Crystals
2022

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High resolution cryo-EM structure of the *Methanocaldococcus jannaschii* small-heat shock protein

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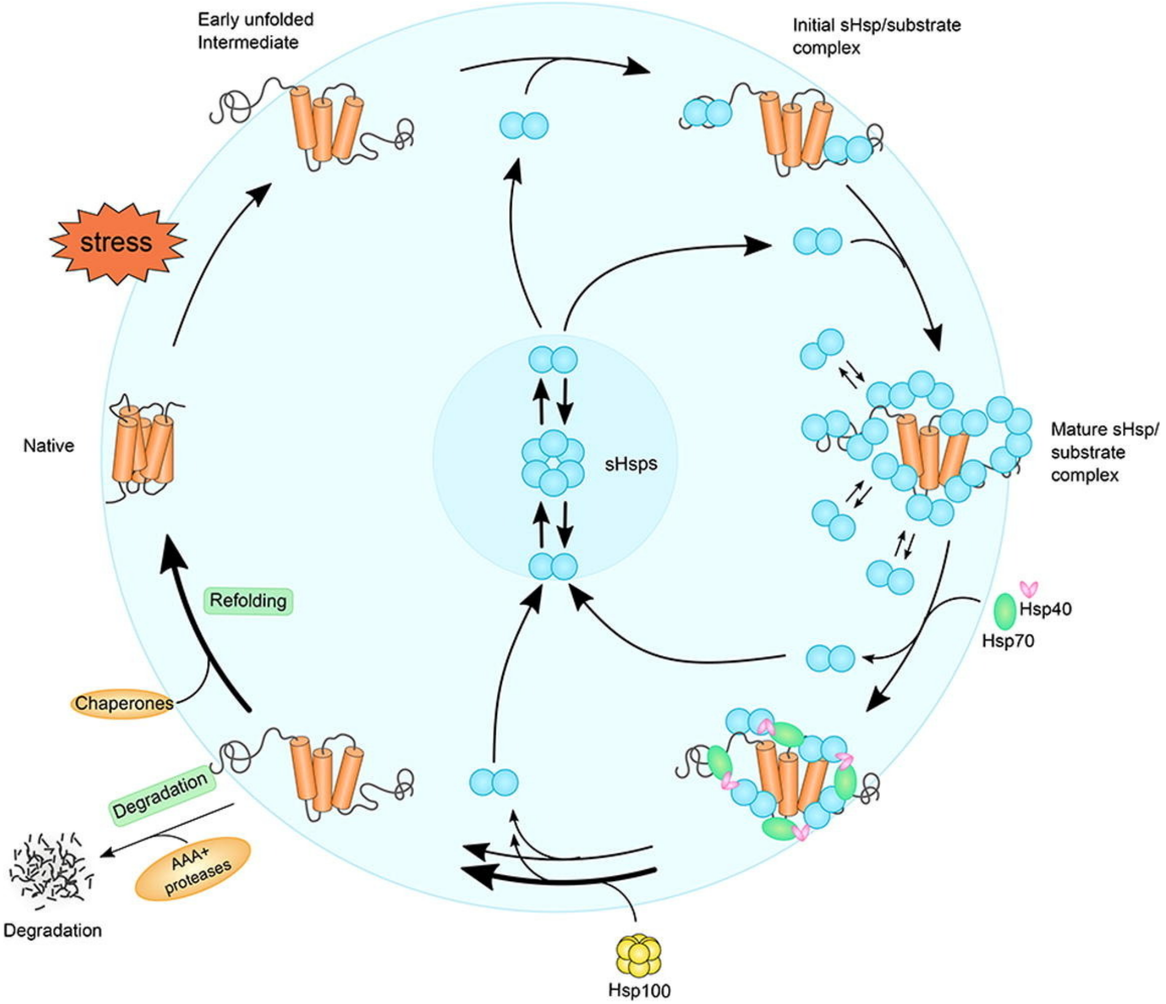
Methanocaldococcus jannaschii, a hyperthermophilic and barophilic methanarchaeon, contains a single gene (MJ0285) encoding a 16.5-kDa polypeptide chain of a small heat-shock protein (sHSP). This sHSP—now called MjsHSP16.5—is upregulated in response to high growth temperature or pressure and functions as an ATP-independent holding chaperone that transiently binds and prevents the misfolded proteins from aggregation. The molecular mechanism is remained to be elucidated, which primarily required a higher resolution of MjsHSP16.5 structure. In this study, we reconstructed the MjsHSP16.5 24-subunit oligomer to a 2.5-Å resolution using the single-particle cryo-electron microscopy (cryo-EM) technique. Despite a similar hollow spherical homo-oligomer, the MjsHSP16.5 cryo-EM structure is slightly bigger than its crystal structure and reveals a loosen subunit-subunit interactions. Furthermore, cryo-EM image reconstruction shows additional N-terminal residues which are absent in most of MjsHSP16.5 crystal structures. These residues likely involve the holding chaperone activity and the oligomer stabilization. Using dynamic light scattering (DLS) and negative-staining transmission electron microscopy (TEM), we observed that MjsHSP16.5 oligomer was shrunk upon heating, suggesting a large conformational change in MjsHSP16.5 at elevated temperature. To our knowledge, MjsHSP16.5 is the first sHSP to have the cryo-EM structure archiving a resolution at 2.5 Å.

Keywords: chaperon, small heat-shock protein, single-particle cryo-electron microcopy

Introduction

Function of small heat-shock proteins



Graphical abstract: Cycle of formation and dissociation of sHsp/substrate complexes

· Chaperones bind their client proteins in stable complexes, thereby inhibiting aggregation.

· Under stress conditions, sHsps bind partially unfolded proteins in ATP-independent manner.

· The substrates are refolded by ATP-dependent chaperone systems.

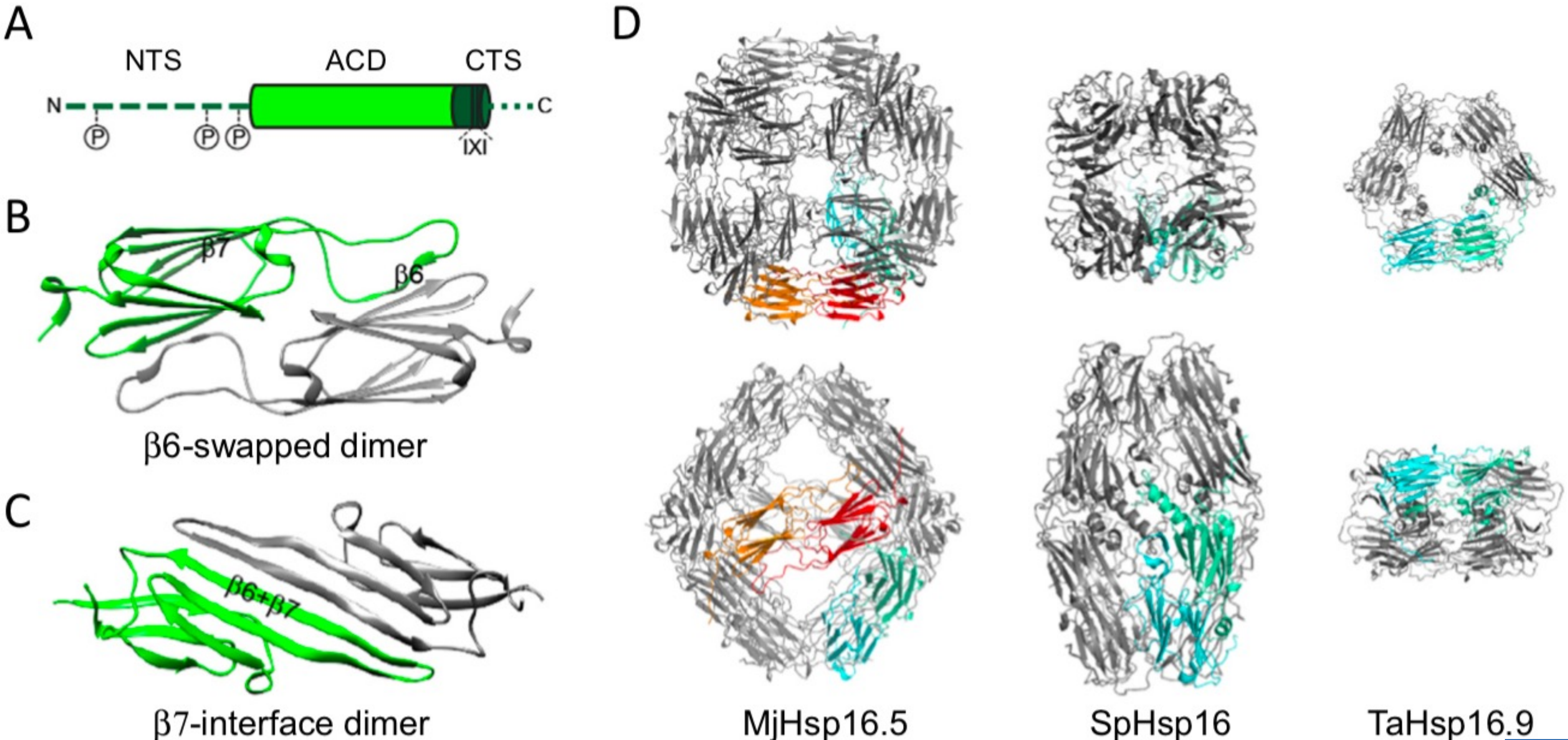
J. Mol. Biol. 427, 1537–1548 (2015)
J. Mol. Biol. 167157 (2021)

