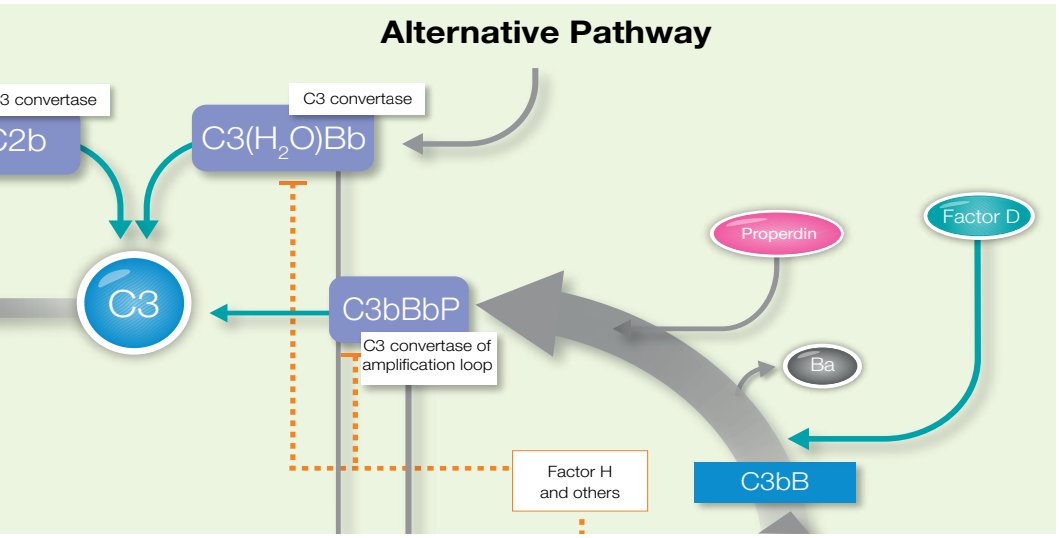


FUNCTIONAL ASSESSMENT OF PROPERDIN OR AP-MODULATION?

Application of a Functional Assay for Complement Therapeutics Development

The complement system's amplification loop presents promising targets for emerging complement therapeutics. These targets have the potential to improve disease outcomes while maintaining the complement system's protective capabilities[2-4]. This potential is reflected in the current landscape of emerging drugs that target the alternative pathway to treat a wide range of diseases.

In this case study, Dr. Cecilia Månsson, Senior Scientist, and Viktoria Kozma, Senior Research Engineer at Svar Life Science, discuss Properdin, a crucial regulator of the amplification loop. They explore novel approaches for correlation of Properdin function and quantitation, and how these methods can support development of next-generation complement therapeutics.

PROPERDIN: A CRUCIAL REGULATOR

Properdin, or Factor P, is a protein crucial for regulation of the Complement Alternative Pathway (AP). As the only known positive regulator of this pathway, properdin plays a key role in the AP amplification loop, a target of interest for researchers and drug developers alike. In the amplification loop, Properdin acts as a stabilizer for the AP C3-convertase, diminishing its rate of degradation, thus enhancing the overall rate of conversion of C3 into C3a and C3b. "Properdin's unique role as a positive regulator sets it apart in complement research," mentions Senior Scientist, Dr. Cecilia Månsson.

The AP amplification loop contributes to significant complement activity, independent on the initial mode of activation. It's important to understand Properdin's contribution and effects, as an immune response regulator, to fully understand associated disease mechanisms, explains Senior Research Engineer, Viktoria Kozma.

Severe health conditions have been associated with Properdin deficiency or dysfunction, but the significance of this protein's normal range and function to patient outcomes has yet to be fully studied.

NOVEL ASSAYS FOR PROPERDIN EVALUATION

A novel functional assay commercialized by Svar Life Science, the Factor P Functional Assay (COMPL FPF), enables a simple and standardized method for the functional assessment of Properdin and the amplification loop of the complement system, along with the accompanying Factor P Quantitative Assay (COMPL FPQ).

