DC1. This project aims at establishing a reproducible protocol to generate Aβ-lipid complexes made from recombinant Aβ and near physiological vesicle preparations. The kinetics of the complex formation will be determined using SPR and RRS. Molecular simulations will be applied to determine the affinity (ΔG) between Aβ and the different lipids, and to determine the effects on the structure and dynamics of Aβ (FZJ), which will involve collaborations with DC 4 who will perform the same kind of simulations for IAPP and αS.

DC2. This project aims at establishing a reproducible protocol to generate αS-lipid complexes made from recombinant αS and near physiological vesicle preparations, the characterisation of their stability using DSC and ITC and their structure at the near atomic resolution using solid state NMR.

DC3. This project focus on the characterization of the islet amyloid polypeptide (IAPP)/lipid complexes formed with lipid mixtures of varying composition, ranging from phospholipids to free fatty acids. The candidate will isolate β-cells from pancreatic islets and determine their lipid composition using lipidomics (in collaboration with a mass spectrometry centre). IAPP fibril formation and structure will be investigated using biophysical techniques, mainly fluorescence spectroscopy, circular dichroism, and microscopy. Finally, the candidate will study the insertion of IAPP–lipid complexes into lipid membranes using Resonance Raman Spectroscopy, as well as perform kinetic and thermodynamic measurements. This will be done in the presence and absence of peptidomimetic inhibitors made by DC11.

DC4. The goal of this project is to design and perform molecular simulations to determine the affinity of binding of the amyloid proteins IAPP and α-Synuclein (αS) to different lipids. The aim of these simulations is to elucidate the effects of the lipids on the structure and dynamics of IAPP and αS. Furthermore, methods of the computer-assisted drug design tool box will be used to design peptidomimetic inhibitors targeting the binding hotspot between IAPP/αS and lipids, which will then be synthesized and tested by other members of the LipAgg network.

DC5. The goal of this project is to determine the structures of both islet amyloid polypeptide (IAPP) and amyloid-β (Aβ) using cryo-electron microscopy (cryo-EM). Amyloid fibrils will first be characterized alone and then in the presence of specific free lipids and model membranes. Both conventional lipids (such as phosphatidylcholine and phosphatidylserine) and less conventional lipids will be selected in collaboration with DC1 and DC3. Smaller aggregate species will also be investigated using electron microscopy, although at lower resolution due to their size and structural heterogeneity. All cryo-EM structural data will be correlated with toxicity assays performed by DC7.

DC6. The project aims to characterize the islet amyloid polypeptide (IAPP)/lipids complexes by NMR and to determination their structure at the near atomic resolution. Structural characterization of the IAPP fibrils grown in the presence of the Amyloid Protein-Lipid (AP-L) complexes will be done using NMR and compared to the structure obtained with cryo-EM

(DC5). IAPP fibril formation kinetics in the absence and presence of AP-L will be studied as well as the incorporation of the AP-L into model membranes. The Doctorate Candidate will also investigate membrane damages and/or perturbations associated with IAPP-lipid complex binding using fluorescence leaking assay and ^{2}H and ^{31}P-NMR. All NMR structural data will be correlated with toxicity assays performed by DC7.

DC7. The Doctorat Candidate (DC) enrolled in this position, will identify mechanisms by which astrocytes spread Amyloid Protein-Lipid (AP-L) complexes and study if the AP-Ls induce astrocytic stress responses that affect their interplay with neurons. The candidate will expose cultures of human iPCS-derived astrocytes to fluorescently labelled AP-L complexes and analyze how the cells accumulate and spread the complexes over time using live cell imaging, immunocytochemistry, ELISA, Western blot, electron microscopy and other techniques. Moreover, the DC will isolate deposits of amyloid complexes from human astrocytes and determination of their lipid composition.

DC8. The DC enrolled in this position, will synthesize peptide mimics to modulate the pathological α-Synuclein protein aggregation as well as α-Synuclein/lipids interaction. The DC will be involved in the synthesis of unnatural scaffolds, i.e. non-natural amino acids and of peptide mimics, designed according to the results of the structural biology results of the consortium. The DC will also perform conformational studies using NMR techniques, and will be involved in some biophysical and biochemical evaluations of the activity and the interaction of the prepared compounds with α-Synuclein or with lipids.

DC9. This research project consists in investigating the cellular toxicity of AP-L complexes (αS, Aβ and IAPP-L) in INS1E cells, iPSC-derived neurons and astrocytes. The cellular toxicity of the AP-L will be compared to that of lipid-free AP complexes. The influence of AP-L inhibitors on cell toxicity will also be investigated.

DC10. This project aims at identifying the lipids which co-assemble with αS in cells using MS and at testing the seeding capacity of ex-cellulo isolated and in vitro prepared (in collaboration with DC2) αS-lipid complexes in the absence and presence of inhibitors (in collaboration with DC4 and DC8) using ThT assays. The PhD student will also investigate membrane damages and/or perturbations associated with αS-lipid complex binding using RRS and fluorescence leaking assays during his/her stay at UNICT.

DC11. The DC enrolled in this position, will synthesize peptide mimics to modulate the pathological Aβ-lipid and IAPP-lipid interaction. The DC will be involved in the synthesis of unnatural scaffolds, i.e. non-natural AAs and of peptide mimics, designed according to the results of the structural biology results of the consortium. The DC will also perform conformational studies using NMR techniques, and will be involved in some biophysical and biochemical evaluations of the activity and of the influence of the prepared compounds on Aβ/lipid effects.

DC12. This research project aims at elucidating whether AP-L complex may seed the aggregation of endogenous IAPP, αS and Aβ in their relevant cells (pancreatic cells, iPSC-derived neurons and astrocytes). The propensity for the AP-L complexes to trigger aggregation in cells will be compared to that of lipid-free AP assemblies. Finally, the potential inhibitory effect of small molecules and peptidomimetics on in cellulo protein aggregation will be studied (in collaboration with DC4 and DC8).

DC13. This project aims at characterizing the aggregation state of amyloid proteins Aβ and IAPP complexed with lipid membranes using FTIR, EPR and NMR spectroscopy. The dynamical properties will be studied by measuring membrane fluidity and order parameters using EPR with spin labelled lipids or site directed spin labelled Aβ. The lipid/proteins contacts will be determined by NMR spectroscopy. The composition of the model membranes investigated will be designed according to the protocols established by research partners (mainly DC1)

DC14. This project aims to elucidate how complexes formed by amyloid proteins (Aβ, IAPP, and α-Synuclein)—hereafter referred to as AP-L complexes—insert into lipid membranes. Using enhanced molecular simulation techniques, the reciprocal effects between AP-L complexes and the membrane environment will be investigated. These simulations will be integrated with EPR experiments to provide a comprehensive assessment of how these complexes modulate lipid membrane integrity.

DC15. This PhD project studies how alpha-synuclein aggregates in the presence of lipids, which is relevant for example to Parkinson's disease. High-resolution structures of lipidic alpha-synuclein fibrils will be determined using cryo-electron microscopy in the presence of aggregation inhibitors, with the aim to characterize how different inhibitors change the fibril structure. The structural data will guide the design of new aggregation inhibitors, and the results will be linked to cellular toxicity. Computational modelling and peptide docking to alpha-synuclein structures to improve inhibitor binding. The project also supports related amyloid-β studies and includes collaboration on inhibitor design and synthesis.