

Structural basis of soluble membrane attack complex packaging for clearance

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18 ONLINE SUPPLEMENTARY INFORMATION

19 SUPPLEMENTARY TABLES

20 Supplementary Table 1.

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22 Cryo-EM data collection, refinement, and validation statistics

	#1 1C9-sMAC (EMD-12649)	#2 2C9-sMAC (EMD-12651) (PDB 7NYD)	#3 3C9-sMAC (EMD-12650) (PDB 7NYC)	#4 2C9-sMAC ^{stalk} (EMD-12648) (PDB 7NYD)	#5 2C9-sMAC ^{arc} (EMD-12647) (PDB 7NYD)	#6 3C9-sMAC ^{arc} (EMD-12646) (PDB 7NYC)
Data collection and processing						
Magnification	130k	130k	130k	130k	130k	130k
Voltage (kV)	300	300	300	300	300	300
Electron exposure (e-/Å ²)	40 D ₁ 42 D ₂	40 D ₁ 42 D ₂	40 D ₁ 42 D ₂	40 D ₁ 42 D ₂	40 D ₁ 42 D ₂	40 D ₁ 42 D ₂
Defocus range (µm)	-1.1 to -2.3 D ₁ , -1.1 to -2.1 D ₂	-1.1 to -2.3 D ₁ , -1.1 to -2.1 D ₂	-1.1 to -2.3 D ₁ , -1.1 to -2.1 D ₂	-1.1 to -2.3 D ₁ , -1.1 to -2.1 D ₂	-1.1 to -2.3 D ₁ , -1.1 to -2.1 D ₂	-1.1 to -2.3 D ₁ , -1.1 to -2.1 D ₂
Pixel size (Å)	1.047 D ₁ 1.048 D ₂	1.047 D ₁ 1.048 D ₂	1.047 D ₁ 1.048 D ₂	1.047 D ₁ 1.048 D ₂	1.047 D ₁ 1.048 D ₂	1.047 D ₁ 1.048 D ₂
Symmetry imposed	None	None	None	None	None	None
Initial particle images (no.)	1,084,293 D ₁ 195,552 D ₂	1,084,293 D ₁ 195,552 D ₂	1,084,293 D ₁ 195,552 D ₂	1,084,293 D ₁ 195,552 D ₂	1,084,293 D ₁ 195,552 D ₂	1,084,293 D ₁ 195,552 D ₂
Final particle images (no.)	99,277	142,499	85,151	142,499	96,118	85,151
Map resolution (Å)	3.8	3.3	3.5	3.6	3.3	3.8
FSC threshold	0.143					
Map resolution range (Å)	3.6-9.1	3.0-10.9	3.2-11.1	3.4-7.7	3.2-6.8	3.6-9.1
Refinement						
Initial model used (PDB code)		6H04, 4A5W, 2WCY, 6H03, 6CXO	6H04, 4A5W, 2WCY, 6H03, 6CXO	6H04, 4A5W, 2WCY, 6H03, 6CXO	6H04, 4A5W, 2WCY, 6H03, 6CXO	6H04, 4A5W, 2WCY, 6H03, 6CXO
Model resolution (Å)		3.2	3.5	3.2	3.2	3.5
FSC threshold		0.5				
Model resolution range (Å)						
Map sharpening B factor (Å ²)	Locally sharpened	Locally sharpened	Locally sharpened	Locally sharpened	Locally sharpened	Locally sharpened
Model composition						
Non-hydrogen atoms		38180	41,094	38,180	38,180	41,094
Protein residues		4,940	5,312	4,940	4,940	5,312
Ligands						
B factors (Å ²)						
Protein		88	98	88	88	98
Ligand						
R.m.s. deviations						
Bond lengths (Å)		0.012	0.013	0.012	0.012	0.013
Bond angles (°)		1.917	1.984	1.917	1.917	1.984
Validation		1.28	1.34	1.28	1.28	1.34

MolProbity					
score	1.84	2.31	1.84	1.84	2.31
Clashscore	0.49	0.68	0.49	0.49	0.68
Poor rotamers					
(%)					
Ramachandran					
plot					
Favored (%)	95.17	95.19	95.17	95.17	95.19
Allowed (%)	4.81	4.81	4.81	4.81	4.81
Disallowed (%)	0.02	0.00	0.02	0.02	0.00

23

24 D₁ and D₂ refer to the two datasets which were combined for image processing. D₁
 25 was collect at 0 degrees and D₂ was taken at a tilt angle of 37 degrees.

26 1C9-sMAC, 2C9-sMAC, and 3C9-sMAC maps refer to the sMAC reconstructions after
 27 3D classification, in which different stoichiometries of C9 in complex were separated.

28 2C9-sMAC^{stalk} is the 2C9-sMAC map after density subtraction and focused refinement
 29 on C5b and the C-terminal domains of C7. 2C9-sMAC^{arc} refers to the 2C9-sMAC map

