

Laboratory of Bioinformatics 1 part B - Capriotti

Saul Pierotti

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Introduction

- There will be a project for the final, to be submitted for May 18
- Protein structure is more conserved than sequence
- When sequence identity is sufficiently high, we can transfer structural information
- A structural alignment is a rigid body transformation of 2 subsets from 2 sets of points that maximizes a given distance metric
 - The subsets need to have the same number of elements and define the correspondence set
 - Finding the correspondence set is an NP-hard problem
 - Finding the optimal rigid transformation of the correspondence set is $\Theta(n)$
- The distance of 2 sequences can be evaluated with a substitution matrix and a gap penalty
- Global alignments are computed with the NW algorithm, local alignments with the SW
- Local alignments are useful for multidomain proteins, when only some domains are conserved
- The significance of an alignment score can be evaluated by comparing with the score distribution for random alignments
- Sanders and Schneider developed a twilight curve before Rost
 - Sander's curve becomes straight after 80 residues
 - Rost's curve does never become straight
- Over 100 residues, under 25% of sequence identity only 10% of the sequences are homologous, while above 20% 90% of them are
- Over 30% identity sequences longer than 100 residues have similar structures, but this does NOT mean that under 30% the structure is necessarily different!
- Proteins with low sequence identity but high structural similarity are referred to as remote homologs
- Structures can be predicted by comparative modeling, threading, ab initio
- If I want to use sequence identity for transferring annotation features, I need to identify the problem-specific twilight region
 - For subcellular localization, the twilight zone is 50% (!)
- The sequence identity needed for transferring subcellular localization is higher than that required for structure
- Function of proteins with really high sequence identity can be completely different
- In remote homologs the sequence alignment is often wrong
- Important residues in a sequence can be identified by comparing conservation levels

Structural alignment

- Structural alignment is different from superimposition
- Superimposition assumes that I already have the correspondence set, and it is relatively easy
- Structural alignment requires the identification of the correspondence set, which is hard
- The definition of domain is often heuristic and questionable
- Proteins with similar spatial distribution but different topology are difficult to align
- Alignment methods can be classified in different ways

- Pairwise or multiple
- Depending on the descriptor used
 - * Backbone
 - * All atoms
 - * Sequence-based
 - * Contact map
 - * Surface
- Rigid body or flexible
- The comparison of torsion angles is $\Theta(n)$
 - They are invariant for rotation and translation
 - It is good for local regions but problematic for whole structures
- A distance matrix is also invariant for rotation and translation
 - Comparing matrices is hard, $\Theta(n^2)$
 - It is not sensitive to chirality
- At the moment, all methods are able to identify obvious similarities
- Remote similarities are detected by a subset of methods, and different methods recognize different similarities
- Speed is an issue in many algorithms
- We want our method to be biologically meaningful, not only geometrically
- The expected score or random pairwise alignments is an extreme value distribution
 - I would have a gaussian if there was no evolution
 - In real databases I have an excess of good-scoring pairs
- When I want to determine the distribution of scores, it is better to have an analytical distribution than an empirical one
 - I don't have tools for working with empirical distributions (!)

CE algorithm

- Compares AFPs composed of 8 residues, stitches them together and finds an optimal path through them with dynamic programming
- It gives a statistical score
- The alignment is the longest continuous path of AFPs in a similarity matrix S
- The similarity matrix S is composed represent all AFPs conforming to a similarity criterion
- The dimensions of S are $(n_a - m)(n_b - m)$, where n_a and n_b are the length of the sequences and m the size of the AFPs
- The matrix is large to compute, therefore we need constraints
- Two consecutive AFPs can be aligned with a gap in protein A, a gap in protein B or without gaps
- The AFP length is set to 6 and the maximum possible gap to 30
- Similarity measures are RMSD, full set of distances, and others
- The best 20 alignments with Z score above 3.5 are compared based on RMSD and the best one is kept
 - I get an error in 1000 comparisons
- Each gap is assessed for relocation up to $m/2$ times
- Iterative optimization with dynamic programming
- It cannot find non-topological alignments
- The unit of comparison was originally the protein chain, but domains are optimal
 - Domains are difficult to define (!)
- The statistical distribution of alignment scores can be used to evaluate the Z score of an alignment

PDBe Fold

- It uses secondary structure elements (SSEs)
- Secondary structure is typically conserved
- SSE are represented as vectors that connected in a graph by edges
 - 2 vertices and an edge describe position and orientation of the SSEs

- SSEs are helices and strands
- Each edge is labelled by a property vector containing information on edge-vertices angles, torsion angles between vertices, length of the edge
- The set of vertices, edges and labels defines the graph that is then matched with an algorithm
- Vertex and edge lengths are compared both in absolute and relative terms
 - In relative terms, the same absolute difference is less significant for longer edges
- Torsion angles are used for distinguishing mirror symmetries
- The SSE matching gives correspondences among SSEs, and can be used to yield an initial sequence alignment
- Connectivity (topology) can be neglected, considered but allow for any number of missing SSEs (soft connectivity) or allow only for an equal number of unmatched SSEs (strict connectivity)

MAMMOTH algorithm

- Matching molecular models obtained from theory (MAMMOTH) is one of the fastest algorithms
- The protein is represented as a set of unit vectors among Ca
- It is based on dynamic programming
- An unit vector is the normalized vector among Ca atoms
 - For each position, k consecutive vectors are mapped into a unit sphere that represents the local structure of k residues
- Each set of unit vectors is compared to all the sets in the other structure, building a matrix
- Each comparison yields a unit root mean square distance (URMS)
 - This is compared against the expected random URMS
 - The alignment score is obtained by normalizing the URMS with its expected value
- The path through the matrix is found with dynamic programming by a global alignment without end-gap penalties

