

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

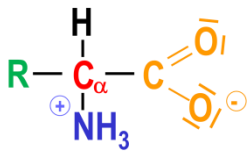
- 1 - Solubilisation – extraction des protéines
- 2 - Précipitation différentielle
 - A - précipitation isoélectrique
 - B - précipitation par des sels
- 3 - Techniques chromatographiques
 - A - échange d'ions
 - B - exclusion / diffusion
 - C - affinité
- 4 - Techniques électrophorétiques
 - A - électrophorèse 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

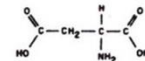


Symbole	Code 3 lettres	Nom
A	Ala	Alanine
C	Cys	Cystéine
D	Asp	Aspartate
E	Glu	Glutamate
F	Phe	Phénylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Méthionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Sérine
T	Thr	Thréonine
V	Val	Valine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

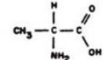
Glycine (Gly)



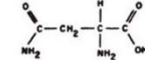
Acide aspartique (Asp)



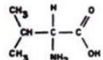
Alanine (Ala)



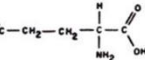
Asparagine (Asn)



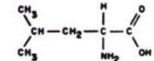
Valine (Val)



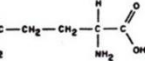
Acide glutamique (Glu)



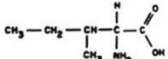
Leucine (Leu)



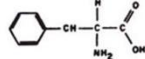
Glutamine (Gln)



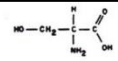
Isoleucine (Ile)



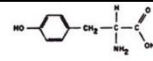
Phénylalanine (Phe)



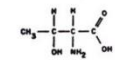
Sérine (Ser)



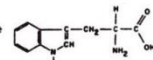
Tyrosine (Tyr)



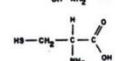
Thréonine (Thr)



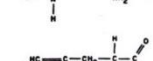
Tryptophane (Trp)



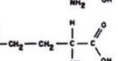
Cystéine (Cys)



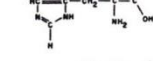
Histidine (His)



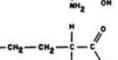
Méthionine (Met)



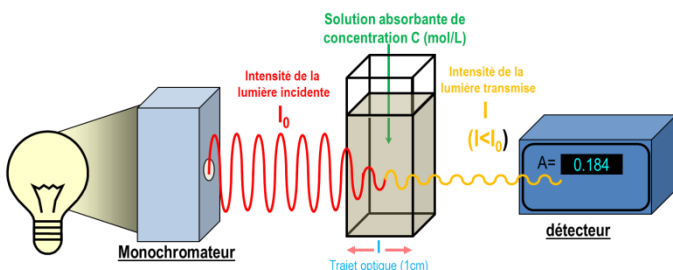
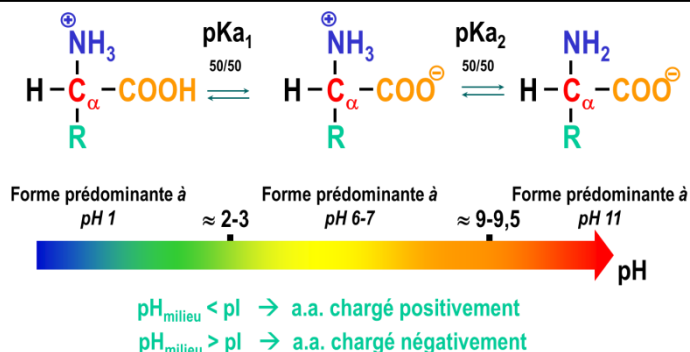
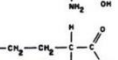
Proline (Pro)



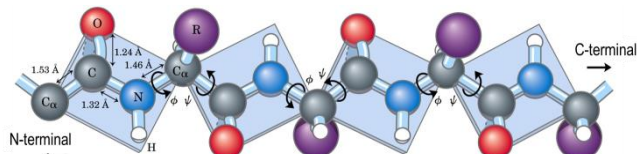
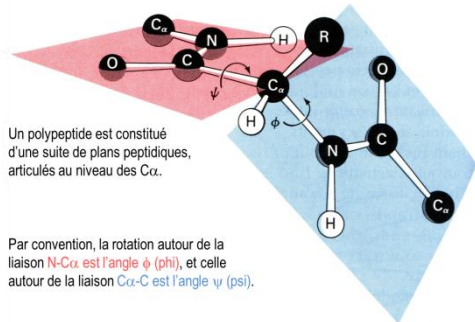
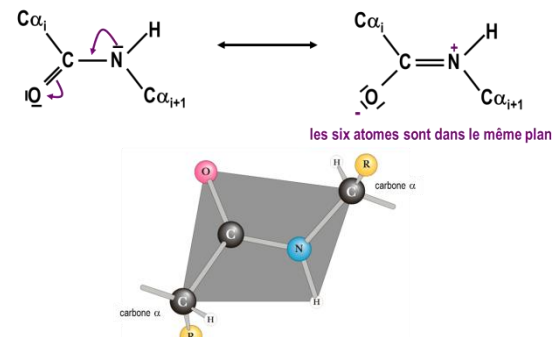
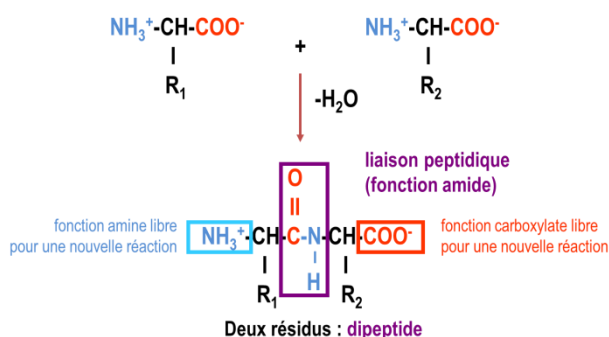
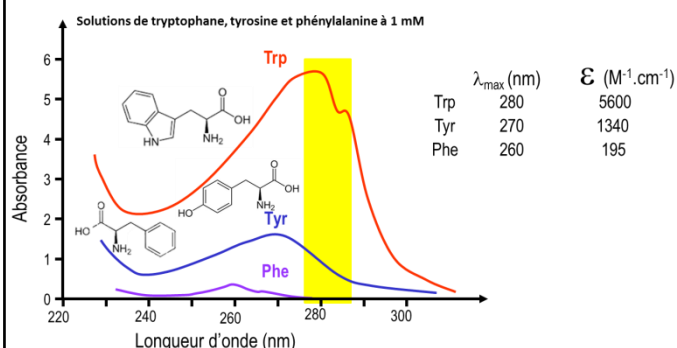
Lysine (Lys)

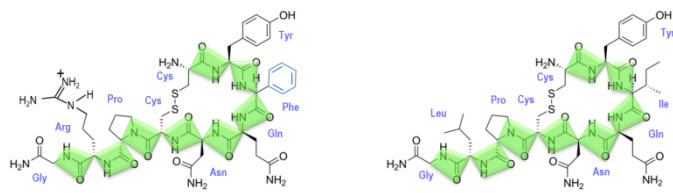


Arginine (Arg)

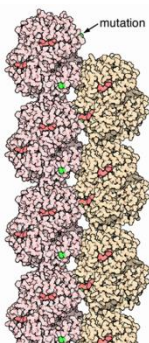
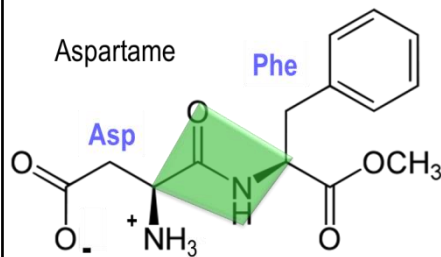


- La fraction de la lumière incidente absorbée par une solution à une longueur d'onde donnée dépend :
- 1- de l'épaisseur de la solution que la lumière doit traverser (trajet optique)
 - 2- de la concentration de la solution en espèces absorbantes





Hormones peptidiques post-hypophysaires

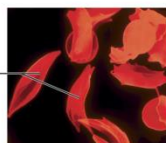


L'anémie falciforme (1^{ère} maladie génétique dans le monde) résulte d'une mutation sur le gène codant l'hémoglobine : l'hémoglobine modifiée (E6V sur la chaîne β) va s'agréger dans la cellule, surtout lorsque la $[O_2]$ est faible.

