Structure et fonction des protéines

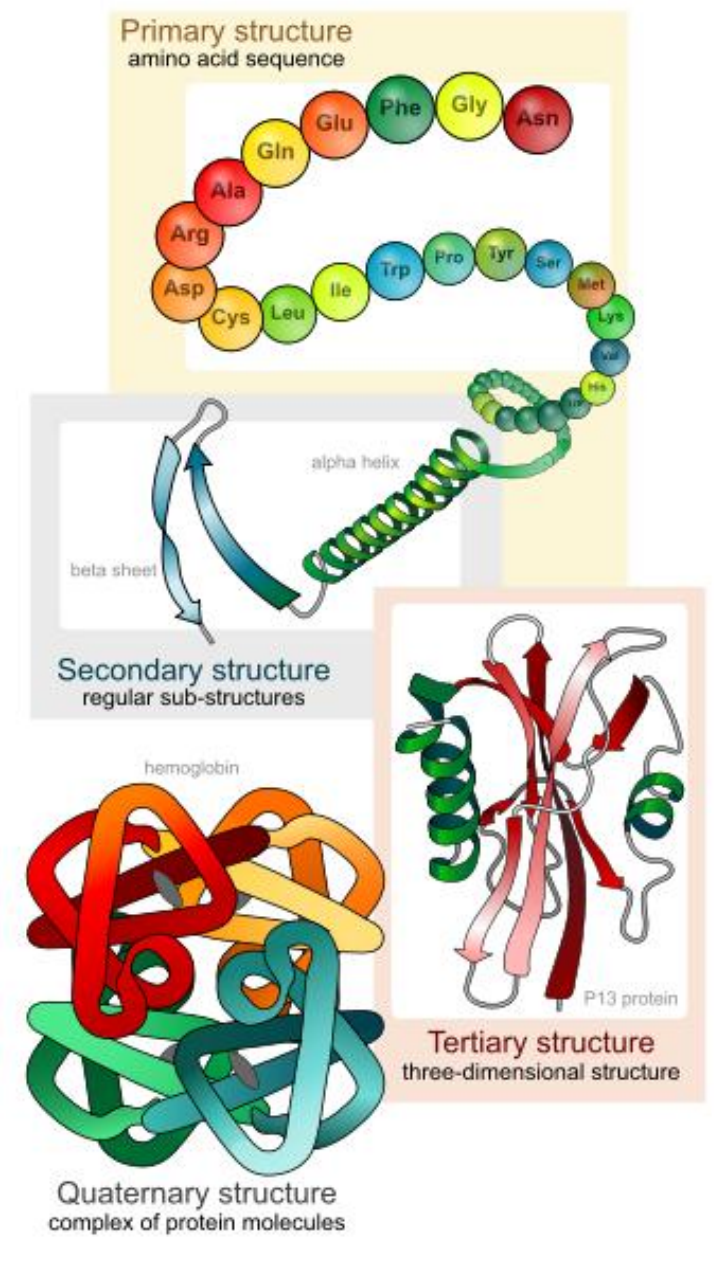
2012-2013

Partie 1 : Repliement et structure tertiaire

marie-line.garron@afmb.univ-mrs.fr

L2

Structures des polypeptides



- Structure Primaire:
  séquence linéaire des acides aminés

- Structure Secondaire:
  Hélices α, brins β, boucles…

- Structure Tertiaire:
  association des éléments de structure secondaire provenant d'une seule chaine polypeptidique

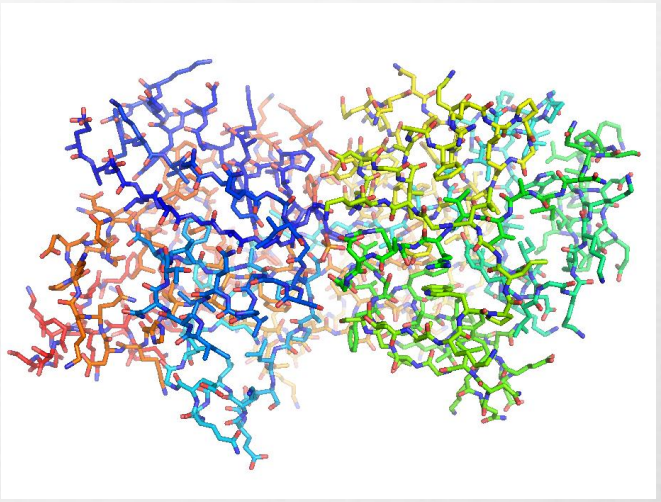
---

- Structure Quaternaire:
  complexe stable de plusieurs protéines (complexe multimèrique)

Les représentations et les conventions

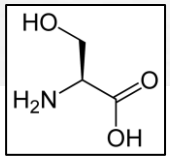
Phosphate binding protein
(1IXH)**EASLTGA**GATFPAPVYAKWADT
YQKETGNKVNYQGIGSSGGVKQIIANTV
DFGASDAPLSDEKLAQEGLFQFPTVIGG
VVL…

du N-terminal vers le C-terminal

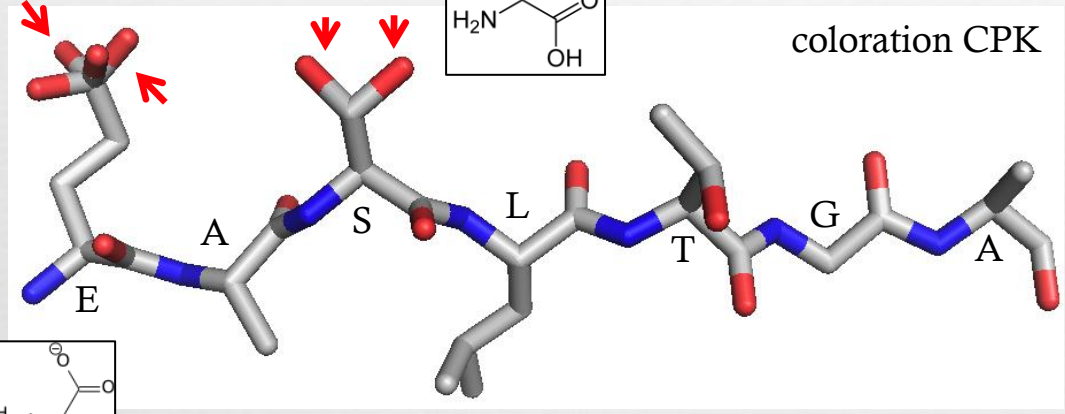


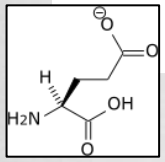
Stick

Rotamères

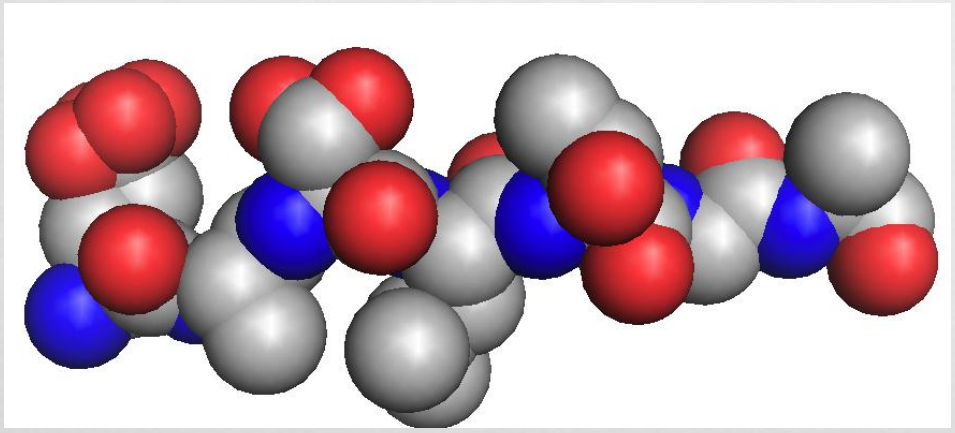


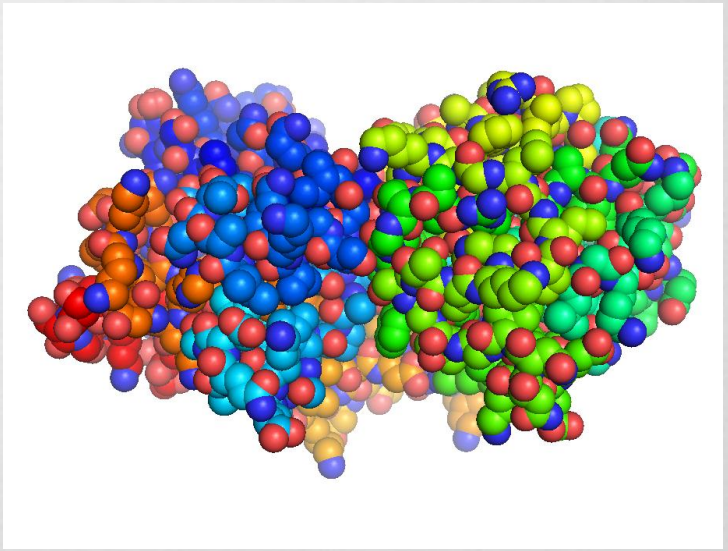
coloration CPK



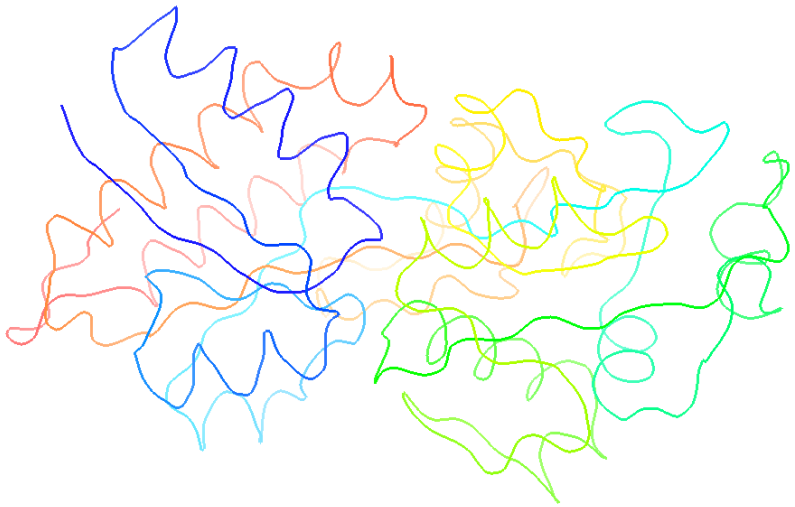


Sphère

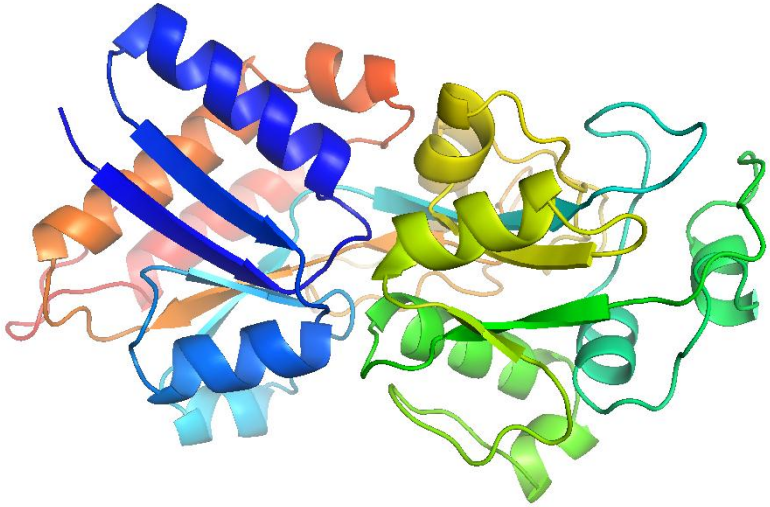




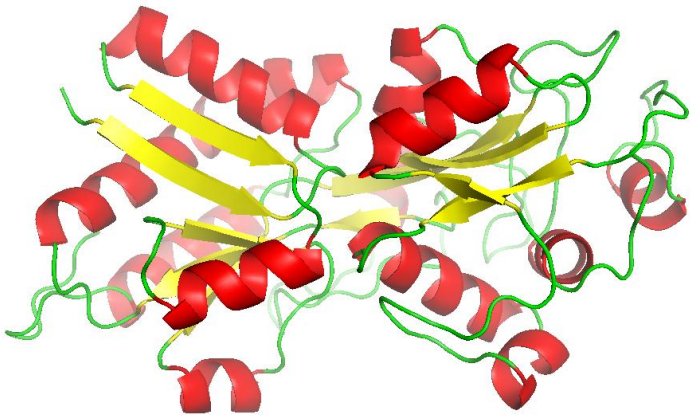
Les représentations et les conventions



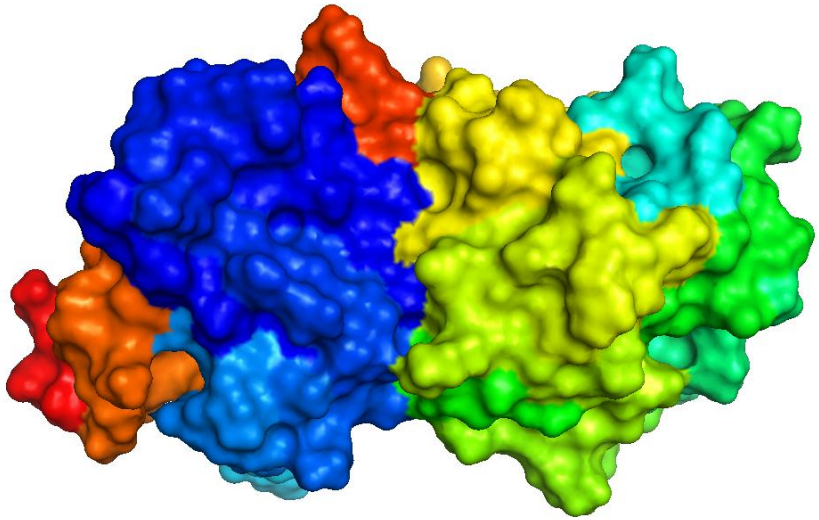
Ribbon



Cartoon



Coloration en fonction des SII



Surface

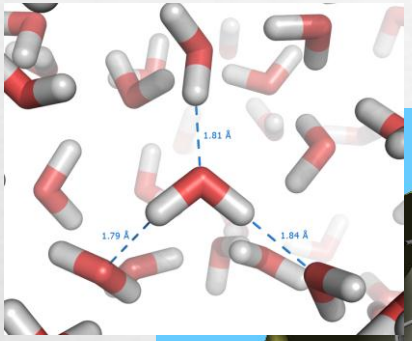
Partie 1 : Repliement et structure tertiaire