RNA structure

- Most RNAs are around 50 bp
- Secondary structure of RNAs is usually represented with parentheses
 - I cannot represent pseudo-knots in this way
- For RNA, the secondary structure is much more informative than for proteins
 - A certain secondary structure constraints a lot the tertiary structure
- There is less variability in RNA structures than in proteins
- The best atom for representing the backbone is C3', since it has the most constant inter-nucleotide distance
- The professor adapted MAMMOTH to work with RNA C3' atoms instead of Ca in proteins: SARA
 - The statistics of the score had to be re-evaluated
 - They still used the extreme value distribution, which is defined by μ and σ
 - They selected how the parameters change when RNA size changes
 - The set of unit vectors was 3 instead of 7
 - The method gives a $-\log(\text{p-value})$ score
 - By comparing RNAs of known function, I can determine a score threshold that gives correct functional annotation
- Another method was developed in Israel: ARTS
- Few people are working in RNA: not so many methods
- The twilight zone of RNA sequence alignment is around 60%
- Secondary structure identity (PSS) correlates well with tertiary structure identity (PSI) but not with sequence identity

Multiple sequence alignment

- In MSA it is easier than in pairwise alignments to identify conserved regions, that could be functionally important
 - We can observe blocks of conservation in MSAs
- I can transform a MSA in a profile of the sequences
- A profile is a matrix with a row for each possible residue and a column for each position
 - The value of each element reflects the frequency of a residue in a specific position
 - Each position is therefore a vector of 20 elements
 - I represent a profile as a matrix containing as many vectors as the number of positions
 - I can also have a row for the presence of a gap in the position
- A sequence logo is a plot showing the entropy of each residue in each position
 - It is obtained from a profile and it is a way to represent it
- Shannon entropy: information content of a message
 - For a single column $S(p) = \sum_{i=1}^{20} -p_i \ln p_i$
 - Total conservation: $S(p) = 0$
 - All residues are equally probable: $S(p) = \ln(20)$
 - There are more sophisticated models that take into account the expected frequency of residues
 - The entropy of an alignment is obtained by summing the Shannon entropy over the all alignment
- Scoring an MSA: sum of pairwise scores or entropy score
 - Not all the positions are equal in an MSA: some conservations are critical, others not
 - Scoring has necessarily to depend on the evolutionary history of the sequences
 - Almost all scoring functions assume positional independence
- I can score each pairwise alignment and sum it
 - $S = \sum_{i < j} S(A_i, A_j)$
- I can score an MSA depending on its entropy
 - The best alignment is the one with the lowest entropy (i.e. the most conserved one)
 - It is the sum over the alignment of the entropy in each position
 - $S = \sum_{j=1}^{N_{cols}} \sum_{i=1}^{20} -p_i \ln p_i$
- I can align a sequence to a profile
 - Each position is aligned to a vector for the position
 - The score for the position of the residue in the sequence with every possible residue is summed and weighted for the frequency encoded in the vector
 - * This is a matrix by vector multiplication (!)
 - These scores can be used with a dynamic programming algorithm

Algorithms for MSAs

- Dynamic programming approaches exist, but they are $O(N^M)$ and they are np-hard
- An MSA method can be evaluated from the functionally important residues that are correctly aligned

Progressive MSA

- ClustalW is an example of progressive MSA
- I align sequences in pairs, one after the other
- The result depends on the order of how I pair sequences (!)
 - I usually pair the most similar sequences first
- Similarity is measured by Kimura distance (see MUSCLE for more info)
- From each pairwise alignment, I build a profile
- I iterate until there are no sequences left, by aligning pairwise sequences and profiles
- In order to do this I need to be able to align profiles (!)
- I want to be conservative with gaps with the initial pairwise alignments, and introduce them later on profiles
 - When I get to profiles I have info about conservation (!)

- Errors in the first alignments are propagated
 - If I am not conservative I can become full of gaps
- I can improve the alignment by changing the sequence tree
 - By default Clustal uses NJ
 - Maybe I have a tree available (!)
- Adding gaps is tricky, since their penalty logically depends on the position and conservation
 - They are usually added in the first alignments
- In ClustalW the penalty is multiplied by a factor which is context specific
 - Gaps in hydrophobic regions are more penalised
 - These coefficients were derived from gaps frequencies in a large number of structural alignments
 - Gaps are discouraged if there is another gap nearby in the MSA
- Low-scoring alignments are postponed for later by adjusting the tree
 - ClustalW aligns them when it has more information deriving from the profiles
- A profile-to-profile alignments involve the pairwise comparison of same-dimensional vectors
 - I do a double sum all against all elements weighted with a substitution matrix
 - This is done via a simple vector to matrix multiplication, followed by a multiplication for the remaining vector (!)
- ClustalW corrects for biased representation of subfamilies
- The scoring matrices used change depending on the similarity of the sequences to be compared
- In general, ClustalW uses an heavily crafted heuristics
- The main problem of progressive alignment: subalignments are frozen in place
 - Once aligned, a group of sequences cannot be re-aligned by taking advantage of the new information deriving from other sequences

