"Structure of Complement C6 suggests a mechanism for initiation and unidirectional, sequential assembly of the Membrane Attack Complex (MAC)"

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SUPPLEMENTAL FIGURES 1-10

Supplemental Fig. 1. Stereo views of C6 and selected Electron Density. *A,* Fragment of the C6 model fitted into the 2Fo-Fc electron density map, drawn at the 2.0-σ level. The tryptophans are glycosylated at C1 atom with α-mannose. *B,* The C6 molecule is shown as a Cα tube with the thickness and the color indicating the B-factors of Cα atoms (dark blue ~60 Å^2 and red ~200 Å^2). MACPF of a crystallographically related molecule (marked with *) is also shown as a gray ribbon. It may mimic C5b in the C5b-C6 complex, in separating the CCP modules and FIMs from the body of the MACPF.

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                                    TS1
C6   1  CFCDHYAWTQWTSCSKTCNSGTQSRHRQIVVDKYYQENFCEQICSKQETRECNWQRCPIN  60
C7   1                                                         SSPVN   5
C8β  1                                                    SVDVTLMPID   10
C8α  1                                                      AATPAAVT    8
C9   1                                            QYTTSYDPELTESSGSASHID  21

                                    TS2
C6  61  CLLGDFGPWSDCDPCIEKQSKVRSVLRPSQFGGQPCTAPLVAFQPCIPSKLCKIEEADCK  120
C7   6  CQWDFYAPWSECNGCTKTQTRRRSVAVYGQYGGQPCVGNAFETQSCEPTRGC-PTEEGCG   64
C8β 11  CELSSWSSWTTCDPCQKKRYRYAYLLQPSQFHGEPCNFSDKEVEDCVTNRPC-GSQVRC-   68
C8α  9  CQLSNWSEWTDCFPCQDKKYRHRSLLQPNKFGGTICSGDIWDQASCSSSTTC-VRQAQCG   67
C9  22  CRMSPWSEWSQCDPCLRQMFRSRSIEVFGQFNGKRCTDAVGDRRQCVPTEPCEDAEDDCG   81

                              LR                        MACPF
C6  121  NKFRC-DSGRCIARKLECNGENDCG-DNSDERDCGRTKAVCTR---KYNPIPSVQLMGNG  175
C7   65  ERFRC-FSGQCISKLVCNGDSDCDEDRCEDSERRPSCDIDK---PPPNIELTGNG       121
C8β  69  EGFVCAQTGRCVNRRLLCNGDNDCG-DQSDEANCRRIYKKCQHEMDQYWGI-GS--LASG  124
C8α  68  QDFQCKETGRCLKRHLVCNGDQDCL-DGSDEDDCEDV-RAIDEDCSQYEPIPGSQKAALG  125
C9   82  NDFQC-STGRCIKMRLRCNGDNDCG-DFSDEDDCESEPRPPCRDR-VVEESELARTAGYG  138

                                   MACPF
C6  176  FHFLAGEPRGEVLDNSFTGGICKTVKSSRTS-----------------NPYRVPANLENV  218
C7  122  YNELTGQFRNRVINTKSFGGQCRKVFSGDGK-----------------DFYRLSGNVLSY  164
C8β 125  INLFTNSFEGPVLDHRYYAGGCSPHYILNTR-----------------FRKPYNVESY   165
C8α 126  YNILTQEDAQSVYDASYYGGQCETVYNGEWRELRYDSTCERLYYGDDEKYFRKPYNFLKY  185
C9  139  INILGMDPLSTPFDNEFYNGLCNRDRDGNTL-----------------TYYRRPWNVASL  181

                                        CH1
C6  219  GFEVQTAEDDLKTDFYKDLTSLGHNENQQGSFSSQGGSSFSVP-----------------  264
C7  165  TFQVK-INNDFNYEFYNSTWSYVKHTSEHTSSSRKRSF-                      203
C8β 166  TPQTQ-GKYEFILKEYESYSDFERNVTEKMASKSGFSFGFKIP-                 207
C8α 186  HFEAL-ADTGISSEFYDNANDLLSKVKKDKSDFGVTIGIGPAG-                 228
C9  182  IYETK-GEKNFRTEHYEEQIEAFKSIIQEKTSNFNAAISLKFTPTETNKAEQCCEETASS  240

                  CH1
C6  265  --IFYSSKRSENINHNSAFKQAIQASHKKDSSFIRIHKVMKVLNF-TTKAKDLHLSDVFL  318
C7  204         FRSSSSSSRSYTSHTNEIHKGKSYQLLVVENTVEVAQFINNPEFLQLAEPFW  255
C8β 208  --GIFELGISSQSDRGKHYIRRTKRFSHTKSVFLHARSDLEVAHY-KLKPRSLMLHYEFL  264
C8α 229  --SPLLVGVSHSQDTSFLNELNKYNEKKFIFTRIFTKVQTAHF-KMRKDDIMLDEGML    285
C9  241  ISLHGKGSFRFSYSKNETYQLFLSYSSKKEKMFLHVKGEIHLGRF-VMRNRDVVLTTTFV  299

                                     MACPF                            CH2
C6  319  KALNHLPLEYNSALYSRIFDDFGTHYFTSGSLGGVYDLLYQFSSEELKNSGLTEEEAKHC  378
C7  256  KELSHLPSLYDYSAYRRLDQYGTHYLQSGSLGGEYRVLFYVDSEKLKQNDFNSVEEKKC   315
C8β 265  QRVKRLPLEYSYGEYRDLFRDFGTHYITEAVLGGIYEYTLVMNKEAMERGDYTLNNVHAC  324
C8α 286  QSLMELPDQYNYGMYAKFINDYGTHYITSGSMGGIYEYILVIDKAKMESLGITSRDITTC  345
C9  300  DDIKALPTTYEKGEYFAFLETYGTHYSSSGSLGGLYELIYVLDKASMKRKGVELKDIKRC  359

