DISCOVERY OF A NOVEL INTERACTION SITE BETWEEN GPIbα AND VWF

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Aim: investigate whether an alternative PPI site exists for GPIbα^{GOF} on VWF

Project background

Von Willebrand factor (VWF) is essential for mediating primary hemostasis. Upon vascular breach (i.e. an injury), VWF elongates, exposing previously cryptic interaction sites [1]. One such site, the VWF-A1 domain, is the canonical interaction site for platelet glycoprotein Ib alpha (GPIbα) [2]. This protein-protein interaction (PPI) is the first in a long series of PPIs that culminate in clot formation. Many bleeding disorders are derived from defects in clotting-related PPIs. To this point, platelet-type von Willebrand disease (PT-VWD) is a rare platelet disorder that results in a hyperactive association of platelets to VWF, mediated by gain-of-function (GOF) mutations within GPIbα [3]. However instead of thrombosis, ***PT-VWD patients counterintuitively suffer from prolonged bleeding***. PT-VWD is both underdiagnosed and does not have an effective therapeutic. We therefore developed a peptide inhibitor called G14 that displays specificity towards GPIbα^{GOF} [4]. While the peptide disrupts the PPI between GPIbα^{GOF} and full-length VWF, it did not disrupt the PPI between GPIbα^{GOF} and the canonical GPIbα interaction domain: VWF-A1. This led us to speculate that an alternative interaction site for GPIbα^{GOF} exists somewhere within full-length VWF.

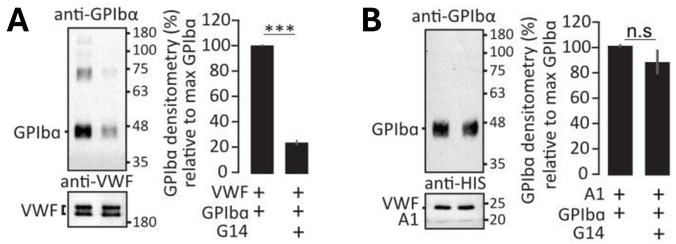
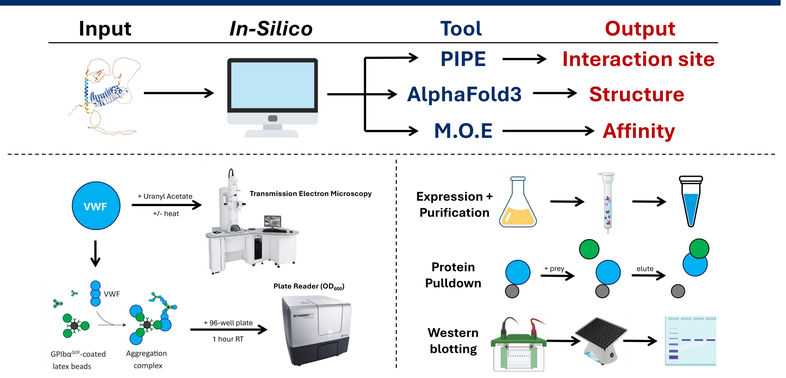


Figure 1: Initial evidence supports the existence of an alternative interaction site for GPIbα^{GOF} on VWF. (**A**) The G14 peptide disrupts the PPI between full-length, recombinant VWF and GPIbα^{GOF} by approximately 80%. (**B**) The G14 peptide does not interfere with the GPIbα^{GOF}-VWF A1 PPI. These experiments were performed in biological and technical triplicate. All errors are derived from the standard deviation, with significance assessed using a two-tailed unpaired T-test. *** indicates a P-value less than or equal to 0.001. "n.s" refers to a P-value that is greater than or equal to 0.05.