Iterative MSA

- Iterative MSA tries to overcome the problem of frozen subalignments
- MUSCLE: multiple sequence comparison by log expectations
- It is based on 3 steps: draft progressive, improved progressive, and refinement
- Draft progressive: create a first progressive MSA
 - Sequence similarity is defined by k-mer distance, not pairwise alignment score
 - * If a rare kmer is present in 2 sequences maybe they are related
 - It creates a distance matrix with all sequences against each other
 - It uses UPGMA instead of NJ for building the tree from the matrix
 - The score is based on log expectations, not pairwise score for profile to profile alignments
 - * It is the entropy score
- Improved progressive: from the draft create a new matrix and from that a new tree and a new alignment
 - The pairwise distances are calculated from the Kimura distance
 - * $K_{dist} = -\ln 1 - D - D^2/5$, where D is the pairwise identity
- Refinement: cut and re-align the tree
 - 1 edge is deleted at random from the tree
 - The 2 resulting profiles are re-aligned to each other to get the full MSA
 - If the score improves, keep the new MSA otherwise keep the previous one
 - This is iterated until convergence on a local minimum

Consistency-based MSA

- Consistency: if residue X is aligned with Y and Y is aligned with Z, then X is aligned to Z
 - This is necessarily true in an MSA
- In reverse, I can use consistency to align two sub-alignments: I take advantage of transitivity of alignments
- MSA are not necessarily consistent with the respective pairwise alignments
 - Progressive MSA methods frequently are not consistent with the pairwise alignments used for building the tree

- T-Coffe (tree-based consistency objective function for alignment evaluation) is an MSA method based on consistency
 - Build the primary library
 - * I do all the possible pairwise alignments and I measure the pairwise sequence identity
 - * Each pairwise alignment is equipped with a weight equal to the average identity of matched residues, ignoring gaps
 - Build the extended library
 - * In order to align sequences A and B, I try all the possible alignment, direct and based on an intermediate sequence C
 - * The weight of each alignment is the minimum of the pairwise weights for the intermediate alignments
 - * The final weight of a position is the sum of the weights of all the possible alignments supporting it
 - Maximise the pairwise alignments from the extended library with dynamic programming
 - * The score of each match corresponds to its weight
 - From the extended pairwise alignments, build a guide tree
 - Do a progressive MSA from this guide tree and the extended pairwise alignments
 - T-Coffe considers both global and local pairwise alignments and it can use information about domains and motifs

MSA benchmark

- BaliBASE was the first large-scale benchmark specifically designed for MSA
 - It is a dataset with manually refined alignments derived from structural superimposition
- BaliBASE is subdivided in several reference datasets
 - 1 - Small number of equidistant sequences
 - * This is further subdivided by identity levels
 - 2 - Families with one or more orphan sequences
 - 3 - Pair of divergent subfamilies with less than 25% reciprocal identity
 - 4 - Sequences with large extensions at the N or C terminal
 - 5 - Sequences with large internal indels
- The evaluation of the benchmark is based on a series of scores
 - The scores are evaluated only for columns that are reliably aligned in the reference (core columns)
 - Sum of pairs score (SP score): proportion of correctly aligned residue pairs in the core columns
 - Total column score (TC score): proportion of completely correctly aligned core columns
 - TC and SP score both are a number between 0 and 1
 - In a pairwise alignment SP and TC score are necessarily equal
 - In an MSA with 3 or more sequences, $SP \geq TC$
 - Both scores encourage sensitivity, but they do not test for specificity
 - * There is no penalty for wrong alignments (!)
- BaliBASE also evaluates time of execution and peak memory usage
- SP, TC, memory and time are reported as Z-scores on a spiderweb plot for each alignment tool
- What comes out of the BaliBASE benchmark?
 - No single method is perfect in all cases (!)
 - On average, consistency-based methods are more accurate but slower
 - T-Coffe suffers with N and C terminal extension
 - ClustalW and MUSCLE are the least resource-heavy tools
 - T-Coffe and MAFFT are well suited for alignments larger than those in BaliBASE
 - Multi-threading can greatly speed-up these softwares, since there is a lot of parallel computing
 - Many algos take advantage of parallel processing

Probabilistic sequence models

- A model is an object producing different outcomes (sequences) from a probability distribution
- The probability distribution in sequence space determines the specificity of the model
- The probability for model M of generating sequence s is $p(s|M)$
- In the reverse, I can see a model as an object that given an outcome computes a probability value
- Models can be trained: I can adjust the probability density function over the sequence space from a set of known sequences
 - If I want to model the globin family, I can train my model with sequences that are known to belong to that family
- After training, I can use the model to compute the probability of an unknown sequence to belong to the globin family
- The model M given a sequence s returns the probability $p(s|M)$
 - This is the probability of the model generating the sequence, not the sequence coming from the model
- Most times I am interested in the probability of a given sequence s to come from the model M
 - This is the probability of a sequence being part of a specific family
 - This is $p(M|s)$
- In order to compute $p(M|s)$ from $p(s|M)$ I need to use Bayes theorem
 - $p(M|s) = p(s|M)p(M)/p(s)$
- The priors $p(M)$ and $p(s)$ need to be estimated to do the conversion
 - $p(M)$ is the a priori probability of any sequence belonging to the model
 - * This is the relative abundance of the class, relative to all possible classes
 - * It can be estimated from the abundance of the known sequences in the family
 - $p(s)$ is the a priori probability of the sequence and cannot be estimated reliably
- In order to avoid specifying $p(s)$ I can compare the probabilities of 2 different models
 - Instead of looking for $p(M_1|s)$, I look for $p(M_1|s)/p(M_2|s)$
 - $\frac{p(M_1|s)}{p(M_2|s)} = \frac{p(M_1|s)p(M_1)}{p(s)} \frac{p(s)}{p(M_2|s)p(M_2)} = \frac{p(M_1|s)p(M_1)}{p(M_2|s)p(M_2)}$
 - In this way, the conditional probabilities of the sequences are easy to estimate from the models themselves
 - The ratio $p(M_1)/p(M_2)$ can be estimated from the relative abundance of the 2 classes
- To make the calculation more standard, I can systematically compare any model to the NULL model
- The NULL model N is a model that generates all the possible sequences with equal probabilities, only depending on the residue frequencies

