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Dynamic Insight into Protein Structure Utilizing Red Edge Excitation Shift

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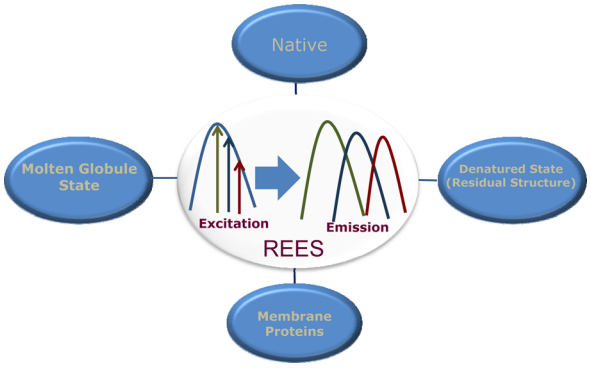
CONSPECTUS

Proteins are considered the workhorses in the cellular machinery. They are often organized in a highly ordered conformation in the crowded cellular environment. These conformations display characteristic dynamics over a range of time scales. An emerging consensus is that protein function is critically dependent on its dynamics. The subtle interplay between structure and dynamics is a hallmark of protein organization and is essential for its function. Depending on the environmental context, proteins can adopt a range of conformations such as native, molten globule, unfolded (denatured), and misfolded states. Although protein crystallography is a well established technique, it is not always possible to characterize various protein conformations by X-ray crystallography due to transient nature of these states. Even in cases where structural characterization is possible, the information obtained lacks dynamic component, which is needed to understand protein function. In this overall scenario, approaches that reveal information on protein dynamics are much appreciated.



Dynamics of confined water has interesting implications in protein folding. Interfacial hydration combines the motion of water molecules with the slow moving protein molecules. The red edge excitation shift (REES) approach becomes relevant in this context. REES is defined as the shift in the wavelength of maximum fluorescence emission toward higher wavelengths, caused by a shift in the excitation wavelength toward the red edge of absorption spectrum. REES arises due to slow rates (relative to fluorescence lifetime) of solvent relaxation (reorientation) around an excited state fluorophore in organized assemblies such as proteins. Consequently, REES depends on the environment-induced motional restriction imposed on the solvent molecules in the immediate vicinity of the fluorophore. In the case of a protein, the confined water in the protein creates a dipolar field that acts as the solvent for a fluorophore in the protein. In this Account, we focus on REES to monitor organization and dynamics of soluble and membrane proteins utilizing intrinsic protein fluorescence.

We discuss here the application of REES in various conformations of proteins. While application of REES to proteins in native conformation has been in use for a long time, our work highlights the potential of this approach in case of molten globule and denatured conformations. For example, we have demonstrated the presence of residual structure, that could not be detected using other methods, by REES of denatured spectrin. Given the functional relevance of such residual structures, these results are very far reaching. We discuss here the application of REES to molten globule conformation and to the green fluorescent protein (GFP). The case of GFP is particularly interesting since the dipolar field in this case is provided by the protein matrix itself and not confined water. We envision that future applications of REES in proteins will involve generating a dynamic hydration map of the protein, which would allow us to explore protein function in terms of local dynamics and hydration.

Proteins are ubiquitous macromolecules involved in a variety of biological functions. They are highly ordered yet exhibit dynamics over a wide range of time scale.[1] The interplay between structure and dynamics is a hallmark of protein organization and is essential for its function. Depending on the context, proteins can adopt a range of conformations such as native, molten globule, unfolded, and misfolded states. Due to transient nature of some of these states, it is not always possible to characterize them by techniques such as X-ray crystallography. Even in cases

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where structural characterization is possible with X-ray crystallography, the information obtained is static in nature. Dynamic information on protein organization leading to function is therefore crucial.

