Protein Fold Recognition Weak Similarities

- why do we do sequence alignments ?
  - find related proteins
    - build models
    - guess at function
- For some interesting protein
  - sequence always available
- What should one do with really weak sequence homology ?
- two ideas
  - how to search for very weak similarities
  - can one take advantage of conserved structures ?

Mission

Problem

- for some protein sequence – find as much as possible
  - function
  - build good model
  - build a bad model

- relatively vague information may be useful
  - which residues are near active site ?
  - which residues are near a dimer interface ?
  - which residues are in weakly structured loops ?
  - overall shape (bad model) may be enough for phasing

Approach

- start with most reliable methods
- add more speculative methods as necessary

- Example
  - simple sequence searches
  - searches for more remote homologues
  - searches for possible structures

- methods so far
  - emphasis on speed (in Georgio's lectures)

alignment methods

	slow	fast
methods	Needleman & Wunsch / Smith-Waterman	seeded – blast, fasta, suffix tree methods
time	O(nm) or O(nm^2) (sequence sizes)	O(nk) – database size
guaranteed to find optimal alignment	yes	no
very remote homologues	may work	less likely to work

- does speed matter ?

Slow methods

- Methods for large databases are
  - fast
  - approximate

- Here
  - ultimate use is often a small database (PDB 7×10^4)
  - computer time does not matter

- In lab you have 1 or 10's of proteins
  - each take weeks or months to work on
  - if each search takes hours ? no problem
- remote searches

Remote searches

When to do this ?

- Assume simple (blast / fasta) search returned
  - related sequences
  - unknown function
  - none of related proteins have known structures

Weak sequence similarities

- Your sequence

yours A B D E F G H I K L M N P Q…

- finds no helpful proteins. Try searching with a related protein

prot_1 A B Q E F G R I S L T N P Q…

- finds a protein whose structure has been solved

prot_2 Q B Q E Q G R Q S L T N P A…

- claim
  - yours & prot_2 are related
  - relationship too weak to see directly
  - prot_2 can be used
    - to make a bad model
    - as a guess for function

Weak sequence similarities

- first idea
- take your protein
- collect related proteins
    - foreach (related protein)
        - do a sequence search
        - see if results change

- not practical
- not very systematic

- what else does one get from homologues ?

Information from related sequences

- usually one finds many related sequences.
- consider details…

```
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
MLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAAWGKVGAHAGEYGAEALEKMFLSFPTTKTYFPHFDLSHGSAQVKGHG
 LSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAAWGKVGAHAGDYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPDDKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
MLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTHVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAAWGKVGAHAGEYGAEAWERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
MLSPADKTNVKAAWGKVGAHAGEYGAEAWERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
MLSPADKTNVKAYWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAHWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSAADKTNVKAGWSKVGGHAGEYGAEALERMFLGFPTTKTYFPHFDLSHGSAQVKAHG
VLSAADKTNVKAFWSKVGGHAGEYGAEALERMFLGFPTTKTYFPHFDLSHGSAQVKAHG
VLSADDKANIKAEWGKIGGHGAEYGAEALERMFCSFPTTKTYFPHFDVSHGSAQVKGHG
MLSPADKTNVKADWGKVGAHAGEYGAEAFERMFLSFPTTKTYFPHFDLSHGSAQVKGQG
VLSPADKTNVKACWGKVGAHAGEYGAEAFERMFLSFPTTKTYFPHFDLSHGSAQVKGQA
VLSAADKSNVKAAWGKVGGNAGAYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
MLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKSNVKATWDKIGSHAGEYGGEALERTFASFPTTKTYFPHFDLSPGSAQVKAHG
VLSPADKSNVKAWWGKVGGHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
MLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTGTYFPHFDLSHGSAQVKGHG
VLSSADKNNVKACWGKIGSHAGEYGAEALERTFCSFPTTKTYFPHFDLSHGSAQVQAHG
VLSAADKSNVKAAWGKVGGNAGAYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAQWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSANDKSNVKAAWGKVGNHAPEYGAEALERMFLSFPTTKTYFPHFDLSHGSSQVKAHG
VLSPADKSNVKAAWGKVGGHAGDYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
    …       …        … …
```

