

Figures

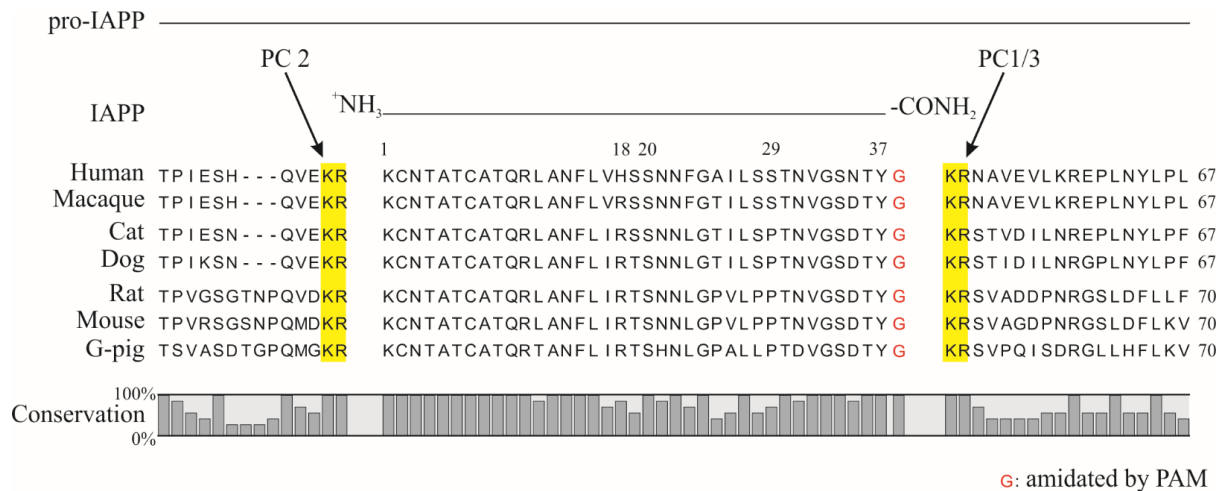


Figure 1. Amino acid sequence of proIAPP in different species: ProIAPP is 67 amino acids long in humans, Macaques, cats and dogs but 70 amino acids in rodents and guinea pigs. The propeptide is cleaved by the enzymes PC2 and PC1/3 and further amidated at the C-terminus by peptidylglycine α -amidating monooxygenase (PAM). This results in the the mature 37 amino acid IAPP.

The conservation of the amino acid components between the different species is shown in the lower panel.

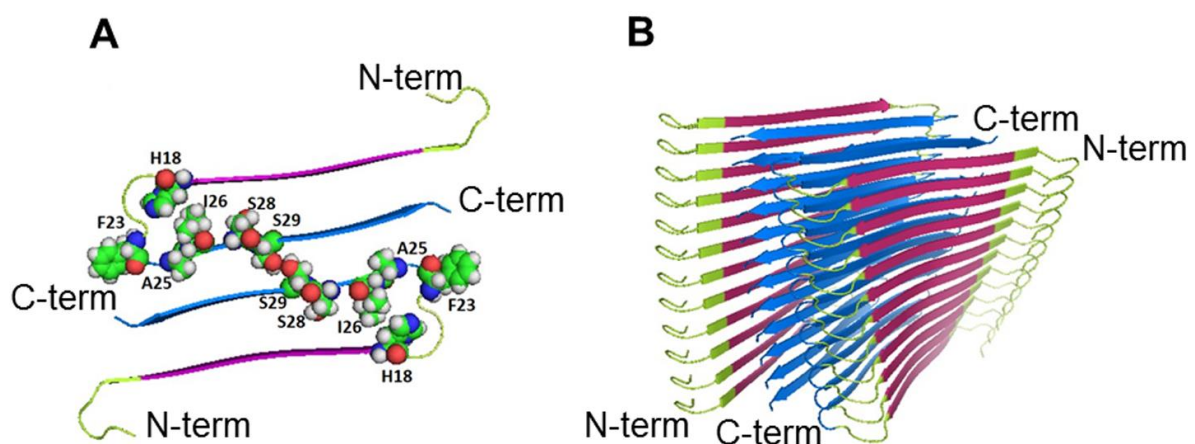


Figure 2. Structural models of the hIAPP protofibril. A. A β -bend in regions 18-27 results in a horseshoe shaped structure with the N- and C-termini (N-term, C-term) on the same face. There are no intra peptide backbone hydrogen bonds in the structure; instead the backbone hydrogen bonding occurs between adjacent monomers in one of the horseshoe shaped stacks. B. The two stacks are aligned so that two molecules lie antiparallel in the same plane and

interact via their sidechains. Protofibrils have a super helical twist, although the β -sheets are still relatively flat by the standards of globular proteins. Protofibrils further assemble into fibrils containing two or more protofibrils (Wiltzius, et al. 2008).

