

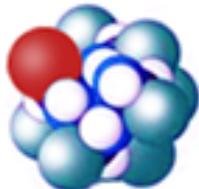
Introduction and Basic Concepts

**Laboratory of Bioinformatics I
Module 2**

March 13, 2018

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<http://biofold.org/>



**Biomolecules
Folding and
Disease**

Department of Pharmacy and
Biotechnology (FaBiT)
University of Bologna



Schedule and Materials

This module is a 60-hour course running for 8 weeks
March 13 - May 3, 2017

Regular schedule:

Tuesday and Thursday 14:00 - 17:00

Friday 10:00 - 13:00

Project submission deadline May 7, 2018

Course website

<http://biofold.org/courses/2018/lb1-2.html>

Main Aims

- Knowledge of tools for sequence and structure analysis and their development
- Protein functional annotation
- Theoretical background of machine learning approaches
- Problem solving skills and development of basic tools.

Topics

- Protein Geometrical Features and Protein Structural Alignment
- Multiple Sequence Alignment
- Hidden Markov Models for Sequence Alignment
- Methods for Building Hidden Markov Models for Proteins
- Protein Structure and Mapping Problems
- Introduction to Statistical Methods and Machine Learning
- Development of Structure Prediction Methods
- Module Project: Model a Protein Domain HMM

Take Home Message

- Protein structure is more conserved than sequence. Proteins sharing high sequence identity usually share similar structures, as proven by pair-wise structural alignment procedures.
- When the identity level is high enough, it is possible to exploit the results of pair-wise sequence alignment for transferring structural information between proteins.

Structural Alignment

Given two sets of points $A = (a_1, a_2, \dots, a_n)$ and $B = (b_1, b_2, \dots, b_m)$ in Cartesian space, find the optimal subsets $A(P)$ and $B(Q)$ with $|A(P)| = |B(Q)|$, and find the optimal rigid body transformation G between the two subsets $A(P)$ and $B(Q)$ that minimizes a given distance metric D over all possible rigid body transformation G , i.e.

$$\min_G \{D[A(P) - G(B(Q))]\}$$

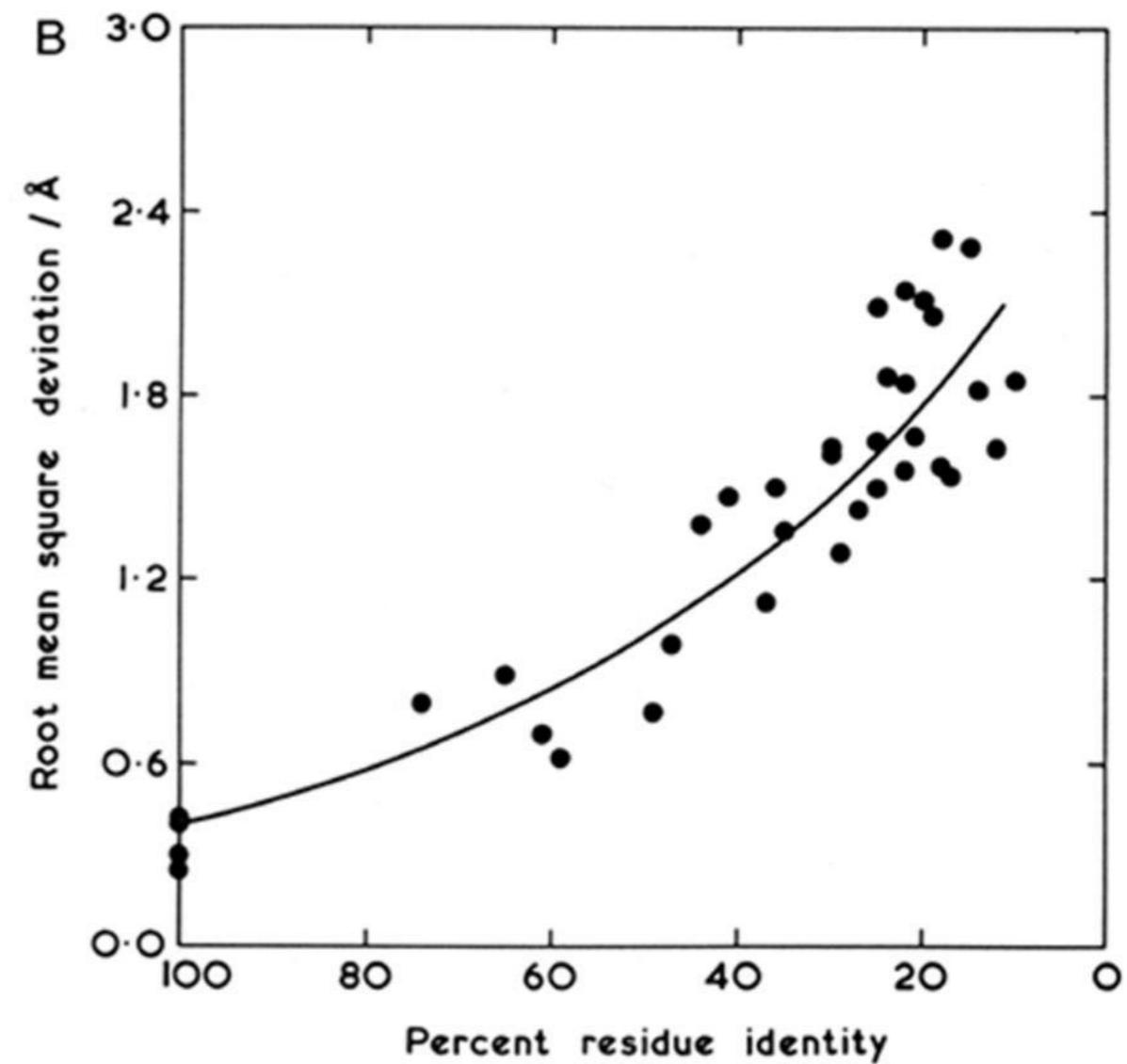
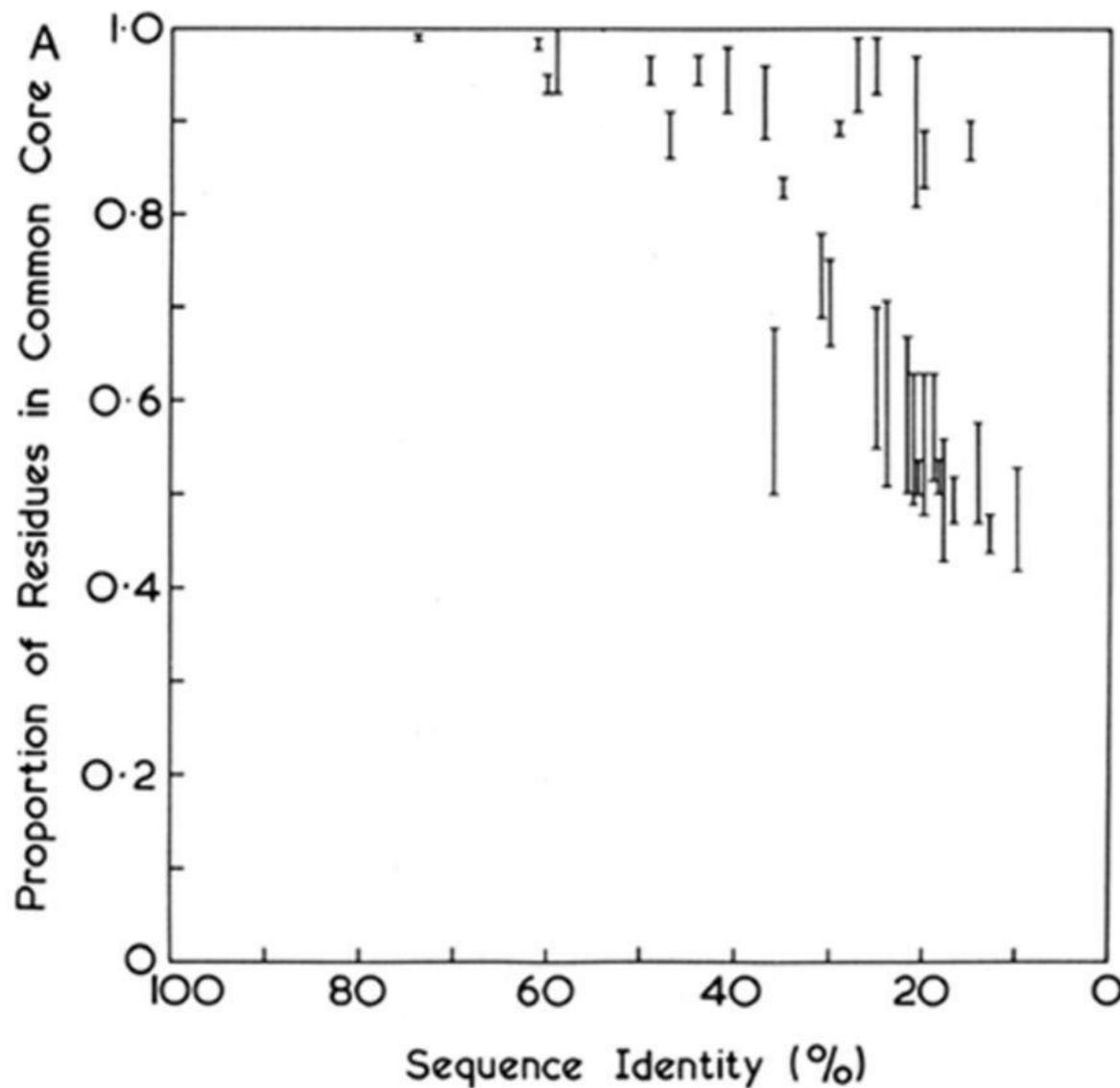
$$\text{RMSD} = \sqrt{\frac{\sum_{i=1}^n (a_i - b_i)^2}{n}}$$

The two subsets $A(P)$ and $B(Q)$ define a “correspondence”, and $p = |A(P)| = |B(Q)|$ is called the correspondence length. Naturally, the correspondence length is maximal when $A(P)$ and $B(Q)$ are similar.

