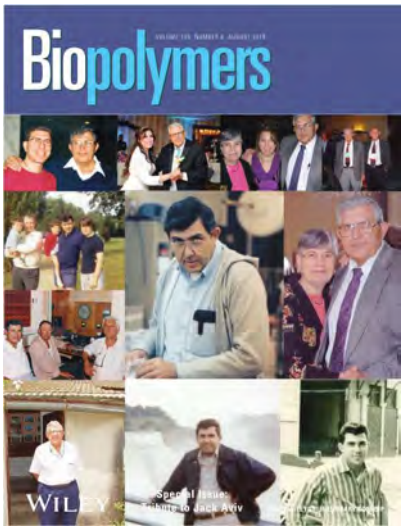
Biopolymers

Special Issue: Tribute to Jack Aviv

Guest Editor: Kenneth J. Breslauer (Rutgers, The State University of New Jersey, U.S.A.)



Received: 4 April 2018 | Revised: 10 July 2018 | Accepted: 13 July 2018

DOI: 10.1002/bip.23235

WILEY Biopolymers

REVIEW

Discovery of the first self-assembling peptide, study of peptide dynamic behaviors, and G protein-coupled receptors using an Aviv circular dichroism spectropolarimeter

Shuguang Zhang

Center for Bits and Atoms E15-401, Massachusetts Institute of Technology, Cambridge, Massachusetts

Correspondence
Shuguang Zhang, Center for Bits and Atoms E15-401, Massachusetts Institute of Technology, 20 Ames Street, Wiesner Media Lab Building, Cambridge, MA 02139-4307.
Email: shuguang@mit.edu

Abstract

Circular dichroism (CD) spectroscopy is a useful technique to study the structure and dynamics of peptides, proteins and nucleic acids. CD is particularly useful because sample volumes may be as low as 50 μL, it provides high precision and sensitivity, and it achieves a good signal to noise ratio. CD characterizes molecular conformational changes in real time by finely controlling temperature, pH, and titrating urea and guanidine·HCl which is necessary for studying protein folding. Although CD does not provide detailed structure at the atomic level, it provides a global structural framework. Researchers use CD to observe molecular phenomena, namely how macromolecules unfold/refold and their overall self-assembly/disassembly. Using CD to monitor a peptide structure, I serendipitously discovered the self-assembling peptide EAK16 from yeast protein *Zuotin*. This unusual peptide formed a new type of nanofiber scaffold hydrogel material. The discovery in 1990 opened a new field in the design and study of numerous self-assembling peptides, thereby launching the area of peptide nanobiotechnology. In this review, I reflect on my personal discoveries of several self-assembling peptides, investigations into the dynamic behaviors of peptides, as well as the impact of the work on society. I also describe studies of natural membrane proteins and engineered membrane proteins using CD. Furthermore, I enjoyed numerous and close interactions with Jack Aviv since 1997. He generously supported 10 high impact workshops (Crete and Mikonos) and meetings in various countries around the world that left fond memories of many young researches who later became leading scientists in their respective fields.

KEYWORDS

beta-sheet to alpha-helix conversion, bi-stable peptides, peptide nanofiber scaffolds, self-assembling peptides, structural dynamics

1 | INTRODUCTION

I was first introduced to circular dichroism (CD) spectroscopy while working on yeast genetics and protein chemistry in the laboratory of the late Alexander Rich at Massachusetts Institute of Technology. At that time, I sought to gain an understanding of the biological roles of the left-handed Z-DNA structure. In 1989, I identified and purified a protein Zuotin[1] for its ability to bind to left-handed Z-DNA in the presence of a 400-fold excess of sheared salmon DNA that contains ubiquitous right-handed B-DNA and other structures. In Zuotin, there is a repeating segment with the sequence n-AEAEAKAKAEAEAKAK-c, which I named EAK16 for its amino acid composition and peptide length (Figure 1).[1] Despite predictions of an α-helix, I was very curious about the actual structure of EAK16. Following several attempts, I finally convinced Alexander Rich to obtain a synthetic peptide encompassing the EAK16 sequence (n-AEAEAKAKAEAEAKAK-c).

Using an Aviv Biomedical, Inc. CD spectrometer, I discovered that the EAK16 peptide formed an exceedingly stable β-sheet structure (Figure 2), rather than the computationally predicted α-helix. On addition of salts, a membrane-like material emerged from the peptide solution that was visible to the naked eye.[4–6] This unexpected discovery opened a new field of self-assembling peptide nanotechnology. Since 1990, CD has become an indispensible technique and played a crucial role in the discovey of several classes of self-assembling peptides and

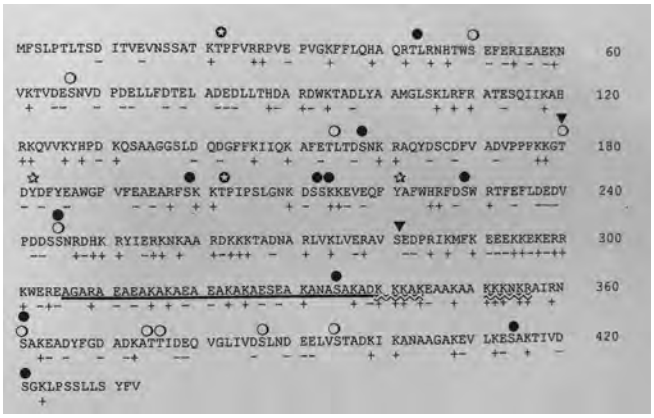


FIGURE 1 The protein sequence of yeast Zuotin. The positive and negative charges are labeled + or -, respectively. The open and closed circles are predicated phosphorylation sites. The thick underline indicates the alternating sequence that contains the AEAEAKAKAEAEAKAK motif. It was predicted to be a typical α-helix because of its *i*, *i* + 3, *i*, *i* + 4 hydrogen bond signature, from both what was the latest computer algorithm and what were the latest publications in 1987.[2,3] However, the reproducible experimental evidence obtained using CD revealed this motif to be a typical β-sheet structure (please see Figure 2).

the study of their dynamic behavior. These peptides have been engineered into diverse biological materials composed of simple amino acids with well-ordered nanostructures, and suitable for a variety of uses because of their materials properties under physiological conditions. We have also used CD spectroscopy to study membrane proteins and design detergent-free membrane proteins.

Self-assembling peptides form various structures, including well-ordered nanofibers, and can form scaffold hydrogel materials that have been used for: (*a*) 3D tissue cell cultures of primary cells and stem cells; (*b*) 3D tissue printing; (*c*) sustained releases of small molecules, growth factors and monoclonal antibodies; (*d*) reparative and regenerative medicine, and tissue engineering; (*e*) human clinical trials for accelerated-wound healing; and (*f*) human clinical trials for siRNA delivery in the treatment of cancers. It is likely that these self-assembling peptides discovered via CD spectroscopy will open new avenues for more diverse applications. Indeed, beyond materials science, research on self-assembling peptides is being carried out in a number of areas including synthetic biology and clinical medicine.

Since the field has expanded beyond that which can be discussed in a single article summary in this article, I only focus on studies using CD by myself, and those performed by other members of my laboratory and my close colleagues.

2 | STRUCTURAL PROPERTIES

I discovered an unusual class of self-assembling peptides that form stable β-sheets that further self-organize to form visible materials (shown in Figure 3).[4–6] The peptides have unusual characteristics, namely, the sequence has alternating repeating clusters of positively (Lys, Arg) and negatively charged (Asp, Glu) amino acids. It is known that hydrophobic residues must be shielded from water, thus alanines interdigitally interact (like interdigital fingers),[7] similarly to the molecular structure found in silk.[4] They are referred to as modulus I, II, III and IV respectively when the charges have -, +; –, –, +, +; –, –, –, +, +, +; –, –, –, –, +, +, +, +, (Table 1).[8]

Several designed peptides with modulus I, II and IV have been systematically studied. These peptides all form β-sheets at room temperature, and self-assemble into well ordered nanofibers by the millions, which further form a nano-scaffold by the billions or trillions (Figure 4). The nanofiber scaffolds can hold enormous amount of

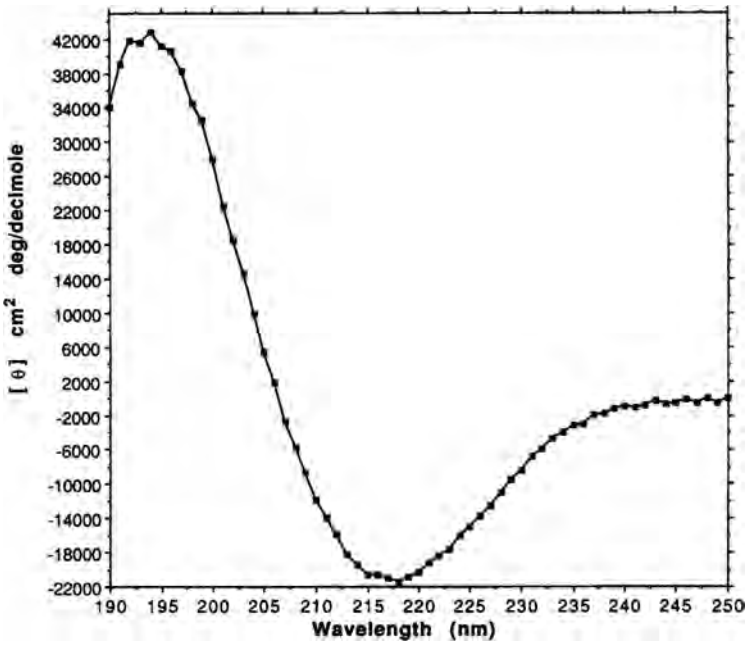


FIGURE 2 CD spectrum of the first self-assembling EAK16 peptide (AcN-AEAEAKAKAEAEAKAK-CONH2). The EAK16 peptide was dissolved in water (10 μM) before recording the CD spectrum. A typical β-sheet CD spectrum with a minimum at 218 nm and a maximum at 195 nm was observed. Copyright 1993 National Academy of Sciences.