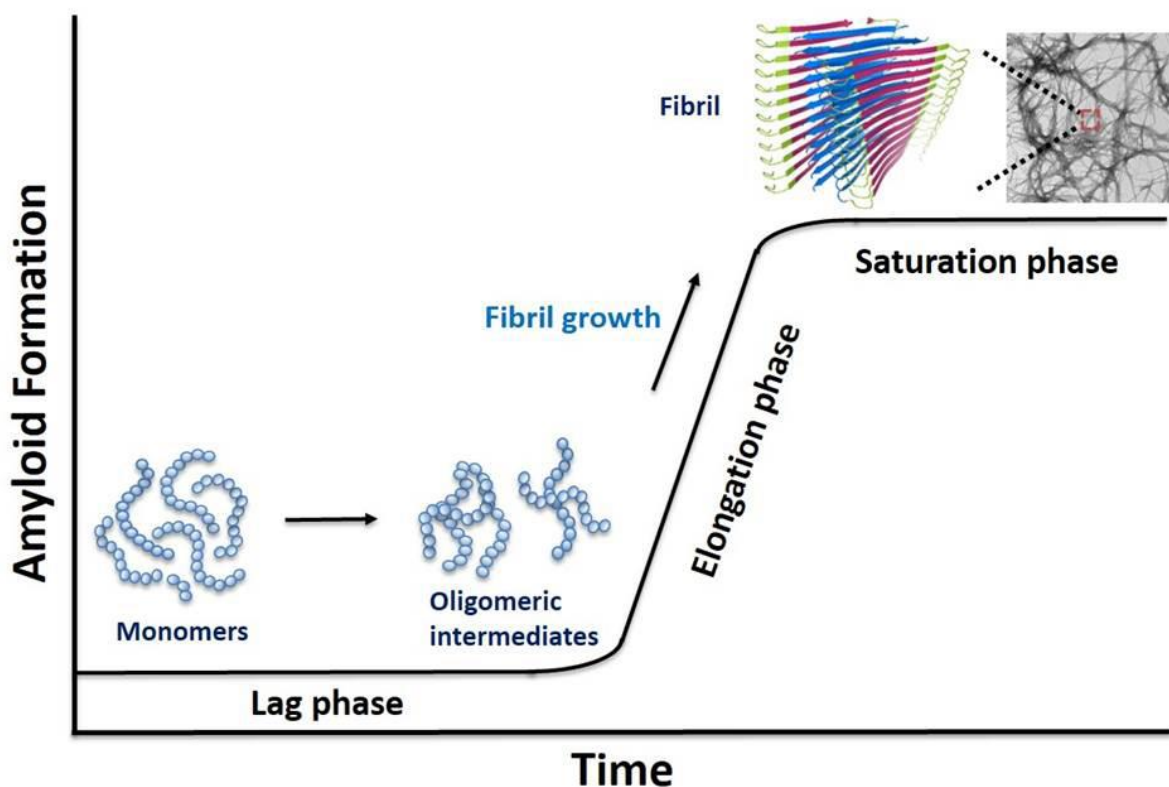


Figure 3: Schematic diagram of amyloid fibril formation from hIAPP. During the initial phase (lag phase) monomeric hIAPP forms oligomers and adopts conformations which differ from the monomeric state. The growth phase involves elongation of protofibrils and assembly into fibrils. A saturation phase, (also called the plateau phase) occurs with synthetic hIAPP in vitro when most of the monomers have become assembled into mature fibrils.