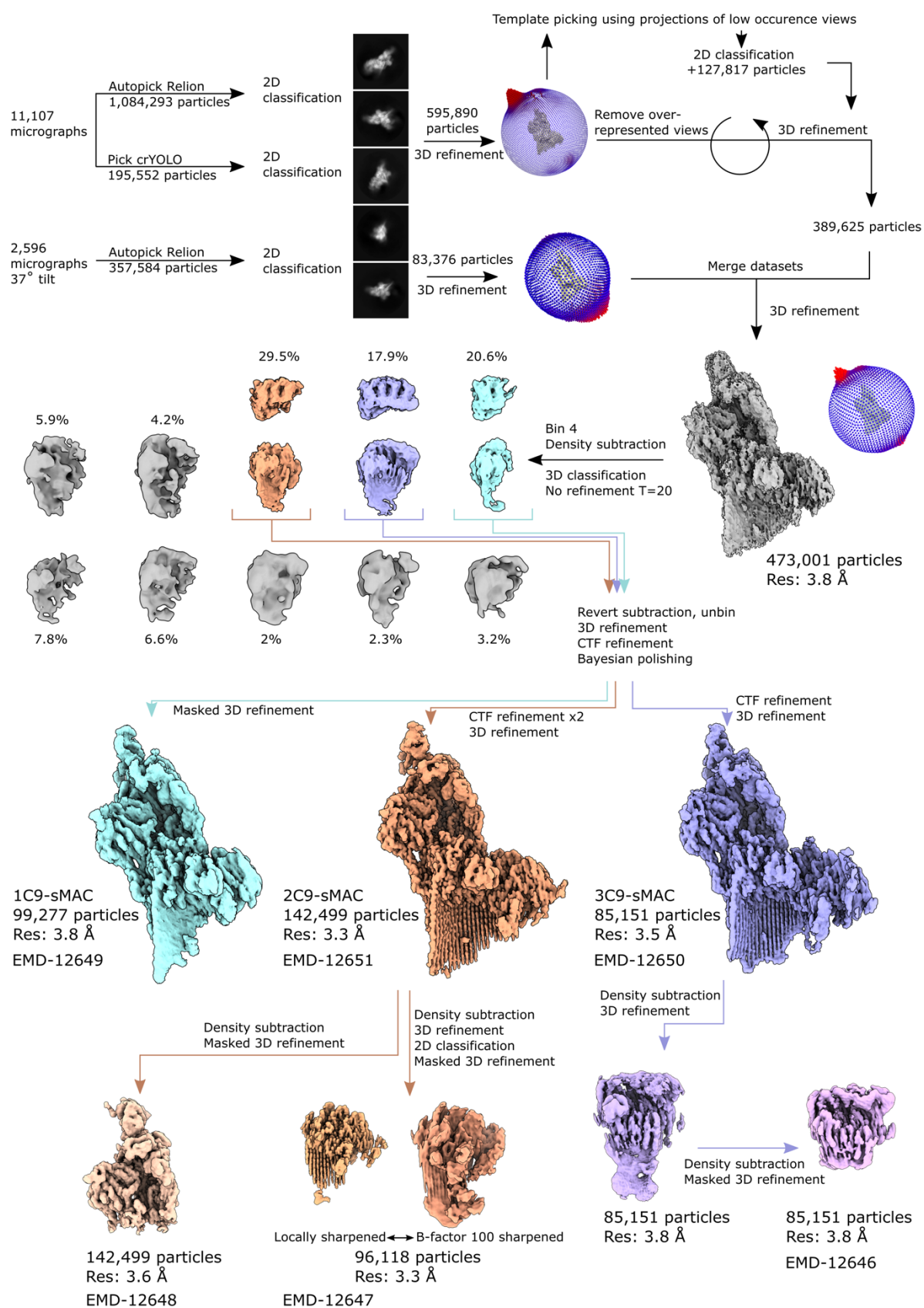
30 after density subtraction and focused refinement on the C9 oligomer. 3C9-sMAC^{arc}

31 refers to the 3C9-sMAC map after density subtraction and focused refinement on the

32 C9 oligomer.

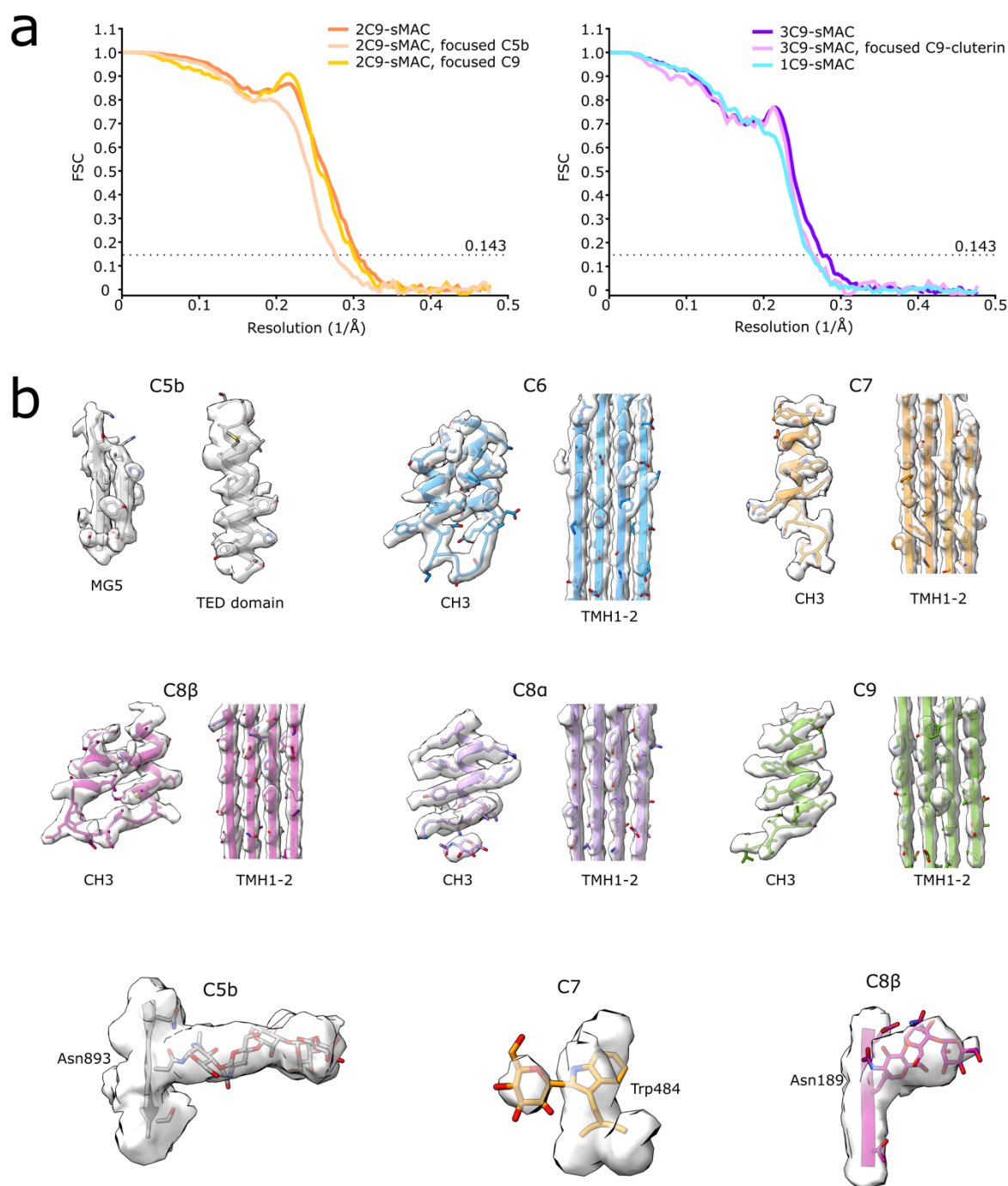
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34 SUPPLEMENTARY FIGURES



