Review

Tryptophan, an Amino-Acid Endowed with Unique Properties and Its Many Roles in Membrane Proteins

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Abstract: Tryptophan is an aromatic amino acid with unique physico-chemical properties. It is often encountered in membrane proteins, especially at the level of the water/bilayer interface. It plays a role in membrane protein stabilization, anchoring and orientation in lipid bilayers. It has a hydrophobic character but can also engage in many types of interactions, such as π–cation or hydrogen bonds. In this review, we give an overview of the role of tryptophan in membrane proteins and a more detailed description of the underlying noncovalent interactions it can engage in with membrane partners.

Keywords: tryptophan; membrane proteins; noncovalent interactions

1. Introduction

Among the naturally coded eukaryote amino acids, tryptophan (Trp) is unique in terms of its physico-chemical properties. Considered as an aromatic residue similarly to tyrosine (Tyr), phenylalanine (Phe) or histidine (His), it is however the sole amino acid that contains two rings in its lateral side-chain, namely the indole moiety composed of a benzene ring fused to a pyrrole ring, making it the largest coded amino acid in the natural series.

Depending on the many different hydrophobic/hydrophilic scales that have been developed over the years by different research groups to rank amino acids, Trp is seen as more or less hydrophobic. The nitrogen of its indole ring can indeed be engaged in hydrogen bonds, which may facilitate the solubility of proteins. Its large quadrupole allows it to engage in intense π–π or π–cation interactions. In addition, Trp has a dipole moment similar to Tyr but not Phe. All these specific physico-chemical properties make Trp unique in biological functions and localizations of proteins. In this review, we develop how these unique Trp properties are crucial for membrane proteins and help to understand their membrane location and functions.

2. Trp in Membrane Proteins

2.1. Trp Localization at the Lipid/Water Interface of Membrane Proteins

The analysis of amino acids distribution in proteins indicates that Trp is mostly located in transmembrane proteins, where it represents 3.3% of amino acid composition, whereas it only represents 1.2% for soluble proteins [1].

In these transmembrane proteins, Trp has a strong preference for the bilayer interface as demonstrated for the first time by Jacobs and White using neutron diffraction [2]. Since then, other techniques have been used in order to determine Trp localization, including X-ray diffraction [3], nuclear magnetic resonance (NMR) [4], fluorescence spectroscopy [5] and molecular simulations [6]. All these studies converge towards the same direction:

Trp is located at the lipid/water interface. Actually, Trp is not the only residue found at the interface, aromatic residues in general have this preferential location in transmembrane proteins in both α helix and β sheet structures, which is called "the aromatic belt" [7] (Figure 1).
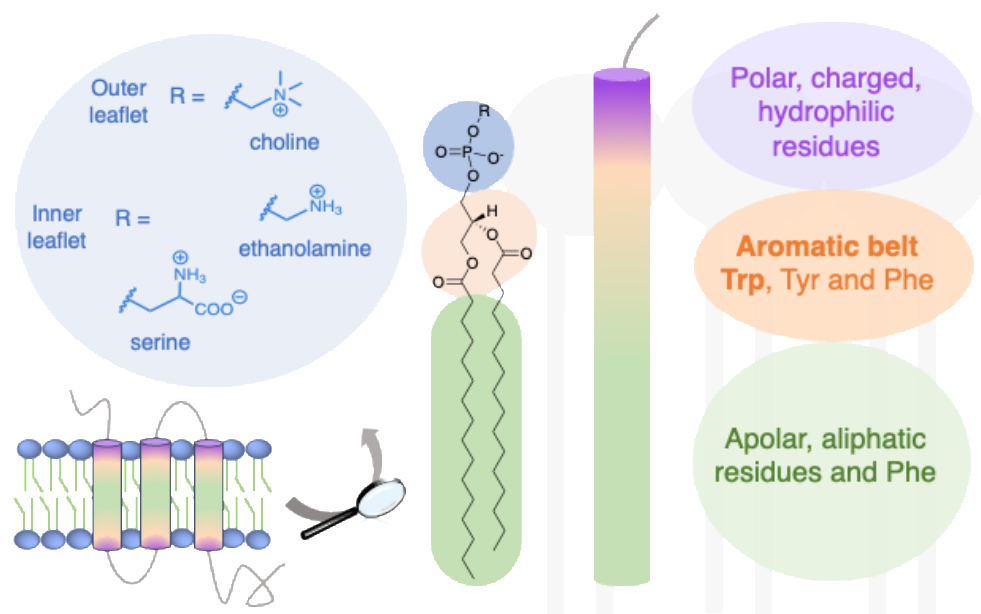

Figure 1. Position of amino acids in transmembrane proteins.

