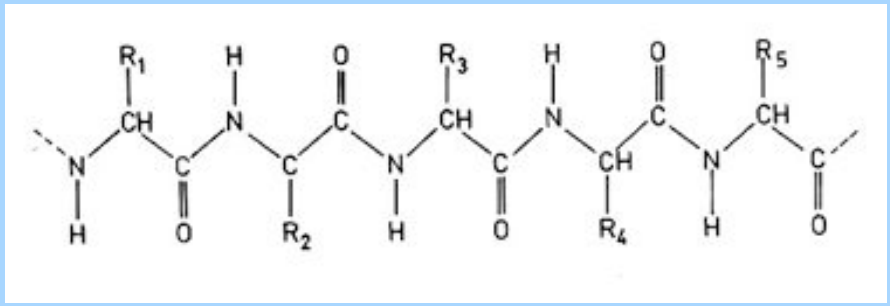
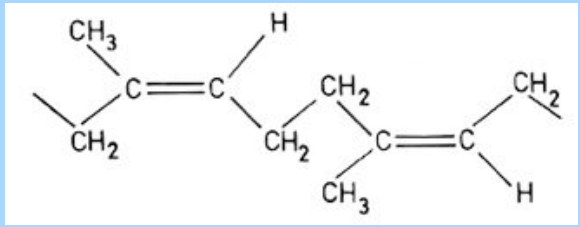
NORCIA CINQUE
LUGLIO 2005, Pier Luigi Luisi

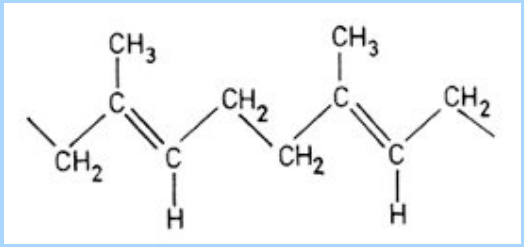
WHY DOES NATURE USE MACROMOLECULES?
WHY ARE ENZYMES SO LARGE?

Or: must enzymes be macromolecules?





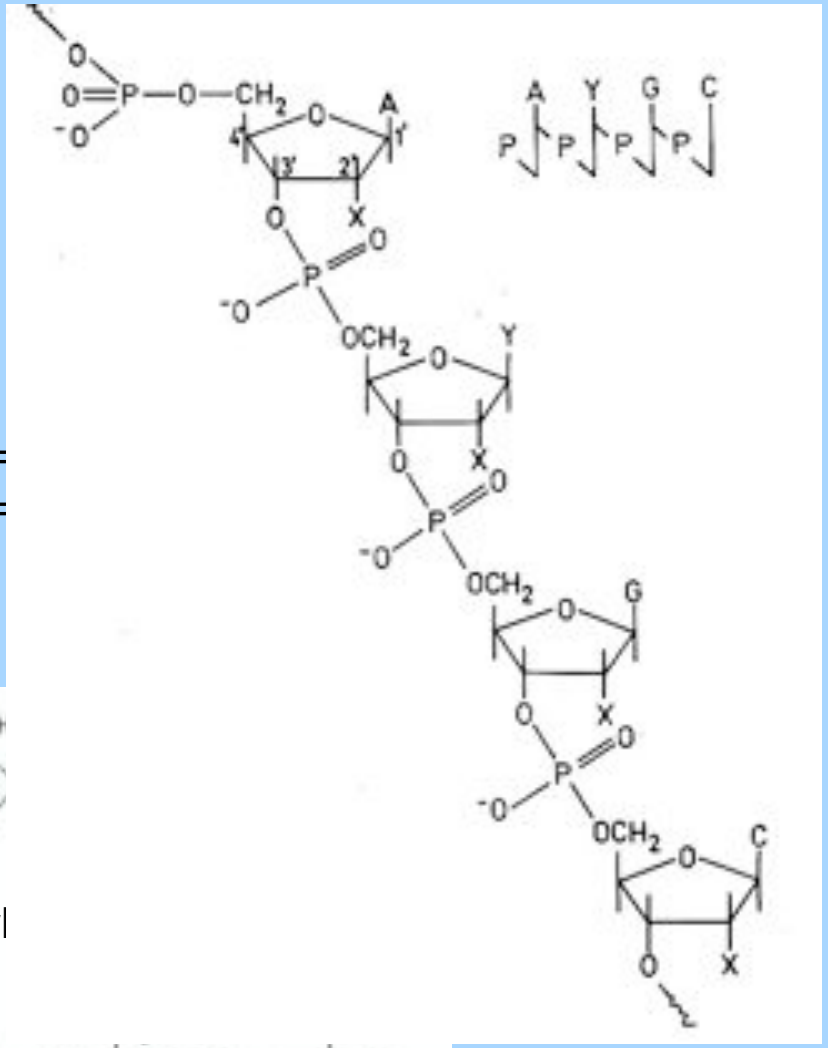
Caoutchouch



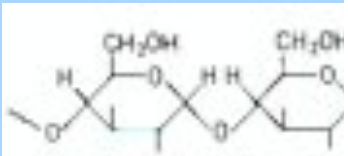
Guttapercha

RNA (X=

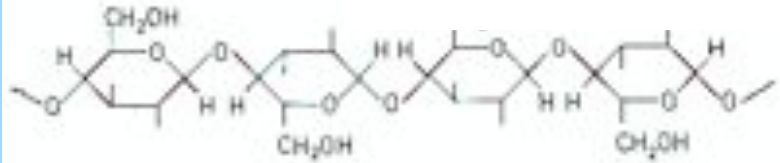
DNA (X=



3`-end
(”tail”)



Amyl



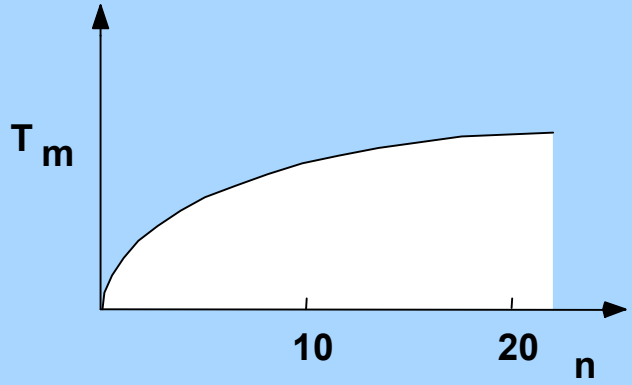
Cellulose

n-butane  gas

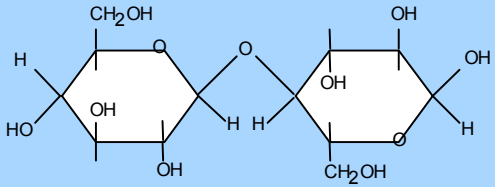
n-hexane  liquid

PE  solid

why is polyethylene solid ?

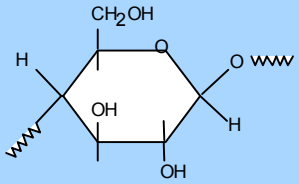


The case of cellulose vs cellobiose



cellobiose

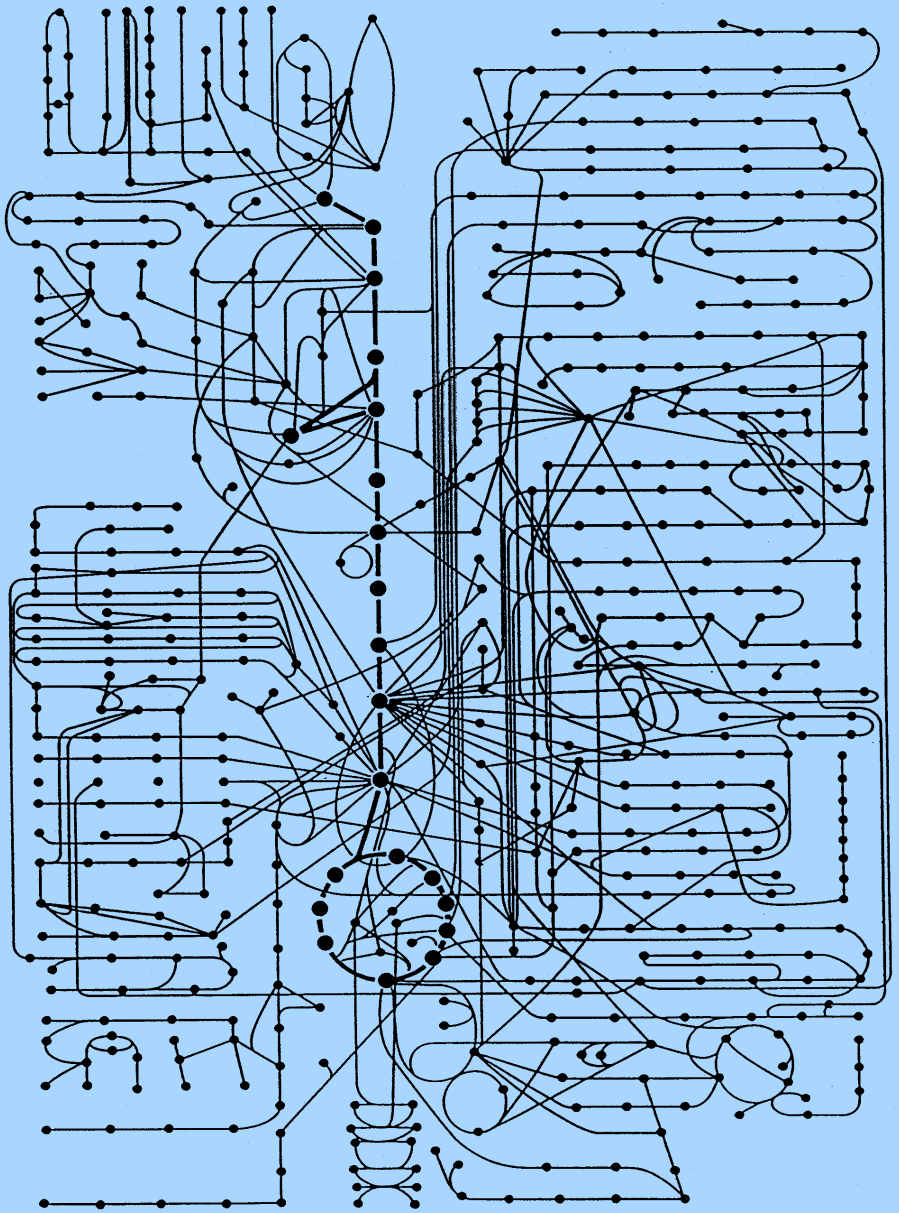
why is cellulose insoluble ?



cellulose

....BUT WHY MUST THE CATALYSTS OF LIFE (THE ENZYMES) BE SO BIG?

A maze illustrating the chemical reactions that interconvert small molecules in cells.



Urease

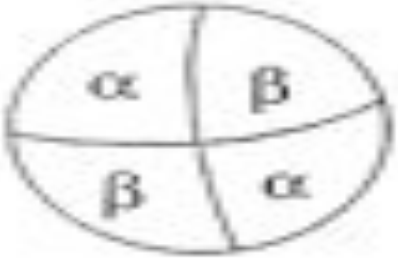
MW 480.000

Urea

MW 60

Hemoglobin

MW 64.000



Oxygen

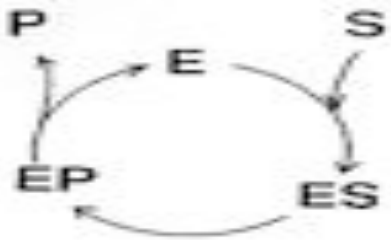
MW 32

1 chain –140 residues

⟶ –1140 atoms

2 atoms

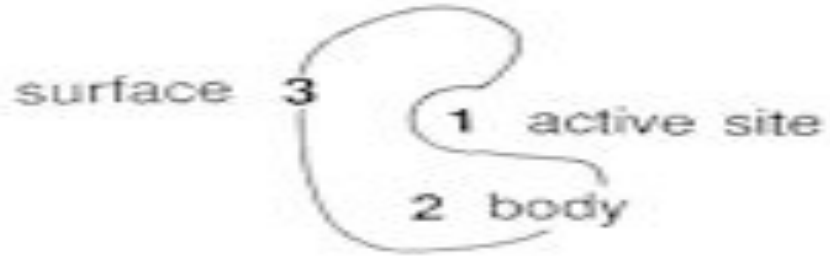
HOW DO ENZYMES WORK?

