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Functional recombinant human complement C1q with different affinity tags

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Abbreviations

CLR	collagen-like region
GR	globular region
NHS	normal human serum
SPR	surface plasmon resonance

Abstract

Complement C1q is a multifunctional protein able to sense pathogens and immune molecules such as immunoglobulins and pentraxins, and to trigger the classical complement pathway through activation of its two associated proteases, C1r and C1s. C1q is a multimeric protein composed of three homologous yet distinct polypeptide chains A, B, and C, each composed of an N-terminal collagen-like sequence and a C-terminal globular gC1q module, that assemble into six heterotrimeric (A-B-C) subunits. This hexameric structure exhibits the characteristic shape of a bouquet of flowers, comprising six collagen-like triple helices, each terminating in a trimeric C-terminal globular head. We have produced previously functional recombinant full-length C1q in stably transfected HEK 293-F cells, with a FLAG tag inserted at the C-terminal end of C1qC chain. We report here the generation of additional recombinant C1q proteins, with a FLAG tag fused to the C-terminus of C1qA or C1qB chains, or to the N-terminus of the C1qC chain. Two other variants harboring a Myc or a 6-His tag at the C-terminal end of C1qC were also produced. We show that all C1q variants, except for the His-tagged protein, can be produced at comparable yields and are able to bind with similar affinities to either IgM, a ligand of the globular regions, or to the C1r₂-C1s₂ tetramer, and to trigger IgM-mediated serum complement activation. These new recombinant C1q variants provide additional tools to investigate the multiple functions of C1q.

Key Words: Complement, C1q, recombinant protein, affinity tag, interaction properties

1. Introduction

The C1q protein is part of the initiating complex of the classical complement pathway (called C1), an important actor of humoral innate immunity in mammals. Besides C1q, C1 comprises two homologous serine proteases, C1r and C1s that are associated in a C1r₂-C1s₂ tetramer. Binding of C1q to danger or damage signals, including antigen-antibody complexes and factors present at the surface of pathogens or infected cells, triggers auto-activation of C1r, which then activates C1s and initiates the complement proteolytic cascade (Reid, 2018). Complement activation leads to target opsonization for enhanced phagocytosis, to the development of an inflammatory response and ultimately triggers the lytic pathway for destruction of complement-sensitive pathogens (Merle et al., 2015; Ricklin et al., 2010). In addition, C1q recognizes altered self-elements including apoptotic cells and contributes to their safe elimination and maintenance of host tissue homeostasis (Lu et al., 2008; Ricklin et al., 2013). C1q is also involved in several non-canonical functions including modulation of immune cells, coagulation, development, and in the pathogenesis of numerous diseases, among which central nervous system disorders, pregnancy complications and cancer (Koussorou et al., 2015; Nayak et al., 2010; Thielens et al., 2017). These functions arise from the binding versatility of C1q for an amazing variety of soluble and cell surface ligands, a feature related to the complexity of its structure.

C1q is a high molecular weight (about 460 kDa) multimeric protein assembled from 18 polypeptide chains of three types: 5 C1qA, 6 C1qB and 6 C1qC chains, each containing a collagen-like N-terminal region and a globular gC1q C-terminal domain. The A and B chains are linked by a disulfide bond and are non-covalently associated with a C chain to form a heterotrimeric collagen-like helix terminating in a trimeric globular region. In addition, a disulfide bond between two C chains results in a basic subunit composed of two heterotrimers and three subunits compose the C1q molecule with an overall characteristic shape of a bouquet of flowers (Fig. 1A). From a functional point of view, C1q contains two regions, the globular regions responsible for C1q binding to its targets, including immunoglobulins, and the collagen-like regions responsible for association with its cognate C1r and C1s proteases and most C1q cell receptors (Reid, 2018).

This complexity of the C1q molecule accounts for the fact that its expression in a recombinant form could be achieved only a few years ago. We were able to produce a full-length functional recombinant C1q molecule using HEK 293-F cells stably transfected with plasmids encoding

each of its three chains (Bally et al., 2013). The generation of different recombinant C1q proteins with point mutations in the collagen-like regions or in the globular regions allowed us to identify important residues for C1q interaction with its associated proteases or for binding to immunoglobulins and pentraxins, respectively (Bally et al., 2013, 2019b; Espericueta et al., 2020). All reported C1q mutants were generated based on a recombinant protein with a FLAG tag inserted at the C-terminal end of C1qC chain. With a view to generate additional recombinant C1q protein tools, we investigated the possibility to produce recombinant C1q either with a different tag (Myc or 6-histidine) located at the C-terminus of C1qC, or with a FLAG tag at different locations (N-terminus of C1qC, C-terminus of C1qA or C1qB). The ability of the purified newly produced variants to interact with IgM, a ligand typical of C1q globular region, and with the C1r₂-C1s₂ tetramer, associated to the collagen-like stalks was investigated, as well as their serum complement activating capacity. The functional properties of these variants were compared with those of the initially produced recombinant C1q with a FLAG tag at the C-terminal end of the C chain.

2. Material and Methods

2.1 Proteins and Reagents

Human IgM and BSA were from Sigma. The molar concentration of IgM was estimated using a Mr of 900,000 and A_{1%, 1cm} at 280 nm of 11.8. L-ascorbic acid and L-ascorbic acid 2-phosphate were from Sigma. Oligonucleotides were from Eurogentec and restriction and modification enzymes from New England Biolabs.

