

# NMR analysis of a 900K GroEL–GroES complex

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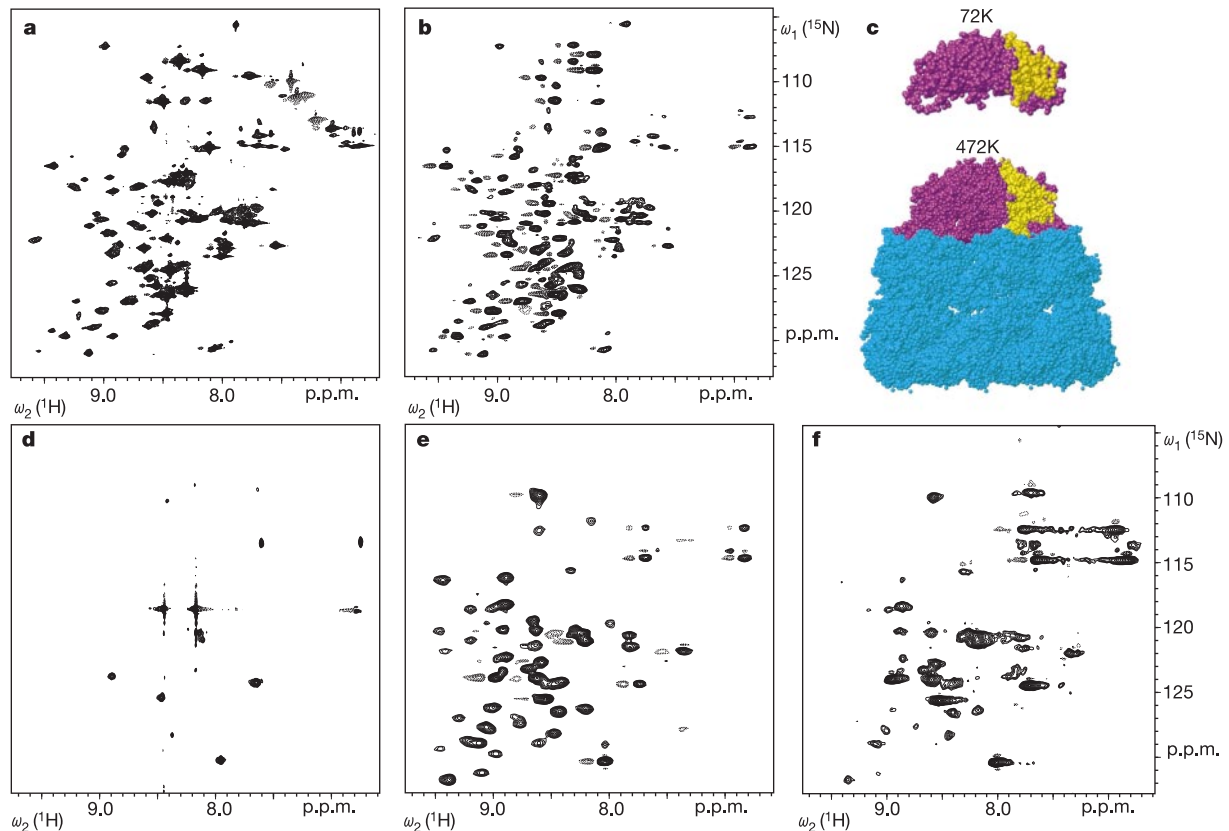
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**Biomacromolecular structures with a relative molecular mass ( $M_r$ ) of 50,000 to 100,000 (50K–100K) have been generally considered to be inaccessible to analysis by solution NMR spectroscopy. Here we report spectra recorded from bacterial chaperonin complexes ten times this size limit (up to  $M_r$  900K) using the techniques of transverse relaxation-optimized spectroscopy and cross-correlated relaxation-enhanced polarization transfer<sup>1–5</sup>. These techniques prevent deterioration of the NMR spectra by the rapid transverse relaxation of the magnetization to which large, slowly tumbling molecules are otherwise subject. We tested the resolving power of these techniques by examining the isotope-labelled homoheptameric co-chaperonin GroES ( $M_r$  72K), either free in solution or in complex with the homotetradecameric chaperonin GroEL ( $M_r$  800K) or with the single-ring GroEL variant SR1 ( $M_r$  400K). Most amino acids of GroES show**

the same resonances whether free in solution or in complex with chaperonin; however, residues 17–32 show large chemical shift changes on binding. These amino acids belong to a mobile loop region of GroES that forms contacts with GroEL<sup>6–10</sup>. This establishes the utility of these techniques for solution NMR studies that should permit the exploration of structure, dynamics and interactions in large macromolecular complexes.

