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Structural and functional insights into the evolution and stress adaptation of type II chaperonins

Jessica J Chaston1,2, Callum Smits1,2, David Aragão3, Andrew S. W. Wong4,5, Bilal Ahsan4, Sara Sandin4,5, Sudheer K. Molugu6, Sanjay K. Molugu6, Ricardo A. Bernal6, Daniela Stock1,2*, Alastair G. Stewart1,7*

1Molecular, Structural and Computational Biology Division, The Victor Chang Cardiac Research Institute, Darlinghurst, NSW 2010, Australia.

2Faculty of Medicine, The University of New South Wales, Sydney, NSW 2052, Australia.

3Australian Synchrotron, Clayton, VIC 3168, Australia.

4School of Biological Sciences, Nanyang Technological University, 637551, Singapore.

5NTU Institute of Structural Biology, Nanyang Technological University, 637551, Singapore.

6Department of Chemistry, University of Texas at El Paso, El Paso, TX 79968, U.S.A.

7School of Molecular Bioscience, The University of Sydney, Sydney, NSW 2006, Australia

*to whom correspondence should be sent: d.stock@victorchang.edu.au; alastair.stewart@sydney.edu.au
Summary
Chaperonins are essential biological complexes assisting protein folding in all kingdoms of life. Whereas homooligomeric bacterial GroEL binds hydrophobic substrates non-specifically, the heterooligomeric eukaryotic CCT binds specifically to distinct classes of substrates. *Sulfolobales*, which survive in a wide range of temperatures, have evolved three different chaperonin subunits (α, β, γ) that form three distinct complexes tailored for different substrate classes at cold, normal and elevated temperatures. The larger octadecameric β-complexes cater for substrates under heat stress, whereas smaller hexadecameric αβ-complexes prevail under normal conditions. The cold-shock complex contains all three subunits, consistent with greater substrate specificity. Structural analysis using crystallography and electron microscopy reveals the geometry of these complexes and shows a novel arrangement of the α and β subunits in the hexadecamer enabling incorporation of the γ subunit.

Introduction
Chaperonins are ubiquitous protein complexes that assist protein folding and prevent detrimental aggregation of un- or misfolded proteins (Lopez et al., 2015; Saibil, 2013; Skjaerven et al., 2015). They are typically up-regulated under conditions of cellular stress, including heat, and are therefore considered to be heat shock proteins. Multiple copies of one or more subunits form molecular cages of around one megadalton that sequester misfolded protein substrates in their inner cavity. Allosteric binding and hydrolysis of ATP is believed to create ratchet-like movements within the cage (Bigotti and Clarke, 2008; Fei et al., 2013; Horovitz and Willison, 2005; Saibil et al., 2013). These movements are thought to trigger changes in the substrate binding surfaces, thereby helping to overcome local energy minima and encouraging proper folding of the substrate. Type I chaperonins are made of 14 copies of a 60 kDa protein arranged in 72 point symmetry (D7) (Figure 1A) (Braig et al., 1994; Horwich et al., 1990). These occur in eubacteria (GroEL) and within the mitochondrial matrix (cpn60/HSP60). Type II chaperonins occur in archaea (thermosome, TF55) and in the evolutionary related eukaryotic cytosol (CCT, TRiC) (Horwich et al., 2007; Lopez et al., 2015; Skjaerven et al., 2015). Some archaecal chaperonins are constructed from a single type of 60 kDa subunit, but the majority consist of two paralogous subunits, α and β (Bigotti and Clarke, 2008; Ditzel et al., 1998). These are arranged alternately in a hexadecameric complex of two eight-membered rings (Figure 1B) and are closely related to the eight paralogous eukaryotic CCT subunits, which are also arranged in pairs of eight-membered rings (Figure 1C) (Cong et al., 2012; Dekker et al., 2011; Kalisman et al., 2013; Munoz et al., 2011). Chaperonins are known to function in a highly cooperative manner mediated by the subunit geometry within the complexes and this differs in type I and type II chaperonins (Bigotti and Clarke, 2008; Lopez et al., 2015; Reissmann et al., 2012; Skjaerven et al., 2015; Yebenes et al., 2011).

Thermophilic Factor 55 (TF55, rosettasome) is a type II chaperonin occurring in *Sulfolobus* (Trent et al., 1991). Unlike most other archaea, which encode two related type II chaperonin subunits arranged in complexes of 42 point symmetry (D4), *Sulfolobus* encodes three paralogous TF55 subunits, α, β, and γ that form a series of different complexes (Figures 1D-F). These complexes have been
analyzed and discussed over the past two decades but their precise structure, stoichiometry and subunit arrangement remained controversial (Ellis et al., 1998; Kapatai et al., 2006; Marco et al., 1994; Trent et al., 1991).

The *Sulfolobus* β subunit was the first type II chaperonin subunit to be described due to its abundance under heat shock (Trent et al., 1990). It was found that its up regulation allows *Sulfolobus* to survive extreme temperatures, up to 92°C. Initially, eight-fold and nine-fold symmetrical particles were discussed (Trent et al., 1991). Subsequent EM studies using material derived from heat-shocked cells confirmed nine-fold symmetry (Knapp et al., 1994; Marco et al., 1994). However when the α subunit was identified it was concluded that *Sulfolobus* TF55 is most likely constructed from α and β subunits in the same manner as observed in other archaeal chaperonins showing eight-fold symmetry (Kagawa et al., 1995; Kapatai et al., 2006). Following discovery of the γ subunit in the *Sulfolobus* genome (Archibald et al., 1999) analysis of *Sulfolobus shibatae* cultures revealed that the expression levels and relative amounts of subunits vary with temperature (Kagawa et al., 2003). Thus the third subunit, γ, is only present under cold shock conditions (60-75°C), while α and β are present at normal growth temperatures (75-79°C). Under heat shock conditions (>86°C) the β subunit predominates.

Here we show that like the *S. shibatae* TF55 subunits, the three corresponding *S. solfataricus* subunits have remarkably different properties in terms of thermal stability and their propensity to form complexes. We show that an all-β complex is formed under heat shock and prevails at extreme temperatures, whereas a mixed αβ complex predominates at normal growth temperatures, and a mixed αβγ complex only forms at low temperatures. Determination of the structures of the three complexes using a combination of X-ray crystallography and electron cryo microscopy (cryo-EM) defines the subunit arrangements within each complex and suggests that the different complexes have different substrate specificities and folding chamber sizes. Different inter-ring interactions point to different cooperativity networks within these complexes compared to GroEL and thermosomes and provide new insight into the evolution of chaperonins. While little is known about *in vivo* substrates of archaeal chaperonins our X-ray data shows a potential substrate interaction between domains of adjacent subunits.
Results

Characterization of TF55 complexes and subunits
Using ion exchange and size exclusion chromatography, we purified two different TF55 complexes from *S. solfataricus* cells grown at 80°C. One complex contains only β subunits (termed prepA) whereas the other contains both α and β subunits (termed prepB). The γ subunit was undetectable in either preparation via MALDI-TOF analysis of in-gel tryptic digests. To establish the role of the γ subunit, we cloned, expressed and purified all three subunits from *E. coli* and analyzed their thermal stability and propensity to form complexes (Figures S2). Recombinant expression levels of the three subunits in *E. coli* vary significantly, preventing co-expression of subunits. Expression levels for the α subunit are low, whereas the β and γ subunits express well. The β subunit forms inclusion bodies that fold upon heat treatment of the crude lysate and subsequently remain folded and in solution (Figure S1).