Interestingly, aromatic residues are not randomly distributed at the interfaces. Indeed, aromatic residues are often mainly located at the lipid–extracellular interface [8]. This observation is reported in the case of the photosynthetic reaction center, where Trp is mainly located in the periplasmic side [1]. Another study points out this particularity for 29 integral proteins, highlighting the fact that Trp is located at the noncytoplasmic interface for α helical transmembrane proteins according to a sequence-based method. The same study shows that this residue is more present in α helical structures than in β sheet structures [7]. However, this tendency is not true for all proteins. For example, in the transmembrane segments of human type I single-span membrane proteins, Trp is found at both ends of the hydrophobic domain, whereas Tyr is only located at the C-terminal boundary and Phe is found inside the hydrophobic domain and at the Tyr position [8].

Trp is found at the interface and, more precisely, in the region of acyl carbonyl groups of the lipid bilayer, as evidenced by the chemical shift change of the signals attributed to the choline group in the presence of Trp [9,10].

Another study shows that Trp is also found in the glycerol region and in the hydrophobic core of lipid bilayers, according to ^1H and ^2H NMR studies. The authors propose that Trp location in the choline region could be mainly governed by cation–π interactions, whereas in the glycerol region, other kinds of interactions could be involved, such as van der Waals interactions, dipolar interactions, entropic contribution or even hydrogen bonding [10,11]. The interfacial localization of Trp allows it to establish interactions with choline moieties, as mentioned above, but it can also be involved in interactions with other molecules present in its environment such as water with which hydrogen bonds are formed. Cationic residues in proteins, such as Arg and Lys, are located near phosphate moieties because a deeper localization inside a hydrophobic core is energetically unfavorable and leads to specific lipid organization [12]. Trp can also interact favorably with these cationic residues through cation–π interactions.

Trp has a preference for the hydrophilic region over the hydrophobic core [13]. If we focus on its orientation in a bilayer, the benzene moiety of the indole prefers the

hydrophobic core, whereas the pyrrole moiety points towards the more hydrophilic part of the lipid bilayer [8,14]

Molecular simulations for indoles in POPC phospholipid bilayers have been carried out and confirm this trend, showing the existence of three weak binding sites for Trp, in the choline region, the glycerol region and hydrophobic core region, suggesting different types of interactions depending on the nature of the binding site [6]. All these interactions will be further discussed in the second part of the manuscript.

2.2. Trp in the Stabilization of Membrane Proteins

Trp is an essential residue that participates in the thermal stability [15], the stabilization of tertiary and quaternary structures [16,17] and in the folding process of proteins in general [18]. It also plays an important role in protein binding sites [19–23].

Focusing on membrane proteins, Trp is often located at the transmembrane helix–helix interface, where it could contribute to protein folding [24]. This residue is found at both ends of α helices, whereas it is only found on one side of β-strand structures in OmpF porin [25,26]. In addition, it has been noticed that in 3.2% of cases, it is linked with symmetrical parts in membrane proteins, which reinforces the idea of a major contribution of this residue in the protein folding process. Moreover, in membrane proteins, Trp interacts with several residues distant from it, suggesting its implication in the stabilization of a tertiary structure. However, contrarily to the case of α-helices structures, its contribution to the free energy of the unfolded state seems to be limited in β-barrel membrane proteins [27]. In fact, this stabilizing effect is highly dependent on Trp position and environment. Indeed, the mutation of one Trp of OmpX by Tyr or Phe alters the folding kinetic and the protein stability, whereas the same mutation in another position has no effect [28]. Some studies show the important contribution of Trp to protein folding and stability when it is located at the interface [28,29], whereas other studies underline its importance when placed in the middle of the bilayer [30]. This ambivalent character is due to the complexity of the bilayer structure that influences the energetic contribution of each amino acid to the stability of the protein, which means that Trp contribution also depends on the sequence to which it belongs [30]. The stabilizing effect can occur through hydrogen bonds between Trp residue and the lipid carbonyl according to its localization at the interface.

Even if Trp contributes to membrane protein stability, it does not appear to be essential for protein integration inside membranes. Indeed, in a study in which Trp residues were systematically mutated to Ala, it was shown that these mutations led to a decrease in the interactions between transmembrane segments but had no impact on the protein ability to be inserted inside a bilayer [31].

The stabilizing effect of Trp in membrane proteins is also due to interactions inside the protein itself, and Trp residues are often enriched in hotspots that determine the interaction strength for protein–protein interactions [32].

