

# **Reduced order modeling and analysis of the human complement system**

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## Abstract

Complement is an important pathway of innate immunity which plays a significant role in inflammation, and many disease processes. However, despite its importance, there has been a paucity of validated mathematical models of complement activation. In this study, we developed an ensemble of experimentally validated reduced order complement models. The modeling approach combined ordinary differential equations with logical rules to produce a complement model with a limited number of equations and parameters. The reduced order model, which described the lectin and alternative pathways, consisted of 18 differential equations with 28 parameters. Thus, the model was an order of magnitude smaller and included more pathways than comparable models in the literature. We estimated an ensemble of model parameters from *in vitro* time series measurements of the C3a and C5a complement proteins. Subsequently, we validated the model on unseen C3a and C5a measurements that were not used for model training. Despite its small size, the model was surprisingly predictive. After validation, we performed global sensitivity and robustness analysis to estimate which parameters and species controlled model performance. These analyses suggested complement was robust to any single therapeutic intervention. The only intervention that consistently reduced C5a formation for all cases was a dual-knockdown of both C3 and C5. Taken together, we developed a reduced order complement model that was computationally inexpensive, and could easily be incorporated into pre-existing or new pharmacokinetic models of immune system function. The model described experimental data, and predicted the need for multiple points of therapeutic intervention to disrupt complement activation.

**Keywords:** Complement system, systems biology, reduced order models, biochemical engineering

## **1 Introduction**

2 Complement is an important pathway in innate immunity. It plays a significant role in  
3 inflammation, host defense as well as many disease processes. Complement was dis-  
4 covered in the late 1880s where it was found to 'complement' the bactericidal activity of  
5 natural antibodies (1). However, research over the past decade has shown that the im-  
6 portance of complement extends well beyond innate immunity. For example, complement  
7 contributes to tissue homeostasis by inducing tissue repair (2). Complement has also  
8 been linked with several diseases including Alzheimers, Parkinson's disease, multiple  
9 sclerosis, schizophrenia, rheumatoid arthritis and sepsis (3, 4). Complement plays both  
10 positive and negative roles in cancer; attacking tumor cells with altered surface proteins  
11 in some cases, while potentially contributing to tumor growth in others (5, 6). Lastly, sev-  
12 eral other important biochemical subsystems are integrated with complement including  
13 the coagulation cascade, the autonomous nervous system and inflammation (6). Thus,  
14 complement is important in a variety of beneficial and potentially harmful functions in the  
15 body.

16 The complement cascade involves over 30 soluble and cell surface proteins, receptors  
17 and regulators. The molecular connectivity of complement is complex, see the review of  
18 Walport (7, 8). The central outputs of complement are the Membrane Attack Complex  
19 (MAC), and the inflammatory mediator proteins C3a and C5a. The membrane attack  
20 complex, generated during the terminal phase of the response, forms transmembrane  
21 channels which disrupt the membrane integrity of targeted cells, leading to cell lysis and  
22 death. On the other hand, the C3a and C5a proteins act as a bridge between innate and  
23 adaptive immunity, and play an important role in regulating inflammation (5). Complement  
24 activation takes places through three pathways: the classical, the lectin binding and the  
25 alternate pathways. Each of these pathways involves a different initiator signal which trig-  
26 gers downstream events in the complement system. The classical pathway is triggered

27 by antibody recognition of foreign antigens or other pathogens. A multimeric protein com-  
28 plex C1 binds to antibody-antigen complexes and undergoes a conformational change,  
29 leading to an activated form with proteolytic activity. This activated complex then cleaves  
30 soluble complement proteins C4 and C2 into C4a, C4b, C2a and C2b, respectively. The  
31 C4a and C2b fragments bind to form the C4bC2a protease, which is also known as the  
32 classical C3 convertase. The lectin pathway is initiated through the binding of L-ficolin or  
33 Mannose Binding Lectin (MBL) to carbohydrates on the surfaces of bacterial pathogens.  
34 These complexes, in combination with the associated mannose-associated serine pro-  
35 teases 1 and 2 (MASP-1/2), also cleave C4 and C2, leading to additional classical C3  
36 convertase. Thus, the classical and lectin pathways, initiated by the recognition of a for-  
37 eign surface, converge at the classical C3 convertase. However, the alternate pathway  
38 works differently. The alternate pathway involves a 'tickover' mechanism in which com-  
39 plement protein C3 is spontaneously hydrolyzed to form an activated intermediate C3w;  
40 C3w recruits factor B and factor D, leading to the formation of C3wBb. C3wBb can cleave  
41 C3 into C3a and C3b, where the C3b fragment can further recruit additional factor B and  
42 factor D to form C3bBbC3b, which is also known as the alternate C3 convertase (9). The  
43 role of classical and alternate C3 convertases is varied. First, C3 convertases encode  
44 an amplification loop by cleaving C3 into C3a and C3b; the C3b fragment is then free to  
45 form additional alternate C3 convertases, thereby forming a positive feedback loop. Next,  
46 C3 convertase activity links complement initiation with the terminal phase of the cascade  
47 through the formation of C5 convertases. Both classical and alternate C3 convertases  
48 can recruit an additional C3b subunit to form the classical C5 convertase (C4bC2aC3b),  
49 and the alternate C5 convertase (C3bBbC3b), respectively. C5 convertases cleave C5  
50 into the C5a and C5b fragments. The C5b fragment, along with the C6, C7, C8 and mul-  
51 tiple C9 complement proteins, form the membrane attack complex. On the other hand,  
52 both C3a and C5a are important inflammatory signals involved in several responses.

Activation of the complement cascade is strongly regulated by many plasma and host cell proteins. The initiation of the classical pathway via complement protein C1 is controlled by the C1 Inhibitor (C1-Inh), a protease inhibitor belonging to the serpin superfamily. C1-Inh irreversibly binds to and deactivates the active subunits of C1, preventing spontaneous fluid phase and chronic activation of complement (10). Regulation of the upstream elements of complement is also achieved through the interaction of the C4 binding protein (C4BP) with C4b, as well as through the interaction of factor H with C3b (11). These regulatory proteins are also capable of binding their respective targets while they are bound in convertase complexes. Membrane cofactor protein (MCP or CD46) possesses a cofactor activity for C4b and C3b, which protects the host from self-activation of complement (12). Decay accelerating factor (DAF or CD55) is also able to recognize and dissociate both C3 and C5 convertases (13). Carboxypeptidase-N, a well known inflammation regulator, cleaves carboxyl-terminal arginines and lysines of the complement proteins C3a, C4a, and C5a rendering them inactive (14). Lastly, the assembly of the MAC complex is inhibited by vitronectin and clusterin in the plasma, and CD59 at the host surface (15, 16). Thus, there are many points of control which influence complement activation across the three activation pathways.

Developing quantitative mathematical models of complement could be crucial to understanding its role in the body. Traditionally, complement models have been formulated as systems of linear or non-linear ordinary differential equations (ODEs). For example, Hirayama et al. modeled the classical complement pathway as a system of linear ODEs (17), while Korotaevskiy and co-workers modeled the classical, lectin and alternate pathways as a system of non-linear ODEs (18). More recently, large mechanistic models of sections of complement have also been proposed. For example, Liu et al. analyzed the formation of the classical and lectin C3 convertases, and the regulatory role of C4BP using a system of 45 non-linear ODEs with 85 parameters (19). Recently, Zewde and co-

79 workers constructed a detailed mechanistic model of the alternative pathway which con-  
80 sisted of 107 ODEs and 74 kinetic parameters and delineated the complement response  
81 of the host and pathogen (16). However, these previous modeling studies involved little  
82 experimental validation. Thus, while these models are undoubtably important theoretical  
83 tools, it is unclear if they can describe or quantitatively predict experimentally validated  
84 complement dynamics. The central challenge is the estimation of model parameters from  
85 experimental data. Unlike other important cascades, such as coagulation for which there  
86 are well developed experimental tools and many publicly available data sets, the data for  
87 complement is relatively sparse. Missing or incomplete data sets, and limited quantitative  
88 data make the identification of mechanistic complement models difficult.