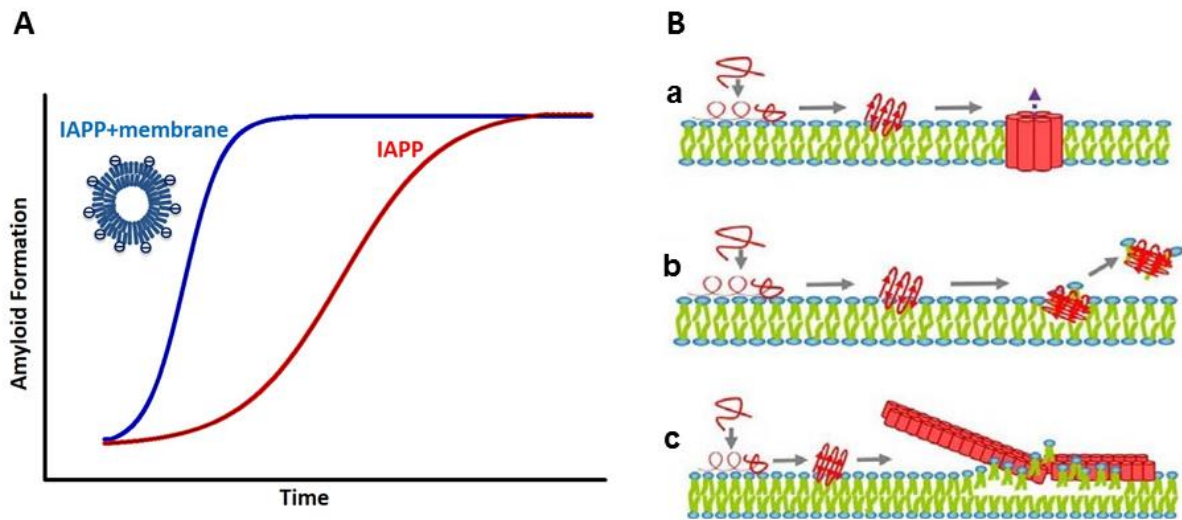


Figure 4: A. Schematic representation of the influence of membranes on the rate of hIAPP fibril formation. Synthetic membrane liposomes (blue line) reduce the lag time and accelerate the growth phase compared to fibrillogenesis in the absence of membrane (red line). B. Diagrammatic representation of potential processes of membrane interactions of hIAPP. (a) hIAPP (red) hydrophobic, monomeric hIAPP adopts a transient helical conformation at the membrane surface which becomes β -sheet leading to oligomer assembly at the membrane. These multimers interact with the lipid bilayer to form a pore. (b) multimers assembled at the membrane bind to components of the outer leaflet of the bilayer and destroy the structure. (c) multimers assembled at the membrane continue to extend into protofibrils and fibril growth occurs with part of the fibril inserted into the membrane and membrane damage.

