I – Les protéines

1- Les acides aminés
   A- Structure générale
   B- Propriétés acido-basiques et optiques
2- Les peptides
   A- Définitions
   B- Structure primaire
   C- La liaison peptidique
   D- Exemples de peptides
3- La structure des protéines

II – Les Enzymes

1 - Introduction – Définitions

2 - Les cofacteurs enzymatiques
   A - biotine (ou vitamine B8)
   B - Nicotinamide Adénine Dinucléotide (NAD^+)

3 - La réaction enzymatique
   A - réaction non-catalysée
   B - catalyse enzymatique
   C - notion de site actif
   D - introduction à la cinétique enzymatique
   E – mesures enzymatiques : quantification d'une biomolécule

III – Techniques de Purification et d'Analyse

1 - Solubilisation – extraction des protéines

2 - Précipitation différentielle
   A - précipitation isoélectrique
   B - précipitation par des sels

3 - Techniques chromatographiques
   A - échange d'ions
   B - exclusion / diffusion
   C - affinité

4 - Techniques électrophorétiques
   A - électrophorèse sur papier
   B - électrophorèse sur gel de polyacrylamide

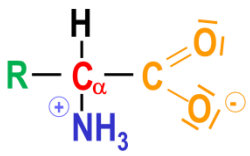
5 - Technique immunoenzymatiques

IV – Les Acides Nucléiques

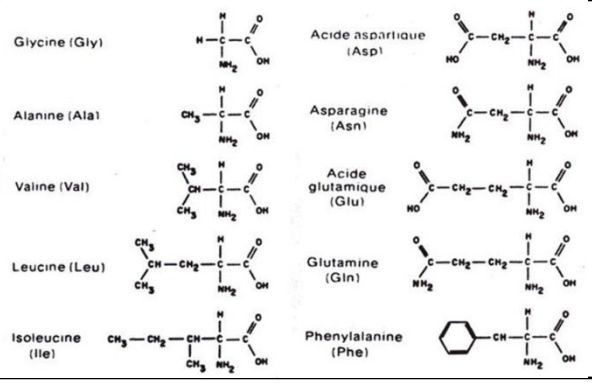
1- Bases azotées
   A- 2 sortes de bases : purines et pyrimidines
   B- Absorbance dans l'U.V.
   C- Densité de charge
2- Nucléosides et nucléotides
   A- Liaison avec 2 types de sucres
   B- Modification avec l'acide phosphorique
   C- Nomenclature
3- Structures spatiales
   A- Association des nucléotides dans un acide nucléique
   B- Complémentarité des bases
   C- Double hélice/Propriétés
   D- Modifications chimiques des acides nucléiques
4- Des nucléotides remarquables : ATP, AMPc et GMP
5- Séquençage de l'ADN et PCR

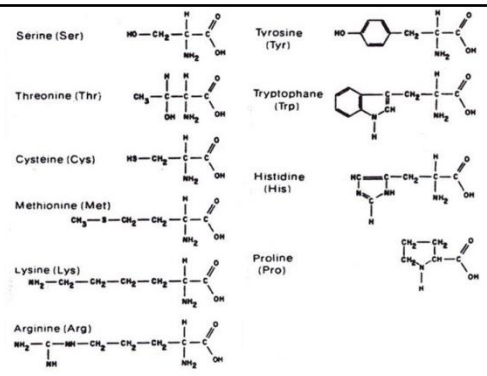
V – Les lipides

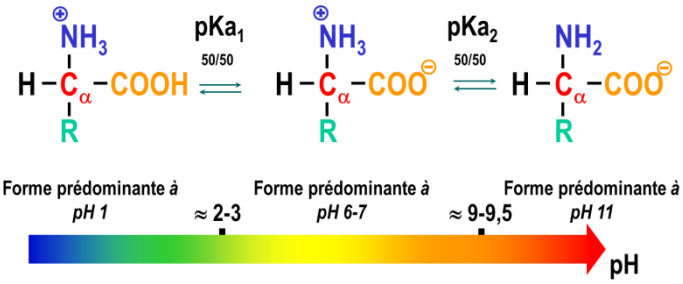
1- Introduction
2- Nature et propriétés des acides gras
3- Lipides contenant des acides gras
4- Lipides dérivés d'acides gras
5- Lipides issus d'unités isopréniques
6- Purification des lipides

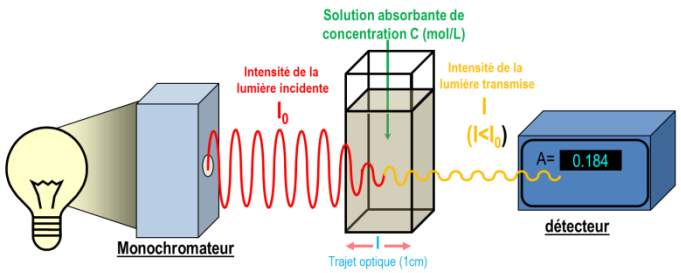


Symbole	Code 3 lettres	Nom
A	Ala	Alanine
C	Cys	Cystéine
D	Asp	Aspartate
E	Glu	Glutamate
F	Phe	Phénylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Méthionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Sérine
T	Thr	Thréonine
V	Val	Valine
W	Trp	Tryptophane
Y	Tyr	Tyrosine

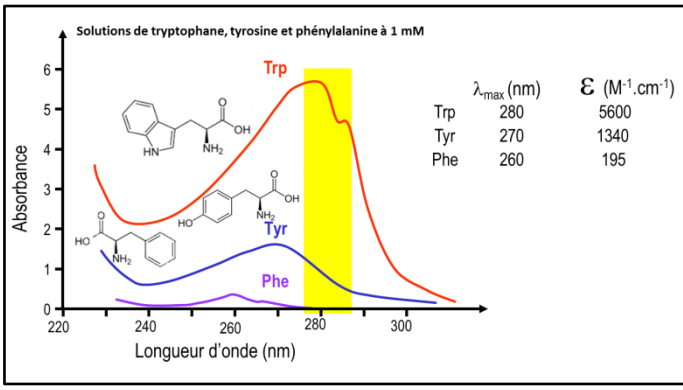


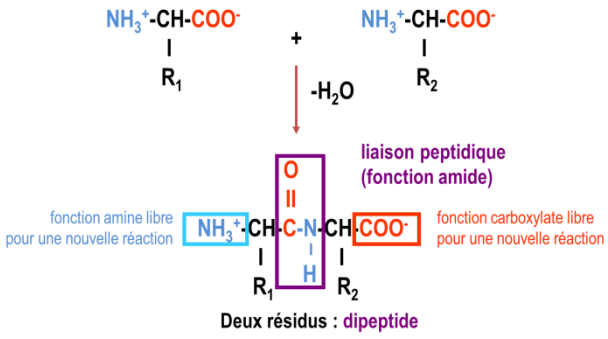


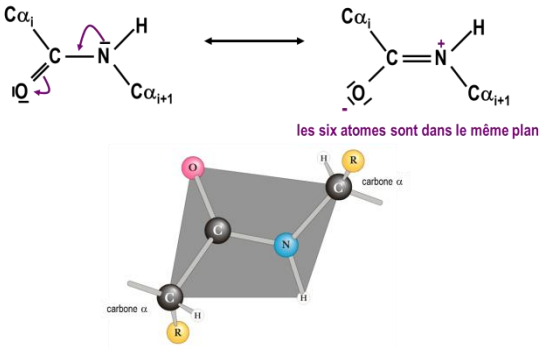


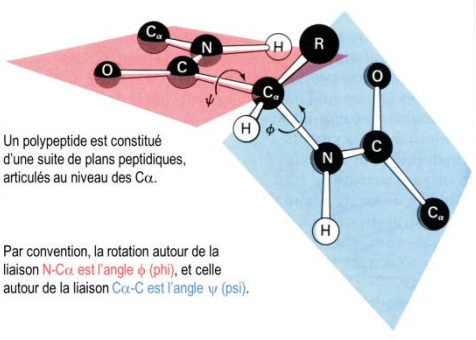


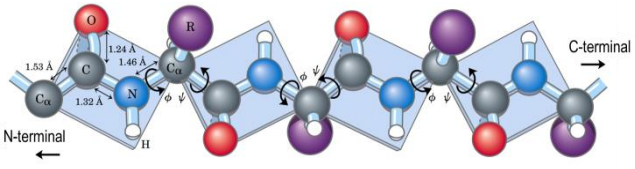
- La fraction de la lumière incidente absorbée par une solution à une longueur d'onde donnée dépend :
  1- de l'épaisseur de la solution que la lumière doit traverser (trajet optique)
  2- de la concentration de la solution en espèces absorbantes