2.2 Expression Vectors

The pcDNA3.1 plasmids coding for the individual C1qA, C1qB and C1qC chains and containing neomycin-, hygromycin-, and zeocin-resistance genes, respectively, have been described previously (Bally et al., 2013). The pcDNA3.1_C1qA and pcDNA3.1_C1qB plasmids were used as templates to generate expression vectors for C1qA and C1qB chain with a C-terminal FLAG epitope (DYKDDDDK) (pcDNA3.1_C1qA-FLAG and pcDNA3.1_C1qB-FLAG) by site-directed mutagenesis (Quick Change XL site-directed mutagenesis kit; Agilent Technologies). The vectors for expression of C1qC chain with an N-terminal FLAG epitope (pcDNA3.1_FLAG-C1qC), a C-terminal Myc epitope (EQKLISEEDL) (pcDNA3.1_C1qC-

Myc), or a C-terminal 6-histidine tag (pcDNA3.1_C1qC-His) were generated in the same way, using the pcDNA3.1_C1qC plasmid as a template. The mutagenic oligonucleotides used are provided in Table S1. All constructs were verified by DNA sequencing (GATC Biotech).

2.3 Stably Transfected Cell Lines

Stable FreeStyle 293-F cell lines producing the individual B and C, or A and C chains of C1q were generated as described previously for the stable transfectants expressing the A and B chains of C1q (Bally et al., 2013). Cells producing the C1qB and C1qC chains, grown in FreeStyle 293 medium supplemented with 100 µg/ml hygromycin (Thermo Fisher), and 10 µg/ml zeocin (Thermo Fisher), were transfected with the C1qA-FLAG-containing plasmid, and stable transfectants producing the three C1q chains were obtained following additional selection with 400 µg/ml G418 (Thermo Fisher). In the same way, stably transfectants producing C1qA and C1qC chains were transfected with the C1qB-FLAG-containing plasmid, and stably transfected cells producing the three C1q chains were obtained following additional selection with 100 µg/ml hygromycin. Stably transfected cells producing C1qA and C1qB chains were transfected with the pcDNA3.1_FLAG-C1qC, pcDNA3.1_C1qC-myc or pcDNA3.1_C1qC-His plasmids and stable transfectants expressing the three chains obtained after selection with 10 µg/ml zeocin. Cells producing C1q with a FLAG-tag at the C-terminal end of the C-chain (rC1q_C-FLAG) have been described previously (Bally et al., 2013).

2.4 Production and purification of C1q variants

The cells expressing each of the six different C1q variants, rC1q_A-FLAG, rC1q_B-FLAG, rC1q_C-FLAG, rC1q_FLAG-C, rC1q_C-myc or rC1q_C-His, were expanded in the Freestyle 293 expression medium containing the three selection antibiotics and supplemented with 250 µM L-ascorbic acid and 450 µM L-ascorbic acid 2-phosphate (Koch et al., 2006). The culture medium was harvested and replaced every 72 h up to three times. All recombinant C1q variants were purified from the culture supernatants using a first step of adsorption on insoluble IgG-ovalbumin aggregates (Arlaud et al., 1979). The extracts containing rC1q with a FLAG or Myc tag were dialyzed against 50 mM Tris-HCl and 150 mM NaCl (pH 7.4). C1q variants with a FLAG-tag were further purified by chromatography on an anti-FLAG M2 affinity column (Sigma-Aldrich) as described previously (Bally et al., 2013). The rC1q_C-Myc extract was

applied to a 2 ml anti-c-Myc agarose affinity column (Pierce) equilibrated in the dialysis buffer. After washing with 20 ml of the equilibration buffer, the bound material was eluted twice with 2 ml of a 1 mg/ml solution of c-Myc peptide (Sigma-Aldrich) in the same buffer. The eluted fractions were dialyzed against 50 mM Tris-HCl and 150 mM NaCl (pH 7.4) and concentrated to 0.3–0.4 mg/ml. The rC1q_C-His extract was dialyzed against PBS containing 10 mM imidazole and applied to a 1.5 ml HIS-Select HF Nickel Affinity column (Sigma) equilibrated in the dialysis buffer. After washing with 20 ml of the equilibration buffer, the bound material was eluted in PBS containing 300 mM imidazole. The eluted fractions were dialyzed against 50 mM Tris-HCl and 150 mM NaCl (pH 7.4) and concentrated to 0.3–0.4 mg/ml. The concentration of purified rC1q was estimated using an absorption coefficient ($A_{1\%, 1\text{cm}}$) at 280 nm of 6.8 and a Mr value of 460,000. Recombinant rC1q variants were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing and reducing conditions using Tris-HCl gels containing 14% polyacrylamide, and colored using Instant Blue (Expedeon).

