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SUBJECT AREAS:  
KINETICS  
BIOLOGICAL PHYSICSReceived  
25 November 2013Accepted  
11 April 2014Published  
29 April 2014Correspondence and  
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# Thermal-triggered Proteinquake Leads to Disassembly of DegP Hexamer as an Imperative Activation Step

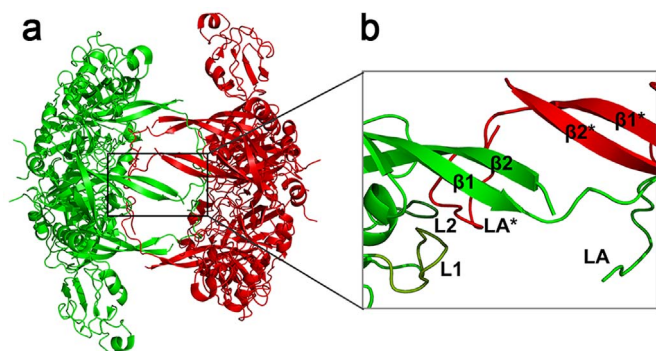
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The *Escherichia coli* DegP has been reported to function both as molecular chaperone and protease for the quality control of outer membrane protein biogenesis. Activation of the inactive DegP hexamers was believed to occur via their disassembly into trimeric units and subsequent reassembly into larger oligomers (12-mers and 24-mers). Here, we analyzed the thermal stability and the unfolding dynamics of the different secondary structure components of the DegP hexamers using Fourier transform infrared spectroscopy and temperature-jump nanosecond time-resolved IR difference absorbance spectroscopy. We found that the interfacial secondary structure components possess a degree of thermal stability, with the disassembly of the DegP hexamers follows a “proteinquake” manner, such that the fully exposed parts of the interfacial  $\beta$ -sheets serving as the temperature sensor and epicenter to drive the sequential unfolding/disassembly process that finishes within about 134 ns at room temperature.

The aggregation of misfolded proteins represents a severe danger for cell viability. To minimize such a hazard, effective quality-control systems, largely involving molecular chaperones and proteases, have evolved to suppress such aggregation and/or to refold or degrade the misfolded proteins<sup>1</sup>. The *Escherichia coli* (*E. coli*) DegP (also designated as HtrA), whose homologs are widely found in all forms of life, is a unique protein that has been reported to possess both chaperone and protease activities<sup>2</sup>. The DegP protein, present in the periplasmic space of *E. coli* cells, was found to be essential for bacterial survival at high temperatures<sup>3,4</sup>. It has been shown that DegP exists as inactive hexamers, which are transformed into the active and cage-like 12- or 24-mers upon substrate binding<sup>5,6</sup>. Therefore DegP provides an excellent example for understanding how an inactive protein becomes activated by readjusting its oligomeric quaternary structures.

Crystal structure determination revealed that the DegP hexamer is formed by a staggered association of two trimeric units in a face-to-face manner<sup>7</sup>. The primary contacts between the two trimeric units consist of six (one from each of the six subunits) two-stranded  $\beta$ -sheets, as illustrated in Fig. 1a. In addition, at the interface of the two trimeric units, the six LA loops (each LA loop connecting two  $\beta$  strands designated as  $\beta 1$  and  $\beta 2$  from the six two-stranded  $\beta$ -sheets) protrude into their opposing subunits and intimately interact with loops L1 and L2 in active sites of the latter, forming six loop triads, LA\*-L1-L2 (Fig. 1b; the asterisk denotes its contribution from an opposing subunit) that completely prevent the unfolded substrate proteins from entering the active sites, deactivating the protease function of the DegP hexamers<sup>6,7</sup>. It has been reported that upon binding unfolded substrate proteins, the DegP hexamers are converted into the larger oligomeric forms of 12 or 24 subunits (i.e., the 12- and 24-mers). Structural analyses of these larger oligomers have revealed that the inhibitory LA loops are liberated from the active sites, enabling DegP to adopt a functional state<sup>6,8</sup>. It follows that the conversion from hexamers to 12- and 24-mers is a crucial step for DegP to be activated<sup>9</sup>. Such conversion has been implicated to occur via oligomeric disassembly and reassembly, with the trimeric units being produced as the intermediate<sup>5</sup>, during which the six LA\*-L1-L2 loop triads would have to be disrupted.

