Understanding the structure and formation of heteromeric amyloid fibrils using tomography, cryo-transmission electron microscopy and correlative microscopy techniques

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Many important human disorders are known to be associated with protein aggregation and the self-assembly of proteins into fibrillar complexes. However, the complexities of these structures and how they are formed have not yet been thoroughly explored. Due to advanced microscopy techniques, such as cryo-transmission electron microscopy (CryoTEM) and correlative light and electron microscopy (CLEM), these protein aggregates can now be more effectively visualised in order to fully understand them. However, these techniques can be quite challenging, therefore protocol optimisation and technique development are essential in order to discover the exact parameters required for optimum specimen preparation and visualisation. CryoTEM is not a high-throughput method, which is why standard techniques, such as negative staining, room temperature transmission electron microscopy (TEM) imaging and TEM tomography are recommended prior to exploring more advanced visualisation methods.

This study focuses on the M45 protein from the murine cytomegalovirus, which inhibits host cell defense mechanisms by sequestering the cellular kinase RIPK3 within heteromeric amyloid fibrils. Previously, negative staining and standard TEM imaging have identified that these proteins co-assemble in a fibrillar network. However, these techniques involve applying purified protein samples onto a formvar-coated copper mesh grid and staining with heavy metal solutions, such as uranyl acetate, which is then followed by air-drying. Evaporation can be quite a destructive process, therefore, in order to thoroughly characterise these structures they ideally need to be studied in their hydrated form. CryoTEM allows us to do this and involves using a FEI Vitrobot™ to rapidly freeze the samples in liquid ethane within a controlled humidity and temperature chamber. The samples are then transferred to a Gatan cryo-tomography holder and inserted into a JEOL 2100 TEM operating at 200kV. By employing a tomography holder, we will be able to create a three dimensional reconstruction of the fibrils, which will give us a better idea of their dynamic shape and structure. In order to complete the picture, correlative techniques will be used to bridge the gap between visualising M45 and RIPK3 proteins fluorescently labelled with mCHERRY and YPet using light microscopy and high resolution TEM images showcasing the detailed structure of these amyloid fibrils.