Water plays a vital role in determining the folding, structure, dynamics, and therefore the function of proteins and peptides.[2−7] It is estimated that a minimum level of hydration is required to fully activate the dynamics and function of globular proteins.[8] Hydration has been shown to be critical in protein folding[7] and water has been shown to act as a catalyst for hydrogen bond exchange in protein folding, thereby acting as a "foldase".[3] Water molecules become confined on the surfaces of proteins and such interfacial confinement destroys the water−water hydrogen bond network and couples the motion of water molecules with the slow moving protein molecules.[9,10] Consequently, the dynamics of water molecules is retarded. In such a scenario, red edge excitation shift (REES) represents a convenient approach to explore the dynamic organization of proteins.[11−15]

REES represents a unique approach that relies on slow solvent reorientation in the excited state of a fluorophore. It can be conveniently used to monitor the environment and dynamics around a fluorophore in an organized molecular environment such as found in biological membranes and proteins.[11−15] REES is operationally defined as the shift in the wavelength of maximum fluorescence emission toward higher wavelengths, caused by a shift in the excitation wavelength toward the red edge of absorption spectrum. REES has its genesis in slow rates (relative to fluorescence lifetime) of solvent relaxation (reorientation) around an excited state fluorophore in an organized assembly. REES therefore depends on the environment-induced motional restriction imposed on the solvent molecules in the immediate vicinity of the fluorophore. This approach allows to assess the rotational mobility of the environment itself, represented by the relaxing solvent molecules, utilizing the fluorophore *merely* as a reporter. It should be noted here that the definition of solvent in this context is rather flexible. Solvent relaxation dynamics could include dynamics of restricted solvent (water), but also the dynamics of the host dipolar matrix such as the peptide backbone in proteins.[16] A comprehensive conceptual photophysical framework of REES is provided in a recent feature article (review) of ours.[15] In this Account, we will focus on the application of REES to monitor organization and dynamics of soluble and membrane proteins utilizing intrinsic fluorescence.

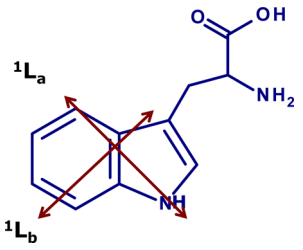

FIGURE 1. Molecular structure of tryptophan showing the transition moment directions for the transitions from the singlet ground state (S_0) to the two lowest excited electronic states, ^1L_a and ^1L_b. The ^1L_a transition density is localized along the atoms (as it is directed through the ring −NH group), whereas the ^1L_b transition density lies along the bonds in the benzene ring. The change in dipole moment associated with S_0 to ^1L_a transition is larger compared to S_0 to ^1L_b transition, making the transition involving ^1L_a state more sensitive to environment. Fluorescence in most proteins arises due to ^1L_a to S_0 transition. In nonpolar environment, ^1L_a has higher energy than ^1L_b. However, the energy level of ^1L_a is lower in polar environment due to favorable dipole−dipole interactions. See text for other details. Adapted and modified from ref 17.

Tryptophan as an Intrinsic Fluorophore in Proteins and Peptides: A Natural Choice

The intrinsic fluorescence of proteins and peptides arises due to the presence of aromatic amino acids tryptophan, tyrosine, and phenylalanine. When all three aromatic amino acids are present in a protein, pure emission from tryptophan can be obtained only by photoselective excitation at wavelengths above 295 nm.[17] Tryptophan is the most widely used amino acid for fluorescence analysis of proteins due to a number of reasons. The indole group of tryptophan (see Figure 1) is mainly responsible for UV absorption and emission in proteins. In a protein containing all three naturally fluorescent amino acids, observation of tyrosine and phenylalanine fluorescence is often complicated due to interference by tryptophan because of resonance energy transfer.[17,18] The application of tyrosine and phenylalanine fluorescence is therefore mostly limited to tryptophan-free proteins (however, there are exceptions[19]). A limitation of tyrosine fluorescence is its poor sensitivity to environmental factors such as polarity (unlike tryptophan).[20] Fluorescence of phenylalanine is weak and seldom used in protein studies.[18] Intrinsic protein fluorescence is therefore mostly associated with tryptophan fluorescence.