89 In this study, we developed an ensemble of experimentally validated reduced order  
90 complement models. The modeling approach combined ordinary differential equations  
91 with logical rules to produce a complement model with a limited number of equations and  
92 parameters. The reduced order model, which described the lectin and alternative path-  
93 ways, consisted of 18 differential equations with 28 parameters. Thus, the model was an  
94 order of magnitude smaller and included more pathways than comparable mathematical  
95 models in the literature. We estimated an ensemble of model parameters from *in vitro*  
96 time series measurements of the C3a and C5a complement proteins. Subsequently, we  
97 validated the model on unseen C3a and C5a measurements that were not used for model  
98 training. Despite its small size, the model was surprisingly predictive. After validation, we  
99 performed global sensitivity and robustness analysis to estimate which parameters and  
100 species controlled model performance. These analyses suggested complement was ro-  
101 bust to any single therapeutic intervention. The only intervention that consistently reduced  
102 C5a formation for all cases was a dual-knockdown of both C3 and C5. Taken together,  
103 we developed a reduced order complement model that was computationally inexpensive,  
104 and could easily be incorporated into pre-existing or new pharmacokinetic models of im-

<sup>105</sup> immune system function. The model described experimental data, and predicted the need  
<sup>106</sup> for multiple points of intervention to disrupt complement activation.

107 **Results**

108 **Reduced order complement network.** The reduced order complement model described  
109 the alternate and lectin pathways (Fig. 1). A trigger event initiated the lectin pathway,  
110 which activated the cleavage of C2 and C4 into C2a, C2b, C4a and C4b respectively.  
111 Classical Pathway (CP) C3 convertase (C4aC2b) then catalyzed the cleavage of C3 into  
112 C3a and C3b. Activation of the alternative pathway was initiated through the spontaneous  
113 hydrolysis of C3 into C3a and C3b. The C3b fragment then recombined with C3 to form  
114 the alternate pathway (AP) C3 convertase. Both the CP and AP C3 convertases catalyzed  
115 the cleavage of C3 into C3a and C3b. A second C3b fragment could then bind with either  
116 the CP or AP C3 convertase to form the CP (or AP) C5 convertase. The C5 convertase  
117 catalyzed the cleavage of C5 into the C5a and C5b fragments. Lectin pathway activation  
118 was approximated using a combination of saturation kinetics and non-linear transfer func-  
119 tions, which facilitated a significant reduction in the size of the model while maintaining  
120 performance. Thus, while the reduced order complement model encoded significant bio-  
121 logical complexity, it was highly compact consisting of only 18 differential equations and  
122 28 model parameters. Next, we estimated an ensemble of model parameters from time  
123 series measurements of the C3a and C5a complement proteins.

124 **Estimating an ensemble of reduced order complement models.** A critical challenge  
125 for any dynamic model is the estimation of model parameters. We estimated the com-  
126 plement model parameters in a hierarchical fashion using two *in vitro* time-series data  
127 sets generated with and without zymosan, a lectin pathway activator (20). The residual  
128 between model simulations and experimental measurements was minimized using the dy-  
129 namic optimization with particle swarms (DOPS) approach, starting from an initial random  
130 parameter guess. A hierarchical approach was taken to determine model parameters in  
131 which the alternate pathway parameters were first estimated and then fixed during the  
132 estimation of the lectin pathway parameters. The reduced order complement model cap-

tured the behavior of the alternative and lectin pathways (Fig. 2). For the alternative pathway, we used the C3a and C5a measurements in the absence of zymosan, and only allowed the alternative parameters to vary (Fig. 2A and B). Lectin parameters were estimated from C3a and C5a measurements in the presence of 1g zymosan (Fig. 2C and D). Taken together, the reduced order model reproduced a panel of lectin pathway initiation data sets in the neighborhood of physiological factor and inhibitor concentrations. However, it was unclear whether the reduced order model could predict new data, without updating the model parameters. To address this question, we fixed the model parameters and simulated data not used for model training.

We tested the predictive power of the reduced order complement model with data not used during model training (Fig. 3). Six validation cases were considered, three for C3a and C5a respectively at different zymosan concentrations. All model parameters were fixed for the validation simulations. The ensemble of reduced order models captured the qualitative dynamics of C3a formation (Fig. 3, left column), and C5a formation (Fig. 3, right column) at three inducer concentrations. However, there were shortcomings, especially for the C3a prediction. First, while the C3a dynamics and concentration peak times were captured, the overall level of C3a was under-predicted in all cases (Fig. 3, inset left column). We believe the C3a under-prediction can be attributed to how we modeled C4BP interactions. C4BP interactions were modeled as irreversible binding steps resulting in completely inactive complexes; however, the binding of C4BP with complement proteins is likely reversible and convertases may have residual activity even in the bound form. Thus, the model may over-predict the influence of C4BP. We also failed to capture the concave down curvature for the 0.001 g and 0.01 g zymosan cases in the C5a validation studies. The decreasing slope of the C5a measurements may indicate decreasing cofactors abundance, or missing biology which we have not explicitly accounted for in the reduced order approach. However, despite these shortcomings, we qualitatively pre-

159 dicted unseen experimental data, including correctly capturing the dynamic time scale of  
160 C3a formation, and the correct order of magnitude for the concentration of C5a for three  
161 inducer levels. Next, we used global sensitivity and robustness analysis to determine  
162 which parameters and species controlled the performance of the complement model.

163 **Global analysis of the reduced order complement model** We conducted sensitivity  
164 analysis to estimate which parameters controlled the performance of the reduced order  
165 complement model. We calculated the sensitivity of the C3a and C5a residuals with and  
166 without zymosan for the ensemble of parameter sets (Fig. 4A - D). In the absence of zy-  
167 mosan (where only the alternative pathway is active),  $k_{f,C3b}$  (formation of C3b) and  $k_{d,C3a}$   
168 (degradation rate constant governing C3a) were largely responsible for the system re-  
169 sponse. Interestingly,  $k_{c,C3}$  (the rate constant governing AP C3-convertase activity) was  
170 not sensitive in the absence of zymosan. Thus, the behavior of the alternative pathway  
171 was more heavily influenced by the spontaneous hydrolysis of C3, rather than AP C3-  
172 convertase activity. On the other hand,  $k_{c,C3}$  was one of the parameters that controlled  
173 C5a formation, in addition to the expected parameters related to AP C5-convertase for-  
174 mation. The AP C3-convertase is required for AP C5-convertase formation, and the for-  
175 mation of the C3b fragment. Thus, changes in the activity of AP C3-convertase will not  
176 drastically change the C3a dynamics, but will effect AP C5-convertase activity and C5a  
177 formation. The sensitivity analysis yielded the expected results for the lectin pathway that  
178 included parameters sensitive to pathway initiation (Fig. 4C and D). One key difference  
179 observed between the sensitivity of C3a and C5a parameters, was their respective degra-  
180 dation constants. The rate constant governing C3a degradation was sensitive, while the  
181 degradation constant for C5a was not. This difference was likely attributable to the mag-  
182 nitude of the degradation parameters and the respective concentrations of C3a and C5a.  
183 Thus, sensitivity analysis identified important indirect parameter interactions that could  
184 have therapeutic significance. However, sensitivity coefficients are a local measure of

185 how small changes in a parameter value effects a performance objective, for example the  
186 abundance of C5a. To more closely simulate a clinical intervention e.g., administration of  
187 an anti-complement antibody, we performed robustness analysis. Robustness coefficients  
188 quantify the response of a marker to a macroscopic structural or operational perturbation  
189 to the network architecture. In this case, we computed how the C3a and C5a trajectories  
190 responded to a decrease in the initial abundance of C3 and C5.

191 Robustness analysis suggested there was no single intervention that inhibited com-  
192 plement activation in the presence of both initiation pathways (Fig. 5). We calculated  
193 robustness indices for C3a and C5a for the 50 parameter sets in the ensemble with and  
194 without the lectin pathway initiator. We simulated the addition of different doses of anti-  
195 complement antibody cocktails by decreasing the initial concentration of C3 or C5 or the  
196 combination of C3 and C5 by 50% and 90%. A  $\log_{10}$  transformed robustness index of  
197 zero indicated no effect due to the perturbation, whereas an index of less than zero in-  
198 dicated decreased C3a or C5a. As expected, a C5 knockdown had no effect on C3a  
199 formation for either the alternate (Fig. 5A, lanes 1 or 3) or lectin pathways (Fig. 5B, lanes  
200 1 or 3). However, C3a abundance and to a lesser extent C5a abundance decreased with  
201 decreasing C3 concentration in the alternate pathway (Fig. 5A or B, lanes 1 or 2). This  
202 agreed with the sensitivity results; changes in AP C3-convertase formation or activity af-  
203 fected the downstream dynamics of C5a formation. Thus, these results suggested that C3  
204 alone would be a reasonable target, especially given that C5a formation was surprisingly  
205 robust to C5 levels in the alternate pathway (Fig. 5A or B, lane 2). Yet, in lectin initiated  
206 complement activation, C5a levels were robust to the initial C3 concentration (Fig. 5A or  
207 B, lane 4). Thus, above some limiting threshold, even small concentrations of C3 and C5  
208 convertases catalyzed the downstream formation of C5a. The only reliable intervention  
209 that consistently reduced C5a formation for all cases was a dual-knockdown. For exam-  
210 ple, a 90% decrease of both C3 and C5 reduced the formation of C5a by over an order of

<sub>211</sub> magnitude (Fig. 5B, lane 4).