Therefore there are essentially two problems in structure alignment:

- Find the correspondence set (which is NP-hard), and
- Find the alignment transform (which is $O(n)$).

The Foundation of Structural Bioinformatics



Why Sequence Alignment?

The measure of sequence similarity allow to make estimation about the structural similarity

Comparison of two sequences for measuring their similarity

- To define a distance between two sequences
- Develop an algorithm for finding the alignment with minimal distance
- To statistically evaluate the significance of the alignment

Sequence Distance Score

Which events do we consider?

Mutation

It is necessary to define a score for the substitution of residue i with residue j
Substitution Matrices $s(i,j)$

A: ALASVLIRLITRLYP
B: ASAVALNRLITRLYP

$$Score(A, B) = \sum s(A^i, B^i)$$

	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W
C	9																			
S	-1	4																		
T	-1	1	5																	
P	-3	-1	-1	7																
A	0	1	0	-1	4															
G	-3	0	-2	-2	0	6														
N	-3	1	0	-2	-2	0	6													
D	-3	0	-1	-1	-2	-1	1	6												
E	-4	0	-1	-1	-1	-2	0	2	5											
Q	-3	0	-1	-1	-1	-2	0	0	2	5										
H	-3	-1	-2	-2	-2	-2	1	-1	0	0	8									
R	-3	-1	-1	-2	-1	-2	0	-2	0	1	0	5								
K	-3	0	-1	-1	-1	-2	0	-1	1	1	-1	2	5							
M	-1	-1	-1	-2	-1	-3	-2	-3	-2	0	-2	-1	-1	3						
I	-1	-2	-1	-3	-1	-4	-3	-3	-3	-3	-3	-3	-3	1	4					
L	-1	-2	-1	-3	-1	-4	-3	-4	-3	-2	-3	-2	-2	2	2	4				
V	-1	-2	0	-2	0	-3	-3	-3	-2	-2	-3	3	2	1	3	1	4			
F	-2	-2	-2	-4	-2	-3	-3	-3	-3	-1	-3	-3	0	0	0	-1	6			
Y	-2	-2	-2	-3	-2	-3	-2	-3	-2	-1	2	-2	-2	-1	-1	-1	3	7		
W	-2	-3	-2	-4	-3	-2	-4	-4	-3	-2	-2	-3	-3	-1	-3	-2	-3	1	2	11
	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W

Other events

Deletion and Insertion: some residues can be inserted or deleted during the evolution

A: ALASVLIRLIT--YP
B: ASAVHL---ITRLYP

$$Score(A, B) = \sum s(A^i, B^i) + \sigma(3) + \sigma(2)$$

The (negative) score of a gap depends only on the length

$$\sigma(n) = -nd \text{ linear}$$

$$\sigma(n) = -d - (n-1)e \quad (d: \text{opening}, e: \text{extension})$$

Alignment Algorithms

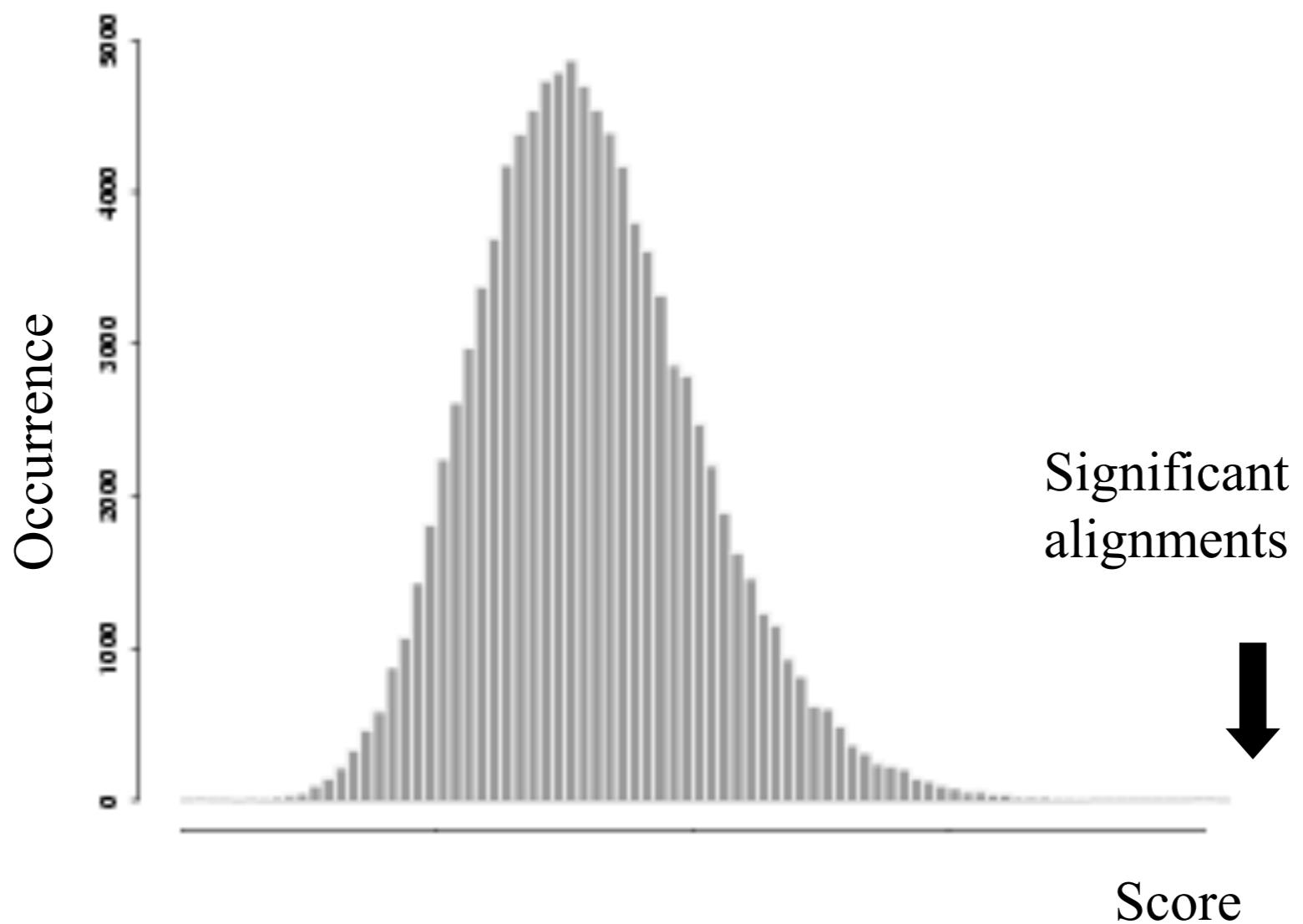
Algorithms for finding the **minimum distance** between two sequences

- **Global alignment:** Needleman-Wunsch: Global alignment-compare pairs of sequences on their whole length
- **Local alignment:** Smith-Waterman: Local alignment-compare pairs of sequences searching the most similar subsequences

Alignment Significance

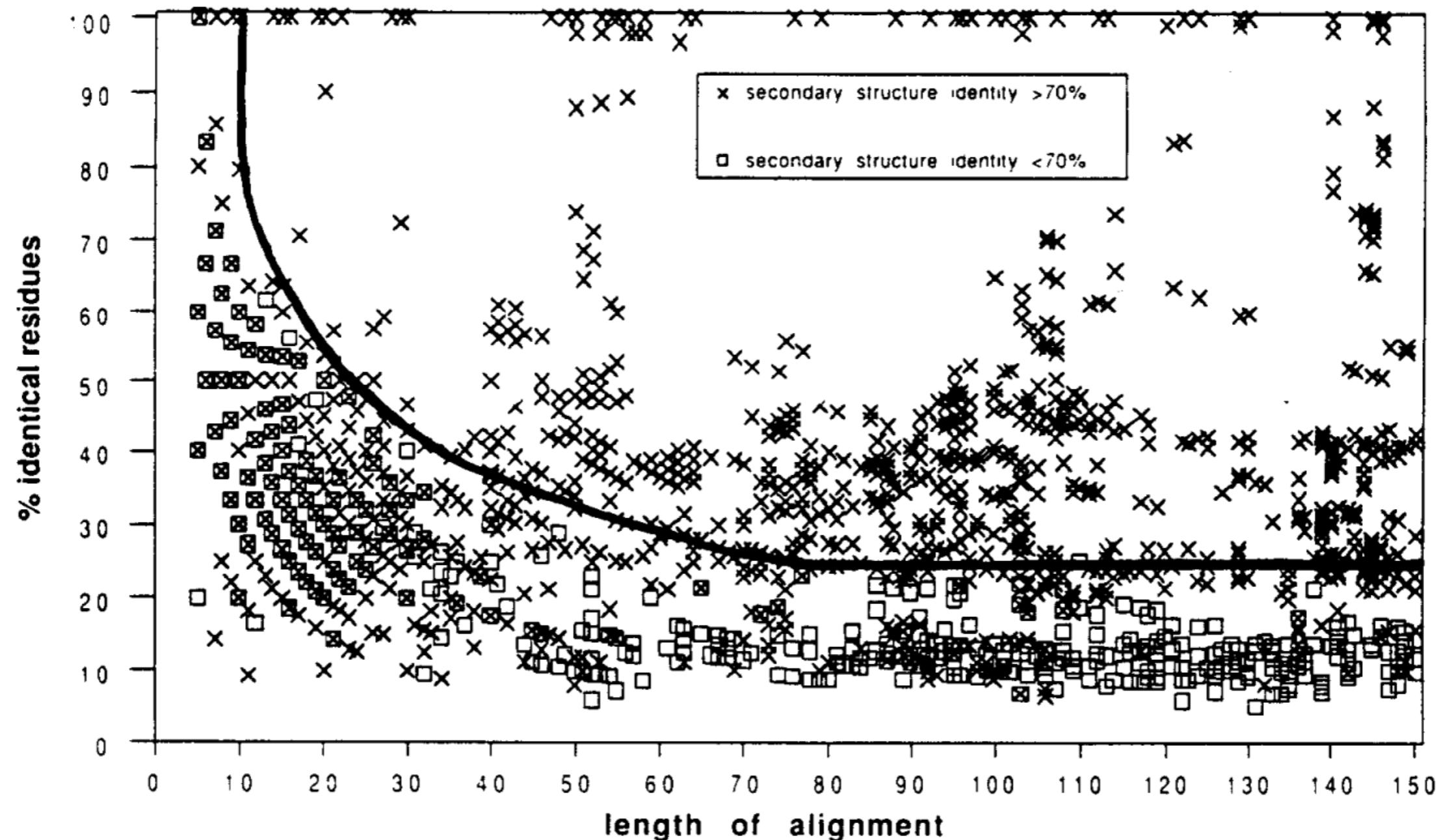
Given an alignment with score S , is it significant?