For the activation of DegP hexamers, outstanding unresolved issues include the following. First, what factor, the presence of unfolded substrate proteins (as proposed in ref. 9), the heat shock temperature (as implicated in ref. 2) or others, triggers the activation process of DegP? With equal importance, how the interactions between the trimeric units are disrupted in the DegP hexamers for the disassembly to occur?



**Figure 1** | Cartoon representation of the crystal structure of the DegP hexamer (PDB ID:1ky9) emphasizing the interfacial linkers between the two trimeric units. Shown in the enlarged view is the anti-parallel  $\beta$ -sheets and the loop triad LA\*-L1-L2 (LA\* comes from a monomer of the opposing trimeric unit). The crystal structure was drawn using Pymol program.

We tried to resolve these issues via an approach of both thermodynamics and dynamics. For these we first performed thermal titration studies using Fourier transform infrared spectroscopy (FTIR) to reveal the varied thermal stability of the different secondary structure components in the DegP hexamer. We then applied temperature-jump time-resolved IR difference spectroscopy to probe the unfolding dynamics of these secondary structure components. Consistently, our thermodynamic and dynamic data unveil a mechanism for the inactive DegP hexamers to disassemble, such that the disruption of the interfacial secondary structure components, having degreed thermal stability, occurs in a proteinquake mode, which eventually leads to the dissociation of the two trimeric units. Remarkably, this occurs even at the room temperature (25°C) and within only about 134 nanoseconds. It is conceivable that similar proteinquake mechanism might be utilized by other oligomeric proteins (e.g., small heat shock proteins) that modulate their activities by changing their oligomeric forms<sup>10</sup>.

## Results

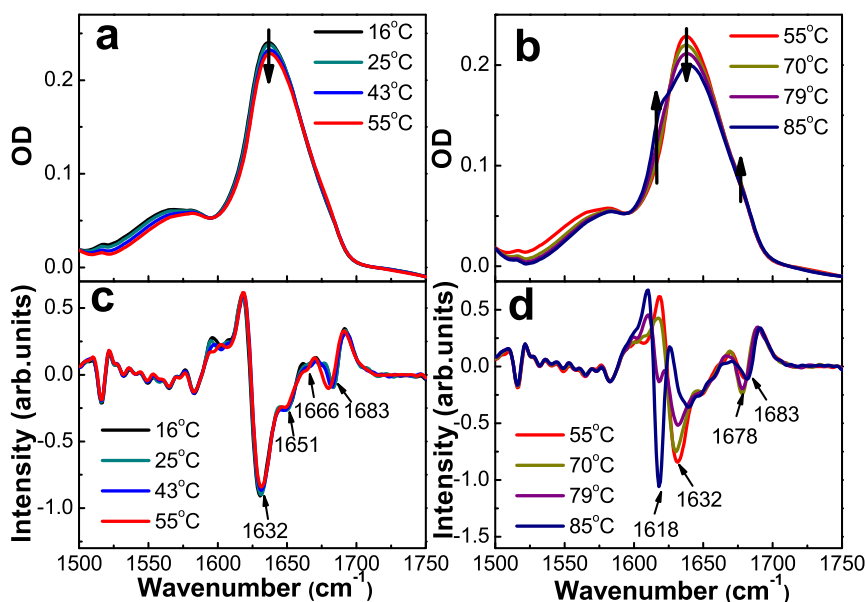
**Temperature-dependent FTIR spectroscopic characterization of the secondary structure components of the DegP hexamer.** To unveil the mechanism for the inactive DegP hexamer to become active, in particular for its disassembly, we first examined the thermal stability of each of its secondary structure components, especially those participating in the interaction between the trimeric units. For this, we first conducted IR spectroscopic analysis, such that the temperature-dependent FTIR spectra with the DegP<sub>S210A</sub> protein sample dissolved in D<sub>2</sub>O, were recorded at 1500 cm<sup>-1</sup>–1750 cm<sup>-1</sup>, including the amide I' (the prime denotes the frequencies of deuterated amide groups) band, i.e., between 1600 cm<sup>-1</sup>–1700 cm<sup>-1</sup>, a region at which each different secondary structure component would have absorption at a characteristic wavenumber. As shown in Figures 2a and 2b, the absorbance in the 1500 cm<sup>-1</sup>–1600 cm<sup>-1</sup> region for the amide II band is low, indicating that the H<sub>2</sub>O content in the protein sample was insignificant<sup>11</sup>. The FTIR spectra of the inactive DegP<sub>S210A</sub> protein were collected in 3°C steps starting from 10°C and up to 85°C, representative ones recorded at the indicated temperatures are displayed in Figures 2a and 2b, and their corresponding second derivatives are presented in Figures 2c and 2d. For clarity, we presented the FTIR spectra as two groups, with those that only change slightly, i.e., between 10°C–55°C, displayed in Fig. 2a, while those that change significantly, i.e., between 55°C–85°C, displayed in Fig. 2b (the corresponding second derivatives displayed respectively in Figures 2c and 2d). For the second derivative spectra in the lower temperature group, the broadband centered at 1632 cm<sup>-1</sup> (Fig. 2c) can be assigned to be produced from  $\beta$ -sheets