212 **Discussion**

213 In this study, we developed an ensemble of experimentally validated reduced order com-  
214 plement models. The modeling approach combined ordinary differential equations with  
215 logical rules to produce a complement model with a limited number of equations and pa-  
216 rameters. The reduced order model, which described the lectin and alternative pathways,  
217 consisted of 18 differential equations with 28 parameters. Thus, the model was an order  
218 of magnitude smaller and included more pathways than comparable mathematical mod-  
219 els in the literature. We estimated an ensemble of model parameters from *in vitro* time  
220 series measurements of the C3a and C5a complement proteins. Subsequently, we val-  
221 idated the model on unseen C3a and C5a measurements that were not used for model  
222 training. Despite its small size, the model was surprisingly predictive. After validation, we  
223 performed global sensitivity and robustness analysis to estimate which parameters and  
224 species controlled model performance. These analyses suggested complement was ro-  
225 bust to any single therapeutic intervention. The only intervention that consistently reduced  
226 C5a formation for all cases was a dual-knockdown of both C3 and C5. Taken together,  
227 we developed a reduced order complement model that was computationally inexpensive,  
228 and could easily be incorporated into pre-existing or new pharmacokinetic models of im-  
229 mune system function. The model described experimental data, and predicted the need  
230 for multiple points of intervention to disrupt complement activation.

231 Despite its importance, there has been a paucity of validated mathematical models  
232 of complement pathway activation. To our knowledge, this study is one of the first com-  
233 plement models that combined multiple initiation pathways with experimental validation  
234 of important complement products like C5a. However, there have been several theoreti-  
235 cal models of components of the cascade in the literature. Liu and co-workers modeled  
236 the formation of C3a through the classical pathway using 45 non-linear ODEs (19). In  
237 contrast, in this study we modeled lectin mediated C3a formation using only five ODEs.

238 Though we did not model all the initiation interactions in detail, especially the cross-talk  
239 between the lectin and classical pathways, we successfully captured C3a dynamics with  
240 respect to different concentrations of lectin initiators. The model also captured the dy-  
241 namics of C3a and C5a formed from the alternate pathway using only seven ODEs. The  
242 reduced order model predictions of C5a were qualitatively similar to the theoretical com-  
243 plement model of Zewde et al which involved over 100 ODEs (16). However, we found  
244 that the quantity of C3a produced in the alternate pathway was nearly 1000 times the  
245 quantity of C5a produced. Though this was in agreement with the experimental data (20),  
246 it differed from the theoretical predictions made by Zewde et al. who showed C3a was  $10^8$   
247 times the C5a concentration (16). In our model, the time profile of C5a generation from the  
248 lectin pathway changed with respect to the quantity of zymosan (the lectin pathway initia-  
249 tor). The lag phase for generation was inversely proportional to the initiator concentration.  
250 Korotaevskiy et al. showed a similar trend using a theoretical model of complement, albeit  
251 for much shorter time scales (18). Thus, the reduced order complement model performed  
252 similarly to existing large mechanistic models, despite being significantly smaller.

253 Global analysis of the complement model estimated potential important therapeutic  
254 targets. Complement malfunctions are implicated in a number of diseases, however the  
255 development of complement specific therapeutics has been challenging (3, 21). Previ-  
256 ously, we have shown that mathematical modeling and sensitivity analysis can be useful  
257 tools to estimate therapeutically important mechanisms in biochemical networks (22–25).  
258 In this study, we analyzed a validated ensemble of reduced order complement models to  
259 estimate therapeutically important mechanisms. In presence of an initiator, C5a forma-  
260 tion was primarily sensitive to the lectin initiation parameters, and parameters governing  
261 the conversion of C5 to C5a and C5b. This result agrees well with the current protease  
262 inhibitors targeting initiating complexes, including mannose-associated serine proteases  
263 1 and 2 (MASP-1,2) (26). The most commonly used anti-complement drug eculizumab

264 (21), targets the C5 protein which is cleaved to form C5a. Our sensitivity analysis showed  
265 that kinetic parameters governing C5 conversion were sensitive in both lectin initiated and  
266 alternate pathways, thus agreeing with targeting C5 protein. The formation of basal C3b  
267 was also a sensitive parameter in the formation of C3a through the alternate pathway.  
268 Thus, this mechanism can act as a target for both C3a and C5a inhibitors. Lectin initiated  
269 C3a formation showed a number of sensitive parameters. This included the lectin initi-  
270 ation parameters that controlled C5a formation, C3 convertase inhibition by C4BP, and  
271 parameters governing C3 convertase activity. All these mechanisms are potential drug  
272 targets.

273 To further validate these results from sensitivity analysis about potential drug targets  
274 we did a robustness analysis. We knocked down C3 and C5 levels and studied their im-  
275 pact on the generation of C3a and C5a. The C3a and C5a levels in the lectin pathway  
276 were strongly influenced by initial levels of C3 and C5. Thus direct inhibition of C3 and  
277 C5, or targeting complexes (MASP complex, C3 and C5 convertases) that act on C3 and  
278 C5 have a direct impact on production of C3a and C5a. This is also in agreement with  
279 sensitivity analysis that C5 is a good drug target. A number of drugs targeting C5 are  
280 being developed. For example LFG316 by Novartis is being used to target C5 in cases  
281 of Age-Related Macular Degeneration (27), Mubodina is an antibody that targets C5 in  
282 the treatment of Atypical Hemolytic-Uremic Syndrome (aHUS) (28), Coversin is a small  
283 molecule targeting C5 (29), Zimura is an aptamer targeting C5 (30), small peptides and  
284 RNAi are also being used to inhibit C5 (31). Another important conclusion that can be  
285 drawn together from sensitivity and robustness analysis is that C3 and C5 convertases  
286 can be important therapeutic targets. Though knockdown of C3 and C5 affects C3a and  
287 C5a levels downstream, the abundance and turnover rate (32, 33) of these proteins make  
288 them difficult targets. Thus targeting C3 and C5 directly will require high dosage of drugs.  
289 It is also well known that eculizumab dosage needs to be adjusted while treating for Atyp-

ical Hemolytic-Uremic Syndrome (aHUS), a disease that is caused due to uncontrolled complement activation (34). The issue of high dosage can potentially be circumvented by targeting convertases or fragile mechanisms that involve C3, C5 or their activated components. Our analysis shows that formation and assembly of these convertases are sensitive mechanisms that strongly impact downstream proteins like C5a. Formation of convertases is inhibited by targeting upstream protease complexes like MASP-1,2 from lectin pathway (or C1r, C1s from classical pathway). For example, Omeros is a protease inhibitor that targets MASP-2 complex and thereby inhibits formation of downstream convertases (35). Lampalizumab (an immunoglobulin) and Bikaciomab (an antibody fragment) target Factor B and Factor D respectively. Factor B and Factor D are crucial to formation alternate pathway convertases (36, 37). Novelmed Therapeutics recently developed antibody, NM9401 against propedin, a small protein that stabilizes alternate C3 convertase (38). Cobra Venom Factor (CVF), an analogue of C3b has been used to bind to Factor B to regulate alternate convertases (39). Thus, analysis of the ensemble of complement models identified potentially important therapeutic targets that are consistent with therapeutic strategies that are under development.

