Final Publishable Summary- MAPNE 252126- Elena Martines

MAPNE (Mechanobiology of *Aplysia* Neurons) has been funded through the FP7-PEOPLE-2009-IEF call to support the move of a postdoctoral researcher, Elena Martines, to University College Dublin (UCD). The researcher at the time of moving had published 6 peer-reviewed journal articles with an h-index of 5 and 220 citations. The fellow leaves with 9 peer-reviewed journal articles, h-index 6 and over 300 citations.

The goal of MAPNE was to understand the molecular and cellular mechanisms of directed movements of neuronal growth cones. This process, known as 'growth cone steering', underlies the establishment of neuronal connectivity in normal development, during axonal regeneration and in pathological conditions. MAPNE aimed at understanding the role of mechanical force in growth cone motility and how neural cell adhesion molecules may act as force transducers between the extra-and intra-cellular space. Neuronal cell adhesion molecules are known to be involved in brain development processes and also contribute to the synaptic alterations connected with memory formation in adults. The research carried out in MAPNE will help to understand the molecular and neurobiological bases of neurodegenerative diseases such as Alzheimer's disease, and thus it will contribute to the search for new therapeutic targets.

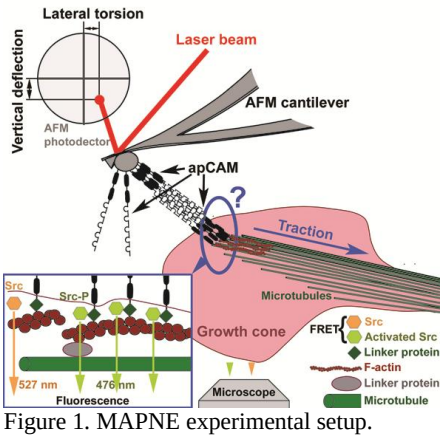

Figure 1. MAPNE experimental setup.

The main instrument necessary to perform this research has been a high-resolution force measurement system, the atomic force microscope (AFM), coupled with Forster Resonance Energy Transfer (FRET) imaging capabilities. MAPNE proposed to induce adhesion-mediated growth cone steering in *Aplysia Californica* neurons, while simultaneously measuring the resulting traction force by AFM and intra-cellular signalling by FRET optical microscopy **(**Figure 1).

apCAM (*Aplysia* cell adhesion molecule) is the *Aplysia* homologue of NCAM (human neural cell adhesion molecule). apCAM is mainly located at the trailing edge (lamellipodium) of *Aplysia* bag cell growth cones (Figure 2**Error! Reference source not found.**). Recent studies have shown that local apCAM clustering and immobilization induce activation of Src protein tyrosine kinase followed by steering of cultured *Aplysia* neuronal growth cones. The aim of this work is to correlate the force transduced by the cell cytoskeleton upon growth cone steering with intra-cellular Src activation. These measurements will be the first direct evidence of mechanotransduction, i.e. the transformation of an external mechanical signal into a chemical signal inside the cell. The research was broken down into three main workpackages (see Figure 3) whereby first the binding kinetics between individual apCAM molecules would be measured in a model system (WP1) and then the binding kinetics between apCAM molecules would be measured on *Aplysia* growth cones (WP2). The traction force exerted by the growth cone would be measured in WP2, and associated to chemical signalling in WP3.

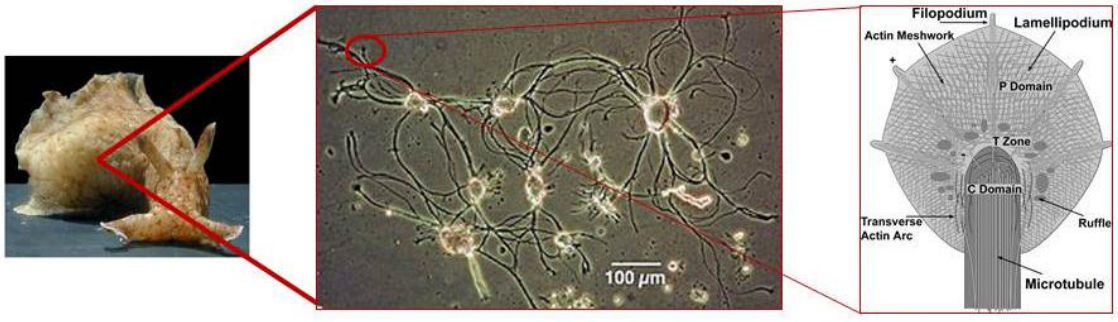

Figure 2. Left: *Aplysia Californica* sea hare. Middle: *Aplysia* bag cell neurons. Right: *Aplysia* neuronal growth cone.

