Introduction / Modelling

- who am I ?
- language .. Deutsch / English .. verhandelbar
- Zettel

  www.bioinformatics.uni-hamburg.de/research/BM/torda/lehre.html
- + stine
- Übungen ebenfalls im web
- heute ? nicht 90 Minuten

Administration

People

- Andrew Torda 1. Stock / 105
  schade@zbh.uni-hamburg.de
  sekr (Annette Schade) 42838 7330

- Marco Matthies und Iryna Bondarenko

Vorlesungen	Mi	16:15 – 17:45
Übungen	Mo	18:15 – 19:45

- aber..
  - Montag 18:00 statt 18:15 ?

Homework / Übungen

- not much

Übungen

- very short report (schriftlich)
- individuelle / eigene

Textbooks

- Folien (viele Fallstudien aus der Literatur)
- nicht zu kaufen
  - any biochemistry book (Stryer/Voet&Voet/Lehninger..)
    - expensive, not used too much

Exams

- any facts that are mentioned in these lectures and Übungen
- schriftliche Klausur
  - 2 Feb 2014 9:00 - 10:30
  - 24 März 2014 9:00 - 10:30

Konten

- Ein Konto auf unserem Rechner
- Können sie ausprobieren, einloggen, …

Backgrounds

- Informatiker – nicht viel von Chemie, Biochemie
- Biologen, Chemiker – nicht viel von Computern
- Some people – know everything

Frühere Jahren.. Mo 20:10 Übungzeit

- Protein structure for informatiker × 2
- The linux command line × 2 for biologists

Ausgleichung

- Informatikers – familiarity with proteins /chemistry /structure
- MLS/Chemiker/.. - some scripting, linux command line

	Informatiker	MLS/Chemiker/..
Mo. 20. Okt	Protein struct 1	Linux command line / Scripting 1
Mo. 27. Okt	Protein struct 2	Linux command line / Scripting 2

Lecture Plans

1	15. Okt. 14	Models
2	22. Okt. 14	Similarity - protein sequences
3	29. Okt. 14	Cluster Analysis
4	5. Nov. 14	Protein Domains
5	12. Nov. 14	Protein Domains
6	19. Nov. 14	Function prediction
7	26. Nov. 14	Function prediction
8	3. Dez. 14	Sequence design RNA
9	10. Dez. 14	Sequence design proteins
10	17. Dez. 14	Sequence design proteins
11	7 Jan. 15	fold recognition
12	14. Jan. 15	fold recognition
13	21. Jan. 15	structure prediction
14	28. Jan. 15	structure prediction

Themes - Applications

Theme of Semester

- given some information about a macromolecule (protein)
  - what can be calculated ? predicted ?
  - how much would you trust predictions ?
    - limitation, applicability, reliability

- typical information
  - a protein sequence (lots known)
  - a protein structure (less known)
  - a DNA sequence (think of genomes)

Today

- meaning of modelling
- similarity is not easy

Specific and general models

Dream

- Feed data to box and have it interpreted
  - given my protein, what is the structure ?
  - given my spectrum where is the centre of the peak ?

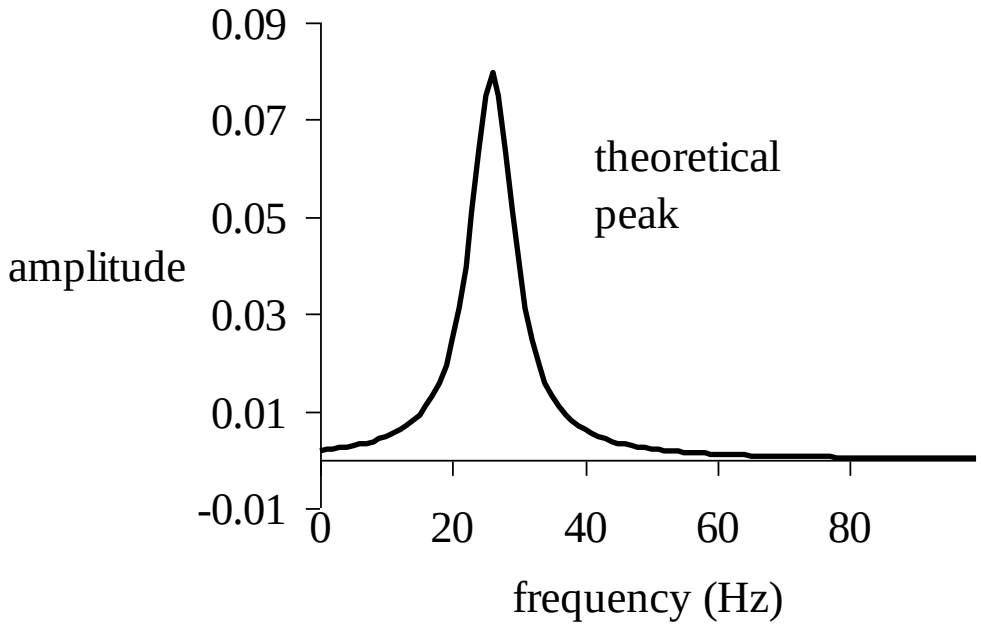
Model types

- Specific
  - you know the structure of your data, fit points to the observations
- General
  - look for some patterns in data – little understanding of the underlying theory

- examples

Interpreting spectroscopic data

- just an example (no spectroscopy in this course)
- many kinds of peaks in spectroscopy look like



