

The Structure of Intrinsic Membrane Proteins

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Intrinsic membrane proteins are embedded in the lipid bilayer so that the polypeptides come in contact with the non-polar region of the bilayer. There are two major types of intrinsic proteins: those with most of their mass outside the cytoplasm (Type I) and those with most of their mass inside the cytoplasm (Type II). In the latter group are the membrane transport systems. The anion exchange system of the human erythrocyte is a dimer of band 3 polypeptides. These polypeptides span the bilayer, have most of their mass in the cytoplasm, and are glycosylated. About 20–25% of the polypeptide, however, is in the bilayer. Arguments are presented to support the view that the intramembrane segments of the protein are α -helical and that the major protein-protein interactions between the subunits are in the cytoplasmic portion of the protein.

Key words: membrane proteins, anion exchange, band 3 polypeptide, red cell membrane, transport

Intrinsic membrane proteins are by definition those proteins which cannot be removed from the membrane without the use of detergents. This property suggests that these proteins are embedded in or interact strongly with the nonpolar region of the bilayer.

This hypothesis is supported by the evidence obtained on a small number of intrinsic membrane proteins. This evidence strongly indicates that all intrinsic proteins examined so far span the bilayer. In these proteins, there clearly must be parts of the polypeptide which are in contact and thus interact with the nonpolar part of the bilayer. Thus, we may start with the assumption that all intrinsic proteins are transmembrane proteins.

There seem to be 2 general types of intrinsic membrane proteins which differ in their properties and arrangement in the membrane. They are called Type I and Type II proteins (Fig. 1).

Type I intrinsic proteins have most of their mass and all of their functional properties in the aqueous environment outside the cytoplasm. The intramembrane portion serves only to anchor these proteins to a particular membrane and it consists of a short amino acid sequence sufficient to span the bilayer as an α helix. Examples of Type I proteins are the major sialoglycoprotein of the red blood cell membrane, several proteins of enveloped viruses like Semliki Forest virus and Sindbis virus, and the histocompatibility antigens of human cells (HL-A). The best studies of these is the major sialoglycoprotein of the human erythrocyte.

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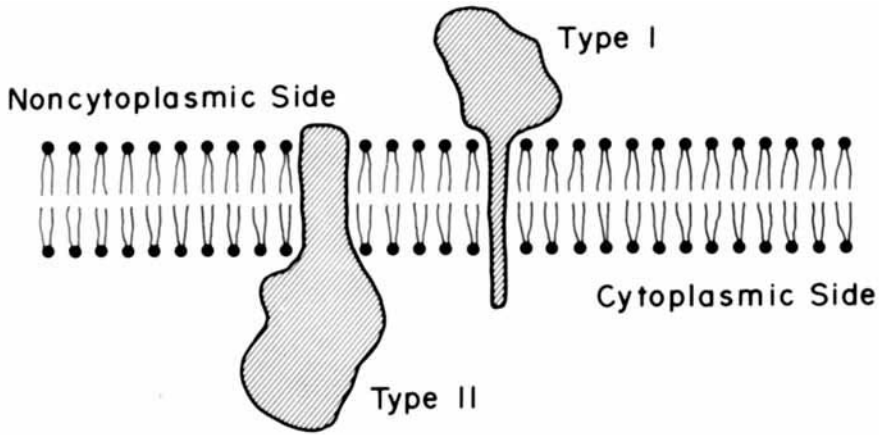


Fig. 1. Arrangement in the membrane of the 2 classes of intrinsic membrane proteins: Type I and Type II.

This protein, whose amino acid sequence and arrangement in the membrane have been described (1), is composed of 131 amino acids of which 72 at the NH_2 -terminal end are located outside the cell, 23 form the intramembrane and transmembrane portion, and the 36 amino acid residues at the COOH -terminal end of the protein are located inside the cell in the cytoplasm. In addition, the protein is a glycoprotein and the carbohydrate comprises two-thirds of the mass of the protein. All the sugar residues, approximately 100 of them, are attached to the amino acid residues on the outside of the membrane. Thus, all the protein-bound carbohydrate is extracellular (1).

The intramembrane portion is 23 amino acids long. Importantly, almost all of these residues are nonpolar and none of them is an acidic or basic amino acid. One can visualize that this stretch of residues could be accommodated in the lipid bilayer. As will be discussed later, the only likely structure for this portion of the protein is an α helix.

