

REPLY TO MORTENSEN ET AL.:

# The zymogen form of complement component C1

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In their letter, Mortensen et al. (1) query our model of zymogen C1. It was assembled from overlapping crystal structures with constraints imposed by known interactions (2). The starting point was the protease subcomponent, C1<sub>r2</sub>C1<sub>s2</sub>, which comprises two antiparallel C1r-C1s dimers (mediated via CUB1-EGF-CUB2 contacts) linked through a central interaction between the CCP1-CCP2-SP domains of C1r. During C1 assembly, C1<sub>r2</sub>C1<sub>s2</sub> folds-up, so the CUB1-EGF-CUB2 domains bind to the collagenous stems of C1q. We propose that C1r-C1r interactions are maintained in zymogen C1, preventing one C1r polypeptide from activating its partner. Activation is driven by separation of C1r arms when C1q binds to a surface. Our model is compatible with solution, structural, and kinetic data, suggesting intracomplex activation (3), and incorporates all known interactions: C1r CCP1-CCP2-SP dimers (4, 5), C1r/C1s CUB1-EGF-CUB2 dimers (2), and CUB-C1q contacts (6, 7).

We used rigid-body modeling of small-angle X-ray scattering (SAXS) data (8) to test the feasibility of our model. Flexibility was incorporated using two or three residue linkers, and constraints were used to fix known interactions with twofold symmetry and 100 iterations. We did not impose the stacked-tetramer, because our solution/structural data show that the CUB-EGF-CUB2 domains form heterodimers (2). We fixed the C1r-C1r contacts (using PDB ID code 1GPZ), because there is sound biophysical evidence for these interactions (4, 5).

Our model is compatible with SAXS data (2). We did not, however, use rigid-body modeling to make additional predictions about C1, because there were too many unknown parameters to have confidence in this approach (e.g., degree of flexibility at domain

boundaries, the relative positions of the CUB1-EGF-CUB2 heterodimers and of the collagenous stems at the base of C1q, and flexibility of CUB-C1q interactions). In preliminary experiments, different starting configurations/constraints yielded (often radically) different outputs, many of which appeared implausible, but all with good  $\chi^2$  values. We did not deposit SAXS-derived coordinates because the approach was insufficiently sensitive to draw firm conclusions, given the limited constraints available. We can provide these coordinates upon request.

Mortensen et al. (1) refer to their earlier analysis of C1 (8). However, this study highlights the dangers of modeling to SAXS data, with insufficient constraints. The authors proposed the stacked-tetramer arrangement for unbound C1<sub>r2</sub>C1<sub>s2</sub> based on excellent fits, despite it being incompatible with preexisting biophysical/structural data, as criticized in Arlaud et al. (9), and acknowledged in Mortensen et al. (10). However, despite being incorrect, this model was then used as a starting point for rigid-body modeling of the much larger C1 complex (8).

Mortensen et al. (8) claim  $\chi^2$  values of 2.4 in fits. However, the fit-data files deposited in the SAXS database (SASDB38) state  $\chi^2$  values of 13.30 and 11.44. Moreover, the coordinate files themselves (in SASDB38) and described in Mortensen et al.'s letter (1) contain significant steric clashes, with considerable overlap between the EGF and CUB2 domains of C1r.

Our model (2) is consistent with available EM data (8). There are potentially nine protruding domains (six C1q heads, two C1s SP domains, and the base of C1q) not six (2). Additional domains in some images could easily reflect separation of the C1r CCP1-CCP2-SP domains, as would occur when C1 autoactivates.

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