Protein Design

An excuse to talk about

- Monte Carlo
- a pruning method on a monster problem
- why we do not have to get everything correct

Protein Design

- What is it ?
- Why ?
- Experimental methods
- What we need
- Computational Methods

Introduce

- Monte Carlo
- a pruning algorithm

What is protein design ?

Assumption

- you can write a protein sequence on a piece of paper
- a molecular biologist can produce it

Most general

- you have a protein which is useful (enzyme, binding, …)
- you want to make it more stable
  - temperature
  - solvents (tolerate organic solvents)
  - pH

  - we concentrate on stability

Experimental approaches

- Bacteria / selection
- For binding
  - phage display
  - in vitro evolution
- stability – more difficult

- computational methods…

Formalising the problem

We have a working structure

- want to make it more stable

native protein

"improved" protein

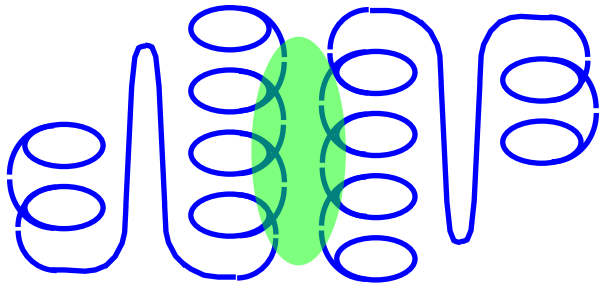
Rules

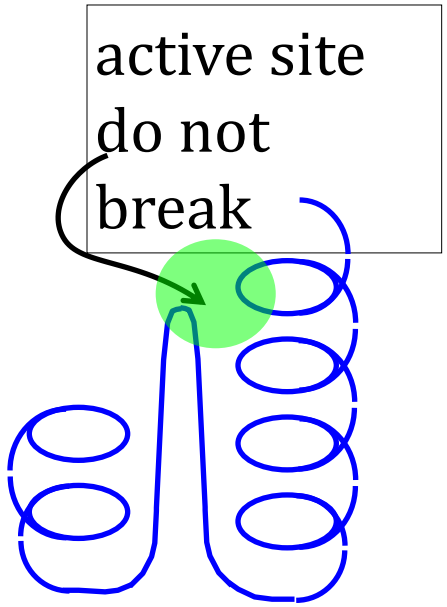
- structure should not change
- should be able to fix some residues (active site, important)..

Fixing / specifying residues

Examples

- lysine (K) often used for binding
  - change a residue to K and protein does not fold
  - mission:
    - adapt the rest of the residues to be stable
- change all residues, but not those in active site
- change some residues at surface to be soluble
- change some residues at surface to stop dimers





Ingredients

- Score function (like energy)
- Search method

Score function

- how does sequence fit to structure ?
- sequence S={s_1, s_2, ..s_N}
- coordinates R = { $\mathbf{r}_1$, $\mathbf{r}_2$, ... $\mathbf{r}_N$}
- score = f (S, R) (different nomenclature soon)
- mission
  - adjust S to as to maximise score (minimise quasi-energy)

Score function

How do amino acids

- suit structure ?
- suit each other ?

$$score = \sum_{i=1}^{N_{res}} score_{struct}(s_i, R) + \sum_{i=1}^{N_{res}} \sum_{j>i}^{N_{res}} score_{pair}(s_i, s_j, R)$$

$score_{struct}$ might have

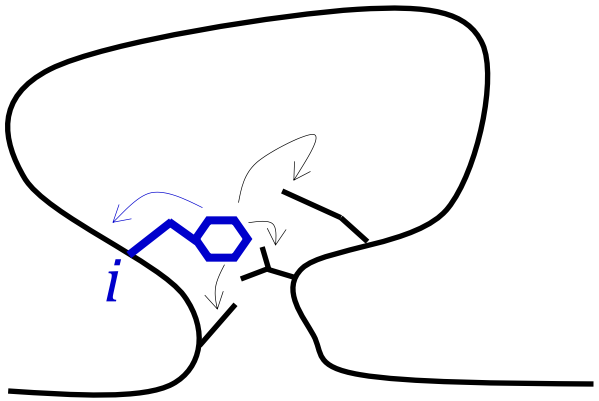
- backbone preferences (no proline in helices, ..)
- solvation (penalise hydrophobic at surface)

$score_{pair}$

- are residues too big (clashing)
- are there holes ? charges near each other ?

Messy functions

- lots of parameters



Searching

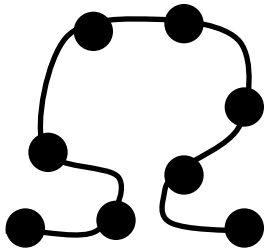
Systematic search – how long ?

