

# Conformational Changes in the Chaperonin GroEL: New Insights Into the Allosteric Mechanism

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Conformational changes are known to play a crucial role in the function of the bacterial GroE chaperonin system. Here, results are presented from an essential dynamics analysis of known experimental structures and from computer simulations of GroEL using the CONCOORD method. The results indicate a possible direct form of inter-ring communication associated with internal fluctuations in the nucleotide-binding domains upon nucleotide and GroES binding that are involved in the allosteric mechanism of GroEL. At the level of conformational transitions in entire GroEL rings, nucleotide-induced structural changes were found to be distinct and in principle uncoupled from changes occurring upon GroES binding. However, a coupling is found between nucleotide-induced conformational changes and GroES-mediated transitions, but only in simulations of GroEL double rings, and not in simulations of single rings. This provides another explanation for the fact that GroEL functions a double ring system.

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## Introduction

The bacterial chaperonin GroEL and its cofactor GroES are among the best characterised molecular chaperones (Fenton & Horwich, 1997; Martin & Hartl, 1997; Horovitz, 1998). X-ray studies (Braig *et al.*, 1994, 1995; Boisvert *et al.*, 1996; Xu *et al.*, 1997) combined with electron microscopy (EM) studies (Langer *et al.*, 1992; Ishii *et al.*, 1992; Chen *et al.*, 1994; Roseman *et al.*, 1996; White *et al.*, 1997) have provided insight into the functional cycle of this chaperonin. GroEL is active as a double heptameric ring (Hendrix, 1979; Hemmingsen *et al.*, 1988), with each ring containing a large central cavity in which substrate protein can be bound (Langer *et al.*, 1992; Braig *et al.*, 1993). The co-chaperonin GroES also exists as a heptamer and adopts a dome-like structure (Hunt *et al.*, 1996)

that can bind to either GroEL ring to form a cap on the central cavity (Chandrasekhar *et al.*, 1986; Saibil *et al.*, 1991; Langer *et al.*, 1992). Figure 1 shows the asymmetric crystal structure of GroEL with GroES bound to one GroEL ring (Xu *et al.*, 1997), showing the packing of the subunits in the assembly and the topology of each subunit.

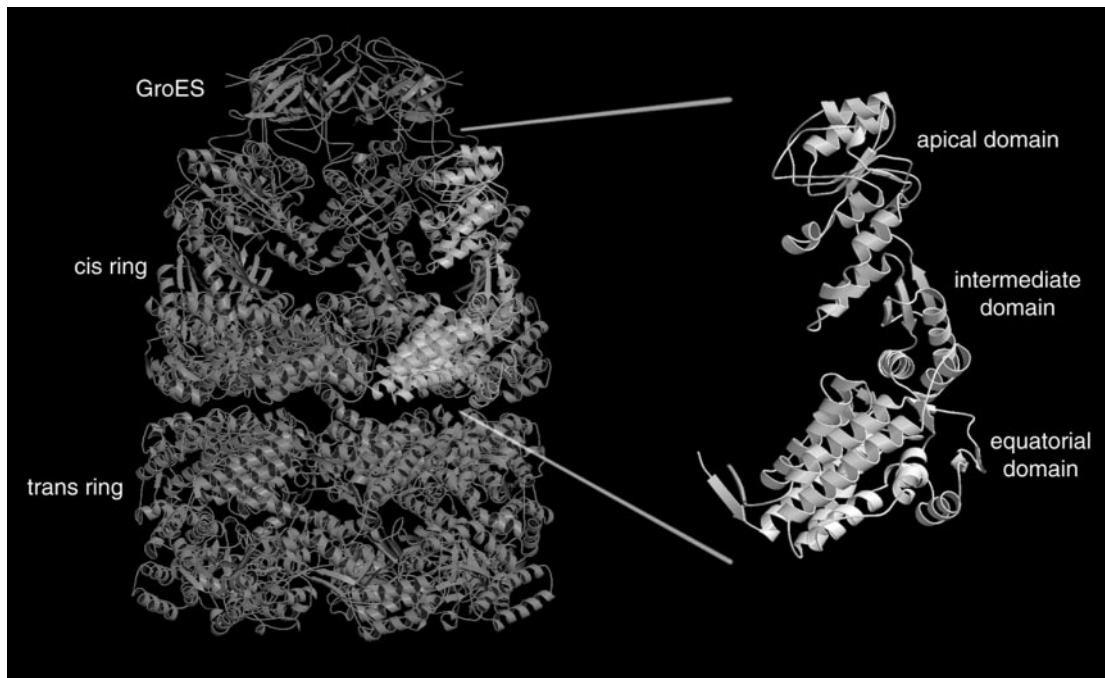
Each subunit of GroEL can be subdivided in three domains (Braig *et al.*, 1994; see Figure 1). The equatorial domains form the backbone of the protein and contain an ATP binding site; they are involved in most intra-ring and all inter-ring subunit contacts. The apical domains are involved in interactions with substrate protein and GroES. The third domain, termed intermediate domain, forms the link between the apical and equatorial domains.

