Chapter 3

Sequence recombination and aggregation:
Formation of amyloids through bleeding of conformational equilibria
Introduction

Naturally occurring polypeptides fold into compact globular structures by burying away most of their hydrophobic residues in their nonpolar core; such hydrophobic associations drive the collapse of the chain into a compact state in what is often referred to as a 'hydrophobic collapse', considered to be one of the major early events in protein folding. However, if circumstances are such that folding occurs only to a limited degree, or in a fraction of a protein population, exposed hydrophobic residues that have failed to become appropriately buried away can cause partially-structured molecules to seek hydrophobic association through inter-molecular rather than intra-molecular contacts [1]. If structured regions of partially-folded aggregation-prone chains were stable enough to survive such association, aggregates could potentially contain native-like elements of structure that would act as elements of 'molecular recognition' causing association to be homomolecular through inter-molecular association of chemically-compatible, shape-complementary surfaces that had evolutionarily developed to associate in intra-molecular fashion during folding. Alternatively, association could also conceivably cause sufficient destabilization of partially-formed structures to allow molecules to reorganize entirely into new 'non-native' structures, e.g., through the non-sequence-dependent formation of intermolecular beta sheets, allowing aggregation to be potentially heteromolecular in mixed protein systems.

Very little is actually known about the structural features of aggregates. In theory, going by the discussion above, aggregates can range from being highly ordered assemblies containing peptides in native-like structural format to quite disordered entities with little or no regular structure present. Likewise the noncovalent intermolecular interactions stabilizing aggregates too can range from being principally of the non-specific hydrophobic or hydrogen bonding varieties, to highly specific interactions amounting to molecular recognition. As intermediate possibilities, aggregates can also contain a mixture of ordered and disordered forms, with order resulting again from either non-native or native-like elements of structure. Our group has been engaged, for some years, in addressing a number of fundamental structural-biochemical issues concerning aggregation. For this thesis, in particular, however, our
interest in aggregation stems mainly from the fact that here we have extensively altered the sequence-structure relationships of naturally-occurring proteins in ways that could cause them to lose the ability to fold rapidly into well-formed three-dimensional structures, if not outright lose the ability to fold. Thus sequence-recombined proteins may be expected to show some tendency for aggregation. The limited aggregation observed with substructure-reshuffled proteins might owe to the less profound nature of the scrambling of folding information effected; such aggregation has been discussed in detail in the chapter dealing with scrambled TIM barrels. The scrambling of folding information effected through backbone-reversal, however, is much more profound, and with a far greater potential of compromising folding ability. In this chapter, therefore, we examine in particular the aggregation-related behavior of the retroproteins.

Our interest was to visually examine whether such aggregation occurs and to see whether aggregates resemble amorphous or amyloiditic aggregates (in other words, ordered or disordered). Furthermore, we wished to examine whether molecular recognition plays any role in such aggregation. More specifically, we were conscious of the possibility that structures formed by backbone-reversed proteins could lack sufficient stability to ensure that every molecule in a population would be structured, even if structure-forming tendencies were otherwise evident. It was demonstrated in chapter 1 that retroproteins can show a tendency to form secondary, tertiary and quaternary structures. However, it was equally evident that only in the case of retro-GroES had folding occurred to a sufficient extent to make the retroprotein highly soluble. Both with retro-HSP12.6 and retro-CspA, protein precipitation was observed above 1.2-1.5 mg/ml, with retro-CspA furthermore being poorly folded. Since in such situations retroprotein populations (especially of retro-CspA) could be expected to exist in an equilibrium of folded, partially-folded and unfolded states, with intermolecular collisions occurring at rates determined by protein concentration, chances would arise for molecules to associate in inter-molecular fashion. It may be noted that the backbone-reversal transformation conserves the beta sheet-forming tendencies of any amino acid sequence; thus, unfolded or partially-folded forms of a polypeptide with beta sheet forming tendency could very well associate through non-sequence-dependent hydrogen bonding interactions to form intermolecular beta sheets. Such structures would have greater kinetic stability than poorly-stable folded
forms. Since the population in solution would constantly maintain an equilibrium between folded and partially-folded, or unfolded forms, molecules depositing on the surface of an aggregate could very well ‘bleed away’ the conformational equilibrium into the aggregated state, at high protein concentrations. Since our studies of retroproteins showed a concentration-dependent tendency for aggregation and precipitation of retro-HSP12.6 and retro-CspA, we decided to separately and combinedly investigate the aggregation of these two proteins and their ability to affect each other’s aggregation using electron microscopy and confocal fluorescence microscopic studies.