Conservation

- as in secondary structure prediction lectures
- if your sequence has a Q here,
    - may not be helpful to use it in sequence searches

```
L D D Q R Q   S T R
L D A Q R A D S T R
V D D Q R R W S T R
A D D Q R C A S S K
I D D Q R D D S T R
L D D Q R E G S T K
L D D Q R F C S T R
```

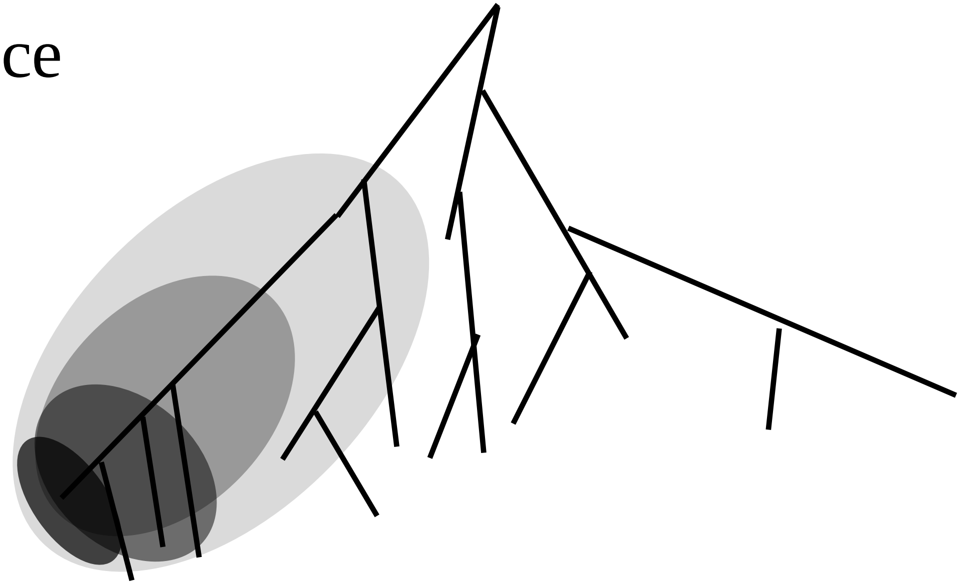
    - better to use the "average" residue at this point
- first have to find the "average" residue
- leads to method

Searching with profiles

- initial average_sequence = your_sequence
- while (step < max_steps)
  - search with blast using average_sequence
  - if interesting result (function / structure..)
    - return results
  - else
    - update average_sequence

- basis of "psi-blast"

- does it work ?



Remote sequence searching

- much more sensitive than simple searches, but

- involves weaker sequence similarities, more errors
- alignment not perfect
- statistical significance harder to estimate
- possibility of finding unrelated sequences (rubbish)

- still relies on some significant sequence similarity

- can one move away from sequence similarity ?

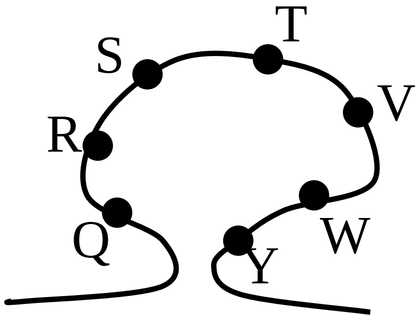
Sequence alignments – implied structures

- From sequence viewpoint

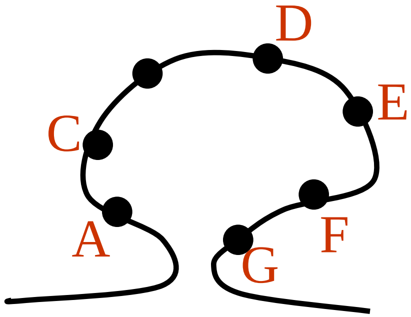
```
..AC-DEFG..
..QRSTVWY..
```

- what if structure of second sequence is known ?

```
..AC-DEFG..   query sequence
..QRSTVWY..   known structure
```