in general, while the other peaks can be assigned to be produced from loops/helices (1651 cm<sup>-1</sup>), turns (1666 cm<sup>-1</sup>), and the high frequency vibration of anti-parallel  $\beta$ -sheets (1678 cm<sup>-1</sup> and 1683 cm<sup>-1</sup>). Our secondary structure assignments of the major peaks are largely consistent to those reported earlier<sup>11,12</sup>. The simultaneous appearance of absorption at 1618 cm<sup>-1</sup> and 1683 cm<sup>-1</sup> at temperatures higher than 70°C (Fig. 2d) has been assigned to be produced from the intermolecular anti-parallel  $\beta$ -sheets present in the aggregate formed by denatured proteins<sup>13,14</sup>. Another feature for the spectra in the higher temperature group is the absorption peak at 1678 cm<sup>-1</sup>, which appears at 61°C but disappears at above 79°C, apparently being produced from a certain  $\beta$ -sheet structure intermediate formed prior to protein aggregation. It should be pointed out that the temperature-induced spectral changes recorded by us for the DegP<sub>S210A</sub> protein at the high temperature range are largely consistent with what were reported by Skorko-Glonek *et al.*, who also observed similar unfolding-related temperature-induced spectral changes from 60°C to 70°C and the aggregation-related changes above 70°C<sup>11</sup>.

We then focused on the broadband centered at 1632 cm<sup>-1</sup> which we assigned to be derived from  $\beta$ -sheet structures. To find out the particular types of  $\beta$ -sheets that contribute to this band, we plotted the FTIR difference spectra of DegP<sub>S210A</sub>, as shown in Fig. 3a, by subtracting the spectrum recorded at a given higher temperature by that recorded at 16°C. We also plotted temperature-dependent changes for the wavenumber of the bleaching peaks, as displayed in Fig. 3b, using the information extracted from the FTIR difference spectra shown in Fig. 3a. The data displayed in Fig. 3b demonstrate that at least three different types of  $\beta$ -sheet structures can be clearly identified as indicated by the fact that the wavenumber curve has three different slopes in three typical temperature ranges, with one absorbing at 1623 cm<sup>-1</sup>–1626 cm<sup>-1</sup> at temperatures lower than 55°C, and being blue shifted from 1623 cm<sup>-1</sup> to 1626 cm<sup>-1</sup> upon temperature rising. Upon raising the temperature from 55°C to 70°C, the bleaching peak wavenumber shifts drastically from 1626 cm<sup>-1</sup> to 1634 cm<sup>-1</sup> (Fig. 3b). Given that for  $\beta$ -sheet structures, the more solvent-exposed the more red shifted for its amide I' absorption peak<sup>15,16</sup>, the  $\beta$ -sheet structures absorbing at 1623 cm<sup>-1</sup>–1626 cm<sup>-1</sup> would be more exposed to the solvent than those absorbing at 1626 cm<sup>-1</sup>–1634 cm<sup>-1</sup>. It follows that the former can be assigned to those fully exposed parts of the  $\beta$ -sheets located at the interface of the trimeric units, while the latter to those partially exposed parts of the interfacial  $\beta$ -sheets. When the temperature elevation exceeds 70°C, the bleaching peak remains at 1634 cm<sup>-1</sup> and no longer shifts, thus to be assigned as the absorption of buried  $\beta$ -sheets.

