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Thermo-Cryo-Electron Microscopy of Macromolecular Complexes

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The forces that stabilize multi-protein complexes and drive their assembly operate on three levels: (i) the folding of individual subunits; (ii) their oligomerization into functional building-blocks; (iii) assembly of the building-blocks into final complete structures. There must be coordination between these respective levels in the hierarchy and coordination must be maintained through the conformational changes that many complexes undergo during their reaction cycles. We have been studying the assembly and maturation of bacteriophage HK97 capsid, considered as a tractable model system, whereby both the energetics (by differential scanning calorimetry) and the structure (by cryo-EM) are addressed. As it matures, the capsid passes through five distinct structural states [1]. When heated, several of these particles exhibit phase transitions [2,3] in which the protein shell remains intact but substantial changes in structure may be incurred [4].

To capture thermally excited conformations, we used a custom-built environmental chamber mounted over a Reichert KF80 cryo-station. In it, specimen temperature and humidity can be controlled at specified levels up to the moment of vitrification. Our first-generation instrument was described earlier [4]: the second-generation instrument (Fig. 1) covers a wider temperature range (up to 80^{o}C), has a more compact design, and improved insulation. Details are available on request. Vitrified specimens were observed in a Philips CM200-FEG and reconstructions were calculated as described [2-4].

HK97 Head I is a capsid that is mature in structural terms but lacks the covalent crosslinks of the mature wild-type capsid, Head II, on account of a point mutation in one of the cross-linking residues. When subjected to thermal scanning, it exhibits an endothermic transition centered on 60^{o}C, prior to the onset of thermal denaturation at 77^{o}C (Fig. 2a). Head I was incubated at 70^{o}C and then rapidly vitrified for cryo-EM observation. The capsids were found to have shrunk in size by >10% (cf. Figs. 2b & 2c) and to exhibit a markedly altered conformation that has not been observed before (cf. Figs. 2d & 2e). Ongoing research aims at leveraging the resolution by fitting crystal structures of the capsid protein in order to distinguish the respective contributions of rigid-body subunit movements and subunit refolding to the observed transition; and at exploring possible physiological implications.

References

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[5] We thank Mr G. Melvin for construction of our environmental chambers. This research was supported by NIAMS Intramural Research Program and NIH Grant 2R01GM047795 (to RH).

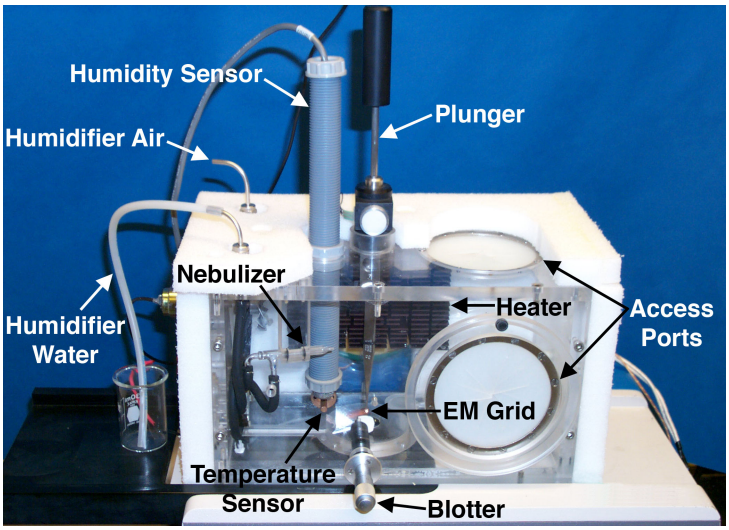

FIG.1: Environmental chamber mounted over freezing unit.

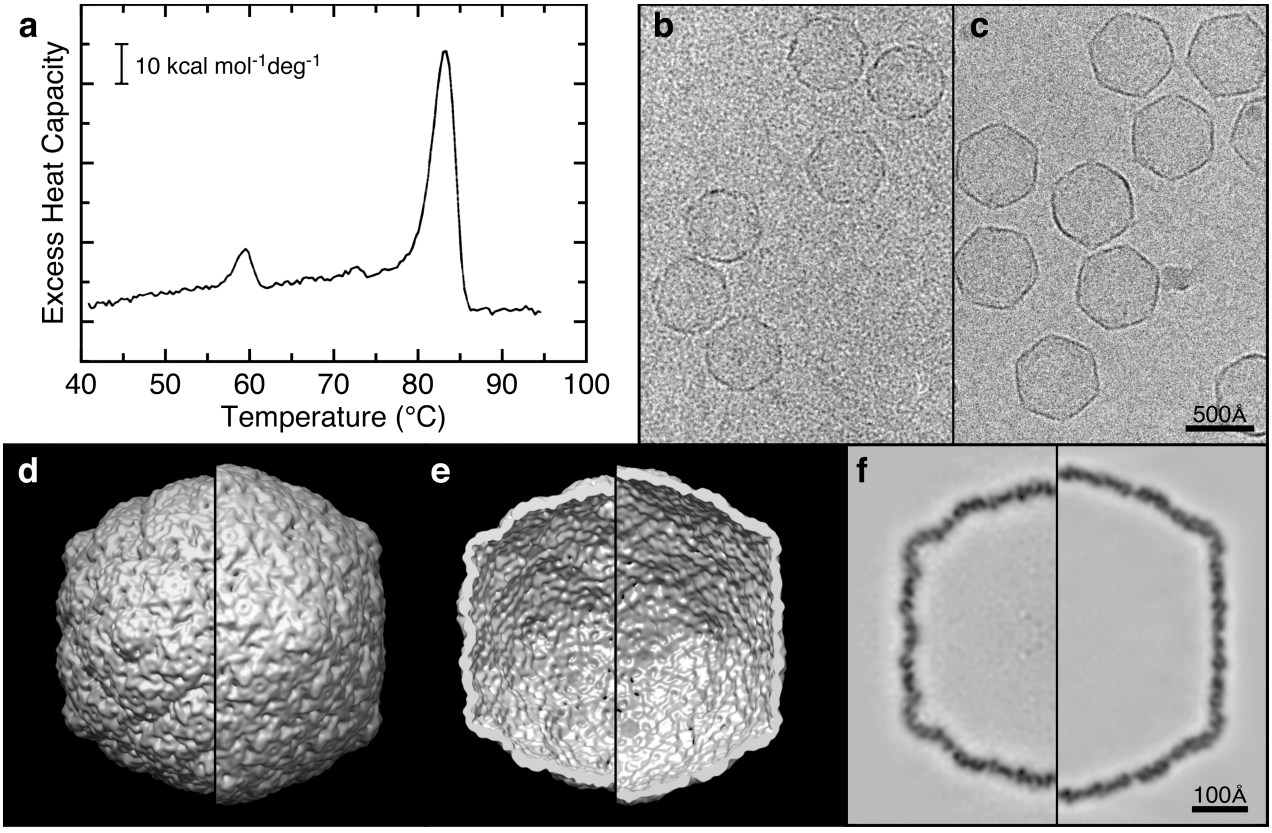

FIG. 2. (a) Calorimetric scan of purified Head I (from [2]). (b & c) Cryo-electron micrographs of Head I capsids after 10 min incubation at 70°C (b) and an unheated control (c). (d - f) Heated Head I (left side) and control (right side), reconstructed at ~ 14 Å resolution. (d) outer surfaces; (e) inner surfaces; (f) central sections The capsid, which observes T=7 icosahedral symmetry, i.e. it is composed of 60 hexamers and 12 pentamers, is viewed along a 2-fold axis.

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