Tryptophans serve as intrinsic, site-specific fluorescent probes for analysis of protein structure and dynamics and are generally present at ∼1 mol % in proteins.[18] The relatively small tryptophan content could be a consequence of the metabolic expense of its biosynthesis. Nonetheless, the low tryptophan content of proteins appears to be a boon since it makes interpretation of fluorescence data less

complicated due to absence of inter-tryptophan interactions. The well documented sensitivity of tryptophan fluorescence to environmental factors such as polarity and mobility makes tryptophan fluorescence a valuable tool in studies of protein structure and dynamics by providing specific and sensitive information of protein structure and its interactions.[17,18] The molecular basis of such interesting spectral properties of tryptophan are attributed to a number of factors. Tryptophan has a large indole side chain that consists of two fused aromatic rings (see Figure 1). Tryptophan has two overlapping $S_0 \rightarrow S_1$ electronic transitions denoted as ^1L_a and ^1L_b. These transition moments are almost perpendicular to each other (Figure 1).[17] Both $S_0 \rightarrow {}^1L_a$ and $S_0 \rightarrow {}^1L_b$ transitions take place in the 260−300 nm range. In nonpolar solvents, ^1L_a has higher energy than ^1L_b. In polar solvents, the energy level of ^1L_a is lowered making it the lowest energy state. This inversion is believed to occur because ^1L_a transition has a higher dipole moment (as it is directed through the −NH group of the indole ring), and can have dipole−dipole interactions with polar solvent molecules. Interestingly, equilibration between these two states is believed to be very fast (of the order of 10^{-12} s), irrespective of whether ^1L_a or ^1L_b is the lowest S_1 state.[21] This implies that emission only from the lower S_1 state is observed in all cases. It is generally believed that ^1L_a is the fluorescing state in all proteins in aqueous solution with the possible exception of Trp-48 of azurin.[22,23]

REES as a Reporter of Protein Conformation: Native, Molten Globule, and Denatured States

REES can be effectively utilized to examine the local dynamics of the protein matrix around a given amino acid residue (usually tryptophan) from the rate at which the matrix responds to (i.e., relaxes around) the excited state dipole of the fluorophore. The magnitude of REES provides a measure of the relative rigidity of the region of the protein surrounding the fluorophore. Early work on REES of proteins was carried out by Demchenko and co-workers using proteins such as β-lactoglobulin, β-casein, and serum albumins using both intrinsic protein (tryptophan) fluorescence and extrinsic fluorescent probes (such as 2-(*p*-toluidinylnaphthalene)-6-sulfonate (TNS)) labeled at specific sites in the protein (reviewed in refs 12 and 13). In an earlier study, we showed that the tryptophan residues in the globular protein tubulin exhibits REES in the native state.[24] As was expected and previously observed for other proteins, REES was absent when tubulin was denatured in urea, thereby implying fast solvent relaxation around the tryptophans in the denatured

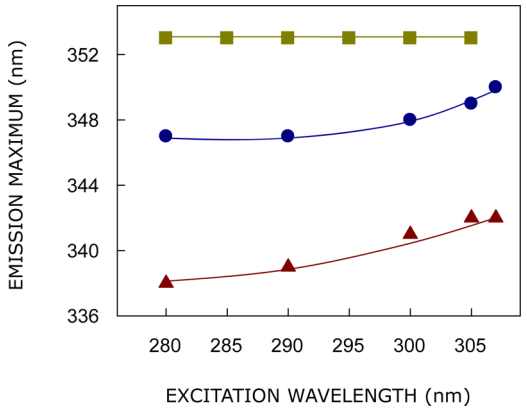

FIGURE 2. Detection of local residual structure by REES. Effect of changing excitation wavelength on the wavelength of maximum emission of the cytoskeletal protein spectrin in native (▲) and denatured (●) conditions. Tryptophan residues in denatured proteins usually do not display REES since they are exposed to bulk water (the figure also shows lack of REES in a representative denatured protein, tubulin (■)). The presence of REES in denatured spectrin therefore demonstrates that spectrin tryptophans are shielded from bulk water even when denatured, thereby implying local residual structure in denatured spectrin. The residual structural integrity that remains in denatured spectrin is considered to be the hallmark of a protein whose main function is to provide a stable scaffold to the cell membrane. See text for details. Data from refs 24 and 26.

