# Plasmin-Clipped Beta-2-Glycoprotein I: Novel Structures Lead to Increased Antibody Binding

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## What is Beta-2-Glycoprotein I (B2GPI)?

- Serum protein circulating at 0.2mg/ml.
- 5 Domain Protein.
  - 4 Sushi/CCP Domains, 1 larger lysine rich domain.
- Regulates Complement and Coagulation cascades.
  - Unique → Can up and down regulate both cascades.
- Main autoantigen in Antiphospholipid Syndrome
  - Leading cause of strokes < 50 years old
  - Leading acquired cause of miscarriage
- Has 2 structures – open J shape and closed O shape
  - Theorised to control changes in function by shifting structure
  - Methods of shifting structure proven: Acetylation, Reduction and pH Change
- Is a substrate for Plasmin in the 5th domain.

### How Does Plasmin Cleavage Alter B2GPI Function?

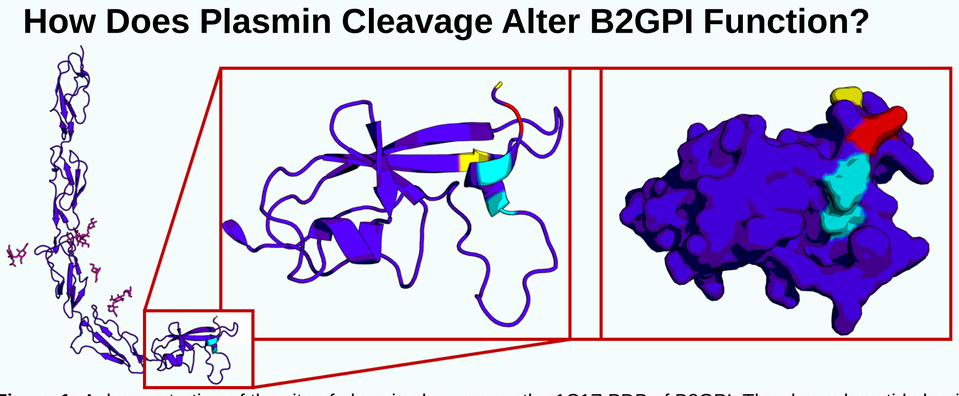


**Figure 1:** A demonstration of the site of plasmin cleavage on the 1C1Z PDB of B2GPI. The cleaved peptide begins at the cyan Aspartic acids and runs through the red lysines to the yellow terminal cysteine. This highlights the change in charge in the local area with the loss of these residues.

## Why is Plasmin Cleavage Important?

- Plasmin cleavage is associated with significant shift in function for B2GPI including:
  - Loss of binding to negative surfaces and plasminogen
  - Loss of inhibition of FXI activation
  - Increased inhibition of VEGF/bFGF
  - Binding to Angiostatin driving anti-angiogenic pathways.
- Plasmin cleaved B2GPI has also been detected in other conditions including foetal heart block and leukaemia.

