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Reply to Harwood et al.: Alternative functional conformations of native human α_2 -macroglobulin

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Harwood et al. (1) challenge our study of human α_2 -macroglobulin ($h\alpha_2M$) by cryogenic electron microscopy (2) based on differences with other α_2M -family members. This discrepancy would result from damaged protein caused by the usage of thawed frozen fresh plasma (TFP) for protein preparation. Instead, “homogeneously native” $h\alpha_2M$ would be purified from fresh, nonfrozen plasma (NP).

To address this issue, we compared $h\alpha_2M$ purified from TFP and NP. Briefly, we found that both preparations were equivalent in electrophoretic migration patterns, ultraviolet absorbance profiles, molar mass distributions, and free thiol groups. No differences in the inhibitory capacity against trypsin and thermolysin with three different substrates were detected. Thus, the preparations were equivalent, active, and functional, as already mentioned in our paper (2). This confirms reports from many groups which have been using frozen fresh plasma over decades (3–6). In particular, ref. 7 reckoned that frozen plasma may be used for native $h\alpha_2M$ preparation.

Recently, a comparative study with NP samples was published by Huang et al. (8), which shows that native preparations contain particles with multiple conformations (figure 2 of ref. 8). This is in agreement with very early studies (9). Thus, purified, native $h\alpha_2M$ from NP does not evince a unique structure as postulated by Harwood et al. (1) but rather a compendium of them, as we found from TFP. In ref. 2, we performed *in silico* separation and analysis of several subclasses of particles (see figures S3 and S9 and table S1 in ref. 2). Specifically, ~46,000 images were included in the native I set, which rules out that the protein was randomly damaged (tables S1 and S4 of ref. 2). In addition, the overall conformation of the fully native tetramer of ref. 8 is very similar to ours (compare figure 1B of ref. 8 with figures 2 and 3 of ref. 2). In particular, the MG7–CUB(TED)–RBD block adopts a similar overall conformation in both “extended” (2) and “expanded” (8) protomers, in which the thioester bond is protected by a hydrophobic circus and the nearby presence of RBD (see figure 4 E and F of ref. 2). There just seems to be a relative rearrangement of the RBDs.

As to native α_2M -family members, the mentioned proteins operate as monomers through completely different mechanisms, except frog ovostatin, which occurs in a physiologically different context from $h\alpha_2M$. In addition, α_2M -family members evince substantially different activated structures. Thus, consistent with disparate functions, there is no structural or functional homogeneity. However, we cannot exclude that the extended protomer of $h\alpha_2M$ may sample alternative conformations while maintaining a functional tetrameric structure. This is consistent with the large flexibility and conformational disparity found in the theoretically homogeneous native preparations. Note also that this variability (figure S2 of ref. 2) is reduced to a single activated tetramer conformation upon treatment with trypsin (figure S4 of ref. 2), which further underpins that our native preparations are functionally competent.

Overall, the claims that our structures be artifacts resulting from damaged samples are not substantiated. Our study (2) and that of Huang et al. (8) complementarily recapitulate the mechanism of function of $h\alpha_2M$.

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The authors declare no competing interest.

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