Introduction

Molecular Architecture of Small Heat Shock Proteins

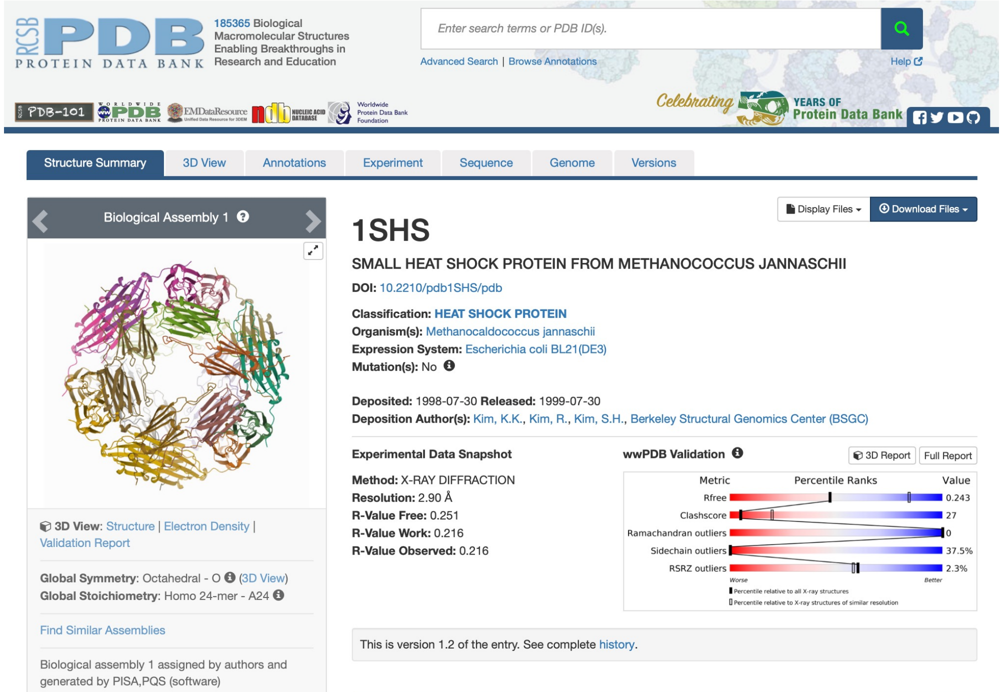
· sHSPs are built around a conserved α-crystallin domain.

- ACD forms a dimeric building block (**α-crystallin dimer**) and assembles into oligomers through interactions in sequence-variable N-terminal domain and C-terminal tail.



J. Mol. Biol. 427, 1537–1548 (2015)

Introduction
The first crystal structure of a small heat shock protein



1SHS

SMALL HEAT SHOCK PROTEIN FROM METHANOCOCCUS JANNASCHII

DOI: 10.2210/pdb1SHS/pdb

Classification: HEAT SHOCK PROTEIN
Organism(s): Methanocaldococcus jannaschii
Expression System: Escherichia coli BL21(DE3)
Mutation(s): No

Deposited: 1998-07-30 **Released:** 1999-07-30
Deposition Author(s): Kim, K.K., Kim, R., Kim, S.H., Berkeley Structural Genomics Center (BSGC)

Experimental Data Snapshot

Method: X-RAY DIFFRACTION
Resolution: 2.90 Å
R-Value Free: 0.251
R-Value Work: 0.216
R-Value Observed: 0.216

wwPDB Validation

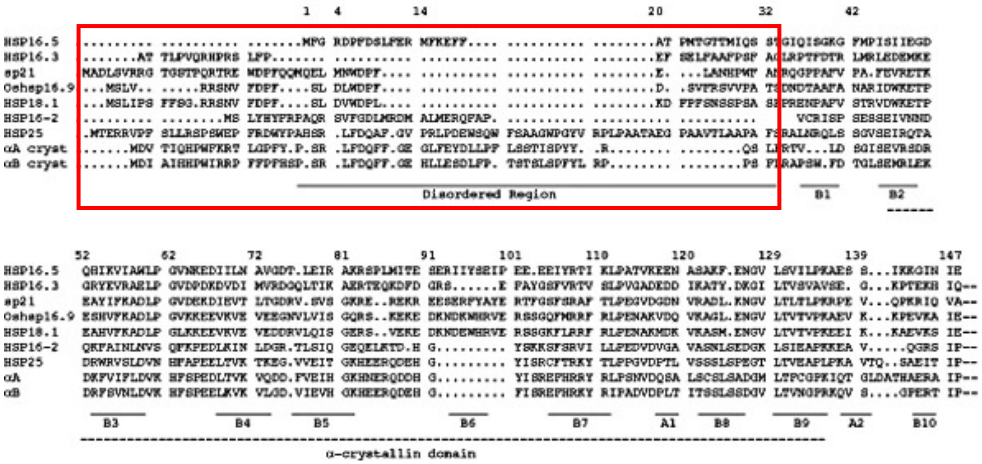
This is version 1.2 of the entry. See complete history.

3D View: Structure | Electron Density | Validation Report

Global Symmetry: Octahedral - O (3D View)
Global Stoichiometry: Homo 24-mer - A24

