Structural studies of the CCT-gelsolin complex

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The eukaryotic cytosolic chaperonin CCT (Chaperoning Containing T-CP1) is a molecular machine involved in assisting the folding of proteins that regulate in important cellular processes. This chaperonin consists of a large cylindrical oligomer formed by two rings each one built by eight different subunits (~60kDa). It was originally thought that the function of CCT was the folding of the cytoskeletal proteins actin and tubulin but subsequent studies have shown that CCT interacts with a wide range of proteins. Some of these proteins bind to CCT but do not requiere interactions with CCT in order to get its proper folding and to be functional. Gelsolin is an actin filament severing protein that increases actin dynamics by generating filament ends for further actin polymerization, and previous studies have shown its interaction with CCT. This binding is slow and gelsolin is accumulated over time on CCT suggesting that this protein is not a real folding substrate of the chaperonin. In fact, although bacteria lack CCT, gelsolin can successfully be produced as a native soluble protein in bacteria. Therefore, CCT could have a regulatory effect on gelsolin, acting indirectly in the actin filament dynamics.
The main aim of this project is the structural characterization of the CCT-Gelsolin complex using electron-microscopy in order to elucidate the binding mechanism that mediates such interaction and whether CCT has an actual role on actin dynamics regulation. To face this goal, our first step has been to carry out binding assays between CCT and Gelsolin adding DTSSP (3,3´-dithiobis[sulfosuccinimidylpropionate]) to crosslink the complex, which was later purified by gel filtration and this sample is being used for further structural characterization which is currently in progress.