This protein illustrates clearly the salient features of the intrinsic proteins of Type I: The proteins are basically aqueous proteins with an anchor which attaches them to a membrane. The anchor comprises a small fraction of the polypeptide chain. All the functional properties of the protein are in the aqueous compartments on the exterior of the membrane.

Type II intrinsic proteins have 2 major characteristics: The major part of their polypeptide mass is in the cytoplasm and a very small fraction of their amino acid residues is exposed to the noncytoplasmic side of the bilayer. This means that over 90% of the amino acid residues are either in the bilayer or in the cytoplasm. Clearly these proteins are drastically different in their membrane arrangement from the Type I proteins. It is also apparent that the mechanism by which the Type I proteins are inserted in the bilayer must be different from that used by Type II proteins.¹

¹Type I intrinsic proteins resemble secreted proteins in all aspects except that a small COOH -terminal segment remains inserted in the bilayer and in the cytoplasm. It is likely that the proteins are synthesized by the same mechanism involved in the synthesis and secretion of extracellular proteins, which has recently been described by Blobel (Blobel G, Dobberstein B: *J Cell Biol* 67:835-851, 1975). On the other hand, Type II proteins are probably made on normal, soluble ribosomes, and fold to produce a structure similar to that shown in Fig. 2. Since the nonpolar area on the outside of the polypeptide is localized in one discrete area, while most of the protein surface is polar, the protein will insert in a distinct way in the bilayer. In this view, Type II proteins will become attached to the membrane after synthesis and there will be no necessary localization of the NH_2 - and COOH -terminal residues.

TABLE I. Properties of Membrane Transport Proteins*

	Na ⁺ ,K ⁺ - ATPase	Ca ²⁺ - ATPase	Anion- exchange protein	Acetyl- choline receptor	Rhodopsin
Molecular weights of component polypeptides	α-90,000 β-40,000	α-100,000	α-90,000	α- 40,000 β- 48,000 γ- 58,000 δ- 64,000 ε-105,000	α-38,000
Glycoproteins	β		α	α(β - ε)?	α
Probable structure and molecular weight of the protein part of the enzyme	α ₂ β ₂ 260,000	(α ₂ ?) (200,000?)	α ₂ 180,000	ε ₂ or α ₂ β ₂ 240,000	(α ₂ to α ₄ ?) (76,000- 152,000?)
Transmembrane arrangement	α		α		α
Detergent binding (mg/mg of protein)	0.28	0.20	0.77	0.7	1.10
Relative hydrophobic surface area of subunit	0.20-0.24	0.2-0.25	0.5-0.65	0.5-0.6	0.54

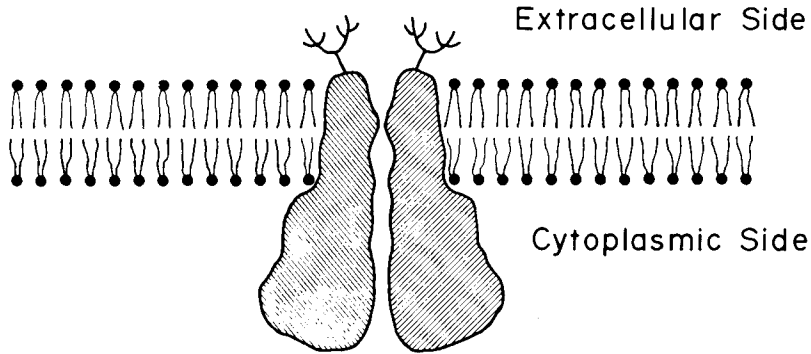
*Reprinted from Guidotti (9), with permission of the publisher.

There appear to be 2 categories of Type II proteins. One group is represented by cytochrome b₅, cytochrome b₅ reductase, and stearyl-CoA desaturase (2). These proteins are similar to the Type I proteins in that their functional domains are entirely in the aqueous compartment, in this case in the cytoplasm. Cytochrome b₅ is attached to the membrane through a hydrophobic region of the molecule which is COOH-terminal and contains 44 amino acids. It has not been established whether or not the intramembrane portion of this polypeptide traverses the bilayer entirely or is localized to the inner leaflet. The other proteins in this category resemble cytochrome b₅ in their arrangement on the membrane. One concludes that the attachment to the membrane of these proteins has the principal function of concentrating the enzymes on the endoplasmic reticulum.