The role of GroEL in the substrate folding process is twofold. First, GroEL prevents substrate proteins from aggregating by binding unproductive folding intermediates and forces them to unfold to states more committed towards correct folding (Weissman *et al.*, 1994; Ranson *et al.*, 1995; Zahn *et al.*, 1996; Corrales & Fersht, 1996; Buckle *et al.*, 1997). Second, it has been proposed that the central cavity works as an Anfinsen cage in which the substrate protein is actively folded (Weissman *et al.*, 1996; Mayhew *et al.*, 1996). The dramatic conformational changes that are involved in the

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Abbreviations used: EM, electron microscopy; MD, molecular dynamics; ED, essential dynamics; CT1, conformational transition 1; SR, single ring; DR, double ring.

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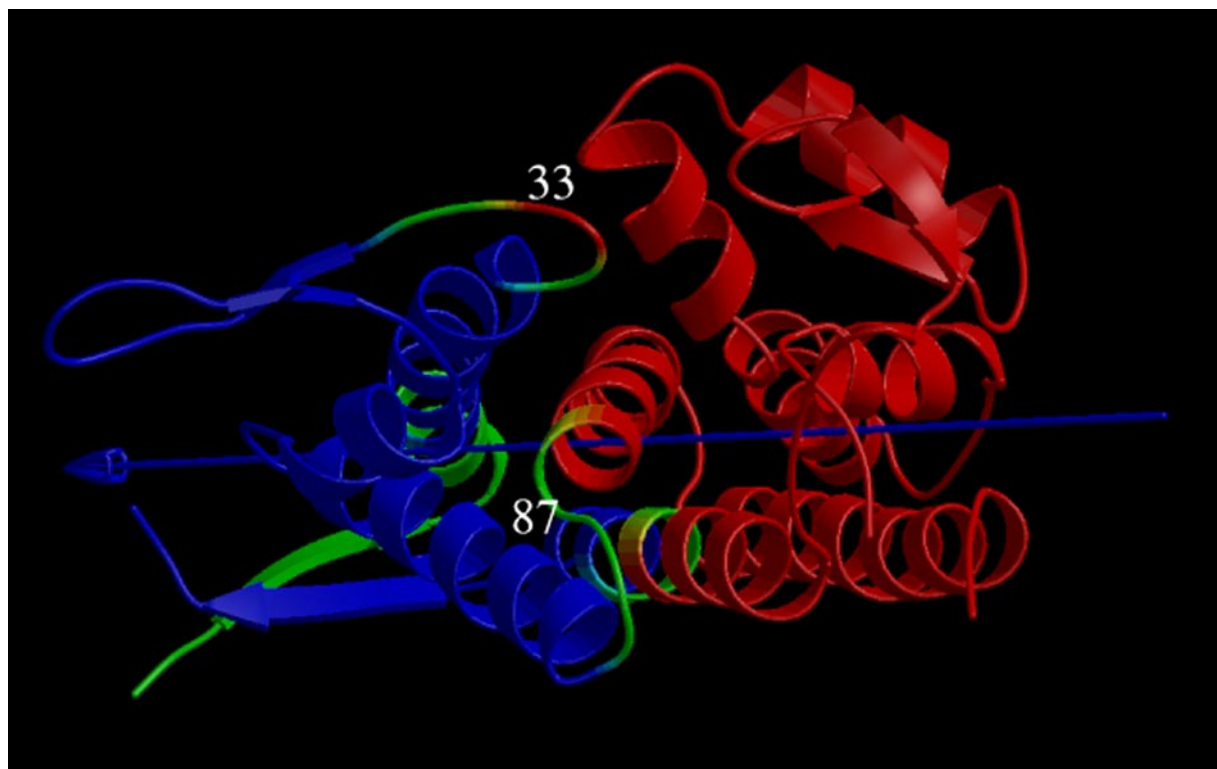
**Figure 1.** Left, the structure of the GroEL/GroES complex (Xu *et al.*, 1997). Right, the topology of a single GroEL subunit. The Figure was generated with Molscript (Kraulis, 1991; Esnouf, 1997) and Raster3D (Merritt & Bacon, 1997)

functional cycle of GroEL are indicative of a highly mobile system and stress the relevance of this flexibility for its biological activity.

