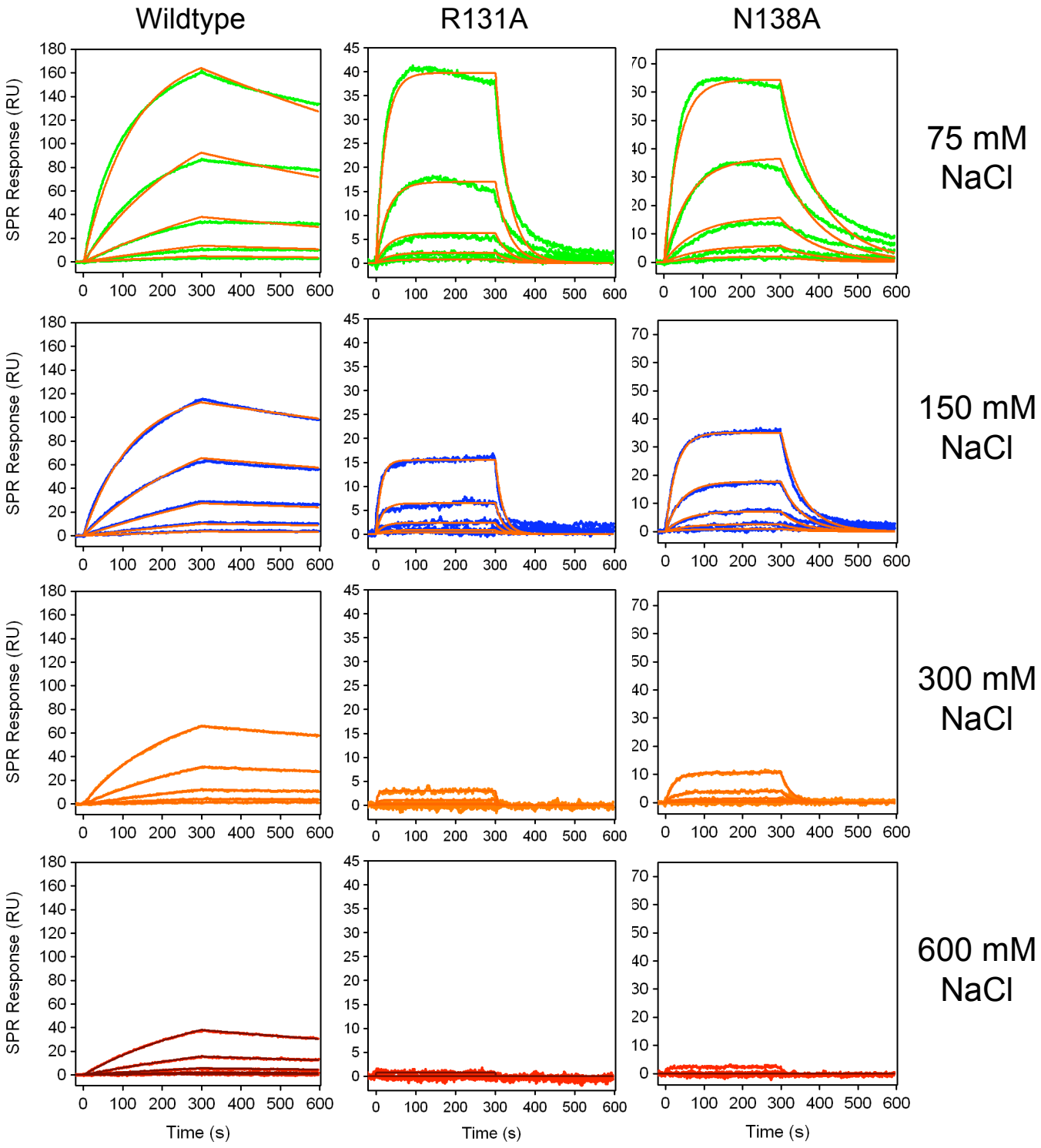
Haspel *et al.*: Electrostatic Contributions Drive the Interaction Between Efb-C and C3d

Supplementary Figure 1



Kinetic evaluation of recombinant C3d (0.3-22 nM) interaction with immobilized Efb-C or its R131A and N138A mutant in PBS buffer of increasing salt concentration. All data sets were processed using Scrubber (v2; BioLogic) and fitted to a 1:1 Langmuir binding isotherm (thin red lines). Sub-physiological salt concentration (75 mM) lead to slight deviations from the 1:1 binding model, most likely due to increased non-specific binding. At high salt concentrations (300-600 mM), both mutants show very low signal-to noise ratios, which render a precise kinetic fit increasingly difficult. The resulting kinetic rate constants are plotted and compared in Supplemental Figure 2.