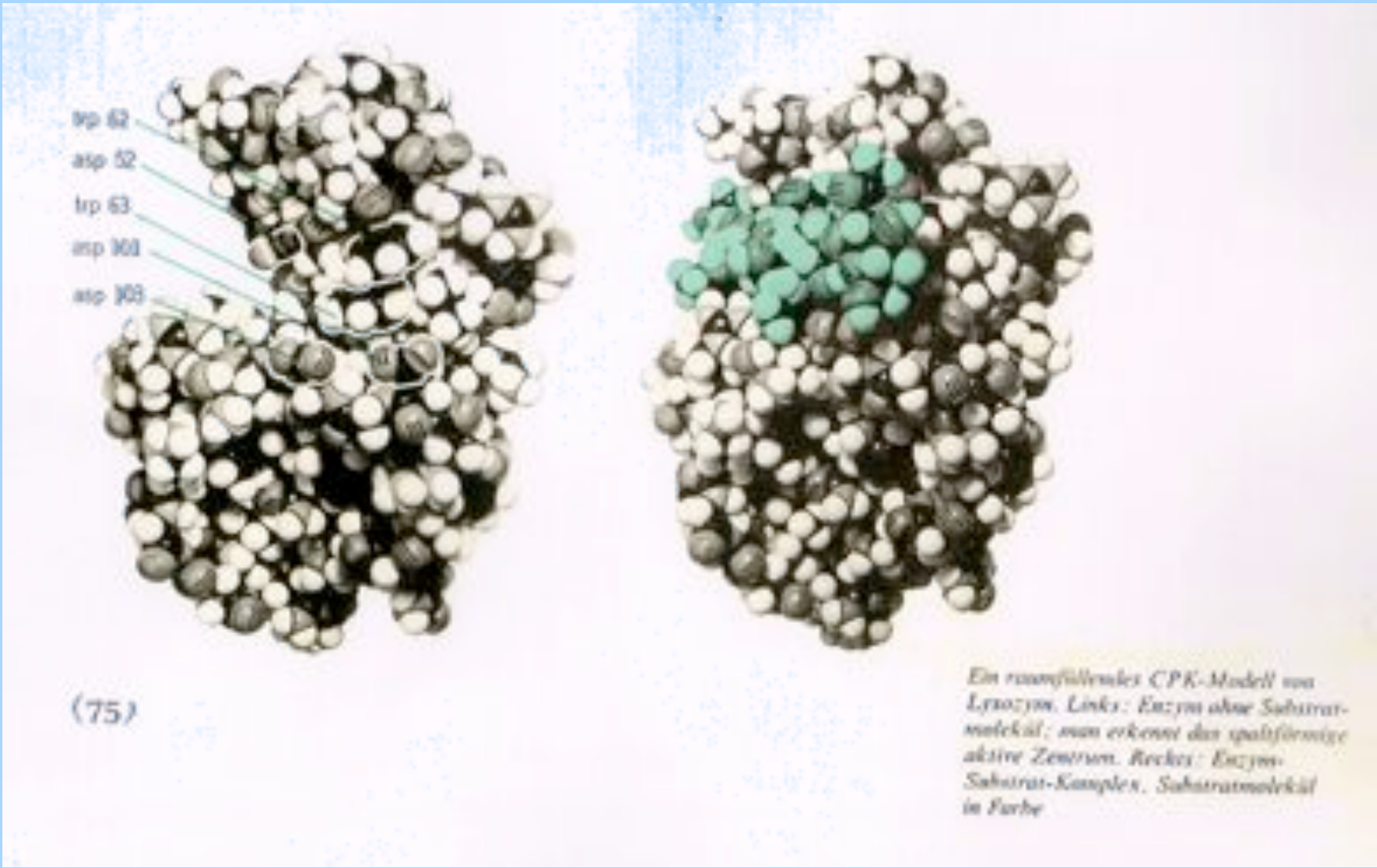


$$E + S \longleftrightarrow ES \longleftrightarrow E + P$$

Recognition (Binding) (fast)

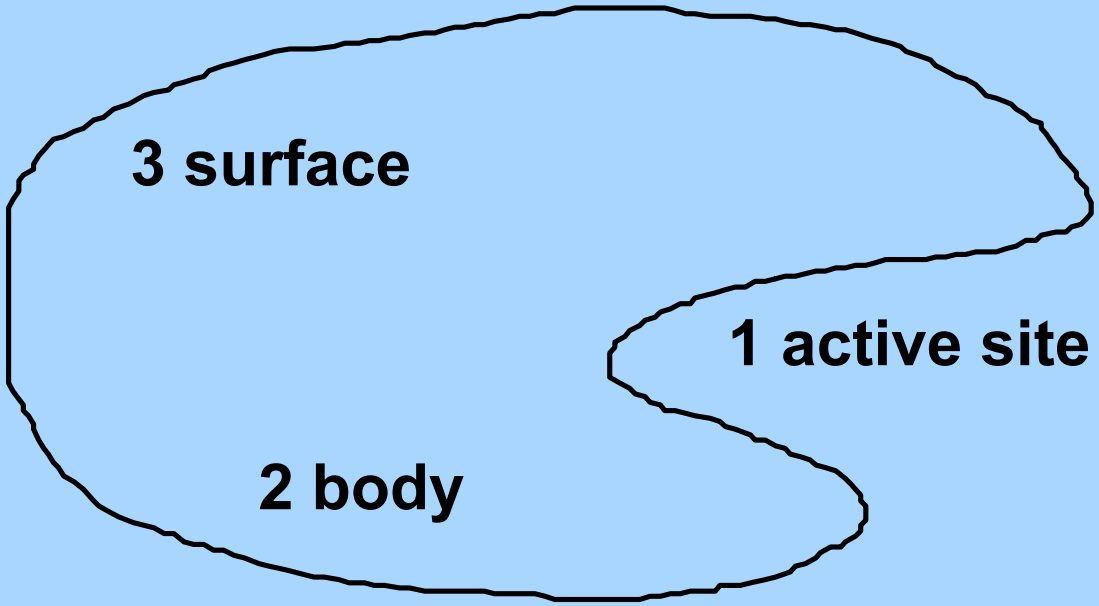
Chemistry (slow)

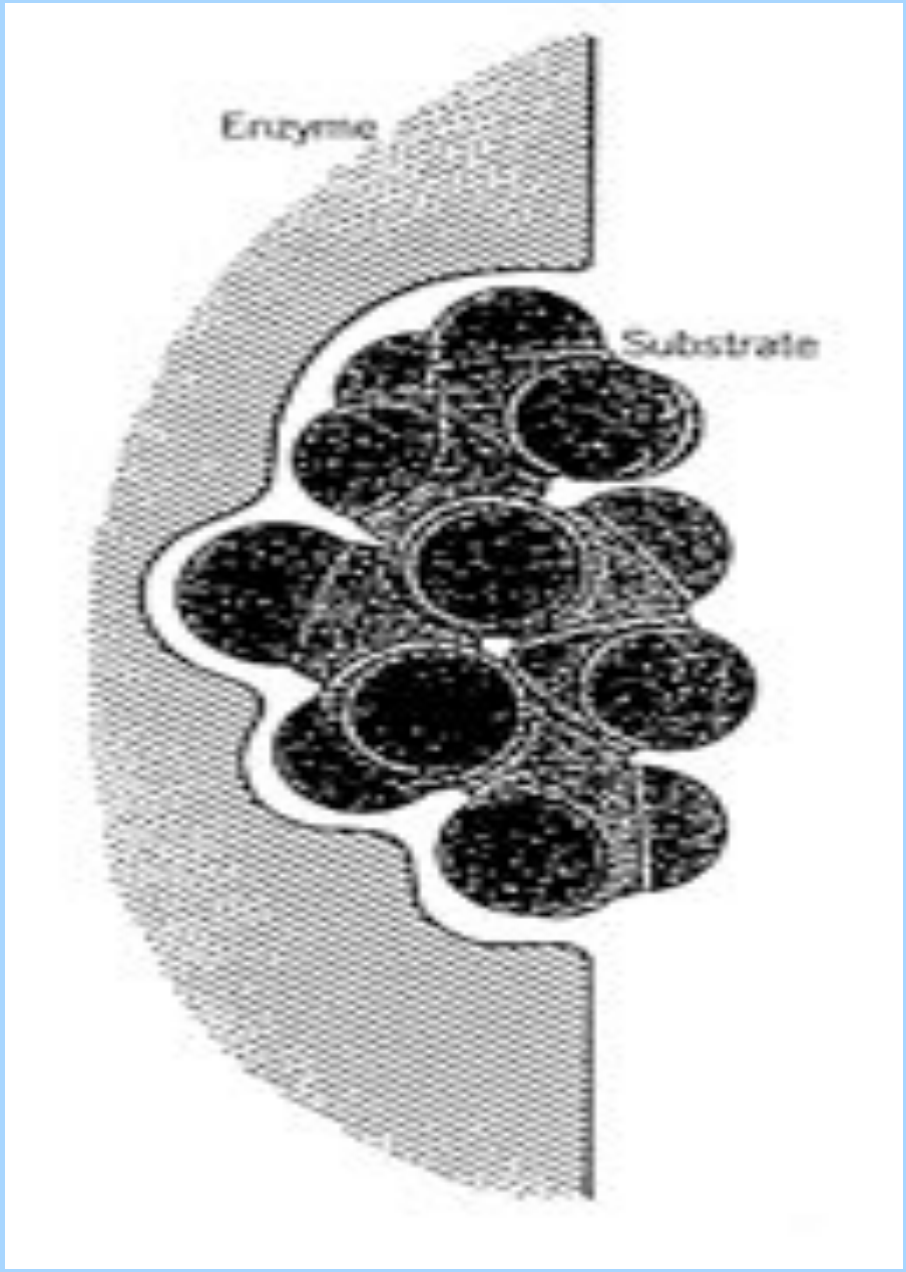


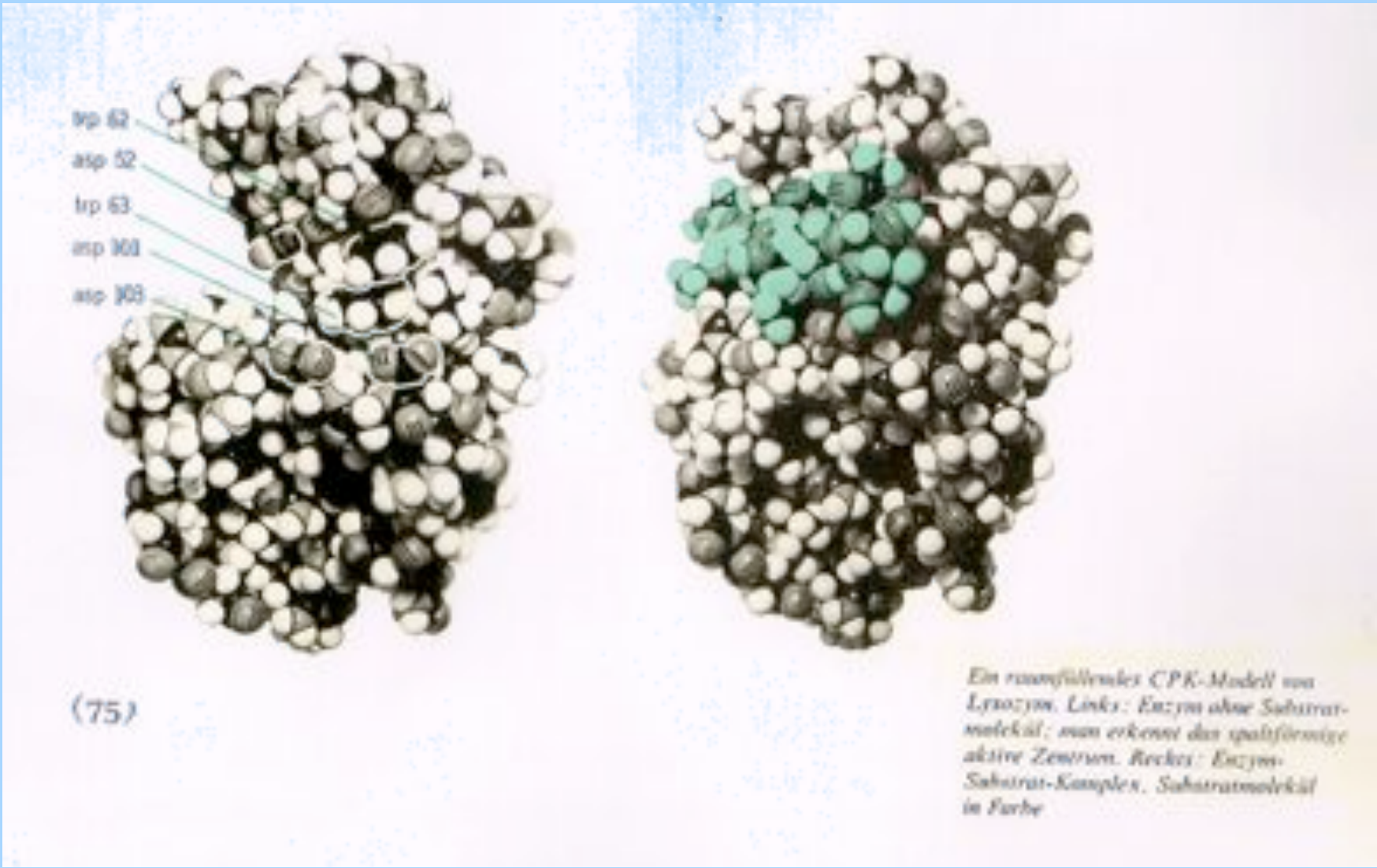


(75)