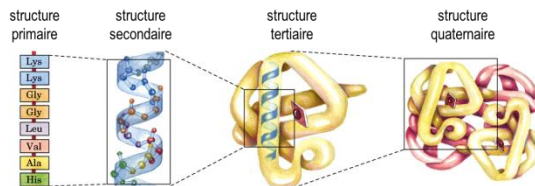
Globules rouges avec hémoglobine normale



Globules rouges avec hémoglobine mutée



Différents niveaux de structuration pour une protéine

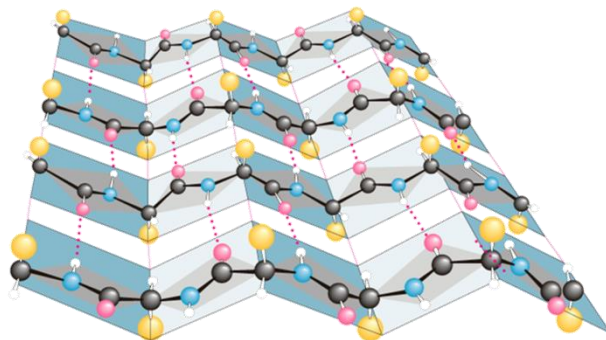
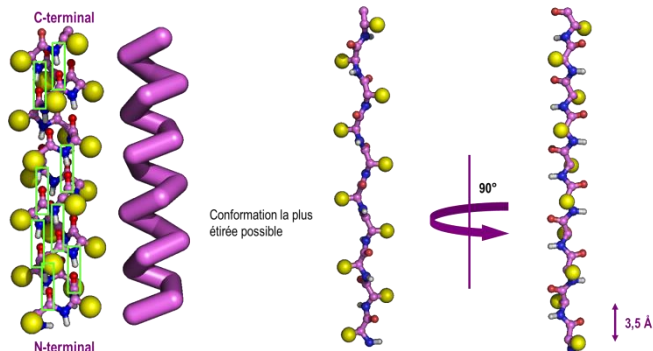


Structure primaire : enchaînement des acides aminés (séquence)

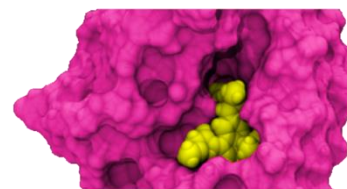
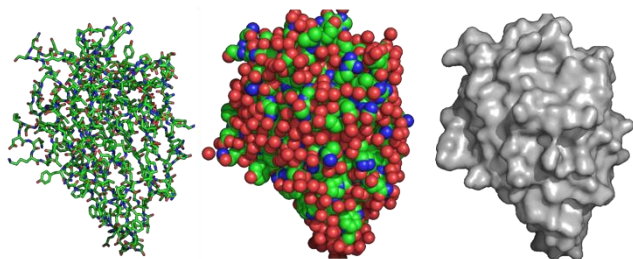
Structure secondaire : repliement local de la chaîne polypeptidique

Structure tertiaire : repliement global de la chaîne polypeptidique

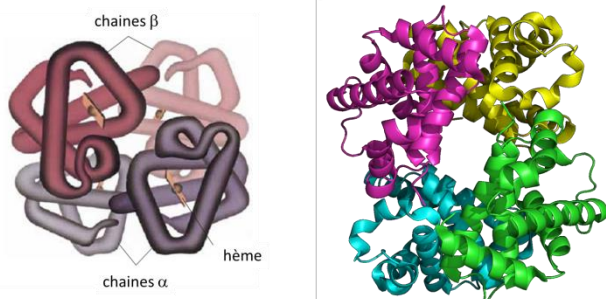
Structure quaternaire : assemblage de plusieurs chaînes polypeptidiques



La concanaviline A

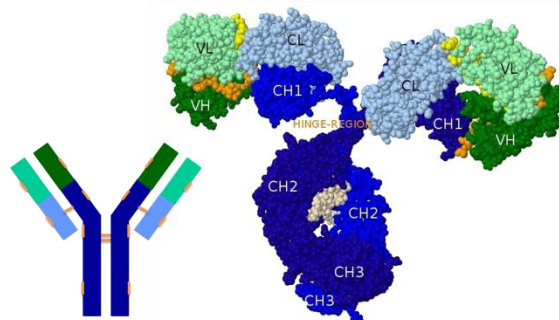


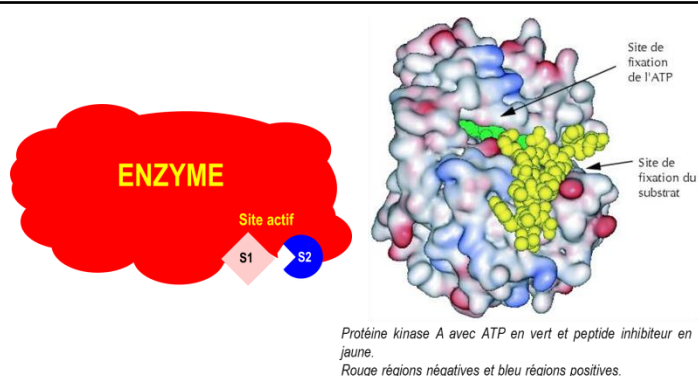
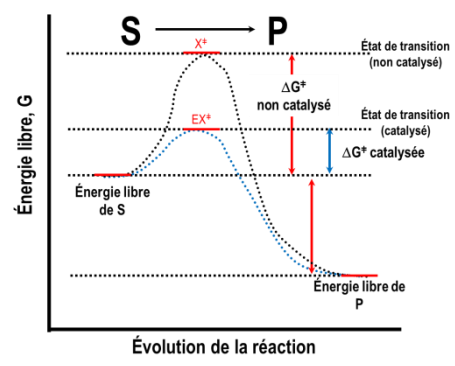
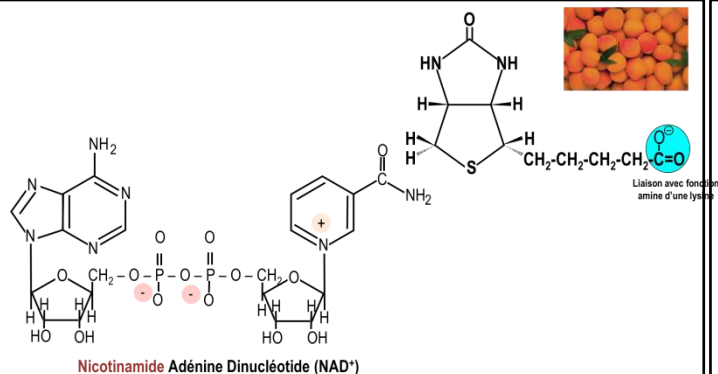
L'hémoglobine



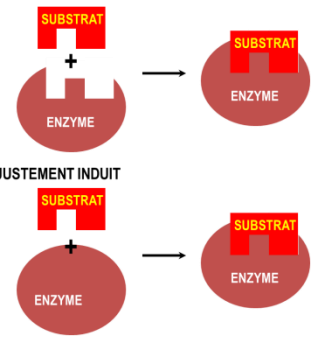
hétérotétramère, environ 100 liaisons H impliquées

Les immunoglobulines

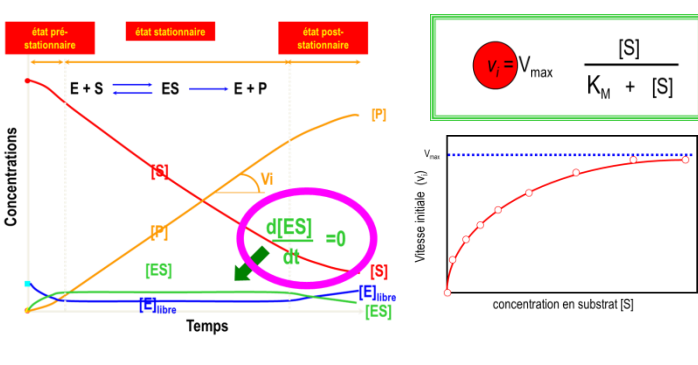
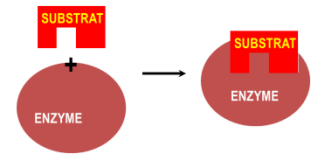