The performance of the complement model was impressive given its limited size. However, there are several questions that should be explored further. A logical progression for this work would be to expand the network to include the classical pathway and the formation of the membrane attack complex (MAC). However, it is unclear whether the addition of the classical pathway will decrease the predictive quality of our existing model. Liu et al have shown cross-talk between the activation of the classical and lectin pathways that could influence model performance (19). One potential approach to address such difficulties would be to incorporate C reactive proteins (CRP) and L-ficolin (LF) into the model, both of which are involved with the initiation of classical and lectin pathways. Liu et al. showed that under inflammation conditions interactions between lectin and classical

pathways was mediated through CRP and LF (19). Thus incorporating these two proteins would help us in modeling cross talk. Time course measurements of MAC abundance (and MAC formation dynamics) are also scarce, making the inclusion of MAC challenging. Next, we should address the under-prediction of C3a. We believe the C3a under-prediction can be attributed to how we modeled C4BP interactions. C4BP interactions were modeled as irreversible binding steps resulting in completely inactive complexes; however, the binding of C4BP with complement proteins is likely reversible and C4BP-bound convertases may have residual activity. We also did not capture the maximum concentration of C3a at low initiator levels. One possible reasons for this could be the C2-by-pass pathway, which was not included in the model. This pathway further accelerates C3a production without the involvement of a C3 convertase. Currently the C3a in the model is generated only through the activity of a C3 convertase. Incorporating this additional step within the reduced order modeling framework would be a future direction that we need to consider. We should test alternative model structures which include reversible C4BP binding, and partially active convertases. Alternatively, we could also perform sensitivity analysis on the C3a prediction residual to determine which parameters controlled the C3a prediction.

333 **Materials and Methods**

334 We used ordinary differential equations (ODEs) to model the time evolution of complement  
 335 proteins ( $x_i$ ) in the reduced order model:

$$\frac{dx_i}{dt} = \sum_{j=1}^{\mathcal{R}} \sigma_{ij} r_j(\mathbf{x}, \epsilon, \mathbf{k}) \quad i = 1, 2, \dots, \mathcal{M} \quad (1)$$

336 where  $\mathcal{R}$  denotes the number of reactions and  $\mathcal{M}$  denotes the number of protein species  
 337 in the model. The quantity  $r_j(\mathbf{x}, \epsilon, \mathbf{k})$  denotes the rate of reaction  $j$ . Typically, reaction  $j$  is  
 338 a non-linear function of biochemical and enzyme species abundance, as well as unknown  
 339 model parameters  $\mathbf{k}$  ( $\mathcal{K} \times 1$ ). The quantity  $\sigma_{ij}$  denotes the stoichiometric coefficient for  
 340 species  $i$  in reaction  $j$ . If  $\sigma_{ij} > 0$ , species  $i$  is produced by reaction  $j$ . Conversely, if  $\sigma_{ij} < 0$ ,  
 341 species  $i$  is consumed by reaction  $j$ , while  $\sigma_{ij} = 0$  indicates species  $i$  is not connected  
 342 with reaction  $j$ . Species balances were subject to the initial conditions  $\mathbf{x}(t_0) = \mathbf{x}_0$ .

343 Rate processes were written as the product of a kinetic term ( $\bar{r}_j$ ) and a control term  
 344 ( $v_j$ ) in the complement model. The kinetic term for the formation of C4a, C4b, C2a and  
 345 C2b, lectin pathway activation, and C3 and C5 convertase activity was given by:

$$\bar{r}_j = k_j^{max} \epsilon_i \left( \frac{x_s^\eta}{K_{js}^\eta + x_s^\eta} \right) \quad (2)$$

346 where  $k_j^{max}$  denotes the maximum rate for reaction  $j$ ,  $\epsilon_i$  denotes the abundance of the  
 347 enzyme catalyzing reaction  $j$ ,  $\eta$  denotes a cooperativity parameter, and  $K_{js}$  denotes the  
 348 saturation constant for species  $s$  in reaction  $j$ . We used mass action kinetics to model  
 349 protein-protein binding interactions within the network:

$$\bar{r}_j = k_j^{max} \prod_{s \in m_j^-} x_s^{-\sigma_{sj}} \quad (3)$$

350 where  $k_j^{max}$  denotes the maximum rate for reaction  $j$ ,  $\sigma_{sj}$  denotes the stoichiometric co-  
 351 efficient for species  $s$  in reaction  $j$ , and  $s \in m_j$  denotes the set of *reactants* for reaction  
 352  $j$ . We assumed all binding interactions were irreversible. The control terms  $0 \leq v_j \leq 1$   
 353 depended upon the combination of factors which influenced rate process  $j$ . For each rate,  
 354 we used a rule-based approach to select from competing control factors. If rate  $j$  was in-  
 355 fluenced by  $1, \dots, m$  factors, we modeled this relationship as  $v_j = \mathcal{I}_j(f_{1j}(\cdot), \dots, f_{mj}(\cdot))$   
 356 where  $0 \leq f_{ij}(\cdot) \leq 1$  denotes a regulatory transfer function quantifying the influence of  
 357 factor  $i$  on rate  $j$ . The function  $\mathcal{I}_j(\cdot)$  is an integration rule which maps the output of regu-  
 358 latory transfer functions into a control variable. Each regulatory transfer function took the  
 359 form:

$$f_{ij}(\mathcal{Z}_i, k_{ij}, \eta_{ij}) = k_{ij}^{\eta_{ij}} \mathcal{Z}_i^{\eta_{ij}} / (1 + k_{ij}^{\eta_{ij}} \mathcal{Z}_i^{\eta_{ij}}) \quad (4)$$

360 where  $\mathcal{Z}_i$  denotes the abundance of factor  $i$ ,  $k_{ij}$  denotes a gain parameter, and  $\eta_{ij}$  denotes  
 361 a cooperativity parameter. In this study, we used  $\mathcal{I}_j \in \{min, max\}$  (40). If a process has  
 362 no modifying factors,  $v_j = 1$ . The model equations were implemented in MATLAB and  
 363 solved using the ODE23s routine (The Mathworks, Natick MA). The complement model  
 364 code and parameter ensemble is freely available under an MIT software license and can  
 365 be downloaded from <http://www.varnerlab.org>.

366 **Estimation of an ensemble of complement model parameters.** We minimized the  
 367 residual between simulations and experimental C3a and C5a measurements using Dy-  
 368 namic Optimization with Particle Swarms (DOPS). DOPS minimized the objective:

$$\min_{\mathbf{k}} \sum_{\tau=1}^{\mathcal{T}} \sum_{j=1}^S \left( \frac{\hat{x}_j(\tau) - x_j(\tau, \mathbf{k})}{\omega_j(\tau)} \right)^2 \quad (5)$$

369 where  $\hat{x}_j(\tau)$  denotes the measured value of species  $j$  at time  $\tau$ ,  $x_j(\tau, \mathbf{k})$  denotes the sim-  
 370 ulated value for species  $j$  at time  $\tau$ , and  $\omega_j(\tau)$  denotes the experimental measurement

variance for species  $j$  at time  $\tau$ . The outer summation is with respect to time, while the inner summation is with respect to state. DOPS is a novel metaheuristic that combines multi swarm particle swarm optimization (PSO) with a greedy global optimization algorithm called dynamically dimensioned search (DDS). DOPS is faster than conventional global optimizers and has the ability to find near optimal solutions for high dimensional systems within a relatively few function evaluations. It uses an adaptive switching strategy based on error convergence rates to switch from the particle swarm to DDS search phases. This enables DOPS to quickly estimate globally optimal or near optimal solutions even in the presence of many local minima. In the swarm search, for each iteration the particles compute error within each sub-swarm by evaluating the model equations using their specific parameter vector realization. From each of these points within a sub-swarm a local best is identified. This along with the particle best within the sub-swarm  $S_k$  is used to update the parameter estimate for each particle using the following rules:

$$z_{i,j} = \theta_1 z_{i,j-1} + \theta_2 r_1 (\mathcal{L}_i - z_{i,j-1}) + \theta_3 r_2 (\mathcal{G}_k - z_{i,j-1}) \quad (6)$$

where  $z_{i,j}$  is the parameter vector,  $(\theta_1, \theta_2, \theta_3)$  were adjustable parameters,  $\mathcal{L}_i$  denotes the best solution found by particle  $i$  within sub-swarm  $S_k$  for function evaluations  $1 \rightarrow j-1$ , and  $\mathcal{G}_k$  denotes the best solution found over all particles within sub-swarm  $S_k$ . The quantities  $r_1$  and  $r_2$  denote uniform random vectors with the same dimension as the number of unknown model parameters ( $K \times 1$ ). At the conclusion of the swarm phase, the overall best particle,  $\mathcal{G}_k$ , over the  $k$  sub-swarms was used to initialize the DDS phase. For the DDS phase, the best parameter estimate was updated using the rule:

$$\mathcal{G}_{new}(J) = \begin{cases} \mathcal{G}(J) + \mathbf{r}_{normal}(J)\sigma(J), & \text{if } \mathcal{G}_{new}(J) < \mathcal{G}(J). \\ \mathcal{G}(J), & \text{otherwise.} \end{cases} \quad (7)$$