GroEL is an allosteric protein. ATP binds cooperatively to the subunits of one ring (Gray & Fersht, 1991; Bochkareva *et al.*, 1992; Jackson *et al.*, 1993), triggering a conformational change that reduces substrate affinity (Staniforth *et al.*, 1994; Yifrach & Horovitz, 1996) in the ATP-bound ring. GroES binding to the ATP-bound ring has been reported to complete this conformational change (Roseman *et al.*, 1996). GroES binding switches the interior surface of the cavity from hydrophobic to hydrophilic, triggering a conformational change in the bound substrate molecule (Xu *et al.*, 1997). Negative co-operativity between rings (Yifrach & Horovitz, 1994, 1995, 1996) also results in a reduced GroES affinity in the ring opposite to the GroES-bound ring. ADP binding to one of the rings does not impair ATP or GroES binding to the other ring (Kad *et al.*, 1998), but ATP binding and hydrolysis in one ring has been proposed to play a role in GroES and substrate release from the other ring (Todd *et al.*, 1994). Communication between the two rings must be responsible for this effect, as supported by the observation that a mutant that impairs dimer formation is defective in GroES release (Weissman *et al.*, 1995, 1996; Llorca *et al.*, 1997b), thereby blocking bound substrates from leaving the GroEL cavities. On the other hand, under different conditions (higher KCl concentration), productive folding has been observed in this single ring mutant (Hayer-Hartl *et al.*, 1996).

Despite the wealth of available experimental information, some aspects of the conformational

changes and allosteric mechanism of GroEL remain unresolved. Knowledge of the mechanism underlying these conformational changes would greatly facilitate interpretation of a number of experimental results. Therefore, we have studied the conformational fluctuations in GroEL, with the aim to learn more about the mechanism(s) that govern these fluctuations. The most common method to study conformational fluctuations in proteins is molecular dynamics (MD), but with a molecular mass of 800 kDa it would be an impossible task to reach biologically relevant time-scales when realistic force-fields are being used. A number of methods exists to speed up the efficiency of conformational sampling in MD (Berne & Straub, 1997; Schlick *et al.*, 1997), and other computational techniques are also available. Ma & Karplus (1998) recently performed normal mode calculations on a minimal subsystem (three subunits) of GroEL that could provide insight into its allosteric mechanism. We have chosen to use CONCOORD (De Groot *et al.*, 1997), which is a method to generate different protein conformations based on distance restrictions. This method has been shown to yield low frequency collective fluctuations for proteins very similar to those that can be extracted from MD simulations, but at a dramatically reduced computational expense (De Groot *et al.*, 1997). To study the allosteric mechanism of GroEL, CONCOORD simulations have been performed of complete GroEL and GroEL/GroES assemblies starting from the different experimentally determined GroEL conformations.



**Figure 2.** Illustration of the main structural changes within the equatorial domains. The results are obtained from DYNDOM (Hayward & Berendsen, 1998) based on an ED analysis of X-ray structures of the equatorial domain. The equatorial domain can be considered to consist of two subdomains (in red and blue). The residues that form the transition regions between the domains are coloured green. The arrow indicates direction of rotation of the red domain relative to the blue domain by the thumb rule of the right hand. The loops containing residues 33 and 87, which are known to interact directly with the nucleotide, are situated at the flexible interface between the domains. The Figure was generated with Molscript (Kraulis, 1991; Esnouf, 1997) and Raster3D (Merritt & Bacon, 1997).

## Results

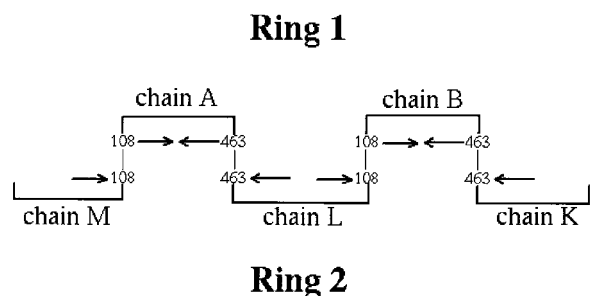
### Conformational changes in the equatorial domain

