Dual-agonist Optical Stimulation of Platelets Results in Increased and Reliable Activation

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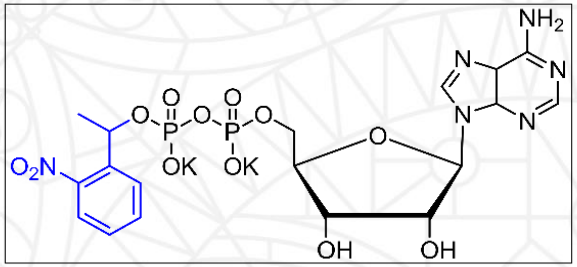
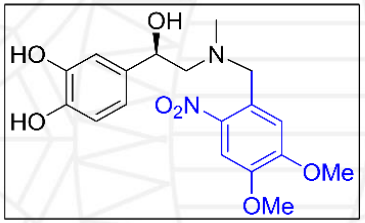
Activation of blood platelets is the main process in normal hemostasis. Unfortunately, it also makes a great contribution to the development of cardiovascular diseases. In this work, we study activation dynamics using cytosolic calcium probe. To increase the accuracy of dynamic measurements, we present a method for optical activation of single platelets in suspension. It is achieved by the use of photolabile "caged" activation agonists. In our earlier works, the use of such compounds resulted in precise measurement of early activation dynamics. Here we show that dual-agonist stimulation dramatically increases the activation probability, resulting in increased and reliable activation with 10-to-50-times less irradiation dose as compared to our previous report.

Photolabile caged agonists of platelet activation:

A. Caged ADP

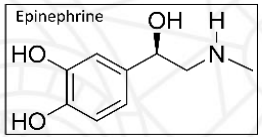


B. Caged Epinephrine

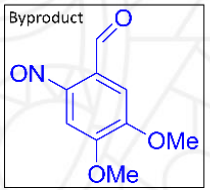


hν, 350 nm

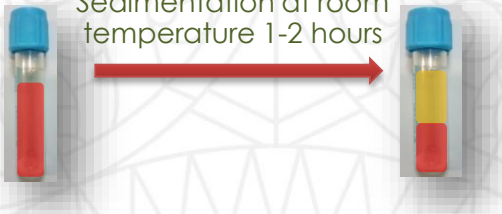
C. Products of photolysis



+



Sample preparation:



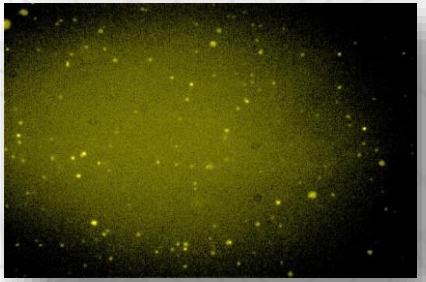
Sedimentation at room temperature 1-2 hours

+ Fluo-4 (10^{-4} M)

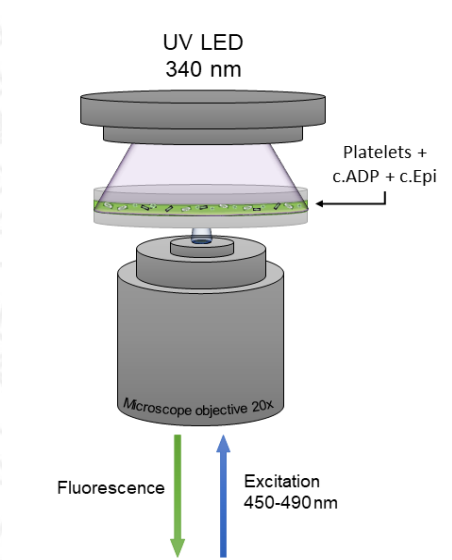
Incubation 30 min in the dark.

