Models of the complement C1 complex

Simon A. Mortensen[a], Bjørn Sander[b], Rasmus K. Jensen[c], Jan S. Pedersen[d,e], Monika M. Golas[a], Steffen Thiel[a], and Gregers R. Andersen[c,1]

Almitairi et al. (1) present structural information on the interaction between the proteases C1r and C1s, both consisting of six domains, called CUB1-EGF-CUB2-CCP1-CCP2-SP. The authors also propose a model for the C1 complex where the $C1r_2s_2$ tetramer is bound to C1q. Using our published and deposited small-angle X-ray scattering (SAXS) data (2) for the C1 complex, Almitairi et al. (1) conduct rigid body modeling and obtain models with a fit to the data (χ^2) in the range 2–4, of which only one model with $\chi^2 = 2.9$ is presented. In this model, the two C1r molecules interact in the center of the C1 complex through a known CCP1-SP contact (3). This model is fundamentally different from our model of the C1 complex based on rigid-body modeling against the same SAXS data and EM micrographs, where the serine protease domains of both C1r and C1s are located at the periphery of C1 (2). If the two C1r SP domains were located centrally in the C1 complex, intramolecular C1r activation would be feasible; in contrast, an exposure of the two C1r SP domains on two opposite sides of C1 favors an intermolecular activation mechanism.

The model of the C1 complex presented in Almitairi et al. (1) is consequently taken as evidence in favor of intramolecular activation of C1r. We find this misleading, as it is not mentioned that our model with the C1r SP domains located at the periphery fits our SAXS data with $\chi^2 = 2.4$. Moreover, the description of Almitairi et al.'s SAXS modeling is incomplete. They have not provided important methodological details, such as symmetry and distance restraints imposed on the C1 model, whether different refinements scenarios were evaluated, and whether the output model presented is representative of multiple refinements. It is also not described whether Almitairi et al. assume the stacked tetramer arrangement of the C1r and C1s CUB1-EGF-CUB2 domains in their modeling of the C1 complex, as previously done (4), or an alternative arrangement. Furthermore, Almitairi et al. (1) have not deposited their resulting model, despite taking advantage of deposited scattering data.

Almitairi et al. (1) do state that the C1r catalytic domains (presumably CCP1-CCP2-SP) were fixed as dimers, which inevitably locks these domains in the center of the molecule. We already comprehensively tested alternative starting models of the C1 complex, including models with the C1r SP domains either in the periphery or in the center of C1, similar to their model. The resulting models after rigid-body refinement always had the C1r SP domains at the periphery (2). In contrast, Almitairi et al. (1) give no information on whether they conducted rigid-body refinement without fixing the C1r catalytic domains in a central dimer. They furthermore claim that their results are in accordance with our low-resolution cryo-EM data and that our negative-stain EM micrographs display a nonnatural C1 conformation. However, the maximal six protruding domains in their model are in disagreement with our cryo-EM data. Moreover, there are no discrepancies between our negative-stain EM and cryo-EM class averages apart from technical differences originating from differences in defocus-induced phase-contrast transfer, as we already stated (2).

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[a]Department of Biomedicine, Aarhus University, DK8000 Aarhus, Denmark; [b]Department of Clinical Medicine, Center for Stochastic Geometry and Advanced Bioimaging, Aarhus University, DK8000 Aarhus, Denmark; [c]Department of Molecular Biology and Genetics, Aarhus University, DK8000 Aarhus, Denmark; [d]Department of Chemistry, Aarhus University, DK8000 Aarhus, Denmark; and [e]Interdisciplinary Nanoscience Center, Aarhus University, DK8000 Aarhus, Denmark

Author contributions: S.A.M., B.S., R.K.J., J.S.P., M.M.G., S.T., and G.R.A. wrote the paper.

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[1]To whom correspondence should be addressed. Email: gra@mbg.au.dk.

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