## Title

You gotta know how to fold 'em: Novel chaperonins are prevalent in the virioplankton and reveal the presence of marine archaeal viruses.

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## **Keywords**

Thermosomes; viral ecology; marine

Chaperonins are an essential class of proteins that utilize ATP to fold nascent polypeptides. The group I chaperonin GroES-EL system, occurs primarily in eubacteria, whereas group II chaperonins occur in archaea (thermosomes) and eukarvotes (TRiC/CCT). During infection, viruses may utilize host chaperonins (e.g., phages  $\lambda$ , T5) or their own chaperonins, such as the GroES paralog gp31 in phage T4; however, the occurrence of chaperonin genes within natural viral populations is unknown. Recognizing the potentially central role that chaperonins may play in the life cycle of viruses, we investigated the prevalence and diversity of these genes within the virioplankton. Group I chaperonin genes were identified within all 14 of the virome libraries investigated, comprising 0.1-0.6% of total reads per library. Surprisingly, thermosomes were also detected in all libraries, albeit at lower abundances. Viral thermosome genes were phylogenetically distinct from archaeal thermosomes and gene neighbor annotation suggests they may belong to a group of viruses infecting a marine euryarchaeal host. Similarly, most virioplankton GroEL genes were not closely related to bacterial chaperonins and formed three distinct clades that corresponded with chaperonin operon organization: a GroEL only clade, a GroEL-ES clade, and a GroES-EL clade. GroES sequences that occurred in GroEL-ES or ES-EL operons clustered separately from other viral GroES sequences, suggesting co-evolution of GroES and GroEL in viruses carrying both genes. Chaperonin genes are likely carried by both temperate and lytic viruses as determined from examination of polymerase A genes on contigs containing GroEL/ES genes. These data indicate that viral chaperonins are diverse and more abundant in marine environments than their occurrence in known viruses would suggest. The sequence conservation of GroEL and thermosome sequences makes them ideal for follow-up marker gene studies and may prove to be useful for characterizing the diversity of dsDNA phages with larger genome sizes.