Supplementary Figure 1: CryoEM image processing workflow. Particles were picked from micrographs using autopicking programs in Relion and cryYOLO to account for differences in ice-thickness across the micrographs. Images were subject to 2D classification and an initial auto-refinement, resulting in a reconstruction with strong preferred orientations. To improve the angular distribution of the dataset, we combined three strategies: 1) We implemented template-based picking procedures using projections of low occurrence views. 2) We pruned particles from over-represented views. 3) We incorporated an additional dataset collected at a tilt angle of 37°. Duplicate particles were removed and datasets were merged. Data was subject to an additional 3D auto-refinement to generate a consensus sMAC reconstruction with an improved angular distribution. To separate sMAC maps that contained different numbers of C9 molecules we subtracted density corresponding to the core complement complex (C5b6, C7, C8) and used 3D classification with no refinement. Maps with clear density for either 1 (cyan), 2 (orange) or 3 (purple) copies of C9 were taken forward. Reconstructions for each of these three classes were then calculated based on the corresponding particles before density subtraction. Data was subjected to Bayesian polishing and multiple rounds of per-particle CTF refinement before a final 3D auto-refinement to generate the sMAC maps: 1C9-sMAC (3.8 Å) (EMD-12649), 2C9-sMAC (3.3 Å) (EMD-12651), and 3C9-sMAC (3.5 Å) (EMD-12650). To better resolve density above the C9 LDL domains, we used particles corresponding to the 3C9-sMAC map (purple) and subtracted density for the core complement complex. The resulting reconstruction (light purple) showed improved density in this region. We next performed an additional density subtraction and focused our 3D refinement on the core of C9 to generate the C9-clusterin focus-refined 3C9-sMAC map (pink, 3.8 Å) (EMD-12646). To better resolve density corresponding to C5b, we used particles

61 corresponding to the 2C9-sMAC map (orange) and subtracted density for the MACPF
62 arc. Using a masked 3D auto-refinement we calculated a map corresponding to C5b
63 and C6/C7 C-terminal domains (tan, 3.6 Å) (EMD-12648). To better resolve the
64 alternative conformation of C9 in sMAC, we used particles corresponding to the 2C9-
65 sMAC map (orange) and subtracted density for the core complement complex.
66 Particles were subjected to a further 2D classification to improve homogeneity of the
67 population and a final reconstruction of the C9 focus-refined map was calculated (light
68 orange, 3.3 Å) (EMD-12647). The locally sharpened map was used to build the
69 intermediate C9 conformation in sMAC. Blurring the map using an ad hoc B-factor
70 (100), we observe density in the vicinity of the hydrophobic β -hairpins of the C9
71 MACPF, which may correspond to vitronectin.