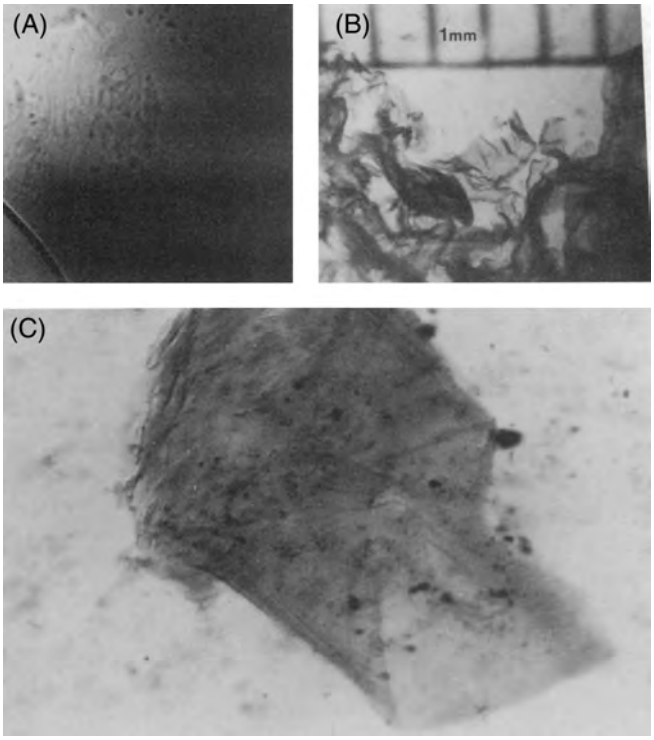


FIGURE 3 Discovery of the first self-assembling peptide scaffold material. A, The structure was formed in phosphate-buffered saline and transferred to a glass slide. The colorless membranous structures are isobuoyant; therefore, the image is not completely in focus (75× Nomarski phase contrast microscope). B, The structure is stained bright red with Congo red and can then be seen by the naked eye (15×, each scale unit = 1 mm). C, A portion of a well-defined membranous structure with layers is clearly visible; the dimensions of this particular membrane are 2×3 mm (20×). Copyright 1993 National Academy of Sciences.

water molecules as molecular capillaries, thus they become hydrogels with only 0.3%–1% solid peptide material.[7–10]

When I first studied the EAK16 peptide using a CD spectropolarimeter in 1990, the EAK16 was first predicted by computer to be an α-helix. But EAK16 CD spectrum exhibited β-sheet structure even at very dilute concentrations and at 90 °C (Figure 5A). Furthermore EAK16 is stable at wide pH ranges from pH 1 to pH 11, with minimal CD spectrum changes. EAK16 also resisted to temperature as high as 90 °C, and stayed stable in the presence of denaturing chemicals including 1% SDS, 8 M urea, and 6 M guanidine·HCl.[5]

Both EAK16 and a similar peptide, RAD16 (AcN-RARADADAR-ARADADA-$CONH_2$), show different β-sheet CD spectra. EAK16 shows the typical characteristics of a β-sheet spectrum with minima and maxima at 218 nm and 195 nm, respectively. Conversely, β-sheet spectrum of RAD16 is atypical, exhibiting a minimum at 219 nm and a lower (Θ) at 203 nm,[6] thus suggesting it may have a different backbone twist (Figure 5B).

3 | SUBTLE BEHAVIOR CHANGES OF PEPTIDES

My colleagues and I systematically studied several modulus IV peptides containing 16 amino acids, they include EAK16-IV, KAE16-IV, DAR16-IV, RAD16-IV, and mixed moduli peptides (Table 1). All 6 modulus IV peptides display typical β-sheet CD spectra at room temperature. However, when the peptide solutions are heated, each shows a different spectrum. For example, both EAK16-IV and KAE16-IV are extremely stable at high temperatures. Their β-sheet CD spectra remain essentially unchanged at 20 °C and at 90 °C (Figure 6). Figure 6A presents CD spectra after 10 minutes heating on the thermal stability of EAK16-IV and KAE16-IV at 20 °C and at 90 °C. These spectra overlap with the exception of minor variations in the 195 nm region. Figure 6B presents CD spectra of peptide RAD16-IV at 20 °C before heating, and after heating to 90 °C for 10 minutes. RAD16-IV exhibits a β-sheet spectrum at 20 °C, but after heating at 90 °C, the CD spectrum ellipticity near 195 nm is decreased, it is perhpas due to a change in twist of the β-sheet backbone.[12]

4 | AN ABRUPT STRUCTURE TRANSITION

A surprising observation is made of DAR16-IV peptide.[12] In water at 20 °C, DAR16-IV self- forms a stable β-sheet, and when stained with Congo red, it is visible like pieces of floating sheets. However, when incubation at increased temperatures to 90 °C, DAR16-IV undergoes a structural transition from a β-sheet directly to an α-helix structure. Thus, the 2° structure of DAR16-IV undergoes significantly changes at higher temperature (Figure 7). The β-sheet to α-helix transition is observed by measuring the CD spectra of the peptide solution at different temperatures in the thermostatted chamber of the Aviv CD instrument. To further study the transition, we heated several identical samples at 75 °C for 1, 2, 4, 8, 16, 32 minutes and measured their spectra at 20 °C (Figure 7B). While no apparent transition is observed on heating for 1, 2, 4, to 8 minutes, spectra changes are observed after 16 minutes of heating, and the β-sheet to α-helix transition is complete after heating for 32 minutes. This observation suggests that the process of thermally disrupting the β-sheet lattice to free peptides and forming the α-helix is rather slow.

It is interesting to note that further heating beyond 32 minutes does not promote further transitions and once the α-helical structure is formed, it is stable. When the DAR16-IV peptide in α-helical form is re-examined by reheating at different temperatures, the α-helical content gradually reduces as a function of incubation temperature but does not return to the β-sheet form nor completely denature into a random coil (Figure 7C). The mean residue ellipticity $[\Theta]_{222nm}$ shows that ~60% of peptide is in the α-helical form at 0 °C and ~30% remains at 90 °C.

Re-examination of DAR16-IV after several weeks reveals that only a small fraction returns to the β-sheet form. This delayed hysteresis suggests that the structural conversion is kinetically irreversible. Our experiments studying the α-helix to β-sheet reverse transition process in DAR16-IV revealed that the reverse transition process is very slow, and only after 6 months of incubation, there was a small amount of higher β-sheet content observed (Zhang, unpublished results). This leads to the possibility that the process is autocatalytic or cooperative, with the presence of β-sheet exhibiting the ability to recruit α-helices into an assembly. Similar findings have been reported that aggregated amyloid proteins can recruit other properly folded amyloid proteins by inducing changes in secondary structure.[13–17]

TABLE 1 Modulus IV and mixed modulus self-assembling peptides

Name	Sequence (N- > C)	Structure	Transition	Matrix formation
	+ + − − + +			
EAK12-a	AKAKAEAEAKAK	r.c.	No	No
	+ − − + +			
EAK12-b	AKASAEAEAKAK	r.c.	No	No
	+ − − − + +			
EAK12-c	AKAEAEAEAKAK	r.c	No	No
	− − − − + +			
EAK12-d	AEAEAEAEAKAK	α/β	Yes	Yes
	− − − − + + + +			
DAR16-IV	n-ADADADADARARARAR	α/β	Yes	Yes
	− − − − + + + +			
DAR16-IV*	n-DADADADARARARARA	α/β	Yes	Yes
	− − − − + + + +			
DAR32-IVc	(ADADADADARARARAR)2	α/β	No	Yes
	+ + + + − − − −			
RAD16-IV	RARARARADADADADA	β	No	Yes
	+ + + + − − − −			
KAE16-IV	KAKAKAKAEAEAEAEA	β	No	Yes
	− − − − + + + +			
EAK16-IV	AEAEAEAEAKAKAKAK	β	Yes	Yes

The + and − denote the positively and negatively charged residues, respectively.
α and β indicate alpha-helical and beta-sheet structures, respectively. r.c, random coil. The number following each name indicates the chain length of the peptides.
N- and C-termini of the oligopeptides are acetylated and amidated, respectively. Direct conversion occurs when there is no detectable intermediate by CD spectroscopy. Matrix formation indicates whether or not the peptide solution forms visible matrices on exposure to saline conditions. Copyright 2000 John Wiley & Sons, Inc.