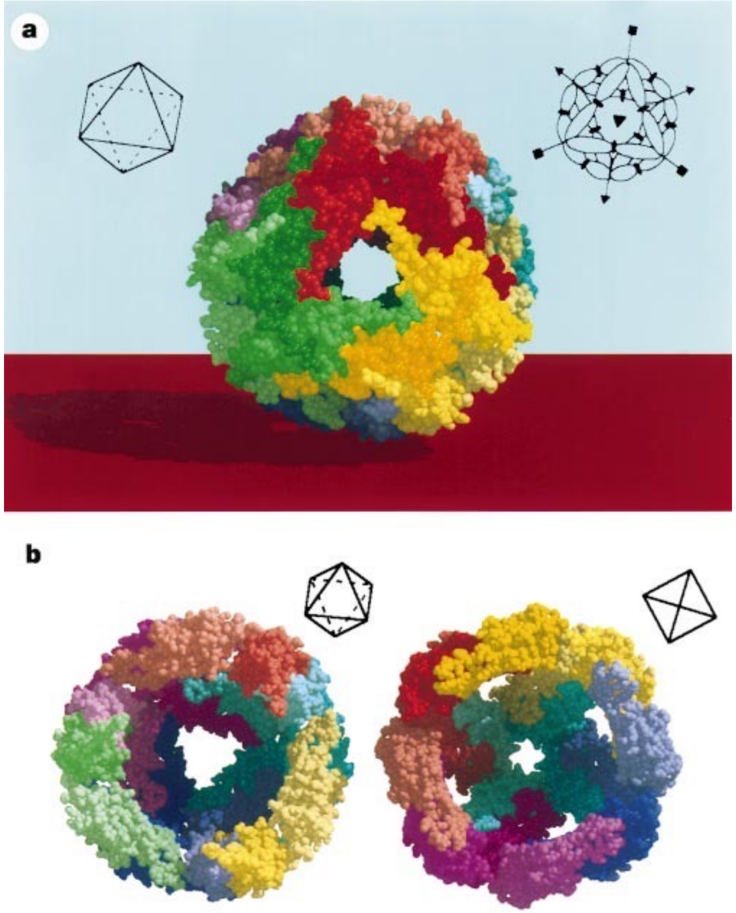
Find Similar Assemblies

Biological assembly 1 assigned by authors and generated by PISA,PQS (software)



```
                                 1  4         14                    20         32         42
HSP16.5    .......... .......... .......MFG RDPFDSLFER MFKEFF.... .......... .........AT PMTGTTMIQS STGIQISGKG FMPISIIEGD
HSP16.3    .......AT TLPVQRHPRS LFP....... .......... .......... .......... .........EF SELFAAFPSF AGLRPTFDTR LMRLEDEMKE
sp21       MADLSVRRG TGSTPQRTRE MDPFQQMQEL MNWDPF.... .......... .......... .........E. ...LANHPMF AMRQGPFAFV PA.FEVRETK
Oshsp16.9  ...MSLV.. ......RRSNV FDPF....SL DLWDPF.... .......... .......... .........D. .SVFRSVVPA TSDNDTAAFA NARIDWKETP
HSP18.1    ...MSLIPS FFSG.RRSNV FDPF....SL DVWDPL.... .......... .......... .........KD PFPFSNSSPSA SSPRENPAFV STRVDWKETP
HSP16-2    .......... ........MS LYHYFRPAQR SVFGDLMRDM ALMERQFAP. .......... .......... ..........      VCRISP SESSEIVNND
HSP25      .MTERRVPF SLLRSPSWEP FRDWYPAHSR .LFDQAF.GV PRLPDEWSQW FSAAGWPGYV RPLPAATAEG PAAVTLAAPA FSRALNRQLS SGVSEIRQTA
αA cryst   ......MDV TIQHPWFKRT LGPFY.P.SR .LFDQFF.GE GLFEYDLLPF LSSTISPYY. .R........ ........QS LFRTV...LD SGISEVRSDR
αB cryst   ......MDI AIHHPWIRRP FFPFHSP.SR .LFDQFF.GE HLLESDLFP. TSTSLSPFYL RP........ ........PS FLRAPSW.FD TGLSEMRLEK
                                 Disordered Region                                                   B1         B2

           52         62         72         81         91         101        110        120        129        139        147
HSP16.5    QHIKVIAWLP GVNKEDIILN AVGDT.LEIR AKRSPLMITE SERIIYSEIP EE.EEIYRTI KLPATVKEEN ASAKF.ENGV LSVILPKAES S...IKKGIN IE
HSP16.3    GRYEVRAELP GVDPDKDVDI MVRDGQLTIK AERTEQKDFD GRS......E FAYGSFVRTV SLPVGADEDD IKATY.DKGI LTVSVAVSE. G...KPTEKH IQ--
sp21       EAYIFKADLP GVDEKDIEVT LTGDRV.SVS GKRE..REKR EESERFYAYE RTFGSFSRAF TLPEGVDGDN VRADL.KNGV LTLTLPKRPE V...QPKRIQ VA--
Oshsp16.9  ESHVFKADLP GVKKEEVKVE VEEGNVLVIS GQRS..KEKE DKNDKWHRVE RSSGQFMRRF RLPENAKVDQ VKAGL.ENGV LTVTVPKAEV K...KPEVKA IE--
HSP18.1    EAHVFKADLP GLKKEEVKVE VEDDRVLQIS GERS..VEKE DKNDEWHRVE RSSGKFLRRF RLPENAKMDK VKASM.ENGV LTVTVPKEEI K...KAEVKS IE--
HSP16-2    QKFAINLNVS QFKPEDLKIN LDGR.TLSIQ GEQELKTD.H G......... YSKKSFSRVI LLPEDVDVGA VASNLSEDGK LSIEAPKKEA V.....QGRS IP--
HSP25      DRWRVSLDVN HFAPEELTVK TKEG.VVEIT GKHEERQDEH G......... YISRCFTRKY TLPPGVDPTL VSSSLSPEGT LTVEAPLPKA VTQ..SAEIT IP--
αA         DKFVIFLDVK HFSPEDLTVK VQDD.FVEIH GKHNERQDDH G......... YISREFHRRY RLPSNVDQSA LSCSLSADGM LTFCGPKIQT GLDATHAERA IP--
αB         DRFSVNLDVK HFSPEELKVK VLGD.VIEVH GKHEERQDEH G......... FISREFHRKY RIPADVDPLT ITSSLSSDGV LTVNGPRKQV S....GPERT IP--
            B3         B4      B5            B6          B7        A1   B8        B9     A2        B10
                               α-crystallin domain
```

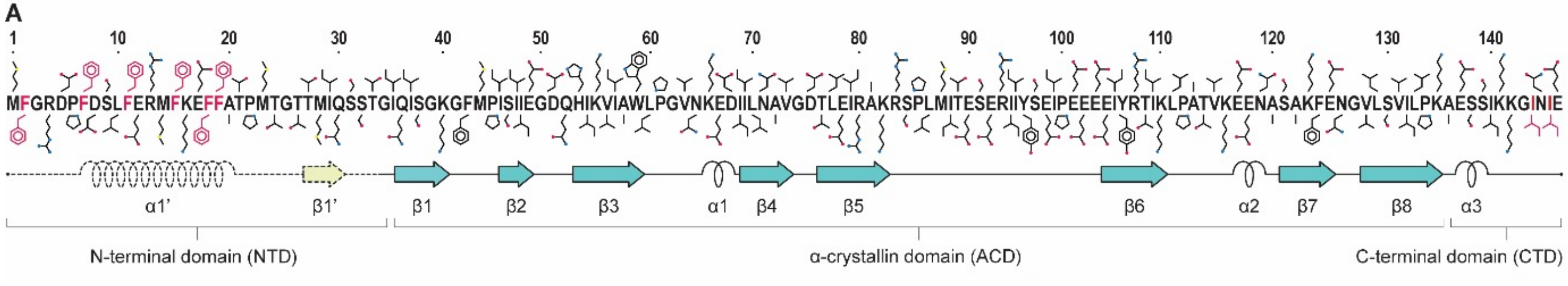


Nature. 394, 595-599 (1998)