Ein raumfüllendes CPK-Modell von Lysozym. Links: Enzym ohne Substratmolekül; man erkennt das spaltförmige aktive Zentrum. Rechts: Enzym-Substrat-Komplex. Substratmolekül in Farbe


3 surface
1 active site
2 body


Enzyme
Substrate



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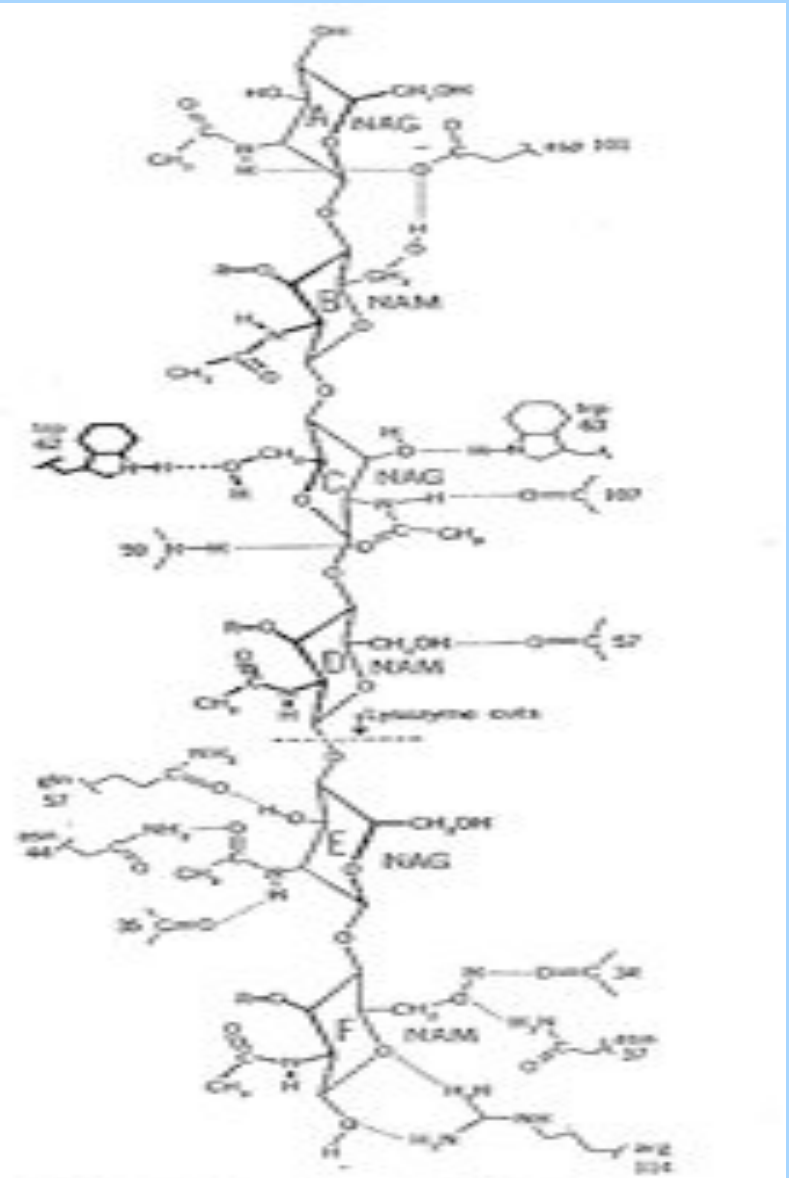
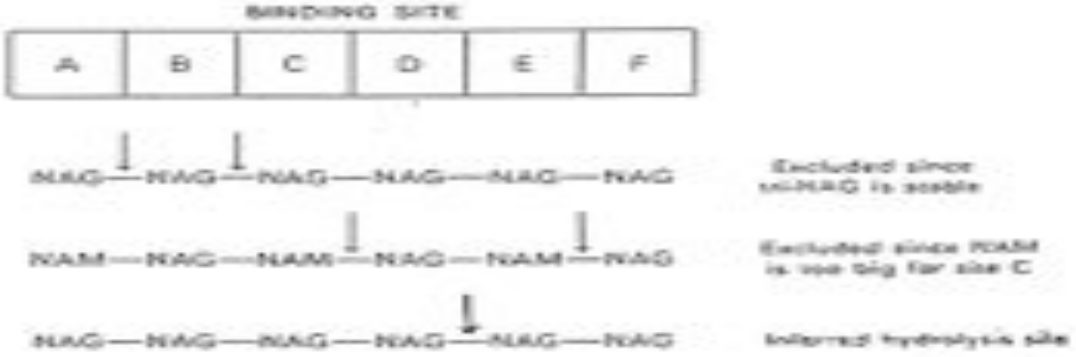
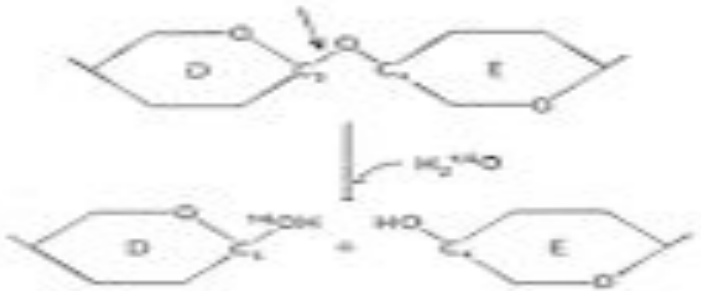


Substrate interactions with lysozyme. The view is into the active crevice, with the darker edges of the rings exposed to the outside, the lighter ones buried at the bottom.—OR = —O—CH(CH₃)—COOH.

Substrate	Relative rate of hydrolysis
NAG_2	0
NAG_3	1
NAG_4	8
NAG_5	4000
NAG_6	30000
NAG_8	30000



Steps in deducing that the glycosidic bond between sugar residues D and E is the one cleaved by lysozyme



Hydrolysis in $H_2{}^{18}O$ water showed that lysozyme cleaves the C_1—O bond rather than the O—C_4 bond. (Only the skeletons of the D and E residues are shown here.)

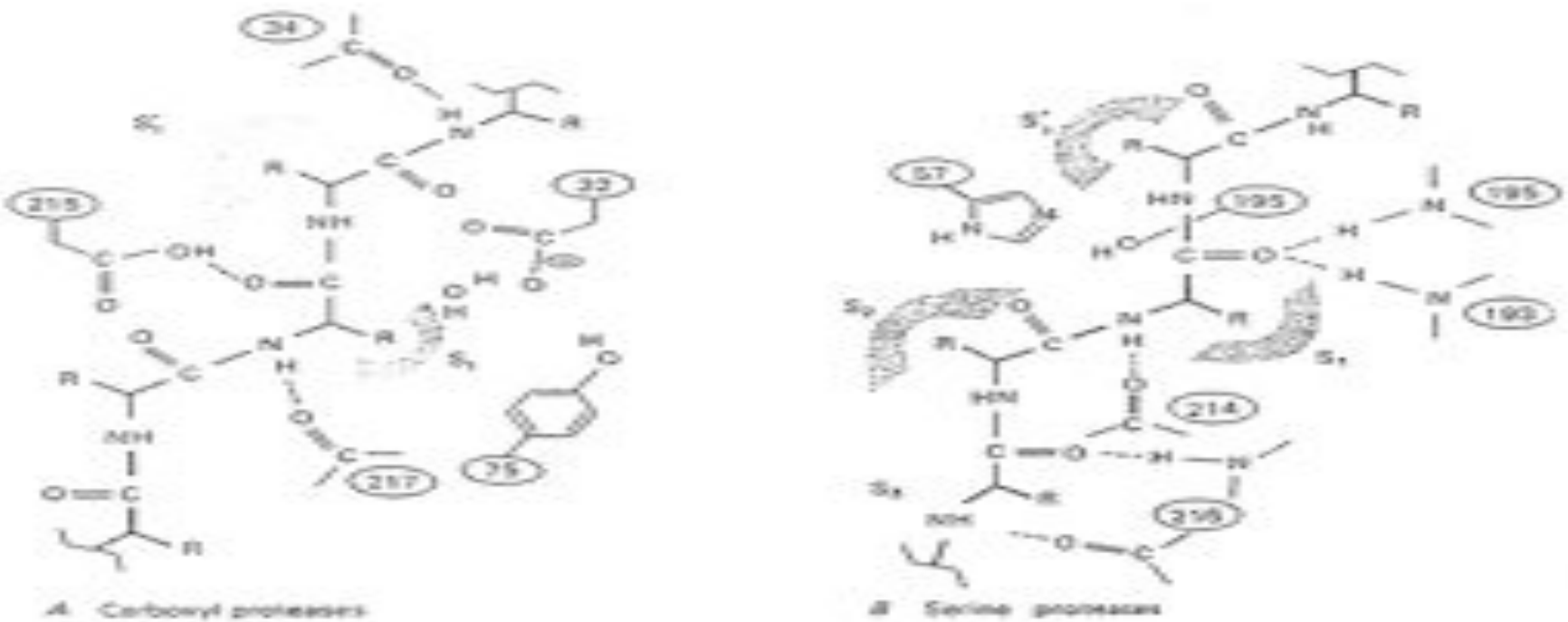


Figure 9–12
Schematic comparison of the active sites of (A) carboxyl and (B) serine proteases. The polypeptide substrates are indicated by the thick lines; functional groups of the enzymes are also included. Hydrogen bond interactions are indicated by dotted lines; nonpolar interactions by the shaded areas. (From M. N. G. James, Can. J. Biochem. 58:231–271, 1980.)

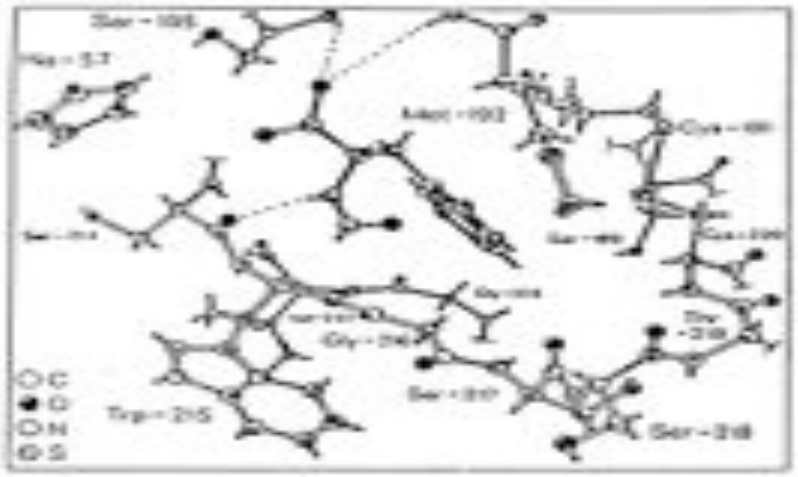


FIG. 1.12. Comparison of the binding pockets in chymotrypsin (top, with N-formyl-L-tryptophan bound) and elastase (bottom, with N-formyl-L-alanyl bound). The binding pocket in trypsin is very similar to that in chymotrypsin; an enzyme that residue 189 is an aspartate or head positively charged side chains. Note the hydrogen bonds between O•• solvent and backbone of the enzyme.

Ladungsübertragungs - Verbund
- System

Ladungstransfer
→ reaktives Ser 195

Figure 8-14
Conformation of the charge relay system in chymotrypsin. [Based on D. M. Blow and T. A. Steitz, X-ray diffraction studies of enzymes, Ann. Rev. Biochem. 39(1970): 86. Copyright © 1970 by Annual Reviews Inc. All rights reserved.]