Molecular chaperones are required for the correct folding, transport and degradation of many proteins *in vivo*. Because of these physiological functions, chaperone proteins have been the subject of intensive studies of their mechanism of action. In the *Escherichia coli* chaperonin system, the double ring-shaped homotetradecamer GroEL ( $M_r$  800K), interacts with the dome-shaped homoheptamer GroES ( $M_r$  72K; Fig. 1c) in the presence of ATP to form a chamber that is the site of productive polypeptide folding<sup>7,11–17</sup>. The homooligomeric nature and high symmetry of GroEL, SR1, GroES and their complexes can facilitate NMR spectral analysis because the number of resonances corresponds to the size of an individual subunit, here 58K for GroEL and 10K for GroES, even though the large functional assembly is investigated.

We first measured NMR correlation spectra with uniformly <sup>15</sup>N-labelled and perdeuterated chaperonin proteins, GroEL and SR1, using refined implementations of the principles of transverse relaxation-optimized spectroscopy (TROSY)<sup>1</sup> and cross-correlated relaxation-enhanced polarization transfer (CRINEPT)<sup>2</sup>. In the resulting <sup>15</sup>N–<sup>1</sup>H correlation spectra of either SR1 or GroEL, the pattern of cross-peaks was similar and limited in number to about



**Figure 1** Two-dimensional [<sup>15</sup>N, <sup>1</sup>H]-correlation spectra at 25 °C of the uniformly <sup>15</sup>N, <sup>2</sup>H-labelled co-chaperonin GroES free in solution and in a complex with the unlabelled GroEL variant SR1. **a, b**, Free [<sup>U-<sup>15</sup>N</sup>; >97% <sup>2</sup>H]GroES recorded with two-dimensional [<sup>15</sup>N, <sup>1</sup>H]-TROSY<sup>1</sup> (**a**) and two-dimensional [<sup>15</sup>N, <sup>1</sup>H]-CRIP-TROSY<sup>2</sup> (**b**); **c**, Top, GroES ( $M_r$  72K) is an oligomeric protein with seven identical subunits<sup>6</sup>, one of which is shown in gold. Bottom, a molecular model of the complex of GroES and SR1 ( $M_r$  472K) based on ref. 7, in which the blue-coloured SR1 is not isotope-labelled and therefore not visible in the current

NMR experiments. **d–f**, [<sup>U-<sup>15</sup>N</sup>; >97% <sup>2</sup>H]GroES bound to natural isotope abundance SR1 recorded with two-dimensional [<sup>15</sup>N, <sup>1</sup>H]-TROSY (**d**), two-dimensional [<sup>15</sup>N, <sup>1</sup>H]-CRIP-TROSY (**e**) and two-dimensional [<sup>15</sup>N, <sup>1</sup>H]-CRINEPT-TROSY<sup>2</sup> (**f**). The concentration of free GroES was 0.3 mM in heptamer. The GroES–SR1 complex was studied in a solution containing 0.15 mM [<sup>U-<sup>15</sup>N</sup>; >97% <sup>2</sup>H]GroES and 0.24 mM natural isotope abundance SR1. The experimental set-up is described in Methods.