**Amyloids and amyloidoses**

Since this chapter focuses on the examination of whether retroproteins can form intermolecular beta sheet structures such as amyloids upon concentration, an introduction to amyloids and amyloidoses would be fitting. Amyloid fibrils have a characteristic cross β structure in which polypeptide chains form β-strands aligned with the long axis of the fibril resulting in β sheets propagating in the direction of the fibril with hydrogen bonds oriented perpendicular to the fibril [2]. Usually fibrils do not develop from native conformations of proteins but from precursors that are partially folded. Electron microscopy suggests that all fibrils are formed from unbranched protofilaments that tend to be 2-5 nm wide [3], suggesting that all amyloids contain some common structural feature of chain organization. Protofilaments associate to form filaments that further associate laterally or twist together to form fibrils of much larger diameter extending up to 120nm. Protofilaments show an axial periodicity of ~20nm and are 3-4nm in diameter[4]. These small protofilaments then elongate by unknown mechanisms to give rise to larger filaments and fibrils that can be quite long. Fibrils have a helical twist and a diameter that is approximately 2-3 times that of a protofibril. Later on branched filaments can also be found forming [5]. Amyloids once formed are kinetically stable and indestructible in physiological conditions, explaining why amyloid plaques made up of Alzheimer’s peptide, or prion protein, reside in the body as long-lived deposits.
An amyloid plaque is usually defined by three features:

1). Binding of amyloid specific dyes such as congo Red and Thioflavin T,
2). A fibrous morphology observed by electron microscope, and
3). A distinctive X-ray fiber diffraction pattern that is characteristic of amyloids.

Recently direct visualization of the aggregate in three dimensions has also been made possible by atomic force microscopy.

Amyloids are associated with a range of human disorders in which the deposition of insoluble protein aggregates in the form of fibrils takes place. Amyloidosis is a clinical condition in which these deposits are found in the extracellular spaces of organs and tissues. These aggregates accumulate in the brains of patients with Alzheimer’s and Parkinson’s diseases, in the pancreata of patients with type II diabetes and systemic inflammatory diseases such as rheumatoid arthritis. They are also found in some less well-known but equally serious disorders such as fatal familial insomnia. Each of the above diseases is a result of the aggregation of different proteins and can be sporadic, inherited or infectious [6]. It has been a point of debate whether these deposits are the final end-products that are tucked away as wastes that are not to come back into the mainstream of cellular physiology or whether these act as triggers to convert more of their kind into amyloids. It appears that the second possibility is feasible since ridding the organism of amyloid plaques gets rid of the associated disease like condition [7]. The fact that only some, and not all, proteins are seen to form amyloids in the cell also supports this view.

An important recent development has been the discovery that amyloid formation is not restricted to the small number of proteins associated with diseases. Most proteins can form amyloid like filaments if the right conditions are found to persuade them to do so. Under in vitro conditions the aggregation of proteins has been achieved using conditions like low pH which keeps proteins in partially-denatured form long enough to allow intermolecular interactions to take place. This is because amyloid fibrils do not develop from the native state of a protein but from precursors that are only partially folded [8-12]. Protein concentrations are important in determining the initiation of aggregation. At higher protein concentrations the conversion of proteins to gelatinous, amorphous aggregates takes place more rapidly than at lower concentrations. These amorphous aggregates have been shown by
kinetic studies using ESR and EM to be the initiation sites for the development of protofilaments and fibrils [13]. These aggregates also bind amyloid specific dyes Congo Red and Thioflavin T and have also been shown to possess extensive β-sheet character by both CD and FTIR spectroscopy for many protein systems [14-15].

Growth of fibrils

As mentioned above, fibrils seem to grow from amorphous aggregates and in vitro studies show that nucleation is an important stage in Aβ fibrillogenesis. Nucleation involves a series of thermodynamically controlled steps leading to the creation of a stable nucleus. It is not clear as to how big the nucleus is but the smallest particles detected in the fibrillogenesis process at low pH have sizes that correspond to the diameter of an Aβ fibril [16,17]. These nuclei can be very short fibrils which can subsequently elongate to generate larger protofilaments and fibrils. Indeed, it has been shown by AFM that spherical units of β(1-40) are present immediately after initialization of fibrillization. The nascent fibrils and protofibrils observed also showed a beaded appearance and thus seem to form by the association of these units [18]. The growth of the fibrils has been said to be bi-directional or unidirectional, and literature exists to show that it can be either. The deposition of protein at both the ends of a growing fibril shows that it might be bi-directional [19]. In another recent observation fluorescently labeled yeast Sup35 (Sup35 NM) has been shown to grow mostly unidirectionally and only a very small fraction of the population of fibres grow in both directions [20].