Chymotrypsin
Trypsin
Elastase

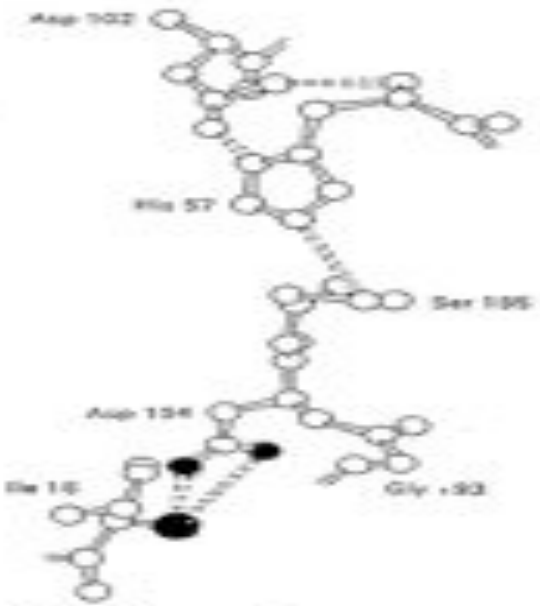
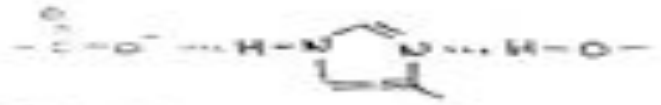


Figure 8-15
Environment of aspartic 194 and isoleucine 16 in chymotrypsin. The electrostatic interaction between the carboxylate of Asp 194 (red) and the α-NH_3 group of Ile 16 (blue) is essential for the activity of chymotrypsin. These groups are adjacent to the charge relay network. [Based on D. M. Blow and T. A. Steitz, X-ray diffraction studies of enzymes, Ann. Rev. Biochem. 39 (1970): 86. Copyright © 1970 by Annual Reviews Inc. All rights reserved.]

* Zymogen - Aktivierung

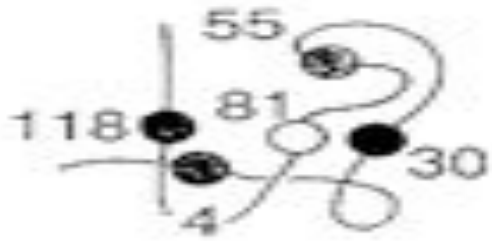


Asp 102 His 57 Ser 195

Asp 102 His 57 Ser 19

A long chain permits "dilution" of active groups:

a long chain is however endowed with an extremely high flexibility:

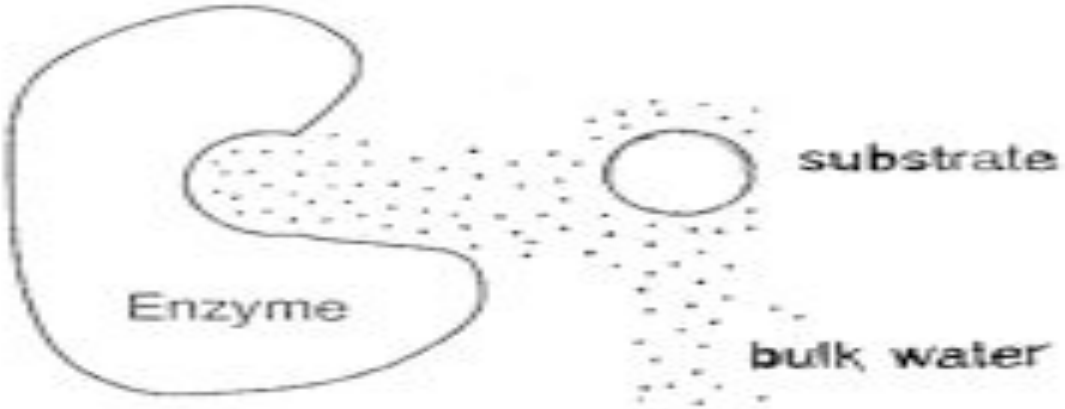


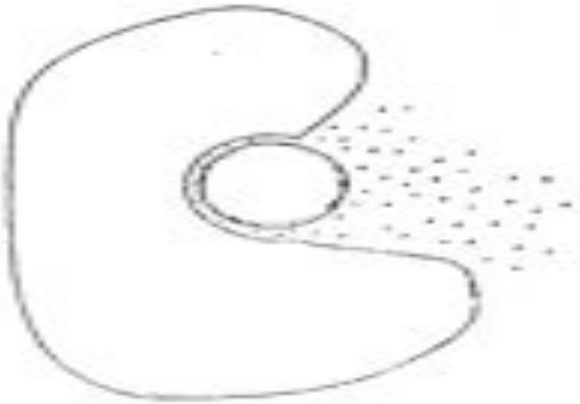
... forced proximity ...

so that groups which are far apart in the primary sequence can come very close together

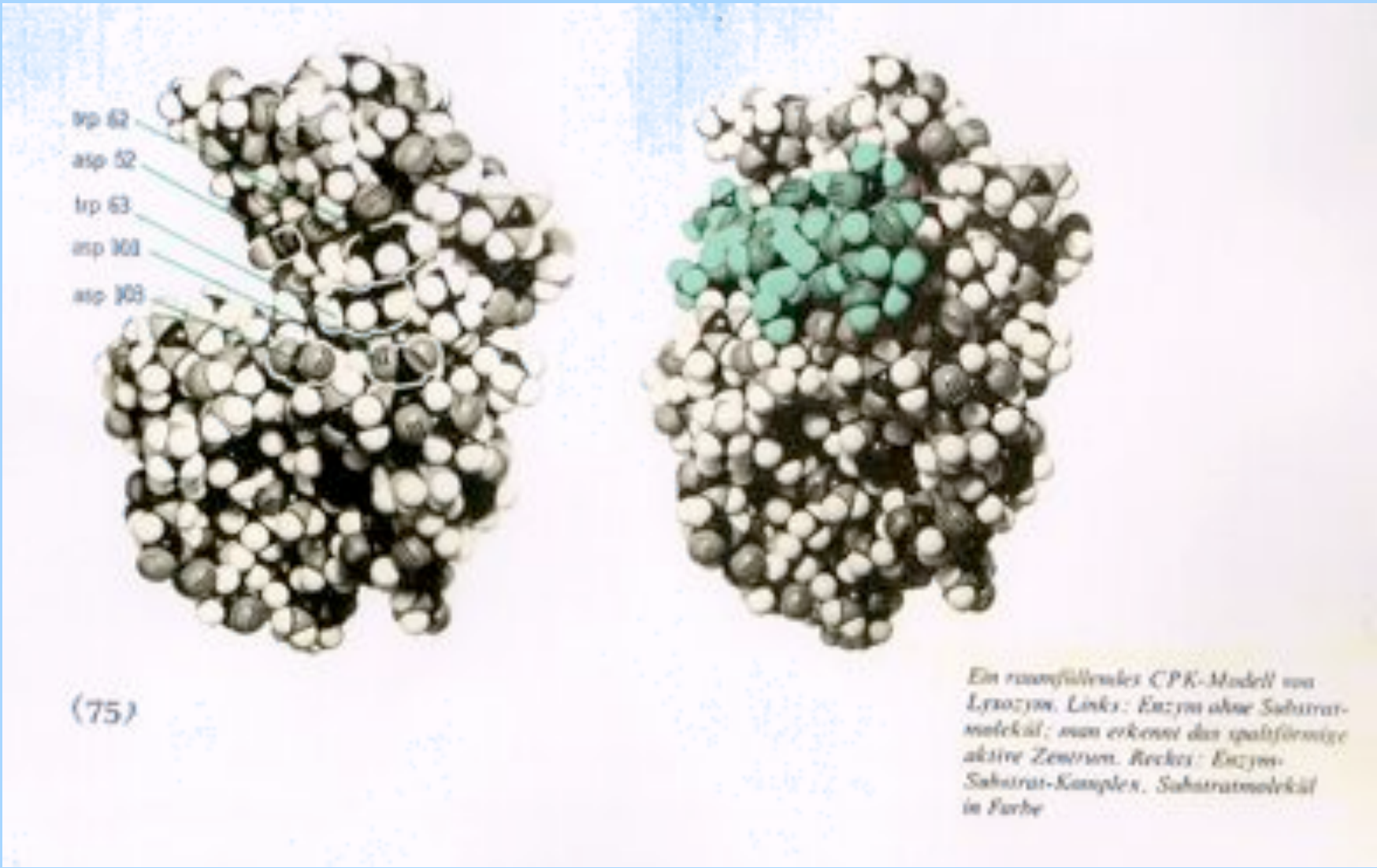
Microenvironement
in the active site:

an important reason for
the overgrowth or enzymes





the enzyme as a
phase transfer
agent



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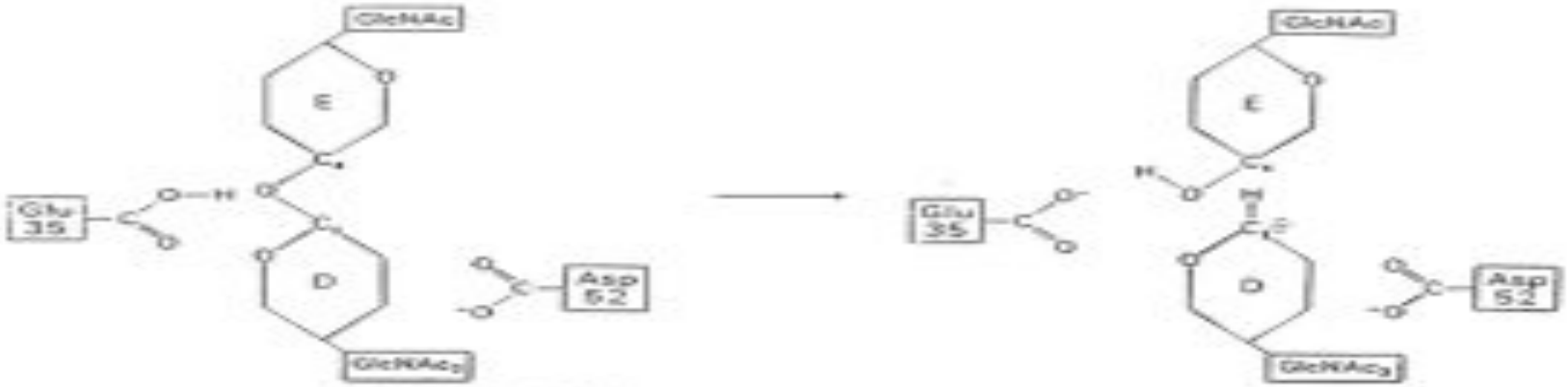
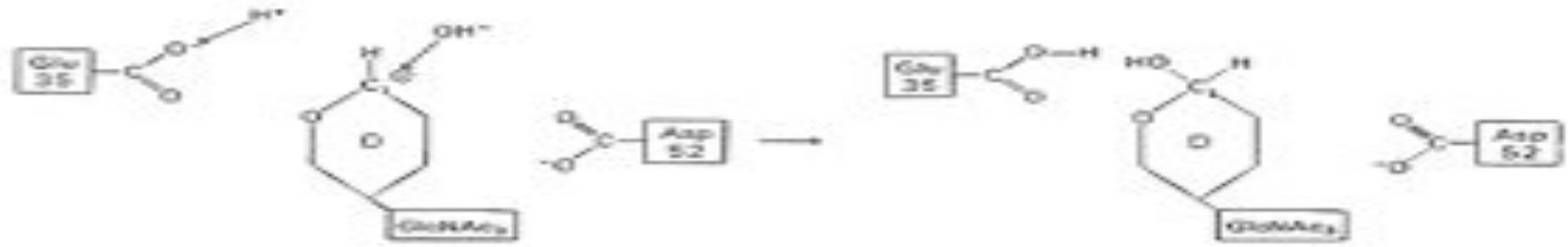
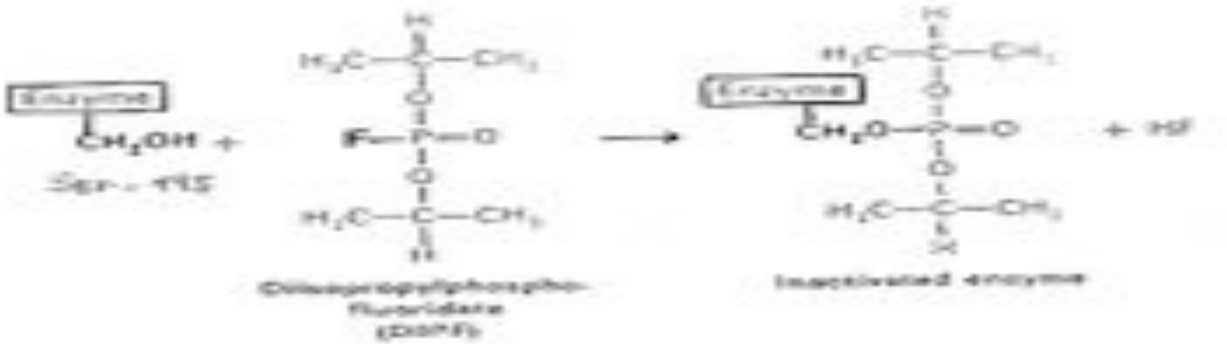


3.12 Der erste Schritt im katalytischen Mechanismus des Lysozyms besteht im Transfer eines H^+ von Glu 35 auf das Sauerstoffatom der glykosidischen Bindung. Dabei wird letztere gespalten und als Zwischenprodukt ein Carbeniumion gebildet.