I/ De la structure primaire à la structure tertiaire

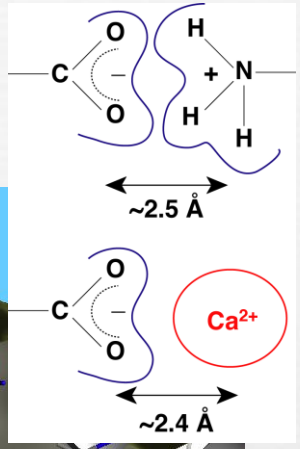
Interaction, Energie et Repliement

Les interactions

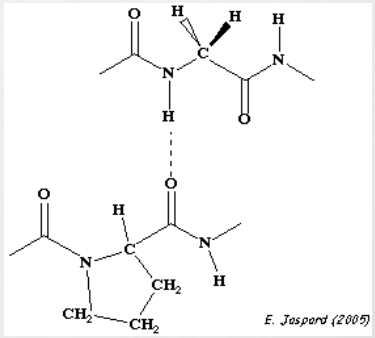
Solvatation

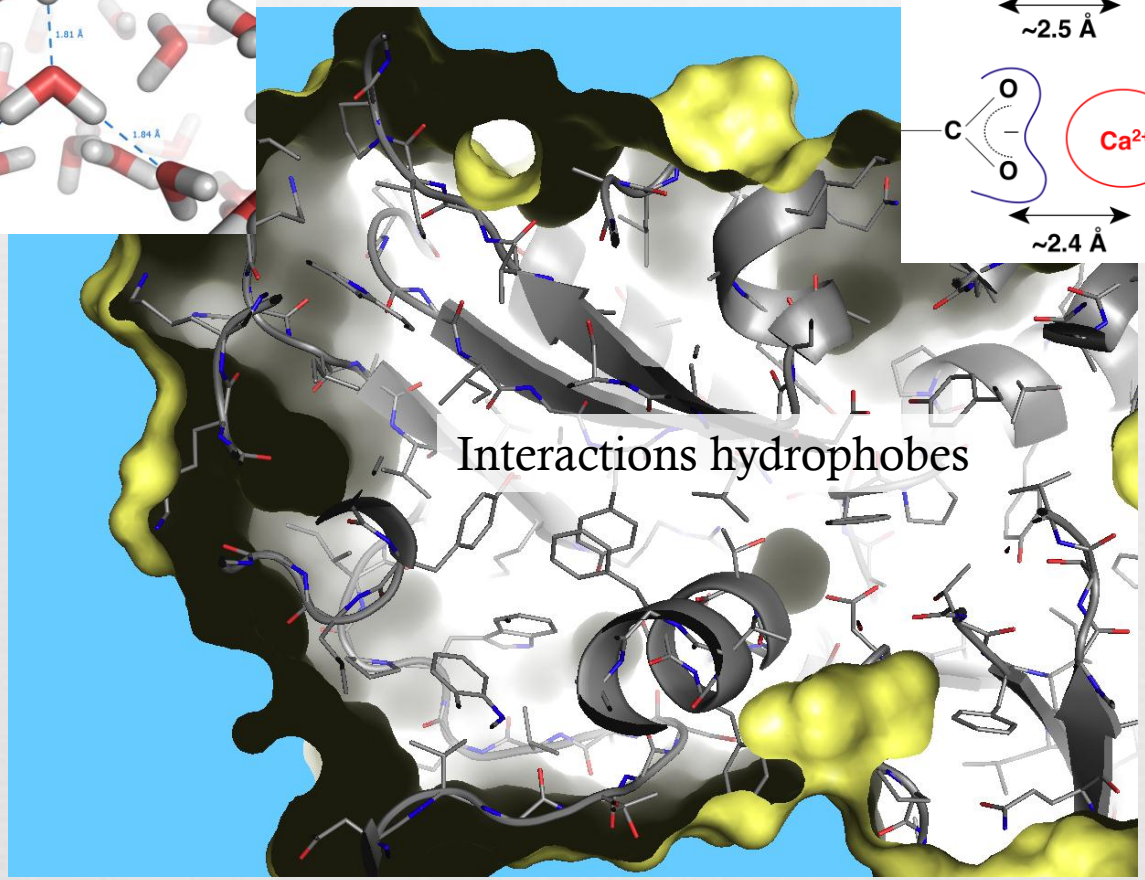


Liaisons électrostatiques



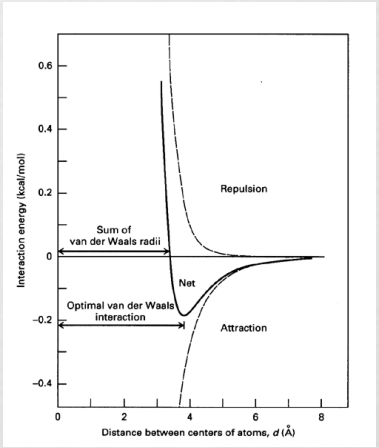
Liaisons hydrogènes





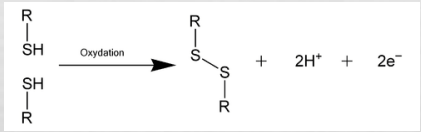
Interactions hydrophobes

Liaisons de Van der Waals

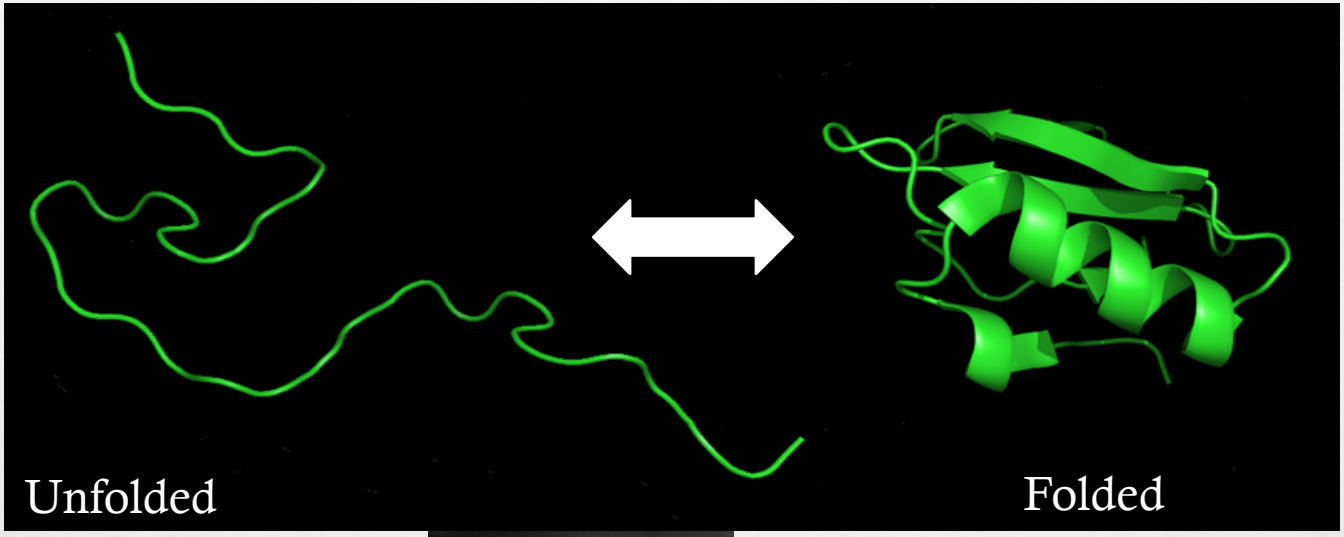


Protéine globulaire soluble

Ponts disulfures

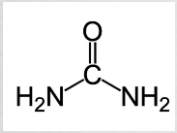


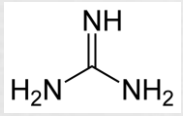
Le repliement



- Agents chaotropes

Urée

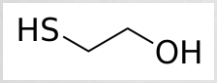




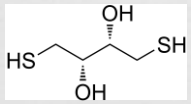
Chlorure de Guanidinium

- Agents réducteurs

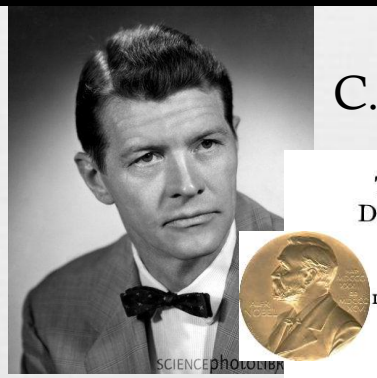
βmercaptoethanol



Dithiothreitol (DTT)



- Température
- pH extrêmes



C. Anfinsen

THE KINETICS OF FORMATION OF NATIVE RIBONUCLEASE DURING OXIDATION OF THE REDUCED POLYPEPTIDE CHAIN

By C. B. Anfinsen, E. Haber,* M. Sela,† and F. H. White, Jr.

Laboratory of Cellular Physiology and Metabolism, National Heart Institute, National Institutes of Health

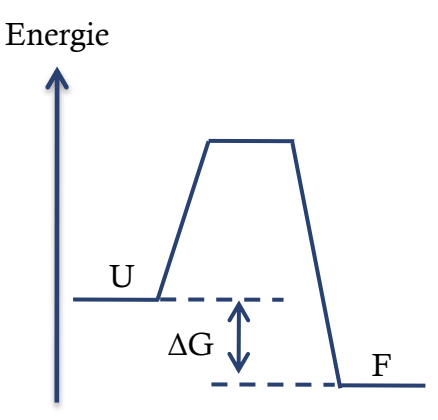
Communicated by John T. Edsall, July 31, 1961

Bovine pancreatic ribonuclease is completely reduced by treatment with mercaptoethanol in 8 *M* urea to yield a randomly coiled polypeptide chain containing eight cysteine residues.[1–3] Under optimal conditions of polypeptide concentration and pH, essentially complete reformation of the disulfide bonds of the native enzyme occurs in the presence of molecular oxygen.[2, 3] From chemical and physical studies of the reformed enzyme, it may be concluded that the information for the correct pairing of half-cystine residues in disulfide linkage, and for the assumption of the native secondary and tertiary structures, is contained in the amino acid sequence itself.

L'information de repliement est contenu dans la séquence primaire

L'information de repliement est contenu dans la séquence

La structure d'une protéine native est unique, stable et thermodynamiquement accessible

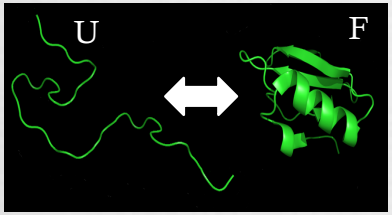


Energie libre

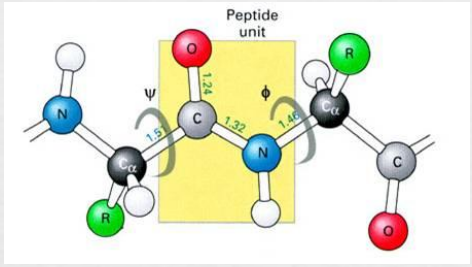
$\Delta G = \Delta H - T\Delta S$

entropie

enthalpie



• Le paradoxe de Levinthal



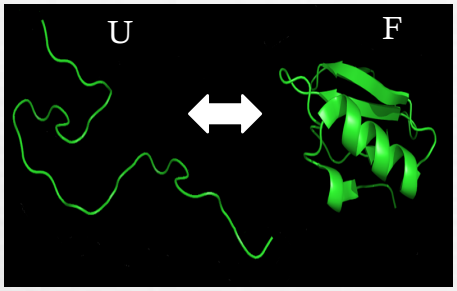
Polypeptides de 100AA
Si chaque AA peut prendre deux conformations
Alors la chaine polypeptidique peut adopter $2^{100} \approx 10^{30}$ conformations
Disons que l'interconversion entre chaque conformations prends 10^{-12}s

10^{30} conformations x 10^{-12}s/conformations = 10^{18} s
1 an = 31 536 000s ≈ 30 10^{6}s

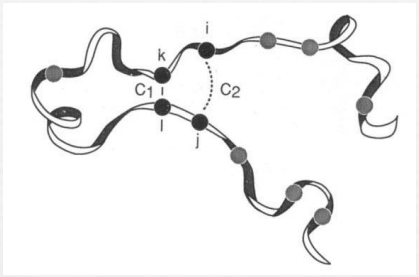
≈3 10^{10} ans
30 milliards d'années…

Rmq : La terre c'est formée il y a 4,5 milliards d'années

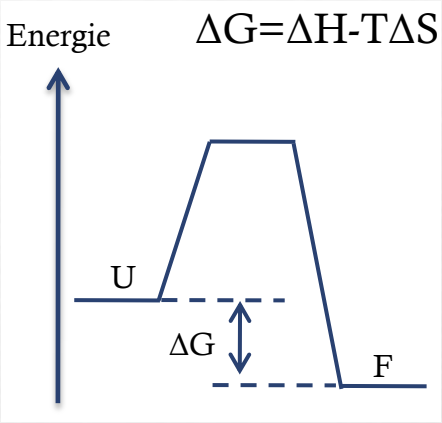
L'information de repliement est contenu dans la séquence

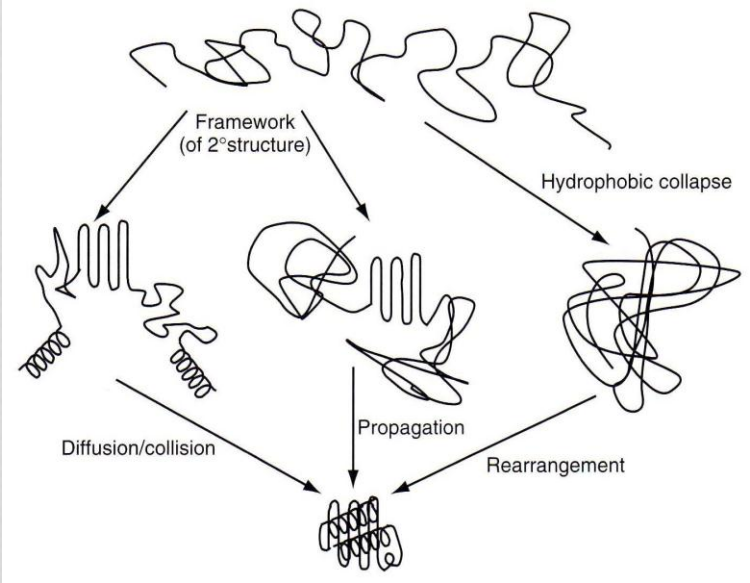


Il existe un « chemin » de repliement et une coopérativité



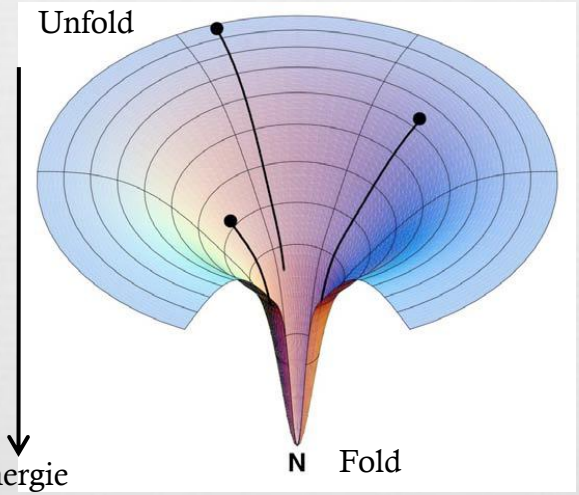
Dill et al., PNAS 1993; 90:1942-6

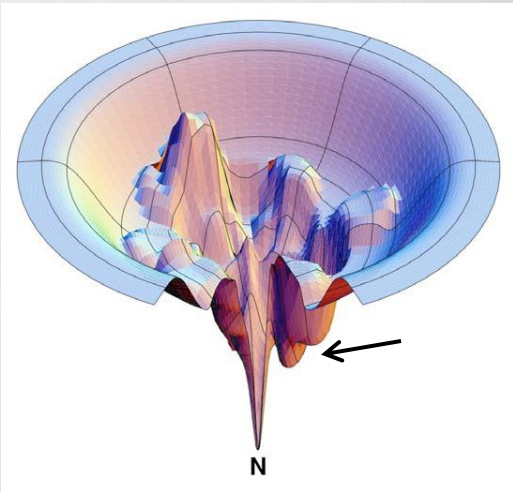




From Alan Fersht. "Structure and Mechanism in Protein Science"

Théorie de l'entonnoir





Dill and Chan, Nature Struct Biol 1997; 4:10-19

Les interactions et le repliement

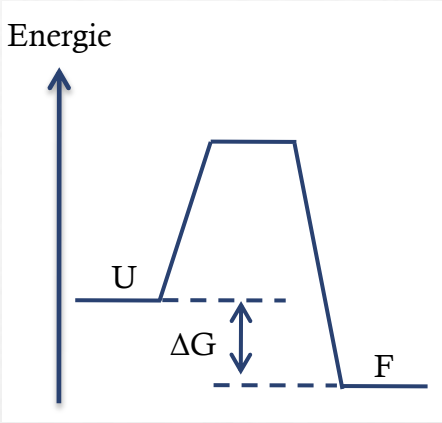
Energie libre

$\Delta G = \Delta H - T\Delta S$

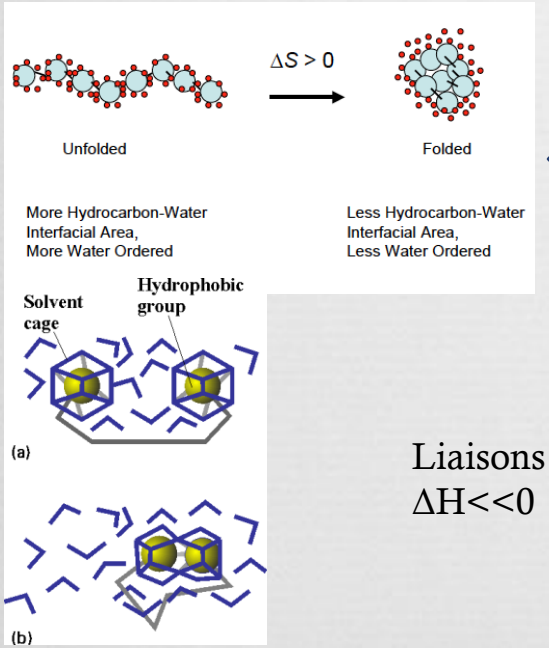
enthalpie

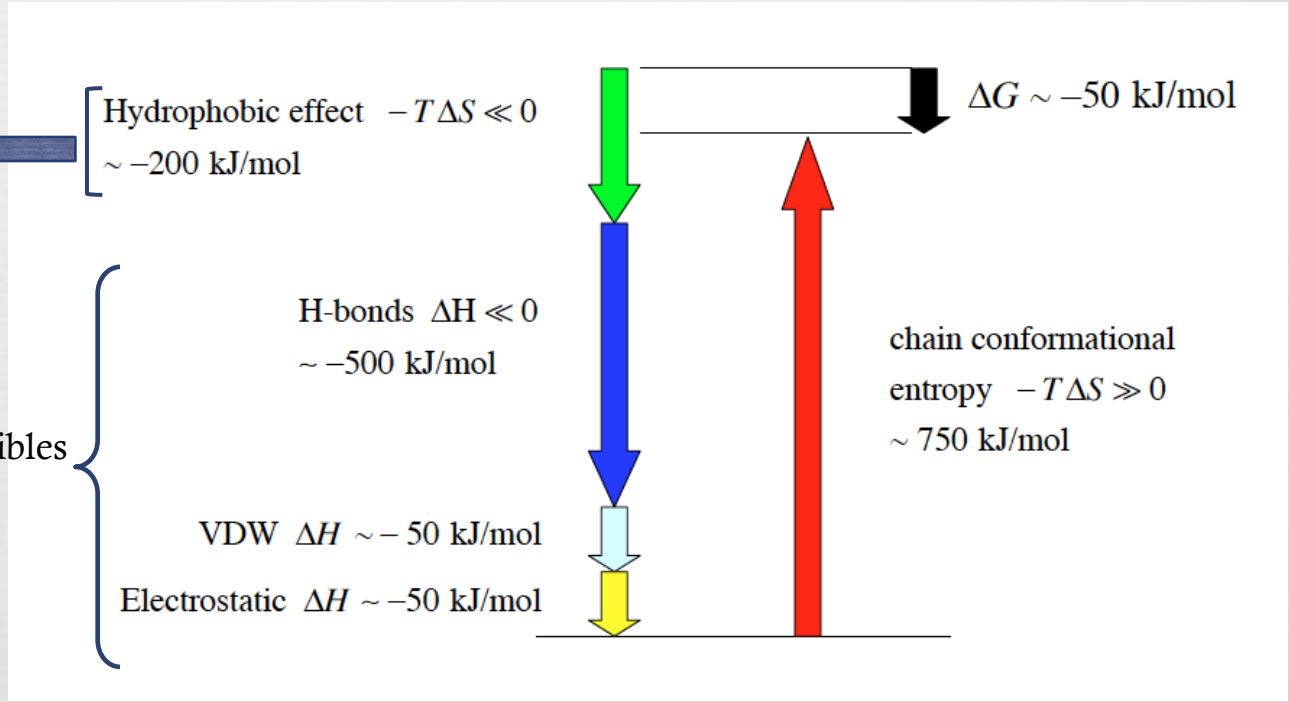
entropie « ordre »

