

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 other 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 the impor-
6 tance of complement extends well beyond innate immunity. For example, complement
7 contributes to tissue homeostasis by inducing growth factors involved in tissue repair (2).
8 Complement malfunctions have been linked with several diseases including Alzheimers,
9 glaucoma, Parkinson's disease, multiple sclerosis, schizophrenia, rheumatoid arthritis
10 and sepsis (3, 4). Complement can also play both a positive and negative role in cer-
11 tain cancers; attacking tumor cells with altered surface proteins in some cases, while
12 potentially contributing to tumor growth in others (5, 6). Several other important biochemical
13 networks are integrated with complement including the coagulation cascade, the au-
14 tonomous nervous system and the ability to regulate inflammation (6). Thus, complement
15 is an important system involved in a variety of both beneficial and potentially harmful
16 functions in the body.

17 Complement is mediated by over 30 soluble and cell surface proteins that are present
18 as inactive forms in the circulation (7). The central output of complement activation is the
19 formation of the Membrane Attack Complex (MAC) and a key protein called C5a. The
20 membrane attack complex forms transmembrane channels which disrupt the cell mem-
21 brane of targeted cells, leading to cell lysis and death. The C5a protein acts as a bridge
22 between innate and adaptive immunity, and plays an important role in regulating inflam-
23 mation and coagulation (5). Complement activation takes places through three pathways:
24 the alternate, the classical and the lectin binding pathway. Each of these pathways in-
25 volves a different initiator signal which leads to a cascade of downstream events in the
26 complement system. The classical pathway is triggered when antibodies form complexes

27 with foreign antigens or other pathogens. A multimeric protein complex C1 binds to the
28 antigen-antibody complex and undergoes a conformational change. This activated com-
29 plex then cleaves complement proteins C4 and C2 into C4a, C4b, C2a and C2b respec-
30 tively. The C4a and C2b fragments combine to form the C4bC2a protease, also known as
31 the classical C3 convertase. The lectin binding pathway is initiated through the binding of
32 L-ficolin or Mannose Binding Lectin (MBL) to carbohydrates on the surfaces of bacterial
33 pathogens. This bound complex in turn cleaves C4 and C2, leading to the formation of
34 C4bC2a. The alternate pathway involves a 'tickover' mechanism in which complement
35 protein C3 is hydrolyzed to form C3b. In the presence of pathogens, the C3b fragment
36 binds foreign surfaces and recruits the additional proteins, factor B and factor D, which
37 lead to the formation of C3bBb, the alternate C3 convertase (8). The formation of clas-
38 sical and alternate C3 convertases on bacterial surfaces is followed by the formation of
39 proteases called C5 convertases. The classical and alternate C3 convertases recruit C3,
40 Factor B and Factor D to form the classical C5 convertase (C4bC2aC3b), and alternate
41 C5 convertase (C3bBbc3B) respectively. The C5 convertases then cleave C5 to form the
42 C5a and C5b fragments. The cleavage of C5 is followed by a series of sequential cleav-
43 age steps involving the C6, C7, C8 and C9 complement proteins which combine with C5b
44 to form the membrane attack complex (2).

45 Complement activation is regulated by many plasma and host cell proteins. The initi-
46 ation of the classical pathway via complement protein C1 is controlled by the C1 Inhibitor
47 (C1-Inh), a protease inhibitor belonging to the serpin superfamily. C1-Inh irreversibly binds
48 to and deactivates the active subunits of C1, preventing spontaneous fluid phase and
49 chronic activation of complement (9). Regulation of the upstream elements of comple-
50 ment is also achieved through the interaction of the C4 binding protein (C4BP) with C4b,
51 as well as through the interaction of factor H with C3b (10). These regulatory proteins
52 are also capable of binding their respective targets while they are bound in convertase

53 complexes. Membrane cofactor protein (MCP or CD46) possesses a cofactor activity for
54 C4b and C3b, which protects the host from self-activation of complement (11). Delay
55 accelerating factor (DAF or CD55) is also able to recognize and dissociate both C3 and
56 C5 convertases (12). Carboxypeptidase-N, a well known inflammation regulator, cleaves
57 carboxyl-terminal arginines and lysines of the complement proteins C3a, C4a, and C5a
58 rendering them inactive (13). Lastly, the assembly of the MAC complex is inhibited by
59 vitronectin and clusterin in the plasma, and CD59 at the host surface (14, 15). Thus,
60 there are many points of control which influence complement activation across the three
61 activation pathways.