The thermostability of individual subunits was assessed by thermal melt analysis (Figure S2A). These confirm that the γ subunit is heat labile and unfolds above 75°C, just below the optimal growth temperature range of *S. solfataricus*. The α and β subunits are substantially more heat resistant, both starting to unfold at around 94°C. This is consistent with observations made for *S. shibatae* subunits and together with varying transcription levels determined by Northern analysis of *S. shibatae* cells grown at different temperatures (Kagawa et al., 2003) (Figure S2B) explains the varying in vivo subunit ratios observed in *Sulfolobus* at different temperatures (Figure S2C).

Native gel electrophoresis combined with Western blot analysis shows that complex formation is triggered by addition of ATP. However the propensity of subunits to form complexes differs significantly as follows. The β subunit forms complexes by itself, whereas the α and γ subunits do not (Figure S2D). The α and β subunits together form complexes, as do all three subunits together. The γ subunit requires the presence of both the α and β subunit to form a complex. To exclude the possibility that all high molecular weight bands on the native gel correspond to all-β complexes, we combined untagged wild type β subunit and αβ complexes with tagged recombinant subunits. A mixed αβ complex is formed by adding recombinant α subunits to wild type β subunits (Figure S2D), but no mixed αγ or βγ complexes are formed. Interestingly, adding recombinant γ subunit to wild type αβ complex results in an αβγ heterotrimeric complex. This suggests that the γ subunit is either able to insert itself into the complex, or that a dynamic equilibrium of complex formation and disassembly exists as previously suggested (Quaite-Randall et al., 1995). Furthermore, when incorporated into the complex, the γ subunit still degrades above 75°C (Figure S2E).

X-ray structure determination of complexes

The all-β TF55 chaperonin structure: The crystal structure of the recombinant all-β complex was determined to 3.8 Å resolution by molecular replacement using the closely related *Acidianus tengchongensis* type II chaperonin structure, 84% sequence identity (Huo et al., 2010), as a search model. The crystallographic symmetry (*P6₃* with six monomers per asymmetric unit, Figure S3A) builds up an
octadecameric chaperonin complex of 92 point symmetry (D9) (Figure 2A)
consistent with that seen in the A. tengchongensis chaperonin structure that
crystallized in a different space group, C2, with nine subunits (one ring) per
asymmetric unit. Despite the different crystal symmetries, the two structures are
remarkably similar (rmsd 0.92 Å) and show an-open conformation with subunits in
register across the equator. The complex has a maximum internal diameter of 78
Å (Figure 2A), suggesting that it is limited to binding proteins with a volume of
around 250 nm³, corresponding to a molecular weight of ~200 kDa.

The αβ TF55 chaperonin structures: Mixed αβ subunit containing PrepB
provided crystals of space group I422, with one heterodimer per asymmetric unit
consistent with a T. acidophilum “thermosome-like” structure (Ditzel et al., 1998)
of 42 point symmetry (Figure 3A,B). Two separate crystal forms were obtained
from the same preparation using the same crystallization conditions. One has a
long c-axis (304.7 Å) whereas the other has a shorter c-axis (248.1 Å), and we
refer to them as “αβlongC” and “αβshortC” respectively. The structure of each crystal
form was solved independently by molecular replacement using the coordinates of
the S. solfataricus β subunit described above (Figure 2B) and the A.
tengchongensis α subunit, 83% sequence identity (Zhang et al., 2013), as
individual search models. Although the resolution of the X-ray data for the αβlongC
crystal form extends to 3.0 Å in the best direction, the data is highly anisotropic
(Table 1). This is reflected in the electron density for the apical domains being so
poor that unequivocal assignment of the residues in this region was not possible.
While the main chain of the β subunit could be traced for all three domains, the
electron density for the α subunit’s apical domain (Figure 2B) disappears into
noise and was therefore omitted from the final model (see Table 1 for missing
residues). The electron density for the equatorial and intermediate domains on the
other hand is remarkably clear and the α and β subunits could be identified using
small differences between the sequences of the subunits (Figure S6A and B). In
addition a Platinum derivative confirmed that we assigned the subunits correctly
(Figure S6C). The data for the αβshortC crystal form extends to only 3.75 Å
resolution, but is less anisotropic and the electron density allowed unequivocal
tracing of all three domains of either subunit with the omission of only few
residues (Table 1). Density corresponding to ADP is clear in the β subunit of the
αβshortC crystal form, whereas no density for nucleotide is detectable in the α
subunit, nor in any subunit of the αβlongC crystal from.

In both crystal forms the crystal symmetry builds up a hexadecameric chaperonin
complex that, in each case is constructed from two rings containing four α subunits
and four β subunits. The subunits alternate within a single ring as seen in the T.
acidophilum αβ thermosome structure (Ditzel et al., 1998). However, most
interestingly, the inter-ring arrangements of the α and β subunits are different in
between the two species; the α and β subunits in the T. acidophilum structure form
homodimers across the equator, whereas in the Sulfolobus TF55 structure the rings
are rotated by 45° thus leading to αβ heterodimers across the equator in a staggered
arrangement (Figures 1B,E and 3A,B).
The αβ\textsubscript{long} crystal structure most likely adopts a near closed conformation when compared to other chaperonin structures (Figure 3C) (Ditzel et al., 1998; Douglas et al., 2011). However, in the αβ\textsubscript{short} structure, which clearly shows the apical domains, the β subunit is in a closed conformation, whereas the α subunit adopts an extremely extended open conformation that has not been seen in any previous chaperonin structure (Figure 3D, Figure S4). The molecular arrangement within the αβ\textsubscript{long} and αβ\textsubscript{short} crystal lattices accounts for the large c-axis difference observed between them (Figure 3 and S3B and C). In the αβ\textsubscript{long} crystal, the asymmetric unit is arranged such that one α subunit is in contact with the outside of the other (Figure 4A). Conversely, in the αβ\textsubscript{short} crystal, the α subunit is highly extended and intercalates with the α subunit of an adjacent complex (Figure 4B). This leads to a shorter distance between asymmetric units (27 Å) and hence a shorter crystallographic c-axis (-57 Å – twice the distance since there are two asymmetric units per unit cell along the c-axis). The maximum internal diameter of the αβ complexes is smaller than that of the all-β complex, being ~65 Å. This suggests that the αβ complex would be limited to binding proteins with a maximum volume of around 145 nm\textsuperscript{3} or ~120 kDa.