In order to better define the role of Trp in helix–helix interactions, heptad repeat motifs corresponding to interfacial residues have been randomized [31]. Sequences involved in high-affinity self-interactions have been found to be enriched in Trp at specific positions. In total, 78% of sequences contained at least one Trp, often localized at the helix–helix interface or helix–lipid interface. The authors suggest that Trp could be implicated in interactions between transmembrane proteins, provided its particular position and physico-chemical properties. Another study also supports this role in multi-membrane-spanning proteins, where Trp may be involved in the side-to-side packing of proteins through interactions with helices distant from each other [33].

2.3. The Role of Trp in Anchoring and Orientation of Membrane Proteins

Trp residues are generally found at the extremity of membrane proteins, where they play the role of anchors (Figure 2). This was exemplified by the WALP peptides family, which have been designed to model membrane proteins [34]. They have a hydrophobic segment composed of different numbers of alternated Ala and Leu residues and two Trp

residues at each extremity. WALP peptides all adopt an α helical transmembrane conformation according to circular dichroism studies [33]. This observation means that Trp residues interact with the interfacial lipid moieties, through cation–π interactions, for instance [35]. These strong interactions define the protein orientation, a longer hydrophobic segment of a WALP peptide compared to the bilayer thickness promotes the adaptation of the lipids constituting the bilayer by their elongation. As a consequence, an increase in the hydrophobic part of the bilayer thickness is observed in order to interact with the hydrophobic part of the protein according to ^{2}H NMR and electron spin resonance spectroscopy experiments [36]. A lipid reorganization rather than that of the peptide supports the idea of the Trp as an anchoring residue [12]. This increased thickness is much less pronounced when Trp is substituted by a Lys in a KALP peptide, which reinforces the anchoring role for Trp [9].

For multiple spanning proteins, Trp residues at each helix–helix interface act as a landmark to insert each transmembrane segment and to adjust its verticality as demonstrated by the glycosylation mapping technique on model poly-Leu segments [37].

This anchoring function is available for α-helices as well as transmembrane β sheets even if in this latter case its contribution depends on its depth [28]. Trp adapts to its environment by readjusting its orientation rather than moving inside the bilayer, which suggests the presence of a specific anchoring site [27]. An interesting point is that a single Trp can be sufficient to anchor an entire protein [27].
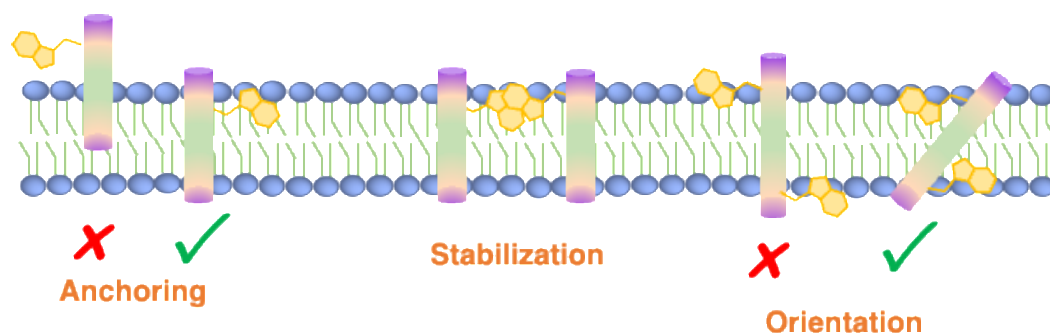

Figure 2. Multiple roles of Trp in membrane protein anchoring, stabilization and orientation in a lipid bilayer.

As discussed above, Trp plays an important role in protein stabilization and anchoring, but it is not its only function. It is also able to direct the protein orientation relative to the lipids without affecting their organization [27,38]. In order to better understand the behavior of Trp in membrane proteins, WALP peptides were again useful. The influence of Trp residue on the orientation of the protein is reported in a study based on GW^{x,y}ALP peptides, which are peptides designed from WALP peptides (acetyl-GGALW^{x}LAL-ALALALALALW^{y}LAGA-ethanolamide with x and y the position of each Trp residue). GWALP peptides adopt a transmembrane α-helix structure in DOPC, DMPC and DLPC phospholipid environment whatever the distance between the two Trp residues as demonstrated by circular dichroism measurements [39]. This structure is maintained more strongly inside the region between the two Trp. Moreover, the distance between Trp inside the GW^{x,y}ALP peptide has an impact on the peptide orientation. Indeed, a decrease in the bilayer thickness induces a more pronounced tilt for the GW^{x,y}ALP peptides with a longer distance between Trp residues. Focusing on the behavior of Trp residues at each terminus, it appears that they do not undergo the same reorientation. NMR experiments reveal that the Trp near the C-terminus changes its orientation faster than the N-terminus one.