Propensity of proteins to form amyloids

Amyloid formation is common to most protein aggregates. Different proteins having no sequential or structural similarity form amyloidic aggregates which are similar in morphology and organization and consist predominantly of β-sheet structures [2,21]. Polypeptide chains of normally folded proteins undergo conformational changes, resulting in β-sheet structured intermediates, which are capable of assembling into highly ordered aggregated fibrils [6,10]. It has been shown that the extent of β-sheet formation of a protein is directly related to the ability
to form amyloid fibrils [22]. Sequence modifications of βA4 peptide fragment characteristic of Alzheimer's disease, which show a decrease in β-sheet conformation, are fibrillogenic in character [23-28]. Point mutations as found in the Dutch type of βA4 (Glu-Gln) result in a higher β-sheet content and accelerated fibril formation [29,30]. Water soluble β-sheet peptides have also been shown to self aggregate into fibrillar structures [31]. Thus a predisposition of natural sequences for β-sheet structures is critical for amyloid formation.

However proteins containing mainly α-helical elements in their native structure have also been seen to form amyloids. This occurs through α-helix to β-sheet transformation occurring either before or during fibril formation. The conversion of the cellular form of the prion protein to its fibrillar scrapie counterpart is accompanied by reduction in helical content and an increase in β-sheet structure [32]. Amyloid β-peptide (Aβ) fibril formation associated with Alzheimer's disease also involves α-helix to β-sheet conversion [33].

Unlike *in vitro* development of amyloid deposits where conditions were designed to convert proteins into amyloids, amyloid diseases mostly occur without known precipitating factors [34] and no explanation has yet been found for de-novo α (for a protein with α-helices) to β conversion in amyloid forming proteins. This conversion may occur in partially denaturing extra-cellular environments [35], which, however does not explain why certain proteins form amyloids since it cannot be generalized for all proteins. However, destabilizing point mutations can cause fibril formation of an otherwise stable protein [10] but point mutation related to inherited forms of human diseases do not induce the prion to scrapie conversion *in vitro* and are not generally destabilizing [36]. Aβ (1-42) peptide is highly fibrillogenic whereas peptides lacking the residues 14-23 are not [37]. It could be that proteins that give rise to amyloid may harbor polypeptide segments that make them more prone to undergo α to β conversion, under slightly destabilizing conditions, than non-amyloidogenic proteins. Therefore, since amyloids are cross β-structures, proteins that are predominantly β-sheet or that can easily undergo a structural transition to a mostly β-sheet structure are likely to form fibrils.
Constitution of aggregates – Homomeric or heteromeric?

Little is known about whether the structural organization of polypeptides within aggregates, with regard to what sort of interactions between polypeptides cause them to come together and whether these interactions tend to be specific. In other words whether aggregates form through the non-specific coagulation of polypeptide chains or whether molecules actually recognize each other as they participate in aggregate formation. With respect to the coming together of polypeptides, aggregation too can be likened to the folding of polypeptides in the cell milieu where the presence of the rest of the proteins does not deter the correct assemblage of oligomeric assemblies.

At the most fundamental level, such a question can be addressed by asking the question whether two proteins caused to aggregate under similar conditions might each aggregate through homo-molecular interactions, or whether, hetero-molecular interactions might occur to create aggregates incorporating any polypeptide of any identity present in the mix. In principle both forms of aggregation can be conceived to occur. Thus eg., (i) aggregates forming through associations of partially structured folding intermediates can be imagined to support the homo-molecular recognition of surfaces, since colliding partially structured molecules of identical structure forming tendencies could potentially support the native like assembly of preformed substructures through intermolecular, rather than intramolecular interactions, to promote aggregation. Likewise, (ii) aggregates forming primarily through association of molecules into intermolecular β-sheets could potentially involve hetero-molecular interactions, since as long as interactions tend to be non-native in character a β-sheet can be imagined to form with equal facility between either different regions of identical polypeptide chains, or different regions of different polypeptide chains. It is quite possible that both forms of aggregation described above do indeed occur. In the literature however, only two instances of a demonstration of homo-molecular interactions-based aggregation have been shown but there is no evidence yet to show that aggregation can also be hetero-molecular. Further, there is a lack of a simple experimental test to address this issue. With our backbone-reversed β-sheet proteins, however, we could query specificity of molecular recognition in aggregate formation, since both retro-CspA and retro-HSP12.6 aggregate under similar conditions.
Questions and approaches

The primary question addressed was whether retro-HSP12.6 and retro-CspA aggregates contain amyloid-like character. To examine this, we independently caused the two proteins to aggregate, by concentrating them in solution, and examined aggregates using negative staining electron microscopy. We also examined aggregates of retro-CspA, which tended to be produced in copious amounts, for Congo Red-binding ability using fluorescence microscopy. Each aggregate showed its own distinct morphological characteristics.

