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Illustrated Abstracts of the 5th EUPLAN International Conference

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ILLUSTRATED REVIEW

Illustrated Abstracts of the 5th EUPLAN International Conference

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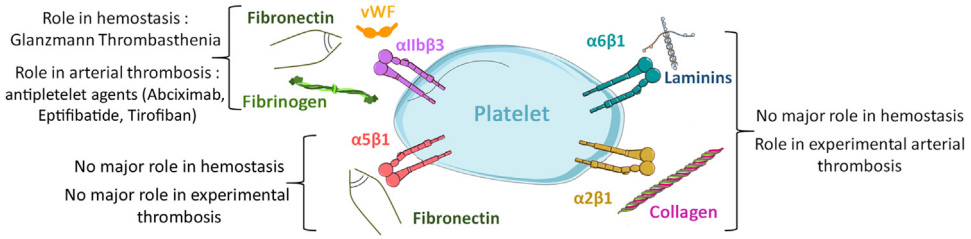
Abstract

These illustrated capsules have been prepared by some speakers of State-of-the-Art talks and of original investigations, presented at the 5th European Platelet Network (EUPLAN) International Conference, which was held at the Università degli Studi di Milano (Italy) on September 28-30, 2022. The programme featured various state-of-the-art lectures and a selection of oral presentations covering a broad range of topics in platelet and megakaryocyte biology, from basic science to recent advances in clinical studies. As usual, the meeting brought together senior scientists and trainees in an informal atmosphere to discuss platelet science in person.

CONTENTS

Role of integrin α5β1 in platelet function, haemostasis and arterial thrombosis in mice

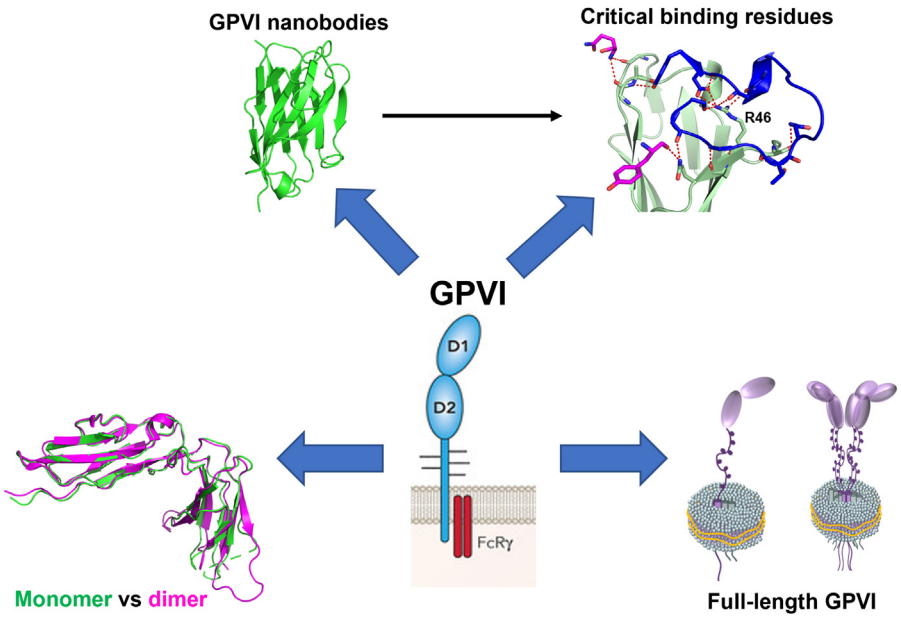
Alexandra Yakusheva



Platelets express five different integrins of the β1 and β3 families. The main β3 integrin is αIIbβ3, playing a major role in hemostasis as evidenced by a hemorrhagic disorder named Glanzmann thrombasthenia and in thrombosis as attested by the clinical use of anti-αIIbβ3 agents (Abciximab, Eptifibatide, Tirofiban). Concerning β1 integrins, α2β1 and α6β1 are involved in the initial step of platelet adhesion and activation and in experimental thrombosis, however, they have no major in hemostasis [1,2]. We and others identified a role for integrin α5β1 in platelet adhesion, activation and aggregation on fibronectin *in vitro*. In $FeCl_3$-, forceps- and laser-injury models of arterial thrombosis, mice invalidated for the platelet α5β1 present a normal profile of thrombus formation [3]. Furthermore, the normal tail-bleeding time favors no major role of this integrin in hemostasis. In conclusion, platelet α5β1 integrin is an important receptor for fibronectin but seems dispensable for hemostasis and experimental thrombosis.