state. The magnitude of REES of tryptophans in tubulin was found to be reduced at higher temperature since the rate of solvent relaxation increases upon increase in temperature and emission is dominated from solvent-relaxed states.[24] In addition, we analyzed the organization and dynamics of tryptophans in the soluble and hemolytic protein α-toxin from *S. aureus* utilizing REES obtained with this protein under various conditions.[25]

REES in Denatured Proteins: Detection of Residual Structure. It became apparent that tryptophans or external probes labeled at specific sites in proteins exhibit REES and this could provide an additional handle to monitor protein conformation and dynamics. An accompanying dogma was that tryptophan residues in denatured proteins usually do not display REES since they are exposed to bulk water characterized with fast solvent relaxation. For example, although tryptophans in tubulin exhibit REES of 7 nm, no REES was observed when the protein was denatured (see the top plot in Figure 2).[24] This dogma was challenged when we observed significant REES for tryptophans in the cytoskeletal protein erythroid spectrin, even when denatured (see Figure 2).[26] Spectrin is the major constituent protein of the erythrocyte cytoskeleton which forms a filamentous network on the cytoplasmic face of the membrane by providing a scaffold for a variety of proteins.[27] The presence of REES in

denatured spectrin demonstrates that spectrin tryptophans are shielded from bulk water under denaturing condition. These results point out to the presence of residual structure in denatured spectrin. *Interestingly, analyses of fluorescence maxima and circular dichroism (CD) measurements were unable to detect the residual structure present in denatured spectrin, thereby implying the uniqueness of the REES approach.*[26] These results were further supported by analysis of fluorescence quenching data using acrylamide as quencher. Interestingly, the residual structural integrity that remains in denatured spectrin is considered to be the hallmark of a protein whose main function is to provide a stable scaffold to the cell membrane. Such residual structure in an unfolded protein is thought to reside predominantly in hydrophobic clusters, where residues like tryptophan stabilize these networks through cooperative long-range non-native interactions.[28] Although residual structure in denatured proteins has been earlier reported,[29,30] our results constituted the first demonstration of slow solvent relaxation, monitored by REES, in a denatured protein.

REES in Molten Globule Proteins. The paradigm of structure–function relationship of proteins has changed considerably since the discovery of intrinsically (partially) disordered yet functional proteins.[31] The molten globule state is considered to be an important intermediate in protein folding, and was initially proposed as a partially folded state with stable native-like secondary structure but lacking a specific tertiary structure.[32] Molten globule states are now considered to be an ensemble of conformations with varying degrees of disorder.[33] The molten globule conformation has acquired greater relevance in cellular processes since proteins in the molten globule state have been shown to be involved in interaction with molecular chaperones, translocation across biological membranes and amyloid formation.[34−36] In this overall context, exploring the organization and dynamics of proteins in the molten globule form utilizing REES assumes greater significance. In one of the early reports on the organization of the molten globule state using REES, we worked with the well-known protein bovine α-lactalbumin (BLA). BLA is a small acidic Ca^{2+}-binding protein present in milk and is known to be present in molten globule form under various conditions.[37,38] It is extensively used to study the molten globule state since it assumes the molten globule state at acidic pH and in the *apo*-state. In order to explore REES of molten globule conformation(s), we examined the organization and dynamics of the tryptophan residues of BLA in the molten globule state, achieved using two different approaches