These observations further stimulated our interest in *de novo* design of several more peptides EAK12 series a, b, c, d (Table 1)[18] that are able to undergo structural transitions after heating (Figure 8) and pH changes. These EAK12 series peptides, similar to DAR16-IV have two surfaces with regular repeats of alternating hydrophilic and hydrophobic side chains (Figure 9). These designs allow ionic and

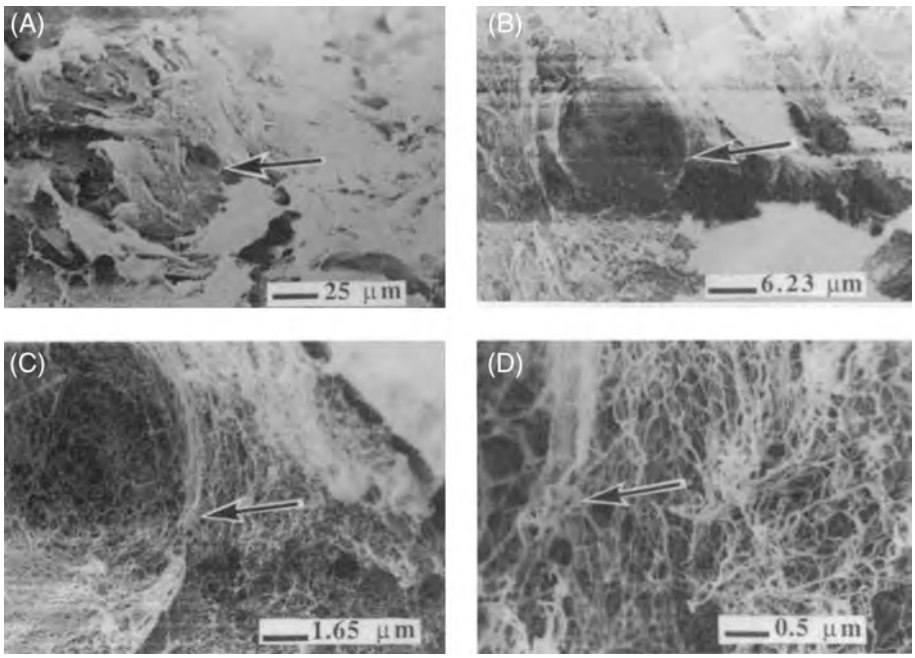


FIGURE 4 SEM of the self-assembling peptide scaffold material. The peptide material was serially dehydrated using ethanol, 20%, 40%, 60%, 80%, 100% for 10 minutes each and finally immersed in liquid carbon dioxide. The prepared sample was examined under a scanning electron microscope. The diameter of the nanofibers is ~10 nm, and the distances between nanofibers are ~50–200 nm. Arrows mark the same location. Magnifications are: A, 300×, B, 1200×, C, 4500×, and d, 15,000×. Copyright 1993 National Academy of Sciences.

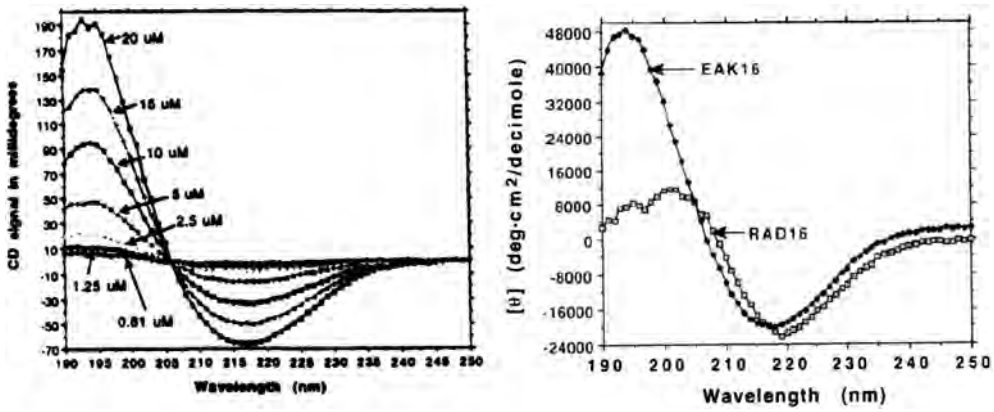


FIGURE 5 Unusual property of self-assembling EAK16 (AcN-AEAEAKAKAEAEAKAK-CONH2) and RAD16 (AcNRARADADADARARADADA-CONH2). A, CD spectra of the serial dilutions of EAK16 peptide as indicated (20, 10, 5, 2.5, 1.25, 0.625 μM) suggesting the β-sheet structure is very stable. B, EAK16 (10 μM) and RAD16 (10 μM) showed β-sheet but with different spectra. EAK16 has the typical 218 nm and 195 nm spectrum but RAD16 has atypical spectrum 219 nm and lower (Θ) at 203 nm thus suggesting it has different backbone twist.[11] Copyright 1994 John Wiley & Sons, Inc.

hydrophobic interactions resulting in the formation of stable β-sheets.

The other defining characteristic of this type of EAK12 series peptide is a cluster of negatively charged aspartic or glutamic acid residues located toward the N-terminus, and positively charged arginine or lysine residues located toward the C-terminus. This arrangement of charge balances the α-helical dipole moment C→N, resulting in a strong tendency to form stable α-helices (Table 1, Figure 10). The amino acid sequences of these peptides therefore allow both stable β-sheet and α-helix formation, with the balance between the two forms governed by environmental conditions. It is interesting to note that this β-sheet to α-helix transition of EAK12 series peptides does not occur readily when the polarity of the polypeptide is reversed, as in the case of RAD16-IV. This suggests that the orientation of the α-helix dipole moment perhaps contributes to stabilization of the α-helix in one case but not in the other.

5 | pH CHANGES INFLUENCE PEPTIDE STRUCTURE

It is well known that pH changes have significant effects on protein and peptide structures. The best-known example is polylysine. Polylysine can undergo a structural change from α-helix to β-sheet near pH 10 and higher when 95% of the lysine residues are de-protonated. This conformational change is reversible when the pH is lowered allowing for re-protonation. In the same manner, the β-amyloid peptide βA1-42 undergoes conformational changes at different pH. At pH 1.3 and pH 8.3, the peptide has an α-helical structure, Conversely, at pH 5.4 the peptide βA1-42 has a β-sheet structure.

We also studied the secondary structural conformational changes of the peptides listed in Table 1 as a function of pH.[12,18] When the β-sheet is destabilized by lowering the pH, destabilization only begins to become evident in the CD spectra when the peptide solution is below pH 4, and is essentially complete at pH 2. It is perhaps that the aspartate residues are gradually protonated thus destablilize ionic self-complementary β-sheet interactions.

Our first study of EAK16-II and several other ionic self-complementary peptides at various pH reveal that their β-sheet conformations are stable over a wide range.[5] This is not seen for DAR16-IV which adopts an α-helical structure when incubated at pH 1 and pH 2. Incubation of DAR16-IV at pH 3 and pH 4 promotes a β-sheet form without a clear isodichroic point, suggesting that there may be some structural intermediates near pH 3. However, when the peptide is incubated in solutions from pH 5 to 11, the CD spectra exhibit a β-sheet form with an isodichroic point at 212 nm rather than the typical 218 nm, and a gradual decrease of ellipticity as a function of increasing pH.

EAK12-d can also undergo conformational changes as a function of pH. At pH 1–3, EAK12-d exhibits a typical β-sheet spectrum (similar to the case of polylysine) with a minimum ellipticity at 218 nm and a maximum at 195 nm (Figure 11).[18] However, when the pH increases from pH 1 to pH 3, there is a noticeable change in the peptide CD spectrum. Although the peptide still forms β-sheets, the backbone twist perhaps changed as suggested by a decreased ellipticity at 195 nm. When EAK12-d is incubated at pH 4, a different spectrum is observed, consisting of a β-type spectrum with greatly reduced β-sheet content and further altered backbone conformation. This spectrum suggests that a β-strand structural intermediate may exist, or both β-sheet and α-helical structures may coexist. When EAK12-d is incubated at pH 5, the molecules are deprotonated and the peptide undergoes a complete structural transition into a conformation with ~30% α-helical content. Additional changes from pH 6–10 do not result in a significant alteration of the peptide conformation suggesting the presence of a stable structure (Figure 11) within this range. Figure 8 summarizes the structural transitions of EAK12-d and its ability to exist in three distinct states due to the influence of pH.

Therefore, although EAK12-d and DAR16-IV both undergo pH-induced conformational changes, their structural transitions follow two different paths: they are almost exactly reversed in their structures at a given pH. Again, this illustrates that the nature of the charge on the different amino acids plays more of a role than sequence alone in determining peptide structure because of the correlation between pH and charge. Further studies are required to gain a better understanding of the conformations that ionic peptides can adopt as a function of charged amino acid combinations.