62 Developing quantitative mathematical models of complement could be crucial to un-
63 derstanding its role in the body. Traditionally, complement models have been formulated
64 as systems of linear or non-linear ordinary differential equations (ODEs). For example,
65 Hirayama et al. modeled the classical complement pathway as a system of linear ODEs
66 (16), while Korotaevskiy and co-workers modeled the classical, lectin and alternate path-
67 ways as a system of non-linear ODEs (17). More recently, large mechanistic models of
68 sections of complement have also been proposed. For example, Liu et al. analyzed the
69 formation of the classical and lectin C3 convertases, and the regulatory role of C4BP
70 using a system of 45 non-linear ODEs with 85 parameters (18). Recently, Zewde and co-
71 workers constructed a detailed mechanistic model of the alternative pathway which con-
72 sisted of 107 ODEs and 74 kinetic parameters and delineated the complement response
73 of the host and pathogen (15). However, these previous modeling studies involved little
74 experimental validation. Thus, while these models are undoubtably important theoretical
75 tools, it is unclear if they can describe or quantitatively predict experimentally validated
76 complement dynamics. The central challenge is the estimation of model parameters from
77 experimental data. Unlike other important cascades, such as coagulation for which there
78 are well developed experimental tools and many publicly available data sets, the data for

79 complement is relatively sparse. Missing or incomplete data sets, and limited quantitative
80 data make the identification of mechanistic complement models difficult.

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

99 **Results**

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

116 **Estimating an ensemble of reduced order complement models.** A critical challenge
117 for any dynamic model is the estimation of model parameters. We estimated the com-
118 plement model parameters in a hierarchical fashion using two *in vitro* time-series data
119 sets generated with and without zymosan, a lectin pathway activator (19). The residual
120 between model simulations and experimental measurements was minimized using the dy-
121 namic optimization with particle swarms (DOPS) approach, starting from an initial random
122 parameter guess. A hierarchical approach was taken to determine model parameters in
123 which the alternate pathway parameters were first estimated and then fixed during the
124 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-

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

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

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

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

²⁰³ magnitude (Fig. 5B, lane 4).

204 **Discussion**

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

223 Despite its importance, there has been a paucity of validated mathematical models
224 of complement pathway activation. To our knowledge, this study is one of the first com-
225 plement models that combined multiple initiation pathways with experimental validation
226 of important complement products like C5a. However, there have been several theoreti-
227 cal models of components of the cascade in the literature. Liu and co-workers modeled
228 the formation of C3a through the classical pathway using 45 non-linear ODEs (18). In
229 contrast, in this study we modeled lectin mediated C3a formation using only five ODEs.

Though we did not model all the initiation interactions in detail, especially the cross-talk between the lectin and classical pathways, we successfully captured C3a dynamics with respect to different concentrations of lectin initiators. The model also captured the dynamics of C3a and C5a formed from the alternate pathway using only seven ODEs. The reduced order model predictions of C5a were qualitatively similar to the theoretical complement model of Zewde et al which involved over 100 ODEs (15). However, we found that the quantity of C3a produced in the alternate pathway was nearly 1000 times the quantity of C5a produced. Though this was in agreement with the experimental data (19), it differed from the theoretical predictions made by Zewde et al. who showed C3a was 10^8 times the C5a concentration (15). In our model, the time profile of C5a generation from the lectin pathway changed with respect to the quantity of zymosan (the lectin pathway initiator). The lag phase for generation was inversely proportional to the initiator concentration. Korotaevskiy et al. showed a similar trend using a theoretical model of complement, albeit for much shorter time scales (17). Thus, the reduced order complement model performed similarly to existing large mechanistic models, despite being significantly smaller.

Global analysis of the complement model estimated potential important therapeutic targets. Complement malfunctions are implicated in a number of diseases, however the development of complement specific therapeutics has been challenging (3, 20). Previously, we have shown that mathematical modeling and sensitivity analysis can be useful tools to estimate therapeutically important mechanisms in biochemical networks (21–24). In this study, we analyzed a validated ensemble of reduced order complement models to estimate therapeutically important mechanisms. In presence of an initiator, C5a formation was primarily sensitive to the lectin initiation parameters, and parameters governing the conversion of C5 to C5a and C5b. This result agrees well with the current protease inhibitors targeting initiating complexes, including mannose-associated serine proteases 1 and 2 (MASP-1,2) (25). The most commonly used anti-complement drug eculizumab