- search space for $N_{res} = 20 \times 20 \times \cdots = 20^{N_{res}}$

Search space complex

- every time you change a residue, affects all neighbours
  - effects neighbours of neighbours

- brute force not a good idea
- two methods here
  1. Monte Carlo / simulated annealing
  2. Pruning / dead end elimination



Monte Carlo

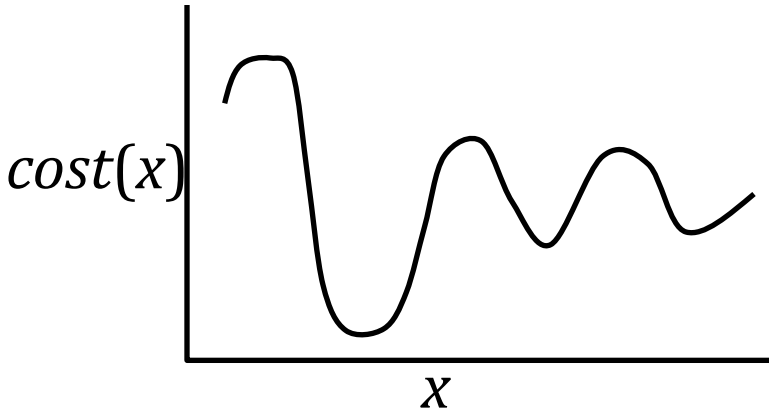
- more formally next semester
- first the problem

The sequence optimisation problem

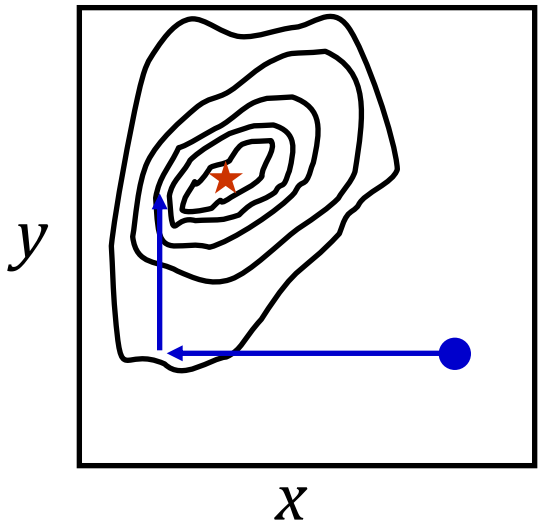
- discrete
- local minima / correlations in surface
- high dimensional

dimensions and correlations

- a 1D problem



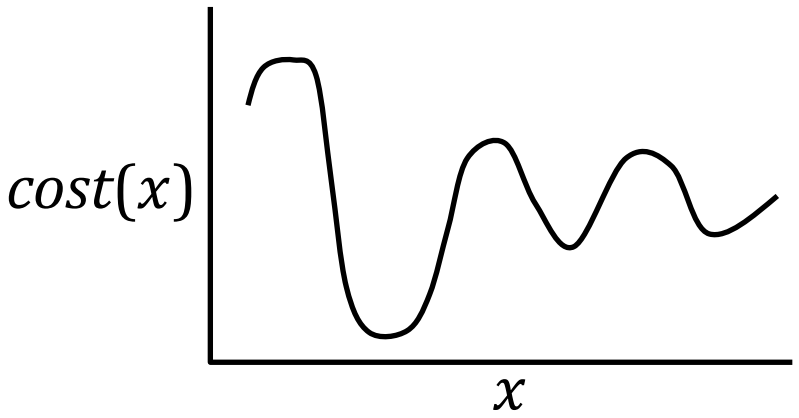
- local minima
- minimum of x depends on y
- cannot optimize x and y independently
- what are correlations in this problem ?



Discrete vs continuous problems

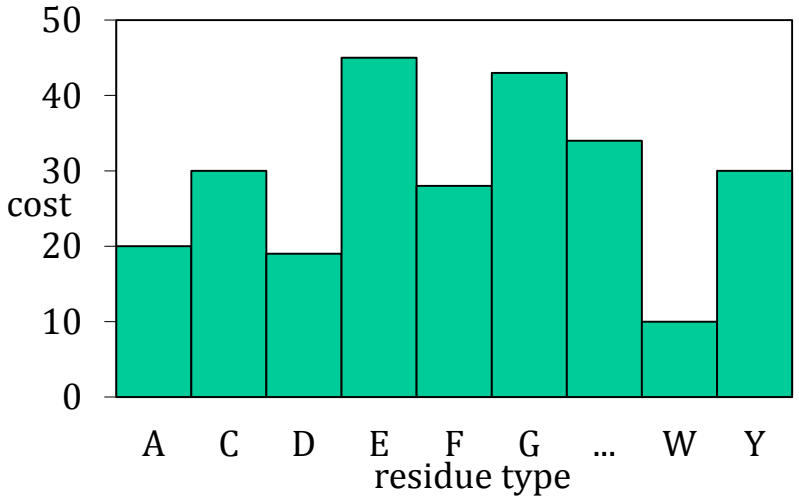
For a continuous function use gradients

- to optimise
- to recognise minima / maxima
- continuous functions
  - step in one direction is good
    - try another in same direction



With a discrete function

- no gradients
- order of labels arbitrary
  - ACDE or ECAD
- discrete
  - step in one direction may be no predictor of best direction



