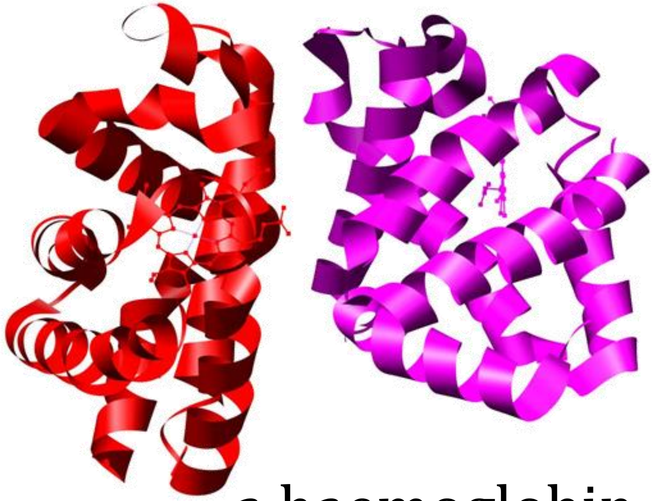
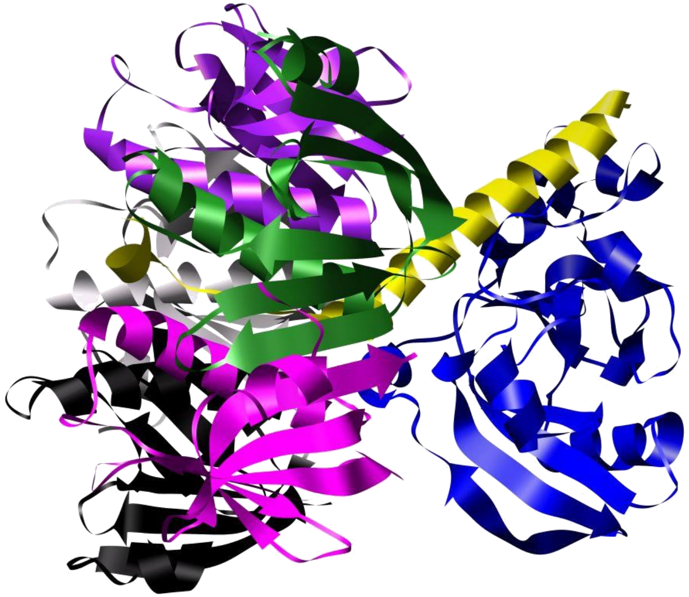
Protein Domains

Two weeks for this topic

- many proteins have separate chains



a haemoglobin (1h97)



enterotoxin (1lts) 7 chains

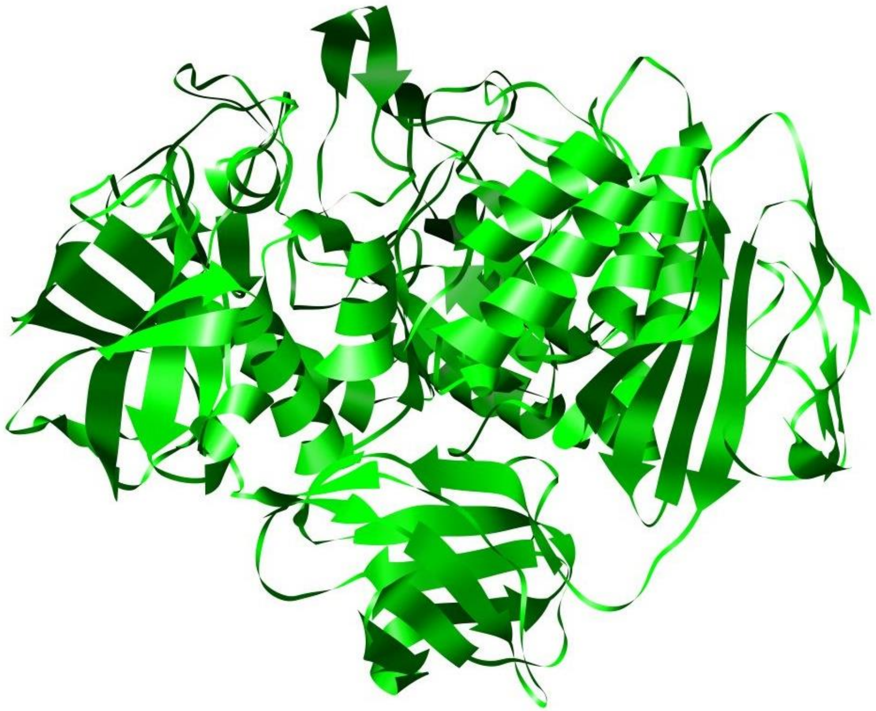
- what about units within one chain ?

4 domain protein

1cxl has 686 residues

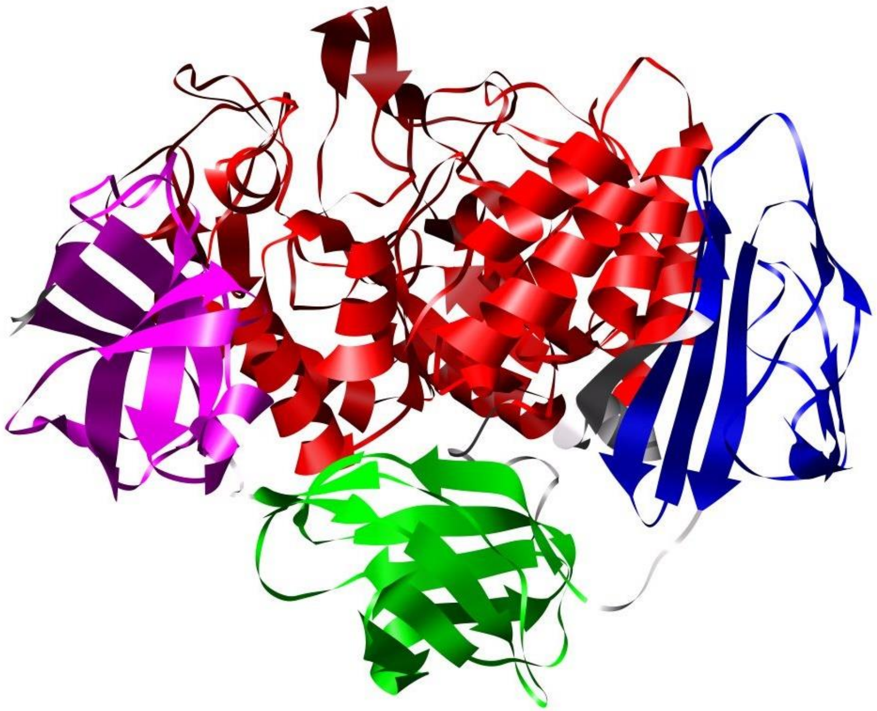
Cleaves carbohydrate bond

- one solid lump but…



4 domain protein

1. α-amylase catalytic
2. α-amylase C-terminal
3. immunoglobulin like domain
4. starch binding



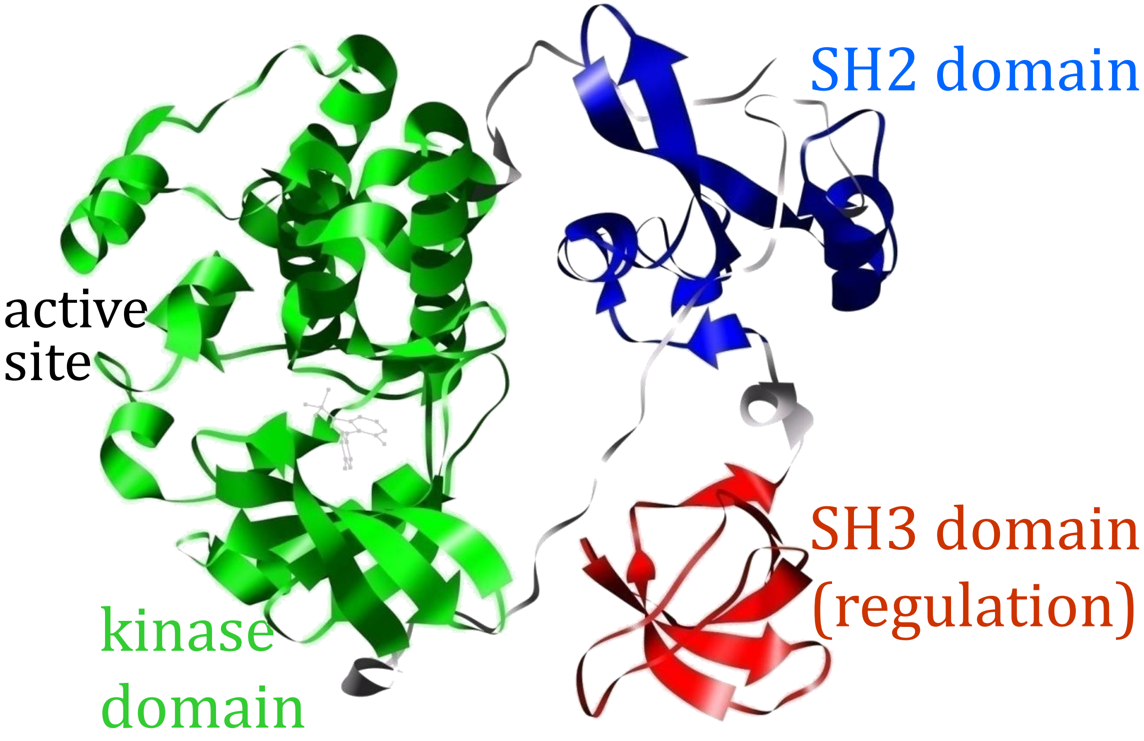
- even clearer example

3 domain protein

1qcf "src tyrosine kinase"

The domains really are common to other proteins

Number of domains is not absolutely defined



Plan

- chemistry, examples
- methods to automatically recognise domains (examples)
- chemistry – how common are domains of different sizes, types, …

Earlier history

Term "domain" used before there were many structures

- Invented example: protein that
  - joins ADP + P_i → ATP
  - performs some oxidation
  - responds to some regulator

  - take protein + protease (splits protein in a few places)
  - cleave / break protein - get a few pieces (2, 3, 4..)
  - purify pieces

  - pieces found that
    - can bind ADP/ATP
    - bind sugars, some regulators

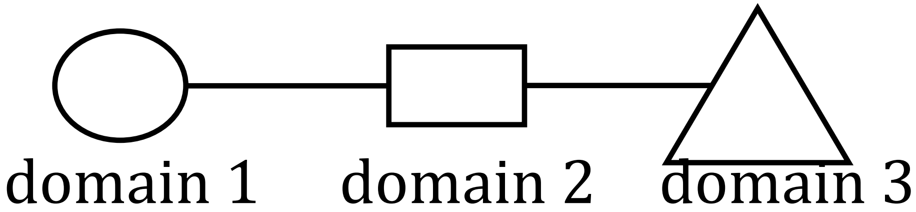
Earlier history

Appeared that for some proteins

- different functions associated with different pieces
- refer to as "functional domains"

