Small heat shock proteins from *Caenorhaptidis elegans*: structural insights obtained by electron-microscopy

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Small heat shock proteins (sHsps), a widespread and diverse class of molecular chaperones, contribute *in vivo* to thermotolerance and maintain protein homeostasis by complexing a variety of nonnative proteins. sHsps lack active refolding properties. They rather act as molecular traps, preventing substrate aggregation efficiently by binding nonnative proteins in a coordinated manner. One of the most conspicuous features of sHsps is their organization in large oligomeric structures. For a few family members, the structures of the oligomeric complexes were determined either by X-ray crystallography or electron microscopy [1]. The presence of diverse sHsps in *C. elegans* enables to address a number of questions concerning their evolution: as many as 17 genes containing an α-crystallin-domain have yet been identified in *C. elegans*. In this work, we analysed in detail the three-dimensional structure of sHsp-oligomers 16.11, 16.2, 16.41, 16.48 and Sip-1 from *C. elegans* by transmission electron microscopy (TEM) with a view to compare their 3D-structures among each other and to the other known sHsp structures from different organisms. As determined by SEC-analyses, all oligomers have masses within a range of 400-500 kDa which would correspond to assemblies of 20-32 monomers, in accordance to the most known sHsp oligomeric sizes/structures.

For the phylogenetically closely related sHsp 16.11 and 16.2 oligomers, no detailed structural information could be extracted from TEM images, as the observed oligomers were heterogeneous and partially dissociated.

TEM images of two closely related *C. elegans* sHsps 16.41 and 16.48 revealed oligomers with well defined structures. Their three-dimensional reconstruction shows that both oligomers are similar, forming roughly spherical assemblies with diameters of about 12.5 nm. The protein shell contains openings at the positions of the 3- and 2-fold axes (Fig. 1). The structural analogy to other known sHsp structures from different organisms may indicate comparable interactions during chaperone activity. Examples for similar sHsp oligomers are the archaeal sHsp 16.5 from *M. jannaschii* and sHsp20.2 from *A. fulgidus* as well as eukaryotic sHsp 26 from *yeast* and human αB-crystallin [2,3,4,5]

Sip-1 is only expressed in the embryonal phase of *C. elegans* and is functionally different when compared to other sHsps from *C. elegans*. Furthermore it assembles in structurally completely different 3D-oligomers, as shown recently by cryo-TEM in our group. Sip-1 oligomers are double discs with a central cavity, a diameter of about 15 nm and a height of about 12 nm (Fig 2). The inherent 8-fold symmetry indicates a 32meric assembly. No analogous structure is known for any studied sHsp.

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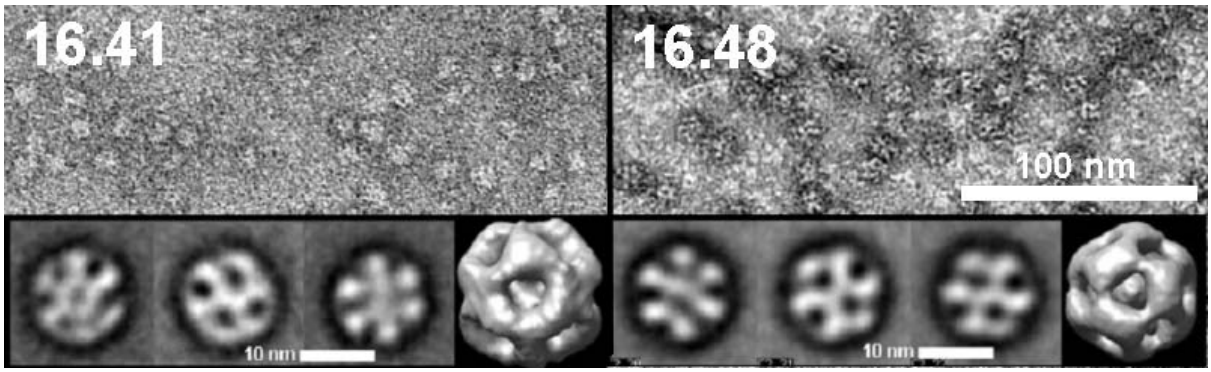

Figure 1: Negatively-stained sHsp oligomers from *C. elegans*. Top: overviews; bottom: 3 typical class averages and a surface presentation of the corresponding 3D-model. Left side: sHsp16.41 (ammonium molybdate). Right side: sHsp16.48 (uranyl formiate).

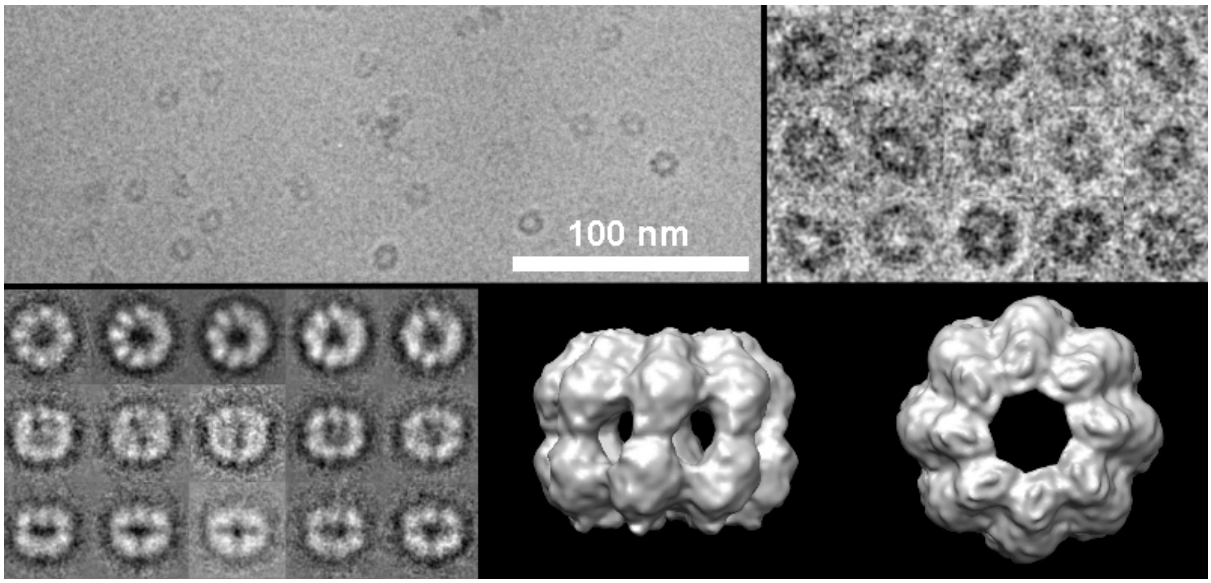

Figure 2: Top left: Cryo-EM images of sHsp Sip-1 from *C. elegans*. Top right: gallery of single Sip-1 images. Bottom left: representative class averages. Bottom right: side-view and top-view surface presentations of the corresponding 3D-model.

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