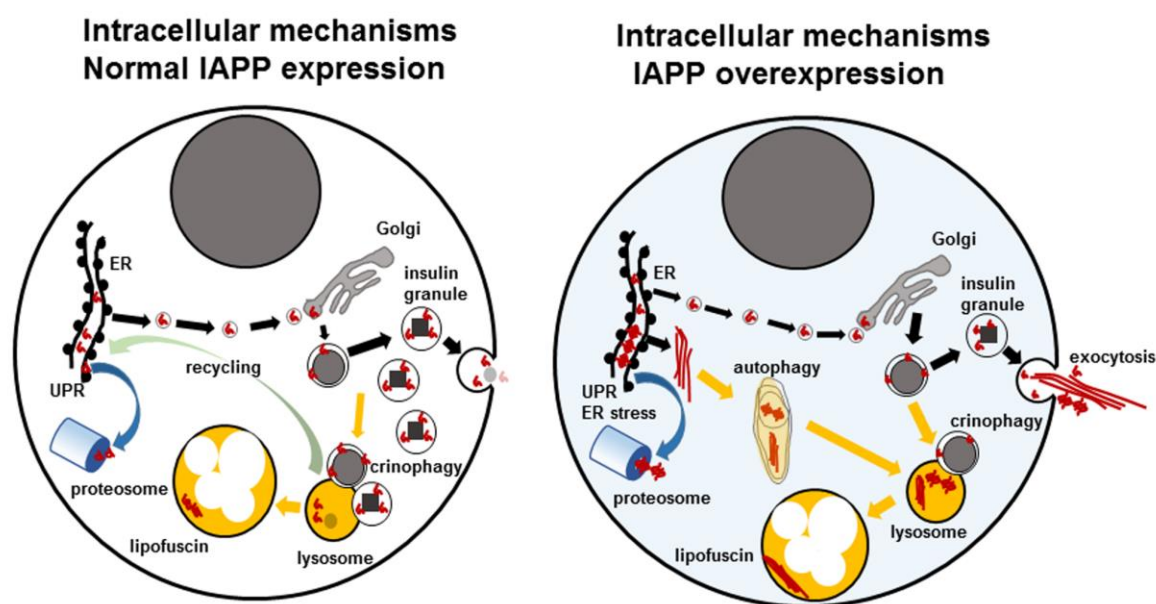


Figure 5: Diagram of intracellular mechanisms contributing to cytotoxicity of hIAPP in β -cells. With normal IAPP expression (left hand diagram) proIAPP (red) synthesised in the endoplasmic reticulum (ER) is transported to the Golgi through the cytoplasm. If aberrant folding or transcription/translation occurs, misfolded molecules are targeted (unfolded protein response, UPR) by ubiquitination for degradation via the proteasome. proIAPP is packaged in the Golgi, together with insulin and processing enzymes, into secretory granules. Maturation and post-translational processing of the peptide occurs in the granule where proIAPP/hIAPP is largely stabilised by interaction with insulin. Some granules are targeted for destruction via the lysosomal system (crinophagy) and fuse with lysosomes. Granule components, including amino acids and lipids, arising from this degradation process are recycled back for re-synthesis. Human IAPP is partially resistant to lysosomal proteases and is retained in the lysosomal storage compartment (lipofuscin bodies). Overexpression (right hand side) of proIAPP affects trafficking through the ER, overloading the UPR and creating ER stress which can lead to cell death. High concentrations of proIAPP will aggregate in the ER or cytoplasm forming fibrils. These are removed by autophagy and targeted to the lysosomal system. Overload of this protective mechanism can result in apoptosis. The high concentrations of proIAPP/hIAPP can result in fibril formation in secretory granules. On release from the cell by exocytosis of the granule, hIAPP can interact with the membrane causing cytotoxicity or be deposited as fibrils in the extracellular space.

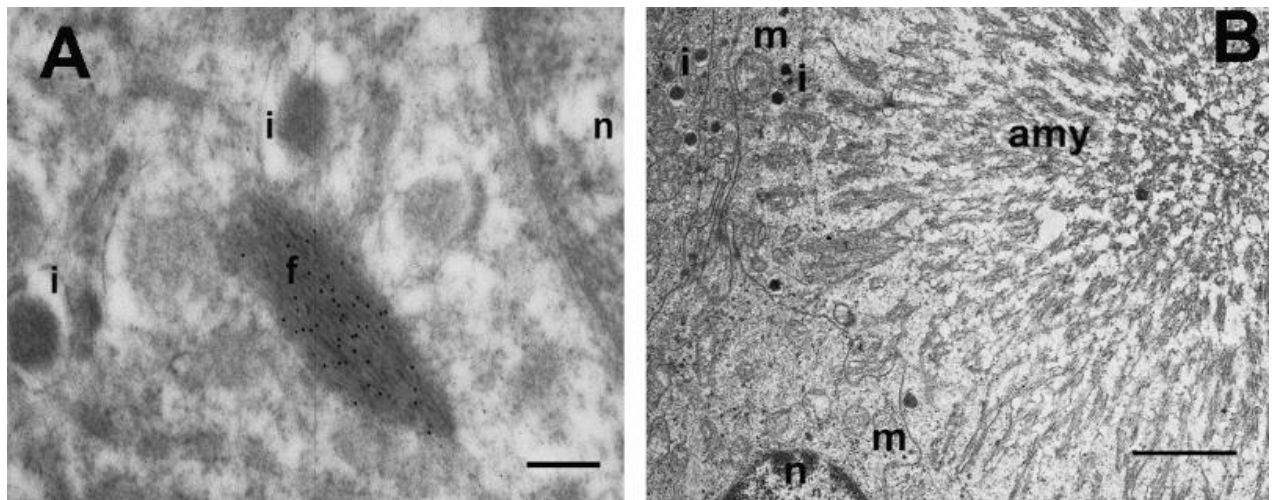


Figure 6: IAPP and proIAPP fibrils in overexpressing hIAPP transgenic mouse and human insulinoma cells viewed by electron microscopy. A. A bundle of fibrils (f), immunogold labelled for IAPP in cytoplasm of a β -cell of a hIAPP transgenic mouse. B. Arrays of fibrils immunoreactive for IAPP in the cytoplasm of a cell from a human insulinoma. i, insulin granules; amy, IAPP amyloid fibrils m, mitochondrion; n, nucleus. Scale bars A, 500nm; B, 1.0 μ m.

