Using artificial intelligence to disrupt the vWF- GPIBα interaction in platelet-type von Willebrand disease

Thomas DD Kazmirchuk[1], Keaun Amani[2], Frank Dehne[3], Maha Othman[4,5,6], and Ashkan Golshani[1]

[1]Department of Biology and the Ottawa Institute of Systems Biology, Carleton University, Ottawa, Ontario, K1V 0V7, Canada
[2]Neurosnap Incorporated, Wilmington, Delaware, USA
[3]School of Computer Science, Carleton University, Ottawa, Ontario, K1S 5B6, Canada
[4]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, K7L 3N6, Canada
[5]School of Baccalaureate Nursing, St. Lawrence College, Kingston, Ontario, K7L 5A6, Canada
[6]Clinical Pathology Department, Mansoura University, Mansoura, 35516, Egypt

BACKGROUND

Platelet glycoprotein-1B alpha (GPIBα) is a surface glycoprotein that is functionally defective ($GPIB\alpha^{FD}$) in platelet-type von Willebrand disease (PT-VWD). Gain-of-function mutations within the β-sheet of the glycoprotein convert GPIBα to an open conformation resulting in the excessive association of $GPIB\alpha^{FD}$ with its receptor von Willebrand clotting factor (vWF), and counterintuitively a bleeding phenotype. The β-sheet mutations (clustered within the Trp230 - Met239 window) therefore represent an attractive site to develop therapeutics. We recently developed the In-Silico Protein Synthesizer (InSiPS) – an artificial intelligence that designs small peptides which interfere with important protein-protein interactions (PPIs).

AIMS

We are using InSiPS to design small peptides that disrupt the interaction between $GPIB\alpha^{FD}$ and vWF.

METHODS

We used InSiPS to generate peptides specific to $GPIB\alpha^{FD}$. The resulting peptides were validated using docking simulations and *in-vitro* binding assays. A total of 23 peptides were generated and assessed for target affinity using AlphaFold2 software. Peptide specificity towards $GPIB\alpha^{G233V, M239V}$ was assessed *in-vitro* using differential scanning fluorimetry (DSF), aggregation assays, and a pulldown assay.

RESULTS

Derived from our InSiPS AI (Figure 1), one peptide (G14) displayed high affinity towards $GPIB\alpha^{G233V, M239V}$ (Figure 2A). Via DSF, G14 was found to increase the melt peak of $GPIB\alpha^{FD}$ (Figure 2B) in a dose-dependent manner. Furthermore, G14 disrupts the PPI between $GPIB\alpha^{G233V, M239V}$ and vWF via a latex bead aggregation assay (Figures 2C and D) in a dose-dependent manner. These results are supported by a pulldown assay, which indicates that G14 disrupts the interaction between $GPIB\alpha^{G233V, M239V}$ and vWF.

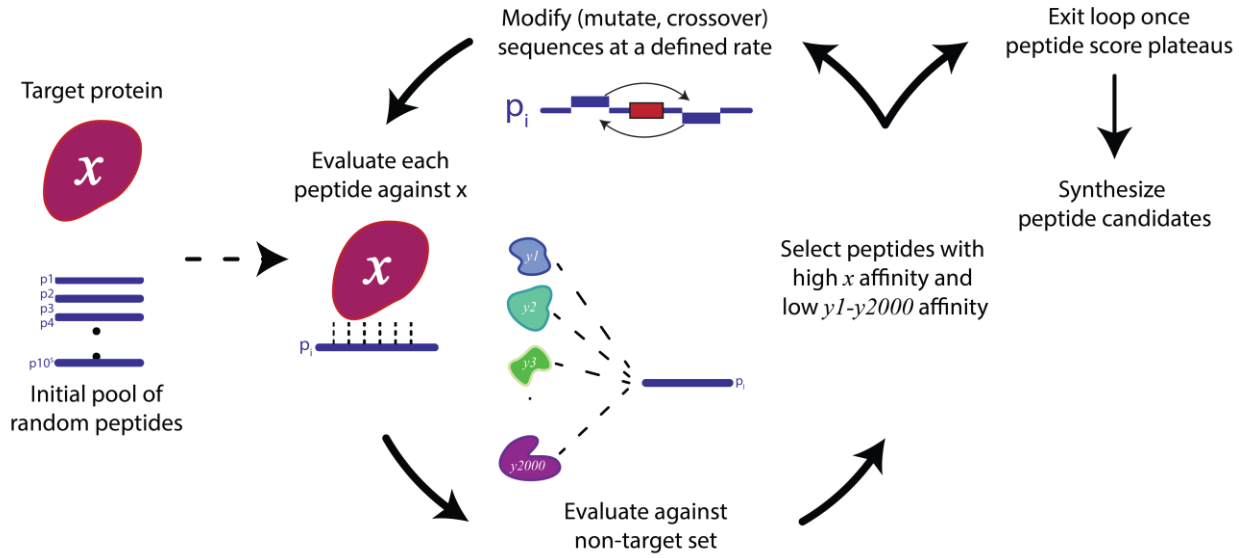

Figure 1: The methodology underlying the *In-Silico Protein Synthesizer* (InSiPS) AI. InSiPS designs peptides to specific regions of a protein based on known PPIs. A total of 10,000 randomly generated peptides are subjected to 1000+ cycles of positive and negative selection until peptides with high target affinity are designed. These peptides are then subjected to mutations and crossovers *in-silico* to maximize predicted affinity. The resulting designed peptides are then synthesized and are subject to *in-vitro* confirmation.