> **"These assays are designed to identify whether AP downregulation results from Properdin deficiency or dysfunction, providing more precise results than a conventional AP functional assay"** says Dr. Månsson.

Dr. Cecilia Månsson
Senior Scientist

Viktoria Kozma
Senior Research Engineer

DEEP-DIVE INTO PROPERDIN

Properdin, also known as Factor P, is a crucial glycoprotein in the immune system, mainly produced by leukocytes. Existing in different forms (di-, tri-, and tetramers); its ability to stabilize complement activity increases with its degree of oligomerization.

It plays a dual role by binding to and stabilizing C3- and C5-convertases and inhibiting the inactivation of these complexes, highlighting its importance in immune regulation.

Deficiencies in Properdin, categorized into three types based on the level and functionality of circulating Properdin, are linked to a range of severe infectious and non-infectious diseases, underscoring its critical role in maintaining immune homeostasis.

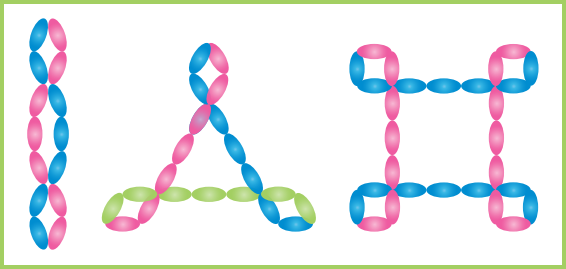


Figure 1:
Structural Variants of Properdin: Dimer, Trimer, & Tetramer Forms

The assays are produced under ISO 13485 standards, delivering fast, accurate, and reproducible results for Properdin and complement regulation studies. They are user-friendly, versatile, and time-efficient. Their 96-well ELISA format also enables easy automation when a high throughput is desirable.

Senior Research Engineer Kozma mentions, “By quantifying the concentration and functionality of Properdin, these assays offer a comprehensive approach to examining Properdin’s role in many scenarios. Since they meet the highest standards, we also ensure reliable results for researchers.”

> ... **these assays offer a comprehensive approach to examining Properdin’s role in many scenarios. Since they meet the highest standards, we also ensure reliable results for researchers.”**

Svar’s innovative FP functional assay leverages a non-inhibitory capture antibody to evaluate the stabilizing effect of Properdin and the activity of the C3-convertase formed on the captured Properdin in vitro. Meanwhile, the quantitative assay utilizes the same capture antibody, ensuring that Properdin function is directly proportional to concentration.

“The integration of functional and quantitative assessments offers a more complete picture of Properdin’s role,” notes Dr. Månsson. This assay represents a cutting-edge technology that offers new insights into Properdin’s role in immune responses.

> **“This assay represents a cutting-edge technology that offers new insights into properdin’s role in immune responses.”**

