "number" (also 48b etc!)
- – RESName, residue name (ALA, VAL...)
- – NAME, atom name (N, CA...)

```
coor select (resid 5 and name hn) ... end
coor select ((resid 5 or resid 7) and name hn) ..
coor select (resid 5:7 and not name h*) ... end
```

- Atoms can also be selected by
  - CHEM (atom type defined in topology)
  - ID (internal number)

# Wildcards and ranges

- wildcards and ranges can be used for
  - SEGId
  - RESId
  - RESName – NAME
  - CHEM
- ranges are lexigraphical order indicated by ":"
  - `coor sele (name ha:hg#) ... end`
  - ... selects ha, hb1, hb2, hg1, hg2
- wildcard hierarchy
  - "*" any string (abcd, 78, 8u)
  - "#" any number (2, 43, 39987)
  - "%" any character (a, 6, j)
  - "+" any digit (0, 1, ... 9)

# Selection by atom property

- ATTRibute selects on any atom property
  - coordinates, derivatives, mass, charge, ...)
  - `coor sele (attribute charge > 0) ... end`
- AROUnd, SAROund select atoms within cutoff of specified atoms
  - `coor sele ((resi 1 and name ca) around 5.0) ... end`
- SAROund selects atoms also in symmetry mates
- POINt ... CUT selects atoms around point
  - `coor sele (point (3.0 4.0 5.0) cut 5.0) ... end`

# Selection by residue etc

- BYREsidue (selection) selects all atoms in a residue

```
coor sele= (byres( point (0 0 0) cut 5.0) )
```

selects all atoms in residues that have at least one atom in a sphere around the origin

```
coor sele= (bygrp(resid 1 and name ca) )
```

# STOREi and RECALLi

- Atom selections can be stored and used later

```
iden (store1) (name ca)
coor sele= (store1) ...
coor sele= (recall1) ...
```

# Vector manipulations

- SHOW and DO allow analysis and manipulation of atom properties and names.
  - SHOW ELEMent (AtomArray) (selection)
    lists elements and defines $result
  - SHOW AVERage (AtomArray) (selection)
  - SHOW RMS (AtomArray) (selection)
  - SHOW SUM (AtomArray) (selection)
  - SHOW NORM (AtomArray) (selection)

```
show element (resid)
 (name ca and (resid 5 and name ca) around 5.0)
show average (x) (name ca)
```

  - DO (expression) (selection)

```
vector do (b = b + x^2 + y^2 + z^2) (all)
```

  - IDEN (STOREi) (selection)
    defines a STORE to be used in atom selection later

# 3D vectors and matrices

- 3D vectors can be defined explicitely, or through atom selections

```
coor translate vector= (1 0 0) end
coor translate
   vector= (head=(resid 1 and name cb)
   tail=(resid 1 and name ca))
distance= 5.0 end
```

- 3x3 matrices can be defined by rotation center, axis, angle

```
coor rotate
   center= (0 0 0)
   matrix= AXIS (head=(resid 1 and name cb)
                 tail=(resid 1 and name ca)) 90.0
```

  - or by Euler angles, Lattman angles, Quaternions, Spherical angles
  - or explicitely

```
coor rotate
    center= (0 0 0)
    matrix= (1 0 0) (0 1 0) (0 0 1) end
```

# Output files

- DISPLAY files
  - for DISPlay statements
  - open with SET DISPlay filename END
- PRINT files
  - for info from PRINt statements (e.g. PRINt ANGLes)
  - open with SET PRINt filename END
- coordinate, structure, parameter files
  - with WRITE COOR (structure...) OUTPut= file- name end
- trajectory files

# Examples

```
set display rmsd.disp end
evaluate ($maxcount = 10)
evaluate ($count = 1)
for $filename in ( @@file.list ) loop fit
   coor @@$filename
   if ($count eq 1) then
      coor copy end
   end if
   coor sele (name ca) fit end
   coor sele (name ca) rms end
   display $count $filename rms $result A
   if ($count ge $maxcount) then
      exit loop fit
end if
   evaluate ($count = $count + 1)
end loop fit
```