In most peptides, such as WALP or gramicidin peptides, the pyrrole moiety of the indole side chain of Trp points toward the extracellular medium with its dipole aligned with the helix axis [9]. This assists the peptide orientation in the bilayer. This peptide

orientation is manifested by a helix tilt, leading to a change in torsion angles of the Trp side chain to adjust the orientation of the indole ring [39].

3. Noncovalent Interactions Engaging Trp

As described above, Trp is a crucial amino acid for maintaining the interactions and structure within a membrane spanning protein or with surrounding membrane partners, such as other protein(s) or lipids. In addition to weak interactions (hydrophobic, van der Waals), stronger noncovalent interactions such as hydrogen bonds, π−π stacking and cation−π interactions are of crucial importance in biological systems and particularly regarding aromatic amino acids, such as Trp.

In terms of binding energy, it is generally considered that π−π stacking interactions are weaker than hydrogen bonding, while cation−π interactions are stronger [40–42]. Theoretical calculations continuously help improve our understanding of noncovalent interactions. Anion−π interactions, for instance, were first evidenced by elegant theoretical works (reviewed in [43]) before the experimental demonstration of their biological and chemical relevance was reported [44–47]. In addition to cation−π and anion−π, ion pair–π interactions have been recently shown to potentiate biological processes, and their existence is supported by theoretical calculations as well [48–50]. In addition, theoretical chemical analyses give reliable information on the energetic aspects of the various conformations of Trp and its ionized counterparts and their possible involvement in biological interactions and processes [51,52].

3.1. Trp Interactions with Lipids

Trp stabilization, anchoring and orientation functions are ensured by various interactions with lipids. Trp can be implicated in electrostatic, dipolar and hydrophobic interactions but also in hydrogen bonds with the lipid headgroups and other molecules of the environment. For example, Trp interactions with water molecules and choline groups on lipids occur through charge–dipole interactions and weak cation−π interactions [53]. Trp can act as a hydrogen-bond donor through its NH but also as a hydrogen-bond acceptor via its aromatic ring [54]. The strength of these interactions is not similar for all lipids, for instance, cation−π interactions are more pronounced for phosphatidylethanolamine [35]. The nature of the interactions Trp engages with lipids depends on its localization inside the bilayer.

In the following paragraphs, we will give a more detailed description of the different types of interactions Trp can establish, summarized in Figure 3b, and how these interactions confer unique properties to Trp.
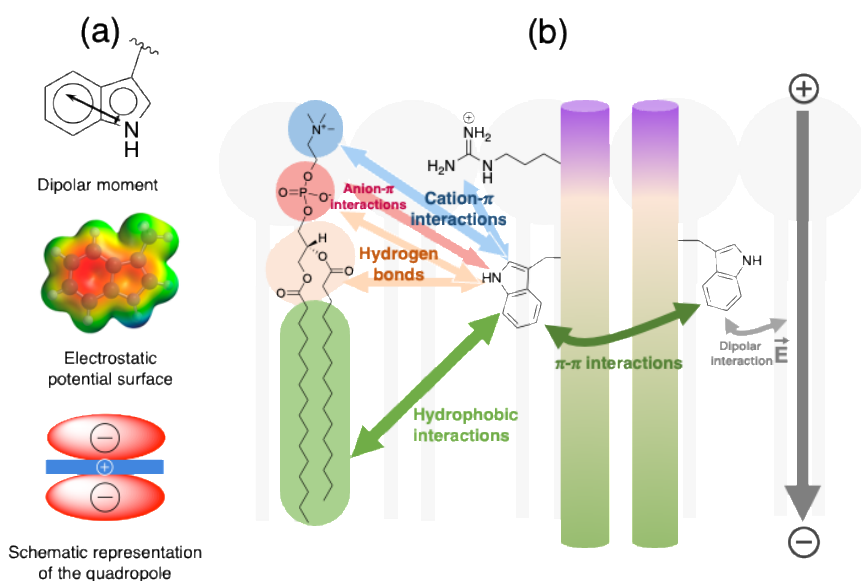
Figure 3. (**a**) Electrostatic properties of Trp as illustrated by its dipolar moment, electrostatic potential surface (red = negative, blue = positive, isovalue 0.001 a.u. credit: Antonio Bauzá) and schematic representation of the quadrupole. (**b**) Summary of the different interactions Trp can establish with membrane partners.