To validate these assignments, we repeated the above experiment on two previous reported trimeric mutants, the DegP ( $\Delta$ PDZ2), having the PDZ2 domain (residues 356–446) deleted, and the broken cage DegP<sub>S210A</sub>, having residues 39 to 78 within the LA loop removed<sup>17</sup>. The DegP ( $\Delta$ PDZ2) mutant protein was demonstrated to exist mainly as trimers, while the broken cage DegP<sub>S210A</sub> mutant existing mostly as trimers and monomers<sup>17</sup>. It follows that the interfacial  $\beta$ -sheets and LA loops present in the hexameric forms would no longer exist in these two mutant forms of DegP.

The FTIR absorption spectra of these two forms of DegP recorded at 25°C are shown in Supplementary Fig. S1 and their corresponding second derivative spectra are displayed in Fig. 3c. Remarkably, instead of detecting a significant peak centered at 1632 cm<sup>-1</sup> for the hexameric DegP, we only detected a peak that is much weaker and centered at 1634 cm<sup>-1</sup> for the DegP ( $\Delta$ PDZ2) and broken cage DegP<sub>S210A</sub> mutants (Fig. 3c). Conceivably, those disappeared absorption, located at the red side in a spectral range of 1623 cm<sup>-1</sup>–1634 cm<sup>-1</sup>, were most likely contributed by the solvent-exposed interfacial  $\beta$ -sheets in the hexamers. Similar plotting of temperature-dependent changes for the wavenumber of the bleaching peaks for the trimeric mutants, also displayed in Fig. 3b, reveals a blue-shift



**Figure 2** | Overall characterization of the secondary structure components in the DegP hexamer via temperature-dependent FTIR absorption spectroscopy. (a)(b) Absorption spectra of DegP<sub>S210A</sub> at 12.5 mg/ml, in D<sub>2</sub>O (pD 7.4). (c)(d) The second derivative spectra corresponding to (a) and (b). Arrows in the absorption spectra denote the direction of the absorbance change with increasing temperature.

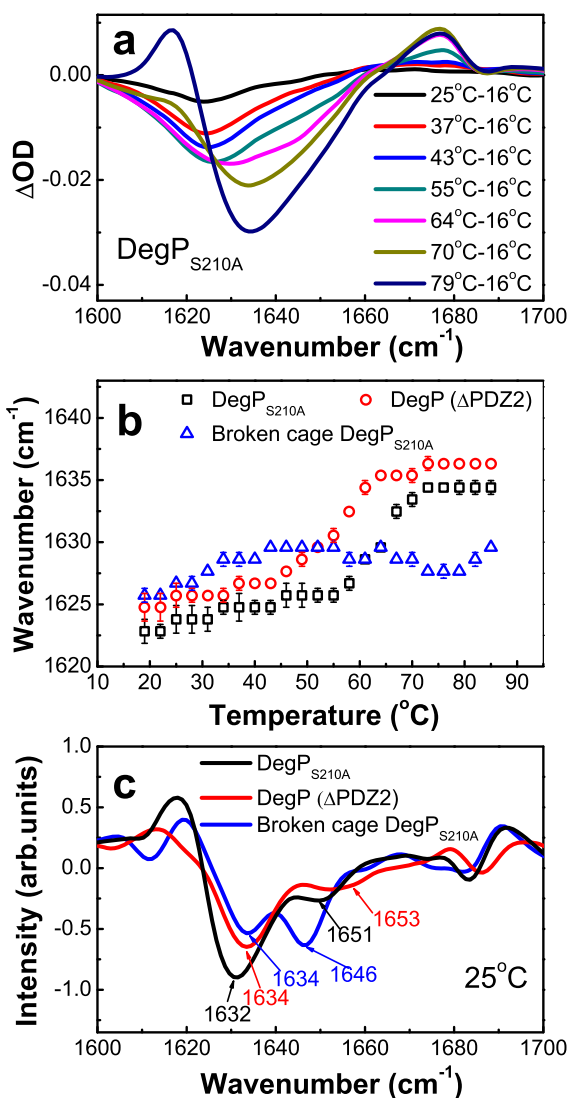
of 2–4 cm<sup>-1</sup> at temperatures lower than 55°C in comparison with that of the DegP hexamer. Taken together, these observations strongly validate our assignment of the IR absorption band at 1623 cm<sup>-1</sup>–1626 cm<sup>-1</sup> to be derived from those fully exposed parts of the β-sheets located at the interface of the trimeric units of the hexamer.