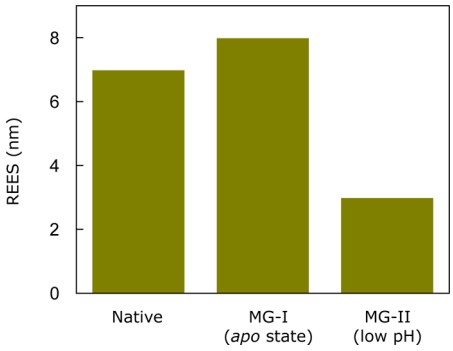

FIGURE 3. Protein conformations and REES: REES exhibited by tryptophan residues of bovine α-lactalbumin (BLA) in native and molten globule states. MG-I refers to the Ca^{2+}-depleted (*apo*-state) molten globule conformation (see ref 39) and MG-II refers to the molten globule state of BLA induced by low pH (ref 40). These results are one of the first reports of REES in molten globule conformation of proteins. Note that the magnitude of REES appears to depend on the manner in which the mollten globule state was achieved, indicating that the molten globule states represent an ensemble of conformations with varying degrees of disorder. The magnitude of REES corresponds to the total shift in emission maximum as the excitation wavelength was changed from 280 to 307 nm. See text and refs 39 and 40 for details.

(i.e., low pH and *apo*-state).[39,40] REES of BLA in the native state served as a control. Interestingly, we observed that BLA exhibits REES in the molten globule states, irrespective of the manner in which the molten globule state was reached (see Figure 3). However, depending on the approach taken to induce molten globule conformation, REES could vary. For example, we observed REES of 8 nm in case of calcium-depleted (*apo*) state of BLA,[39] while REES exhibited in the molten globule state generated by low pH was 3 nm[40] (Figure 3). This is supported by the proposition that the molten globule states represent an ensemble of conformations with varying degrees of disorder.

"Solvent" Relaxation in Green Fluorescent Protein. Green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* and its variants have become popular reporter molecules for monitoring protein expression, localization, and dynamics of membrane and cytoplasmic proteins in the last two decades.[41−43] The reasons for the immense popularity of GFP (culminating in the award of Nobel Prize in Chemistry in 2008)[42] include its intrinsic, cofactor-independent fluorescence which exhibits remarkable stability in the presence of denaturants and over a wide range of pH. GFP is a compact barrel-shaped protein consisting of 11 β strands with an α helix running through the central axis of the cylindrical structure, sometimes termed as a *molecular lantern* (see Figure 4). The fluorophore of GFP (*p*-hydroxybenzylideneimidozolidinone) responsible for its green fluorescence (see Figure 4) is

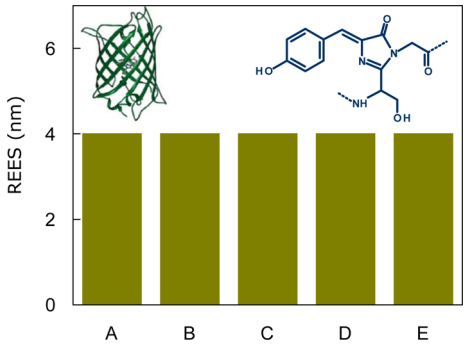

FIGURE 4. REES of the enhanced green fluorescent protein (EGFP) due to dipolar relaxation within the protein matrix is independent of bulk viscosity and dynamics. REES of EGFP in bulk water (A), glycerol (B), AOT reverse micelles of increasing hydration (C–E, corresponding to $w_o = 2$, 15 and 30, respectively). The figure also shows the GFP fluorophore (*p*-hydroxybenzylideneimidozolidinone) and the β-barrel structure of GFP. The observed REES of EGFP is due to the constrained environment experienced by the EGFP fluorophore in the rigid protein matrix, rather than the dynamics of the surrounding medium, and therefore independent of the viscosity of the medium. See ref 16 and text for details. Adapted and modified from ref 45. Copyright 2012 Springer.

localized at the center of the cylindrical structure and is formed spontaneously (as a result of post-translational modification) upon folding of the polypeptide chain by internal cyclization and oxidation of the residues Ser65-Tyr66-Gly67 in the α helix. The fluorophore in GFP is in a highly constrained environment, protected from the bulk solvent by the surrounding rigid β strands. Although photophysical aspects of GFP have been extensively studied, information on solvent dipolar relaxation around the GFP fluorophore is relatively scant.[44]