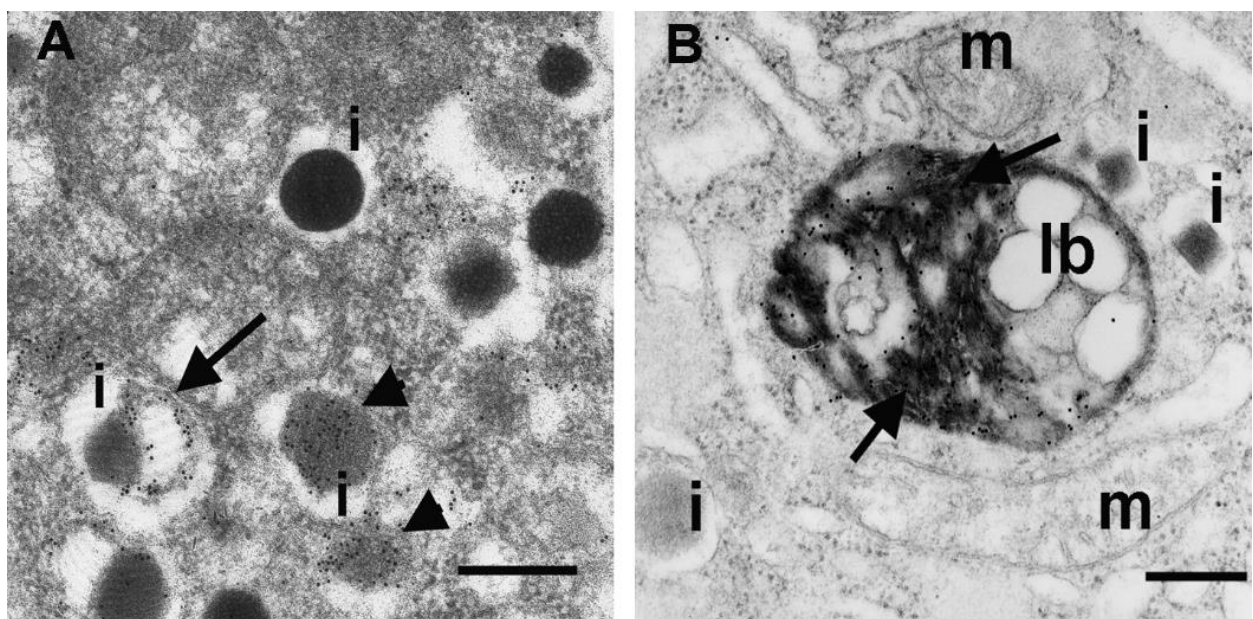


Figure 7: IAPP in insulin secretory granules of a hIAPP transgenic mouse and in a lipofuscin body of a non- diabetic human subject. A. Immunogold labelling with an antibody against hIAPP with N-terminal of proIAPP intact demonstrates fibrillar IAPP/proIAPP in mature insulin granules (i) (with halos) (arrow) and in immature granules (without halos)(arrowhead) suggesting that fibrils are formed at this site from unprocessed proIAPP. Scale bar 200nm. B. Immunoreactivity for IAPP in a lipofuscin body (arrows) of a non-diabetic subject. M, mitochondrion; I,insulin secretory granule. Scale bar 200nm.