Significance can be evaluated by comparing with the score distribution of random alignments



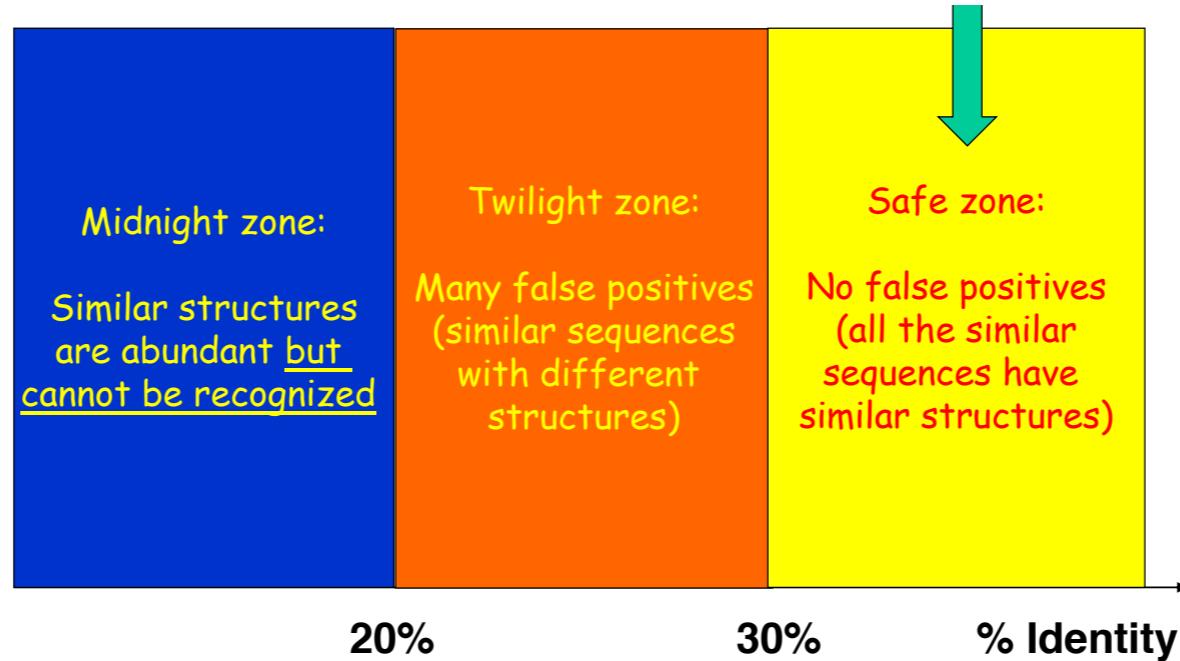
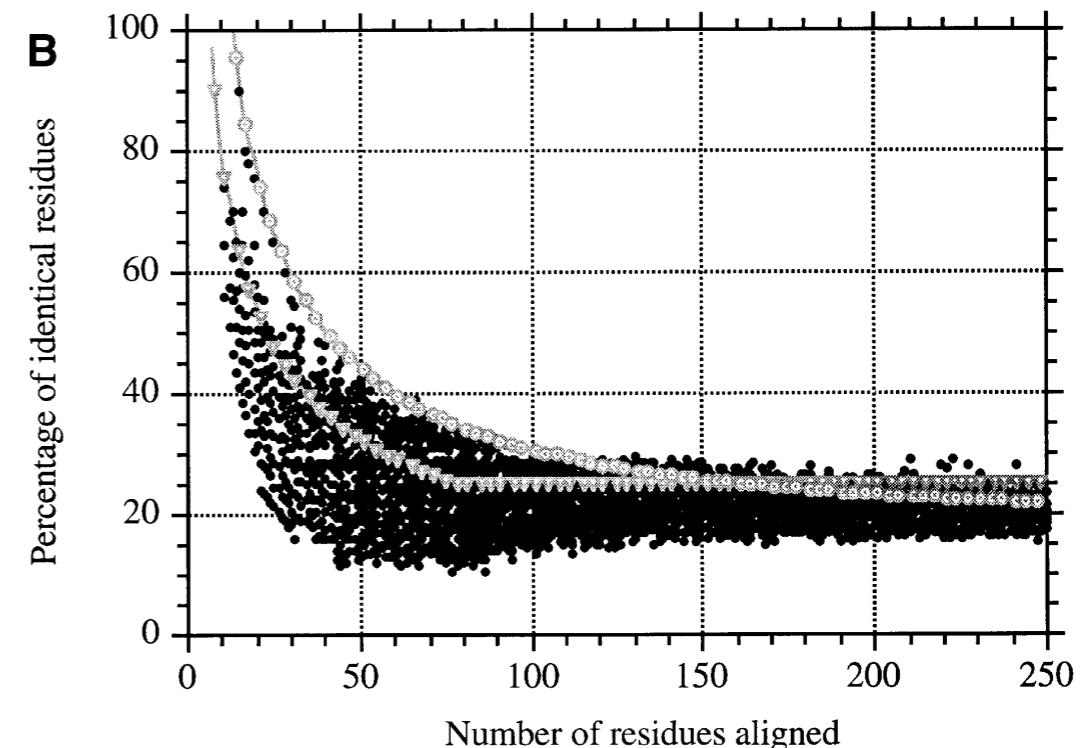
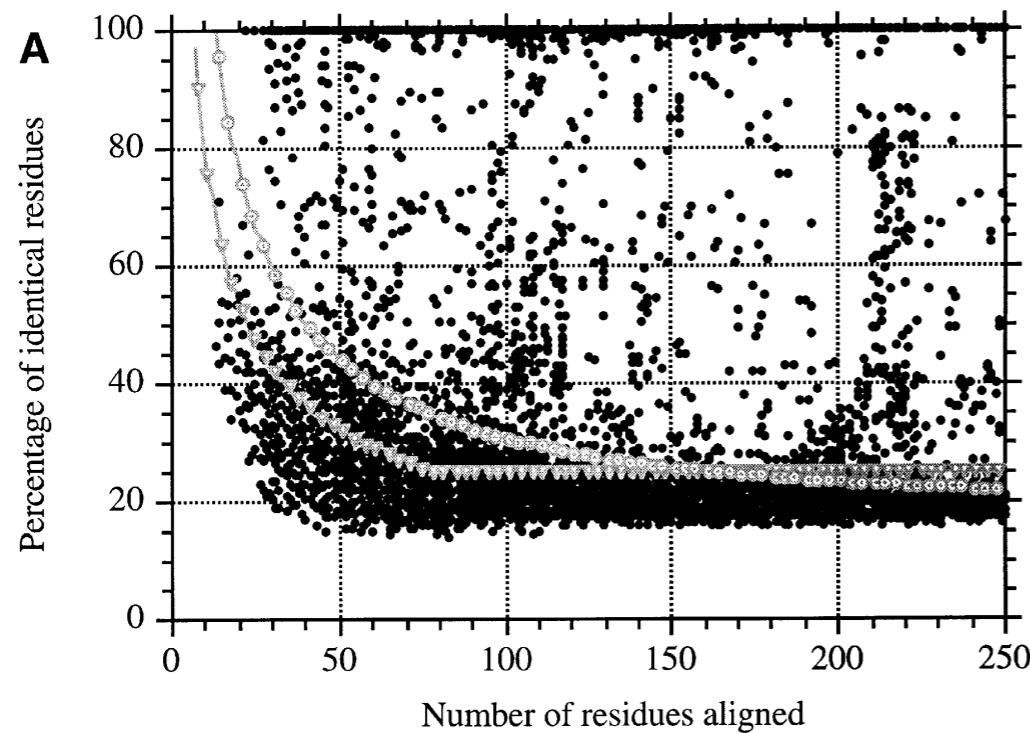
Structural Homology

Based on the database of homology-derived secondary structure of proteins (HSSP).
Define the **relation between sequence similarity, structure similarity, and alignment length**.