Detailed analysis of the crystal contacts between the α subunits in the αβ\textsubscript{short} crystal form highlights an interesting feature; the N-terminal part of the helical protrusion of the α subunit (Figure 2B) has unwound to create an extended β-sheet with the sensor loop in the equatorial domains of an adjacent complex reminiscent of the structural conversion seen in prion formation (Prusiner, 2012) (Figure 4C).

**Electron microscopy**

Complexes were analyzed by negative stain and cryo-EM (Figures S5). As expected, classification of all-β complexes shows a homogeneous distribution of nine-membered rings in top views (Figure S5). Classification of wild type αβ complexes shows predominantly eight-membered rings with a small fraction (5-6\%) of nine-membered rings, presumably consisting of all-β complexes (Figure S5). Addition of recombinant γ subunit to this sample converts the eight-membered rings into nine-membered rings (Figure S5). This not only corroborates the observation made by native gel analysis, that the γ subunits can insert themselves into preformed αβ complexes, but also confirms that the nine-membered rings observed in images of recombinant αβγ complexes represent indeed the ternary complex rather than an all-β complex.

A 16 Å resolution reconstruction of the unstained ternary complex made from recombinant α, β and γ subunits was obtained via cryo-EM and shows an octadecameric complex consisting of two nine-membered rings of alternating α, β and γ subunits with 32 point symmetry (D3) (Figure 5, S5). Attempts were made to obtain higher resolution data using a direct electron detector, however data converged at 16 Å resolution indicating that the sample limited resolution rather than the detector. In order to identify the correct symmetry of the complex, we used the multi-model refinement algorithm (multirefine) of the EMAN software suite (Ludtke, 2010). The dataset was refined using models without symmetry as well as all possible symmetries for a cylindrical octadecamer up to D9. The resulting D3 subset converged into a substantially better resolved map compared to the others, indicating that the symmetry of the complex is D3 (Figure S5). At
the current resolution it is not possible to establish the exact sequence of subunits in a ring to show whether α follows β follows γ or α follows γ follows β. However we suggest that the subunits can be assigned to the EM density according to the following considerations: (i) the hexadecameric TF55 structures have alternating α and β subunits that form heterodimers across the equator. Assuming that as many contacts as possible remain preserved, the only way an octadecameric complex can be assembled with an additional subunit, γ, is for γ to form a homodimer across the equator. (ii) Due to the chiral nature of proteins, the 32 point symmetry of the 18-mer dictates an arrangement of one homodimer sitting on the two-fold axis that is surrounded by two heterodimers on either side generated by the two-fold axis (Figure 1D, 5B and 6). The octadecameric all-β crystal structure fits well into the EM density indicating that this map shows the complex in a near open conformation.

**Discussion**

**Chaperonin evolution – jack of all trades versus specialists**

Cellular stress impairs protein folding, leading to cytotoxic protein aggregation (Gregersen et al., 2006; Tam et al., 2006; Tam et al., 2009). All kingdoms of life therefore employ chaperonins for protein quality control and rescue, yet with very different degrees of complexity. Bacterial type I chaperonin complexes (GroEL) consist of homooligomeric heptadecamers that bind to virtually all unfolded proteins via unspecific hydrophobic interactions (Saibil, 2013). Since both average protein size and genomes are larger in archaea and eukaryotes, these organisms have evolved larger chaperonins (hexa- or octadecamers) consisting of two to eight different subunits that originated from gene duplication (Figure 1). These paralogous subunits recognize their substrates specifically via a combination of hydrophilic and hydrophobic interactions (Joachimiak et al., 2014). Extremophiles are under particular pressure to protect their protein repertoire under a very wide range of conditions. Whereas the eight paralogous subunits in eukaryotes have likely evolved by duplications that occurred early and only once, the two to five different subunits found in different species of archaea most likely originated from various independent and lineage specific duplications for adaptation to extreme environments (Archibald et al., 2001; Bigotti and Clarke, 2008). In contrast to eukaryotes, where the eight paralogous subunits constitute one eight-membered ring, the three paralogous TF55 chaperonin subunits found in *Sulfolobales* exhibit considerable plasticity with regards to the complexes they can form. This is achieved by a combination of different expression levels and thermostability of subunits that leads to formation of three distinct complexes with different properties suitable to rescue different classes of substrates under different forms of stress.

**TF55 complex formation and geometry**

In this study we used *S. solfatarius* cells grown at 80°C, which is slightly above normal growth temperatures (75-79°C) and significantly above the thermostability range for subunit γ. We isolated intact TF55 complexes consisting of hexadecameric αβ complexes. EM analysis of this preparation showed that 5-6% of particles consist of nine-fold symmetrical, most likely all-β complexes (Figure
S5). This suggests that αβ complexes are the most stable and predominate species at normal growth temperatures. A previous study using heat shocked (85°C) *S.
shibatae* cells and similar purification protocols had isolated intact, exclusively
nine-fold symmetrical TF55 complexes (Schoehn et al., 2000). Thus, while the
mixed αβ complexes found at normal growth temperatures are similar to
thermosomes of other archaea, the octadecameric all-β complexes appear to be
unique to *Sulfolobales* and prevail under heat shock conditions. We confirm that
the β subunit forms homooligomers readily when ATP is added (Kagawa et al.,
2003), whereas the α subunit forms homooligomers only to a limited extent and
the γ subunit does not oligomerize on its own into larger complexes. This
somewhat varies to the chaperonins of other species. For example the α subunits
from *T. acidophilum* and from *A. tengchongensis* do form stable complexes of
either eight or nine-fold symmetry (Waldmann et al., 1995; Zhang et al., 2013). A
common theme for thermostability and the propensity to oligomerize seem to be
the extreme N- and C-termini of the subunits (Luo and Robb, 2011; Zhang et al.,
2013). While the β subunit has an extended N-terminus, the α subunit has an
extended C-terminus, but the γ subunit has neither (Figure S6A), and this might
explain its lack of self-oligomerization. The structure of the chaperonin subunit
from the psychrophilic Antarctic methanogen *Methanococcoides burtonii* is the
only example of a monomeric chaperonin structure and the construct used for
structural analysis lacks its termini (Pilak et al., 2011).

