# Evolution and Structure of Biomolecules

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# Introduction

## Which molecules are we interested in?

- *Evolving molecules*: DNA, RNA, Proteins
- Several structures are resolved: we have the set of coordinates for almost all atom positions
- There are several levels of structure organization

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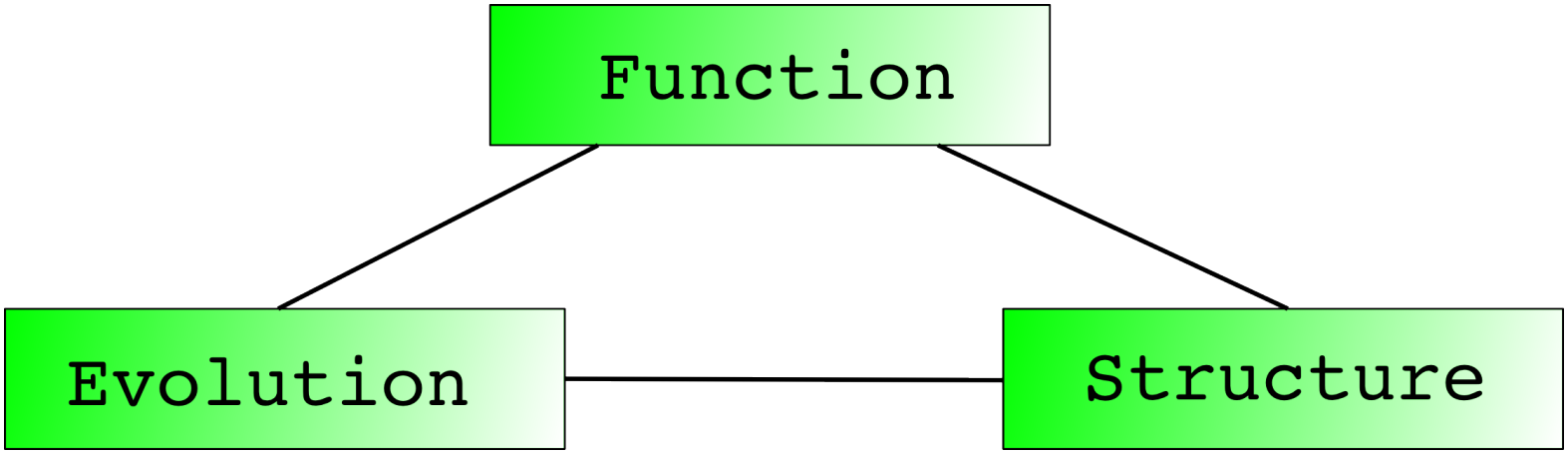
| | DNA | RNA | Proteins |
|---|---|---|---|
| I | desoxyribonucleotides sequence | ribonucleotides sequence | amino-acids sequence |
| II | double-helix | loop and stem (double stranded regions) | loop, helices, turns, strands and sheets |
| III | — | yes, for rRNA and tRNA | domain organization |
| IV | — | rRNA | a lot of protein complexes |

# Why should we study their structure?




- The function of a molecule is tightly linked to its structure
- Improve models of sequence evolution, for better inference of evolutionary processes (including phylogeny)
- Use evolutionary information to study/predict the structure of molecules

# Outline of the lecture

1. On the non-homogeneity of the substitution process
   - Rate across site variation
   - Rate across site co-variation
   - Accounting for secondary structure
2. On the non-independence of substitution events
   - The success story of RNA structure prediction
   - Accounting for phylogeny: substitution mapping
   - Detecting coevolution in proteins
   - Non-independence and models of evolution

# All positions are not equal

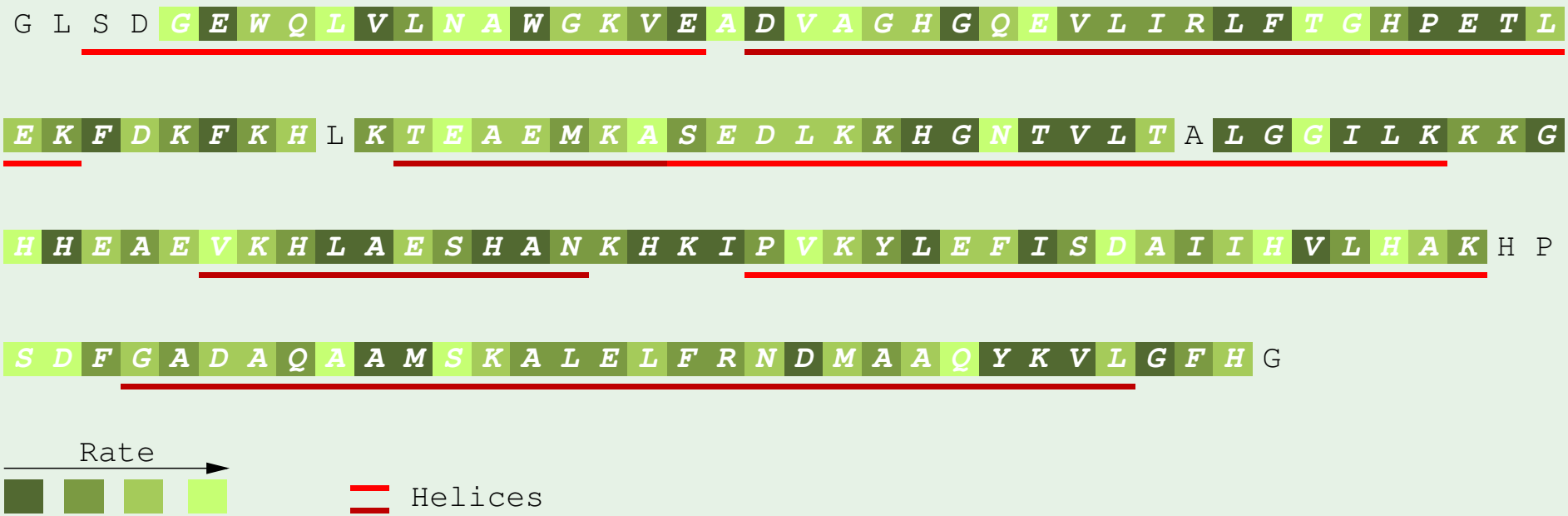
- The structure of a molecule determines a set of constraints acting on individual sites
- The neutral theory states that the level of constraint determines the rate of substitution
- $\Rightarrow$ All positions do not evolve at the same rate

## Estimating site-specific rates

- Site variability (entropy, information): do not account for phylogeny! May be very inaccurate...
- Parsimony score
- Empirical Bayesian estimation (work of Ziheng Yang)