$\Delta G < 0$

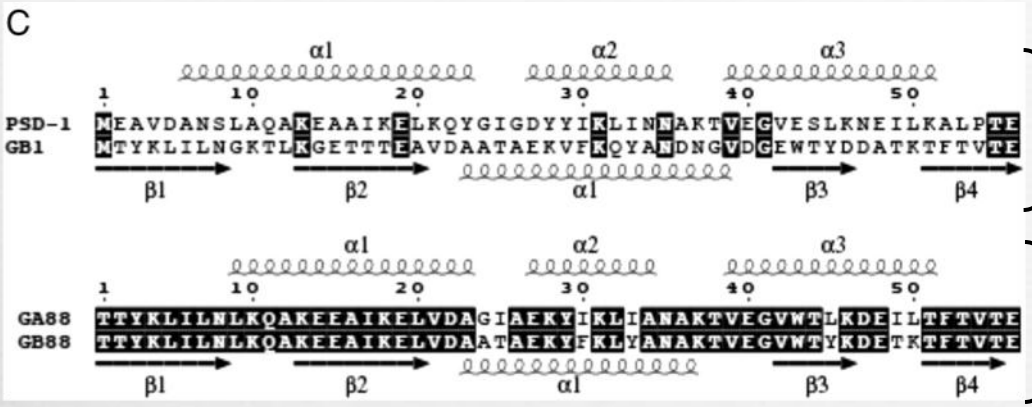


Plus un système est désordonné plus son entropie est grande





L'information de repliement est contenu dans la séquence

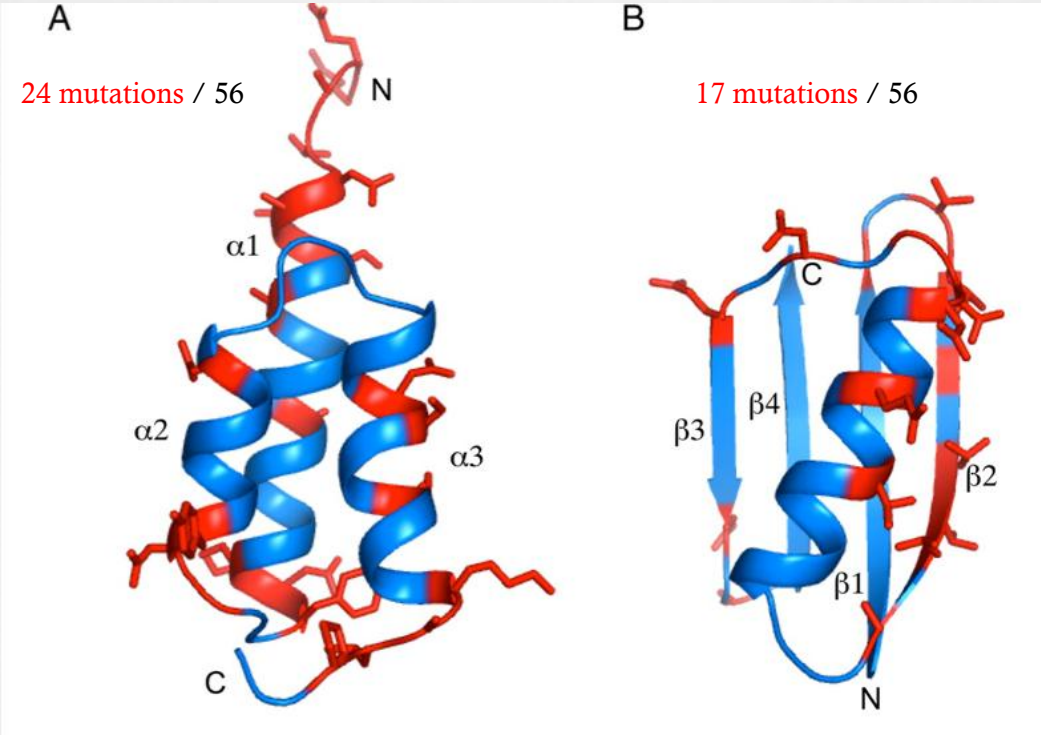


16% d'identité

Mutagenèse

88% d'identité

Domaine Ga
HSA binding(PSD-1)



Domaine Gb
IgG binding domain
(GB1)

He Y et al. *PNAS* 2008

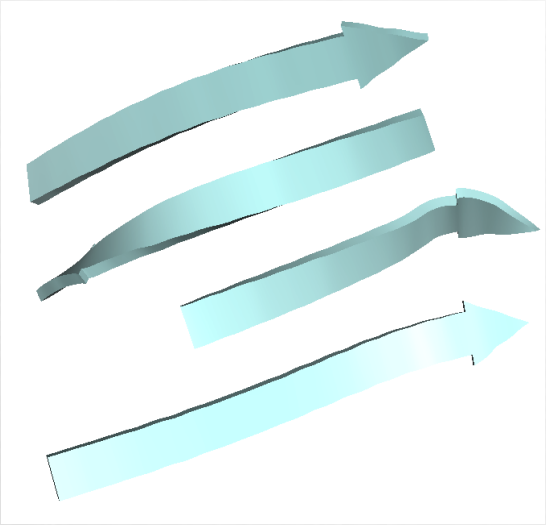
Partie 1 : Repliement et structure tertiaire

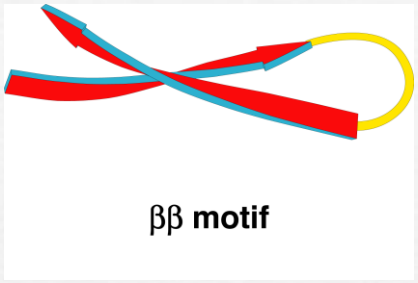
II/ Les motifs structuraux

Super structure secondaire et connectivité

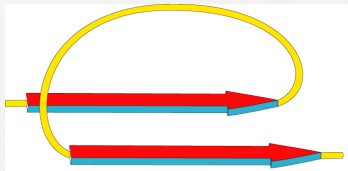
Les motifs en brin β

Feuillet β

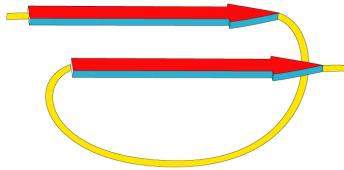




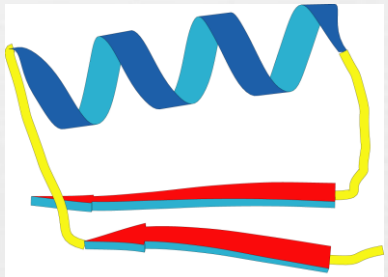
β hairpin



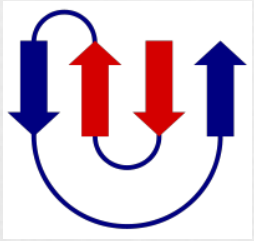
Right-handed crossover (observed)



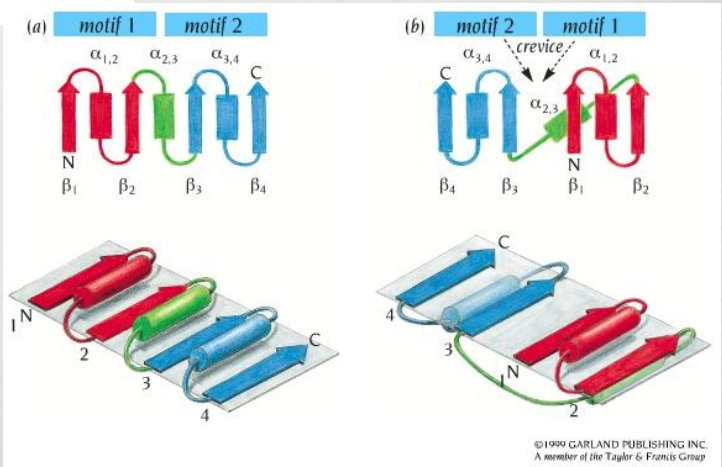
Left-handed crossover (exceedingly rare)



Ψloop



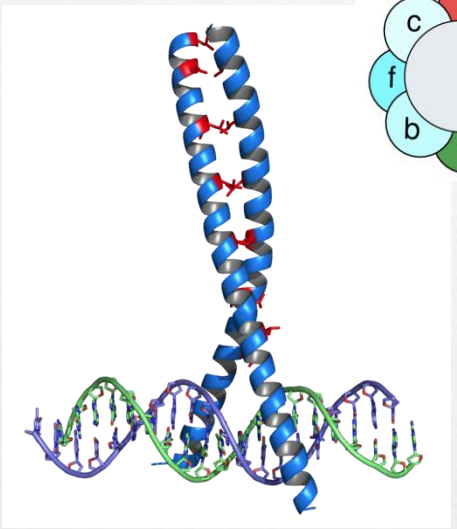
La clé grecque

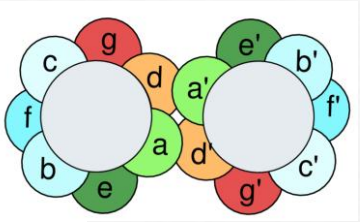


Les motifs en hélice α

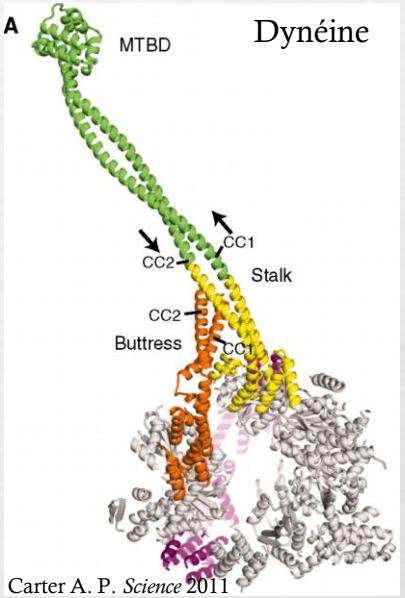
- Hélice Tour Hélice

- Leucine zipper



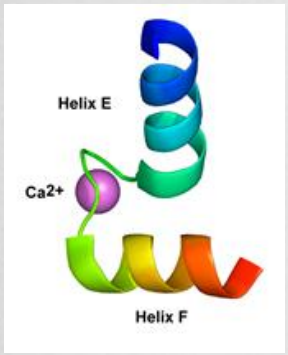


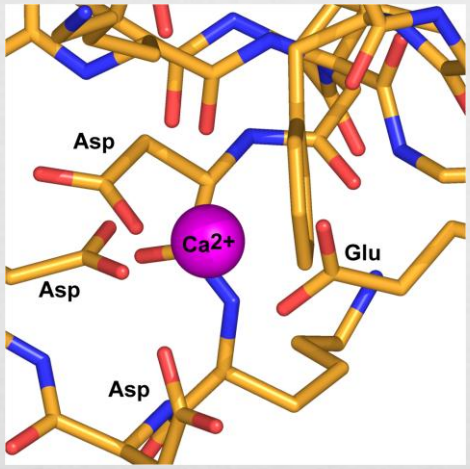
- Coiled-coil

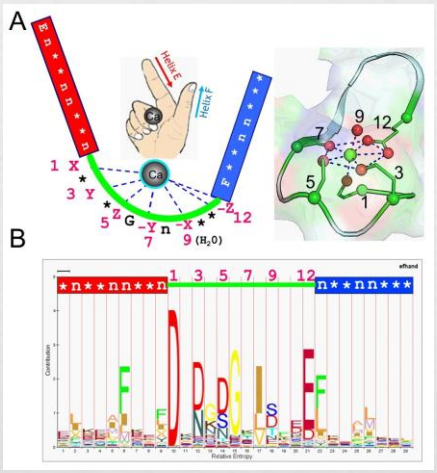


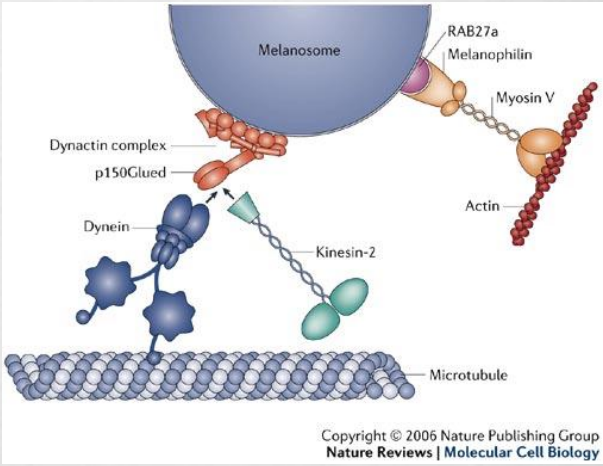
Carter A. P. *Science* 2011

- La main EF





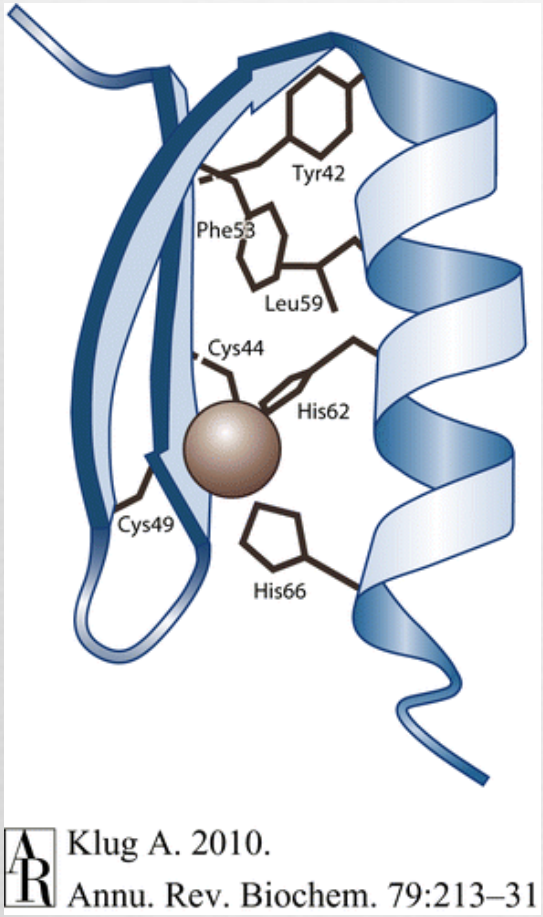




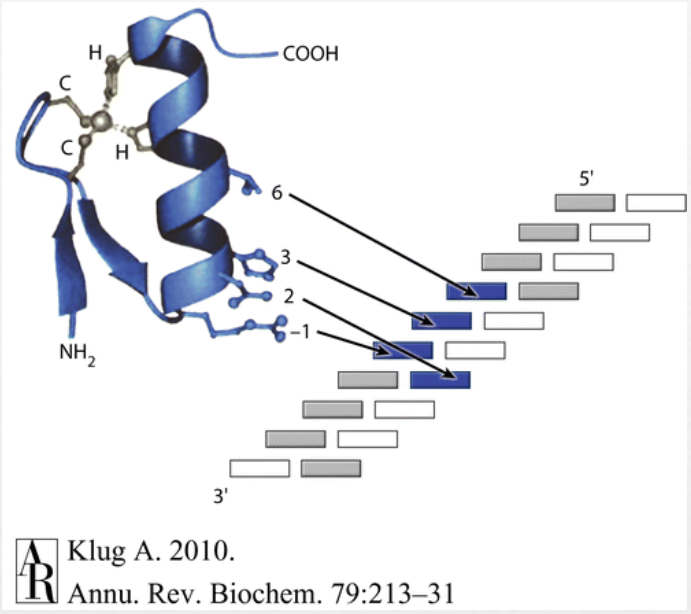
Calcium Binding protein (CBP)

Connaître, comprendre et utiliser

Doigts de Zinc



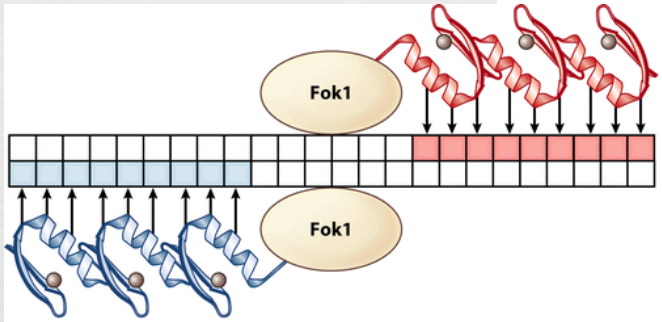
Klug A. 2010.
Annu. Rev. Biochem. 79:213–31



Klug A. 2010.
Annu. Rev. Biochem. 79:213–31

$C\text{-}X_{2,4}\text{-}C\text{-}X_{12}\text{-}H\text{-}X_{3/5}\text{-}H$

ZFNs (Zinc Finger Nucleases)



Klug A. 2010.
Annu. Rev. Biochem. 79:213–31

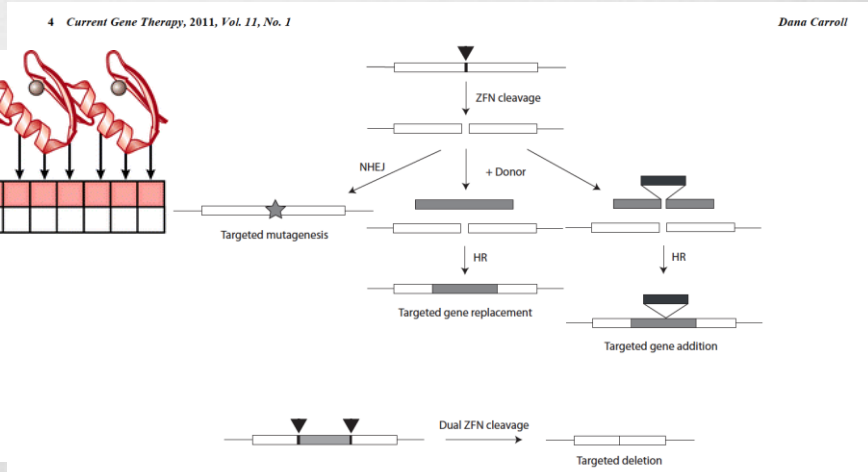
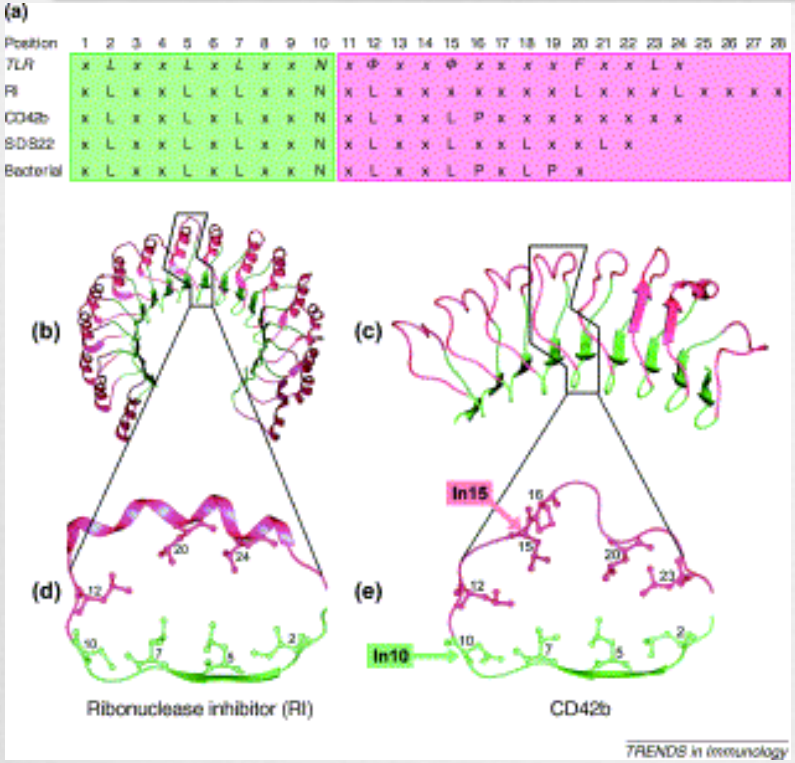
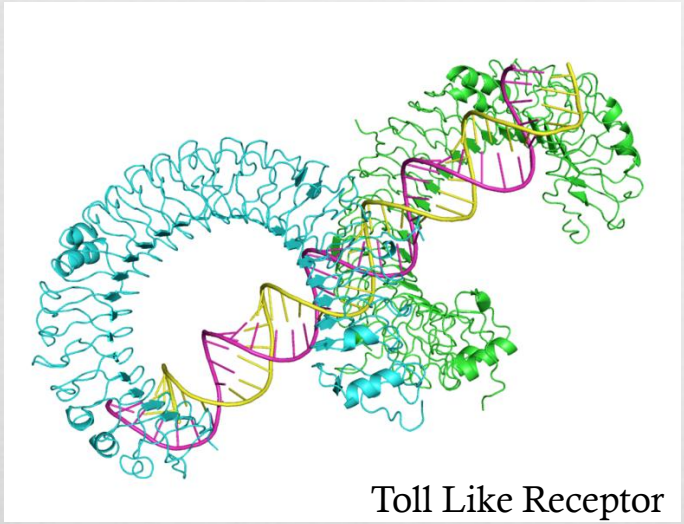


Fig. (2). HR and NHEJ outcomes after ZFN-induced cleavage. Following a single break (top, black triangle), repair can occur by NHEJ to create a sequence change at the target site (left). Alternatively, a donor DNA provided by the experimenter can be used as a template for repair by HR. This can result in gene replacement (center) or sequence addition (right), if the donor carries an insertion. The lower line shows that a deletion can occur between two sites (black triangles) that are both targeted by ZFNs [64].

