

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 Complement is mediated by over 30 soluble and cell surface proteins and regulators.
17 The molecular connectivity of complement is complex, and has been extensively reviewed
18 by Walport (7, 8). The central outputs of complement are the Membrane Attack Complex
19 (MAC), and the inflammatory mediators C3a and C5a. The membrane attack complex
20 forms transmembrane channels which disrupt the membrane integrity of targeted cells,
21 leading to cell lysis and death. On the other hand, the C3a and C5a proteins act as
22 a bridge between innate and adaptive immunity, and play an important role in regulat-
23 ing inflammation and coagulation in the case of C5a (5). Complement activation takes
24 places through three pathways: the alternate, the classical and the lectin binding path-
25 way. Each of these pathways involves a different initiator signal which leads to a cascade
26 of downstream events in the complement system. The classical pathway is triggered by

27 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 combine to form the C4bC2a protease, which is also known as
32 the classical C3 convertase. The lectin binding pathway is initiated through the binding of
33 L-ficolin or Mannose Binding Lectin (MBL) to carbohydrates on the surfaces of bacterial
34 pathogens. These complexes also cleave C4 and C2, leading the formation of additional
35 classical C3 convertase. Thus, the classical and lectin pathways converge at the clas-
36 sical C3 convertase, and are initiated by the recognition of a foreign surface. However,
37 the final pathway, known as the alternate pathway, works differently. The alternate path-
38 way involves a 'tickover' mechanism in which complement protein C3 is spontaneously
39 hydrolyzed to form an activated intermediate C3w; C3w recruits factor B and factor D,
40 leading to the formation of C3wBb which cleaves C3 into C3a and C3b. The C3b frag-
41 ment can further recruit additional factor B and factor D to form C3bBbC3b, otherwise
42 known as the alternate C3 convertase (9). The role of classical and alternative C3 con-
43 vertases is varied. First, the C3 convertases encode an amplification loop by cleaving C3
44 into C3a and C3b, the latter is then free to form additional alternate C3 convertase. Next,
45 they link complement initiation with the terminal response of the cascade through the for-
46 mation of C5 convertases. The classical and alternate C3 convertases recruit C3b, form
47 the classical C5 convertase (C4bC2aC3b), and alternate C5 convertase (C3bBbC3b) re-
48 spectively. The C5 convertases cleave C5 to form the C5a and C5b fragments. The C5b
49 fragment, along with the C6, C7, C8 and multiple C9 complement proteins, form the mem-
50 brane attack complex. On the other hand, both C3a and C5a are important inflammatory
51 signals involved in several responses.

52 Complement activation is regulated by many plasma and host cell proteins. The initi-

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

69 Developing quantitative mathematical models of complement could be crucial to un-
70 derstanding its role in the body. Traditionally, complement models have been formulated
71 as systems of linear or non-linear ordinary differential equations (ODEs). For example,
72 Hirayama et al. modeled the classical complement pathway as a system of linear ODEs
73 (17), while Korotaelevskiy and co-workers modeled the classical, lectin and alternate path-
74 ways as a system of non-linear ODEs (18). More recently, large mechanistic models of
75 sections of complement have also been proposed. For example, Liu et al. analyzed the
76 formation of the classical and lectin C3 convertases, and the regulatory role of C4BP
77 using a system of 45 non-linear ODEs with 85 parameters (19). Recently, Zewde and co-
78 workers constructed a detailed mechanistic model of the alternative pathway which con-

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

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

¹⁰⁵ for multiple points of intervention to disrupt complement activation.

106 **Results**

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

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

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

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

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

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

²¹⁰ magnitude (Fig. 5B, lane 4).

211 **Discussion**

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

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

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

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

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

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

332 **Materials and Methods**

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

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

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

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

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

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

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

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

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

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

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

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

367 where $\hat{x}_j(\tau)$ denotes the measured value of species j at time τ , $x_j(\tau, \mathbf{k})$ denotes the simulated
 368 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 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:

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

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

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

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

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

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

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

420 Robustness coefficients quantify the response of a marker to a structural or opera-
 421 tional perturbation to the network architecture. Robustness coefficients were calculated
 422 as shown previously (44). Robustness coefficients denoted by $\alpha(i, j, t_o, t_f)$ are defined
 423 as:

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

424 Here t_o and t_f denote the initial and final simulation time respectively, while i and j denote
 425 the indices for the marker and the perturbation respectively. A value of $\alpha(i, j, t_o, t_f) >$
 426 0, indicates increased marker abundance, while $\alpha(i, j, t_o, t_f) < 0$ indicates decreased
 427 marker abundance following perturbation j . If $\alpha(i, j, t_o, t_f) \sim 0$ the j th perturbation did not
 428 influence the abundance of marker i . In this study, we perturbed the initial condition of C3
 429 or C5 or a combination of C3 and C5 by 50% or 90% and measured the area under the
 430 curve (AUC) of C3a or C5a with and without lectin initiator. Robustness coefficients were
 431 calculated for every member of the ensemble, where the mean $\pm 1 \times$ standard-deviation
 432 are reported.