                        CH2                                   CH3
C6  379  VRIETKKRVL----FAKKTKVEHRCTTNKLSEKHEGSFIQAEKSISLIRGGRSEYGAAL   434
C7  316  KSSGWHF---------VVKSSHGCKELENALKAASGTQNNVLRGEPFIRGGGAGFISGL   365
C8β 325  AKNDFKIGGAIEEVYVSLGVSVGKCRGILNEIKDRNKRDTMVEDLVVLVRGGASEHITTL  384
C8α 346  FGGSLGIQYE-DKINVGGGLSGDHCKKFGGGKTERARKAMAVEDIISRVRGGSSGWSGGL  404
C9  360  LGYHLDVSLAFSEISVGAEFNKDDCVKRGEGRAVNITSENLIDDVVSLIRGGTRKYAFEL  419
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3
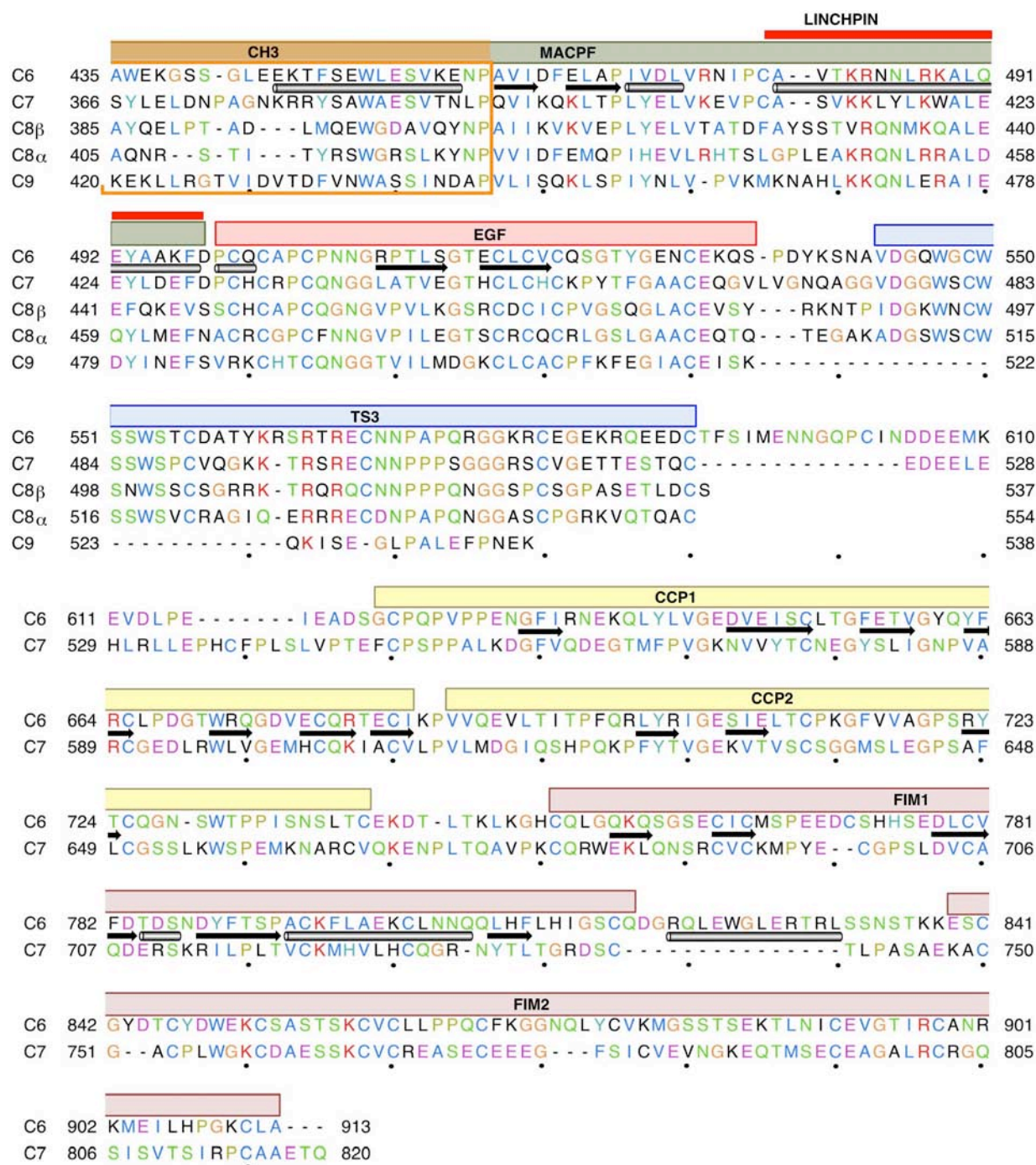
Supplemental Fig. 2. Multiple sequence alignment of MAC components C6-C9 (2 pages). Arrows (β-sheet) and cylinders (α-helix) indicate the secondary structure elements of C6. Colored bars above sequences indicate the C6 domains/modules. Aligned residues are colored according to the ClustalX scheme. In CH1 and CH2, the hydrophobic residues of the putative transmembrane hairpins are highlighted by pink backgrounds, and the regions predicted to lie on the periplasmic side are highlighted by cyan backgrounds. Ca^{2+}-coordinating residues in the LR domain are marked with asterisks.
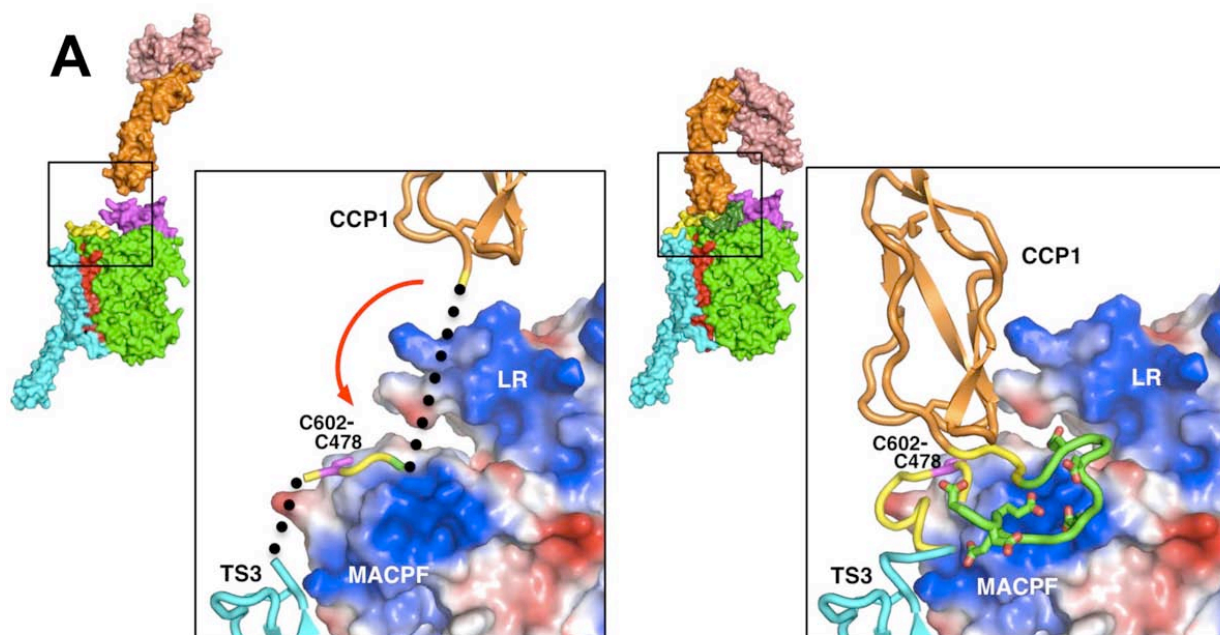
CH3 / MACPF / LINCHPIN

```
C6  435 AWEKGSS-GLEEKTFSEWLESVKENPAVIDFELAPIVDLVRNIPCA--VTKRNNLRKALQ 491
C7  366 SYLELDNPAGNKRRYSAWAESVTNLPQVIKQKLTPLYELVKEVPCA--SVKKLYLKWALE 423
C8β 385 AYQELPT-AD---LMQEWGDAVQYNPAIIKVKVEPLYELVTATDFAYSSTVRQNMKQALE 440
C8α 405 AQNR--S-TI---TYRSWGRSLKYNPVVIDFEMQPIHEVLRHTSLGPLEAKRQNLRRALD 458
C9  420 KEKLLRGTVIDVTDFVNWASSINDAPVLISQKLSPIYNLV-PVKMKNAHLKKQNLERAIE 478
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EGF