An essential dynamics (ED) analysis of conformations of single subunits extracted from the different experimentally determined structures confirmed the observations by Xu *et al.* (1997) that domain motions occur upon GroES binding. Two modes of collective fluctuation were found to dominate the conformational transitions of isolated subunits. The first, most prominent, mode describes differences between subunits extracted from the *cis* and *trans* rings from the asymmetric GroEL/ADP/GroES complex. Apical domains make a rotation of about  $90^\circ$  with respect to the intermediate domains, while the equatorial domains are involved in a closure motion of about  $30^\circ$  with respect to the intermediate domains (see Figure 2(c) by Xu *et al.*, 1997). The second mode displays the largest difference between the rings from the ATP $\gamma$ S-bound structure and the other structures. Internal fluctuations within the equatorial and apical domains dominate along this second mode.

In contrast to the structural changes of the domains with respect to each other, the internal fluctuations of the equatorial domain are for a

large part similar along the first and second mode. Residues involved in nucleotide binding show large displacements along this common mode, suggesting that structural changes necessary to accommodate ATP (or to a lesser extent ADP or analogues) dominate the internal dynamics of the equatorial domains (Figure 2). Along this common mode, the DYNDOM method (Hayward *et al.*, 1997; Hayward & Berendsen, 1998) identifies two subdomains. The first subdomain consists of residues 12-30, 37-83, 510-521 and the second subdomain of 32-34, 90-137, 411-506. Several residues directly involved in binding nucleotide (Val31-Pro33, Asp87, Thr91; Boisvert *et al.*, 1996; Fenton *et al.*, 1994) are situated at the interface between the two subdomains (Figure 2). Both groups have two glycine residues in their proximity (32, 35 and 85, 88) that allow for the conformational flexibility needed to adapt to the structural constraints imposed by the bound nucleotide.

Both subdomains of the equatorial domain also exhibit internal fluctuations. The subdomain which is coloured red in Figure 2 contains the two regions forming the most extensive contacts with the other ring (around Ala108 and Ser463). Upon nucleotide and GroES binding, the distance between these inter-ring contact-forming residues changes significantly. In each subunit, the distance between the



**Figure 3.** Illustration of how the internal fluctuations of the equatorial domains may be involved in the negative co-operativity between the two GroEL rings. Two subunits (A and B) of ring 1 and three subunits (K, L and M) of ring 2 are shown (chain identifiers after Boisvert *et al.* (1996)). The arrows indicate displacements of the residues involved in inter-ring contacts (around residues 108 and 463). A displacement of the two main sites of inter-ring contacts in the subunits in one ring has to be compensated by a displacement in the opposite direction in the subunits of the other ring to preserve inter-ring contacts.

$C^\alpha$  atoms of residues Ala108 and Ser463 is more than 2 Å smaller in the subunits of the *cis* ring than in those of the *trans* ring in the asymmetric GroEL-GroES complex (Xu *et al.*, 1997). These internal fluctuations have a direct effect at the interface and could play a role in the communication between the rings. These observed changes are consistent with the known negative co-operativity between the two GroEL rings, as depicted in Figure 3. A motion of the residues around 108 and 463 towards each other in the equatorial domains of one ring must result in an opposite displacement in the other ring if the integrity of the interface is to be maintained. The largest displacements of the residues forming the inter-ring contacts are found to take place in the plane of the rings, but as Ma & Karplus (1998) pointed out, fluctuations perpendicular to this plane may also play a role in inter-ring communication.

The residues directly involved in inter-ring contacts show displacements both upon GroES binding and upon nucleotide binding (although with a smaller amplitude). The X-ray structures show a conformational change of the stem loop (Lys34 to Asp52) only between GroES-bound subunits and subunits from GroES-free rings. This stem loop displacement is correlated with the reorientation of the intermediate domain with respect to the equatorial domain. This stem loop displacement also induces a motion of the subunits with respect to each other, resulting in the *en bloc* tilt of the equatorial domains in the *cis* ring with respect to the *trans* ring that has been reported by Xu *et al.* (1997). It has also been suggested that the stem loop is involved in the co-operative binding of ATP (and accompanied tertiary structural changes) in one ring from normal mode analysis (Ma & Karplus, 1998). Our results suggest that these resi-