Belief / claim

- bigger proteins are made from units, combined over evolutionary time scales



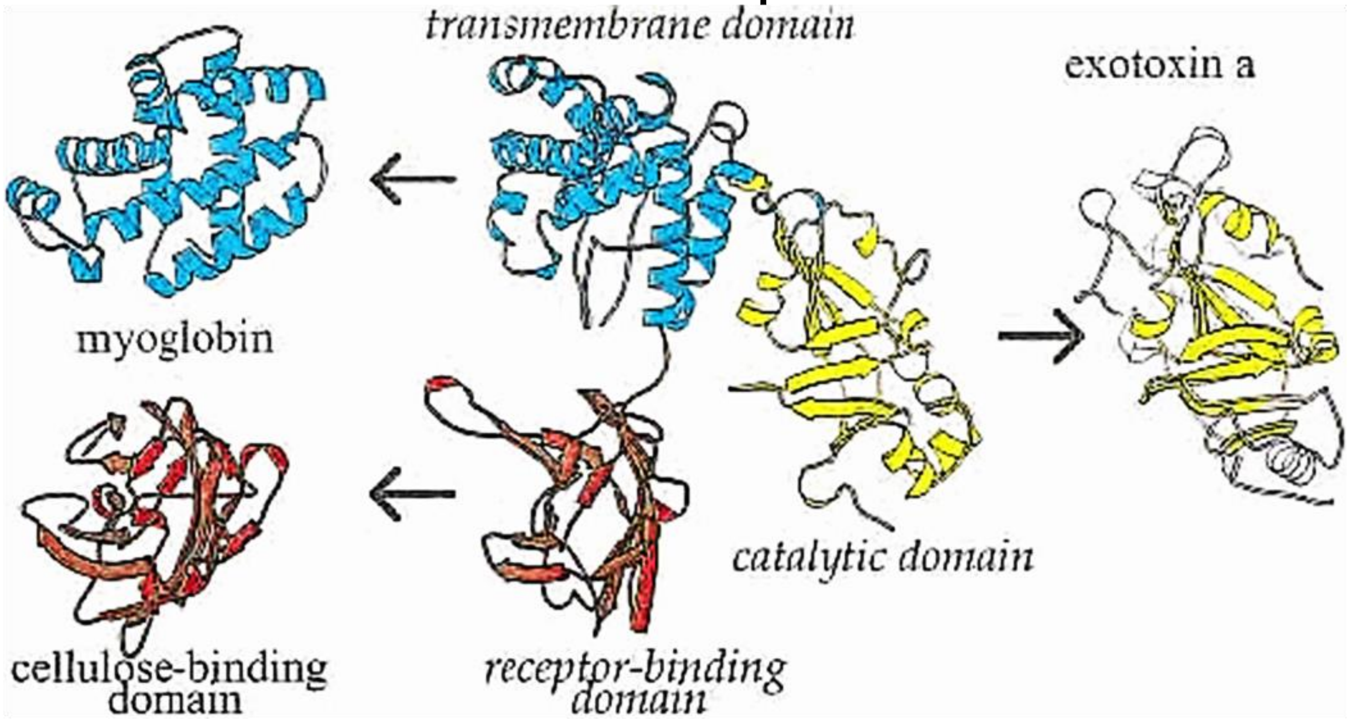
domain 1 domain 2 domain 3

- an example…

modular protein

Diptheria toxin (1ddt) middle of picture

- 3 domains
  - each similar to some different protein

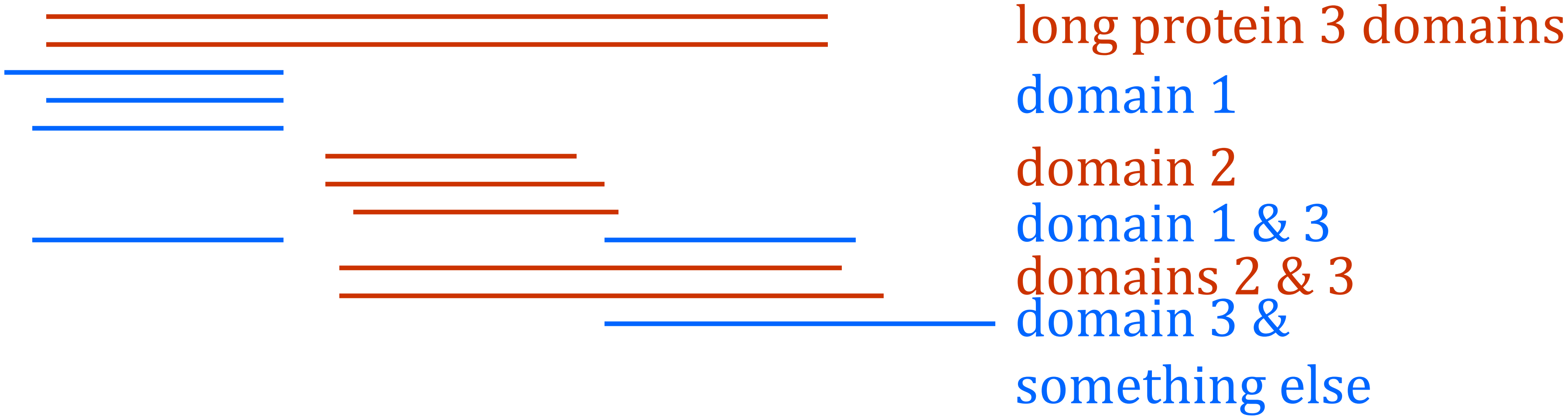


- appears as if modules are mixed together
- should be visible at sequence level…

picture from Holm, L & Sander, C. Proteins, 33, 88-96 (1998) Dictionary of recurrent domains in protein structures

Sequence level domains

Align a group of sequences



long protein 3 domains

domain 1

domain 2

domain 1 & 3

domains 2 & 3

domain 3 & something else

- appears to have 3 or 4 domains
- no reference to structures or function

Domain definitions summary

	structure	sequence	biochemistry
functional	not necessary	not necessary	yes
sequence-based	not necessary	yes	no
structure	yes	usually known	no

How important ?

- > ⅔ proteins have 2 or more domains
- part of definition
  - a piece of a protein which can fold and is stable

Now

- methods based on structure

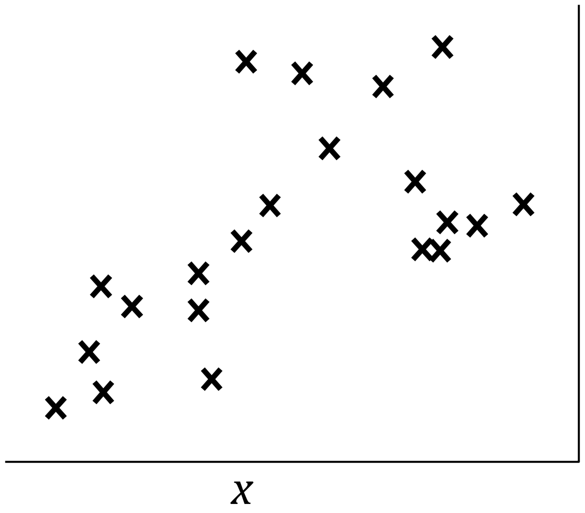
Finding Domains

A definition leads to methods

- domain is a compact unit

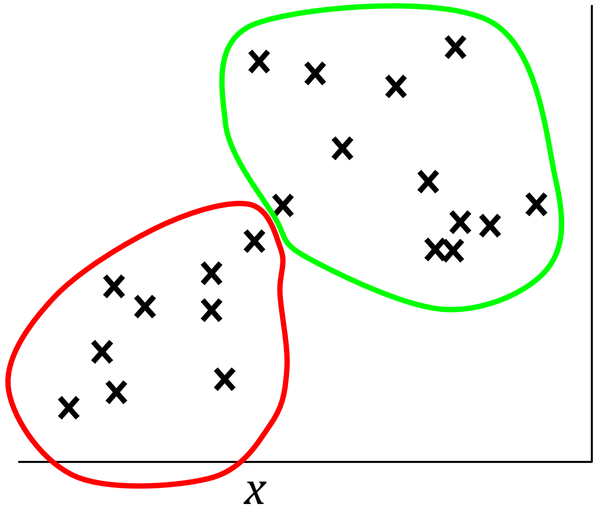
Objective way to look for dense units ?

- cluster analysis



Philosophy in cluster analysis

- look for dense groupings

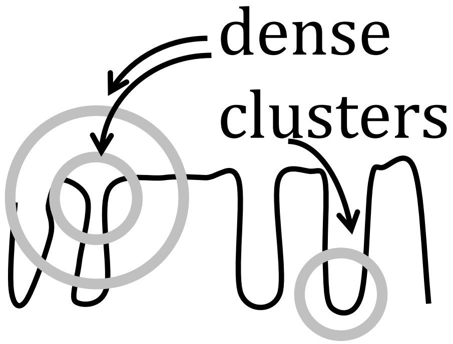


Leads to dendrogram

Clustering

Approach

- need a (dis)similarity matrix between every object
- here: distance between C^{α} atoms



residue	1	2	⋯	N
1	0	…	…	…
2		0	…	
…			⋱	
N				0

- does this work ?