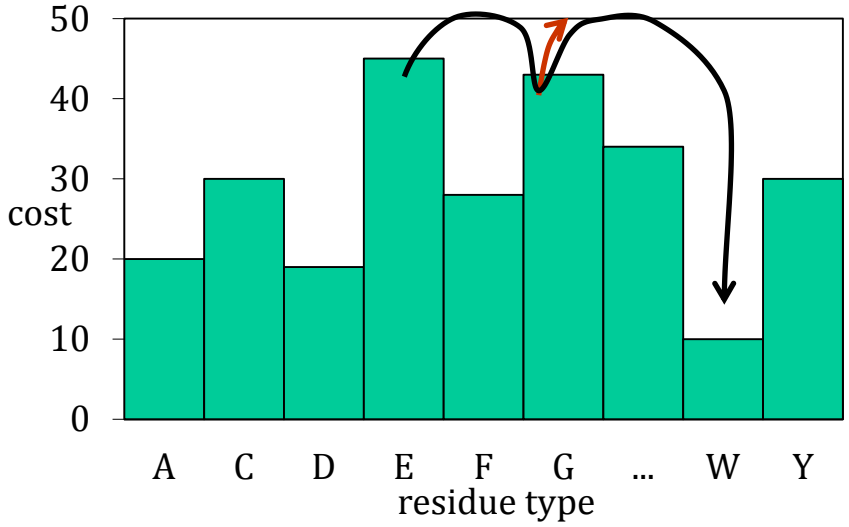
what do we want ?

From step to step (sequence to sequence)

- be prepared to move in any direction
- if the system improves, try not to throw away good properties
- must be willing to go uphill sometimes

Philosophy

- take a random move
- if it improves system
  - keep it
- if cost becomes worse
  - sometimes keep it
  - sometime reject



Acceptance /rejection

- for convenience, write $cost(S_n)$ - neglect coordinates R

Sign convention

- system (sequence) at step n is S_n
- after a random step, cost changes from $cost(S_n)$ to $cost(S_{n+1})$
- $\Delta c = cost(S_{n+1}) - cost(S_n)$
- our sign convention: if $\Delta c < 0$, system is better

When to accept ?

- if $\Delta c < 0$ accept
- if Δc is a bit > 0, maybe OK
- if $\Delta c >> 0$, do not accept

Formal acceptance rule

- $-\Delta c < 0$, system has become worse, $e^{-\Delta c}$ is between 0..1
- $-\Delta c \approx 0$ then $e^{-\Delta c} \approx 1$ as $\Delta c \to \infty$ then $e^{-\Delta c} \to 0$

formalise this rule

```
set up S=S0 and cost(S0)
while (not finished)
    Strial = random step from S
    Δc = cost(Strial)-cost(S)
    if (Δc < 0)                          /* accept */
          S = Strial
    else
          r = rand (0..1)
          if (e^-Δc ≥ r)
                S= Strial
```

vorsicht ! not the final method

why we need temperature

As described

- system will run around
- try lots of new configurations
- sometimes accept bad moves
- always take good moves
- may never find best solution
  - imagine you are at a favourable state
  - most changes are uphill (unfavourable)
  - many of the smaller ones will be accepted
    - if we were to find the best sequence, the system would move away from it

- how to fix ?

why we need temperature

- Initial sequence is not so good
  - let the system change a lot and explore new possibilities
- after some searching, make the system less likely to go uphill
- introduce the concept of temperature T
- initially high T means you can go uphill (like a high energy state)
- as you cool the system, it tends to find lowest energy state
- change acceptance criterion to $e^{\frac{-\Delta c}{T}}$ as

$$T \to \infty, \qquad e^{\frac{-\Delta c}{T}} \to 1$$

$$T \to 0, \qquad e^{\frac{-\Delta c}{T}} \to 0$$

- put this into previous description

why we need temperature

```
set up S = S0 and cost(S0)set T = T0
while (not finished)
    Strial = random step from S
    T = εT                          /* ε bit smaller than 1 */
    Δc = cost(Strial) - cost(S)
    if (Δc < 0)
          S= Strial
    else
          r = rand (0..1)
          if ( exp(-Δc/T)≥ r)
                S= Strial
```

Name of this procedure

- "simulated annealing"

Final Monte Carlo / annealing

History applications

- discrete problems – travelling salesman, circuit layout
- deterministic ? No
- convergence ? Unknown

Practical issues

- what is a random step ?
  - change one amino acid ? change interacting pairs ?
- easy to program
- lots of trial and error
- statistical properties next semester

- can we reduce the search space ?

Pruning

Are there elements of sequence which are impossible ?

- at position 35, no chance of Y, W, I, L, …

Can one find impossible combinations

- reduce the search space so it can be searched systematically (brute force)

… dead end elimination method

- use an energy-like nomenclature

Nomenclature

- we are not dealing with
  - free energy G or F or potential energy U or E
- but let us pretend
  - score is E
- rule : more negative E , better the system
- structure is fixed so neglect R / **r** terms
- define a function $s_i(a)$ as the residue type at site i
  - can take on 20 values of "a" why ?

```
foreach (a in A, C, D, E.., W, Y)
   evaluate energy corresponding to a
```

- our energies ?
  - two parts – pairwise and residue with backbone

Nomenclature

E is (quasi-energy) of whole system

- label E_1 as the terms that depend on residue + fixed environment
- E_2 as the energy terms that depend on pairs

$$E = \sum_{i=1}^{N_{res}} E_1(s_i) + \sum_{i=1}^{N_{res}} \sum_{j \neq i}^{N_{res}} E_2(s_i, s_j)$$

If we are interested in site i and being in state a what do we have to look at ?

$$\sum_{i=1}^{N_{res}} E_1(s_i(a)) + \sum_{i=1}^{N_{res}} \sum_{j \neq i}^{N_{res}} E_2(s_i(a), s_j(b))$$

- there are 20 (N_{type}) residues
- which fits best to the fixed environment ? $\min_a E_1(s_i(a))$
- implies testing each of the N_{type} for a
- best energy type a at site i could have, interacting with one site j ?