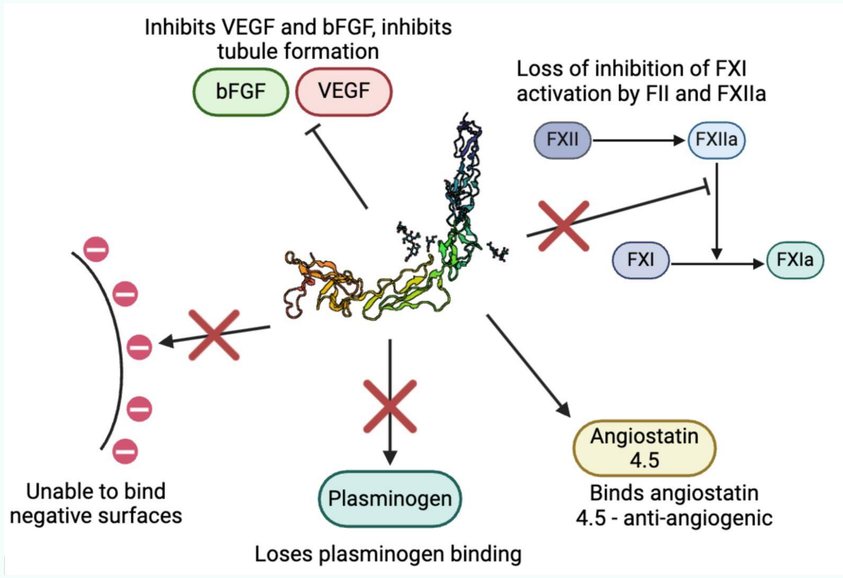



**Figure 2:** A summary of the changed functions of B2GPI post cleavage, with its loss of binding to surfaces, loss of regulation for coagulative purposes and seemingly a gain in anti-angiogenic properties.

## Does Plasmin Cleavage Alter Function Through Structural Change?

## Dynamic Light Scattering Indicates Smaller Diameter Post Cleavage

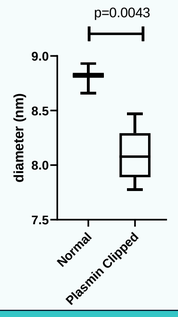



**Figure 3:** Dynamic Light Scattering shows both a significantly smaller diameter for plasmin clipped B2GPI but also a greater variation. This may suggests an increased flexibility in the cleaved molecule.

## Ion Mobility Mass Spectrometry Reveals Structural Shift Post Cleavage

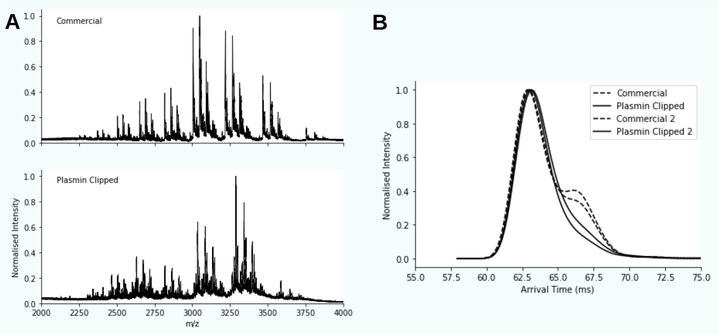



**Figure 4:** Initial mass spectrometry showed extremely similar profiles for the clipped and non-clipped (commercial) species with similar glyco-isomers identified (A). Cyclic Ion Mobility Mass Spectrometry revealed a change in structure, with the late arriving shoulder absent in the clipped protein (B).