The energetic landscape of membrane protein insertion inside the lipid bilayer is favorable thanks to the presence of the aromatic residues, Trp, Tyr and Phe. However, these three residues do not have the same contribution to the protein stability in lipid membranes. For example, studies performed with transmembrane and integral β-barrel proteins suggest that Trp and Tyr are less stabilizing than Phe [55] because the hydrophobic contribution of Phe exceeds its aromatic contribution.

Trp contribution to protein stability in bilayers is highly dependent on its local environment. Different studies suggest that minimal free energy is obtained when Trp is placed near the bilayer mid-plane [30] or when Trp is localized at the interface [29]. Furthermore, the stability effect could result from aromatic–aromatic interactions occurring in the "aromatic-belt", although these interactions are highly dependent on the local environment [29].

In order to determine Trp and Tyr affinities for the interface between water and the bilayer, partitioning of small model peptides that cannot adopt a secondary structure has been determined by Wimley and White [56]. These results show that the presence of aromatic residues at the interface is very energetically favorable. Water/octanol partitioning studies show the same trend, which suggests that hydrophobicity plays an important role in this partitioning [12,13,57]. However, studies using both neutron diffraction and simulations on indole in water and methanol indicate a preference for indoles to interact with water and methanol through electrostatic interactions. Interactions between water and indole only occur in an amphiphilic environment, i.e., in the presence of methanol in this study. More generally, both electrostatic contact and hydrophobic contacts are involved in Trp localization [54].

3.2. Hydrophobicity and Hydrophobic Effects

All aromatic residues possess a cyclic planar structure with π bonds in resonance at the origin of their hydrophobic character. The hydrophobic character of Trp allows it to be involved in protein folding through hydrophobic interactions [58]. However, it is difficult to nail Trp on a hydrophobic scale because of its amphipathic character.

There are several scales evaluating the hydrophobicity of amino acids, but they differ, depending on the way hydrophobicity is evaluated. Some of these are presented in Figure 4 [2,56,59–65]. Two classes of scales coexist. On the one hand, there are scales based on

experimental measurements such as solubility in organic solvents or partitioning in model membranes or apolar solvents. Usually, these scales imply acetylated amino acids, or amino acids inserted into short host peptides, as free amino acids are too polar. On the other hand, there are scales based on biological observations such as amino acid position in the tertiary structure of proteins, or on the ability of full proteins to insert within membranes. Finally, Kyte and Doolittle have proposed a hydropathy scale based on an amalgam of experimental observations, as the authors themselves qualify it [63].

Some scales place Trp as the most hydrophobic residue, which can be explained by its largest hydrophobic area. However, if we consider the contribution of each carbon to hydrophobicity, aliphatic residues appear as more hydrophobic than the aromatic ones [59].

A common feature between the scales placing the Trp as the most hydrophobic residue is that they all consider solvents with hydrogen bonding properties: octanol, ethanol or dioxane. Interestingly, Trp is also capable of hydrogen bonding (see below), which could explain more favorable interactions with these solvents [66]. Another interesting point is that water/octanol ($logK_{ow}$ or $logP$) and water/membrane partition coefficients follow the same trend, but membrane hydrophobicity values are half of the octanol values. That could be explained by a smaller solvation parameter [56]. This observation points to the choice of solvents for experimental measurement as an important parameter to take into account [66]. Simulation studies have also been performed to estimate amino acids hydrophobicity, and these results have been compared with experimental scales [67]. The simulations are usually in good agreement with experimental measurements.