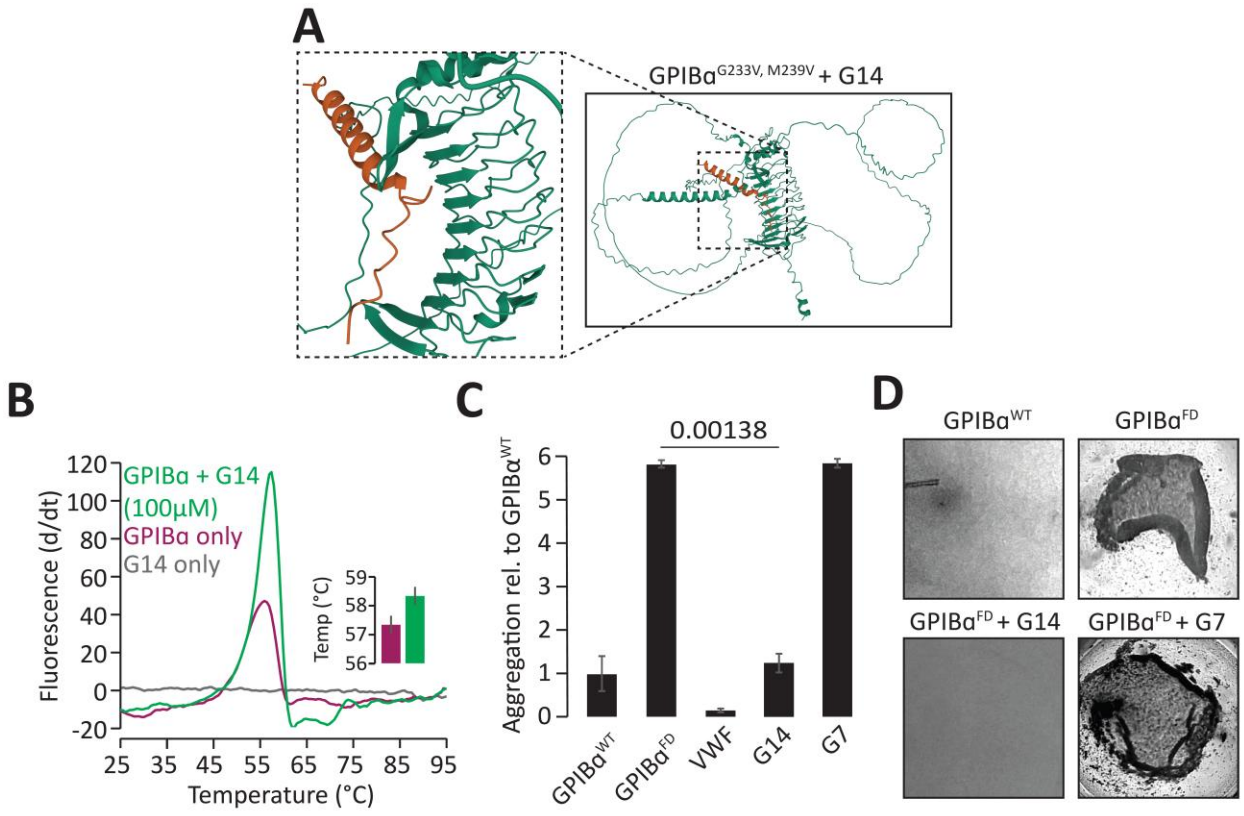

Figure 2: Peptide G14 disrupts the interaction between GPIBα$^{G233V, M239V}$ and vWF. (A) AlphaFold2 docking software predicts G14 interacts with the β-sheet of GPIBα$^{G233V, M239V}$. (B)

Peptide G14 shifts the melt peak of GPIBα$^{G233V, M239V}$ by 1.5°C at a concentration of 100μM. (C) The G14 peptide prevents the aggregation between GPIBα$^{G233V, M239V}$-coated latex beads and vWF. The random G7 peptide has no effect on the aggregation of GPIBα$^{G233V, M239V}$ to vWF. (D) Representative images for the aggregation assay in “C”.

CONCLUSIONS

This study demonstrates that the AI-generated G14 peptide can bind to and disrupt the interaction between GPIBα$^{G233V, M239V}$ and vWF. Consequently, G14 is a potential drug candidate for PT-VWD. Future work will assess the efficacy of the G14 candidate in mammalian systems.