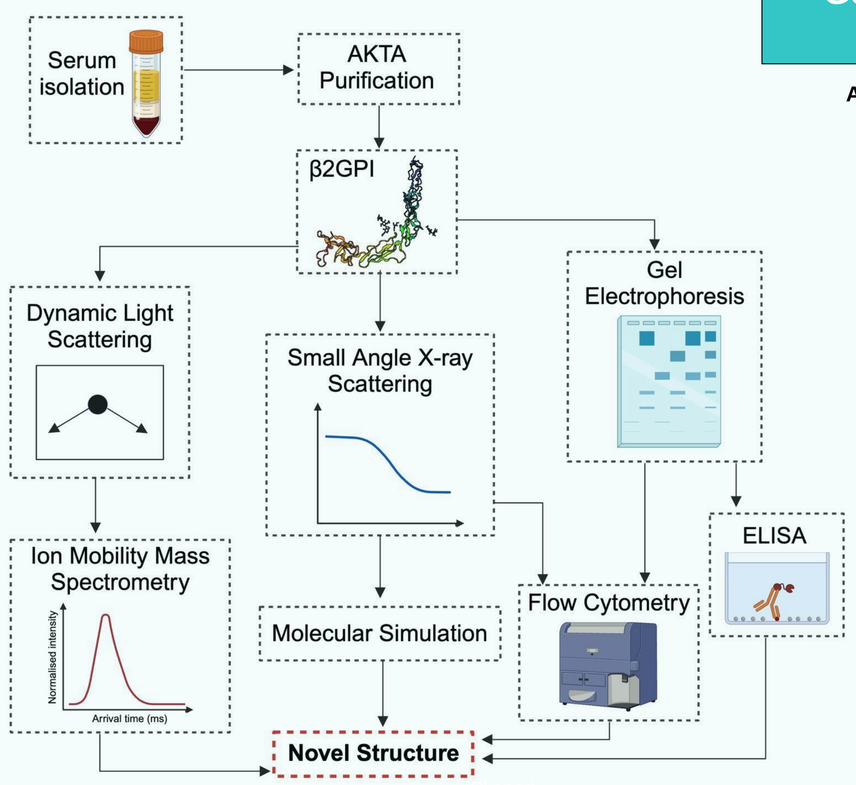



**Figure 8:** A diagram to identify the orthogonal methods used during the project.

## Gel Electrophoresis Demonstrates Altered Reduction Mobility and Altered Flexibility

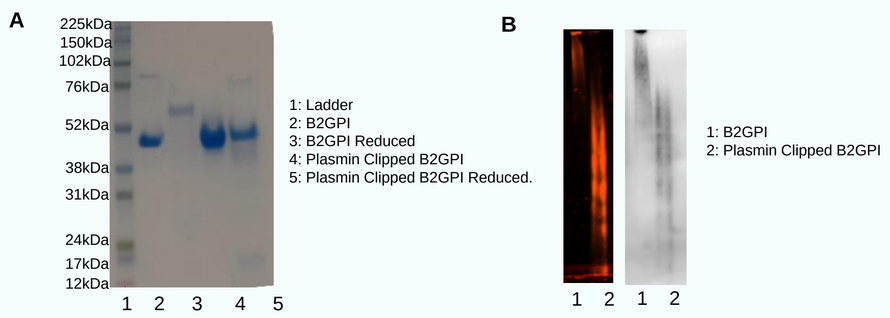
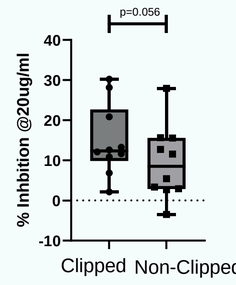



**Figure 6:** SDS-PAGE under reducing and non reducing conditions showed a change in electrophoretic mobility for the non-clipped but non the clipped species (A), as is in keeping with literature. Use of fluorescent glycan labelling and western blotting showed a change in electrophoretic mobility under native conditions, with the clipped species migrating quicker, suggesting a smaller, or more flexible structure (B).

## Inhibition ELISAs Show Increased Antibody Binding for Plasmin Clipped B2GPI

**Figure 7:** Inhibition ELISAs using patient serum (n=10) showed a strong trend (P=0.054) towards higher inhibition for plasmin clipped B2GPI. Plates were coated with commercial B2GPI and patient serum challenged for 2 hours with either lipped or non clipped B2GPI at 20µg/ml. Signal with and without inhibitor were compared for % inhibition. This suggests a structural change allowing greater antibody binding has taken place after cleavage.