Markov Models

- HMM have their most frequent application in speech recognition
- A simple Markov Model, or Markov chain is a collection of states associated with probabilities for all the possible transitions between them
- It is useful for modeling the probability of a sequence of states that only depend on the preceding state in the sequence
- I can consider each residue as a state, and I can assume that its state depends only on the previous residue
- The Markov model will contain all the possible residues and their transition probabilities
- In this framework, the transition probability is the probability that residue B follows residue A in position x_i of a sequence
- The transition probability a_{AB} is the conditional probability of the position $i+1$ being B given that position i is A
 - $a_{A,B} = p(x_{i+1} = B | x_i = A)$
- The probability of a sequence x of length n is the product of all the transition probabilities at the various positions
 - Here I am assuming independence of each transition

- $p(x) = p(x_n|x_{n-1}) * p(x_{n-1}|x_{n-2}) * \dots * p(x_2|x_1) * p(x_1)$
- $p(x) = p(x_1) * \prod_{i=2}^n a_{x_{i-1}, x_i}$
- I can also add a BEGIN and an END state to my model for avoiding irregularities
 - * In this case the transition probability from BEGIN to a state is the probability of starting with that state
 - * This is symmetrical for transitions from a state to the END state
 - * We treat both BEGIN and END states as the same state 0 so a_{0k} and a_{j0} are transitions from BEGIN and to END
 - There is no ambiguity since transitions are only from BEGIN and only to END
- Let's say I want to model the probability that a given sequence is a CpG island
 - In such sequence, a_C, G would be much higher than elsewhere
 - I can create 2 different Markov chains M_+ and M_- for modelling the 2 sequences: CpG island and non CpG island
 - The 2 models will have the same states but different transition probabilities
 - To determine the likelihood S of a sequence x being a CpG island, I can compare the log-odds of the 2 models
 - * $S(x) = \log \frac{P(x|M_+)}{P(x|M_-)} = \sum_{i=1}^n \log \frac{a_{x_{i-1}, x_i}^+}{a_{x_{i-1}, x_i}^-}$
- I can define the probability of a sequence s_i to be generated by a family described by the model M
 - $p(s_i|M)$
- In a Markov model, the sum of probabilities going out of a state is always 1
 - It is certain that I will go out of the state
- When I have only 2 possible mutually exclusive models, I can have a measure for $p(s)$
 - $p(s|M_1) + p(s|M_2) = p(s)$
- From this, I can recover $p(M_1|s)$ and $p(M_2|s)$
 - $p(M_1|s) = \frac{p(s|M_1)p(M_1)}{p(s)} = \frac{p(s|M_1)p(M_1)}{p(s|M_1) + p(s|M_2)}$
 - $p(M_2|s) = \frac{p(s|M_2)p(M_2)}{p(s)} = \frac{p(s|M_2)p(M_2)}{p(s|M_1) + p(s|M_2)}$
- We always work with Markov models of order 1: every state depends only on the previous 1 state
 - There are also MM of order 0 or >1

Training the model

- The parameters for a model can be estimated from a set of training data
- For any sequence s and model M , I can express $p(s|M)$ as the Markov chain that can produce s
 - $p(s|M) = \prod_{j=0}^{n+1} \prod_{k=0}^{n+1} a_{jk}^{n_{jk}}$
 - In this representation 0 is the BEGIN state and $n+1$ the END state
 - The probability is the product of the transition probability for all the possible transitions to the power of how many times they do occur
- The model is always under the normalization constraint
 - $\forall j \sum_{k=1}^{n+1} a_{jk} = 1$
 - The sum of outgoing transitions from any state must sum up to 1
- Maximum likelihood estimation: the value of the parameter θ is the one that maximises the probability of the dataset D given the model and the parameter
 - $\theta_{ML} = \operatorname{argmax}_{\theta} P(D|M, \theta)$
 - The solution for any parameter θ can be obtained
 - * $\theta = a_{ik} = \frac{n_{ik}}{\sum_j n_{ij}}$
 - * The optimal value of the parameter is the frequency of occurrence of the transition in the dataset
 - * The normalization constraint forces to divide the count of transitions for the total number of outgoing transitions
- Maximum a posteriori estimation: the Bayesian correction of the ML approach
 - $\theta_{MAP} = \operatorname{argmax}_{\theta} (p(\theta|M, D))$
 - $p(\theta|M, D) = p(D|M, \theta)p(\theta)$