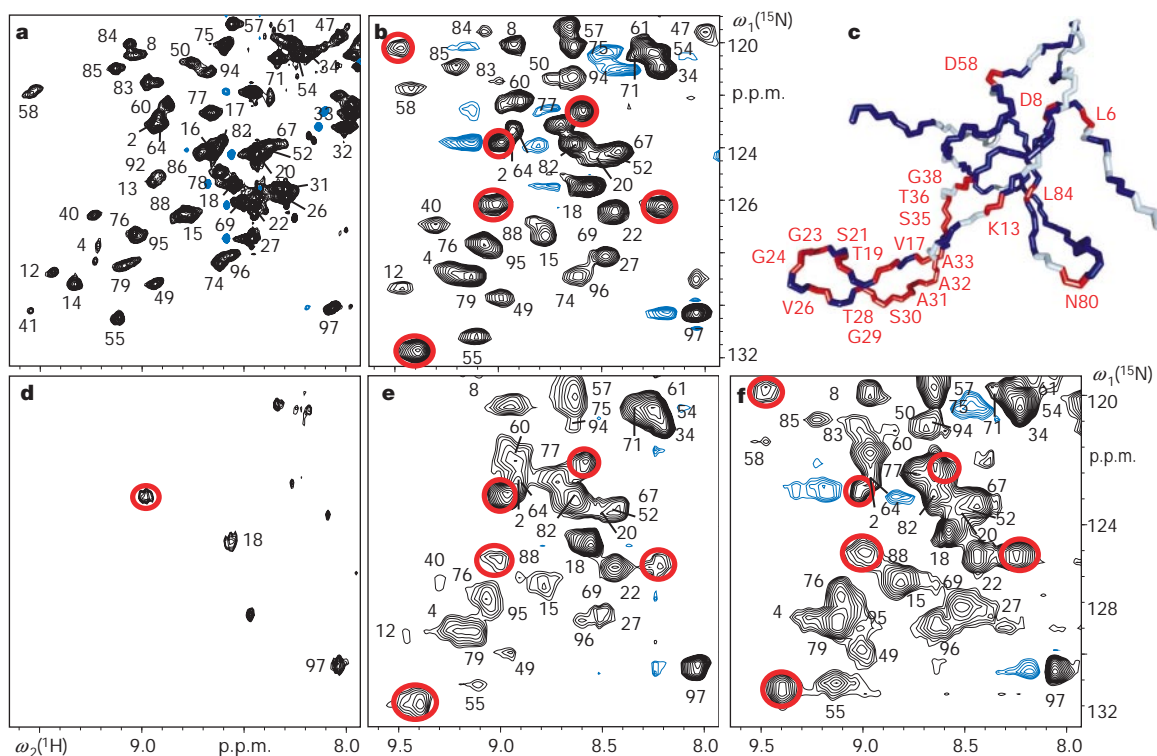
20% of the expected resonances (data not shown). Peaks may be missing from these spectra for many reasons, including incomplete N–D/N–H exchange because the proteins were produced in perdeuterated medium, overlapping resonances, or line broadening as the result of intramolecular conformational exchange. In contrast to these results for the labelled chaperonins, virtually complete  $^{15}\text{N}$ – $^1\text{H}$  correlation spectra were obtained for  $^{15}\text{N}$ -labelled and perdeuterated GroES (Fig. 1a, b). Studies of GroES in complex with GroEL or SR1 thus provided an opportunity for unambiguous testing of the potential of TROSY/CRINEPT-based NMR techniques<sup>1,2</sup> for structures with an  $M_r$  of up to 900K.

For free GroES, we detected 89 out of 94 expected backbone amide  $^{15}\text{N}$ – $^1\text{H}$  cross-peaks in two-dimensional  $^{15}\text{N}$ ,  $^1\text{H}$ -TROSY and two-dimensional  $^{15}\text{N}$ ,  $^1\text{H}$ -CRIPT-TROSY experiments (Fig. 1a, b), and we obtained sequence-specific backbone resonance assignments using TROSY triple-resonance experiments<sup>18–20</sup>. Complexes of  $^{15}\text{N}$ ,  $^2\text{H}$  GroES with chaperonin were then produced in four experiments, by its addition either to perdeuterated and  $^{15}\text{N}$ -depleted GroEL or SR1, or to natural isotope abundance GroEL or SR1. We compared  $^{15}\text{N}$ -depleted with natural isotope abundance chaperonin to obtain experimental proof that all the resonance lines observed in the complexes originated from GroES. Perdeuteration of the chaperonin was carried out to assess whether this would improve the observation of GroES in the complexes. No significant effect of chaperonin deuteration on the  $^{15}\text{N}$ ,  $^1\text{H}$  GroES correlation

spectra was detected, and therefore the spectra shown were recorded using GroES complexes with unlabelled chaperonins.

Complex formation during stepwise addition of the deuterated chaperonins was evident from a decrease of the GroES TROSY cross-peak intensities (data not shown). After adding one equivalent of either SR1 or GroEL, nearly all of the TROSY resonances observed with free GroES had disappeared (Figs 1a, d and 2a, d), almost certainly because transverse relaxation in the complexes was too fast to allow observation of the NMR signals with the TROSY experimental scheme. Yet the TROSY spectra of the complexes did contain a few peaks, which we attributed to regions of GroES that remained mobile (Figs 1d and 2d). One of these resonances was identifiable as Ala 97, the carboxy-terminal residue of GroES (Fig. 2d, peak 97).

