Spatial and Temporal Dynamics of Signalling Pathways

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Cardiovascular disease (CVD) carries a high mortality with 4.3 million deaths in Europe and over 2.0 million deaths in the European Union (EU). CVD causes nearly half of all deaths in Europe (48%) with an estimated cost of €169 billion per annum. This makes CVD one of the most important diseases in Europe, and innovations is this area will have great impact on the EU and its citizens.

Atherosclerosis, which underlies the CVD related-mortality, has been associated with risk factors (eg. hypercholesteraemia, hypertension, diabetes and others) that are associated with progression of the disease. These risk factors are important as they determine diagnostic developments and day-to-day treatment of the disease. Remarkably, these risk factors predict a random or a homogeneous distribution of plaques over the arterial system, but this differs from clinical observations that indicate plaques are confined to curved vessels, bifurcations and side branches. It has been postulated that disturbances of the blood flow pattern at these sites could induce atherosclerosis there, either through a direct effect on the endothelial cells or by remote transport effects (for reviews see [1-3]). While early studies focussed on the initiation of the disease, recent studies strengthened the role of blood flow in *advanced* atherosclerosis by showing that blood flow plays a role in human advanced atherosclerotic disease [4], vulnerable plaques [4-7], in in-stent restenosis [8,9] and in inflammation and plaque formation [7, 10, 11]. As a consequence, shear stress theories have entered the clinical arena and are a topic of clinical trials [4, 10]. Despite strong evidence relating blood flow to all stages of atherosclerotic disease, the underlying mechanism is largely unknown, and consequently a *specific* therapy is lacking.

Endothelial cells sense the local blood flow by detecting the shear stress, although the mechanism by which they do this is not fully understood. Shear stress is the in-plane drag force exerted by the movement of blood with respect to the non-moving endothelial layer. Due to the mechanical deformation of the (endothelial) cell, several putative sensors are activated, including caveolae [12,13], ion-channels [13], G-proteins[14], trans-membrane proteins[ref] and focal adhesion complex [15]. After stimulation by shear stress a combination of these receptors is activated, leading to clustering of a series of membrane bound proteins which then activate down stream signalling pathways eventually leading to gene expression. Approximately 7 pathways are modified by mechanical stimulation, and these regulate 8 acknowledged transcription factors, which leads to a total of ~2000 genes responsible for the response to shear stress [16]. The large complexity that results from the interactions of so many molecules prohibits an intuitive and coherent understanding of processes involved. Mathematical modelling is often used to solve these large networks, and predict signalling pathway dynamics (for reviews see [17, 18]). Indeed, for known shear stress-sensitive pathways new emerging properties have been identified like oscillatory behaviour (IP3-pathway, MAPK-pathway, NF-κB-pathway), bi-stability (MAPK-pathway) and memory (MAPK-pathway). However, it is currently unknown which signalling pathway is dominant under flow conditions and what controls this activity. It has been suggested that specificity of signalling pathways resides in their spatial organisation and indeed for the MAPK pathways, one of the most fundamental for mechanotransduction, signalling specificity is by scaffolding proteins. These proteins increase local concentration and enhance interaction between signalling molecules. In addition, endothelial cells polarise under the influence of flow and it has been shown that regulators of the MAPK pathway upstream of the nucleus are different from downstream of the nucleus identifying an extra spatial complexity to the organisation of the MAPK pathway under the influence of flow.

Spatial modelling of MAPK pathways is in its infancy, despite multiple biochemical studies showing its importance [19-21]. The few modelling studies that have incorporated spatial control showed different dynamics for spatially controlled than for non-spatial controlled models [22, 23]. The models presented in these studies were too simple and underscore the fact that spatial simulation of signalling pathways is still in its infancy. The aim of this project is to develop a model of an endothelial cell (dimensions LxHxB 10X2X2μm) exposed to flow, including the activation of the MAPK pathway through RAS signalling. In the first instance, activation of the MAPK pathway (Figures 1 and 2) will be simulated without spatial control, and then spatial control will be incorporated through scaffolds to be included in the model. Finally, if time

permits, the polarisation of endothelial cells (RAS activation upstream and RAC activation downstream) will be considered.