```
C6  492 EYAAKFDPCQCAPCPNNGRPTLSGTECLCVCQSGTYGENCEKQS-PDYKSNAVDGQWGCW 550
C7  424 EYLDEFDPCHCRPCQNGGLATVEGTHCLCHCKPYTFGAACEQGVLVGNQAGGVDGGWSCW 483
C8β 441 EFQKEVSSCHCAPCQGNGVPVLKGSRCDCICPVGSQGLACEVSY---RKNTPIDGKWNCW 497
C8α 459 QYLMEFNACRCGPCFNNGVPILEGTSCRCQCRLGSLGAACEQTQ---TEGAKADGSWSCW 515
C9  479 DYINEFSVRKCHTCQNGGTVILMDGKCLCACPFKFEGIACEISK---------------- 522
```

TS3

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C6  551 SSWSTCDATYKRSRTRECNNPAPQRGGKRCEGEKRQEEDCTFSIMENNGQPCINDDEEMK 610
C7  484 SSWSPCVQGKK-TRSRECNNPPPSGGGRSCVGETTESTQC--------------EDEELE 528
C8β 498 SNWSSCSGRRK-TRQRQCNNPPPQNGGSPCSGPASETLDCS 537
C8α 516 SSWSVCRAGIQ-ERRRECDNPAPQNGGASCPGRKVQTQAC 554
C9  523 -----------QKISE-GLPALEFPNEK 538
```

CCP1

```
C6  611 EVDLPE-------IEADSGCPQPVPPENGFIRNEKQLYLVGEDVEISCLTGFETVGYQYF 663
C7  529 HLRLLEPHCFPLSLVPTEFCPSPPALKDGFVQDEGTMFPVGKNVVYTCNEGYSLIGNPVA 588
```

CCP2

```
C6  664 RCLPDGTWRQGDVECQRTECIKPVVQEVLTITPFQRLYRIGESIELTCPKGFVVAGPSRY 723
C7  589 RCGEDLRWLVGEMHCQKIACVLPVLMDGIQSHPQKPFYTVGEKVTVSCSGGMSLEGPSAF 648
```