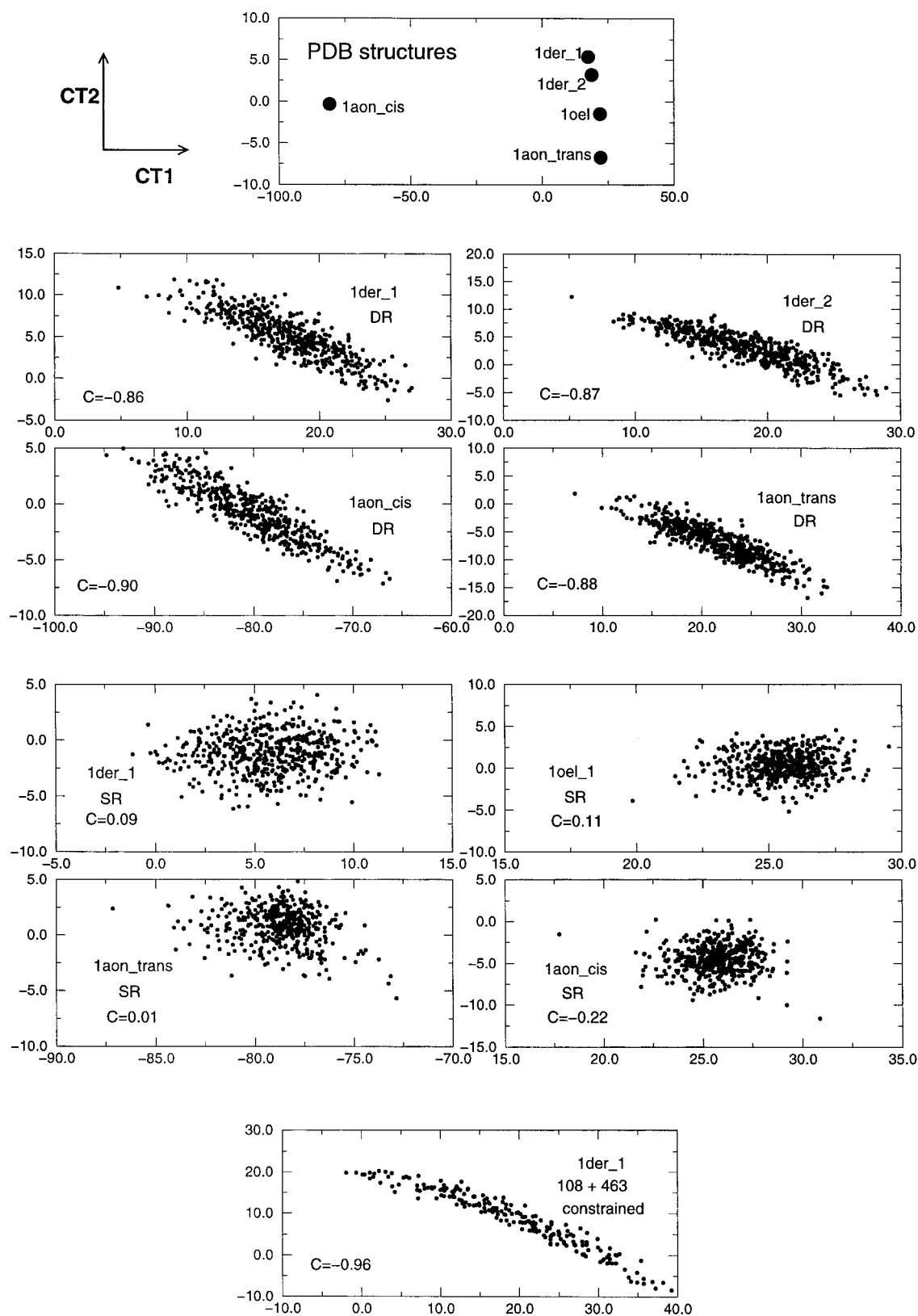
dues may be indirectly involved in inter-ring communication, in which the equatorial domains from one ring directly transmit structural changes associated with GroES binding (and to a lesser extent nucleotide binding) to the other ring.