Répétition et motif : exemple des Leucin Rich Repeat





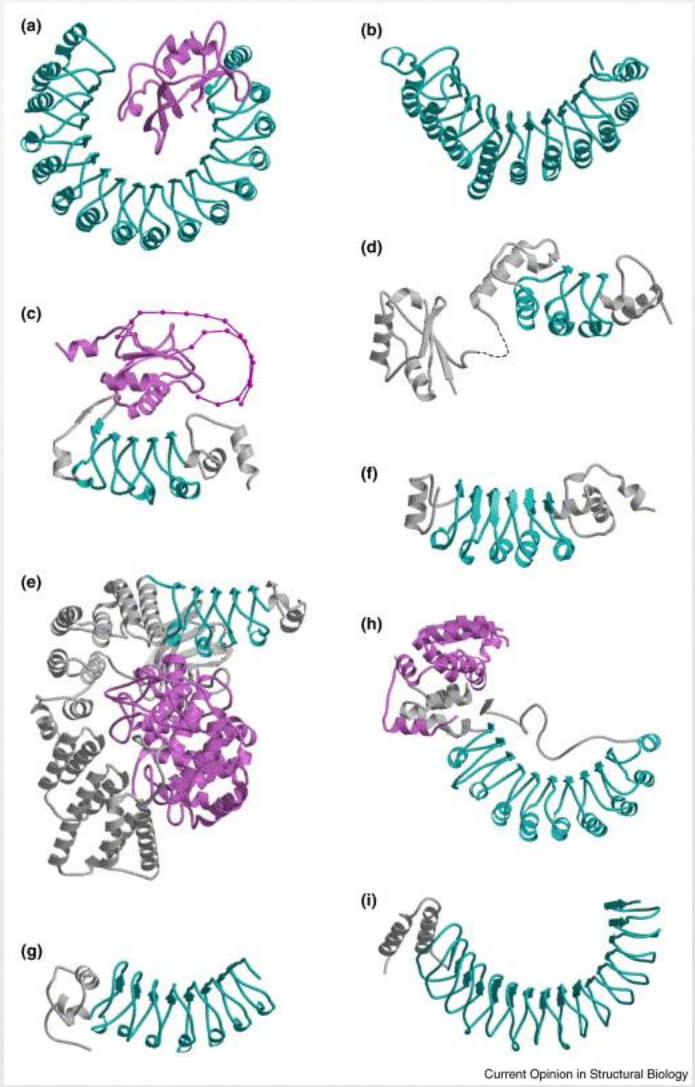
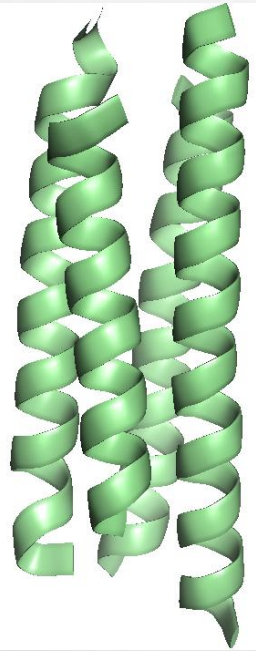
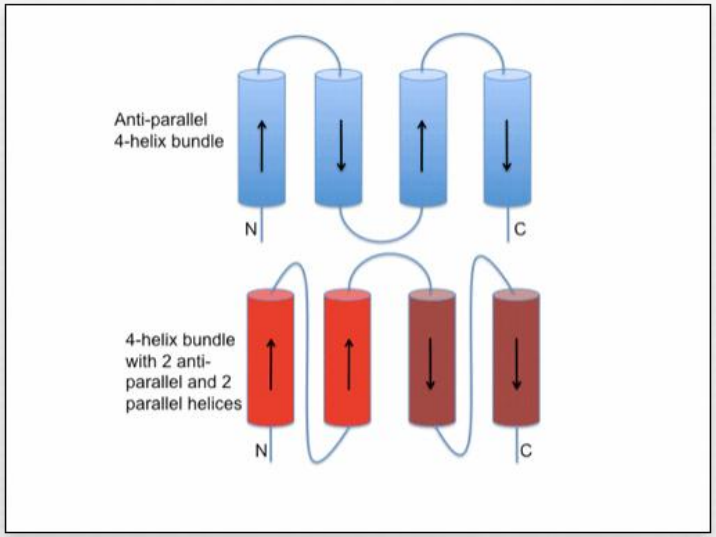
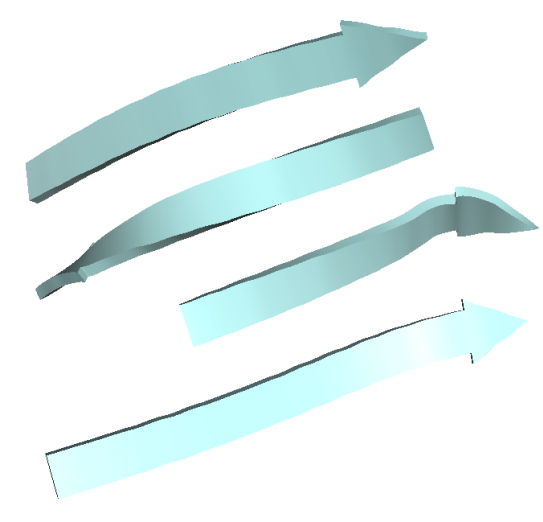


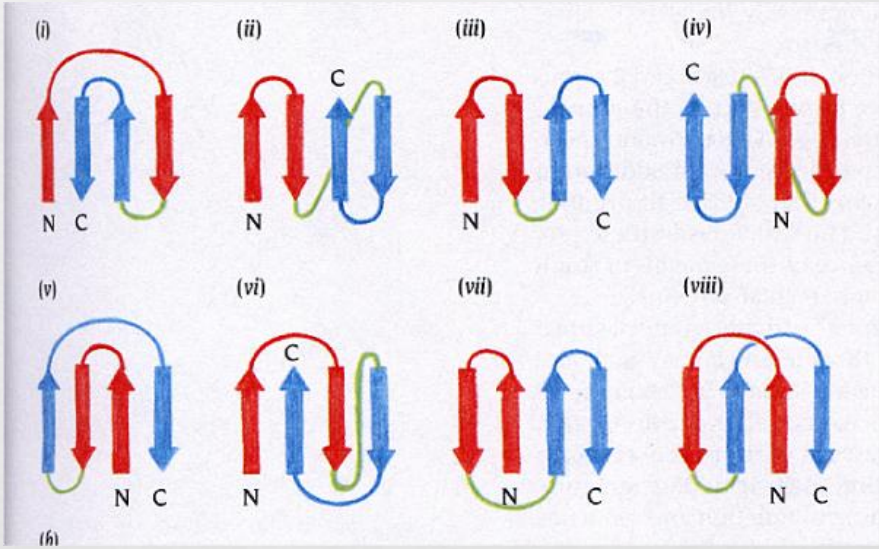
Fig. 1. 3D structures of LRR proteins. The LRR domains are shown in cyan, the flanking regions that are an integral part of the LRR domain but do not correspond to LRR motifs are shown in gray, and the other domains/subunits in the structure are shown in magenta. **(a)** RI (PDB code 2BNH), **(b)**rna1p (PDB code 1YRG), **(c)** U2A′–U2B″ (PDB code 1A9N), **(d)** TAP (PDB code 1FO1), **(e)** RabGGT (PDB code 1DCE), **(f)**dynein LC1 (PDB code 1DS9), **(g)** InlB (PDB code 1D0B), **(h)** Skp2–Skp1 (PDB code 1FQV) and **(i)** YopM (PDB code 1G9U).

Topologie et connectivité









http://www.proteinstructures.com/index.html

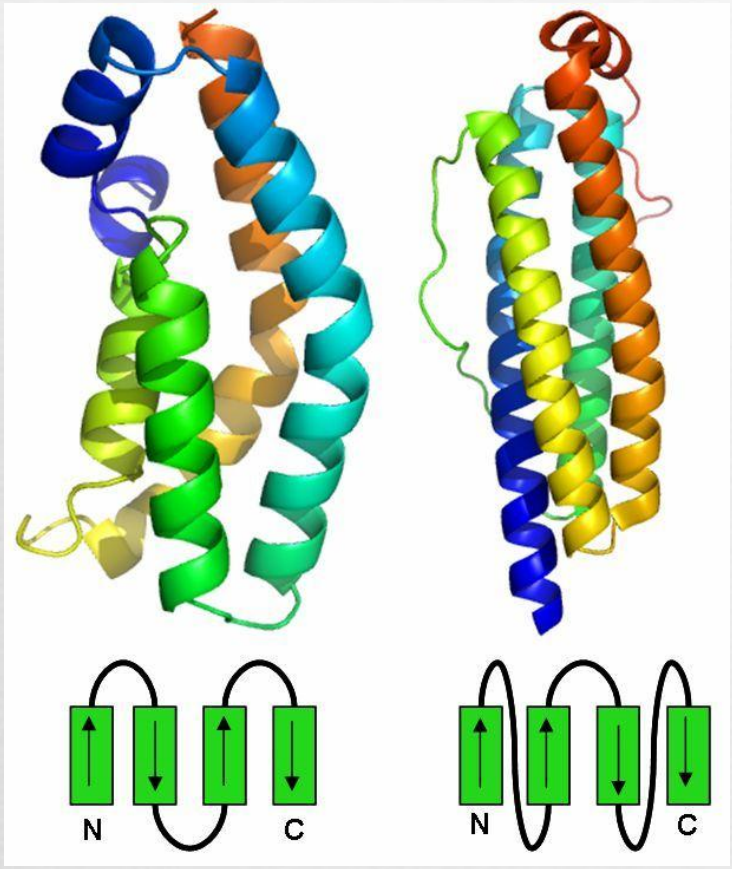
Partie 1 : Repliement et structure tertiaire

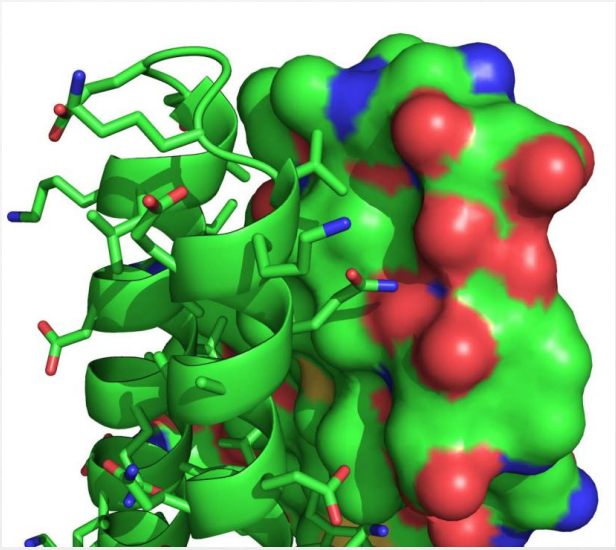
III/ Structure tertiaire

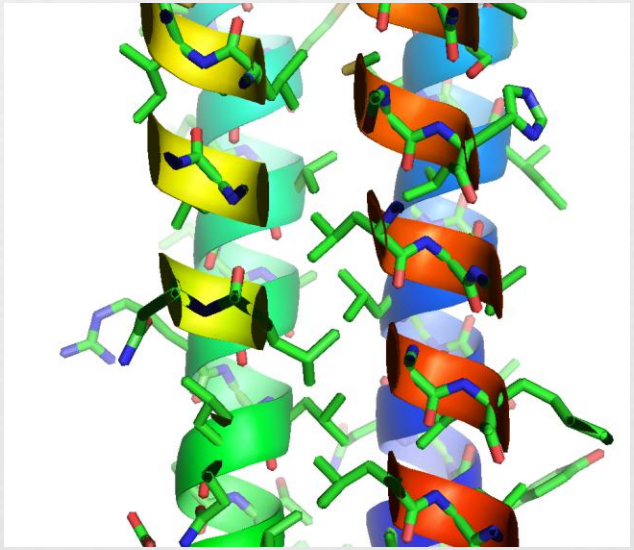
Conservation, classification et repliements remarquables

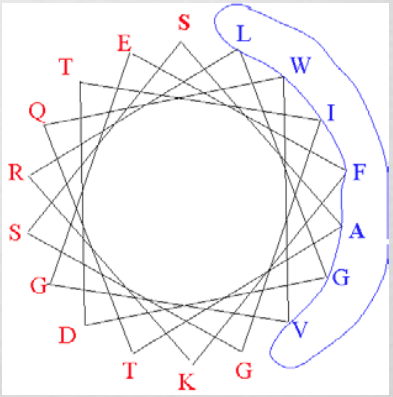
Topologies remarquables

Helix bundle





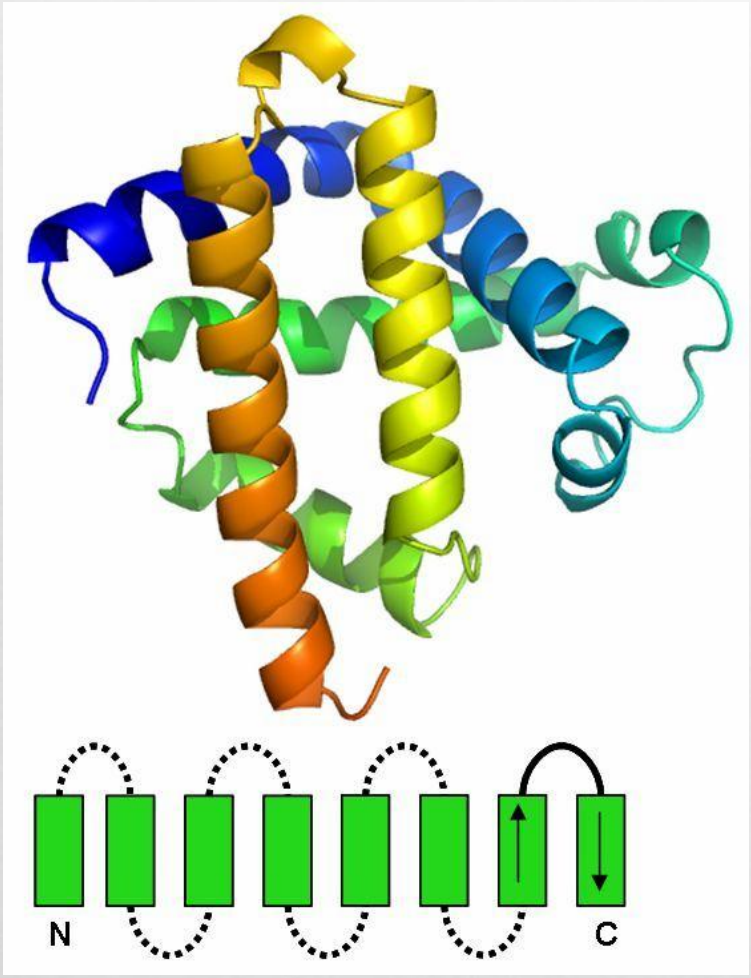


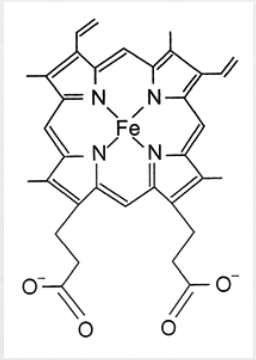


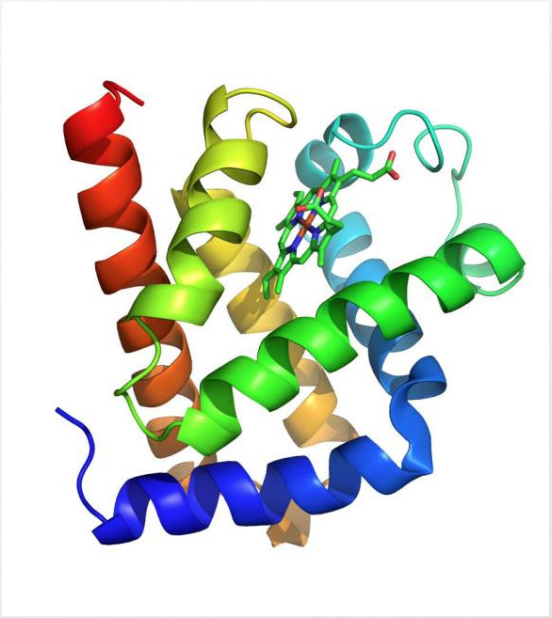
Topologies remarquables

Hélices α perpendiculaires : Globin fold

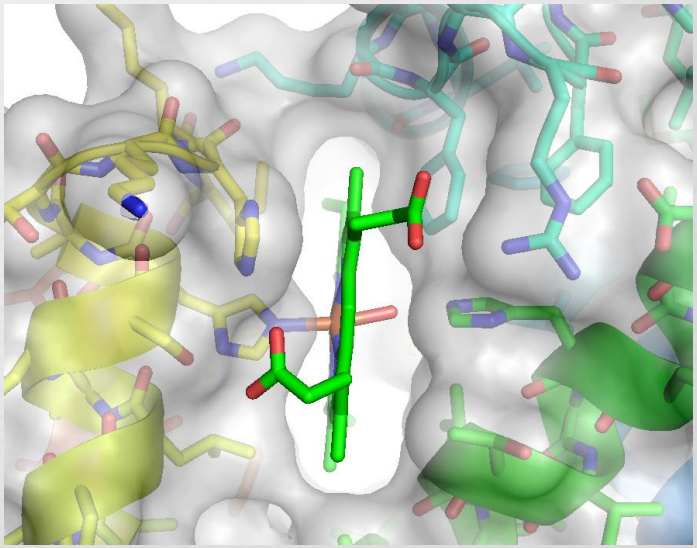
- généralement huit hélices α
- repliement commun à la myoglobine et à l'hémoglobine
- angles 20°, 50° ou 90° (dépendent de la séquence primaire)
- conservation des patchs hydrophobes