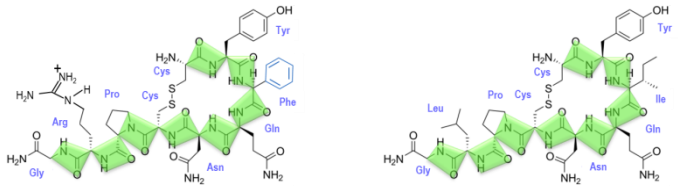




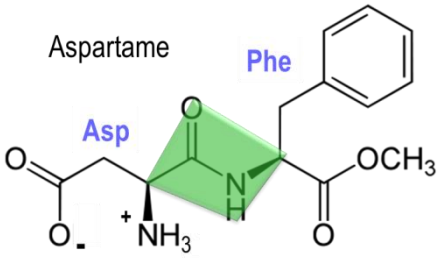


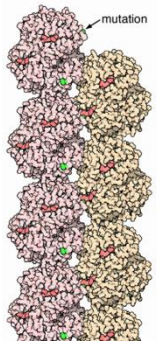






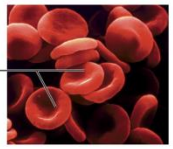
Hormones peptidiques post-hypophysaires



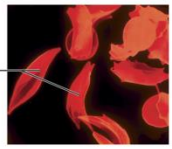


L'anémie falciforme (1ère maladie génétique dans le monde) résulte d'une mutation sur le gène codant l'hémoglobine : l'hémoglobine modifiée (E6V sur la chaine β) va s'agréger dans la cellule, surtout lorsque la [O_2] est faible.

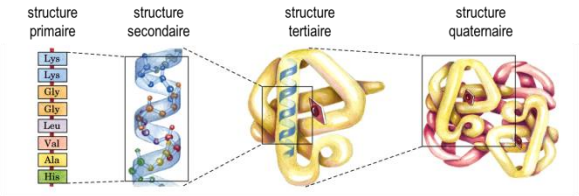
Globules rouges avec hémoglobine normale



Globules rouges avec hémoglobine mutée



Différents niveaux de structuration pour une protéine

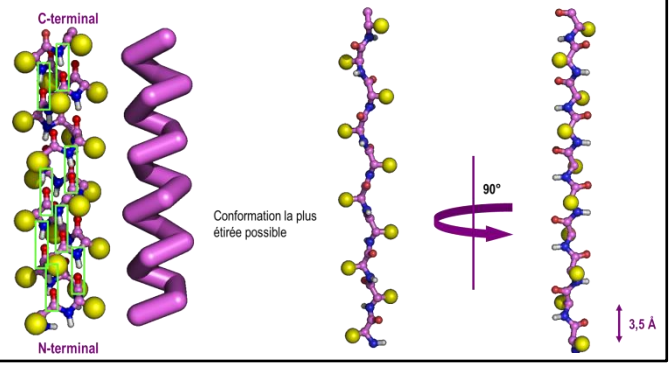


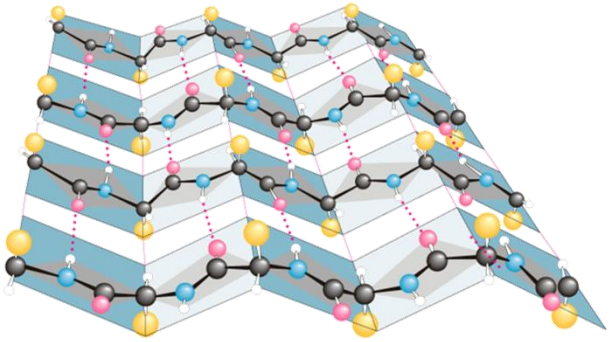
Structure primaire : enchaînement des acides aminés (séquence)

Structure secondaire : repliement local de la chaîne polypeptidique

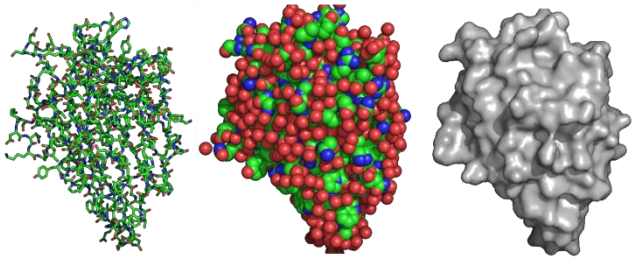
Structure tertiaire : repliement global de la chaîne polypeptidique

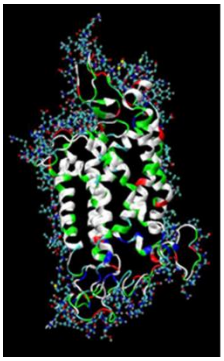
Structure quaternaire : assemblage de plusieurs chaînes polypeptidiques