**The secondary structure components of the DegP hexamers possess a degreed thermal stability.** We next studied the thermal stability of these secondary structure components identified above (Fig. 2 and 3). To avoid the potential overlapping between the absorption spectra of the fully and partially exposed interfacial β-sheets (absorbing at 1623 cm<sup>-1</sup>–1626 cm<sup>-1</sup> and 1626 cm<sup>-1</sup>–1634 cm<sup>-1</sup> respectively), we decided to perform Singular Value Decomposition (SVD) analysis, being a powerful mathematical tool commonly used for multicomponent spectral resolution<sup>18,19</sup>, on the temperature-dependent FTIR spectra between 1610 cm<sup>-1</sup> and 1632 cm<sup>-1</sup> that we acquired in the low temperature region between 10°C–55°C (for details, see Supplementary Note and Fig. S2). The plotting of intensity against wavenumber, as shown in Fig. 4a, clearly resolves two absorption peaks centered at 1623 cm<sup>-1</sup> and 1629 cm<sup>-1</sup>, which can be respectively assigned to those fully exposed and those partially exposed parts of the interfacial β-sheets. Meanwhile, the SVD-resolved population thermal titration curve for the secondary structural component absorbing at 1623 cm<sup>-1</sup>, as shown in Fig. 4b, can be fitted by a two-state unfolding model, with the transition midpoint temperature ( $T_m$ ) being at 23.6 ± 2.3°C. It should be pointed out that a highly similar  $T_m$  value of 23.9 ± 0.8°C was also obtained by analyzing the absorbance thermal titration curve at 1623 cm<sup>-1</sup> (see Supplementary Note and Fig. S3). This relatively low  $T_m$  value strongly indicates that the fully exposed interfacial β-sheet structure is actually quite unstable, with its thermal-induced disruption starting at least from such a low temperature as 10°C. A similar SVD-resolved population thermal titration curve at 1629 cm<sup>-1</sup>, also presented in Fig. 4b, failed to be fitted by a two-state unfolding process, suggesting that this absorption band is contributed from β-sheets of varied thermal stability. Nevertheless, the data presented in Fig. 4b clearly indicate that these partially exposed β-sheet structures absorbing at 1629 cm<sup>-1</sup> are more stable than the fully exposed β-sheets absorbing at 1623 cm<sup>-1</sup>.

We subsequently analyzed the thermal stability of the loop/helical structures that absorb at 1651 cm<sup>-1</sup> also in the low temperature region (between 10°C–55°C). The crystal structure of DegP indicates that most of the α-helices are located far away from the trimer-trimer interface in the hexamer, and are thus expected to be more stable than the loops that are largely located closer to the trimeric interface<sup>7</sup>. In view of this, we assumed that the thermal-induced IR absorbance change at the above low temperatures largely reflects the structural change of the loops, although the absorption spectra from α-helices and loops in proteins may overlap in this spectral region<sup>20</sup>. The thermal titration curve for IR absorbance at 1651 cm<sup>-1</sup>, displayed in Fig. 4c, shows that the absorption begins to decrease at 25°C and reaches a plateau at 40°C, giving a  $T_m$  value of 31.5 ± 1.5°C, reflecting the unfolding process of a certain kind of loop structures. Upon further increase of the temperature, no plateau appears till 55°C, indicating a continuously opening of the loop structures. It has been previously reported that the LA loop undergoes a significant thermal-induced opening at a temperature of 25°C–30°C, the L2 loop at a temperature exceeding 35°C, and the L1 loop at a temperature higher than 40°C<sup>12</sup>. In view of these, the observed  $T_m$  value of 31.5 ± 1.5°C should be assigned to the unfolding process of the LA loop. It should be pointed out that the loops absorbing at 1651 cm<sup>-1</sup> are apparently more stable than the partially exposed β-sheet structures absorbing at 1629 cm<sup>-1</sup>, as indicated by our plotted SVD-resolved population thermal titration curve presented in Fig. 4c.