433 **Competing interests**

434 The authors declare that they have no competing interests.

435 **Author's contributions**

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

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446 **References**

- 447 1. Nuttall G (1888) Experimente über die bacterienfeindlichen Einflüsse des thierischen
448 Körpers. *Z Hyg Infektionskr* 4: 353-394.
- 449 2. Ricklin D, Hajishengallis G, Yang K, Lambris JD (2010) Complement: a key system
450 for immune surveillance and homeostasis. *NAT IMMUNOL* 11: 785–797.
- 451 3. Ricklin D, Lambris JD (2007) Complement-targeted therapeutics. *NAT BIOTECHNOL*
452 25: 1265-1275.
- 453 4. Rittirsch D, Flierl MA, Ward PA (2008) Harmful molecular mechanisms in sepsis. *NAT*
454 *REV IMMUNOL* 8: 776–787.
- 455 5. Sarma JV, Ward PA (2011) The complement system. *Cell Tissue Res* 343: 227–235.
- 456 6. Ricklin D, Lambris JD (2013) Complement in immune and inflammatory disorders:
457 pathophysiological mechanisms. *J Immunol* 190: 3831–3838.
- 458 7. Walport MJ (2001) Complement. first of two parts. *N Engl J Med* 344: 1058-66.
- 459 8. Walport MJ (2001) Complement. second of two parts. *N Engl J Med* 344: 1140-4.
- 460 9. Pangburn MK, Müller-Eberhard HJ (1984) The alternative pathway of complement.
461 *Semin Immunopathol* .
- 462 10. Walker D, Yasuhara O, Patston P, McGeer E, McGeer P (1995) Complement c1 in-
463 hibitor is produced by brain tissue and is cleaved in alzheimer disease. *Brain Res*
464 675: 75–82.
- 465 11. Blom AM, Kask L, Dahlbäck B (2001) Structural requirements for the complement
466 regulatory activities of c4bp. *J Biol Chem* 276: 27136–27144.
- 467 12. Riley-Vargas RC, Gill DB, Kemper C, Liszewski MK, Atkinson JP (2004) Cd46: ex-
468 panding beyond complement regulation. *Trends Immunol* 25: 496–503.
- 469 13. Lukacik P, Roversi P, White J, Esser D, Smith G, et al. (2004) Complement regulation
470 at the molecular level: the structure of decay-accelerating factor. *Proc Natl Acad Sci*
471 *USA* 101: 1279–1284.

- 472 14. Liszewski MK, Farries TC, Lublin DM, Rooney IA, Atkinson JP (1995) Control of the
473 complement system. *Adv Immunol* 61: 201–283.
- 474 15. Chauhan A, Moore T (2006) Presence of plasma complement regulatory proteins
475 clusterin (apo j) and vitronectin (s40) on circulating immune complexes (cic). *Clin
476 Exp Immunol* 145: 398–406.
- 477 16. Zewde N, Gorham Jr RD, Dorado A, Morikis D (2016) Quantitative modeling of the
478 alternative pathway of the complement system. *PLoS one* 11: e0152337.
- 479 17. Hirayama H, Yoshii K, Ojima H, Kawai N, Gotoh S, et al. (1996) Linear systems analy-
480 sis of activating processes of complement system as a defense mechanism. *Biosys-
481 tems* 39: 173–185.
- 482 18. Korotaevskiy AA, Hanin LG, Khanin MA (2009) Non-linear dynamics of the comple-
483 ment system activation. *Math Biosci* 222: 127–143.
- 484 19. Liu B, Zhang J, Tan PY, Hsu D, Blom AM, et al. (2011) A computational and experi-
485 mental study of the regulatory mechanisms of the complement system. *PLoS Comput
486 Biol* 7: e1001059.
- 487 20. Morad HO, Belete SC, Read T, Shaw AM (2015) Time-course analysis of c3a and
488 c5a quantifies the coupling between the upper and terminal complement pathways in
489 vitro. *J Immunol Methods* 427: 13–18.
- 490 21. Morgan BP, Harris CL (2015) Complement, a target for therapy in inflammatory and
491 degenerative diseases. *NAT REV DRUG DISCOV* 14: 857-877.
- 492 22. Luan D, Zai M, Varner JD (2007) Computationally derived points of fragility of a human
493 cascade are consistent with current therapeutic strategies. *PLoS Comput Biol* 3:
494 e142.
- 495 23. Nayak S, Salim S, Luan D, Zai M, Varner JD (2008) A test of highly optimized tol-
496 erance reveals fragile cell-cycle mechanisms are molecular targets in clinical cancer
497 trials. *PLoS One* 3: e2016.