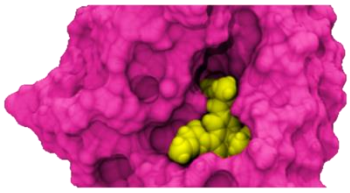




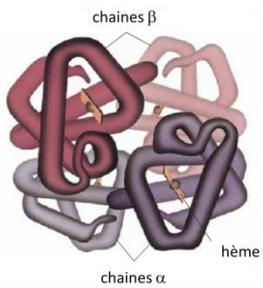
La concanavaline A

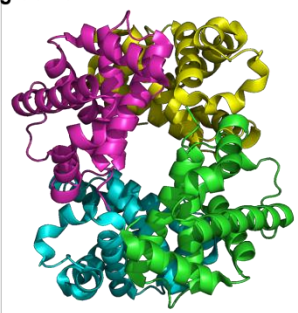






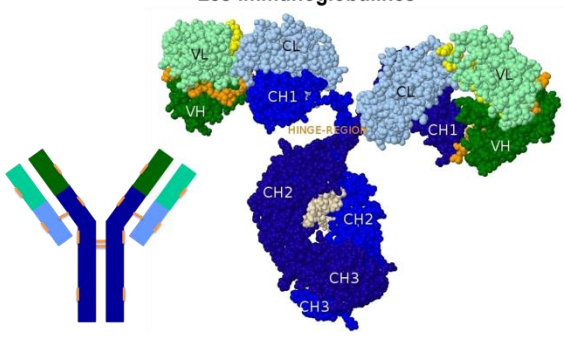
L'hémoglobine

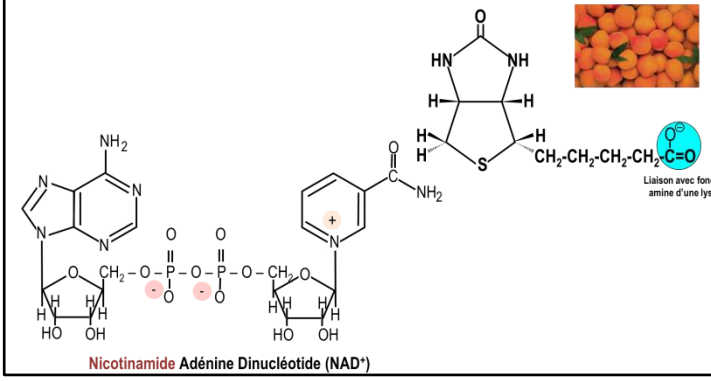


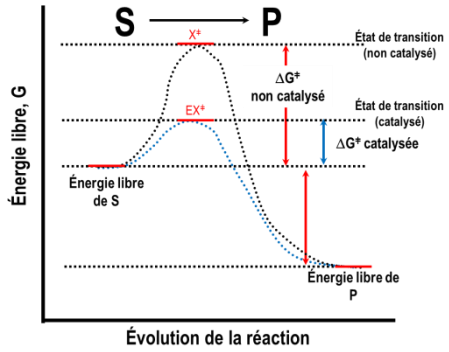


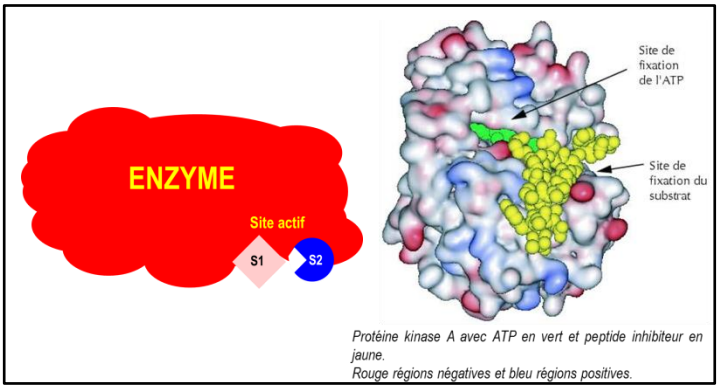
hétérotétramère, environ 100 liaisons H impliquées

Les immunoglobulines

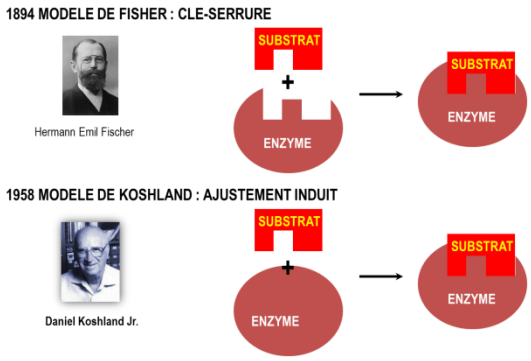


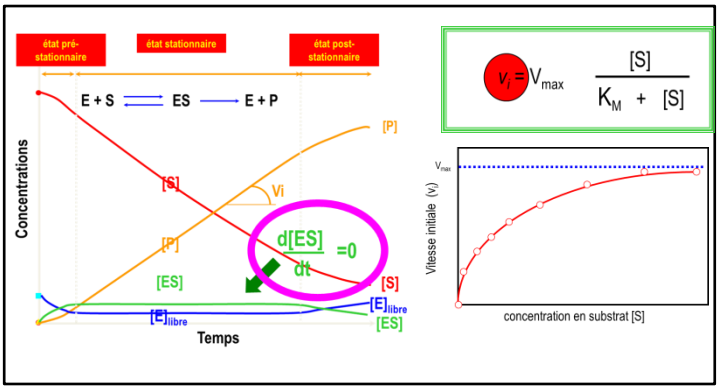






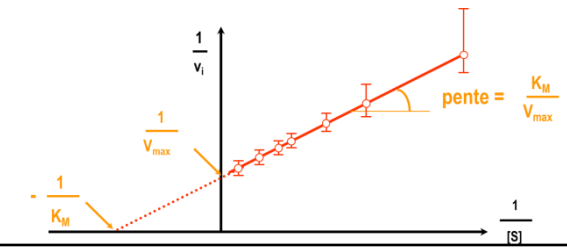
Protéine kinase A avec ATP en vert et peptide inhibiteur en jaune.
Rouge régions négatives et bleu régions positives.





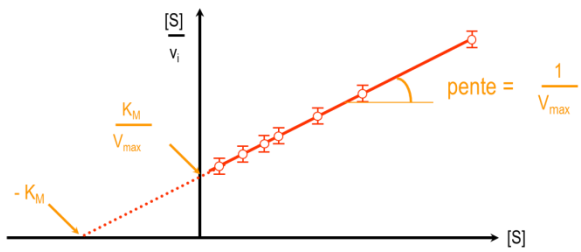
Lineweaver - Burk

$$\frac{1}{v_i} = \frac{K_M}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$



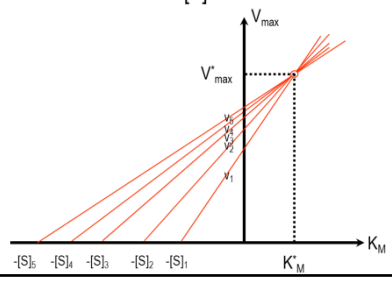
Hanes

$$\frac{[S]}{v_i} = \frac{K_M}{V_{max}} + \frac{1}{V_{max}} \times [S]$$



Eisenthal - Cornish-Bowden

$$V_{max} = v_i + \frac{v_i}{[S]} \times K_M$$



REACTIFS BIOLABO
www.biolabo.fr
FABRICANT : BIOLABO SA.
02160, Maizy, France

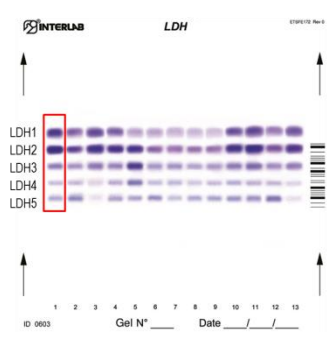
CHOLESTEROL
Méthode CHOD-PAP

Réactif pour le dosage quantitatif du cholestérol total dans le plasma ou le sérum humains

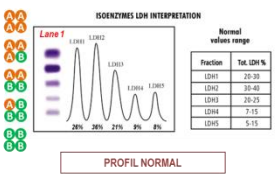
Cholestérol estérifié —CE→ Cholestérol + acides gras libres

Cholestérol + O_2 —CO→ Cholesten 4 one 3 + H_2O_2

2 H_2O_2 + Phénol + PAP —POD→ Quinonéimine (rose) + 4 H_2O

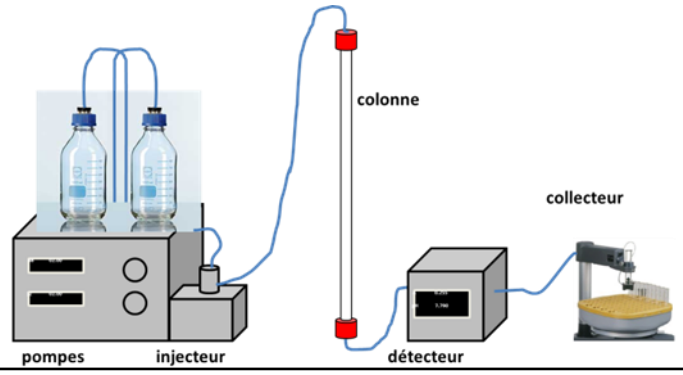


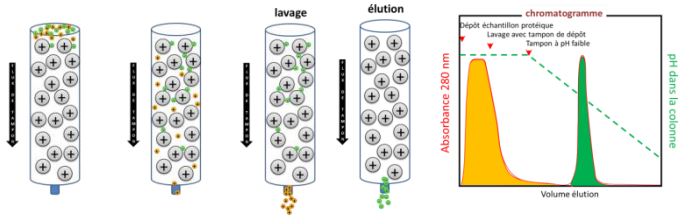
La LDH sérique est un marqueur d'AVC et d'infarctus.