# All positions are not equal

## Example (A vertebrate myoglobin data set)



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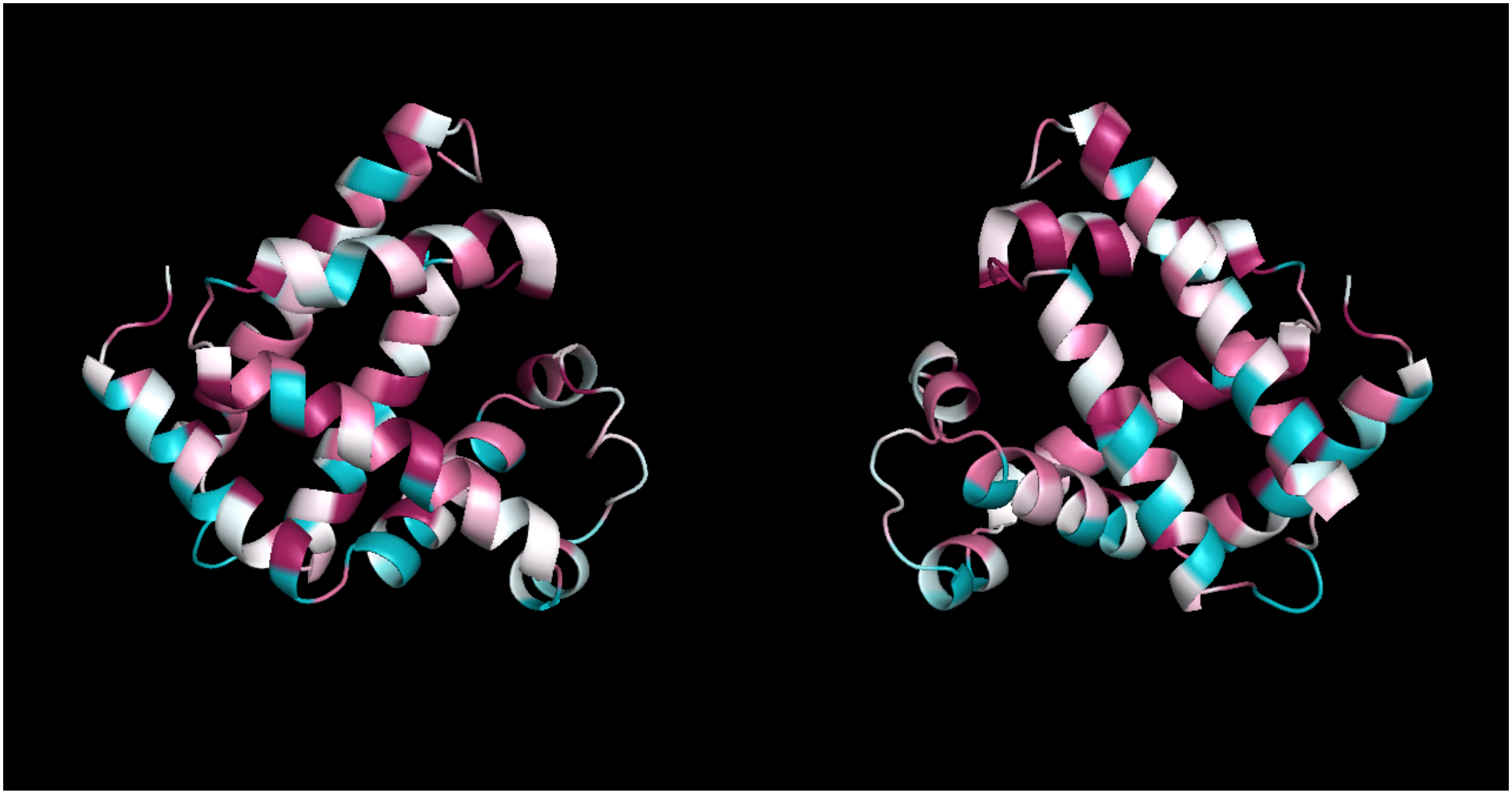

Purple (lent) $\longrightarrow$ Cyan (fast)

Made with ConSurf `http://consurf.tau.ac.il/` [Glaser et al., 2003]

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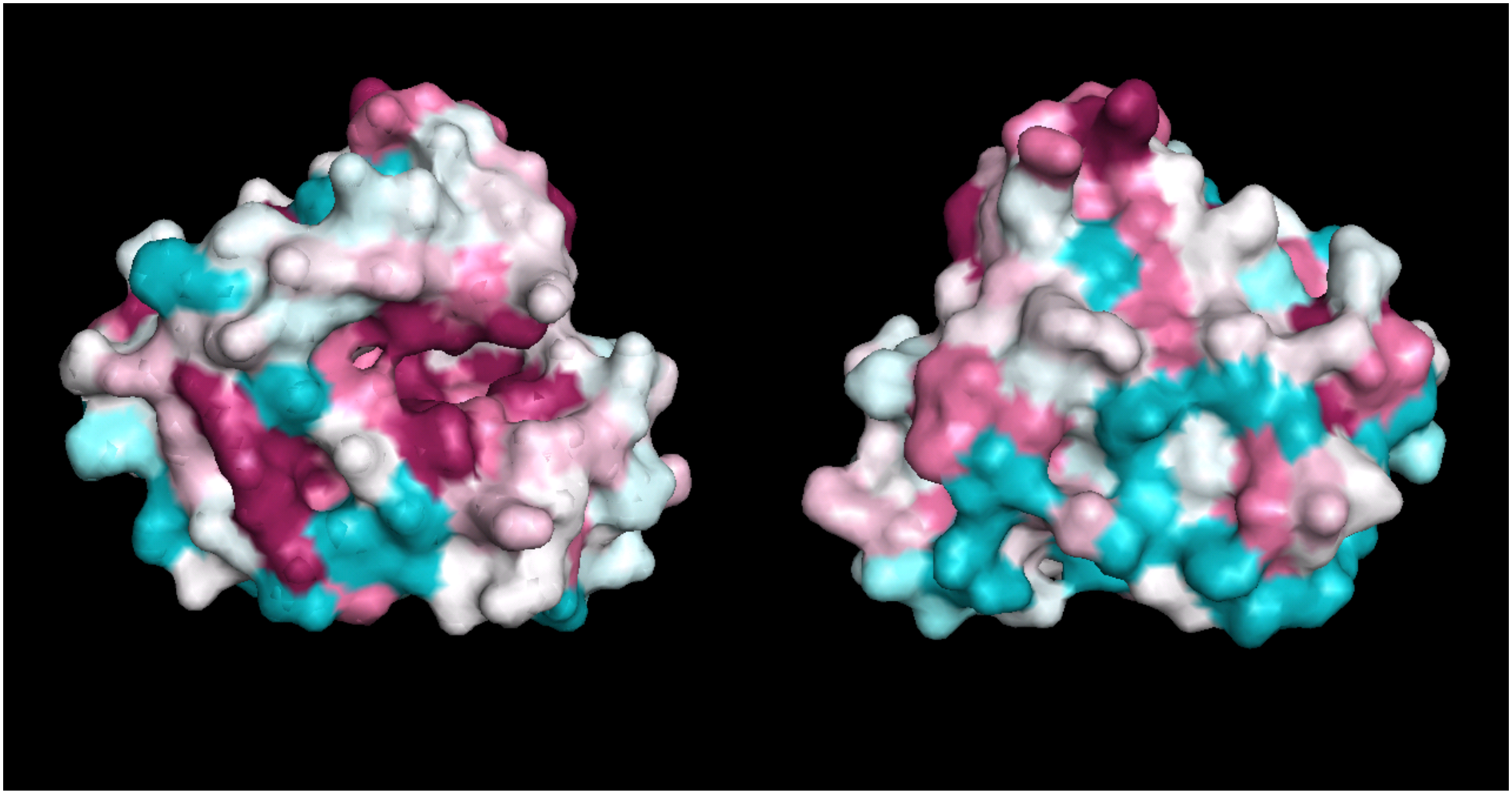