- my mission
- find centre (≈24) and height (≈0.08)
- but they have noise

noisy data

Real world has noise

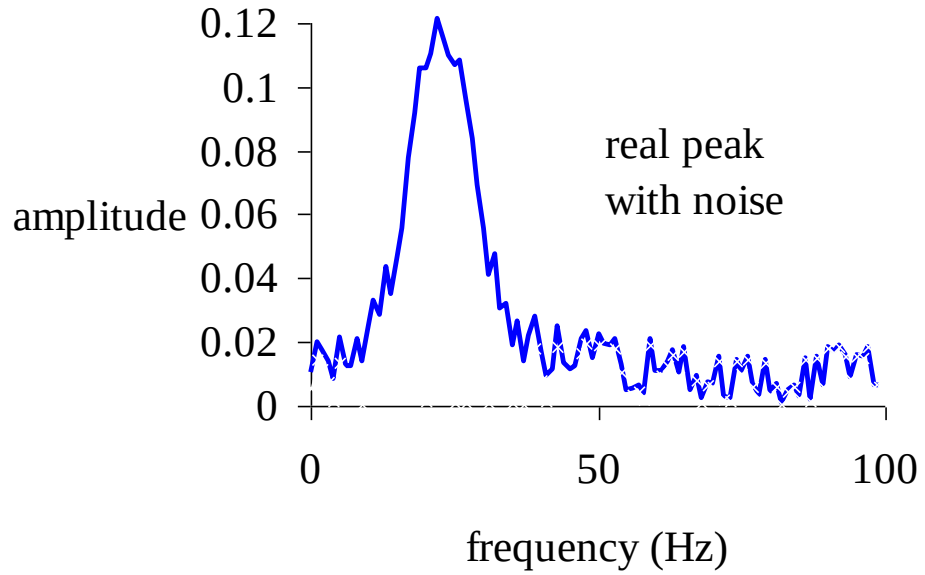
- we still want centre, height

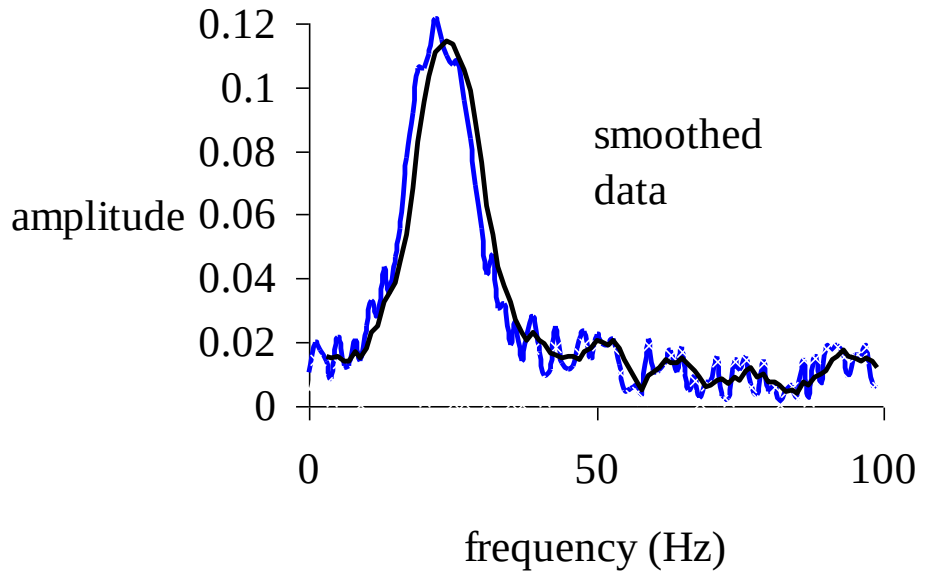
Try simple smoothing

- no assumptions about data

Claim

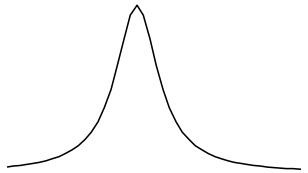
- centre around 23
- looks believable





Using prior knowledge

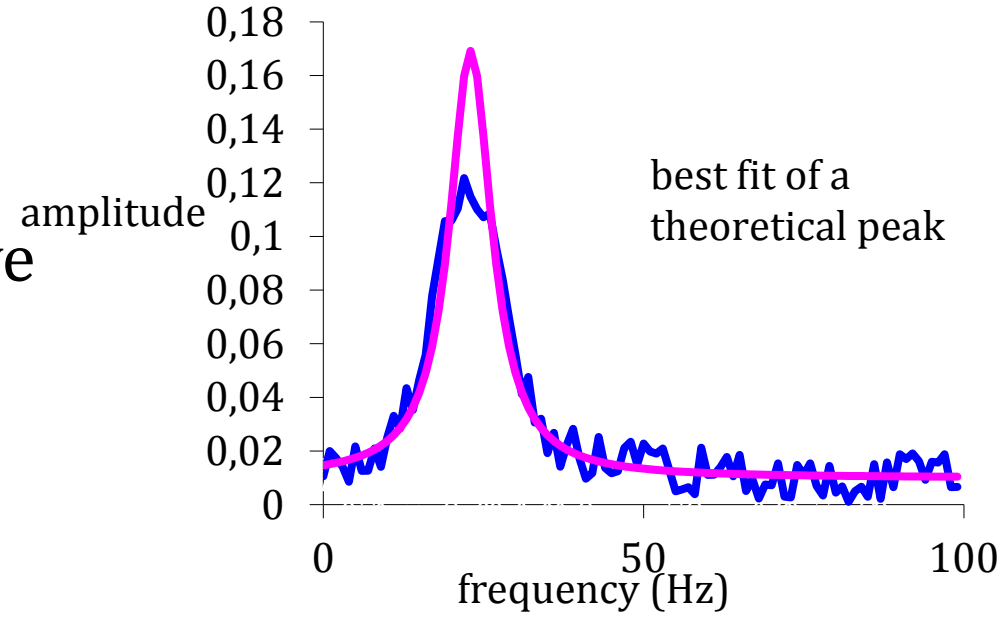
Imagine we expect peaks like $\frac{a^2}{a^2+x^2}$



A fit of a calculated peak…

- something is clearly wrong
  - if peak has a certain width it must have an appropriate height