known structure

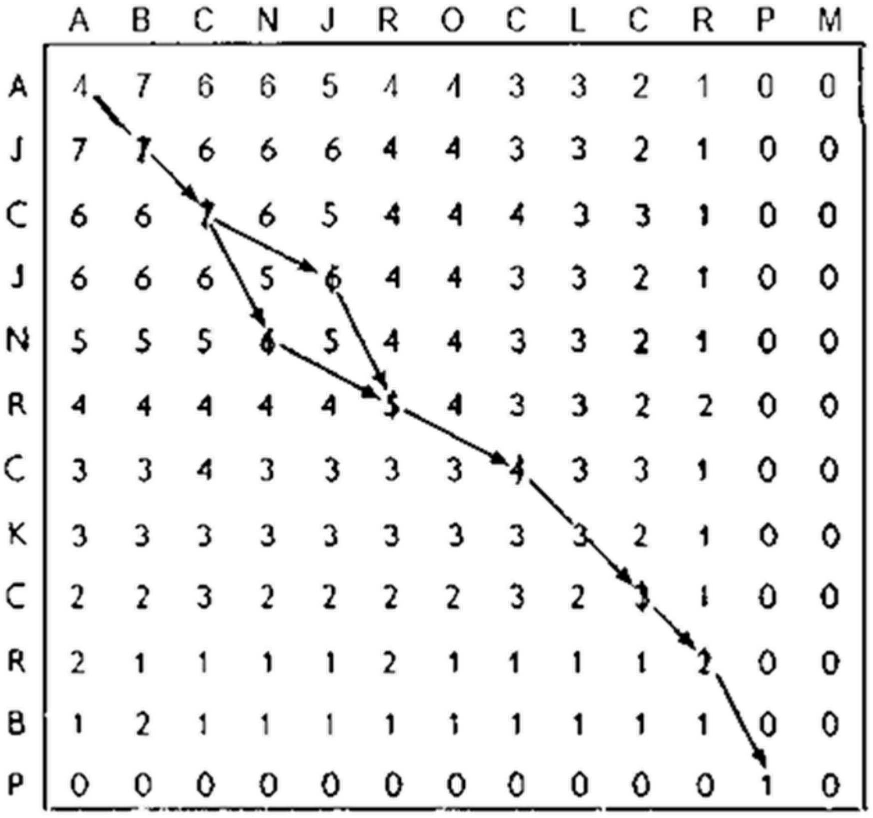


model implied

Sequence to structure alignments

- Remember how sequence alignments work
  - similarity / substitution scores
  - fill out score matrix
  - find best path

- Can we use this for sequenc to structure alignments ?



	A	B	C	N	J	R	O	C	L	C	R	P	M
A	4	7	6	6	5	4	4	3	3	2	1	0	0
J	7	7	6	6	6	4	4	3	3	2	1	0	0
C	6	6	7	6	5	4	4	4	3	3	1	0	0
J	6	6	6	5	6	4	4	3	3	2	1	0	0
N	5	5	5	6	5	4	4	3	3	2	1	0	0
R	4	4	4	4	4	5	4	3	3	2	2	0	0
C	3	3	4	3	3	3	3	4	3	3	1	0	0
K	3	3	3	3	3	3	3	3	3	2	1	0	0
C	2	2	3	2	2	2	2	3	2	3	1	0	0
R	2	1	1	1	1	2	1	1	1	1	2	0	0
B	1	2	1	1	1	1	1	1	1	1	1	0	0
P	0	0	0	0	0	0	0	0	0	0	0	1	0

Needleman, SB & Wunsch, CD, (1970) J. Mol. Biol. 48, 443-453

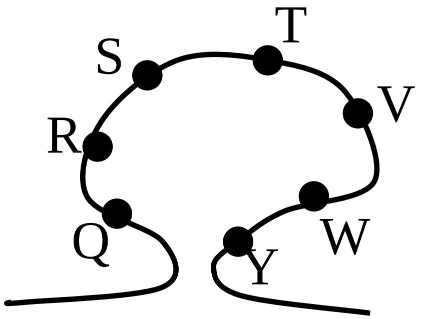
more exotic scoring

- From sequence viewpoint

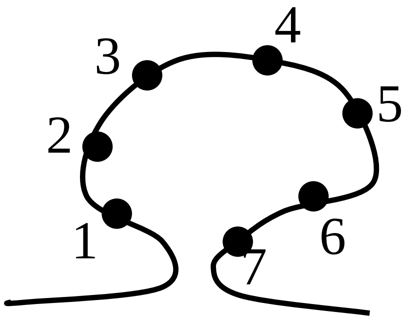
  `..AC-DEFG..` my sequence

  `..QRSTVWY..` a protein of known structure

- rather than just align sequences, could I use the structure ?



known structure



forget sequence

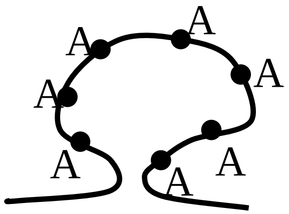
- score matrix ?