We observed REES of enhanced green fluorescent protein (EGFP,[46] a variant of GFP) in buffer and in glycerol due to slow rate of solvent dipolar reorientation around the excited state fluorophore. Interestingly, the term "solvent" here refers to the dipolar protein matrix[16] (see Figure 4). REES of EGFP in buffer (a nonviscous medium) was surprising and provided the hint that the fluorophore may not sense the bulk solvent. To explore this further, we examined REES of EGFP incorporated in reverse micelles of sodium bis(2-ethylhexyl)-sulfosuccinate (AOT). We found that EGFP displays REES when incorporated in AOT reverse micelles. Interestingly, REES of EGFP in reverse micelles appears to be independent of the reverse micellar hydration state (w_o) (see Figure 4). Neither entrapment in a reverse micelle nor the hydration state of the reverse micelle influenced the magnitude of REES of EGFP. This clearly implies that the extent of REES of EGFP is independent of the viscosity and hydration of the surrounding medium, implying that the *dynamics of the protein matrix, rather than the dynamics of the surrounding medium plays an important role.*

Conformation-Dependent REES of Membrane Proteins and Peptides

Biological membranes are complex, two-dimensional, anisotropic assemblies of lipids and proteins that allow cellular compartmentalization, and provide an interface for cells to communicate with each other and with the external milieu. Membrane proteins are major players involved in a variety of important cellular processes such as signaling across the membrane, cell–cell recognition, and membrane transport. They represent prime candidates for the generation of novel drugs in all clinical areas.[47] Membrane proteins are difficult to crystallize in their native conditions because of their intrinsic dependence on surrounding membrane lipids.[48]

Membranes provide a unique environment to membrane-spanning proteins and peptides. Membrane-spanning proteins have characteristic stretches of hydrophobic amino acids which form the membrane-spanning domain and are reported to have a significantly higher tryptophan content than soluble proteins.[49] The role of tryptophan residues in the structure and function of membrane proteins and peptides has been a topic of considerable discussion.[15,50–54] Tryptophan residues in membrane proteins and peptides are not uniformly distributed, but tend to be localized toward the membrane interface (see Figure 5). The interfacial region in membranes has unique motional and dielectric characteristics distinct from both the bulk aqueous phase and the hydrocarbon-like interior of the membrane.[55] Tryptophan has the polar-NH group which is capable of forming hydrogen bonds and it also has the largest nonpolar accessible surface area among the naturally occurring amino acids.[56] This makes tryptophan a unique amino acid since it is capable of both hydrophobic and polar interactions. It has been shown that the experimentally determined interfacial hydrophobicity of tryptophan is highest among the naturally occurring amino acids based on partitioning of model peptides to membrane interfaces.[57] This could account for its specific interfacial localization in membrane peptides and proteins. We have utilized REES to monitor the conformation and dynamics of a variety of membrane peptides and proteins such as melittin,[58,59] gramicidin,[60,61] the pore-forming α-toxin from *S. aureus*,[25] and the N-terminal domain of CXC chemokine receptor (CXCR1).[62] We will discuss below the application of REES to conformational analysis of a representative ion channel peptide gramicidin.