  $E_1(s_i(a)) + \min_b E_2\big(s_i(a), s_j(b)\big)$
- what is the best energy that type a at i could have considering all neighbours

$$E_1(s_i(a)) + \sum_{j \neq i} \min_b E_2\big(s_i(a), s_j(b)\big)$$

- for each a – can work out what is the best score it could yield
  - loop over b
  - within loop over j

Dead-end elimination method

worst energy that type c at i could have considering all neighbours ?

$$E_1(s_i(c)) + \sum_{j \neq i} \max_d E_2\big(s_i(c), s_j(d)\big)$$

when can one eliminate (rule out) residue type a at site i ?

for any residues a, c

a is worse than the worst for c

a cannot be part of the optimal solution … if

$$E_1(s_i(a)) + \sum_{j \neq i} \min_b E_2\big(s_i(a), s_j(b)\big) > E_1(s_i(c)) + \sum_{j \neq i} \max_d E_2\big(s_i(c), s_j(d)\big)$$

Desmet, J, de Maeyer, M., Hazes, B, Lasters, I, (1992), Nature, 356, 539-542, "… dead-end elimination"

Dead-end elimination method

$$E_1(s_i(a)) + \sum_{j \neq i} \min_b E_2\big(s_i(a), s_j(b)\big) > E_1(s_i(c)) + \sum_{j \neq i} \max_d E_2\big(s_i(c), s_j(d)\big)$$

using this approach

```
for (i = 0; i <  N_res ; i++)
    foreach a in N_type
        calculate worst score for a
        calculate best score for a
for (i = 0; i <  N_res ; i++)
    foreach a in N_type
        foreach b in N_type
            if best(a) > worst (b)
                remove a from candidates
```

How strong is this condition ?

DEE condition

- much of the time
  - cannot really rule out type *a*
- example ?
  - initial
    - 2×10^{27}
  - final
    - searchable in 90 cpu hr
- deterministic

Dahiyat, B.I, Mayo, S.L. (1997), Science 278, 82-87

Combining ideas

- use DEE to get a list of candidate residues at each position
- search remaining space with Monte Carlo / simulated annealing
- not deterministic

Success

Method

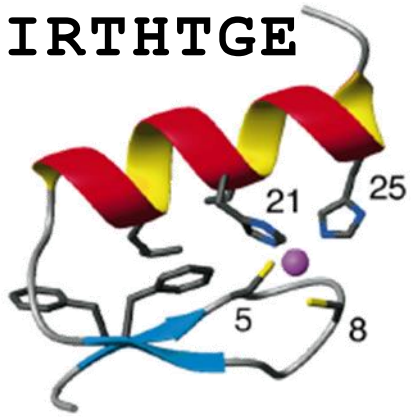
- Dead end elimination + systematic search

designed **QQYTAKIKGRTFRNEKELRDFIEKFKGR**

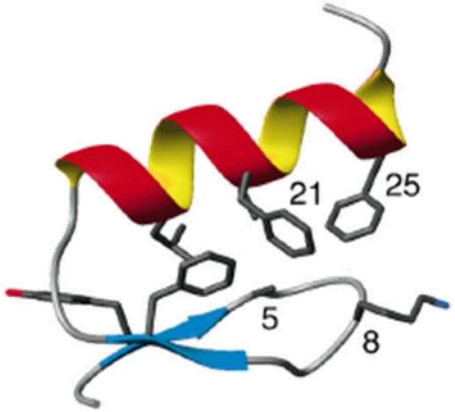
native **KPFQCRICMRNFSRSDHLTTHIRTHTGE**

New sequence

- about 20 % similar to start
- not related to any known protein (still)