1894 MODELE DE FISHER : CLE-SERRURE

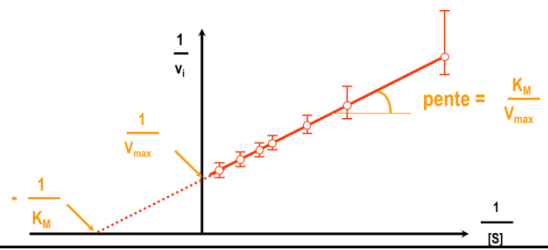


1958 MODELE DE KOSHLAND : AJUSTEMENT INDUIT



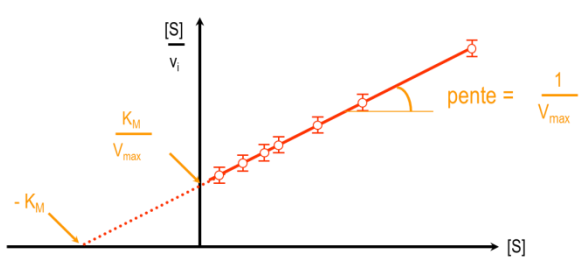
Lineweaver - Burk

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



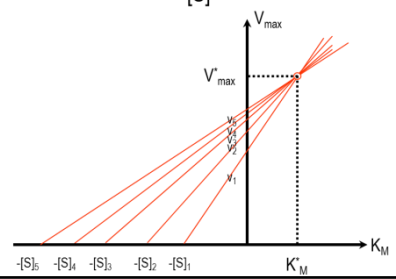
Hanes

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

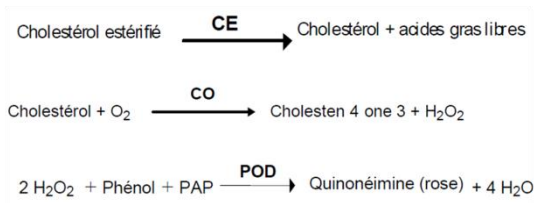


Eisenthal - Cornish-Bowden

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

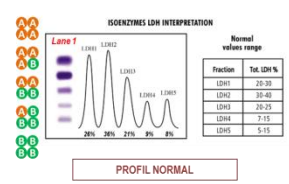
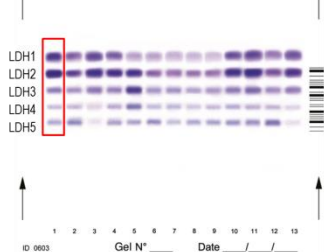


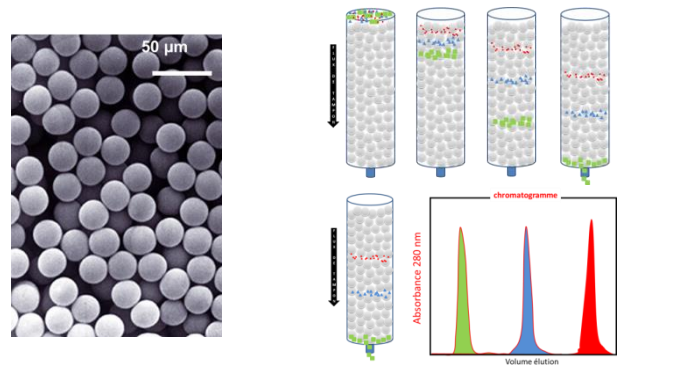
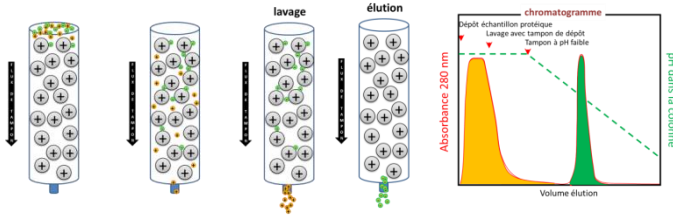
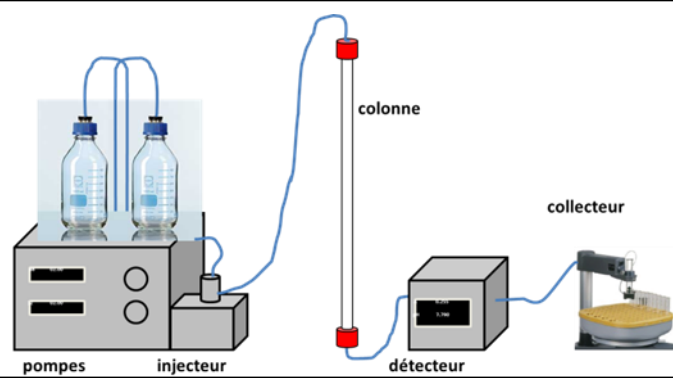
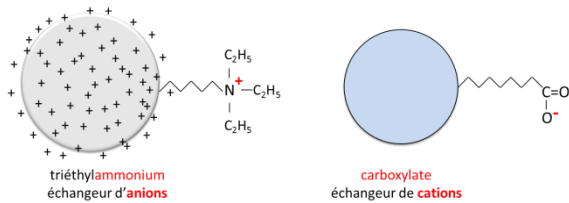
CHOLESTEROL
Méthode CHOD-PAP



LDH

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



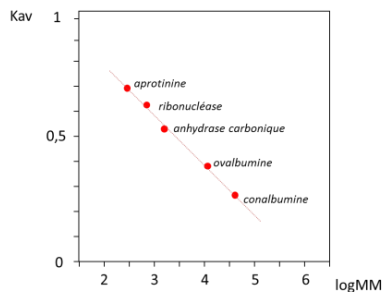


K_{av} = constante de volume accessible → estimer la MM d'une protéine inconnue
(fraction des billes accessible aux molécules)

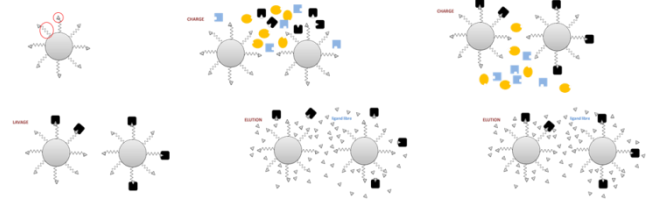
Mélange injecté (témoins)

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

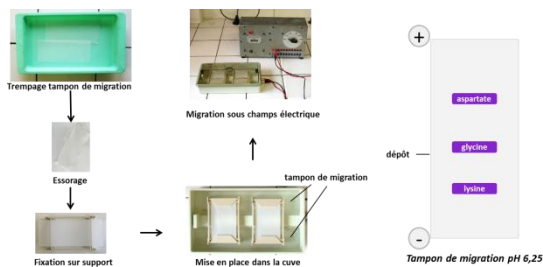
- MM connue
- K_{av} calculé



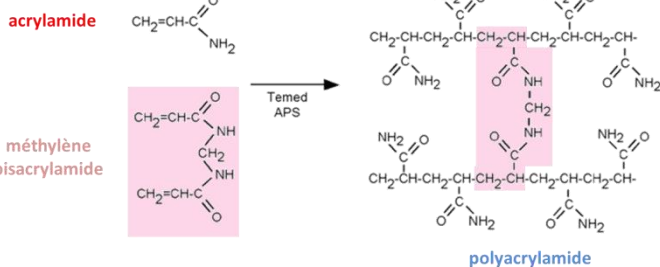
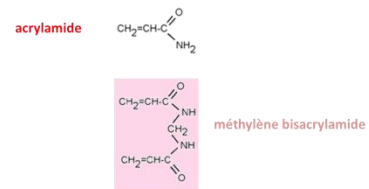
CHROMATOGRAPHIE D'AFFINITÉ



ELECTROPHORESE SUR PAPIER



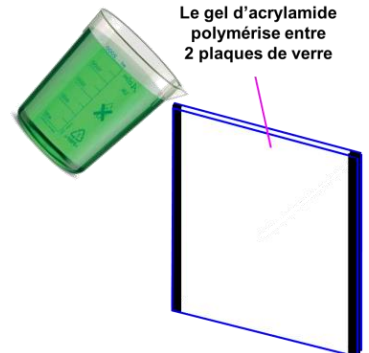
ELECTROPHORESE SUR GEL ACRYLAMIDE



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



Le gel d'acrylamide polymérise entre 2 plaques de verre

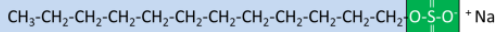


Préparation des échantillons protéiques

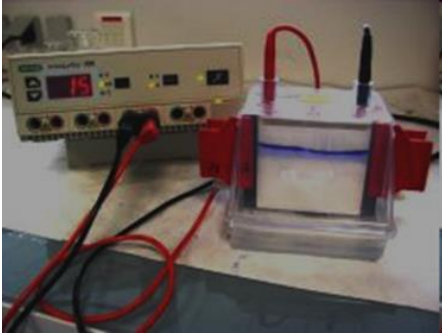
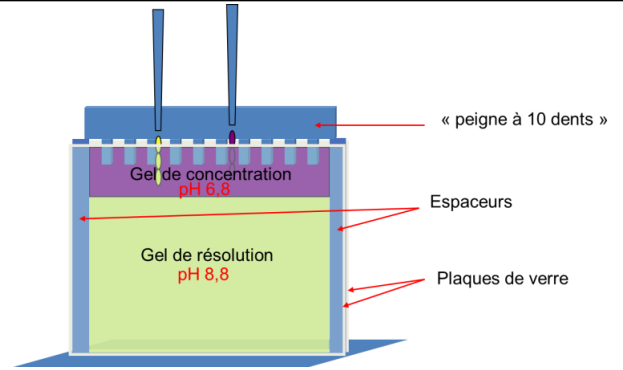
Les protéines sont incubées en présence de :

- 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.

