

ORIGINAL ARTICLE

Exploring the function of factor XIII free B subunit: Interactions with complement factors and a novel approach to identify potential binding partners

Bojun Li PhD¹ | Clément Bechtler PhD² | Lorenz Jenny PhD¹ | Daniel Ricklin PhD² | Verena Schroeder PhD¹ 

¹Experimental Haemostasis Group, Department for BioMedical Research DBMR, University of Bern, Bern, Switzerland

²Molecular Pharmacy Group, Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland

Correspondence

Verena Schroeder, Experimental Haemostasis Group, Department for BioMedical Research, University of Bern, Murtenstrasse 40, 3008 Bern, Switzerland.
Email: verena.schroeder@dbmr.unibe.ch

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Abstract

Background: The factor XIII (FXIII)-B subunit has a critical function as a carrier protein to stabilize FXIII-A in plasma and supply it to its main substrate, fibrinogen. However, the function of the excess free FXIII-B circulating in plasma is still elusive.

Objectives: In the present study, we explored potential interactions of free FXIII-B with complement factors and searched for novel binding partners.

Methods: We tested for cofactor activity in the degradation of complement C3b and C4b and used ELISA- and surface plasmon resonance-based binding assays to investigate interactions between FXIII-B and complement components. We performed immunoprecipitation and mass spectrometry analysis to identify potential binding partners of free FXIII-B in freshly drawn plasma samples.

Results: FXIII-B did not exhibit cofactor activity in the degradation of C3b and C4b similar to factor H and C4b-binding protein, nor did it bind to complement factors to a relevant extent. Identification of proteins potentially binding to free FXIII-B revealed high interindividual variation. We confirmed α_2 -macroglobulin (α_2 MG) as a candidate, although direct interactions or functional effects remain to be validated.

Conclusions: Our study reveals that free FXIII-B has no direct role in regulating the complement system, despite a structural similarity to major complement regulators. Further studies are needed to validate α_2 MG as a binding partner and explore potential functional consequences of this binding.

KEYWORDS

α_2 -macroglobulin, coagulation factor XIII, complement system, factor XIII B-subunit

Essentials

- Coagulation factor XIII (FXIII)-A, which stabilizes fibrin, circulates in complex with its carrier protein, FXIII-B.
- The function of the large excess free FXIII-B circulating in plasma is not known.
- We show that free FXIII-B does not interact with the complement system.
- α_2 -macroglobulin remains a candidate binding partner to free FXIII-B.

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1 | INTRODUCTION

Coagulation factor XIII (FXIII) circulates in plasma as a heterotetramer consisting of two catalytic A subunits (FXIII-A) and two protective/inhibitory B subunits (FXIII-B). The transglutaminase FXIII-A is synthesized by cells of bone marrow origin and is present in platelets and monocytes/macrophages in dimeric form (FXIII-A₂), also referred to as cellular factor XIII. While FXIII-B is mainly expressed in the liver and secreted by hepatocytes,¹ it rapidly binds FXIII-A in plasma to create a stable complex (FXIII-A₂B₂) that circulates at an average concentration of 21.6 µg/ml.²

FXIII-B consists of 641 amino acids, with a molecular mass of ~80 kDa, and is assembled from 10 short consensus repeat (SCR) domains, also termed complement control protein or sushi domains. Each SCR domain contains about 60 amino acids held together by disulfide bonds.¹ In plasma, about half of the total amount of FXIII-B circulates in complex with FXIII-A, serving as carrier and regulatory protein.³ The B subunit protects the A subunit from proteolysis, prolonging its circulating half-life. Consequently, patients with congenital FXIII-B deficiency show significantly decreased FXIII-A levels and moderate bleeding symptoms.^{4,5} FXIII-B also mediates the interaction between the FXIII heterotetramer and fibrinogen. By facilitating the formation of a ternary complex between proenzyme (FXIII-A), substrate (fibrinogen), and activator (thrombin), FXIII-B accelerates fibrin crosslinking.⁶

While plasma FXIII-A is almost exclusively present as part of the FXIII heterotetramer, only half of the total FXIII-B present in plasma is complex bound.³ Approximately 50% of FXIII-B exists in free form in plasma, presumably as a homodimer.^{3,7} Importantly, the functional implications of this significant amount of free FXIII-B subunit remain unknown. In patients with congenital FXIII-A deficiency, the total amount of FXIII-B can decrease, but the concentration of free FXIII-B remains constant.¹ This may indicate that free FXIII-B may have other functions in addition to binding and regulating FXIII-A.

The gene encoding FXIII-B is part of the regulator of complement activation gene cluster, which also comprises 15 genes related to the complement system.^{8,9} The proteins encoded by these genes share significant similarities in amino acid sequence and structural characteristics, as they are all composed of SCR domains. Complement factor H (FH) and C4b-binding protein (C4BP) are the major complement regulators in the fluid phase.¹⁰ FH serves as cofactor in the factor I (FI)-dependent inactivation of C3b to iC3b and prevents alternative pathway amplification by accelerating the decay of the C3 convertase complexes.¹¹ C4BP is the major soluble inhibitor of the classical and lectin pathways, with cofactor activity in the degradation of C4b and decay acceleration activity toward C3 convertases of the classical/lectin pathway.¹² Whereas FH is a single-chain protein with 20 SCR domains, the major isoform of C4BP is composed of seven identical α -chains, each containing 8 SCR domains, and a single β -chain of 3 SCR domains.¹³ Intriguingly, FH is more closely related to FXIII-B

than to the other complement regulators, and individual SCR domains of FXIII-B and FH share sequence homologies of 30%–40%.^{13,14} This could suggest a role of FXIII-B in the regulation of the complement system, which is even more conceivable when considering the numerous crosstalk functions between the complement and coagulation systems. A recent study employed proteomics to identify potential interaction partners for FXIII-B and reported that FXIII-B bound to complement C1q and C3; it also found FH to be present in FXIII concentrates purified from human plasma.¹⁵ However, another study from the same group suggested that the FXIII-B subunit has no effect on the rate of complement activation and, hence, exerts no regulatory functions in the complement system.¹⁶

In the present study, we aimed to address the open questions on potential functions of free FXIII-B and the partly conflicting results reported so far. We tested for cofactor activity of free FXIII-B in the degradation of complement C3b and C4b and used ELISA- and surface plasmon resonance (SPR)-based binding assays to investigate the interaction profile between free FXIII-B and complement components. We also developed a novel approach to specifically identify potential binding partners of free FXIII-B in fresh human plasma samples.

2 | METHODS

2.1 | Protein reagents and plasma samples

Recombinant FXIII-A and FXIII-B were purchased from Zedira and α 2-macroglobulin (α 2MG) purified from human plasma and bovine serum albumin (BSA) from Sigma-Aldrich/Merck (Burlington, MA, USA). Plasma-purified C3b, C4b, FH, C4BP, and factor I (FI) were from Complement Technology Inc.