2.5 Production of the recombinant proenzyme C1r₂-C1s₂ tetramer

The recombinant proenzyme C1r₂-C1s₂ tetramer was obtained by co-transfection with plasmids encoding C1r and C1s serine proteases, each stabilized in a zymogen form by mutation of the active site serine residues 637 (C1r) and 617 (C1s) to alanine (mature proteins numbering). The tetramer was produced in the Freestyle 293 Expression System (Thermo Fisher), using a pcDNA3.1/Zeo(+) plasmid encoding human C1r with the Ser637Ala mutation (Bally et al., 2019b) and a pcDNA3.1/Neo(+) plasmid encoding human C1s with the Ser617Ala mutation. The latter was generated by site-directed mutagenesis using the pcDNA3.1/Neo(+) plasmid encoding human C1s with a C-terminal FLAG tag (Bally et al., 2019a) as a template and the mutagenic primers described in Table S1. Generation of stably co-transfected 293-F cells and purification of the recombinant proenzyme tetramer from the culture supernatant by anti-FLAG affinity chromatography were performed as described in (Bally et al., 2013). The molar concentration of the tetramer was estimated using a Mr value of 344,500 and an absorption coefficient ($A_{1\%, 1\text{cm}}$) at 280 nm of 13.45.

2.6 SPR analyses and data evaluation

Analyses were performed at 25 °C using a Biacore T200 instrument (Cytiva). The C1q variants and BSA, diluted at 50 µg/ml in 10 mM sodium acetate pH 4.5 and 25 µg/ml in 10 mM sodium acetate pH 4, respectively, were immobilized on CM5 sensor chips (Cytiva) using the amine coupling chemistry in 10 mM Hepes, 150 mM NaCl, 3 mM EDTA, 0.05% surfactant P20, pH 7.4. Binding was measured at a flow rate of 20 µl/min in 50 mM Tris-HCl, 150 mM NaCl, 2 mM CaCl₂, 0.05% surfactant P20, pH 7.4. The specific binding signal was obtained by subtracting the signal over the BSA reference surface. The surfaces were regenerated by 10 µl injections of 1M NaCl, 10 mM EDTA, pH 7.4. Kinetic data were analyzed by global fitting to a 1:1 Langmuir binding model for six concentrations simultaneously, using the Biacore T200 evaluation 2.0 software (Cytiva). Buffer blanks were subtracted from the data sets used for kinetic analyses. The apparent equilibrium dissociation constants (K_D) were calculated from the ratio of the dissociation and association rate constants (k_d/k_a). Chi2 values were between 2.7 and 5.8 in all cases.

2.7 Classical complement pathway activation assays

Microtiter plates (Maxisorp Nunc) were coated with IgM (2 µg/ml) in 35 mM NaHCO₃, 15 mM Na₂CO₃, pH 9.6 overnight at 4 °C. Wells were saturated for 1 h at 37 °C with 2% BSA (w/v) in PBS and washed with PBS containing 0.05% Tween 20 (PBS-T). C1q-depleted serum (CompTech), diluted 1:25 in 5 mM Na veronal, 145 mM NaCl, 5 mM CaCl₂, 1.5 mM MgCl₂, pH 7.5 and reconstituted with the recombinant C1q variants (4 µg/ml), was added to the wells and incubated for 1 h at 37 °C. The wells were washed with 5 mM Na veronal, 145 mM NaCl, 5 mM EDTA, pH 7.5 before addition of a rabbit anti-C4 polyclonal antibody (diluted 1:1,000 in PBS-T with 0.2% BSA (w/v)) (Siemens Healthcare Diagnostics) and incubation for 1 h at 37 °C. After washing with PBS-T and incubation with a peroxidase-conjugated goat anti-rabbit polyclonal antibody (diluted 1:20,000 in PBS-T with 0.2% BSA (w/v)) (Jackson ImmunoResearch) for 1 h at 37 °C, plates were washed with PBS-T and developed with 3,3',5,5'-tetramethylbenzidine (Tebu). The reaction was stopped with 1 N H₂SO₄ and absorbance was read at 450 nm. Each assay was performed in duplicate. Normal human serum (NHS) was obtained from the Etablissement Français du Sang Rhône-Alpes (agreement number 14-1940 regarding its use in research) and used as a positive control.

3. Results

3.1 Production and biochemical characterization of the C1q variants

The original recombinant C1q (C1q_C-FLAG) and five new variants have been produced, harboring fusion tags differing in nature (FLAG, Myc or His) and location (C-terminal end of C1qA, C1qB or C1qC, or N-terminal end of C1qC), named herein C1q_A-FLAG, C1q_B-FLAG, C1q_FLAG-C, C1q_C-Myc and C1q_C-His. The C1q variants were produced in 293-F cells stably transfected with the three C1q chains (two untagged and one tagged chains). The recombinant proteins were purified from the cell culture supernatants based on C1q affinity for insoluble IgG-ovalbumin aggregates, as described previously for C1q_C-FLAG (Bally et al., 2013). Further purification was achieved based on the nature of the fusion tag, using a resin functionalized with anti-FLAG or anti-Myc antibodies, or with immobilized nickel ions, aiming at elimination of the contaminating IgG molecules arising from the preceding step. The amount of purified concentrated protein recovered from 1 l of culture supernatant varied from 0.35 mg (variants with the FLAG-tag at the C-terminus of C1qA, C1qB or C1qC chain), to 0.4 and 0.55 mg for the C-ter Myc- and N-ter FLAG-tagged C1qC variants, respectively. A very low amount of protein was recovered in the case of the variant harboring a C-terminal His-tagged C1qC chain (less than 4 µg from 400 ml culture supernatant) which proved in addition to be still contaminated with IgG (Fig. 1B, C). SDS-PAGE analysis of the five other variants yielded typical C1q band patterns, with characteristic A-B and C-C dimers under non-reducing conditions (Fig. 1A, B) and the three A, B and C chains under reducing conditions (Fig. 1A, C), with small differences between the variants accounting for the presence of the tags on the respective chains. A slightly higher apparent molecular weight of the C chain and its dimer was observed for the three variants with a tagged C-chain, independently of the tag nature (FLAG or Myc) or location. In the same way, the apparent molecular weights of the FLAG-tagged A and B chains were slightly higher than those of their non-tagged counterparts, a difference only visible under reducing conditions (Fig. 1C) since the A-B dimers migrated similarly (Fig. 1B). It should be noted that the untagged A chain and FLAG-tagged B chain could not be separated under reducing conditions (Fig. 1C). Indeed, addition of a tag to the B chain induces a slight increase in its apparent molecular weight, resulting in migration at the same level as untagged A chain and merging of both bands. SDS-PAGE and Western blot analysis of the culture supernatant corresponding to C1q_C-His, using a polyclonal anti-C1q antibody, revealed the presence of the