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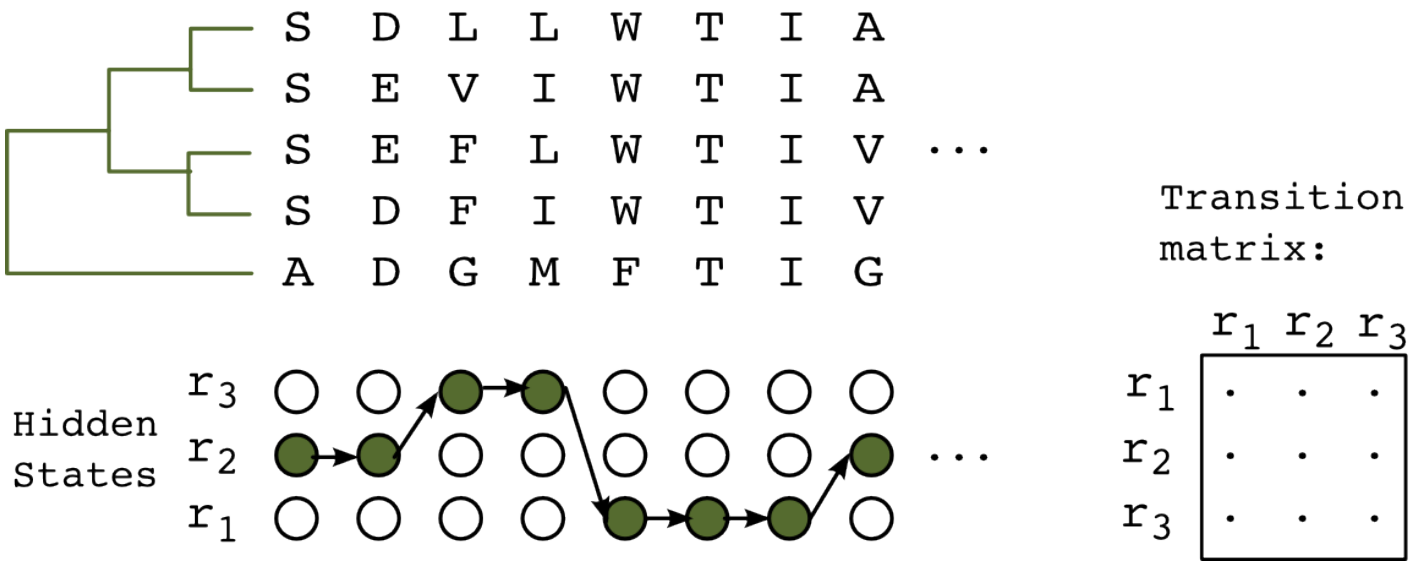
# Do constraints act on individual sites?

- Several motifs cover whole regions of the sequence (helices, strands)
- We then expect the rates of substitutions to be somehow correlated along the sequence
- Model which allow consecutive sites to be correlated: Hidden Markov Models

# Hidden Markov Models: Felsenstein and Churchill [1996]




- The rate of a specific site is unknown, be we can compute the likelihood of a given site according to a given rate
- Each rate is a (hidden) state, and we have a (Markov) model of transitions between states along the alignment
- We can compute the likelihood of the whole data set according to the transition model, estimate transition parameters, estimate the most likely hidden state for each site, etc

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## Hidden Markov Models: Felsenstein and Churchill [1996]'s model

- Several rate classes like in Yang's model
- Model of substitution between rates
- Developed for DNA sequences, application to hemoglobin: helices evolve more rapidly than coils, but large variations

# Distinct structural constraints, distinct substitution processes

## Goldman, Thorne and Jones's model [Thorne et al., 1996, Goldman et al., 1996, 1998]

- Four types of secondary structure: Helix, Sheet, Turn and Coil
- Position dependent states $(1,2,3,\ldots,n-2,n-1,n)$
- Two solvent accessibility classes: Exposed or Buried $\Rightarrow 38 \neq$ states

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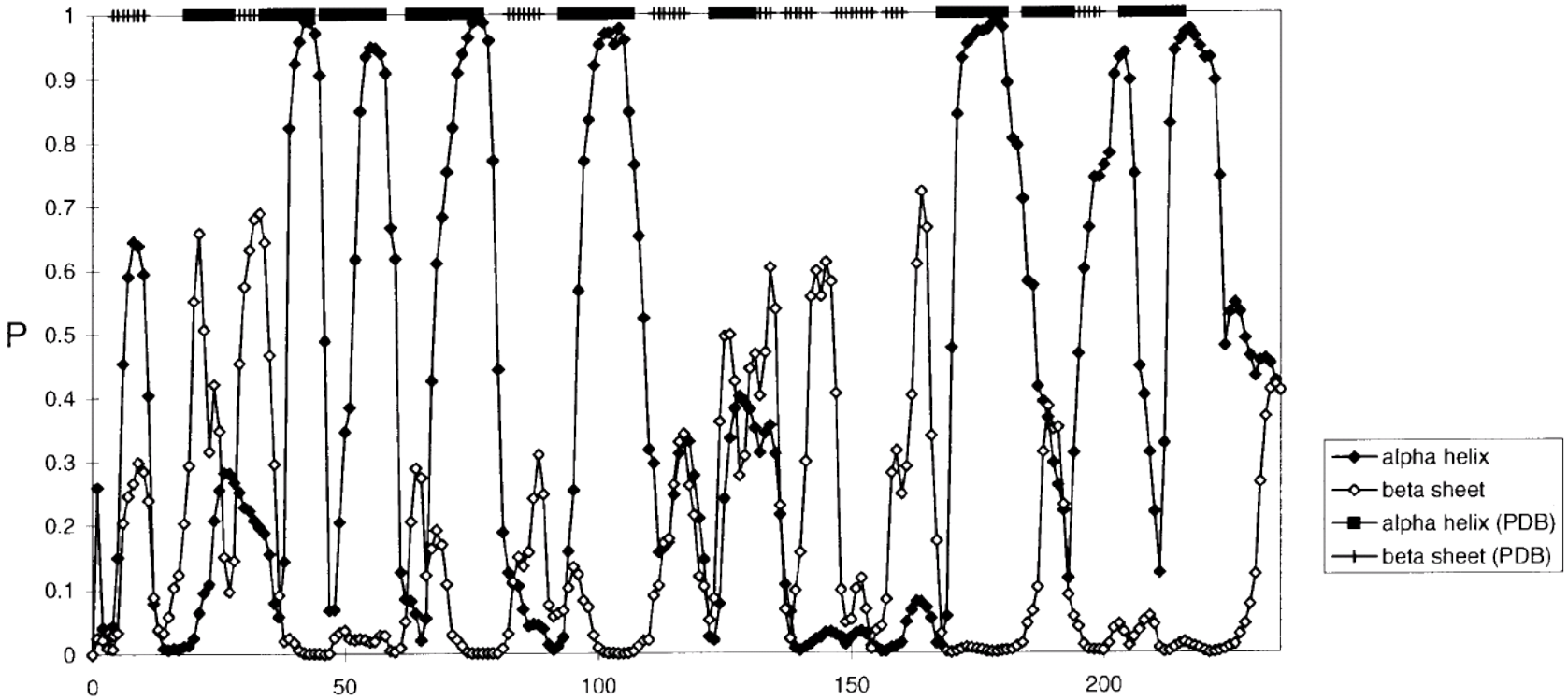
- A secondary structure specific replacement matrix is estimated from a set of protein data set with known structure
- This model provides a significantly better fit than independent, homogeneous models
- These matrices can be used with a protein with unknown structure, and allow to predict the most likely motif for each residue