Here we have also demonstrated that as soon as α subunits are added to β subunits,
mixed αβ complexes are formed. This implies that the α subunit must have a
greater affinity for the β subunit than the β subunit has for itself. Similarly, the γ
subunit must have the highest affinity to bind to the α subunit on one side and to
the β subunit on the other, since addition of the γ subunit to preformed αβ
complexes results in incorporation of the γ subunit. Interestingly the TF55 αβ
complex geometry differs to that found previously in the αβ thermosome from *T.
acidophilum* (Ditzel et al., 1998). In the *T. acidophilum* thermosome, subunits
homodimerize between the rings of the complex (Figure 1B, 6A) whereas in the
TF55 complex the subunits heterodimerize between the rings (Figure 1E, 6B).
This concept has interesting implications for the αβγ complex. When two αβ rings
form a complex, they can be arranged either to form homodimeric interactions (α-
α or β-β) between the subunits across the equator (as seen in the *T. acidophilum*
thermosome), or heterodimeric (α-β) interactions (as seen in the TF55 αβ
complex). This would be of little consequence provided that the number of
different subunits was two. However, when moving to a three subunit system, the
chiral nature of the rings dictates that not all subunits can homodimerize. Instead
only one subunit can homodimerize and the others must heterodimerize (Figure
1D, 4C, 6C). Thus the homodimeric α-α and β-β interactions between subunits in
opposite rings in the *T. acidophilum* thermosome would not easily accommodate
the incorporation of the γ subunit, whereas the αβ heterodimeric interactions in the
TF55 complex does. The same considerations apply to the eukaryotic CCT
complex: the chiral nature of the subunits dictates an antiparallel arrangement of
subunits between rings with two homodimers (subunits β and γ) along the two-fold
symmetry axis and the other subunits arranged as heterodimers clockwise in one
ring and anti-clockwise in the other (Figure 6D).
Conformations of subunits and complexes
Superposition of individual subunits from the protein data bank on their structurally conserved equatorial domains gives an indication of the conformational space that the subunits can occupy (Figure S4). Conformational changes fall into two classes; first there is the helical protrusion, which is the most flexible part of the structure that moves along a trajectory reminiscent of tentacles fishing for prey. The other movement is around a different axis at the interface of the intermediate and equatorial domains (Booth et al., 2008; Clare et al., 2008; Zhang et al., 2010). Comparison of all archaeal chaperonin subunit structures indicates that the α subunit of the αβlongC structure forms the most extended of all conformations seen so far and the β subunit the most closed one (Figure S4).

Opening and closing of the substrate chamber also involves relative movement of subunits and this is best looked at by superimposing subunits in context of the intact complexes (Figure 2C, 3C and S4). This confirms that the αβlongC structure is in an almost closed conformation, whereas the αβshortC complex is made of extreme open α subunits and closed β subunits. It is interesting to note that although the αβ complex was purified from source, without the addition of nucleotide, differences in nucleotide binding exist between the two crystal forms. Only the β subunit of the αβshortC crystal form contains ADP in the nucleotide binding site (Figure S7B) whereas all other subunits are nucleotide free. This implies that the two subunits have different nucleotide affinities and provides structural validation of previous biochemical analysis of the *T. acidophilum* thermosome (Bigotti and Clarke, 2005). Here eight molecules of ATP were seen to hydrolyze in a rapid first phase followed by a slow phase where a further eight molecules are hydrolyzed.

Whether the αβshortC structure represents a physiologically relevant conformation or not is unclear. However the conformation of the extended α subunit shows some interesting features that might point to possible interactions with substrates. The helical protrusion is partially unwound forming a β strand that contributes to an extended β sheet formed by the sensor loop of another α subunit and the C-terminus of the neighbouring β subunit (Figure 4C). These observations confirm that the secondary structure of the helical protrusion is weak and prone to unfolding, but in this case might also mimic a substrate that can be recognized by the sensor loop of another α subunit or *vice versa*. A similar conformation of the helical protrusion could be seen in an NMR-derived model of the apical domain of subunit CCT3 (Joachimiak et al., 2014). Here the helical protrusion has unwound to form a similar β strand. Residues at the base of the protrusion were suggested to participate in both substrate recognition and in modulating the conformation of the protrusion for substrate release upon lid closure. Our crystal structure may show this substrate recognition region in complex with a surrogate substrate, that being the hydrophobic sensor loop of the open α subunit. This region in itself has been implicated in substrate recognition (Skjaerven et al., 2015; Yebenes et al., 2011).

Allosteric networks and subunit cooperativity within complexes
Chaperonins are intricate molecular machines. GroEL has been shown to have negative cooperativity with one chamber preferentially closed by GroES and the other open, giving rise to “bullet”-shaped structures (Xu et al., 1997). Subunit interactions in GroEL across rings are out of register (i.e. one subunit in one ring contacts the gap in between two subunits in the other) and these interactions are
likely to confer the negative cooperativity between rings (Lopez et al., 2015; Skjaerven et al., 2015; Yebenes et al., 2011). The reaction cycle and cooperativity between subunits and rings in type II chaperonins is more complex and less clear. In contrast to type I chaperonins, subunits across the equator are in register suggesting different modes of cross-talk. However, in type II chaperonins with more than one subunit there are further differences: with two different subunits the subunits can either homodimerize across the equator (as in the *T. acidophilum* thermosome) or heterodimerize (as in the *Sulfolobus* TF55 αβ complex). As mentioned above, different subunits have been shown to have different nucleotide affinities and show different kinetics in ATP turnover (Bigotti and Clarke, 2008; Cong et al., 2012; Reissmann et al., 2012). The different geometries of subunits will therefore likely influence the kinetics of ATP hydrolysis and substrate refolding. This is enhanced in complexes with more than two subunits and the antiparallel arrangement of subunits in the TF55 αβγ complex and even more so in CCT will lead to very complex kinetics that is likely to be important for substrate-specific refolding (Cong et al., 2012; Lopez et al., 2015).

**Contributions**

DS and AGS designed and performed experiments, analysed data and wrote manuscript, JJC performed the majority of the biochemical experiments, CS refined X-ray structures and processed EM data, DA collected X-ray data, AW, BA, SS, SKMs and RAB collected and processed EM data.

**Acknowledgments**

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Coordinates and structure factors were deposited at the protein data bank. Accession codes: TF55 all-β: 4XCD, TF55 αβshortC: 4XCG and TF55 αβlongC: 4XCI. The 16 Å cryo-EM reconstruction obtained for the αβγ complex was deposited in the EMDDataBank, accession code EMD-6291.

**Experimental Procedures**
Purification of wild type TF55 complexes from S. solfataricus cells

_Sulfolobus solfataricus_ (DSM1617) cell paste was purchased from the University of Georgia fermentation facility. Cells were grown at 80°C, pH 3.7 and harvested at an OD_{600} of 1.2. The cell paste was kept at -80°C until use. TF55 complexes were prepared by using ion exchange and size exclusion chromatography as described in Supplemental Information.