The second category of Type II proteins is represented by proteins involved in transmembrane processes, for example, transport. There are only a few well-characterized proteins in this group: the Na⁺,K⁺-ATPase (3), the Ca²⁺-ATPase (4), band 3 of the human erythrocyte membrane which is involved in anion exchange (5,6), vertebrate rhodopsin (7), and the acetylcholine receptor (8). All of these intrinsic proteins have explicit or putative transport functions and thus are involved in the transfer of material across the bilayer. It is likely that all transmembrane transport of both material and information (i.e., hormone reception) is catalyzed by proteins that have properties similar to those of the proteins described above. These proteins are all transmembrane oligomers which are glycoproteins if attached to the plasma membrane, as is shown in Table I and discussed in a previous publication (9).²

Let us focus now on a particular transport system — the anion exchange system of human erythrocytes. The diagram shown in Fig. 2 indicates the relevant features of this protein with regard to its arrangement in the membrane. The transport system is composed of dimers of Type II polypeptides: There is very little protein outside the cytoplasm; there is a substantial amount in the bilayer; the major fraction of the protein is inside the cytoplasm.

²It is likely that transport across bacterial membranes is catalyzed by oligomeric transmembrane proteins which resemble eukaryotic systems. This suggestion is supported by recent evidence that bacterial rhodopsin, which is a light-activated proton pump, probably is a transmembrane protein which may be oligomeric. [Henderson R, Unwin PNT: Nature 257:28-32 (1975)].



Anion Exchange System (Band 3)

Fig. 2. Oligomeric structure and arrangement in the membrane of the anion exchange systems of human erythrocyte membranes (band 3 polypeptide).

The evidence for this arrangement is fairly straightforward. In the first place, proteolysis of the exterior surface of intact red blood cells causes no degradation of the protein (trypsin) or at best cleavage of a single peptide bond (6). This experiment shows that while a portion of the polypeptide must span the bilayer, very few amino acid residues are present on the outer surface.

The fraction of the polypeptide present in the bilayer is between 0.2 and 0.4. Two experiments bear on this point. Clarke (10) has shown that in some cases the extent of Triton X-100 binding to a protein gives an estimate of the surface area of the protein which is nonpolar and thus presumably in contact with the nonpolar part of the bilayer. The estimate is likely to be correct if the protein binds detergent below the critical micellar concentration (cmc) of the detergent, and thus can be shown not to insert into a micelle. The band 3 polypeptide behaves in this way. Thus the data shown in Table II can be interpreted as giving an estimate of the hydrophobic surface of the protein: It is approximately 40% of the total.

The second experiment involves the removal by extensive proteolysis of all segments of the protein which are not in the bilayer, and then isolation and characterization of the intramembrane segments. The data are shown in Table III, and they indicate that approximately 20% of the polypeptide remains in the bilayer. The fraction of nonpolar residues in these segments is very high and it substantiates the view that they are located in the bilayer.

Finally, the localization of the major part of the polypeptide inside the cytoplasm, which can be deduced by exclusion, is compatible with the experiments of many investigators which show that there is extensive destruction of the polypeptide by proteolytic enzymes which can attack the inner surface of the membrane (11).

Let us consider now the structure of the oligomers. There are 3 areas of interest: the symmetry of the oligomer, the protein-protein interactions which stabilize the oligomers, and the structure of the intramembrane part.

Symmetry

The evidence shown in Table II, along with the cross-linking data of Yu and Steck, indicate that the band 3 polypeptide is a dimer (12).

The oligomeric arrangement of the subunits together with the membrane asymmetry of polypeptide arrangement means that the axis of symmetry of the oligomer must be

TABLE II. Size of the Triton X-100 Complex of Band 3 Protein*

$\bar{v}_{20,w}$	6.9S	f/f_0	1.7
\bar{v}	0.81 cm ³ /g	g Triton/g protein	0.77
$D_{20,w}$	2.7×10^{-7} cm ² /sec	g carbohydrate/g protein	0.08
M_r complex	320,000	M_r protein portion	175,000
Moles Triton/Moles protein	208	M_r protein in SDS	90,000
Fraction of surface area covered by detergent ^a	0.41	M_r (Triton)/ M_2 (SDS)	1.95

*Data taken from Clarke (10).