Structural studies of glycoprotein VI: From extracellular domains to full-length

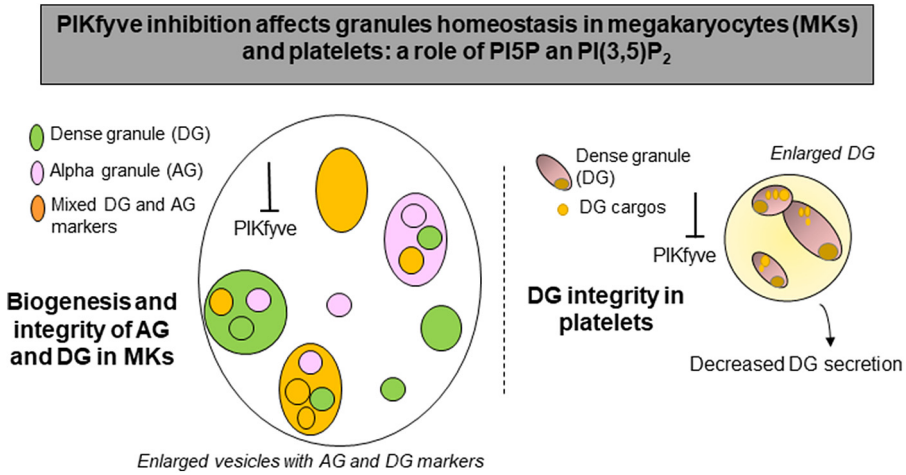
Alexandre Slater, PhD



Glycoprotein VI (GPVI) is the major signaling receptor for collagen on platelets and a promising anti-thrombotic target [4]. We mapped the binding sites of two inhibitory anti-GPVI nanobodies using x-ray crystallography and show they both bind to the same binding pocket within the D1 domain [5] with GPVI residue arg46 being critical for binding. Unlike previous dimeric structures, one of our crystal structures revealed a novel monomeric conformation of GPVI. We show that the conformation of the extracellular domains between the monomer and dimer is the same. This provides evidence against the proposed concept that GPVI dimerization induces a conformational change required for ligand binding [6]. Using styrene maleic acid co-polymer, we extract and purify the full-length GPVI-FcRγ complex from the membrane in a series of oligomeric forms. We aim to use this for full-length structural determination to gain further insight into GPVI structure and new ways to inhibit function.

Phosphoinositides in the regulation of platelet generation and function

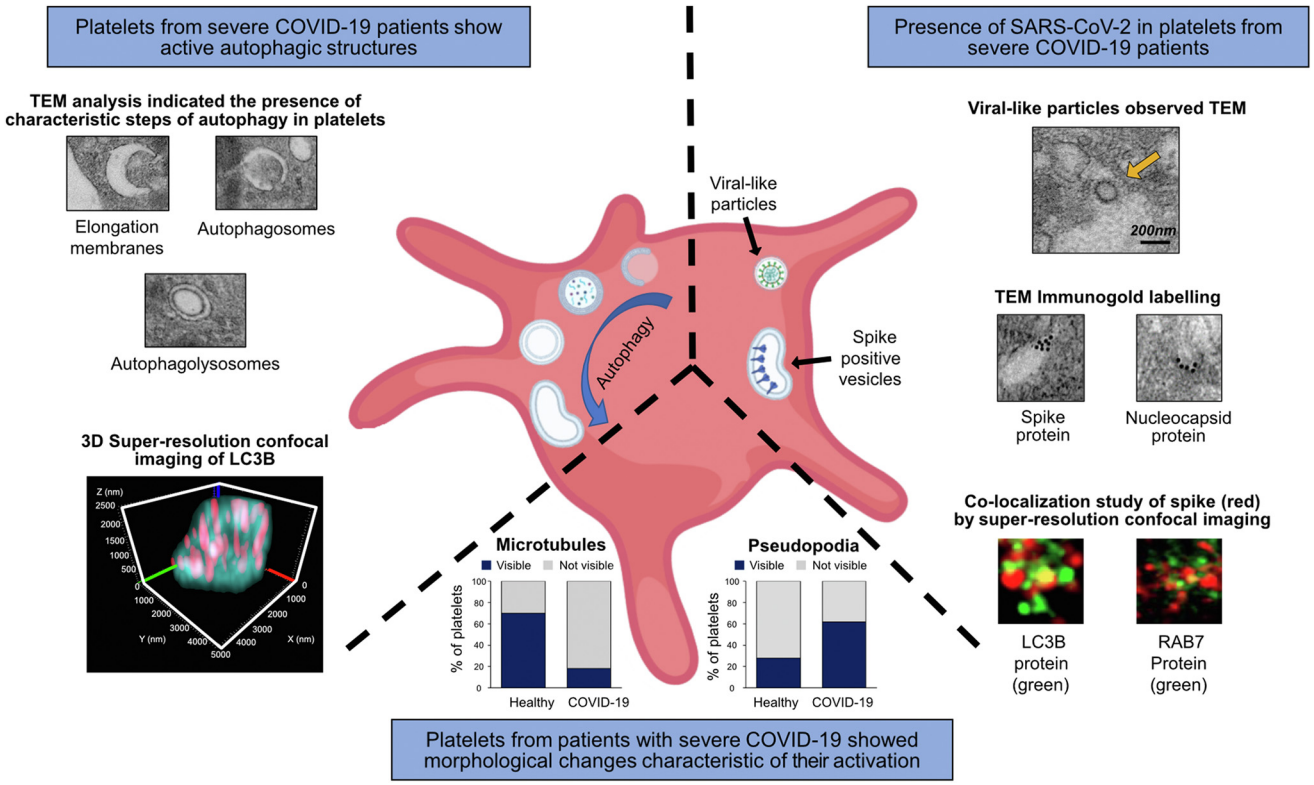
Bernard Payrastre, PhD



Phosphoinositides are a family of lipids playing major roles in cell signaling, membrane remodeling or cytoskeleton reorganization. Their metabolism is highly controlled by a set of specific kinases, phosphatases and phospholipases. Through interactions with protein domains (such as PH, PX, FYVE, etc) these lipids are considered as major spatiotemporal regulators of protein complexes formation. For instance, in platelets, PI 3-kinases and their products have been shown to play major roles [7]. Recently, new mass spectrometric methods (LC-MS/MS) have been developed to accurately quantify the different isomers of phosphoinositides and their fatty acid composition from biological samples with a high sensitivity [8]. Using these methods as well as biochemical and cell biology approaches we found that the phosphoinositide 5-kinase PIKfyve and its products, PI5P and $PI(3,5)P_2$, are involved in the control of granule biogenesis and integrity maintenance in megakaryocytes and platelets [9].

