

■ MOLECULAR BIOLOGY

A Catalogue of Structural Motifs in Amyloid Fibril Organization

Zheng J, Ma B, Tsai CJ, and Nussinov R. Structural stability and dynamics of an amyloid-forming peptide GNNQQNY from the yeast prion sup-35. *Biophys J* 91: 824–33, 2006.

A wide variety of proteins with no sequence similarity or structural homology may form linear, unbranched fibrils sharing specific cross- β sheet structures. Many of these are associated with neurodegenerative diseases. Determination of high-resolution molecular structures of amyloid fibrils is an important first step toward understanding the pathogenesis and aggregation mechanism of amyloid diseases, yet it is still a highly challenging task due to the noncrystalline and insoluble nature of amyloid fibrils. Nevertheless, the combination of computational predictions (Ma B and Nussinov R. *Proc Natl Acad Sci U S A* 99: 14126–31, 2002; Zheng J et al. *Biophys J* 91: 824–33, 2006) and experimental results (Nelson R et al. *Nature* 435: 773–8, 2005; Luhrs T et al. *Proc Natl Acad Sci U S A* 102: 17342–7, 2005; Petkova AT et al. *Biochemistry* 45: 498–512, 2006) allows derivation of the emerging structural motifs in amyloid organization (summarized in [Table 1](#)). A catalogue of these structural motifs is expected to be enormously useful in drug design for prevention and treatment of amyloid-related diseases.

Table 1. General Structural Features in Amyloid Fibrils

Feature	Characteristics
Sheet-to-sheet recognition via steric zipper	Between β sheets, an inter-sheet zipper can be characterized by complementarity—of shape, hydrophobicity, charge, and hydrogen bonding.
Twisted cross- β sheet	The twist angle involved in single and multiple β sheets can range between approximately 5 and 20 degrees.
Identical/similar residue ladder in a parallel in-register organization	Asn and Gln ladders; aromatic stacking (Phe, Tyr, Pro, and His); hydrophobic stacking (Val, Ile, or Leu).
β -strand-loop- β -strand	Two adjacent β strands in the primary sequence oriented in an antiparallel arrangement and linked by a short loop of two to five amino acids.

Steric Zipper. There is no universal driving force that associates and stabilizes β sheets into amyloid fibrils: Hydrophobic interactions govern some cases ($A\beta$, human islet amyloid, and Syrian hamster prion protein), whereas polar interactions govern others (GNNQQNY and human calcitonin amyloid). Yet, by examining crystal structures of amyloid peptides, including GNNQQNY, $A\beta$, and human CA150, we observed a similar steric zipper arrangement in those protofilaments. The remarkable GNNQQNY crystal structure from the yeast protein Sup35 presents a dry, tightly self-complementing steric zipper between two β sheets. Simulations of mutational variants show that substitutions of N2, Q4, or N6 by Ala at the dry interface knock down the steric zippers, destroy sheet-sheet packing, and thus inhibit fibril formation. Similarly, $A\beta$ and human CA150 amyloidogenic peptides form steric zippers by the interdigitation of side-chains (i.e., M35-M35 contacts for $A\beta$ and T13-T18, V5-R24, V5-L26, and T3-S28 contacts for CA150). Since the shape-complementary zipper optimizes side-chain and main-chain interactions common to amyloid fibrils *regardless* of their sequences, the steric zipper may be a general feature in amyloid fibrils. The zipper can be hydrophobic or polar and the interactions within or between molecules.

Twisted Cross- β Sheets. The simulations indicated that the GNNQQNY β sheets twist by about 15 degrees. The twisted sheets are not unique to GNNQQNY; rather, similar twisted β sheets were observed in other amyloid peptides such as $A\beta_{16-22}$ (KLVFFAE), $A\beta_{21-30}$, the human islet amyloid polypeptide₂₂₋₂₇ (NFGAIL), KFFE, KVVE, KLLE, KAAE, the human calcitonin hormone₁₅₋₁₉ (DFNKF), and NHVTL SQ from human β_2 -microglobulin. Since twisted β sheets optimize the hydrogen bonds, side chain stacking, and electrostatic interactions, it is commonly accepted that twisted sheets are more stable than flat sheets. Interestingly, the pairs of β sheets, while twisting, are still compatible with the steric zipper.

Parallel β Sheet Organization. Many amyloid fibrils consist of parallel β sheet structures, at least for longer protein chains or peptides. Such a parallel organization, observed for GNNQQNY, $A\beta$, the human CA150 WW domain, β_2 -microglobulin, and other peptides, as well as in β -helices, allows a ladder-like stacking of chemically similar side chains on top of each other (e.g., Asn or Gln ladders, aromatic stacking, and hydrogen bonding zipper). Unlike in antiparallel organization, shuffling the sequence is not likely to disrupt those residue pairs and thus has little impact on parallel β sheet structures.

β -Strand-Loop- β -Strand. The β -strand-loop- β -strand motif is formed by two β strands of non-native register linked by a flexible loop. As first predicted by the simulations of $A\beta$ (Ma B and Nussinov R. *Proc Natl Acad Sci U S A* 99: 14126–31, 2002), the β -strand-loop- β -strand motif was recently discovered in the amyloid protofilaments of human CA150 and the β_2 -microglobulin. This motif consists of two sheets whose side chains zip against each other in an antiparallel fashion, where each sheet consists of a parallel arrangement of the β strands. The loop is stabilized by a salt bridge in $A\beta$ and covalent bonds in CA150 and β_2 -microglobulin. Thus, this motif resembles those of shorter peptides, illustrating the advantage of the tight packing.

Although our current work has made progress in understanding the dynamics and structure (thermodynamics) of amyloid formation, it behooves us to remember that the details of peptide organization and preference among possible conformational states depend on amino acid composition, sequence, chain length, and environment. Further, for a given sequence, amyloids are likely to exist as different phenotypic strains; that is, there may be meta-stable conformational states obeying the same conformational principles. Moreover, beyond the motifs, the crucial questions of the kinetics and pathways of amyloid formation and the mechanism of amyloid toxicity still remain; our studies of these key issues are under way.

Jie Zheng, PhD

Scientist

CCR Nanobiology Program

jzheng@ncifcrf.gov

Tsai Chung-Jung, PhD

Programmer Analyst

CCR Nanobiology Program

tsai@ncifcrf.gov

Buyong Ma, PhD

Senior Computational Scientist

CCR Nanobiology Program

mab@ncifcrf.gov

Ruth Nussinov, PhD

Senior Investigator

CCR Nanobiology Program

NCI-Frederick, SAIC-Frederick, Bldg. 469/Rm. 151

Tel: 301-846-5579

Fax: 301-846-5598

ruthn@ncifcrf.gov
