

FIGURE 16.1 Ribbon diagram of the crystal structure of β_2m taken from the class I HLA complex (1DUZ) [3]. Secondary structural elements are labeled A through G. The side chain of the *cis*-proline (P32) is shown in ball-and-stick representation.

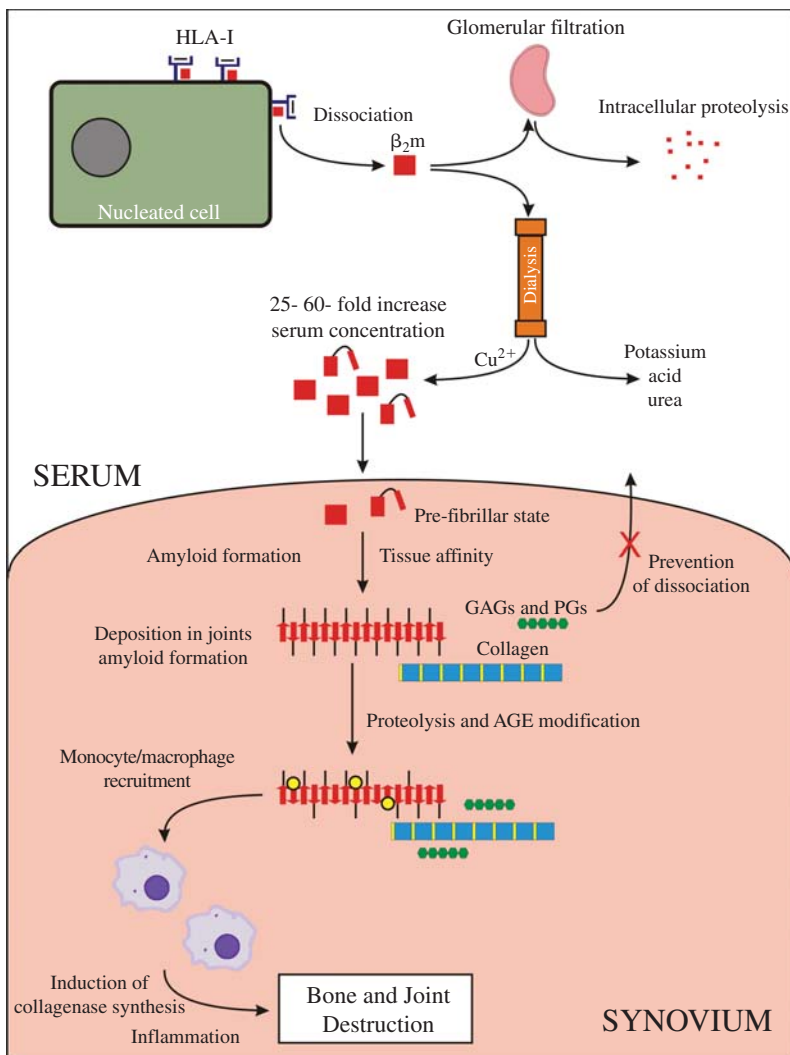


FIGURE 16.2 Events involved in the development of HDRA. Note that the precise details of the structure of the precursor(s) are unknown, as are the details of the amyloid fibrils that form.