THE PROPERDIN FUNCTIONAL ASSAY
HOW IT WORKS

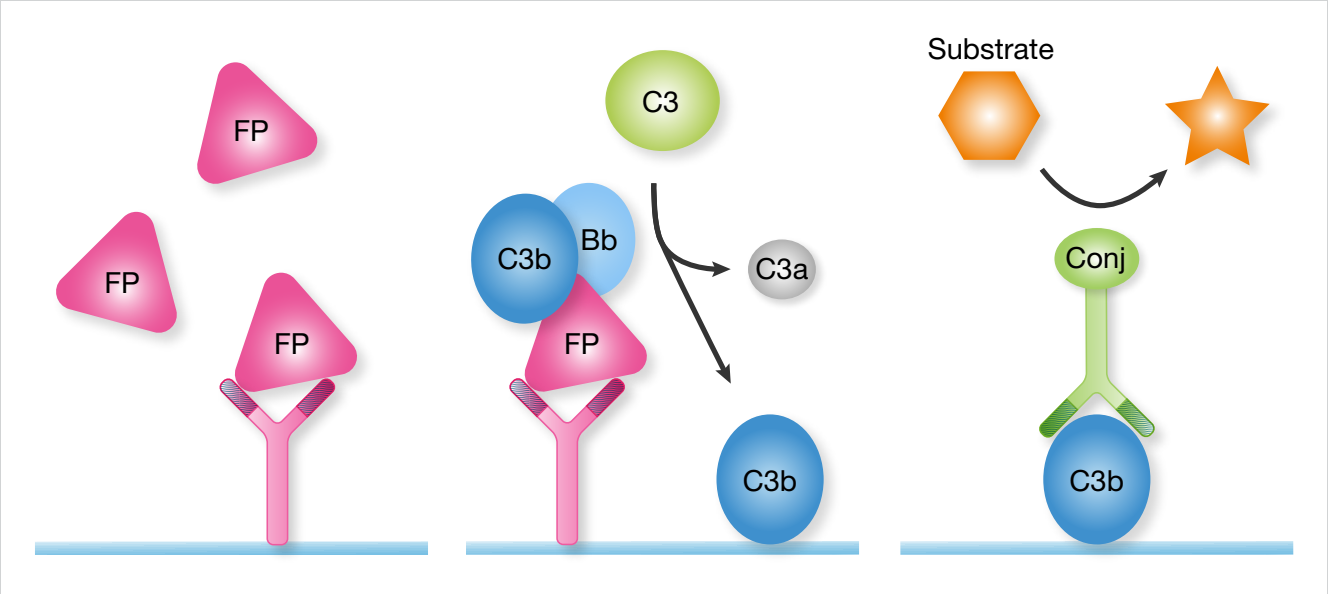


Figure 2:
Schematic representation of the Factor P ELISA-based assay for assessment of Properdin function. The assay involves a three-step process. First, Properdin samples are added after dilution to 200 ng/ml. Then, a Properdin-depleted reference serum is added, allowing the formation of a functional convertase and cleavage of C3 to C3b. Finally, an enzyme-conjugated detection antibody is added, producing a signal proportional to the deposited C3b.

PROPERDIN IN TWO SCENARIOS

Here, we will discuss how the assays can be used as valuable tools in assessing Properdin in cohort testing and alternative assay use.

1. The Potential of Assessing Properdin in Clinical Scenarios: Insights from Healthy Control Testing

In a study involving 120 healthy subjects, the two assays were used to assess Properdin activity and concentration. Substantial variability in Properdin activity was observed among subjects, indicating that Properdin function isn't solely dependent on its concentration. "The observed variability highlights the complexity of complement regulation and underscores the significance of understanding individual differences in immune responses," notes Sr. Research Engineer Kozma. "Further studies are required to fully understand the underlying factors contributing to the concentration-independent diversity within the cohort and its implications for health".

These findings suggest that functional assessments of Properdin can provide valuable insights beyond mere concentration measurements. Understanding individual differences in Properdin activity can help elucidate disease causes and evaluate the efficacy of complement-targeted therapies, thus enabling personalized treatment strategies.