# Example of posterior decoding:

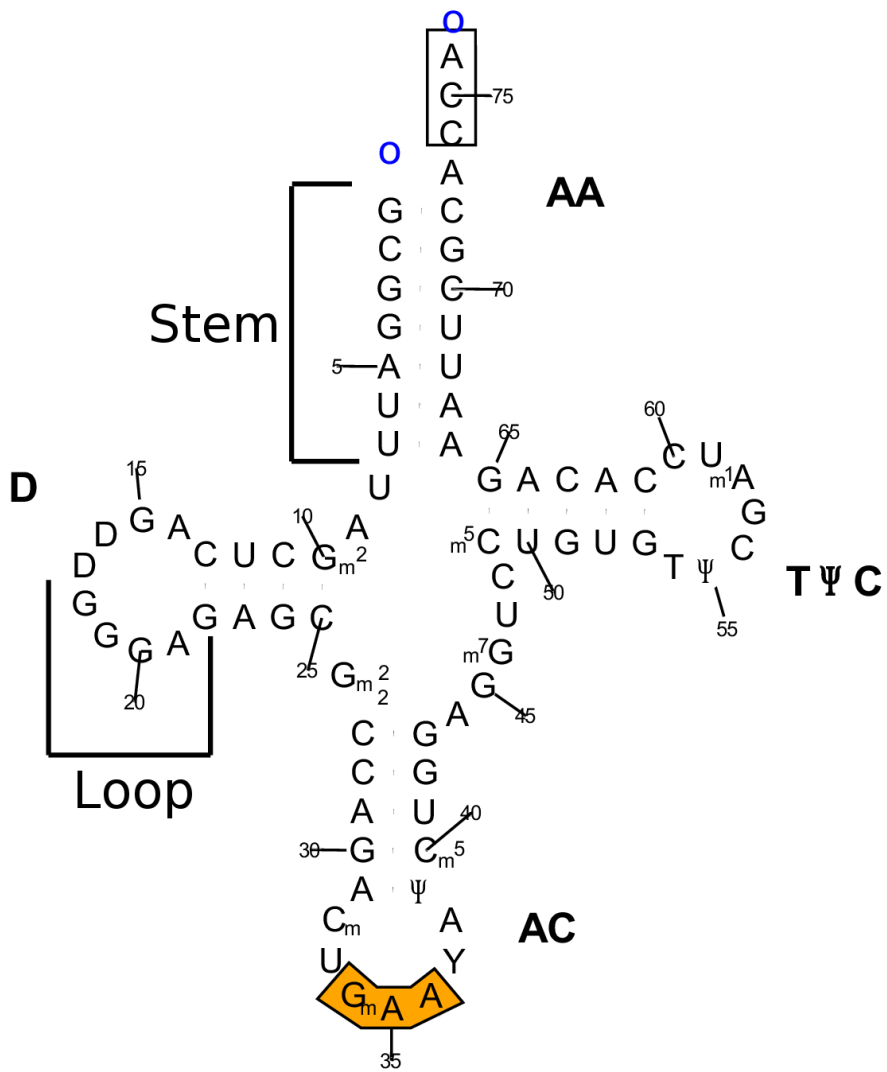


# RNA structure

- Two motifs in RNA: stems and loops
- Constraints on Watson-Crick pairs within stems
- $\Rightarrow$ Find the sequence folding that maximizes the number of W-C pairs ( 80s)

Improvements:

- Use a sequence alignment ( 90s)
- Take into account the underlying phylogeny ( 90s +)
- Use complex models that account for spatial dependency (2000)

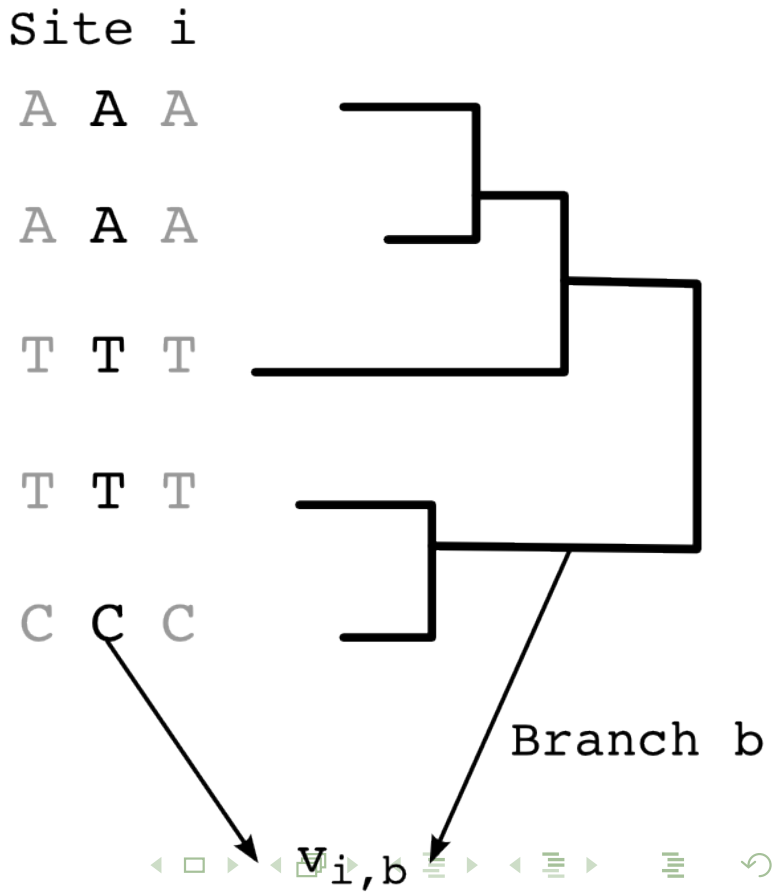


# Using phylogenetics to detect coevolving pairs

## Mapping the substitution events

**Goal:**

Locate the substitution events on a phylogenetic tree for each position of a sequence alignment