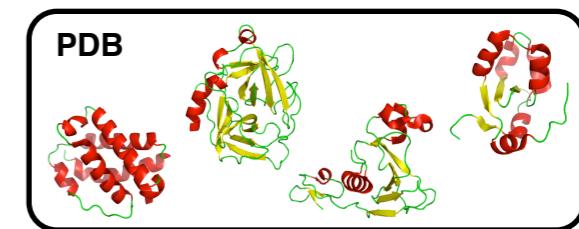
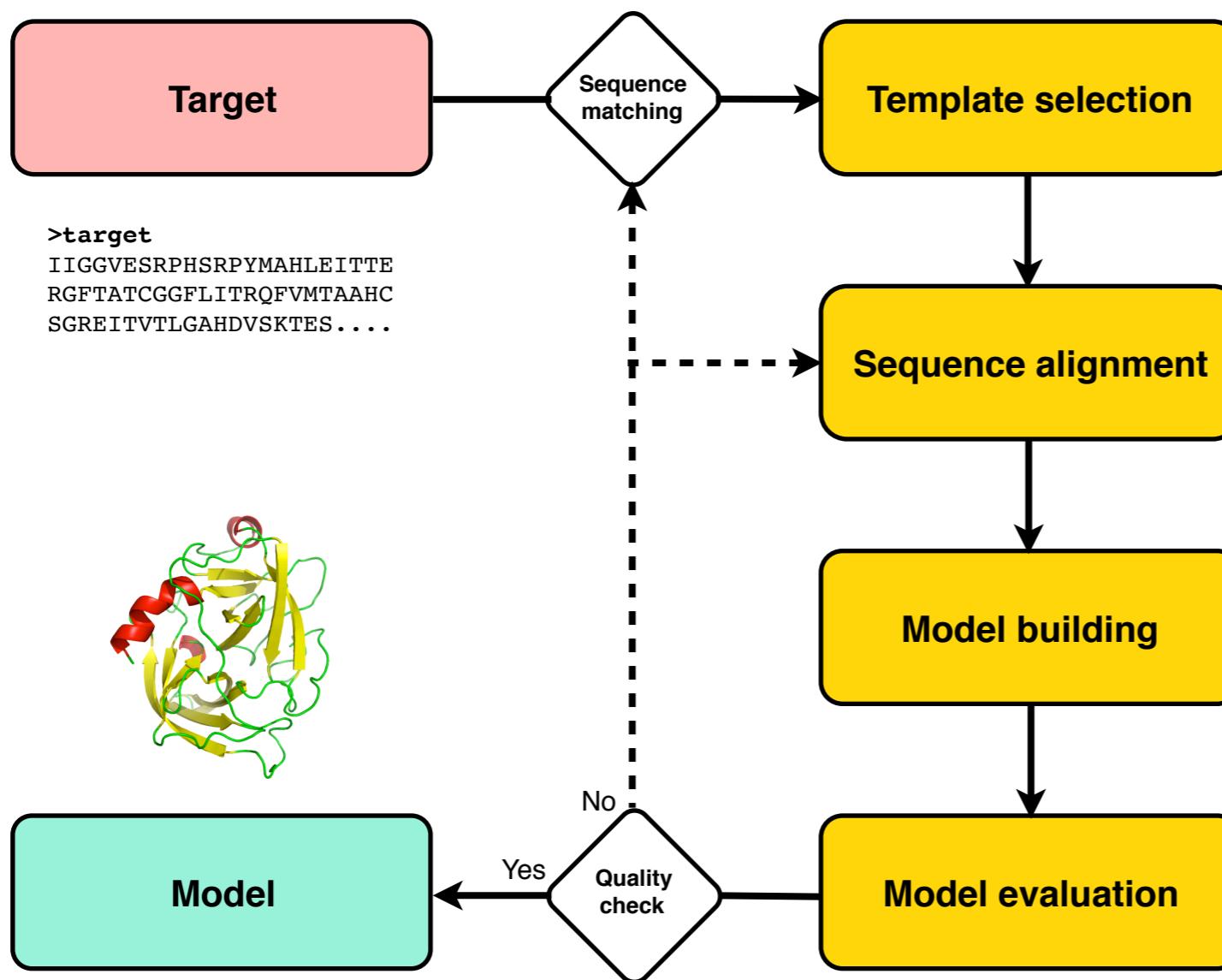
Twilight Zone

In the region above 20% of sequence identity, 90% of alignments correspond to homologous protein; while below 25% only 10%.

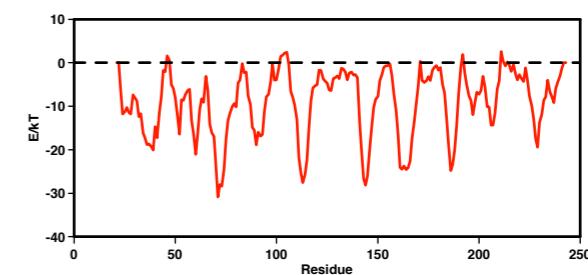
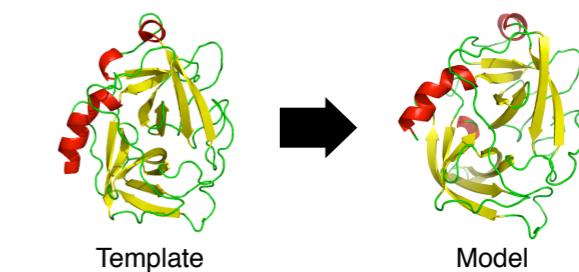


Comparative Modeling

Flow chart of Comparative Modeling



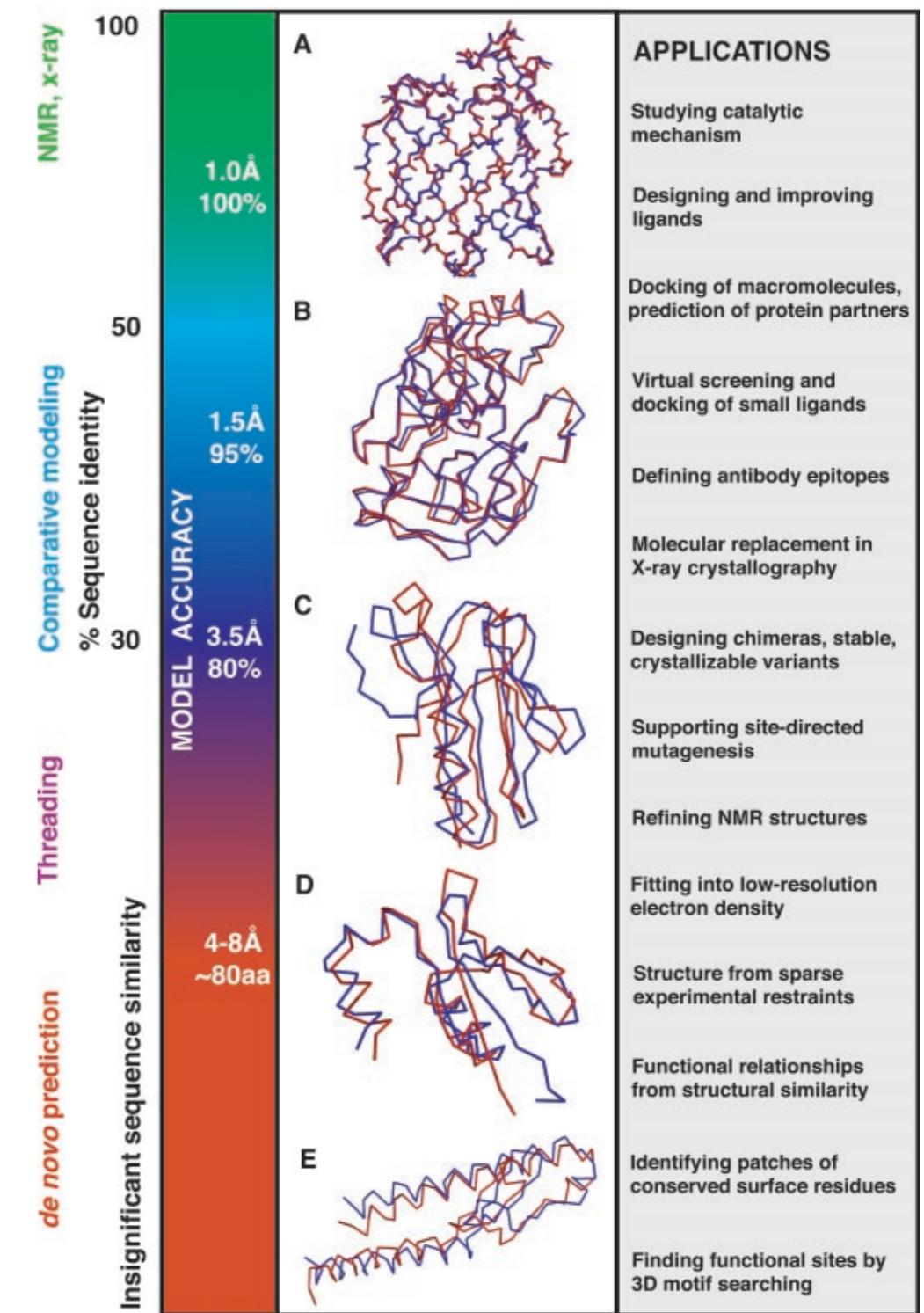
target IIGGVESRPHSRPYMAHLEI
3RP2A IIGGVESIPHSPYMAHLDI
target TTERGFTATCGGFLITRQ..
3RP2A VTEKGLRVICGGFLISRQ..



Use of Predicted Structures

Depending off the sequence similarity with the template the predicted structure can be used for different purposes

- Comparative Modeling
- Threading
- *Ab initio* or De novo predictions



Remote homologs

Sequences longer than 100 residues and sharing more than 30% of residues have similar structures (for shorter sequences the level of identity must be higher).