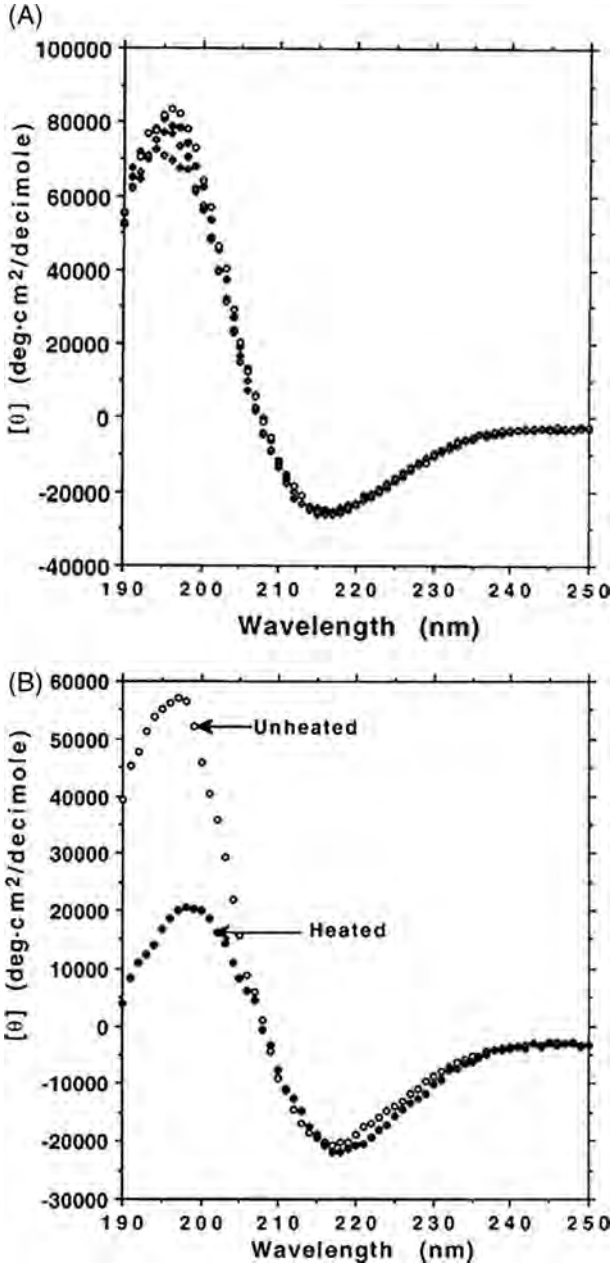


FIGURE 6 Thermal stability of ionic self-complementary peptides. A, CD spectra of EAK16-IV (AEAEAEAEAKAKAK) and KAE16-IV (KAKAKAKAEAEAEAEA) at 20 °C and 90 °C after heating for 10 minutes. These spectra are superimposable except slight variations in the 195 nm region. B, CD spectra of RAD16-IV (RARARARADADADADA) at 20 °C before heating and at 20 °C after heating at 90 °C for 10 minutes. It has a β-sheet spectrum at 20 °C (open circles); however, after heating at 90 °C (filled circles), the CD spectrum of RAD16-IV has a reduced ellipiticity near 195 nm, probably due to a change in the twist on the β-sheet backbone. Copyright 1997 National Academy of Sciences.

6 | PROPOSED STRUCTURE OF β-SHEET ASSEMBLY

Almost all ionic self-complementary β-sheet peptides self-assemble to form macroscopic materials in the presence of monovalent salts.[4–10] The structural feature of this class of ionic β-sheet peptides is characterized by alanine on one side of the β-sheet and + charged arginine or lysine and - charged asparate or glutamate on the other side adapting an alternating repeating pattern.

Monomeric peptides with extended backbone β-strands are inherently unstable and therefore must be stabilized on both faces to form an assembly. In addition to conventional backbone hydrogen bonding between the peptides, one face of the peptides is stabilized by intermolecular ionic interactions while the other is stabilized by intermolecular hydrophobic interactions. A proposed assembly structure of DAR16-IV provides a possible explanation of the observed physical properties of the peptides. These include strong resistance to proteases and denaturing agents because of unexposed side chains, β-sheet thermostability because of extensive ionic and hydrophobic bonding, and the formation of hydrogel materials on exposure to salts when these assemblies emerged out of solution.

7 | PROPOSED MECHANISM FOR A β-SHEET TO α-HELIX TRANSITION

We showed that some changes in the twist of the β-sheet backbone may occur a few minutes after increasing the temperature thus suggesting β-sheet structure is destabilized before there is any evidence of α-helical transition. These observations lead to the proposal of two possible models for the structural changes: 1) a possibility of a well-defined monomeric intermediate, 2) a possible complex intermediate.

For the first model, when the β-sheets in solution are heated, a monomeric β-strand breaks away from the assembly. Once free from intermolecular forces, intramolecular forces could cause this β-strand to coil into an α-helix. The change in β-sheet backbone twist, detected by CD spetra might be associated with the β-sheet to β-strand conversion. This situation may common in proteins. For example, interactions between different parts of the protein, or with other molecules during catalysis or transport, may cause one of the β-strands in a β-sheet to break away from the pack.

In the second model, part of the peptide begins to transition to α-helix while the other part of it remains bound to the β-sheet assembly. This perhaps results in an intermediate that may be partially α-helix and a section that is partially extended remaining in the assembly. Over time, the entire molecule becomes helical and separates from the assembly. In this case, the observed change in backbone twist may be accounted for by a peptide of mixed secondary structure. Both are reversible, and the reverse pathways can result in the reassembly of β-sheet structure over time.

8 | STRUCTURAL PLASTICITY

α-helices have a helical dipole moment along the backbone that has a directionality C→N. It has been suggested that the α-helix may be an array of dipoles arranged in such a way that these orient along the axis of the α-helix with the negative pole at the C-terminus and the positive pole at the N-terminus.[19] Therefore, it is presumed that amino acids with - charges at the N-terminus and amino acids +