- 498 24. Tasseff R, Nayak S, Salim S, Kaushik P, Rizvi N, et al. (2010) Analysis of the molecular
499 networks in androgen dependent and independent prostate cancer revealed fragile
500 and robust subsystems. *PLoS One* 5: e8864.
- 501 25. Rice NT, Szlam F, Varner JD, Bernstein PS, Szlam AD, et al. (2016) Differential con-
502 tributions of intrinsic and extrinsic pathways to thrombin generation in adult, maternal
503 and cord plasma samples. *PLoS One* 11: e0154127.
- 504 26. Héja D, Harmat V, Fodor K, Wilmanns M, Dobó J, et al. (2012) Monospecific inhibitors
505 show that both mannan-binding lectin-associated serine protease-1 (masp-1) and-2
506 are essential for lectin pathway activation and reveal structural plasticity of masp-2. *J
507 Biol Chem* : 20290–20300.
- 508 27. Roguska M, Splawski I, Diefenbach-Streiber B, Dolan E, Etemad-Gilbertson B, et al.
509 (2014) Generation and characterization of Ifg316, a fully-human anti-c5 antibody for
510 the treatment of age-related macular degeneration. *Investigative Ophthalmology &
511 Visual Science* : 3433–3433.
- 512 28. Melis JP, Strumane K, Ruuls SR, Beurskens FJ, Schuurman J, et al. (2015) Com-
513 plement in therapy and disease: Regulating the complement system with antibody-
514 based therapeutics. *Molecular immunology* : 117–130.
- 515 29. Weston-Davies WH, Nunn MA, Pinto FO, Mackie IJ, Richards SJ, et al. (2014) Clin-
516 ical and immunological characterisation of coversin, a novel small protein inhibitor of
517 complement c5 with potential as a therapeutic agent in pnh and other complement
518 mediated disorders. *Blood* : 4280–4280.
- 519 30. Epstein D, Kurz JC (2007). Complement binding aptamers and anti-c5 agents useful
520 in the treatment of ocular disorders. US Patent App. 12/224,708.
- 521 31. Borodovsky A, Yucius K, Sprague A, Banda NK, Holers VM, et al. (2014) Aln-cc5, an
522 investigational rnai therapeutic targeting c5 for complement inhibition. *Complement
523 20:* 40.

- 524 32. Sissons J, Liebowitch J, Amos N, Peters D (1977) Metabolism of the fifth component
525 of complement, and its relation to metabolism of the third component, in patients with
526 complement activation. *J Clin Invest* 59: 704.
- 527 33. Swaak A, Hannema A, Vogelaar C, Boom F, van Es L, et al. (1982) Determination of
528 the half-life of c3 in patients and its relation to the presence of c3-breakdown products
529 and/or circulating immune complexes. *Rheumatol Int* : 161–166.
- 530 34. Noris M, Galbusera M, Gastoldi S, Macor P, Banterla F, et al. (2014) Dynamics of
531 complement activation in ahus and how to monitor eculizumab therapy. *Blood* : 1715–
532 1726.
- 533 35. Schwaeble HW, Stover CM, Tedford CE, Parent JB, Fujita T (2011). Methods for
534 treating conditions associated with masp-2 dependent complement activation. US
535 Patent 7,919,094.
- 536 36. Katschke KJ, Wu P, Ganesan R, Kelley RF, Mathieu MA, et al. (2012) Inhibiting al-
537 ternative pathway complement activation by targeting the factor d exosite. *Journal of*
538 *Biological Chemistry* : 12886–12892.
- 539 37. Hu X, Holers VM, Thurman JM, Schoeb TR, Ramos TN, et al. (2013) Therapeutic in-
540 hibition of the alternative complement pathway attenuates chronic eae. *Mol Immunol*
541 : 302–308.
- 542 38. Bansal R (2014). Humanized and chimeric anti-properdin antibodies. US Patent
543 8,664,362.
- 544 39. Vogel CW, Fritzinger DC, Hew BE, Thorne M, Bammert H (2004) Recombinant cobra
545 venom factor. *Molecular immunology* : 191–199.
- 546 40. Sagar A, Varner JD (2015) Dynamic modeling of the human coagulation cascade
547 using reduced order effective kinetic models. *Processes* 3: 178.
- 548 41. Sobol I (2001) Global sensitivity indices for nonlinear mathematical models and their
549 monte carlo estimates. *MATH COMPUT SIMULAT* 55: 271 - 280.