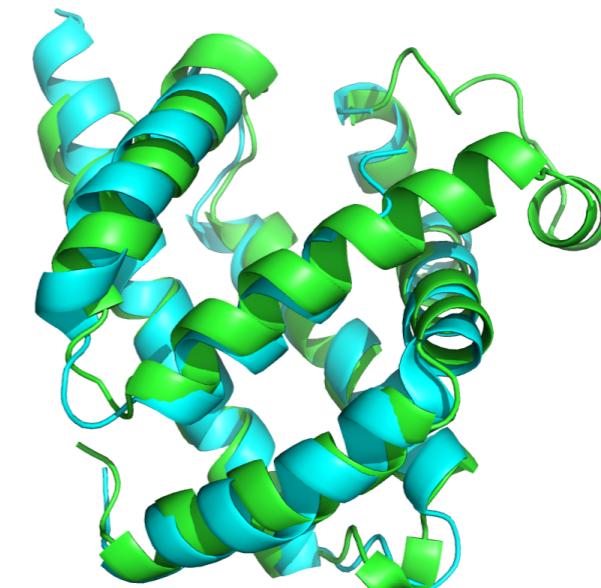
This **DO NOT** exclude that sequences sharing lower identity have similar structures.

Example:

Sperm Whale Myoglobin (1JP6:A)

Bacterial Haemoglobin (1VHB:A)

RMSD = 0.18 nm, Identity: 12%



Pairs of proteins with similar structure and low sequence identity are referred as “remote homologs”

aligned by TM-align

Sequence Identity Inference

Can we use sequence similarity to predict other features of an unknown protein?

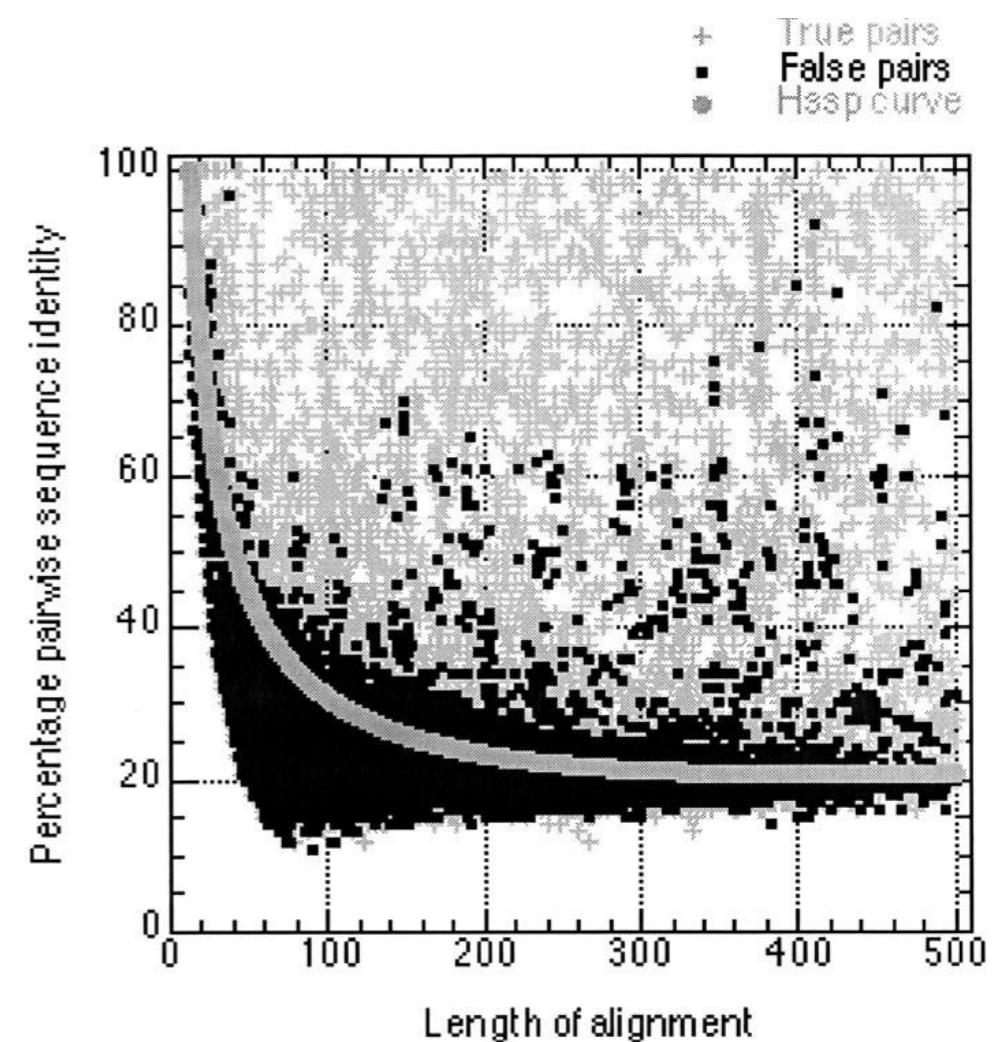
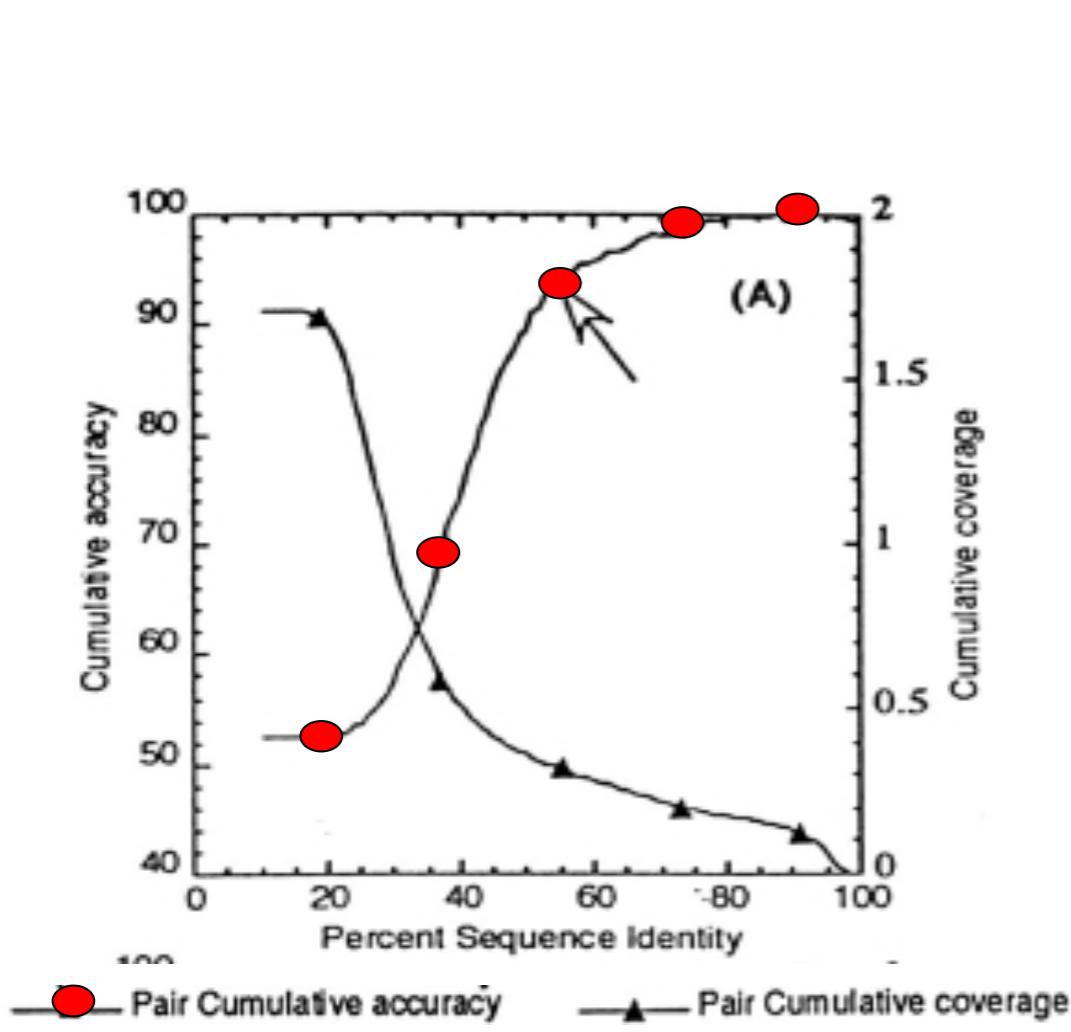
Solution: Define a the sequence similarity threshold that allow a reliable transfer of annotation features.

In other words we need to find the problem specific twilight region



Subcellular Localization

Sequence identity for reliably transferring **subcellular localization** is higher than that required for transferring structure.



A false positive

sp Q9S	MEFEKIKVINP ::	VVEMDGDEM ::	TRVIWKFI ::	KDKLIFPF ::	LELDIYF ::	DLGLPNRDFT ::	DDKV ::	TI
10	20	30	40	50	60			
sp Q9S	MAFEKIKVAN ::	PIVEMDGDEM ::	TRVIWKS ::	SIKDKLIT ::	PVELDIYF ::	DLGLPHR ::	DATDDK ::	V ::
10	20	30	40	50	60			
sp Q9S	ETAEATL ::	KYNVAIK ::	CATITP ::	DEARVREF ::	GLKKMWRSP ::	PNGTIRN ::	NILNGTVF ::	REPIICRN ::
70	80	90	100	110	120			
sp Q9S	ESAEATKK ::	YNVAIK ::	CATITP ::	DEGRVTEF ::	GLKQMWRSP ::	PNGTIRN ::	NILNGTVF ::	REPIICKN ::
70	80	90	100	110	120			
sp Q9S	RLVPGWT ::	KPKICIGR ::	HAFGDQYR ::	ATDLIVNE ::	PGKLKL ::	VFEPSGS ::	SQKTEFEV ::	FVNFTG-GGV ::
130	140	150	160	170				
sp Q9S	KLVPGWT ::	KPKICIGR ::	HAFGDQYR ::	ATDAVIKG ::	PGKLTMTF ::	--GKDGT ::	ETEVFTFT ::	GEGGV ::
130	140	150	160	170				
sp Q9S	180	190	200	210	220	230		
sp Q9S	ALAMYNT ::	DESIRAF ::	AESSMYT ::	AYQKKW ::	PLYLST ::	KNTILKI ::	YDGRFKD ::	IFQE ::
180	190	200	210	220	230			
sp Q9S	240	250	260	270	280	290		
sp Q9S	YEAA ::	GIWYE ::	HRLIDDM ::	VAYAMK ::	SEGGYV ::	WACKNY ::	DGDVQSDF ::	LAQGYG ::
sp Q9S	YDAAGI ::	WIWYE ::	HRLIDDM ::	VAYALK ::	SEGGYV ::	WACKNY ::	DGDVQSDF ::	LAQGFG ::
240	250	260	270	280	290			
sp Q9S	300	310	320	330	340	350		
sp Q9S	DGKTIE ::	AEAAHGT ::	TVTRHY ::	RHQKG ::	GETSTNS ::	IASIFAWS ::	RGLAHR ::	AKLDSNA ::
300	310	320	330	340	350			
sp Q9S	360	370	380	390	400	410		
sp Q9S	LEAACMGT ::	VESGK ::	MTKDL ::	ALLIHG ::	AKVRRD ::	QYVNTE ::	EFIGDA ::	VAWELK ::
360	370	380	390	400	410			