Recombinant cloning and expression of TF55 subunits in _E. coli_

The genes of the α, β and γ subunits were amplified from _S. solfataricus_ genomic DNA and cloned into _E. coli_ expression vectors as described in Supplemental Information. Initial attempts to co-express the subunits (with the α subunit in MCS1 and the β subunit in MCS2 of a PETDUET expression vector) failed because of different expression levels and properties of the subunits. Expression of the α subunit is low (1.5 mg / L culture), whereas the β and γ subunits express at much higher levels (14 and 12 mg / L culture respectively). The β subunit forms insoluble inclusion bodies whereas the γ subunit is soluble. Heat shock treatment of _E. coli_ crude lysate containing insoluble β subunit denatures most _E. coli_ proteins whereas the β subunit becomes soluble (Figure S1). Once refolded by heat shock, the β subunit remains stable and in solution. All three subunits were individually purified to homogeneity as monomers (Figure S1) and mixed in equimolar amounts for complex formation (Figure S1) as described in Supplemental Information.

Crystallization of αβ and all-β complexes and structure determinations

_all-β complex:_ Both recombinant and native all-β complexes (PrepA) crystallized. Crystals of recombinant protein were easier to reproduce and therefore used for structure determination. Fractions from the Superose 6 column were concentrated to ~15 mg/ml and subjected to crystallization trials. Large single crystals (0.15 - 0.25 mm) grew in Linbro plates with 2 µl protein solution and 2 µl reservoir mixed in hanging drops over 500 µl reservoir containing 2 M sodium formate. These crystals were cryo-protected with 4 M trimethylamine N-oxide (TMAO), pH 8.0 before being plunge frozen in liquid N₂. A native data set was collected at beamline MX2 at the Australian synchrotron. Data was processed using XDS (Kabsch, 2010) and scaled using Aimless (Evans and Murshudov, 2013). Crystals appear to belong to space group P6₁22. However after careful analysis using Phenix xtriage (Adams et al., 2010) and reprocessing of the data (Collaborative_Computational_Project_Number_4, 1994), it became apparent that the crystals are twinned and instead belong to spacegroup P6₁, with a twinning fraction close to 50% and a twinning operator of K, H, -L. The structure was subsequently solved by molecular replacement with Phaser (McCoy et al., 2007) using the _A. tengchongensis_ chaperonin coordinates (pdb entry 3KO1, chain A, 84 % sequence identity) as the search model, finding 6 monomers in the asymmetric unit. This model was iteratively refined using Phenix/Rosetta (DiMaio et al., 2013) and refmac5 (Vagin et al., 2004), with manual model building using coot (Emsley and Cowtan, 2004) and final R-factors of R_{work}: 24.37% and R_{free}: 27.93% (Table 1). The nucleotide bound to the _A. tengchongensis_ structure was initially omitted from the model, but strong difference density (peaks >5 σ for all 6 sites) appeared in the known nucleotide-binding site during refinement. This
difference density was interpreted to be ADP (as in the *A. tengchongensis*
structure) and refined well (Table 1).

**αβ complex**: Wild type αβ complex (PrepB) crystallized more readily than recombinant αβ complex and was therefore used for structure determination. Fractions from the Superose 6 column were concentrated and subjected to crystallization trials. Large single crystals were produced using hanging drop Linbro plates with 1 μl protein solution at 3.3 mg/ml and 1 μl mother liquor consisting of 0.1 M Tris pH 7.9, 62.5 % PEG 2000, 10 % 2-propanol and 40 μM SrCl₂. Crystals were cryo-protected by gradual addition of 4 M TMAO, pH 8.0 to the drop over 20 mins, before being plunge frozen in liquid N₂. Data sets were collected at beamline MX2 at the Australian synchrotron. Data were processed to 3.0 Å and 3.75 Å (αβₙₒₙ₉ and αβₚₒᵣᵢₙ₉ respectively) using XDS and scaled using Aimless (merging two datasets taken from the same crystal at different positions in the case of the αβₚₒᵣᵢₙ₉ crystal) in space group *I*422. The data from the αβₙₒₙ₉ crystal form is severely anisotropic and an anisotropy corrected dataset from the Diffraction Anisotropy Server (Strong et al., 2006) was used truncating the *c* axis to 5.37 Å. The structure of each crystal form was solved independently by molecular replacement with Phaser using the coordinates of the *S. solfatarius* β subunit described above and the *A. tengchongensis* α subunit (pdb 3J1B, chain A, sequence identity 83 %) as individual search models, finding a single monomer of each in the asymmetric unit. These models were iteratively refined using Phenix/Rosetta and refmac5, with manual model building using coot and omit maps with final R<sub>work</sub> and R<sub>free</sub> of 22.83 % and 26.66 % for αβₙₒₙ₉ and 24.74 % and 29.20 for αβₚₒᵣᵢₙ₉ (Table 1). In both crystal forms the equatorial domain is very well ordered, and side chains are easily visible for the majority of residues (Figure S3D and S6B). However, the density corresponding to the apical domain is much weaker and the model presented may contain side chain errors in this region. Moreover, in the αβₙₒₙ₉ crystal form the apical domain of the α subunit was not visible even after the final stages of refinement and model building. The αβₙₒₙ₉ model was therefore truncated as shown in Table 1.

**Cryo-EM sample preparation and imaging (αβγ complex)**

Samples for cryo-EM were applied onto grids using a 3 μl droplet of the αβγ complex (at 2 mg/ml). The drop was deposited onto a (400-mesh) copper grid with holey carbon film (Quantifoil R2/2) that was glow discharged for 30s. Excess protein solution was blotted away using Whatman #1 filter paper and then rapidly plunged into liquid ethane to flash freeze it. Single particle EM data was collected on a JEOL 3200FS transmission electron microscope (JEOL, USA) equipped with a US4000 CCD detector (Gatan, USA). The images were recorded at 300 keV at liquid nitrogen temperature with a nominal magnification of 60,000x (calibrated magnification, 69,000x) corresponding to a pixel size of 2.174 Å.

**Particle selection, 2D class averaging and refinement**

Particle selection was performed using the *e2boxer.py* component of the EMAN2 software package (Ludtke, 2010). From an initial 32,240 particles in the dataset, 16,534 particles were used in the final reconstruction. The defocus for each of the CCD frames was calculated using the program CTFIND3 to assess drift, astigmatism and used for CTF correction. The EMAN program CTFIT was used for flipping the phases in each of the micrographs. An initial model was generated
with startcsym program imposing a C3 symmetry that was then refined for 8 iterations using refine algorithm of EMAN. Later, the multi-refine program was used to sort the particles in the dataset. The highest quality particles were extracted from the dataset and refined to a final resolution of 16 Å.