### Overall structural changes

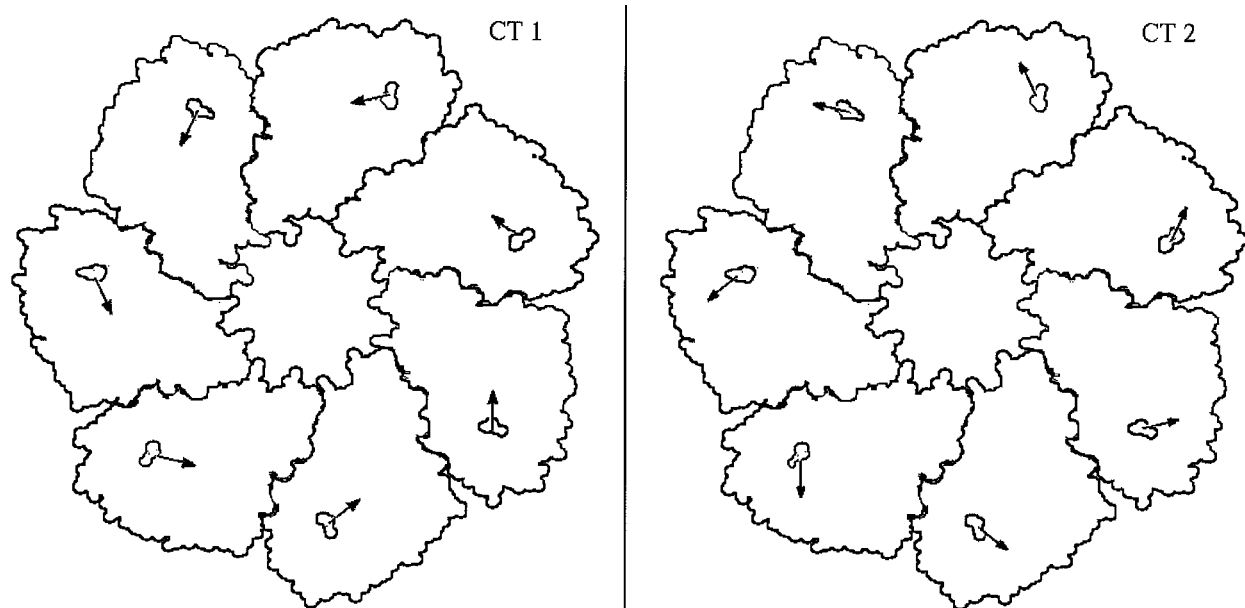
Analysis of crystallographic structures reveals dramatic conformational differences between GroES-free rings and GroEL rings bound to the cochaperonin GroES (Xu *et al.*, 1997). Previous comparisons between X-ray structures of free GroEL and GroEL bound to ATP $\gamma$ S showed much more modest conformational differences (Boisvert *et al.*, 1996). Figure 4 schematically shows the main conformational differences between the different experimentally characterised GroEL rings. The largest difference is observed between the GroES-bound *cis* ring and the different GroES-free rings (horizontal direction, first mode; from now on referred to as conformational transition 1 or CT1). The GroES-free rings differ most from each other along the mode with second-largest amplitude (CT2). The largest difference along CT2 is observed between the GroEL rings bound to ATP $\gamma$ S (pdb entry 1der; Boisvert *et al.*, 1996) and the other ring from the asymmetric GroEL-GroES complex (the *trans* ring of the complex, pdb entry 1aon). CT2 is likely to be connected with nucleotide binding and/or affinity, since it describes the main difference between the rings from the 1oel and 1der X-ray structures which only differ from each other by the presence of ATP $\gamma$ S.

X-ray structures of GroES-free rings have similar positions along CT1, indicating that conformational changes upon nucleotide binding are distinct from those upon GroES binding. The ring *trans* to the GroES-bound ring in the asymmetric GroES-bound structure is shifted with respect to the nucleotide-free symmetric GroEL structure along CT2 and not along CT1. GroES binding, therefore, causes a shift along the mode presumably connected with nucleotide binding (in the direction of nucleotide release) in the ring *trans* to GroES.

CONCOORD simulations starting from the different experimental structures sample both CT1 and CT2 with a significant amplitude (Figure 4) and are among the largest amplitude fluctuations in the simulations. Interestingly, there is a clear correlation between the fluctuation along CT1 and CT2 in the different double ring simulations (Figure 4). For GroES-free rings, this correlation links conformational changes in the direction of the change taking place upon GroES binding, with changes presumably happening upon nucleotide binding. Therefore, this connection between the two modes of conformational change displays a mechanism by which nucleotide binding in one ring would result in a conformational shift corresponding to a larger GroES affinity in the same ring.



**Figure 4.** Essential dynamics analysis of conformational differences between ring conformations obtained from different experimental (X-ray) structures. Projection of individual rings onto the CT1-CT2 plane. Upper panel, ring conformations from crystallographic structures. Next four panels, CONCOORD-generated double ring structures. Next four panels, CONCOORD-generated single ring structures. Lower panel, CONCOORD-generated single ring structures with the residues involved in inter-ring contacts (residues 108 and 463 were taken as representative) constrained. The values of C denote the correlation coefficient between the displacements along the two modes. SR, single ring; DR, double ring.



