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# Influence of membrane surface charge on adsorption of complement proteins onto supported lipid bilayers

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ABSTRACT

The complement system is an important part of the innate immune response, and there is great interest in understanding how complement proteins interact with lipid membrane interfaces, especially in the context of recognizing foreign particulates (e.g., liposomal nanomedicines). Herein, a supported lipid bilayer platform was employed in order to investigate the effect of membrane surface charge (positive, negative, or neutral) on the adsorption of three complement proteins. Quartz crystal microbalance-dissipation (QCM-D) experiments measured the real-time kinetics and total uptake of protein adsorption onto supported lipid bilayers. The results demonstrate that all three proteins exhibit preferential, mainly irreversible adsorption onto negatively charged lipid bilayers, yet there was also significant variation in total uptake and the relative degree of adsorption onto negatively charged bilayers versus neutral and positively charged bilayers. The total uptake was also observed to strongly depend on the bulk protein concentration. Taken together, our findings contribute to a broader understanding of the factors which influence adsorption of complement proteins onto lipid membranes and offer guidance towards the design of synthetic lipid bilayers with immunocompetent features.



## 1. Introduction

The complement system is the first line of host defense that responds to pathogenic invaders[1–3] (e.g., bacteria, viruses) and damaged host cells[4–6] (e.g., apoptotic or necrotic cells) by promoting their clearance. A complex and highly orchestrated sequence of complement proteins, typically soluble and cell surface proteins found in extracellular fluids, act in coordinated fashion to mediate a rapid and vital immune response. The initiation of complement activation is a critical first step of recognition and occurs through one of three distinct pathways [7,8]; antibody-antigen interaction on the target surface (*classical pathway*) [9], binding of a mannan-binding lectin (MBL) to terminal sugar molecules on the surface of microorganisms (*lectin pathway*) [10], or nonspecific deposition of complement proteins on the surface of foreign particulates (*alternative pathway*) [11,12].

In contrast to the highly specific initiatory steps involved in the classical and lectin pathways, the alternative pathway of the complement system is continuously and spontaneously activated at a low and regulated level [13]. When a foreign particulate is detected, the level of activation is amplified through the deposition of pattern recognition proteins from the alternative pathway onto the target surface [14,15]. The deposited proteins have multiple functions, including acting as opsonins which facilitate clearance by the reticuloendothelial system (RES) as well as initiators which initiate a proteolytic cascade to generate potent effector molecules that stimulate (*recruit*) immune cells or induce complement-mediated lysis [16–18]. For these reasons, there is great interest in understanding the factors which influence the adsorption of complement proteins onto target surfaces.

While conventional assays to study the complement system focus on measuring hemolysis [19,20] or generation of soluble complement products [21–25], there is growing attention to surface-sensitive measurement approaches to measure the deposition of complement proteins on target surfaces due to their distinct functional properties over generated proteins. In particular,

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surface-sensitive analytical tools such as ellipsometry, surface plasmon resonance (SPR) and quartz crystal microbalance-dissipation (QCM-D) are popular tools to measure the adsorption of complement proteins in a label-free and real-time measurement format [26,27]. Such measurements are typically performed in one of two ways. The most common option is to incubate a model surface with serum, followed by exchange with an aqueous buffer solution. Then, adsorbed proteins at a model surface are identified by addition of a specific antibody that recognizes a complement protein [27–29]. Antibody binding to the serum treated-surface infers the presence of the deposited complement protein which reveals surface-mediated complement activation [29–31]. To date, this approach has enabled the study of the adsorption of certain complement proteins onto many medically relevant model surfaces such as inorganic substrates [27,32–34], protein-coated substrates [27], polymeric surfaces [35–38] and self-assembled monolayers (SAMs) [39–41]. Another option is to add purified complement proteins and directly monitor their binding to the surface, however, there are few studies in this direction despite the utility of this measurement format for analyzing protein adsorption [42–45]. Indeed, direct measurement of protein adsorption onto target surfaces would be advantageous because, in many previous studies, the C3b pattern recognition protein was the main focus and it was covalently attached to the target surface beforehand in order to monitor its interaction with other complement proteins [46,47]. The covalent attachment of complement proteins to the substrate was generally chosen because it permits efficient use of protein reagents [48–50]. However, this approach prevents monitoring of real-time target recognition through deposition of the complement proteins. Therefore, understanding the factors which influence the noncovalent adsorption of complement proteins onto target surfaces is still an outstanding subject due to its important role in target recognition [40]. Moreover, there has been limited exploration of the deposition of complement proteins in general on more biologically relevant interfaces such as lipid membranes, with only one investigation relating to the deposition of C3 protein on zwitterionic lipid bilayers [51].