We used polyclonal goat anti-human C3, goat anti-human C4, goat anti-human FH antibodies (Complement Technology Inc., Tyler, TX, USA), rabbit anti-FXIII-B polyclonal antibody (Calbiochem, Merck, San Diego, CA, USA), a monoclonal murine anti-human C4BP antibody (Quidel, San Diego, CA, USA), and secondary peroxidase-labeled anti-goat IgG, anti-rabbit IgG, anti-mouse IgG, and goat anti-rabbit alkaline peroxidase-labeled antibody (Sigma-Aldrich/Merck). A mouse monoclonal IgG antibody specific for free FXIII-B was a kind gift from László Muszbek and Eva Katona, University of Debrecen, Hungary.³

Fresh citrated human plasma was obtained from anonymous healthy blood donors (Blood Donation Center Bern, Bern, Switzerland, where informed consent was obtained). FXIII-deficient (immunodepleted) plasma (FXIII-DP) was purchased from Affinity Biologicals (Ancaster, ON, Canada) and CoaChrom Diagnostica (Maria Enzersdorf, Austria). FXIII deficiency in FXIII-DP was confirmed by ELISA.¹⁷ We could not detect any FXIII-A and the FXIII-B level was <5%. Normal reference plasma was from Precision BioLogic (Dartmouth, NS, Canada).

2.2 | Influence of free FXIII-B on the degradation of complement C3b and C4b

We tested for cofactor activity of FXIII-B in the FI-mediated degradation of C3b and C4b using protocols as described earlier.^{18,19} In a final reaction volume of 30 μ l, C3b (final concentration, 50 μ g/ml) was incubated with FI (10 μ g/ml) in the presence of FH (4 μ g/ml) and/or FXIII-B (10 μ g/ml) for up to 20 minutes at 37°C. Similarly, C4b (267 μ g/ml) was incubated with FI (10 μ g/ml) in the presence of C4BP (4 μ g/ml) and/or FXIII-B (10 μ g/ml). The reactions were stopped with Laemmli buffer (NuPAGE LDS sample buffer). The samples were boiled and run in MES-SDS running buffer on a 4%–12% NuPAGE Novex Bis-Tris Mini Gel (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) together with a Precision Plus Protein Dual Color Standard (Bio-Rad Laboratories, Hercules, CA, USA). The gels were stained with Coomassie (Bio-Rad Laboratories).

2.3 | Binding of free FXIII-B to complement C3b and C4b, and α 2MG, investigated by ELISA-based binding assays

ELISA-based binding assays with FXIII-B, C3b, and C4b were performed according to published protocols,^{18,19} with purified FH and C4BP as positive controls and BSA as negative control. Proteins were coated onto 96-well microplates (Nunc Immuno Maxisorp; Thermo Fisher Scientific) overnight. The proteins to bind were added in a concentration range of 0.3–50 μ g/ml and detected with the respective primary and secondary antibodies.

To investigate potential binding between α 2MG and free FXIII-B, we coated 96-well microplates with 100 μ l α 2MG (10 μ g/ml). After washing and blocking steps, we added 100 μ l FXIII-B (final concentrations 4–0.0625 μ g/ml). For detection, rabbit anti-FXIII-B antibody followed by goat anti-rabbit AP-labeled antibody were added. The plates were developed with p-nitrophenyl phosphate (Sigma), and absorbance was read at 405 nm on a microplate reader (Spark™ 10M; Tecan, Männedorf, Switzerland).

2.4 | SPR analysis of FXIII-B interactions

The interaction pattern between FXIII-B, FXIII-A, FH, C3b, C4b, and α 2MG was elucidated by SPR at 20°C on a Biacore T200 instrument using a CMD200M sensor chip (Xantec Bioanalytics GmbH, Düsseldorf, Germany). Amine coupling (National Health Service/Equality and Diversity Council) was used for protein immobilization on separate flow cells (Fc). Fc1 served as a mock-activated reference surface. FXIII-B (6546 response units; RUs) was immobilized on Fc2, C3b (8202 RUs) on Fc3 and iC3b (14,582 RU) on Fc4. The analytes were injected in a concentration range of 0.22–500 nM in 1:2 serial dilutions. The flow rate was 10 μ l/min and the association and dissociation phases were measured for 600 s. The running buffer consisted of PBSTE (10 mM Na₂HPO₄, 140 mM NaCl, 2.7 mM KCl,

50 μ M EDTA, 0.005% Tween20, pH 7.4, 0.03% sodium azide; Xantec) if not mentioned otherwise. Other running buffers included HBST++ (10 mM HEPES, 150 mM NaCl, 1 mM MgCl₂, 2.5 mM CaCl₂, 0.005% Tween20, pH 7.4; Xantec), and for analysis of α 2MG PBSTE+glycine was used (20 mM Na₂HPO₄, 140 mM NaCl, 2.7 mM KCl, 4.8 mM glycine, 50 μ M EDTA, 0.005% Tween20, pH 7.0, 0.03% sodium azide; Xantec). Regeneration was performed with 0.1 M Na₂CO₃ for 30 s followed by a 120-s stabilization period. Data analysis was performed with BiaEvaluation (Cytiva, Marlborough, MA, USA) and Scrubber software (BioLogic) after reference subtraction (Fc1). For single normalization, the SPR signal was divided by the molecular weight of the corresponding ligand. For double normalization, correction for ligand molecular weight and target density was calculated according to the formula:

$$\text{Normalised binding} = \frac{\text{analyte binding [RU]}}{\frac{\text{ligand loading [RU]}}{\text{ligand molecular weight [Da]}}}$$

2.5 | Immunoprecipitation of free FXIII-B₂ from human plasma using magnetic beads

According to the manufacturer's protocol, we transferred 50 μ l (about 1.5 mg) of protein G-coated magnetic beads (Dynabeads Protein G, Invitrogen) to a 1.5-ml test tube and placed the tube on the magnetic stand (6-Tube Magnetic Stand, Ambion, Thermo Fisher Scientific) to separate the beads from the solution and removed the supernatant. The beads were washed three times with 200 μ l of washing buffer (PBS, pH 7.4, with 0.02% Tween20) by gentle pipetting. We diluted about 9 μ g of mouse monoclonal IgG antibody specific for free FXIII-B in 200 μ l of PBS with 0.02% Tween20 and added the antibody to the magnetic beads. The beads and antibody were incubated under rotation for 10 min at room temperature followed by a washing step and incubation for 4 h at 4°C with either 300 μ l fresh citrated human plasma obtained from four healthy individuals or with FXIII-DP. The beads were washed four times and stored at –20°C until further analysis. As a negative control, we used nonimmune mouse IgG to coat the magnetic beads before incubation with fresh human plasma.

2.6 | SDS PAGE and western blotting

In a first step, we analyzed the beads with SDS-PAGE and western blotting. The beads were resuspended in Laemmli buffer (Bio-Rad Laboratories) and boiled, and the supernatant was separated by electrophoresis on Bolt 8% Bis-Tris Plus Gels (Invitrogen) with 1X Bolt™ MES-SDS Running Buffer (Invitrogen). Gels were stained with Coomassie Imperial Protein Stain (Thermo Fisher Scientific). For Western blotting, the proteins were transferred onto Immun-Blot® PVDF Membrane (Bio-Rad) with 1X Bolt Transfer Buffer (Invitrogen). The membranes were incubated with mouse monoclonal anti-free FXIII-B IgG antibody, followed by a horseradish peroxidase-conjugated goat anti-mouse secondary antibody, and