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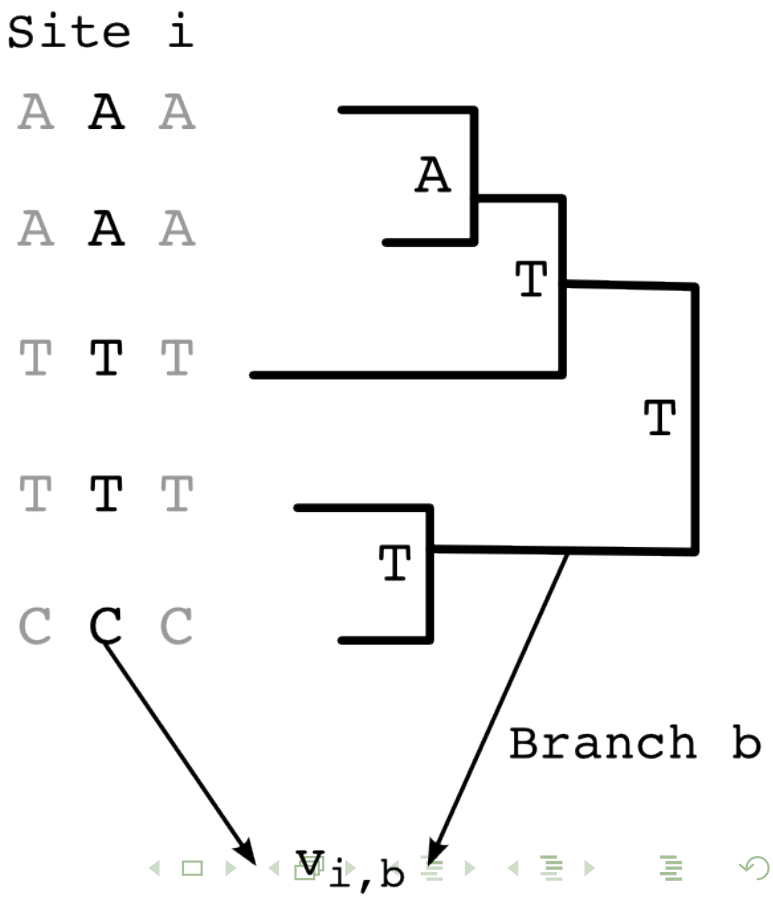

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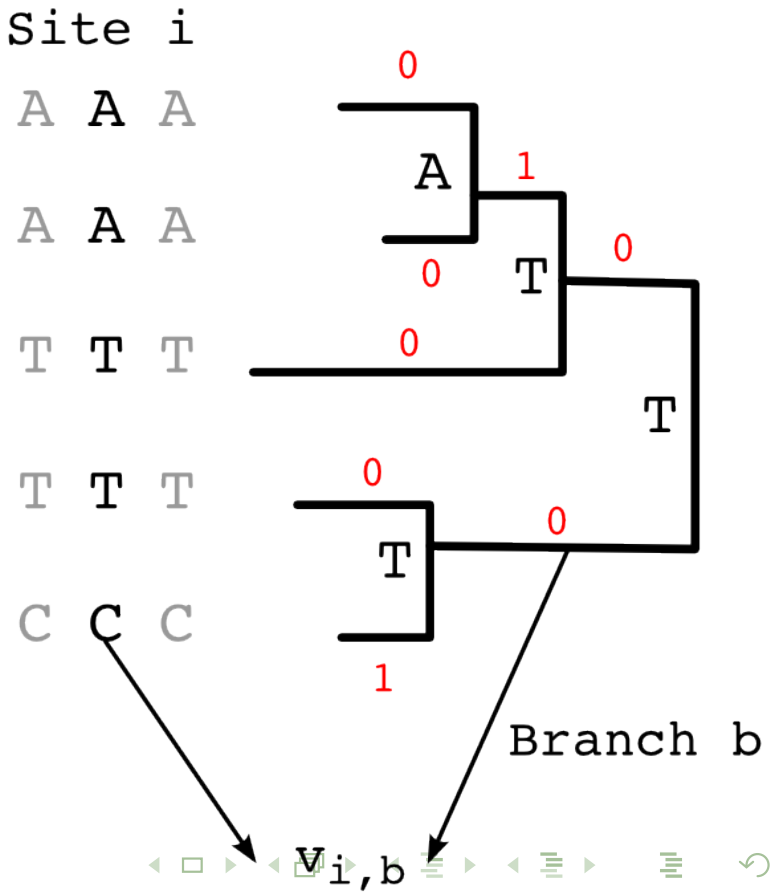

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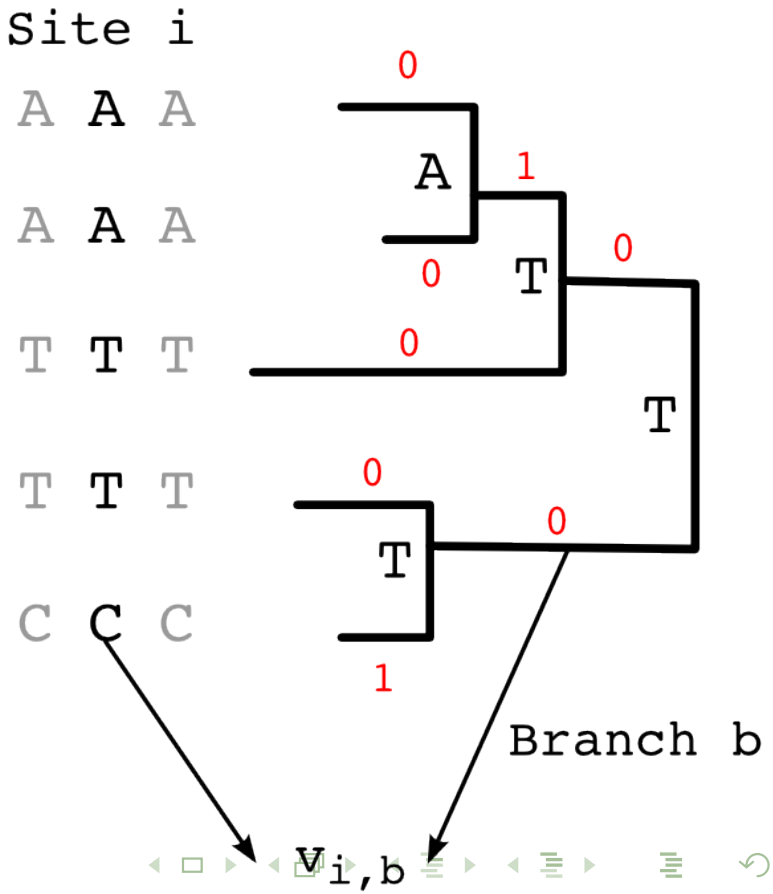
- Improvements: take into account uncertainty on ancestral states and branch lengths