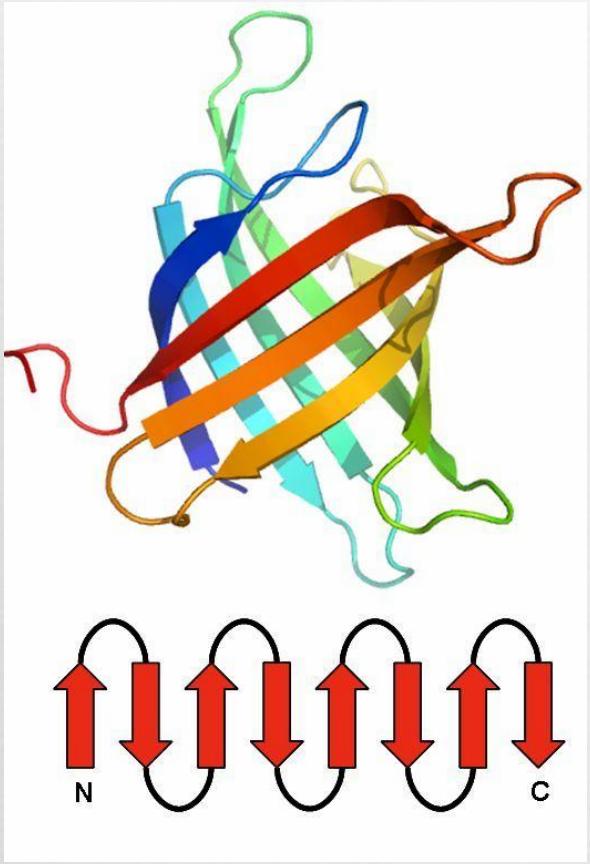
Myoglobine avec hème



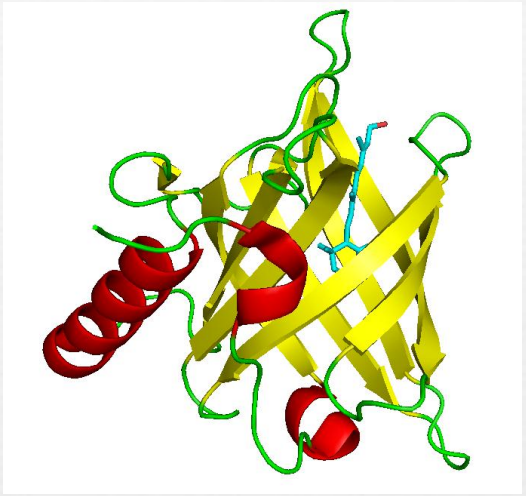
Topologies remarquables

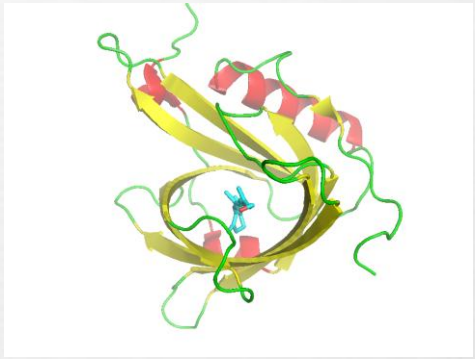
Up-and-down β-barrel

- De 4 à plus de 20 brin antiparallèles

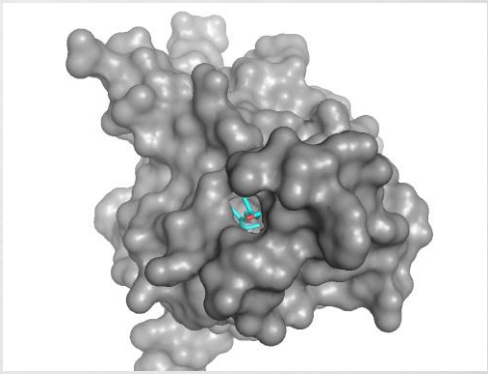


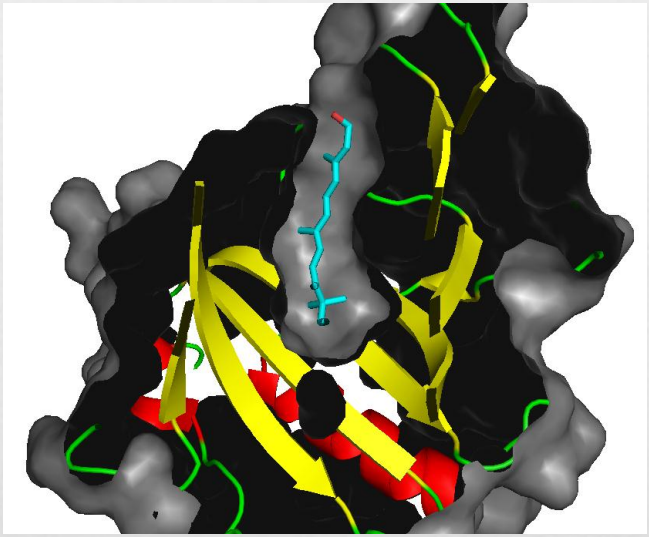
Retinol Binding Protein



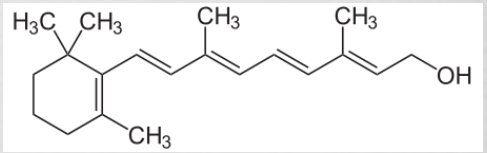


Encombrement stérique





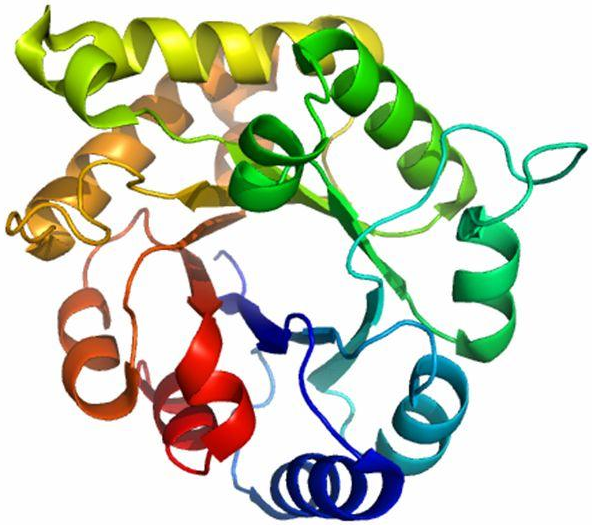
Représentation surface

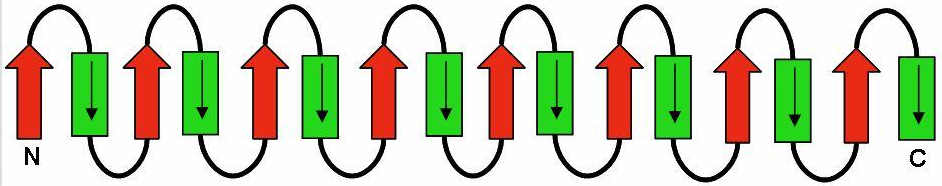


Rétinol

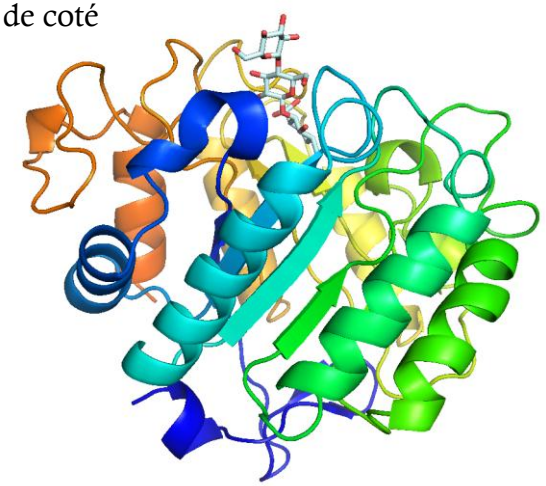
Topologies remarquables

le Tim barrel

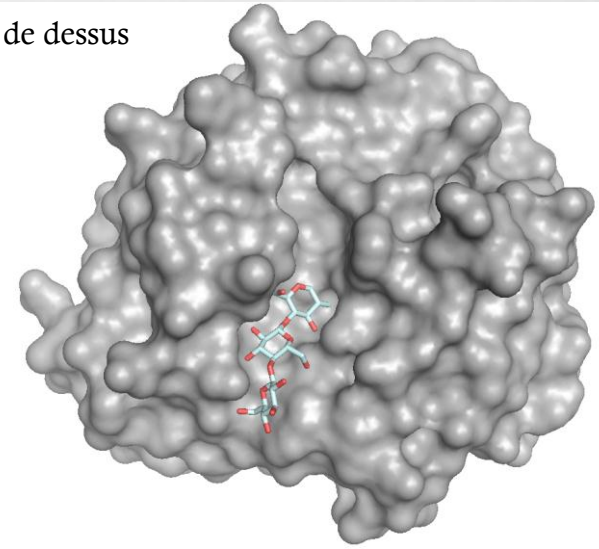




Vue de coté



Vue de dessus

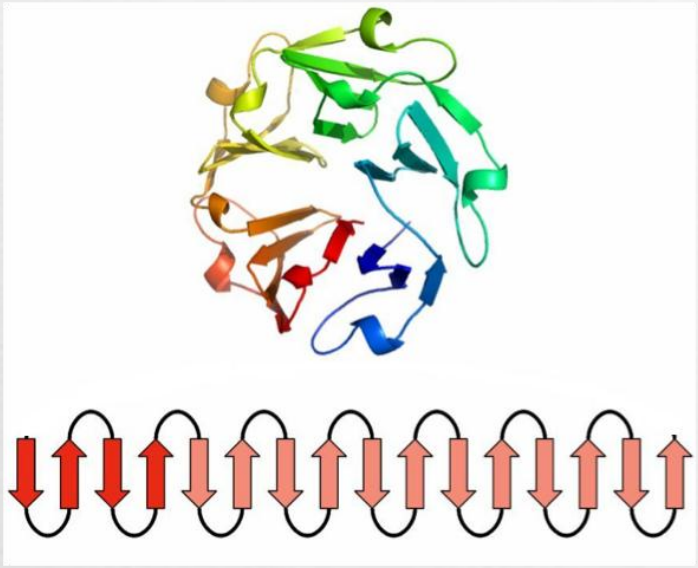


Topologies remarquables

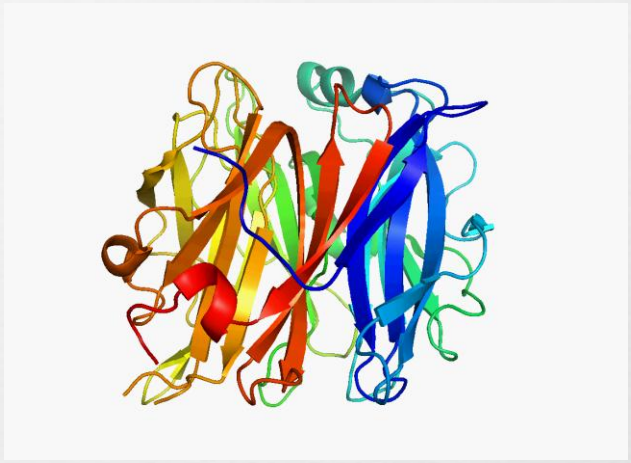
β-propeller (hélice)

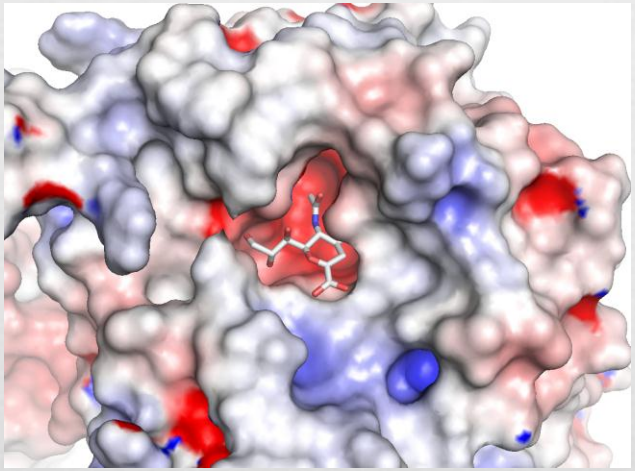
Répétition de 4 à 8 fois du motif (blade/pale) constitué de 4 brins antiparallèles

5-bladed β-propeller



Neuraminidase (sialidase) (1VCU)

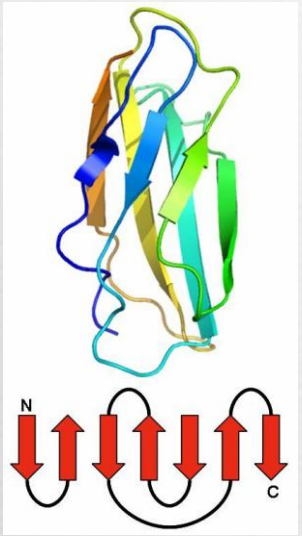




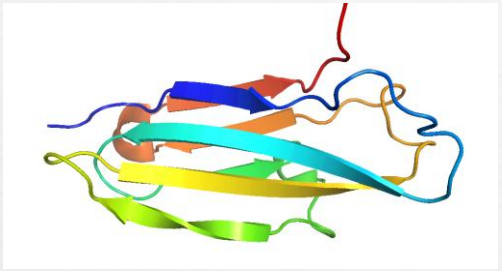
Topologies remarquables

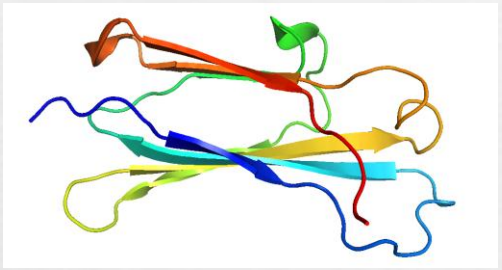
Les sandwichs β:

Ig fold

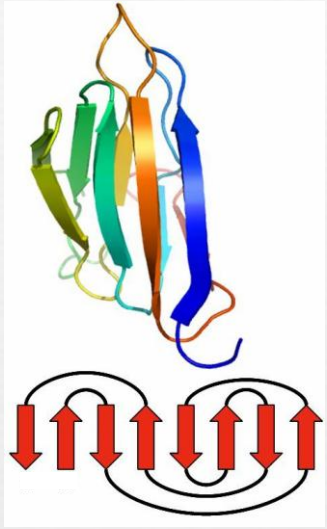


IgG (1FDL)

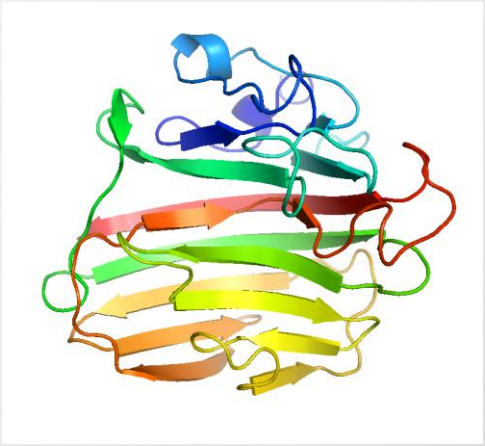


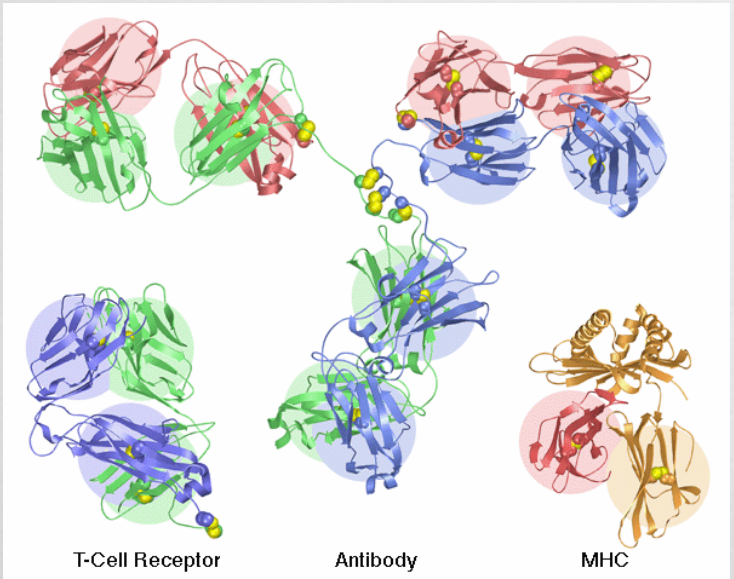


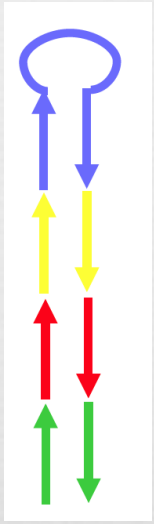
βjelly roll

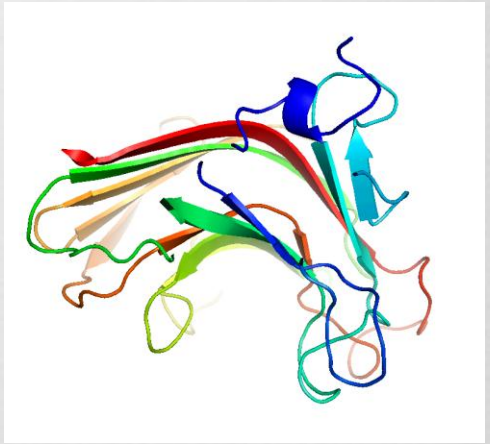


Alginate lyase (1UAI)