^aThis calculation was done in the following way. The total surface area of the protein was determined as that of a sphere with a mass of the polypeptide chain and a \bar{v} of 0.73 cm³/g. The surface area occupied by the measured number of bound detergent molecules was calculated from the data in this Table assuming that each detergent molecule occupies an area of 0.5 nm² (data for an air to water interface: Technical bulletin, Rohn and Haas).

TABLE III. Membrane-Bound Proteolytic Fragments of Band 3*

Material	% of total	% nonpolar amino acids
Intact band 3	100	52
Tryptic fragments (mol. wt. 7,000)	20	63
Papain fragments (mol. wt. 8,000)	18	61

*Proteolysis of membrane-bound band 3 was done at 23°C for 24 h. The membranes were separated from the solution, washed, and the fragments isolated by SDS gel electrophoresis.

perpendicular to the plane of the membrane. If the oligomer is a dimer, homologous bonding between the subunits can satisfy this requirement. It should be emphasized that homologous bonding is the only arrangement which can close at a dimer stage. On the other hand, if the oligomer is larger than a dimer, say a tetramer or hexamer (for example, the Ca²⁺-ATPase of sarcoplasmic reticulum has been envisaged as a tetramer (13)), then the requirements stated above can only be satisfied by heterologous bonding between the subunits. This means that an n-mer will have an n-fold axis of symmetry perpendicular to the bilayer. This also means, since heterologous bonding is less frequent than is homologous bonding, that the most likely structure of membrane proteins is dimeric.

PROTEIN-PROTEIN INTERACTIONS

The possible interactions between subunits in oligomeric membrane proteins can involve the 3 parts of the polypeptide – that part which is exposed outside the membrane to the extracellular fluid, the intramembrane part, and the major cytoplasmic component. Since evidence suggests that very little of the polypeptide is exposed to the outer environment, extracellular interactions should be negligible. On the other hand the intramembrane part and the cytoplasmic portion are likely to have a major role.

The intramembrane piece of the polypeptide, which, as we shall discuss, is likely to be α helical, will interact with the lipid by nonpolar interactions, but will interact with the segments of the polypeptide and with another polypeptide by hydrogen bonds: These

clearly are more stable in a nonpolar environment and will strongly stabilize protein-protein interactions. This means that any contact surface between 2 polypeptides will be stabilized by polar interaction; i.e., there is likely to be a polar surface at the contact between membrane polypeptides. This is the likely surface which will interact with polar solutes. Thus, I suggest that the active site of membrane transport systems is at the interface between 2 subunits. Necessarily this means that the number of active sites is less than the number of polypeptides, indeed the requirement for half-of-the-sites activity is a necessity for membrane transport. If the intramembrane segments of the polypeptide are the main ones involved in subunit-subunit interactions and these are necessarily polar interactions, they should vanish if these areas of the helices are exposed to water. Thus, under conditions which eliminate the lipid bilayer, for example with detergents like Triton X-100 or deoxycholate, transmembrane oligomers stabilized by the intramembrane segments should dissociate into protomers. This is not the case with the band 3 oligomer (10). Therefore one might conclude that the major part of the stabilizing interactions between the monomers does not involve the intramembrane portion. However, the cytoplasmic portion of the protein, which is large for the band 3 polypeptide, can have a major role in the intersubunit interactions. These can be both polar and nonpolar interactions, and can contribute the main stabilization for the oligomeric structure. Since the cytoplasmic portion of the protein is normally present in the aqueous environment, removal of the protein from the bilayer with detergents should have no effect on the oligomeric structure of the protein.

In fact, the band 3 polypeptide exists at least as a dimer in the membrane, and as a dimer in solutions containing certain detergents (Triton X-100) (10). This fact suggests that the main stabilizing energy is not affected by weak detergents any more than are the usual water-soluble proteins.

INTRAMEMBRANE PART

Approximately 25% of the mass of the band 3 polypeptide is in the bilayer (10). Results obtained from this laboratory by K. Drickamer (6) show the location in the linear sequence of the polypeptide of at least one region which must span the bilayer (Fig. 3): It is located between the NH₂-terminal 10,000 dalton fragment which is on the cytoplasmic side of the membrane and the 7,000 dalton fragment which is 30,000 daltons from the COOH-terminal end and on the outside of the membrane. Since Steck's group (11) has suggested that there is a 17,000 dalton fragment which is embedded in the membrane, this piece must be located in the region described above and illustrated in Fig. 3. Evidence from another laboratory (14, 15) suggests that the polypeptide of band 3 has at least 2 different regions which span the bilayer. One concludes that a substantial fraction of this protein is in the bilayer.