three chains under reducing conditions, but of only very low amounts of A-B and of virtually no C-C dimers under non-reducing conditions (not shown). This observation strongly suggested that most of this recombinant variant was not correctly assembled, and did not interact with immune complexes in the first purification step. The functional characterization of the C1q variants was next performed with all samples, except for C1q_C-His.

3.2 Interaction properties of the C1q variants with IgM and C1r and C1s proteases

The functionality of the C1q variants was first investigated by SPR analysis of their interaction with two emblematic physiological ligands of C1q globular and collagen-like regions, IgM and the C1r₂-C1s₂ tetramer, respectively. IgM bound to immobilized C1q_C-FLAG variant, in accordance with our previous data (Bally et al., 2019b) (Fig. 2A), and also to the four other variants (Fig. 2B-E). Kinetic analyses of the interactions yielded association rate constants (k_a) in the same range (1.10 - $1.78 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$) and similar dissociation rate constants (k_d) (1.75 - $1.85 \times 10^{-3} \text{ s}^{-1}$) for all C1q variants (Table 1). The deduced apparent K_D values were in the nM range (1.0 - 1.6 nM), reflecting a comparable high affinity of the five C1q variants for IgM. In the same way, the C1r₂-C1s₂ tetramer interacted with immobilized C1q_C-FLAG variant (Fig. 2F), as reported in our previous studies (Bally et al., 2019b, 2013) and with the four other C1q variants (Fig. 2G-J). Kinetic analyses yielded association and dissociation rate constants in the same range ($k_a = 5.55$ - $9.40 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $k_d = 2.90$ - $3.66 \times 10^{-3} \text{ s}^{-1}$), resulting in comparable apparent dissociation constants ($K_D = 3.7$ - 5.2 nM) (Table 1). These data indicated that the presence of the tags had no significant impact on the functionality of both globular and collagen-like regions within the recombinant C1q molecules.

3.3 IgM-dependent serum complement activation by the C1q variants

The capacity of the C1q variants to trigger activation of the classical complement pathway was next analyzed in a C4b deposition assay, using wells coated with IgM and C1q-depleted human serum as a source of complement components (C1r/C1s proteases and C4). As expected, the five C1q variants yielded amounts of deposited C4b comparable to those obtained with NHS whereas no significant signal was observed with C1q-depleted serum (Fig. 3). These results confirmed the functionality of the C1q variants, in accordance with the SPR interaction analyses.

4. Discussion

The only recombinant full-length C1q protein currently available has been engineered with a FLAG-tag fused to C1qC chain (rC1q_C-FLAG) and could be used since as a template for the generation of mutants to map ligand binding sites in the collagen-like and globular regions (Bally et al., 2019b, 2013). Our initial tag choice had been motivated by our unpublished observations suggesting that the formation of the disulfide-linked C-C dimer was a limiting factor in the assembly of the full-length molecule.

We show here that the location of the tag does not appear as a limiting factor for production of a functional recombinant C1q protein. Indeed, fusion of the FLAG tag at the C-terminal end of C1qA or C1qB chain allowed production of recombinant C1q with expected binding properties for IgM, a major ligand of its globular regions. This lack of interference can likely be explained by the position of the C-terminal tags in the 3-D structure of the globular regions of C1q. Indeed, as revealed by the X-ray crystal structure of the globular region of C1q (Gaboriaud et al., 2003; Moreau et al., 2016), the N- and C-termini of the three gC1q modules emerge at the base of the trimer. Thus, addition of a FLAG-tag at C1qA, C1qB or C1qC chain results in the location of the tag in a common area at the base of the GR, close to the junction with the collagen-like region of C1q (Fig. 4). The fact that the interaction properties with the associated proteases are intact confirms the correct assembly of the collagen stalks of the C1q variants. Interestingly enough, fusion of the tag with the N-terminus of C1qC had also no detectable impact on C1q ability to interact either with IgM or with the C1r/C1s proteases. Although the corresponding variants harboring the FLAG tag at the N-terminus of C1qA or C1qB were not produced in the present study, it may be assumed that they would be functional since the N-terminal extremities of the three chains are also in close proximity (Fig. 4). Indeed, a cysteine residue engaged in a disulfide bond (A-B and C-C) is present at the fourth position of each mature C1q chain.