**Figure 5.** Schematic representation of the displacements of the inter-ring contact forming residues ( $C^{\alpha}$  displacements of residues 463 from each subunit were chosen as representative) along the two dominating modes of ring fluctuation. The point of view is along the cylindrical axis formed by the double ring. The arrows indicate the (exaggerated) displacements of residues 463 from each subunit upon GroES release (left, CT1) and nucleotide release (right, CT2).

No significant correlation is detected between CT1 and CT2 in the single ring simulations (Figure 4). Apparently, interactions between the rings induce a conformational restriction on both rings which accomplishes the coupling between the two modes. Indeed, when the effect of the CT1 and CT2 on the packing of the equatorial domains is examined in detail, a mechanism emerges which explains the coupling. In the equatorial domains, the major site of contacts with the other ring are formed by residues 461-467. Significant displacements of these residues are observed in both CT1 and CT2 (Figure 5). Looking along the cylindrical axis, the effect of a displacement along CT1 is an inward motion of these residues, whereas displacement along CT2 corresponds to an outward motion. Any steric restrictions that inhibit an overall inward or outward motion would therefore generate a coupling between CT1 and CT2.

To check if the observed coupling is a direct result of extra restrictions of the residues involved in inter-ring contacts in the double rings with respect to the single rings, a CONCOORD simulation was started on a single ring with these residues constrained. As can be seen in Figure 4, CT1 and CT2 are even more strongly coupled than in the case of the double ring simulations. This indicates indeed the existence of a mechanism that correlates CT1 to CT2 (GroES binding to nucleotide binding) in one half of a double ring, induced by restrictions formed by the other ring.

## Discussion and Conclusions

The results presented here provide new insight into the mechanism underlying the conformational changes of GroEL upon nucleotide and GroES binding.

First, an ED analysis of GroEL subunits extracted from X-ray structures shows that within equatorial domains, a direct effect on the inter-ring interface is observable upon both GroES and nucleotide binding which may play a role in the observed negative cooperativity between GroEL rings. This mechanism may enhance (or co-operate with) an earlier observation that nucleotide binding affects the Glu434-Lys105 inter-ring contact (Roseman *et al.*, 1996).

Second, an ED analysis of the crystallographic ring conformers has shown that structural changes that take place upon GroES binding are not an extension (completion) of the changes induced by ATP $\gamma$ S. Based on EM data, where larger overall structural changes are observed upon nucleotide binding, such a completion mechanism has been proposed (Roseman *et al.*, 1996). The crystal structure of the ATP $\gamma$ S-bound GroEL structure indicates that nucleotide can be bound to GroEL with only very modest changes in the nucleotide-binding pocket. These structural changes are also sampled in the CONCOORD simulations presented here, and are therefore likely to be significant. The conformational changes upon nucleotide binding in the equatorial domains are apparently not necessarily coupled to the larger, overall, nucleotide-induced structural changes as observed from EM

data, although such a coupling is likely to be present in the functional cycle of GroEL. Indeed, the overall structural changes that are observed from the crystallographic structures upon nucleotide binding (CT2) and upon GroES binding (CT1), are described by two perpendicular modes of motion, along which the displacements are not necessarily coupled. However, a coupling between the modes is observed in CONCOORD simulations of the double ring, correlating shifts towards GroES binding to shifts that happen upon nucleotide binding. Since such a coupling is not or hardly present in the simulations of single rings, this leads to the conclusion that the source of this coupling must be provided by the interface between the two rings. This finding is confirmed by the observation that this coupling is present in single ring simulations in which the inter-ring contact-forming residues are constrained. This coupling mechanism may provide an additional explanation for the fact that GroEL acts as a double ring. The double ring has previously been proposed to play a role in substrate release (Inbar & Horovitz, 1997; Behlke *et al.*, 1997), and to provide enhanced efficiency under stress conditions (Azem *et al.*, 1995; Llorca *et al.*, 1997a; Sparrer *et al.*, 1997).

Furthermore, the results show that CONCOORD, despite a few obvious restrictions, is a powerful tool for studying protein conformational freedom for molecular masses and time-scales that are currently beyond the scope of explicit dynamic simulation techniques.