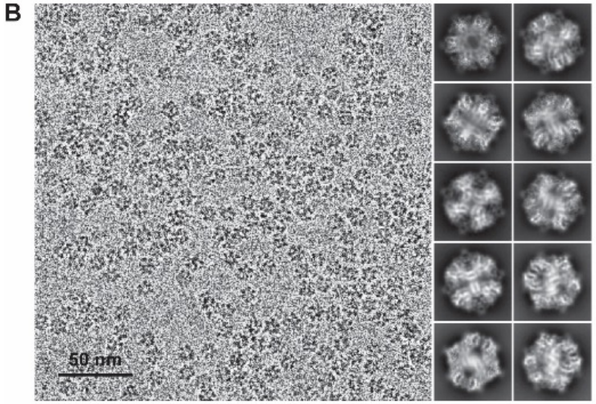
Results and Discussion

1. The cryo-EM structure of MjsHSP16.5

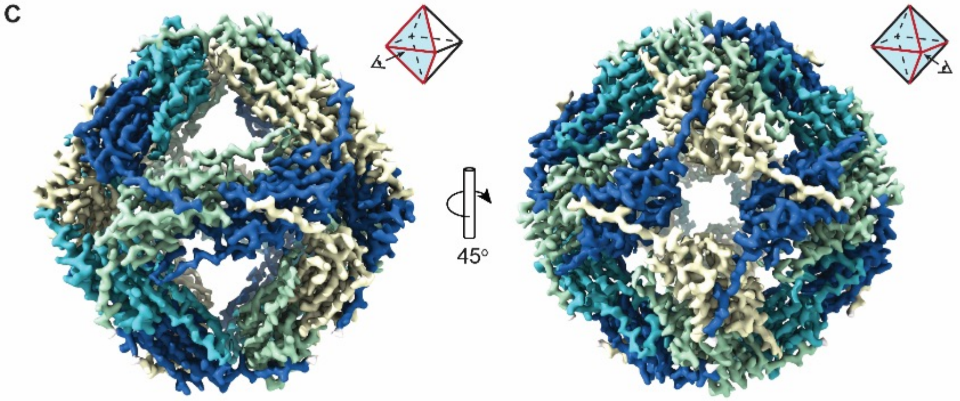
Primary sequence of MjsHSP16.5



Cryo-EM micrograph
with the 2D class averages



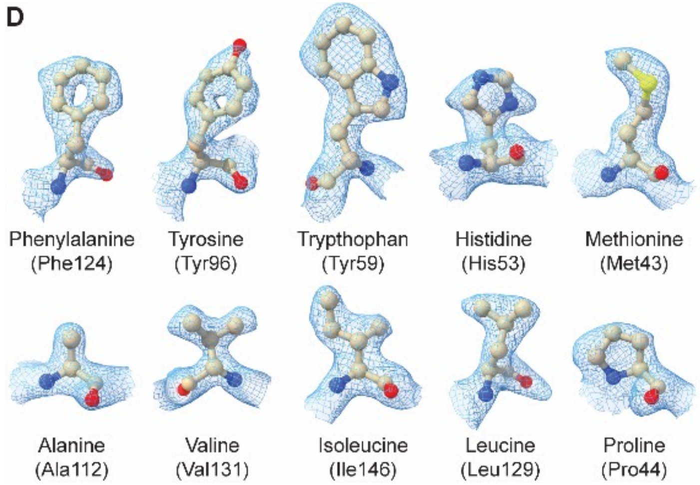
Cryo-EM structure of MjsHSP16.5



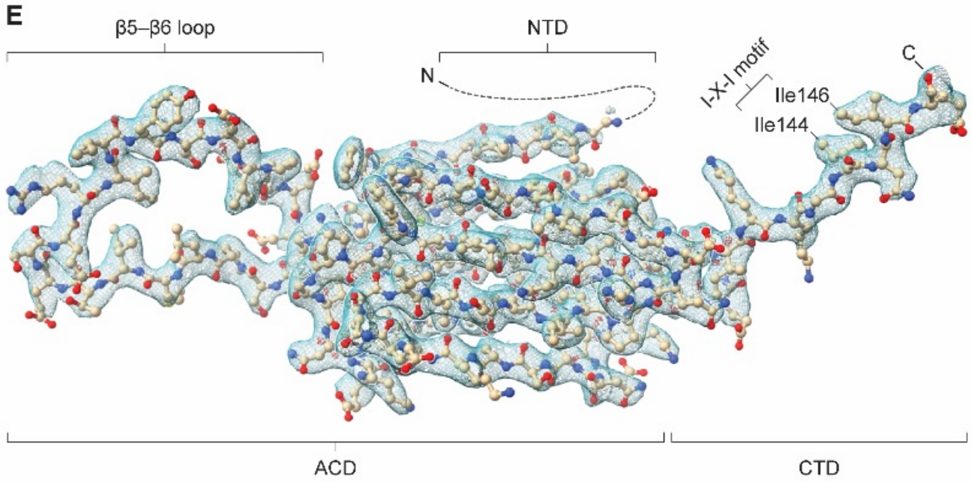
Results and Discussion

1. The cryo-EM structure of MjsHSP16.5

Representative hydrophobic residues fitted into the cryo-EM map



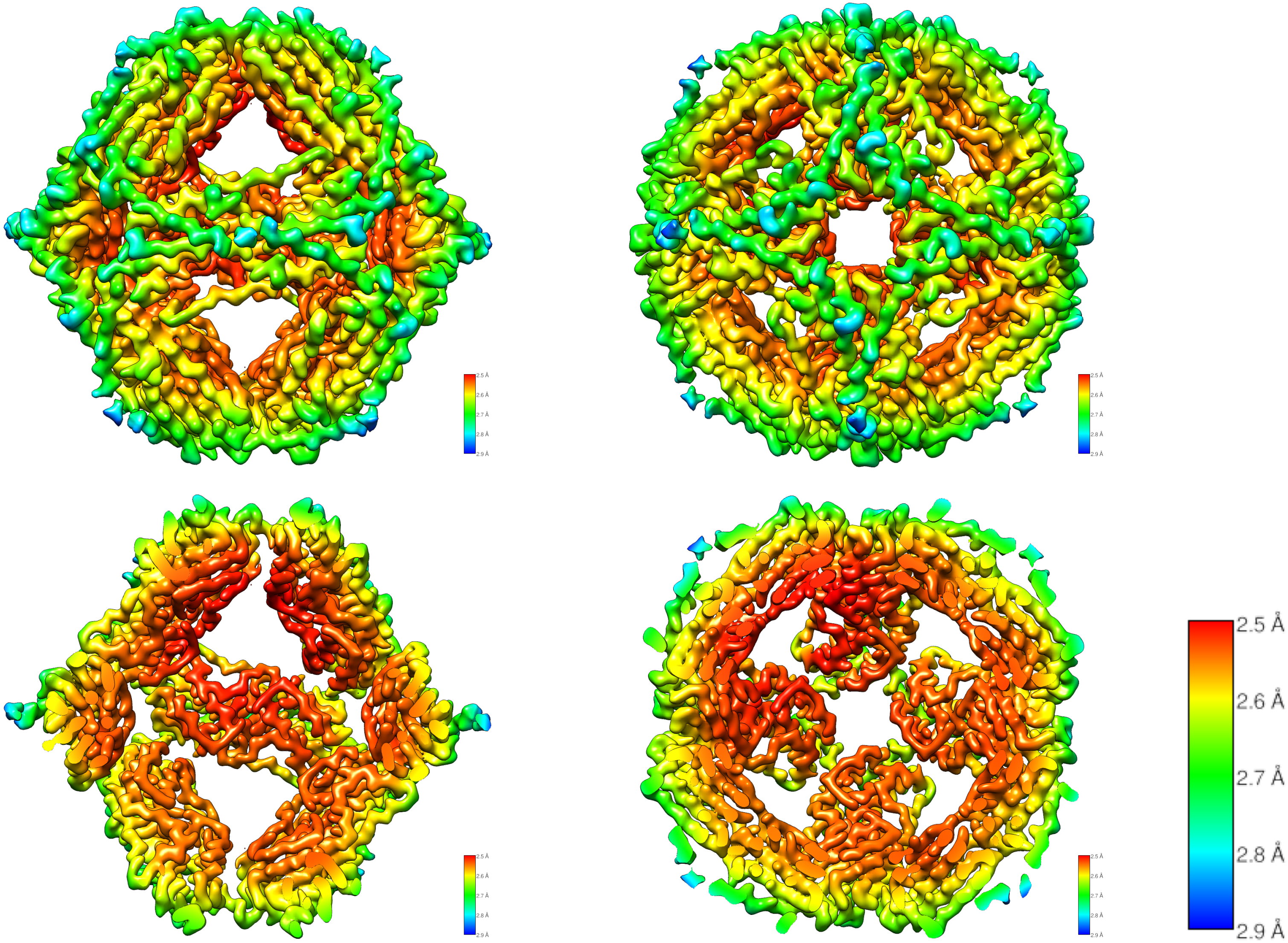