Materials and methodologies



PPI prediction software suggest GPIbα^{GOF} can interact with an alternative VWF domain

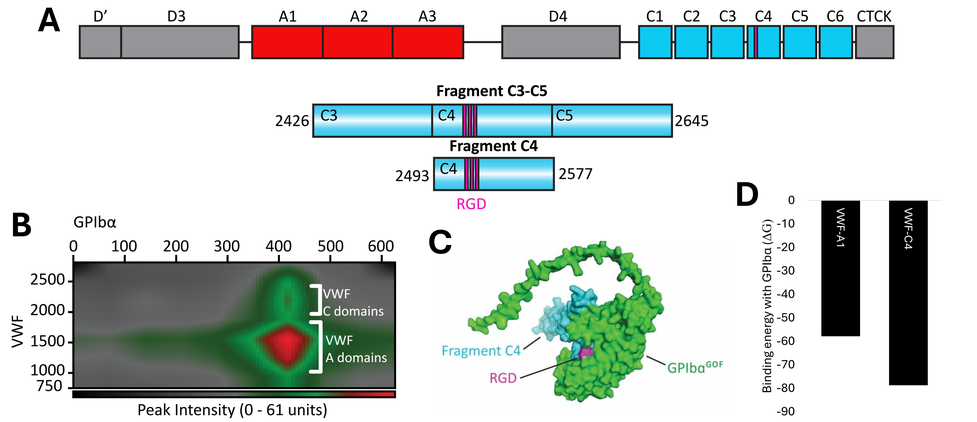


Figure 2: PPI prediction suggests that VWF-C4 might act as an alternative interaction site for GPIbα^{GOF}. (**A**) A schematic of the VWF domains. The three "A" domains are highlighted in red, whereas the six "C" domains are highlighted in blue. (**B**) PIPE [5] predicts that GPIbα interacts with two independent sites on VWF – an A domain and a C domain (indicated by red colours). Green and grey colours denote little or no predicted interaction. (**C**) GPIbα^{GOF} (green) is predicted to dick with the C4 fragment (blue) via AlphaFold3 [6]. The RGD domain is highlighted in magenta. (**D**) Molecular operating environment (MOE) software predicts that GPIbα^{GOF} has a greater affinity for the C4 domain when compared to the canonical VWF-A1 domain.

GPIbα^{GOF} binding to VWF persists even when the VWF-A1 canonical GPIbα binding domain is hidden

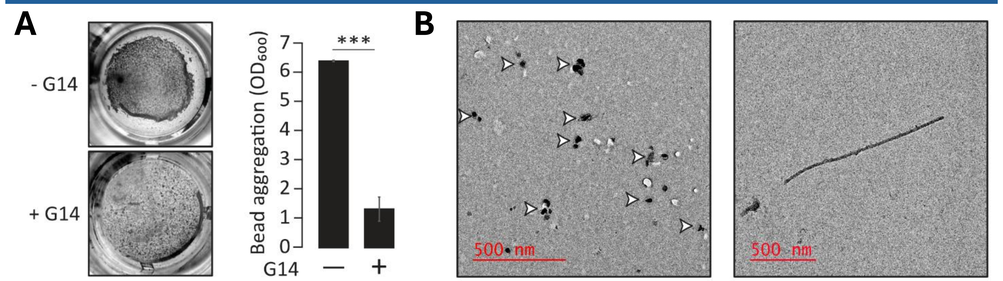


Figure 3: GPIbα^{GOF} interacts with globular VWF. (**A**) An aggregation assay demonstrating that GPIbα^{GOF}-coated latex beads interact with recombinant VWF. These aggregates are disrupted by the presence of G14. (**B**) Transmission electron micrographs of recombinant VWF at room temperature or 1 hour at 95°C. The white arrows depict VWF globules. Scale bar = 500 nm. These experiments were performed in technical triplicate. All errors are derived from the standard deviation, with significance assessed using a two-tailed unpaired T-test. *** indicates a P-value less than or equal to 0.001.