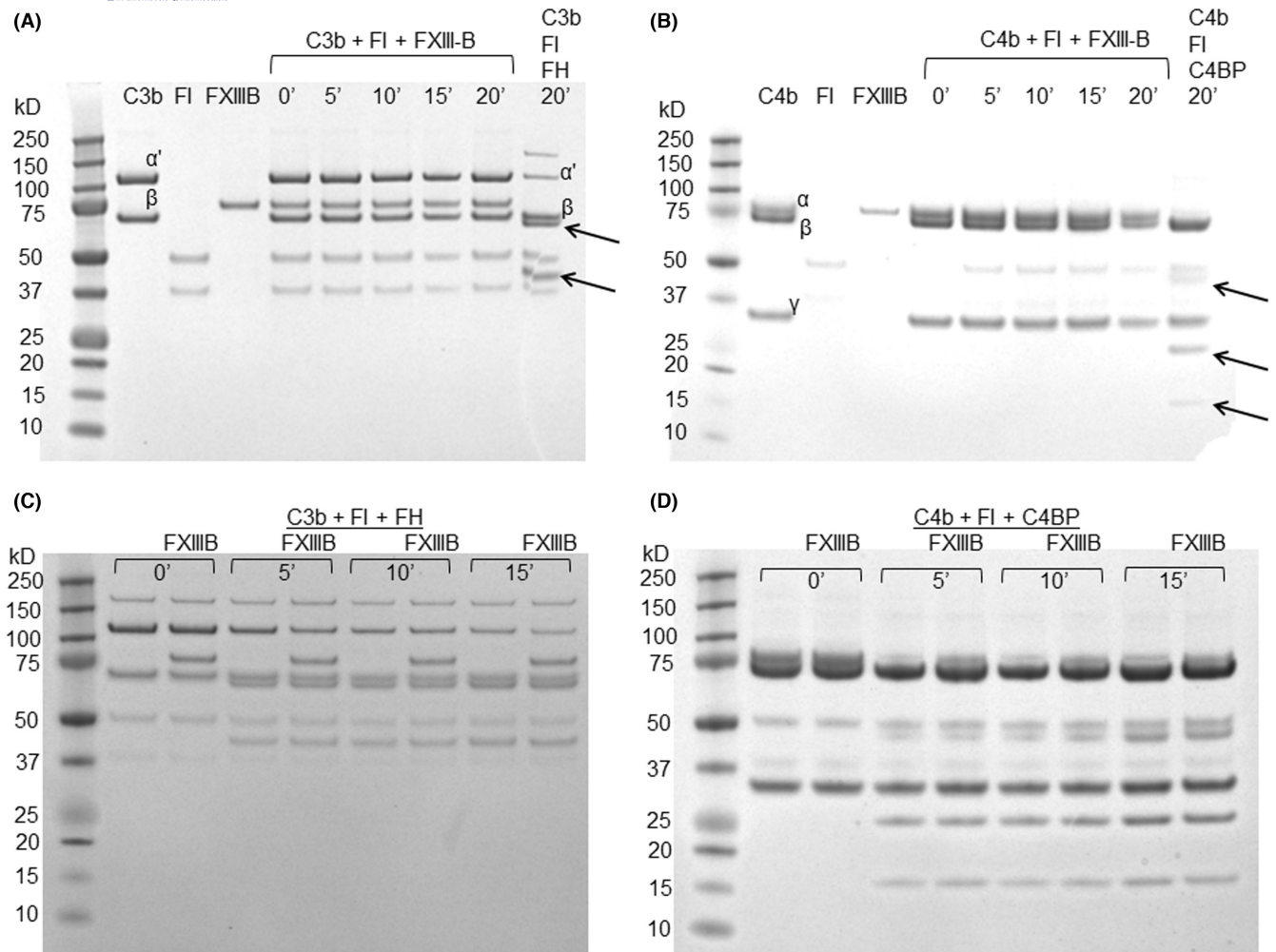


FIGURE 1 Cofactor activity in the degradation of complement C3b and C4b. C3b and C4b were incubated with FI in presence of cofactors FH and C4BP, respectively, and/or free FXIII-B. (A) The individual proteins C3b, FI, and FXIII-B are shown, followed by a time course of the incubation with FXIII-B. The last lane shows as positive control the degradation of C3b by FI in the presence of FH and the degradation products are indicated with arrows. (B) The individual proteins C4b, FI, and FXIII-B are shown, followed by a time course of the incubation with FXIII-B. The last lane shows as positive control the degradation of C4b by FI in the presence of C4BP and the degradation products are indicated with arrows. (C) C3b was incubated with FI and FH over 15 min, and in every second sample FXIII-B was added. (D) C4b was incubated with FI and C4BP over 15 min, and in every second sample FXIII-B was added. C4BP, C4b-binding protein; FH, factor H; FI, factor I; FXIII-B, factor XIII-B

developed with WesternBright Quantum HRP chemiluminescent substrate (Advansta, San Jose, CA, USA). We visualized the membranes with a Fusion Solo S imaging system (Vilber, Eberhardzell, Germany).

2.7 | Mass spectrometric analysis of proteins bound to the magnetic beads

Mass spectrometric analysis of the magnetic beads was performed by the Proteomics Mass Spectrometry Core Facility, Department for BioMedical Research, University of Bern. Proteins captured on the beads were analyzed essentially as described in Brügger et al.²⁰ with a few modifications: digestion with trypsin was done

overnight at room temperature, and peptides were separated with a 60-min gradient at a flow rate of 350nl/min. Fragment spectra data were converted to peak list files (mgf format) with ProteomeDiscoverer 2.4 and peptide identification made with EasyProt software, searching against the forward and reversed UniprotKB SwissProt Human protein database (Release 2017_12) with the following parameters: parent mass error tolerance of 10ppm, trypsin cleavage mode allowing three missed cleavages, static carbamidomethylation on Cys, variable oxidation on Met and acetylation on the protein's N-terminus. On the basis of reversed database peptide spectrum matches, a 1% false discovery rate was set for acceptance of target database peptide spectrum matches, and a minimum of two peptides were required for a positive protein identification.