References


Evans, P.R., and Murshudov, G.N. (2013). How good are my data and what is the resolution? Acta crystallographica 69, 1204-1214.


Figure 1. Chaperonin architecture across taxonomic systems. Type I chaperonins (GroEL, HSP60, cpn60) consist of 14 copies of the same 60 kDa subunit arranged in a cylindrical particle with two seven-membered rings arranged back to back (A). Type II chaperonins (archaea: thermosomes; eukaryotic cytosol: CCT) generally consist of two eight-membered rings; thermosomes typically have two evolutionarily related (paralogous) subunits that are arranged alternately within one ring (B), eukaryotic CCT/TRiC has eight paralogous subunits per ring (C). Sulfolobales have three paralogous subunits that as we show here can form three types of complexes: two species of nine-membered rings (either homomeric all-β or heterotrimeric αβγ) arranged in pairs to form octadecameric complexes (D and F), or hexadecameric αβ complexes (E).

Figure 2. S. solfataricus TF55 all-β chaperonin structure. (A) Cartoon representation of the biological unit. β subunits are shown in blue and nucleotide in yellow. (B) Cartoon representation of an individual β subunit with domains labelled; SL: sensor loop, Ct: C-terminus. See also Figures S1, S2, S3, S7 and Table S1.

Figure 3. S. solfataricus TF55 αβ chaperonin crystal structures. Cartoon representation of the biological unit of the αβ_longC and αβ_shortC hexadecameric structures (A and B respectively). β subunits are shown in blue, α subunits in green and nucleotide in yellow. (C and D) Silhouette of individual subunits with respect to the closed hexadecameric structure (pdb1A6D - darker grey) and open hexadecameric structure (pdb3IZH – lighter grey). The subunits were superposed to the biological complex (Figure S11 and S12), rather than individual subunits (Figure S11), so that the relative position within the complex as well as conformational changes can be assessed. See also Figures S3, S4, S6 and S7, and Table S1.

Figure 4. Crystal contacts between the α subunits of the αβ_shortC crystal structure. (A and B) Silhouettes of the α subunits show how the different contacts cause a large change in the c axis dimension between the αβ_longC (A) and the αβ_shortC (B). (C) Cartoon representation of the major crystal contact of the αβ_shortC structure; the helical protrusion (HP) has been partially unwound into a β-strand to extend the β-sheet formed by the sensor loop (SL) and the C-terminus (Ct) of α and β subunits of an adjacent complex. See also Movie S1.

Figure 5. Cryo-EM reconstruction of the αβγ complex. (A) 3D reconstruction of the recombinant αβγ complex to 16 Å resolution in side and top views (grey density). One set of three symmetry-unrelated subunits (labelled 1,2,3) is highlighted in a red box (B) Rigid body docking of the symmetrical all-β 18-mer structure into the EM density. The α and β equivalent subunits are coloured light or dark cyan (indicating they cannot be assigned) and the γ subunit across the two-fold symmetry axes is coloured in red. See also Figures S5.
Figure 6. Mercator projections of chaperonin complexes. Planar projections (in analogy to (Cong et al., 2012)) highlight the differences in subunit arrangement within chaperonin complexes. (A) thermosome [α - green and β - blue] (B) αβlongC [α - green and β - blue] (C) αβγ model [α/β - light/dark cyan, γ - red] and (D) CCT [α - yellow, β - red, γ - white, δ - orange, ε - green, ζ - black, η - indigo and θ - purple] (pdb - 4v94). Two-fold symmetry axes are indicated. Note the different locations within the T. acidophilum and S. solfataricus structures, causing the differences in subunit arrangement.
Figure 1

A: Bacteria: GroEL
    1 subunit
    72 symmetry

B: Archaea: Thermosome
    2 different subunits
    42 symmetry

C: Eukarya: CCT
    8 different subunits
    2-fold symmetry

D: Sulfolobus TF55
    3 different subunits
    temperature dependent symmetry

60°C

E: 75°C

F: 85°C
### Data Collection and Refinement Statistics

#### Table 1:

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</table>

| Refinement Statistics | | | |
|-----------------------|------------------|------------------|
| Resolution range (Å)  | 64.93-3.79 (3.89-3.79) | 41.91-3.00 (3.12-3.00) | 47.60-3.74 (4.03-3.74) |
| No. of reflections R_work/R_free | 34788/3645 | 24929/1273 | 14206/718 |
| Data completeness (%) | 71 (18) | 73 (20) | 100 (99) |
| Atoms (protein/ligands) | 22955/162 | 11776/0 | 15026/27 |
| R_work (%) | 24.9 (33.5) | 22.8 (32.7) | 22.7 (25.9) |
| R_free (%) | 27.9 (35.8) | 26.7 (40.1) | 28.5 (32.8) |
| Rmsd bond length (Å) | 0.006 | 0.004 | 0.003 |
| Rmsd bond angle (°) | 0.978 | 0.761 | 0.642 |
| Mean B value (Å²) | 178 | 95 | 121 |
| Ramachandran plot (%) | 96.6/3.4/0.0 | 94.9/4.6/0.5 | 94.7/5.1/0.1 |
| Maximum likelihood coordinate error | 0.37 | 0.39 | 0.54 |
| Missing residues | T7 tag and linker, 1-29, 255-256, 541-557 | | |
| Alpha chain: 1-16, 166-174, 203-394, 531-559 |
| T7 tag and linker, 1-29, 255-256, 541-557 | | |
| Alpha chain: 1-18, 251-267, 532-559 |

* Data for the outermost shell are given in parenthesis
* As suggested by Aimless (Evans and Murshudov, 2013).
* As defined by MolProbity (Chen et al., 2010).
FIGURE S1. Recombinant expression and purification of TF55 complexes (related to Figure 2).

(A) Vectors for heterologous expression of TF55 subunits in E. coli. (B) SDS PAGE of soluble (S) and insoluble (I) fractions after centrifugation of crude lysate. Left: no heat treatment shows large amounts of the β subunit in the insoluble fraction (red square). Right: 10 min of heat treatment at 65°C denatures most E. coli proteins and shifts the β subunit towards the soluble fraction (red square). This is confirmed in the Western blot shown on the right, using anti-T7 antibody. (C) Purification of α, β and γ monomers and complexes. (A) Lane 1: SeeBlue Plus2 marker, Lane 2: concentrated α subunit before size exclusion, Lane 3+: fractions from size exclusion (Superdex 75). (B) same for the β subunit. (C) same for the γ subunit. (D) Left: OD_{280} chromatogram of all-β complex from size exclusion column (Superose 6). Middle: same for αβ complex. Right: same for αβγ complex. “m”: monomer peaks, “ATP”: excess ATP.
FIGURE S2. Temperature stability and denaturation of TF55 monomers and complexes (related to Figure 2).