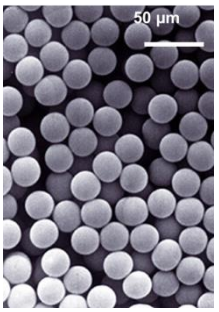


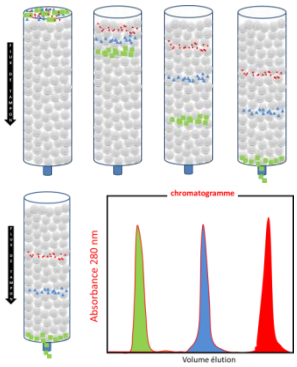
triéthylammonium
échangeur d'**anions**

carboxylate
échangeur de **cations**









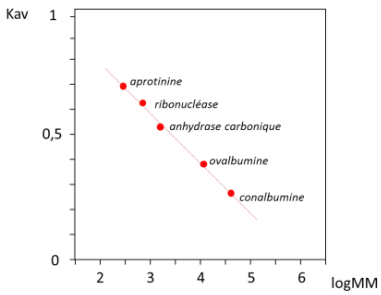
Kav = **constante de volume accessible** → estimer la **MM** d'une protéine inconnue

(fraction des billes accessible aux molécules)

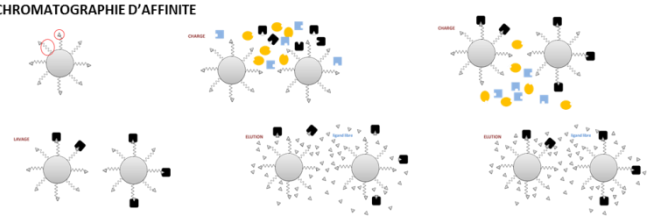
Mélange injecté (témoins)

NOM	kDa
Aprotinine	6,5
Ribonucléase	13,7
Anhydrase carbonique	29
Ovalbumine	43
Conalbumine	75

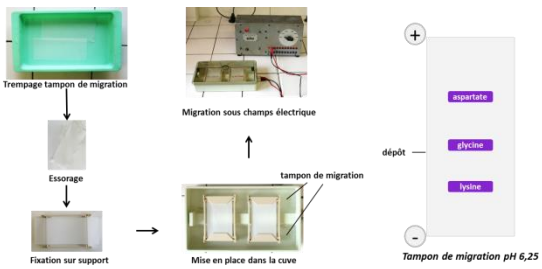
- MM connue
- K_{av} calculé



CHROMATOGRAPHIE D'AFFINITE

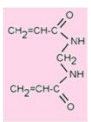


ELECTROPHORESE SUR PAPIER



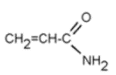
ELECTROPHORESE SUR GEL ACRYLAMIDE

acrylamide

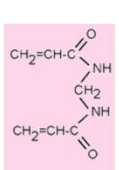


méthylène bisacrylamide

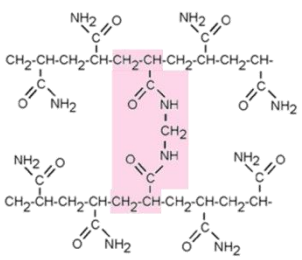
acrylamide



méthylène bisacrylamide

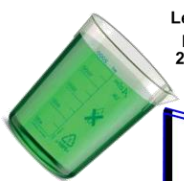


Temed
APS

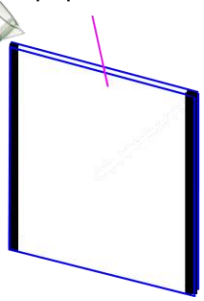


polyacrylamide

Acrylamide/bisacrylamide
Tampon Tris pH6,8 ou 8,8
SDS
Persulfate d'ammonium
TEMED



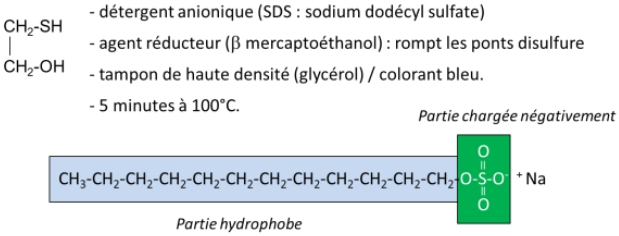
Le gel d'acrylamide polymérise entre 2 plaques de verre

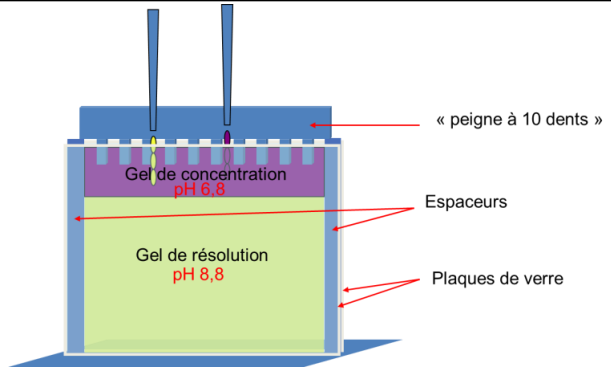


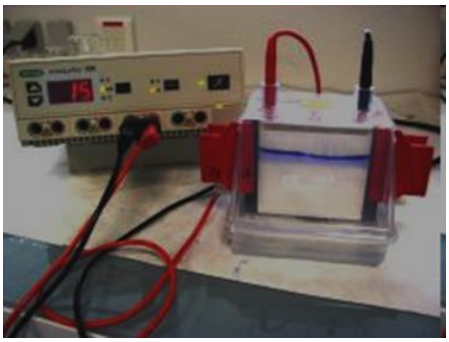
Préparation des échantillons protéiques

Les protéines sont incubées en présence de :

- détergent anionique (SDS : sodium dodécyl sulfate)
- agent réducteur (β mercaptoéthanol) : rompt les ponts disulfure
- tampon de haute densité (glycérol) / colorant bleu.
- 5 minutes à 100°C.

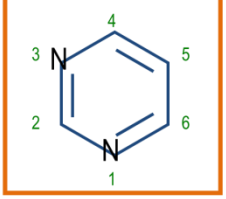




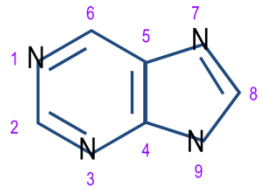


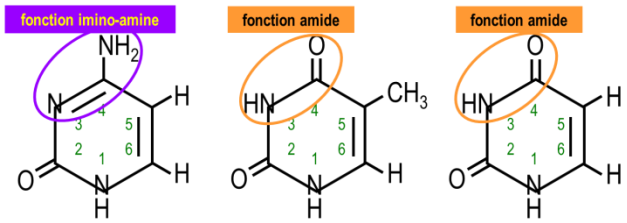
Il existe 2 sortes de bases azotées hétérocycliques :

bases pyrimidiques (noyau pyrimidine)

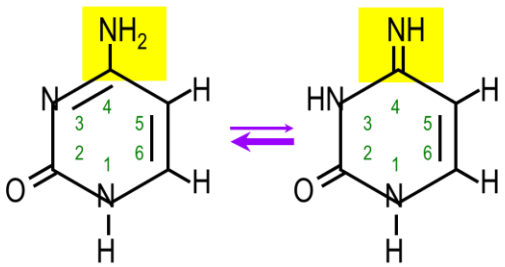


bases puriques (noyau purine)

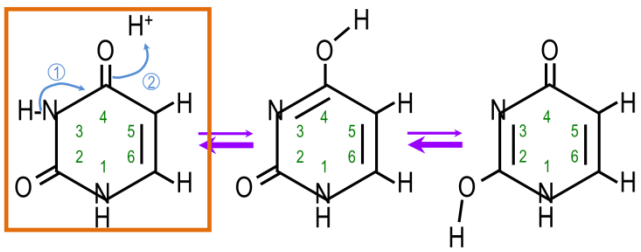




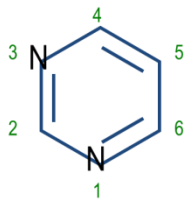
Équilibre amino-imino (cytosine)



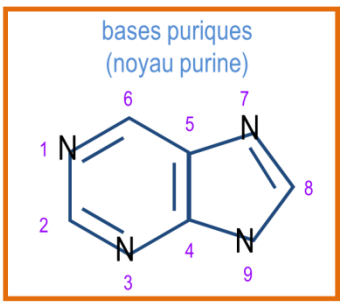
Équilibre énol-cétone (thymine-uracile)

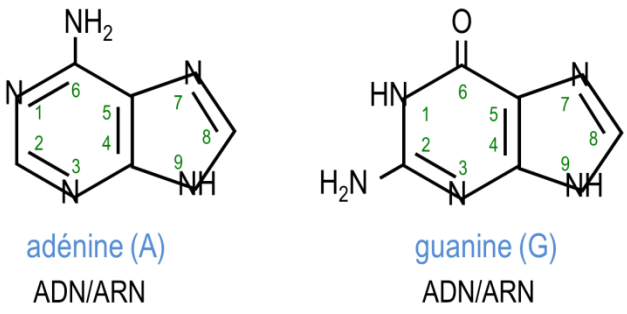


bases pyrimidiques (noyau pyrimidine)