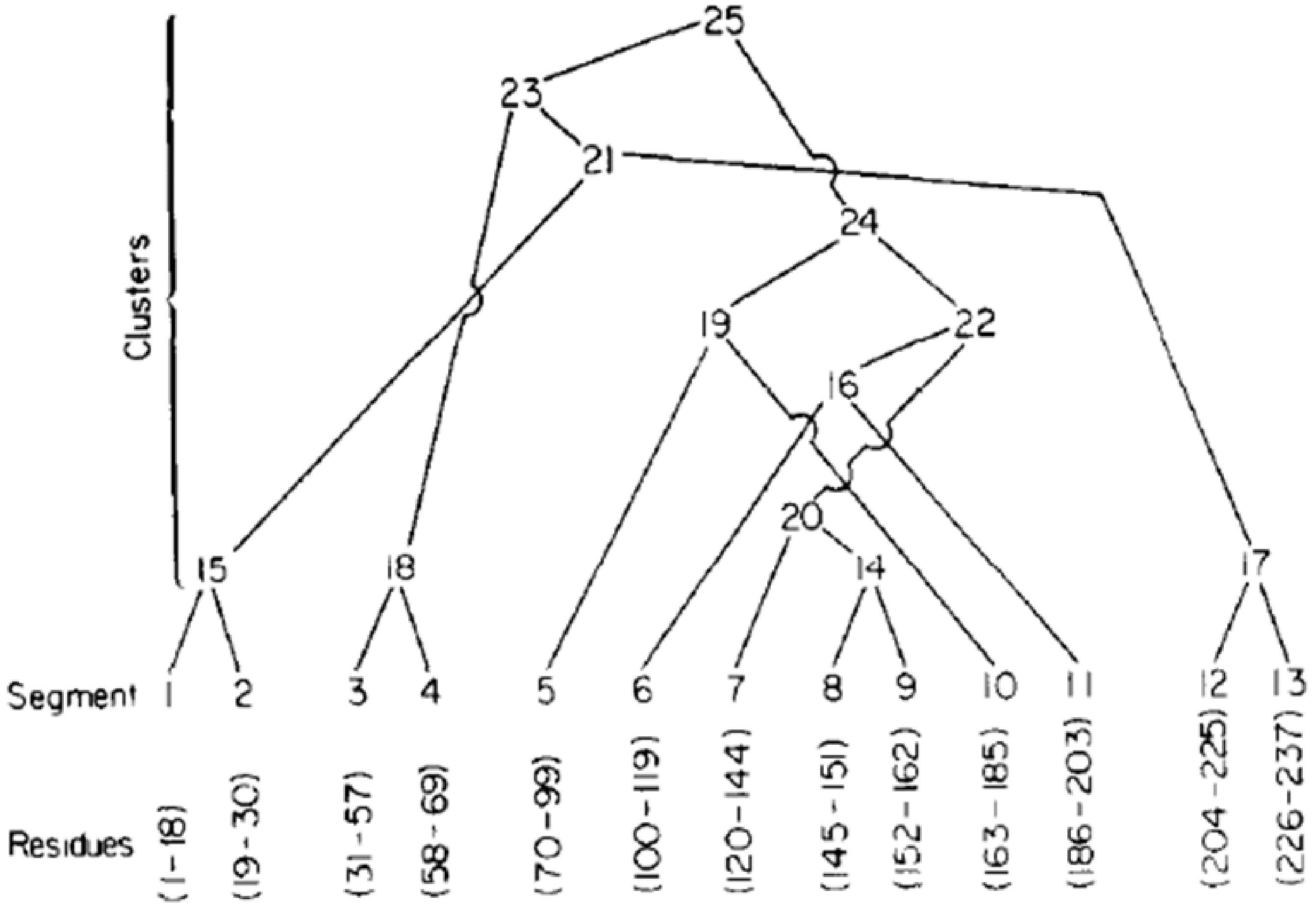
Clustering

Clustering applied to concanavalin A

- bottom - small compact pieces
- higher – compact units
- looks like natural 3 domains

Number of domains is not absolutely determined

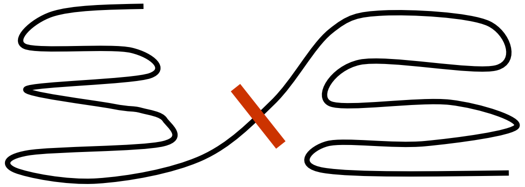
Very very very old method



Crippen, G.M. J. Mol. Biol. 136, 315-332 (1978) The tree structural organization of proteins

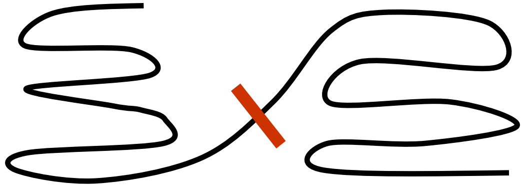
Cuts / Surface area / volume

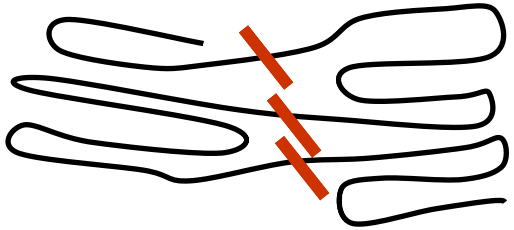
- Simple idea - cut chain in two pieces
- density of part 1 / versus part 2



- cut so as to maximise density

Problems - one cut is not enough





A method should be able to split with 1, 2, 3, … cuts

- For 3 cuts with N_{res} positions: $N_{res} \times N_{res} \times N_{res}$

really $(N_{res})^{N_{cut}}$

Problems - density

I want to maximise density

- density of protein ?
  - number of residues in a volume ?
  - volume ? not sphere

Contacts are easier than density

- within a domain there are many contacts
- between domains - few contacts
- an approximation

Counting contacts

Do I have many contacts compared to the number of atoms ?

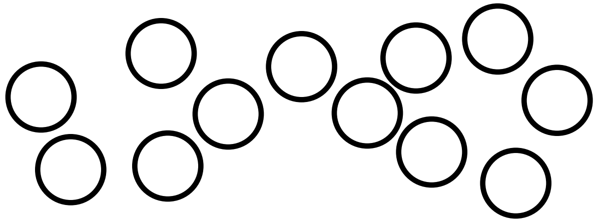
- calculate distance between each $C_i^{\alpha}C_j^{\alpha}$ atoms = d_{ij}
- if d_{ij}< 4 Å, set p_{ij}=1 else p_{ij} = 0
- for a given set of N_{res} atoms (not whole protein)

$$\frac{\sum_{i=1}^{N_{res}}\left(\sum_{j>i}^{N_{res}} p_{ij}\right)}{N_{res}}$$

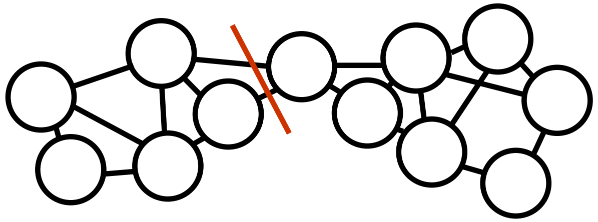
- not accurate, but easy to calculate

Cutting / contacts

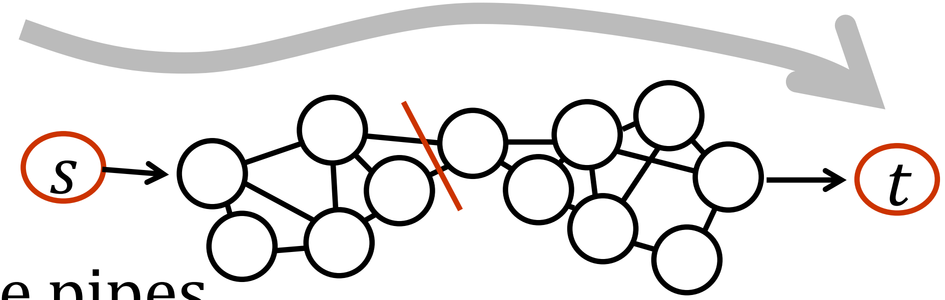
A protein



Find close contacts



How can one find the best place(s) to cut ?

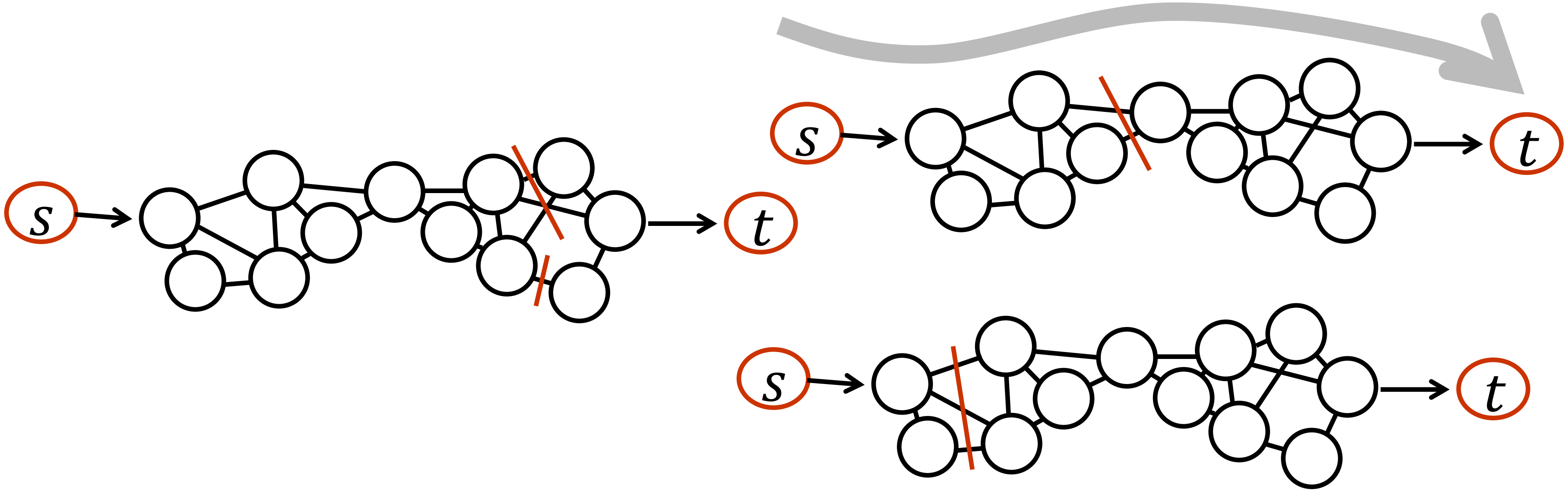


Feed water into s (to t)

- find the most blocked restrictive pipes
- not one, but all that are restrictive