- Structure solved by NMR



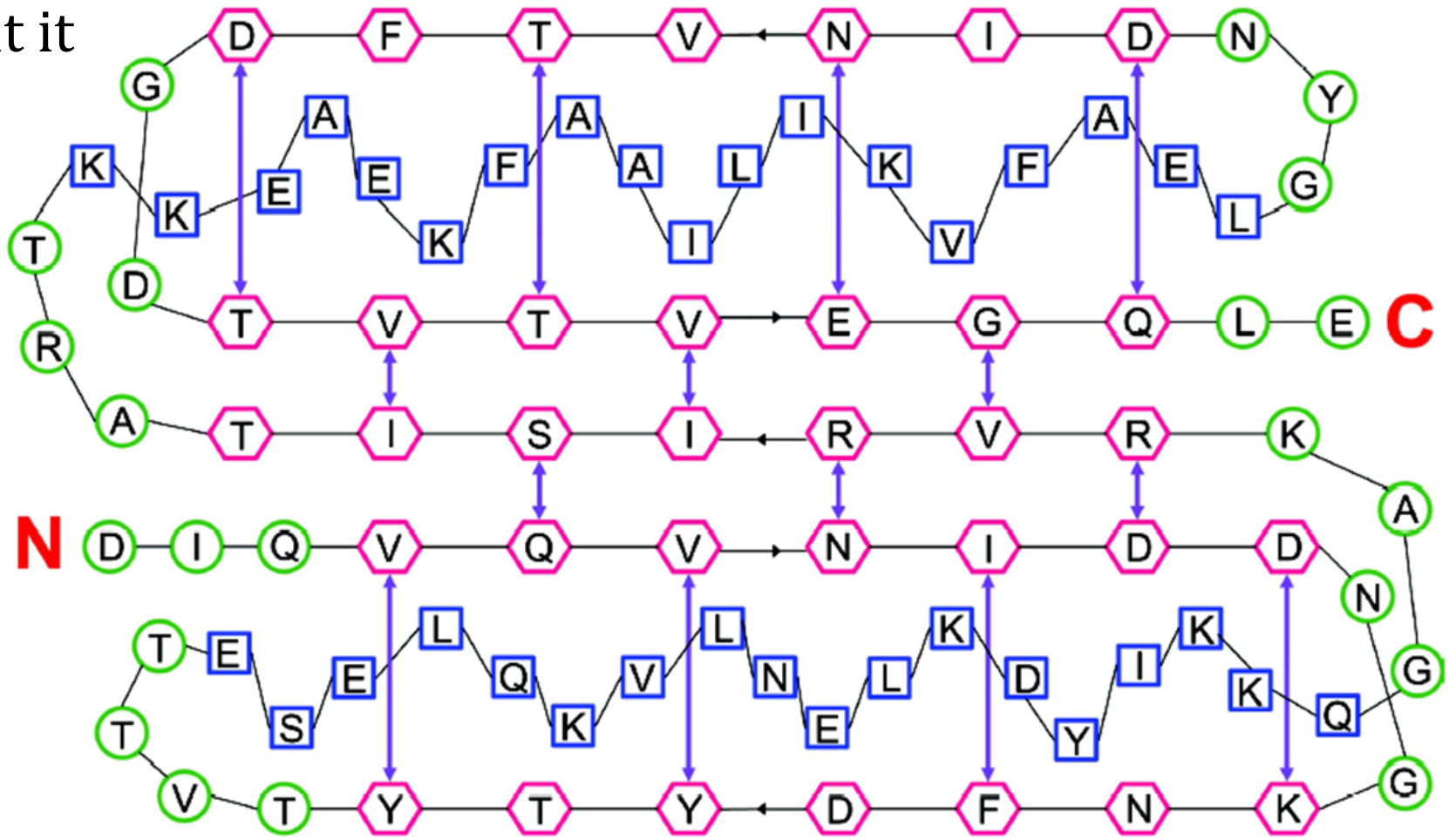
- Problem solved ?
  - maybe not

Dahiyat, B.I, Mayo, S.L. (1997), Science 278, 82-87

Success

Mission

- sketch a new protein topology
- build a sequence to fit it



Kuhlman, B.; Dantas, G.; Ireton, G.C.; Varani, G.; Stoddard, B.L.; Baker, D. Science 2003, 302, 1364-1368.

Success

Methods

- pure Monte Carlo

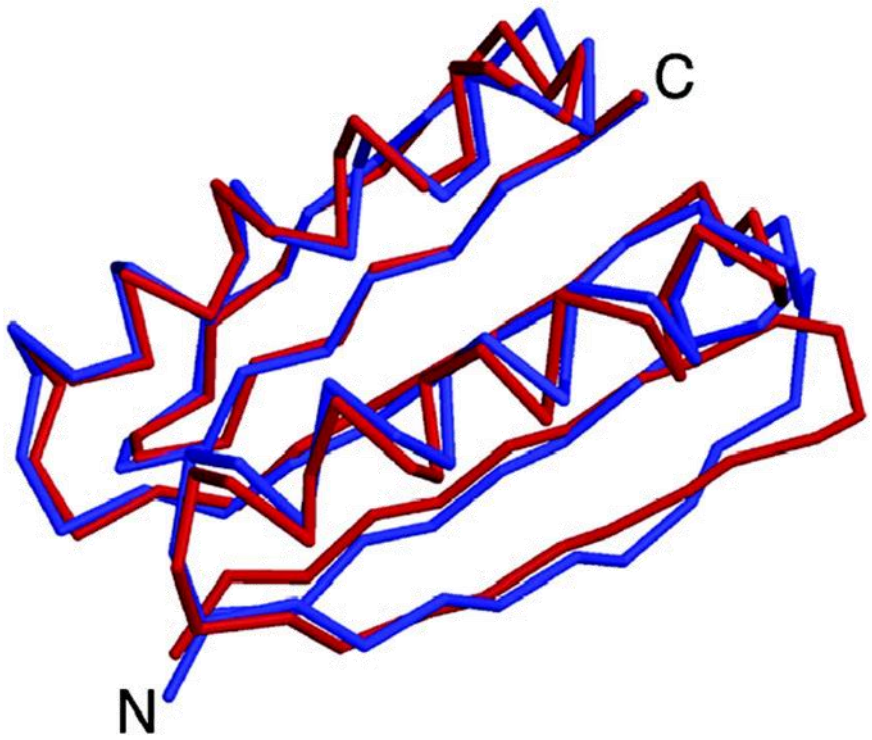
Result

- apparently new sequence

Structure

- as predicted
- solved by X-ray
  - phasing story

- Problem solved
  - unclear (how many failures ?)