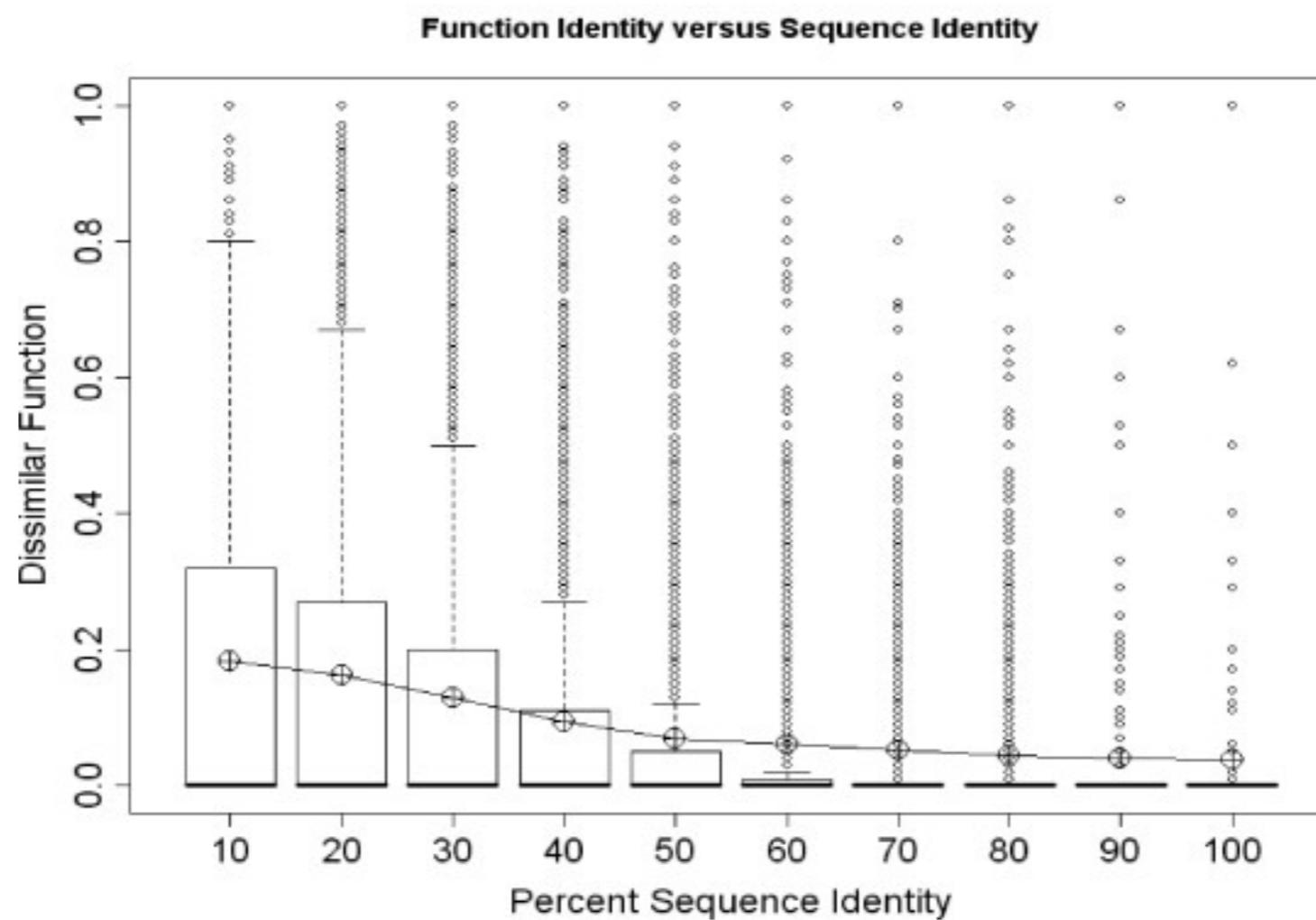
Q9SLK0 (ICDHX_ARATH):
Peroxisomal isocitrate dehydrogenase

Q9SRZ6 (ICDHC_ARATH):
Cytosolic isocitrate dehydrogenase

84.2% identity (93.3% similar) in 417
aa overlap

Functional Annotation

Sequence identity for can be used for functional annotation measuring the identity and similarity between Gene Ontology terms.



Dissimilar functions

	10	20	30	40	50	60
sp P04	MTKSHSEEVIVPEFNSAKELPRPLAECPSIICKFISAYDAKPDFVARSPGRVNLLIGEH					
	:: .	:: : :	... :: :	. . :	... :: :	. . :: :
sp P13	MNTN-----VPIFSSPVRLPLRSFEQKHLAVVDAFFQTYHVKPDFIARSPGRVNLLIGEH					
	10	20	30	40	50	
	70	80	90	100	110	120
sp P04	IDYCDFSVLPLAIDFDMLCAVKVLNEKNPSITLINADPKFAQRKFDLPLDGSYVTIDPSV					
	:: : :	:: : :	. . :: :	:: : :	:: : :	. . :: :
sp P13	IDYCDFSVLPLAIDVDMLCAVKILDEKNPSITLTNAADPKFAQRKFDLPLDGSYMAIDPSV					
	60	70	80	90	100	110
	130	140	150	160	170	180
sp P04	SDWSNYFKCGLHVAHSFLKKLAPERFASAPLAGLQVFCEGDVPTGSGLSSAAFICAVAL					
	:: : :	:: : :	. . :: :	:: : :	:: : :	. . :: :
sp P13	SEWSNYFKCGLHVAHSYLKKIAPERFNNTPLVGAQIFCQSDIPTGGGLSS--AFTCAAAL					
	120	130	140	150	160	170
	190	200	210	220	230	240
sp P04	AVVKANMGPGYHMSKQNLMRITVVAEHYVGVNNGMDQAASVCGEEDHALYVEFKPQLKA					
	:: : :	. . :: :	:: : :	:: : :	:: : :	. . :: :
sp P13	ATIRANMGKNDISKKDLTRITAVAEEHYVGVNNGMDQATSVYGEEDHALYVEFRPKLK					
	180	190	200	210	220	230
	250	260	270	280	290	300
sp P04	TPFKFPQLKNHEISFVIANTLVVSNKFETAPTNYNLRVVEVTTAANVLAATYGVVLLSGK					
	:: : :	:: : :	:: : :	:: : :	:: : :	. . :: :
sp P13	TPFKFPQLKNHEISFVIANTLVKSNKFETAPTNYNLRVIEVTVAAANALATRYSVALPSHK					
	240	250	260	270	280	290
	310	320	330	340	350	360
sp P04	EGSSTNKGNLRDFMNVYYARYHNISTPWNGDIESGIERLTKMLVLVEESLANKKQGFSVD					
	. . :: :	. . :: :	. . :: :	. . :: :	. . :: :	. . :: :
sp P13	DNSNSERGNLRFMDAYYARYENQAQPWNLDIGTGIERLLKMLQLVEESFSRKKGFTVH					
	300	310	320	330	340	350
	370	380	390	400	410	420
sp P04	DVAQSLNCSREEFTRDYLTTSPVRFQVLKLYQRAKHVYSESLSRVLKAVKLMTTASFTADE					
	. . :: :	. . :: :	. . :: :	. . :: :	. . :: :	. . :: :
sp P13	EASTALNCSREEFTRDYLTTFPVRFQVLKLYQRAKHVYSESLSRVLKALKMMTSATFHDE					
	360	370	380	390	400	410
	430	440	450	460	470	480
sp P04	DFFKQFGALMNESQASCSDKLYECSCPEIDKICSIALSNGSYGSRLTGAGWGGCTVHLVPG					
	. . :: :	. . :: :	. . :: :	. . :: :	. . :: :	. . :: :
sp P13	DFFTDFGRLMNESQASCSDKLYECSCIETNQICSIALANGSGSRLTGAGWGGCTIHLVPS					
	420	430	440	450	460	470
	490	500	510	520		
sp P04	GPNGNIEKVKEALANEFYKVKYPKITDAELENIAIVSKPALGSCLYEL					
	. . :: :	. . :: :	. . :: :	. . :: :	. . :: :	. . :: :
sp P13	GANGNVEQRKALIEKFYNVRYPLDTDEELKDIAIIVSKPALGTCLYEQ					
	480	490	500	510	520	

P04385 (GAL1_YEAST) Galactokinase

Catalytic activity

ATP + alpha-D-galactose = ADP + alpha-D-galactose 1-phosphate.