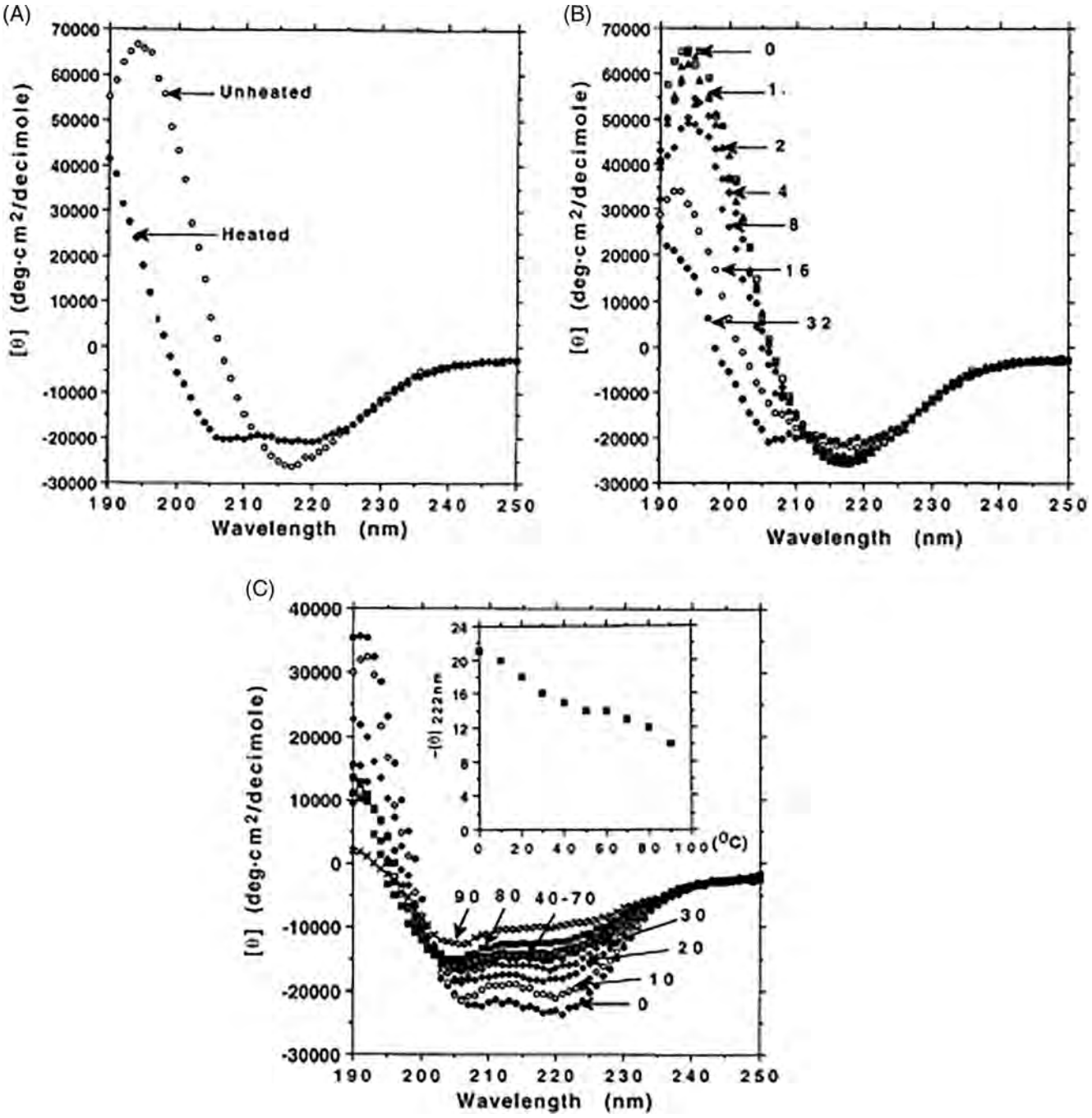


FIGURE 7 Temperature-induced secondary structural transition in DAR16-IV. A, CD spectra were measured at 20 °C with an identical sample heated at 90 °C for 10 min. The heated sample has an apparent α-helical CD spectrum as indicated. B, Seven identical samples of DAR16-IV were heated at 75 °C for different time periods in minutes, as indicated, and returned to 20 °C. Small differences are seen in the spectra between unheated and those heated for 1, 2, and 4 minutes. The transition started after 8 minutes of heating and was completed after 32 minutes of heating. No further changes were observed for longer periods of heating. C, Stability of the α-helical structure. The helical form of peptide DAR16-IV was heated and the spectra were measured at 10 °C intervals up to 90 °C. The peptide solution remained at each temperature for 10 minutes, and the temperature was held constant for each spectrum. (Inset) Decrease of the mean residue ellipiticity at [Θ]222 nm (103 deg/cm^2/decimole) as a function of temperature. At 0 °C, ~60% of the peptide was in the helical form, while at 90 °C, it was reduced to 30%. Thus the helical form was not completely denatured at the highest temperature. Copyright 2000 John Wiley & Sons, Inc.

charges at the C-terminus may enhance and stabilize the helical dipole moment, and in turn the α-helix itself. This phenomenon is significant and many α-helices in proteins conform with this prediction.[19–24]

Systematic protein engineering has accelerated our understanding of protein structures and provided us with a great deal of detailed information regarding their critical biological functions. The rapid accumulation of protein sequences and structures deposited in the databases will likely provide us with vital information to improve and refine our knowledge of protein structure, stability, plasticity, and complex conformational behavior.[25–27]

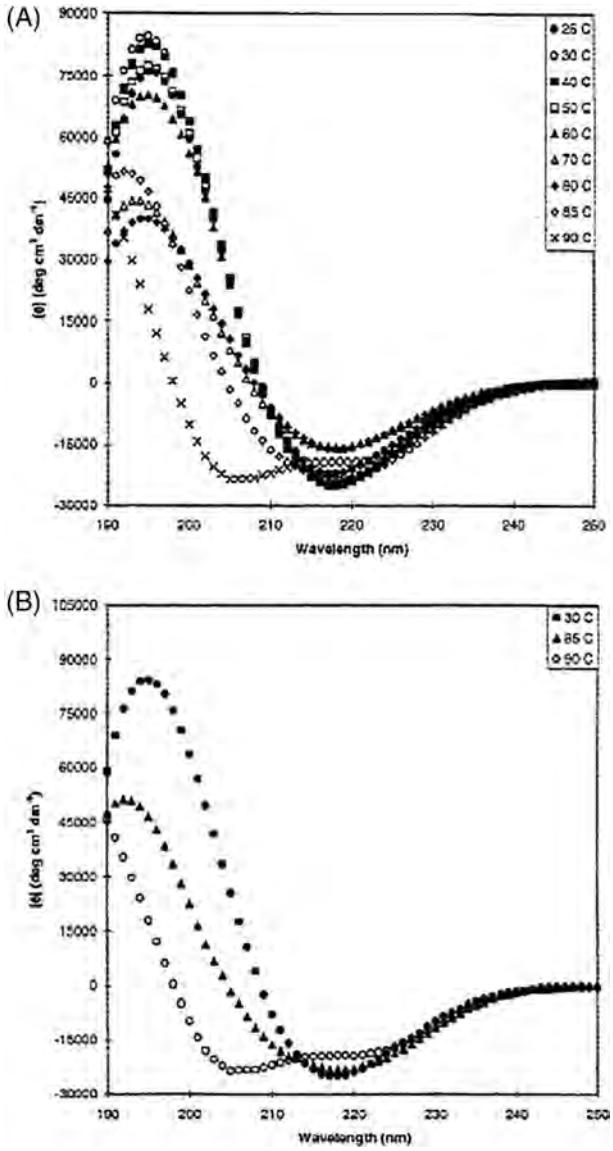


FIGURE 8 Temperature effect on self-assembling peptide EAK12-d (AEAEAEAEAKAK) structural transitions. EAK12-d peptide solution was divided into nine identical samples. The samples were incubated at indicated temperatures for 10 minutes and the CD spectrum was measured at the same temperature. A, CD spectrum of EAK12-d from 25 °C to 90 °C. At 25 °C, it forms a stable β-sheet. This β-sheet structure is stable until 70 °C. It then undergoes two distinctive transitions. During the first transition, the β-sheet content is not only reduced, but also changes its degree, which may be related to changes in right-handed twist.[11] The second structural transition is drastic and occurs between 85 °C and 90 °C. Here, the β-sheet structure is converted to an α-helical structure. B, EAK12-d exhibits three distinctive structures at 3 different temperatures: 30 °C, 85 °C, and 90 °C. The isodichroic point is at 213 nm. Copyright 2000 John Wiley & Sons, Inc.

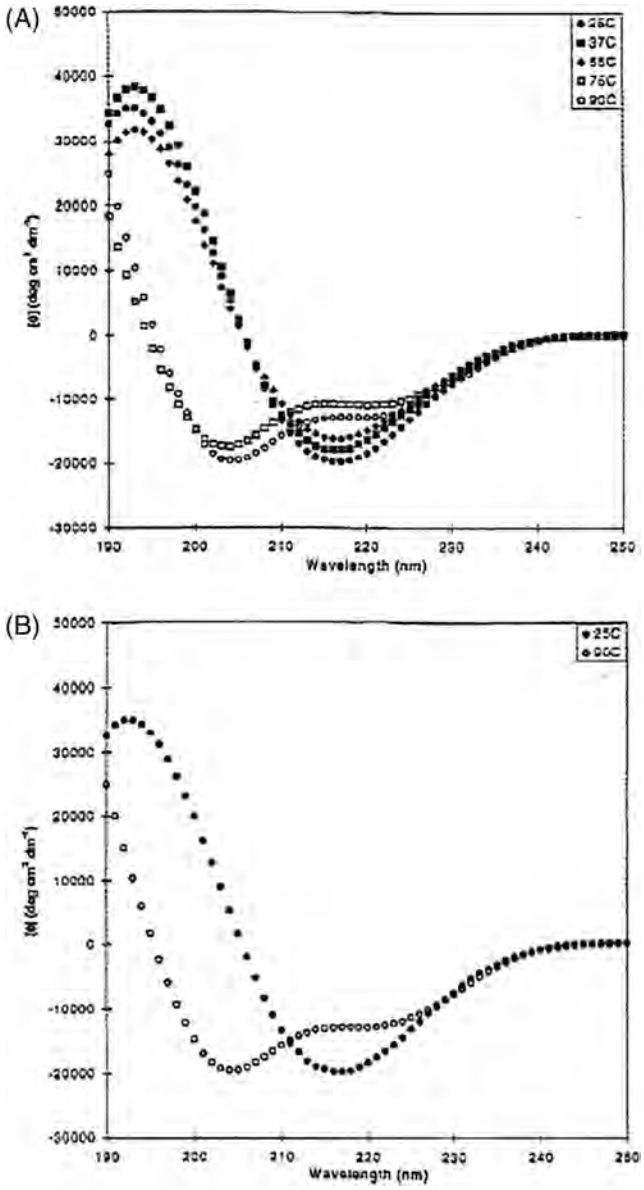


FIGURE 9 Temperature effect on DAR16-IV* structural transition. DAR16-IV* (see Table 1) was incubated at various temperatures for 10 minutes and measured at 25 °C. A, Structures of DAR16-IV* from 25 to 90 °C. At 25 °C, it forms a stable β-sheet. This β-sheet structure is stable until 75 °C. Here, the β-sheet structure is abruptly converted to an α-helical structure without detectable intermediates. The conversion in DAR16-IV* is much more abrupt than in EAK12-c, as only two distinct spectra are observed. B, DAR16-IV exhibits two distinctive spectra at two different temperatures, 25 °C and 90 °C. Copyright 2000 John Wiley & Sons, Inc.