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

265 To further validate these results from sensitivity analysis about potential drug targets
266 we did a robustness analysis. We knocked down C3 and C5 levels and studied their im-
267 pact on the generation of C3a and C5a. The C3a and C5a levels in the lectin pathway
268 were strongly influenced by initial levels of C3 and C5. Thus direct inhibition of C3 and
269 C5, or targeting complexes (MASP complex, C3 and C5 convertases) that act on C3 and
270 C5 have a direct impact on production of C3a and C5a. This is also in agreement with
271 sensitivity analysis that C5 is a good drug target. A number of drugs targeting C5 are
272 being developed. For example LFG316 by Novartis is being used to target C5 in cases
273 of Age-Related Macular Degeneration (26), Mubodina is an antibody that targets C5 in
274 the treatment of Atypical Hemolytic-Uremic Syndrome (aHUS) (27), Coversin is a small
275 molecule targeting C5 (28), Zimura is an aptamer targeting C5 (29), small peptides and
276 RNAi are also being used to inhibit C5 (30). Another important conclusion that can be
277 drawn together from sensitivity and robustness analysis is that C3 and C5 convertases
278 can be important therapeutic targets. Though knockdown of C3 and C5 affects C3a and
279 C5a levels downstream, the abundance and turnover rate (31, 32) of these proteins make
280 them difficult targets. Thus targeting C3 and C5 directly will require high dosage of drugs.
281 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 (33). 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 (34). 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 (35, 36). Novelmed Therapeutics recently developed antibody, NM9401 against propedin, a small protein that stabilizes alternate C3 convertase (37). Cobra Venom Factor (CVF), an analogue of C3b has been used to bind to Factor B to regulate alternate convertases (38). 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 (18). 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 (18). 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.

325 **Materials and Methods**

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

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

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

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

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

338 where k_j^{max} denotes the maximum rate for reaction j , ϵ_i denotes the abundance of the
 339 enzyme catalyzing reaction j , η denotes a cooperativity parameter, and K_{js} denotes the
 340 saturation constant for species s in reaction j . We used mass action kinetics to model
 341 protein-protein binding interactions within the network:

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

342 where k_j^{max} denotes the maximum rate for reaction j , σ_{sj} denotes the stoichiometric coefficient
 343 for species s in reaction j , and $s \in m_j$ denotes the set of *reactants* for reaction j .
 344 The control terms $0 \leq v_j \leq 1$ depended upon the combination of factors which influenced
 345 rate process j . For each rate, we used a rule-based approach to select from competing
 346 control factors. If rate j was influenced by $1, \dots, m$ factors, we modeled this relationship
 347 as $v_j = \mathcal{I}_j(f_{1j}(\cdot), \dots, f_{mj}(\cdot))$ where $0 \leq f_{ij}(\cdot) \leq 1$ denotes a regulatory transfer function
 348 quantifying the influence of factor i on rate j . The function $\mathcal{I}_j(\cdot)$ is an integration rule which
 349 maps the output of regulatory transfer functions into a control variable. Each regulatory
 350 transfer function took the form:

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

351 where \mathcal{Z}_i denotes the abundance of factor i , k_{ij} denotes a gain parameter, and η_{ij} denotes
 352 a cooperativity parameter. In this study, we used $\mathcal{I}_j \in \{min, max\}$ (39). If a process has
 353 no modifying factors, $v_j = 1$. The model equations were implemented in MATLAB and
 354 solved using the ODE23s routine (The Mathworks, Natick MA). The complement model
 355 code and parameter ensemble is freely available under an MIT software license and can
 356 be downloaded from <http://www.varnerlab.org>.

357 **Estimation of an ensemble of model parameters.** We minimized the residual between
 358 simulations and experimental C3a and C5a measurements using Dynamic Optimization
 359 with Particle Swarms (DOPS). DOPS minimized the objective:

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

360 where $\hat{x}_j(\tau)$ denotes the measured value of species j at time τ , $x_j(\tau, \mathbf{k})$ denotes the simulated
 361 value for species j at time τ , and $\omega_j(\tau)$ denotes the experimental measurement

variance for species j at time τ . 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 \mathcal{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 \mathcal{S}_k for function evaluations $1 \rightarrow j-1$, and \mathcal{G}_k denotes the best solution found over all particles within sub-swarm \mathcal{S}_k . The quantities r_1 and r_2 denote uniform random vectors with the same dimension as the number of unknown model parameters ($\mathcal{K} \times 1$). 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:

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

382 where \mathbf{J} is a vector representing the subset of dimensions that are being perturbed, \mathbf{r}_{normal}
383 denotes a normal random vector of the same dimensions as \mathcal{G} , and σ denotes the pertur-
384 bation amplitude:

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

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

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

401 **Sensitivity and robustness analysis of model performance.** We conducted global
402 sensitivity and robustness analysis to estimate which parameters and species controlled
403 the performance of the reduced order model. We computed the total variance-based sen-
404 sitivity index of each parameter relative to the training residual for the C3a alternate, C5a
405 alternate, C3a lectin, and C5a lectin cases using the Sobol method (40). The sampling

406 bounds for each parameter were established from the minimum and maximum value for
407 that parameter in the parameter ensemble. We used the sampling method of Saltelli *et*
408 *al.* to compute a family of $N(2d + 2)$ parameter sets which obeyed our parameter ranges,
409 where N was the number of trials per parameters, and d was the number of parameters
410 in the model (41). In our case, $N = 200$ and $d = 28$, so the total sensitivity indices were
411 computed using 11,600 model evaluations. The variance-based sensitivity analysis was
412 conducted using the SALib module encoded in the Python programming language (42).