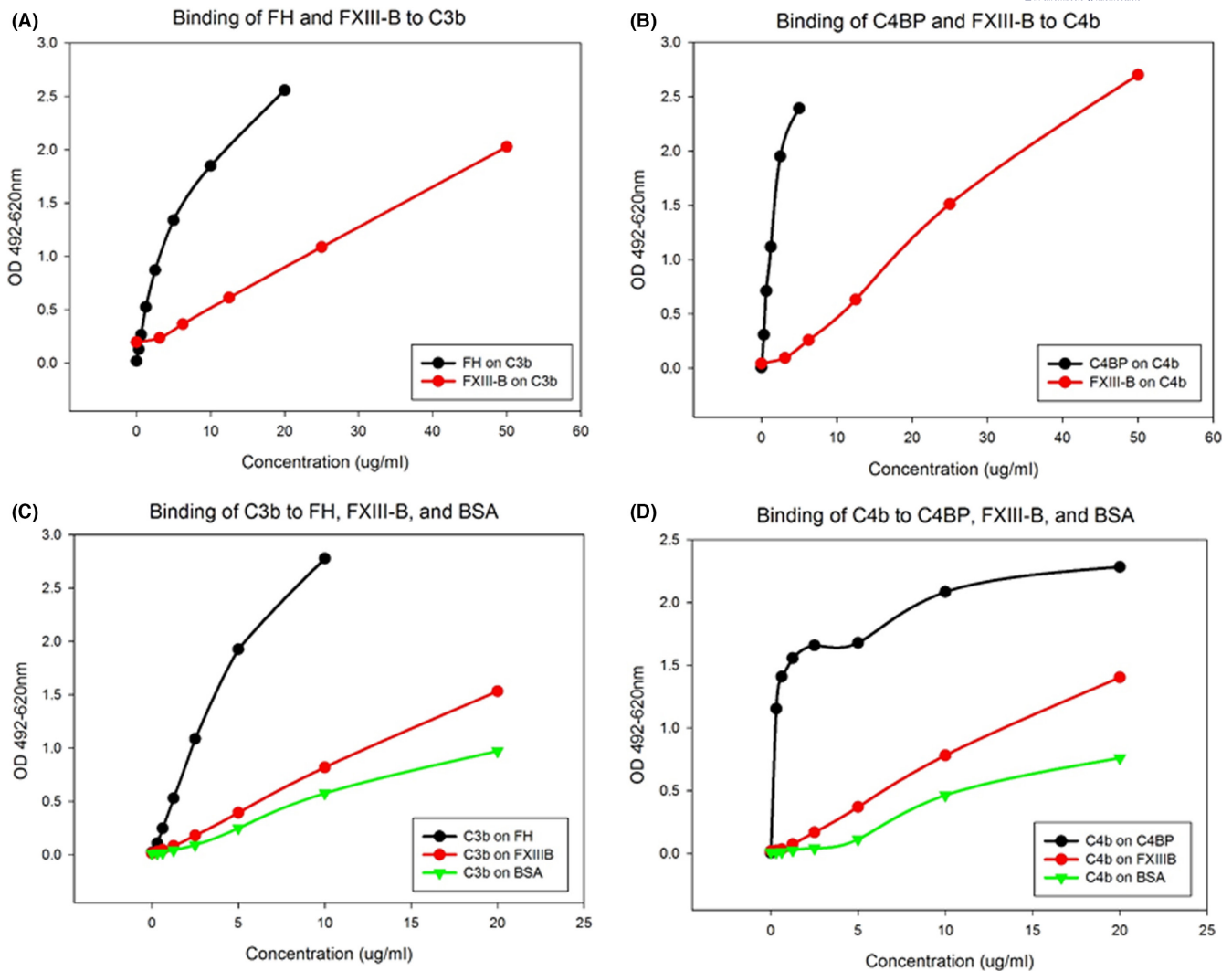


FIGURE 2 ELISA-based binding assays. (A) Dose-dependent binding of FH and free FXIII-B to C3b coated onto a microplate. (B) Dose-dependent binding of C4BP and free FXIII-B to C4b coated onto a microplate. (C) Dose-dependent binding of C3b to FH, FXIII-B and BSA coated onto a microplate. (D) Dose-dependent binding of C4b to C4BP, FXIII-B, and BSA coated onto a microplate. BSA, bovine serum albumin; C4BP, C4b-binding protein; FH, factor H; FXIII-B, factor XIII-B

3 | RESULTS

FXIII-B shows a high degree of structural homology with complement FH and C4BP, which are major fluid-phase inhibitors of C3b and C4b. We therefore studied possible interactions between FXIII-B and C3b and C4b, respectively.

3.1 | Free FXIII-B does not act as cofactor in the degradation of complement C3b and C4b

Complement activation fragments C3b and C4b are degraded in the fluid phase by FI in the presence of cofactors FH and C4BP, respectively. Here, we tested for potential cofactor activity of FXIII-B due to its similarities with FH and C4BP. As shown in [Figure 1](#), C3b and C4b cleavage products generated by FI in the presence of FH or C4BP could be clearly detected and appeared as early as 5 min

of incubation. In the presence of FXIII-B, however, no C3b and C4b cleavage products were generated even after 20 min of incubation. Furthermore, FXIII-B did not affect the cofactor activity of FH and C4BP: addition of FXIII-B to the digestion of C3b in presence of FH and to the digestion of C4b in presence of C4BP did not impair the digestion either, so there was no competition for binding sites between FXIII-B and FH or C4BP.

3.2 | Free FXIII-B does not specifically bind to complement factors

We performed ELISA-based binding assays to investigate potential interactions between free FXIII-B and complement C3b and C4b. As shown in [Figures 2A,B](#), FXIII-B exhibited dose-dependent binding to C3b and C4b, albeit to a lesser extent compared with FH and C4BP. However, when the orientation was reversed as shown in

Ligand Binding (0.2 - 500 nM) to FXIII-B Surface

Ligand Binding (0.2 - 500 nM) to C3b Surface

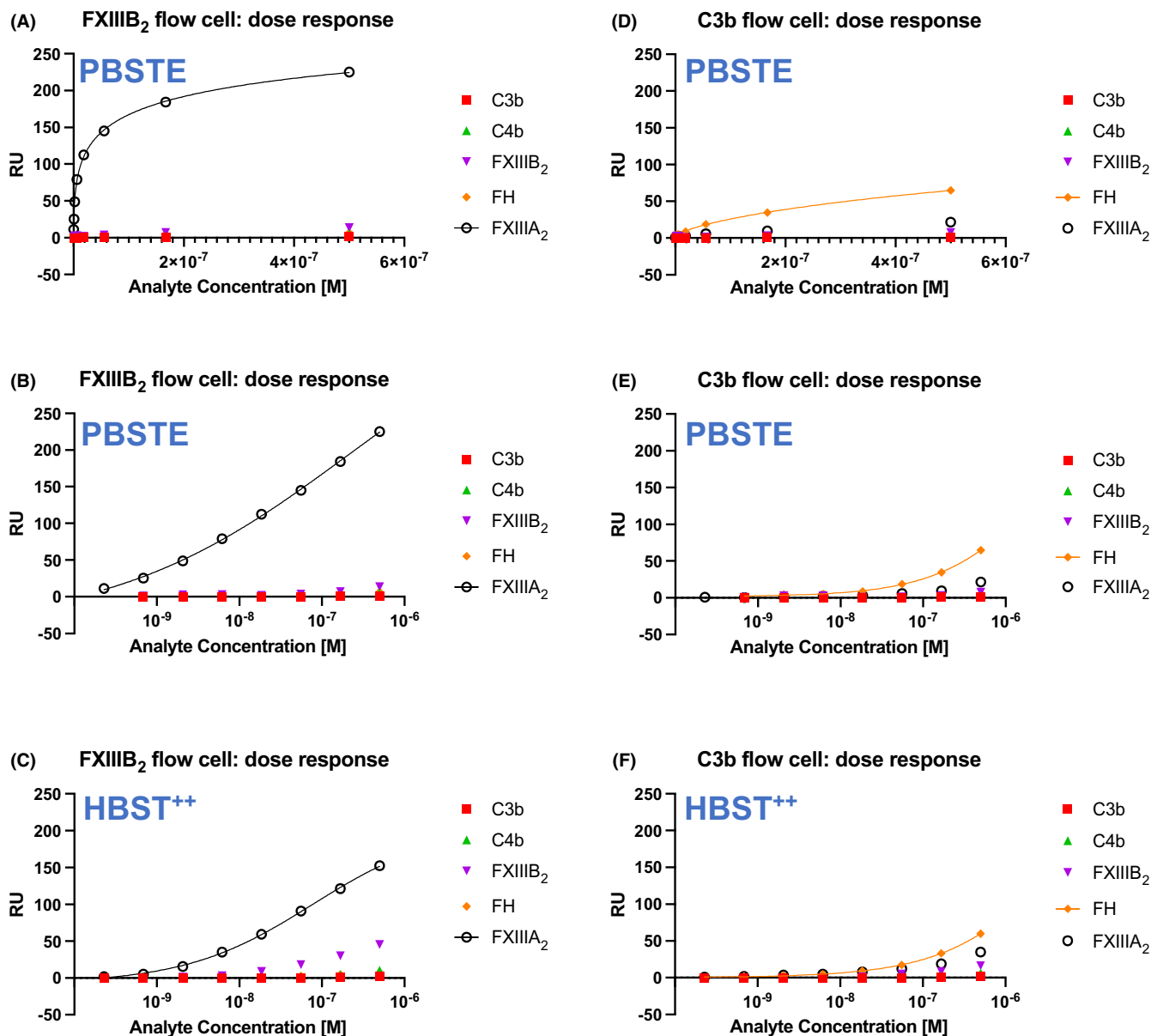


FIGURE 3 Dose-response curves obtained with SPR. Panels A–C show ligand binding to the FXIII-B surface. Panels D–F show ligand binding to the C3b surface. Panels A and D show normalized dose response curves in PBSTE buffer with linear concentration scale, panels B, C and E, F show logarithmic concentration scales. FXIII-B, factor XIII-B; SPR, surface plasmon resonance

Figures 2C,D, C3b and C4b showed binding to FXIII-B to a similar extent as to the negative control BSA, suggesting nonspecific binding.