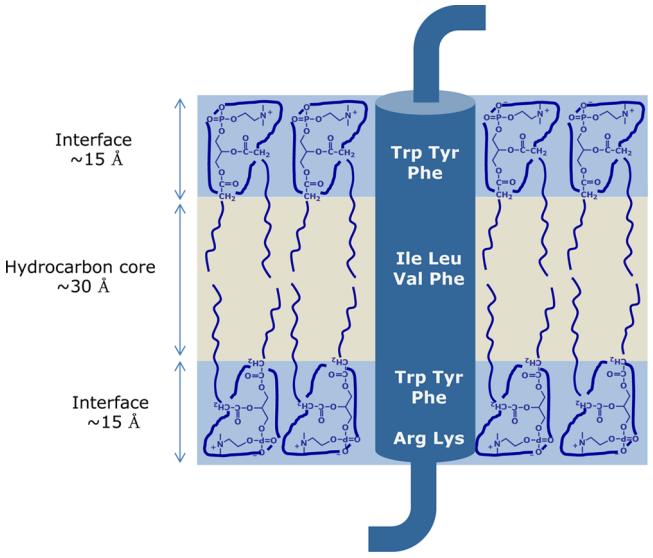

FIGURE 5. Schematic representation of biological membranes showing various regions of the bilayer with a membrane-embedded transmembrane domain. The membrane interface is represented by a heterogeneous chemical environment and relatively slow dynamics. It constitutes ~50% of the thermal thickness of the bilayer and is characterized by unique organization, dynamics, hydration and functionality. Amino acids in transmembrane helical domains display a quasi-random distribution along the bilayer normal. The preferred locations of various amino acids present in a membrane-spanning transmembrane domain are shown in the figure. It should be noted that the tryptophan residues are localized in the membrane interface region, a feature shared by many membrane proteins.[52] See text for details. Adapted and modified from ref 14.

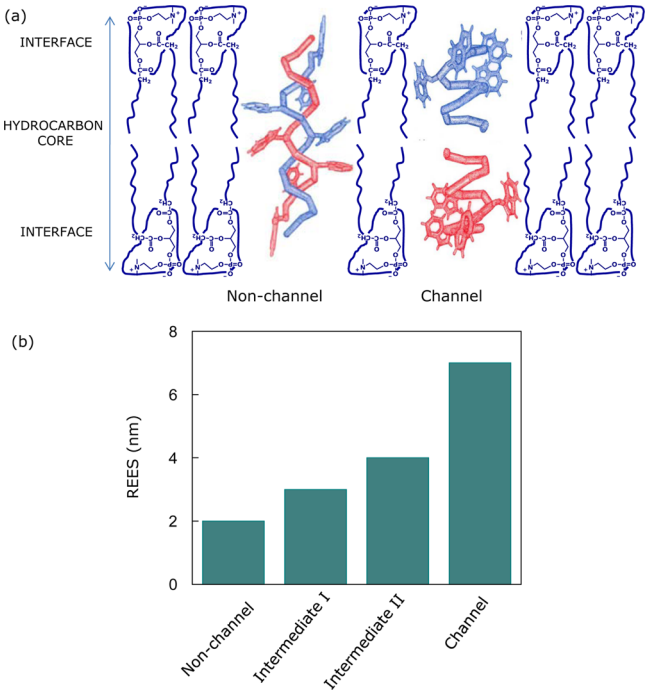

FIGURE 6. Conformation-dependent REES of membrane proteins. (a) A schematic representation of the non-channel and channel conformations of the ion channel peptide gramicidin indicating the localization of tryptophan residues in the membrane bilayer. Tryptophan residues are clustered toward the membrane interface in the channel conformation, whereas they are distributed along the membrane *z* axis (bilayer normal) in the non-channel conformation. Gramicidin is a popular membrane peptide and is extensively used to explore the principles that govern the folding and function of membrane proteins (see ref 64). Adapted with permission from ref 15. Copyright 2011 American Chemical Society. (b) REES is sensitive to the conformation of membrane-bound gramicidin. Intermediates I and II represent intermediates in the folding pathway of the channel conformation from non-channel conformation. The magnitude of REES corresponds to the total shift in emission maximum as the excitation wavelength was changed from 280 to 307 nm. Data from ref 61.