3.13 Die Hydrolysereaktion wird durch Addition von OH^- an das Carbeniumion und von H^+ an die Seitenkette von Glu 35 beendet.



... die anderen 27 Ser-Reste werden nicht angegriffen ...

Stichwörter:

- besondere Reaktivität von Ser-195
- kovalente Bindung mit E
- Serinproteasen

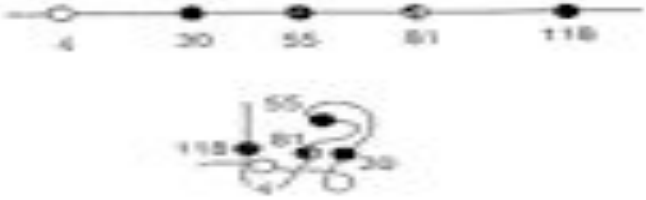
ACTIVE REGION: FOUR GOOD REASONS FOR AN ENZYME TO BE A MACROMOLECULE

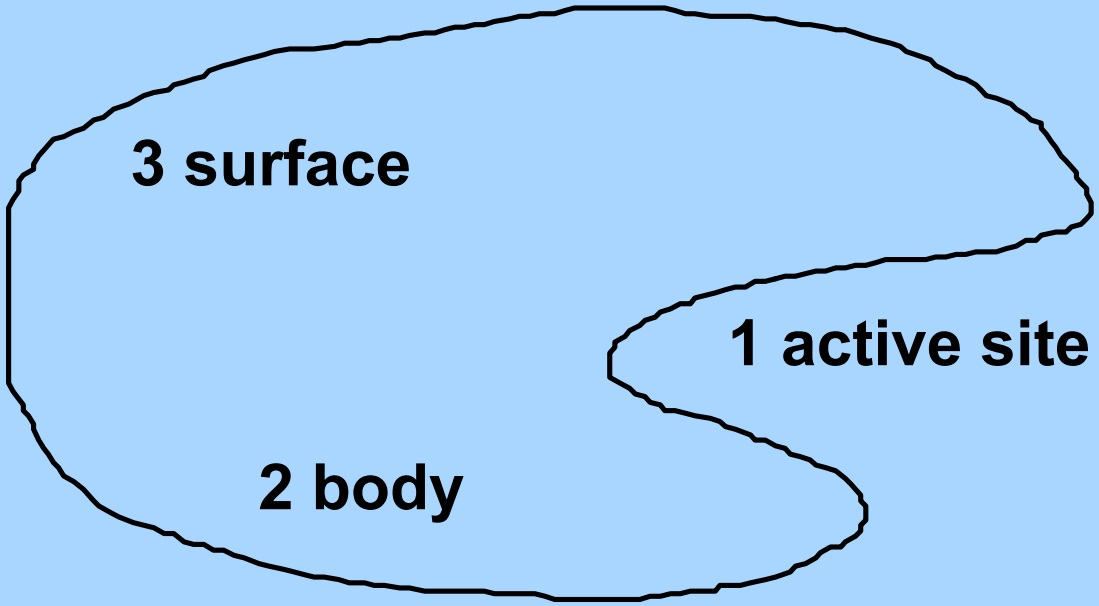
1. ***Binding:*** a long chain can give rise to a long high stability of the ES complex via non-covalent, weak interactions.

2. ***Stereochemical complementarity:*** only with a long chain can tortuous walls be built, which permit the good fit of the substrate

3. ***Microenvironment:*** a long chain can create its own environment for the reaction

4. ***Forced proximity of active residues:*** the active residues may be far apart in the primary sequence, which permits a high degree of freedom for the final adjustments required for catalysis

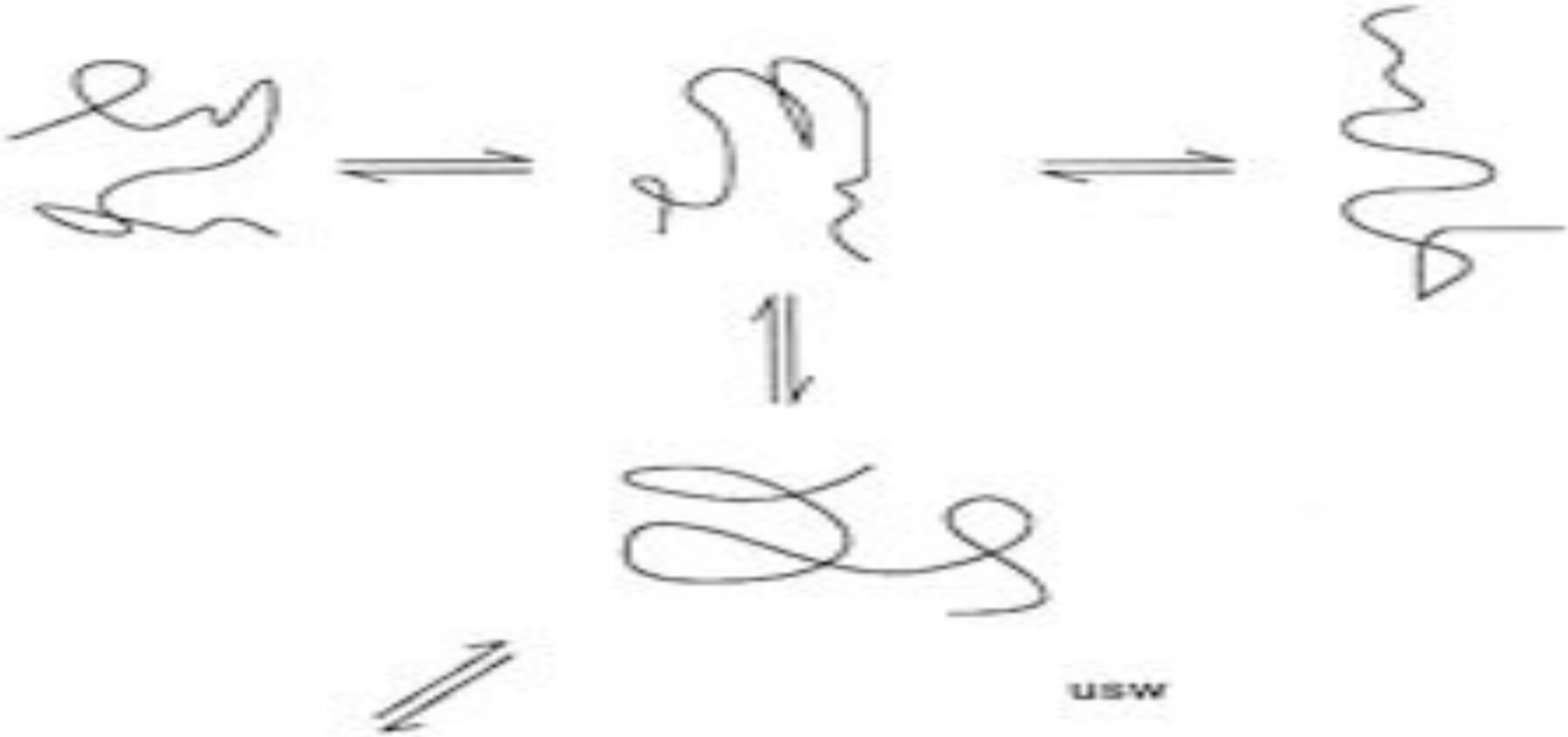




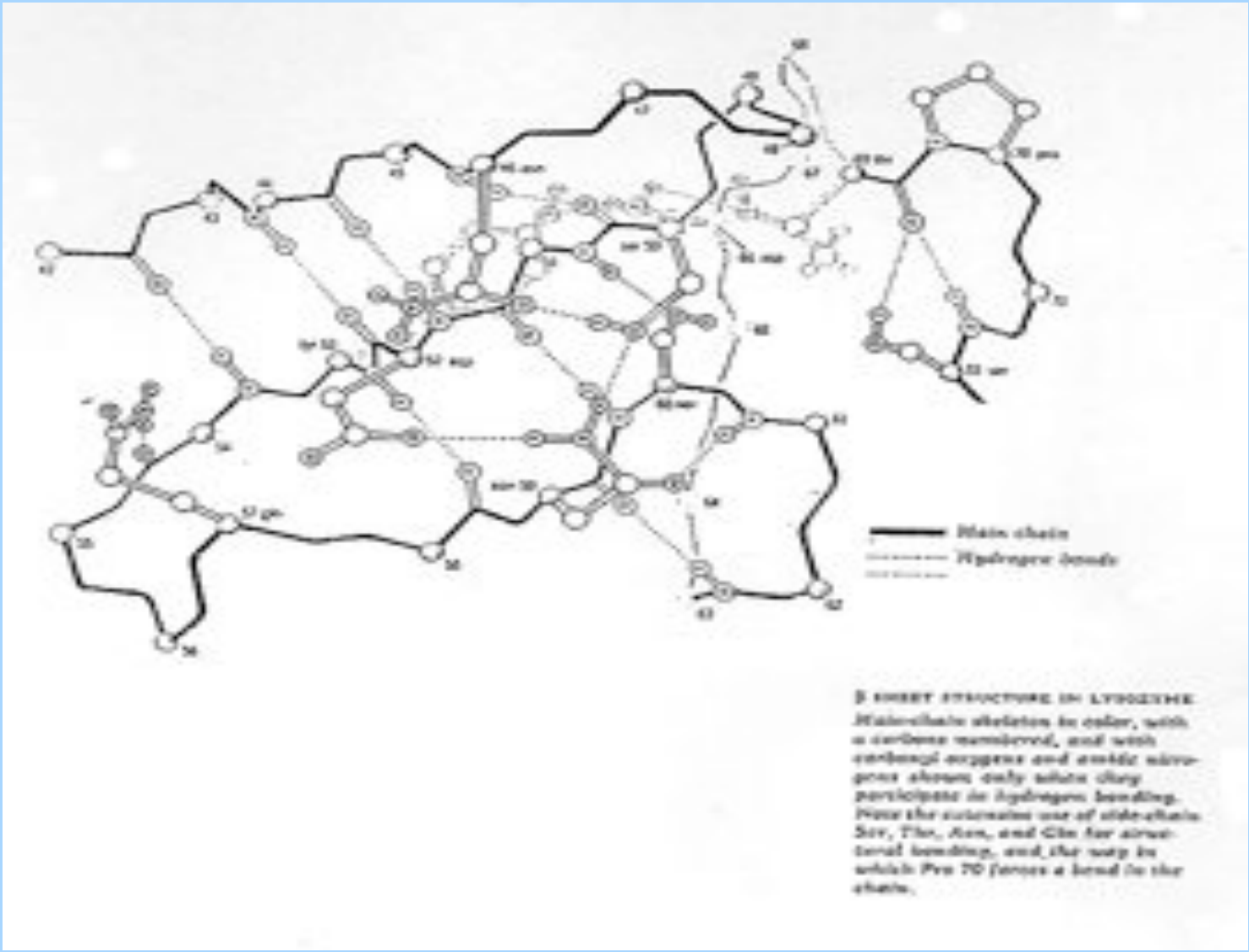
body:

- conformation
- folding
- stability, rigidity
- cooperativity

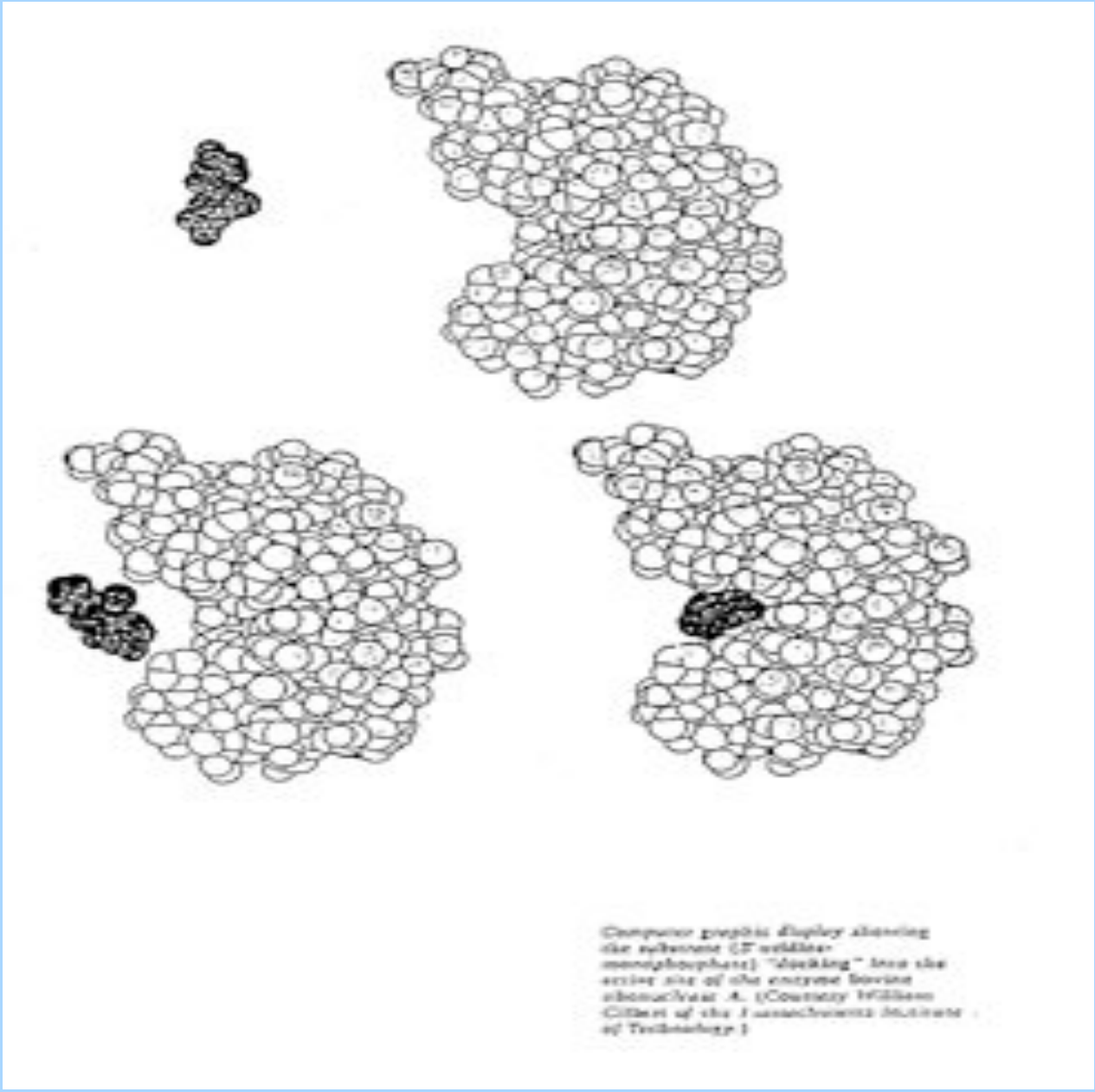
Synthetic Polymers in solution are "random coils"



Transformation rate $\sim 10^8 - 10^9$ /sec (C-C-C)



β SHEET STRUCTURE IN LYSOZYME
Main-chain skeleton is color, with
a carbons numbered, and with
carbonyl oxygens and amide nitro-
gens shown only where they
participate in hydrogen bonding.
Note the extensive use of side-chain
Ser, Thr, Asn, and Gln for struc-
tural bonding, and the way in
which Pro 70 forces a bend in the
chain.



Computer graphic display showing the substrate (3'-cytidine-monophosphate) "docking" into the active site of the enzyme bovine ribonuclease A. (Courtesy William Gilbert of the Massachusetts Institute of Technology.)

PROTEINS ARE TIGHTLY PACKED AS GOOD MOLECULAR CRYSTALS.

The observed local packing densities of proteins vary between 0.68 and 0.82 ... In comparison, equal-sized hard spheres in closest packing have a packing densitiy of 0.74. Crystals of small molecules that are held together bv van der Waals forces have values between 0.70 and 0.78. Glasses, oils ... have values below 0.70 or even below 0.60.

Therefore proteins are indeed as densly packed as small molecules in van der Waals crystals.

(from G.E. Schulz & R.H. Schirmer, Principles of Protein Structure, Springer-Verlag, p. 43, 1979)

the long chains builds a high packing density region actualizing an ordered(*) tridimensional cavity and a surface which positively interacts with the physiological environment

(*) ...do not confuse "order" with symmetry. A protein is actually a case of "aperiodic order", the highest type of structural order.

order: a situation in which each constituting element of the system has the position in space and time precisely assigned by the programmer

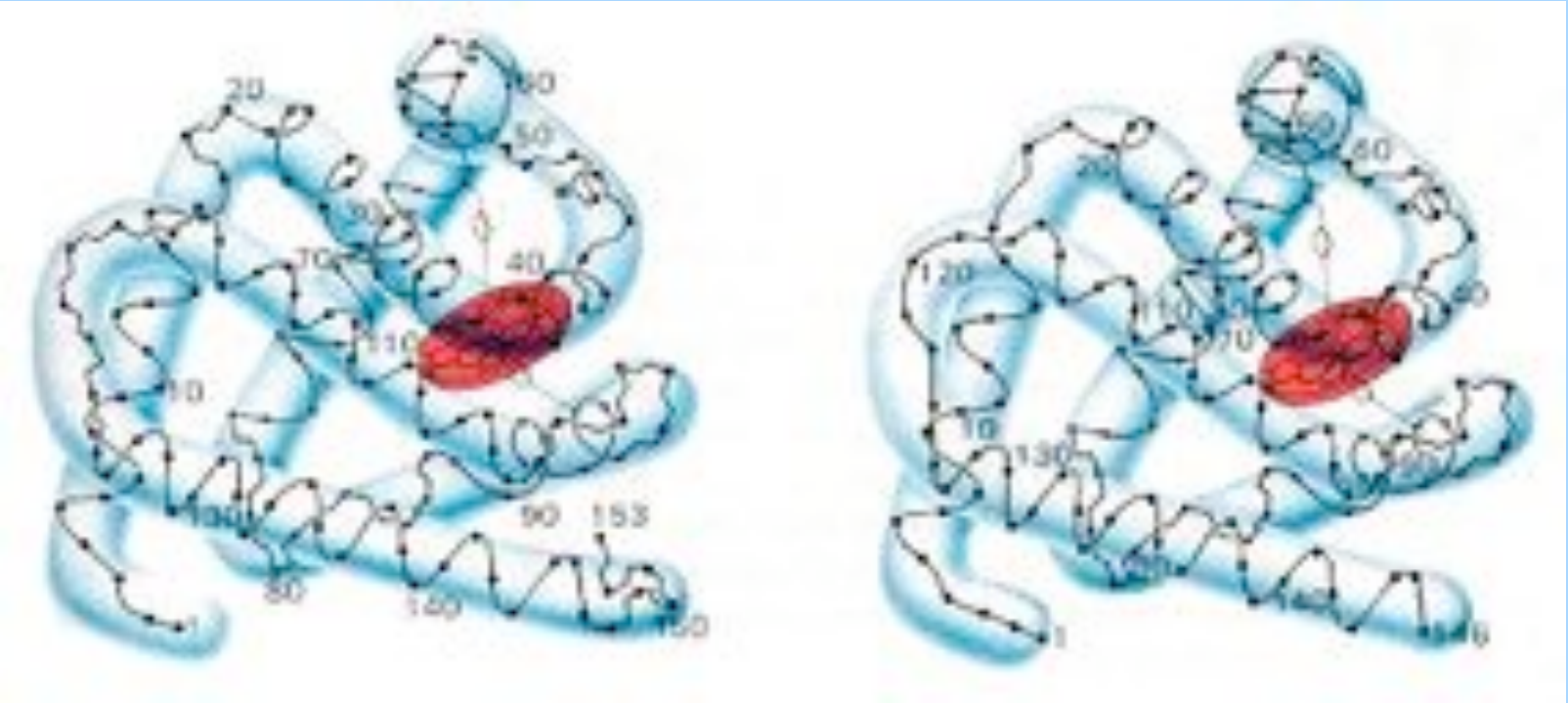
Conformational rigidity
and
conformational flexibility
(must coexist in enzymes)

*

conformational rigidity to warrant individuality of form and specificity of binding

*

conformational flexibility to warrant fine tuning in the function (allosterism, regulation, probably catalysis)

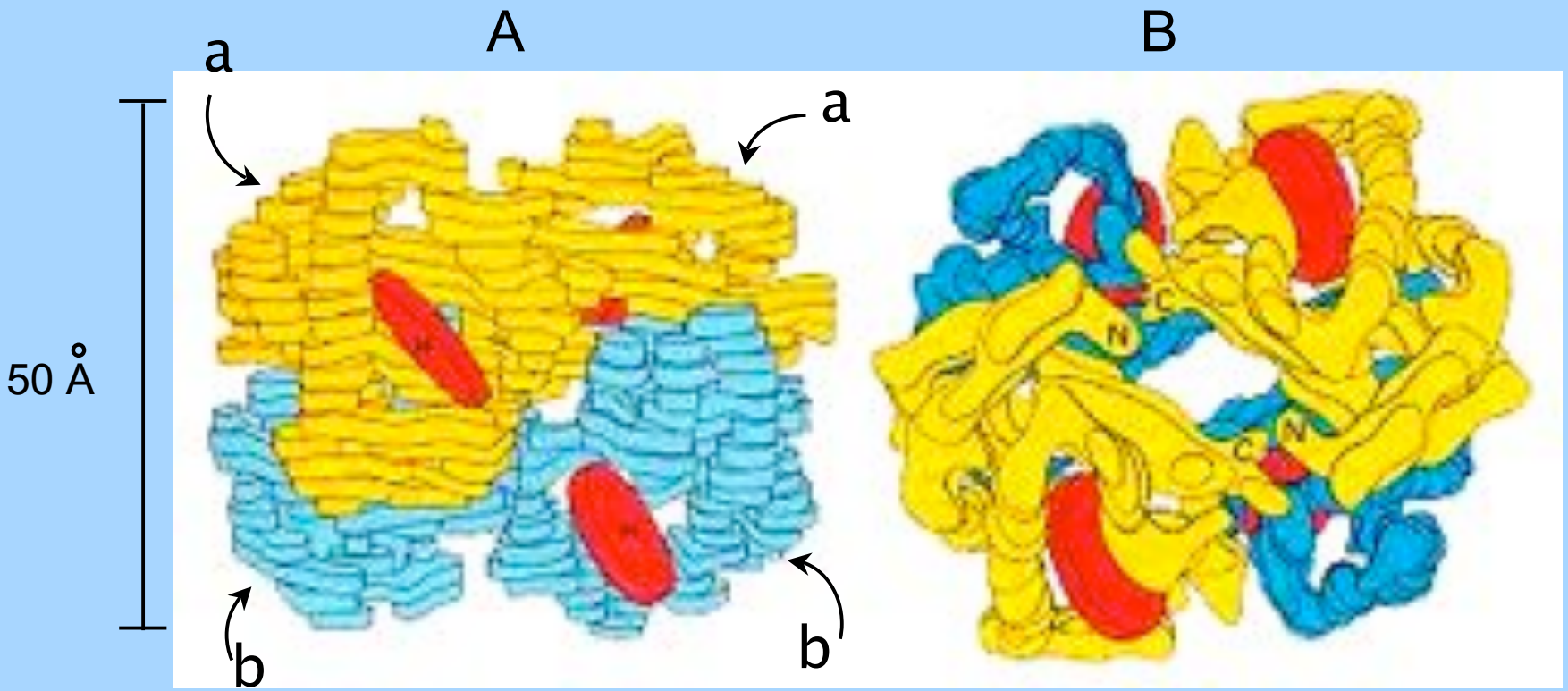


Myoglobin

b chain of hemoglobin

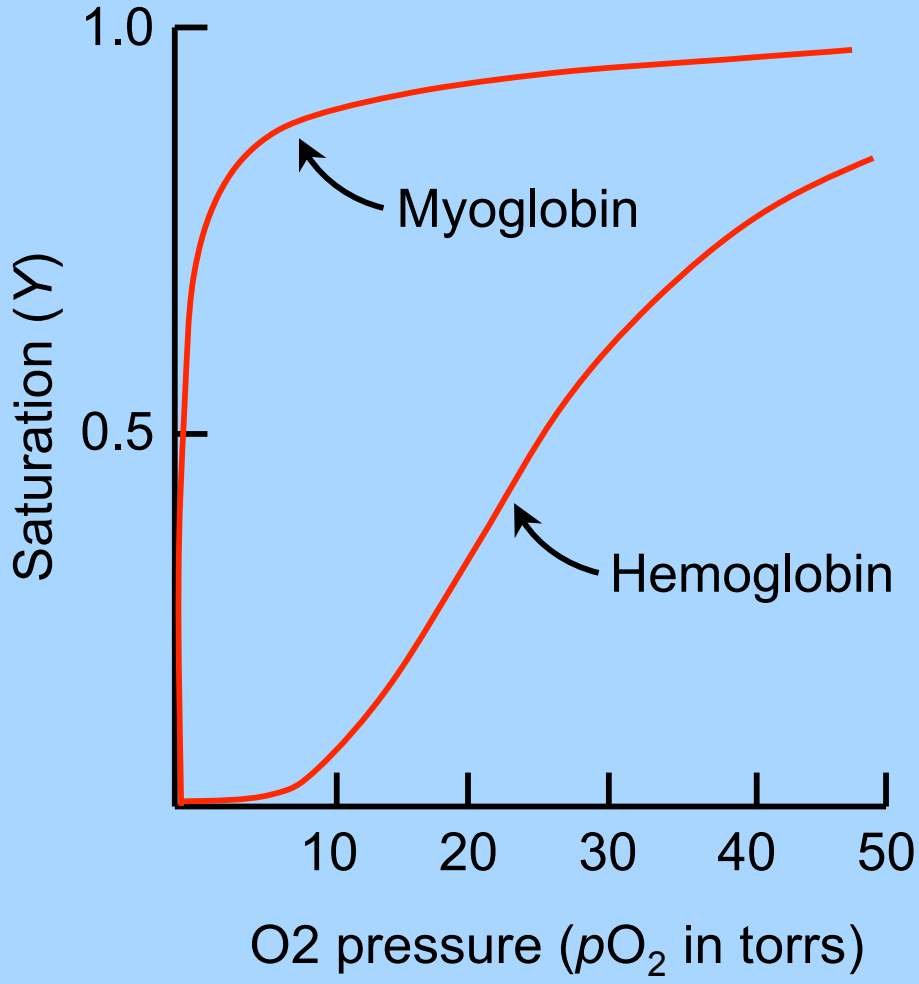
Comparison of the conformations of the main chain of myoglobin and the b chain of hemoglobin.
The similarity of their conformations is evident.