- 550 42. Saltelli A, Annoni P, Azzini I, Campolongo F, Ratto M, et al. (2010) Variance based
551 sensitivity analysis of model output. design and estimator for the total sensitivity index.
552 Comput Phys Commun 181: 259–270.
- 553 43. Herman J. Salib: Sensitivity analysis library in python (numpy). con-
554 tains sobol, morris, fractional factorial and fast methods. available online:
555 <https://github.com/jdherman/salib>.
- 556 44. Tasseff R, Nayak S, Song SO, Yen A, Varner JD (2011) Modeling and analysis
557 of retinoic acid induced differentiation of uncommitted precursor cells. Integr Biol
558 (Camb) 3: 578-91.

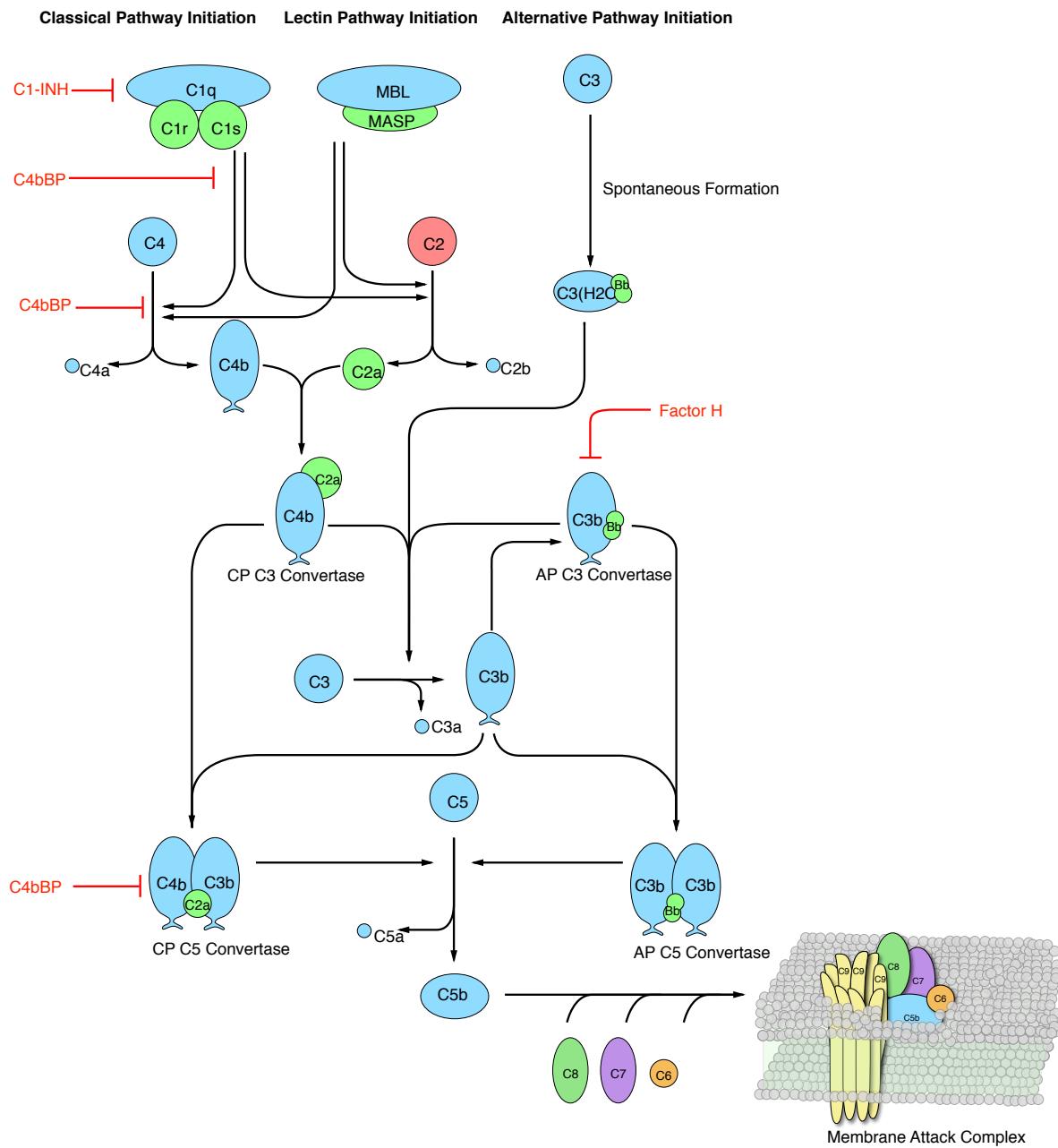