The incorporation of GroES into a much larger structure was also readily apparent in  $^{15}\text{N}$ ,  $^1\text{H}$ -CRIPT-TROSY spectra (Figs 1b, e, 2b, e, f and 3a–d). In contrast to the TROSY experiment, most of the 89  $^{15}\text{N}$ – $^1\text{H}$  cross-peaks seen in free GroES (Fig. 1b) remained resolved in the  $^{15}\text{N}$ ,  $^1\text{H}$ -CRIPT-TROSY spectra of the complexes. Moreover, for nearly all cross-peaks, only the most slowly relaxing one of the four components of the multiplet pattern (Fig. 3e) was observable (Figs 1e, 2b, e, f and 3b, d). The other three components were broadened beyond detection in the large complexes<sup>2</sup>. The few cross-peaks that were also present in the  $^{15}\text{N}$ ,  $^1\text{H}$ -TROSY spectra of the complexes (Figs 1d and 2d) showed one or two of the other multiplet components in the  $^{15}\text{N}$ ,  $^1\text{H}$ -CRIPT-TROSY spectra



**Figure 2** Chemical shift changes in  $[U\text{-}^{15}\text{N}; >97\% \text{ } ^2\text{H}]$ GroES on binding to natural isotope abundance SR1 or GroEL. **a**,  $^{15}\text{N}$ ,  $^1\text{H}$ -TROSY spectrum of free GroES at 25 °C, which provides the GroES reference chemical shifts (same data set as in Fig. 1a). **b**,  $^{15}\text{N}$ ,  $^1\text{H}$ -CRIPT-TROSY spectrum of GroES bound to SR1 in the presence of ADP (same data set as in Fig. 1e). **c**, Molecular model of one subunit of GroES in the crystal structure<sup>7</sup>, coloured according to the chemical shift changes observed on complex formation with GroEL or SR1. The chemical shift variations were evaluated as  $\Delta\delta_{av} = \{0.5 \times [\Delta\delta(^1\text{H})^2 + (0.2 \Delta\delta(^{15}\text{N}))^2]\}^{1/2}$  (ref. 21). Red indicates  $\Delta\delta_{av} \geq 0.1$  p.p.m.; this includes the peaks for which no corresponding peak was assigned in bound GroES. All the residues in this class are identified by red lettering, which indicates the amino acid and the sequence position. Dark blue indicates  $\Delta\delta_{av} < 0.1$  p.p.m. Light blue indicates no information because of spectral overlap or absence of the resonances in free GroES. **d**,  $^{15}\text{N}$ ,  $^1\text{H}$ -

TROSY spectrum of GroES bound to GroEL at 25 °C. **e**,  $^{15}\text{N}$ ,  $^1\text{H}$ -CRIPT-TROSY spectrum of GroES bound to GroEL at 25 °C. **f**, Same as **e** but at 35 °C. In **a**, the blue peaks represent antiphase magnetization, which is not fully suppressed because of the short INEPT transfer delay of 3.4 ms. In **d**–**f**, the sample contained 0.13 mM  $[U\text{-}^{15}\text{N}; >97\% \text{ } ^2\text{H}]$ GroES and 0.15 mM natural isotope abundance GroEL. In all spectra, positive contour lines are black and negative contour lines are blue. The sequence-specific assignments for free GroES (black numbers in **a**) were obtained from TROSY triple-resonance experiments<sup>18–20</sup> with a  $^2\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ -labelled protein sample (unpublished data). Tentative assignments for the two complexes indicated in **b**, **e** and **f** have been obtained by transferring assignments of free GroES to nearby peaks of bound GroES. Peaks of bound GroES that appear in different chemical shift regions when compared with free GroES are circled in red and have not been assigned individually.

(Figs 1e and 2b, e, f), which is consistent with the idea that these residues are mobile in the complex. Finally, the [ $^{15}\text{N}, ^1\text{H}$ ]-CRINEPT-TROSY spectrum<sup>2</sup> (Fig. 1f) also contained the cross-peaks from both the structured and the more mobile regions of the chaperonin-bound GroES, but this experiment had lower sensitivity than the [ $^{15}\text{N}, ^1\text{H}$ ]-CRIP-T-TROSY scheme.

Comparison of the [ $^{15}\text{N}, ^1\text{H}$ ]-CRIP-T-TROSY spectra of GroES in the free form with that bound to either SR1 or GroEL showed that complex formation caused significant chemical shift changes for a discrete set of resonance lines, and that there were several cross-peaks in bound GroES that could not be correlated with any of the

peaks in free GroES (Fig. 2, red circles). Because nearly identical numbers of  $^{15}\text{N}$ - $^1\text{H}$  correlation peaks were observed for free and bound GroES, and most cross-peaks were in very similar positions in free and chaperonin-bound GroES, we chose the following validated approach<sup>21</sup> for a systematic comparison of free and bound GroES.