72

73 Supplementary Figure 2: CryoEM map validation. (a) Fourier Shell Correlation (FSC)

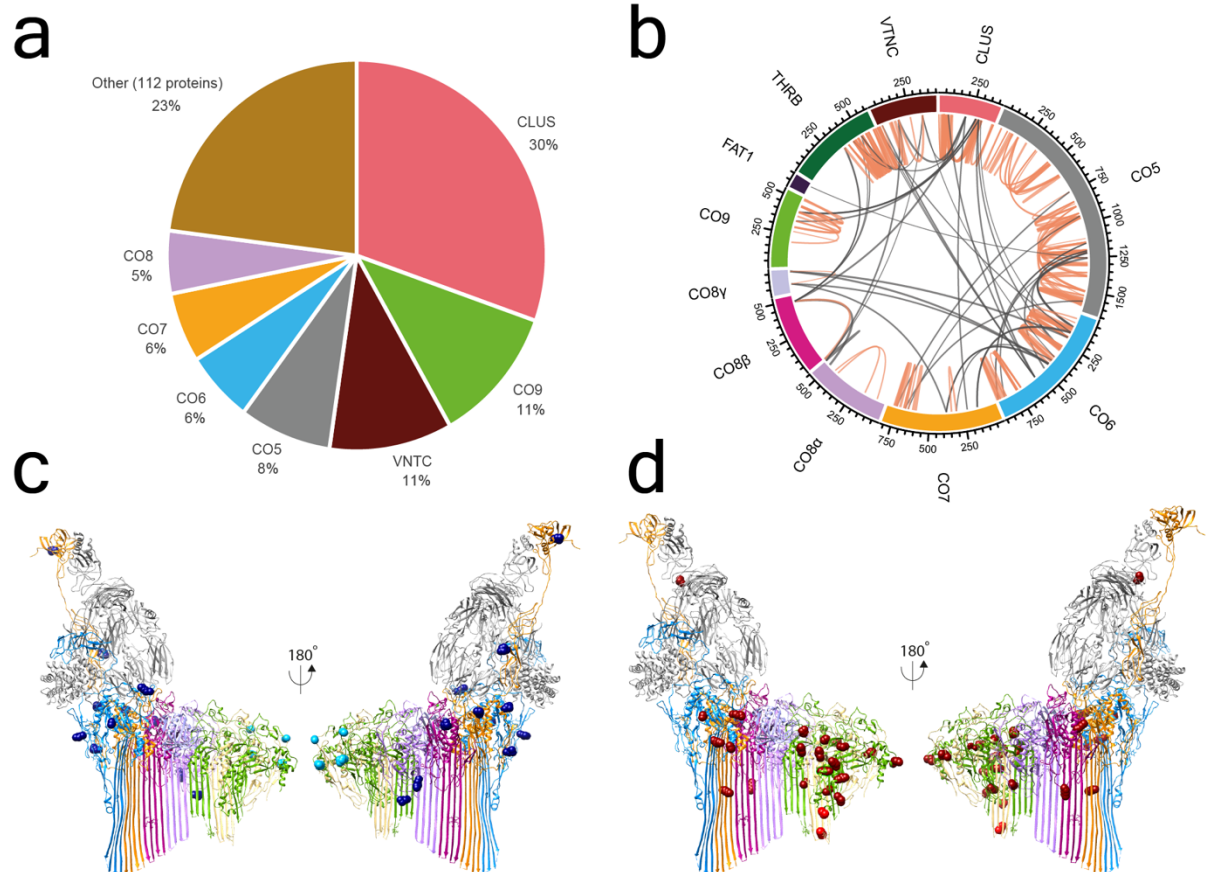
74 curves for all deposited maps: 1C9-sMAC (EMD-12649), 2C9-sMAC (EMD-12651),

75 3C9-sMAC (EMD-12650), C5b-focused 2C9-sMAC (EMD-12648), C9-focused 2C9-

76 sMAC (EMD-12647), and C9-clusterin focused 3C9-sMAC (EMD-12646). (b) cryoEM

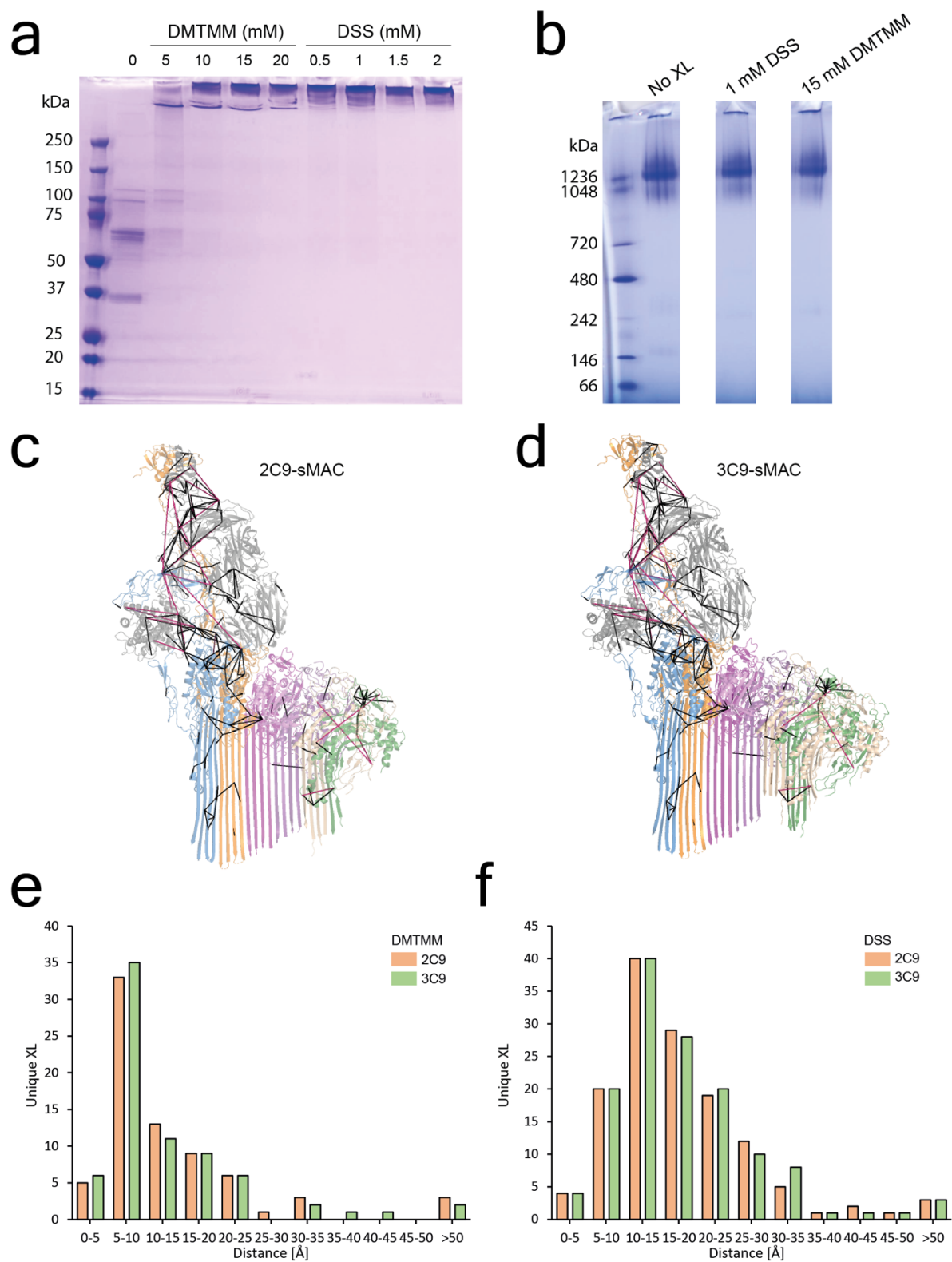
77 density maps (transparent surface) overlaid with atomic models for complement

proteins (colored according to protein composition). Representative densities for modeled glycans are shown in the bottom panel.