413 Robustness coefficients quantify the response of a marker to a structural or opera-
414 tional perturbation to the network architecture. Robustness coefficients were calculated
415 as shown previously (43). Robustness coefficients denoted by $\alpha(i, j, t_o, t_f)$ are defined
416 as:

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

417 Here t_o and t_f denote the initial and final simulation time respectively, while i and j denote
418 the indices for the marker and the perturbation respectively. A value of $\alpha(i, j, t_o, t_f) >$
419 0, indicates increased marker abundance, while $\alpha(i, j, t_o, t_f) < 0$ indicates decreased
420 marker abundance following perturbation j . If $\alpha(i, j, t_o, t_f) \sim 0$ the j th perturbation did not
421 influence the abundance of marker i . In this study, we perturbed the initial condition of C3
422 or C5 or a combination of C3 and C5 by 50% or 90% and measured the area under the
423 curve (AUC) of C3a or C5a with and without lectin initiator. Robustness coefficients were
424 calculated for every member of the ensemble, where the mean $\pm 1 \times$ standard-deviation
425 are reported.

⁴²⁶ **Competing interests**

⁴²⁷ The authors declare that they have no competing interests.

⁴²⁸ **Author's contributions**

⁴²⁹ J.V directed the study. A.S developed the reduced order complement model and the
⁴³⁰ parameter ensemble. W.D and M.M analyzed model simulations and generated figures
⁴³¹ for the manuscript. The manuscript was prepared and edited for publication by A.S, W.D,
⁴³² M.M and J.V.

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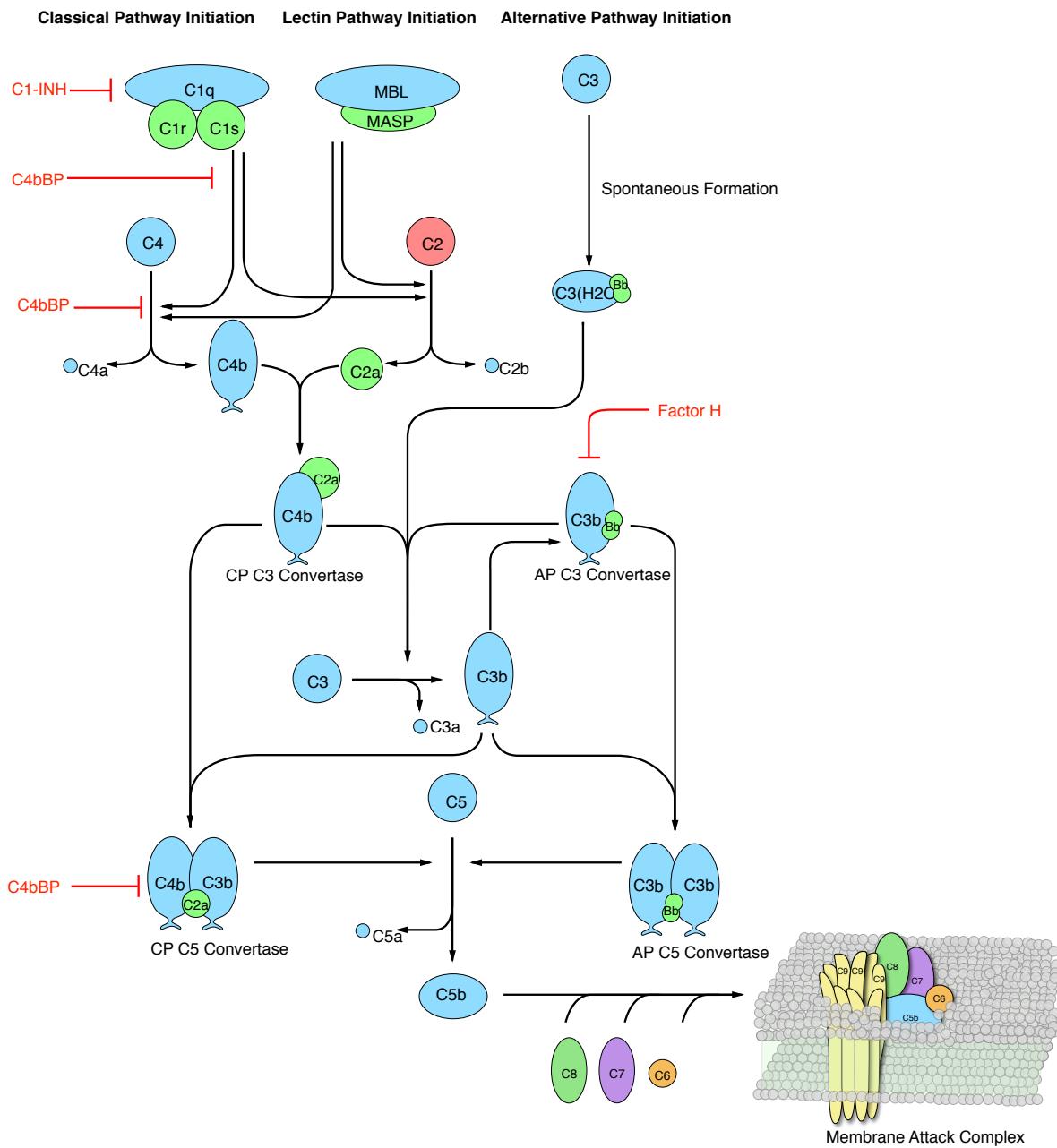