FIM1

```
C6  724 TCQGN-SWTPPISNSLTCEKDT-LTKLKGHCQLGQKQSGSECICMSPEEDCSHHSEDLCV 781
C7  649 LCGSSLKWSPEMKNARCVQKENPLTQAVPKCQRWEKLQNSRCVCKMPYE--CGPSLDVCA 706
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```
C6  782 FDTDSNDYFTSPACKFLAEKCLNNQQLHFLHIGSCQDGRQLEWGLERTRLSSNSTKKESC 841
C7  707 QDERSKRILPLTVCKMHVLHCQGR-NYTLTGRDSC---------------TLPASAEKAC 750
```

FIM2

```
C6  842 GYDTCYDWEKCSASTSKCVCLLPPQCFKGGNQLYCVKMGSSTSEKTLNICEVGTIRCANR 901
C7  751 G--ACPLWGKCDAESSKCVCREASECEEEG---FSICVEVNGKEQTMSECEAGALRCRGQ 805
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C6  902 KMEILHPGKCLA--- 913
C7  806 SISVTSIRPCAAETQ 820
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Supplemental Fig. 3. Comparison of TS modules from C6 (in blue) and thrombospondin-1 (TSP-1) (in magenta). *A,* Despite differences in crystal contacts, pairs of TS modules from C6 and thrombospondin-1 (PDB entry 1LSL) have similar rod-like conformations, presumably due to the presence of conserved prolines (Pro58 and Pro472), disulfide bonds (Cys18-Cys57 and Cys433-Cys471), and O-glycosylation (Thr15 in C6) in their linkers. TS modules are often C1-mannosylated at the first two tryptophans of a WxxWxxW motif, as seen in TS3 of C6. Although TS1 of C6 has only 2 of the 3 Trps of the canonical linear motif, it is still mannosylated at two tryptophans, presumably because Trp54 occupies the physical position of the "missing" Trp14. This would