Kuhlman, B.; Dantas, G.; Ireton, G.C.; Varani, G.; Stoddard, B.L.; Baker, D. Science 2003, 302, 1364-1368.

Methods so far

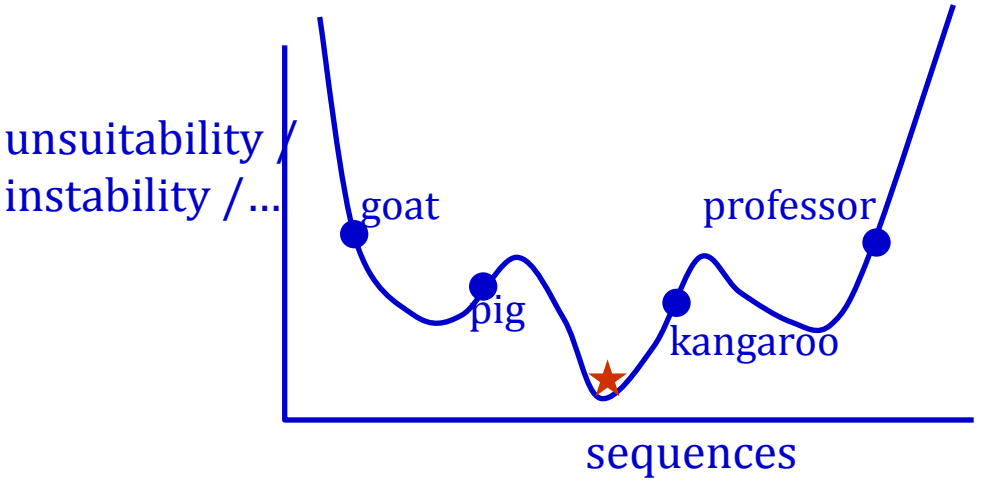
	Monte Carlo	Dead-end elimination
guaranteed global optimum	no	does not try
deterministic	no	yes

Only one answer ?

May not matter

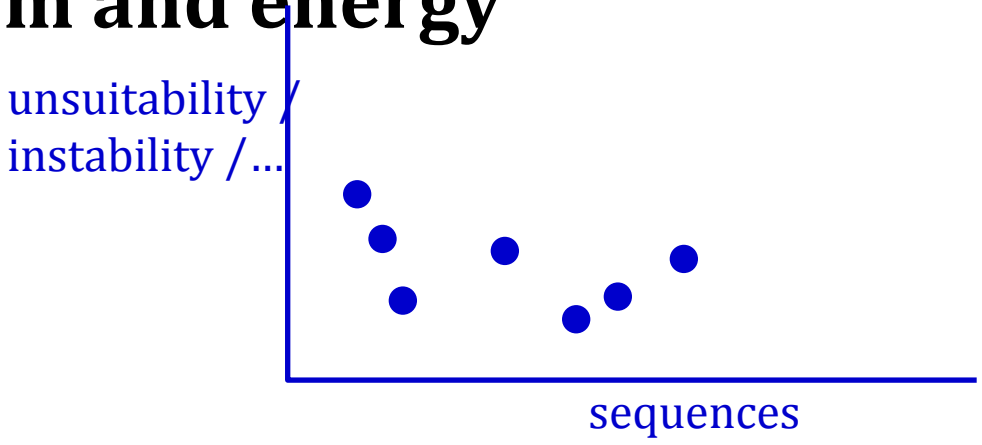
- consider real proteins – compare human, goat, …
  - all stable – all slightly different
- implication
  - there may be many solutions which are equally good

- How good are our energy functions ?



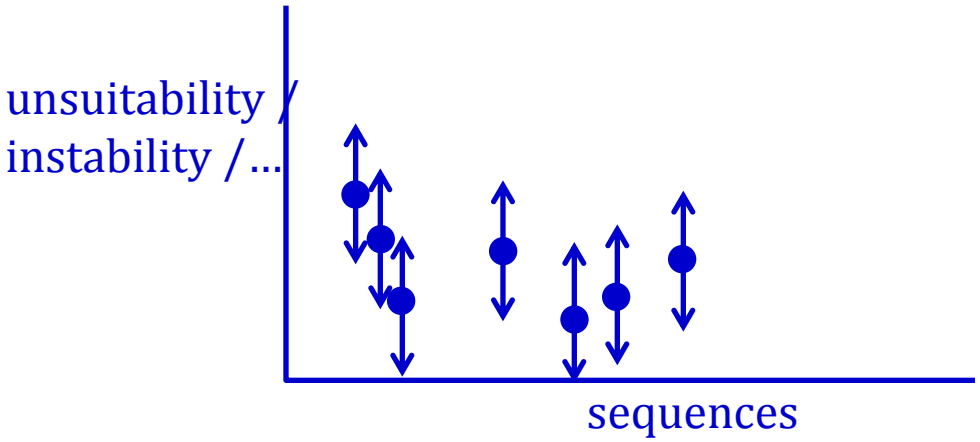
Determinism and energy

I have a perfect score / energy function



I have errors / approximations

- best answer could be any one

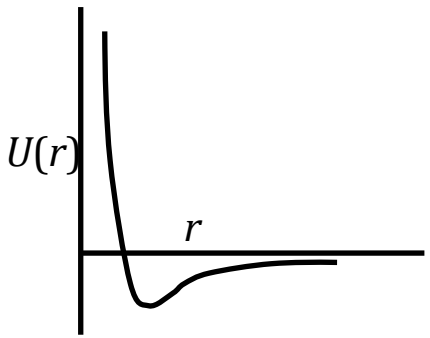


Problems – stability / energy

What do we mean by energy ?