Fig. 1: Simplified schematic of the human complement system. The complement cascade is activated through any one, or more, of the three pathways: the classical, the lectin, and the alternate pathways. The classical pathway is activated by the binding of C1 complex through the C1q subunit to the IgG or 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 ($C_3bB_bC_3b$). The C5 convertases cleave C5 into C5a, and C5b that undergoes a series of reactions to form the membrane attack complex (MAC).

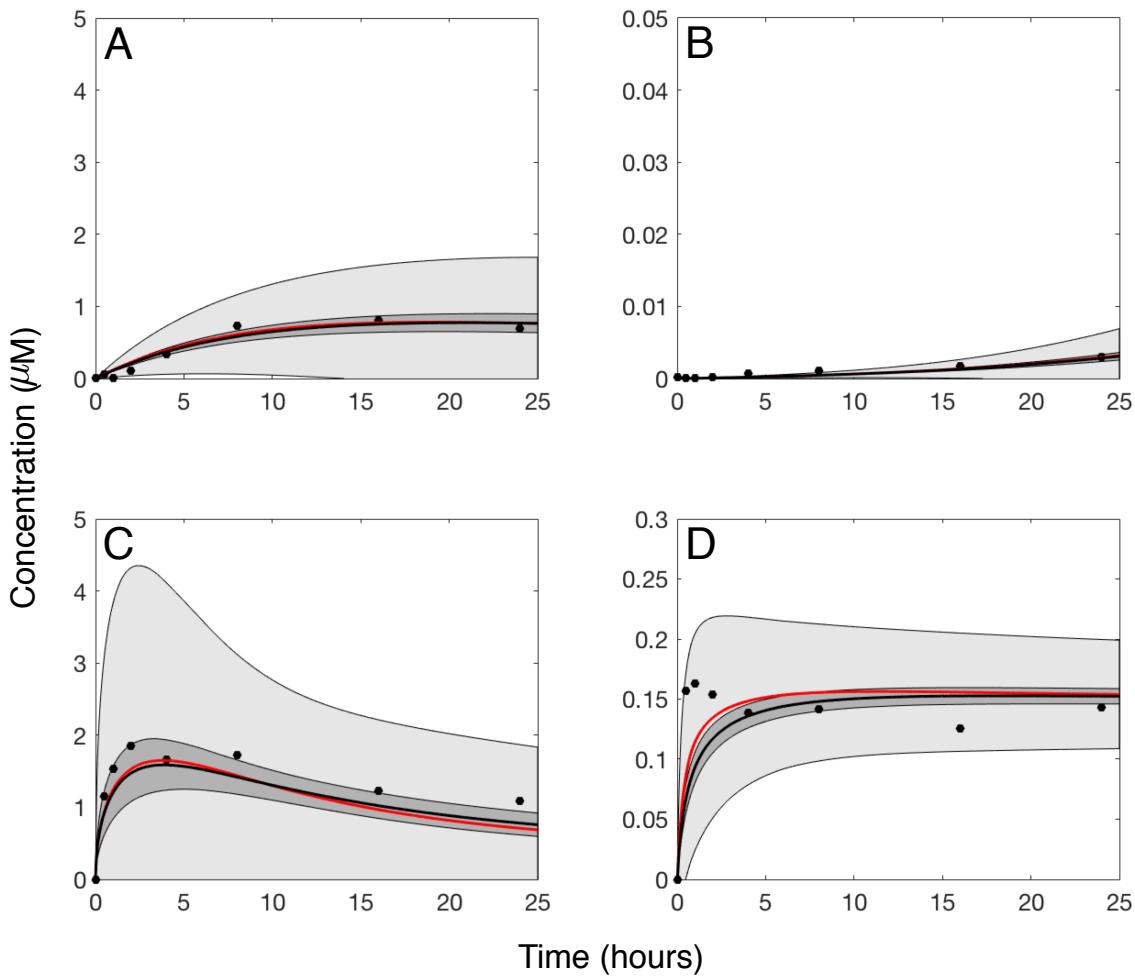


Fig. 2: Reduced order complement model training simulations. Reduced order complement model parameters were