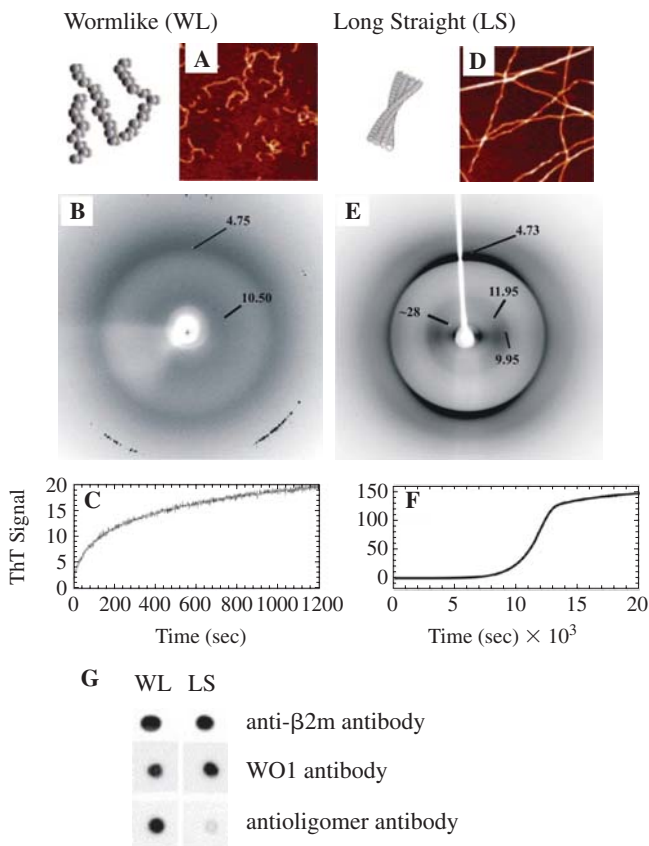


FIGURE 16.3 Characterization of different types of amyloidlike fibrils formed from β_2m in vitro. Wormlike (WL) fibrils formed in vitro at pH 3.6 at an ionic strength of 0.4 M (left-hand panels) and long, straight (LS) fibrils formed at pH 2.5 at an ionic strength of 50 mM (right-hand panels). (A,D) Atomic force microscopy images and cartoon representation of fibrils formed under each condition. The samples were dried onto mica. (B,E) X-ray fiber diffraction image: The major reflections on the meridian and equator are labeled in angstroms. (C,F) Growth kinetics at 37°C monitored by the fluorescence of thioflavin T; (C) at pH 3.6, 0.4 M ionic strength (no agitation) and (F) pH 2.5, 50 mM ionic strength (agitation at 1400 rpm). (G) Dot-blot analysis of the WL and LS fibrils probed using an anti- β_2m antibody (positive control), antibody WO1 [79], and the generic antioligomer antibody A11 [80]. (Adapted from [54,70].)