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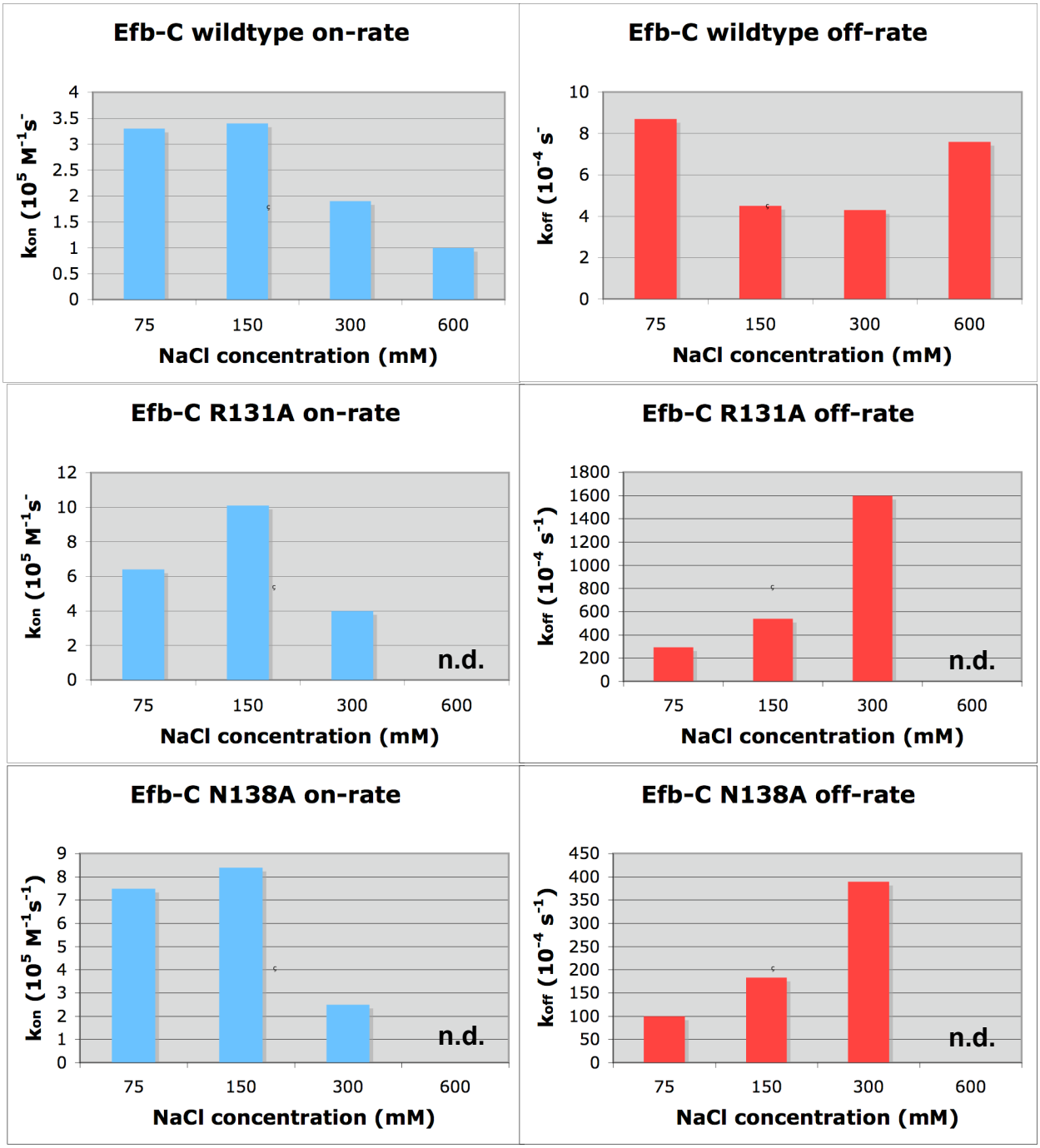
Supplementary Figure 2



Comparison of the kinetic rate constants of the C3d interaction with Efb-C and its mutants R131A and N138A (based on the fitting results as seen in Supplemental Figure 1). In case of wildtype Efb-C, the effect of increasing buffer salt concentration is more pronounced on the kinetic on-rate (I.e. complex formation) than on the off-rate (complex stability). For the mutants, this trend is less clear and both rates seem to be affected. Note that the unfavorable signal-to-noise ratio, especially at high NaCl concentrations, make the kinetic evaluation increasingly imprecise (no rate constants could be extracted at 600 nM NaCl for both mutants). As a consequence, these results represent a semi-quantitative trend analysis rather than a quantitative assessment of kinetic profiles.