Cutting / contacts

- Flow problem
- Many ways to cut the flow from *s* to *t*



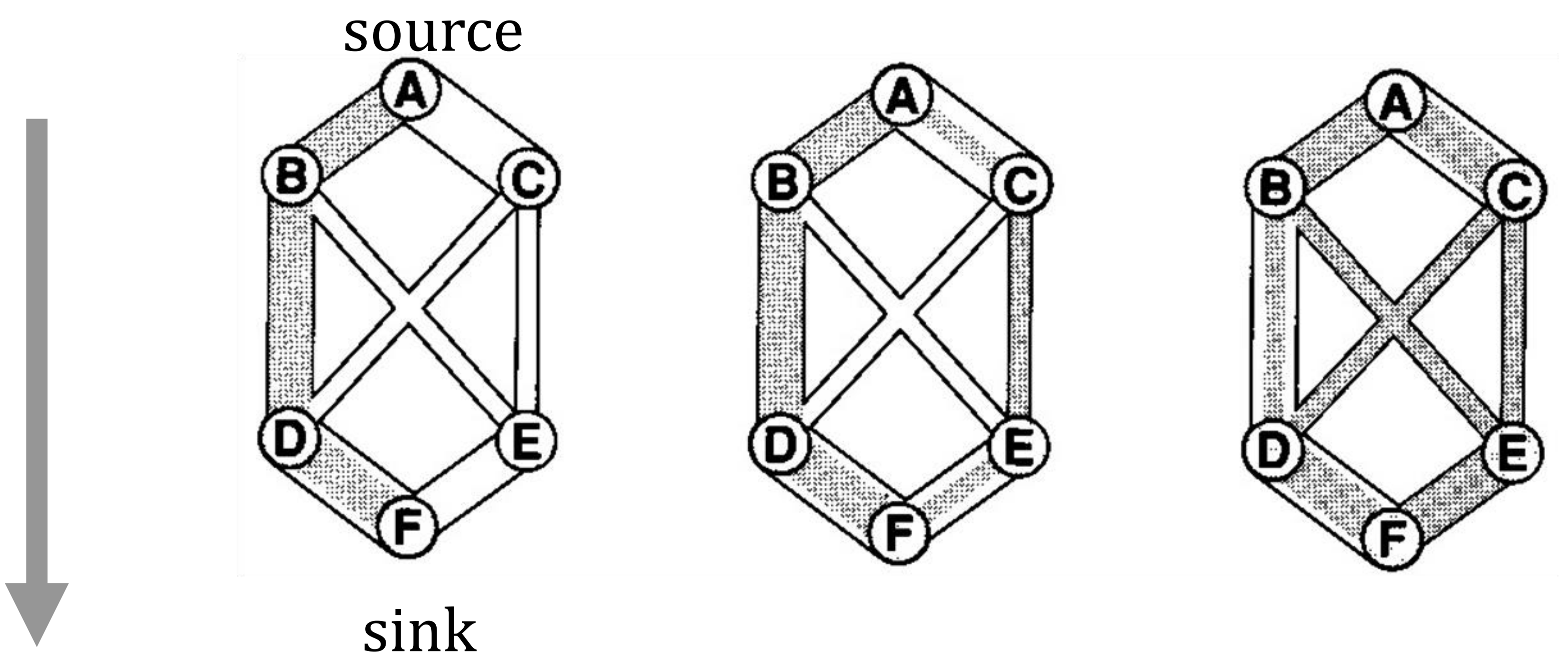
- of all these "*st* cuts" find the one with smallest capacity (flow)
- more interesting - make the pipes different flow capacity
  - how are the residues really touching ? C^{α} C^{α} or C^{α} sidechain

Xu, Xu and Gabow, Bioinformatics, 16, 1091-1104, (2000), Protein decomposition using...

Cutting / contacts

Two steps

1. find the maximum flow from *s* to *t*
2. cut *s* from *t* at the few most filled pipes



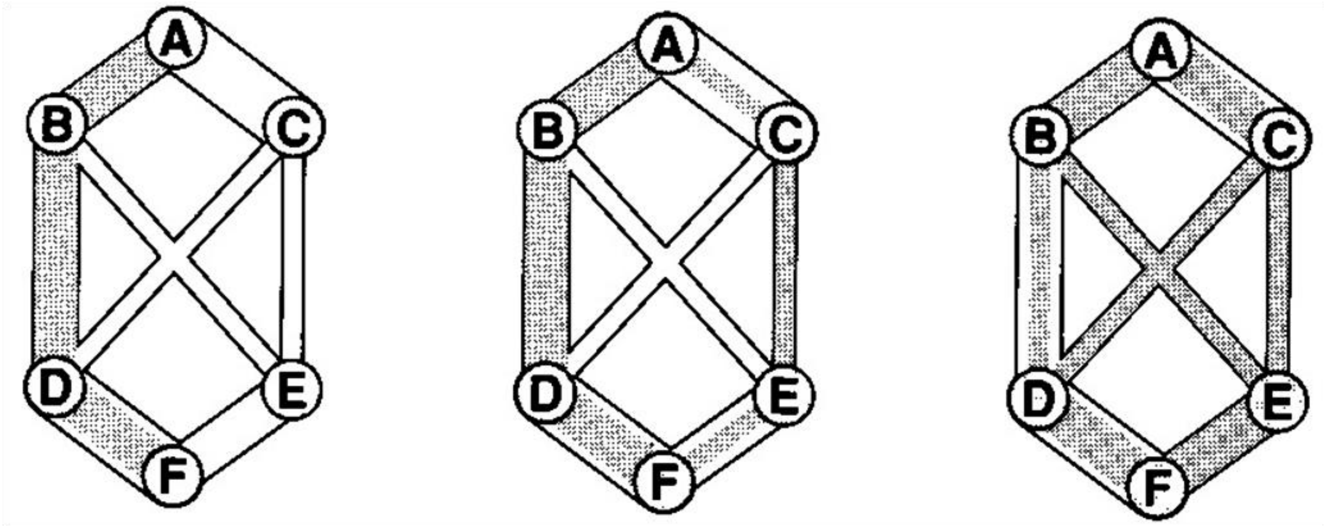
source

sink

Maximum Flow

rule

- if every path from source to sink has one full edge
  - flow is maximum

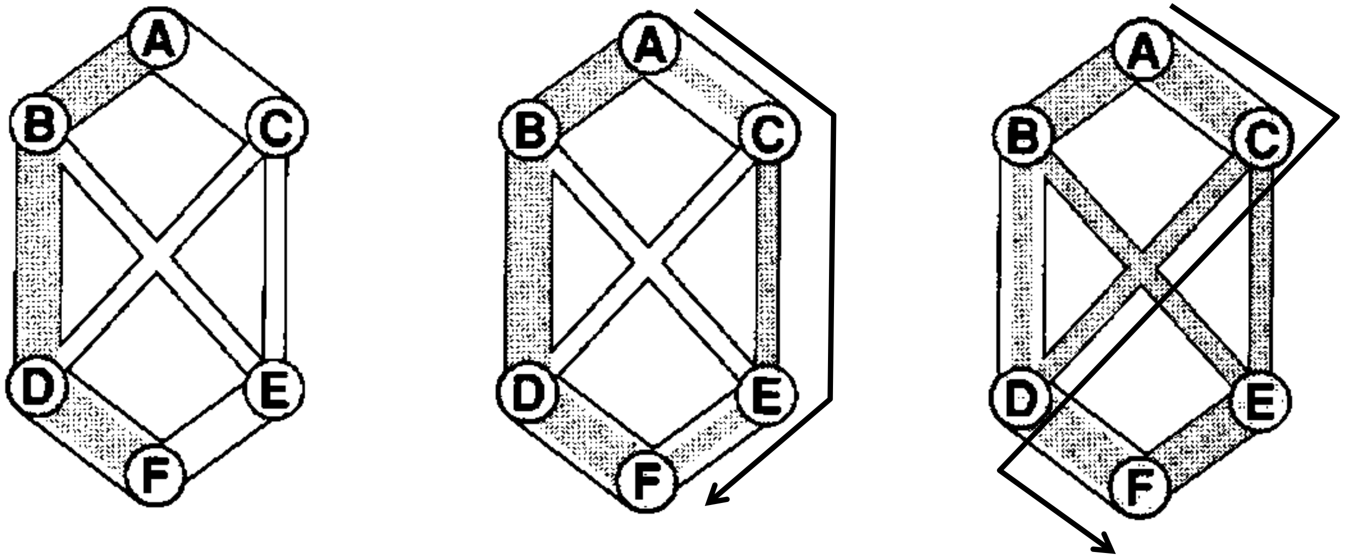


- keep trying every possible path, look to see if there is unused capacity
- we can go backwards

diagram from Sedgewick, R, Algorithms, Addison-Wesley, Reading, (1989)

Maximum Flow

- A B D F   note DF is not quite full
- add some A C E F   AC, EF are not full
- look at C, switch some capacity to CD (DF)



- with some numbers on the edges

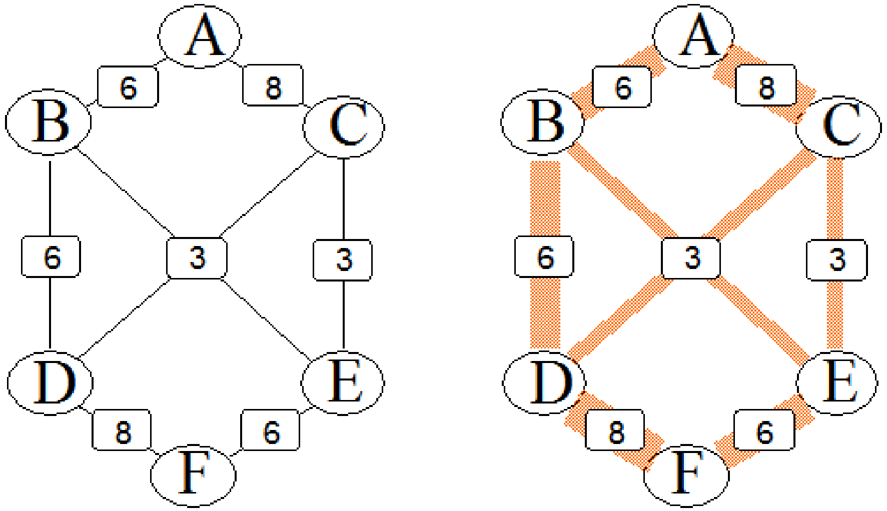
diagram from Sedgewick, R, Algorithms, Addison-Wesley, Reading, (1989)