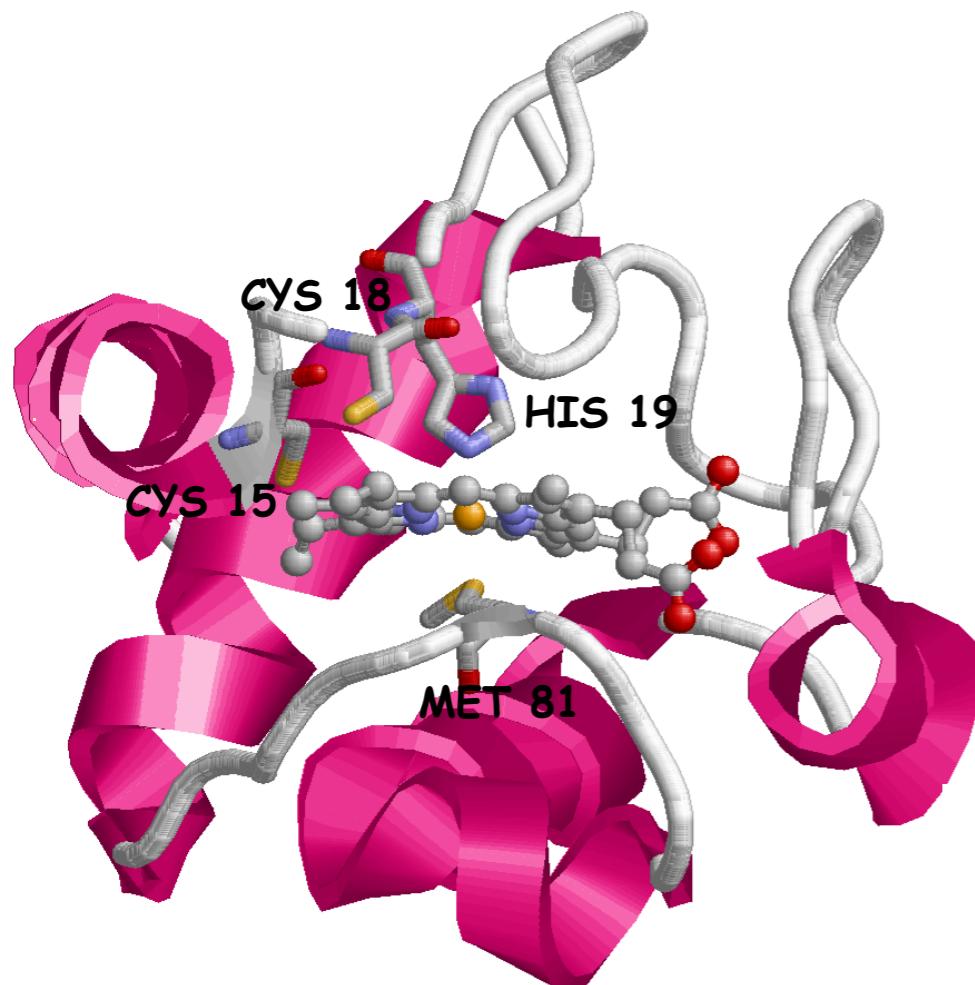
P13045 (GAL3_YEAST) Protein GAL3

The GAL3 regulatory function is required for rapid induction of the galactose system.

72.9% identity (90.5% similar) in 528 aa overlap

Case Study

Electron carrier protein. The oxidized form of the cytochrome c heme group can accept an electron from the heme group of the cytochrome c1 subunit of cytochrome reductase. Cytochrome c then transfers this electron to the cytochrome oxidase complex, the final protein carrier in the mitochondrial electron-transport chain.



Feature key	Position(s)	Length	Description
Binding site ⁱ	15 – 15	1	Heme (covalent)
Binding site ⁱ	18 – 18	1	Heme (covalent)
Metal binding ⁱ	19 – 19	1	Iron (heme axial ligand)
Metal binding ⁱ	81 – 81	1	Iron (heme axial ligand)

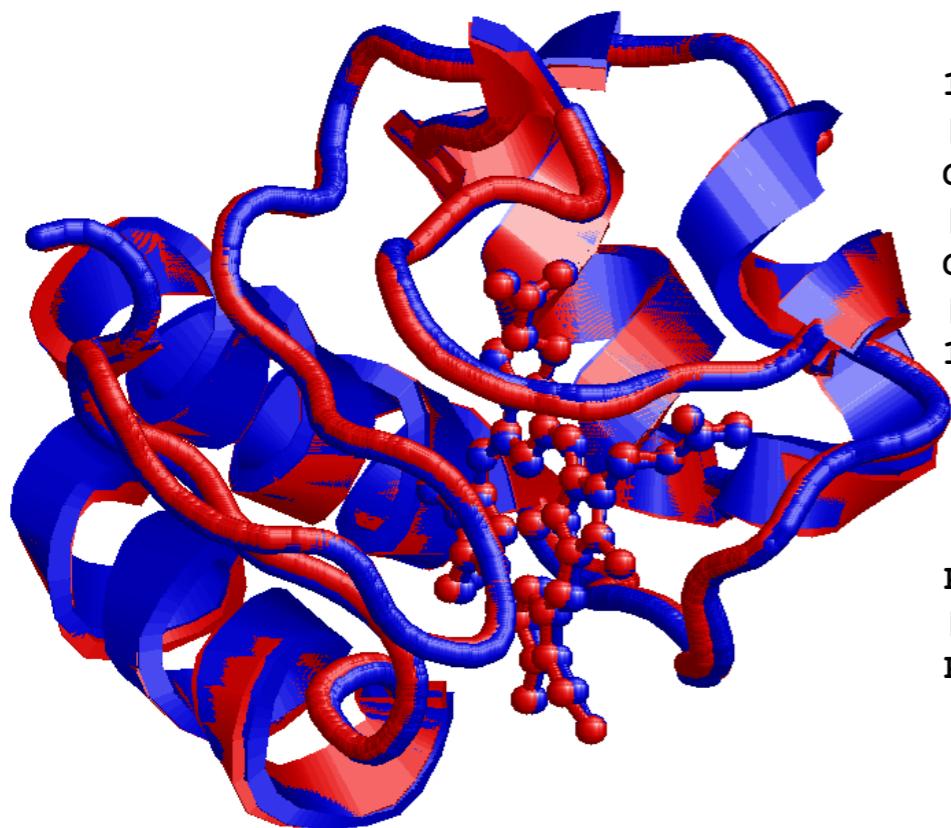
PDB: 3zcf:A

Homo vs Horse

Human Cytochrome C – Uniprot:P99999. PDB: 3ZCF:A

Equine Cytochrome C – Uniprot: P00004. PDB 3O20:A

Structural alignment:
RMSD= 0.035 nm
88% sequence identity



1 : A	20 : A	40 : A	60 : A
	.	.	.
GDVEKGKKIFIMK CSQCH TVEKGGKHKTGPNLHGLFGRKTGQAPGYSYTAANKNKGIIWG	EDETLMEYLEN		
: : .	: .	: .	: .
GDVEKGKKIFVQ KCAQCH TVEKGGKHKTGPNLHGLFGRKTGQAPGFTYTDANKNKGITW	KEETLMEYLEN		
	.	.	.
80 : A	100 : A		
.	.	.	.
PKKYIPG TKM IFVGIKKKEERADLIAYLKKATNE			
: : .	: .	: .	: .
PKKYIPG TKM IFAGIKKKTEREDLIAYLKKATNE			
.	.	.	.
80 : A	100 : A		

Sequence vs Structure

In this case the sequence alignment is the same of the structural alignment and the **positions of the binding sites are conserved**.

Sequence alignment:
88% sequence identity
IDENTICAL TO STRUCTURAL ALIGNMENT

88.6% identity (95.2% similar) in 105 aa overlap (1-105:1-105)

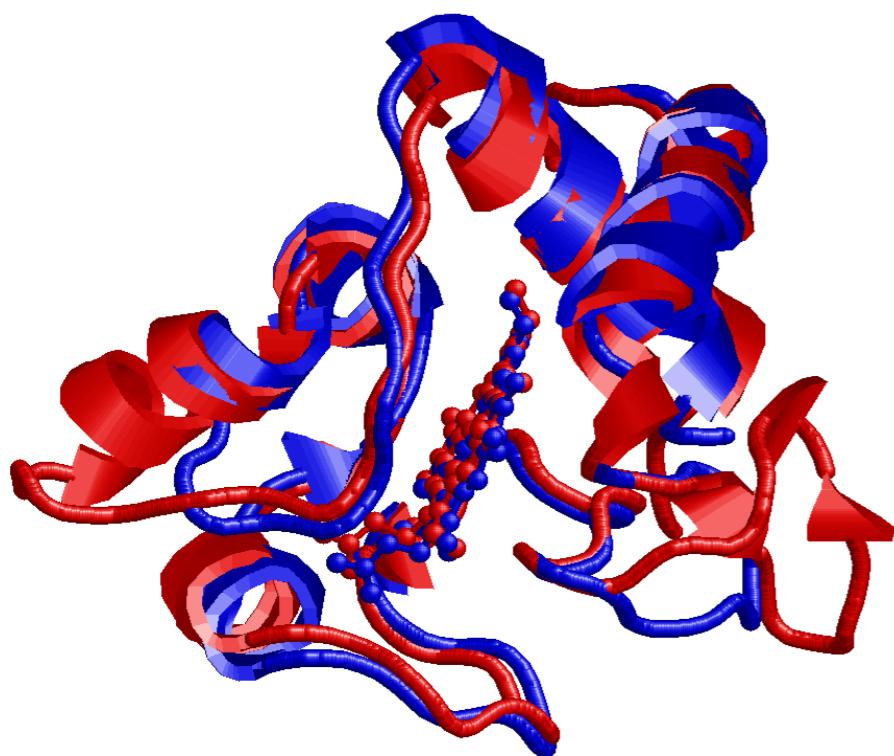
	10	20	30	40	50	60
Homo	MGDVEKGKKIFIMK <u>CSQCH</u> TVEKGGKHKTGPNLHGLFGRKTGQAPGYSYTAANKNKGIW					
	:::::::	:::::::	:::::::	:::::::	:::::::	:::::::
Horse	MGDVEKGKKIFVQK <u>CAQCH</u> TVEKGGKHKTGPNLHGLFGRKTGQAPGFTYTDANKNKGITW					
	10	20	30	40	50	60
	70	80	90	100		
Homo	GEDTLMEYLENPKKYIPGTM <u>I</u> FVGIKKKKEERADLIAYLKKATNE					
	:::::::	:::::::	:::::::	:::::::	:::::::	:::::::
Horse	KEETLMEYLENPKKYIPGTM <u>I</u> FAGIKKKTEREDLIAYLKKATNE					
	70	80	90	100		

Homo vs Rhodobacter Sph.