Fig. 1: Simplified schematic of the human complement system. The complement cascade is activated through any one, or more, of the three pathways: the classical, the lectin, and the alternate pathways. The classical pathway is activated by the binding of C1 complex through the C1q subunit to the IgG or 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 ($C_3bB_bC_3b$).²⁷ The C5 convertases cleave C5 into C5a, and C5b that undergoes a series of reactions to form the membrane attack complex (MAC).

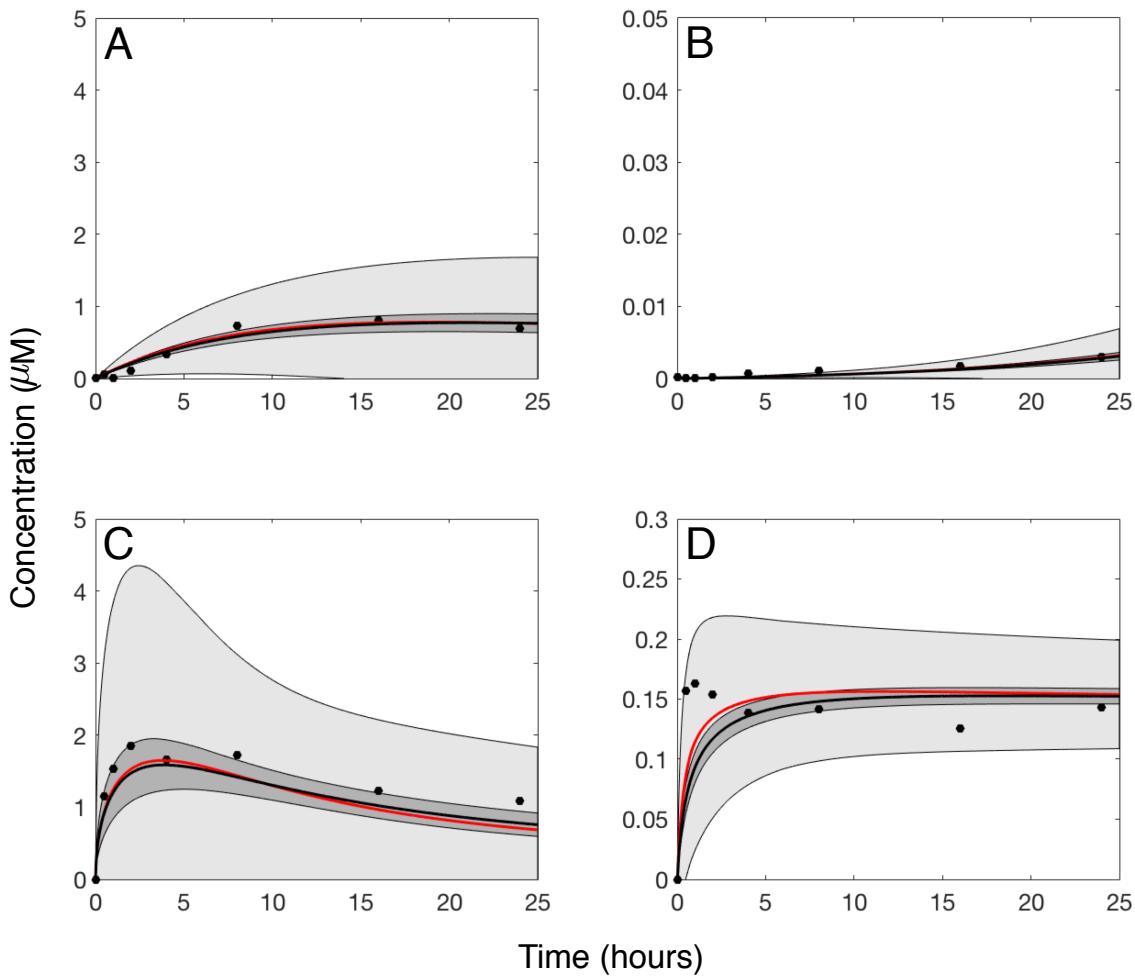


Fig. 2: Reduced order complement model training simulations. Reduced order complement model parameters were estimated using Dynamic Optimization with Particle Swarms (DOPS). The model was trained against experimental data from Shaw and co-workers (20) in the presence and absence of zymosan. The model 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.