	A	C	D	E	F	G
1	?	...				
2						
3						
4						
5						
6						
7						

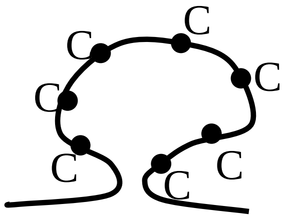
sequence to structure scoring

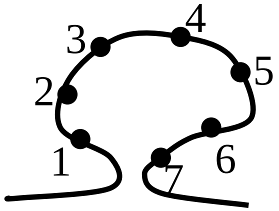
	A	C	D	E	F	G
1	?	...				
2						
3						
4						
5						
6						
7						

- I have to be able to place (A, C, D..) at each position and get a suitability score



then

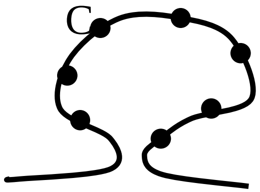


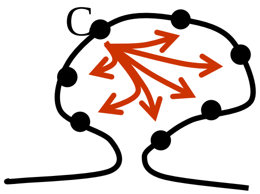


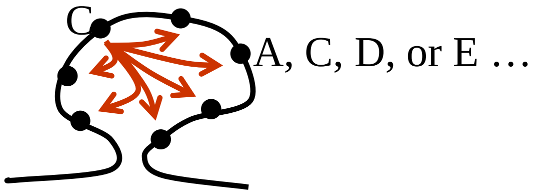
- then it would be easy to do sequence to structure alignments
- advantage:
  - we claim that structure is more conserved than sequence
  - can find appropriate/fitting/suitable structures for a sequence
  - very remote, but homologues
- vorsicht !!!!

sequence to structure scoring

- define an energy function
  - depends on interaction of residue with structure
    - easy
  - depends on interaction with neighbours
    - but who are the neighbours ?



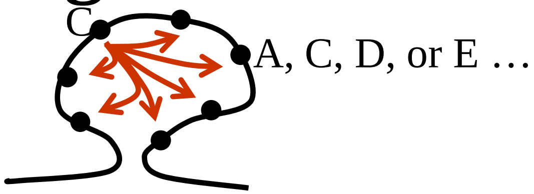




- bad news
  - we cannot even fill out a column in the score matrix
  - to test every combination of neighbours
    - NP-complete
- an excuse to try some approximations

	A	C	D	E	F	G
1		?				
2		?				
3		?				
4		?				
5		?				
6		?				
7		?				

approximations for scoring



- two problems
  - we do not know where all the atoms are – side chain coordinates
  - to score "C" at each position we need to know neighbours
- side-chains : ignore / average
  - use a score / energy function which averages over all conformations
- neighbour positions : much harder
  - environment description
  - frozen approximation

Environment description

- an example of profiles (case study)
- we know
  - certain sites are hidden from solvent (middle of protein)
    - only compatible with trp, phe, ile, … (hydrophobic)
  - some sites are involved in "salt bridges"
  - some secondary structures are preferred by certain residues
- can one count the probabilities of residue types ?

- overview
  - collect list (parameterisation set) of proteins
  - classify sites (18 types)
  - collect probability of each residue type in each site type