Reference	Type of scale	Hydrophobicity criterion	Hydrophobicity of aromatic residues	
Nozaki and Tanford, *J. Biol. Chem.*, 1971 **[45]**	Experimental measurement	Δf_t *(group contribution to free-energy) for transfer of amino-acid side chain from 100% organic solvent to water* Solubilities of amino-acids in ethanol and dioxane	Trp (Δf_t = 3400 cal/mol) Phe (Δf_t = 2500 cal/mol) Tyr (Δf_t = 2300 cal/mol)	**Trp is the most hydrophobic**
Fauchere and Pliska, *Eur. J. Med. Chem.*, 1983 **[46]**	Experimental measurement	*Hansch side chain parameter* π_R Partitioning of N-acetyl-amino-acid amides in octanol/water	Trp (π_R=2.25) Phe (π_R=1.79) Tyr (π_R=0.96)	
Jacobs and White, *Biochemistry*, 1989 **[2]**	Experimental measurement	ΔG_t *for transfer of short hydrophobic peptides from water to DMPC vesicle* Partitioning of Ala-X-Ala-OtBu peptides in which X = Gly, Ala, Leu, Phe or Trp in DMPC bilayers	Trp (ΔG_t =−5.12 kcal/mol) Phe (ΔG_t =−4.24 kcal/mol)	
Wimley and White, *Nat. Struct. Biol.*, 1996 **[42]**	Experimental measurement	$\Delta G_x^{residue}$ *from bilayer interface to water* Partitioning Ac-WL-X-LL peptides (with X the studied amino acid) into POPC vesicles	Trp (ΔG_t = 1.85 kcal/mol) Phe (ΔG_t = 1.13 kcal/mol) Tyr (ΔG_t = 0.94 kcal/mol)	
Chothia, *J. Mol. Biol.*, 1976 **[47]**	Biological scale	*Proportion of residues 95% buried* Residue accessibility in 12 folded proteins, 95% buried meaning 5% or less of potential accessible surface available to solvent contact	Phe (0.50) Trp (0.27) Tyr (0.15)	**Trp is moderately hydrophobic**
Wolfenden et al., *Biochemistry*, 1981 **[48]**	Experimental measurement	*Equilibrium constant for transfer of amino-acid side chains from water to the vapor phase*	Phe (K=$10^{-0,56}$) Trp (K=$10^{-4,33}$) Tyr (K=$10^{-4,49}$)	
Kyte and Doolittle, *J. Mol. Biol.*, 1982 **[49]**	Experimental measurement/ Biological scale	*Hydropathy index* Hybride scale based on [47] and [48]	Phe (2.8) Trp (−0.9) Tyr (−1.3)	
Hessa et al., *Nature*, 2005 **[50]**	Biological scale	ΔG_{app} *derived from apparent the equilibrium constant between membrane-inserted and non membrane- inserted forms of a protein* Membrane integration of designed transmembrane segments into dog pancreas rough microsomes	Phe (ΔG_{app} = −0.32 kcal/mol) Trp (ΔG_{app} = 0.30 kcal/mol) Tyr (ΔG_{app} = 0.68 kcal/mol)	**Trp is very moderately hydrophobic**
Moon and Fleming, *Proc. Natl. Acad. Sci. USA*, 2011 **[51]**	Biological scale	$\Delta\Delta G^{\circ}_{w,l}$ *difference in the Gibbs free energy of unfolding of each amino-acid variant at position 210 compared to wild-type OmpLA* Substitution of Ala^{210} by the studied amino-acids in OmpLA protein and determination of the Gibbs free energy of unfolding in the presence of water or lipid bilayers	Phe ($\Delta\Delta G^{\circ}_{w,l}$= −2.20 kcal/mol) Tyr $\Delta\Delta G^{\circ}_{w,l}$= −1.09 kcal/mol) Trp ($\Delta\Delta G^{\circ}_{w,l}$= −0.38 kcal/mol)	

Figure 4. Different hydrophobicity scales derived from either direct experimental measurements or biological observations. The hydrophobicity of Trp, Phe and Tyr when available is detailed.

This hydrophobic character is responsible for Trp exclusion from water, but it cannot explain its localization at the water/membrane interface. Indeed, regarding its hydrophobic properties, one can imagine a deep localization inside the bilayer due to the aromatic ring interacting with lipids fatty acid chains. However, this could be accompanied by an unfavorable entropy due to lipids chains reorganization [10], which could partly explain the interfacial localization. Indeed, a major difference between Trp and the other aromatic residues lies in the fact that the indole ring is more rigid than the benzene ring of Phe or Tyr. This implies an important lipid reorganization in the case of a deeper Trp insertion inside the hydrocarbon core, which is an entropically unfavorable process [10]. The transfer of the indole to the membrane interface is enthalpy rather than entropy-driven, whereas indole transfer from aqueous to a bulky nonpolar phase is characterized by a larger positive entropy. This phenomenon is called the "nonclassical "hydrophobic effect because the bilayer does not behave as a bulky nonpolar phase according to its ability to reorganize [68]. Another expression found in the literature to describe this phenomenon is the "bilayer effect". Wimley and White distinguish the hydrophobic effect from the "bilayer effect" by heat capacity measurements. They propose that favorable enthalpy could be due to van der Waals interactions and/or dipolar interactions [69]. Ladokhin et al. also studied these effects using model large unilamellar vesicles (LUVs), and they concluded that the hydrophobic effect contribution is the same regardless of the lipid composition, thus suggesting that these two expressions correspond to a similar phenomenon responsible for the larger enthalpic contribution [70].