Each sequence-specific resonance assignment obtained for free GroES (unpublished data) was transferred to the nearest peak in the spectra of bound GroES, provided that the two peak positions were within a distance of 1.0 p.p.m. along the  $^{15}\text{N}$  chemical shift axis and within 0.2 p.p.m. along the  $^1\text{H}$  chemical shift axis. If no peak was observed in this tolerance range in the spectrum of the bound GroES, then no assignment was transferred from free to bound GroES. The peaks thus assigned in bound GroES are identified in Fig. 2b and d-f with numbers that indicate the sequence positions. There are several observations to be made about this result.

First, the cross-peaks assigned to residues 17–32 in free GroES could not be assigned in the complexes by the above procedure, whereas for most of the other residues the chemical shifts in free and bound GroES could be correlated. The ‘new’ cross-peaks in the complexes (Fig. 2, red circles) are thus likely to correspond to the mobile loop, that is, residues 17–32 of GroES<sup>6,8</sup>.

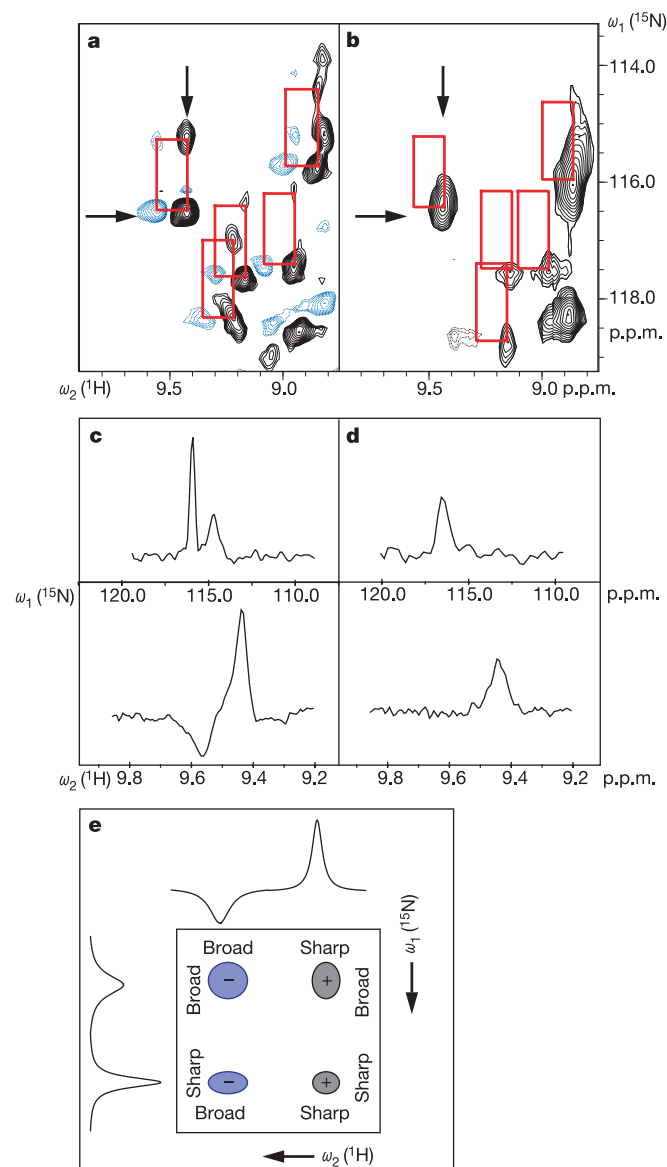
Second, in free GroES the peaks of the mobile loop have random coil chemical shift values, which reflects a disordered conformation. By contrast, the ‘new’ peaks appearing on complex formation show a large chemical shift dispersion, which indicates that the mobile loop of GroES has become ordered on binding to GroEL. The other parts of GroES do not seem to undergo a large conformational change.

Third, most of the [ $^{15}\text{N}, ^1\text{H}$ ]-CRIP-T-TROSY cross-peaks in the spectra of the complexes consist of only the most slowly relaxing fine-structure component (Fig. 3e), which shows that the corresponding residues of GroES are immobilized relative to SR1 or GroEL, respectively, and that their brownian motions are restricted to those of the complex. But a few of the backbone  $^{15}\text{N}$ - $^1\text{H}$  cross-peaks of bound GroES that are located in different positions when compared with free GroES show two or three fine-structure components in the [ $^{15}\text{N}, ^1\text{H}$ ]-CRIP-T-TROSY spectra (Figs 1e and 2b, e, f). These peaks also appear in the [ $^{15}\text{N}, ^1\text{H}$ ]-TROSY spectra (Figs 1d and 2d). This indicates that at least some regions of the GroES mobile loop that undergo conformational changes on binding to the chaperonins nevertheless retain significant mobility in the complex. Fourth, virtually identical results were obtained for GroES in complexes with either SR1 or GroEL (Figs 1 and 2).