suggest that mannosylation occurs after folding into the final 3-D structure. Note that while both TS modules of thrombospondin-1 contain the canonical motif, the protein was expressed in insect cells, which lack the mannosylation pathway. Sugars are shown as green/red sticks, disulfide bonds as yellow sticks. For clarity, sugars/disulfides are not shown on the superposed C6/TSP-1 pair. *B,* TS1 of C6 has an unusual helical conformation at its tip (shown in 3 boxes). Two inserts present hydrophobic patches (lower boxes show surface models colored by hydrophobicity, increasing from blue to orange), which may participate in membrane binding.
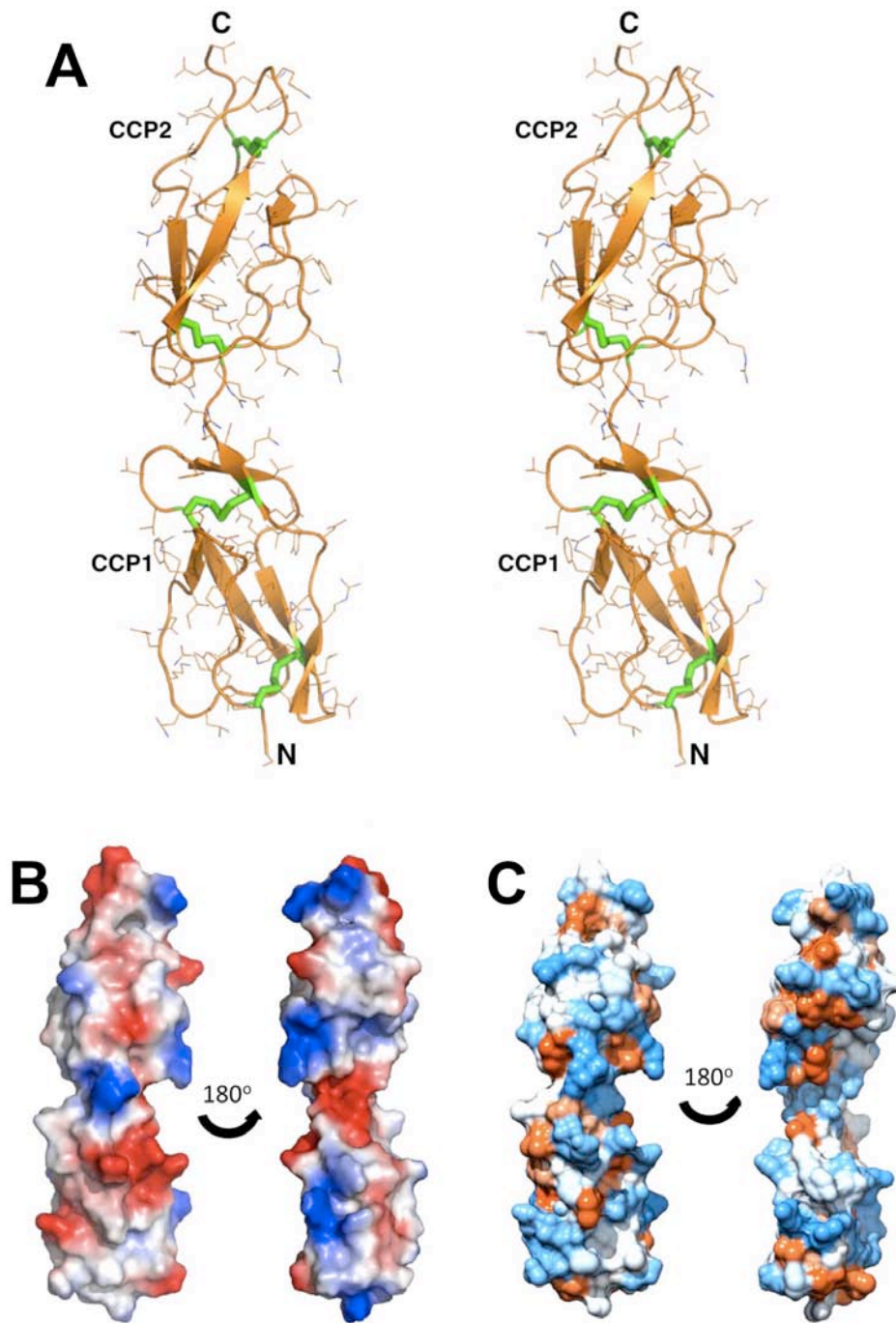
Supplemental Fig. 4. Stereo and surface presentations of the CCP module of C6. *A,* Stereo pair of the 2 domains comprising the CCP module, shown as secondary structure cartoon with side-chains (thin sticks) and disulfide bonds (thick green sticks). *B,* Solvent accessible surface colored according to electrostatic potential (positive is blue, negative is red). First view is same as in A; second is rotated by 180° about a vertical axis. Note the opposite/complementary charges. *C,* Surface hydrophobicity (increasing from blue to orange). Views are the same as in *B*.
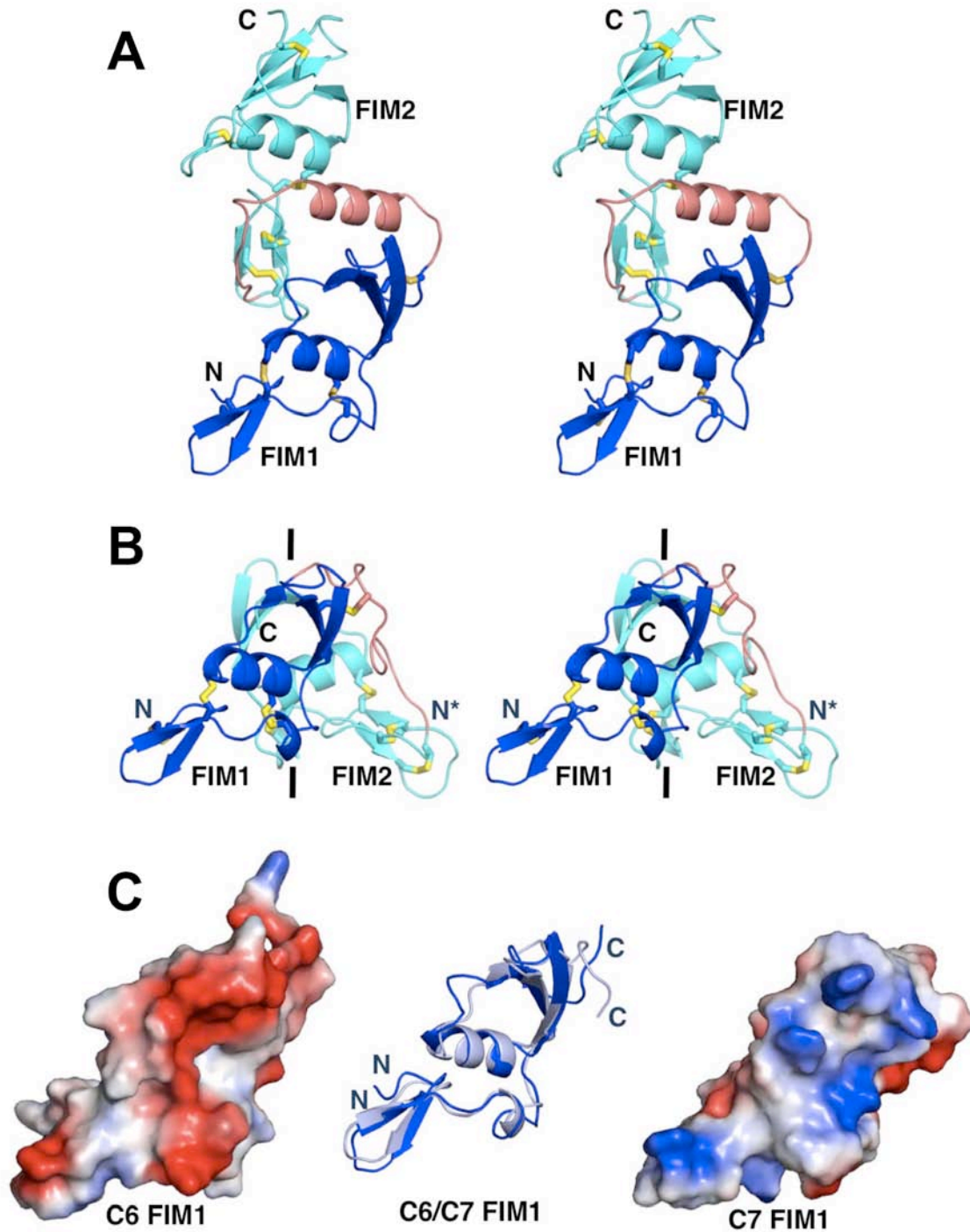
