Protein Design

Andrew Torda, wintersemester 2010 / 2011, AST

- 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

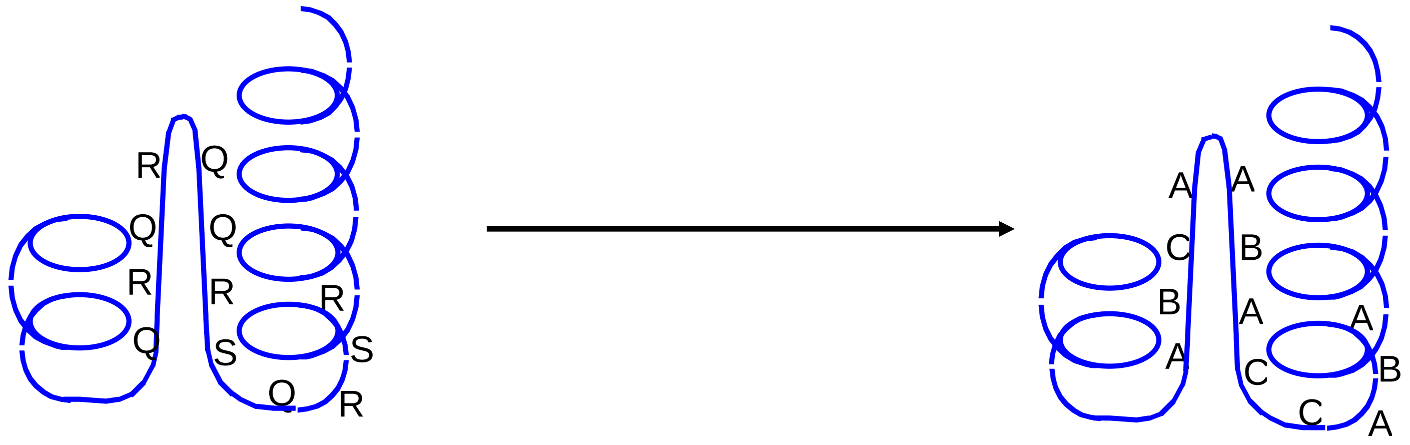
Experimental approaches

- 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

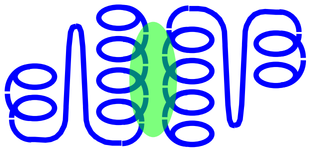
"improved" protein

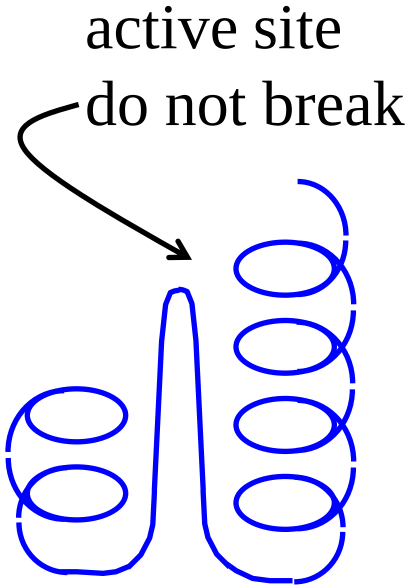
- Rules
  - structure should not change
  - should be able to fix some residues (active site, important)..

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

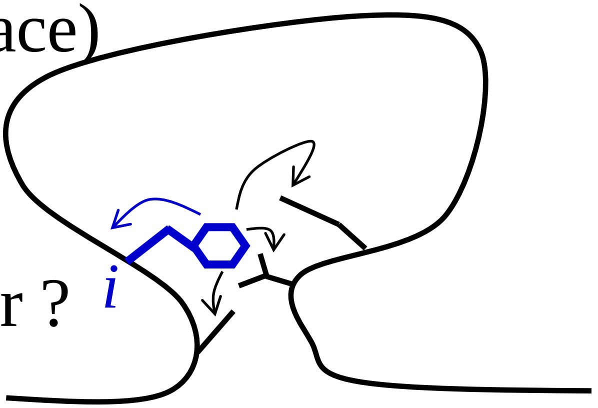
- how does sequence fit to structure ?
- sequence S={s_1, s_2, ..s_N}
- coordinates R = { $\mathbf{r}_1$, $\mathbf{r}_2$, … $\mathbf{r}_\mathrm{N}$}
- score = f (S, R)   (diffferent nomenclature soon)
- mission
    - adjust S to as to maximise score (minimise quasi-energy)

Score function

- how do amino acids
  - suit structure ?
  - suit each other ?