estimated using Dynamic Optimization with Particle Swarms (DOPS). The model was trained against experimental data from Shaw and co-workers (19) in the presence and absence of zymosan. The model 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.

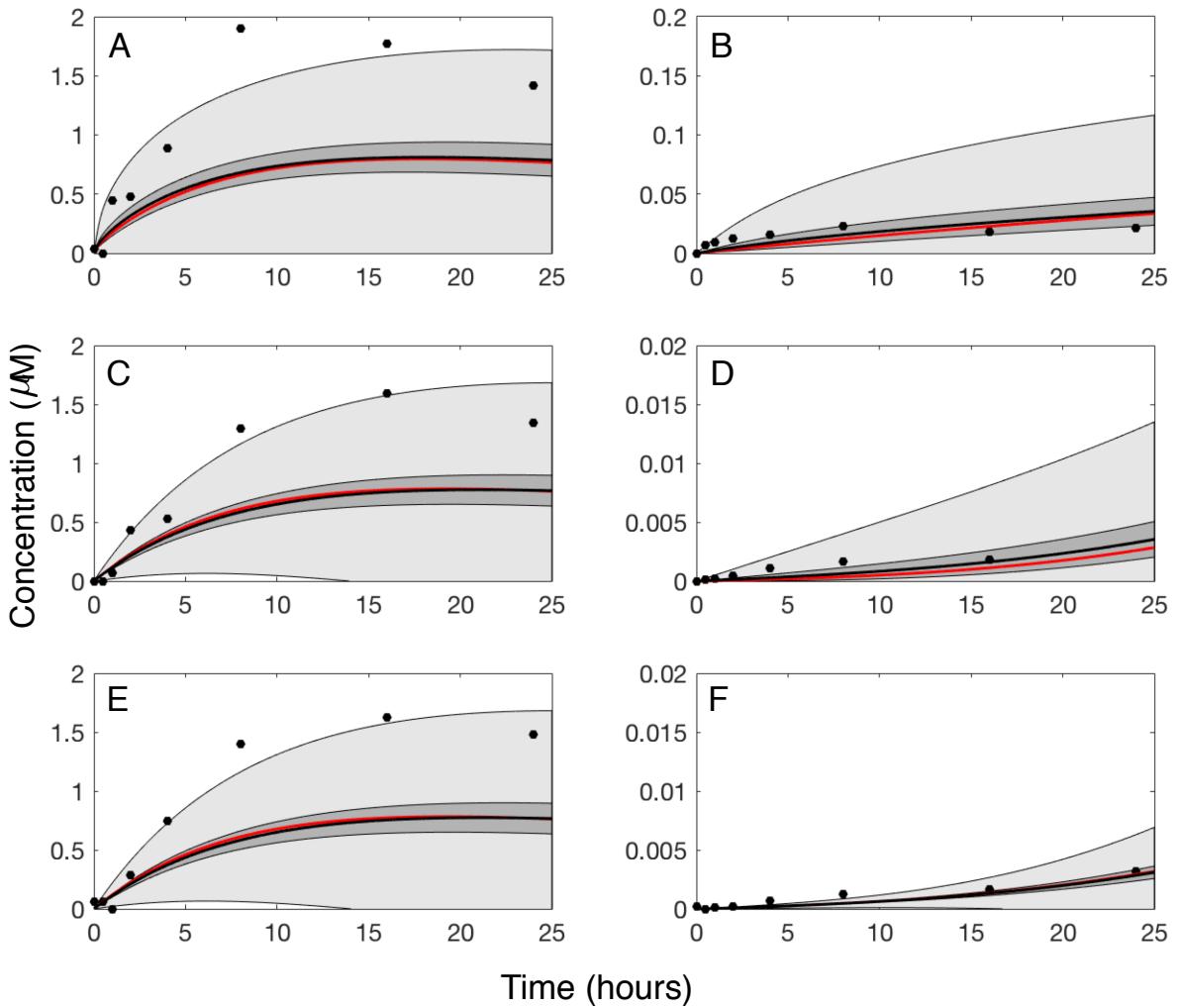


Fig. 3: Reduced order complement model predictions vs experimental data for C3a and C5a generated in the lectin pathway. The reduced order coagulation model parameter estimates were tested against data not used during model training. Simulations of C3a and C5a generated in the lectin pathway using different 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.