HMM

- Let's now try to model the presence of a CpG island in a larger sequence
 - I can integrate both models M_+ and M_- in a single model
 - I will have 2 states for each nucleotide, one for each model
 - The transition probabilities inside states of the + and - models will be similar to before
 - In addition I will have a small probability of going from a state of one model to any state of the other model
 - It will be more probable to go from - to + than vice versa
 - * This means that I will be most of the time in -, so most of the sequence is not a CpG island
- This is an Hidden Markov Model since for every position the sequence itself I cannot know which state generated it
 - For each possible nucleotide I have 2 states, and I do not know which one it came from
- Differently from Markov chains, in HMM we need to distinguish the sequence of states from the sequence of observables (symbols here)
- The sequence of states, which is hidden to us, is called the path π and it is a simple Markov chain
 - The path has transition probabilities $a_{jk} = p(\pi_i = k | \pi_{i-1} = j)$
- A symbol can correspond to multiple states but also a state can generate different symbols (!)
 - In general, the outcome of a single state derives from a probability distribution
 - We define the emission probability of symbol b from state k as $e_k(b) = p(x_i = b | \pi_i = k)$
 - The sum of emission probabilities from a state is always 1, so the state always produces something
- I can write the probability of observing the sequence x of length L under the path π
 - $p(x, \pi) = a_{0\pi_1} \prod_{i=1}^L e_{\pi_i}(x_i) a_{\pi_i \pi_{i+1}}$
 - In this equation $\pi_{L+1} = 0$, so the last transition is to the END state
 - The probability of a character x_i being generated by the model is the product of the probability deriving from the markov chain and the emission probability for that character under the current state
 - The path is typically hidden, so this equation is not useful in practice
- The probability of the above equation under a model M can be rewritten as $p(x, \pi | M)$
 - This can be decomposed as $p(x, \pi | M) = p(x | \pi, M) * p(\pi | M)$
- Naive approach: if I want to obtain $p(x | M)$ under an HMM I need to sum over all the possible paths
 - $p(x | M) = \sum_{\pi} p(x, \pi | M)$
 - The number of possible paths is the number of states elevated to the length of the sequence
 - * No way you can do that brute force
 - The time complexity is $O(tn^t)$ where n is sequence length and t the number of different symbols
 - * Danger! NP-hard!
- There are different algorithms for computing $p(x | M)$ under an HMM
 - In general, my aim is to decode the path from the sequence, so that I can assess the true probability
- Viterbi algorithm: dynamic programming for finding the most probable path
 - If I need to choose just 1 path the most probable one is the most logical choice
 - Let's define the most probable path $\pi^* = \text{argmax}(p(x, \pi))$
 - I can find π^* recursively
 - * I suppose that the probability of π^* having state k in position i is $v_k(i)$ and it is known for all the states k
 - This means that I know the probability of each state in each position of the most probable path
 - * I can calculate recursively the probability of state l for position $i+1$
 - $v_l(i+1) = e_l(x_{i+1}) * \max_k (v_k(i) a_{kl})$
 - The first term is the emission probability of the state l for the observed symbol x_{i+1}
 - The second term is the probability of having state k in position i times the transition probability from k to l
 - In the second term I take the max in k , so I choose the k that maximises the quantity
 - The problem then recurses in calculating $v_k(i)$ and so on
 - * All sequences need to start at some point: the recursion ends in $v_0(0) = 1$

- It is certain that the beginning of the sequence comes from state 0
- Given this framework, I can create a dynamic programming matrix that finds the optimal path
 - * Initialization
 - $i = 0, v_0(0) = 1, v_k(0) = 0$ for $k > 0$
 - * Recursion with $i = 1$ to L (length of sequence)
 - $v_l(i) = e_l(x_i) * \max_k(v_k(i-1)a_{kl})$
 - $pointer_l(i) = \operatorname{argmax}_k(v_k(i-1)a_{kl})$
 - * Termination
 - $p(x, \pi^*) = \max_k(v_k(L)a_{k0})$
 - $\pi^*(L) = \operatorname{argmax}_k(v_k(L)a_{k0})$
 - * Traceback with $i = L$ down to 1
 - $\pi^*(i-1) = pointer_i(\pi_i^*)$
- The probabilities obtained with the Viterbi algorithm are really small and give underflow errors
 - * It is better to operate in log space
 - I use $\log v_l(i)$
 - * This makes also the products become sums
- Forward algorithm: why only the most probable path, if I can have all of them?
 - Using only π^* as in the Viterbi algorithm is a huge approximation, but it works surprisingly well
 - Actually we don't need to do so, since we can calculate the complete probability of x for all paths
 - We can just replace the maximizations of the Viterbi algorithm with sums
 - We can define $f_k(i)$ as the forward parallel of the Viterbi quantity $v_k(i)$
 - * It is the probability of state k in position i under the forward algorithm
 - The probability of state k in position i is the joint probability of the sequence up to position i and the fact that the current state is k
 - * $f_k(i) = p(x_1..x_i, \pi_i = k)$
 - The recursion equation is therefore
 - * $f_l(i+1) = e_l(x_{i+1}) * \sum_{k=0}^i (f_k(i)a_{kl})$
 - Like the Viterbi approach, the forward algorithm can give underflow errors
 - * I can correct by operating in log space or scaling the probabilities
 - * In log space the math is not as clean as with the Viterbi
 - The time complexity is $O(L^2)$
- Backward algorithm: same as forward, but starting from the end
 - The quantities that I consider here is $b_k(i)$
 - It is the probability of state k in position i
 - I don't use the backward algorithm for calculating $p(x)$, since usually I get it with the forward algorithm
- The backward algorithm is useful for calculating posterior probabilities
- What I am really interested in is not $p(x, \pi_i = k|M)$, but $p(\pi = k|x, M)$
 - I want now the probability of the hidden state k being at work given the sequence
 - $p(x, \pi_i = k)$ can be decomposed as $f_k(i)b_k(i)$
 - * This is the joint probability of having $\pi_i = k$ when the sequence up to i and from i to the end is equal to the respective portions of x
 - From this I can get the posterior probabilities
 - * $p(\pi = k|x) = p(x, \pi_i = k)/p(x) = f_k(i)b_k(i)/p(x)$
 - * $p(x)$ is the result of the forward algorithm here (!)
- A posteriori decoding: when choosing the most probable path is not justified
 - In some situations just choosing the most probable path (Viterbi decoding) is not legitimate
 - * I can have many paths with similar probabilities, and a posteriori decoding evaluates all of their contributions for any state
 - For position i the a posteriori estimate for state π_i is $\hat{\pi}_i$, as compared to π_i^* of Viterbi decoding
 - * $\hat{\pi}_i = \operatorname{argmax}_k(p(\pi_i = k|x))$
 - * This probability includes all the possible paths that can bring me in position i at state k
 - This definition is not very useful for determining the path, I can only decode a single state
 - If I use this equation for the whole path, it can be non-sensical (!)