9 | IMPLICATIONS IN AMYLOID FORMATION

The process of converting from an α-helix to β-sheet structure are extensively investigated because of its implications in the molecular basis of a number of disorders, including amyloid formation in neurological tissues for diseases such as scrapie, bovine spongiform encephalitis, or Alzheimer disease.[28–32] It is suggested that 2° structural conformation changes occur in which a cleaved segment of a protein that is normally α-helical is converted to a stable β-sheet. Many believe that these protein conformational changes are central to the disease process.[28–32] Thus, progression of the disease may be related to changes in protein 2° structure leading to the formation of insoluble β-sheet plaques. In infectious diseases, for example scrapie, a segment of the protein in the β-sheet form is believed to further catalyze the conversion of segments from α-helix to β-sheet.[28–32]

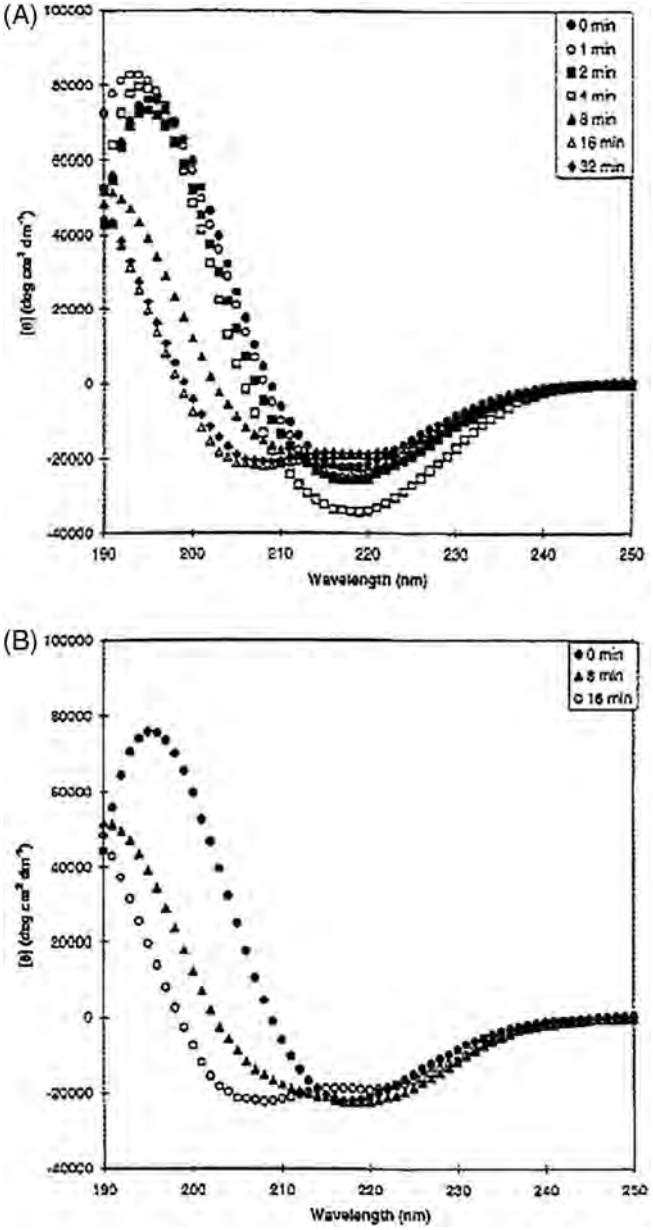


FIGURE 10 Incubation at various time points induces EAK12-d structural transition. A, EAK12-d was incubated at 85 °C for 0, 1, 2, 4, 8, 16, and 32 minutes. It also underwent two-phase transitions. Incubation from 0, 1, 2, and 4 minutes did not result in drastic structural changes. The first transition occurs at 8 minutes when the stable β-sheet becomes less stable and shows signs of structural change. After further incubation at 85 °C, it abruptly converts from a β-sheet to an α-helix by 16 minutes. Further incubation for 32 min did not change the structure. B, EAK12-d exhibits three distinctive structures during incubation for different time periods. Copyright 2000 John Wiley & Sons, Inc.

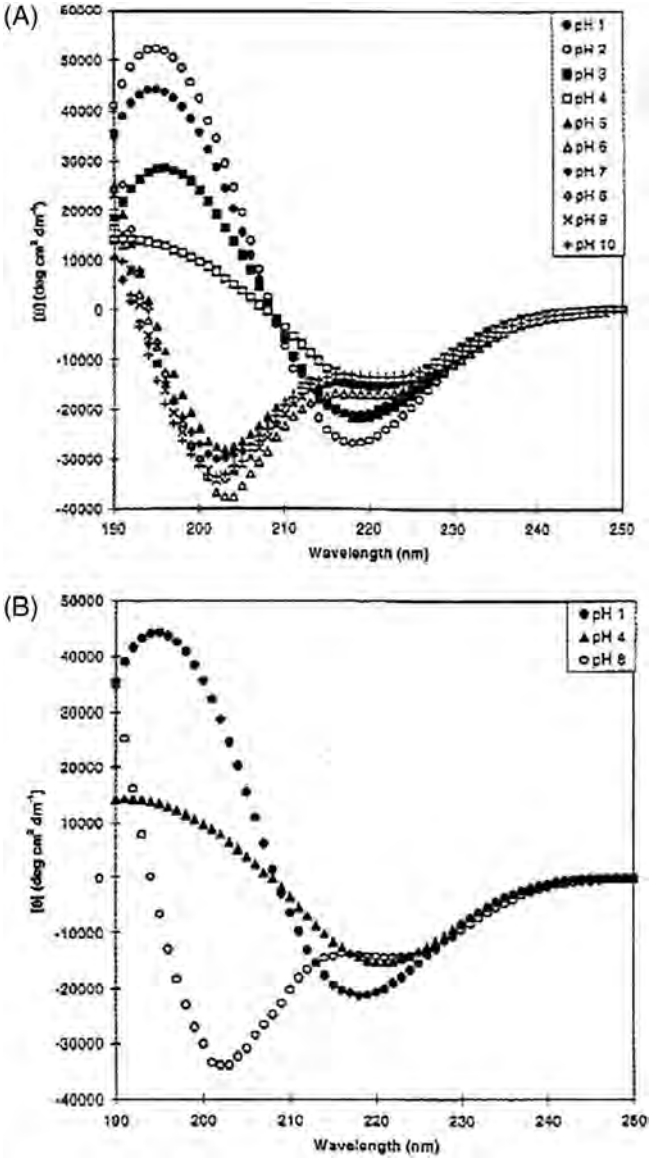


FIGURE 11 pH effect on the EAK12-d structural transition. EAK12-d was incubated in solutions of various pH: from pH 1 to pH 10. A, The structural properties suggested by the spectra of EAK12-d in different pH are variable. The β-sheet content is different at pH 1 and 2-3. EAK12-d at pH 2 has a high β-sheet content. The β-sheet content is essentially identical at pH 1 and pH 3. There exists a difference in the backbone twist among pH 1, 2, and 3 as shown in the 195 nm region. EAK12-d at pH 4 exhibits a very different spectrum. There seems to be an intermediate structure, with both helical and sheet character. When EAK12-d is incubated from pH 5 to pH 10, it exhibits an a-helical spectrum with about 30% helical content. There is an isodichroic point at 214 nm except at pH 4. B, The spectra of EAK12-d suggest 3 different structures at pH 1, pH 4 and pH 8. Copyright 2000 John Wiley & Sons, Inc.

From our systematic studying DAR16-IV, EAK12-d, and their derivatives, it is possible to provide some insight into causes that influence these conformational changes and might have relevance in the pathogenesis of these diseases. For instance, it is interesting that these diseases seem to be characterized by a slow conversion of soluble protein into an insoluble β-sheet form, similar as DAR16-IV that exhibits comparable slow kinetics of conversion. In contrast, the β to α conversion is fast during heating, acting like a molecular switch with a dramatic conformational change. This type of conformational switch may also occur in proteins, and indeed the β to α conversion might represent an element in protein folding or occur in some proteins during the mechanism of their action.

An example of a large conformational change is observed in the hemagglutinin molecule whereby membrane fusion is accomplished via a pH drop in endosomes accompanied by conversion of an unstructured peptide segment into an α-helix.[33] The β to α

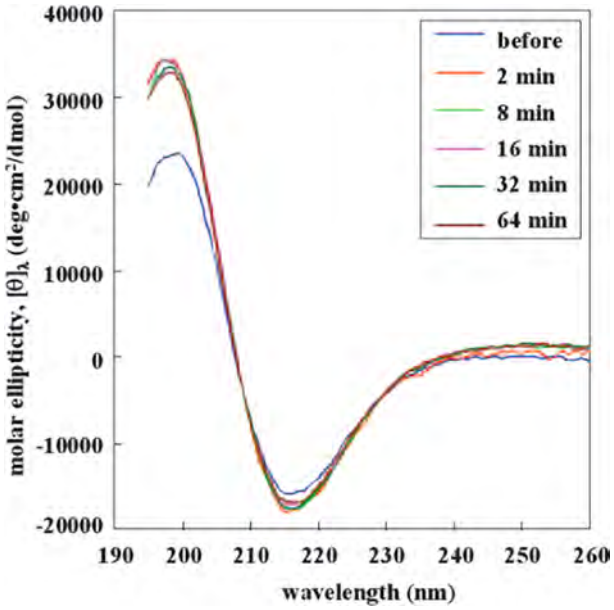


FIGURE 12 CD examination of the peptide structures at various times before and after sonication. Sample time points at 2, 8, 16, 32, and 64 minutes were taken after sonication. The typical β-sheet spectra were observed at all time point experiments, indicating the molecular structure and the integrity of the peptides were unchanged before and after sonication. Furthermore, the β-sheet contents (216 nm) remain nearly unchanged in all time points and slightly higher than the sample before sonication, suggesting tight β-sheet packing. However, the degree of β-sheet twist (195 nm region) before sonication is different, suggesting different β-sheet packing. Copyright 2005 National Academy of Sciences.

conversion that we are describing results in a considerable geometrical change, namely DAR16-IV has an extended β-sheet length of 5 nm compared with an α-helical length of 2.5 nm. Thus, changes of this type might occur in protein mechanisms with significant shape changes.