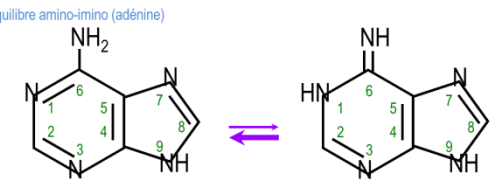


bases puriques (noyau purine)

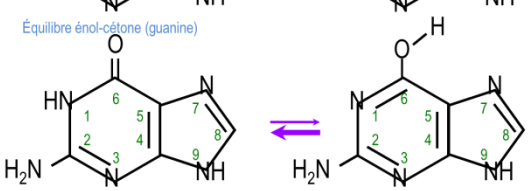


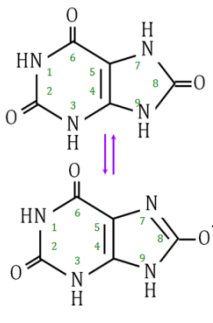


Équilibre amino-imino (adénine)

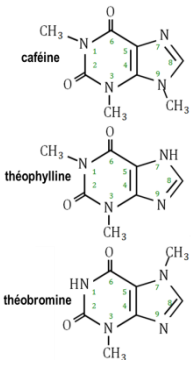


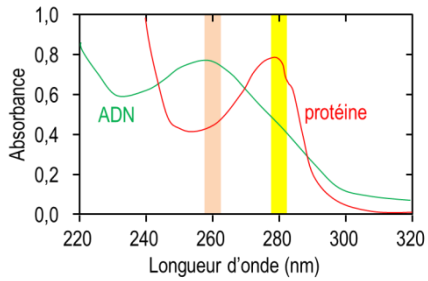
Équilibre énol-cétone (guanine)

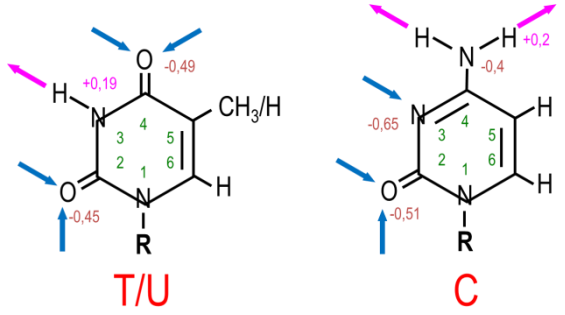


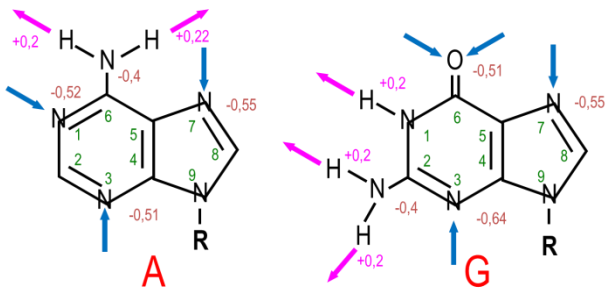


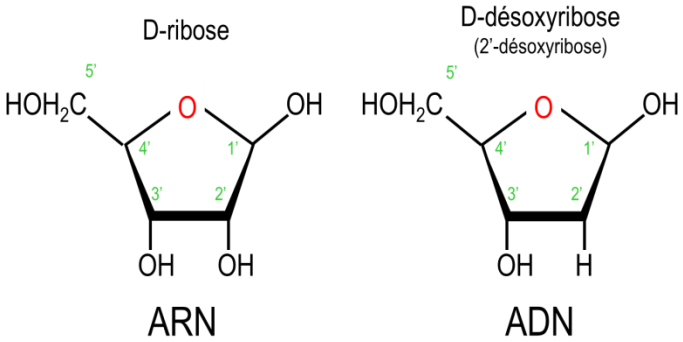
acide urique

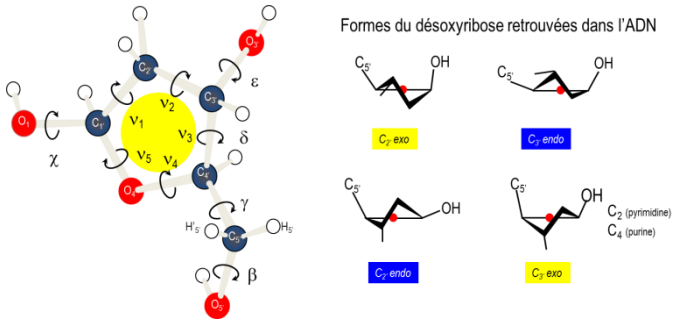


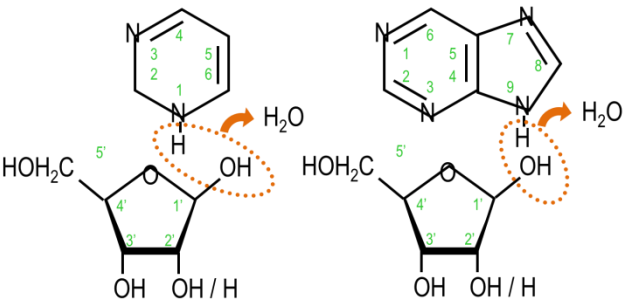


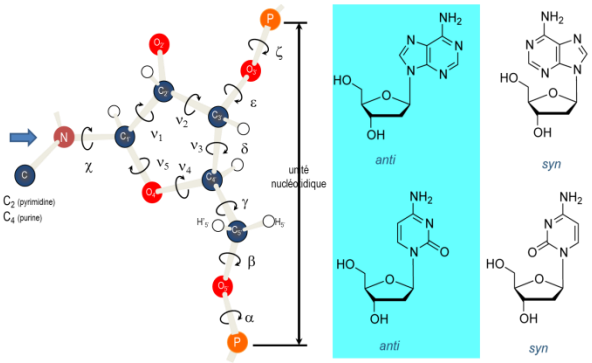


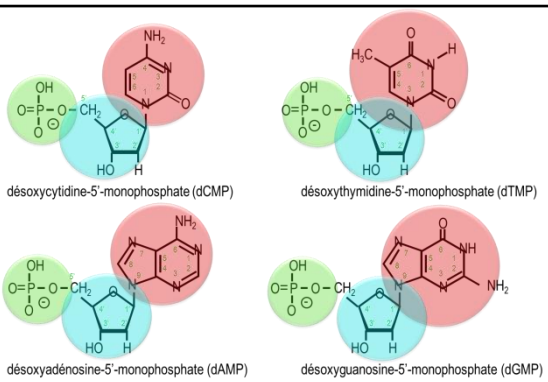


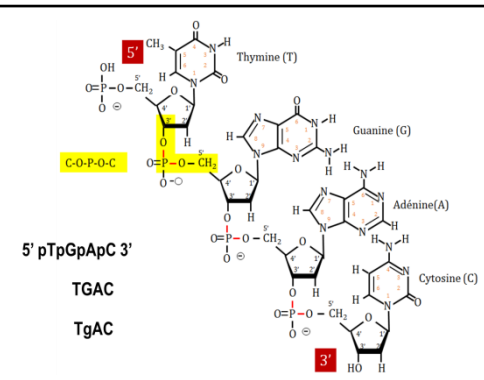








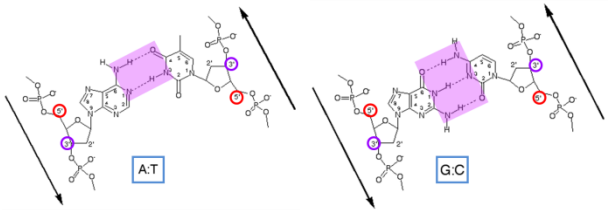


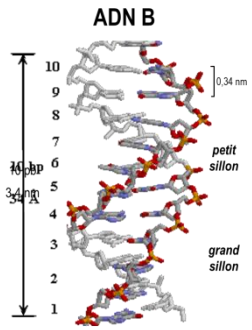


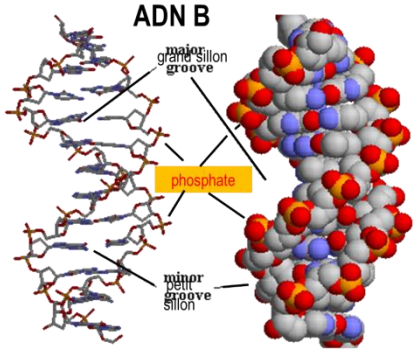
- nombre de A = nombre de T
  nombre de C = nombre de G
- une chaîne d'ADN polyA s'hybride avec une chaîne d'ADN polyT
- une chaîne d'ADN polyG s'hybride avec une chaîne d'ADN polyC