Atomic model built into the cryo-EM map



Results and Discussion

1. The cryo-EM structure of MjsHSP16.5

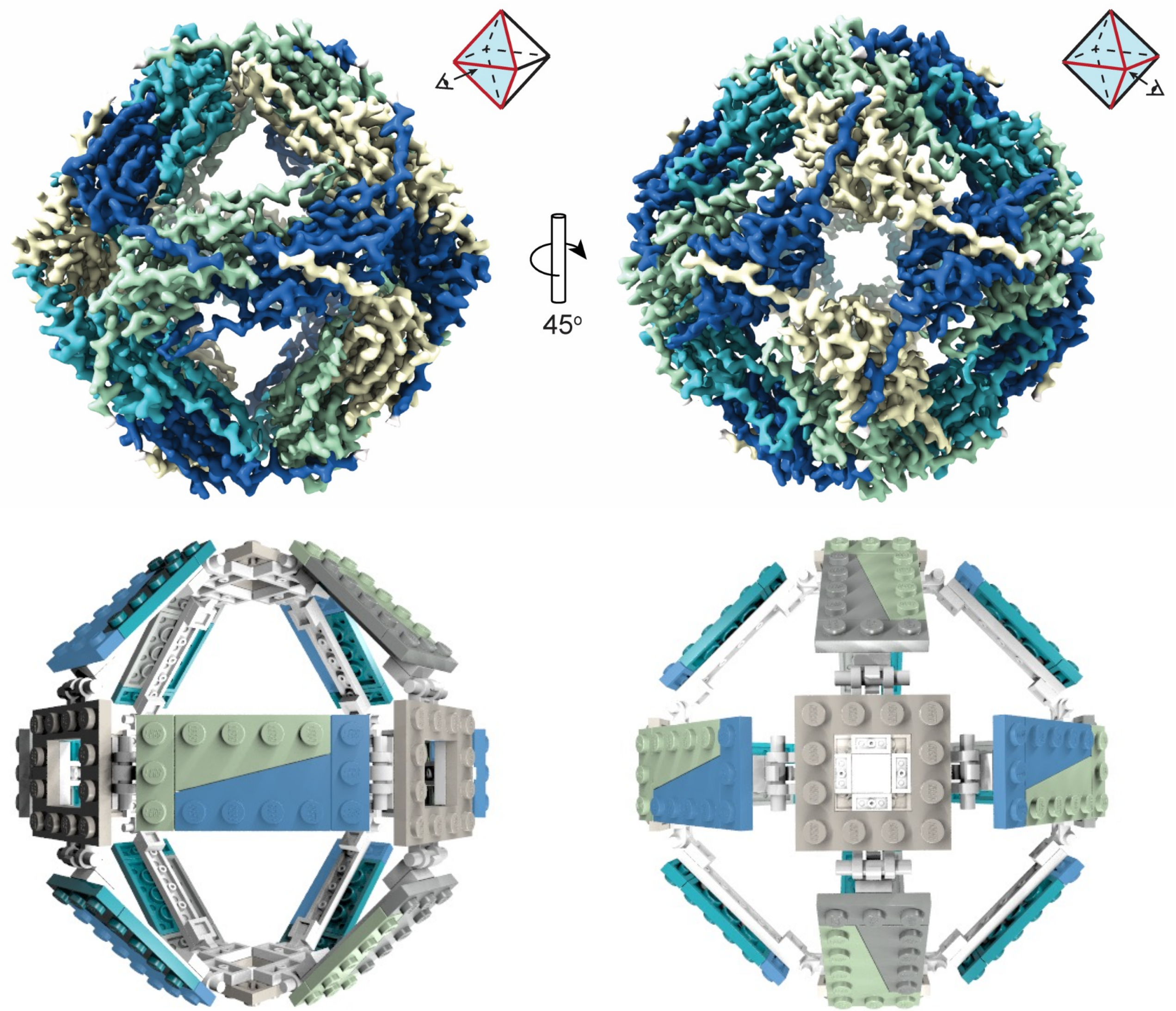
Local resolution of the cryo-EM structure



Results and Discussion

1. The cryo-EM structure of MjsHSP16.5

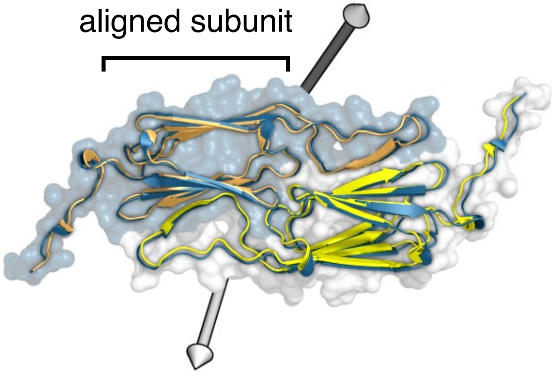
Oligomerization of MjsHSP16.5

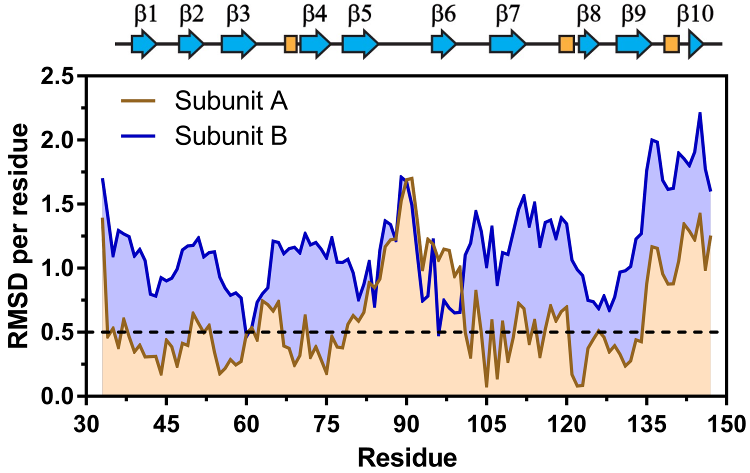


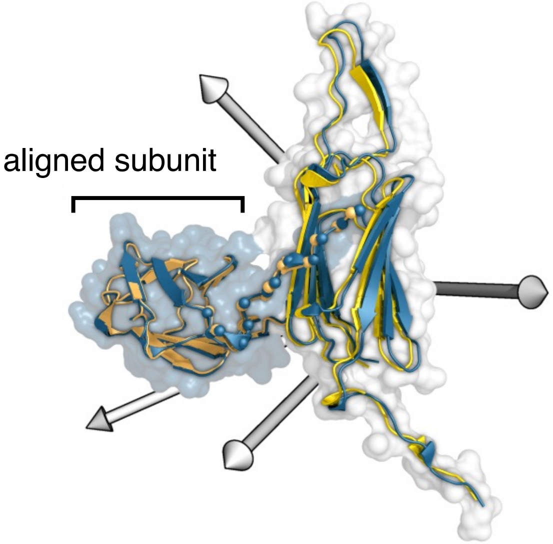
Results and Discussion

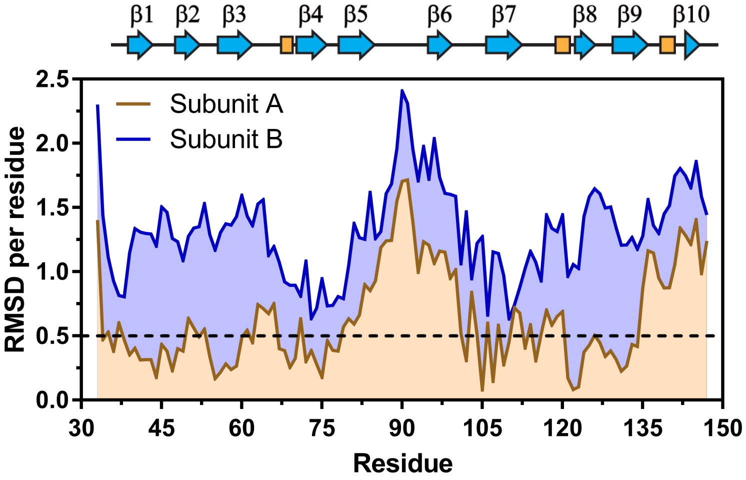
2. Comparison of the cryo-EM structure with crystal structure