10 | OTHER ASPECTS OF STUDYING MEMBRANE PROTEINS USING CD

We also used CD to follow the re-assembly process of self-assembling peptide RADA16-I nanofiber scaffold hydrogel following exposure to mechanical disruption via sonication (Figure 12).[7] CD has been used to examine the peptide structures at various times before and after sonication. Sample time points at 2, 8, 16, 32, and 64 minutes were monitored after sonication. Typical β-sheet spectra are observed at all time points, thereby indicating that the molecular structure and integrity of the peptides are unchanged before and after sonication. Furthermore, the β-sheet content (216 nm) remains nearly unchanged at all time points. Interestingly, the CD spectra are higher than the sample before sonication, suggesting tight β-sheet packing after sonication. However, the degree of β-sheet twist (195 nm region) before sonication is different, suggesting an alternate β-sheet packing. It is possible that the sonication process removes loose β-sheet packing packing on the out edge of the sheets.

In other work, we used CD for studying integral membrane proteins, particularly G protein-coupled receptors (GPCRs), including olfactory receptors, formyl peptide receptors, and trace amine

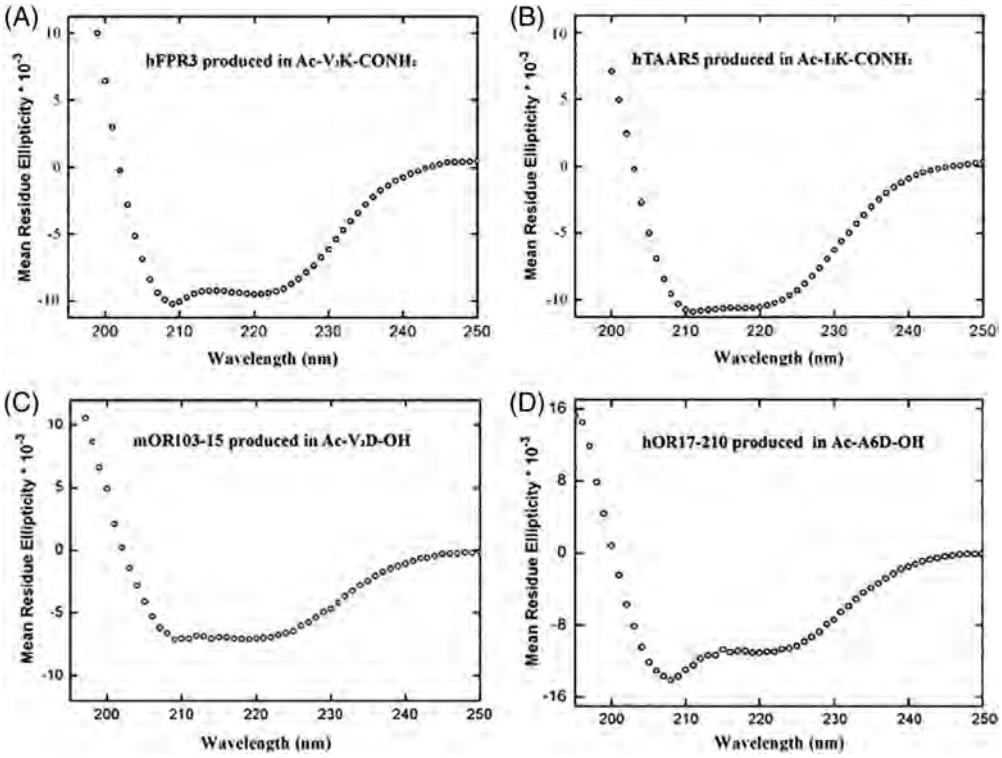


FIGURE 13 CD spectra of four GPCRs produced in the presence of peptide surfactants using a commercial *E. coli* cell-free system. A, hFPR3 produced in Ac-VVVK-CONH2. B, hTAAR5 produced in Ac-IIIK-CONH2. C, mOR103-15 produced in Ac-VVVD-OH. D, hOR17-210 produced in Ac-AAAAAAD-OH. These receptors all have characteristic α-helical spectra with valleys at 208 nm and 222 nm. GPCRs have 7-transmembrane α-helical domains, therefore, the CD spectra indicate that the receptors are properly folded. Copyright 2011 National Academy of Sciences.

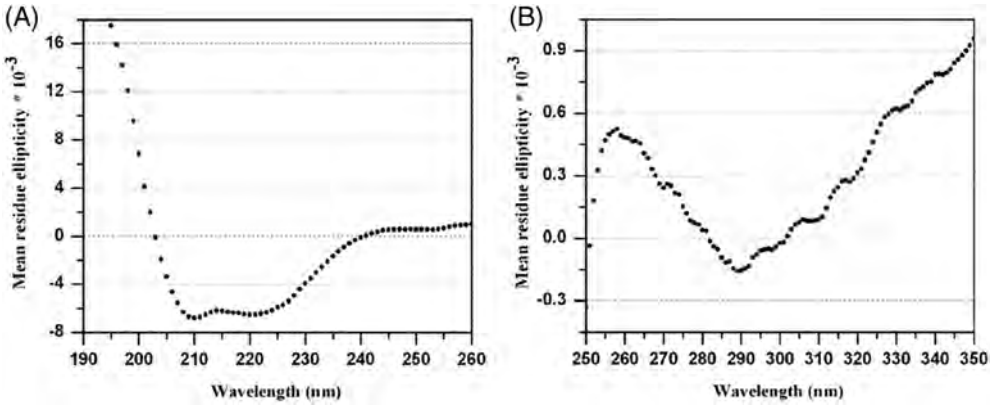


FIGURE 14 Secondary structural study of the purified human GPCR FPR3 receptor using both far-UV and near-UV CD spectroscopy. Mean residue ellipticity [Θ] has units of $103^{\circ}/cm^2/dmol$. A, Far-UV CD spectrum of FPR3 displaying secondary structure of ~57% α-helix. B, Near-UV CD spectrum of FPR3 showing distinct tertiary structure profiles. Spectra are the average of 3 replicate scans.

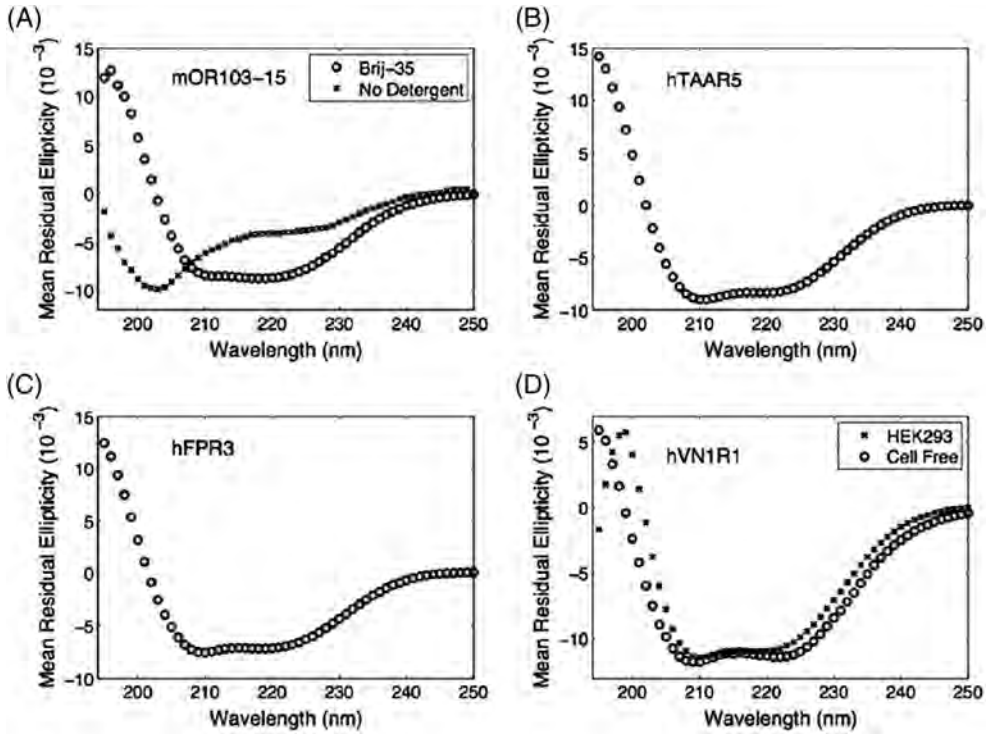


FIGURE 15 CD spectra of purified GPCRs. A, Cell-free expressed mOR103-15 made with Brij-35 or without detergent, B, cell-free expressed hTAAR5, C, cell-free expressed hFPR3, and D, cell-free and HEK293 expressed hVN1R1. All purified GPCRs have characteristic α-helical spectra, except mOR103-15 made without detergent. Since GPCRs have 7-transmembrane helices, and an overall α-helix content of ~50%, the CD spectra. The near overlap of the spectra for cell-free and HEK293 expressed hVN1R1 suggests that both receptors retained their α-helical structure, and further indicates that cell-free produced GPCRs are comparable to those expressed in mammalian cells.

associated receptors (Figures 13–15).[34–43] These proteins are predominately composed of seven transmembrane α-helical segments. They are always water-insoluble following removal from the lipid membrane environment thus requiring the use of detergents for their stabilization.