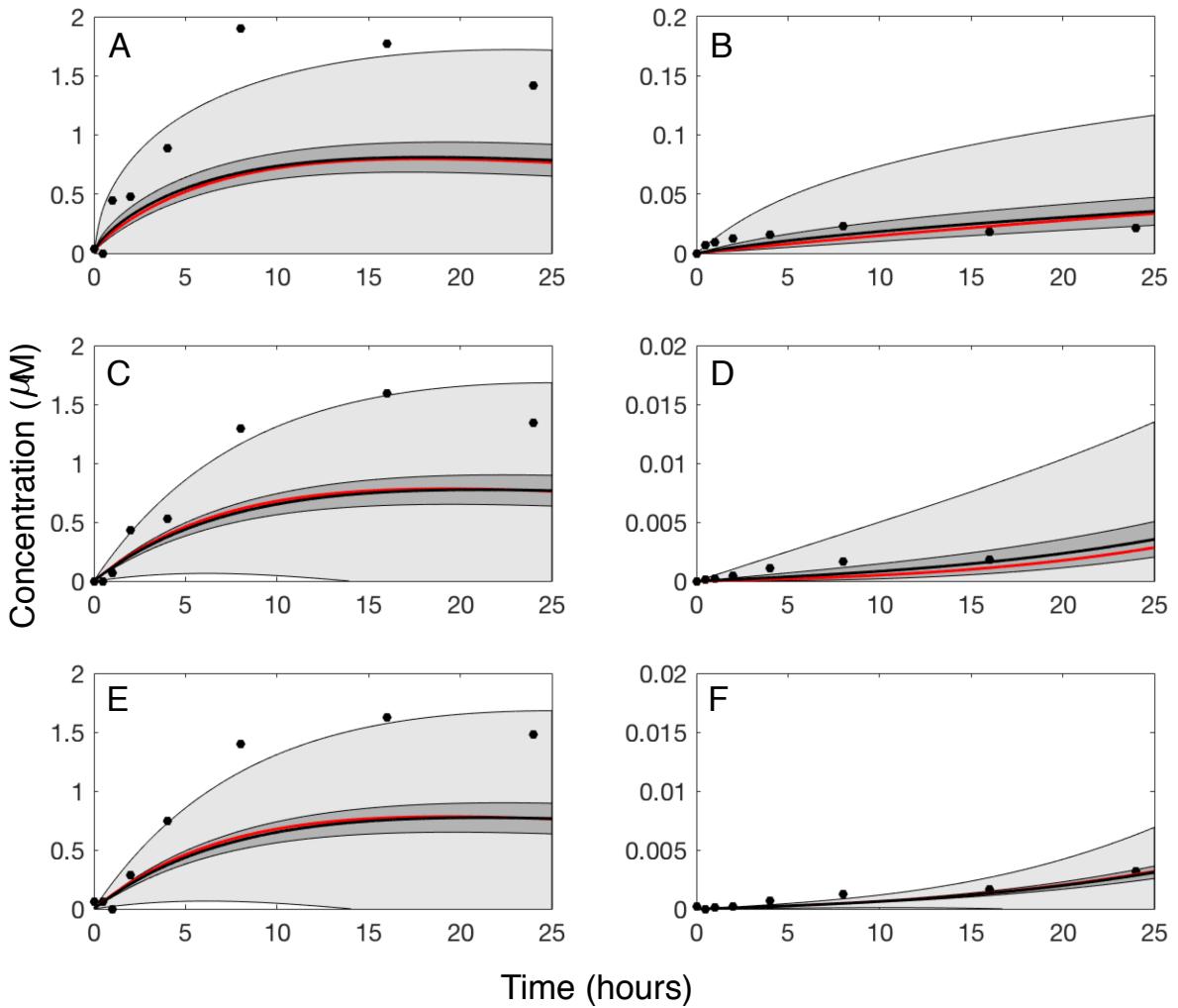


Fig. 3: Reduced order complement model predictions vs experimental data for C3a and C5a generated in the lectin pathway. The reduced order coagulation model parameter estimates were tested against data not used during model training. Simulations of C3a and C5a generated in the lectin pathway using different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