In addition, the present study points out that the nature of the tag rather than its position has likely a bigger impact on recombinant C1q production. Indeed, fusion of C1qC chain to a C-terminal His tag strongly diminished the recovery of correctly folded material and yielded contaminated purified material, precluding functional characterization of this C1q variant. In contrast, insertion of a C-terminal Myc tag yielded a C1q molecule with functional properties undistinguishable from its FLAG-tagged counterpart. The reason of this differential behavior of the recombinant proteins might result from the negatively charged nature of either FLAG

(DYKDDDDK) or Myc (EQKLISEEDL) tag, by comparison with 6-His tag, possibly influencing the tag orientation through electrostatic interactions with neighboring protein residues. The low recovery of the His-tagged variant could also be related to the presence in the GR of a solvent exposed calcium ion, likely contributing to the stability of the GR (Gaboriaud et al., 2003) and possibly removed by the presence of imidazole and nickel ions in the metal chelate chromatography step (Kutyshenko et al., 2019). The lack of interference with C1q variants interaction with IgM can be explained by the tag location at the junction of the GR and CLR, an area not involved in the interaction with immune ligands such as immunoglobulins and pentraxins. Both interactions were shown previously to involve preferentially B chain Arg residues that line the side of the gC1q heterotrimer, with a minor participation of a Tyr residue located at the apex of gC1q trimer (Bally et al., 2019b; Gadjeva et al., 2008; Roumenina et al., 2006; Zlatarova et al., 2006). (Fig. 4)

In conclusion, we have produced novel functional C1q variants with affinity tags differing in the tag nature and location, which open new possibilities for future studies where use of a Myc tag rather than a FLAG tag would be desirable, for example in pull-down experiments. These variants proved to be functional regarding their capacity to interact with two canonical C1q ligands involving either the globular or collagen-like regions. However, a possible interference of the C-terminal tags cannot be excluded in case of a ligand binding site located at the junction of the CLR and GR, as proposed previously for fibronectin (Reid and Edmondson, 1984). This possible issue could be overcome by using a tag fused to the N-terminus part of C1q chains. These recombinant full-length molecules represent additional tools to study the functional versatility of the C1q protein, together with the recombinant single chain globular regions (Moreau et al., 2016) and the recently produced recombinant CLRs obtained by substitution of C1q GR by the nc2 domain of type IX collagen (Fouët et al., 2020).

Declaration of Competing Interest

IB and NMT are co-inventors of a patent describing a method for preparing C1q recombinant protein.

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Figure Legends

Figure 1. Schematic representation of C1q chains pattern and SDS-PAGE analysis of the C1q variants. (A) C1q comprises three polypeptide chains (A, B and C) each containing an N-terminal collagen-like sequence and a C-terminal gC1q module. A particular inter-chain disulfide pattern (A-B and C-C) results in a basic subunit comprised of two heterotrimeric collagen-like stalks prolonged by globular domains. Three subunits associate non-covalently to yield the full-length protein with a typical shape of a bouquet of six flowers. (B, C) Four μg of each variant were analyzed by SDS-PAGE and Coomassie blue staining, under non-reducing (B) and reducing (C) conditions, allowing visualization of the A-B and C-C dimers, and of the three individual chains, respectively.

Figure 2. Kinetic analyses of the interaction of IgM and the C1r₂-C1s₂ tetramer with immobilized C1q variants. Sixty μl of IgM or of the C1r₂-C1s₂ tetramer at the indicated concentrations were injected over rC1q_C-FLAG (12,400 RU) (A, F), rC1q_A-FLAG (12,900 RU) (B, G), rC1q_B-FLAG (11,800 RU) (C, H), rC1q_FLAG-C (13,700 RU) (D, I) and rC1q_C-Myc (13,400 RU) (E, J) in 50 mM Tris-HCl, 150 mM NaCl, 2 mM CaCl₂, 0.05% surfactant P20, pH 7.4 at a flow rate of 20 $\mu\text{l}/\text{min}$. The binding signals shown were obtained by subtracting the signal over the BSA reference surface and further subtraction of buffer blanks. Fits are shown as black lines and were obtained by global fitting of the data using a 1:1 Langmuir binding model. Each kinetic analysis shown is representative of two to four experiments performed on separate sensor chips.

Figure 3. IgM-dependent complement activation by the C1q variants. C1q-depleted human serum (1:25 dilution) was reconstituted with the recombinant C1q variants (4 $\mu\text{g}/\text{ml}$) and added to microwells coated with 2 $\mu\text{g}/\text{ml}$ IgM. The resulting C1-cleaving activity was measured by a C4b deposition assay as described under Material and Methods. Deposited C4b was detected with an anti-human polyclonal antibody, and results are expressed as absorbance at 450 nm (OD₄₅₀), (means \pm SEM of two independent experiments each performed in duplicate). Normal human serum (NHS, 1:25 dilution) is shown as a positive control. Ctrl: signal obtained without IgM coated.