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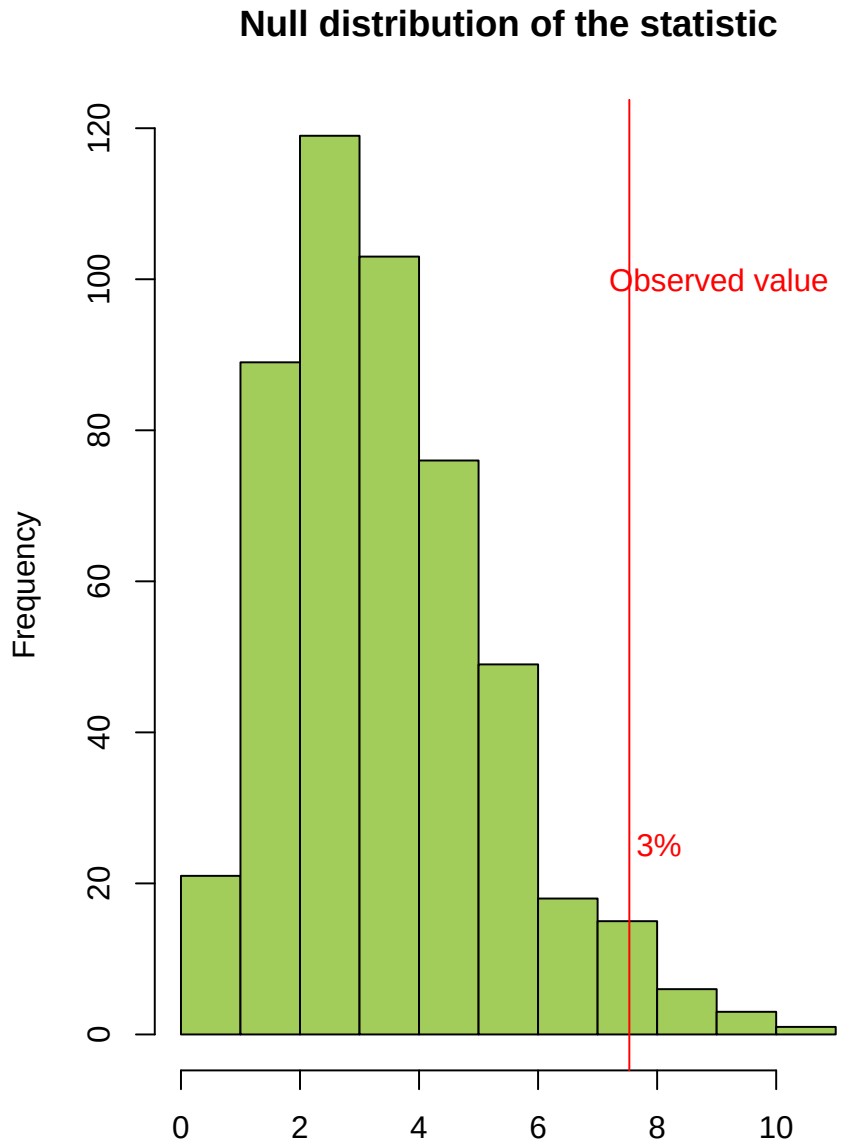
3. Compare the value of the coevolution for real groups and the ones obtained from simulations



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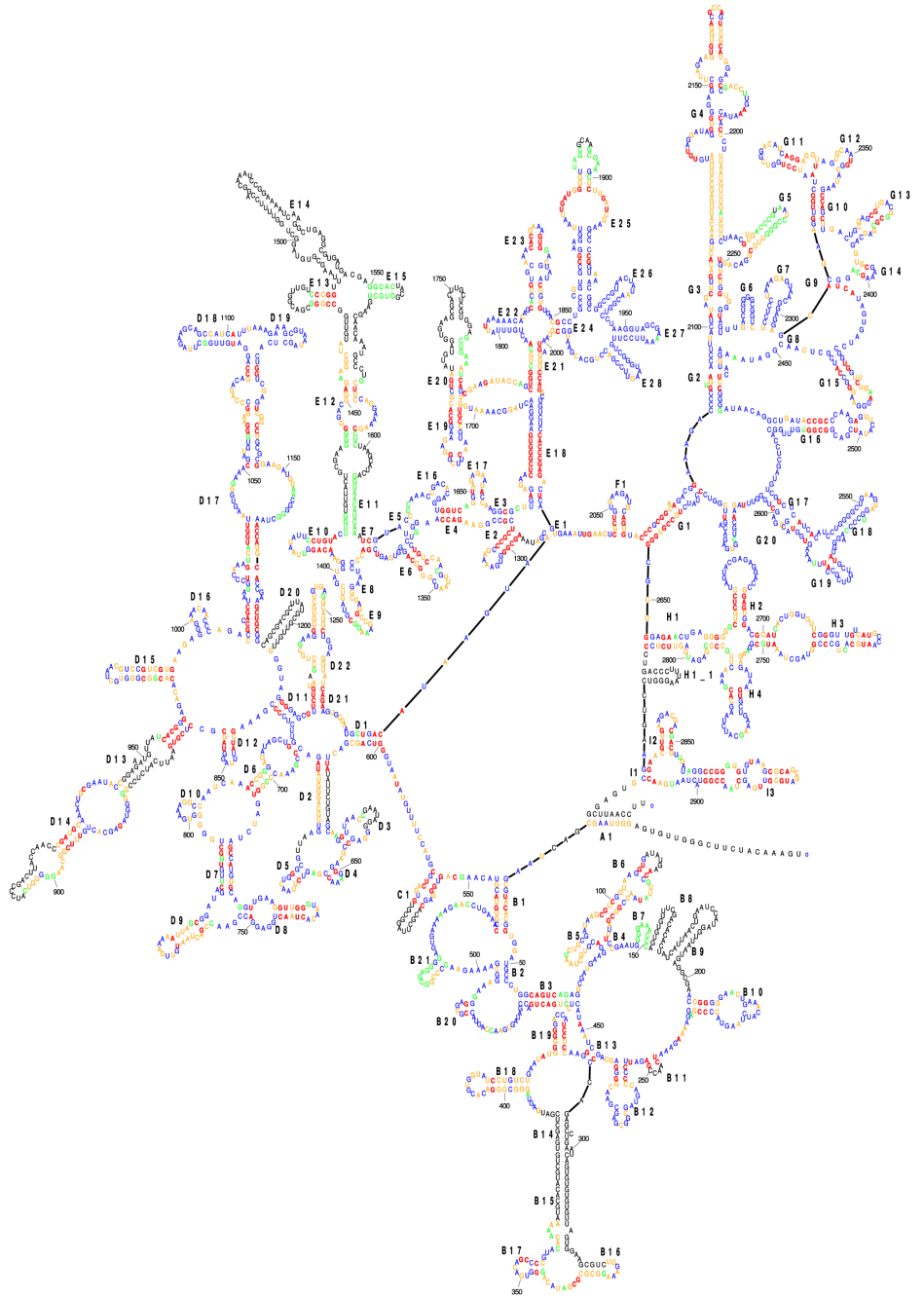

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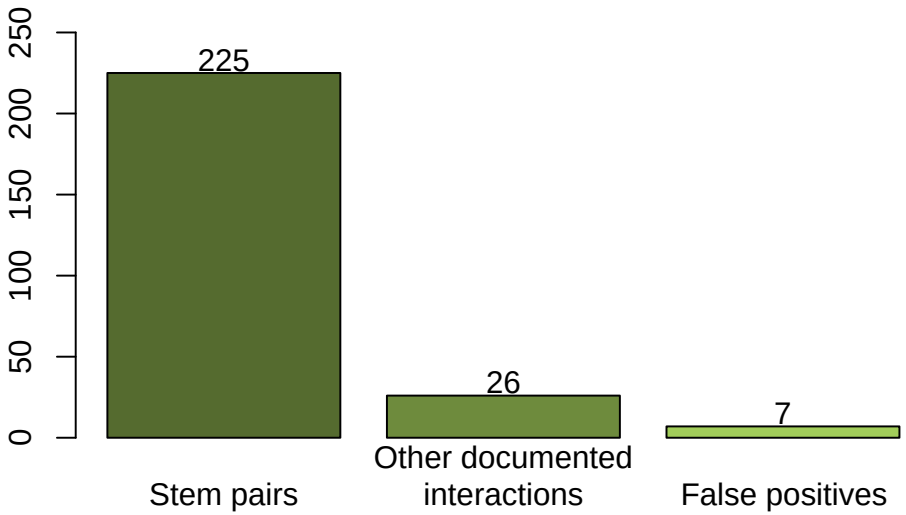