Adding agonist

Measurement with agonists: cADP(10^{-4} M)+cEpi ($1,4 \cdot 10^{-6}$ M)

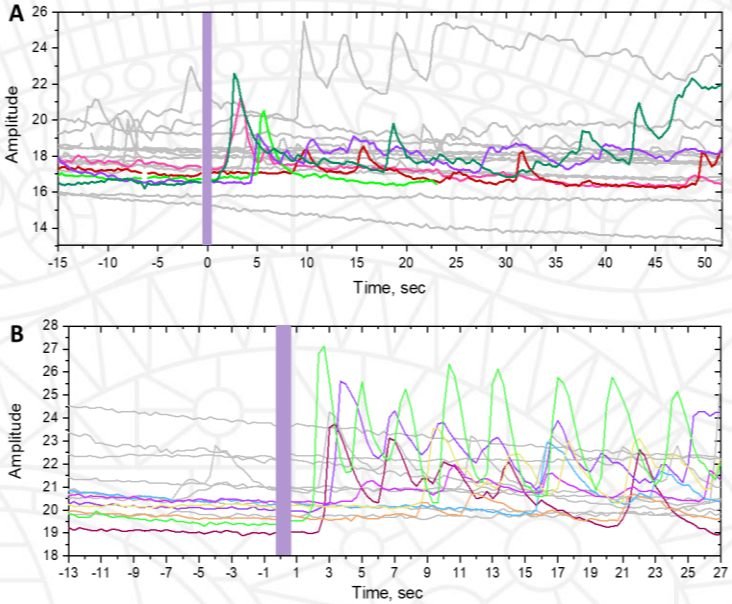


Optical setup:

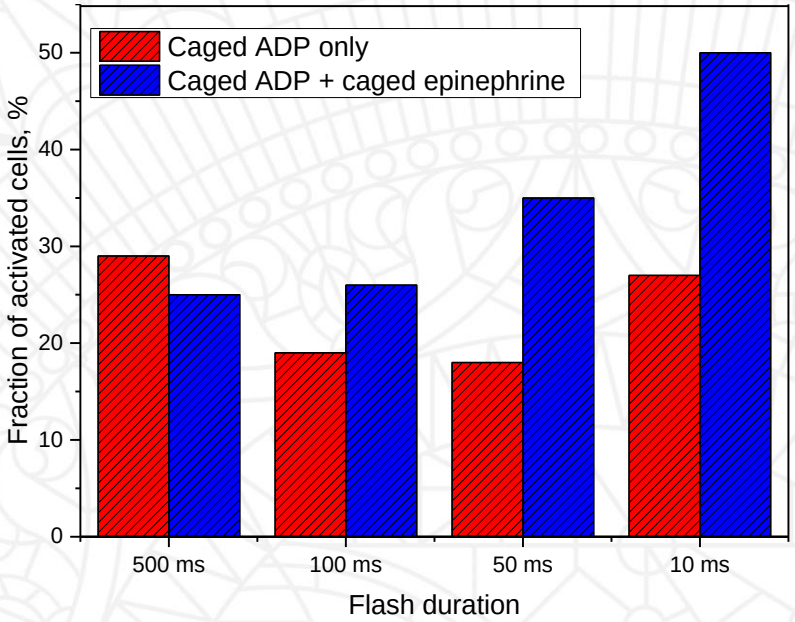


The schematic model of an experimental setup for optical platelet activation. An inverted microscope (Carl Zeiss AxioVert. A1) is used to monitor the intracellular calcium level during platelet activation. Activation is triggered by cleaving of the c.ADP and c.Epi mixture under the UV irradiation.

Results:



A The graph shows several typical calcium responses obtained during experiments with caged ADP alone. There are numerous calcium transients after the UV flash (violet line) which cleaves the caged ADP. B. The graph shows several typical calcium responses obtained during experiments with dual agonists, using caged ADP + caged epinephrine. There are more profound calcium transients after the UV flash (violet line).



The graph shows the activated cells percentage for two methods: red columns correspond to the activation with usage of caged ADP, whereas the blue columns correspond to the activation with usage of caged ADP + caged Epinephrine.

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