These data show that only the mobile loop of GroES undergoes a large conformational change on binding to GroEL, which is compatible with previous spectroscopic and crystallographic studies of GroES interactions with GroEL<sup>7–10</sup>. Additional information comes from the observation that the individual  $^{15}\text{N}$ - $^1\text{H}$  cross-peaks appearing in different chemical shift positions on complex formation (Fig. 2, red circles) have variable dynamic properties, indicating a binding mode in which some of the mobile loop residues are immobilized in the complex whereas others retain a relatively high degree of mobility.

In summary, our results establish that TROSY/CRINEPT-based  $^{15}\text{N}$ - $^1\text{H}$  correlation experiments<sup>1,2</sup> can be used to collect informative solution NMR spectra of structures with an  $M_r$  of up to 900K. Whereas previous NMR studies on large structures have focused on regions with inherent domain mobility<sup>8,22–24</sup>, our experiments can also observe structured regions. In particular, we have shown that chemical shift mapping can be used to characterize protein–protein interactions in complexes of such large size.

Further applications of this approach, with *E. coli* chaperonins and other supramolecular systems, may be facilitated greatly by the observation that deuterium labelling is needed only for the NMR-observed macromolecular component in such complexes, as shown by the fact that exactly the same cross-peaks were observed in the



**Figure 3** Multiplet patterns in [ $^{15}\text{N}, ^1\text{H}$ ]-CRIP-T-TROSY spectra of structures with an  $M_r$  of 72K and 472K. **a**, Experimental spectrum of [ $^{15}\text{N}, ^1\text{H}$ ]-CRIP-T-TROSY (same data set as in Fig. 1b). **b**, Corresponding spectral region of [ $^{15}\text{N}, ^1\text{H}$ ]-CRIP-T-TROSY bound to SR1 (same data set as in Fig. 1e). **c, d**, Cross-sections from **a** and **b** at the frequencies indicated by the arrows. **e**, Theoretical multiplet pattern for [ $^{15}\text{N}, ^1\text{H}$ ]-CRIP-T-TROSY cross-peaks. The four components caused by the  $^1\text{H}$ - $^{15}\text{N}$  scalar coupling have different linewidths, which reflect their different transverse relaxation rates. Because the  $^1\text{H}$  magnetization is detected in antiphase, the two components along the horizontal  $^1\text{H}$  dimension have opposite signs, whereas the two components along the  $^{15}\text{N}$  dimension have equal signs. For very large structures all but the ‘sharp/sharp’ fine-structure component are broadened beyond detection because of fast relaxation. In all spectra, positive contour lines are black and negative contour lines are blue.

$^{15}\text{N}$ - $^1\text{H}$  correlation spectra of complexes with deuterated or protonated (unlabelled) chaperonin. The high amount of protonation in the unlabelled chaperonins also ensures that signals from these components are suppressed<sup>1,2</sup>. For the *E. coli* chaperonin system, the observation that conformational changes in GroES associated with its binding to GroEL are manifested in the solution NMR spectra opens avenues for studying the functional interactions in this system, particularly for substrate polypeptide bound to GroEL. More generally, other large macromolecular assemblies should be accessible to the types of study carried out here, which would allow the interactions and dynamics of selectively labelled subunits or regions to be examined. This may considerably expand the scope of our understanding of macromolecular machines that carry out many essential cellular functions. □

Methods

Protein preparation

GroEL and GroES were expressed from a pET expression vector in strain BL21(DE3). Cells were grown on a minimal medium in 99%  $^2\text{H}_2\text{O}$  supplemented with  $^{15}\text{NH}_4\text{Cl}$  or  $^{14}\text{NH}_4\text{Cl}$  as the sole nitrogen source, and either  $^{2}\text{H}_3\text{Acetate}$  for the fully deuterated  $^{15}\text{N}$ -labelled proteins or  $^{13}\text{C}$  glucose for the ~80% deuterated  $^{13}\text{C}$ ,  $^{15}\text{N}$ -labelled proteins as the sole carbon source. We induced protein expression by adding 1 mM isopropylthiogalactoside in the mid-exponential growth phase and collected the cells after 8–12 h.