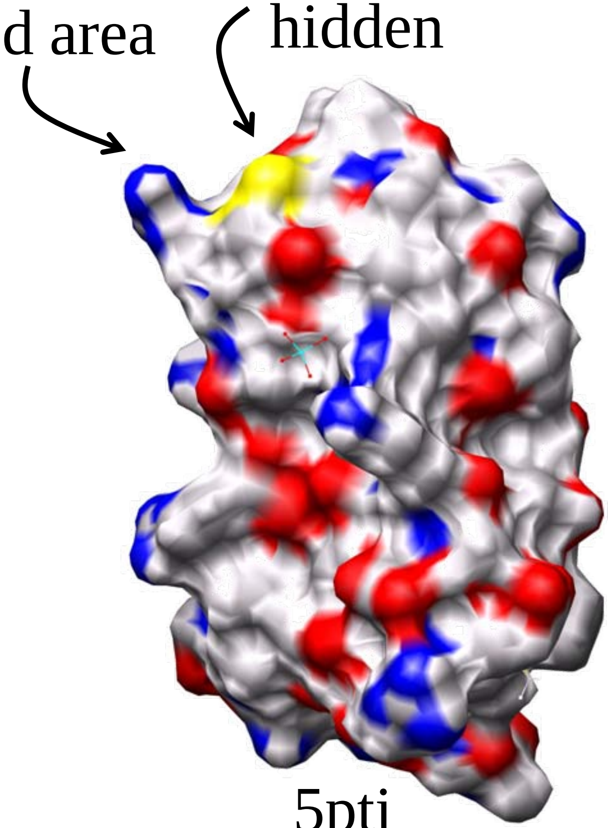
Bowie, J.U., Lüthy, R, Eisenberg, D. (1991) Science 253, 164-170

Environment description

- for each site measure the Å^2 exposed to solvent

- maybe sometimes one has charges / polar groups touching others
  - measure fraction of buried area covered by polar groups

- define environments…

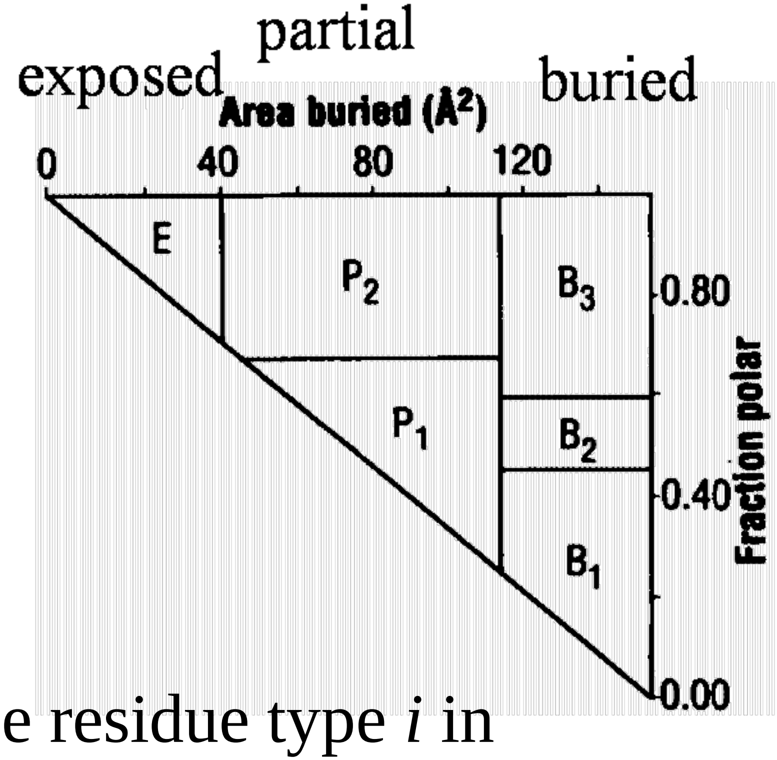


5pti

Environment description

- 6 environment types
- 3 secondary structure types
  - α, β, others
- = 18 environments

- data collection
  - 16 proteins
  - find environment of each site
  - count
    - how many times does one see residue type *i* in environment *j* = *N*(*i*,*j*)
  - count – how many times does one see residue type *i* = *N*(*i*)



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Environment description

- how unusual is a residue *i* in environment *j* ?