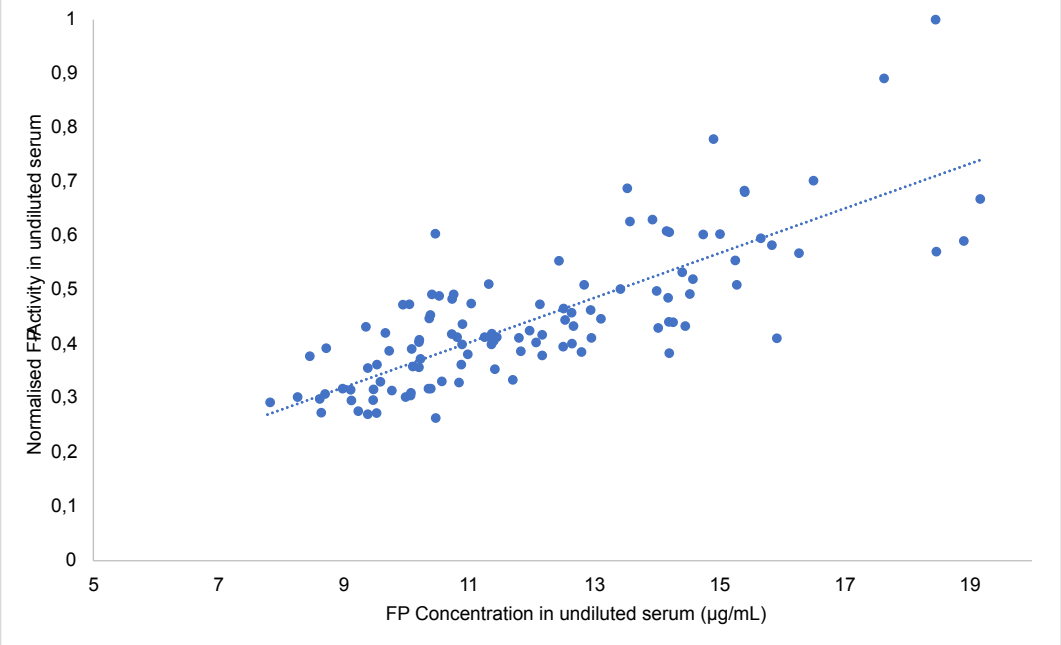
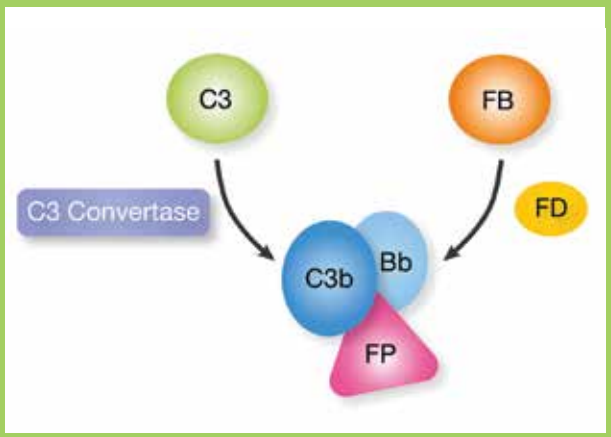


Figure 3:
FP activity against concentration in healthy individuals' undiluted serum, demonstrating that FP activity is not solely dependent on FP concentration in a subject. Further studies would be needed to fully explain this observation. Properdin concentration was determined with a Factor P Quantitative assay, and samples were diluted to 200 ng/mL for functional assessment.

FUNCTIONAL MODULATION OF THE ALTERNATIVE PATHWAY AMPLIFICATION LOOP

C3bBb, also known as the C3 convertase of the alternative pathway, plays a crucial role in the complement cascade; through cleavage of C3 into C3a and C3b, it initiates and amplifies a series of downstream events, including opsonization, inflammation, and cell lysis, which are essential components of the immune response against pathogens.

As emerging therapeutic targets, the individual components (e.g., C3, FB, and FD) and regulators (FP, FH, and FI) of the amplification loop are under scrutiny as modulators of complement dysregulation.



2. Assessing Loss- & Gain-of-Function through Complement Modulation

The reactions within the Svar functional Factor P Assay mimic the assembly and function of the stabilized C3-convertase C3bBbP, and the assay signal is proportional to deposited C3b after in vitro cleavage. Activity-modulating compounds targeting individual components of the convertase, including regulators, thus affect convertase activity and enable the study of Loss- or Gain-of-Function of individual components with minor modification to the assay protocol.