Cells expressing isotope-labelled GroEL were resuspended in extraction buffer I ( $\text{H}_2\text{O}$  solution of 100 mM Tris-HCl, pH 8.1, 10 mM dithiothreitol (DTT), 0.1 mM EDTA and 0.1 mg ml<sup>-1</sup> DNase I) and lysed by sonication. After adding 30% ammonium sulphate the lysate was centrifuged. We precipitated the soluble fraction with 80% ammonium sulphate and dissolved it in chromatography buffer ( $\text{H}_2\text{O}$  solution of 50 mM Tris-HCl, pH 7.2, 2 mM DTT and 0.1 mM EDTA). After overnight dialysis against the same buffer, the extract was loaded onto a DEAE Sepharose fast-flow (Pharmacia) column and eluted with a 0–500 mM NaCl gradient. We concentrated the fractions containing GroEL and applied them to a Superdex 200 column (Pharmacia) equilibrated in 25 mM potassium phosphate buffer, pH 6.15, plus 20 mM KCl, which yielded 24 mg of purified fully deuterated  $^{15}\text{N}$ GroEL from 1 l of culture in deuterated minimal medium. Unlabelled GroEL was expressed using the same system in LB medium and purified as described<sup>25</sup> with a yield of 100 mg of purified protein from 1 l of culture.

For GroES purification, we resuspended 1 g of cells (wet weight) in extraction buffer II (10 ml of B-PER bacterial protein extraction reagent (Pierce), pH 8.0, plus 10% sucrose, 1 mg ml<sup>-1</sup> DNase I, 10 mM MgCl<sub>2</sub>, 5 mM isopropylfluorophosphate and a cocktail of protease inhibitors) and stirred them at 4 °C for 45 min. The lysate was cleared by centrifugation, and the pellet extraction was repeated twice at 4 °C and four times at room temperature. The extraction fractions 4–7 were pooled and applied to Source Q (Pharmacia) equilibrated in 50 mM bisTris-HCl pH 6.2 and eluted with a 0–500 mM NaCl gradient. The pooled fractions were concentrated and purified on Superdex 200 in 25 mM potassium phosphate buffer at pH 6.15 containing 20 mM KCl. The yield from 1 l of culture in deuterated minimal medium was 20 mg of purified  $^{15}\text{N}$ ,  $^2\text{H}$ GroES, or 65 mg of purified  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^2\text{H}$ GroES, respectively; the larger yield for the triply labelled protein is because glucose rather than acetate was used as the carbon source.

The single-ring variant of GroEL, SR1 (ref. 11), was expressed from a pET vector in BL21(DE3) in minimal medium in 99%  $^2\text{H}_2\text{O}$  supplemented with deuterated acetate and either  $^{15}\text{NH}_4\text{Cl}$  or  $^{14}\text{NH}_4\text{Cl}$ , and purified as described<sup>11</sup>, yielding 10 mg of purified labelled SR1 from 1 l of culture. Natural isotope abundance SR1 was expressed in LB medium, yielding 100 mg from 1 l of culture.

The GroES–SR1 complex was prepared by adding 4.2 mg of [ $^{15}\text{N}$ ; >97%  $^2\text{H}$ ]GroES to 34 mg of natural isotope abundance SR1 in the presence of 10 mM ADP. The GroES–GroEL complex was prepared by adding 4.4 mg of [ $^{15}\text{N}$ ; >97%  $^2\text{H}$ ]GroES to 55 mg of natural isotope abundance GroEL in the presence of 10 mM ADP.

NMR spectroscopy

TROSY/CRINEPT-based solution NMR spectroscopy has been described<sup>1,2</sup>, and these experiments have been refined for use with very large structures (R. Riek, J.F., E.B.B., A.L.H. and K.W., unpublished data). Here, three experimental schemes were used for obtaining two-dimensional  $^{15}\text{N}$ - $^1\text{H}$  correlation spectra with uniformly  $^{15}\text{N}$ -labelled and perdeuterated proteins. Scheme 1, two-dimensional [ $^{15}\text{N}$ ,  $^1\text{H}$ ]-TROSY includes the widely used INEPT polarization transfer<sup>26</sup> from  $^1\text{H}$  to  $^{15}\text{N}$ , and vice versa, and includes transverse relaxation-optimization during the  $^{15}\text{N}$  evolution period and the  $^1\text{H}$  detection period. It is used for structure sizes of about 50K to 150K<sup>14,5</sup>, but it is not suitable for use with larger structures because of loss of magnetization during the polarization transfers. Scheme 2, two-dimensional [ $^{15}\text{N}$ ,  $^1\text{H}$ ]-CRIPT-TROSY includes use of TROSY as in scheme 1. The polarization transfers are based completely on CRIPT<sup>27</sup>, which is highly efficient for large structures but has very low efficiency for spin systems with short effective correlation times, and thus tends to suppress resonance lines from such spin systems. This scheme does not select among the four fine-structure components of the  $^{15}\text{N}$ - $^1\text{H}$  correlation peaks (Fig. 3), and therefore has a very short overall duration and correspondingly high sensitivity. In very large structures, the undesired three fine-structure components will be suppressed by rapid transverse relaxation (Fig. 3). Scheme 3, two-dimensional [ $^{15}\text{N}$ ,  $^1\text{H}$ ]-CRINEPT-TROSY includes use of TROSY as in scheme 1. The polarization transfers are based on a combination of CRIPT and INEPT. TROSY is active during the transfers, and