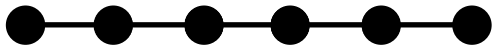
$$score = \sum_{i=1}^{N_{res}} score_{struct}(s_i, R) + \sum_{i=1}^{N_{res}} \sum_{j>i}^{N_{res}} score_{pair}(s_i, s_j, R)$$

- $score_{struct}$ 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



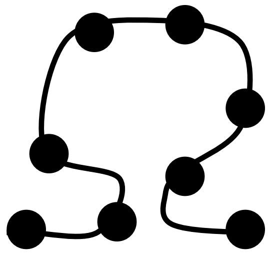
Searching

- systematic search – how long ?
- search space for N_{res} = 20 ×20 ×… = 20^{Nres}



- 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

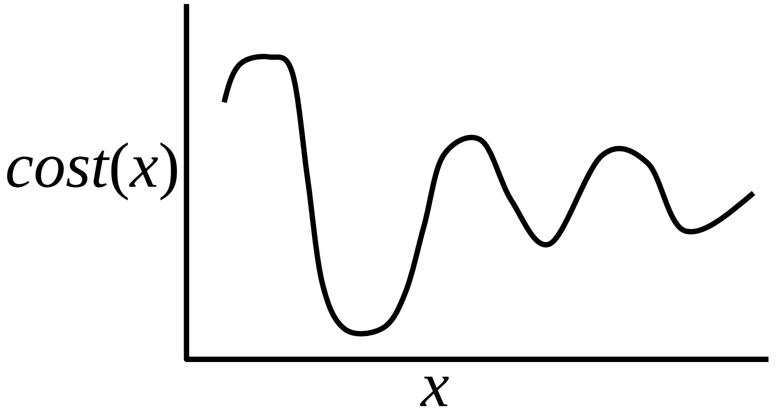
- more formally next semester
- first the problem

The sequence optimisation problem

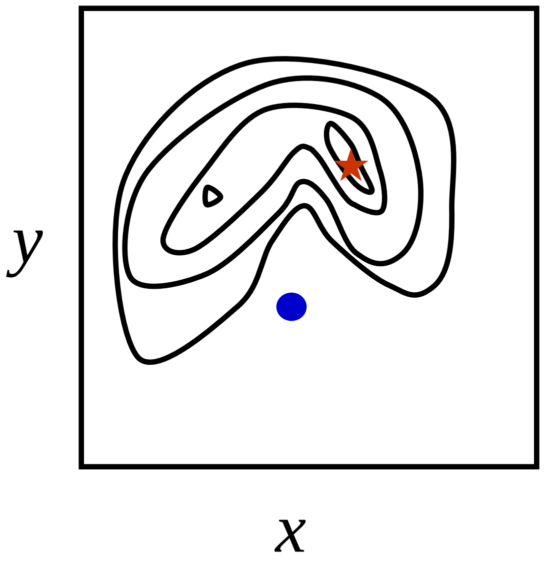
- discrete
- local minima / correlations in surface
- high dimensional