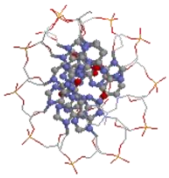
Appariement de Watson et Crick

A T
C G

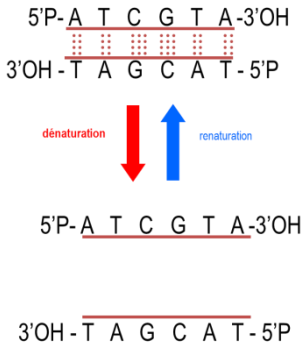


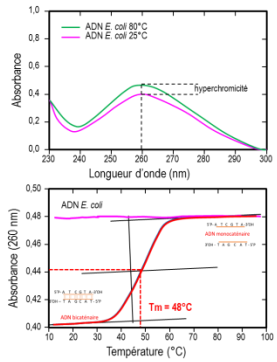


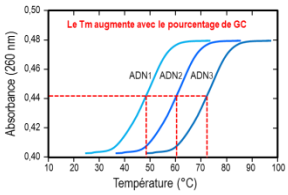


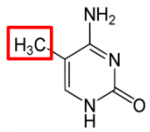


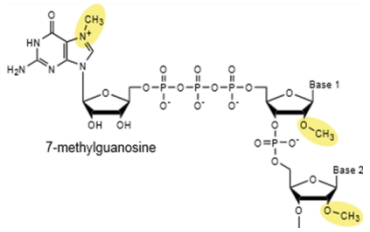
Vue de dessous

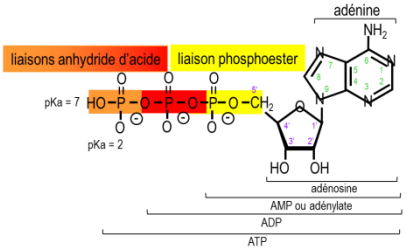


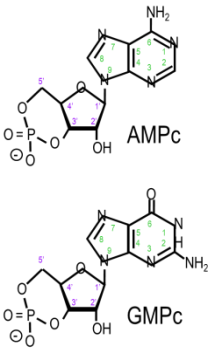


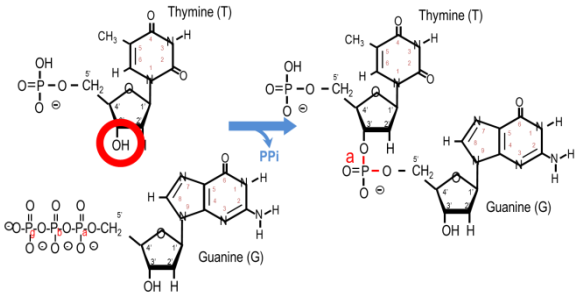




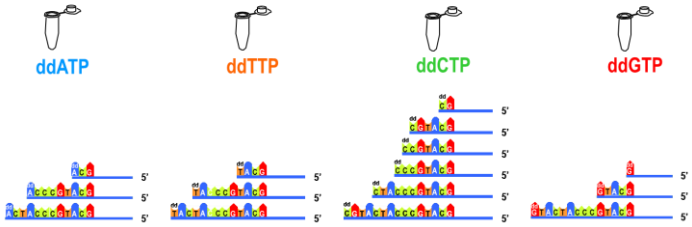


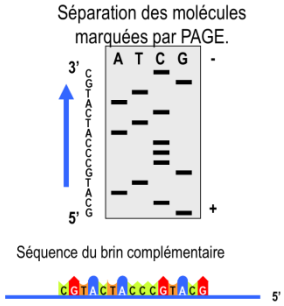


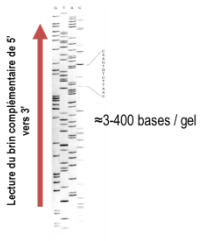




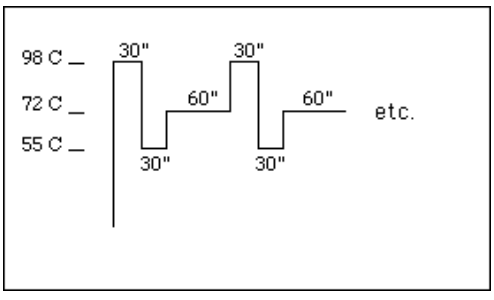
Séquences obtenues dans chaque tube.

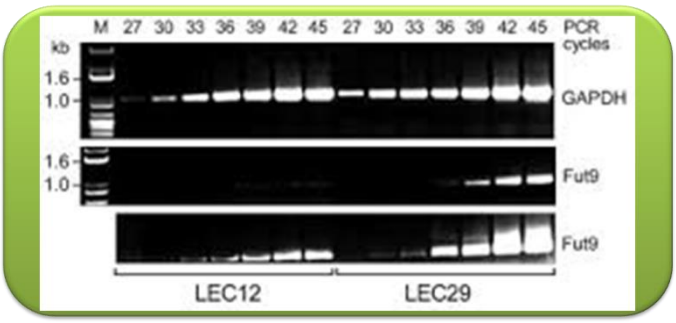






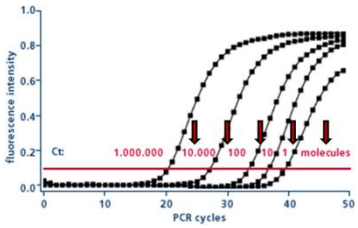
Autoradiogramme d'un gel de séquence d'ADN (polyacrylamide)

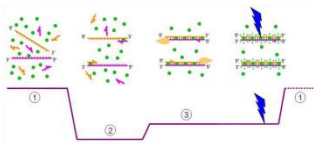


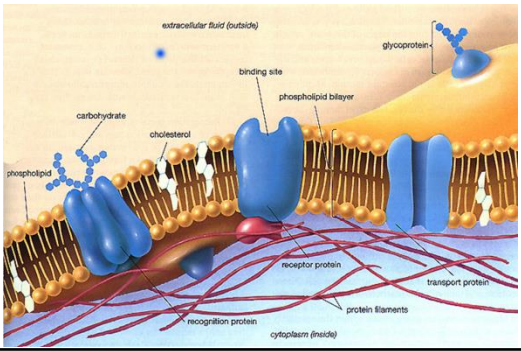


PCR en temps réel utilisant du SYBR Green comme sonde.