Ultrastructural changes in platelets associated with viral xenophagy in severe COVID-19 patients

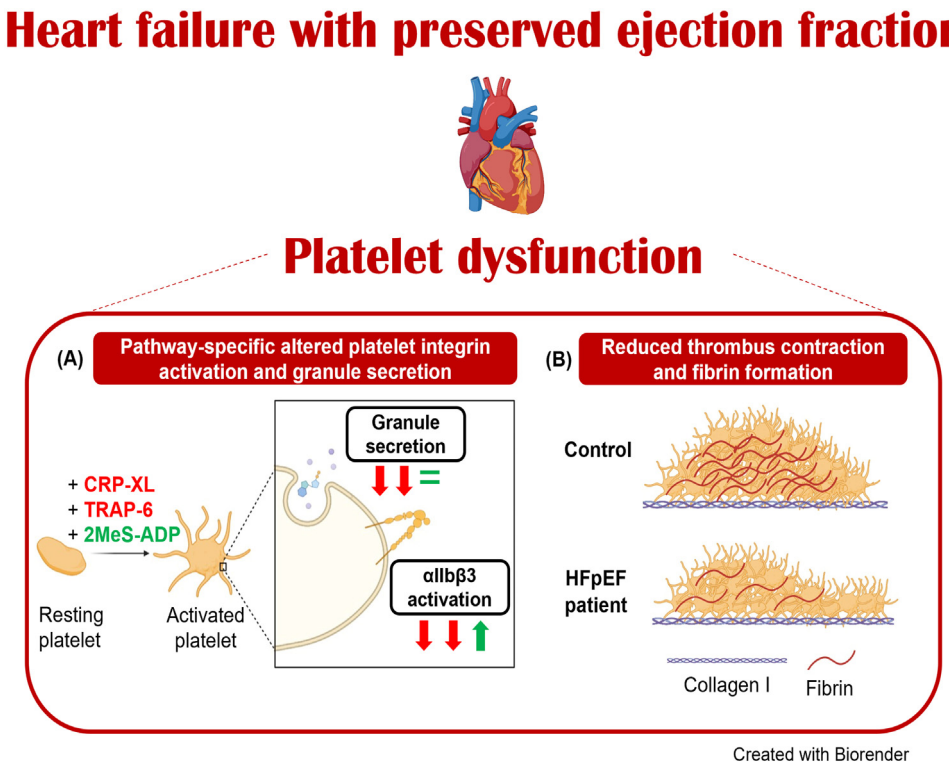
Cédric Garcia



Twenty-nine severe COVID-19 patients were included in the study after their hospitalization in the intensive care unit of the Toulouse hospital [10]. Using transmission electron microscopy (TEM), we observed the presence of virus-like particles in 22% of the platelet population. Immunogold labeling with antibodies targeting spike and nucleocapsid proteins confirmed the presence of the viral material. SARS-CoV-2 is known to manipulate the autophagy system in infected cells [11]. We observed several active autophagic structures in about 20% of platelets. A significant colocalization of spike protein with LC3B and a partial colocalization with RAB7 was observed, suggesting the presence of SARS-CoV-2 material in autophagosomes and late endosomes. TEM analysis also indicated a decrease in microtubule number and an increase in pseudopodia which are signs of platelet activation. Overall, our results showed the presence of the virus in platelets associated with active xenophagy of SARS-CoV-2, a process that may contribute to viral clearance.

Platelet dysfunction in HFpEF patients: reduced platelet activation and thrombus formation under flow