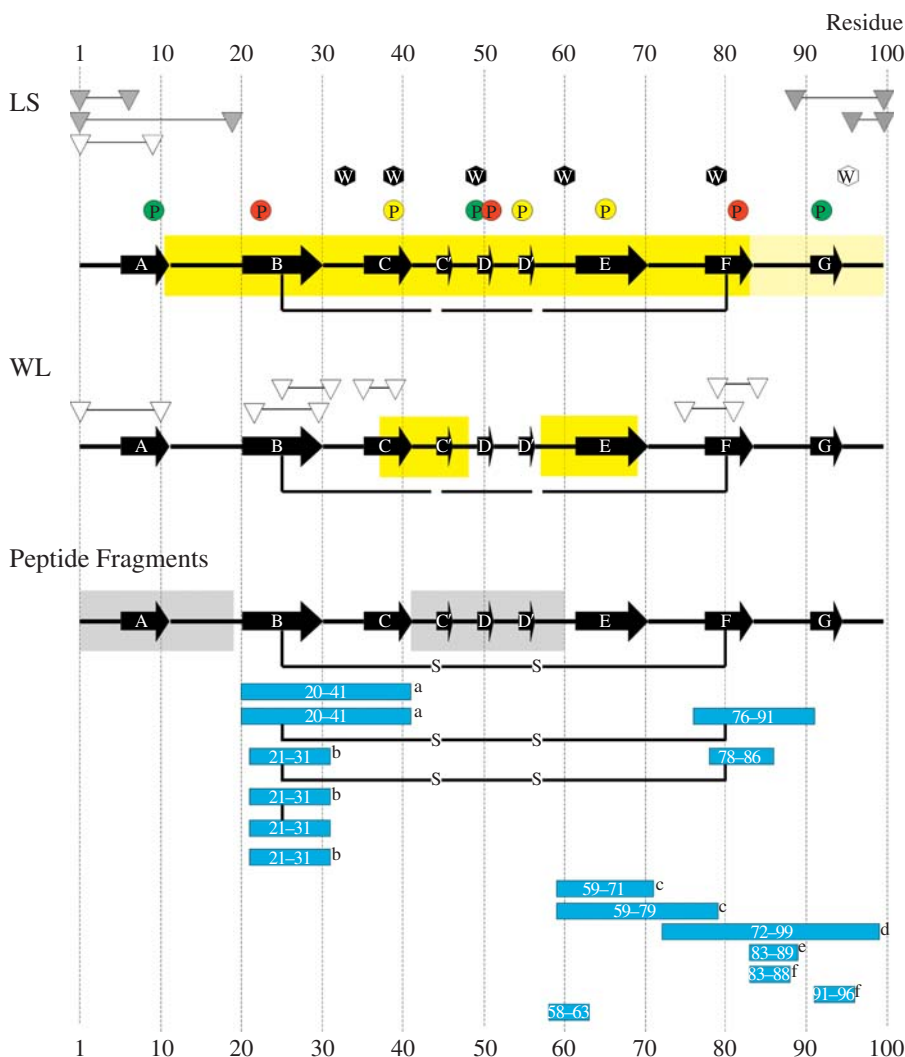


FIGURE 16.4 Schematic diagram outlining current knowledge about the structure of β_2m amyloid fibrils gained to date from biochemical and biophysical analyses. *Top panel:* Hydrogen exchange (HX) and limited proteolysis results are shown for long, straight (LS) fibrils grown at pH 2.5 de novo; approximate regions of high protection from HX (yellow), regions of partial protection (pale yellow) [77,78]. Open triangles, limited proteolysis cut sites observed at pH 2.5 [75,77]; gray filled triangles, limited proteolysis cut sites observed using fibrils formed by extending seeds of ex vivo fibrils at pH 4.0 [76]. Hexagons; Trp residues buried in the core of the fibrils (black), Trp residues exposed to solvent (white) [87]. Proline mutations displaying significant (red), moderate (yellow), and no effect (green) on fibril extension [88]. *Middle panel:* Wormlike (WL) fibrils produced under high-salt conditions at pH 2.5 [75,78]. Regions of HX protection are shown in yellow [78], limited proteolysis cut sites are shown as open triangles [75,77]. *Lower panel:* Peptide fragments deriving from β_2m known to form fibrils in vitro. Regions in gray are not known to form fibrils in isolation. [From (a) [90], (b) [91], (c) [92], (d) [93], (e) [94], (f) [95].]

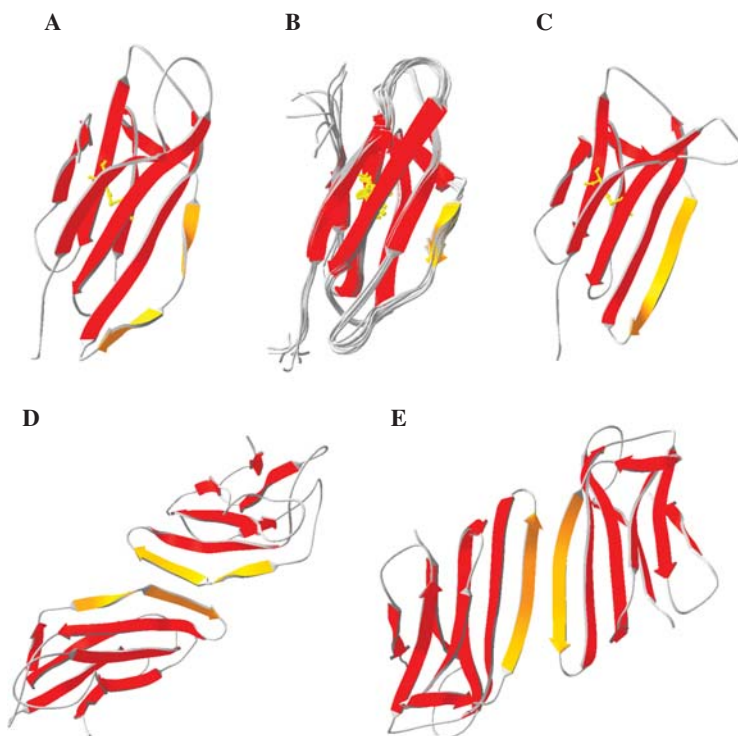


FIGURE 16.5 Selection of different crystal structures of β_2m . Strand D is shown in orange in all cases. (A) β_2m in complex with the heavy chain of the HLA-1DUZ [3]. (B) NMR solution structure of β_2m -1JNJ [5]. (C) Crystal structure of β_2m at pH 5.6 (note the straight D strand)-1LDS [4]. Note that the crystal structure of β_2m at pH 7.0 (2YXF) is essentially the same as at pH 5.6 [6]. (D) Crystal structure of H31Y. The crystal lattice packing shows the occurrence of an antiparallel pairing of the short D2 strand-1PY4 [7]. (E) Crystallographic dimer of P32A related by a twofold axis yielding an eight-strand β -sheet comprised of strands ABED-DEBA-2F8O [65].