🟦 Cryo-EM 🟧 X-ray





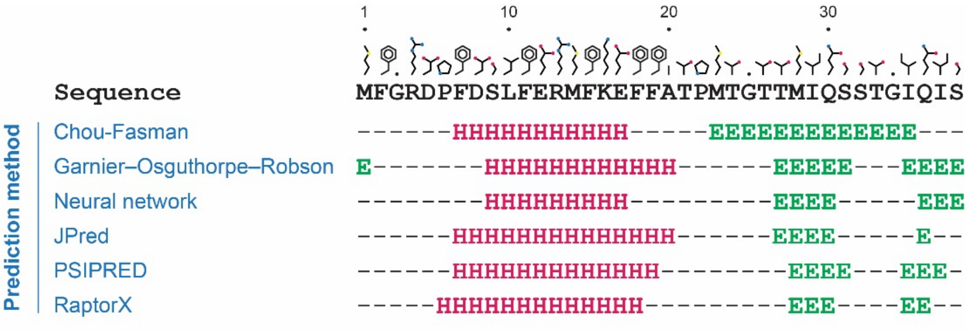




Results and Discussion

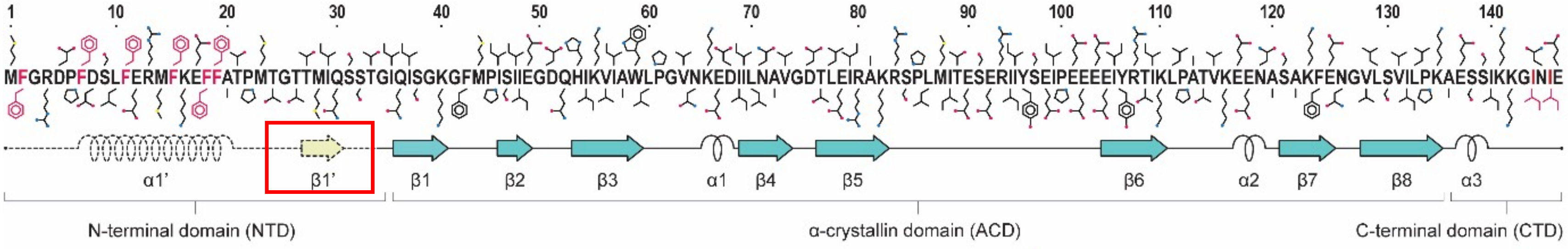
3. Conformations of the cryo-EM N-terminal regions invisible in the crystal structure of MjsHSP16.5

- Secondary structure predictions using different algorithms show that the N-terminal region of MjsHSP16.5 has a tendency to form a β strand.



		1 · 10 · 20 · 30
	Sequence	MFGRDPFDSLFERMFKEFFATPMTGTTMIQSSTGIQIS
Prediction method	Chou-Fasman	------HHHHHHHHHHH-----EEEEEEEEEEEEE---
	Garnier–Osguthorpe–Robson	E-------HHHHHHHHHHHH------EEEEE---EEEE
	Neural network	--------HHHHHHHHH---------EEEE-----EEE
	JPred	------HHHHHHHHHHHHHH------EEEE-----E--
	PSIPRED	------HHHHHHHHHHHHH--------EEEE---EEE-
	RaptorX	-----HHHHHHHHHHHHH---------EEE----EE--

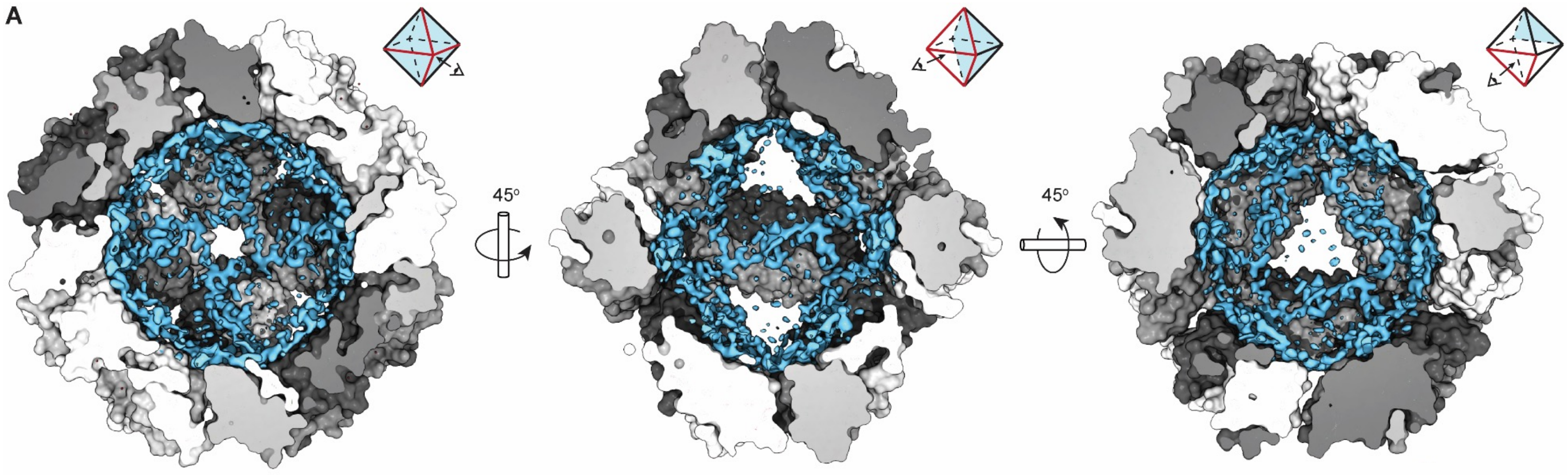
- Cryo-EM image reconstruction shows additional N-terminal residues.