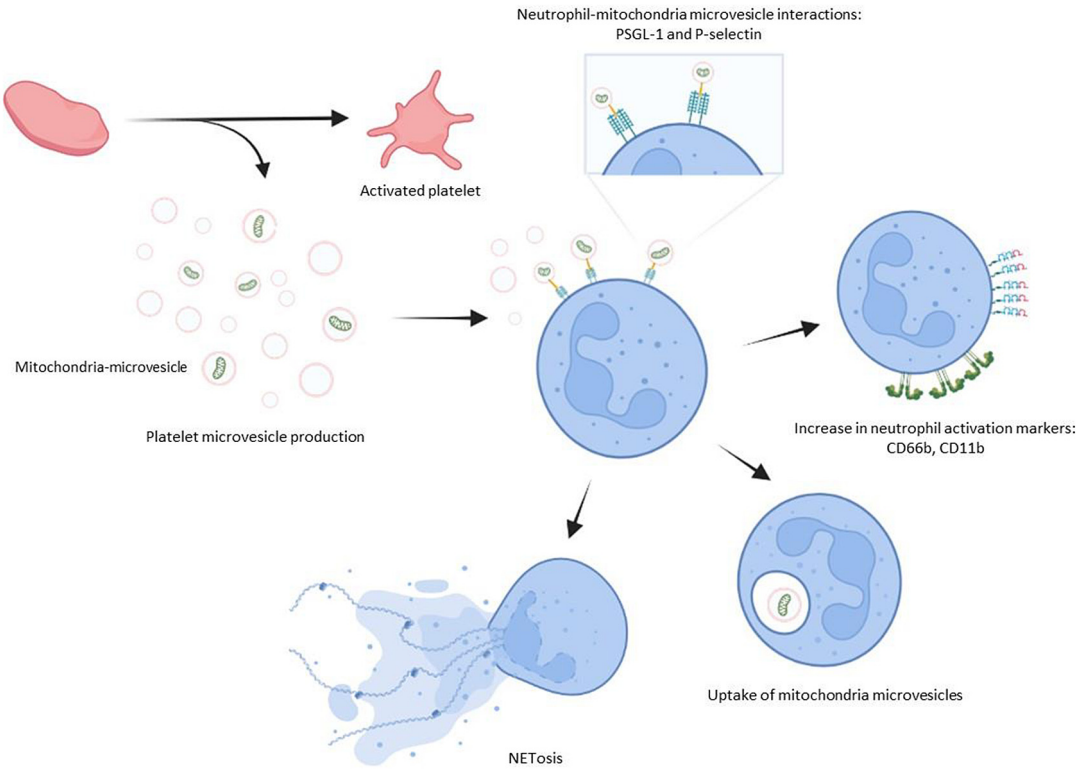
Giorgia D'Italia, MA



Heart failure with preserved ejection fraction (HFpEF) is the result of a complex interplay of systemic syndromes characterized by chronic low-grade inflammation and microvascular dysfunction [12]. Recently, platelets emerged as players in vascular inflammation and endothelial dysfunction, however, the role of platelets in HFpEF is still poorly examined [13]. Platelet functional measurements in more than 100 HFpEF patients reveal a general platelet dysfunction in HFpEF patients compared to age- and sex-matched controls. HFpEF patients show a reduction in platelet activation in response to agonists CRP-XL and TRAP6, an increase in 2MeS-ADP-induced αIIbβ3 integrin activation (A) and an overall reduction in thrombus contraction, density and fibrin formation (B) (Details in the abstract [14]). These alterations in platelet function may suggest a possible contribution of platelets in the complex pathophysiology of HFpEF.

Platelet-released mitochondria interact with neutrophils causing alterations in their phenotype

Harriet Allan, PhD



The production of microvesicles during platelet activation is well documented [15]. More recent research has shown that a small subpopulation of platelet microvesicles contain mitochondria [16]. These mitochondria-containing microvesicles have high levels of surface P-selectin expression and readily interact with isolated neutrophils. This interaction causes upregulation of activation markers, with the neutrophils simultaneously taking on a phagocytic phenotype. In confirmation of this, platelet-mitochondria are detectable within the neutrophil cytoplasm indicating the presence of phagocytic or endocytic pathways. Furthermore, the presence of mitochondria-containing platelet microvesicles enhances the neutrophil's propensity to form neutrophil extracellular traps, with the formation of large elaborate DNA protrusions. This work highlights mitochondrial transfer from platelets and provides insights into their potential role as intercellular communicators. Image created using BioRender.com.

Platelets in atherosclerosis: from experimental models to clinical evidence

Judith M.E.M. Cosemans, PhD

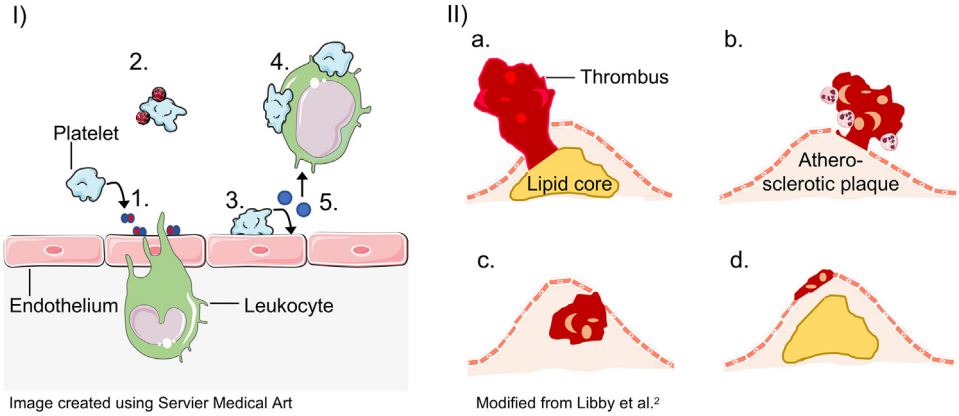


Image created using Servier Medical Art

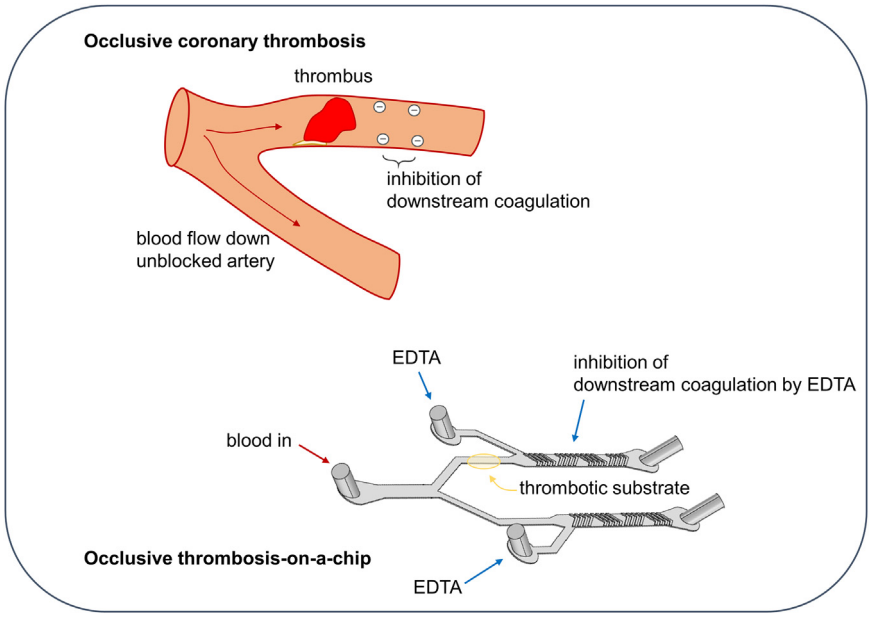
Modified from Libby et al.[2]