Supplemental Fig. 5. Ribbon and surface presentations of FIM modules from C6 and C7. *A* and *B*, Stereo views of FIM modules from *A,* C6 and *B,* C7 (PDB entry 2WCY). FIM1 is blue, the linker is brown, and FIM2 is cyan. All views have FIM1 in the same orientation. The dimer of C7 FIMs has 2-fold pseudo-symmetry (axis indicated by black bars). *C,* Superposition of FIM1 from C6 and C7 (at center) illustrates close structural homology (RMS deviation is 1.0 Å for 40 of 60 Cα atoms). Nevertheless, their putative C5b-binding faces (shown as electrostatic surface potentials (red/blue are negative/positive)) have distinct shapes and opposite charges.

Supplemental Fig. 6. Modeling the position of CCP/FIMs in native C6. ***A,*** On the left, CCP/FIMs are dissociated from each other and from MACPF as seen in the crystal structure of C6; on the right, CCP/FIMs and the TS3-CCP linker are modeled to attach to MACPF near a conserved disulfide bond, Cys478-Cys602. This position of CCP/FIMs corresponds to the electron microscopic images of C6 and C7. ***B,*** Sequence comparison of the TS3-CCP1 linkers in C6 and C7 from different vertebrates. The colors from pink to blue indicate hydrophobicity/charge, and their intensity indicates amino acid conservation (prepared with ClustalW). The yellow fragments of linkers (L1 and L3) in both C6 and C7 make a disulfide bond to MACPF, but the conserved cysteines (marked with a star) have different positions with respect to a conserved negatively charged fragment L2. This rearrangement of the linker fragments