

A data-driven human platelet calcium calculator trained by pairwise agonist scanning (PAS) for predicting combinatorial GPVI, PAR-1/4, P2Y1/P2Y12, TP, IP, and GC signaling.

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Background: Platelet activation occurs through multiple signaling pathways in which agonists bind specific receptors on the platelet to trigger signaling in a dose-dependent manner. During a clotting episode, platelets respond to exposed surface collagen, released ADP and synthesized thromboxane, and the protease thrombin, all while being simultaneously modulated by endothelial derived nitric oxide and prostacyclin. These receptor mediated signaling pathways are not independent and significant crosstalk occurs between these signaling pathways. During blood clotting, numerous agonists bind surface and intracellular platelet receptors to regulate intracellular calcium mobilization, $[Ca(t)]_i$. Since platelet $[Ca(t)]_i$ controls granule release, COX-1 and integrin activation, shape change, and phosphatidylserine exposure, the accurate prediction of clotting requires prediction of platelet $[Ca(t)]_i$ in response to multiple agonists.

Method: Pairwise Agonist Scanning (PAS) deployed all single and pairwise combinations of 6 agonists (ADP, convulxin, thrombin, U46619, iloprost and GSNO used at 0.1, 1, and 1x EC_{50} ; 154 conditions) to stimulate platelet P_2Y_1/P_2Y_{12} , GPVI, PAR-1/PAR4, TP, IP receptors, and guanylate cyclase (GC), respectively, in apixaban-treated dilute platelet rich plasma (Fluo-4 loaded for $[Ca(t)]_i$ measurement).¹ This assay allows the contribution of SOCE and includes the pathophysiologic signaling distal of thrombin. Apixaban-treatment of the platelet rich plasma also enables the use of exogenously added thrombin as an agonist in this plasma based assay. Iloprost and GSNO were also included in this assay to recapitulate endothelial-derived prostacyclin and nitric oxide effects on platelet function.

Results: PAS on 10 healthy donors (50 % male) in duplicate allowed training of 10 neural networks (NN, 2-layer/12-nodes). Additionally, trinary stimulations were conducted for the donors at all 0.1x and 1x EC_{50} doses (160 conditions). The NN accurately predicted $[Ca(t)]_i$ for binary stimulation ($R = 0.994$) as expected for successful NN training, but also accurately predicted the traces of $[Ca(t)]_i$ for trinary stimulation ($R = 0.924$), a prediction beyond the PAS training set. The trinary synergy score, a normalized metric of signaling crosstalk based upon integrated $[Ca(t)]_i$, was also well predicted ($R = 0.850$) for the 160 trinary conditions. The NN also reliably predicted $[Ca(t)]_i$ for a random sampling of conditions with 4,5 and 6 agonists (45 conditions total, 1 donor) ($R = 0.76561$). The NN was also able to predict the corresponding synergy scores for these higher order combinations ($R = 0.54293$). Furthermore, the NN was able to predict with reasonable accuracy ($R = 0.8809$), $[Ca(t)]_i$ of all sequential additions of pairs of ADP, convulxin, and U46619 at low medium and high doses (54 conditions).

Conclusions: For computations of clotting, a user can impose time-dependent combinations of the agonists at varying concentrations to estimate $[Ca(t)]_i$ responses that are predictive of those found in healthy human blood. The 10-donor average NN represents a computationally fast and data-trained calcium calculator for platelet responses to 6 critical pathways that regulate coagulation.

References:

1. Chatterjee, M. S., Purvis, J. E., Brass, L. F., & Diamond, S. L. (2010). Pairwise agonist scanning predicts cellular signaling responses to combinatorial stimuli. (Nature Biotechnology).