(from Biochemistry / L.Stryer, 4th ed.)



Model of hemoglobin at low resolution.
The a chains in this model are yellow, the b chains blue, and the heme groups red.
View (A) is at right angles to view (B); the top of (A) is visible in (B).

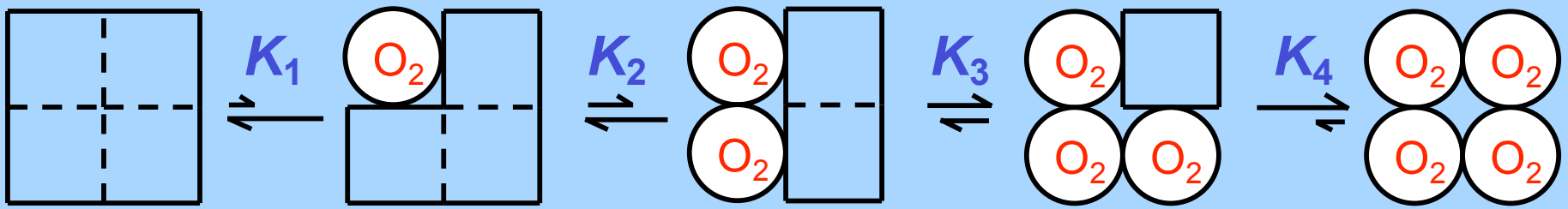
(from Biochemistry / L.Stryer, 4th ed.)



Oxygen dissociation curves of myoglobin and hemoglobin.

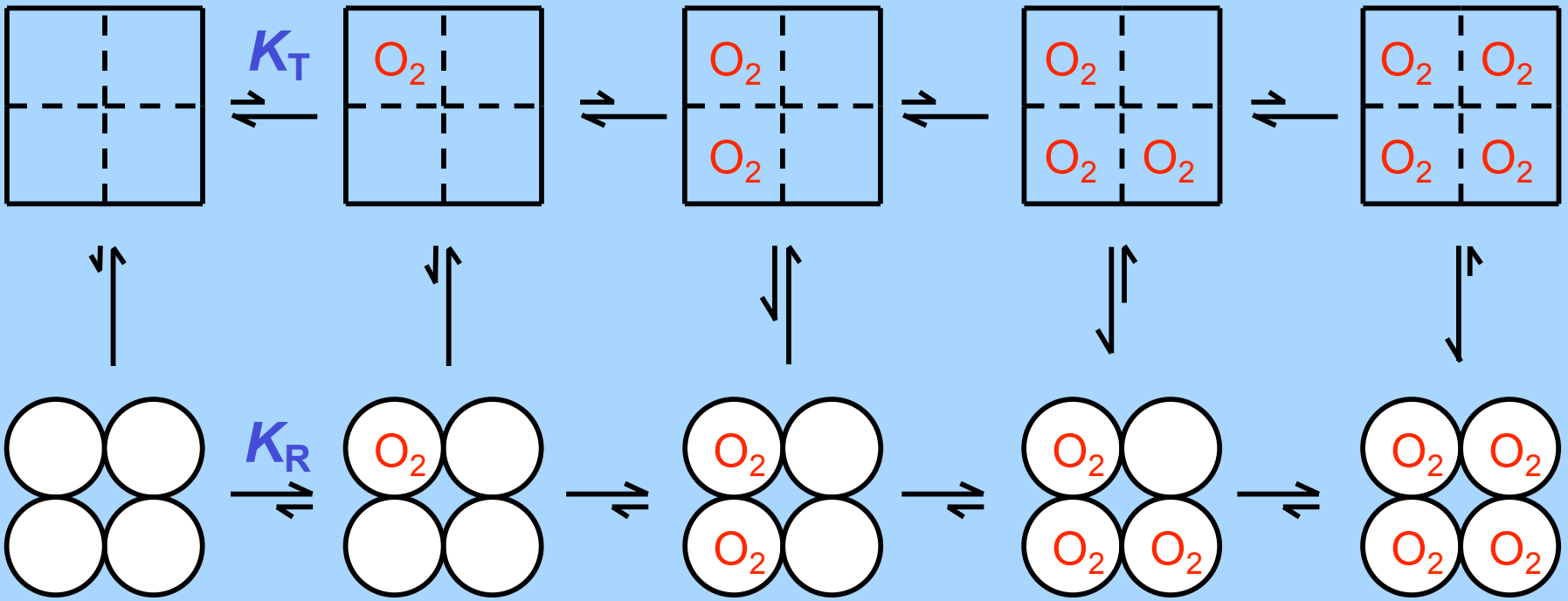
Saturation of the oxygen-binding sites is plotted as a function of the partial pressure of oxygen surrounding the solution.

(from Biochemistry / L.Stryer, 4th ed.)



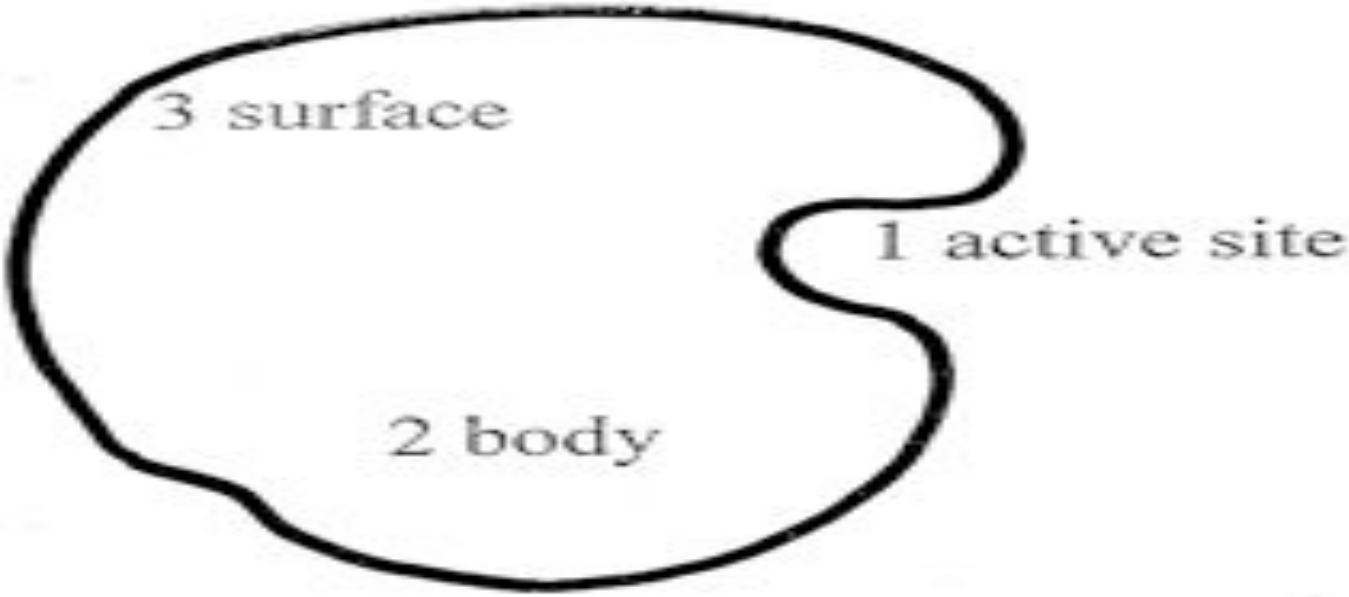
Simple sequential model for a tetrameric allosteric protein.
The binding of a ligand to a subunit changes the conformation of that particular subunit from the T (square) to the R (circle) form.
This transition increases the affinity of the other subunits for the ligand.

(from Biochemistry / L.Stryer, 4th ed.)



Concerted (Monod-Wyman-Changeux, or MWC) model for a tetrameric allosteric protein. The squares denote the T form, and the circles denote the R form. The ratio of T to R forms in the absence of ligand is L. The dissociation constants for the binding of ligand to the T and R states are K_T and K_R.

(from Biochemistry / L.Stryer, 4th ed.)