dimensions and correlations

- a 1D problem

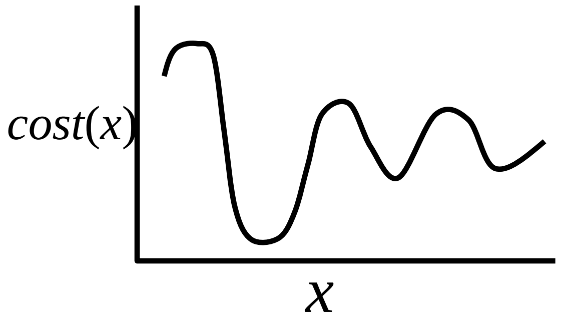


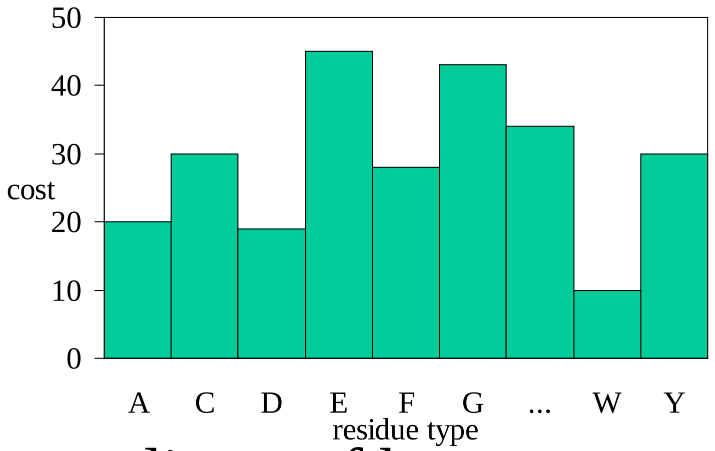
- local minima
- minimum of *x* depends on *y*
- cannot optimize *x* and *y* independently
- what are correlations in this problem ?



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

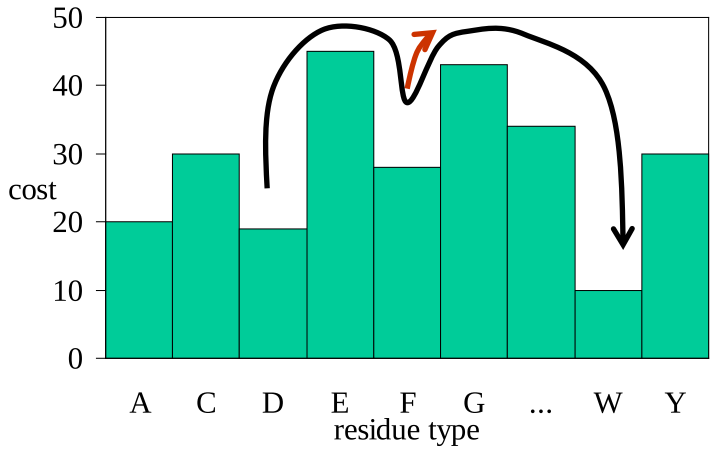




what do we want ?

- 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 the 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})
- Δc= *cost*(S_{n+1})- *cost*(S_n)
- our sign convention: if $\Delta c < 0$, system is better

When to accept ?

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

Formal acceptance rule

- $-\Delta c < 0, \quad e^{-\Delta c}$ is between 0..1
- $-\Delta c \approx 0$ then $e^{-\Delta c} \approx 1$ as $\Delta c \rightarrow \infty$ then $e^{-\Delta c} \rightarrow 0$
- formalise this rule

```
set up S=S_0 and cost(S_0)
while (not finished)
    S_trial = random step from S
    Δc = cost(S_trial)-cost(S)
    if (Δc < 0)                                  /* accept */
          S= S_trial
    else
          r = rand (0..1)
          if (e^-Δc ≥ r)
                S= S_trial
```

- 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 down, it tends to find lowest energy state
- change acceptance criterion to $e^{\frac{-\Delta c}{T}}$
  - as

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

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

- put this into previous description

why we need temperature

```
set up $S=S_0$ and $cost(S_0)$ set $T=T_0$
while (not finished)
    $S_{trial}$ = random step from S
    T = εT                                  /* ε bit smaller than 1 */
    $\Delta c = cost(S_{trial}) - cost(S)$
    if ($\Delta c < 0$)
        $S = S_{trial}$
    else
        r = rand (0..1)
        if ( $\exp(-\Delta c/T) \geq r$ )
            $S = S_{trial}$
```

- 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

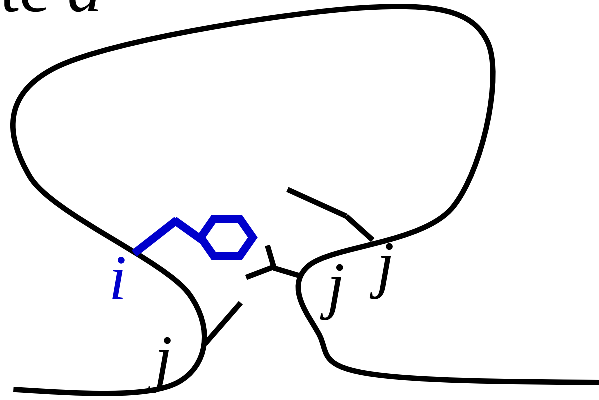
Nomenclature

- *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 ?