"C3bBb inhibitors hold promise in the development of novel therapeutics for conditions where complement dysregulation contributes to pathology," notes Dr. Månsson. Specifically targeting this key complex to intervene in the complement cascade at a crucial juncture holds great potential to provide more targeted and effective treatment options for a range of complement-mediated diseases.

A modified assay protocol was employed to assess C3bBb activity inhibition, wherein the standardized FP calibrator served as the source of Properdin for the functional assay. An experiment that involved mixing a Properdin-depleted serum was performed to assess relative activity. The Properdin-depleted serum served as the assay activator, with various AP inhibitors and down-regulators. These included an anti-FP antibody that inhibits activity, Compstatin (a C3 cleavage inhibitor), an anti-Bb antibody also serving as an inhibitor, and down-regulators such as Factor H (FH) and mini-FH, the latter provided by C. Schmidt from Martin-Luther University. "All inhibitors were effective in decreasing the apparent convertase activity through different modes of action," Sr. Research Engineer Kozma explains, "thus quenching the assay signal."

This inhibition analysis not only validates the assay's utility in drug development but also sheds light on potential therapeutic strategies for modulating complement activity in various disease states.

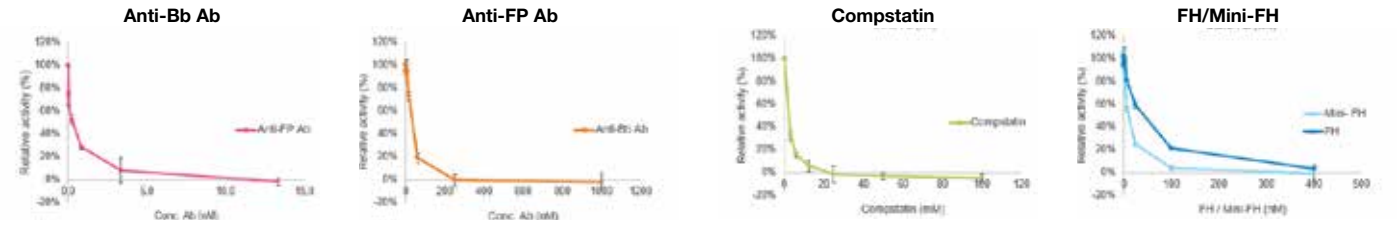


Figure 5.
Activity is reported as % relative activity to sample without the addition of any inhibitor. The error bars represent ± coefficient of variation (%) of duplicates. All inhibitors, including the anti-FP antibody, C3 inhibitor Compstatin, anti-Bb antibody, Factor H, and mini-FH, were efficient in decreasing the apparent convertase activity through different modes of action.

FUTURE DIRECTIONS

Looking ahead, these assays can be applied in various scenarios, from disease models to identify potential therapeutic targets in the amplification loop to potential use in the clinics. "Our goal is to provide tools to support the development of effective complement therapeutics," Sr. Engineer Kozma concludes. "The future of complement therapeutics is bright, and we're excited to be part of this exciting field," Dr. Månsson adds.

> **"The future of complement therapeutics is bright, and we're excited to be part of this exciting field"**
> Dr. Månsson

As research progresses in the growing field of complement therapeutics, these assays provide potential not only for a more in-depth understanding of Properdin but also as versatile tools in drug development. There are several promising avenues for improving patient care and outcomes in this arena. "The future of complement therapeutics is bright, and we're excited to be part of this exciting field," Dr. Månsson concludes.

CONCLUSIONS

The Complement system is a vital part of our immune defense. Properdin plays multiple roles in immune regulation, contributing to the terminal complement pathway activity essential for immune responses.

The assays presented in this context are valuable tools that help to characterize the functionality of Properdin and assess its modulating activity. They enable researchers to study the interaction of Properdin with other complement proteins, identify potential therapeutic targets, and develop new drugs aimed at remedying complement dysregulation.

> **"Our goal is to provide tools to support the development of effective complement therapeutics"**
> Sr. Research Engineer. Kozma

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