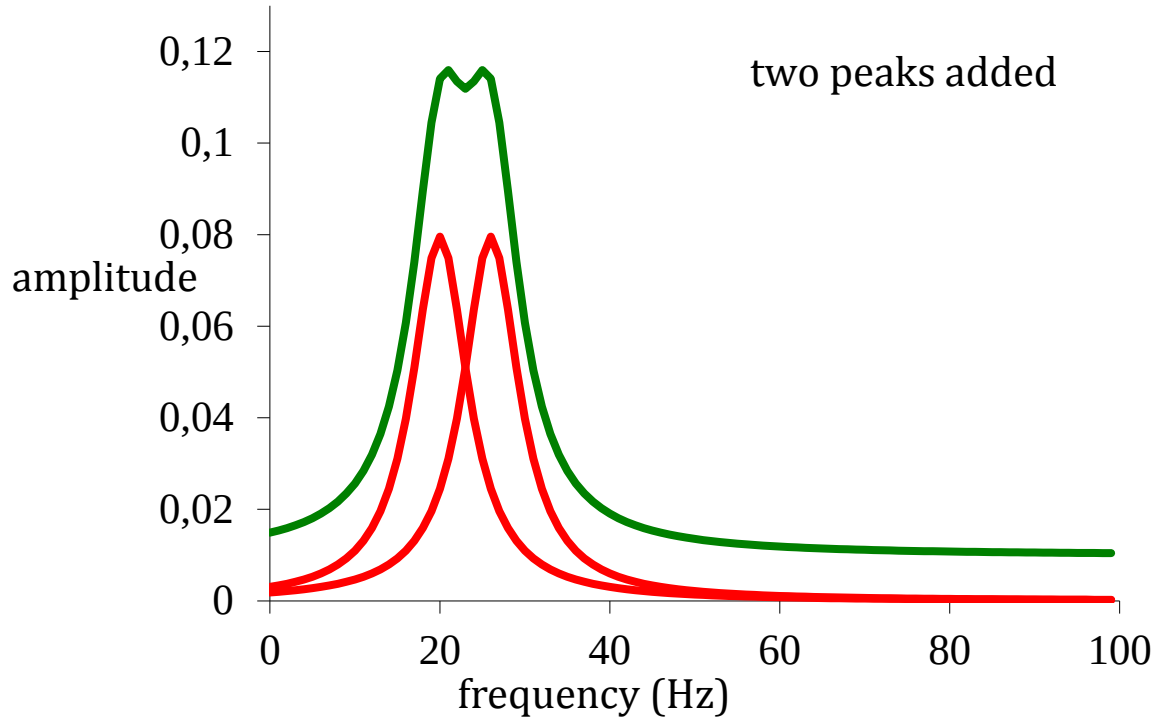
$$\frac{a^2}{a^2 + x^2}$$



What looked good is not the correct form

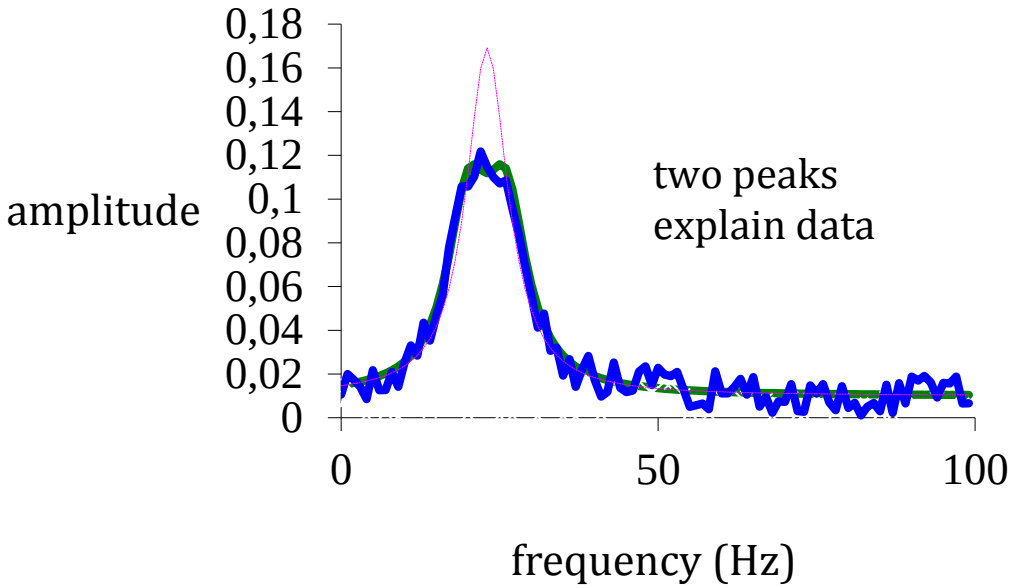
More appropriate fitting

What if we used two peaks ?



Peaks centred at 20 and 26

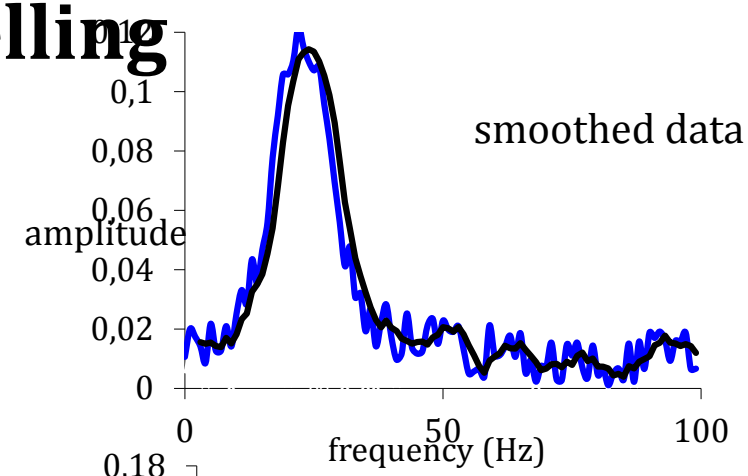
- very different explanation of data



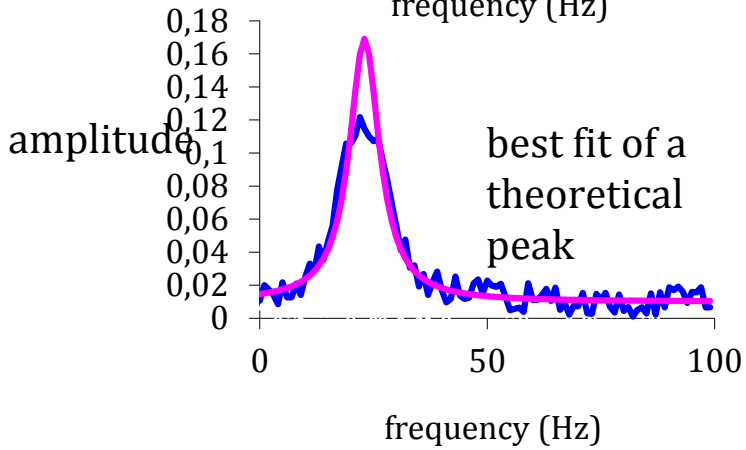
General vs appropriate modelling

General smoothing method suggested one peak

- looks good
- appears to explain observations
- generally applicable

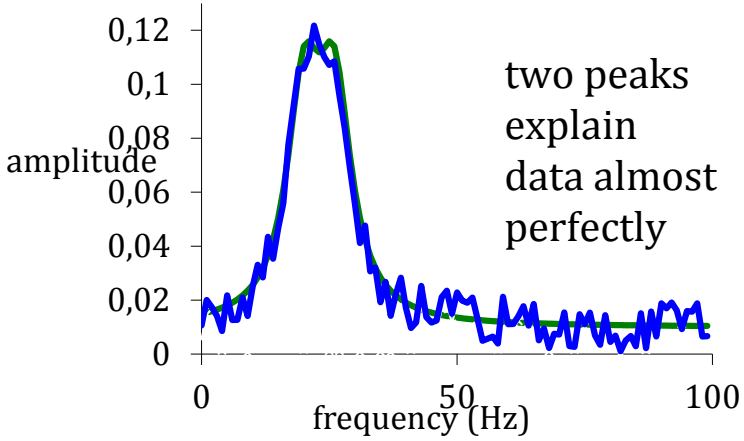


Testing with correct model suggested this is wrong



Fitting with best model (two peaks)

- near perfect



Summary

- if you know the underlying model, use it
- always applicable ?
- back to biological questions