## Methods

### CONCOORD simulations

Principal component analyses of MD simulations of proteins have indicated that collective degrees of freedom dominate protein conformational fluctuations (Garcia, 1992; Amadei *et al.*, 1993). These large-scale collective motions have been shown to be essential to protein function in a number of cases (Van Aalten *et al.*, 1995a,b; De Groot *et al.*, 1998). The notion that internal constraints and other configurational barriers restrict protein dynamics to a limited number of collective degrees of freedom has led to the design of the CONCOORD method to predict these modes without doing explicit MD simulations. The CONCOORD method has been described in detail elsewhere (De Groot *et al.*, 1997) and will only be summarised briefly here, together with some recent modifications.

The CONCOORD method of prediction of protein conformational freedom generates protein structures within a set of predefined distance bounds. Distance bounds are defined on the basis of interatomic interactions within the starting configuration of the protein, and the difference between upper and lower distance bounds depend on the strength of the interaction. A discrete number of categories of interactions has been defined, among which covalent bonds are the least flex-

ible, and weakly interacting non-bonded pairs have the largest freedom in distance. Starting from random coordinates, distance and chirality corrections are applied until all distances fulfil their distance bounds. Resulting structures are uncorrelated and hence the technique does not suffer from sampling problems as do techniques like MD in which such correlation is present.

Since the first implementation of CONCOORD (De Groot *et al.*, 1997), a number of improvements have been made.† First, the original algorithm which required all distances to be restricted has been modified to make the method suitable for large systems. Only the distances between atoms involved in pair interactions are now defined. In order to reach convergence, however, it is necessary to include a fixed number (typically  $20N$ , with  $N$  the number of atoms) of random pairs with significantly more distance freedom. This way, only up to a few per cent of the whole distance matrix needs to be evaluated. Second, categories of distance limits and the difference between upper and lower distance bound for each category were re-evaluated based on crystallographic conformers of T4 lysozyme as well as on distance fluctuations of a number of proteins in MD simulations. The parameters obtained in this way resulted in structures of slightly better quality than those obtained with the previous set. Finally, non-bonded pairs are defined in a different way, depending on the number of contacts within a group of residues. Isolated non-bonded interacting atom pairs will have more distance freedom (maximally 4 Å) than pairs which are part of an intensive network of interactions (e.g. pairs contained in clusters of more than 50 interactions maximally obtain 1.5 Å of distance freedom).

CONCOORD simulations were performed on each of the three currently available crystallographic double ring structures: the symmetrical (both rings are identical) nucleotide-free structure (pdb entry 1oel; Braig *et al.*, 1994, 1995), the pseudo-symmetric ATP $\gamma$ S-bound structure (the inter-ring contact plane is a plane of pseudo-symmetry; pdb entry 1der; Boisvert *et al.*, 1996), and the asymmetric ADP/GroES bound structure (one ring has ADP and GroES bound, the other is empty; pdb entry 1aon; Xu *et al.*, 1997). Additionally, isolated single rings extracted from each of these structures were simulated individually.

### Essential dynamics analysis

Essential dynamics analysis is equivalent to a principal component analysis of atomic displacements in an ensemble of structures (Garcia, 1992) and is related to the so-called "quasi-harmonic" analysis of protein motions (Levy *et al.*, 1984). In practice, ED involves diagonalisation of the covariance matrix of positional fluctuations (after removal of the overall rotation and translation). Resulting eigenvectors describe modes of collective fluctuation of which the corresponding eigenvalue is a measure of the mean square fluctuation along that mode (Amadei *et al.*, 1993).

ED analyses were applied to the ensemble of crystallographic structures to assess the main modes of collective fluctuation in GroEL. Ring conformational changes were analysed (inter-subunit fluctuations) by applying ED to the five unique ring conformations from the three double ring conformers determined by X-ray crystallography (the two rings of the unliganded GroEL structure 1oel are symmetry related). The 35 subunit conformations extracted from these structures were subjected to ED

† The latest version of the CONCOORD program is freely available from the internet (<http://rugmd0.chem.rug.nl/~degroot/conCOORD.html>).

analysis to study conformational changes within subunits (intra-subunit fluctuations). CONCOORD structures were projected onto the modes determined from the crystallographic structures to compare the fluctuations predicted by CONCOORD to the differences between crystallographic structures. The way the CONCOORD structures are situated along the collective coordinates derived from the X-ray structures indicate potential dynamic pathways between the experimentally determined conformers.

## Dyndom

Modes of collective fluctuation were analysed for the presence of clear domain motions as described (Hayward & Berendsen, 1998; Hayward *et al.*, 1997). This method analyses structural differences in terms of rigid body rotations. The rigid bodies are identified by clustering each residue's rotation vector during a conformational transition.

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