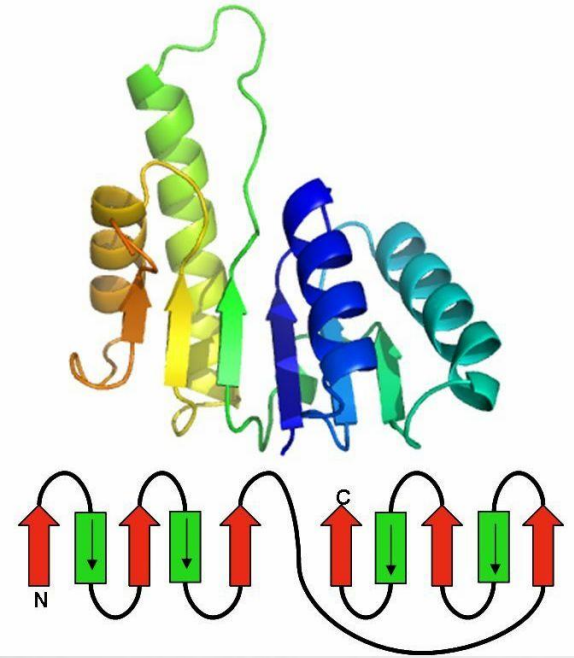






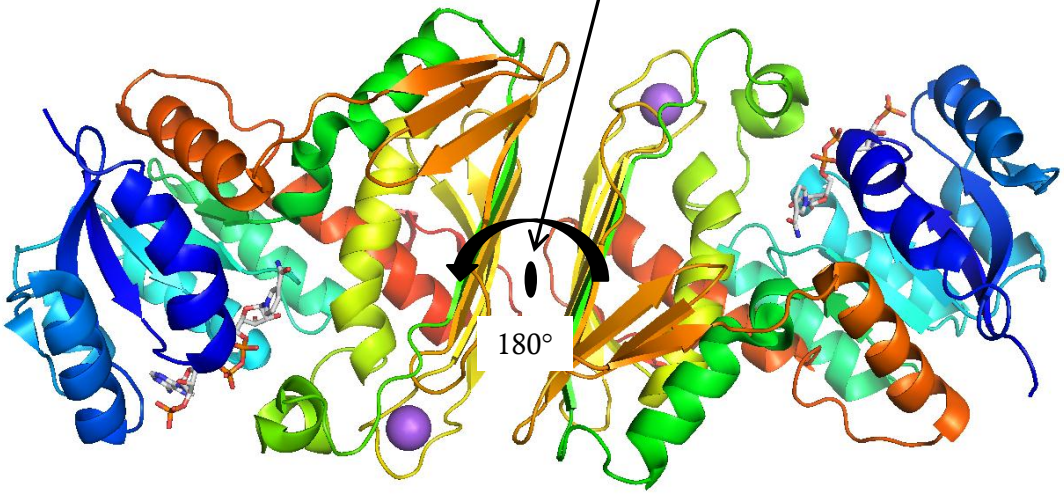
Topologies remarquables

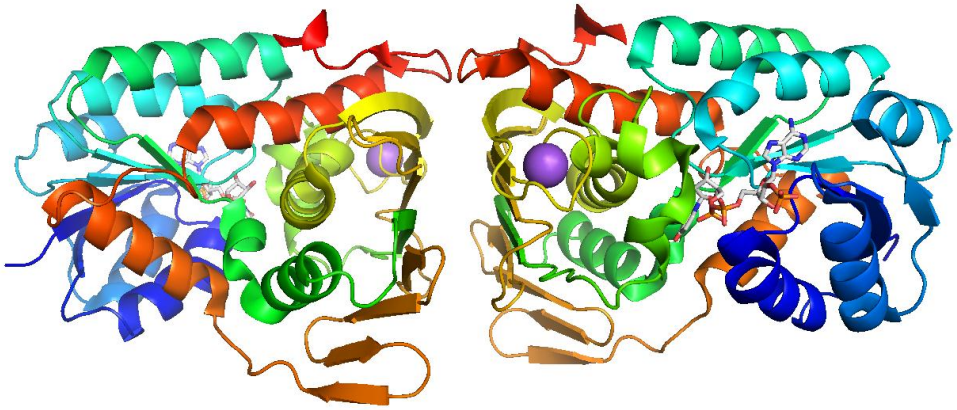
le Rossman fold



Fixe co-facteur

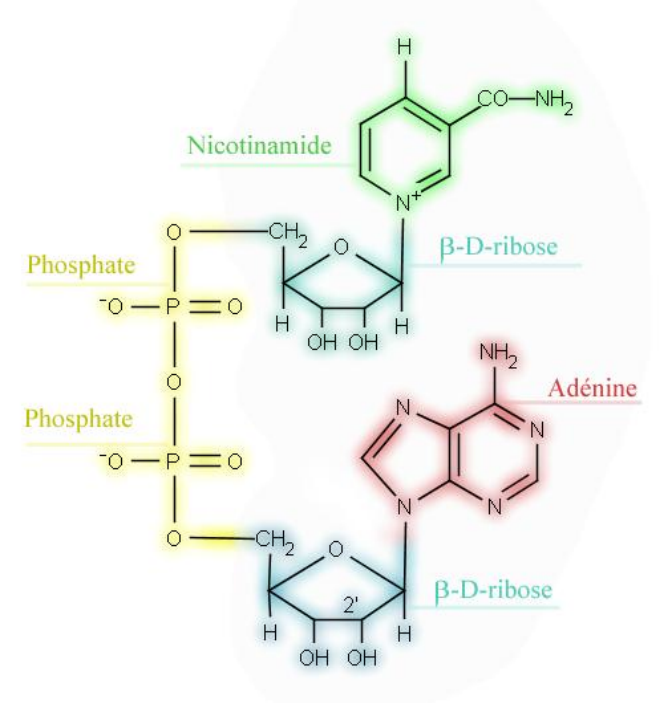
Oxydoreductase (1ZH8)



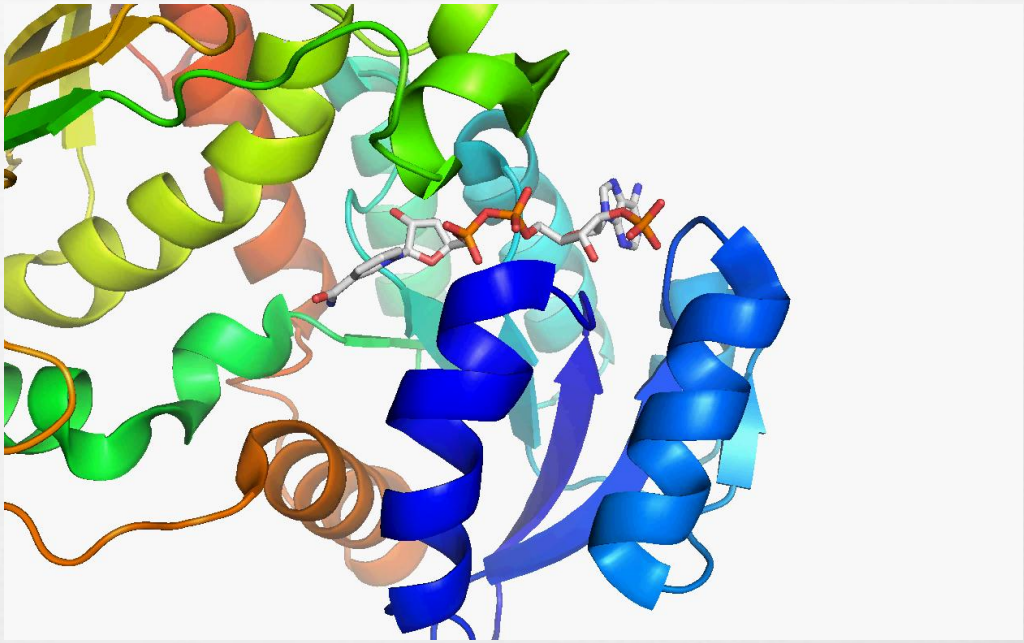


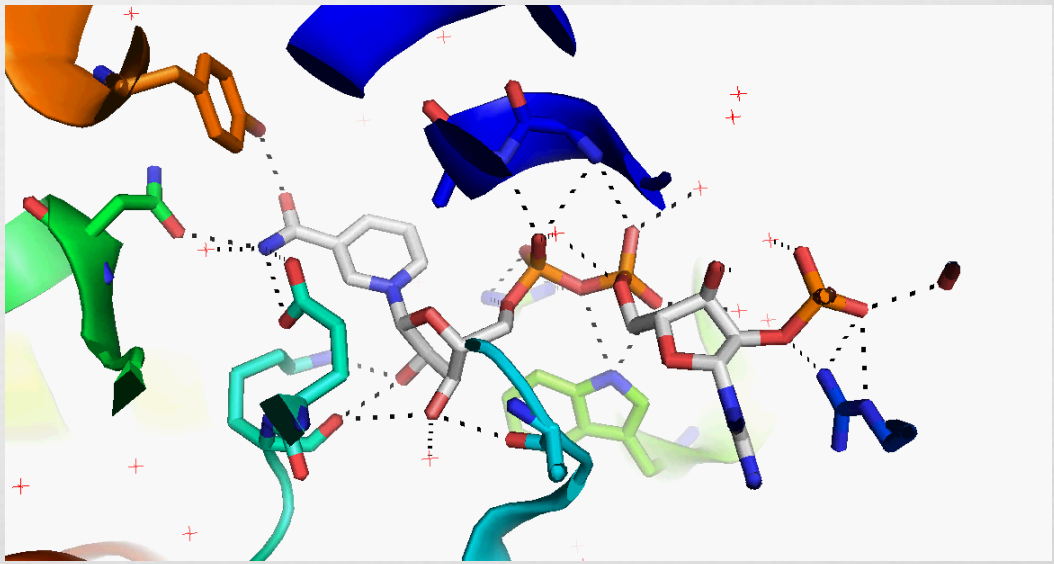
Topologies remarquables

le Rossman fold



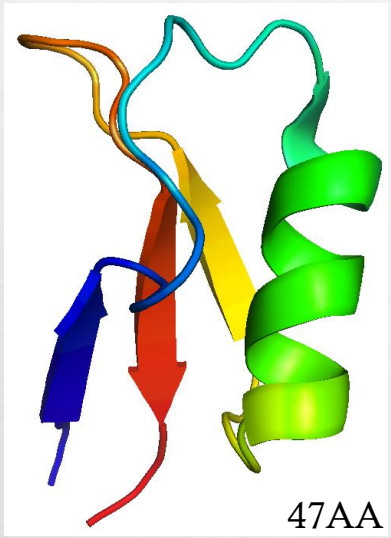
Nicotinamide Adénine dinucléotide



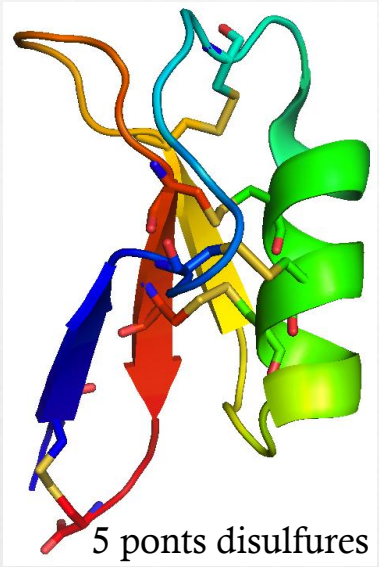


Pont disulfure et petites protéines

Plant Defensin (1N4N)

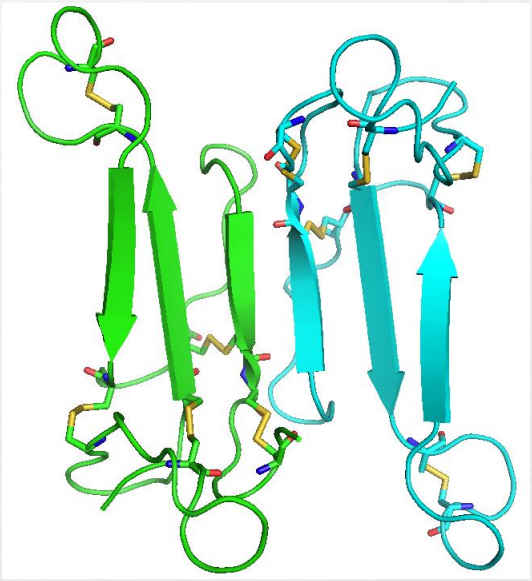


47AA

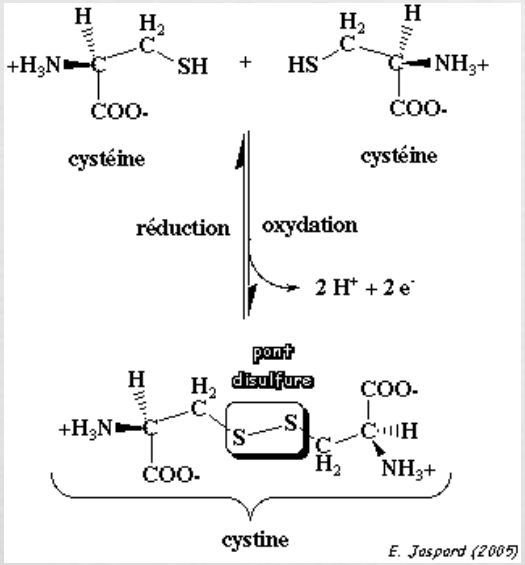


5 ponts disulfures

Bungarotoxine (toxine de serpent) 2NBT



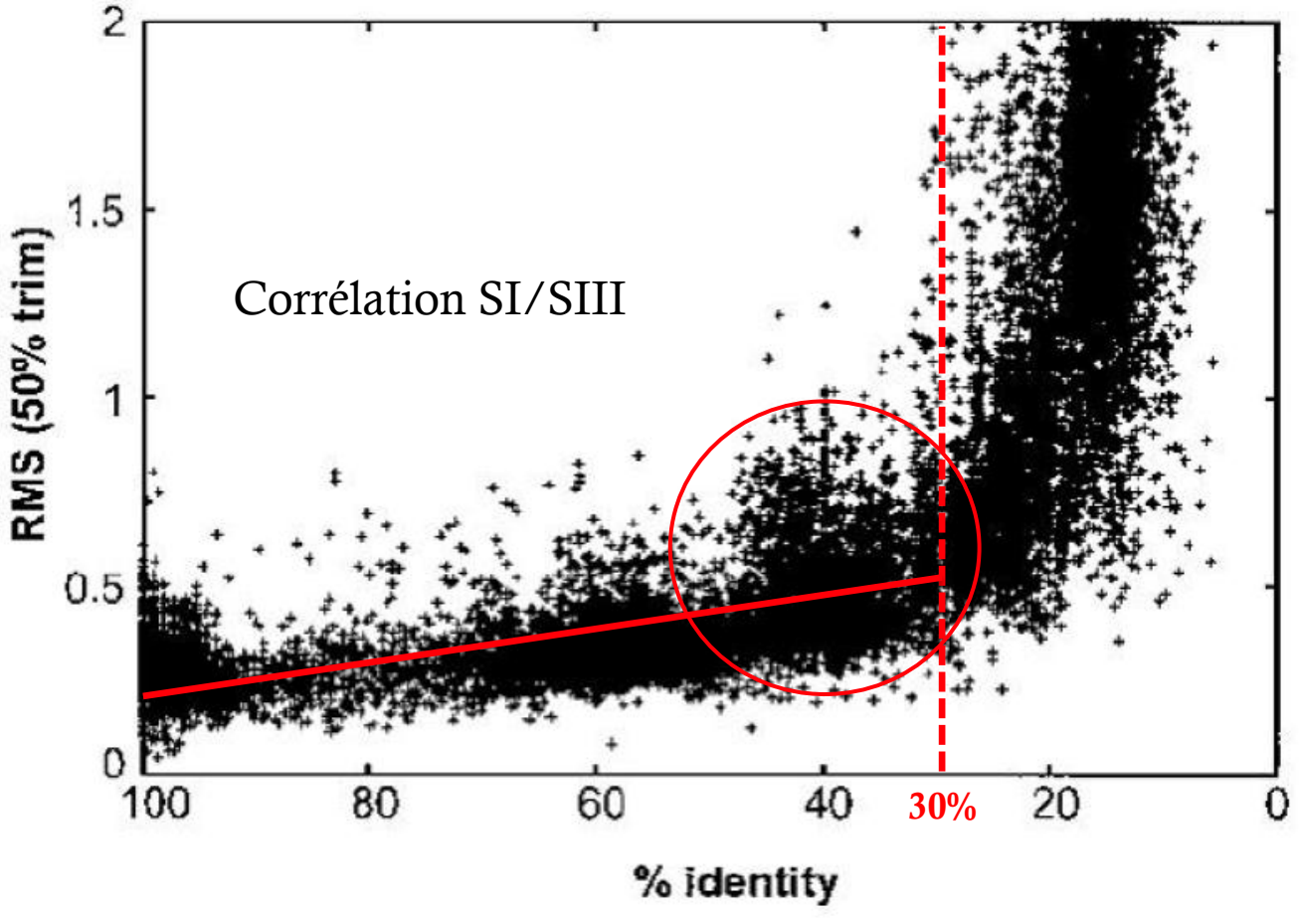
66AA
5 ponts disulfures



Stabilise les petites protéines (intra-caténaires)
Stabilise les structures quaternaires (inter-caténaires)
Typique des protéines secrétées
Rare dans les protéines cytoplasmiques

Identité de séquence et structure

Superposition de la structure III



Conservation de la séquence

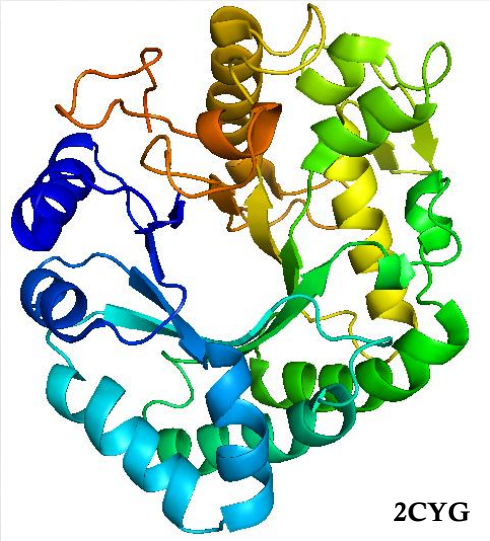
Séquence A (336AA)