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



Nomenclature and rules

- there are 20 (N_{type}) residues
- which fits best to the fixed environment ?
- implies testing each of the N_{type} for a

$$\min_a E_1(s_i(a))$$

- what is the best energy type a at site i could have, interacting with one site j ?

$$E_1(s_i(a)) + \min_b E_2(s_i(a), s_j(b))$$

- what is the best energy that type a at i could have considering all neighbours ?

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

- 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 ?

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

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

- for any residues *a*, *c*
- if the best energy for *a* is worse than the worst for *c*
  - *a* cannot be part of the optimal solution … if

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

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

Dead-end elimination method

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

- 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
    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

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

- deterministic

Combining ideas

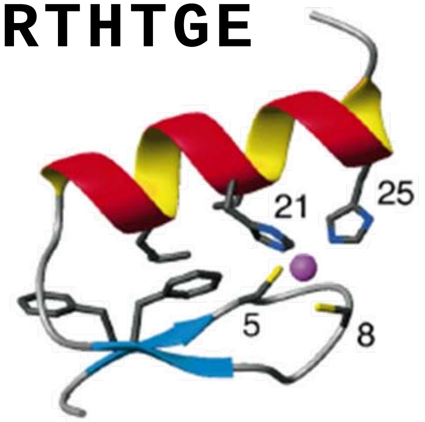
- use DEE to get a list of candidate residues at each position
- search remaining space with Monte Carlo / simulated annealing
- not deterministic