It is interesting and important that careful studies of the structure of the proteins of erythrocyte membrane by optical rotatory dispersion (ORD) and circular dichroism measurements indicate fairly conclusively that the proteins exist as random coils and α helices and that there is very little if any β structure (16). Accordingly, the intramembrane sections of band 3 are most likely in an α -helical conformation.

There is a rationale for this observation on the intramembrane portions of the band

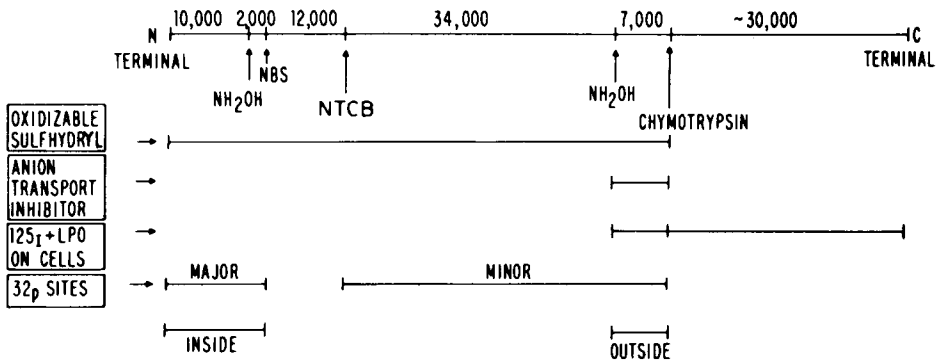


Fig. 3. The sites of chemical and enzymatic cleavage of the 95,000 dalton polypeptide from the human erythrocyte membrane (Band 3 polypeptide). The regions of the polypeptide which are located on the outer surface of the bilayer and on the inner surface of the bilayer are indicated, as are the regions to which specific labels have been attached. NBS) N-Bromosuccinimide; LPO) lactoperoxidase; NTCB) 2-nitro 5-thiocyanobenzoic acid. (Reprinted from Drickamer [6], with permission.)

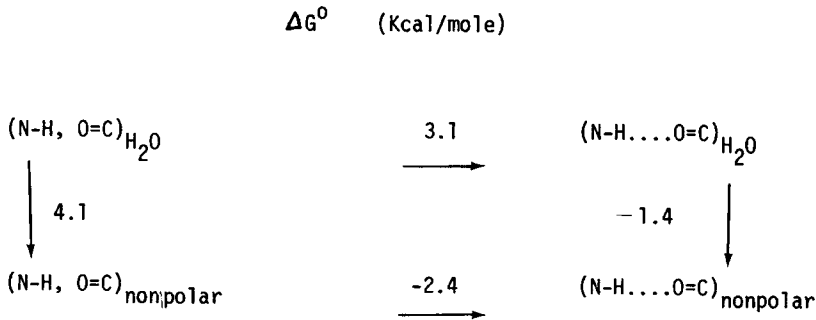


Fig. 4. Free energy changes for the formation of a hydrogen bond between the atoms of an amide group (peptide bond) and for the transfer of the group from water into a nonpolar solvent.

3 polypeptide. A polypeptide chain is composed of 2 parts: the peptide units which make up the backbone and the R groups attached to the α carbons of the backbone. While several R groups are hydrophobic and can interact with the nonpolar part of the bilayer, the peptide group is extremely polar and essentially insoluble in the bilayer. The polarity of the peptide group can be decreased if it is hydrogen-bonded. This is demonstrated by the results obtained by Klotz on the association of substituted acetamides in CCl_4 (17). The results are depicted in Fig. 4, which shows the standard free energy changes for the transfer of a hydrogen-bonded and a non-hydrogen-bonded peptide group from water to a nonpolar solvent. Thus, even a nonpolar amino acid residue will not be stable in a nonpolar environment if its peptide group is not hydrogen-bonded. This can be accomplished in 2 ways: The polypeptide can have an α -helical structure or a β structure. In the former case, the hydrogen bonds are formed between residues in the same segment of the polypeptide chain, while in the β structure the hydrogen bonds are between different strands of the polypeptide. As mentioned above, however, there is no appreciable β structure in the pro-

teins of the erythrocyte membrane. I conclude, therefore, that the transmembrane regions of the band 3 polypeptide are in the α -helical conformation.