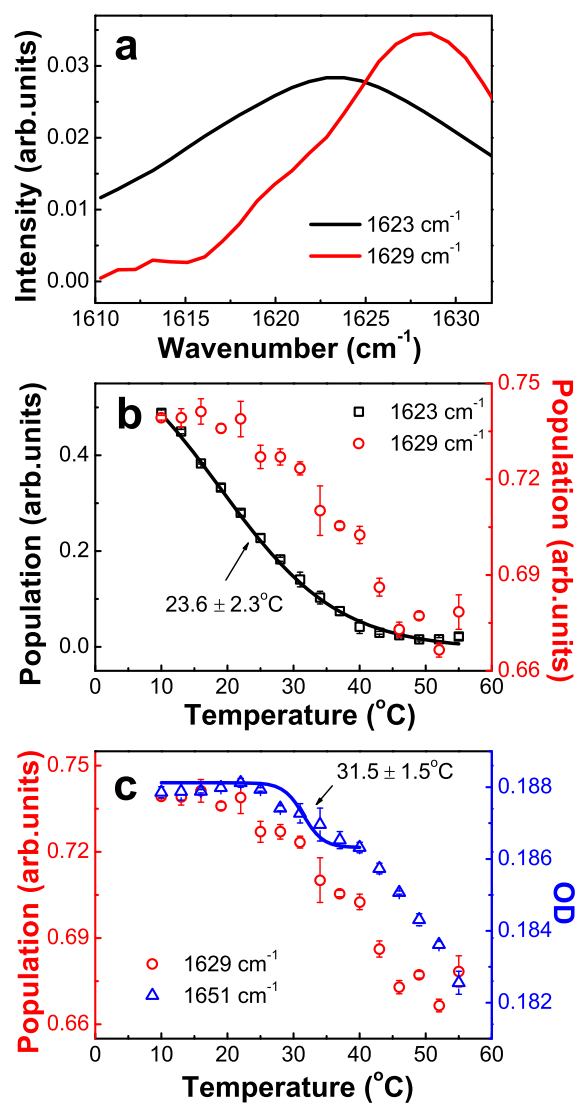
The assignment of the peak at 1651 cm<sup>-1</sup> to the LA loop is again validated by our FTIR spectroscopy analysis of the broken cage DegP<sub>S210A</sub>, for which the residues from positions 39 to 78 within the LA loop were removed and thus existing predominantly as trimers and monomers<sup>17</sup>. As indicated by the second derivative spectrum shown in Fig. 3c, for the broken cage DegP<sub>S210A</sub>, the absorption at 1651 cm<sup>-1</sup> was no longer detectable, instead, a new peak centered at 1646 cm<sup>-1</sup> was recorded, most likely due to the formation of random coil structures resulted from the deletion of the LA loop. Taken together, these observations confirm that the absorption peak centered at 1651 cm<sup>-1</sup> is indeed derived from the LA loop in the DegP hexamer.

Last but not least, we analyzed the thermal stability of the anti-parallel β-sheet structures that absorb at 1683 cm<sup>-1</sup> and are located within the trimeric units. In the second derivative spectra (Fig. 2d),



**Figure 3** | Detailed assignments of the interfacial secondary structure components in the DegP hexamer via the comparison between three mutants. (a) Absorption difference spectra of DegP<sub>S210A</sub> between the spectrum acquired at the indicated temperature and that of 16°C. (b) The plot of the bleaching peak wavenumber against the temperature for DegP<sub>S210A</sub>, DegP ( $\Delta PDZ2$ ), and broken cage DegP<sub>S210A</sub> ( $\Delta 39-78$ ). (c) The second derivative spectra of DegP<sub>S210A</sub>, DegP ( $\Delta PDZ2$ ) and broken cage DegP<sub>S210A</sub> ( $\Delta 39-78$ ) at 25°C.