the experiment includes a selection scheme for components of the cross-peak four-line fine structure. As compared with scheme 2, scheme 3 has lower sensitivity but yields a more complete spectrum because slowly relaxing resonances are not suppressed.

All NMR samples were prepared in  $\text{H}_2\text{O}$  solutions of 25 mM potassium phosphate, pH 6.15, plus 20 mM KCl, 5%  $^2\text{H}_2\text{O}$ , and 0.02% NaN<sub>3</sub>. The NMR experiments were carried out on a Bruker DRX 750 spectrometer at a  $^1\text{H}$  resonance frequency of 750 MHz. The sample temperature was either 25 °C or 35 °C for some experiments with the GroEL complexes. The spectra were processed with the program PROSA<sup>28</sup> and analysed with the program XEASY<sup>29</sup>.

The [ $^{15}\text{N}$ ,  $^1\text{H}$ ]-TROSY spectrum of free [ $^{15}\text{N}$ ,  $^2\text{H}$ ]GroES (Figs 1a and 2a) was recorded for 3 h with a data size of 150 × 1,024 complex points,  $t_{1\text{max}} = 37.5$  ms,  $t_{2\text{max}} = 98$  ms, INEPT transfer time = 3.4 ms. The corresponding [ $^{15}\text{N}$ ,  $^1\text{H}$ ]-CRIPT-TROSY experiment (Figs 1b and 3a) was recorded for 10.5 h, with a data size of 100 × 1,024 complex points,  $t_{1\text{max}} = 25$  ms,  $t_{2\text{max}} = 98$  ms, CRIPT transfer time = 2.8 ms. The recycle delay was 1 s. The [ $^{15}\text{N}$ ,  $^1\text{H}$ ]-TROSY (Fig. 1d) and [ $^{15}\text{N}$ ,  $^1\text{H}$ ]-CRIPT-TROSY (Figs 1e, 2b and 3b) spectra for the GroES–SR1 complex were recorded with the same parameters as the corresponding experiments for free GroES. The [ $^{15}\text{N}$ ,  $^1\text{H}$ ]-CRINEPT-TROSY experiment (Fig. 1f) was recorded for 10.5 h, with a data size of 100 × 1,024 complex points,  $t_{1\text{max}} = 25$  ms,  $t_{2\text{max}} = 98$  ms, CRINEPT transfer time = 2.8 ms. A recycle delay of 0.6 s was used for the three experiments with the GroES–SR1 complex.

For the GroES–GroEL complex, the [ $^{15}\text{N}$ ,  $^1\text{H}$ ]-TROSY spectrum (Fig. 2d) was recorded for 14 h, with a data size of 120 × 1,024 complex points,  $t_{1\text{max}} = 30$  ms,  $t_{2\text{max}} = 98$  ms. The INEPT transfer time was 3.4 ms. The [ $^{15}\text{N}$ ,  $^1\text{H}$ ]-CRIPT-TROSY spectra (Fig. 2e, f) were recorded for 15 h each, with a data size of 64 × 1,024 complex points,  $t_{1\text{max}} = 16$  ms,  $t_{2\text{max}} = 98$  ms, recycle delay = 0.3 s. The CRIPT transfer time was 1.4 ms. For all [ $^{15}\text{N}$ ,  $^1\text{H}$ ]-CRIPT-TROSY and [ $^{15}\text{N}$ ,  $^1\text{H}$ ]-CRINEPT-TROSY data, a sine bell function shifted by 10° (ref. 30) was applied before Fourier transformation in the indirect dimension, and an empirically optimized exponential function was used in the direct dimension.

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#### Competing interests statement

The authors declare that they have no competing financial interests.

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**erratum**

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## Optical pulsations from the anomalous X-ray pulsar 4U0142+61

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 In the second sentence of this Letter, the word ‘neuron’ appears incorrectly. It should read ‘neutron’. □