suggests that in native C6 and C7, their linkers should have compact folds that interact with similar fragments of MACPF. We propose that the negatively charged fragment L2 binds to the positively charged area of MACPF. In the C5b-C6 complex, L2 may bind to the positively charged surfaces of LR or C5b.

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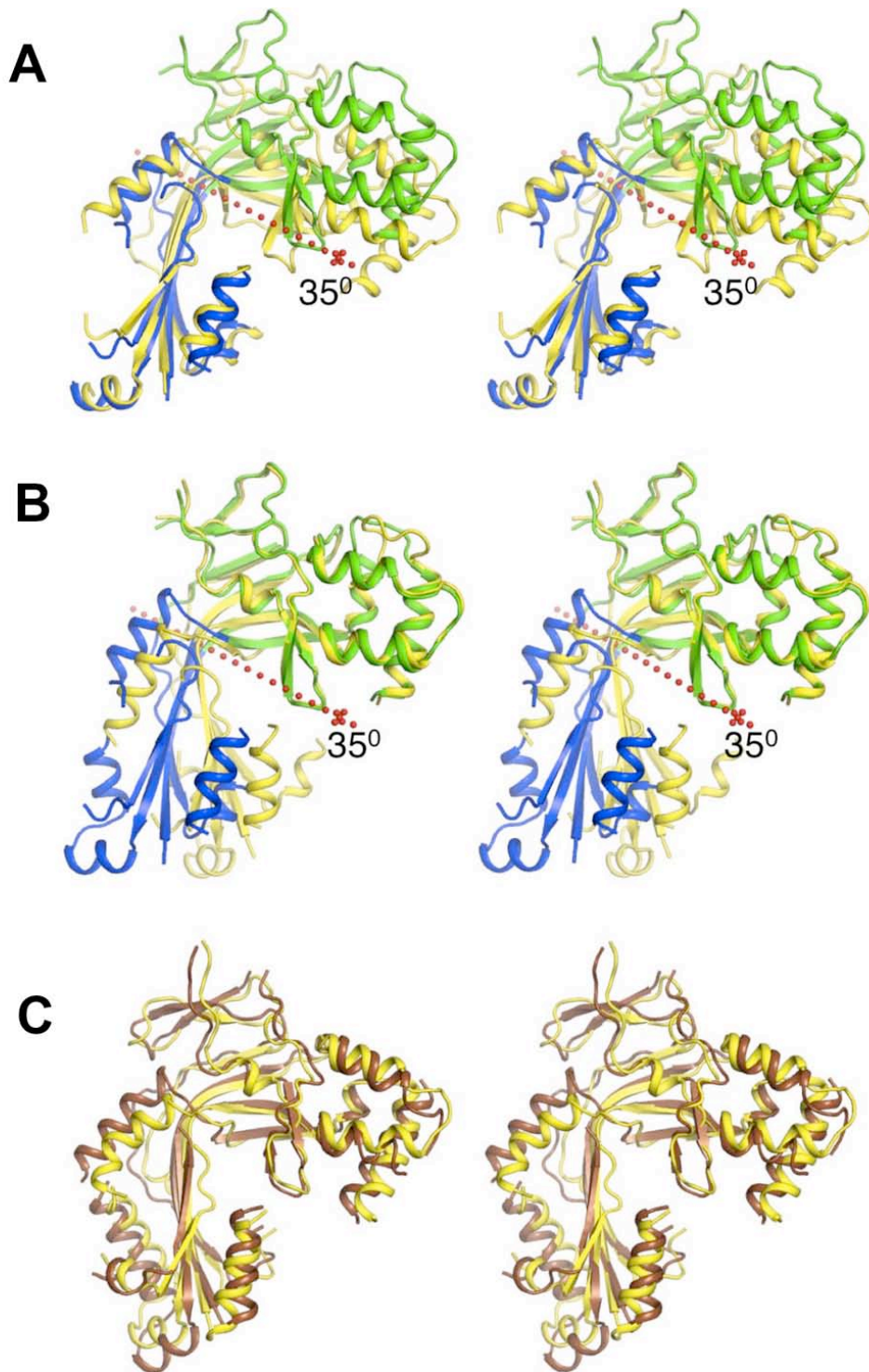
Supplemental Fig. 7. Stereo views of superposed MACPFs in different reference frames and overlay with perforin. *A* and *B*, MACPFs from C6 (yellow) and C8α (green/blue) (pdb entry3OJY) are superposed via either the upper or lower segments of MACPF. The red dotted line illustrates the apparent rotation axis. Rigid body motions were identified using the program DynDom (see Experimental Procedures). *C*, MACPFs from C6 (yellow) and perforin (brown) are superposed, showing close alignment of their β-sheets and flanking elements.
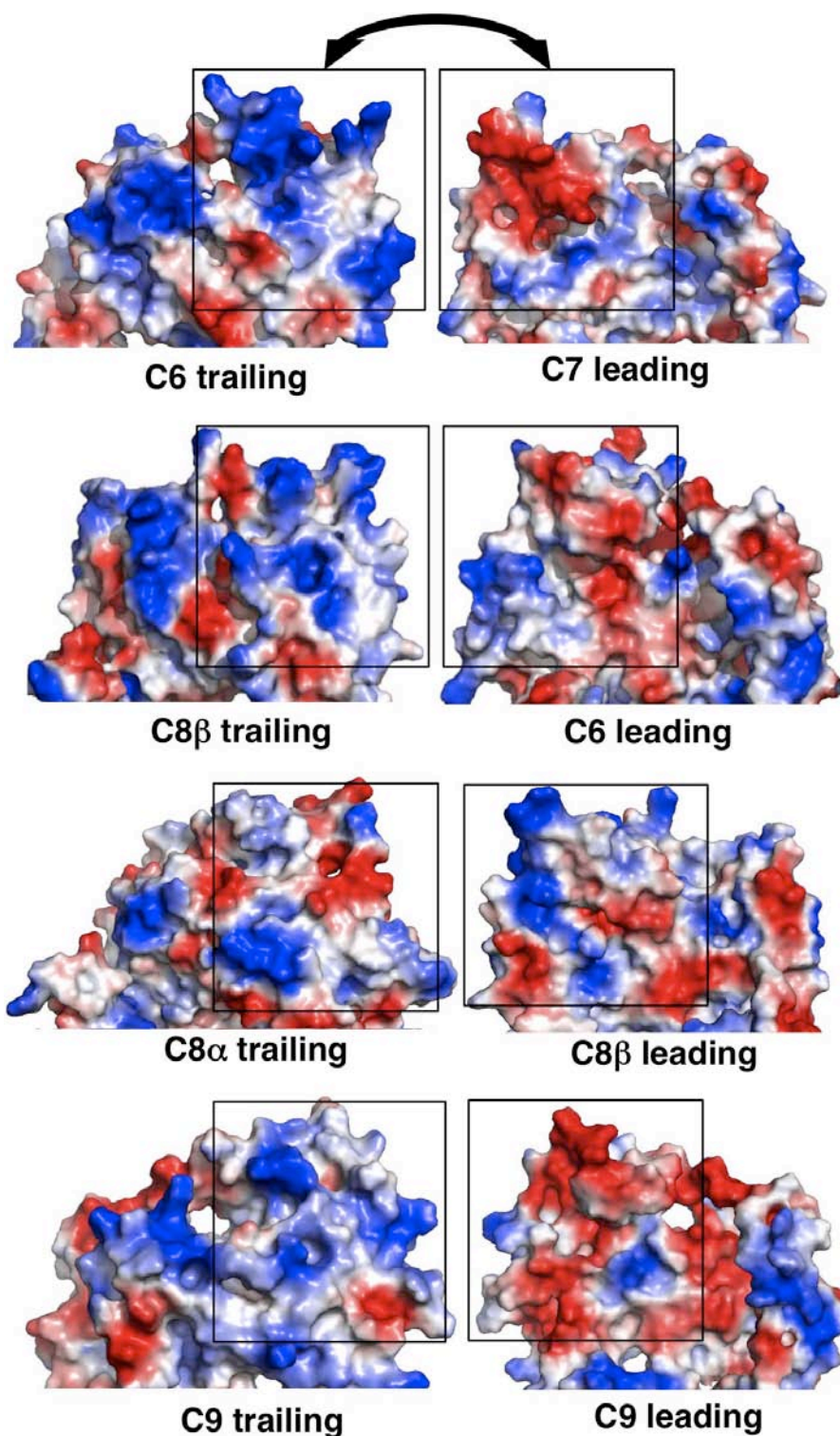
Supplemental Fig. 8. Complementary charges on putative interacting surfaces of MAC components. The black boxes indicate structurally conserved areas in MACPF and LR (the sides of the "Wedge") that form the real (C8α-C8β) or putative interfaces in the mature MAC (surfaces colored according to charges - red/blue = negative/positive). Note the complementary charges in each case. C7 and C9 were modeled by threading their sequences on the C6 structure.

CH1