Subsequently, we co-aggregated the two proteins in the same solution, starting with roughly equimolar proportions, and examined the mixed aggregates electron microscopically. The idea was to see whether the co-aggregates would show the two proteins to have independently sought out likenesses of themselves, resulting in observation of both morphologies that were seen previously, or whether the proteins would interact during aggregation to generate a new morphology and appearance. Such examination of interactions was also performed by labeling the two proteins independently with different fluorescent dyes and examining confocal fields, within mixed aggregates, for colocalization of the two proteins.

Results

Since the two retro-proteins, namely retro-CspA and retro-HSP12.6, readily aggregate and precipitate upon concentration, the precipitated states of the constructs RETCSPA-1 and RETHSP-1 were independently examined to see whether these deposits show amyloid-like character, irrespective of whether or not macroscopic appearances were suggestive of an amorphous character. As already mentioned in Chapter 1, the concentration beyond which the two proteins precipitate had turned out to be approximately the same for both RETCSPA-1 and RETHSP-1. In addition, the molecular weights of the two constructs two were not very different. However, the aggregates differ starkly in their characteristics.
SECTION A – Characteristics of aggregates

1) Microscopic examination of RETCSPA-1 for congo red binding. The precipitated form of retro-CspA displays both amorphous and fibre-like morphologies, as shown in Figure 1, Panel A. Both forms readily bind the dye Congo Red when it is allowed to diffuse in from the sides of the cover slip, causing the protein aggregates to display the pink-red fluorescence characteristically shown by protein-bound Congo Red upon illumination with UV light (Figure 1, Panel B). Congo Red binding and fluorescence is taken to be indicative of the presence of amyloid-like beta structural organization at the molecular level. To start with the protein aggregate was bluish in color but with the uptake of congo red the color gradually changed to red/orange. This suggested the amyloid-like nature of the aggregate.

2) Electron microscopic examination of RETCSPA-1 and RETHSP-1 for the presence of amyloid fibers. Aggregated RETCSPA-1 and RETHSP-1 were observed under an electron microscope through negative staining with uranyl acetate and phosphotungstic acid (PTA), with nickel chloride added to obtain greater contrast through the low-affinity binding (in the absence of the NTA moiety) of nickel to the N-terminal 6xHis histidine tags of individual peptides. RETHSP-1: RETHSP-1 aggregates appeared to constitute a field of amorphous protein aggregates that had 20 nm diameter amyloid filaments associated with them, with the occasional field displaying 20 nm diameter beaded (globular, spherical) structures coalescing to form fibers. A collage of images is shown in Figure 2a and Figure 2b. The ends of some fibers displayed a hemispherical appearance, supporting the likelihood of the fibers arising from the coalescence of the 20 nm beaded structures. In all cases, the filaments were seen associated with amorphous aggregate at one end. The beaded structures were also seen within the amorphous aggregate. This lead us to think that the amorphous aggregates could be functioning as incubators for the creation of the beaded entities, with amyloid fibers growing out of the amorphous aggregates through addition of the beaded structures at the end embedded within the aggregates. It appeared, therefore, that the growth of the fibrils could be unidirectional. Interestingly, the amyloid fibrils showed the
Figure 1: Both the panels show the binding of Congo Red to aggregated RETCSPA-1, amyloidal nature of the aggregates. Note the bluish color of the aggregate turning to red as the protein aggregate takes up more dye with time. Panel A shows fields of fibrous aggregates of RETCSPA-1, while Panel B shows amorphous aggregates. The binding of Congo Red by both forms suggests that the molecular organization into beta structure is similar in both forms.
Figure 2b: Typical amyloid fibrils formed by RETHSP-1. Panels A, B and C show the fibrils associated at one end with amorphous aggregates. Panel D shows the characteristic striations of amyloid fibrils. Panel E shows the rounded tip of a fibril. Note that the tip has approximately the same diameter as the 20nm beads, further indicating that beads coalesce into fibres. Panel F shows the broken tip of a fibril. Panel G shows a much larger assembly, that was very rare in the field. This structure was about 80nm in diameter and also had a different structural organization as shown by the striations on the fibril.
characteristic striations shown by numerous other amyloid filaments. In addition to the regular 20nm amyloid filaments there was one more kind of a filament which was much larger and had different markings on its surface (Figure 2b, Panel F). This morphology was very rare in the fields observed and had a diameter of approximately 80nm.