(A) Thermal melt curves for isolated α, β, and γ subunits. (B) The β and γ subunit mRNA levels from S. shibatae cultures grown at 76°C and incubated for 20 h at 60°C followed by incubation at the indicated temperatures for 30 min from (Kagawa et al., 2003). (C) Relative α, β, γ subunit ratios in vivo. (D) Blue native PAGE of subunits mixed with either each other and/or ATP as depicted. (E) Native PAGE Western blot; tag bearing subunit probed by antibody is underlined (recombinant α: His-tag; recombinant γ: T7 tag; r: recombinant; wt: wild-type). Taken together this suggests that the upper band in lane 9 (red box) consists of only the β subunit. (G-J) Schematic explanation of findings (F) Left: Denaturing SDS PAGE of purified (αβ)γ heterotrimeric complex after 10 min heat treatment at increasing temperatures as indicated. Right: Western blot of the same sample using anti-T7 antibody to monitor the ratio of recombinant γ subunit at increasing temperatures. (G) Corresponds to Fig. S3D lane 6. (H) Corresponds to Fig. S3D lane 5. (I) Corresponds to Fig. S3E lane 4. (J) Corresponds to Fig. S3E lane 5.
FIGURE S3. Crystal packing and density (related to Figures 2 and 3).

(A-C) PyMol (Schrödinger LLC) rendered arrangement of molecules in the crystals; The α subunit is shown in green and the β subunit in blue. The crystallographic c axis is running from left to right in the left panels and perpendicular to the page in the right panels. (A) all-β, (B) αβ_shortC and (C) αβ_longC. (D) Electron density around residues 19-39 of the αβ_shortC crystal structure. 2FoFc map contoured at 1 σ.
The protein data bank was mined for structures with similar sequence to TF55 subunits and all available archaeal type II chaperonin structures were superimposed (Table S1). (A) Each subunit was individually superimposed onto the “heel” (grey box) of the $\alpha\beta_{\text{shortC}}$ subunit. The $\alpha\beta_{\text{shortC}}$ subunit is shown in green, the $\alpha\beta_{\text{shortC}}$ subunit in blue. The previous most open conformation (PDB 3IZH chain A) is shown in yellow cartoon and the $T.\ acidophilum$ $\beta$ (PDB 1A6D) subunit is shown in red. The right panel is 90° rotated from the left panel. (B) Superposition of subunits in the context of the intact complexes they belong to. The structures of the closed $T.\ acidophilum$ thermosome (1a6d; red monomer within black complex), TF55 $\alpha\beta_{\text{longC}}$ $\beta$ subunit (lighter blue, lighter grey complex), TF55 $\alpha\beta_{\text{shortC}}$ $\beta$ subunit (darker blue, lighter grey complex), $M.\ maripaludis$ open D386A cpn complex (3izh; yellow monomer, lighter grey complex), TF55 $\alpha\beta_{\text{shortC}}$ $\alpha$ subunit (green, lightest grey complex).
FIGURE S5. Symmetry analysis of the complexes by electron microscopy (related to Figure 5).

(A) Negative stain electron microscopy of recombinant all-β complex shows exclusively nine-fold symmetrical particles. Approximately 800 particles were picked manually and classified. The class average shown contains ~100 particles. (B) cryo-EM of wildtype “PrepB”. ~4500 particles were picked automatically and subjected to 2D classification revealing an eight-fold object. (C) same grid preparation as (B), however ~1000 top views were picked manually. Classification revealed top views with both eight-fold and nine-fold symmetry, with the eight-fold class containing 622 particles and the nine-fold containing 37 particles (corresponding to 5.6%). (D) cryo-EM of the αβγr complex shows a nine-fold symmetrical object with ~3700 particles picked automatically. (E) Symmetry determination of the 16 Å cryo-EM reconstruction. Initial reconstructions were obtained via multirefine in EMAN (Ludtke, 2010). The D3 symmetry resulted in the highest quality map at this stage and was further refined. (F) Fourier Shell Correlation (FSC) based determination of resolution of the final map using a cutoff value of 0.5 (Chen et al., 2013).
FIGURE S6. TF55 subunit sequence alignment and confirmation of assignment (related to Figure 3).

(A) Sequence alignment (http://www.ebi.ac.uk/Tools/msa/clustalw2/) edited with ALINE (Bond and Schuttelkopf, 2009) showing the secondary structure of the $\alpha$ subunit from the $\alpha\beta_{\text{longC}}$ structure on top and the sequence identities on the bottom right. Major differences in between the $\alpha$ and $\beta$ subunits that were used to confirm the correct assignment of the subunits in the electron density are highlighted in yellow. Locations of peaks in an anomalous difference density map of a platinum derivative are highlighted in orange. (B) Omit maps of highlighted residues (5% omitted, 5000K annealing, 1 $\sigma$) (C) Anomalous difference density of a low resolution data set (10 Å resolution cut off, contoured at 5.5 $\sigma$) of a Dichloro(ethylenediamine)platinum(II) derivative. Left: peaks within asymmetric unit, centre: $\beta$ Met512, right: $\alpha$ Met 34.
FIGURE S7. The nucleotide binding sites of TF55 subunits (related to Figure 2 and 3).

Corresponding intact structures are depicted on the left. Model shown in stick representation and 2FoFc electron density map contoured at 1 σ in grey. (A) αβ_{longC}; the β subunit is shown in blue and the α subunit in green. (B) αβ_{shortC}; the β subunit is shown in blue, ADP in yellow and the α subunit in green. (C) all-β; chain A is shown in blue and ADP in yellow.
### Table S1: List of chaperonin monomers used in Figure S4 (related to Figure 2 and 3).

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Supplementary Movie 1. Morph between TF55 $\alpha\beta_{\text{short}C}$ and $\alpha\beta_{\text{long}C}$ crystal structures (relates to Figure 4).

Cartoon representation showing how the helical protrusion partially unwinds into a $\beta$-strand to extend the $\beta$-sheet formed by the sensor loop.
Supplementary Experimental Procedures:

Purification of wild type TF55 complexes from *S. solfataricus* cells

TF55 complexes were prepared by resuspending 20g of cell paste in 350 ml of *S. solfataricus* lysis buffer (50 mM Tris/Cl pH 8.0, 5 mM MgCl₂, 0.001% PMSF, 100 mM NaCl) to which 1% dodecyl maltoside (DDM) was added before sonication (3 min, 1 sec pulses, scale 7). The lysate was then centrifuged in a Beckman 45Ti rotor (100,000 × g, 30 min, 4ºC) and the supernatant applied to a 26/20 Q-HiLoad column (GE Life Sciences) equilibrated in 10% buffer B (buffer A: 20 mM Tris/Cl pH 8.0, 2 mM MgCl₂, 1 mM EDTA, 0.001% PMSF, 0.05% DDM; buffer B: same as A with 1 M NaCl). After washing the column in 10% buffer B a 400 ml 10-50% buffer B gradient was applied. Fractions containing TF55 were pooled, concentrated to less than 10 ml and applied to a 320 ml HiPrep S300 column equilibrated in buffer C (20 mM Tris/Cl pH 8.0, 2 mM MgCl₂, 1 mM EDTA, 150 mM NaCl). Two peaks eluted, one at high molecular weight containing the αβ complex and a second one at much lower molecular weight containing the β monomer. These separate peaks were pooled, and in the case of the β monomer ATP was added to a concentration of 2 mM in order to form the complex. Both samples were concentrated to 0.5 ml and applied separately to a Superose 6 10/300 GL column equilibrated in buffer C. The fractions containing the β (PrepA) and the αβ (PrepB) complexes were pooled and concentrated for further use.