General purpose modelling

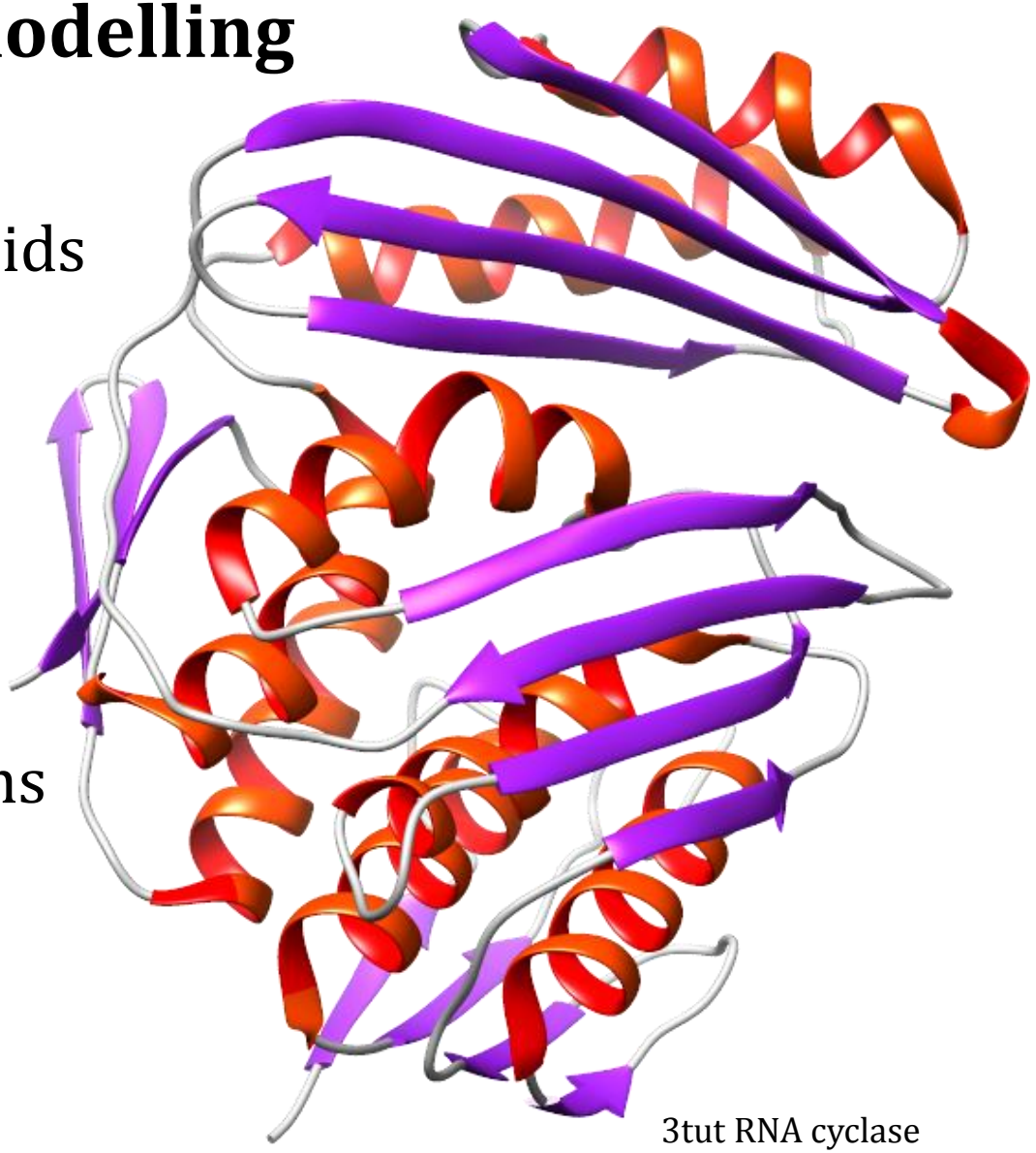
Proteins have "secondary structure"

- It appears to reflect the sequence of amino acids
  - what is the rule ?
  - 20 amino acids, N positions,
    - 20^N sequences, patterns not clear

What to do ?

- correct model – think of all atomic interactions
  - see where atoms should be placed
    - not practical

  or
- forget physics
  - use dumb statistics / machine learning approaches



3tut RNA cyclase

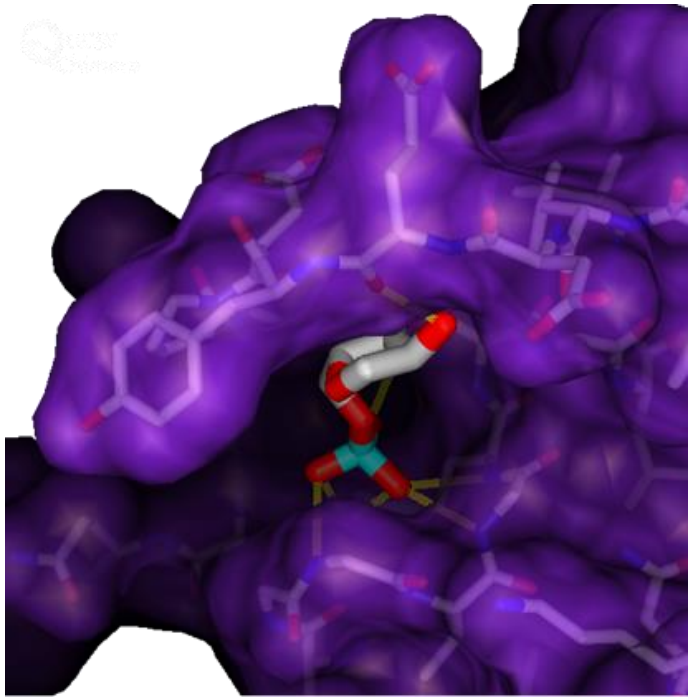
Mixtures of specific and general

Will a ligand (Wirkstoff) bind to a protein ?

- with physics
  - model all atomic interactions, best physical model
  - calculate free energy (ΔG)
    - difference in solution / bound
- more generally
  - gather idea of important terms (H-bonds, overlap, ..)
  - try to find some function which often works
  - do not stick to real physics

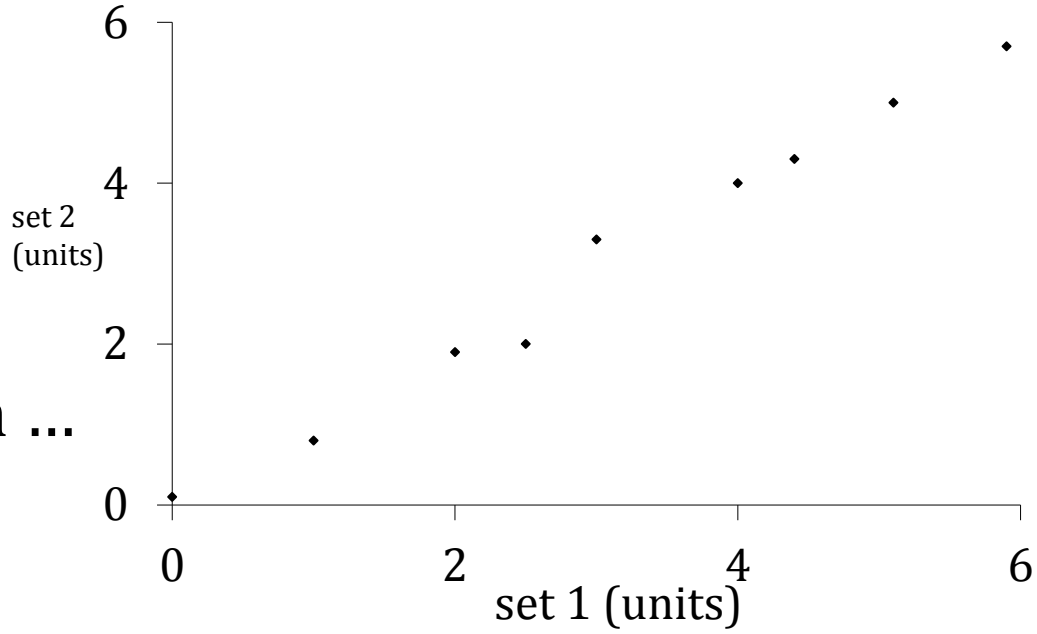
Will my drug dissolve in water or oil (lipid) ? (important)