391 where  $\mathbf{J}$  is a vector representing the subset of dimensions that are being perturbed,  $\mathbf{r}_{normal}$   
392 denotes a normal random vector of the same dimensions as  $\mathcal{G}$ , and  $\sigma$  denotes the pertur-  
393 bation amplitude:

$$\sigma = R(\mathbf{p}^U - \mathbf{p}^L) \quad (8)$$

394 where  $R$  is the scalar perturbation size parameter,  $\mathbf{p}^U$  and  $\mathbf{p}^L$  are  $(\mathcal{K} \times 1)$  vectors that  
395 represent the maximum and minimum bounds on each dimension. The set  $\mathbf{J}$  was con-  
396 structed using a monotonically decreasing probability function  $\mathcal{P}_i$  that represents a thresh-  
397 old for determining whether a specific dimension  $j$  was perturbed or not. DDS updates  
398 are greedy;  $\mathcal{G}_{new}$  becomes the new solution vector only if it is better than  $\mathcal{G}$ . At the end of  
399 DDS phase we obtain the optimal vector  $\mathcal{G}$  which we use for plotting best fits against the  
400 experimental data, and for generating a parameter ensemble.

401 An ensemble of parameters was obtained by randomly perturbing the optimal param-  
402 eter set within bounds established by perturbing each parameter and measuring the in-  
403 crease in the residual. Thereafter, the optimal parameter vector was perturbed within  
404 these bounds for approximately 100,000 iterations. Within each iteration the quality of  
405 perturbed vector was measured using goodness of fit (model residual). If the residual was  
406 too high or the perturbed vector generated a numerical error, the vector was rejected. We  
407 selected an ensemble of  $N = 50$  parameter sets for this study using this sampling proce-  
408 dure. The DOPS routine was implemented in MATLAB (The Mathworks, Natick MA) and  
409 can be downloaded from <http://www.varnerlab.org> under an MIT software license.

410 **Sensitivity and robustness analysis of complement model performance.** We con-  
411 ducted global sensitivity and robustness analysis to estimate which parameters and species  
412 controlled the performance of the reduced order model. We computed the total variance-  
413 based sensitivity index of each parameter relative to the training residual for the C3a  
414 alternate, C5a alternate, C3a lectin, and C5a lectin cases using the Sobol method (41).

415 The sampling bounds for each parameter were established from the minimum and maxi-  
 416 mum value for that parameter in the parameter ensemble. We used the sampling method  
 417 of Saltelli *et al.* to compute a family of  $N(2d + 2)$  parameter sets which obeyed our pa-  
 418 rameter ranges, where  $N$  was the number of trials per parameters, and  $d$  was the number  
 419 of parameters in the model (42). In our case,  $N = 200$  and  $d = 28$ , so the total sensitivity  
 420 indices were computed using 11,600 model evaluations. The variance-based sensitivity  
 421 analysis was conducted using the SALib module encoded in the Python programming  
 422 language (43).

423 Robustness coefficients quantify the response of a marker to a structural or operational  
 424 perturbation to the network architecture. Robustness coefficients were calculated as  
 425 shown previously (44). Log-transformed robustness coefficients denoted by  $\hat{\alpha}(i, j, t_o, t_f)$   
 426 are defined as:

$$\hat{\alpha}(i, j, t_o, t_f) = \log_{10} \left[ \left( \int_{t_o}^{t_f} x_i(t) dt \right)^{-1} \left( \int_{t_o}^{t_f} x_i^{(j)}(t) dt \right) \right] \quad (9)$$

427 Here  $t_o$  and  $t_f$  denote the initial and final simulation time, while  $i$  and  $j$  denote the indices  
 428 for the marker and the perturbation, respectively. A value of  $\hat{\alpha}(i, j, t_o, t_f) > 0$ , indicates  
 429 increased marker abundance, while  $\hat{\alpha}(i, j, t_o, t_f) < 0$  indicates decreased marker abun-  
 430 dance following perturbation  $j$ . If  $\hat{\alpha}(i, j, t_o, t_f) \sim 0$ , perturbation  $j$  did not influence the  
 431 abundance of marker  $i$ . In this study, we perturbed the initial condition of C3 or C5 or  
 432 a combination of C3 and C5 by 50% or 90% and measured the area under the curve  
 433 (AUC) of C3a or C5a with and without lectin initiator. Log-transformed robustness coeffi-  
 434 cients were calculated for every member of the ensemble, where the mean  $\pm 1 \times$  standard-  
 435 deviation are reported.

<sup>436</sup> **Competing interests**

<sup>437</sup> The authors declare that they have no competing interests.

<sup>438</sup> **Author's contributions**

<sup>439</sup> J.V directed the study. A.S developed the reduced order complement model and the  
<sup>440</sup> parameter ensemble. A.S, W.D and M.M analyzed the model ensemble, and generated  
<sup>441</sup> figures for the manuscript. The manuscript was prepared and edited for publication by  
<sup>442</sup> A.S, W.D, M.M and J.V.