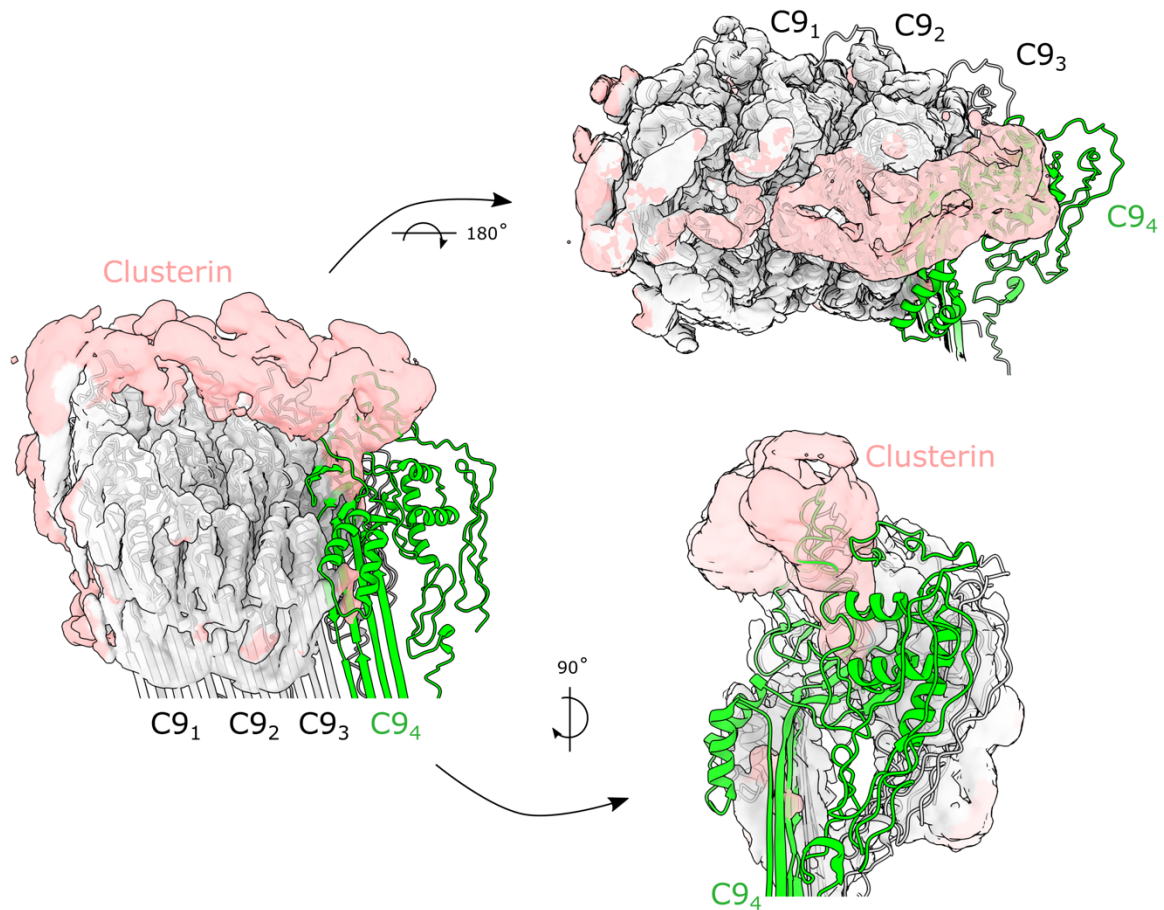


Supplementary Figure 3: Chaperone interactions of sMAC. (a) Label-free quantification (iBAQ) of proteins in the sMAC sample by LC-MS/MS. (b) sMAC circos plot of identified disuccinimidyl sulfoxide (DSS) cross-links. The complement components are cross-linked to thrombin (THRB), cadherin 8 domain of protocadherin FAT1 (FAT1) and the chaperones vitronectin (VTNC) and clusterin (CLUS). Intra-links are shown as orange lines and inter-links are shown as black lines. (c) Residues of sMAC uniquely cross-linked to vitronectin are shown as dark blue spheres plotted on the 3C9-sMAC model. Light blue spheres are not resolved in all three C9 molecules. (d) Residues of sMAC uniquely cross-linked to clusterin are shown as dark red

92 spheres plotted on the 3C9-sMAC model. Residues that are not resolved in all three
93 C9 molecules are colored light red. For panels c and d complement proteins are
94 colored as in Fig. 1: C5b (grey); C6 (blue); C7 (orange); C8 α (light purple); C8 β (dark
95 purple); C8 γ (lilac); C9 (alternating green and tan). For panels b-d source data are
96 provided as a Source Data file.