References

[1] Belyaev AV. 2021. *Biophys J.* **120**(5):899-911.
[2] Bonazza, A., *et al.* 2022. *eLife.* **11:**e75760.
[3] Fu, A., *et al.* 2025. *STH.* **51**(02):219-226.
[4] Kazmirchuk, TDD, *et al.* 2024. *RPTH.* **8**(Suppl. 2):e102503.
[5] Pitre, S., *et al.* 2006. *BMC Bioinf.* **7**:365.
[6] Abramson, J., *et al.* 2024. *Nature.* **630**: 493-500.

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GPIbα^{GOF} forms a strong physical interaction with VWF-C4 which interferes with the VWF-$\alpha_{IIb}\beta_3$ PPI

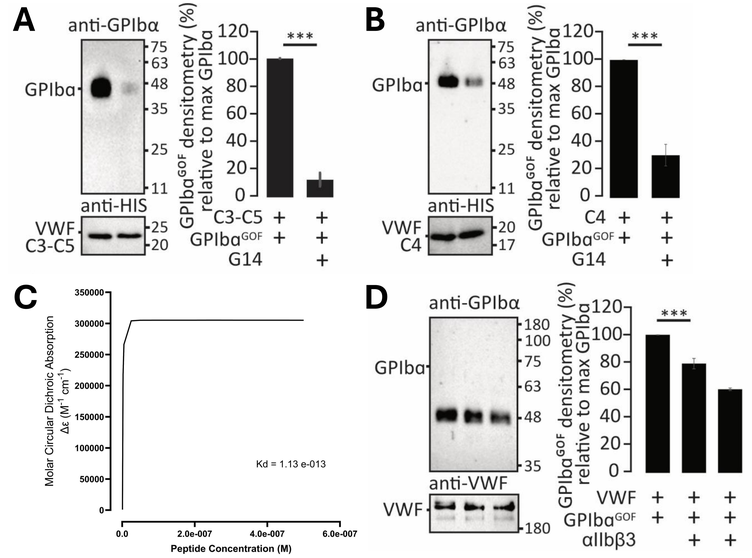

Figure 4: GPIbα^{GOF} forms a strong PPI with both the VWF C3-C5 and VWF-C4 protein fragments, which can be disrupted by G14. (**A**) The G14 peptide disrupts the PPI between VWF C3-C5 and GPIbα^{GOF}, reducing the PPI by approximately 90%. (**B**) The G14 peptide disrupts the GPIbα^{GOF}-VWF C4 PPI, reducing the PPI by approximately 70%. (**C**) Affinity curve for the VWF-C4- GPIbα^{GOF} interaction. VWF-C4 appears to display a dissociation constant (Kd) of 0.1 pM (**D**) GPIbα^{GOF} disrupts the $\alpha_{IIb}\beta_3$-VWF interaction. These experiments were performed in biological and technical triplicate. All errors are derived from the standard deviation, with significance assessed using a two-tailed unpaired T-test. *** indicates a P-value less than or equal to 0.001.

Conclusions

GPIbα^{GOF} binds to VWF-C4 domain, thus implicating VWF-C4 as a novel interaction site for the mutated platelet protein. This work supports new insights into the physiological interaction between VWF and platelet as well as the pathology of platelet disorders, particularly PT-VWD.

Future directions

1) Determine the effect of RGD point mutations on the GPIbα^{GOF} - C4 PPI
2) Introduce C4 mutations into full-length VWF and assess GPIbα^{GOF} PPI
3) Assess this interaction using PT-VWD patient derived platelets

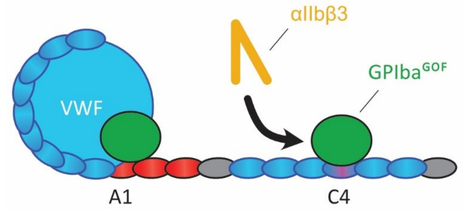

Figure 5: A proposed implication for GPIbα^{GOF} interacting with VWF-C4.