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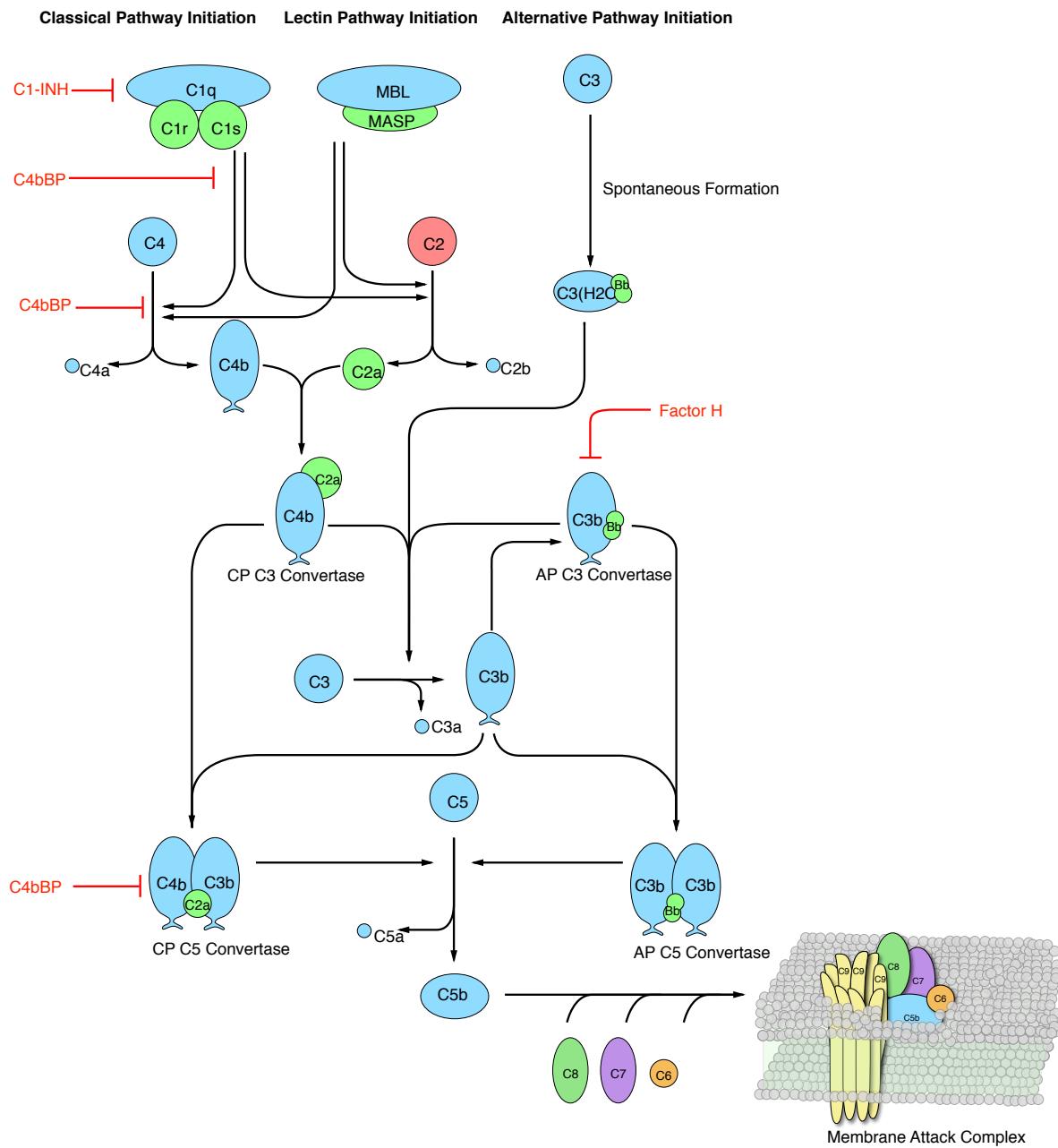
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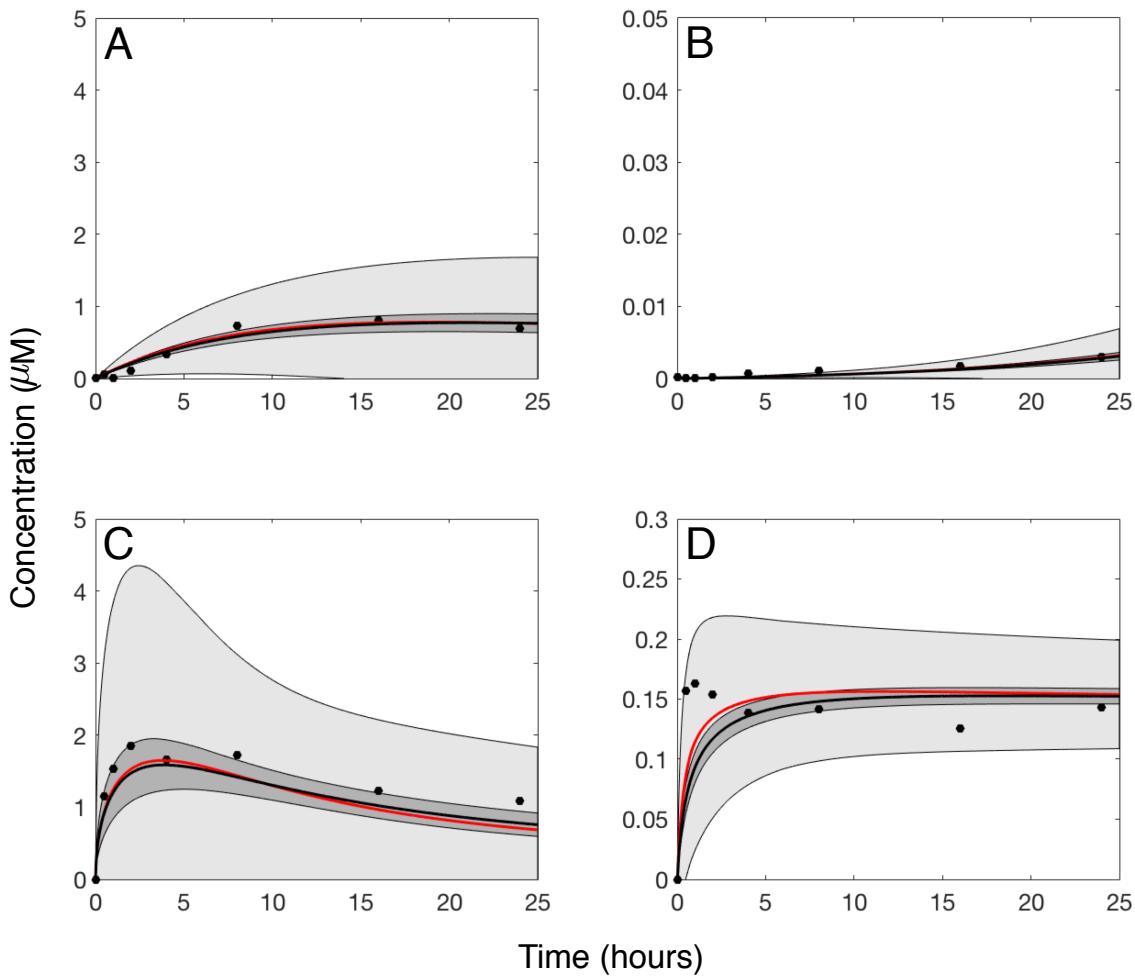
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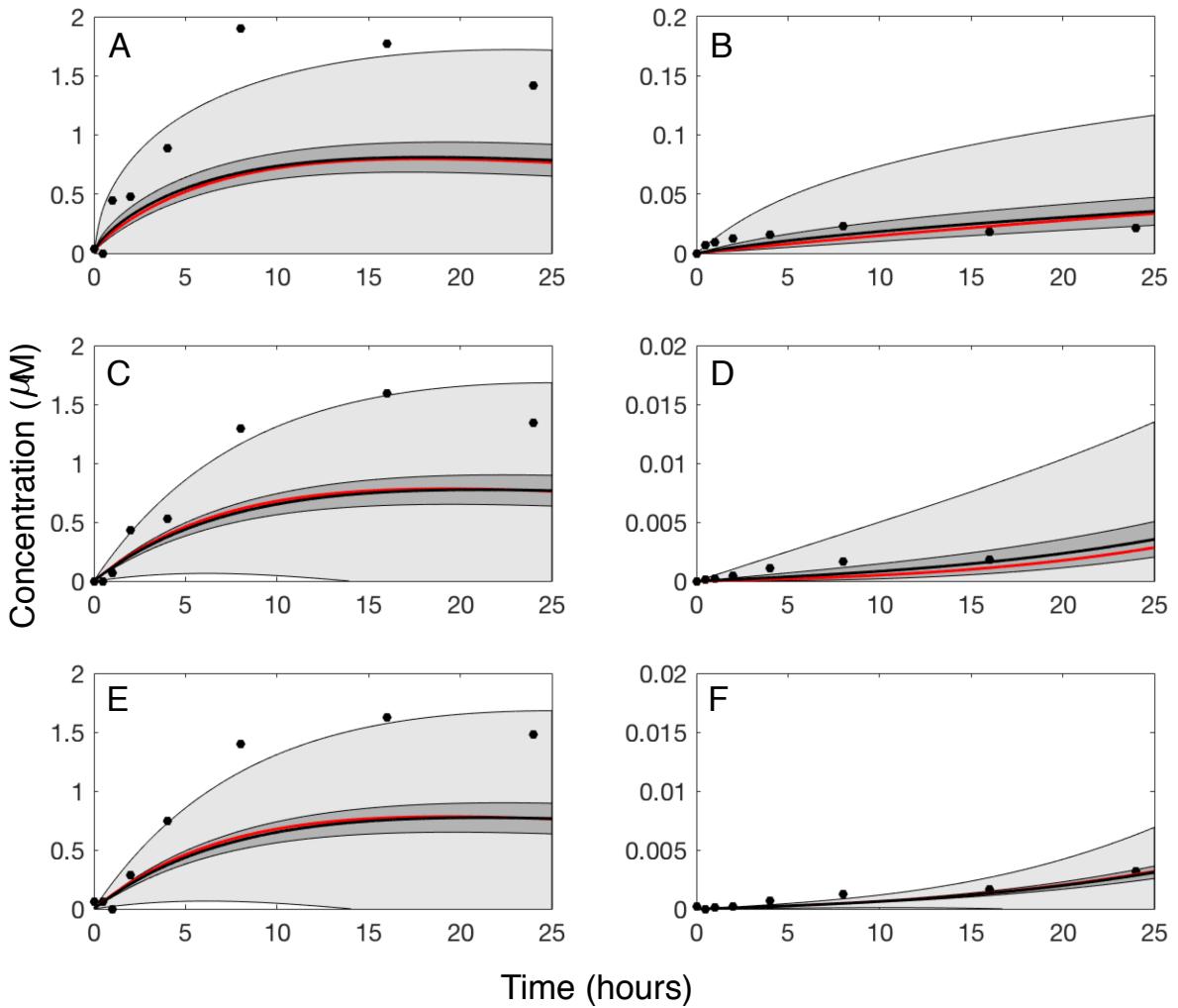
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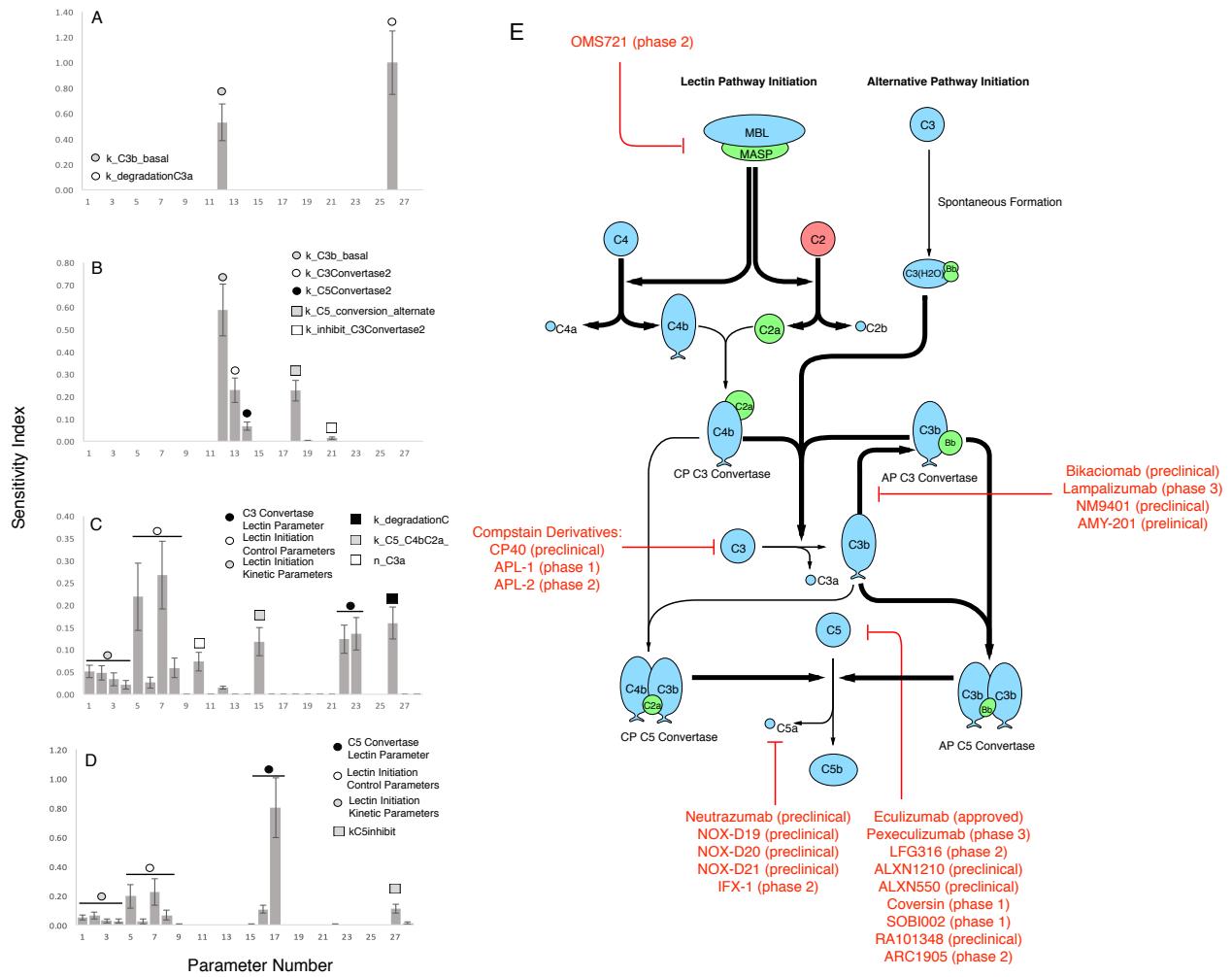
**Fig. 1:** Simplified schematic of the human complement system. The complement cascade is activated through any one, or more, of the three pathways: the classical, the lectin, and the alternate pathways. The classical pathway is activated by the binding of C1 complex through the C1q subunit to the IgG or IgM immune complex. This binding leads to conformational changes in the C1 complex that leads to the activation of C1r and C1s subunits. Activated C1-antibody complex cleaves C4 and C2 to form the classical C3 convertase. The lectin pathway is initiated by the binding mannose-binding lectins (MBL) and ficolins to carbohydrate moieties on the pathogen surfaces. This results in the formation mannose-binding lectin-associated serine proteases (MASPs). The MBL-MASP complex cleaves C4 and C2 to form the lectin C3 convertase. The alternative pathway is activated through a spontaneous tick-over mechanism by the hydrolysis of C3 to form fluid phase C3 convertase. The C3 convertases cleaves C3 into C3a, and C3b. C3b combines with C4b and C2a to form classical C5 convertase ( $C_4bC_3aC_3b$ ). The C3b binds with Factor B to form the alternate C5 convertase ( $C_3bB_bC_3b$ ).<sup>27</sup> The C5 convertases cleave C5 into C5a, and C5b that undergoes a series of reactions to form the membrane attack complex (MAC).