- The file “file.list” contains a list of files, for example ordered by
- energy

```
"sa_1.pdb"
"sa_6.pdb"
...
"sa_67.pdb"
```

- The display file rmsd.disp will look like this

```
1 sa_1.pdb rms 0 A
2 sa_6.pdb rms 1.245 A
...
10 sa_67.pdb rms 1.87 A
```

```
set display rmsfluc.disp end
for $loopid in id (name ca) loop rms
   vector show element (resid) (id $loopid)
   evaluate ($resid = $result)
   vector show element (resn) (id $loopid)
   evaluate ($resn = $result)
   vector show norm (b) (byresidue(id $id) and not
                              hydrogen)
   evaluate ($rmsfluc = $result)
   display $resn $resid $rmsfluc
end loop rms
```

# Energy minimization and molecular dynamics

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# Energy minimization (conjugate gradient)

- Conjugate gradient minimization (Powell method)
  - uses gradient information
  - a "complete" minimization is a series of one– dimensional minimizations (one for each degree of freedom)

- General syntax of minimization command
- started by MINImize POWEll
- Minimization is performed until one convergence criterion is met.
  - NSTEp: maximum number of steps
  - TOLGradient: target norm of gradient
- Other parameters:
  - DROP: expected initial drop in energy (default 0.001, optimal value 10...100)
  - NPRInt: Information is printed every NPRINT steps
- Minimization defines variables $ener, $grad, $bond... of energy terms that are turned on with FLAG state- ment
- Minimization often terminates with "Line search abandoned".

# An energy minimization script

```
structure @@protein.psf end
coor @@protein.pdb
parameters @@TOPPAR:parallhdg.pro
  nbonds repel= 0.78 rcon = 5.0 end
end
flags exclude elec include harm end
evaluate ($kharm = 10)
while ($kharm ge 0) loop mini
  vector do (harm = $kharm) (all)
  mini powell
    drop 10 nstep 100 nprint 10
  end
  evaluate ($kharm = $kharm - 1)
end loop mini
write coor output = protein_m.pdb end
stop
```

# Rigid body minimization

- started by MINImize RIGID
- Minimization is performed until one convergence criterion is met.
- same parameters as POWELL:
  - NSTEp, TOLGradient, DROP, NPRInt
  - rigid groups are defined by
    group = <selection>, for example

```
mini rigid
    nstep 100
    group (segid A) group (segid B)
end
```

# Example script using rigid body minimization

```
structure @@protein.psf end
structure @@DNA.psf end
coor @@protein.pdb
coor @@dna.pdb
NOE
   @@dock.tbl
end
flags exclude * include NOE end
constraints fix (segid "PROT") end
minimize rigid
  group (segid "1BNA")
  nstep 100
end
write coor
   sele= (segid "1BNA") output = dna_dock.pdb
end
```

# Cartesian molecular dynamics

- Invoked by
  - dynamics cartesian ... end
- Important parameters:
  - nstep: number of steps
  - timestep: time step in ps
  - tcoup: switch on Berendsen's method? true/false
  - tbath: temperature of heat bath
  - nprint: print frequency
  - cmremove: remove COM movement? true/false
  - cmperiodic: period of COM movement removal
- initial velocities defined with "do (vx = maxwell(300)) (all)"
- coupling parameter fbeta defined with "do (fbeta = 10) (all)"

# A slow cooling script

```
evaluate ($bath = 1000)
do (fbeta = 10) (all)
do (vx = $bath) (all)
do (vy = $bath) (all)
do (vz = $bath) (all)
while ($bath > 50) loop cool
  evaluate ($bath = $bath - $tempstep)
  dynamics  verlet
     nstep=1000 time=0.005
     tcoup=true temperature=$bath
     nprint=$nstep
     cmremove=true cmperiodic=0
  end
end loop cool
```