The α -helical transmembrane segments are a simple and economical way to anchor a protein in the bilayer. Furthermore, specific aggregates of helices are well suited to form ordered structures, as happens, for example, in the protein tropomyosin, that have the unique feature of forming a hydrophilic active site which is not in contact with the nonpolar part of the lipid bilayer. This view derives from the following arguments. One would expect that the helix-helix interactions in the bilayer would be mainly polar ones since these interactions would be strong ones in the nonpolar environment of the bilayer. Thus the complex of helices would be stabilized by 2 types of interactions: Polar interactions for the helix-helix contacts, and nonpolar ones to stabilize the helix complex in the nonpolar region of the bilayer. Obviously, such an arrangement would require a very specific amino acid sequence for the regions of the polypeptide that span the bilayer. For example for a 2 helix complex in the bilayer the sequence should be nonpolar-nonpolar-polar-nonpolar-nonpolar-nonpolar-polar. A simple extension of this view suggests that a polar channel which spans the bilayer can be formed in the membrane by the association of 2 or more polypeptides which are in contact at the transmembrane helical segments. The stabilizing interactions between the transmembrane segments of the polypeptides would certainly be polar ones, and would thus create a well-defined polar region which spans the bilayer, i.e., a transmembrane polar active site for the catalysis of transport.

The view that α helices are the main, if not the only, ways in which a polypeptide traverses the bilayer is supported by a consideration of the free energy of interaction of these structures with the nonpolar region of the bilayer. If indeed all peptide bonds and polar residues are neutralized in the bilayer by the formation of hydrogen bonds, then the only parts of the polypeptide exposed to the solvent (the lipid) are the nonpolar ones. One can calculate the free energy of interaction of a helix or a group of helices with the nonpolar region of the bilayer by estimating the surface area of the structure in the bilayer and using the value of 25 calories mole⁻¹ A⁻² for the stabilizing energy which has been obtained by Clothia (18). The stabilizing free energy is -30 Kcal/mole for one helix and -300 Kcal/mole for a group of 7 helices. Clearly, the stabilizing energy is sufficient to prevent the polypeptide from dissociation from the membrane.

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REFERENCES

1. Marchesi VT, Furthmayr H, Tomita M: *Annu Rev Biochem* 45:667-698, 1976.
2. Enock HG, Catala A, Strittmatter P: *J Biol Chem* 251:5095-5103, 1976.
3. Dahl JL, Hokin LE: *Annu Rev Biochem* 43:327-356, 1974.
4. McLennan DH, Holland DC: *Annu Rev Biophys Bioeng* 4:377-404, 1975.
5. Ho MK, Guidotti G: *J Biol Chem* 250:675-683, 1975.

6. Drickamer LK: *J Biol Chem* 251:5115–5123, 1976.
7. Cone RA: In Schmitt FO, Schneider DM, Crothers DM (eds): “Functional Linkage in Biomolecular Systems.” New York: Raven Press, 1975, pp 234–246.
8. Karlin A: *Life Sci* 14:1385–1415, 1974.
9. Guidotti G: *Trend Biochem Sci* 1:11–13, 1976.
10. Clarke S: *J Biol Chem* 250:5459–5464, 1975.
11. Steck TL, Ramos B, Strapozon E: *Biochemistry* 15:1154–1161, 1976.
12. Yu J, Steck TL: *J Biol Chem* 250:9176–9184, 1975.
13. Murphy AJ: *Biochem Biophys Res Commun* 70: 160–166, 1976.
14. Jenkins RE, Tanner MJA: *Biochem J* 147:393–399, 1975.
15. Jenkins RE, Tanner MJA: *Biochem J* 161:134–147, 1976.
16. Schneider AS, Schneider MJT, Rosenheck K: *Proc Natl Acad Sci USA* 66:793–798, 1970.
17. Kresheck GC, Klotz IM: *Biochemistry* 8:8–12, 1969.
18. Clothia C: *J Mol Biol* 105:1–14, 1976.