Figure 4. Three-dimensional model of C1q highlighting the location of the affinity tags and the IgM and C1r₂-C1s₂ tetramer binding sites. The model was assembled as described previously (Gaboriaud et al., 2004, 2003; Pflieger et al., 2010). The C1q chains are colored dark blue (A), green (B), and cyan (C). Red arrows indicate the location of the N-terminal and C-terminal tags. The positions of the side chains of the three lysine residues involved in the interaction with C1r₂-C1s₂ and of the two arginine and the tyrosine residues important for IgM binding are shown.

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TABLE 1. Kinetic and dissociation constants for binding of IgM and the C1r₂-C1s₂ tetramer to immobilized C1q variants

C1q ligand	Constants	Immobilized C1q variants				
		CFlag-Cter	AFlag-Cter	BFlag-Cter	Cmyc-Cter	N-ter-CFlag
IgM	k_a (M ⁻¹ s ⁻¹)	1.34 ± 0.05 × 10 ⁶ (9.19 ± 2.08 × 10 ⁶)	1.78 ± 0.02 × 10 ⁶	1.65 ± 0.01 × 10 ⁶	1.20 ± 0.02 × 10 ⁶	1.12 ± 0.03 × 10 ⁶
	k_d (s ⁻¹)	1.84 ± 0.11 × 10 ⁻³ (1.54 ± 0.19 × 10 ⁻³)	1.75 ± 0.01 × 10 ⁻³	1.83 ± 0.01 × 10 ⁻³	1.85 ± 0.04 × 10 ⁻³	1.82 ± 0.02 × 10 ⁻³
	K_D (M)	1.38 ± 0.13 × 10 ⁻⁹ (1.92 ± 0.68 × 10 ⁻⁹)	9.84 ± 0.07 × 10 ⁻¹⁰	1.11 ± 0.01 × 10 ⁻⁹	1.54 ± 0.07 × 10 ⁻⁹	1.63 ± 0.06 × 10 ⁻⁹
C1r ₂ -C1s ₂	k_a (M ⁻¹ s ⁻¹)	8.07 ± 0.99 × 10 ⁵ (4.35 ± 0.15 × 10 ⁵)	5.55 ± 0.05 × 10 ⁵	6.11 ± 0.15 × 10 ⁵	5.40 ± 0.55 × 10 ⁵	7.41 ± 0.57 × 10 ⁵
	k_d (s ⁻¹)	3.66 ± 0.12 × 10 ⁻³ (2.88 ± 0.19 × 10 ⁻³)	2.90 ± 0.09 × 10 ⁻³	3.20 ± 0.12 × 10 ⁻³	3.44 ± 0.05 × 10 ⁻³	3.50 ± 0.22 × 10 ⁻³
	K_D (M)	4.58 ± 0.44 × 10 ⁻⁹ (6.36 ± 0.42 × 10 ⁻⁹)	5.22 ± 0.12 × 10 ⁻⁹	5.27 ± 0.37 × 10 ⁻⁹	3.67 ± 0.21 × 10 ⁻⁹	4.72 ± 0.06 × 10 ⁻⁹

Values are the means ± SD of two to four separate experiments.

The values in parentheses for the CFlag-Cter variant correspond to previously published values (Bally et al., 2019b)

- Recombinant C1q can be produced with a FLAG or Myc tag fused to A, B or C chain
- A C-terminal His-tag fused the C chain impedes recombinant C1q production
- The FLAG- or Myc-tagged C1q variants interact with IgM and the C1r/C1s proteases
- The newly produced C1q variants trigger IgM-mediated serum complement activation
- The tag location is not a limiting factor for functional recombinant C1q production

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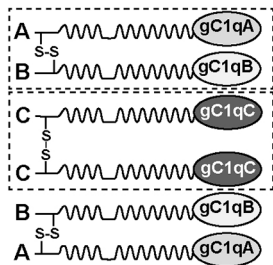
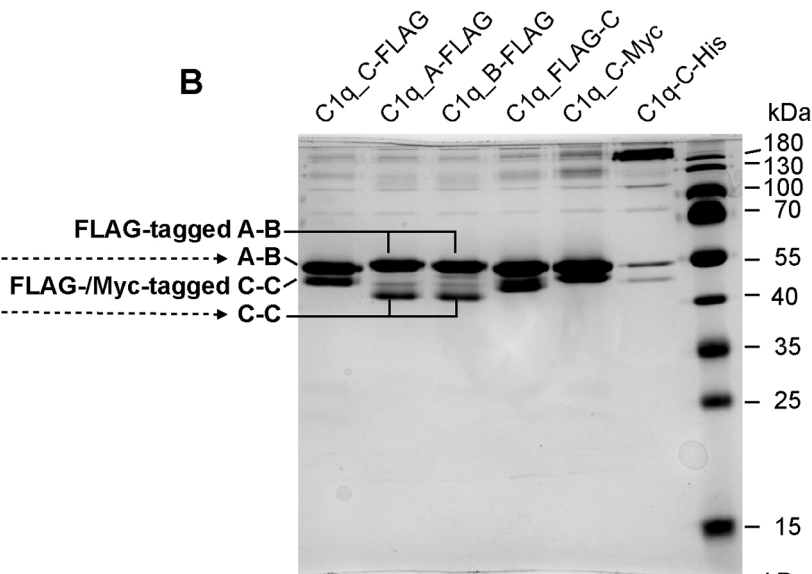
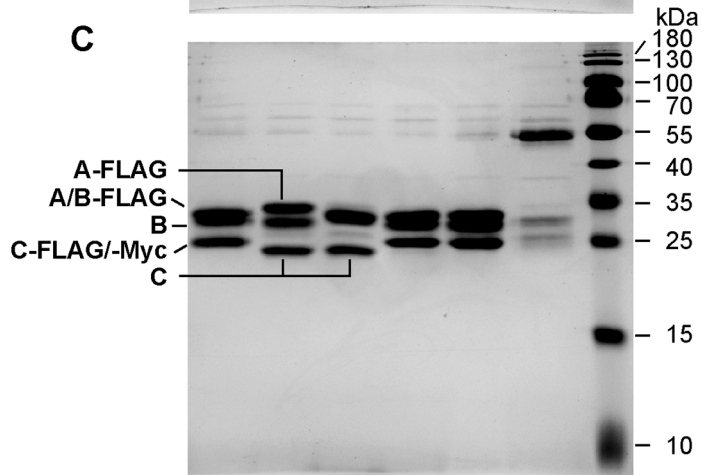
A**Full-length C1q molecule****B****C**