To further investigate whether there were notable, even if transient, interactions between FXIII-B and complement proteins, we employed an SPR-based direct binding assay. The SPR sensorgrams can be found in Appendix S1. Figures 3A–C show the dose-response curves for the binding of ligands to immobilized FXIII-B. The complement components C3b, C4b, and FH did not bind, with the presence of Mg²⁺ and Ca²⁺ (Figure 3C) buffer having no influence on the complement protein signals. As expected, FXIII-A showed strong binding to FXIII-B in all experiments. Since recombinant FXIII-A and FXIII-B

proteins usually exist as dimers, partial dissociation of immobilized FXIII-B into monomers may explain the signal of the soluble FXIII-B ligand to the surface. Next, the orientation was reversed, and the ligands were added to immobilized C3b (Figures 3D–F) and its degradation product iC3b. Only FH showed strong binding to C3b, as expected. Interestingly, FXIII-A showed residual binding, which is plausible, as we have shown earlier that C3 is a substrate of FXIII-A and can be incorporated into a fibrin clot by FXIII-A.²¹ A weak binding signal could also be observed for FXIII-B. In the presence of Ca²⁺ and Mg²⁺, the binding signals of FXIII-A and FXIII-B to C3b increased slightly, but were still substantially lower when compared to FH.

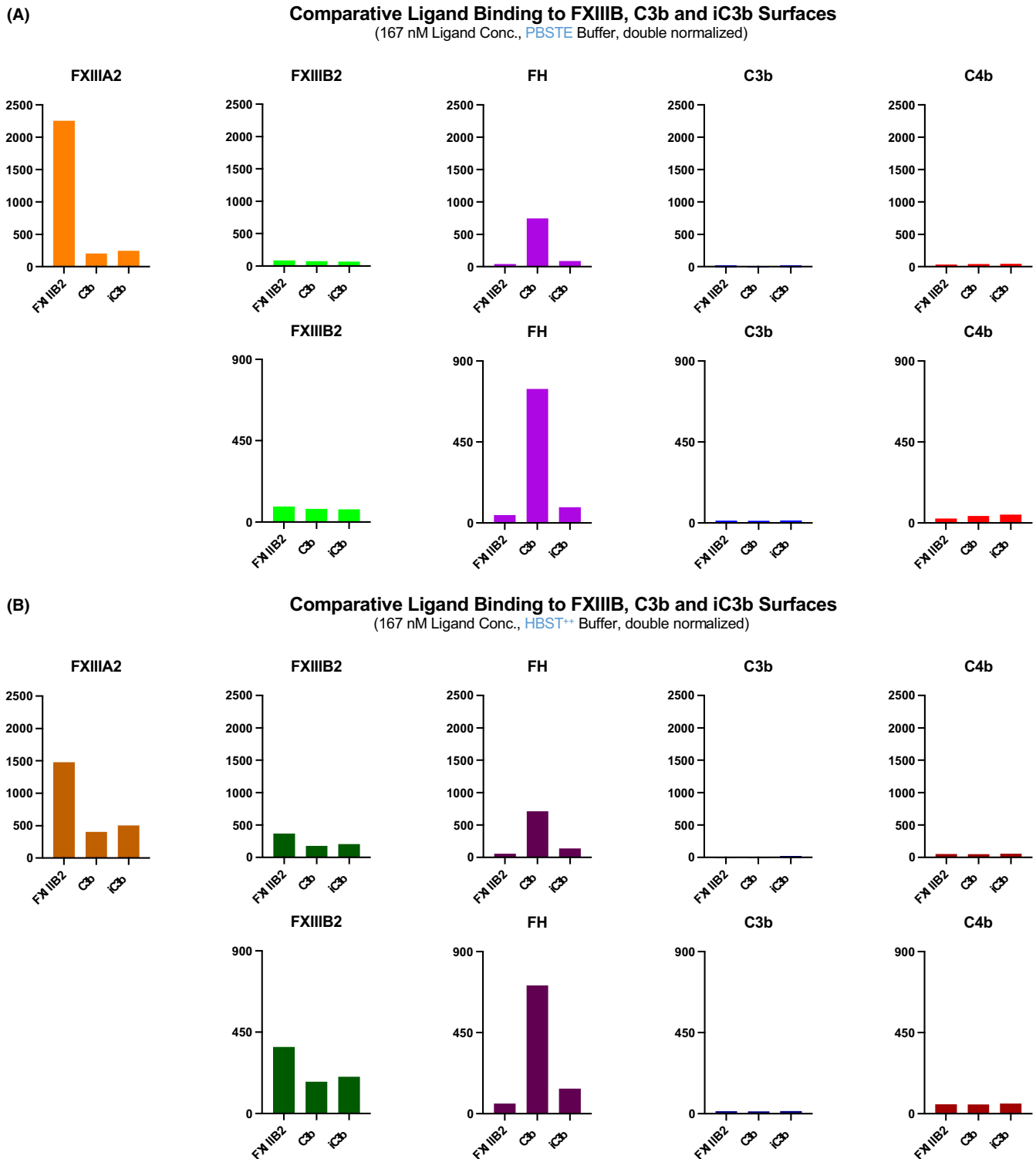


FIGURE 4 Comparative ligand binding to FXIII-B, C3b, and iC3b surfaces obtained with SPR. Panel A shows the binding experiments performed in PBST-EDTA buffer, panel B in HBST buffer containing Ca^{2+} and Mg^{2+} . The upper rows show the scale up to 2500 RU, the rows below up to 900 RU to see smaller differences. FXIII-B, factor XIII-B; SPR, surface plasmon resonance

Figure 4 shows the comparative ligand binding to FXIII-B, C3b and iC3b surfaces in the absence (Figure 4A) and presence of Ca^{2+} and Mg^{2+} (Figure 4B). The direct comparison showed strong and dose-dependent binding only between FXIII-A and FXIII-B and between FH and C3b. As expected, FH bound strongly to C3b and weakly to

iC3b. C3b did not bind to immobilized FXIII-B and although some signals were observed for the binding of FXIII-B to immobilized C3b, the detection of similar signals on the FXIII-B and iC3b surfaces indicated nonspecific binding. Also, in contrast to FH, no signal drop between C3b and iC3b could be observed for binding of FXIII-B,