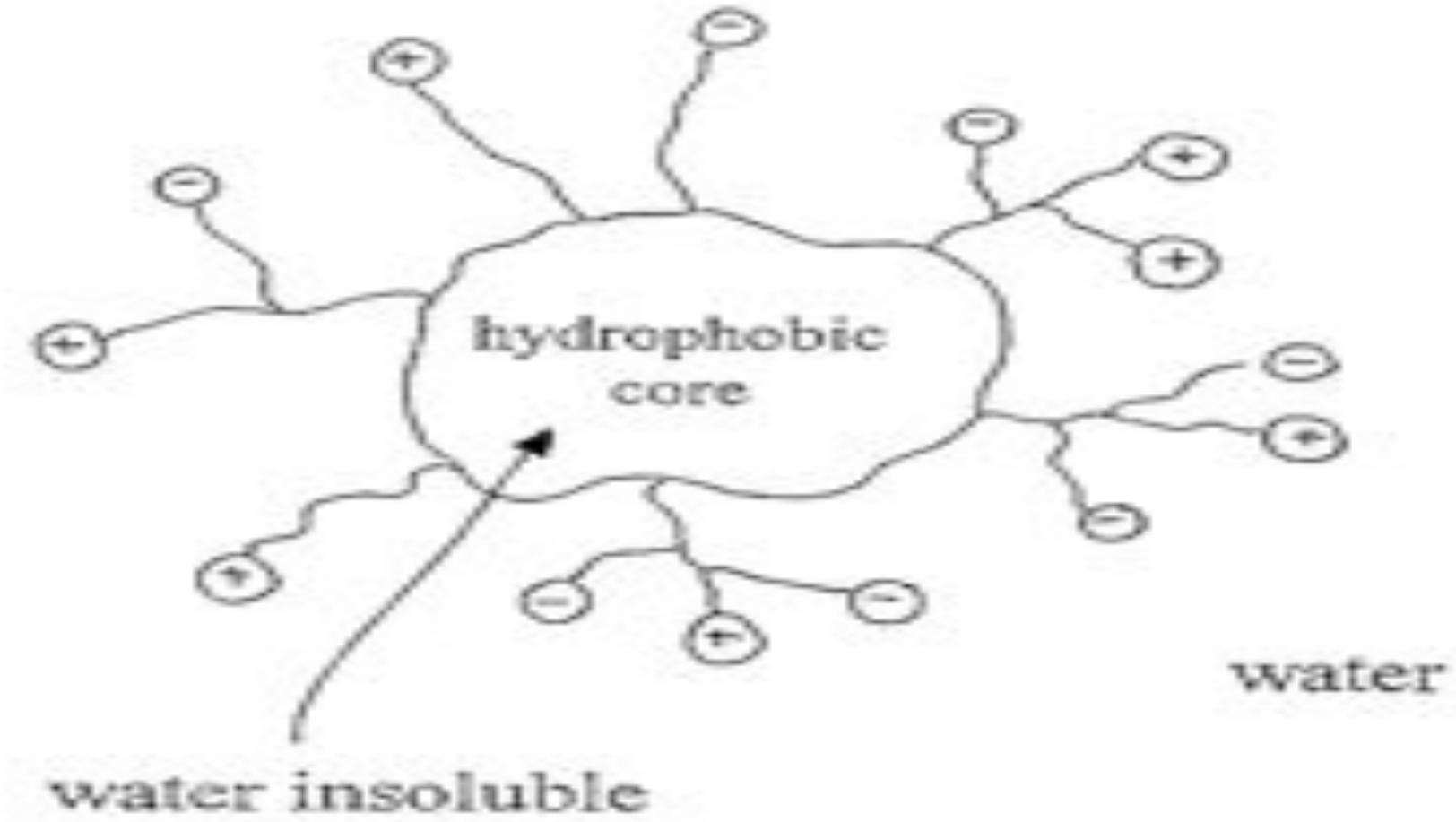


surface:
- contact with the environment
- solubility
- fit with the milieu

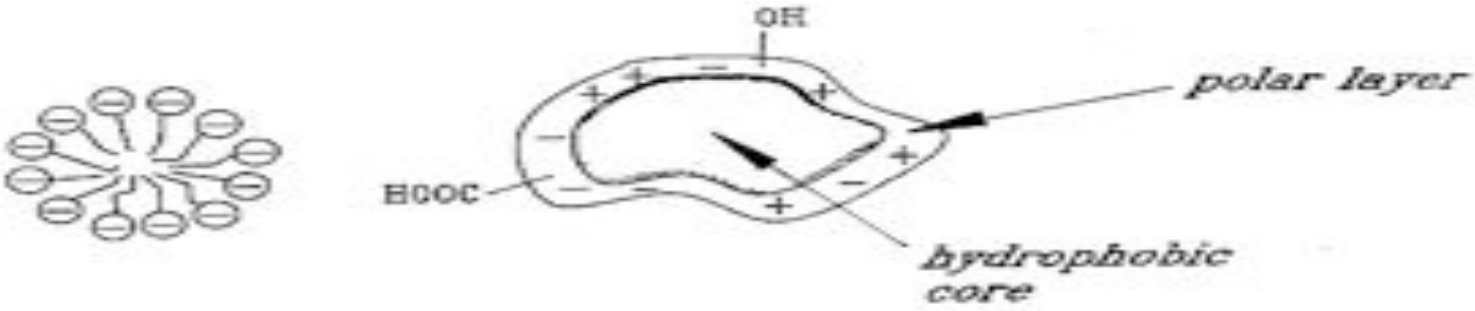
NOTICE: the three "regions" cannot really be separated from each other. Only for a didactic exercise.

OIL DROPLET MODEL

Perutz, 1965
Kendrew, 1972



FIT WITH THE ENVIRONMENT

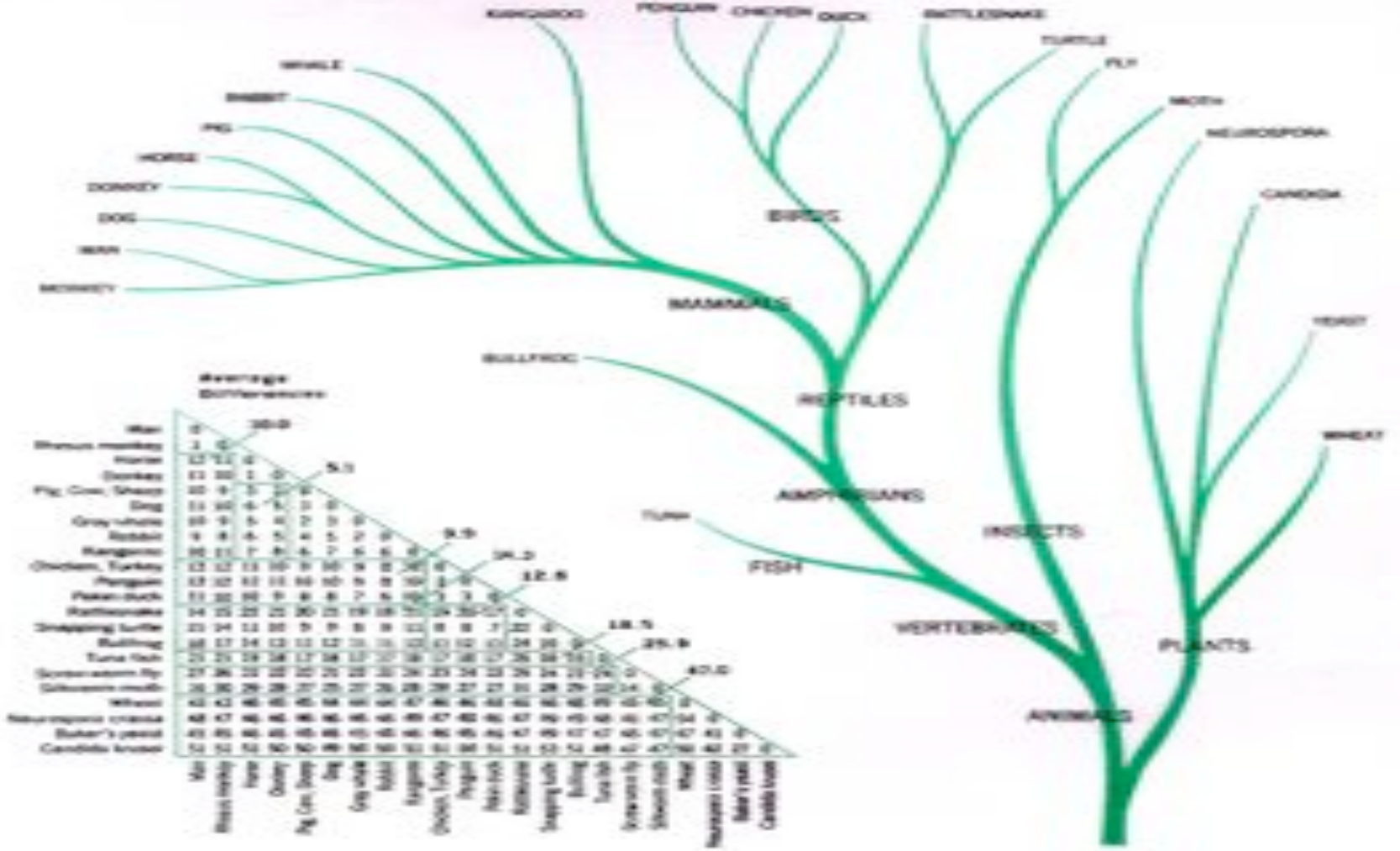


Simple-minded picture of the solubilization of enzymes in water - thanks to the macromolecular support

Fit with membranes, receptors; transport across different milieux

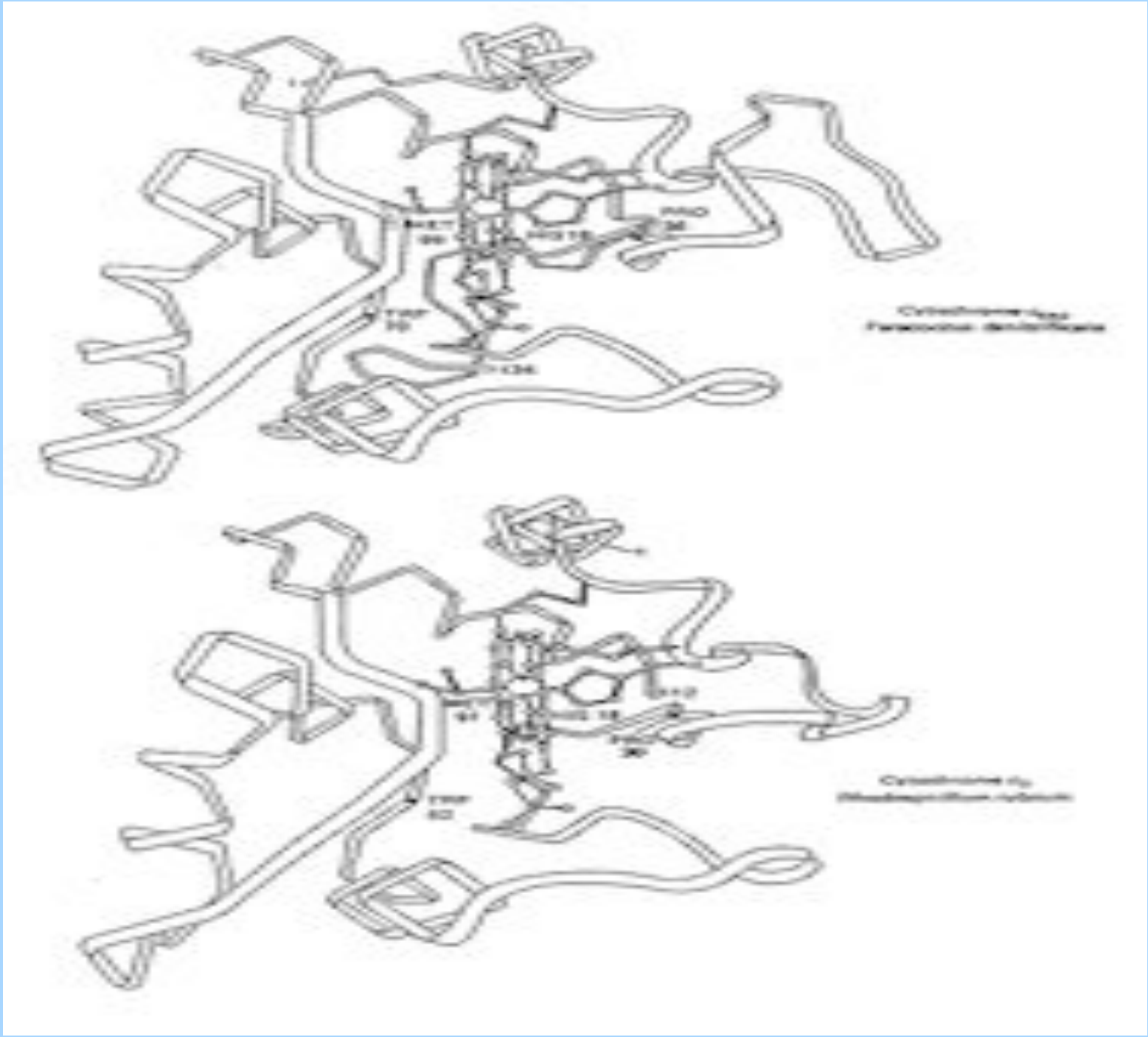
Thermophilic enzymes; halophilic, and other enzymes working in specialized environments

Fit with the environment - stability -
... but protein **turnover** and degradation



THE FAMILY TREE OF THE CYTOCHROMES c

The species differences shown in the table above left lead to a tree of family relatedness. Note that there is no ascending hierarchy. From the viewpoint of a yeast (if it had one), and therein lies a real if anthropocentric distinction), a moth, a man, and a bullfrog are equally far away. Note also how protected is the view that we usually take of the living kingdoms. The differences between fungi are greater than those between insects and vertebrates.



Additional reason for enzymes to be large

- multiple binding (coenzyme, two substrates, metal ions, prosthetic groups)
- multiple functions
- binding to other biopolymers
- viscosity and hydrodynamic properties
- secretion from the cell
- signal proteins (pre-, pro-proteins)
- protein turnover

Why are enzymes macromolecules
(Must enzymes be macromolecules?)

- An enzyme is a coordinate ensemble of operational units (binding, specificity, microenvironment, conformational rigidity, conformational changes, stability, fit with the environment, allostery, turnover, molecular tinkering, fossil sequences, ...)

- Each operational unit requires per se' a long sequence of amino acid residues to be functional. The sum of all operational units is then necessarily a very long chain. One also needs buffer regions to link the operational units to each other.

- Caution: the various operational units cannot be really separated from one another. This division is valid only as a didactic exercise.

An enzyme as an equilibrium - a compromise - among many pairs of opposite properties.

BUT

• hydrophobic active region	soluble in water •
• rigidity	conformational changes •
• stability	turnover •
• high efficiency	feedback •
• high atomic density	fluctuations •
• complex folding	order •
• present day compatibility	evolution •

... to much of only one quality is incompatible with the enzyme's functionality. (Thus, synthetic polymers which are either too stiff, or too flexible, cannot work as an enzyme).

PROJECT / QUESTION

Can one have a mini-enzyme, with only basic binding + catalysis, and without all fancy biology-extra?

100 residues

30 residues ??

..., COULD AN
ENZYME BE DIFFERENT FROM WHAT IT IS,
FOR EXAMPLE SOMEWHAT SMALLER
OR SOMEWHAT LARGER
OR WITH A DIFFERENT FORM
A DIFFERENT NUMBER OF SWUBUNITS?

AND, MORE GENERALLY MUST
THE THINGS OF NATURE BE
EXACTLY THE WAY THEY ARE?

darwinism and molecular evolution

.....The various structures built by Nature are the result of chance. There is no aim, no pre-determination- only chance determined assembly processes, and random structures.

If one of these structures happens to perform an useful function for the organism, it may be codified and preserved. Then Chance becomes encoded in DNA, it becomes Necessity, i.e. the hard law of genetic invariance.

from Monod' Chance and Necessity