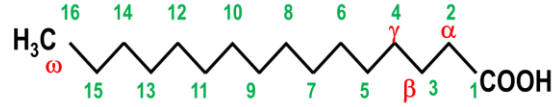
Méthode des ΔΔ Ct





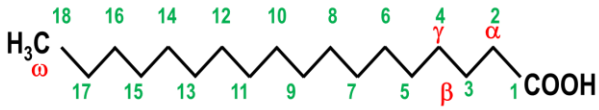


- Acide palmitique ($C_{16:0}$)
- Acide *n*-hexadécanoïque



Acide stéarique ($C_{18:0}$)

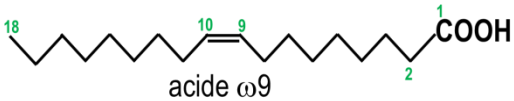
- Acide *n*-octadécanoïque



- Acide palmitoléique $C_{16:1\ \Delta^{9}}$

acide ω7

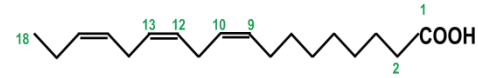
- Acide oléique $C_{18:1\ \Delta^{9}}$



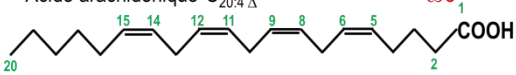
acide ω9

- Acide linoléique $C_{18:2\ \Delta^{9,12}}$ ω6

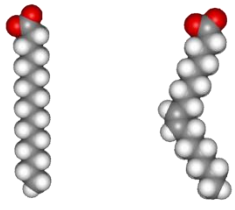
- Acide linolénique $C_{18:3\ \Delta^{9,12,15}}$ ω3



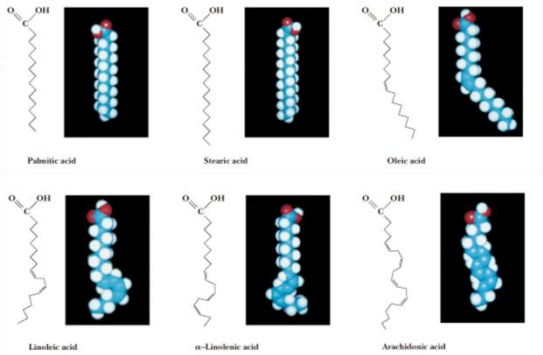
- Acide arachidonique $C_{20:4\ \Delta^{5,8,11,14}}$ ω6



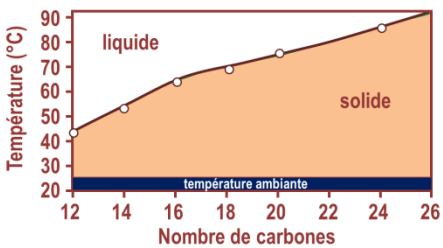
La configuration *cis* de la double liaison induit une courbure rigide de la chaîne aliphatique



$C_{16:0}$ $C_{16:1\ \Delta^{9}}$

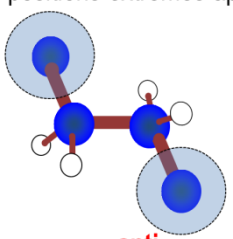


- Point de fusion : température de passage de **l'état gel à l'état fluide.**
  - dépend de la longueur de la chaîne aliphatique



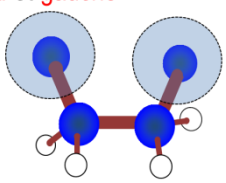
Liberté de rotation des liaisons simples C-C :

2 positions extrêmes appelées anti et gauche



anti

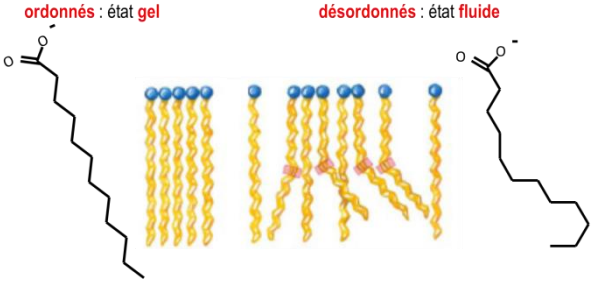
la plus stable



gauche

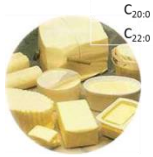
Basse température : position **anti** prédominante. Les acides gras sont **ordonnés** : état **gel**

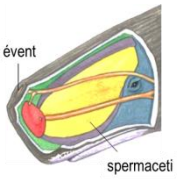
Température élevée : position **gauche** prédominante. Les acides gras sont **désordonnés** : état **fluide**



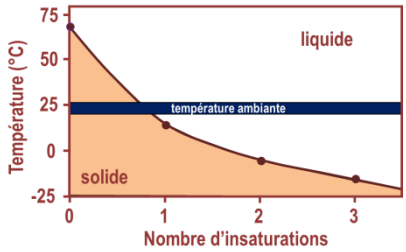
Acide gras saturé	Point de fusion (°C)	Acide gras insaturé	Point de fusion (°C)
$C_{12:0}$	44,2	$C_{16:1}$	-0,5
$C_{14:0}$	53,9	$C_{18:1}$	13,4
$C_{16:0}$	63,1	$C_{18:2}$	5
$C_{18:0}$	69,6	$C_{18:3}$	-11
$C_{20:0}$	76,5	$C_{20:4}$	-49,5
$C_{22:0}$	86,0		

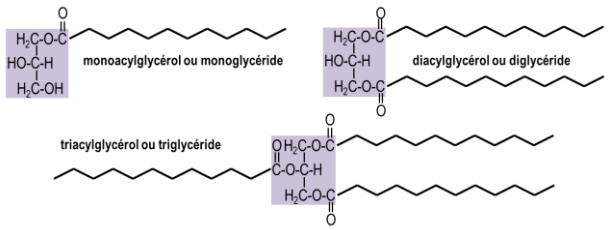
acides gras essentiels



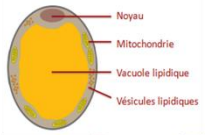


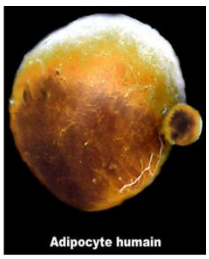
4°C ➔ état solide et dense, l'animal plonge
37°C ➔ état liquide et léger, l'animal remonte