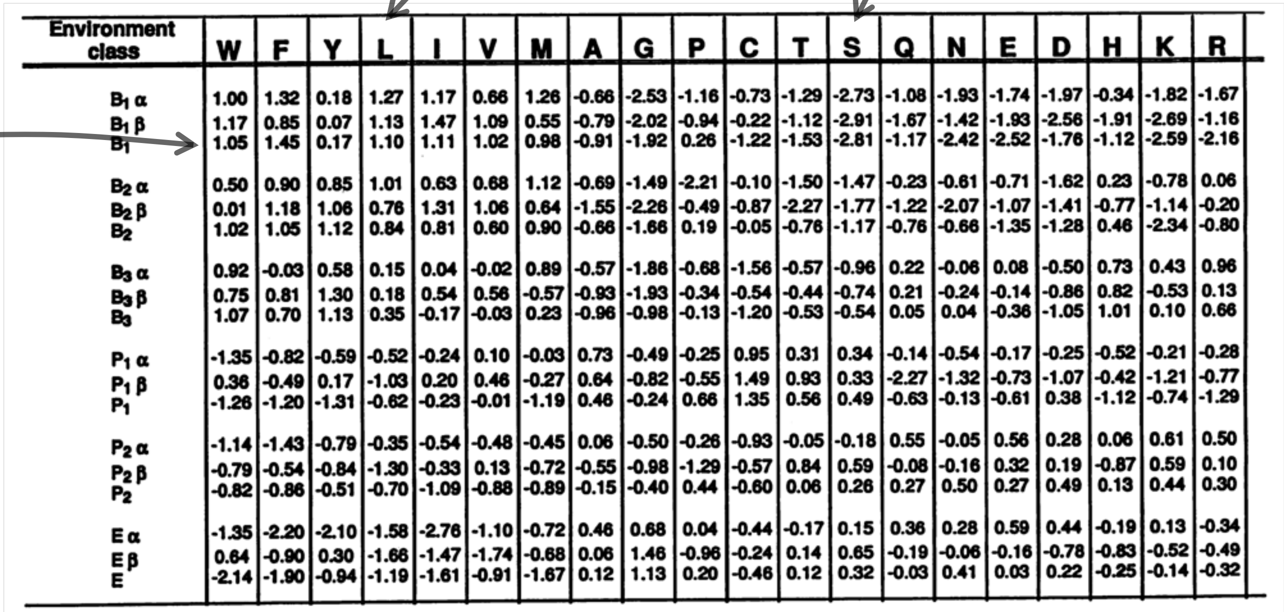
$$score(i, j) = \ln\left(\frac{N(i, j)}{N(i)}\right)$$