Success

- Method
  - Dead end elimination + systematic search

designed **QQYTAKIKGRTFRNEKELRDFIEKFKGR**

native **KPFQCRICMRNFSRSDHLTTHIRTHTGE**

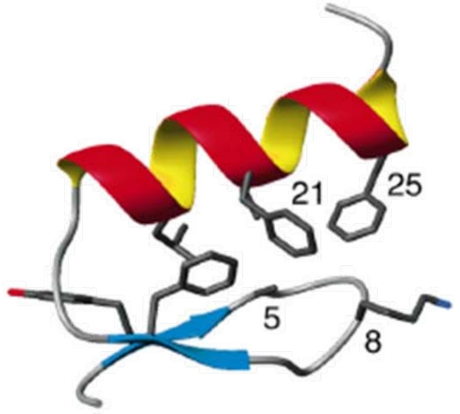


New sequence

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

- Structure solved by NMR

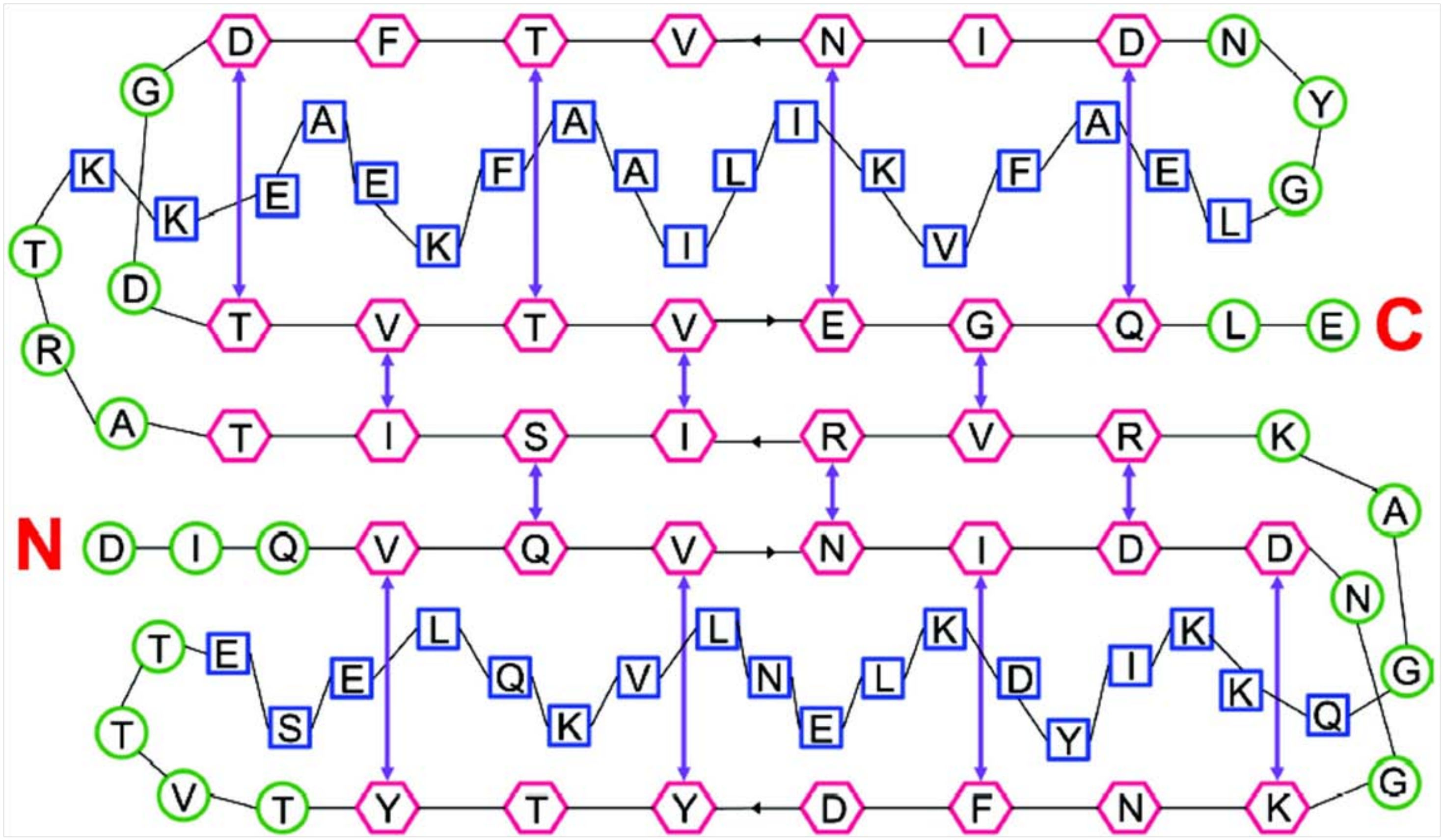
- Problem solved ?
  - maybe not



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

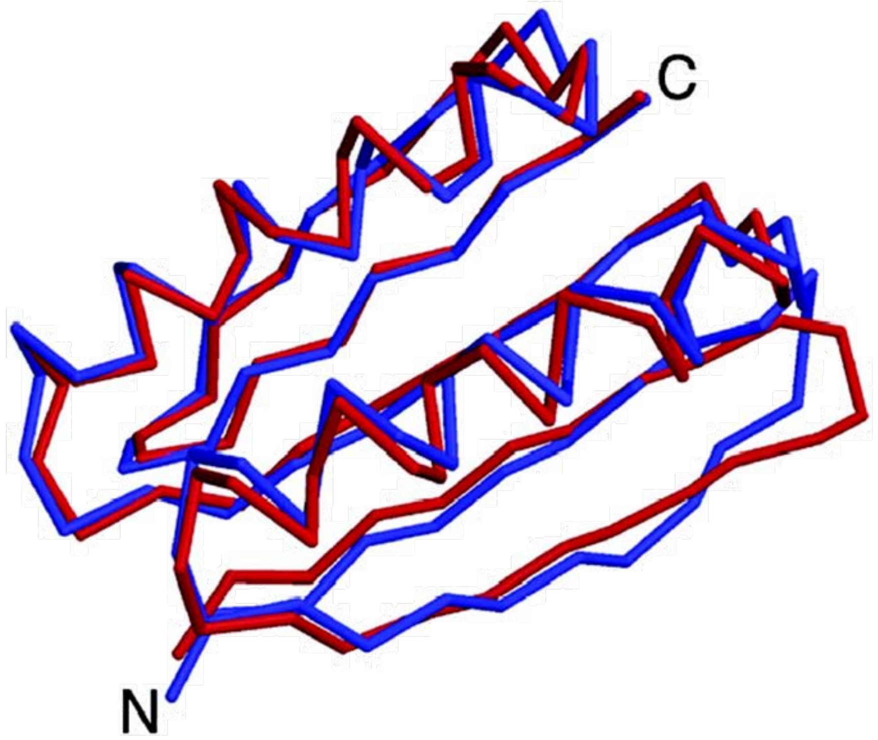
Result

- apparently new sequence

Structure

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

- Problem solved
    - unclear (how many failures ?)