- * It could include forbidden transitions
- * It can give the most probable state in position i given path, and then a state in position $i+1$ given a different path, when the transition from the 2 is impossible

HMM for pairwise alignments

- I can see a gapped alignment as a finite state automaton (FSA) with a match state M , and two state for the respective insertions, X and Y
 - In this FSA I have score changes at every state transition
- I can similarly create a probabilistic HMM with the same states
 - It does not emit a sequence, but an alignment (!)
 - It is called pair HMM
- The pair HMM has the following properties
 - The state M has emission probabilities for all possible matches $x_i : y_i$
 - State X has emission probabilities for all possible single character insertions in X (or deletions in Y) such as $x_i : gap$
 - State Y has emission probabilities for all possible single character insertions in Y (or deletions in X) such as $y_i : gap$
 - We introduce a BEGIN and END state
 - The transition probabilities are called
 - * δ for $M \rightarrow X, Y$
 - * ϵ for $Y \rightarrow Y$ and $X \rightarrow X$
 - * θ for $M, Y, X \rightarrow END$
 - * The other probabilities can be derived from the complements to 1
 - Every position in the pair HMM has two indexes instead of 1
- The Viterbi path of the pair HMM is the optimal FSA alignment (!)
 - It is the one I would recover from the NW algorithm

Profile HMMs

- Profile HMMs are the most important application of HMMs to bioinformatics
 - They were first done by Krogh (the one from Denmark)
- In the ungapped case, I just want to model the propensity of the position for a symbol
 - The profile HMM will have for each state M emission probabilities deriving from the profile vector at that position
- Introducing gaps, we see how their penalty shouldn't be the same across the alignment
- I can introduce the insert (I) state for modelling insertions in my sequence with respect to its family profile
- The I state has emission probabilities deriving from background distribution
 - We need a transition from M_i to I_i , a loop from I_i to itself and a transition from I_i to M_{i+1}
 - This model is essentially an affine gap
- The deletion could be modelled by a series of transitions from M_i to all M_{i+k} , all the subsequent states
 - This requires $n(n-1)/2$ transitions, and I need probabilities for all of them
- To avoid this I insert silent states D for modelling deletions
 - A silent state is a state that does not emit any symbol
 - Now I have transitions from M_i to D_{i+1} and from D_{i+1} to D_{i+k}
 - * I can enter in a delete state (and not add anything to my sequence) and continue there
 - I have also transitions D_i to M_{i+1} for modelling when the deletion ends
 - In this way I have $4n-8$ parameters
- This delete model has different transitions in different positions, so I can include a position-specific gap penalty (!)
- In my insert model I cannot do this, since I have loops for long insertions in the same I state
 - This makes sense since The insertion only matters where it starts and how long it is

- For deletions it matters which residues are missing from the family profile (!)
- As a final refinement, I can include transitions between delete and insert states
 - They are quite unlikely and usually they do not affect much the alignment
 - These are D_i to I_i and I_i to D_{i+1}
- Any profile HMM is able to produce any possible sequence in sequence space
- Parameterising a profile HMM means to make the probability distribution of the produced sequences peak around members of the modelled family
 - We can play with transition and emission probabilities, and with the length of the model itself
- Modelling the length of the model means to decide which MSA columns to assign to match states, and which to insert states
 - A heuristic rule is to assign to insert states the columns that have more than half gaps
- Probabilities can be estimated from the transition and emission frequencies of the sequences in the MSA
 - For this to be meaningful, I need a big training set
 - There can be transitions or emissions with 0 probability due to sampling limitations
 - * We can add pseudocounts for coping with this
- I can evaluate the score of an alignment to a profile with the profile HMM
 - I can calculate $p(x, \pi^* | M)$ with Viterbi or $p(x | M)$ with the forward algorithm
- I can use the log-likelihood $LL = -\log p(x | M)$ as the alignment score
 - It is strongly length dependent and in a not linear fashion (!)
 - I can normalize it obtaining a Z-score
 - For the normalization I need a μ and a σ for the length-dependent score distribution
- I can also use the log-odds against the NULL model
 - This has usually a 3 times better signal-to-noise ratio in discriminating families
 - The NULL model is obtained from the residue composition of the training set
- The accuracy of a prediction can be evaluated on the confusion matrix
 - It is a simple 2*2 matrix that relates true and false positives and negatives
 - The variables under consideration are the true condition and the test outcome
- The accuracy ACC can be evaluated from the confusion matrix
 - $ACC = (TP + TN) / (TP + TN + FP + FN)$
 - This measure can be biased if the classes (positive and negatives or prediction 1 and prediction 2) are highly unbalanced
- A better approach is to evaluate sensitivity (True positive rate, TPR) and specificity (Positive predicted value, PPV)
 - $TPR = TP / (TP + FN)$
 - $PPV = TP / (TP + FP)$
- The Matthews correlation coefficient (MCC) is the analogous of Pearson for categorical predictions
 - $MCC = \frac{(TP * TN) - (FP * FN)}{\sqrt{(TP + FP)(TP + FN)(TN + FP)(TN + FN)}}$
 - It measures the correlation among predictions and real classes
 - It is not affected by class unbalance
- The ROC curve (receiver operating characteristics) is a plot of FPR (false positive rate) against TPR (true positive rate) when a parameter varies
 - This can be a tuning parameter
 - The area under the ROC curve is 0.5 for random predictions and 1 for a perfect prediction