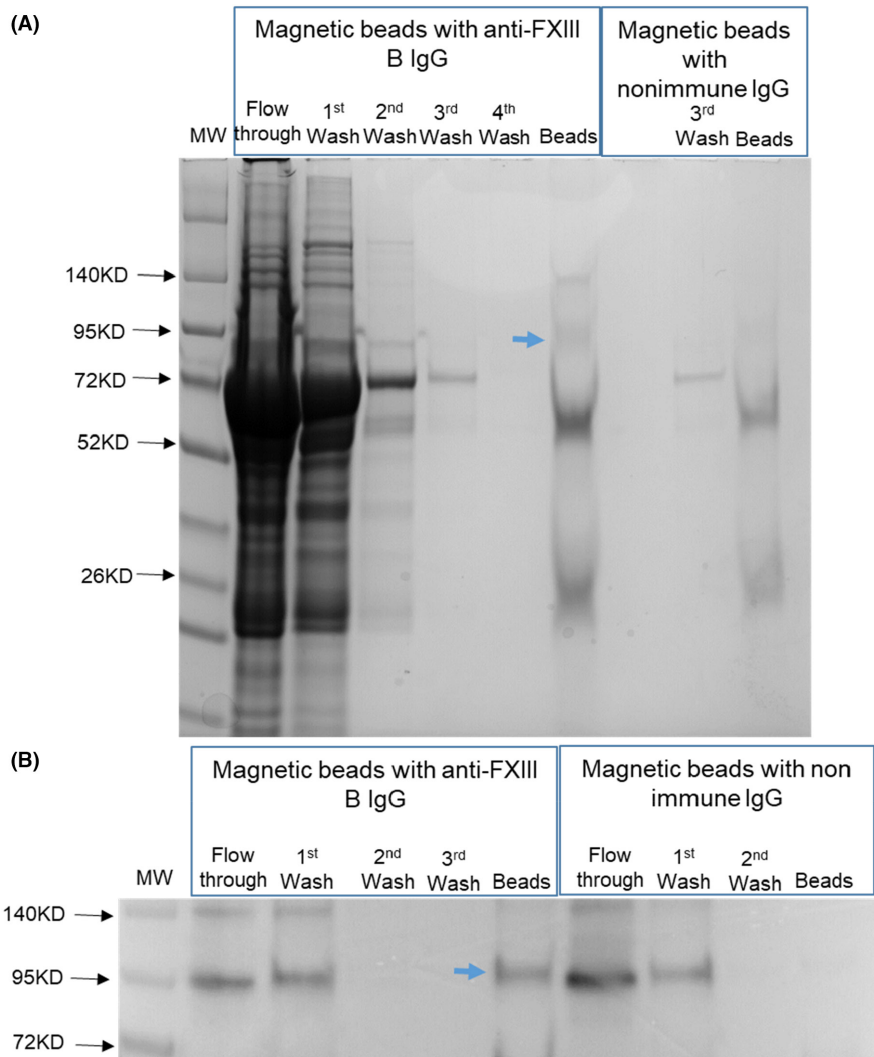


FIGURE 5 SDS-PAGE and western blot analysis of proteins binding to IgG conjugated magnetic Dynabeads. Human plasma was incubated with magnetic Dynabeads with anti-free-FXIII-B monoclonal IgG or Dynabeads with nonimmune IgG (negative control). We analyzed the beads using SDS-PAGE (A) and western blot (B). The arrow indicates the FXIII-B band. FXIII-B, factor XIII-B; MW, a molecular weight marker

which would be expected if FXIII-B behaved similarly to FH. Taken together, the SPR analysis suggested that FXIII-B does not act as FH-type ligand for C3b.

3.3 | Identification of potential interaction partners specific for free FXIII-B with a novel immunoprecipitation and mass spectrometry approach

Since we had ruled out the obvious complement components as likely binding and interaction partners for free FXIII-B, we developed a novel approach to search for other potential binding partners. An immunoprecipitation and mass spectrometry approach was used before¹⁵; however, as laid out in more detail in the discussion, there were important methodological differences. Crucially, we used a monoclonal antibody specific for free FXIII-B to specifically capture free FXIII-B together with its potential binding partners from freshly drawn individual human plasma samples. This seemed important to us, because in a pilot experiment with frozen commercial normal plasma, we were not able to detect any proteins bound to FXIII-B (shown in Appendix S2).

Specific binding of free FXIII-B to magnetic beads coated with anti-free-FXIII-B monoclonal antibody was confirmed by SDS-PAGE and Western blotting (Figure 5). Excess unbound FXIII-B and other proteins that did not bind to the beads appeared in the flow-through and the supernatants from the washing steps. After four washing steps, nonspecific binding of proteins to the beads could no longer be detected. FXIII-B was confirmed to bind to the beads coated with anti-free-FXIII-B monoclonal IgG but not to the beads with nonimmune IgG.

The beads were subjected to mass spectrometry analysis. FXIII-B was detected on the beads coated with anti-free-FXIII-B antibody in all four experiments but not found on the beads coated with nonimmune IgG. No FXIII-A subunit was detected on the beads, confirming that only free FXIII-B, but not FXIII-A₂B₂ tetramer, was able to bind to the beads coated with anti-free-FXIII-B antibody. The detailed results of all proteins detected in the four separate analyses using fresh plasma from four individual healthy blood donors are shown in Appendix S2 and summarized in Table 1. These proteins were detected only on the beads coated with anti-free-FXIII-B antibody incubated with fresh human plasma from healthy volunteers, and neither detected in the control samples,

TABLE 1 Proteomic analysis of proteins binding to anti-free-FXIII-B IgG conjugated beads

| Protein ID | Description | Number of detections in four independent experiments |
|-------------|---------------------------------------------------|------------------------------------------------------|
| F13B_HUMAN | Coagulation factor XIII B | 4 |
| A2MG_HUMAN | α_2 -macroglobulin | 2 |
| HORN_HUMAN | Hornerin | 1 |
| HMGN2_HUMAN | Nonhistone chromosomal protein HMG-17 | 1 |
| APOH_HUMAN | β_2 -glycoprotein 1 | 1 |
| KNG1_HUMAN | Kininogen-1 | 1 |
| ITIH1_HUMAN | Inter- α -trypsin inhibitor heavy chain H1 | 1 |
| HRG_HUMAN | Histidine-rich glycoprotein | 1 |
| MYH9_HUMAN | Myosin-9 | 1 |
| K1C13_HUMAN | Isoform 2 of Keratin, type I cytoskeletal 13 | 1 |
| FINC_HUMAN | Isoform 10 of fibronectin | 1 |
| CAMP_HUMAN | Cathelicidin antimicrobial peptide | 1 |
| PPIA_HUMAN | Peptidyl-prolyl cis-trans isomerase A | 1 |
| H32_HUMAN | Histone H3.2 | 1 |
| H31_HUMAN | Histone H3.1 | 1 |
| SYUA_HUMAN | Isoform 2–4 of α -synuclein | 1 |
| HPTR_HUMAN | Haptoglobin-related protein | 1 |
| A1AT_HUMAN | Isoform 2 of α_1 -antitrypsin | 1 |
| TRFE_HUMAN | Serotransferrin | 1 |
| FETUA_HUMAN | α_2 -HS-glycoprotein | 1 |
| K22E_HUMAN | Keratin, type II cytoskeletal 2 epidermal | 1 |

that is, on beads coated with nonimmune IgG, nor beads coated with anti-free-FXIII-B antibody incubated with FXIII-DP. Some proteins including fibrinogen – although well known to bind to FXIII-B – were detected on both beads with anti-free-FXIII-B and beads with nonimmune IgG, but this binding was judged as non-specific, and those proteins were therefore not listed in [Table 1](#). In line with our results described above, no complement proteins were detected. Besides FXIII-B, that was detected in all four experiments, only α 2MG was detected in two experiments, and all other identified proteins were detected only once, suggesting a high interindividual variation. Since α 2MG had been reported earlier¹⁵ as a binding partner for free FXIII-B, we analyzed this potential binding further.