- example – two charges $U(r) = \frac{q_1 q_2}{Dr}$

- example – two argon atoms $U(r) = 4\varepsilon\left(\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right)$



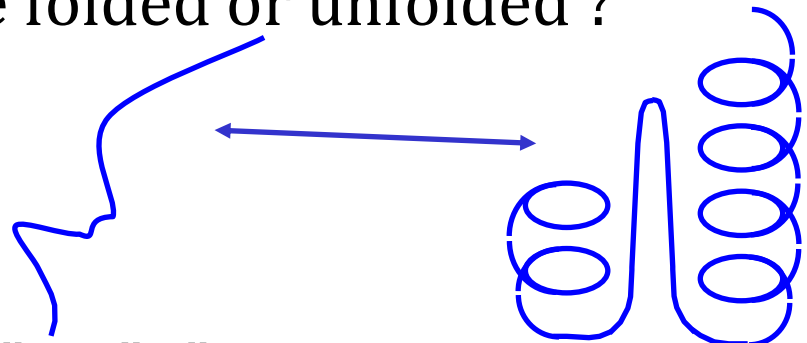
Make energy better ?

- replace every amino acid by a larger one
  (more contacts – more negative energy)
- silly – proteins are not full of large amino acids

What determines stability ?

Problems – stability / energy

- stability – does a molecule prefer to be folded or unfolded ?
- what is unfolded ? or ?



My energy function tells me to change "X" to "Y"

- it affects both the good  and bad 
- has it affected the energy difference ?
  - no guarantee

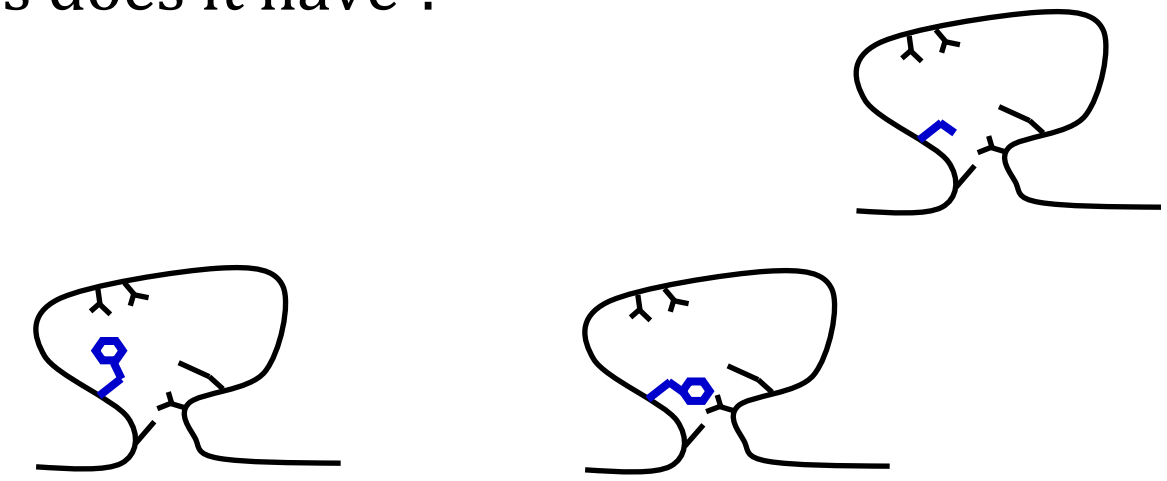
Current score functions ?

- some pure potential energy
- very difficult to estimate ΔG

Problems - sidechains

Side chain positions

- can I ever calculate the energy if I change X to Y ?
- insert a phe into this structure
- what interactions does it have ?

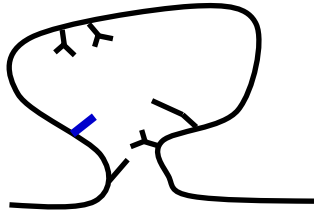


How to cope with side chain positions in a practical way

- optimise location of sidechains
- use average
- explicit rotamers

Sidechains – optimise at each step

Start with known protein



- change A →F
- use an energy minimiser / optimiser to find best position for F

Sensible ?

- we have a gigantic search space
- explicit optimisation of one side chain would be expensive

Silly ?