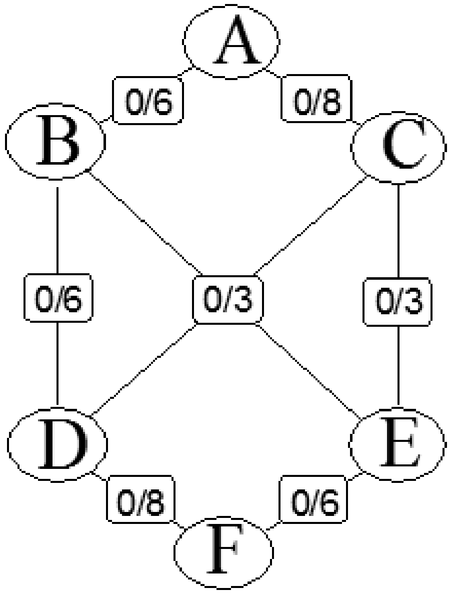
Maximum Flow

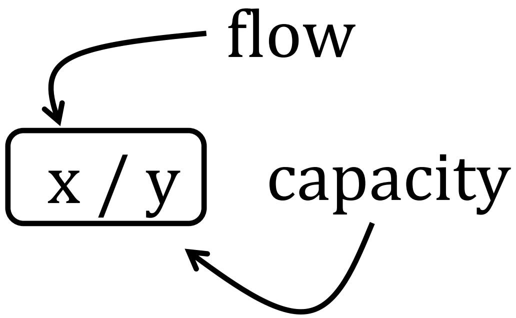
Define an example system

- flow into A
- out of F

- capacities at each edge vary

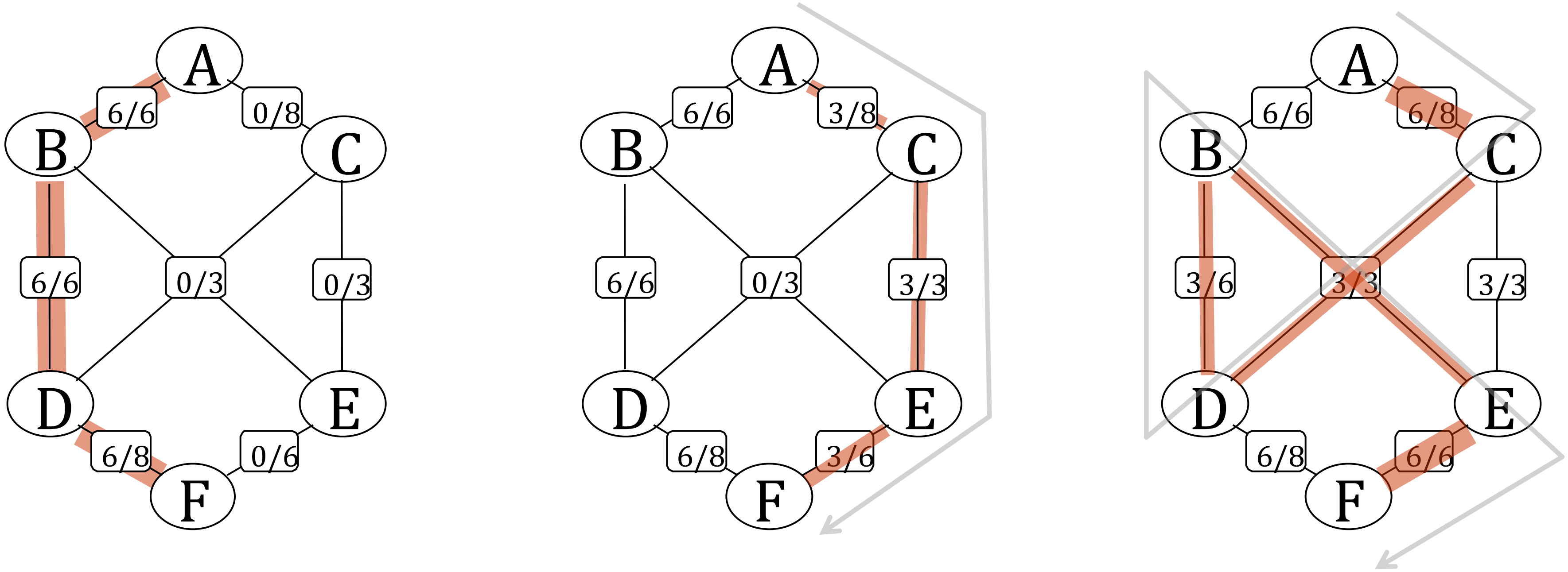






nice java applet: www.cse.yorku.ca/~aaw/Wang/MaxFlowStart.htm

Maximum Flow

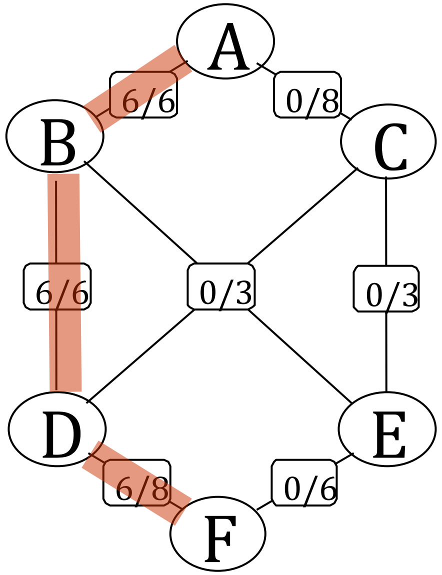


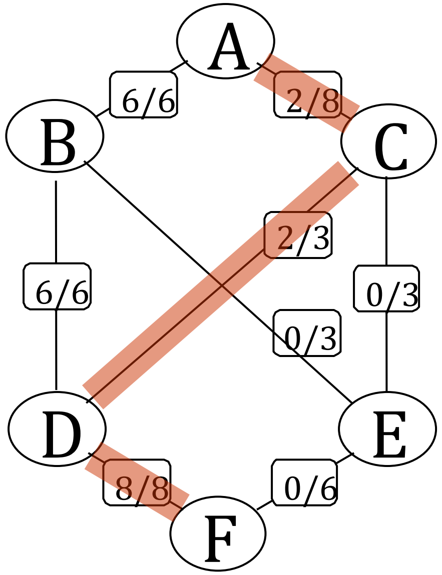
Find a new path (possibly with backwards flow)

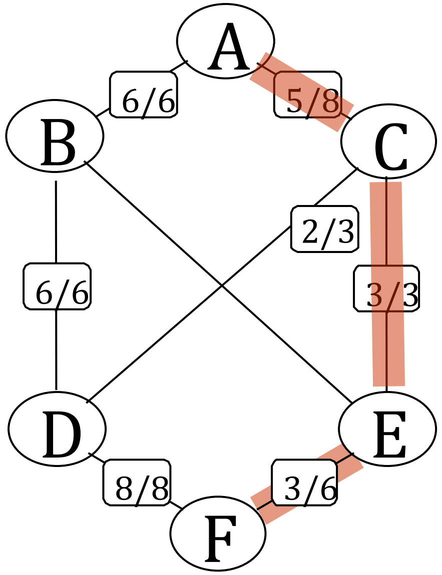
- what is the smallest unused capacity on the path ? Δf

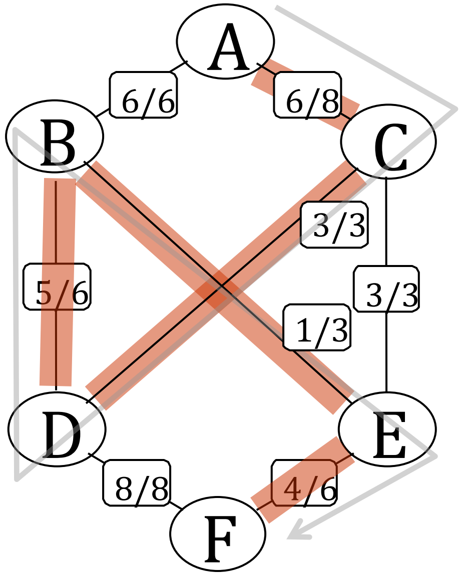
  > 0 ? send flow Δf in this path

Alternative







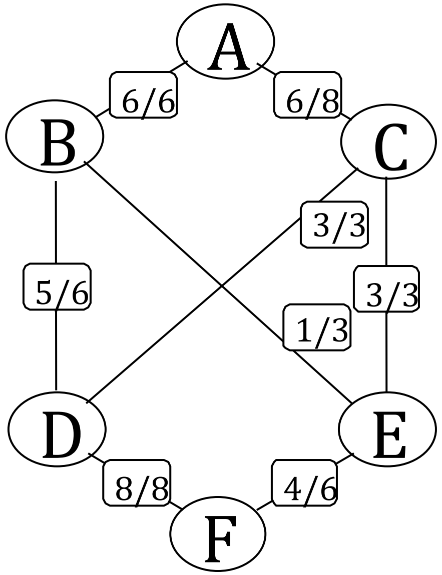


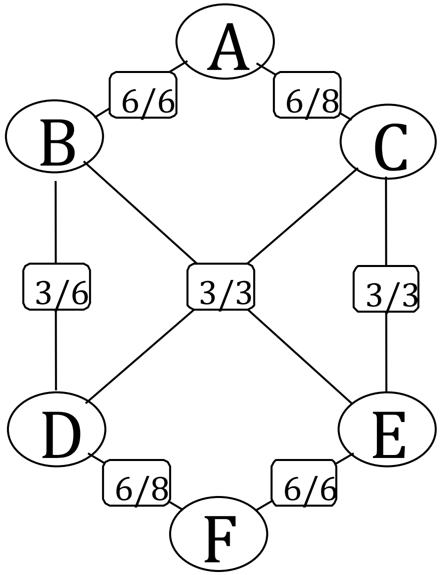
- also ends with flow of 12
- look why this this is definitely time to stop ...

Alternative

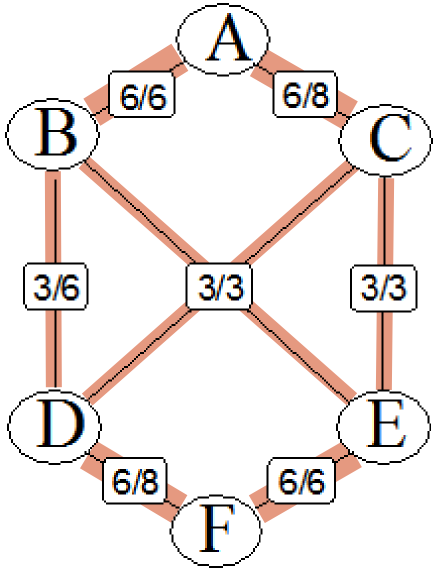
- are there any paths with unfilled pipes ?
- start at A
  - to the left is filled
  - try A→ C
    - both routes out of C are filled

- more solutions ?
- definitely different ways to find solutions
  - different order of visiting paths





Maximum Flow



- path = any route from A to F
- is there any path where all edges have extra capacity ?
  - finished - flow is maximum
- algorithm (not optimal)

```
while (flow not maximum / path found)
    add flow to path
```

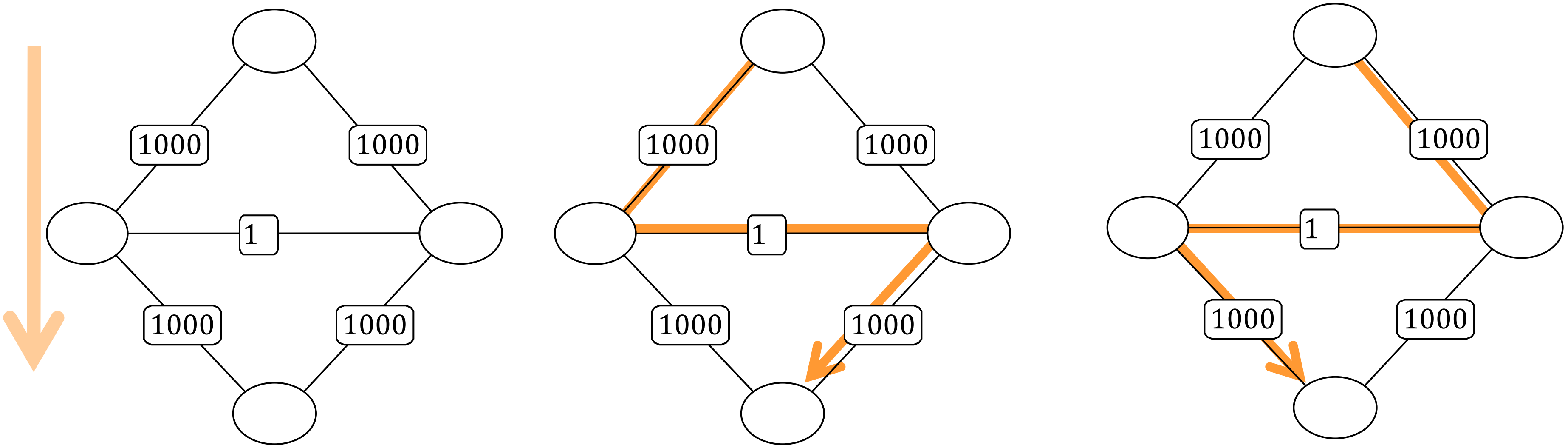
Our definition - finished when

- every path from source to sink has at least one edge (pipe) which is full

Is this efficient ?