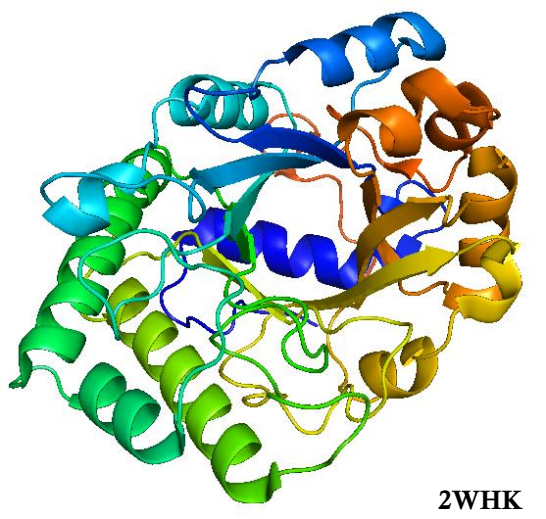
IGVCYGMLGNNLPPPSEVVSLYKSNNIARMRLYDPNQAAL
QALRNSNIQVLLDVPRSDVQSLASNPSAAGDWIRRNVVAY
WPSVSFRYIAVGNELIPGSDLAQYILPAMRNIYNALSSAGL
QNQIKVSTAVDTGVLGTSYPPSAGAFSSAAQAYLSPIVQFL
ASNGAPLLVNVYPYFSYTGNPGQISLPYALFTASGVVVQD
GRFSYQNLFDAIVDAVFAALERVGGANVAVVVSESGWPS
AGGGAEASTSNAQTYNQNLIRHVGGGTPRRPGKEIEAYIF
EMFNENQKAGGIEQNFGLFYPNKQPVYQISF

Séquence B (312AA)

HTVSPVNPNAQQTTKTVMNWLAHLPNRTENRVLSGA
FGGYSHDTFSMAEADRIRSATGQSPAIYGCDYARGW
LETANIEDSIDVSCNGDLMSYWKNGGIPQISLHLANPA
FQSGHFKTPITNDQYKNILDSATAEGKRLNAMLSKIAD
GLQELENQGVPVLFRPLHEMNGEWFWWGLTSYNQK
DNERISLYKQLYKKIYHYMTDTRGLDHLIWVYSPDAN
RDFKTDFYPGASYVDIVGLDAYFQDAYSINGYDQLTA
LNKPFAFTEVGPQTANGSFDYSLFINAIKQKYPKTIYFL
AWNDEWSAAVNKGASALYHDSWTLNKGEIWNGDSL
TPIVE

14% identité





TIM Barrel $(\beta/\alpha)_8$

TIM Barrel $(\beta/\alpha)_8$

Clan Structural

Glycoside Hydrolase 17
β-1,3-glucanase (3.2.1.39)
Eucaryote

Glycoside Hydrolase 26
β-mannanase (3.2.1.78)
Procaryote

Classification des structures

Niveau 1: **C**lasse
Composition en structure secondaire

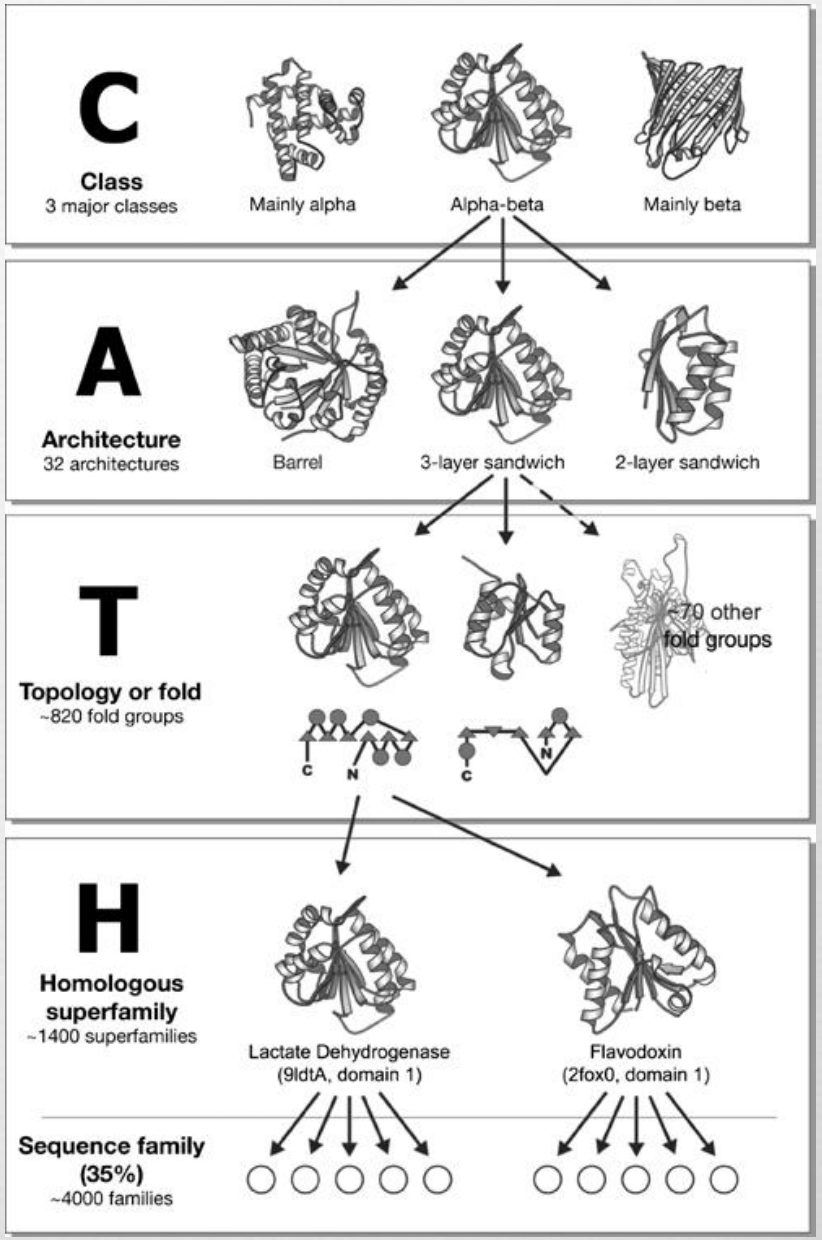
Niveau 2 : **A**rchitecture
Arrangement de structure secondaire sans regarder les connexions

Niveau 3 : **T**opologie
On regarde les connexions entre les éléments de structure secondaire

Niveau 4 : **H**omologie
Recherche d'identité de séquence (ancêtre commun)

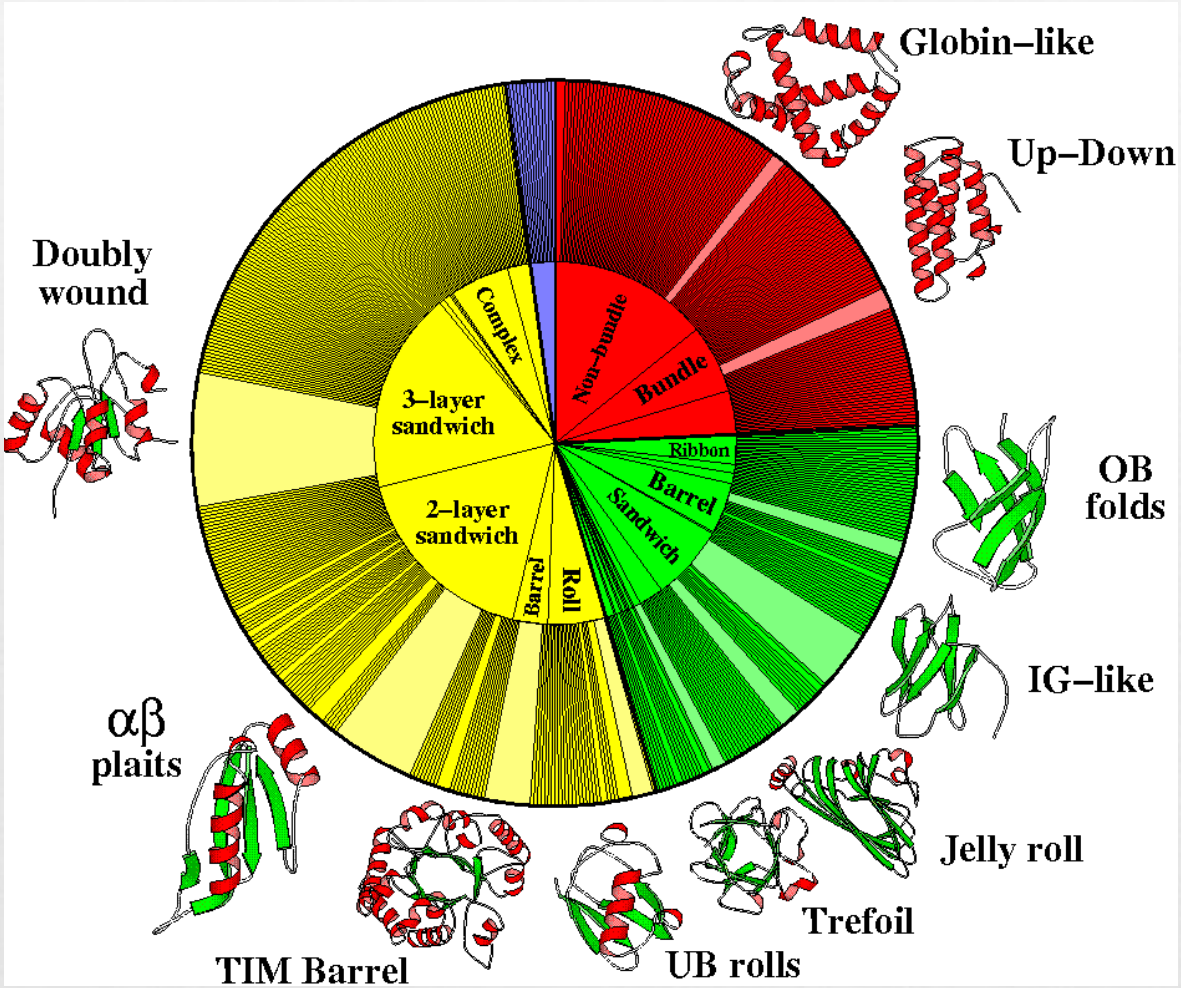
Niveau 5 : Séquence
Sélection stricte dans l'identité de séquence

http://www.cathdb.info/



Annu Rev Biochem. 2005;74:867-900.
Protein families and their evolution-a structural perspective.
Orengo CA, Thornton JM.

Classification des structures



α/β

Majoritairement α

Majoritairement β

SCOP (http://scop.mrc-lmb.cam.ac.uk/scop)
CATH (http://www.biochem.ucl.ac.uk/bsm/cath/)

Partie 1 : Repliement et structure tertiaire

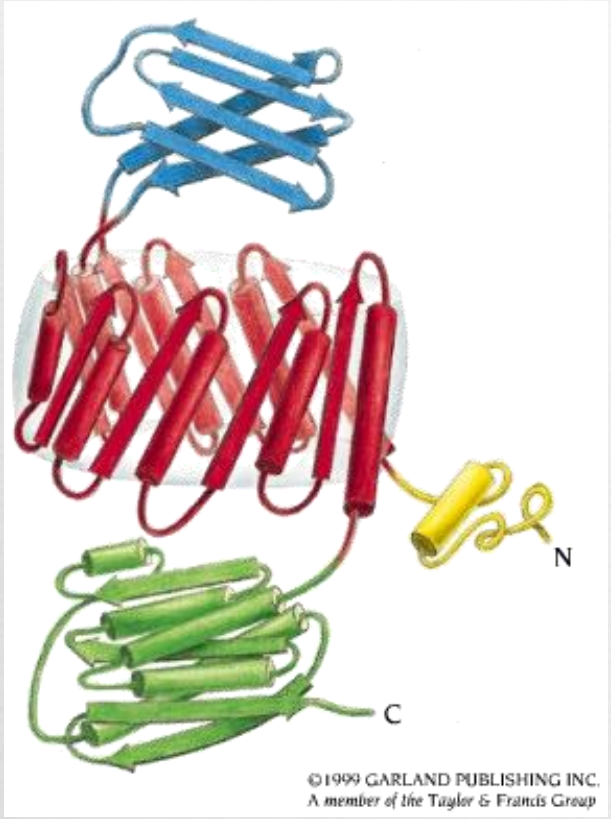
IV/ Modularité

Les domaines d'interactions

ubiquité et spécificité

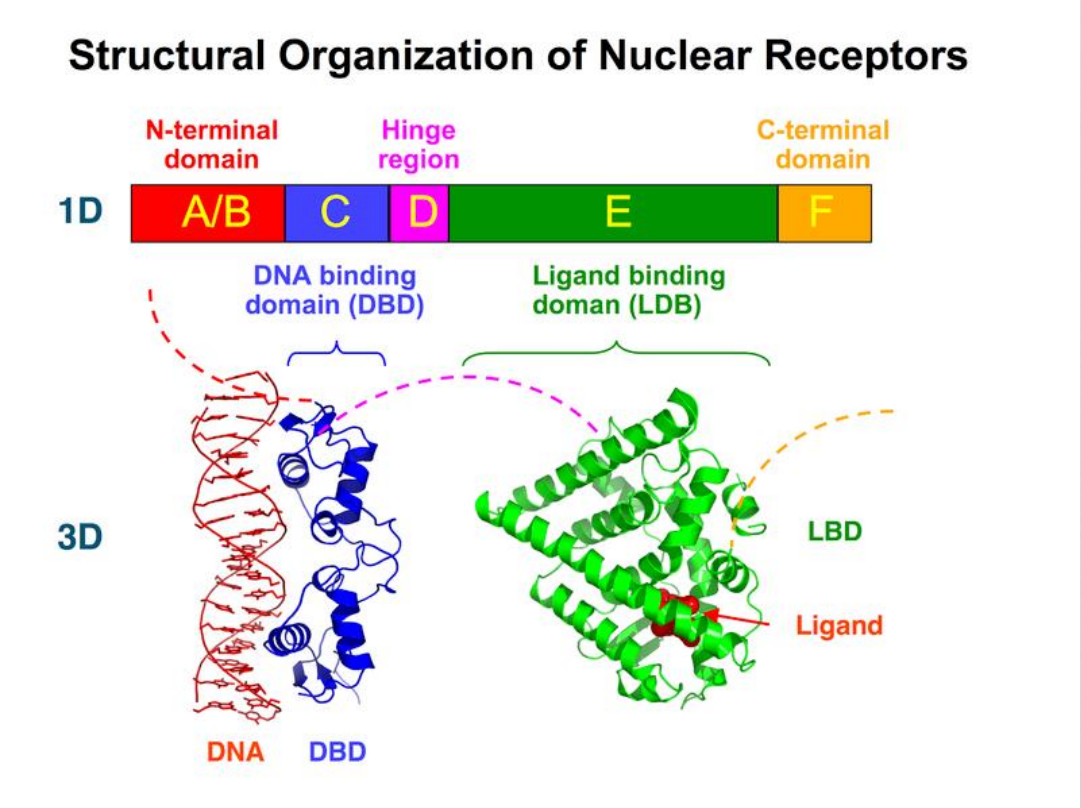
Structure III et modularité

Certaines protéines peuvent être modulaires et présentées plusieurs domaines



Domaine : repliement qui présente une structure (fonction) indépendante du reste de la protéine

Exemple des récepteurs nucléaires :



Les domaines d'interaction

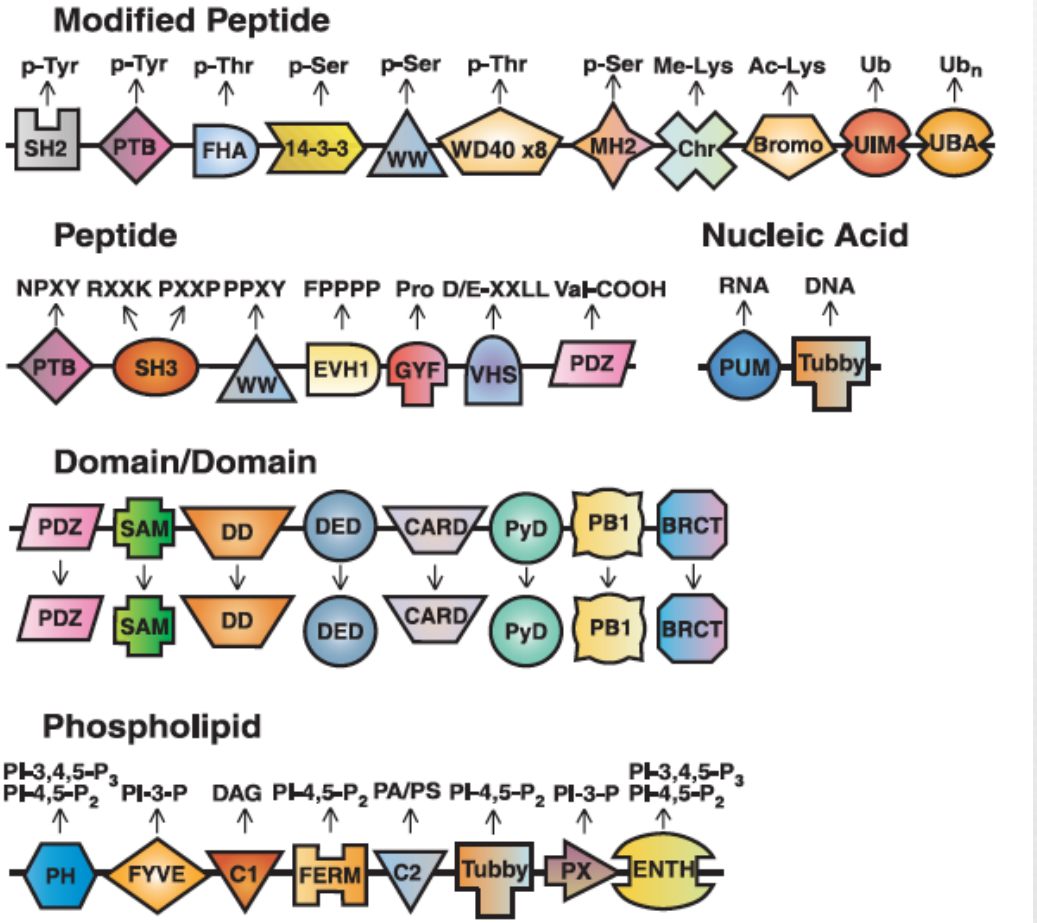


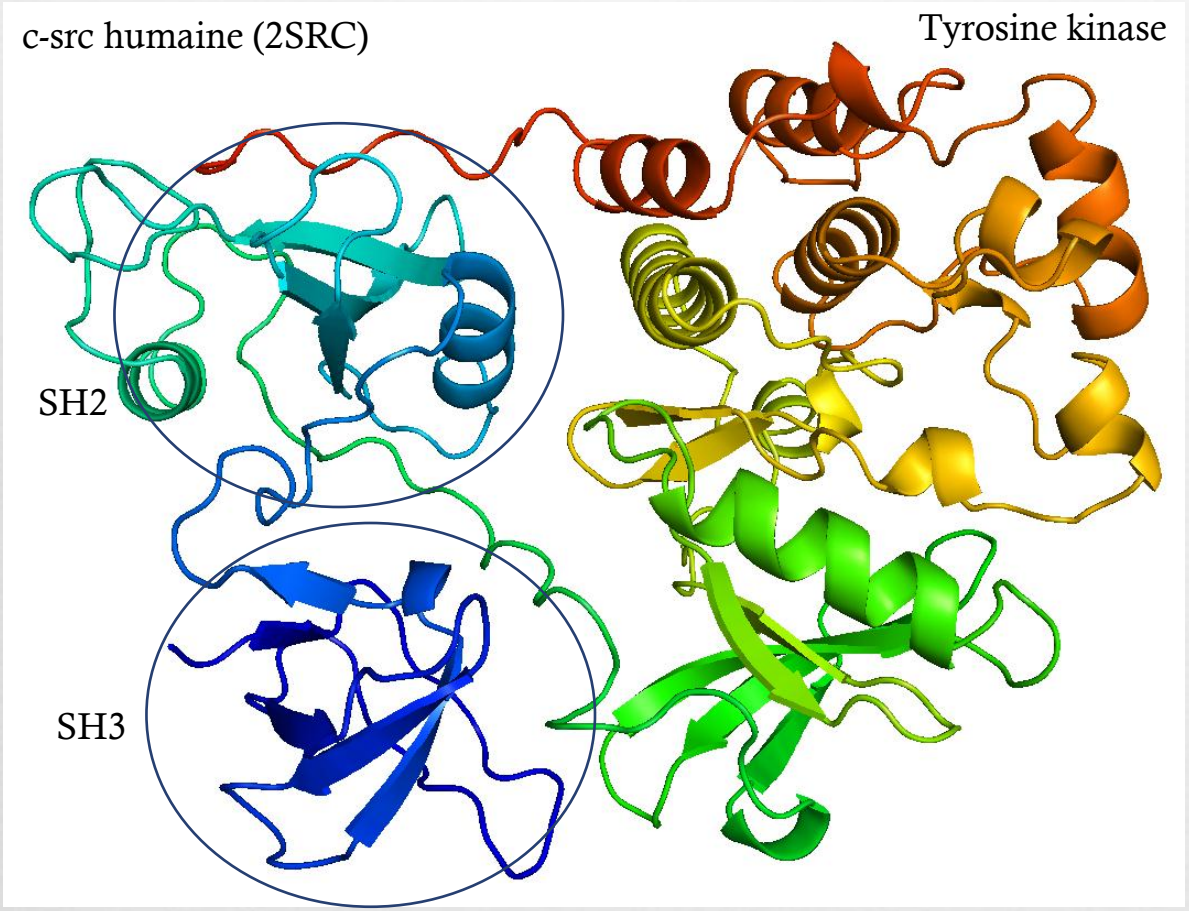
Fig. 1. The building blocks—modular interaction domains in signal transduction. Interaction domains bind proteins, phospholipids, or nucleic acids. A subset of such domains is illustrated and their general binding functions are indicated. For more information, see www.mshri.on.ca/pawson/research and http://smart.embl-heidelberg.de.