Methods so far

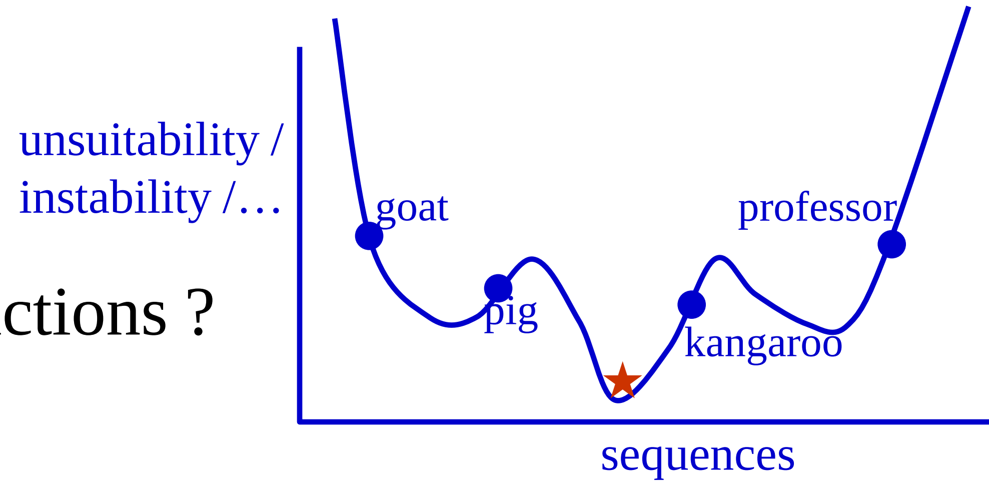
	Monte Carlo	Dead-end elimination
guaranteed global optimum	no	does not try
deterministic	no	yes

Only one answer ?

May not matter

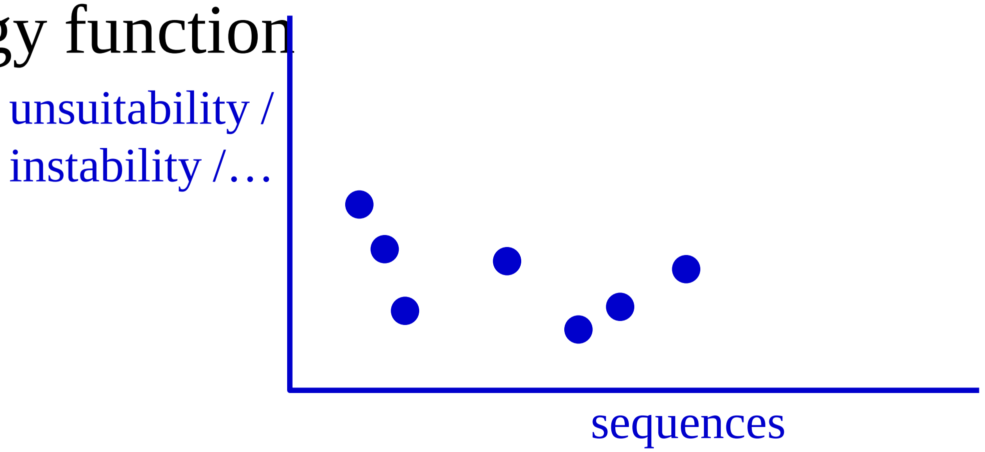
- consider real proteins – compare human, goat, …
  - all stable – all slightly different
- implication
  - there may be many solutions which are equally good

- How good are our energy functions ?

