Final Publishable Summary Report

The **main goal** of the project was to understand the **molecular mechanisms by which proteins equilibrate under the effect of a constant stretching force**. We are using the newly developed single molecule force-clamp spectroscopy technique to elucidate the conformational dynamics of a single refolding protein during its individual folding trajectory from highly extended states. This experimental approach will allow us, for the first time, to **quantitatively reconstruct the free energy landscape of an individual folding protein** and also **the effect of a mechanical force on the free energy surface governing a chemical reaction**. In particular, we aim at experimentally probing the transition state structure of an S_N2 reaction at the single bond level. Within a multidisciplinary approach, we are conducting a series of innovative experiments to directly probe the effect of force on the function of an individual folding polypeptide and also the mechanisms by which mechanical forces modulate chemical reactions, of common occurrence in nature.

In this vein, this project first enabled the Fellow to move from the US (Columbia University) to Europe (Department of Physics, King's College London) to set up a new single molecule force spectroscopy laboratory. This laboratory is presently housing four state-of-the-art single molecule force clamp spectrometers; the first is home-made, and the other three have been purchased from the German Company, Luigs & Neumann, GmbH, and the Fellow's laboratory has been the beta-tester of the first set-up commercialized in Europe. These spectrometers achieve a force resolution of 2 pN and sub-nanometer length resolution. The laboratory is specially designed to perform these high-resolution experiments, provided with acoustic and vibration isolation, and temperature and air-flow control.

Crucial to the success of the scientific agenda was the establishment, parallel to the single molecule laboratory, of a molecular biology laboratory able to conduct recombinant polyproteins, which are crucial to perform state of the art single molecule spectroscopy experiments. Such molecular biology laboratory has been established in the Randall Division of Cell and Molecular Biophysics of KCL, and is fully equipped with the required instrumentation to conduct polyprotein DNA engineering, protein expression in bacteria and protein purification.

After the set-up of both laboratories during the first two years of the project, in the following two years we have focused on conducting the experiments depicted in the proposal. In particular, we have engineered the polyproteins required to conduct the experiments aiming at **(i) understanding the conformational dynamics of individual proteins as they fold**. In the framework of these experiments, we have measured the folding rates and the conformational dynamics of a verity of topologically distinct proteins. **(ii) Capturing misfolding trajectories that lead to protein aggregation**. In this vein, we have mostly focused on the study of the initial stages of gamma-crystallin aggregation. Using our single molecule approach, we have identified a misfolded, domain swapped conformation involving two individual protein monomers that are likely to be the molecular seed of protein aggregation. **(iii) The molecular platforms to explore the effect of mechanical force on the**

outcome of a chemical reaction, at the single bond level. In these experiments, we have mainly studied the mechanochemistry of individual organometallic bonds, whereby an individual sulfur atom binds to a copper center (in the case of blue copper proteins) and also to a zinc moiety, in the context of a zinc finger. Collectively, these experiments highlighted the surprisingly low mechanical stability of the sulphur organometallic covalent bond. Finally, **(iv)** we have also extended our experiments to study **the effect of mechanical force on the mechanical properties of the lipid bilayers of live cells**. These experiments have demonstrated that, during cell division, a set of well defined lipid moieties are up-regulated, greatly increasing the mechanical stability of the cell membrane.

These experiments have elicited 6 scientific publications in high impact journals, such as *Cell*, *Physical Review Letters*, the *Journal of Physical Chemistry Letters*, *Current Opinion in Chemical Biology* and *Nature Communications*. The results stemming from this research might have impact on the academic world, especially in the fields of Physics, Chemistry and Molecular and Cell Biology. The protein aggregation outcome of this grant (Aim 2) might have an important contribution to the molecular understanding of the etiology of the eye cataract disease.

From the training perspective, the Fellow has been able to train 5 postdoctoral researchers, alongside 4 PhD students and 1 Master's student. Moreover, a highly dedicated laboratory technician, in part paid under the auspices of this grant, has been also trained in the molecular biology part of the research agenda.

From the development perspective, the present proposal has allowed the Fellow to establish a new dedicated single molecule laboratory in an outstanding university in Europe. This has allowed him to secure further funding to ensure his feasibility of his research agenda. The establishment of his group has allowed the Fellow to obtain his tenured position, being promoted to Reader in Biophysics in 2014.

The results stemming from this grant have been disseminated, apart from the scientific publications, in a variety of scientific conferences and workshops, together with a variety of seminars in different European and American universities.

Finally, the fellow has co-organised a Single Molecule Workshop within the umbrella of the Francis Crick Institute in King's College London, in October 2015. This workshop was attended by a large number of young students and researchers in the London and UK area, with the idea of fostering the interest in the interdisciplinary single molecule research field, lying just at the interface between physics, chemistry and biology.

Details on the results and scientific outcome of the project are described in our group website (http://garcia-manyeslab.org/).

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