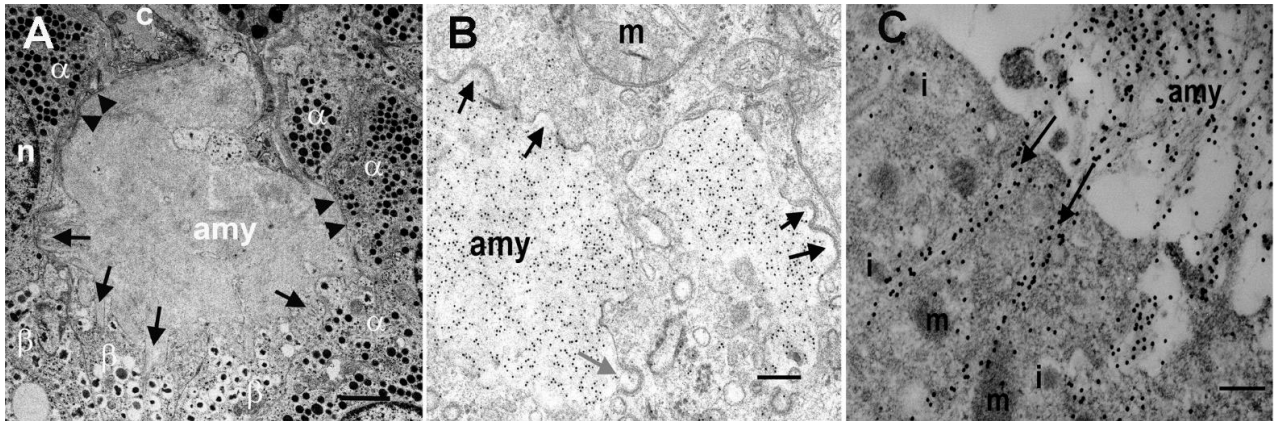


Figure 8: Extracellular IAPP fibrils in plasma membrane invaginations of β -cells seen with electron microscopy. A. Islet with extracellular amyloid immunogold labelled for IAPP from a Type 2 diabetic subject. The amyloid (amy) is situated between the capillary (c) and the β -cell (β) and in deep invaginations of the β -cell membrane (arrows). Adjacent alpha cells (α) although in close contact with the amyloid (arrowhead) do not have membrane invaginations or show nuclear (n) signs of apoptosis. Scale bar 1.0 μ m. B. Extracellular amyloid fibrils immunogold labelled for IAPP (amy) from an islet of an hIAPP transgenic mouse. The fibrils lie in invaginations of the β -cell plasma membrane (arrows). Some of these membrane invaginations show thickening characteristic of clathrin coating suggesting an arrested process of membrane recovery/endocytosis (e.g red arrow). m, mitochondrion. Scale bar 200nm.

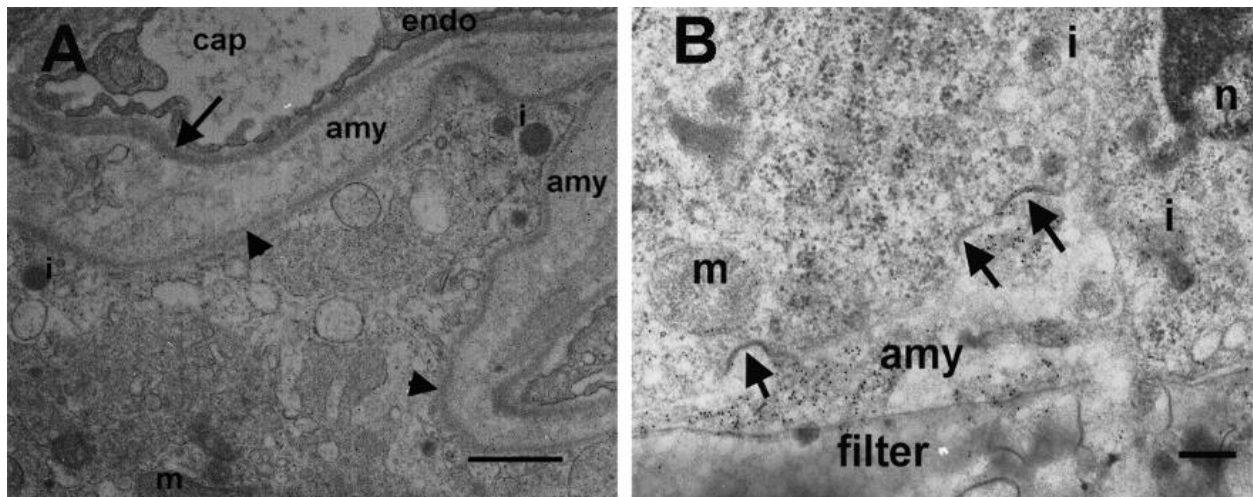


Figure 9: Localisation of IAPP amyloid fibrils in an islet of diabetic macaque monkey and in hIAPP expressing β -cells cultured on a matrix. A. Extracellular amyloid fibrils in humans and all animal models are initially deposited *in vivo* between islet cells and islet capillaries. Gold labelled IAPP fibrils (amy) are situated between the two basement membranes (arrows) in the islet; one adjacent to the islet capillary (cap) and a second adjacent to the β -cell. i, insulin granule. Scale bar 1.0 μ m. B. Electron micrograph of hIAPP expressing β -cells cultured on a filter culture insert. Section taken through the filter (filter) shows the base of the cells with invaginations containing fibrils gold labelled for IAPP (amy). Some of these invaginations (arrows) have a thickened membrane suggesting clathrin coating. m, mitochondrion; n, nucleus, i, insulin granule. Scale bar 200nm.

Reference:

Wiltzius JJ, Sievers SA, Sawaya MR, Cascio D, Popov D, Riek C & Eisenberg D 2008 Atomic structure of the cross-beta spine of islet amyloid polypeptide (amylin). *Protein Sci* **17** 1467-1474.