- I change A→F, but the rest of the side chains may move

Bad idea

Sidechains – use averaging

Ignore the problem of sidechain geometry

- at room temperature, side chains move
  - small (middle of protein) to big (surface)
- we cannot expect Å accuracy anyway

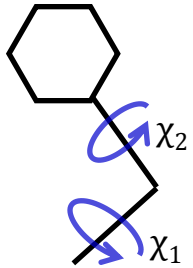
- rather fast

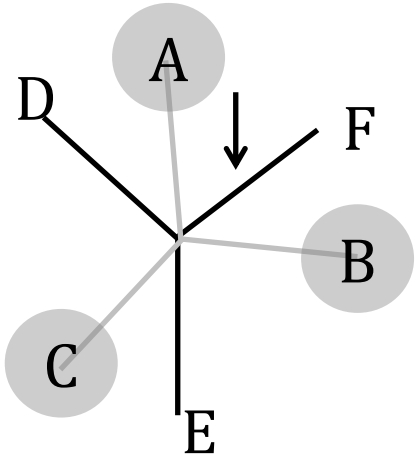
- what if we want to worry about atoms ?

Sidechains – use rotamers

Sidechains can move anywhere but

- there are preferences
  in diagram – three more likely states

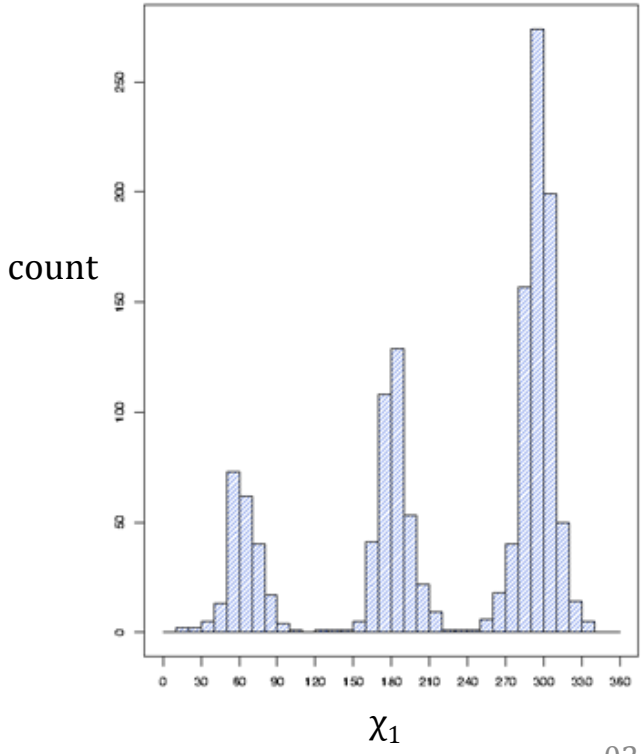




How many times is the first angle (χ_1) seen at each value ?

How to use this ?

- look for most popular angles (60, 180, 300)



histogram from Dunbrack's group http://dunbrack.fccc.edu/bbdep/figures/cys0_x1.gif

Sidechains – use rotamers

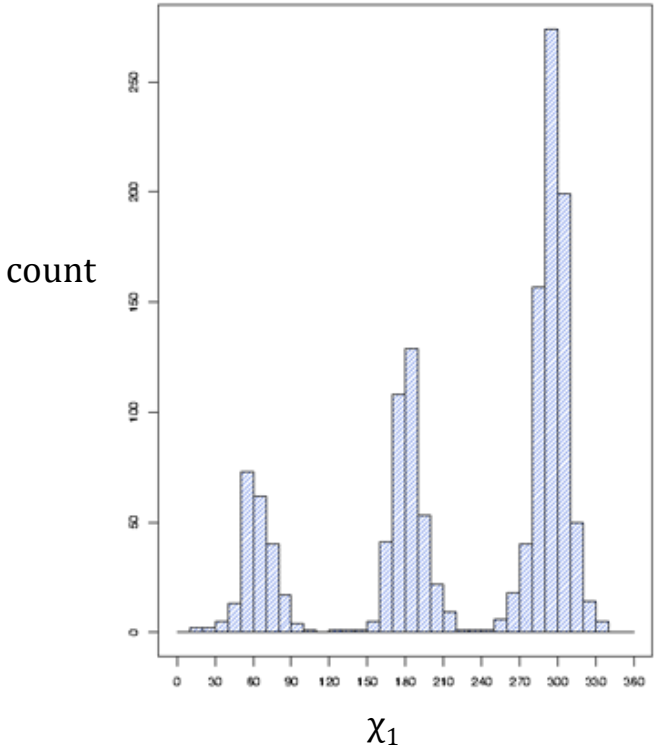
For this example

- do not have 1 cys residue
- replace with cys1, cys2, cys3
- treat all amino acids similarly
- more complicated because of more angles

Consequence

- N_{type} of amino acids >> 20

Requires that you have a pre-built rotamer library



Fits to

- Monte Carlo (random moves between residues or rotamers)
- dead end elimination (will remove impossible rotamers)

histogram from Dunbrack's group http://dunbrack.fccc.edu/bbdep/figures/cys0_x1.gif

Problems – viability

Designed sequences must

- fold
- be expressed + produced

Summary

- Nature of the problem - discrete (not continuous)
- Optimisation methods (MC, DEE)

- Score functions
  - not energy, not free energy, not potential energy

- Success / state of the art
  - not many examples from literature
  - failure rate ?
  - cost
- Definitely not a routine method