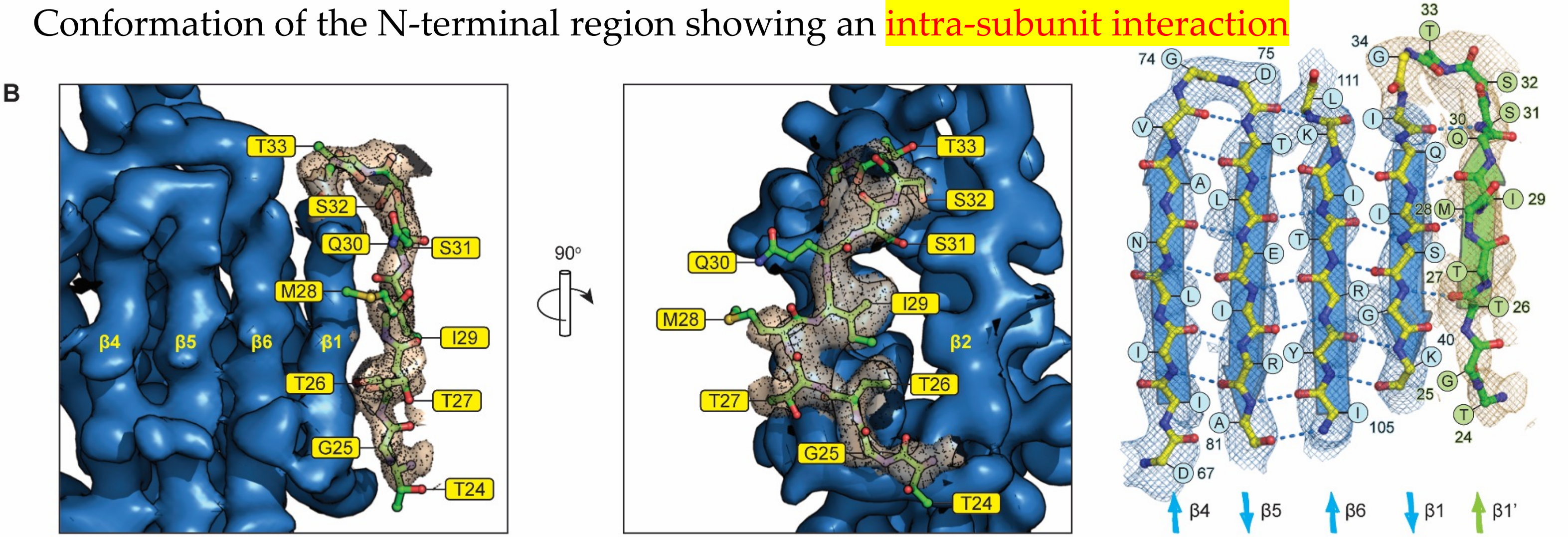
Results and Discussion

3. Conformations of the cryo-EM N-terminal regions invisible in the crystal structure of MjsHSP16.5

Cryo-EM maps of the N-terminal regions beneath the α-crystallin domains



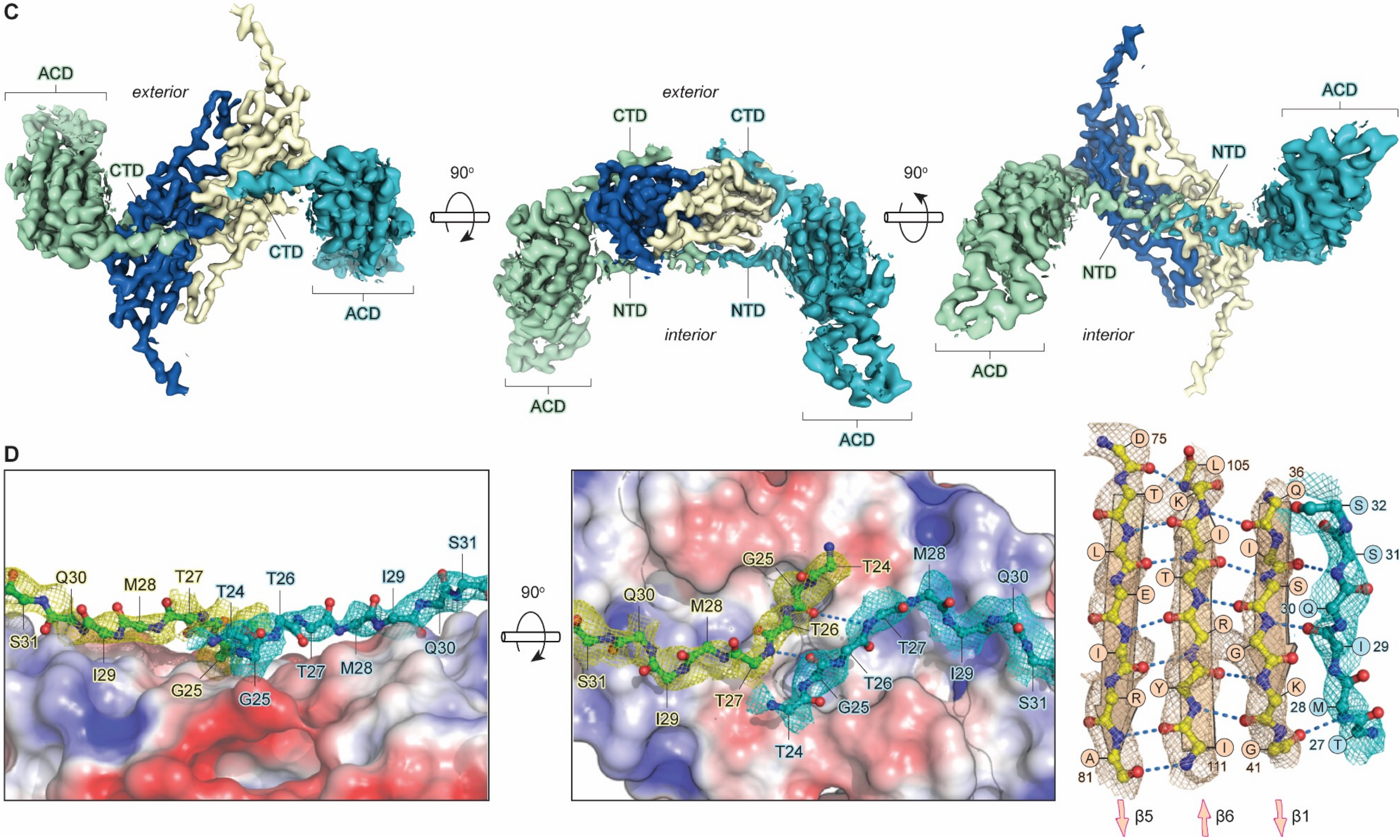
Conformation of the N-terminal region showing an intra-subunit interaction



Results and Discussion

3. Conformations of the cryo-EM N-terminal regions invisible in the crystal structure of MjsHSP16.5

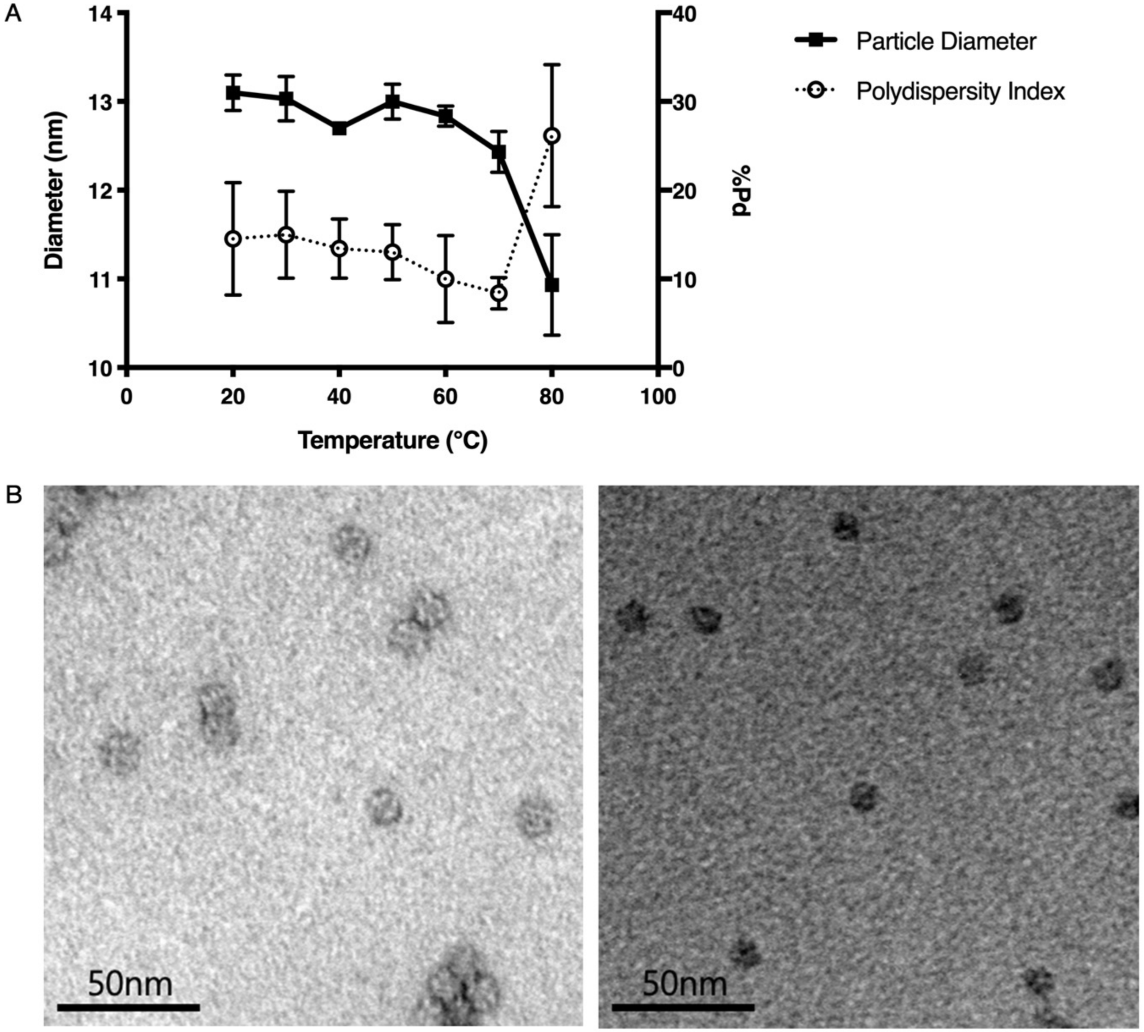
Conformations of the N-terminal regions showing the inter-subunit interactions