**Fig. 2:** Reduced order complement model training simulations. Reduced order complement model parameters were estimated using Dynamic Optimization with Particle Swarms (DOPS). The model was trained against experimental data from Shaw and co-workers (20) in the presence and absence of zymosan. The model was trained using C3a and C5a data generated from the alternative pathway (**A–B**) and lectin initiated pathway with 1g zymosan (**C–D**). The solid red line shows the simulation with the best-fit parameter, the solid black lines show the simulated mean value of C3a or C5a for 50 independent particles. The dark shaded region denotes 99 % confidence interval of the simulated mean concentrations of C3a or C5a , while the light shaded region is the 99 % confidence interval of the best prediction. All initial concentrations of complement proteins are at human serum levels unless otherwise noted.

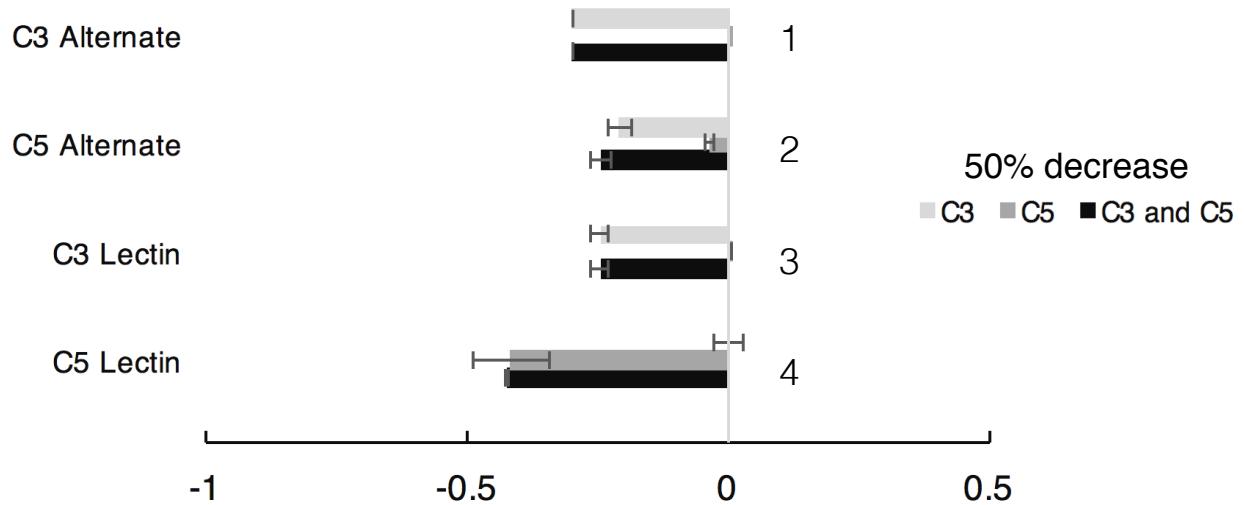


**Fig. 3:** Reduced order complement model predictions vs experimental data for C3a and C5a generated in the lectin pathway. The reduced order coagulation model parameter estimates were tested against data not used during model training. Simulations of C3a and C5a generated in the lectin pathway using different levels of zymosan (0.1, 0.01, and 0.001 grams of zymosan) were compared with the corresponding experimental data (A–F). The solid red line shows the simulation with the best-fit parameter, the solid black lines show the simulated mean value of C3a or C5a for 50 independent particles. The shaded region denotes 99 % confidence interval of the simulated mean concentrations of C3a or C5a, while the light shaded region is the 99 % confidence interval of the best prediction. All initial concentrations of complement proteins are at human serum levels unless otherwise noted.