I) Platelets can promote murine atherosclerotic plaque formation by facilitating leukocyte adhesion and transmigration via depositing chemokines (1), via direct interaction with oxidized low-density lipoprotein (2), the endothelium (3) or leukocytes (4), and via releasing extracellular vesicles (5) [17]. The relevance hereof for human atherogenesis is incompletely understood [17].

II) (Post-)Thrombotic complications of a plaque. Thrombi formed on human ruptured plaques are found to be rich in red blood cells and fibrin (a), whereas eroded plaques appear to give rise to platelet- and neutrophil-rich thrombi (b) [18]. In time, thrombi formed on disrupted coronary plaques may expel platelets while the red blood cells in the thrombus can become polyhedrocytes [19]. The mechanisms underlying this heterogeneity in thrombus composition in space and time are only partially uncovered. Human plaques can grow by incorporating thrombi (c). In mouse models, platelet-thrombi have been demonstrated to also actively promote local plaque growth (d) [17].

Occlusive thrombosis-on-a-chip

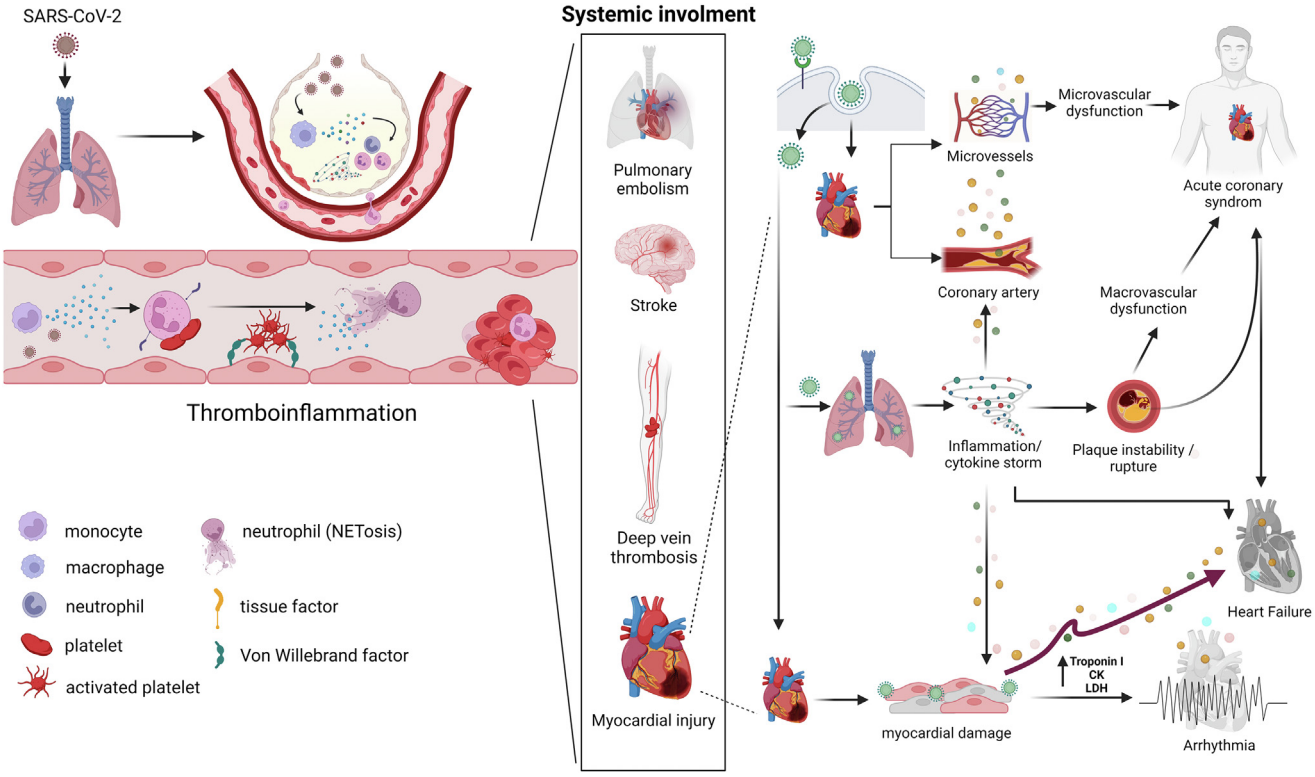
Matthew Harper, PhD



Microfluidic devices are versatile tools to study a wide range of diseases, including arterial thrombosis. Although they are usually a simplification of the in vivo disease process, the differences between the microfluidic disease model and the in vivo situation are themselves useful, as they can reveal important biological aspects that may otherwise have been overlooked. We and others [20-22] have used a 'pressure-relief' design to model occlusive arterial thrombosis, mimicking a thrombus within the branching arterial system. Our recent development of this model uses the divalent cation chelator, EDTA, to quench coagulation downstream of the thrombus initiation site. This new model has revealed the importance of anti-coagulation downstream of a thrombus, presumably by the endothelium, in localising thrombosis to the site of plaque rupture.