- final result ? a big scoring table

likely

unlikely

what one expects



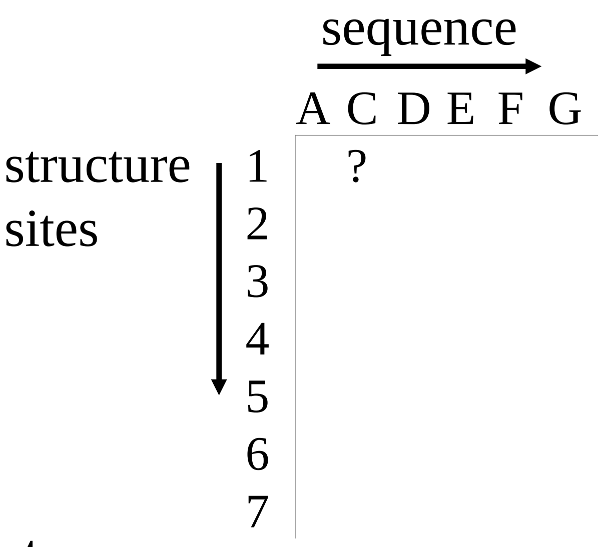
Environment class	W	F	Y	L	I	V	M	A	G	P	C	T	S	Q	N	E	D	H	K	R
$B_1 \alpha$	1.00	1.32	0.18	1.27	1.17	0.66	1.26	-0.66	-2.53	-1.16	-0.73	-1.29	-2.73	-1.08	-1.93	-1.74	-1.97	-0.34	-1.82	-1.67
$B_1 \beta$	1.17	0.85	0.07	1.13	1.47	1.09	0.55	-0.79	-2.02	-0.94	-0.22	-1.12	-2.91	-1.67	-1.42	-1.93	-2.56	-1.91	-2.69	-1.16
B_1	1.05	1.45	0.17	1.10	1.11	1.02	0.98	-0.91	-1.92	0.26	-1.22	-1.53	-2.81	-1.17	-2.42	-2.52	-1.76	-1.12	-2.59	-2.16
$B_2 \alpha$	0.50	0.90	0.85	1.01	0.63	0.68	1.12	-0.69	-1.49	-2.21	-0.10	-1.50	-1.47	-0.23	-0.61	-0.71	-1.62	0.23	-0.78	0.06
$B_2 \beta$	0.01	1.18	1.06	0.76	1.31	1.06	0.64	-1.55	-2.26	-0.49	-0.87	-2.27	-1.77	-1.22	-2.07	-1.07	-1.41	-0.77	-1.14	-0.20
B_2	1.02	1.05	1.12	0.84	0.81	0.60	0.90	-0.66	-1.66	0.19	-0.05	-0.76	-1.17	-0.76	-0.66	-1.35	-1.28	0.46	-2.34	-0.80
$B_3 \alpha$	0.92	-0.03	0.58	0.15	0.04	-0.02	0.89	-0.57	-1.86	-0.68	-1.56	-0.57	-0.96	0.22	-0.06	0.08	-0.50	0.73	0.43	0.96
$B_3 \beta$	0.75	0.81	1.30	0.18	0.54	0.56	-0.57	-0.93	-1.93	-0.34	-0.54	-0.44	-0.74	0.21	-0.24	-0.14	-0.86	0.82	-0.53	0.13
B_3	1.07	0.70	1.13	0.35	-0.17	-0.03	0.23	-0.96	-0.98	-0.13	-1.20	-0.53	-0.54	0.05	0.04	-0.36	-1.05	1.01	0.10	0.66
$P_1 \alpha$	-1.35	-0.82	-0.59	-0.52	-0.24	0.10	-0.03	0.73	-0.49	-0.25	0.95	0.31	0.34	-0.14	-0.54	-0.17	-0.25	-0.52	-0.21	-0.28
$P_1 \beta$	0.36	-0.49	0.17	-1.03	0.20	0.46	-0.27	0.64	-0.82	-0.55	1.49	0.93	0.33	-2.27	-1.32	-0.73	-1.07	-0.42	-1.21	-0.77
P_1	-1.26	-1.20	-1.31	-0.62	-0.23	-0.01	-1.19	0.46	-0.24	0.66	1.35	0.56	0.49	-0.63	-0.13	-0.61	0.38	-1.12	-0.74	-1.29
$P_2 \alpha$	-1.14	-1.43	-0.79	-0.35	-0.54	-0.48	-0.45	0.06	-0.50	-0.26	-0.93	-0.05	-0.18	0.55	-0.05	0.56	0.28	0.06	0.61	0.50
$P_2 \beta$	-0.79	-0.54	-0.84	-1.30	-0.33	0.13	-0.72	-0.55	-0.98	-1.29	-0.57	0.84	0.59	-0.08	-0.16	0.32	0.19	-0.87	0.59	0.10
P_2	-0.82	-0.86	-0.51	-0.70	-1.09	-0.88	-0.89	-0.15	-0.40	0.44	-0.60	0.06	0.26	0.27	0.50	0.27	0.49	0.13	0.44	0.30
$E \alpha$	-1.35	-2.20	-2.10	-1.58	-2.76	-1.10	-0.72	0.46	0.68	0.04	-0.44	-0.17	0.15	0.36	0.28	0.59	0.44	-0.19	0.13	-0.34
$E \beta$	0.64	-0.90	0.30	-1.66	-1.47	-1.74	-0.68	0.06	1.46	-0.96	-0.24	0.14	0.65	-0.19	-0.06	-0.16	-0.78	-0.83	-0.52	-0.49
E	-2.14	-1.90	-0.94	-1.19	-1.61	-0.91	-1.67	0.12	1.13	0.20	-0.46	0.12	0.32	-0.03	0.41	0.03	0.22	-0.25	-0.14	-0.32

Bowie, J.U., Lüthy, R, Eisenberg, D. (1991) Science 253, 164-170

Environment description - application

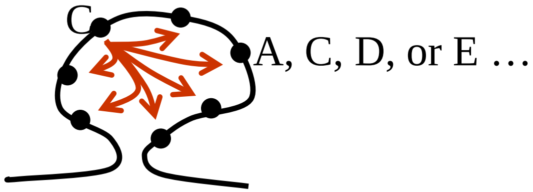
- given these descriptions – use them
- take a protein structure label each site
- take sequence of interest
- for each residue
  - score at each site of protein
- score matrix
- find best path
  - sequence to structure alignment

- final application
  - take protein databank
  - try to align your sequence to every structure