- sounds like chemistry
  - usually approached by machine learning
    - number of atoms, types of atoms, …



http://www.cgl.ucsf.edu/chimera/ImageGallery/, bluetongue virus capsid protein

Similarity



Important in all bioinformatics

- I have a protein of unknown
    - structure / function / cell localisation
- is it similar to one of known structure, function …

Similarity seems obvious

- two sets of numbers (above)
- two protein sequences

  ACDEACDE rather similar - but quantified ?

  ADDEAQDE
    - how many positions differ ? how long are proteins ?
    - could the similarity be by chance ?

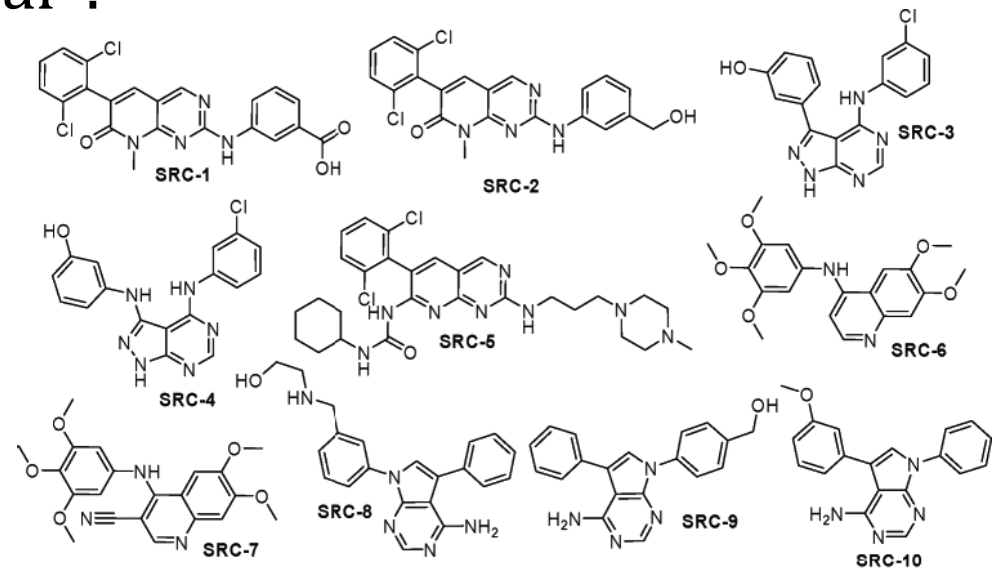
Similarity

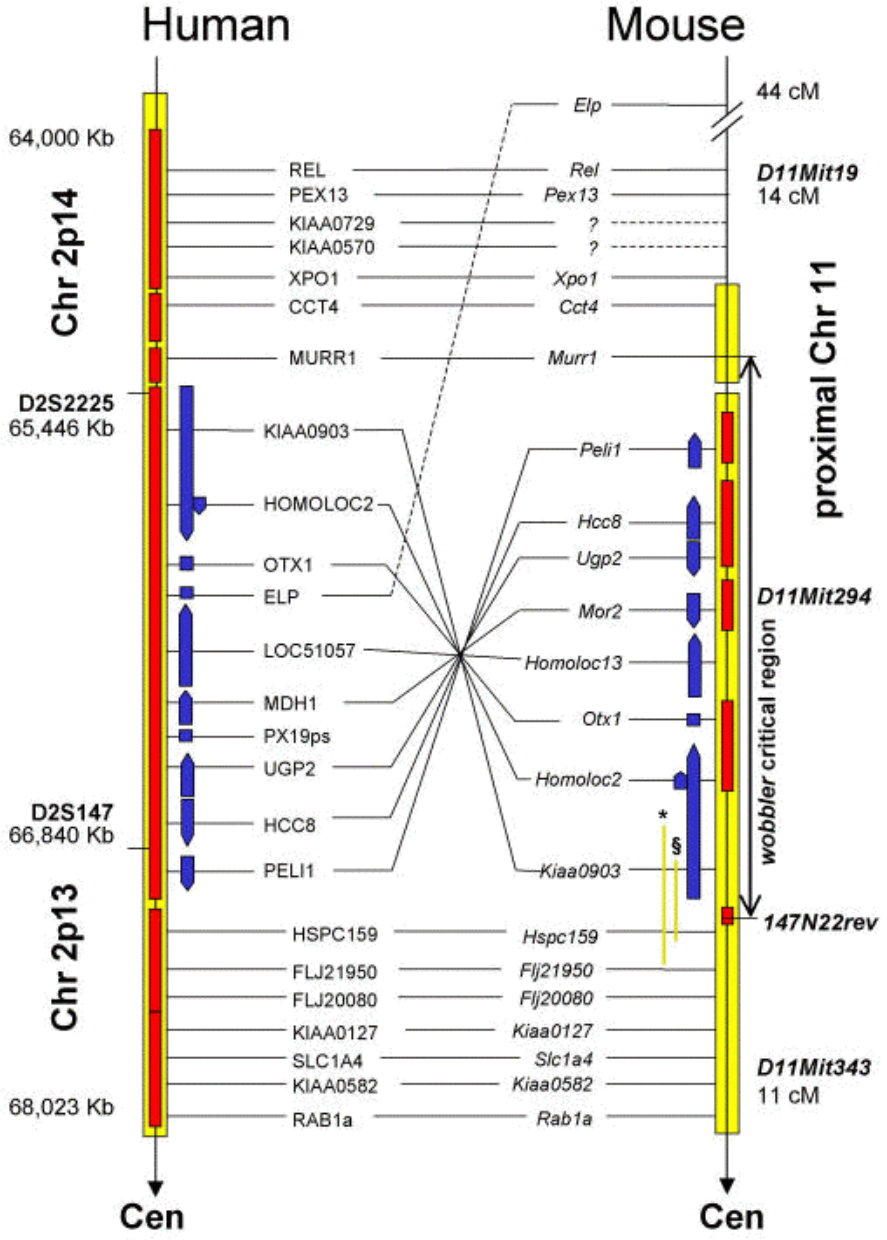
Two genomes similarity

- what are the descriptors ?
- how many genes are common ?
- is the order preserved ?