HMMER

- It is a widely used tool for creation of HMMs from MSAs
- Its HMMs are based on a domain structure
 - They present a domain chain defined by M, I and D states
 - The domain chain is connected by 2 states that model the C and N terminal regions of the domain
 - A J state models inter-domain regions
- The score of a sequence is calculated in the Bayesian frame against the NULL model
- HMMER takes the trained model and scores 200 randomly generated sequences with it in the Bayesian

framework

- The Log odd of the sequences is fitted with a Gumbell distribution
- This allows to estimate the distribution parameters μ and λ
- Once I have the score distribution of random sequences, I can get the p-value of a sequence that I score against the model
 - $p(s > t) = 1 - \exp(-e^{-\lambda(t-\mu)})$
- From the p-value I can obtain the e-value, the number of random sequences expected to have score $>t$
 - $p = 1 - e^{-E}$
 - E is the average number of rare events, modelled under the Poisson distribution
- The default input format is Stockholm, but it accepts many common formats
 - It can include info on secondary structure and can mask some characters
- It is composed of several tools
 - `hmmbuild` takes an msa and gives an HMM
 - `hmmemit` generates a sample sequence from the HMM
 - `hmmsearch` searches a database with the HMM returning a list ordered by e-value
 - `hmmalign` aligns sequences to a given model
 - `hmmcompress` converts one or more concatenated HMMs in binary for subsequent searches
 - `hmmScan` uses a binary HMM db to scan a sequence

Kunitz domain project

- We want to produce an HMM of the Kunitz domain
- The Kunitz domain is a protease inhibitor
 - APP (Alzheimer) and TFPI (Tissue factor inhibitor) have this domain
- Aprotinin is a drug that act as an anti-fibrinolytic
 - It is actually the bovin pancreatic protease inhibitor
 - A good structure is 3TGI, that also shows the mechanism of inhibition
 - It has 6 cysteines
 - Uniprot: P00974
- The domain is included in single-domain and multiple-domain proteins
 - I prefer to build my HMM with single-domain proteins if available in enough number
 - * Other domains nearby could influence the structure of my domain
- We want to build our HMM of the Kunitz domain starting from structural information and use the model for annotating SwissProt

Retrieving the structures

- Several options available
 - We can use PDBfold searching with a prototype structure
 - We can search on CATH
- Potential problems
 - PDBs with multiple chains
 - Chains with multiple domains
 - Redundant PDB structures: I can get more copies of the same protein
 - Mutated proteins for experimental reasons
 - Variable resolution
- Possible solutions
 - Accept only structures with resolution above 2.5-3 Å
 - * Interaction and $C\alpha - C\alpha$ distances are on this range
 - H bonds are around 3 Å long
- I select a set of seed proteins and I refine them on the basis of the structural alignment
- I convert the alignment in Stockholm format with Jalview or with a script

Creating and testing the model

- I use `hmmbuild` to get the model from the alignment
- I scan the original seed set with my model with `hmmsearch` to see if I get a significant E-value
- I do a real benchmark with non-seed proteins that I know to have the Kunitz domain
 - Since SwissProt is manually annotated, I can define a subset containing all the Kunitz-containing proteins
 - I also need a set of negatives to test the specificity of the model
- I need to define the confusion matrix for my model
- I can use the Matthews correlation to give a quality score to my model
- I can plot a ROC curve
- The Kunitz domain is really well defined and annotated, so I expect few false negatives and positives
 - I can manually review all of them and understand where the model failed
- It is important to understand the limitations of my method!

Project report

- To be done following the structure of the Oxford Bioinformatics journal
- I should put an abstract with information about the problem and summary of the results
 - Should be short!
- Introduction should contain info on the domain, previous work, etc.
 - It will contain most of the citation
 - It should describe the state of the art
- Materials and methods: in bioinformatics mostly only methods
 - Defines the reproducibility of the work
 - I should describe procedures, algorithms, validation
 - I should describe the dataset
- Results: what I did with the methods and what I got out of it
- I should include subsections in all parts
 - People can be interested only in some aspects!
- Conclusion/discussion: summarise, discuss the limitations and future directions
- I should start from materials and methods and results
- At the end I write conclusion and introduction
- Reference: always to be included
- Supplementary materials can be included
- Note: there will still be a final exam
- Deadline: May 18 and then 3 weeks after it for the final version
- To be sent by mail at emidio.capriotti@unibo.it with object should be “project lb1b - Saul Pierotti”