3.4 | Binding of α 2MG to free FXIII-B

In an ELISA-based binding assay shown in [Figure 6A](#), recombinant FXIII-B bound to microplate wells coated with α 2MG in a concentration-dependent manner, but did not bind to the control wells. We also performed binding analysis with SPR, which was impacted by a buffer mismatch because commercial α 2MG preparations contain glycine as stabilizer. By adjusting the running buffer, the raw signals could be largely improved. Under these conditions, no binding to FXIII-B at up to 500 nM of α 2MG was observable,

although the FXIII-B surface was still active under these conditions as FXIII-A as positive control still bound strongly ([Figure 6B](#)). Thus, based on the results of the ELISA-based binding assay, binding may be possible, but we were not able to confirm binding of α 2MG to FXIII-B by SPR.

4 | DISCUSSION

The FXIII-B subunit has a critical function as carrier protein to stabilize FXIII-A in plasma and supply it to its main substrate fibrinogen. Therefore, the body must ensure that a sufficient plasma concentration of FXIII-B, which is produced at a different site than FXIII-A, is maintained. However, the large excess of FXIII-B over FXIII-A has puzzled FXIII experts for a long time, leading to the hypothesis that free FXIII-B may have other and hitherto unknown functions.

Considering the structural similarity with complement regulators FH and C4BP, an interaction between free FXIII-B and components of the complement system with regulatory or other crosstalk implications had emerged as one of the functional hypotheses. In the present study, we investigated whether FXIII-B may exert a functional profile similar to the cofactor activity of FH and C4BP in the degradation of C3b and C4b by FI. However, we observed no influence of FXIII-B. We also investigated the interaction of FXIII-B with C3b and C4b by ELISA- and SPR-based binding assays but could not

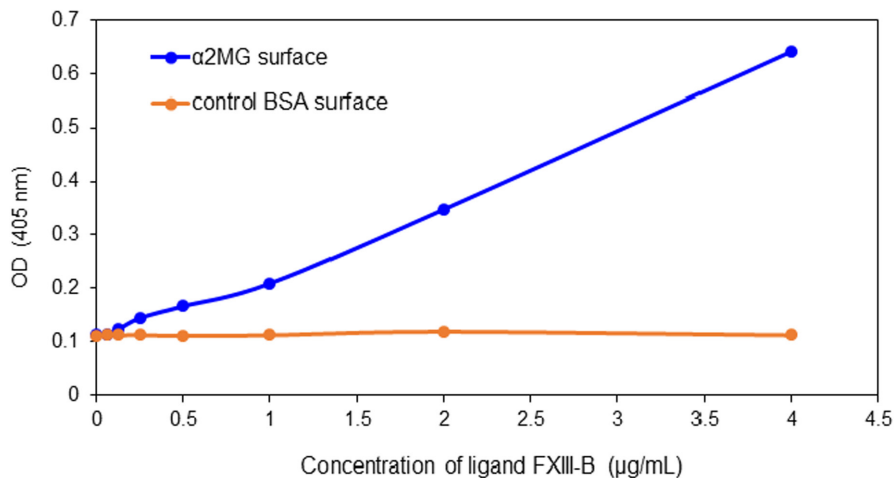
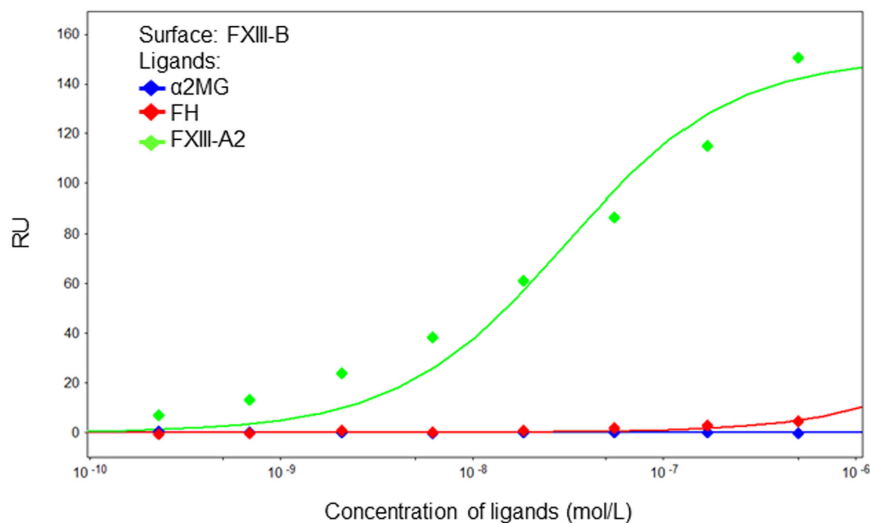
(A) ELISA-based binding assay**(B) SPR-based binding assay**

FIGURE 6 Binding between $\alpha 2\text{MG}$ and FXIII-B. (A) In an ELISA-based binding assay, recombinant FXIII-B₂ bound to $\alpha 2\text{MG}$ in a concentration-dependent manner. (B) In SPR analysis, no binding of $\alpha 2\text{MG}$ to a FXIII-B₂ surface was observed $\alpha 2\text{MG}$, α_2 -macroglobulin; FXIII-B, factor XIII-B; SPR, surface plasmon resonance

observe notable specific binding to those complement proteins. We therefore conclude that, despite structural relation to complement regulators, FXIII-B has no role in the regulation of the complement system that is analog to FH or C4BP.

Thus, the search for other potential binding partners of free FXIII-B continued. With the same aim, Singh et al.¹⁵ had also chosen an interesting approach employing mass spectrometry analysis but with substantial differences to our approach. First, Singh et al.¹⁵ analyzed the commercial FXIII concentrate (Fibrogammin, CSL Behring) by size exclusion chromatography and mass spectrometry analysis and identified albumin, complement FH, and $\alpha 2\text{MG}$. Singh et al.¹⁵ also performed immunoaffinity-based pull-down assays, in which a monoclonal antibody against FXIII-B coupled to a resin captured recombinant FXIII-B that was then exposed to FXIII-deficient plasma. Mass spectrometry analysis revealed the presence of $\alpha 2\text{MG}$, complement C3 and C1q, and fibrinogen.

In our approach, we used a monoclonal antibody that specifically targeted free FXIII-B (not in complex with FXIII-A) from the plasma and fresh human plasma obtained from four healthy

blood donors. We therefore believe that our approach may best represent physiological conditions. Interestingly, we observed a large interindividual variation between the four blood donors in the proteins that were bound to free FXIII-B. This may suggest that a unique physiological binding and interaction partner of the excess free FXIII-B does not exist, and that free FXIII-B may rather coincidentally bind to various proteins depending on the current environment.

On the other hand, in agreement with the results by Singh et al.¹⁵ and despite of the differences in our approaches, we also detected $\alpha 2\text{MG}$ in two of four plasma samples. We could show some binding between $\alpha 2\text{MG}$ and FXIII-B in an ELISA-based but not SPR-based binding assay. Confirmation and further insights into the nature of a potential interaction and physiological relevance are certainly needed as it may be an interesting candidate. $\alpha 2\text{MG}$ is a large (720 kDa) protein with a high plasma concentration ($272.9 \pm 76.5 \text{ mg/dl}$ in healthy individuals).²² It has many functions and is able to inactivate an enormous variety of proteinases without the direct blockage of the protease active site.²³ $\alpha 2\text{MG}$ has been shown to affect

coagulation and fibrinolysis by inhibiting factor Xa, thrombin, or plasmin,^{24,25} and it is a substrate for FXIII-A.²⁶ By bringing α 2MG and fibrinogen together, free FXIII-B might have another role in coagulation and fibrinolysis.