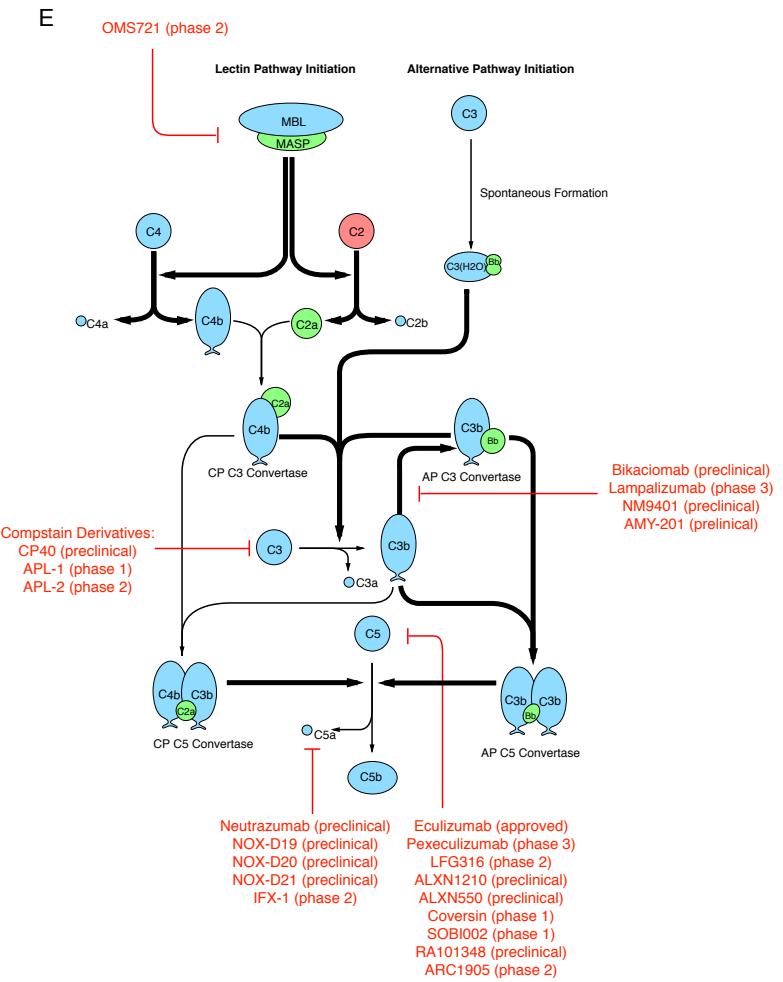
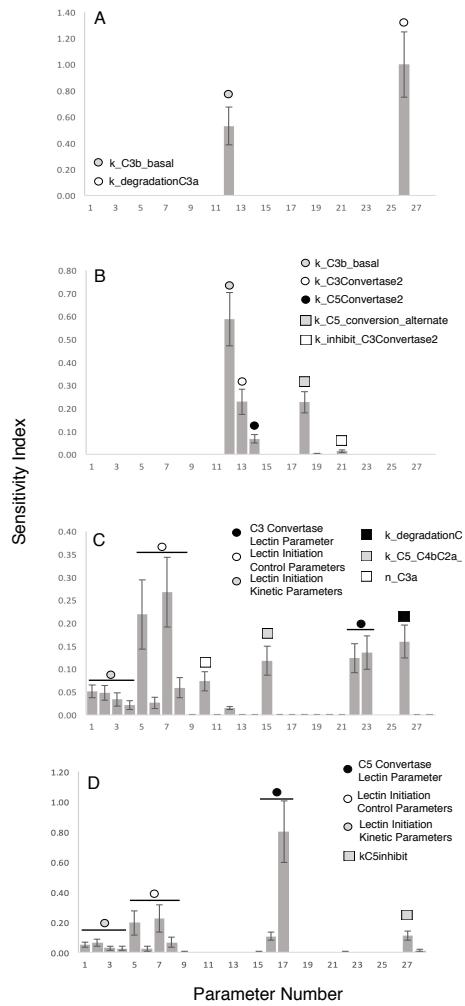
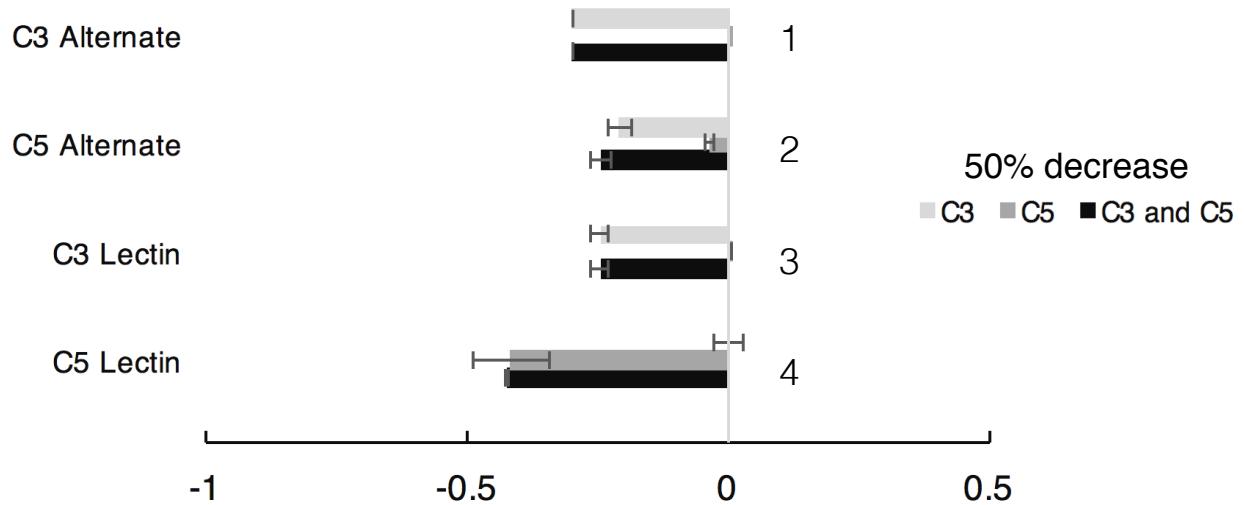


