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Utilisation of EF-Tag Technology for Protein Purification and High Throughput Screening

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Raf1 and Mst2 kinases are a point of specific crosstalk between a mitogenic (Raf/MEK/ERK) and a pro-apoptotic (Mst2/Hippo) signalling pathway in mammalian cells. The complex made by Raf1 and Mst2 has previously been studied by this group using co-immunoprecipitation and FRET/FLIM based assays. The strength of Raf1/Mst2 interaction and its finely tuned regulation participates significantly in cell fate decisions and the balance between cell proliferation and survival, which is of therapeutic significance in cancerous cells. We have performed preliminary kinetic screening of small molecule drugs that are effective in inhibiting Raf1/Mst2 activity in cancer cells. To identify to which protein the drugs are directly binding to we immobilised recombinant Mst2 and Raf1 on individual surfaces of sensor chips using standard amine coupling chemistry and have reproducibly determined that this approach fails. This failure is most likely due to compromised protein structure through the amine coupling immobilisation.

We have developed a novel affinity tag system based on the calcium-dependent reconstitution of protein subdomains from a small calcium binding protein, calbindin D9k. This high specificity, high affinity reconstitution (pM) forms the basis of the tag system that facilitates stable, oriented display of tagged proteins on the biosensor in a completely regenerable system. Raf1 and Mst2 genes were cloned into novel proprietary expression vectors for expression of EF1 fusions under the control of the CMV promoter in HEK293 cells. The expressed proteins were then purified using a novel proprietary EF2 agarose resin. Purified proteins are then displayed on the biosensor surface through specific calcium based coupling of EF1 fusion protein to immobilised EF2 on the sensor surface.

Drug screening studies of the known drug inhibitors will be presented. The establishment of the screening protocols with a model drug target, carbonic anhydrase I, will be shown.