Figure 1

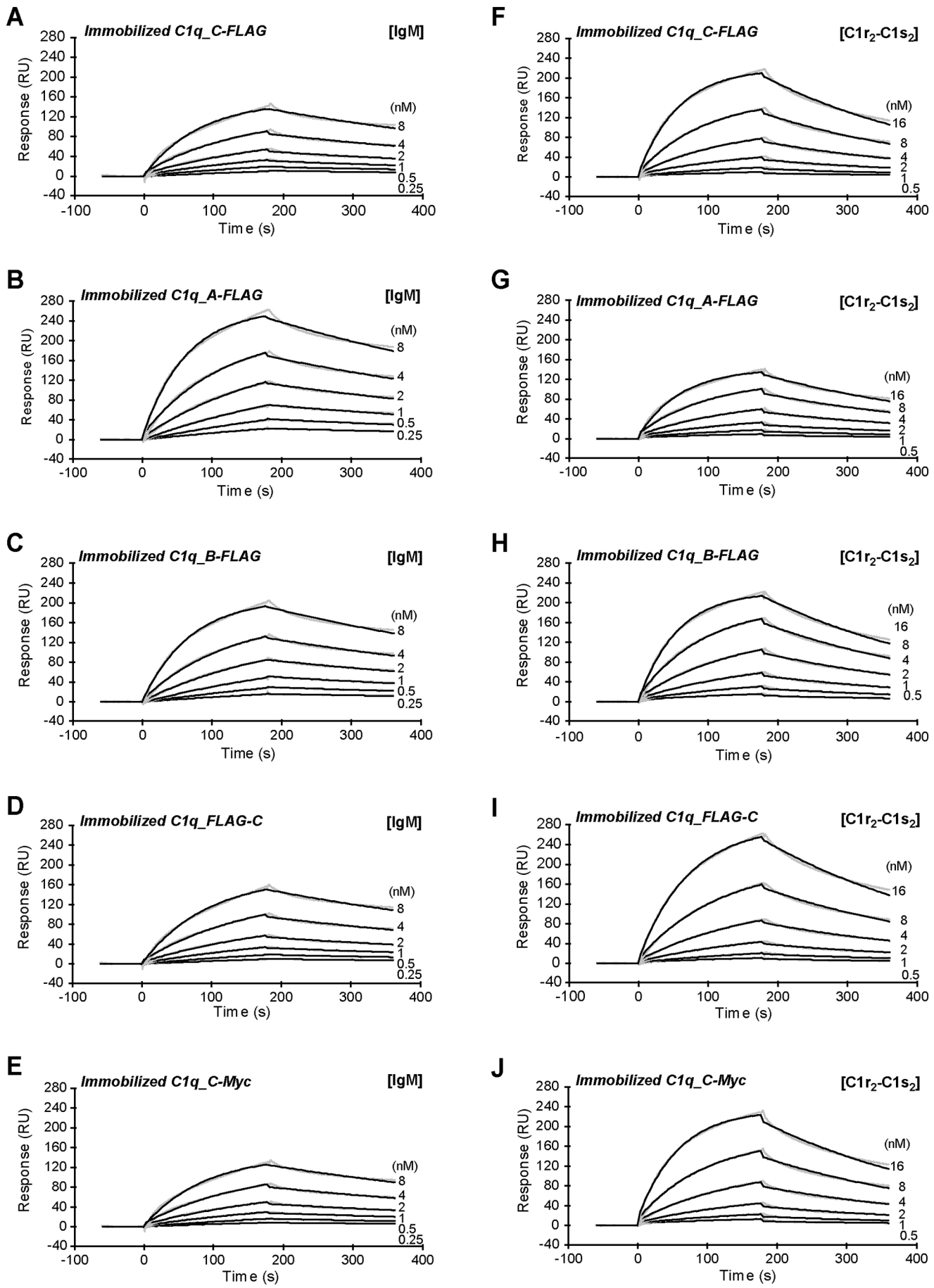


Figure 2

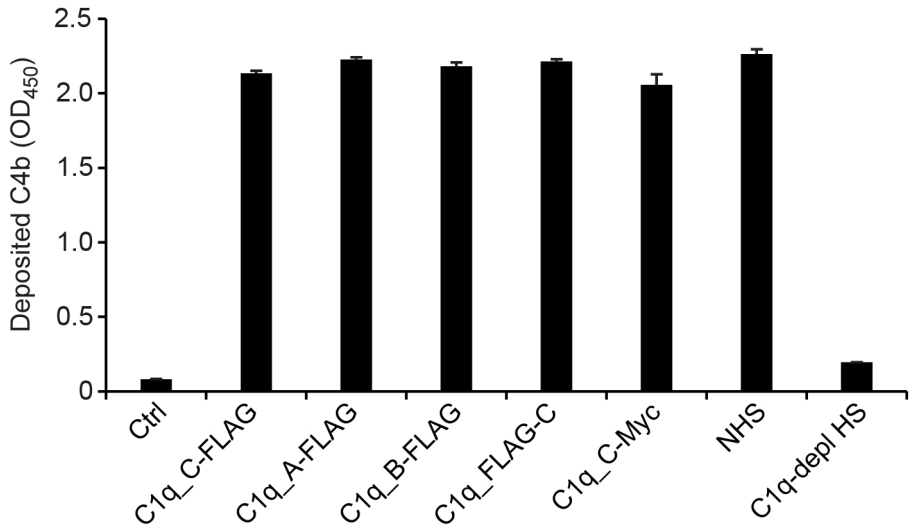