RETCSPA-1: The aggregates of RETCSPA-1, on the other hand, showed a completely different morphology of amyloid filaments. A collage of images is shown in Figure 3. In this case, the filaments were much more prominent and were in almost all the cases, present as twisted rope like assemblies. In the fields observed, there was very little amorphous protein aggregate visible and fields consisted mainly of filaments of various diameters, with enough visual evidence to suggest that the different diameters seen were a result of the association and twisting of the filaments of smaller diameter. The amyloid fibrils in this case were very well formed and some of them present as small 20nm diameter fibrils while most of them showed the formation of twisted structures that were as much as ~70-80nm in diameter though, unlike RETHSP-1 fibrils, the fibrils in this case were not seen associated with the amorphous protein aggregate of which there was very little amount visible. It seems to suggest that more than RTEHSP-1, RETCSPA-1 has a greater tendency to aggregate and form higher order amyloid fibrils.

3) Microscopic examination of retro-CspA aggregates for exposed histidine tags. To investigate whether the N-terminal 6xHis tags of individual molecules were exposed and accessible to reagents, we used a 'pseudo-Western' staining technique. Instead of horse radish peroxidase (HRP) conjugated anti-His antibodies, we used a reagent in which Ni-NTA had been conjugated to HRP. This reagent, along with all necessary additives [including H$_2$O$_2$ for generating brown colour through enzymic action of HRP, but excluding diaminobenzidine (DAB)], was pre-mixed with aggregated RETCSPA-1 and deposited on slides. Subsequently, the substrate DAB was allowed to diffuse in through the side of the cover slip, and allowed to interact with aggregated retro-CspA presumed to be pre-bound to Ni-NTA-HRP. Aggregates turned brown immediately, as shown in Figure 4. Thus,
Figure 3: Twisted rope like fibrillar assemblies of RETCSPA-laggregates. In all these cases the larger \(~80\text{nm}\) diameter fibers seem to develop from the coming together and assemblage of smaller \(~20\text{nm}\) diameter fibrils.
Figure 4: Aggregates of RETCSPA-1 as observed under a microscope. The aggregates were examined for the presence of exposed 6XHis tags by incubating the aggregated protein with Ni-NTA HRP conjugate. In the presence of the substrates to HRP the aggregates turned brown, indicating that the histidine tags remained accessible after aggregation.
Figure 5: RETHSP-1 and RETCSPA-1 coaggregated by concentrating the two proteins together. Both of them are seen to affect each other's amyloïd fibril-forming abilities. Though some small filaments and beads are seen to form, these do not resemble any of the filaments or beads seen independently with either protein.
starting amounts and concentrations, to obtain aggregates containing both labeled forms of RETCSPA-1. When these aggregates were laid out on a slide, the population containing both FITC-labeled RETCSPA-1 and TRITC-labelled RETCSPA-1 displayed a color that like the expected color of a mixture of FITC and TRITC Figure 6. Since RETCSPA-1 could be expected to aggregate with RETCSPA-1 regardless of the nature of the covalently-linked fluorescent label, this molecular-level ‘paint-mixing’ approach was found to work sufficiently well to allow us to test it with different proteins. Thus, RETHSP-1 was labeled with TRITC and RETCSPA-1 with FITC and the two were coaggregated. Figure 6 shows a microscopic field containing pure aggregates of FITC-labeled RETCSP-1 and TRITC-labelled RETHSP-1 as well as mixed aggregates of both species. Figure 7 shows more coaggregates of the two proteins. From the colour, and texture of the mixed aggregate, it would appear as if both proteins were present within every section of the aggregate. The resolution of this microscopic approach was tentatively examined in fields in which the two labeled proteins were mixed post-aggregation through sonication (Figure 8). As can be seen clearly, three different colours can be distinguished, which correspond to two types of pure regions supporting homomeric interactions, and mixed regions of the same overall colour but varying hues which support heteromeric interactions of differing degrees.