Since 2011, I ventured into membrane protein engineering by inventing a QTY Code using hydrophilic polar residues without any charges. Gln (Q), Thr (T) and Tyr (Y) are used to specifically replace hydrophobic residues Leu (L), Ile (I), Val (V) and Phe (F), thus rendering the 7 transmembrane α-helical segments detergent-free and water-soluble following removal from the cell membrane. These GPCR chemokine receptors not only retain their secondary α-helical structure and overall-fold, but also retain their ligand-binding activities.[43] CD played a key role in monitoring the systematic changes and ensuring that the engineered synthetic receptor proteins maintain their structures.

11 | CONCLUDING REMARKS

Proteins and peptides are dynamic entities that frequently undergo conformational changes to perform biological functions. Although

studies using X-ray crystallography can provide high resolution structures, it is more difficult to examine their dynamic properties because the molecules are already packed in tight lattices. Both NMR and CD are useful tools to undertake dynamic analyses. However, NMR instrumentation is substantially more expensive and difficult to access for routine studies. Circular dichroism spectroscopy, particularly that performed using the high precision Aviv CD spectropolarimeter, provides an excellent alternative. It is no surprise that most leading protein and peptide scientists around the world use the instrument to conduct their studies.

ORCID

Shuguang Zhang http://orcid.org/0000-0002-3856-3752

REFERENCES

[1] S. Zhang, C. Lockshin, A. Herbert, E. Winter, A. Rich, *EMBO J.* **1992**, *11*, 3787.
[2] S. Marqusee, R. L. Baldwin, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 8898.
[3] S. Marqusee, V. H. Robins, R. L. Baldwin, *Proc. Natl. Acad. Sci. USA* **1987**, *86*, 5386.
[4] S. Zhang, T. Holmes, C. Lockshin, A. Rich, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3334.
[5] S. Zhang, C. Lockshin, R. Cook, A. Rich, *Biopolymers* **1994**, *34*, 663.
[6] S. Zhang, T. Holmes, M. DiPersio, R. O. Hynes, X. Su, A. Rich, *Biomaterials* **1995**, *16*, 1385.
[7] H. Yokoi, T. Kinoshita, S. Zhang, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 8414.
[8] S. Zhang, *Biotechnol. Adv.* **2002**, *20*, 321.
[9] S. Zhang, *Nat. Biotechnol.* **2003**, *21*, 1171.
[10] S. Zhang, *Interface Focus* **2017**, *7*, 20170028.
[11] G. Fasman, personal communication (**1994**).
[12] S. Zhang, A. Rich, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 23.
[13] J. H. Come, P. E. Fraser, P. T. Lansbury Jr.. , *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 5959.
[14] N. M. Kad, N. H. Thomson, D. P. Smith, D. A. Smith, S. E. Radford, *J. Mol. Biol.* **2001**, *313*, 559.
[15] I. L. Derkatch, S. M. Uptain, T. F. Outeiro, R. Krishnan, S. L. Lindquist, S. W. Liebman, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 12934.
[16] M. Arimon, V. Grimminger, F. Sanz, H. A. Lashuel, *J. Mol. Biol.* **2008**, *384*, 1157.
[17] O. Szczepankiewicz, B. Linse, G. Meisl, E. Thulin, B. Frohm, C. Sala Frigerio, M. T. Colvin, A. C. Jacavone, R. G. Griffin, T. Knowles, D. M. Walsh, S. Linse, *J. Am. Chem. Soc.* **2015**, *137*, 14673.
[18] M. Altman, P. Lee, A. Rich, S. Zhang, *Protein Sci* **2000**, *9*, 1095.
[19] W. G. Hol, *Prog. Biophys. Mol. Biol.* **1985**, *145*, 149.
[20] P. Y. Chou, G. D. Fasman, *Biochemistry* **1974**, *13*, 211.
[21] D. E. Blagdon, M. Goodman, *Biopolymers* **1975**, *14*, 241.
[22] L. G. Presta, G. D. Rose, *Science* **1988**, *240*, 1632.
[23] R. Parthasarathy, S. Chaturvedi, K. Go, *Prog. Biophys. Mol. Biol.* **1995**, *64*, 1.
[24] R. Aurora, G. Rose, *Protein Sci.* **1998**, *7*, 21.
[25] B. C. Cunningham, D. J. Henner, J. A. Wells, *Science* **1990**, *247*, 1461.
[26] T. L. Blundell, *Trends Biotechnol.* **1994**, *12*, 145.
[27] E. I. Shakhnovich, *Fold Des.* **1998**, *3*, 108.
[28] C. J. Barrow, M. G. Zagorski, *Science* **1991**, *253*, 179.
[29] D. A. Kirschner, H. Inouye, L. K. Duffy, A. Sinclair, M. Lind, D. J. Selkoe, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 6953.
[30] C. Hilbich, B. Kisters-Woike, J. Reed, C. L. Masters, K. Beyreuther, *J. Mol. Biol.* **1991**, *50*, 149.
[31] J. Safar, P. P. Roller, D. C. Gajdusek, C. J. Gibbs Jr. , *Protein Sci.* **1993**, *2*, 2206.
[32] D. C. Gajdusek, *Mol. Neurobiol.* **1994**, *8*, 1.
[33] C. M. Carr, P. S. Kim, *Cell* **1993**, *73*, 823.
[34] L. Kaiser, J. Graveland-Bikker, D. Steuerwald, M. Vanberghem, K. Herlihy, S. Zhang, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 15726.
[35] B. C. Cook, D. Steuerwald, L. Kaiser, J. Graveland-Bikker, M. Vanberghem, K. Herlihy, H. Pick, H. Vogel, S. Zhang, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 11925.
[36] X. Wang, S. Zhang, *PLoS One* **2011**, *6*, e23076.
[37] X. Wang, K. Corin, C. J. Wienken, M. Jerabek-Willemsen, S. Duhr, D. Braun, S. Zhang, *Proc. Natl. Acad. Sci. USA* **2011**, *118*, 9049.
[38] X. Wang, K. Corin, C. Rich, S. Zhang, *Sci. Rep.* **2011**, *1*, e102.
[39] K. Corin, P. Baaske, D. B. Ravel, J. Song, E. Brown, X. Wang, C. J. Wienken, M. Jerabek-Willemsen, S. Duhr, D. Braun, S. Zhang, *PLoS One* **2011**, *6*, e23036.
[40] K. Corin, P. Baaske, D. B. Ravel, J. Song, E. Brown, X. Wang, C. J. Wienken, M. Jerabek-Willemsen, S. Duhr, D. Braun, S. Zhang, *PLoS One* **2011**, *6*, e25067.
[41] K. Corin, P. Baaske, S. Geissler, C. J. Wienken, S. Duhr, D. Bruan, S. Zhang, *Sci. Rep.* **2011**, *1*, e172.
[42] K. Corin, H. Pick, P. Baaske, B. Cook, S. Duhr, C. J. Wienken, D. Bruan, H. Vogel, S. Zhang, *Mol. Biosyst.* **2012**, *8*, 1750.
[43] S. Zhang, F. Tao, R. Qing, H. Tang, M. Skuhersky, K. Corin, L. Tegler, A. Wassie, B. Wassie, Y. Kwon, B. Suter, T. Schubert, J. Kubicek, B. Maertens, *Proc. Natl. Acad. Sci. USA* **2018**. https://doi.org/10.1073/pnas.1811031115.

AUTHOR BIOGRAPHY

SHUGUANG ZHANG earned his B.S. from Sichuan University (China) in 1980, and Ph.D. from the University of California, Santa Barbara (U.S.A.) in 1988. He went on to be an American Cancer Society Postdoctoral Fellow and a Whitaker Foundation Investigator. He is currently a foreign corresponding member of the Austrian Academy of Sciences, and a Fellow of the National Academy of Inventors and of the American Institute for Medical and Biological Engineering. In 2006, he won both a Guggenheim Fellowship and the Wilhelm Exner Medal.

How to cite this article: Zhang S. Discovery of the first self-assembling peptide, study of peptide dynamic behaviors, and G protein-coupled receptors using an Aviv circular dichroism spectropolarimeter. *Biopolymers*. 2018;109:e23235. https://doi.org/10.1002/bip.23235