Figure 3

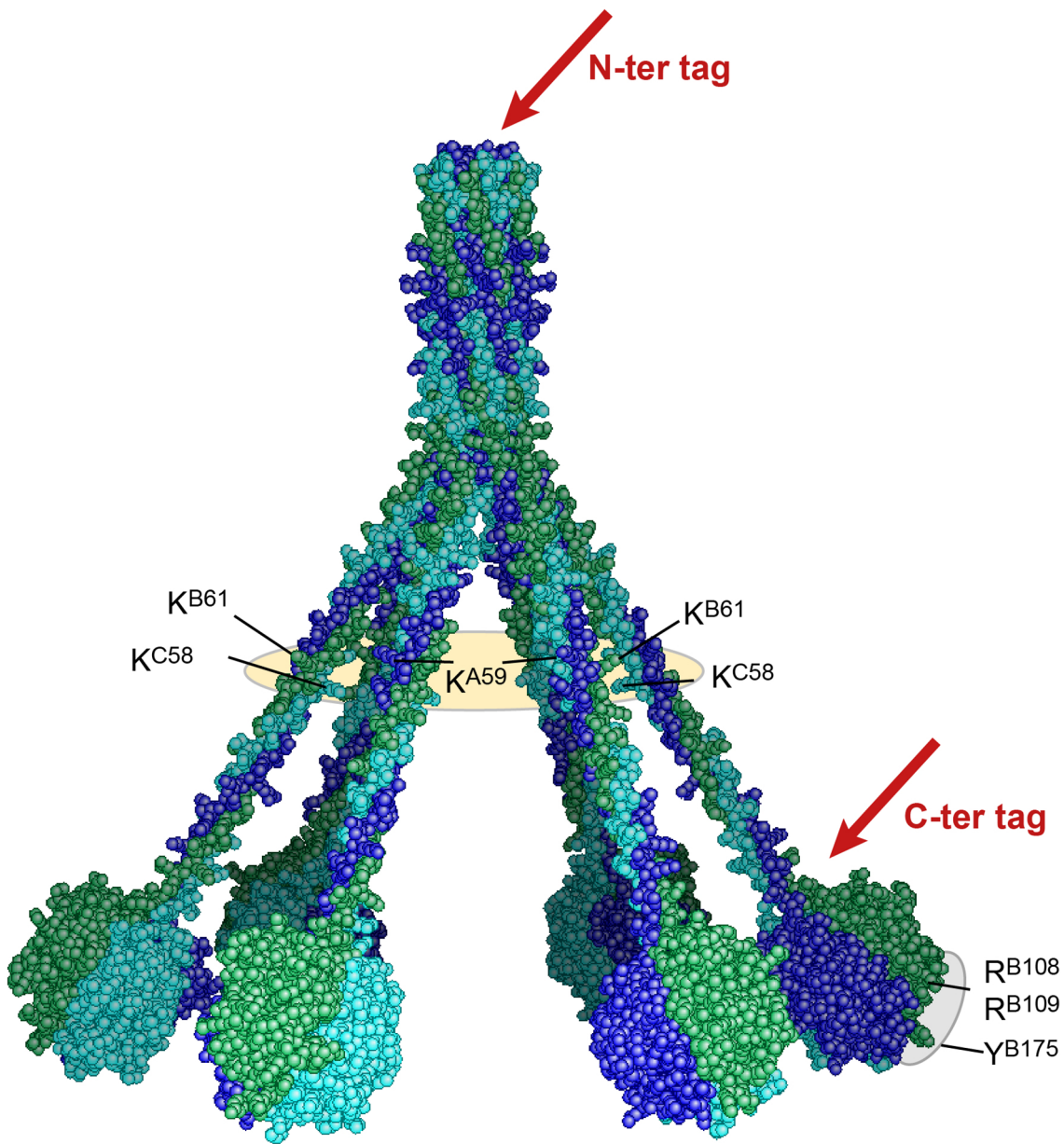


Figure 4