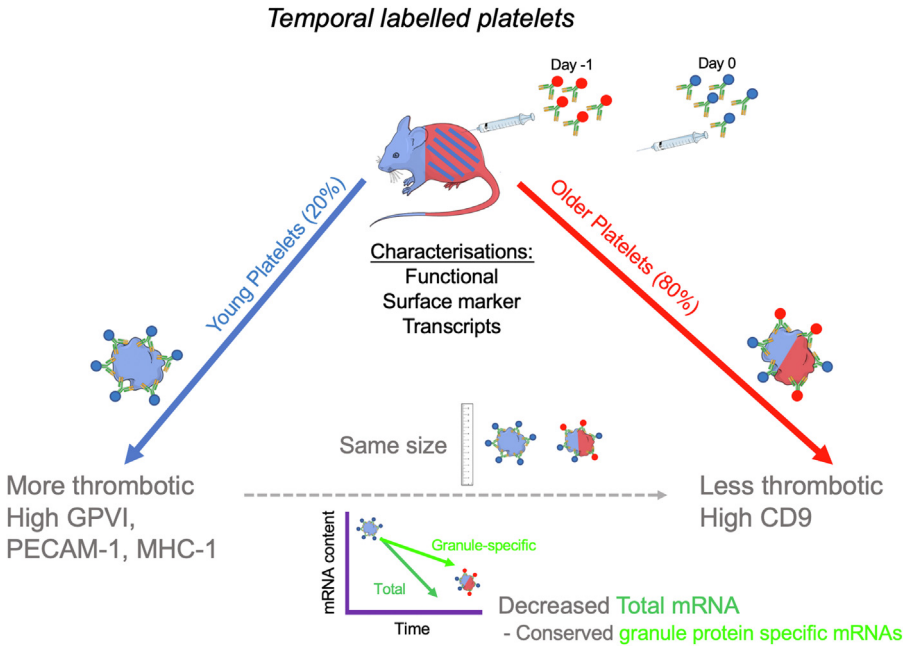
Platelets, thromboinflammation and COVID-19

Meinrad Gawaz



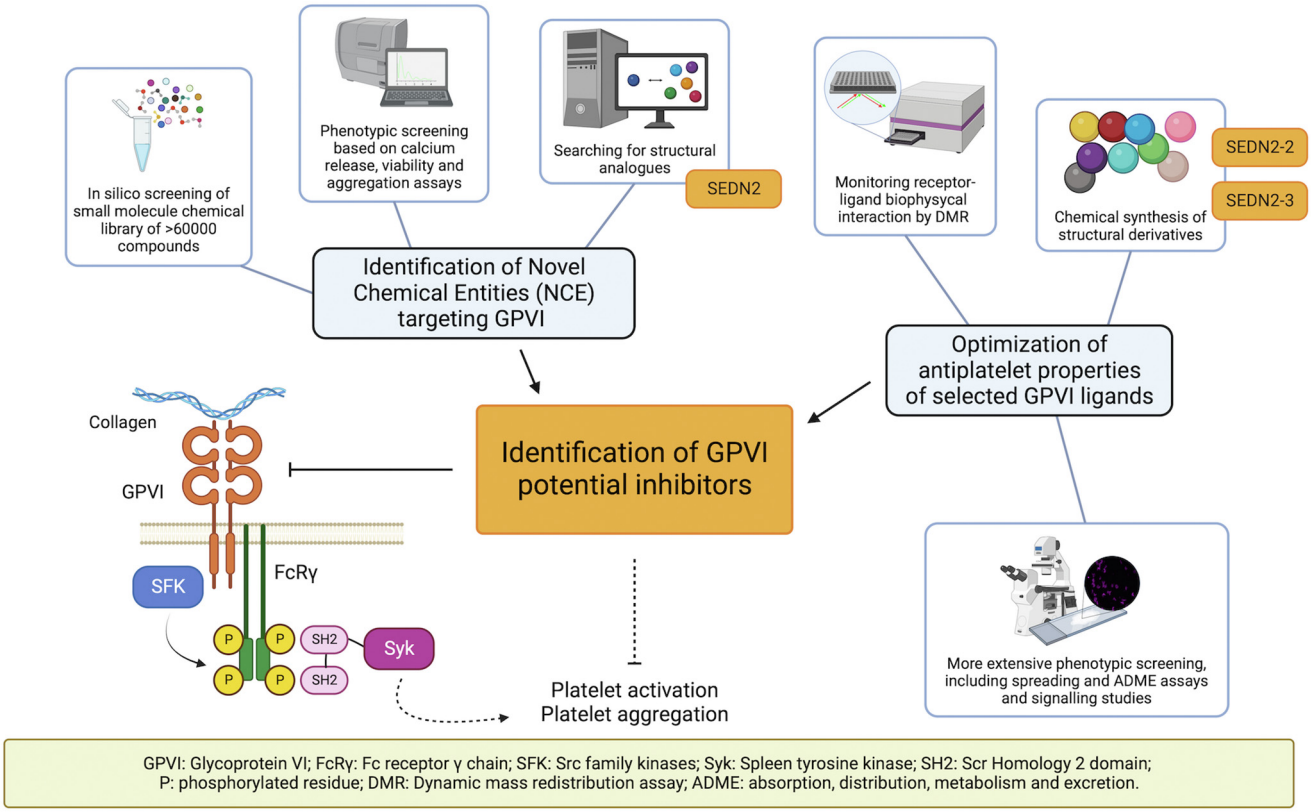
Temporal labelling uncovers functional and molecular changes of platelets as they age in the circulation