electrophoresis (SDS-PAGE) was used to determine optimal cross-linker concentration. sMAC (10 μ g) was cross-linked with 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) (5-20 mM) or disuccinimidyl sulfoxide (DSS) (0.5-2 mM) and quenched before running on the SDS-PAGE (b) Blue native-PAGE (BN-PAGE) of cross-linked sMAC (10 μ g) using optimal DSS (1 mM) or DMTMM (15 mM) concentrations. sMAC sample with no crosslinker present (no XL) is also indicated for reference. The gels in panels a-b were repeated twice with similar results. (c-d) Identified cross-links plotted on the sMAC structural models containing (c) two C9 or (d) three C9 molecules. Cross-links below the distance restraints (DSS <30Å and DMTMM < 20Å) are shown as black lines and distances above the restraints are shown as pink lines. Complement proteins are colored as in Fig. 1: C5b (grey); C6 (blue); C7 (orange); C8 α (light purple); C8 β (dark purple); C8 γ (lilac); C9 (alternating green and tan). (e-f) Histogram of C α -C α distances of (e) DMTMM and (f) DSS cross-links mapped on the 2C9- and 3C9-sMAC atomic models. Displayed cross-links were identified in all three replicates. For panels c-f source data are provided as a Source Data file.



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119 Supplementary Figure 5: Clusterin sterically blocks C9 oligomerization. An oligomer

120 comprised of four C9 molecules was extracted from the MAC structure (PDB ID: 6H04)

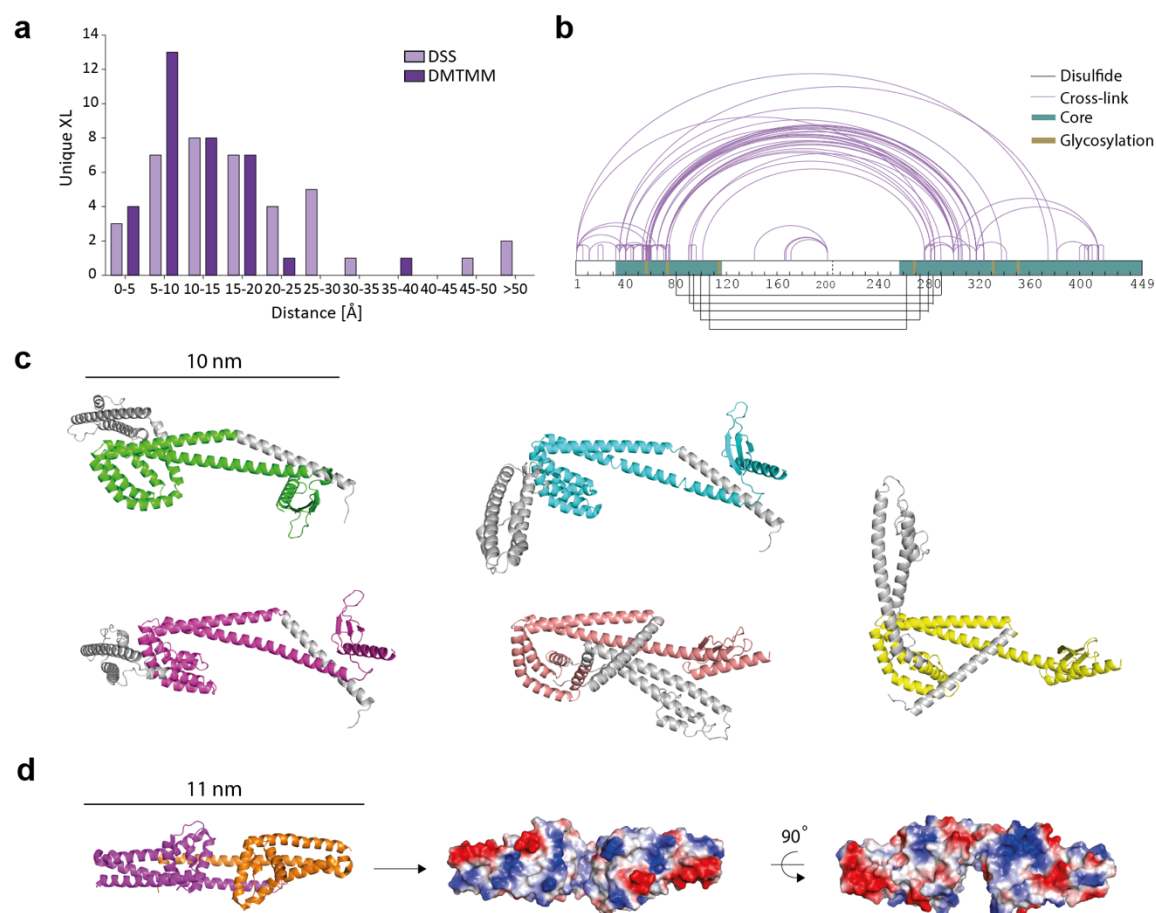
121 and fit into the EM density of the 3C9 focus refined map (transparent surface). While

122 the first three C9 molecules (C9₁, C9₂, C9₃: grey ribbons) fit the density occupied by

123 C9 in sMAC (grey transparent surface), the fourth C9 molecule (C9₄: green ribbons)

124 clashes with density occupied by clusterin in sMAC (pink transparent surface).