Partie chargée négativement

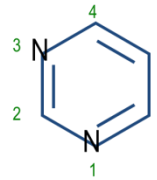


Partie hydrophobe

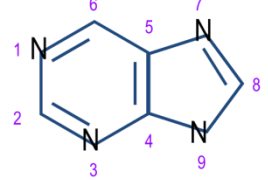


Il existe 2 sortes de bases azotées hétérocycliques :

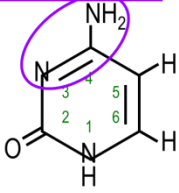
bases pyrimidiques
(noyau pyrimidine)



bases puriques
(noyau purine)



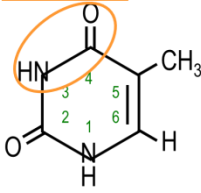
fonction imino-amine



cytosine (C)

ADN/ARN

fonction amide

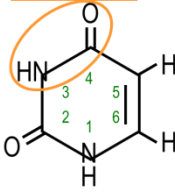


thymine (T)

5-méthyl-uracile

ADN

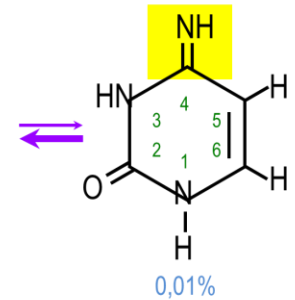
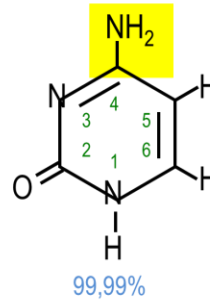
fonction amide



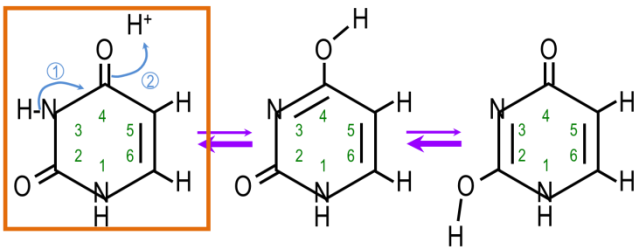
uracile (U)

ARN

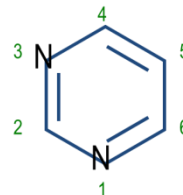
Équilibre amino-imino (cytosine)



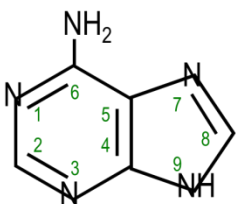
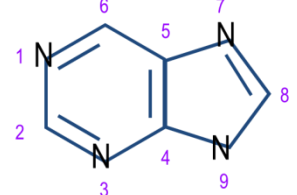
Équilibre énol-cétone (thymine-uracile)



bases pyrimidiques
(noyau pyrimidine)

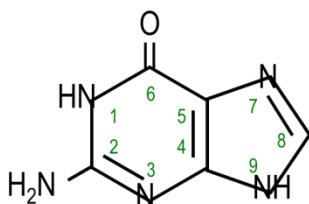


bases puriques
(noyau purine)



adénine (A)

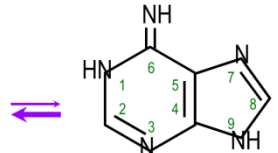
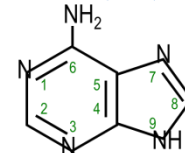
ADN/ARN



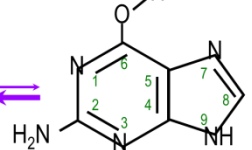
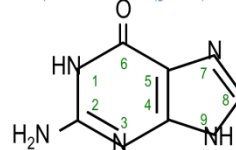
guanine (G)

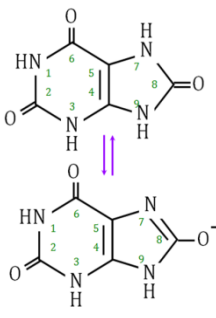
ADN/ARN

Équilibre amino-imino (adénine)

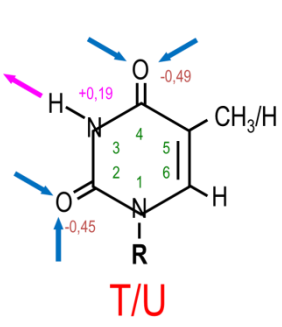
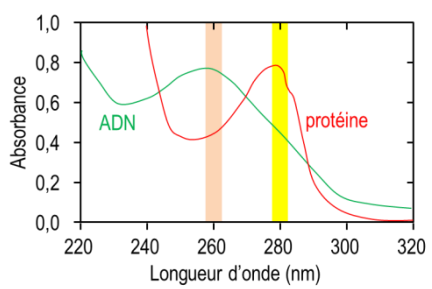
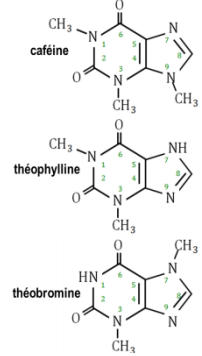


Équilibre énol-cétone (guanine)

