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CHARACTERISATION OF INTRACELLULAR CALCIUM OSCILLATIONS DURING PLATELET ACTIVATION

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Background: Following platelet stimulation calcium signalling is a common secondary messenger. The calcium oscillations of the platelets are dependent on the type and degree of stimulation, different agonist giving rise to specific patterns.

By using calcium-sensitive dyes the monitoring of these patterns is possible. However, it is often a choice between measuring the response of the bulk or a few individual platelets. By using high rate timelapse microscopy combined with image analysis our aim is to follow the calcium oscillations of hundreds of individual platelets. Such an analysis may give information on heterogeneities in the platelet population and allow for detailed studies of the response of specific agonists and inhibitors.

Methods: Washed platelets adhered to a fibrinogen surface were activated by specific agonist and the calcium response was detected by Cal-520 fluorescence. High-rate image acquisition allowed for the detection of each calcium oscillation. Images were processed so that the oscillations of all individual platelets could be characterised and visualised, determining the height, width and the number of the peaks during activation.

Results: Individual platelets were identified, and the calcium oscillations could be tracked throughout experiments, max cal-520 fluorescence of each platelet was determined for every time-point. The individual oscillations patterns were different depending on the agonist or agonist/inhibitor combination. Activation by 1U thrombin caused an increase in the base calcium fluorescence, with a variability in the oscillation pattern between individual platelets. Inhibition of specific receptors such as Par1 and Par4 resulted in decreased fluorescence and oscillation frequencies

Conclusion: By identifying the calcium oscillations of hundreds of platelets it is possible to find heterogeneities in the platelet populations and trends in platelet populations. Combined with evaluation of platelet morphology and activation markers it is possible to detect specific subpopulations and new activation patterns.