Efficiency

Worst possible selection of path order would require 2000 iterations



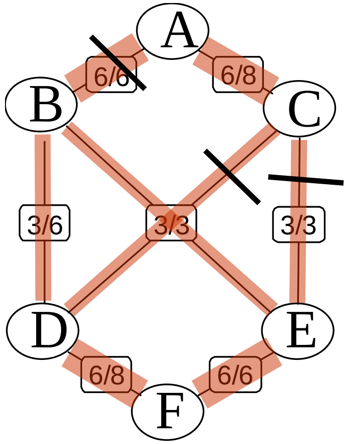
First part of procedure finished

- flow is maximum
- next
  - where to cut graph

Cutting graph

Find ways to cut network, max flow = 12

- AB, AC capacity =14
- BD, BE, AC capacity = 17
  - both bigger than flow (12)



Better

- for each path
  - find first full pipe - cut
  - AB, CD, CE capacity = 12
  - = max flow
  - best cut

Cutting graph

If the capacity across our set of cuts = maximum flow

- it is a "minimum cut"

- smallest connection between two parts of graph
- graph / network / protein is broken into two parts / domains

Useful yet ?

- no mention of finding source s and sink t
- details - efficiency not mentioned

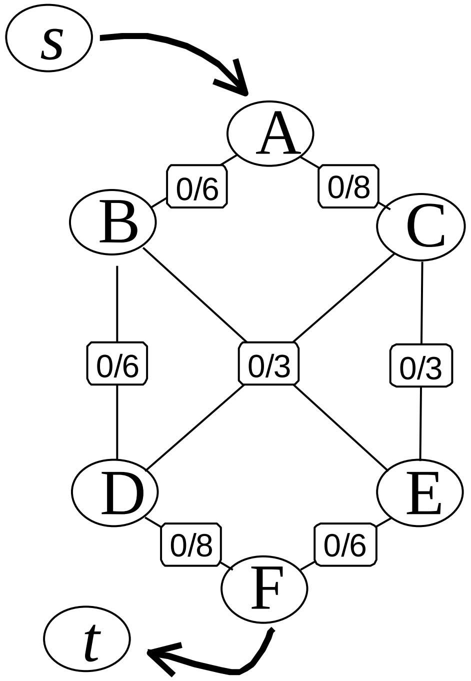
Network flow and proteins

Source

- find a surface residue
- connect an s
- connect to nearby surface residues

Sink

- find a surface residue far away, connect to *t*



Ad hoc ? arbitrary ? optimal ?

- maybe not critical

Multiple domains ?

```
while (domains not too small)
   keep trying to split
```

Xu, Xu and Gabow, Bioinformatics, 16, 1091-1104, (2000), Protein decomposition using…

Splitting - near neighbours / Ising spins

↓↓↓↓↓↓↓↓

Background story - Ising spin model

- energy of spin i depends on $i, i+1$
- energy can be good ↓↓ or ↑↑

  or bad ↓↑

For lots of spins ↓↓↓↑↑↑↑↑↑↓↓↓↑↑↑↑

- islands of same spin
- can be generalised to 2D, 3D

Finding low energies ? Simplest method

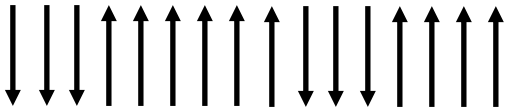
- try to flip a random spin
  - accept flip if energy improves
  - sometimes accept if energy goes up (probabilistic)

Taylor, W. Prot. Eng. (1999) 12, 203-216, Protein structural domain identification

Splitting - near neighbours / Ising spins

Slightly better method

```
while (energy still high)
    for each spin
        change to be same as average of $n$ neighbours
```



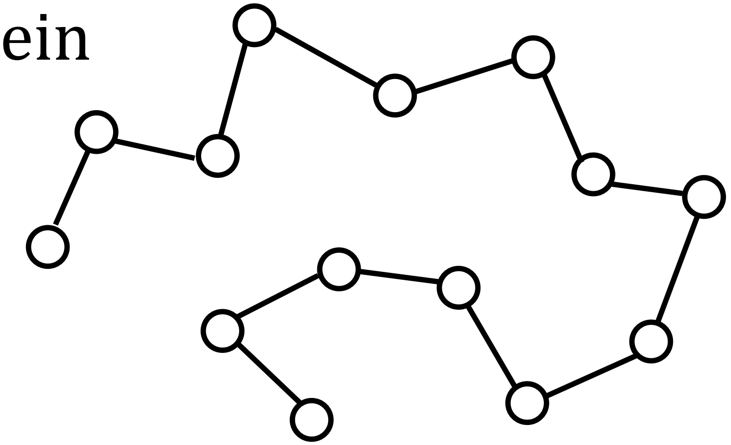
Protein version

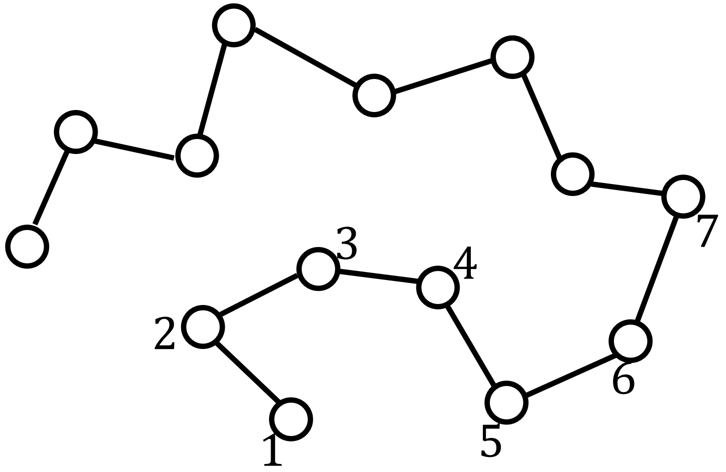
- for any known structure
    - easy to make list of neighbours of each residue

- residues close in space should be in similar domains

Splitting - near neighbours / Ising spins

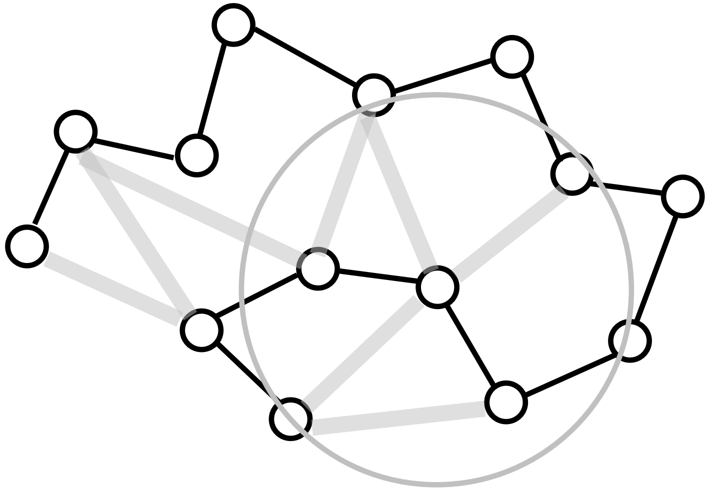
protein





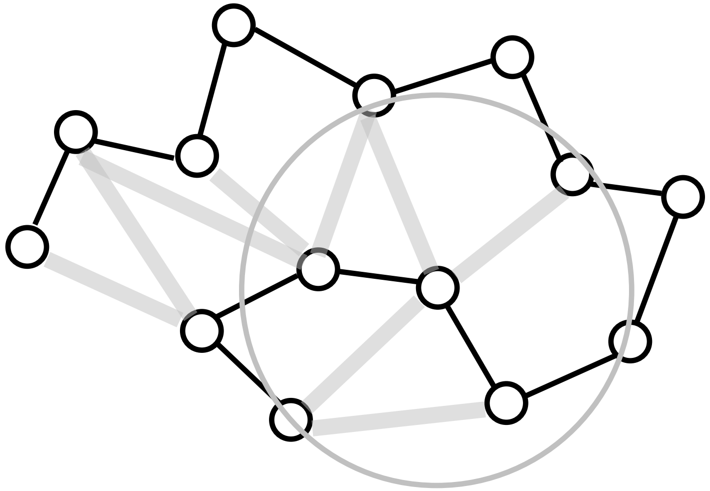
label all points with a number

make a list of neighbours for each residue



Splitting - near neighbours / Ising spins

```
label of a residue is $m_i$
 while (labels changing)
      for each residue $j$
           $m_{av} = \frac{\sum_{i \in neighbours} m_i}{n_{neighbour}}$
           if ($m_{av} > m_j$)
               $m_j$ ($new$) = $m_j$ ($old$) + 1
           else if ($m_{av} < m_j$)
               $m_j$ ($new$) = $m_j$ ($old$) - 1
```



step	residue number								
0	1	2	3	4	5	6	7	8	...
1	2	3	3	4	6	7	8	8	...
2	2	2	4	3	5	8	9	9	
...									

Splitting - near neighbours / Ising spins

Properties of Taylor / Ising spin-inspired method

Optimism

- will converge and become stable

Requires threshold - what is a neighbour

- can use sophisticated averaging - distance dependent
- may converge to 2, 3, … domains

Methods so far

1. simple - look for single cut points and maximise density

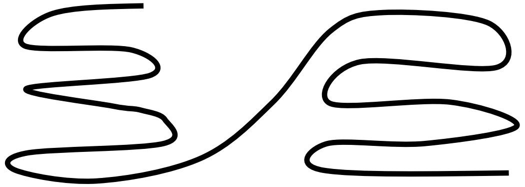
2. Crippen / hierarchical clustering

3. Network flow

4. Ising spin / Taylor

- All methods have arbitrary numbers

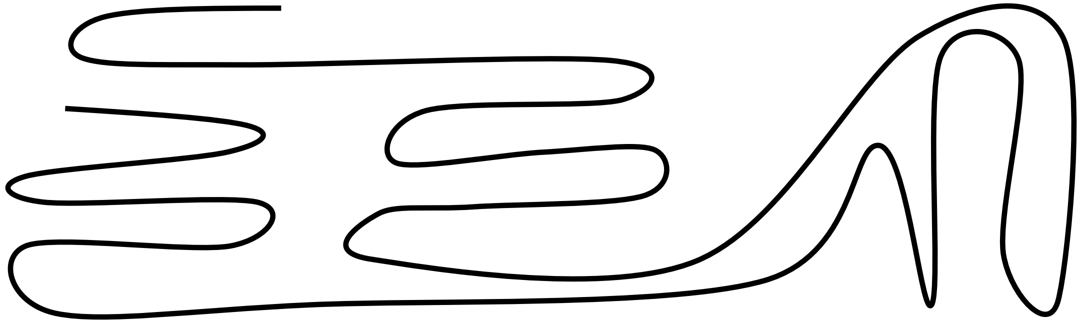
Why are methods so complicated ?