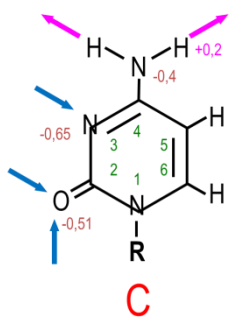




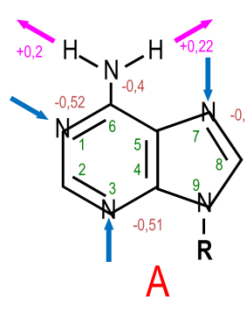
acide urique



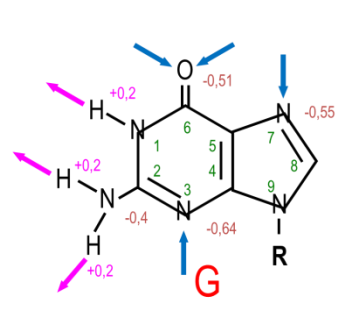
T/U



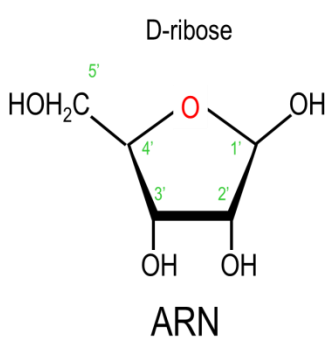
C



A

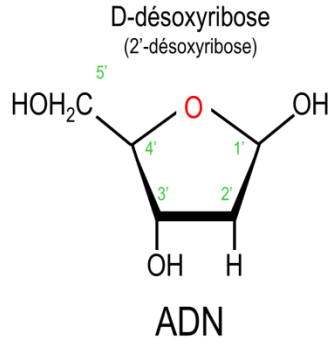


G



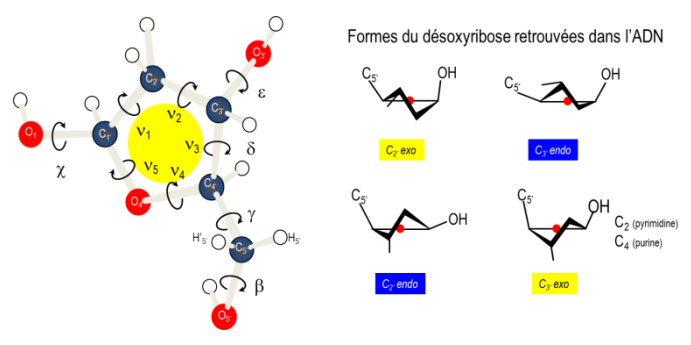
D-ribose

ARN

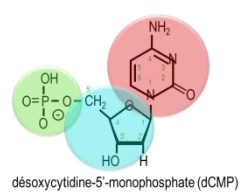
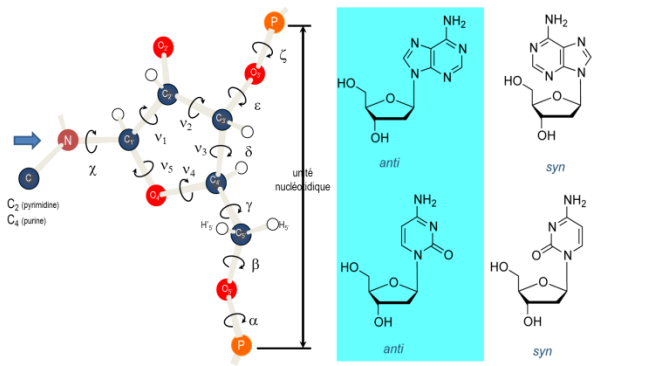
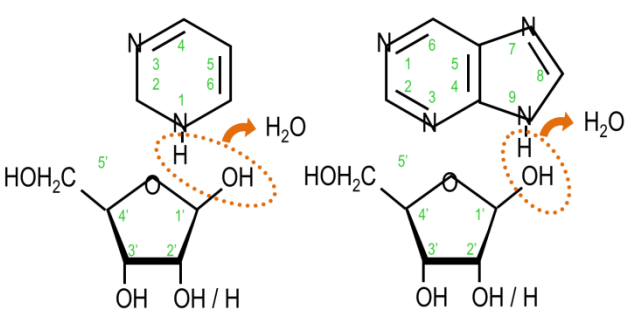


D-désoxyribose
(2'-désoxyribose)

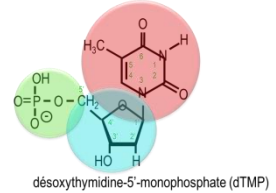
ADN



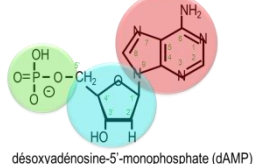
Formes du désoxyribose retrouvées dans l'ADN



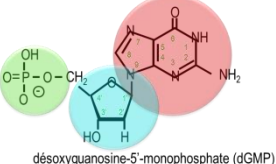
désoxycytidine-5'-monophosphate (dCMP)



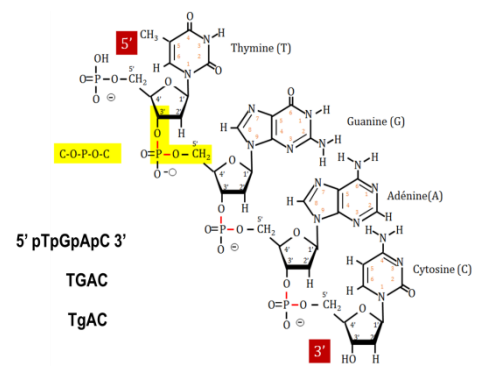
désoxythymidine-5'-monophosphate (dTMP)



désoxyadénosine-5'-monophosphate (dAMP)



désoxyguanosine-5'-monophosphate (dGMP)



5' pTpGpApC 3'

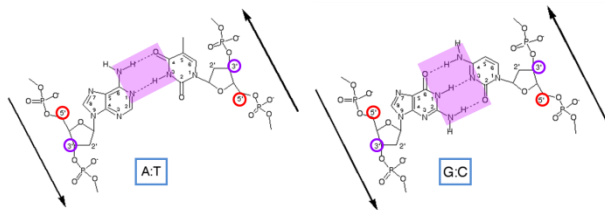
TGAC

TgAC

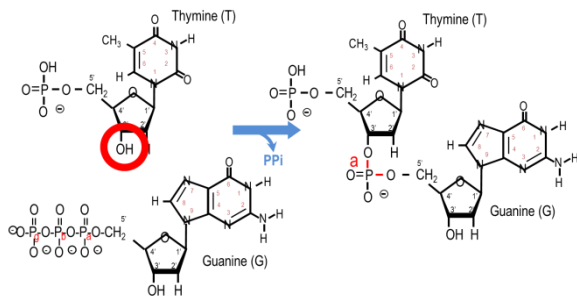
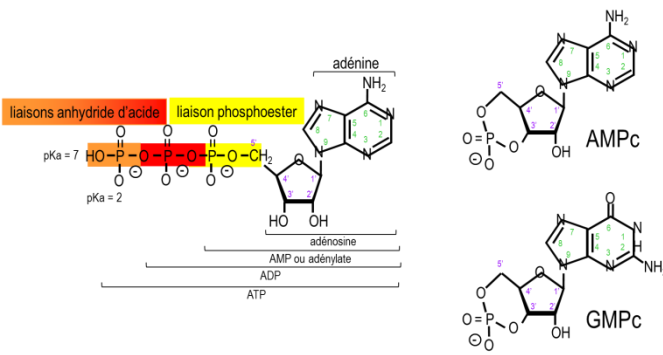
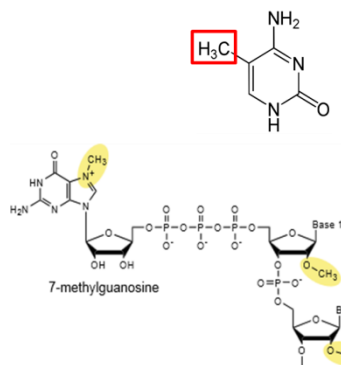
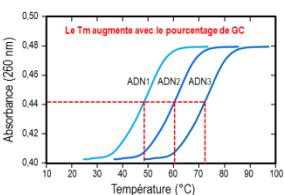
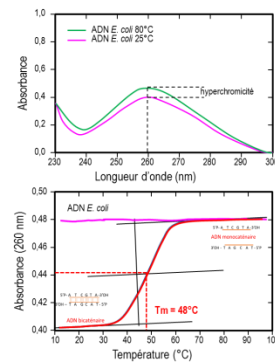
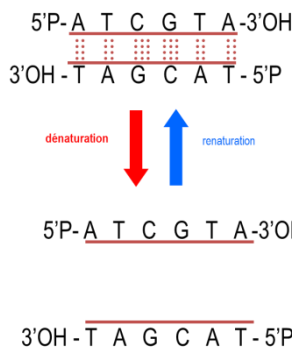
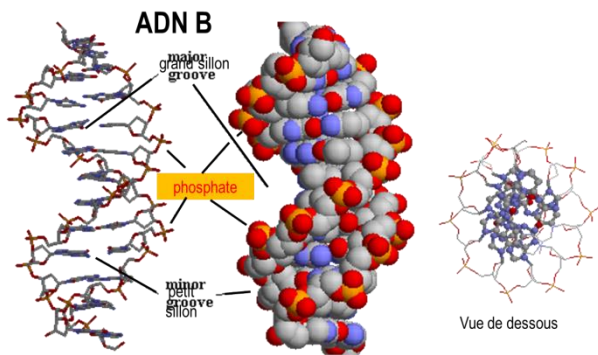
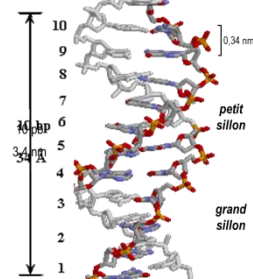
- nombre de A = nombre de T
nombre de C = nombre de G
- une chaîne d'ADN polyA s'hybride avec une chaîne d'ADN polyT
- une chaîne d'ADN polyG s'hybride avec une chaîne d'ADN polyC

Appariement de Watson et Crick

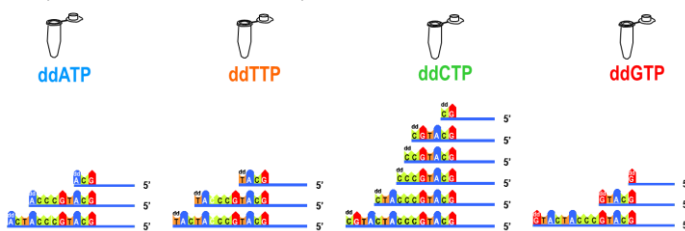
A C T G



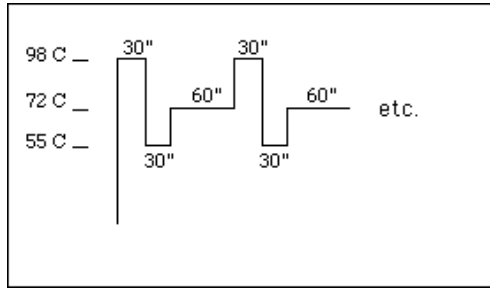
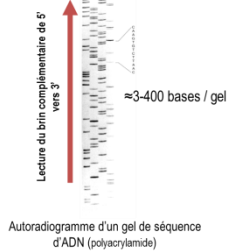
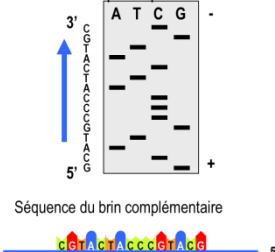
ADN B