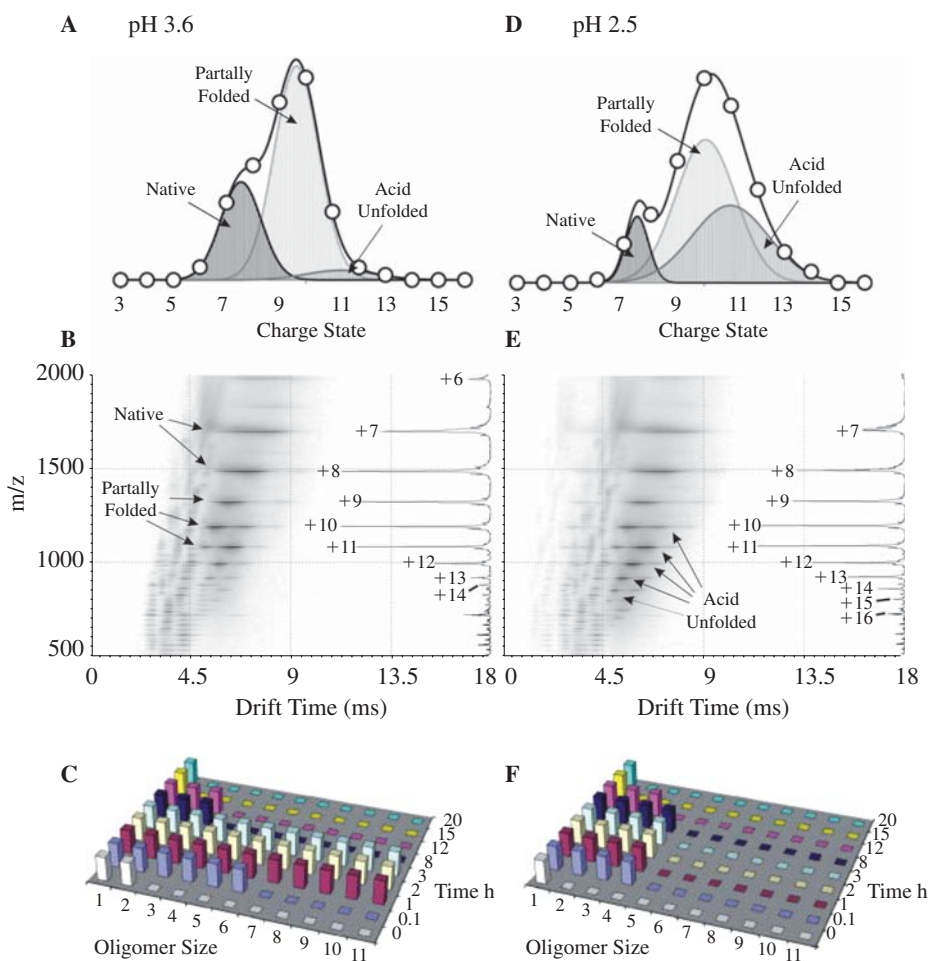


FIGURE 16.6 ESI-MS data collected using β_2m at pH 3.6 (left-hand panels) and pH 2.5 (right-hand panels). (A,D) Co-populated conformational ensembles of β_2m uncovered quantitatively by ESI-MS [105]. (B,E) ESI-IMS-MS driftscope plots showing drift time (x axis) versus m/z (y -axis) for wild-type β_2m under each condition. Insets at the right-hand side of each plot: the summed, full-scan m/z spectra of wild-type β_2m for each data acquisition, showing the charge-state ions detected [106]. (C,F) Oligomer distributions observed during β_2m fibril assembly measured by nanoESI-MS under each condition over a range of m/z 3200 to 5500 [108].