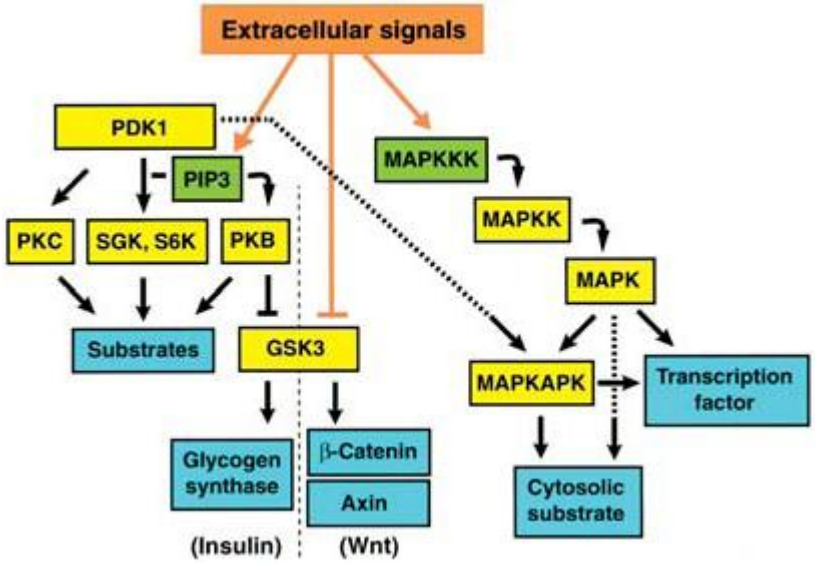

Figure 1: The signalling pathway involved in spatial organisation and controlling MAPK-pathway flux.

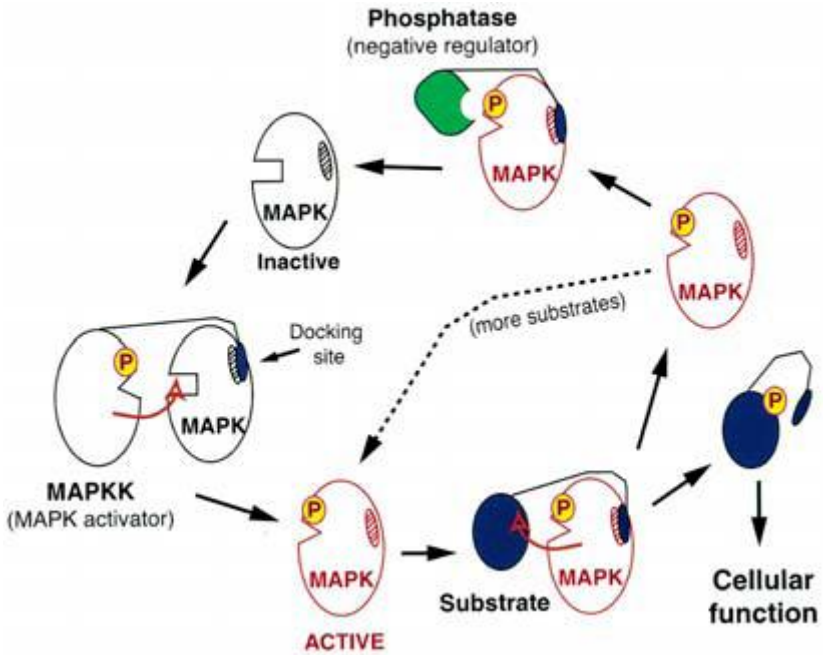

Figure 2: Docking mechanism in MAPK signalling.

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