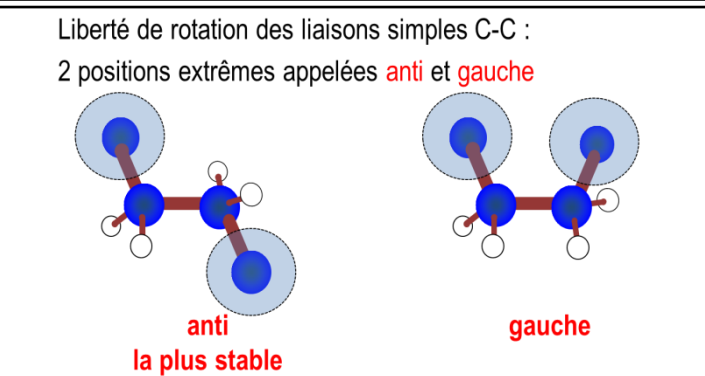
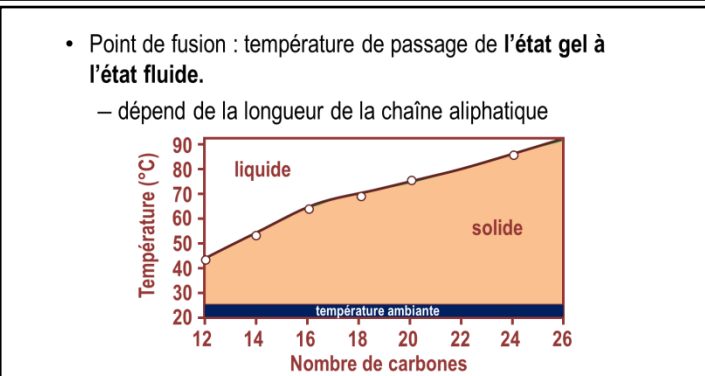
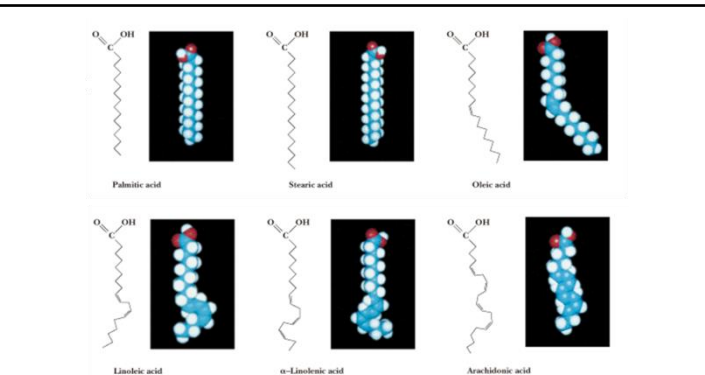
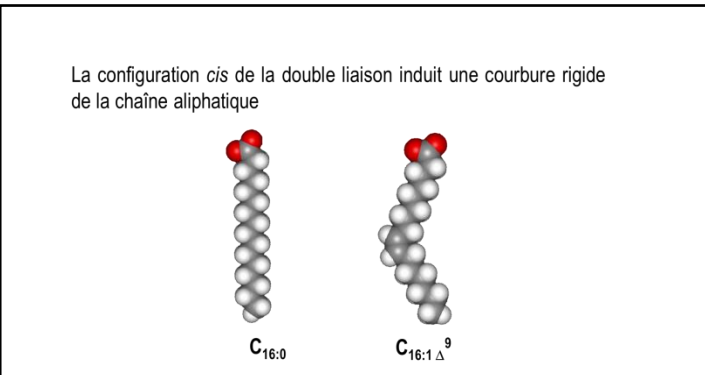
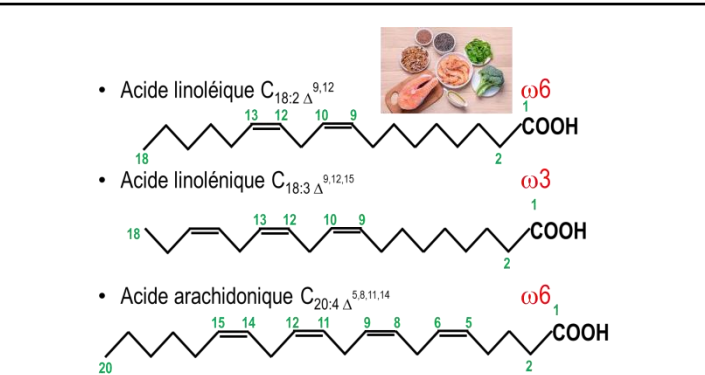
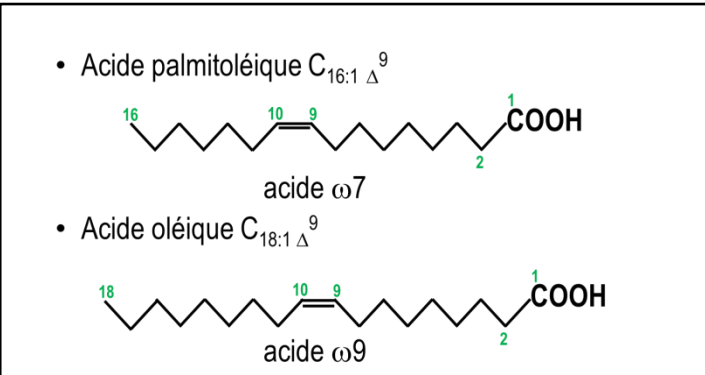
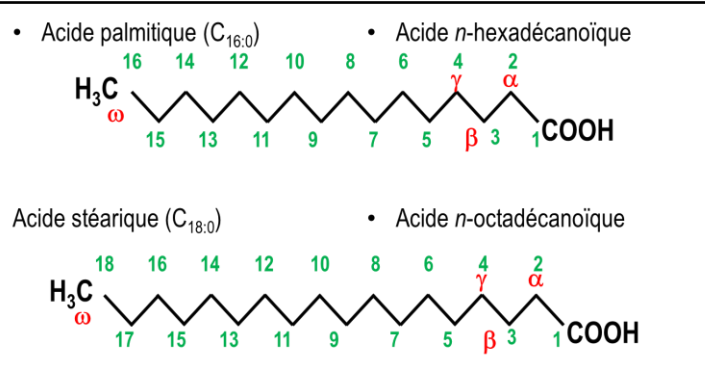
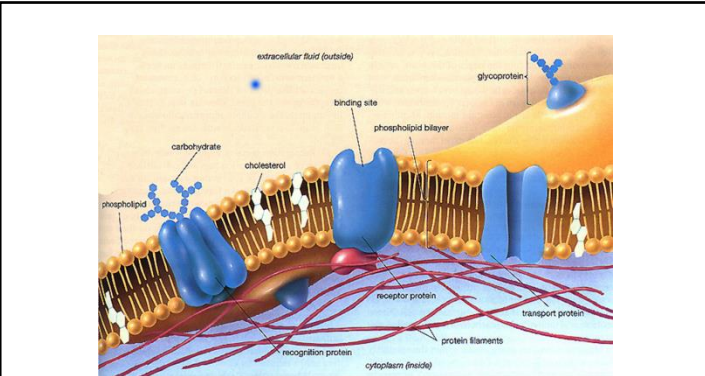
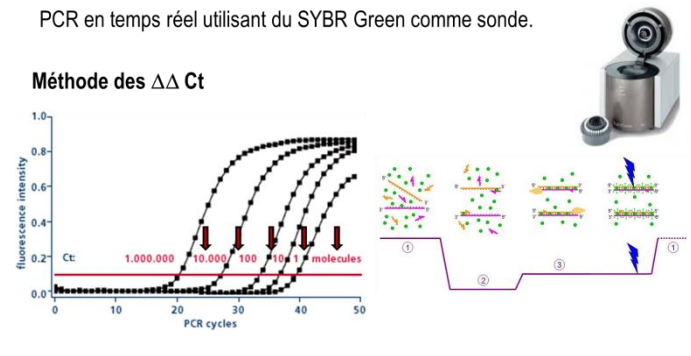
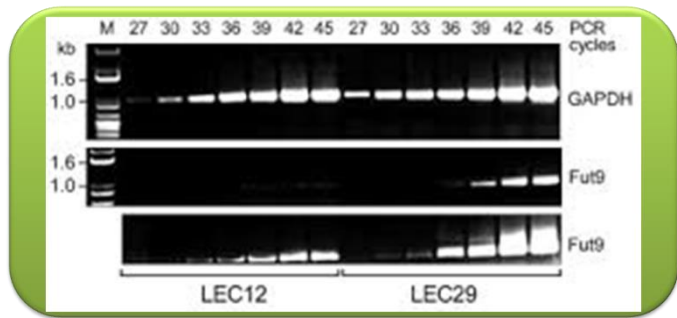


Séquences obtenues dans chaque tube.