Ab initio structural predictions

- It is used when there are no structures in the PDB that can be used as templates in modelling my sequence
- Rosetta (David Becker) is the most used method
 - It is based on short fragments that are used as building blocks of the global fold
 - An energy landscape is built for these fragments
 - All proteins in the PDB were collected and fragmented in blocks of 5 residues
 - Similar fragments were clustered based on RMSD
 - A sequence profile was built from each cluster
 - When modelling a new sequence, this was broken in fragments and aligned to the profiles
 - Some heuristics were used for choosing the best match
 - The structure was refined with molecular dynamics
- Force fields are typically used with these methods

- CHARMM is a traditionally used force field for proteins
 - It was developed by the Nobel prize winner Martin Carplus
- The conformational space is huge and because of this it cannot be used above 150 aa
 - AlphaFold from Google seems to perform better
- *Ab initio* ignores the action of chaperons
- The CASP evaluation is for *Ab initio* methods

Threading and fold recognition

- It is used for sequences that have a suitable template in the PDB but we cannot find it based on sequence alone
 - Proteins with different sequences can have similar structures
- Anna Tramontano was a researcher involved in evaluating the CASP challenge that died 2 years ago
- I can fit (thread) a sequence of unknown structure onto a known structure and evaluate the goodness of fit with a scoring function
- It is a generalization of comparative modelling
 - I try to model against a database of known basic folds
 - I align to a whole set of structures instead of just 1
- The goodness of fit for each aminoacid is evaluated from observed preferences in known structures
- It is possible because
 - There is a limited number of folds in nature
 - Aminoacid preferences for different structural environments allow to choose the best fit
- Fold recognition: even if I cannot align the sequences I can still align secondary structure elements and other features
- For threading I need to first align the sequences through fold recognition
- For doing fold recognition I need to assess the features of the sequence
- Predicting sequence properties is a mapping problem
 - Secondary structure prediction: from a sequence of aminoacids to a sequence of secondary structures
 - * I am mapping a 20-letter alphabet to a 3-letter alphabet
 - Transmembrane regions: I want to map the sequence to the states in the membrane/out of the membrane
- The simplest approach is to use a propensity scale for each residue
- The AAindex is a database of propensity scales for residues
 - AAindex1 contains properties at the residue level (hydrophobicity, ecc...)
 - AAindex2 contains substitution matrices
 - AAindex3 contains propensities for interactions among aminoacids
 - It shows for each entry also the correlation with other scales
- The propensity scale for secondary structure is the Chou-Fasman:
 - First they calculated how frequently a certain residue was found in each secondary structure ($P(A, h)$ with A being the residue and h the secondary structure)
 - Then they corrected it for the independent probabilities for the residue and for the structure ($P(A), P(h)$)
 - The propensity is therefore $P(A, h)/(P(A)P(h))$
 - * If there is no correlation among $P(A)$ and $P(h)$, then $P(A, h) = P(A)P(h)$ and therefore the propensity is 1
 - * If the propensity is bigger than 1 the residue tends to be in that structure more than expected
 - * If it is smaller than 1 the residue tends to avoid that structure
- The original Chou-Fasman was built with only 19 proteins, but the propensity scales did not change much also with the current dataset
 - An updated version is available at AAindex
- The Chou-Fasman is not accurate, it is around 50/60%
 - It is 3-class classification, so it is not so bad but also not so useful in practice
 - It is possible to improve the result by evaluating the average propensity of a sliding window

- * The original algorithm used a 4 residue window
 - We can get better results with neural networks
- The Kyte-Doolittle scale for hydrophobicity: partition coefficient octanol/water combined with the propensity for a residue to being found in a transmembrane helix
 - It is positive for hydrophobic residues and negative for hydrophilic ones
- The GOR method was developed after Chou Fasman for the prediction of secondary structure
 - It uses 8 residues sliding windows
 - It is more accurate than Chou Fasman but it is still not comparable with modern methods

Protein structure analysis

- Anfinsen's hypothesis: there is a native structure
- Levinthal's paradox: the conformational space for a protein is huge and it cannot be explored by the protein in the age of the universe
- Anfinsen's dogma
 - Unicity of structure: same structure in the same condition
 - * I mean fluctuations, but in general it is like that
 - Stability: the structure is conserved with small fluctuations of the environment
 - * The native structure is at the bottom of a funnel
 - Kinetic accessibility: small kinetic barrier for reaching the folded state
 - * It should be possible to reach the folded state in reasonable time
- The solution to protein folding: thermodynamics, kinetics and conformation
- Thermodynamics: ΔG of folding is -5/-15 kcal/mol
 - It is less than the energy of a covalent bond!
 - More or less the energy of a salt bridge
- The alpha-helix is right handed, with $\phi = -60^\circ$ and $\psi = -50^\circ$
 - Side chains are projected backwards and outwards
 - The pitch of the helix is 5.4Å
 - There are 3.65 residues per turn
 - The core is tightly packed
 - The N-H of one aminoacid forms an H bond with the C=O of 4 residues earlier
 - The H bonding pattern gives to the helix a dipole moment
- The beta-strand has $\phi = 140^\circ$ and $\psi = -130^\circ$
 - The side chains project alternating above and below the strand
 - The conformation is more extended than in helices
 - Beta-strands can form parallel or anti-parallel beta-sheets
 - Parallel beta-sheets are less stable than the anti-parallel ones
- DSSP: obtain ϕ and ψ angles from a structure and solvent accessibility
 - It is both available as a web server and as a package
 - For each residue in a PDB structure it calculates the dihedral angles and the solvent accessibility
 - * From these pieces of information I can determine the secondary structure using the Ramachandran plot