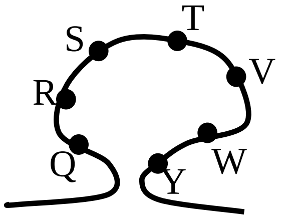


Frozen approximation

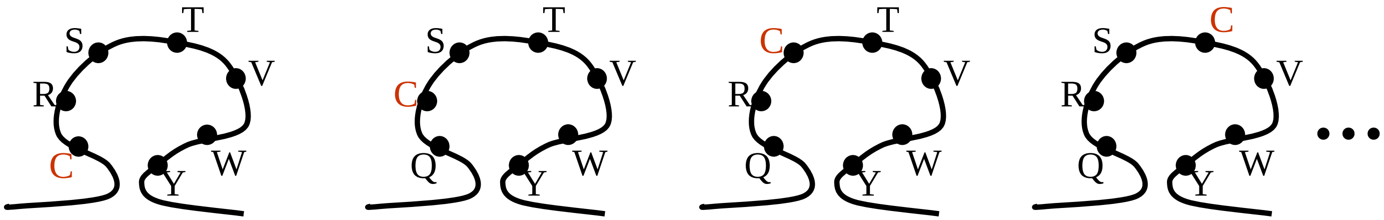
- original problem
  - we want to use a score function which
    - sensitive to sequence
    - sensitive to structure



- remember – original structure did have a sequence
- belief
  - if two proteins are related, the sequences will have similar properties
  - score with the residues of the original sequence



known structure



Frozen approximation

- I can score my sequence in the environment of the known structure
- good
  - the environment is well characterised
    - if my structure has polar residues here, they will go into the scoring function
- bad ?
  - we use the sequence of template (known structure)
  - it may only allow very related residues
  - original aim was to move away from close sequences

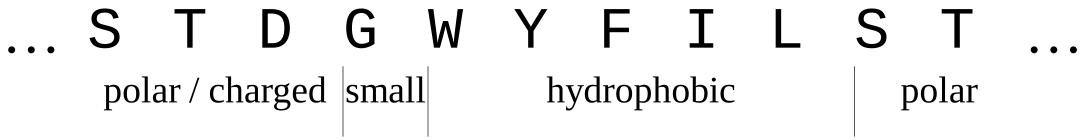
		sequence					
		A	C	D	E	F	G
structure	1		?				
sites	2						
	3						
	4						
	5						
	6						
	7						

Summary so far

- look for closely related templates
- try sequence based methods
- sequence to structure methods are definitely possible

- can I make better scoring schemes ?

Scoring schemes



… S T D G W Y F I L S T …

polar / charged | small | hydrophobic | polar

- how much structural information is hidden in sequence ?
- look at a sequence
- I already have labels for sites
  - implicit in substitution matrices
- does the structure contain extra information ? …

Extra information from structures

Residues exist in a protein for different reasons

- gly is easy to substitute – look at diagonal in blosum matrix
- in some turns, gly is essential
  - can only be seen from structure
- cys
  - sometimes a normal hydrophic residue
  - sometimes the geometry says it must form a disulfide bond
  - structure can say if there is another cys near in space
- …

- it should be useful to combine sequence and structure information

Extra information from structures

- Claim – hope
  - combination of methods has better signal / noise

- implementation ? easy in principle

  - for each residue *i* in your query sequence
    - for each site *j* in template
      - calculate sequence score s_1 based on profile of *i*
      - calculate structural score s_2 based on fitting residue type *i* into site *j*
      - score for alignment matrix = $s_1 + k\, s_2$
  - for some constant *k*

In practice

- most fold recognition programs combine sequence terms and structural scores
- results may or may not be better than best pure sequence methods

- problems..

Problems with clever methods

- Simple sequence searches
  - good models for statistical significance
    - (is a related protein really related)
- Remote sequence searches (psi-blast)
  - statistics OK, but less reliable

- Structure / Sequence+structure methods ?
  - no good model for scores
  - no good model for statistical significance

  - how will score grow with
    - size of query
    - size of alignment
    - sequence composition ?

Principle

- If you have extra information (structure)
  - must be a good idea to use it

	sequence	structure based
database size	10^6-10^7	10^4
	fast	slow
scores	good models	weaker
statistical significance	good or almost good	weaker

Summarise and stop

- Use sequence information when possible
- use adventurous sequence methods when necessary
- use very speculative methods (sequence to structure) when necessary