```
C6 Human    FYKDLTSLGHNENQQGSFSSQGGSSFSVPIFYSSKRSENINHNSAFKQAIQASHKKDSS
C6 Bovine   FYDDLIPLEDNKDQEALGSGLATSSFRVPIFYSSKRSQSSSHSSAFKQAIQASQKKASS
C6 Mice     FYKNLISFEKNKNEDSLSVDERTKFFPIPIFHFSEKNEHSHYSSAFNKVIKASHKKDSS
C6 Xenopus  LHKSLIPFSDENKRHGLSVGRWSGSSGIPWLWHTGTSTKSWASSSFREAITATRQKSSK
C7 Human    FYNSTWSYVKHTSTEHTSSSRKR----SFFR----SSSSSSRSYTSHTNEI-HKGKSYQ
C7 Bovine   FYNSTWAYVKETSTEHSSSSKGR----FLFF----SSSSSSHGYSSNTNIL-TKKKSYQ
C7 Mouse    FYNSSWSYIKHTSTEQNTFYSWK----GLF-----SHSRNTYGHGSAKEEIDTKMKSYK
C7 Xenopus  FYSSSWSYVKETESR---[.]--------------TTSNHGYYHYHKDTQSNTKEKNYQ
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CH2

```
C6 Human    QFSSEELKNSGLTEEEAKHCVRIETK-KRVLFAKKTKVEHRCTTNKLSEKHEGSFIQGAE
C6 Bovine   QFSKEELKNSGLTQEEAKNCIRIETK-KRFLFVKKTKVEHRCTTNKLSEKYEGSFMQGSE
C6 Mice     QFSRQELQNSGLTEEEAQNCVQYETKKLKFLYMEIHKEDTCTKNKLSEKYGGSFLQGSE
C6 Xenopus  QYSTENVQSSGLTDQEMAHCVFEEIRTRRFFFFVKTTHRTTCTTNKMTERYSGSFLQSSE
C7 Human    YVDSEKLKQNDFNSVEEKKCKS---SGWHFVVKFSS---HGCKELENALKAASGTQNNVL
C7 Bovine   HMDSEKVKKFDFHSEDKRKCAS---SHFQFLFTSSK---QKCTTMEEVLKSVSENEGNLL
C7 Mouse    YVDSGSAKETGFQSDQDNACSS---ADFQFLFTSSAD--QRCMKQLETEKSTSGNKGRLL
C7 Xenopus  YLDSEKITANGVTKRDMQSCTS---SSKNFFFVKYSS--KECKALNEVIRQSSGSTDREV
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Supplemental Fig. 9. Sequence alignment of CH1 and CH2 (putative membrane-inserting elements) from vertebrate orthologs of C6 and C7. The red boxes indicate hydrophobic patches at the predicted tips of β-hairpins that may attach the C5b-7 complex to the membrane. The CH2 hairpins of most C7s are 5 residues shorter than in C6. Note, however, that CH1 of Xenopus C7 is 20 residues shorter than its C6, and thus appears to lack a membrane-binding hairpin altogether. The shorter C7 is consistent with a model in which C7 is located at the trailing edge where growth of the immature MAC pore does not occur. The background colors correspond to the ClustalX scheme.
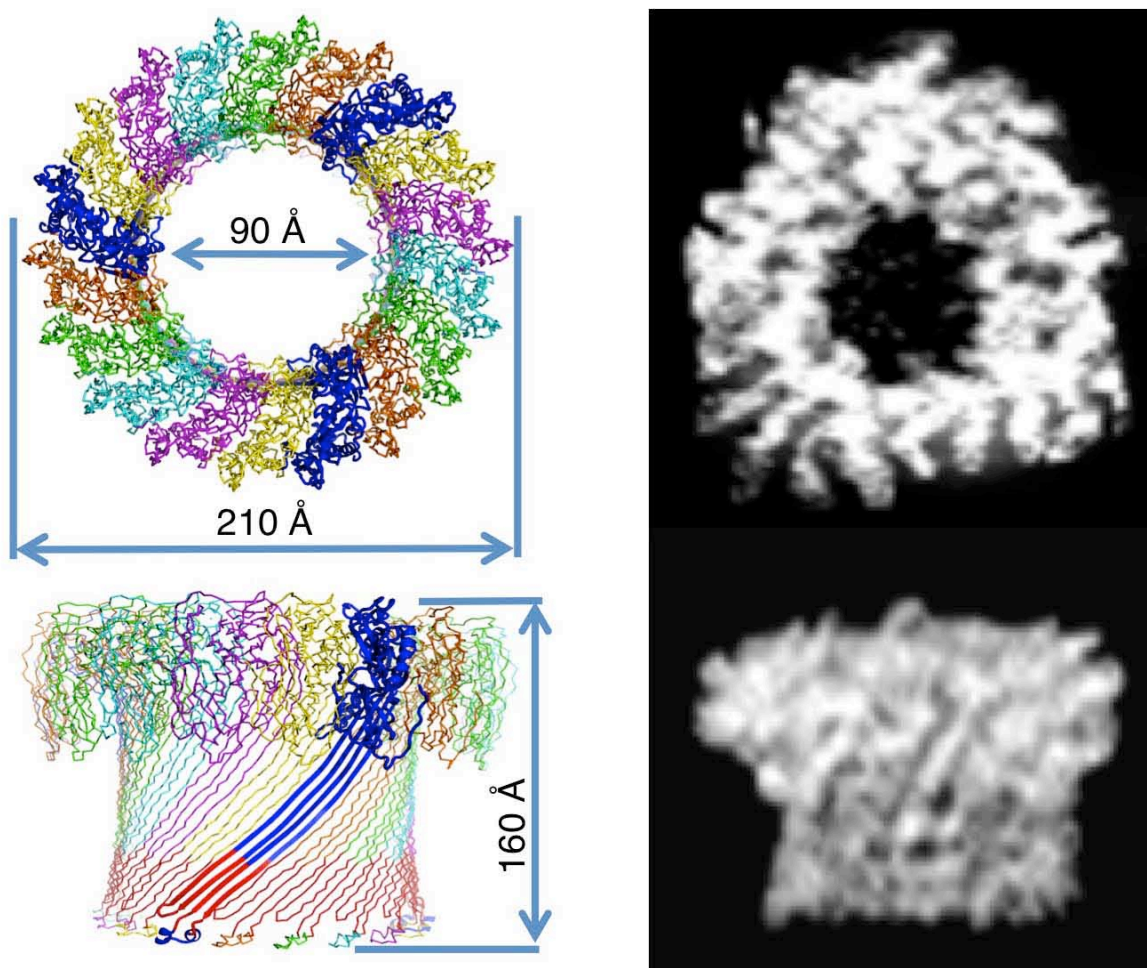
Supplemental Fig. 10. Atomic model of the poly-C9 pore containing 18 subunits The atomic model (at left) is compared with EM images of poly-C9 (at right). The protrusions evident in the upper micrograph may be TS2 domains. Note the tilt of the β-hairpins evident in the barrel (lower micrograph)