The linear peptide gramicidin forms prototypical ion channels specific for monovalent cations and has been used extensively to explore the organization, dynamics, and function of membrane-spanning channels.[63,64] Gramicidin is a multitryptophan protein (Trp-9, 11, 13, and 15) which serves as an excellent model for transmembrane channels due to a number of reasons.[63,64] Gramicidin assumes a wide range of environment-dependent conformations due to its unique sequence of alternating L- and D-chirality. Two major conformations adopted by gramicidin in various media are (i) the single stranded $\beta^{6.3}$ helical dimer ("channel" form) and (ii) the double stranded intertwined helix (collectively known as the "non-channel" form) (see Figure 6a). The amino terminal-to-amino terminal single-stranded $\beta^{6.3}$ helical dimer form represents the channel conformation of gramicidin in membranes. In this conformation, the tryptophan residues remain clustered at the membrane–water interface.[64] We previously showed that the tryptophan residues of gramicidin in the channel conformation exhibit REES, implying that the tryptophan residues are localized in the interfacial region and experience motional restriction.[60,61] Tryptophan residues in gramicidin channels are crucial for maintaining the structure and function of the channel.[64] We explored the structural basis for the reduction in channel-forming propensity using single and double tryptophan analogues of gramicidin utilizing REES and other fluorescence approaches.[65,66] Our results showed that the both single and double tryptophan analogues adopt a predominantly non-channel conformation in membranes.

In case of gramicidin, we conveniently utilized REES to monitor conformational changes (see Figure 6b).[61] The basis of this lies in the fact that while the tryptophan residues are clustered toward the membrane interface in the channel conformation of gramicidin, they are distributed along the membrane axis in the non-channel conformation. Consequently, the environment of the tryptophans in these conformations are different, giving rise to difference in REES (see Figure 6b).[61] More importantly, we were able to detect

REES displayed by intermediates in the folding pathway of membrane-bound gramicidin from the initial non-channel to the final channel conformation (denoted as intermediates I and II). The progressive increase in REES from the non-channel conformation to the channel conformation through the intermediate conformations corresponds to the gradual conversion of the non-channel form to the channel form of gramicidin. These results offer an excellent example of how REES can be conveniently employed to monitor membrane protein folding.

Conclusion and Future Perspectives

In this Account, we have focused on the application of REES in exploring protein organization and dynamics. REES is a sensitive tool to monitor conformation of soluble and membrane proteins. The advantage of REES lies in the fact that it is capable of detecting subtle conformational changes (e.g., residual structure in denatured proteins) in proteins that other conventional techniques cannot detect.[26] A potentially exciting application is to obtain a detailed dynamic hydration map of proteins using a combination of site-specific mutation and labeling.[67,68] This assumes relevance in view of recent reports, based on crystal structures of membrane proteins such as rhodopsin and β_2-adrenergic receptor, that there are motionally restricted water molecules that could be important in inducing conformational transitions in the transmembrane domain of the receptor.[69]

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BIOGRAPHICAL INFORMATION

Amitabha Chattopadhyay received his Bachelor's degree with Honors in Chemistry from St. Xavier's College (Calcutta) and Master's degree in Chemistry from the Indian Institute of Technology, Kanpur. He obtained his Ph.D. from the State University of New York at Stony Brook, and was a Postdoctoral Fellow at the University of California, Davis. He subsequently joined the Centre for Cellular and Molecular Biology (CCMB) in Hyderabad, where he is currently an Outstanding Scientist. Prof. Chattopadhyay's work is focused on monitoring organization, dynamics, and function of biological membranes utilizing fluorescence-based spectroscopic and microscopic approaches. His other major interest is the role of membrane lipids in the organization and function of G protein-coupled receptors. Prof. Chattopadhyay was awarded the prestigious Shanti Swarup Bhatnagar Award by the Prime Minister of India. He is an elected Fellow of all the Indian Academies of Science, and the Royal Society of Chemistry, and serves on the editorial boards of a number of reputed journals.

Sourav Haldar received his Bachelor's degree from the Department of Chemistry, Jadavpur University, Kolkata in Chemistry (Honors) and Master's degree from the Department of Chemistry, Indian Institute of Technology, Madras (Chennai). He obtained his Ph.D. from the Centre for Cellular and Molecular Biology, Hyderabad, and currently is a Postdoctoral Fellow at the National Institutes of Health, Bethesda. His major interest is on fluorescence spectroscopy, membrane organization, dynamics, and lipid–protein interactions.

FOOTNOTES

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The authors declare no competing financial interest.

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