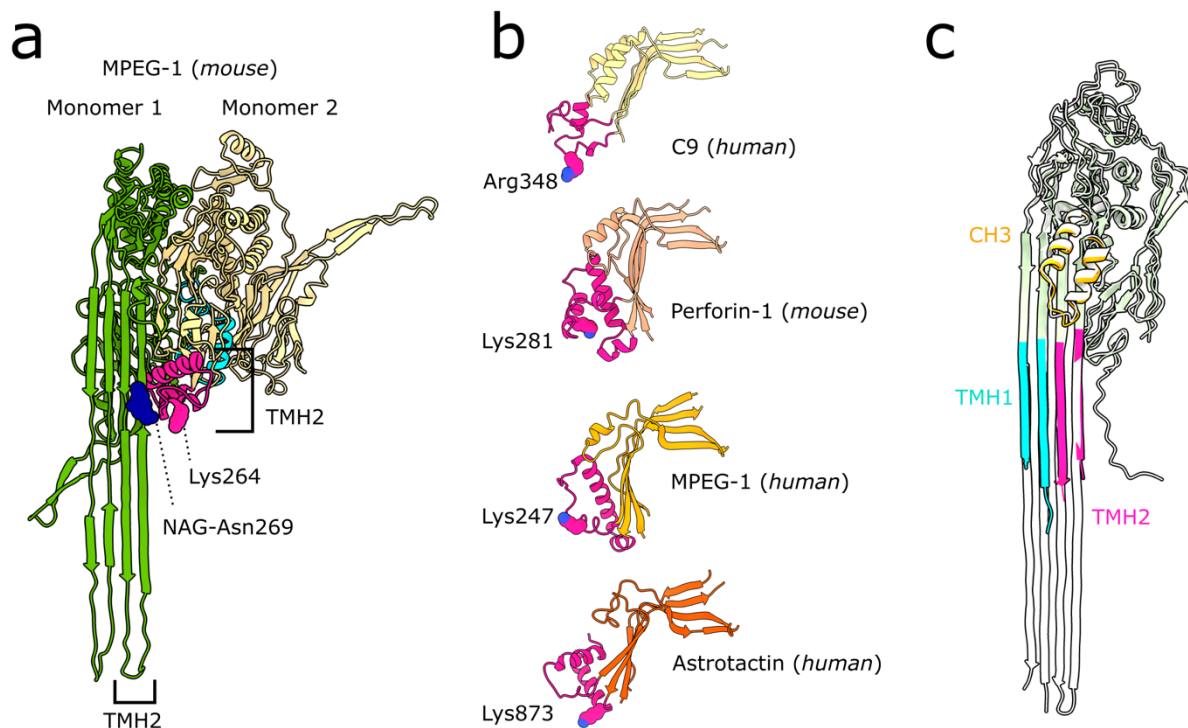
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128 Supplementary Figure 6: Structural model for clusterin. (a) Histogram of Ca-Ca
 129 distances of disuccinimidyl sulfoxide (DSS, light purple) and 4-(4,6-Dimethoxy-1,3,5-
 130 triazin-2-yl)-4-methylmorpholinium chloride (DMTMM, dark purple) cross-links plotted
 131 on the clusterin core model. (b) Identified clusterin inter-links are mapped onto the
 132 clusterin sequence shown with purple lines. The five disulfide bridges are shown as
 133 black lines. The common core is colored teal and reported glycosylation sites are
 134 marked in sand. For panels a-b source data are provided as a Source Data file. (c)
 135 Panel of structural models generated by trRosetta. The consensus core region is
 136 uniquely colored by model, while long helical extensions that flexibly hinge from the
 137 core are grey. (d) Structure of dimeric CspA from *Borrelia burgdoferi* (PDB ID: 1W33)
 138 shown as ribbons where each monomer is individually colored (purple and orange,

respectively) (left panel). Middle and right panels show the Coulombic electrostatic potential of the CspA dimer ranging from -64 (red) to 64 (blue) kcal/(mol·e).

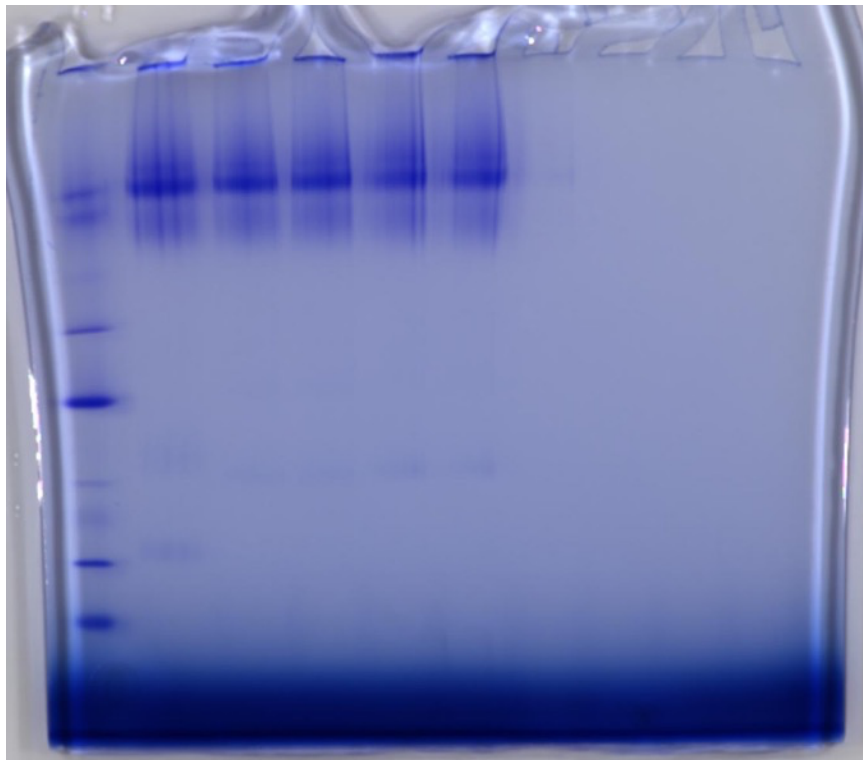


Supplementary Figure 7: MACPF structural transitions. (a) Model of a potential intermediate confirmation of macrophage expressed gene 1 (MPEG-1) generated by superposing a single monomer from a soluble conformation (PDB ID: 6SB3) onto the MPEG-1 pore structure (PDB ID: 6SB5). Two adjacent MPEG-1 monomers are shown. Monomer 1 (green) is from the transmembrane conformation. Monomer 2 (yellow) is the superposed soluble conformation. This superposition places a positively charged residue (Lys264, pink surface) within TMH2 of monomer 2 (pink) near a glycan (blue surface) on the extended β -hairpins of the preceding monomer (monomer 1, green). TMH1 of the soluble monomer (cyan) is shown for reference. (b) Central kinked β -sheet and TMH2 helical bundle of the MACPF domains of C9 (intermediate conformation in sMAC), perforin-1 (PDB ID: 3NSJ), MPEG-1 (PDB ID: 4OEJ) and astrotactin-2 (PDB ID: 5J68). In each case, a similarly oriented positively charged