Results and Discussion

4. Molecular sizes of MjsHSP16.5 at different temperatures

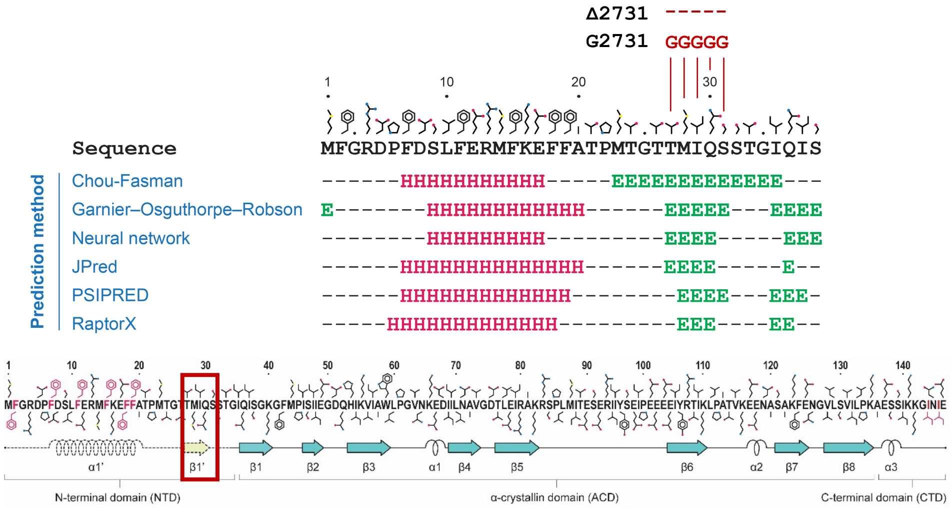
Molecular sizes of MjsHSP at different temperatures
analyzed by Dynamic Light Scattering and transmission election microscopy.



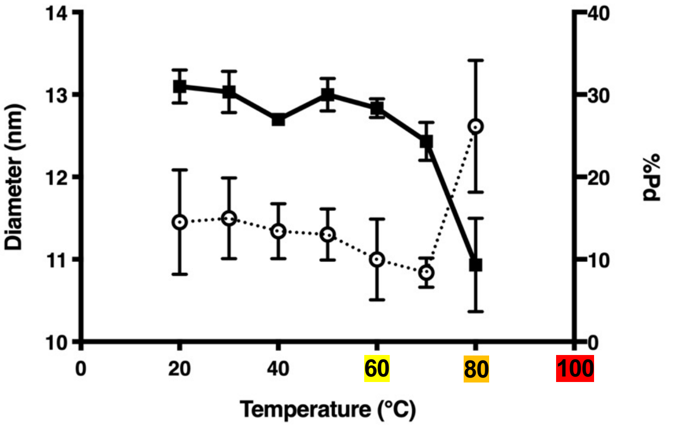
Results and Discussion

5. Functional identification of N-terminal domain

MjsHSP16.5 mutants carrying changes at the β-strand forming region.

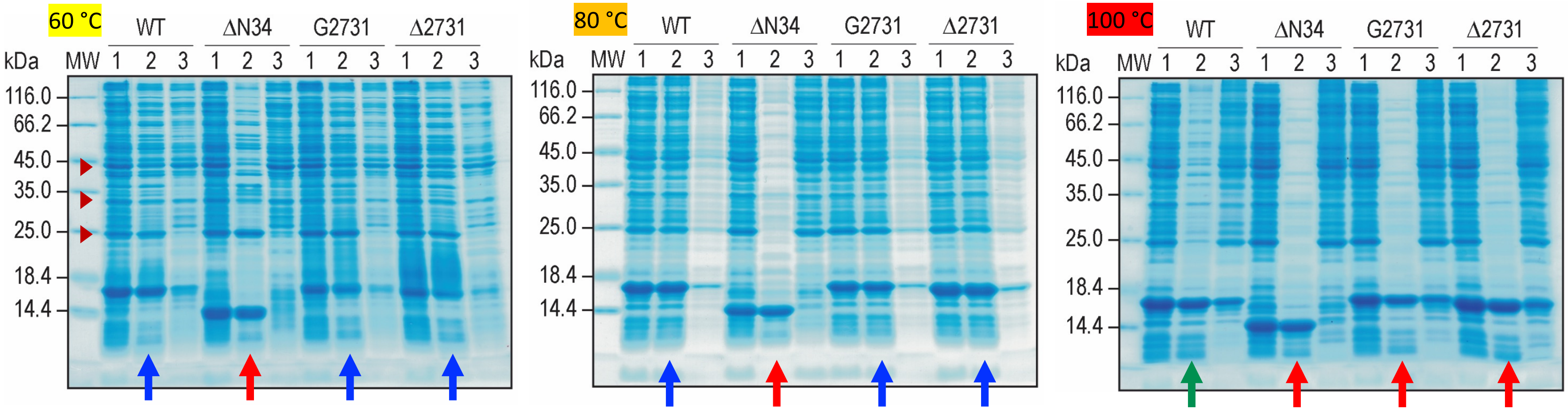


Sequence	MFGRDPFDSLFERMFKEFFATPMTGTTMIQSSTGIQIS
Chou-Fasman	------HHHHHHHHHHH-----EEEEEEEEEEEEE---
Garnier–Osguthorpe–Robson	E-------HHHHHHHHHHHH------EEEEE---EEEE
Neural network	--------HHHHHHHHH---------EEEE-----EEE
JPred	------HHHHHHHHHHHHHH------EEEE-----E--
PSIPRED	------HHHHHHHHHHHHH--------EEEE---EEE-
RaptorX	-----HHHHHHHHHHHHH---------EEE----EE--



E. coli cell lysate thermal protection assay

1: before heating 2: supernatant after heating 3: pellet after heating



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Conclusions

· Using the single-particle cryo-electron microscopy (cryo-EM) technique,

we reconstructed the MjsHSP16.5 24-subunit oligomer to a 2.5-Å resolution.

· The cryo-EM image reconstruction reveals additional N-terminal residues

which are absent in most of MjsHSP16.5 crystal structures.

- Orientation of an extra β-strand is identified in N-terminal domain.
- N-terminal and C-terminal regions help stabilize the oligomeric form of MjsHSP16.5.
- N-terminal domain has an important role in the holding chaperone activity of MjsHSP16.5.

Acknowledgments

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KOREA BASIC SCIENCE INSTITUTE

Korea Basic Science Institute

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