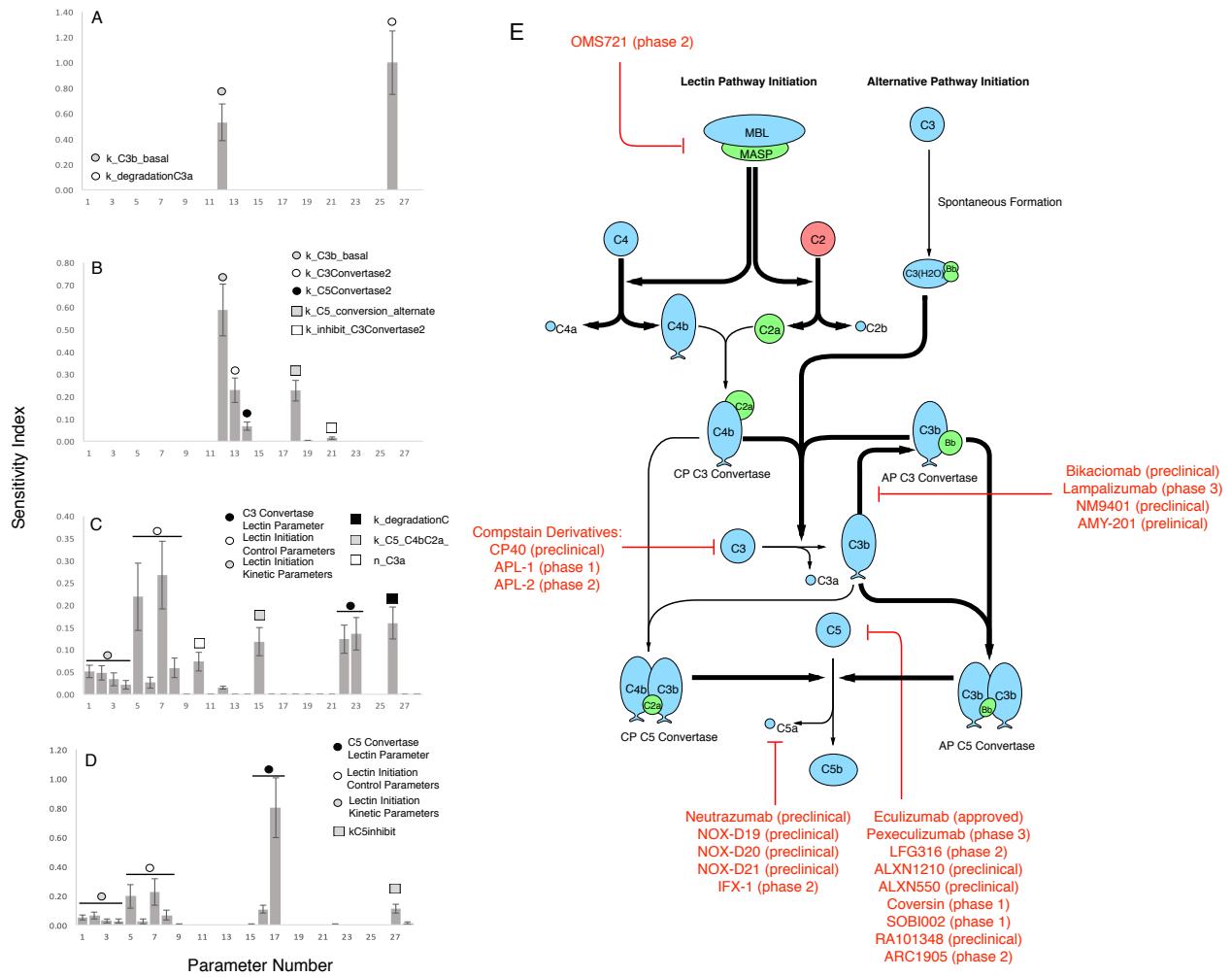


Fig. 4: Sobol's sensitivity analysis of the reduced order complement model with respect to the modeling parameters. Sensitivity analysis was conducted on the four cases we used to train our model: (A) C3a at 0g zymosan, (B) C5a 0g zymosan, (C) C3a 1g zymosan, and (D) C5a 1g zymosan. The bars denote total 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.