Potential drugs

- drug 1 binds, will drug 2 ?
- how similar ?





synteny plot: http://home.cc.umanitoba.ca/~umlawda/39.769/presentation/presentation.html, Fristensky, B.
ligands from, Wang, N., DeLisle, R. K. and Diller, D.J. (2005), J. Med. Chem., 48, 6980-6990

Detection and Quantification

Models for prediction and interpretation

- often not well justified

Similarity in these applications

- detection (finding / recognising)
- quantification

- Each in the context of applications

- first protein structure …

Summary so far

A model can explain observations, make predictions or both

A model may be based

- on a belief of the underlying chemistry / physics
- purely mathematical, probabilistic

Similarity

- we have objects with some information (proteins, ligands, genomes, sequences, …)
- we want to find similar objects and hope they have the same properties
- simlarity has a different meaning in different areas

What should one have learnt

- Modelling versus fitting
- examples of dumb fitting, model-specific fitting
- problems with simple general fitting
- benefits of simple fitting

STOP

Sequence Similarity

What is the easiest information to find about a protein ?

- sequence
  - history - amino acid sequencing
  - today - DNA / mRNA sequencing

Consequence

- lots of sequences
  - want to find similar proteins

Mission

  - similarity of sequences – ways to estimate

Similarity of sequences

Problem

A**C**DEA**C**DE..

A**D**DEA**Q**DE..

How similar ?

A**C**DQRSTSRQDCAEA**C**DE..

A**D**DQRSTSRQDCAEA**Q**DE..

Size counts - longer sequences are more similar

- probabilistically - more chances to mutate

- a measure of (di)similarity – evolutionary distance

Too Simple Estimate

- difference / distance
  - time t
- rate of mutation λ
- few mutations
  - A→C but not A→C →A (OK ?) if p(mutation) small
- sequence length n_{res}
- number mutations n_{mut}

- $n_{mut} = t\lambda n_{res}$ so $t = \frac{n_{mut}}{\lambda\ n_{res}}$

  - too simple

Jukes – Cantor distance

Simplification

- work with 4 base types (like DNA)

Rules and nomenclature

- probability of a specific mutation A→C or G→C
  - in time Δt is α
  - set $\alpha = \frac{\lambda}{4}$
- probability of seeing type A at time t is $p_{\mathrm{A},t}$
- probability of seeing type A at time 1 is $p_{\mathrm{A},1}$
- initial probability at $t = 0$ is $p_{\mathrm{A},0} = 1$

- Remember α is very very small (10^{-10})

Jukes – Cantor distance

- probability of change in $\Delta t = 3\alpha$
- probability of no change $p_{\mathrm{A},1} = 1 - 3\alpha$
- probability of $\mathtt{A} \rightarrow \ ? \rightarrow \mathtt{A}$

$$\left(1 - p_{\mathrm{A},t}\right)\alpha$$

Fear not - slower detailed explanation in Übung

- what is the probability of seeing type $\mathtt{A}$ at a time $t + 1$?
  - (no change) + ($\mathtt{A} \rightarrow \ ? \rightarrow \mathtt{A}$)
  - $p_{\mathrm{A},t+1} = p_{\mathrm{A},t}(1 - 3\alpha) + \alpha(1 - p_{\mathrm{A},t})$
- what change has occurred in time Δt ?

$$\begin{aligned}\frac{\Delta p_{\mathrm{A},t}}{\Delta t} &= p_{\mathrm{A},t+1} - p_{\mathrm{A},t} \\ &= p_{\mathrm{A},t}(1 - 3\alpha) + \alpha\left(1 - p_{\mathrm{A},t}\right) - p_{\mathrm{A},t} \\ &= -4\alpha p_{\mathrm{A},t} + \alpha\end{aligned}$$

Jukes – Cantor distance

$$\frac{dp_{\mathrm{A},t}}{dt} = -4\alpha p_{\mathrm{A},t} + \alpha$$

- we want an estimate of t
- like any differential equation

$$\frac{dt}{dp_{\mathrm{A},t}} = \frac{1}{-4\alpha p_{\mathrm{A},t} + \alpha}$$

$$t = \int \left(\frac{1}{-4\alpha p_{\mathrm{A},t} + \alpha} \right) dp_{\mathrm{A},t}$$

Jukes – Cantor distance

- from $t = \int \left(\frac{1}{-4\alpha p_{A,t} + \alpha} \right) dp_{A,t}$
- we get $p_{no\_change} = \frac{1}{4} + \frac{3}{4} e^{-4\alpha t}$ $p_{change} = \frac{3}{4} - \frac{3}{4} e^{-4\alpha t}$
- but this is for one site
- important what fraction of sites has changed ? $\frac{n_{mut}}{n_{res}}$
- estimate time $t \propto -\ln\left(1 - \frac{4}{3} p_{change}\right)$

$$t \propto -\ln\left(1 - \frac{4}{3} \frac{n_{mut}}{n_{res}}\right)$$

Simplifications made

- We have only worried about relative distances
  - no attempt to speak of years
- What is time ?
  - generations
  - years
- 4 bases for DNA (easy to change to 20 amino acids)

Comments on

- base composition equal at $t = 0$
- a residue can mutate to any other
- gaps / alignment quality
- uniform mutation rates

- some details on these issues…

Base Composition

Not a problem

- think back to slide on integration - constant c
- solved by assuming $p_{A,0} = 1$ but could be any value

Different kinds of mutations

We assumed

- $p_{XY} = \alpha$ for all XY types

Wrong:

- DNA: A→G not as bad as A→C or A→T
- proteins: some changes easy (D → E) some hard (D → W)

Different kinds of mutations

Can be fixed with more parameters

- simple case DNA
  - rate α for purine →purine, β for purine → pyrimidine
- protein:
  - 19 different probabilities (for each amino acid type)

Gaps

- so far ignored
- more generally
  - we have assumed proteins / DNA can be aligned

Gaps and Alignments

- gaps ignored
- more generally - assumption that sequences can be aligned

  A**C**DQRSTSRQDCAEA**C**DE..

  A**D**DQRSTSRQDCAEA**Q**DE..
- but what about

  ACDQRATSRQDQRSTSRQ..

  ADDQRSTSRQDCAEAQDE..
- or

  ACDQRATSRQDQRSTSRQ..