Fig. 4: Sobol's sensitivity analysis of the reduced order complement model with respect to the modeling parameters. Sensitivity analysis was conducted on the four cases we used to train our model: (A) C3a at 0g zymosan, (B) C5a 0g zymosan, (C) C3a 1g zymosan, and (D) C5a 1g zymosan. The bars denote total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

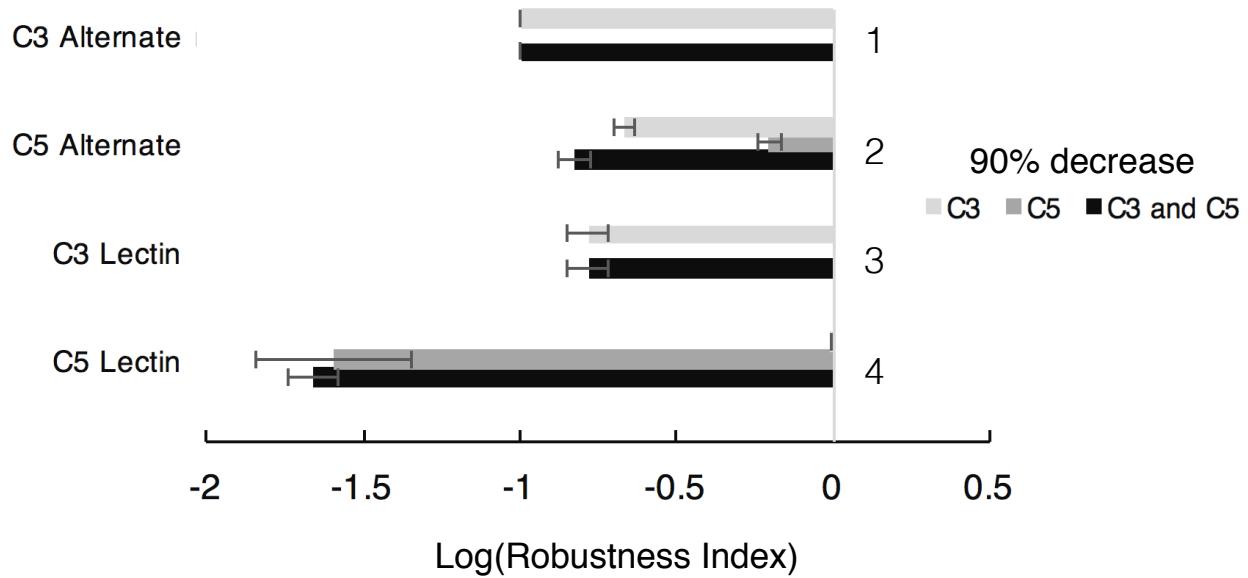


Fig. 5: Robustness analysis of the reduced order complement model with respect to the C3 and C5 initial concentrations using 50 parameter sets. Robustness analysis was conducted on the four cases we used to train our model, C3a alternate (0 zymosan), C5a alternate (0 zymosan), C3a lectin (1 g zymosan), 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.

559 **Supplemental materials.**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

$$(\text{S19})$$

⁵⁶² where the rate equations are given by:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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