If we cut protein chain once

- methods are easy - use density criterion

Cut protein twice ? more ? remember $(N_{res})^{N_{cut}}$

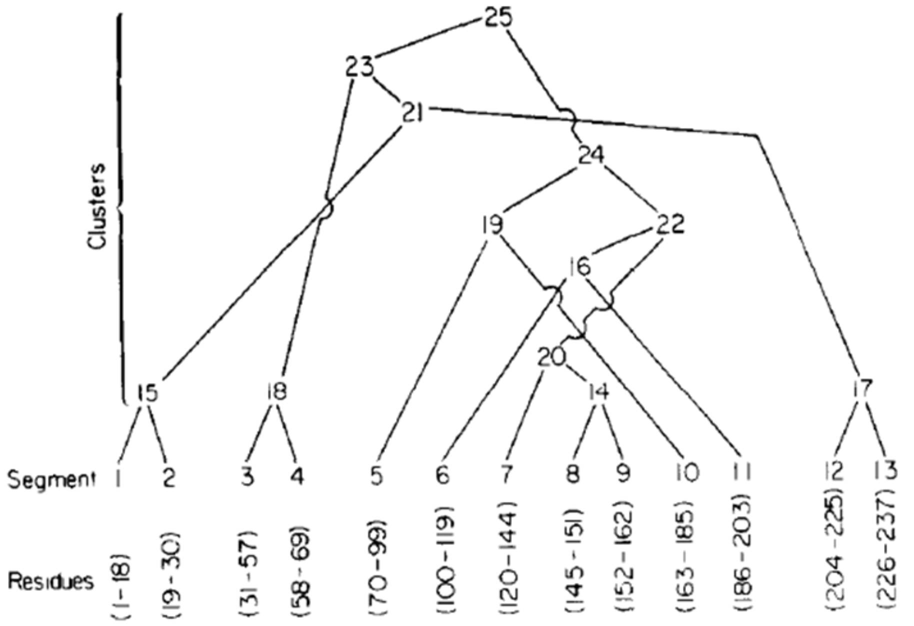


How many domains ?

- Crippen / clustering method - whatever you want
- Network flow - repeatedly split and eventually stop
- Taylor / Ising - may converge to > 2 domains

Crippen / hierarchical clustering

At what level of hierarchy do I cut tree ?



Network flow

- what constitutes a contact ? (any 2 atom < 4 Å ?)
- give pipes (edges) more weight for different kinds of contacts
- are solutions unique ?
  - probably in practice
- when do we stop splitting domains ?

Taylor / Ising spin method

- what constitutes a contact ? how many Å ?
- type of averaging to get m_{av} ?
- when does one converge ?

Elegance

Do methods work as described ? not really

All authors report problems - example

- Taylor finds different results for α-helical and β-sheet regions
- simple explanation ? distances within / between secondary structure are very different

Do methods work ?

With many fixes and tuning - yes

- distance criteria, thresholds

Do methods agree ?

Only ask question if you agree to think in terms of structure

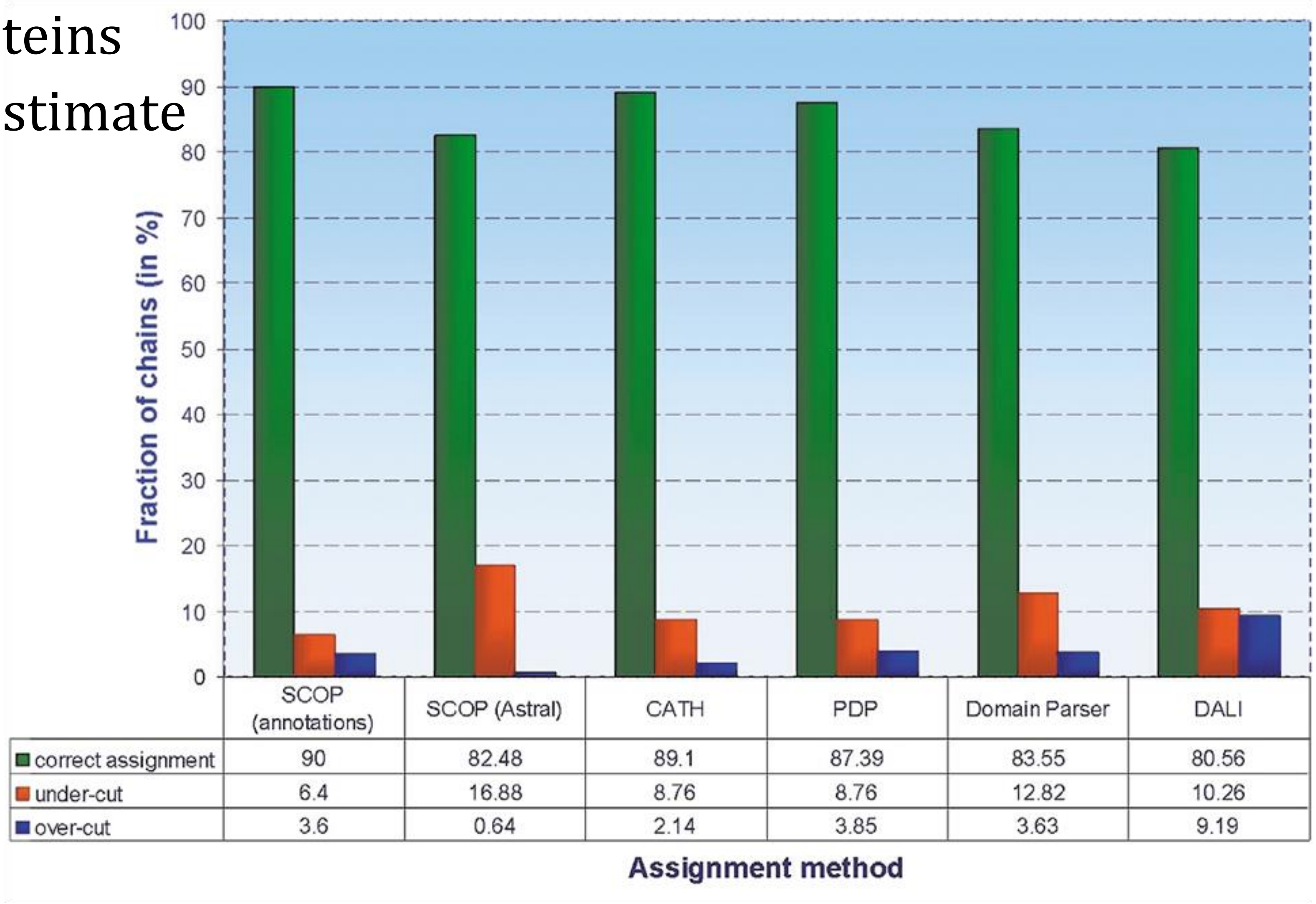
- answer will be different in terms of evolution or sequences

Criteria

- how many domains inside a protein ?
- where are the domain borders ?

Number of domains

- test set of few hundred proteins
- compare against author's estimate
- 80-90 % agreement

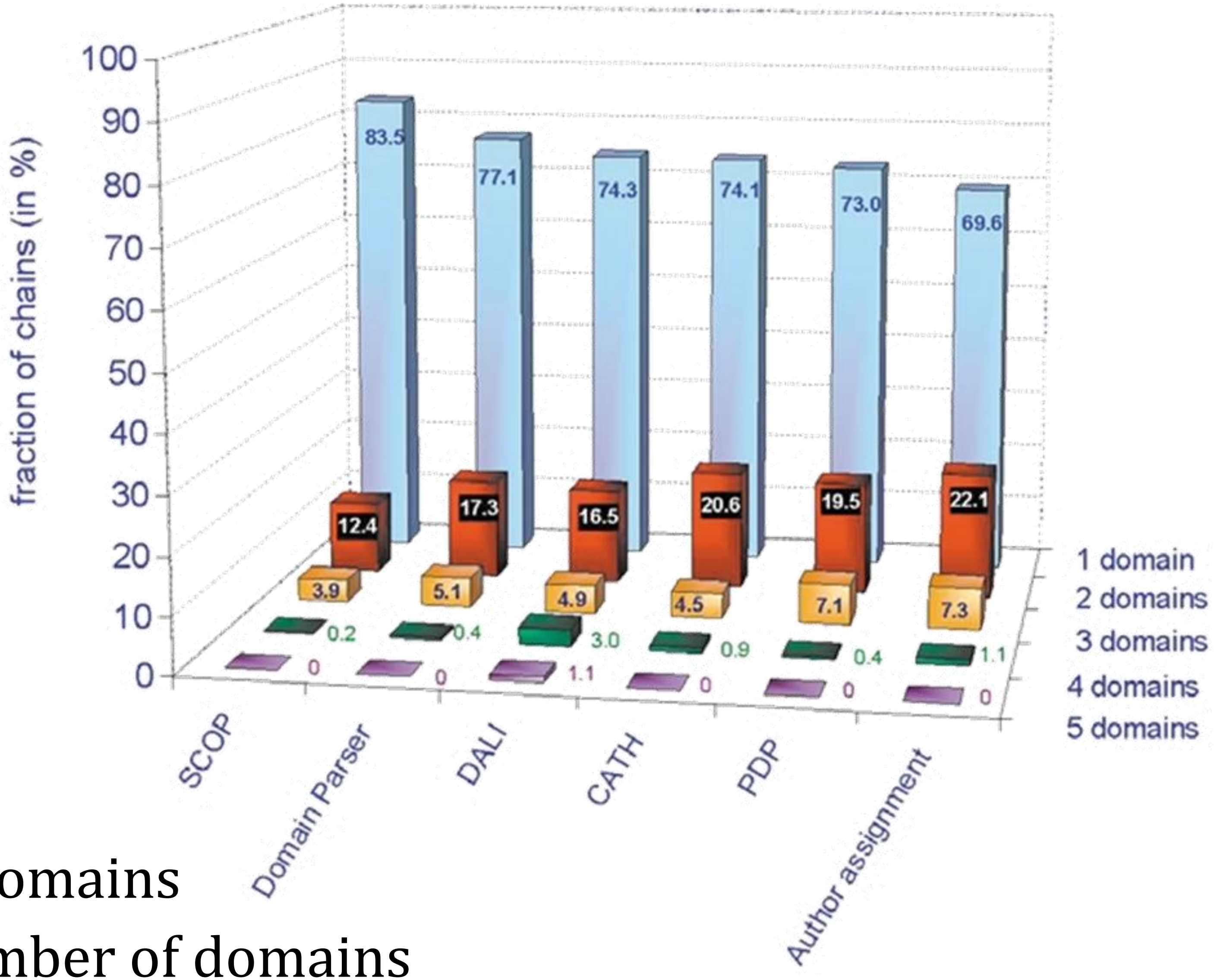


	SCOP (annotations)	SCOP (Astral)	CATH	PDP	Domain Parser	DALI
🟩 correct assignment	90	82.48	89.1	87.39	83.55	80.56
🟥 under-cut	6.4	16.88	8.76	8.76	12.82	10.26
🟦 over-cut	3.6	0.64	2.14	3.85	3.63	9.19

Veretnik, S., Bourne, P.E., Alexandrov, N.N., Shindyalov, J. Mol. Biol. (2004) 339, 647-678, Toward… domains in proteins

How many domains per protein ?