3) Confocal spectral imaging of mixed labeled aggregates. To further test the likelihood of heteroaggregation, the two pure aggregates and the mixed aggregate were subjected to confocal microscopic examination on a Carl Zeiss LSM-510 Meta laser scanning microscope. After selecting a particular mixed aggregate, a field that was roughly 30μm x 30μm x 0.9μm (in the x, y and z axes) was scanned using 364 nm UV illumination and monitoring for fluorescence. The optical bench of the instrument was manipulated in such a manner as to spectrally separate the two emissions and prevent cross-contamination. As shown in Figure 9 and Figure 10, it was possible to demonstrate that the smallest volume element in the aggregate contained both fluorescent dyes, which could only be possible if both retro-proteins were
Figure 7: Coaggregates of RETCSPA-1 (labeled with FITC) and RETHSP-1 (labeled with TRITC) in higher magnifications of 40X (Panel A) and 100X (Panel B) respectively.
Figure 8: The three different types of aggregates, namely RETCSPA-1 labeled with FITC, RETHSP-1 labeled with TRITC and coaggregates of these two labeled proteins.
Figure 9: RETCSPA-1 (labeled with FITC) and RETHSP-1 (labeled with TRITC) coaggregated. The field was viewed with different excitation wavelengths to specifically excite only FITC (panel A), only TRITC (panel C), or both dyes (panel B). The arrows mark out a single piece of coaggregate, in the middle of which a circular region of interest (ROI; 1 micron diameter) was selected for examination. Fluorescence emissions from this ROI are separately shown, through excitation of only FITC or TRITC, to establish that both labels (and hence, both proteins) colocalize in the co-aggregate. In other words, molecules of both proteins are present within a region expected to contain about a 1000 molecules in the x-y plane. Details of specific excitation are in the methods section.
Figure 10: A line drawn across the field (from left to right) shows that every piece of co-aggregate shown both emissions form FITC and TRITC, confirming their colocalization.
together present in that volume element. Such colocalization established that aggregating RETCSP-1 influences and interacts with aggregating RETHSP-1, to generate heteroaggregates of these beta sheet forming proteins. This finding has implications relating to whether amyloids *in vivo* might be expected to be pure if more than one aggregation-prone proteins are simultaneously present in a diseased organ/system.

**Conclusions**

RETHSP-1 and RETCSPA-1 were both chosen to see if they assemble into amyloids. Whereas RETCSPA-1 principally formed amyloids, RETHSP-1 appeared to fold into structured entities containing beta sheet structure that further assembled upon concentration into aggregates that resemble amyloids. The morphologies of the solid forms of RETCSPA-1 and RETHSP-1 were very different. It appeared that filaments are assembled within amorphous aggregates, both from the fact that amorphous aggregates contained small beads that had the same diameter as that of the amyloid fibril, and from the fact that some beads could actually be seen to be arranged in a manner suggesting that they coalesce into protofilaments after lining up. The fully developed RETHSP-1 amyloid filaments were rounded at one end with the rounded tip resembling a single bead. Development of amyloid fibre-like structures form beaded entities has been seen previously [2,16-18, 21,22]; however, arguably the most visually impressive evidence for such assembly comes from our study of RETHSP-1 upon concentration and precipitation/aggregation.

We were able to demonstrate that the 12 extra residues at N-terminus of the protein, containing the 6xHis tag, do not participate in aggregation. This was partly expected since the sequence is a random assembly of amino acids that is not likely to form structure.

Another question that we asked in this section was that of whether molecular recognition operates during aggregation, i.e., do individual protein species seek out likenesses of themselves, or do they also have the potential to coaggregate. Of course, the answer to such a question would change from system to system. In
independent work from our laboratory, evidence for homomolecular aggregation involving molecular recognition has actually been obtained in a system in which aggregation occurs through the intermolecular assembly of partially-unfolded molecules retaining sub-structures. However, since we were dealing with at least one protein that did not display much of a propensity to fold into a stable structure in solution (retro-CspA) despite possessing beta sheet-forming potential, we reckoned that chances were good for our obtaining evidence of sequence-independent intermolecular aggregation through both homomolecular and heteromolecular interactions. We showed by confocal and fluorescence microscopic examination of labeled aggregates, and electron microscopy, that retro-CspA and retro-HSP12.6 can coaggregate and affect each other’s individual aggregation characteristics. These findings have implications for amyloid formation in vivo, in that they suggest that if unstable proteins are available they might join growing aggregates formed principally through intermolecular sequence-independent beta sheet formation.
References


the prion protein H1 peptide as determined by time-dependent ESR. 