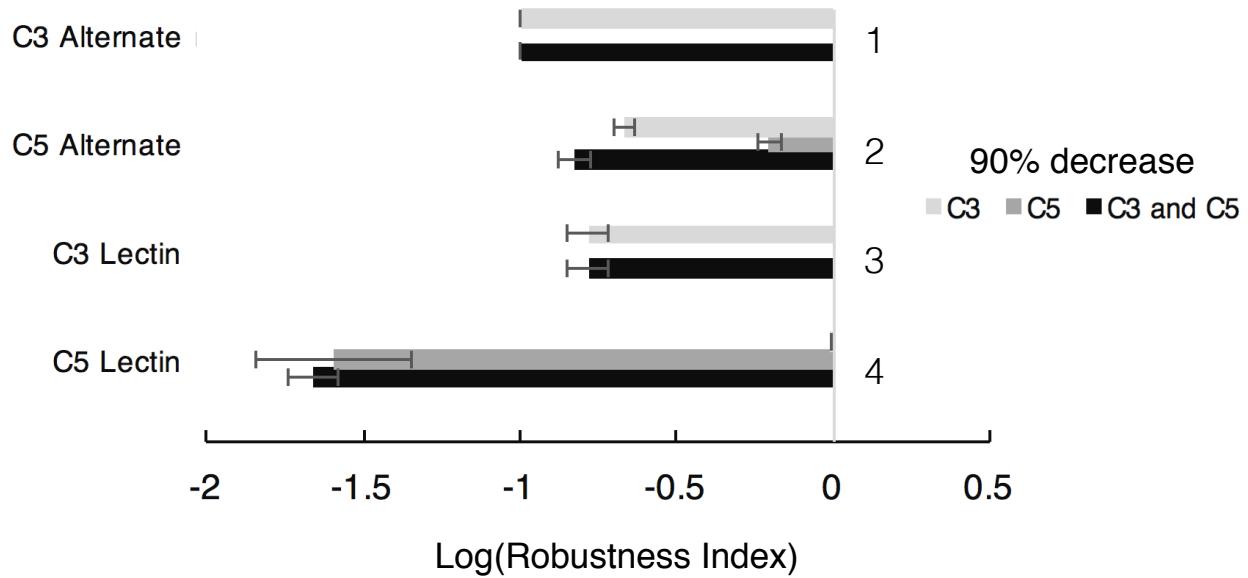


**Fig. 4:** Sobol's sensitivity analysis of the reduced order complement model with respect to the modeling parameters. Sensitivity analysis was conducted on the four cases we used to train our model: (A) C3a at 0g zymosan, (B) C5a 0g zymosan, (C) C3a 1g zymosan, and (D) C5a 1g zymosan. The bars denote total sensitivity index which includes local contribution of each parameter and global sensitivity of significant pairwise interactions. The error bars are the 95 percent confidence interval. Pathways controlled by the sensitivity parameters (E): Bold black lines indicates the pathway is governed by one or more sensitive parameters and the red lines shows some of the current therapeutics targets. Red indicates current complement therapeutics.

A



B



**Fig. 5:** Robustness analysis of the reduced order complement model with respect to the C3 and C5 initial concentrations using 50 parameter sets. Robustness analysis was conducted on the four cases we used to train our model, C3a alternate (0 zymosan), C5a alternate (0 zymosan), C3a lectin (1 g zymosan), and C5a lectin (1 g zymosan), by reducing the initial concentration of C3 and/or C5 by (A) 50 % and (B) 90 %. The bars denote robustness index which a measure of system changes from the perturbation of initial concentration that defined by the ratio of the area under the concentration curve of perturbed case and that of the unperturbed case. The error bars represent one standard deviation. At unity, the perturbed initial concentration has no impact on the measured output, and a robustness index lesser than or greater than one indicates a negative or positive relation between the perturbed initial concentration and the measured output respectively.

562 **Supplemental materials.**

563 **Model equations.** The reduced-order complement model consisted of 18 ordinary dif-  
 564 ferential equations, 12 rate equations, and two control equations:

$$\frac{dx_1}{dt} = -r_1 f_1 \quad (\text{S1})$$

$$\frac{dx_2}{dt} = -r_2 f_2 \quad (\text{S2})$$

$$\frac{dx_3}{dt} = r_1 f_1 \quad (\text{S3})$$

$$\frac{dx_4}{dt} = r_1 f_1 - r_6 \quad (\text{S4})$$

$$\frac{dx_5}{dt} = r_2 f_2 - r_6 \quad (\text{S5})$$

$$\frac{dx_6}{dt} = r_2 f_2 \quad (\text{S6})$$

$$\frac{dx_7}{dt} = r_3 - r_4 - r_5 \quad (\text{S7})$$

$$\frac{dx_8}{dt} = r_3 + r_4 + r_5 - k_{deg,c3a} * C3a \quad (\text{S8})$$

$$\frac{dx_9}{dt} = r_3 + r_4 + r_5 - r_7 \quad (\text{S9})$$

$$\frac{dx_{10}}{dt} = r_6 - r_{10} - r_8 \quad (\text{S10})$$

$$\frac{dx_{11}}{dt} = r_7 - r_{11} - r_9 \quad (\text{S11})$$

$$\frac{dx_{12}}{dt} = r_{10} - r_{14} \quad (\text{S12})$$

$$\frac{dx_{13}}{dt} = r_{10} \quad (\text{S13})$$

$$\frac{dx_{14}}{dt} = -r_{12} - r_{13} \quad (\text{S14})$$

$$\frac{dx_{15}}{dt} = r_{12} + r_{13} - k_{deg,c5a} \quad (\text{S15})$$

$$\frac{dx_{16}}{dt} = r_{12} + r_{13} \quad (\text{S16})$$

$$\frac{dx_{17}}{dt} = -r_8 - r_{14} \quad (\text{S17})$$

$$\frac{dx_{18}}{dt} = -r_9 \quad (\text{S18})$$

$$(\text{S19})$$

565 where the rate equations are given by:

$$r_1 = \frac{k_{i1}(C4)}{(K_{1s} + C4)} \quad (\text{S20})$$

$$r_2 = \frac{k_2(C2)}{(K_{2s} + C2)} \quad (\text{S21})$$

$$f_1 = \frac{Zymo^{\eta_1}}{(Zymo^{\eta_1} + \alpha_1^{\eta_1})} \quad (\text{S22})$$

$$f_2 = \frac{Zymo^{\eta_2}}{(Zymo^{\eta_2} + \alpha_2^{\eta_2})} \quad (\text{S23})$$

$$r_3 = k_3(C3) \quad (\text{S24})$$

$$r_4 = \frac{k_4(C3C_L)(C3^{\eta_3})}{(K_{4s}^{\eta_3} + C3^{\eta_3})} \quad (\text{S25})$$

$$r_5 = \frac{k_5(C3C_A)(C3)}{(K_{5s} + C3)} \quad (\text{S26})$$

$$r_6 = k_6(C4b)(C2a) \quad (\text{S27})$$

$$r_7 = k_7(C4b)(C2a) \quad (\text{S28})$$

$$r_8 = k_8(C3C_L)(C4b)(C4BP) \quad (\text{S29})$$

$$r_9 = k_9(C3C_A)(FactorH) \quad (\text{S30})$$

$$r_{10} = k_{10}(C3C_L)(C3b) \quad (\text{S31})$$

$$r_{11} = k_{11}(C3C_A)(C3b) \quad (\text{S32})$$

$$r_{12} = \frac{k_{12}(C5C_L)(C5^{\eta_4})}{(K_{12s}^{\eta_4} + C5^{\eta_4})} \quad (\text{S33})$$

$$r_{13} = \frac{k_{13}(C5C_A)(C5)}{(K_{13s} + C5)} \quad (\text{S34})$$

$$r_{14} = k_{14}(C5C_L)(C4BP) \quad (\text{S35})$$