  ADDQRSTSRQDCAEAQDE..
- the more distant the sequences, the less reliable the alignment

Uniform mutation rates

- Between organisms
  - fruit flies have short generations
  - bacteria have very short generations
  - within one class of organisms rates vary (DNA repair)
- Neglect of
  - duplication, transposition, major re-arrangements
- Different proteins mutate at different rates
  - essential – DNA copying
  - less essential
  - copied proteins (haemoglobins)
- Functional changes
  - similar proteins in different organisms – different functions
- Within one protein
  - some sites conserved, some mutate fast
- Complete neglect of selection pressure

Similarity of sequences so far

- For very related sequences, not many back mutations
  - even simple mutation count (n_{mut}/n_{res}) OK
- Better to allow for back mutations
- Jukes-Cantor (and related) models
  - can include some statistical properties (base composition)
  - can be easily improved to account for other properties (different types of mutation occur with different frequencies)
  - hard to calibrate in real years, but may not matter
  - will be less reliable for less related species / proteins

Statistical approach to similarity

- Completely different philosophy
- Are proteins A and B related ?
  - how is A related to all proteins (100 000's) ?
  - how strong is the AB relation compared to A-everything ?

What we need

- BLAST / fasta (more in Dr Gonnella's lectures)
- idea of distributions
- measure of significance

Significance

e-value (expectation value)

- I have a bucket with 10 numbered balls (1 .. 10)
- I pull a ball from the bucket (and replace it afterwards)
- how often will I guess the correct number ?
  - *e*-value = 0.1
- you guess the number and are correct 0.25 of the time
  - much more than expected
  - what is the probability (*p*-value) of seeing this by chance ?
  - example distribution.. binomial

Binomial example

- we have 100 attempts (n=100)
- probability p = 0.1 of success on any attempt
- what is the probability that we are always wrong ?

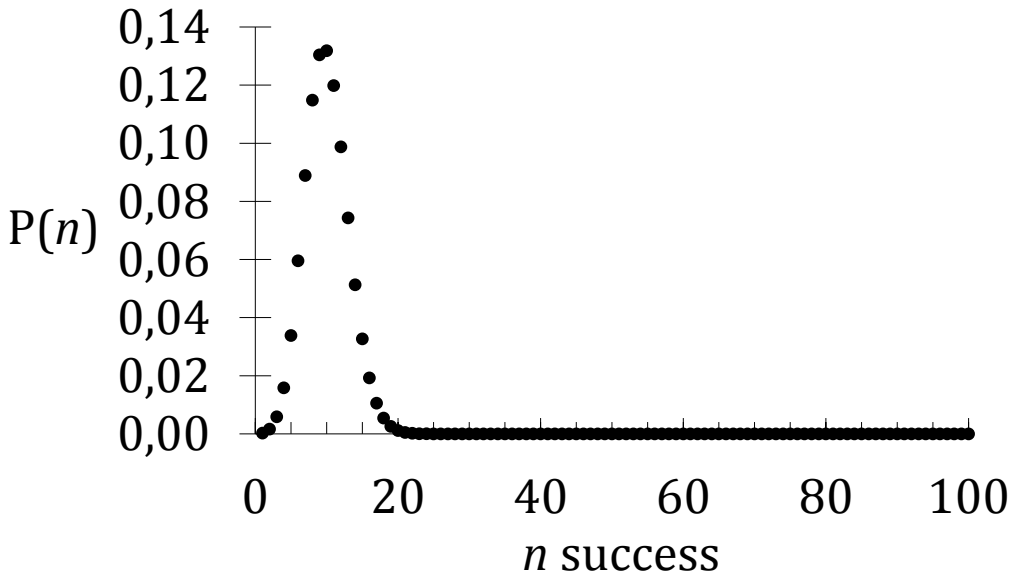
  - $P(0) = 0.9 \times 0.9 \times 0.9 \ldots = 2.7 \times 10^{-5}$

- probability that we make one correct guess
  - $P(1) = 0.1 \times 0.9 \times 0.9 \ldots +$
    $0.9 \times 0.1 \times 0.9 \ldots +$
    $0.9 \times 0.9 \times 0.1 \ldots + \ldots = 3.0 \times 10^{-4}$
  - $P(25) = 9.0 \times 10^{-6}$ my original question
  - $P(10) = 0.13$ what you would guess

Binomial example

- probability that we make one correct guess
  - $P(1) = 0.1 \times 0.9 \times 0.9 \ldots +$
    $0.9 \times 0.1 \times 0.9 \ldots +$
    $0.9 \times 0.9 \times 0.1 \ldots + \ldots = 3.0 \times 10^{-4}$
  - $P(25) = 9.0 \times 10^{-6}$ my original question
  - $P(10) = 0.13$ what you would guess

- this formula not for exams

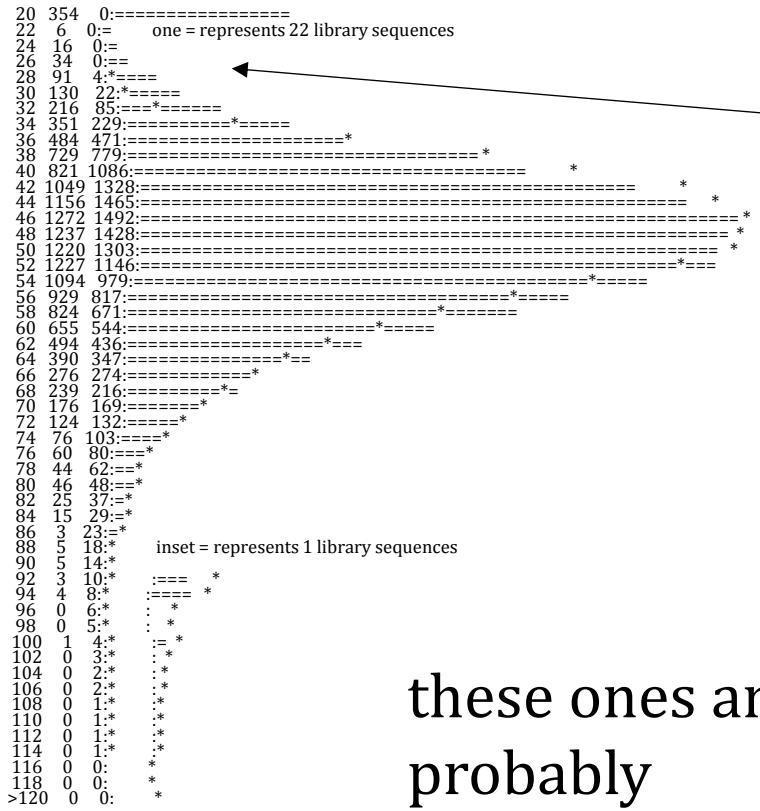


formally
x number of success
n number trials
p probability per trial

$$P(x) = \binom{n}{x} p^x (1-p)^{n-x}$$

Distributions and sequences

- If I align two proteins, sometimes they will be similar (by chance)
- Take a protein and align to a large database
  - there will be a distribution of scores