## Small Angle X-Ray Scattering Identifies Novel Structure of B2GPI

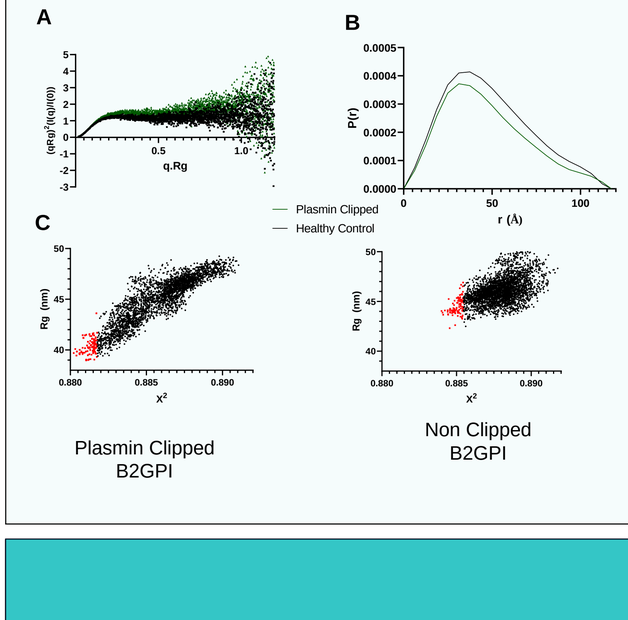
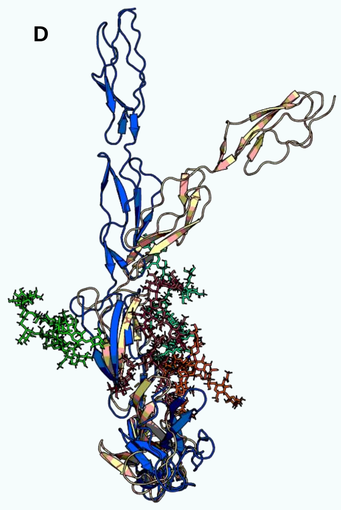



**Figure 5:** Small Angle X-Ray Crystallography revealed an increased flexibility for the cleaved protein as demonstrated by the higher values in the dimentionless Kratky plot (A), and the P(r) plot (B), with a characteristic high peak with extended tail shown in more rod shape structures, whilst the uneven shoulder shows the multi-domain nature of the protein. Analysis of >6000 frames of molecular simulation and their associated SAXS curves showed again those with the best fit had a lower radius of gyration (C). The frames with the best fit are highlighted red. Overlaying the two frames of best fit showed a novel S shape structures for the plasmin clipped (beige) while the classical J shape (blue) was seen for non-clipped B2GPI (D).




## Flow Cytometry Confirms Loss of Cellular Binding Post Cleavage

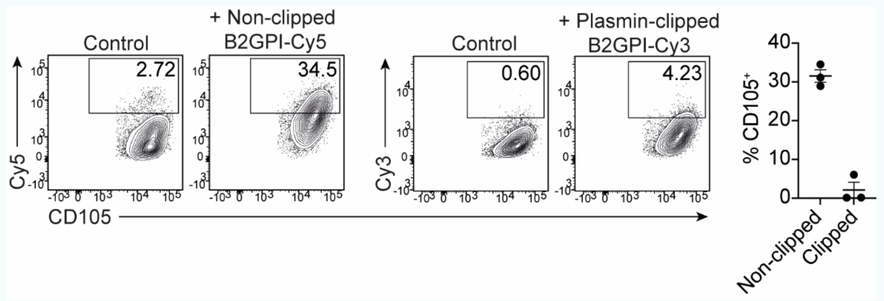



**Figure 8:** Flow Cytometry demonstrates a high binding of non-clipped B2GPI to HUVEC cells (34%) whilst cleaved B2GPI binds at a much lower rate (<5%). This fits with literature confirming the B2GPI is cleaved and behaves as expected.

## Plasmin Cleavage Induces Significant Structural Change in B2GPI

Plasmin cleavage of B2GPI significantly alters the function of the protein, with altered association with binding partners and new processes previously unseen for B2GPI. Here we show for the 1st time the structure of B2GPI post cleavage which has a smaller diameter and appears to form more of an S shape. It is unknown what the effect of this structure may be on the pathogenic process of antiphospholipid syndrome, however, given its increased antibody binding it may well lead to alterations in disease progression. This represents the first novel structure of B2GPI identified in a decade.

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