Given the scope of lipid membranes involved in triggering complement activation, there is significant potential for investigating how lipid membrane properties influence complement protein deposition. To date, lipid membrane investigations have been principally limited to solution-based liposome systems, as studied in many in vitro and in vivo studies that focus on measuring the generation of complement products [52–59]. In the context of opsonization, these studies have shown that negatively charged liposomes are cleared more rapidly from the circulation through RES uptake due to binding of serum proteins (opsonins) [57,60,61]. Moreover, it is known that both negatively and positively charged liposomes activate the complement system significantly more than neutral liposomes [62,63]. While the functional consequences of membrane surface charge are relatively well understood, the influence of membrane surface charge on the deposition of complement proteins remains to be studied. Indeed, there is growing evidence that the role of electrostatic forces in governing protein adsorption can be complex and the electric dipole of the protein itself should also be considered in terms of its binding affinity and orientation to a target surface [64–69].

The goal of this study is to investigate how membrane surface charge influences the adsorption of three different pattern recognition proteins – C3b, C3 and properdin – onto lipid bilayer membranes. Historically, C3 and C3b proteins are the most widely studied pattern recognition proteins and properdin (Factor P) is a newly identified one [15,70]. In order to achieve this goal, we sought to extend the capabilities of supported lipid bilayers which have been recently employed to study other aspects of the innate immune response, including membrane association of membrane attack complex proteins and inhibition thereof [71,72]. Supported lipid bilayers with positive, neutral or negative surface charges were fabricated by the solvent-assisted lipid bilayer (SALB) method, and the quartz crystal microbalance-dissipation (QCM-D) measurement technique was utilized in order to track protein adsorption in a real-time, label-free format. The membrane surface charge of the supported lipid bilayer was varied based on tuning the lipid composition. Taken together, the findings in this work help to establish a platform to study the adsorption of pattern recognition proteins onto lipid bilayer membranes.

## 2. Materials and methods

### 2.1. Protein reagents

Purified human C3, C3b and properdin (Factor P) proteins were purchased from Complement Technology (Tyler, TX). As presented in Table S1 (see in the online version at DOI: http://dx.doi.org/10.1016/j.colsurfb.2016.08.036), C3 is the most abundant complement protein in plasma and has a molar mass of 185 kDa. C3b protein is a 176 kDa fragment of the C3 protein that is obtained through proteolytic conversion. Both C3 and C3b proteins have isoelectric points below 6 and possess negative net charges under neutral pH conditions [73,74]. By contrast, properdin is a much smaller protein with a molar mass of 53 kDa and has an isoelectric point around 9.5 [75–77]; hence, it has a positive net charge under neutral pH conditions. Also, properdin monomers are known to self-associate forming dimers, trimers, and tetramers in the relative portion of 26:54:20 [78–80]. The stock aliquots of complement proteins were prepared in a 10 mM sodium phosphate [pH 7.2] buffer solution with 145 mM NaCl, and were stored at −80 °C until the experiment. Immediately before experiment, the proteins were diluted in a 10 mM Tris [pH 7.5] buffer solution with 150 mM NaCl to the appropriate test concentration.

### 2.2. Lipid reagents

1,2-dioleoyl-*sn*-*glycero*-3-phosphocholine (DOPC), 1,2-dioleoyl-sn-*glycero*-3-ethylphosphocholine (chloride salt) (DOEPC), 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt) (DOTAP) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (sodium salt) (POPG) were obtained in lyophilized powder form from Avanti Polar Lipids (Alabaster, AL). The lipid powders were stored at -20 °C until experiment and were then diluted in the appropriate organic solvent. The chemical structures of the phospholipid molecules are presented in Fig. 1. The DOEPC and DOTAP lipids are positively charged lipids while POPG is a negatively charged lipid and DOPC is a zwitterionic lipid.

### 2.3. Quartz crystal microbalance with dissipation (QCM-D) monitoring

A Q-Sense E4 instrument (Q-sense AB, Gothenburg, Sweden) was utilized in order to monitor lipid and protein adsorption processes by tracking changes in resonance frequency and energy dissipation of a silicon oxide-coated quartz crystal (model no. QSX303) as a function of time. The QCM-D measurement data were collected at the 3rd, 5th, 7th, 9th, and 11th odd overtones, and the reported QCM-D data were all obtained at the 5th overtone and normalized accordingly ($\Delta f_{n=5}/5$). Immediately before the experiment, the QCM-D sensor substrates were sequentially rinsed with 2% SDS, water, and ethanol followed by drying with nitrogen gas. The substrates were then subjected to oxygen plasma treatment (Harrick Plasma, Ithaca, NY) at the maximum radiofrequency (RF) power. All QCM-D measurements were conducted under continuous flow conditions, with the flow rate defined as 100 μL/min for