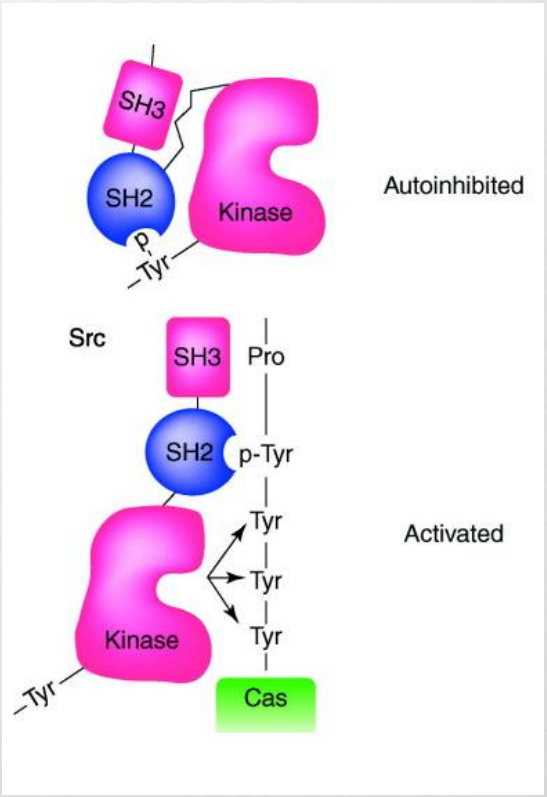
Pawson *et al. Science* 300, 445 (2003)

Src

Protéine tyrosine kinase
Src : sarcome ⟶ proto-oncogène

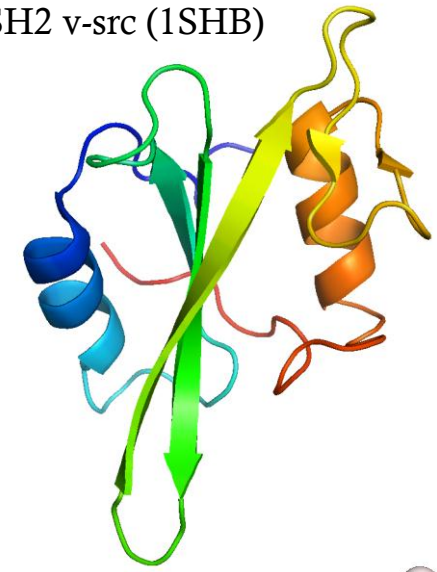
Src **H**omology



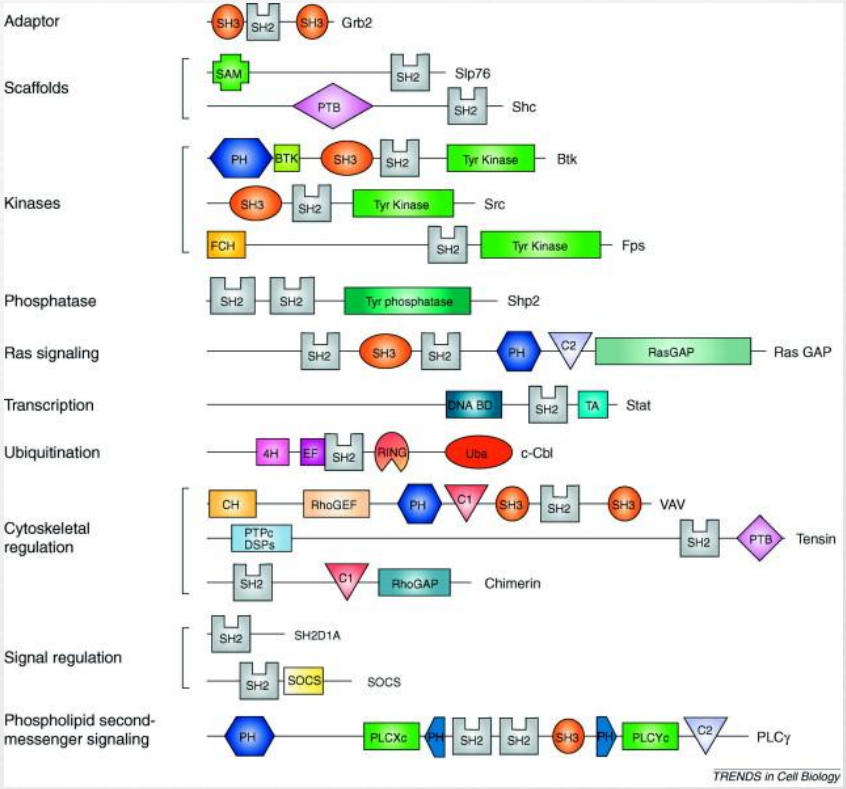


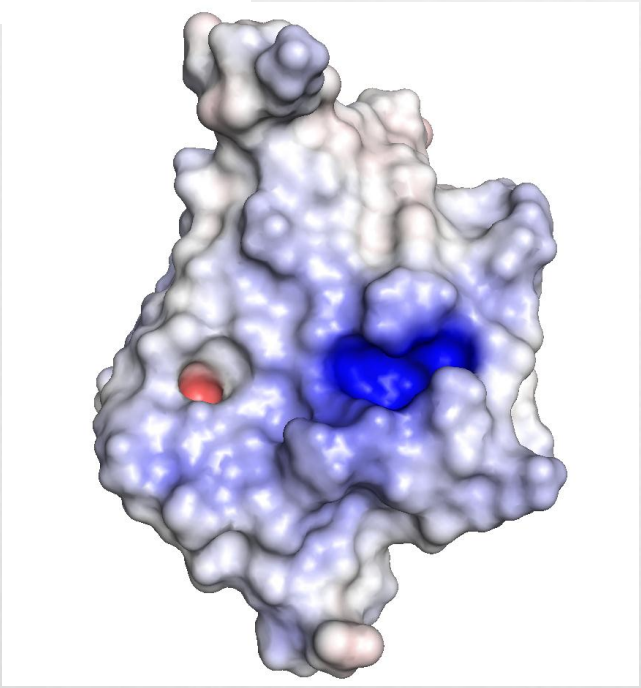
SH2

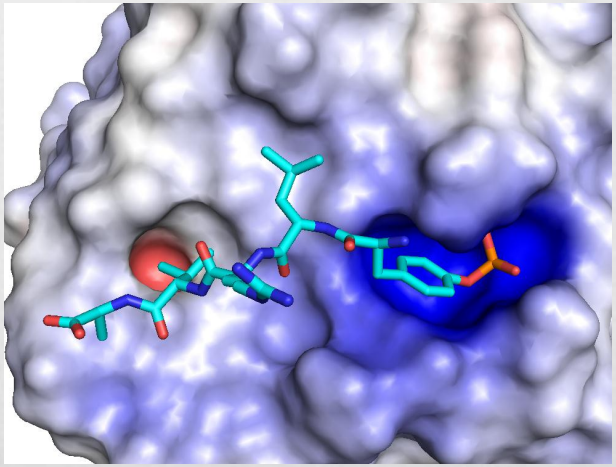
SH2 v-src (1SHB)



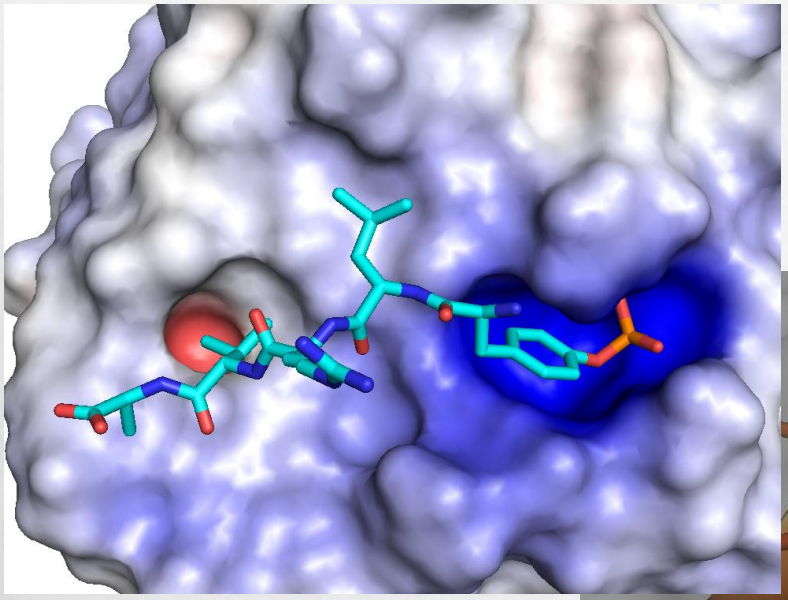
100aine AA
> 100 copies chez l'homme

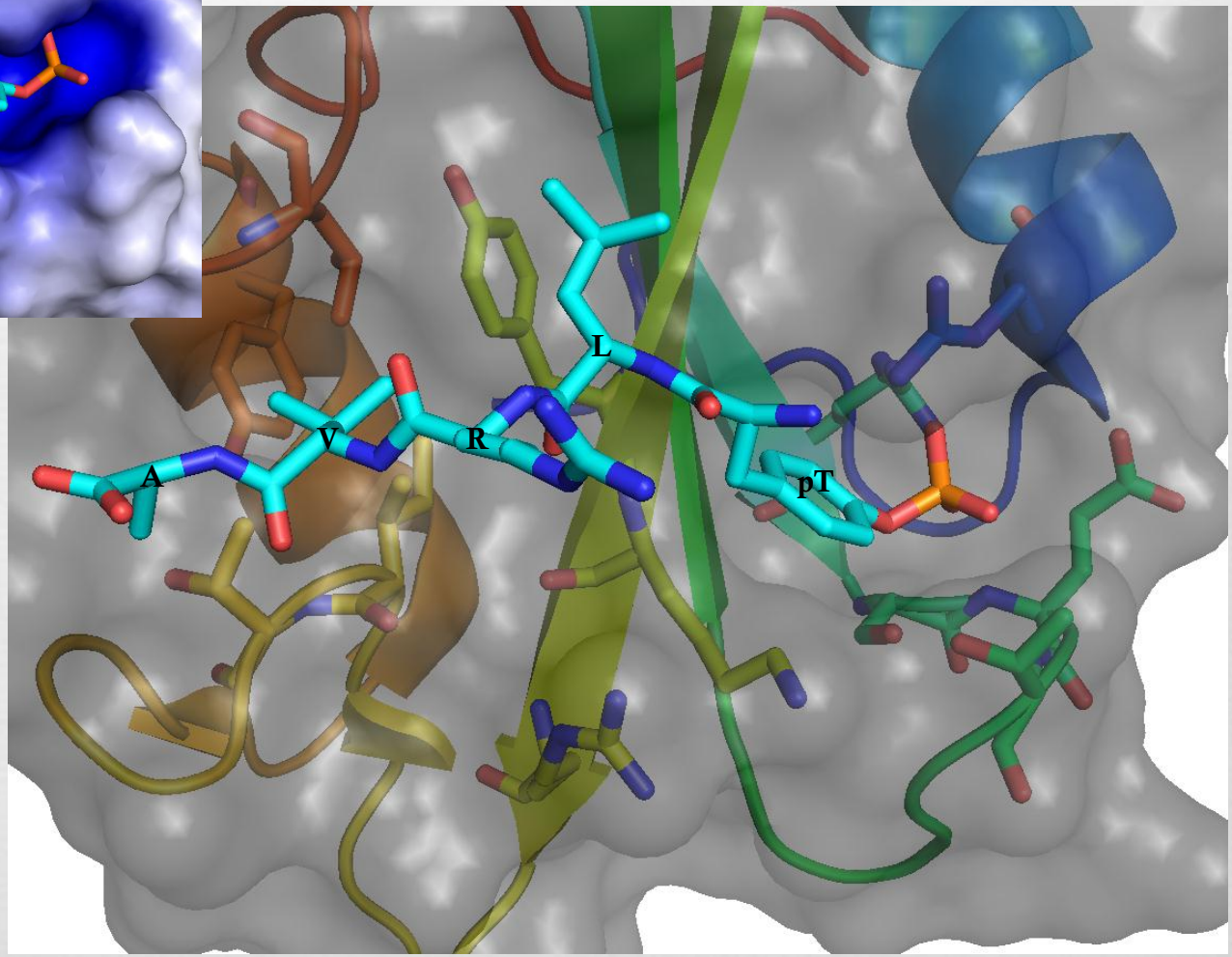






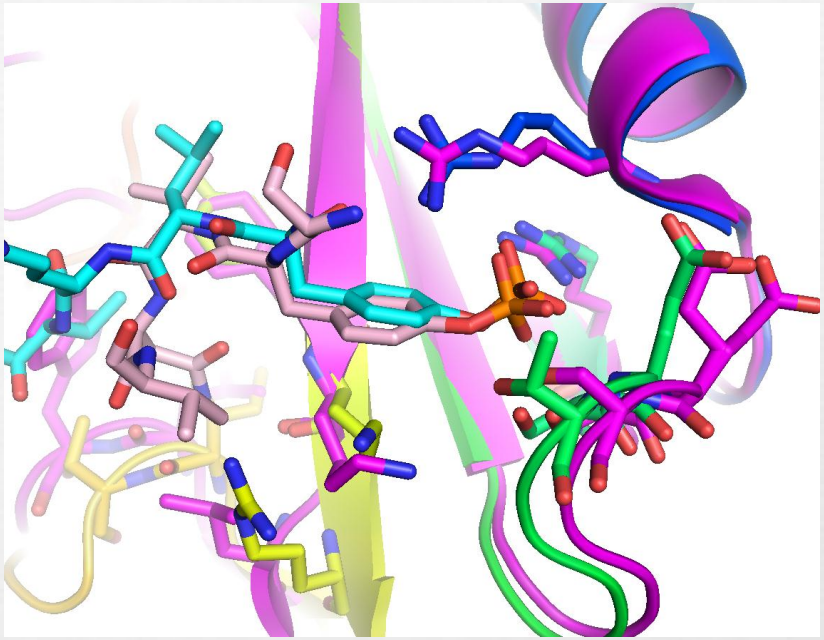
SH2

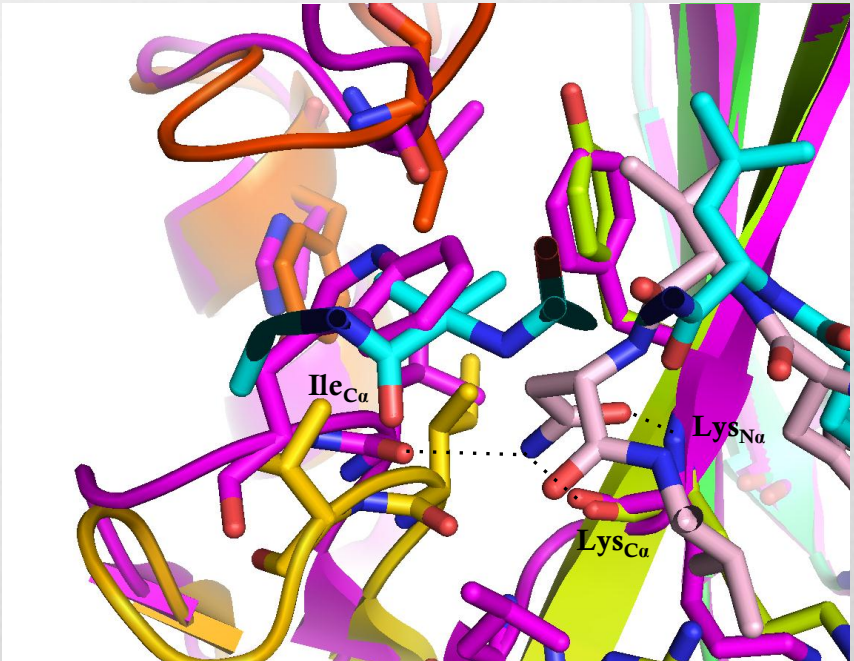




SH2-src : pTyr-Glu-Glu-Ile

SH2





SH2-src : pTyr-Glu-Glu-Ile

SH2-Grb2 : pTyr-X-Asn