Same set of 467 proteins



- authors split into several domains
- "SCOP" prefers smaller number of domains

Veretnik, S., Bourne, P.E., Alexandrov, N.N., Shindyalov, J. Mol. Biol. (2004) 339, 647-678, Toward… domains in proteins

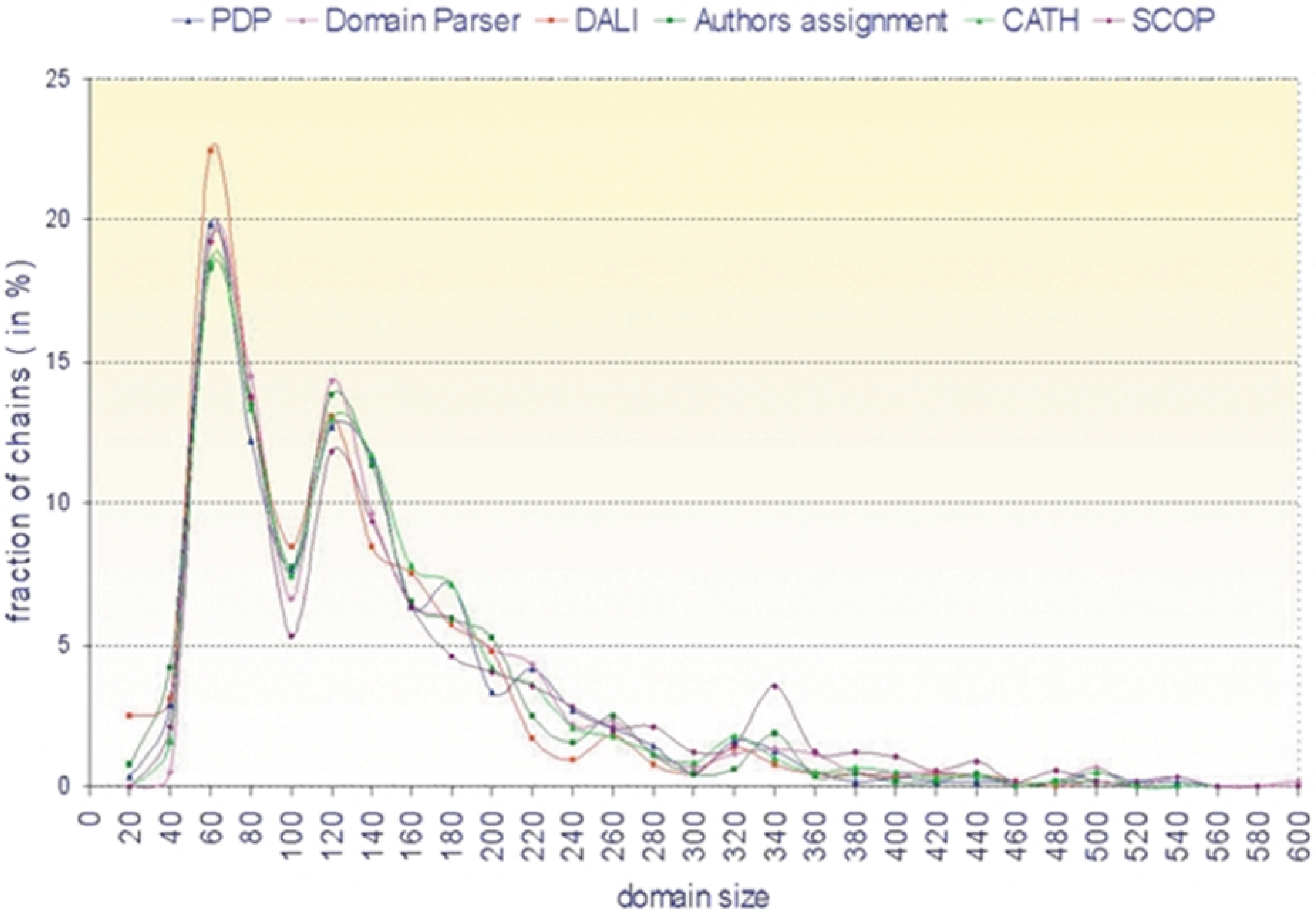
Agreement ?

Lots of room for differences

Some statistics

How big is a protein domain ?

Peaks near 60 and 130 residues



Veretnik, S., Bourne, P.E., Alexandrov, N.N., Shindyalov, J. Mol. Biol. (2004) 339, 647-678, Toward… domains in proteins

How complicated are domains ?

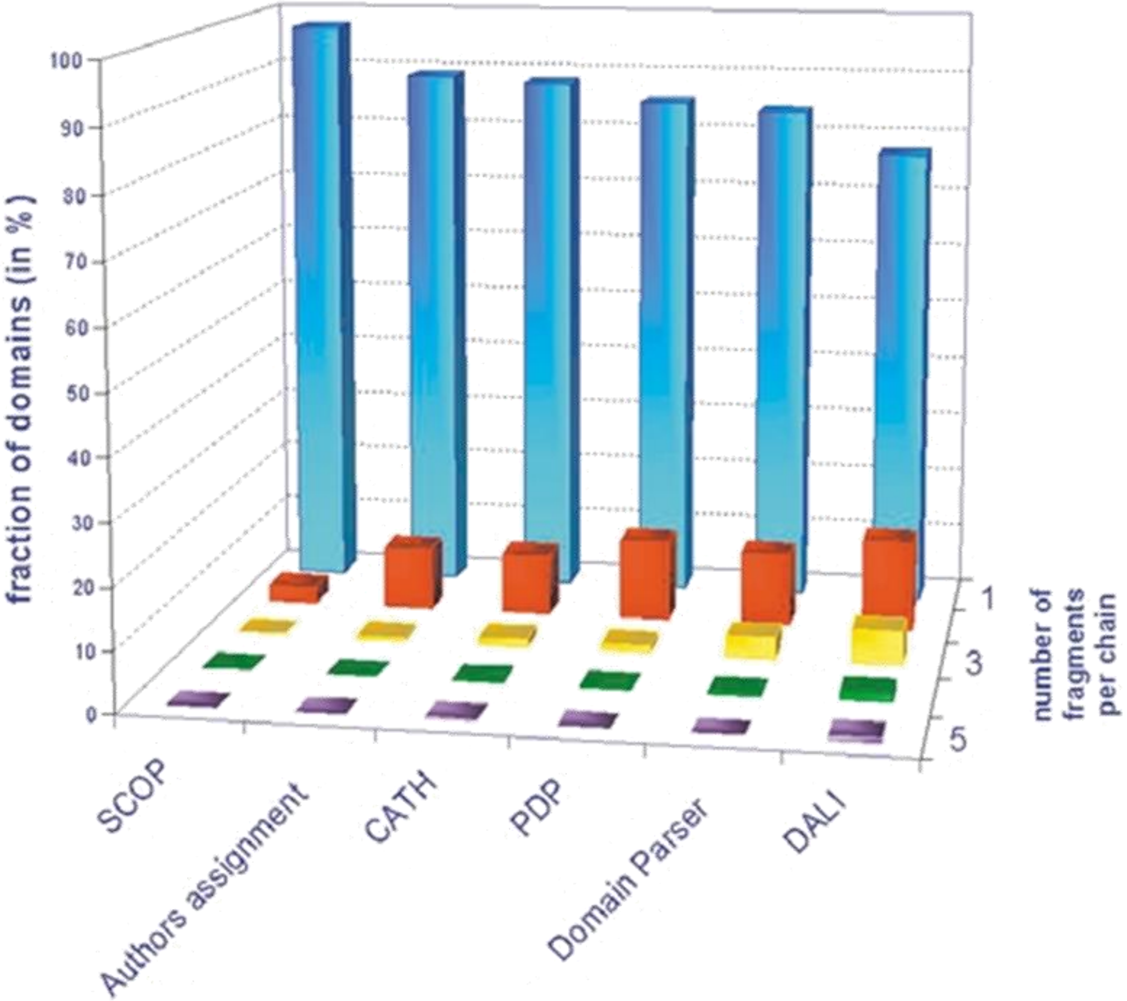
Justification for complicated domain recognition

- single cuts, double cuts in chains are not enough

What percentage of domains are built from

- 1 chain ?
- 2 chains ? …

In "DALI", 23 % of domains are not continuous (multiple crosses of chain)

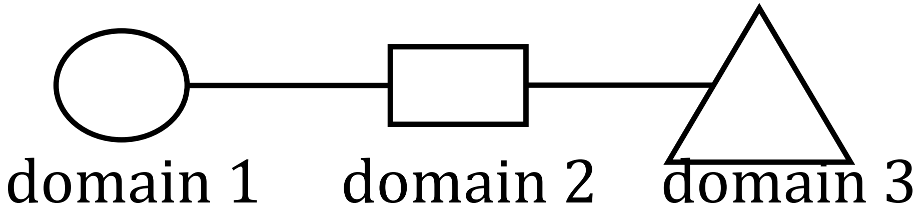


Veretnik, S., Bourne, P.E., Alexandrov, N.N., Shindyalov, J. Mol. Biol. (2004) 339, 647-678, Toward… domains in proteins

Evolutionary picture

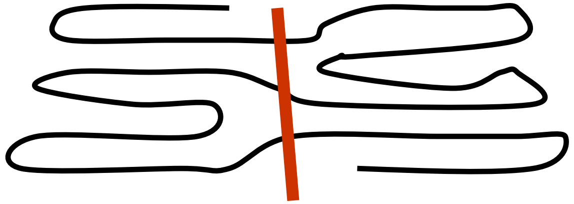
Original claim

- domains are units that move as a module in evolution



If we see multiple cuts 10-20 % of time

- picture is much less clear



Summary

Domain definitions

- functional, structural, sequence based

Finding domains

- relies on contacts, density
- method must be able to handle multiple crossings of chain

We considered

- clustering / hierarchical
- network flow
- Taylor / Ising spin-inspired

- Methods do not agree with each other
- Some trends in size and number of domains
- Real proteins are not as simple as evolutionary picture