So far WP1 has been successfully taken to completion, and has been the subject of a peer-reviewed scientific publication in the Biophysical Journal, a highly regarded journal in the field of biophysics Martines E, Zhong J, Muzard J, Lee AC, Akhremitchev BB, Suter DM, Lee GU. *Single-molecule force spectroscopy of the Aplysia cell adhesion molecule reveals two homophilic bonds*. Biophys J. 2012 Aug 22;103(4):649-57. WP2 is currently being completed in UCD. Elena Martines has set up a shared BSL2 laboratory in UCD that is allowing the *Aplysia* bag cell dissection to be carried out routinely. Elena Martines has also set up all the additional technology required to carry out WP3, in particular the customisation of the AFM microscope to perform simultaneously AFM and FRET microscopy in real time.

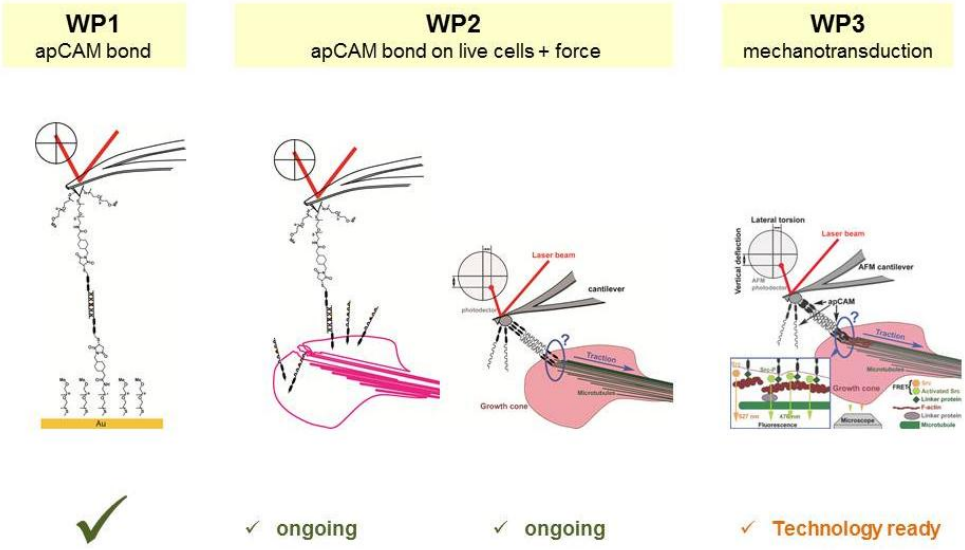

Figure 3. Workpackages in MAPNE.

Impact of MAPNE

Elena Martines has used single molecule force spectroscopy to demonstrate for the first time that apCAM molecules bind to each other, e.g. exhibit homophilic binding, like its vertebrate homolog, NCAM. These results are a fundamental pre-requisite to investigate more complex mechanotransduction mechanisms and this research is currently underway in UCD. Developing novel methods to study neuronal mechanotransduction and its involvement in growth cone steering is necessary to understand the molecular mechanisms of failed neuronal connectivity. In turn, this information can help develop new drugs to treat neurodegenerative diseases and dementia. In 2006, 7.3 million Europeans were suffering from different types of dementia, and some current forecasts project a doubling of the number of persons affected every 20 years. Dementias are very expensive for society as a whole: according to the Dementia in Europe Yearbook (2008), the total direct and informal care costs of Alzheimer's disease and other dementias in 2005 amounted to €130 billion for the EU 27 region; 56% of costs were generated by informal care (an estimated 19 million Europeans are thereby directly affected by dementias). The specific socio-economic characteristics of Alzheimer's disease and other dementias single them out as areas where actions taken at EU level can bring added value in supporting Member States.

The establishment of a new research line on mechanobiology in Ireland will clearly contribute in the longer term to attract the best scientists worldwide and to exploit the European research potential, while supporting the policy of structuring the European Research Area in such a way to prevent the "brain drain" problem. The scientific gain at Community level has been very high, as the fellow and the host group have collaborated closely with the Suter group in Purdue University USA, which is the world-leader in the field of growth cone nanobiology. MAPNE will contribute to establish a European leadership in this area of research, which will raise the attractiveness of Europe as a place to do research. This in turn is expected to increase the European potential to tackle the major socio-economic problems that can affect the competitiveness and the lifestyle of all European citizens.