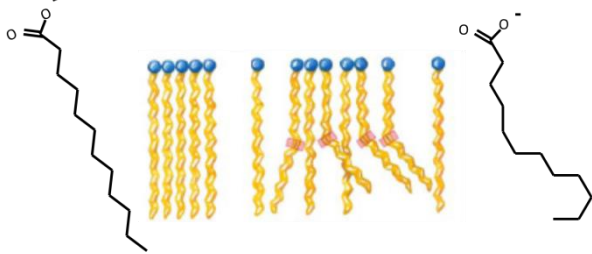
Séparation des molécules marquées par PAGE.





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

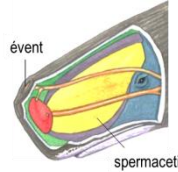
Température élevée : position **gauche** prédominante. Les acides gras sont **désordonnés** : état **fluide**



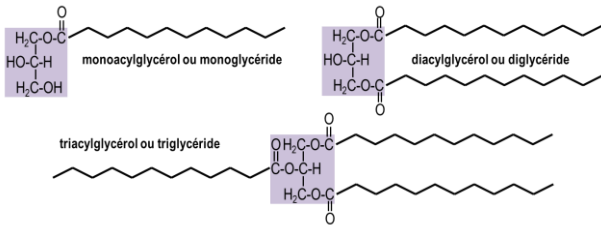
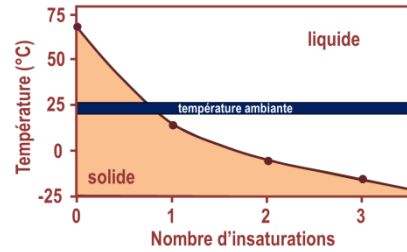
Acide gras saturé	Point de fusion (°C)	Acide gras insaturé	Point de fusion (°C)
C _{12:0}	44,2	C _{16:1}	-0,5
C _{14:0}	53,9	C _{18:1}	13,4
C _{16:0}	63,1	C _{18:2}	5
C _{18:0}	69,6	C _{18:3}	-11
C _{20:0}	76,5	C _{20:4}	-49,5
C _{22:0}	86,0		



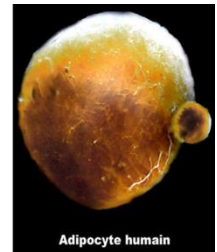
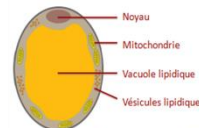
acides gras essentiels



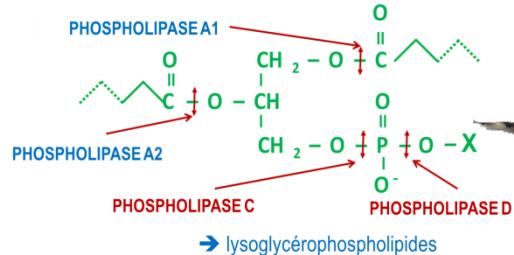
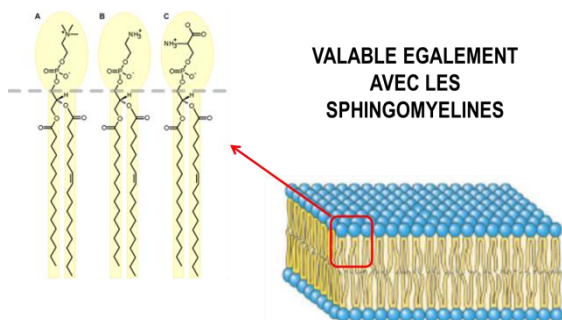
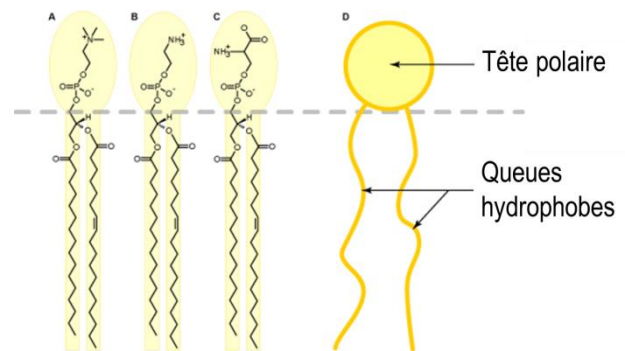
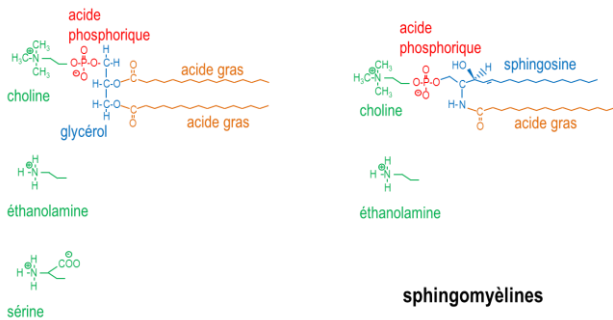
4°C → état solide et dense, l'animal plonge
37°C → état liquide et léger, l'animal remonte