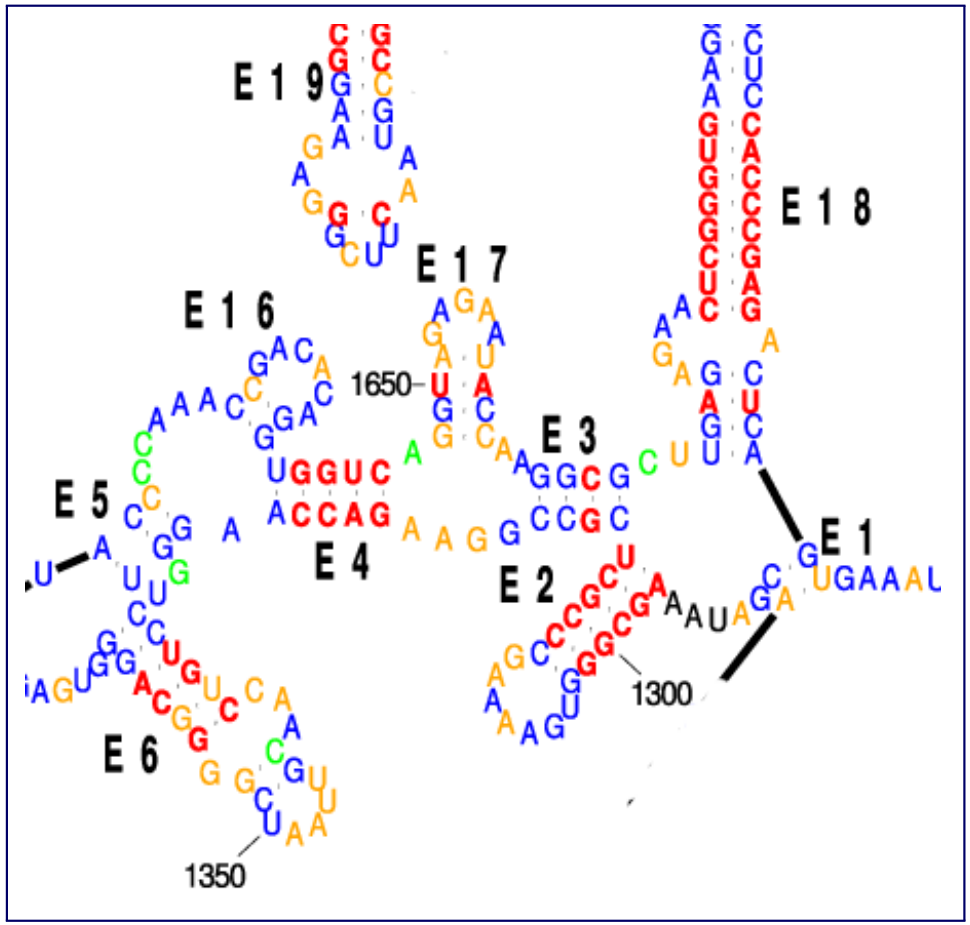


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- 225 belong to secondary structure, 26 to tertiary structure

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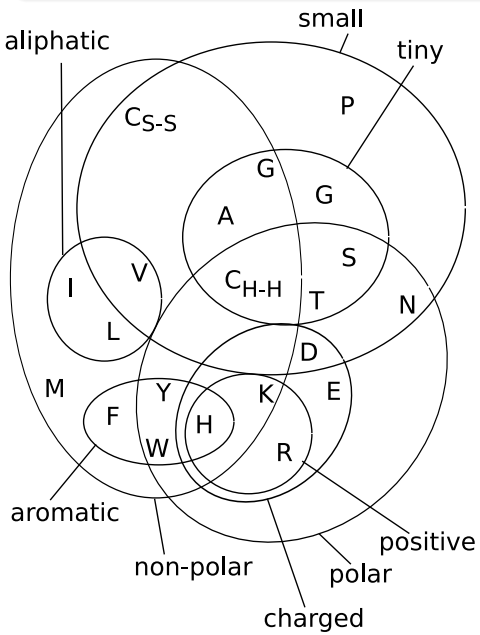

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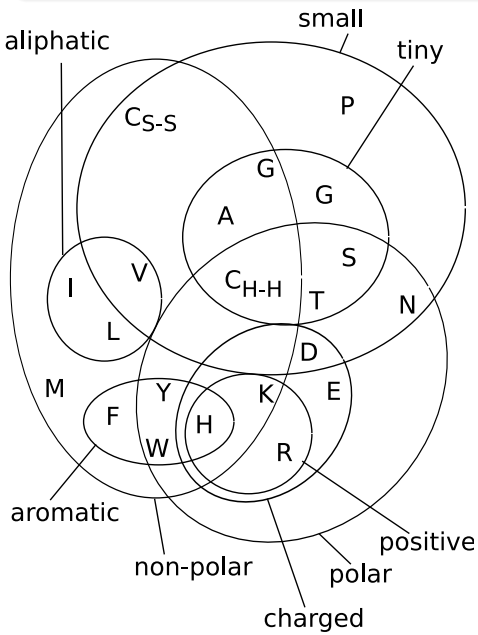


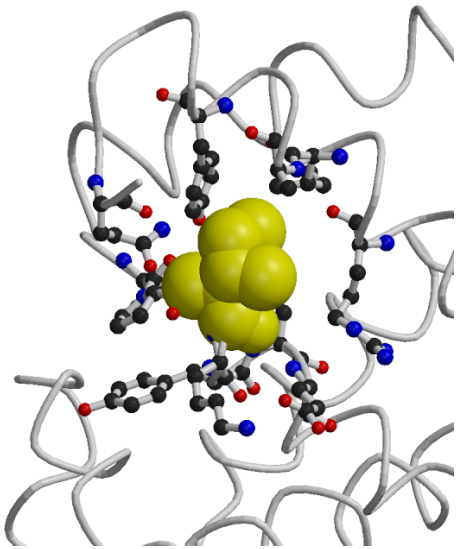

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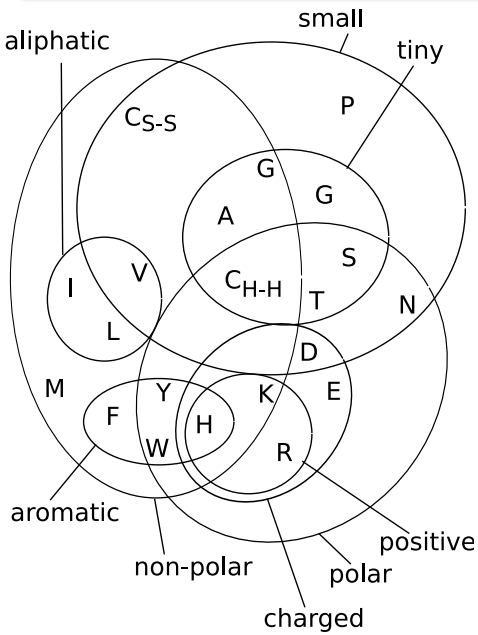