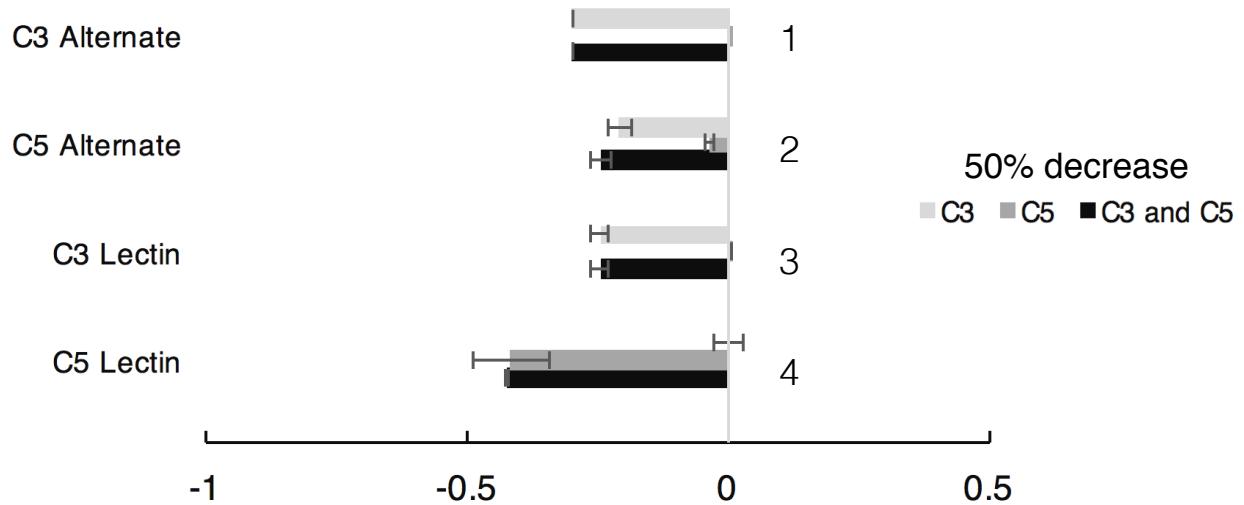
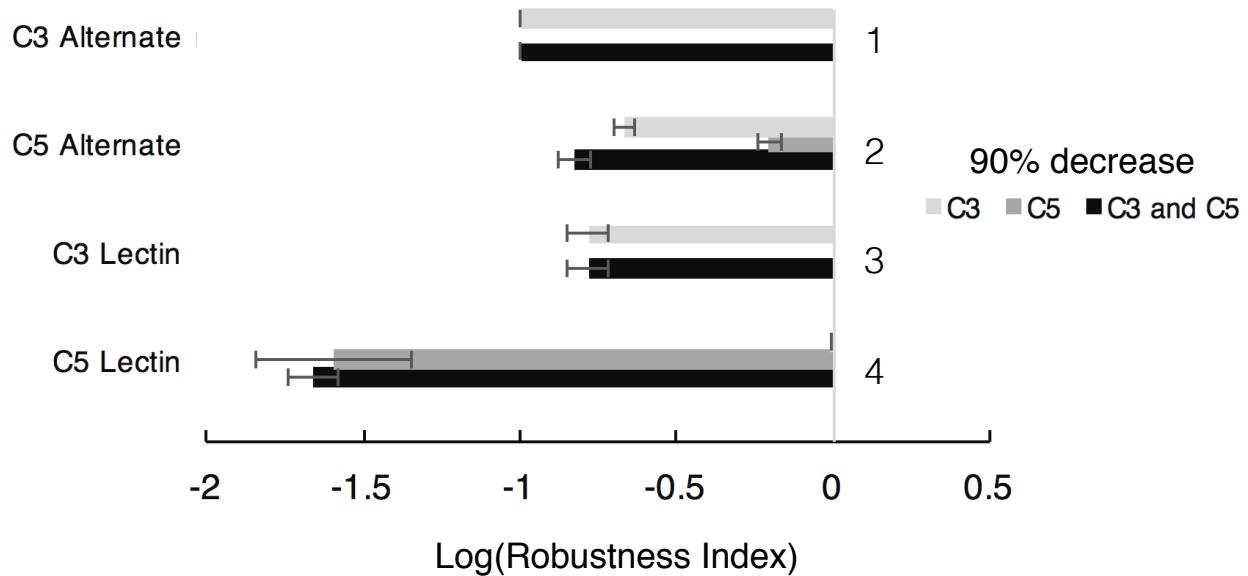
A**B**

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.

551 **Supplemental materials.**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

$$(\text{S19})$$

554 where the rate equations are given by:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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