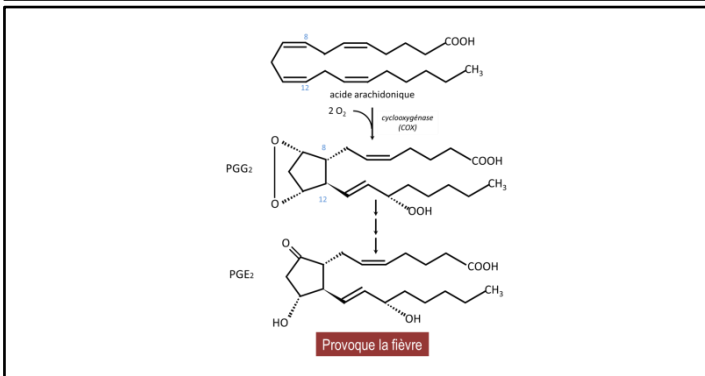
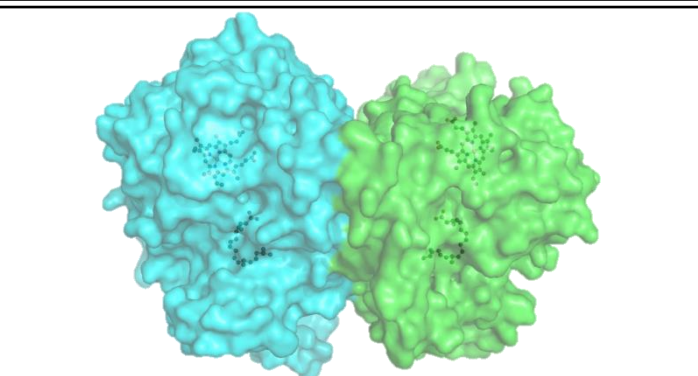
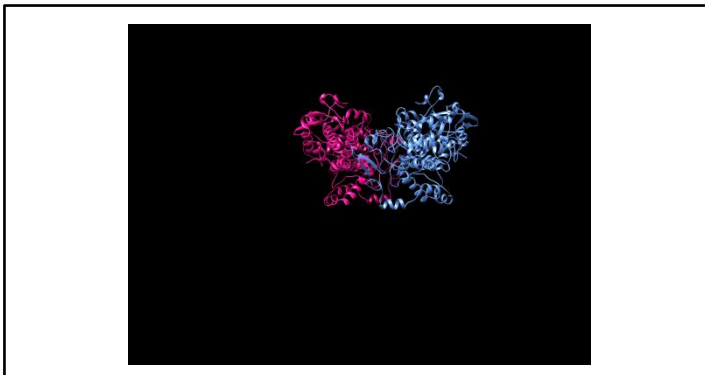
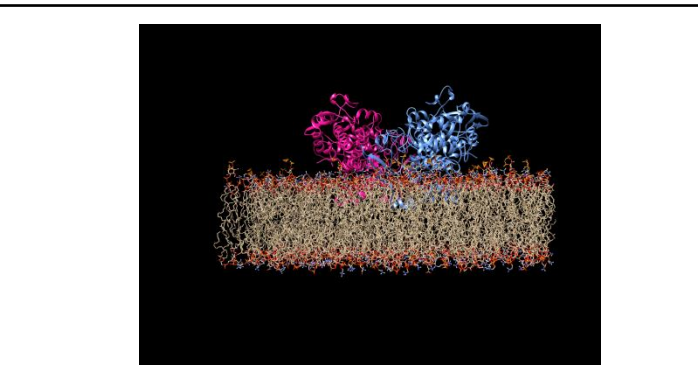
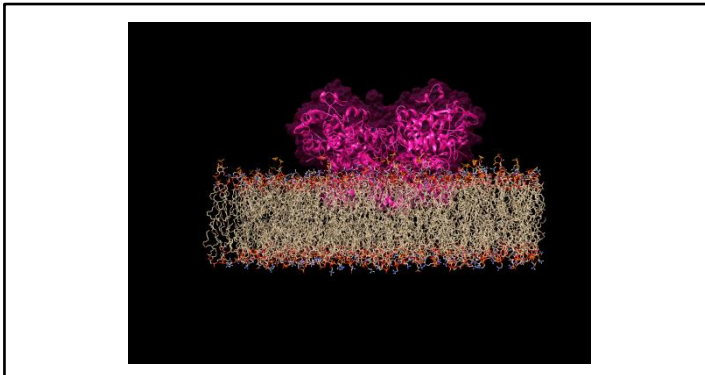
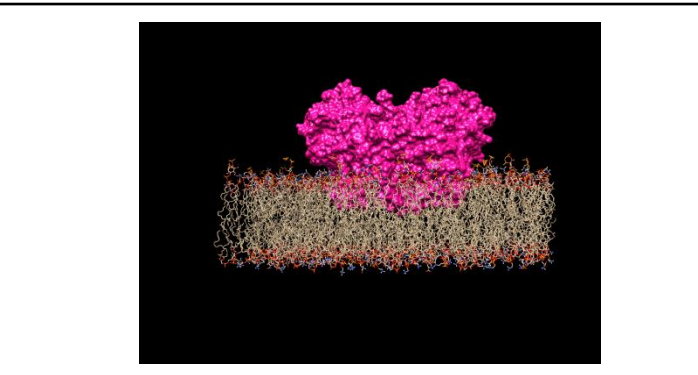
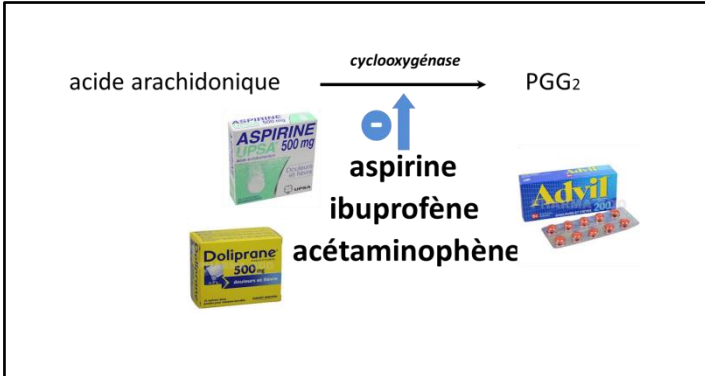
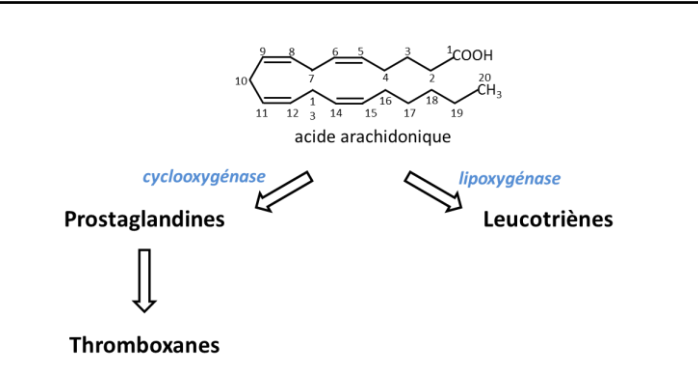
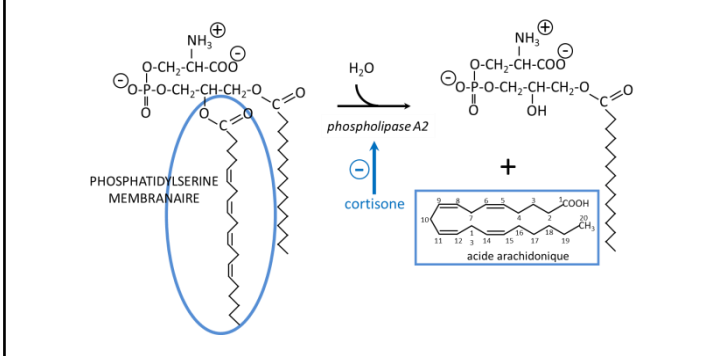
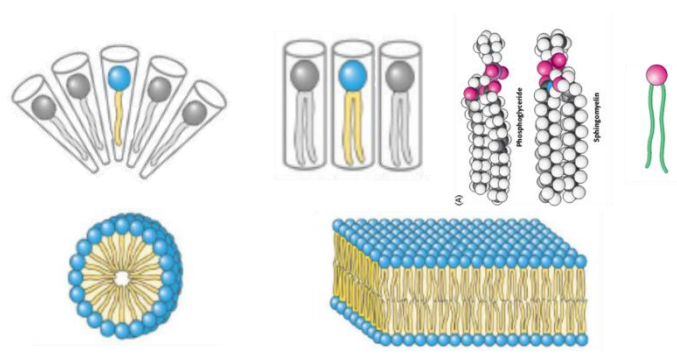


Adipocytes

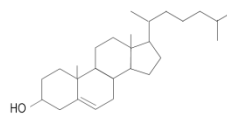
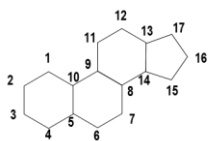


Adipocyte humain

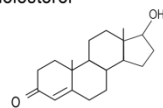




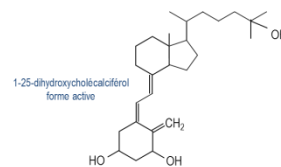
noyau stérol



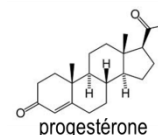
cholestérol



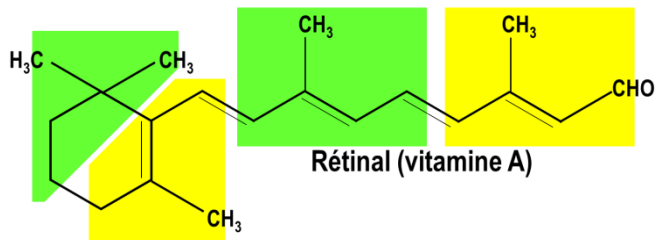
testostérone



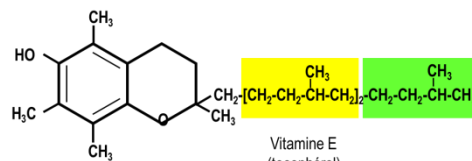
1-25-dihydroxycholecalciférol
forme active



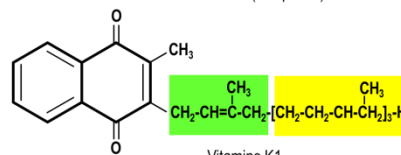
progestérone



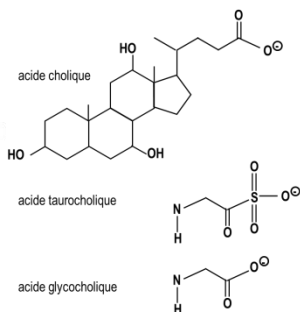
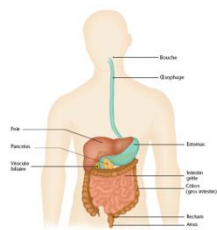
Rétinal (vitamine A)



Vitamine E
(tocophérol)



Vitamine K1
(phylloquinone)



1

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



2



3



4

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