Electrostatic Contributions Drive the Interaction Between *Staphylococcus aureus* Protein Efb-C and its Complement Target C3d

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Supplementary Tables

Table 1: Energetic contribution of each residue on the wildtype Efb-C and two mutants to the complex binding. Notable contributions are marked in boldface. All the energies are expressed in kCal/mol.

Res. No.	WT	N138A	R131A	Res. No.	WT	N138A	R131A	Res. No.	WT	N138A	R131A
101	0.07	0.28	1.38	123	-0.13	-0.17	-0.14	145	-2.57	0.18	0.28
102	0.36	0.34	-1.88	124	0.10	0.06	0.07	146	-0.03	0.03	0.04
103	0.06	-1.00	-1.65	125	-0.71	0.02	0.04	147	0.10	0.12	0.13
104	-0.15	-1.34	-1.08	126	-4.30	-0.06	0.00	148	-0.28	-1.46	-1.85
105	-0.2	-0.97	-0.10	**127**	**-7.58**	**-4.03**	**-2.33**	149	-0.73	-0.04	-0.08
106	**-4.12**	**-3.88**	**-2.21**	128	0.13	-3.00	-0.38	150	0.11	0.10	0.09
107	-0.80	-1.55	-1.13	129	0.07	-0.02	0.01	151	0.10	0.09	0.11
108	0.36	0.32	0.31	**130**	**-1.63**	**-3.39**	**-3.12**	152	-0.62	0.24	-0.04
109	0.12	0.19	0.14	**131**	**-10.33**	**-11.03**	**-1.31**	153	0.31	0.20	0.21
110	**-3.16**	**-3.63**	**-3.02**	132	-0.12	-0.14	-0.60	154	0.06	0.05	0.09
111	-0.10	-0.06	-0.05	133	0.0	0.05	-0.05	155	-0.22	-0.05	-0.27
112	-0.01	-0.00	-0.00	**134**	**-2.41**	**-2.89**	**-2.79**	156	-1.35	-0.49	-0.80
113	0.14	0.13	0.06	**135**	**-3.56**	**-2.93**	**-4.49**	157	0.11	0.12	0.10
114	-0.03	-0.04	-0.02	136	0.01	0.00	-0.24	158	0.08	0.07	0.09
115	0.03	0.03	0.03	137	0.14	-0.98	-0.24	**159**	**-3.51**	**-2.82**	**-2.82**
116	0.03	0.07	0.05	**138**	**-2.81**	**-2.49**	**-5.75**	**160**	**-1.32**	**-1.52**	**-1.81**
117	0.04	0.04	0.04	**139**	**-3.83**	**-2.92**	**-3.69**	161	-0.26	0.05	0.01
118	0.06	0.10	0.08	140	-2.52	-0.84	-0.40	162	-2.76	0.01	-0.70
119	-0.16	-0.12	-0.11	**141**	**-1.25**	**-3.02**	**-3.24**	**163**	**-4.04**	**-2.54**	**-2.23**
120	0.33	0.57	0.39	**142**	**-5.16**	**-2.09**	**-2.82**	164	-0.10	-0.31	-0.15
121	-0.24	-0.17	-0.17	143	0.63	0.16	-0.20	**165**	**-10.53**	**-8.43**	**-8.25**
122	0.30	0.26	0.23	144	0.12	-0.04	-0.00				

Table 2: Energetic contribution of each residue on the wildtype C3d to the complex binding. Notable contributions are marked in boldface. All the energies are expressed in kCal/mol.

Res. No.	Energy	Res. No.	Energy	Res. No.	Energy
39	**-2.62**	53	-3.22	**106**	**-2.35**
40	**-2.62**	54	-0.04	**107**	**-2.73**
41	-0.01	55	0.10	**108**	**-2.03**
42	**-6.80**	56	-0.80	**166**	**-1.94**
43	0.09	57	-0.09	167	-0.89
44	-1.05	58	0.03	168	-0.53
45	**-2.07**	59	0.08	**169**	**-1.88**
46	0.63	60	-0.51	**170**	**-3.47**
47	0.01	**100**	**-1.65**	**171**	**-4.95**
48	0.10	**101**	**-1.76**	**172**	**-1.86**
49	**-2.32**	**102**	**-1.60**	173	-0.17
50	-1.32	**103**	**-1.89**	174	0.07
51	0.57	**104**	**-2.90**	175	0.00
52	**-2.78**	**105**	**-3.54**	176	-0.01