many sequences match a bit

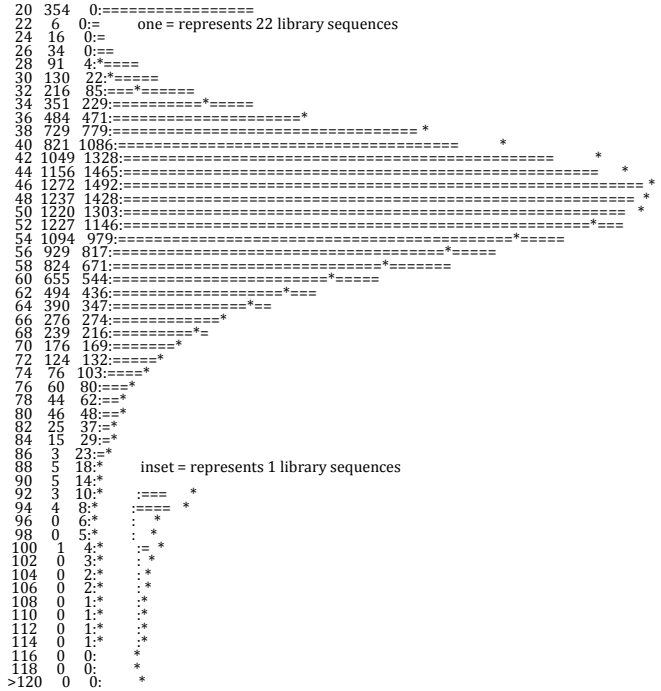
very few are radically different

these ones are probably related

Distributions and sequences

- Can we put numbers on this ?
- model for the distribution
  - "extreme value distribution"
- Probability of score $S \geq x$
- $P(S \geq x) = 1 - \exp\left(-kMNe^{-\lambda x}\right)$

- MN reflect sequence length



Two Distance Measures

One question

- what is the similarity of two sequences ?

Two answers

1. Given two sequences
   - estimate evolutionary t
2. Given two sequences (one is in database)
   - estimate whether they are really related

When are they used ?

Two Distance Measures

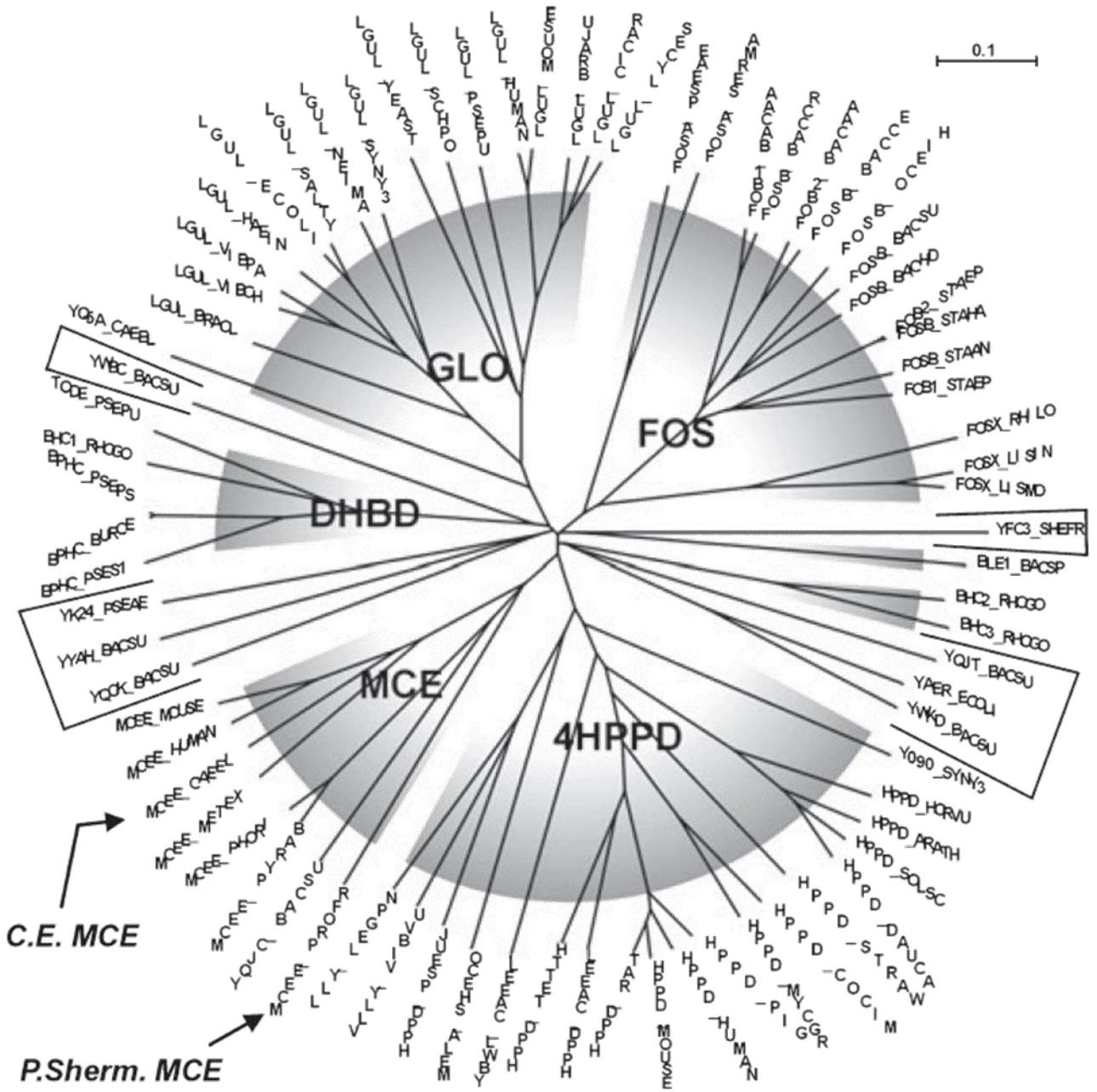
Common uses

- Collection of sequences and want a phylogenetic tree ..
  - each sequence has mutated from another
  - use a measure like Jukes-Cantor

- One sequence
  - which are possibly related sequences ?
  - rank the similarities

An example phylogeny

- metabolic enzyme from a set of parasites



Kühnl... Liebau, FEBS Journal 272 (2005) 1465–1477

Two Distance Measures

Collection of sequences and want a phylogenetic tree ..

- each sequence has mutated from another
- use a measure like Jukes-Cantor

One sequence

- which are possibly related sequences ?
- rank the similarities

- model types …

Model types

Connection to first lecture

- statistical approach
  - very little biology – sequences are objects + distribution
- Jukes - Cantor
  - problem-specific model (mutations, probabilities…)

- next topic – using these similarities - clustering

Probleme von voriges Jahr

- Betrachtet die Jukes-Cantor Beziehung Ruckwärtsmutationen ?

- Was passiert als $t \to \infty$?

What one should have learnt

- distances between sequences – 2 different estimates, one model based
- when would you use one and when would you use the other
- could you summarise the philosophical differences ?