3.3. Electrostatic Interactions

Trp possesses a particular electronic structure allowing it to be involved in electrostatic interactions through its π-conjugated electron cloud (Figure 3a). In this regard, Trp can be involved either in cation$-\pi$ or anion$-\pi$ interactions [43]. Cation$-\pi$ interactions are crucial in biological systems and essentially electrostatic in nature, since they involve a positively charged ion and negatively charged electron cloud of a π-system [71].

Benzene rings do not have a dipolar moment but have carbon sp_2 atoms that are more electronegative than hydrogens, which leads to six aligned dipolar moments. Furthermore, due to the geometry of the molecule, it forms a large negative quadripolar moment. Ions can interact with a quadrupole, the same way as for dipoles. This quadrupole can be visualized through electrostatic potential surfaces with negatively charged regions on each side of the benzene ring due to π electron delocalization [72,73]. This quadrupole can be involved in different types of interactions such as $\pi-\pi$, CH$-\pi$, S$-\pi$, OH$-\pi$, and NH$-\pi$, as previously reviewed [19].

Trp has both a benzene ring and a pyrrole ring, which endow the indole ring with a permanent dipole moment pointing from the nitrogen (N1) in the five-membered ring to the carbon C5 in the six-membered ring. Compared to benzene, a stable ring with homogenously delocalized charges, indole contains a cyclic secondary amine surrounded by a more positively charged area (Figure 3a) because of its slightly acidic properties. This unique π system of indole can lead to strengthened cation$-\pi$ (electrostatic) interactions [73] on the one hand, and anion$-\pi$ (ion-induced polarizability and electrostatic) interactions on the other hand [43,74]. Nearly a quarter of Trp residues of the Protein Data Bank are involved in cation$-\pi$ interactions that occur approximately every 77 residues with an energy of 10 kJ/mol and importantly participates in protein stability. This has been characterized by Gallivan and Dougherty according to an energy criterion [75]. The most favorable configuration is obtained when the interaction occurs between the Trp and the *i* + 4 residue in an α helical structure. Partners involved in this interaction are usually Arg and Lys. A total of 70% of Arg residues are located near an aromatic residue, suggesting a particularly favorable interaction between these two residues. A question that could be raised is why Trp interaction with Arg is more favorable than interaction with Lys while both residues are positively charged [19]. Arg side chains tend to be more available than Lys side chains, due to its larger size but also because it is less solvated by water leading

to better van der Waals interactions. Arg and Lys do not adopt the same geometry during the interaction. Trp interacts mostly through its benzene ring and the steric hindrance caused by the indole ring prevents the Lys side chain approach. These interactions usually occur at the protein surface with a planar stacking geometry when involving an Arg residue [75]. This particular geometry allows the guanidinium moiety of Arg to form hydrogen bonds while interacting with Trp through cation–π interactions [76]. Trp cation–π interactions can also occur with positively charged moieties present at the membrane interface, such as lipid headgroups. Ethanolamine is more likely to interact via cation–π interactions with Trp compared to choline, as shown using molecular dynamic simulations [35].

There is more and more evidence of anion–π interactions in biological systems [77]. Recently, anion–π interactions at the lipid–water interface of the Ca^{2+}-ATP pump SERCA1 involving Trp residues and phospholipid head groups have been suggested [78]. Such anion–π interactions could also occur in more complex assemblies between aromatic residues and charged residues in transmembrane regions [79].

An indole has a dipolar moment of 2.1 D [80] and is able to interact with the bilayer electric field. According to this electrical gradient, an indole is preferentially located in the lipid headgroup region. Thus, this dipolar moment also participates to Trp interfacial localization but is not sufficient to explain it entirely.

Indole polarization as well as its interaction strength remains sensitive to its environment [81]. NMR experiments performed on Trp and analogs 3-methylindole, N-methylindole, and indene, which cannot be involved in dipole interaction, present the same localization at the interface. This result converges with another study that showed that benzene presents an interfacial localization by increasing fictitiously its partial charges in molecular dynamic simulations [6]. These studies suggest that there is a lack of dipolar contribution for the interfacial localization of Trp.

Nevertheless, this dipolar moment seems to play a major role for ion permeation in membrane-spanning gramicidin A channels. Indeed, Trp substitution by Phe decreases the channel conductance, whereas the substitution by N-methyl-Trp does not significantly impact this function. Trp and its analog N-methyl-Trp differ by their hydrogen bonding ability and their hydrophobicity, but they have a similar dipolar moment. It thus appears that the dipolar moment is involved in ion permeation [80].