Future studies could therefore explore if free FXIII-B itself may directly and/or indirectly affect clot formation and lysis. As Souri et al.⁶ had shown, recombinant FXIII-B could directly bind to fibrinogen and suppress its digestion by plasmin by possibly masking cleavage sites. As an indirect mechanism, FXIII-B could also bind and recruit other proteins that in turn affect fibrin formation and lysis. In our immunoprecipitation and mass spectrometry approach, we have detected α 2MG and β 2-glycoprotein 1, which both are suggested to interact with fibrin formation and lysis.²⁴⁻²⁷

5 | CONCLUSION

The function of the excess free FXIII-B circulating in plasma is still elusive. Here, we largely eliminate free FXIII-B as functional contributor to the regulation of the complement system, despite its genetic association and structural similarity to specialized complement regulators such as FH. A novel approach of immunoprecipitation using a monoclonal antibody specific for free FXIII-B and fresh individual human plasma samples followed by mass spectrometry analysis revealed great interindividual differences in potential interaction partners of free FXIII-B. Among those, α 2MG remains the most likely candidate, although direct binding to FXIII-B with subsequent effects on clot formation and lysis could not yet be validated.

AUTHOR CONTRIBUTIONS

BL performed ELISA-based binding assays, developed the approach and performed the experiments to test for binding partners of free FXIII-B, and wrote the manuscript. CB performed and analyzed the SPR experiments. LJ performed the complement cofactor activity experiments. DR designed and analyzed the SPR experiments. VS designed the research, analyzed the results, and wrote the manuscript. All authors reviewed the manuscript.

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RELATIONSHIP DISCLOSURE

The authors declare no conflicts of interest.

ORCID

Verena Schroeder  <https://orcid.org/0000-0001-6508-3271>

REFERENCES

- Muszbek L, Bereczky Z, Bagoly Z, Komaromi I, Katona E. Factor XIII: a coagulation factor with multiple plasmatic and cellular functions. *Physiol Rev*. 2011;91:931-972.
- Schroeder V, Kohler HP. New developments in the area of factor XIII. *J Thromb Haemost*. 2013;11:234-244.
- Katona E, Penzes K, Csapo A, et al. Interaction of factor XIII subunits. *Blood*. 2014;123:1757-1763.
- Rodeghiero F, Tosetto A, Di Bona E, Castaman G. Clinical pharmacokinetics of a placenta-derived factor XIII concentrate in type I and type II factor XIII deficiency. *Am J Hematol*. 1991;36:30-34.
- Saito M, Asakura H, Yoshida T, et al. A familial factor XIII subunit B deficiency. *Br J Haematol*. 1990;74:290-294.
- Souri M, Osaki T, Ichinose A. The non-catalytic B subunit of coagulation factor XIII accelerates fibrin cross-linking. *J Biol Chem*. 2015;290:12027-12039.
- Souri M, Kaetsu H, Ichinose A. Sushi domains in the B subunit of factor XIII responsible for oligomer assembly. *Biochemistry*. 2008;47:8656-8664.
- Rodriguez de Cordoba S, Rey-Campos J, Dykes KK, McAlpine PJ, Wong P, Rubinstein P. Coagulation factor XIII B subunit is encoded by a gene linked to the regulator of complement activation (RCA) gene cluster in man. *Immunogenetics*. 1988;28:452-454.
- Rey-Campos J, Baeza-Sanz D, Rodriguez de Cordoba S. Physical linkage of the human genes coding for complement factor H and coagulation factor XIII B subunit. *Genomics*. 1990;7:644-646.
- Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and immune control. *Nat Immunol*. 2010;11:785-797.
- Ferreira VP, Pangburn MK, Cortes C. Complement control protein factor H: the good, the bad, and the inadequate. *Mol Immunol*. 2010;47:2187-2197.
- Blom AM, Villoutreix BO, Dahlbäck B. Functions of human complement inhibitor C4b-binding protein in relation to its structure. *Arch Immunol Ther Exp (Warsz)*. 2004;52:83-95.
- Krushkal J, Bat O, Gigli I. Evolutionary relationships among proteins encoded by the regulator of complement activation gene cluster. *Mol Biol Evol*. 2000;17:1718-1730.
- Biswas A, Thomas A, Bevans CG, Ivaskevicius V, Oldenburg J. In vitro secretion deficits are common among human coagulation factor XIII subunit B missense mutants: correlations with patient phenotypes and molecular models. *Hum Mutat*. 2013;34:1490-1500.
- Singh S, Akhter MS, Dodt J, et al. Identification of potential novel interacting partners for coagulation factor XIII B (FXIII-B) subunit, a protein associated with a rare bleeding disorder. *Int J Mol Sci*. 2019;20:2682.
- Akhter MS, Singh S, Yadegari H, Ivaskevicius V, Oldenburg J, Biswas A. Exploring the structural similarity yet functional distinction between coagulation factor XIII-B and complement factor H sushi domains. *J Thromb Thrombolysis*. 2019;48:95-102.
- Ariens RA, Kohler HP, Mansfield MW, Grant PJ. Subunit antigen and activity levels of blood coagulation factor XIII in healthy individuals. Relation to sex, age, smoking, and hypertension. *Arterioscler Thromb Vasc Biol*. 1999;19:2012-2016.
- Tortajada A, Montes T, Martinez-Barricarte R, Morgan BP, Harris CL, Rodriguez de Cordoba S. The disease-protective complement factor H allotypic variant Ile62 shows increased binding affinity for C3b and enhanced cofactor activity. *Hum Mol Genet*. 2009;18:3452-3461.

19. Happonen KE, Sjöberg AP, Mörgelin M, Heinegard D, Blom AM. Complement inhibitor C4b-binding protein interacts directly with small glycoproteins of the extracellular matrix. *J Immunol.* 2009;182:1518-1525.
20. Brügger V, Duman M, Bochud M, et al. Delaying histone deacetylase response to injury accelerates conversion into repair Schwann cells and nerve regeneration. *Nat Commun.* 2017;8:14272.
21. Richardson VR, Schroeder V, Grant PJ, Standeven KF, Carter AM. Complement C3 is a substrate for activated factor XIII that is cross-linked to fibrin during clot formation. *Br J Haematol.* 2013;160:116-119.
22. Yoshino S, Fujimoto K, Takada T, et al. Molecular form and concentration of serum α_2 -macroglobulin in diabetes. *Sci Rep.* 2019;9:12927.
23. Rehman AA, Ahsan H, Khan FH. α -2-Macroglobulin: a physiological guardian. *J Cell Physiol.* 2013;228:1665-1675.
24. Meijers JC, Tijburg PN, Bouma BN. Inhibition of human blood coagulation factor Xa by alpha 2-macroglobulin. *Biochemistry.* 1987;26:5932-5937.
25. Lin H, Xu L, Yu S, Hong W, Huang M, Xu P. Therapeutics targeting the fibrinolytic system. *Exp Mol Med.* 2020;52:367-379.
26. Nikolajsen CL, Dyrland TF, Poulsen ET, Enghild JJ, Scavenius C. Coagulation factor XIIIa substrates in human plasma: identification and incorporation into the clot. *J Biol Chem.* 2014;289:6526-6534.
27. McDonnell T, Wincup C, Buchholz I, et al. The role of beta-2-glycoprotein I in health and disease associating structure with function: more than just APS. *Blood Rev.* 2020;39:100610.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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