Recombinant cloning and expression of TF55 subunits in *E. coli*

The α subunit was cloned into the first multiple cloning site (MCS1) of a pETDUET vector (Novagen) with an N-terminal hexahistidine-tag and ampicillin resistance. Both the β and γ subunits were cloned into pET24a (Novagen) with a C-terminal T7-tag and kanamycin resistance (Figure S1).

Purification of recombinant α, β and γ subunits

All vectors were transformed into T7 express/BL21 cells. 1 L of LB medium in a 2 L baffled flask was inoculated with 1 ml of overnight culture and induced with 0.5 mM IPTG at OD₆₀₀ = 0.6, and then left shaking overnight at 37ºC. Cells were harvested in a 12 × 1 L Sorvall RC12BP centrifuge at 4,000 rpm for 30 mins and the pellet was frozen in liquid nitrogen and stored at -20ºC until further use.

Subunit α: 10 L of culture were typically used per preparation. Cells were resuspended in 100 ml buffer D (20 mM Tris/Cl pH 8.0, 2 mM MgCl₂, 1 mM EDTA, 50 mM NaCl, 20 mM imidazole) complemented with 2 Complete protease inhibitor tablets (Roche), 50 µl of 10 mg/ml lysozyme and 50 µl 2 mg/ml DNase, followed by lysis in a Constant Systems (Benchtop TS Series) continuous flow cell disruptor at 18,000 PSI. Heat incubation in a heat block (70ºC for 30 min) was followed by centrifugation at 27,500 × g for 30 min at 4ºC. The supernatant was applied to a nickel NTA column, eluted with a linear gradient of buffer D to buffer E (buffer D with 1 M imidazole). Fractions were analyzed by SDS PAGE. Subunit α containing fractions were concentrated and applied to a Superdex 200 10/300 GL column equilibrated in buffer F (20 mM Tris/Cl pH 8.0, 2 mM MgCl₂, 1 mM EDTA, 150 mM NaCl).

Subunit β: 2 L of culture were typically used per preparation. After resuspension and lysis in 50 ml buffer G (20 mM Tris/Cl pH 8.0, 2 mM MgCl₂, 1 mM EDTA), the suspension was incubated at 75ºC for 30 min. The supernatant was then applied to a 50 ml Q-sepharose HiLoad column, washed in buffer G and eluted in an 8 column volume gradient from 0% to 50% buffer H (20 mM Tris/Cl pH 8.0, 2 mM MgCl₂, 1 mM EDTA, 1 M NaCl). Subunit β containing fractions were concentrated and then purified by gel filtration (as above) in buffer F.

Subunit γ: Typically 1 L of culture was used per preparation. After resuspension in 30 ml buffer G cells were lysed (as above) and then subjected to heat incubation at 65ºC for 20 min. The supernatant of a centrifugation at 27,500 × g, 30 min, 4ºC was then applied to a Q-sepharose column followed by gel filtration as described above for the purification of the β subunit.
Heat treatments and refolding of subunit β

Subunit β bearing pET24a vector was transformed into E. coli T7 express BL21 cells. 10 ml Luria Bertani medium (LB) was inoculated with a single colony, grown at 37°C, induced at OD_{600} = 0.5 with 0.5 mM isopropyl thiogalactoside (IPTG) and further grown overnight. Cells were harvested at 2,600 × g and lysed in 1 ml buffer C (supplemented with 10 µl lysozyme (10 mg/ml) and 10 µl DNase (2 mg/ml) by freeze thawing in liquid nitrogen (4x). The lysate was then split in half. The first half was spun at 27,500 × g for 10 min, the other half was heat treated for 30 min at 65°C, spun at 27,500 × g for 10 min and the soluble and insoluble fractions were loaded on SDS PAGE and compared by Western blot analysis using HRP coupled anti-T7 antibody (Figure S2).

Complex formation

All samples were concentrated prior to complex formation and equimolar amounts of protein were added to form the complexes. For the αβγ heterotrimeric complex the γ subunit was added first, then the α subunit, and subunit β last, in an attempt to avoid αβ and β complex formation. To make the complexes, 25 mM MgCl₂ and 5 mM ATP (in that order) were added to the solution containing the protein. For all complexes upon addition of ATP a cloudy precipitate forms that quickly dissolves when mixing gently with a pipette. The complexes were then separated from remaining monomers using a Superose 6 10/300 GL column equilibrated in buffer F. Mixed αβ and all-β complexes were made accordingly (Figure S1D).

Thermal melt curves

SYPRO orange was used to monitor denaturation of subunits upon heat treatment (Figure S2A). The dye becomes fluorescent when bound to hydrophobic patches of the protein as it denatures. Fluorescence was measured in triplicate using a real–time PCR machine (LightCycler 480 II, Roche) over a temperature gradient from 20 to 99°C. Each reaction contained 1 µl SYPRO Orange (20X), 5 µl protein (at 1 mg/ml) and 14 µl of buffer (20 mM Tris/Cl pH 8.0, 150 mM NaCl).

Heat stability of complexes

Purified complexes were subjected to heat treatment in a PCR machine at varying temperatures for each complex. 20 µl of sample of each complex at 1 mg/ml was heated for 10 min and then centrifuged at 27,500 × g for 10 min to separate soluble and insoluble material. Soluble fractions were run on SDS PAGE and stained by Coomassie brilliant blue or Western blotting using either anti His-tag (subunit α) or anti T7-tag (subunit β or γ) HRP-coupled antibodies (Figure S2).

Cryo-EM sample preparation, imaging, particle selection and 2D class averaging and refinement (αβ and αβγ’ complexes)

CryoEM data shown in Figure S5 was collected on a FEG transmission electron microscope (Arctica, FEI Company) equipped with a direct electron detector (Falcon II, FEI Company). The nominal magnification was 53,000× corresponding to an object pixel size of 2.0 Å. Single particle class averages, shown in Figure S5, were processed in Xmipp (Scheres et al., 2008).
Supplementary References


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