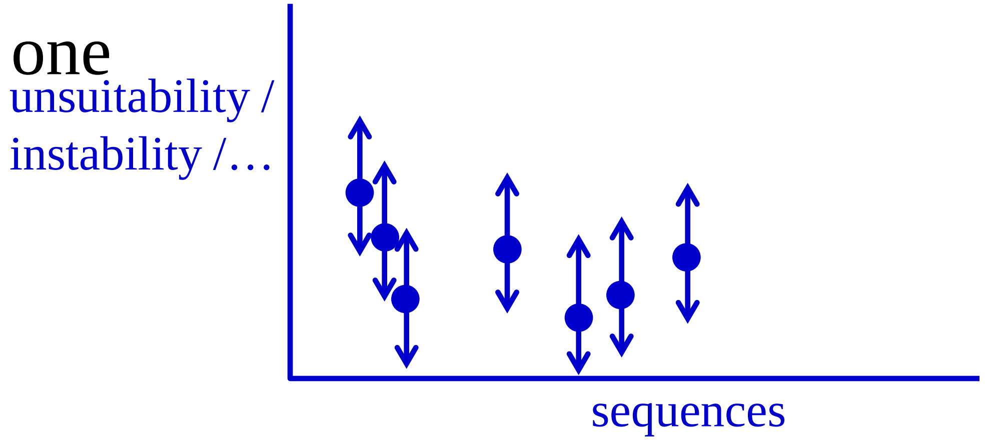


Determinism and energy

- I have a perfect score / energy function

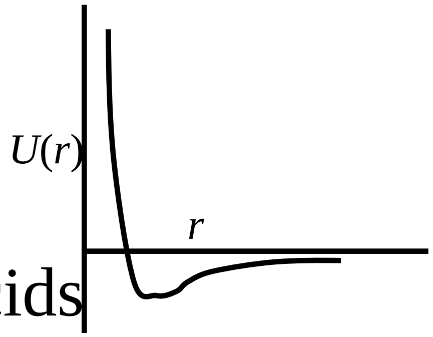


- I have errors / approximations
  - best answer could be any one

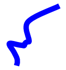


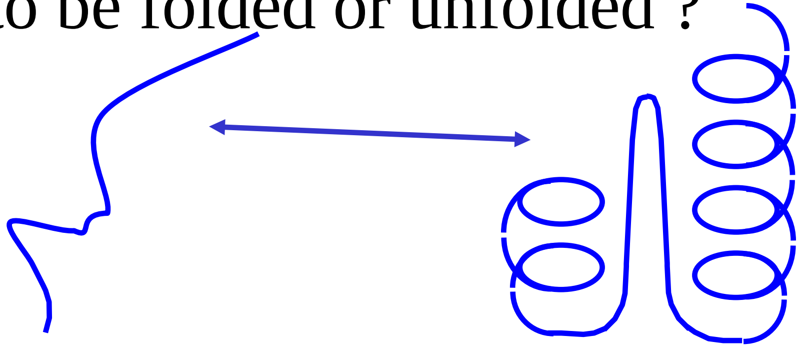
Problems – stability / energy

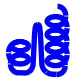
- energy functions
- what do we mean by energy ?
- example – two charges $$U(r) = \frac{q_1 q_2}{Dr}$$
- example – two argon atoms $$U(r) = 4\varepsilon\left(\sigma^{12} r^{-12} - \sigma^{6} r^{-6}\right)$$
- make energy better ?
  - replace every amino acid by a larger one (more contacts – more negative 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 ?

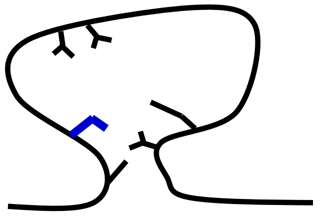


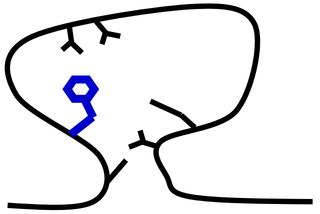
- my energy function tells me to change "X" to "Y"
  - it affects both the good  and bad
  - has it affected the energy difference ?
    - no guarantee

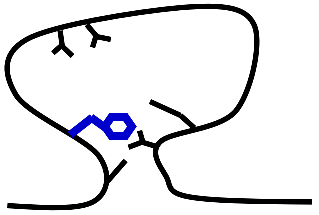
- current score functions ?
  - some pure potential energy
  - very difficult to estimate ΔG

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 ?