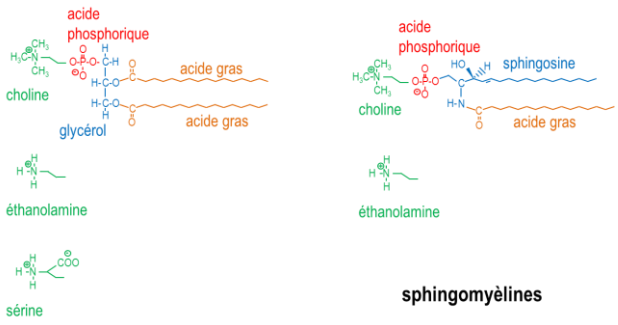




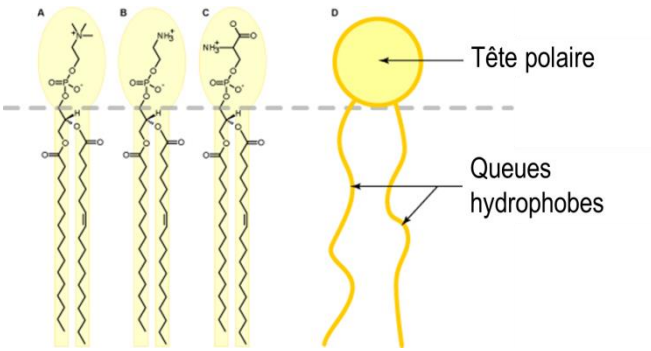
Adipocytes

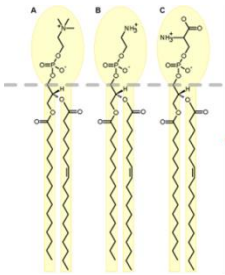




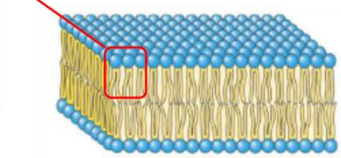


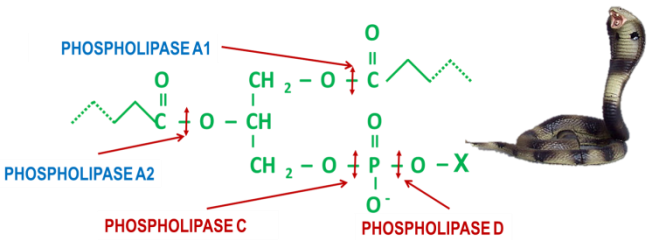
sphingomyèlines



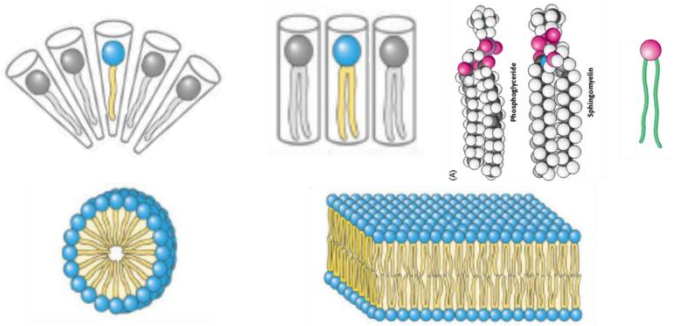


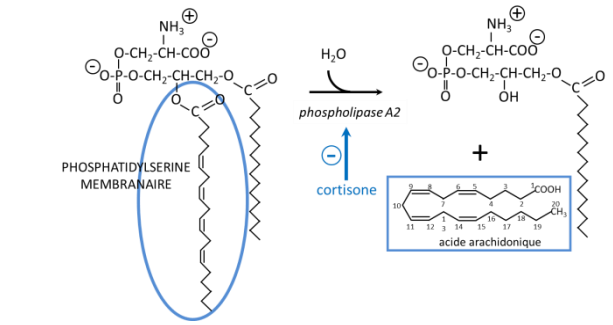
VALABLE EGALEMENT AVEC LES SPHINGOMYELINES

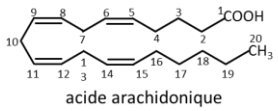




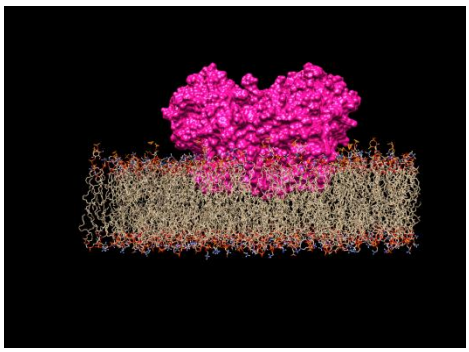
➔ lysoglycérophospholipides

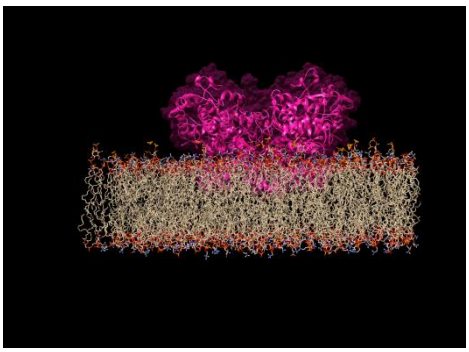

Phosphoglycéride
Sphingomyéline

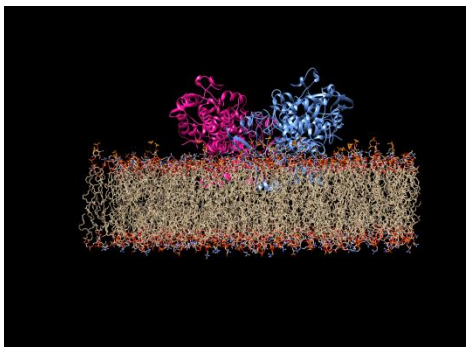

PHOSPHATIDYLSERINE
MEMBRANAIRE
phospholipase A2
cortisone
acide arachidonique

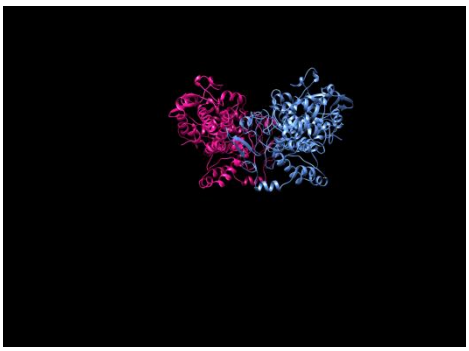

acide arachidonique
cyclooxygénase
lipoxygénase
Prostaglandines
Leucotriènes
Thromboxanes

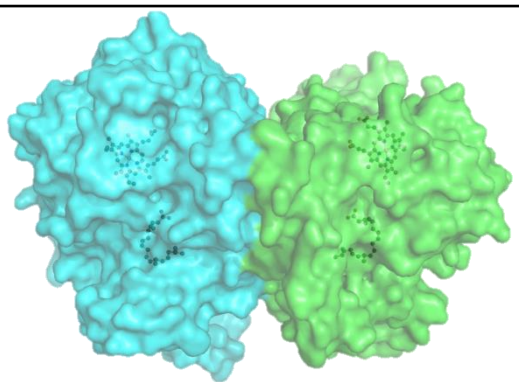

acide arachidonique
cyclooxygénase
PGG2
aspirine
ibuprofène
acétaminophène

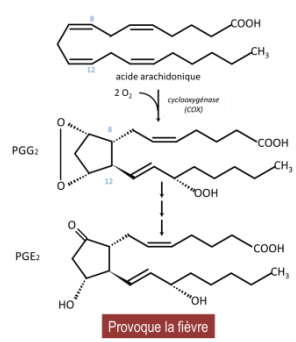




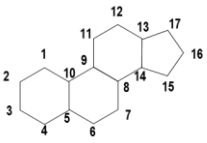


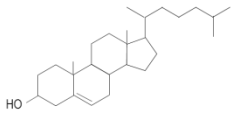




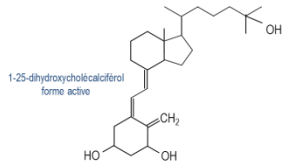

acide arachidonique
cyclooxygénase (COX)
PGG2
PGE2
Provoque la fièvre

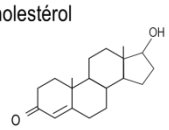
noyau stérol



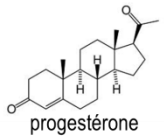


cholestérol

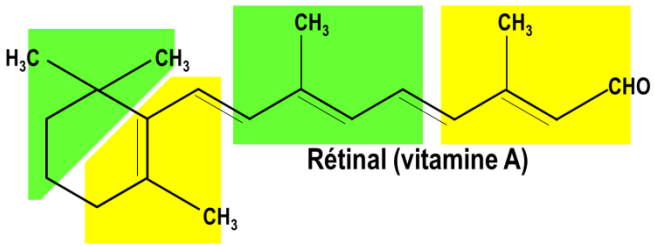




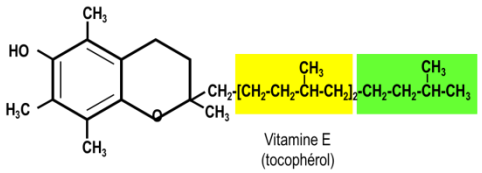
testostérone



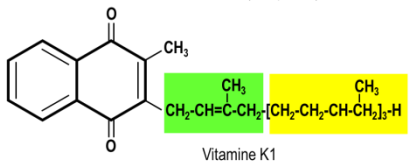
progestérone



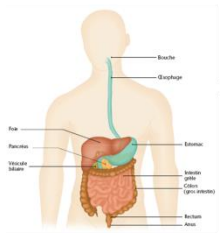
Rétinal (vitamine A)

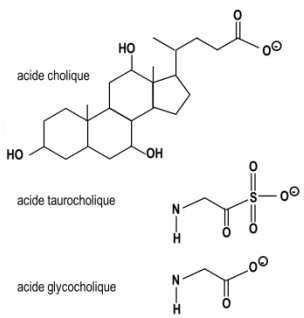


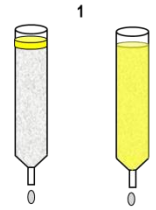
Vitamine E
(tocophérol)



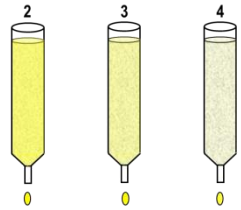
Vitamine K1
(phylloquinone)







① les lipides sont déposés et se fixent sur la colonne.



Les lipides sont élués avec des solutions de plus en plus hydrophobes : ②méthanol, ③acétonitrile, ④acétone.