436a Enzyme Assay Microarrays for Biomarker Detection

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We have developed a slide-based microarray platform for functionally phenotyping multiple enzyme activities within complex biological fluids such as human plasma. Using specific fluorogenic substrates for various coagulation enzymes and a 361 compound fluorogenic library of the general format Ac-Ala-X-X-Arg-coumarin (X = all natural amino acids except cysteine) we have mapped the transient states (t = 1 min, 10 min) of the blood coagulation cascade when perturbed with different activators (kaolin, tissue factor, plasmin, uPA). The library was microarrayed along with fluorescent calibration standards to provide inter and intra slide normalization. Reference maps were plotted for 10 purified human coagulation enzymes (plasma kallikrein, factor XIIa, factor XIa, factor IXa, factor VIIa, factor Xa, factor IIa, activated protein C, uPA and plasmin). Human platelet free plasma (PFP) was treated with various activators, and the enzymatic activities were mapped in the transient states. Using the transient state reference maps generated from the fluorogenic library, we have quantitatively deconvoluted the relative activity or weight function of each enzyme in the complex milieu of PFP and its inclusive activators. The predictive weight functions generated from the limited reference maps of purified enzymes were used to generate regressed maps of the different perturbations in PFP. This primary initial application for this technology is in the field of diagnostic biomarker discovery.

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