Human Cytochrome C — Uniprot:P99999. PDB: 3ZCF:A

Cytochrome C2 Rhodobacter Sph. — Uniprot: P0C0X8. PDB 1CXC:A

Structural alignment:
RMSD= 0,18 nm
28% sequence identity



1 : A	20 : A	40 : A
	.	.
GDVEKGKKIFIMK CSQCH TVEKGG-----KHKTGPNLHGLFGRKTGQAPGYS-YTAANKNKG---IIW		
. :....	.. ::	
GDPEAGAKAFN-Q CQTCH VIVDDSGTTIAGRNAKTGPNLYGVVGRTAGTQADFKGYGEGMKEAGAKGLAW		
	.	.
3 : A	20 : A	40 : A
60 : A	80 : A	100 : A
.
GEDTLMEYLENPKKYI-----PGTK M IFVGIKKKEERADLIAYLKKATNE		
. :...: .: . :: ..:....: 	
DEEHFVQYVQDPTKFLKEYTGDAKAGK MTF -KLKKEADAHNIWAYLQQVAVR		
.	.	.
80 : A	100 : A	120 : A

Sequence vs Structure (I)

In this case the sequence alignment is thecae be used for homology modeling after a refinement of the alignment because **one binding site is not conserved**.

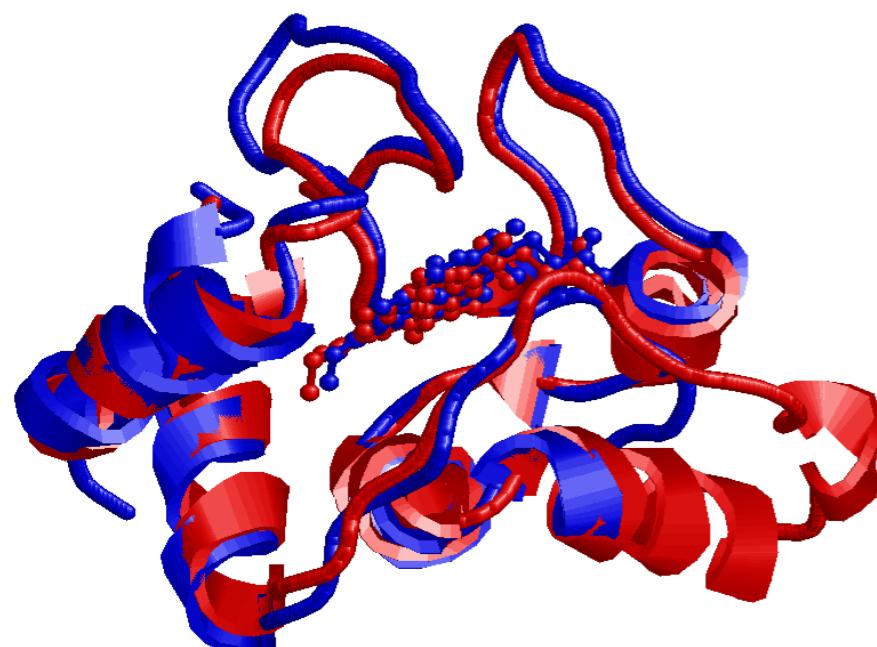
Structural alignment:
RMSD= 0,18 nm
28% sequence identity

Homo vs Rhodobacter Pal.

Human Cytochrome C - Uniprot:P99999. PDB: 3ZCF:A

Cytochrome C2 Rhodopseudomonas pal. – Uniprot: P00091. PDB 1I8O:A

Structural alignment:
RMSD= 0,13 nm
29% sequence identity



Sequence vs Structure (II)

In this case the sequence alignment needs to be fixed homology to because all the **binding site shifted**.

Structural alignment:
RMSD= 0,13 nm
29% sequence identity

Global without end-gap score: 152; 28.7% identity (63.0% similar) in 108 aa

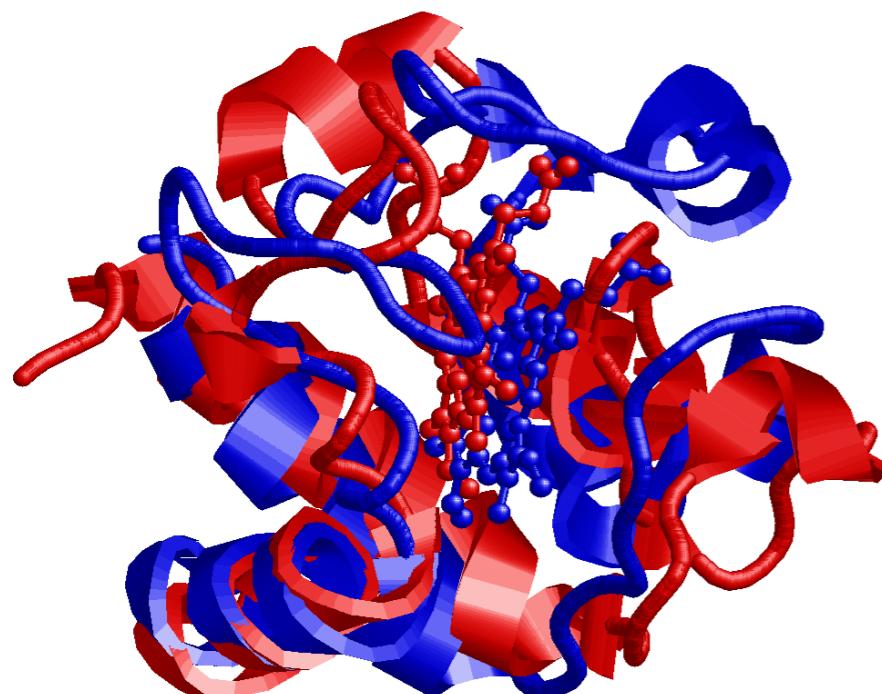
10	20	30			
sp P99	<u>MGDVEKGKKIFIMKCSQCHTVEKGGKHKTGPNLHGL</u>				
	... : . . : . . : . . . : . . . : . . .				
sp P00	<u>MVKKLLTILSIAATAGSLSIGTASAQDAKAGEAVF</u> ---- <u>KQCMTCHRADKNMVGPALGGV</u>				
10	20	30			
40	50	60	70	80	90
sp P99	<u>FGRKTGQAPGYSYTAANKNKG</u> --- <u>IIWGDEDTLMEYLENPKKYIPGTKMIFVGIGKKKEERA</u>				
	: . . : : . . . : . . . : . . : . . . : . . . : . . .				
sp P00	<u>VGRKAGTAAGFTYSPLNHNSGEAGLVWTADNIINYLNDPNAFL</u> --- <u>KKFLTDKGKADQAV</u>				
60	70	80	90	100	110
100					
sp P99	DLIAYLKKATNE				
	. . : . . :				
sp P00	GVTKMTFKLANEQQRKDVVAYLATLK				
120	130				

Homo vs Arabidopsis

Human Cytochrome C - Uniprot:P99999. PDB: 3ZCF:A

Cytochrome C6A Arabidopsis Thaliana – Uniprot: Q93VA3. PDB 2CE0:A

Structural alignment:
RMSD= 0,35 nm
13% sequence identity



Sequence vs Structure (III)

In this case the sequence alignment is significantly different from the structural alignment.

Structural alignment:
RMSD= 0,35 nm
13% sequence identity

Global without end-gap score: 3; 20.0% identity (43.8% similar) in 105 aa

	10	20	30
Homo	<u>MGDVEKGKKIFIMKCSQCH</u>	TVEKGGKHKTG	
	: . . : . : : . . : . :		
A.Thal	DFILLKKLAPPLTAVLLAVSPICFPPE <u>SLGQTLDIQRGATLFNRA</u> CIGCHDT -GGNIIQPG		
	50 60 70 80 90 100		
	40 50 60 70 80 90		
sp P99	<u>PNLHGLFGRKTGQAPGYSYTAANKNKGIIWGEDTLMEYLENPKKYIPG</u> TKMIFVGIKKKE		
	. : . . : : : : . : . : . : . . .		
sp Q93	<u>ATLFTKD</u> LERNGVD-----TEEEIYRVTYFGKGR MPGFGE ---KCTPRGQCTF-GPRLOQD		
	110 120 130 140 150		
	100		
;			
sp P99	ERADLIAYLKKATNE		
 : . .		
sp Q93	EEIKLLAEFVKFQADQGWPTVSTD		
	160 170		

Search for Better Alignment

Why is it not sufficient to align sequences (when identity is low) to recover information, not even for “important” residues?

Sequence alignments are «general» and treat each position in the same way
There is no knowledge on the «important» sites

How can we detect the “important” residues starting from protein structures
(even when information on catalytic sites is not available)?

Compare multiple structures and analyze the conservation of residues

How can we align sequences constraining the alignment of important residues?

Compare multiple sequences and check for the conservation of patterns
Use alignment frameworks able to introduce positional dependences.