3.4. Hydrogen Bonds

Trp has a hydrogen-bond donor NH group. When Trp is involved in a hydrogen bond, the system adopts a planar configuration due to the planar aromatic ring with a typical distance between the donor and the acceptor of about 3 ± 0.2 Å [82].

Lipid headgroups can play the role of hydrogen bond acceptor, which could partly explain Trp interfacial position. Gramicidin A has extensively been used as a model to study hydrogen bonding. For example, the hydrogen-bonding properties of Trp were compared with Phe and N-methyl Trp derivatives of gramicidin A. A deeper insertion inside DOPC vesicles was observed for both Phe and N-methyl Trp derivatives. This result suggests a role of hydrogen bonds for Trp localization at the interface [80]. Clear evidence of hydrogen bonds between the NH group and the lipid carbonyl groups has also been provided by Raman spectroscopy [83]. Another way to study hydrogen bonding between a protein and lipids is to use lipids in which the ester bonds were substituted by ether bonds, and thus cannot be involved in hydrogen bonds. This has been used to identify the presence of hydrogen bonds between gramicidin A and lipids. A comparison of the interaction between gramicidin A and DPPC or its ether analog based on molecular modeling led to the conclusion that a stronger hydrogen bond is formed in the case of DPPC. This interaction is accompanied by a conformational change leading to a modification of the protein activity [84].

Molecular dynamics simulations have been performed on gramicidin A to study the impact of different lipids on hydrogen bonding. The formation of hydrogen bonds was

deduced from the distance (< 3.5 Å) between atoms obtained in MD simulations [35]. A similar tendency to form hydrogen with Trp for both POPE and POPC was observed, with a comparable lifetime for these bonds. Hydrogen bonds can be established with both lipid carbonyl and phosphate groups. The latter seem to be involved especially in the outer membrane interface, but interactions with carbonyl remain dominant and constitute the main reason for Trp localization at the interface according to molecular dynamics studies [38].

The substitution of Trp-15 by Phe at the C terminus of Gramicidin A supports the presence of a hydrogen bond involving Trp-15, important for the nonchannel (double stranded) to pore (single stranded) transition of Gramicidin A. However, this hydrogen bond does not appear to be the driving force of the double-stranded to single-stranded conformational change. Indeed, unlike the previous studies, dissociation rate measurements of gramicidin A and its Phe analog in DPPC and DHPC are similar, which indicate a low contribution of this hydrogen bond, but the contribution of other hydrogen bonds cannot be excluded [85].

Regarding free amino acids, a comparison of the effect of N-methyl indole and Trp in ester and ether lipids by NMR leads to a similar molecular ordering suggesting a minor contribution of hydrogen bonds. In addition, another NMR study based on localization of Trp analogs in POPC membrane supports this idea. It demonstrates that whatever their ability to form hydrogen bonds, all analogs are located at the interface [10]. Only 27% of Trp residues in 180 proteins from the Brookhaven Protein Data Bank are not involved in hydrogen bonds, which demonstrates the importance of this interaction, not for Trp localization, but for other phenomena, such as protein stabilization [19]. An interpretation for this phenomenon is that Trp is found to be less polar than expected, thus involved in weak hydrogen bonds, resulting in the low contribution of this kind of interaction for Trp localization. This conclusion has been drawn from free energies of partitioning of 3-methylindole and N-methylindole between water and cyclohexane, where corrections of interaction with water in cyclohexane have been taken into account [86].

4. Conclusions

In summary, Trp is a very special amino acid, with unique and versatile physicochemical properties that allow it to engage in multiple types of noncovalent interactions, which explains its importance in membrane protein stabilization, anchoring and orientation in the lipid bilayer. Trp has many more interesting properties that were not discussed herein. Trp has unique fluorescent properties that are strongly dependent on its local environment, with a marked shift towards the shorter wavelength of its emission peak, usually accompanied by a change in intensity upon transfer form an aqueous to apolar environment [87]. Trp also has a rich light-induced redox-type reactivity, and recently, it has been shown that Trp could be selectively modified in proteins by photoinduced electron transfer [88]. Trp is involved in electron transfer reactions through its amino group that favors or disfavors proton transfer according to the local microsurroundings and conformation changes [89]. Trp also has many synthetic analogs with interesting properties, such as the blue-colored fluorescent β-(1-azulenyl)-L-alanine, which can be biosynthetically incorporated in proteins [90]. Such chemical biology approaches create exciting perspectives for the future study of Trp and membrane proteins. Finally, the unique properties of Trp are not limited to membrane proteins. In particular, this residue is of crucial importance in the mechanisms of action of membrane active peptides, such as antimicrobial or cell-penetrating peptides.

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