Paul Armstrong



Identification of novel inhibitors of the platelet collagen receptor GPVI by a phenotypic screening assay

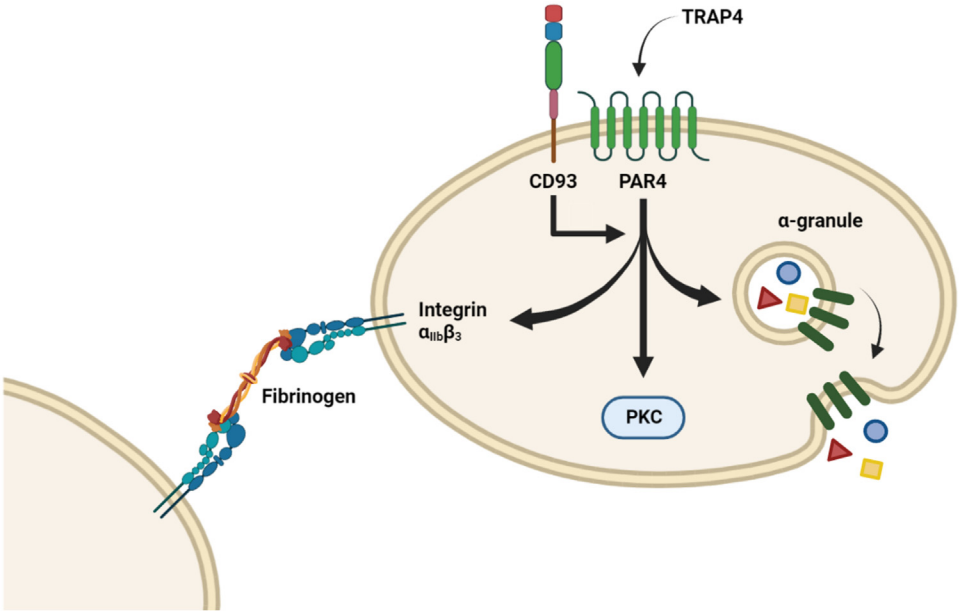
Sara Troitiño, MSc



Due to the significant risk of bleeding associated to current antiaggregant therapy, finding antiplatelet drugs modulating thrombus formation without altering haemostasis is needed. The collagen receptor glycoprotein VI (GPVI) is a promising target, since not only it is uniquely expressed in platelets and megakaryocytes, but its blockade inhibits thrombosis without compromising haemostasis [23]. Moreover, high surface levels of GPVI and platelet hyperactivation were observed in pathologies involving alterations in platelet function, such as severe obesity [24]. By a phenotypic screening including a biophysical ligand-receptor binding assay, intracellular calcium release and aggregation studies, coupled with chemical synthesis strategies of structural analogues, SEDN2 and its derivatives SEDN2-2 and SEDN2-3 were identified as effective molecules inhibiting platelet aggregation and spreading selectively via blocking GPVI. Pending further functional assays and new modification strategies to improve their pharmacological properties, these data profile this family of molecules as a source of promising candidates to be taken into pre-clinical trials.