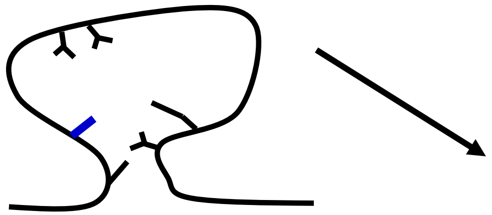






- how to cope with side chain positions in a practical way
  - optimise location of sidechains
  - use average
  - explicit rotamers

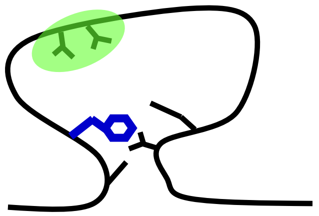
Sidechains – optimise at each step



- I 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

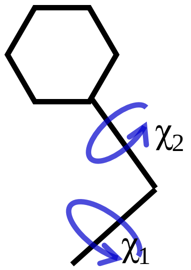


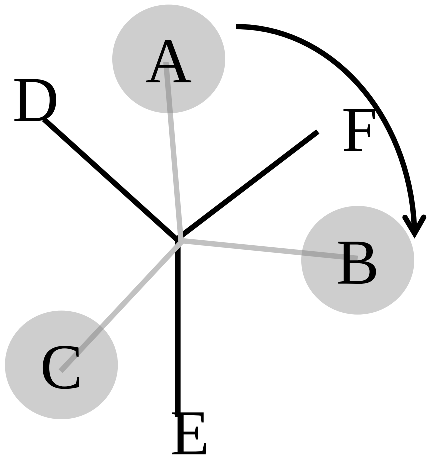
Sidechains – use averaging

- 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 ?

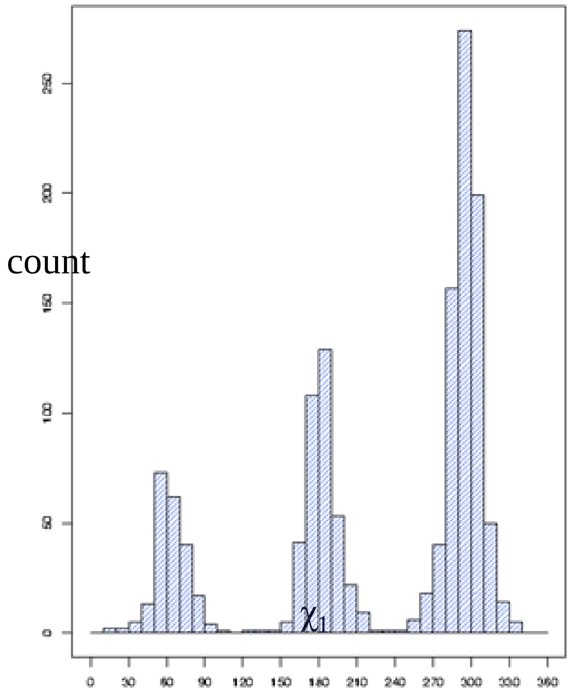
Sidechains – use rotamers

- 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 angle ?



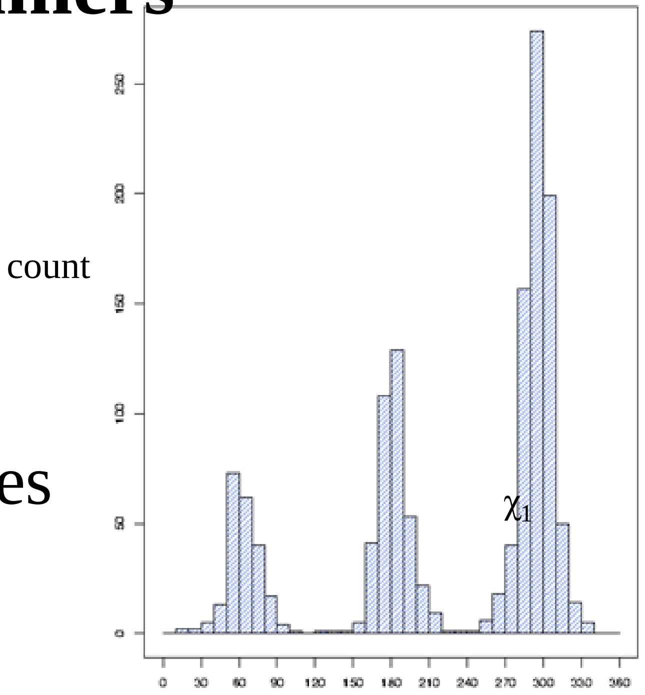
- how to use this ?
  - look for most popular angles (60, 180, 300)

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

- Experimental approaches
- 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