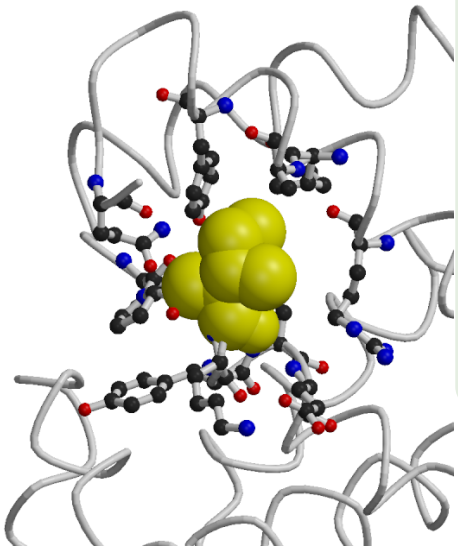

## Main results:

- Signal is scarcer, needs more data to detect
- Positions in close proximity tend to coevolve more than distant positions
- Relation with secondary structure is unclear
- Coevolution within domains is higher than between domains

# Non-independence and models of evolution

## Phylogenetic inference:

- ML is robust to departures from independence, NJ more sensitive:

**Neighbor Joining and Maximum Likelihood with RNA Sequences: Addressing the Interdependence of Sites**

*Elisabeth R. M. Tillier* and Richard A. Collins*

*Department of Botany and Department of Molecular and Medical Genetics, University of Toronto and Canadian Institute for Advanced Research Program in Evolutionary Biology

- Inter-dependence artificially increases the bootstrap support:

*Syst. Biol.* 53(1):38–46, 2004

ISSN: 1063-5157 print / 1076-836X online
DOI: 10.1080/10635150490264680

**Sampling Properties of the Bootstrap Support in Molecular Phylogeny: Influence of Nonindependence Among Sites**

NICOLAS GALTIER

*CNRS UMR 5000, Génome, Populations, Interactions, Université Montpellier 2, CC 63, Place E. Bataillon, 34095 Montpellier, France; E-mail: galtier@univ-montp2.fr*

# A simple model of non-independent evolution: RNA

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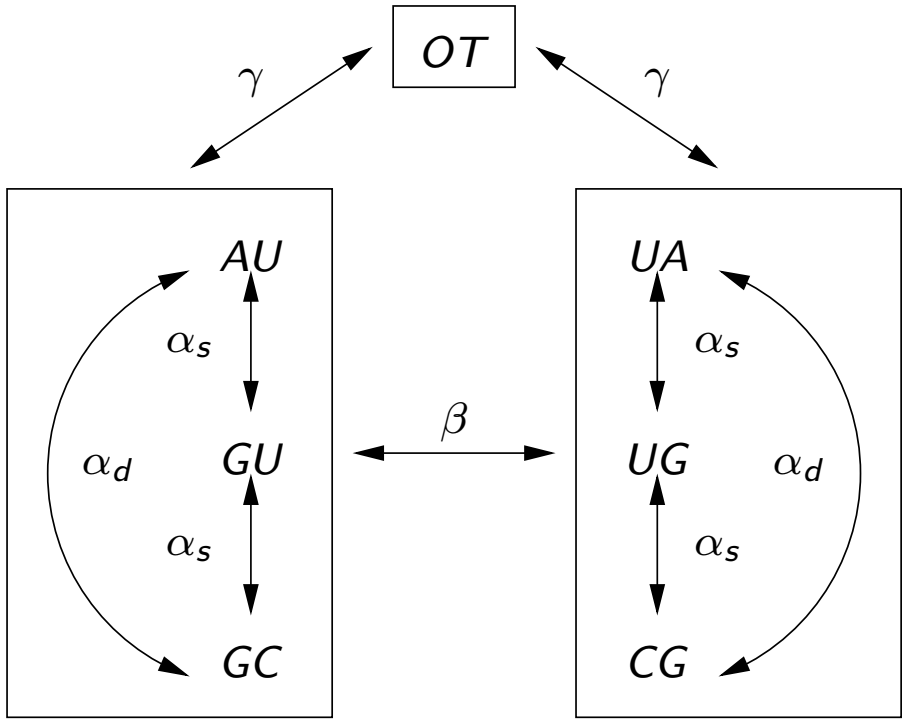

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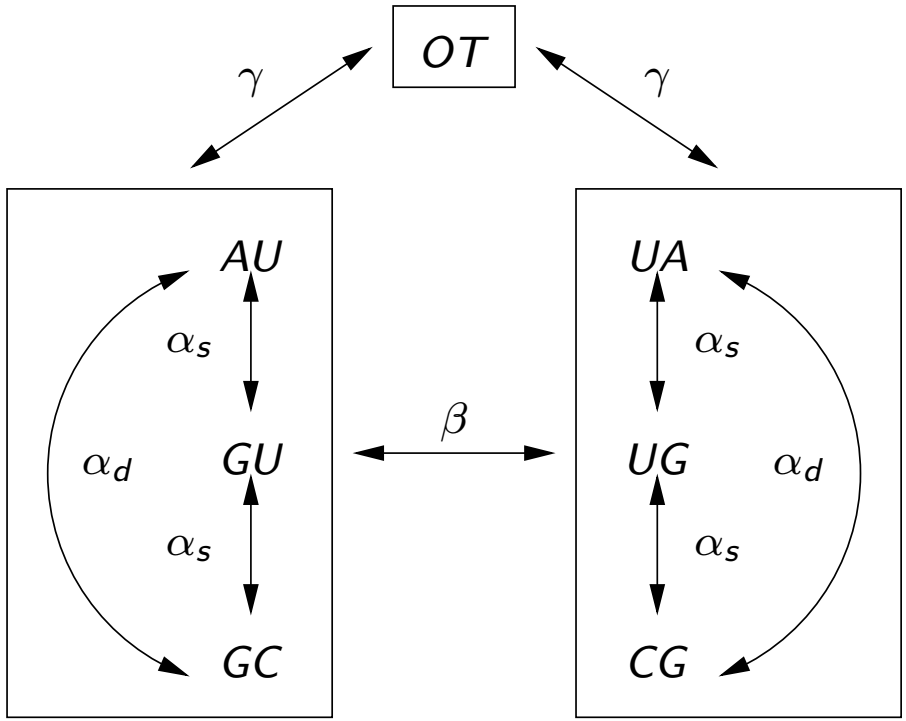
- Results: high substitution rate, compensating mutations appear almost simultaneously



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- Computationally demanding: used only to detect significant coevolving pairs by model comparison.

# A more complex model of non-independent evolution

Rodrigue et al. [2005, 2006]

- Sites are treated simultaneously: sequence of size $N \Rightarrow 20^N$ states!

$$R_{xy} = \begin{cases} 0 & \text{if sequence } x \text{ and } y \text{ differ} \\ & \text{by more than one position} \\ Q_{kl} & \text{if sequence } x \text{ and } y \text{ have states} \\ & l \text{ and } m \text{ at positions } i \text{ and } j \\ -\sum_{y \neq x} R_{xy} & \text{i} = \text{j} \end{cases}$$

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- Add a fitness function that takes into account the whole sequence. This function is computed using measures on real data: statistical potentials. $p$ is a free parameter, estimated from the data.
- Bayesian sampling procedure to estimate parameters.
- Usage limited to small data sets due to the computational load, but provides a better description of the data.

# Conclusions

- Large effort during the last 10 years
- Methodological improvements are limited by the large complexity of structural constraints
- The large amount of sequence and structure data opens a way to characterize these constraints at a large scale, and maybe help the design a new tractable models
- These studies are also a good example of tight interactions between evolutionary biology, molecular biology and bioinformatics.

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