154 residue with TMH2 is highlighted (spheres). TMH2s are indicated in pink; TMH1s are
155 removed for clarity. (c) Superposition of the transmembrane conformation of C9 as
156 seen in MAC (white, PDB ID: 6H03) with the penultimate C9 in 2C9-sMAC (colored
157 ribbons: CH3 is yellow; TMH1 is cyan; TMH2 is pink; the remainder of C9 is green).

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159 SOURCE DATA

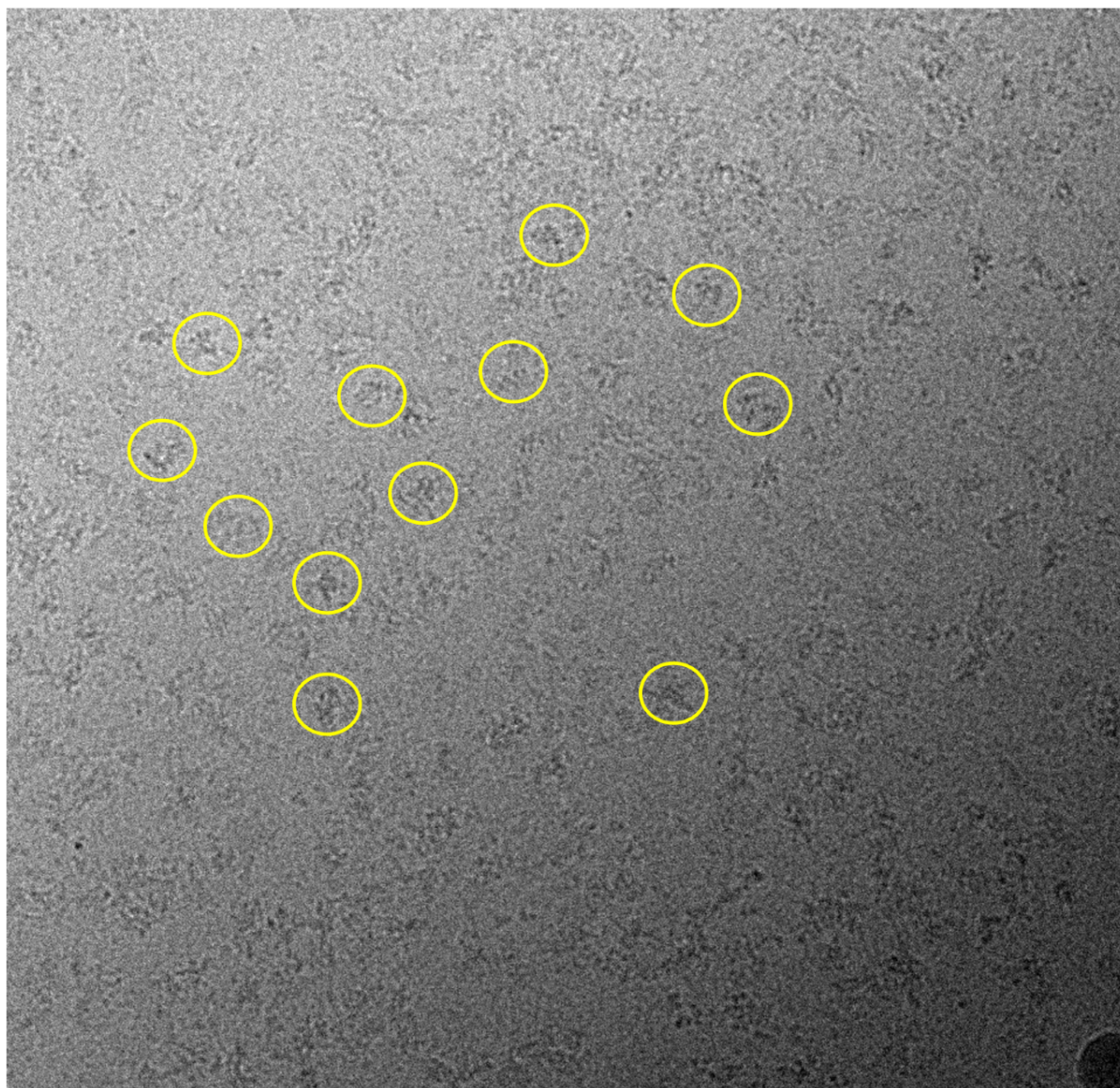
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163 Supplementary Figure 4b: Lane 1 is the marker, lane 2 is the sMAC sample with no
164 crosslinker added, lane 3 is the sMAC sample with the addition of 0.5 mM DSS, lane
165 4 is the sMAC sample with the addition of 1 mM DSS, lane 5 is the sMAC sample
166 with the addition of 10 mM DMTMM, lane 6 is the sMAC sample with the addition of
167 15 mM DMTMM.



168

169 Supplementary Figure 2: Representative raw electron micrograph from the data set
170 underlying the cryo EM maps in Supplementary Figure 2. Representative individual
171 particles are circled.

172