The C-type lectin CD93 regulates platelet activation induced by par4 stimulation

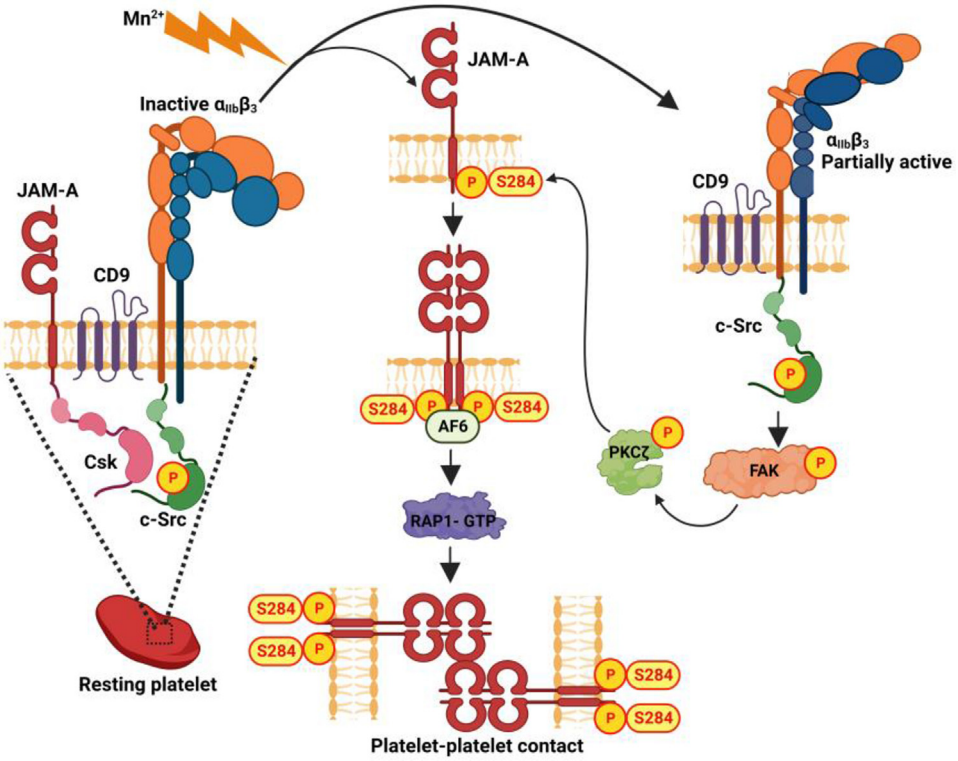
Silvia Maria Grazia Trivigno, PhD



CD93 is a member of C-type lectin superfamily which plays a prominent role in inflammation, vascular diseases, angiogenesis and cancer [25,26] and has never been studied in the context of platelet function. Platelets from CD93-knockout (KO) mice display a defective aggregation upon selective stimulation of PAR4 receptor, using the PAR4-activating peptide TRAP4. Moreover, PKC activation, α-granule release and integrin αIIbβ3 activation induced by TRAP4 are significantly reduced in CD93KO platelets. PAR4 is equally expressed on WT and CD93KO platelet surface in resting conditions but, upon stimulation with TRAP4, CD93KO platelets show a marked reduction of PAR4 expression on their surface. This is associated to a more pronounced desensitization of CD93-deficient platelets compared to WT controls upon stimulation with subthreshold doses of TRAP4, suggesting a possible role of CD93 in the prevention of PAR4 early desensitization.

Conformational change in platelet $\alpha_{IIb}\beta_3$ induce PKC-dependent serine 284 phosphorylation of junctional adhesion molecule-A and Rap1 activation

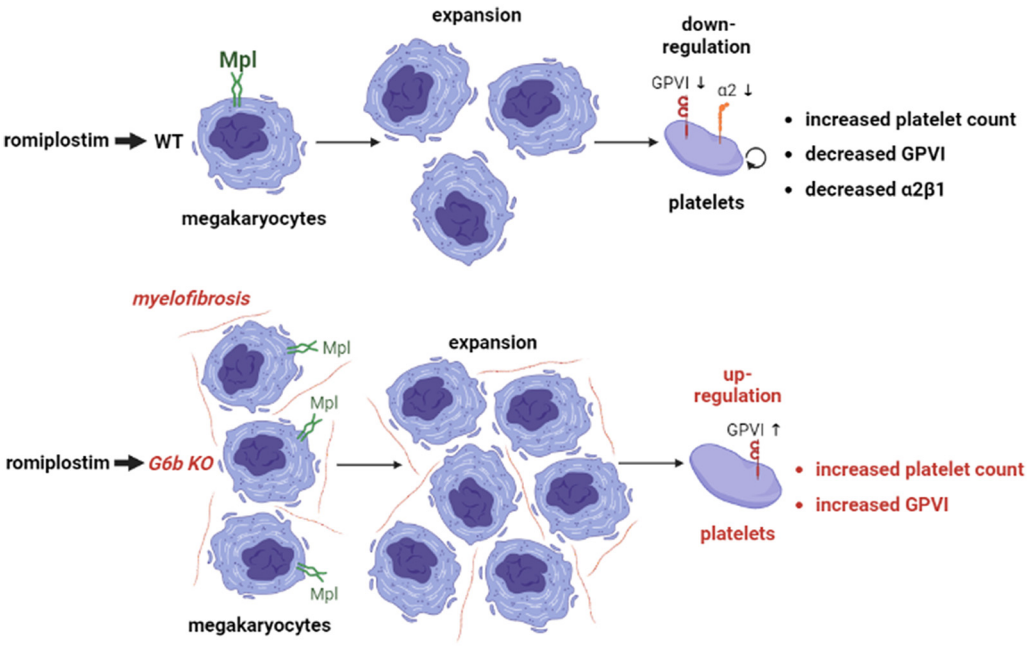
Ulhas P. Naik, PhD



JAM-A suppresses platelet activation by recruiting CSK to αIIbβ3-Src and CD9 complex in resting platelets [27,28]. Agonist-induced inside-out signaling results in the dissociation of JAM-A from αIIbβ3 -Src and CD9 complex. Platelet activation results in rapid phosphorylation of JAM-A S284. Agents such as Mn2+, RGDS, and DTT that are known to induce partial change in the confirmation of αIIbβ3 in the absence of inside-in signaling [29] also induce phosphorylation of JAM-A S284. Partial conformation change in αIIbβ3 is although not sufficient in ligand-binding, it is sufficient in inducing outside-in.

Molecular insights into the cause and treatment of congenital thrombocytopenia in mice lacking the co-inhibitory receptor G6b-B

Yotis A. Senis



Mice lacking the immunoreceptor tyrosine-based inhibition motif-containing co-inhibitory receptor G6b-B (*Mpig6b*, *G6b* knockout, *KO*) are born with a complex megakaryocyte/platelet phenotype characterized by severe macrothrombocytopenia, MK expansion and myelofibrosis [30]. Platelets are almost completely devoid of the GPVI-FcR γ-chain collagen receptor complex and a subset have increased surface immunoglobulins. A similar phenotype was recently reported in patients with null and loss-of-function mutations in *MPIG6B* [31]. In our study, we show that genetically ablating or inhibiting the immune response with either intravenous immunoglobulin or the Syk tyrosine kinase inhibitor BI1002494 only partial rescues aspects of the platelet phenotype of *G6b KO* mice, whereas treatment with the thrombopoietin mimetic romiplostim rescues platelet count, GPVI expression and collagen reactivity [32]. Intriguingly, GPVI and α2β1 expression are significantly downregulated in romiplostim-treated wild-type (WT) mice, suggesting a cell intrinsic feedback mechanism that auto-regulates platelet reactivity in a context dependent manner [32].

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