Title: Development of a platelet calcium flux assay using Fura-2, AM on the FlexStation 3 Multi-Mode Microplate Reader

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Abstract: Platelets are small, anucleate blood cells that mediate haemostasis by aggregating at sites of blood vessel injury to form a thrombus (or clot) that limits blood loss. When platelets respond to vessel damage inappropriately this can lead to thrombotic disease such as heart attacks and ischaemic stroke. Three main steps underlie thrombus formation; stage one is adhesion (platelets attach to exposed matrix proteins in the damaged vascular endothelium), stage two is activation (receptor mediated events such as shape change and secretion of chemical messengers) and stage three is aggregation (platelet-to-platelet adhesion). During platelet adhesion platelets are initially activated by collagen via the glycoprotein VI receptor (GPVI) and then a variety of secreted agonists such as adenosine diphosphate (ADP) and thromboxane A_2 (TxA_2) that activate G-protein coupled receptors (GPCRs). These receptors couple to one of two phospholipase C (PLC) isoforms expressed in platelets; PLCβ or PLCγ2 and subsequently to the release of calcium (Ca^{2+}) into the cytosol from the dense tubular system (DTS). Depletion of Ca^{2+} from the DTS triggers an influx of extracellular Ca^{2+} via a mechanism known as store-operated Ca^{2+} entry. Elevation of cyctosolic Ca^{2+} via these mechanisms underpins all aspects of platelet function, including adhesion, shape change and aggregation. Therefore, understanding how different agonists modulate this critical regulator of platelet function in health and disease will guide development of novel, safe anti-thrombotic drugs.

In this poster we show how it is feasible to easily measure Ca^{2+} flux in human platelets, a primary human tissue, in a microplate format using the high affinity, fluorescent calcium indicator Fura2, AM. We demonstrate that miniaturizing the assay in 96-well half area microplates, and using the flexible liquid transfer features of the FlexStation® 3 reader allows rapid and reliable measurement of EC_{50} values for agonists and IC_{50} values for antagonists.