we noticed that when the temperature exceeds 55°C, the peak at 1683 cm<sup>-1</sup> is first converted to one at 1678 cm<sup>-1</sup> and then back to 1683 cm<sup>-1</sup> upon further temperature elevation. To gain detail information on the structure species attributing to these two absorption peaks, we carried out SVD analysis on the temperature-dependent FTIR spectra between 1670 cm<sup>-1</sup> and 1690 cm<sup>-1</sup> (for details, see Supplementary Fig. S4). The results reveal that two absorption bands with a peak centered at 1678 cm<sup>-1</sup> and 1683 cm<sup>-1</sup> respectively are resolved clearly (Fig. 5a). The SVD-resolved population thermal titration curves for the two different structure species that absorb at 1683 cm<sup>-1</sup> and 1678 cm<sup>-1</sup> respectively, as shown in Fig. 5b, show that the disruption of the anti-parallel  $\beta$ -sheet structures that absorb at 1683 cm<sup>-1</sup> starts at 40°C by first converting to an intermediate form that absorbs at 1678 cm<sup>-1</sup>. This red shift in peak position (from 1683 cm<sup>-1</sup> to 1678 cm<sup>-1</sup>) suggests that these anti-parallel  $\beta$ -sheets somehow become loosened. The disruption apparently completes at around 70°C, with the fitted  $T_m$  value being at  $56.7 \pm 0.4^\circ\text{C}$ . Our

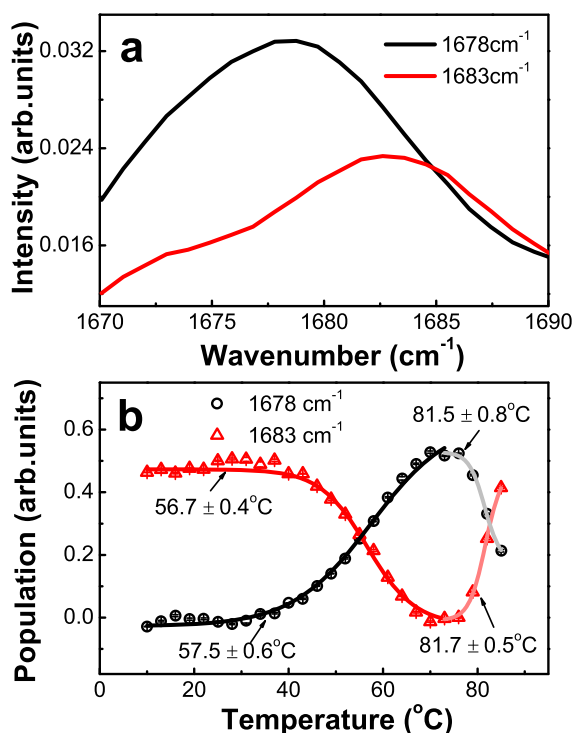


**Figure 4** | The thermal stability analysis of the interfacial secondary structure components in the DegP hexamer. (a) The SVD-resolved two species-associated spectra centered at 1623 cm<sup>-1</sup> and 1629 cm<sup>-1</sup>. (b) The population thermal titration curves of the structure components (1623 cm<sup>-1</sup>, 1629 cm<sup>-1</sup>) retrieved by SVD analysis. (c) The absorbance thermal titration curve at 1651 cm<sup>-1</sup> and the population thermal titration curve of the structure component at 1629 cm<sup>-1</sup>.

conclusion that the structure species absorbing at 1678 cm<sup>-1</sup> is derived from that initially absorbing at 1683 cm<sup>-1</sup> is strongly supported by the fact that the population decrease of the latter is accompanied by an increase of the former in an inverse and proportional manner upon temperature elevation from 40°C to 70°C.

The results of the thermal stability analysis described above clearly suggest that the different secondary structure components of the DegP hexamer possess a degreed thermal stability. In particular, their relative stability increases in the following order, the fully exposed parts of the interfacial  $\beta$ -sheets ( $T_m = 23.6 \pm 2.3^\circ\text{C}$ ), the partially exposed parts of the interfacial  $\beta$ -sheets, the LA loops ( $T_m = 31.5 \pm 1.5^\circ\text{C}$ ), the buried anti-parallel  $\beta$ -sheet structures within the interior of the trimeric unit ( $T_m = 56.7 \pm 0.4^\circ\text{C}$ ).

**The unfolding dynamics of the secondary structure components of the DegP hexamers as revealed by temperature-jump time-resolved IR difference absorbance spectroscopy.** To find out how these secondary structure components that possess a degreed

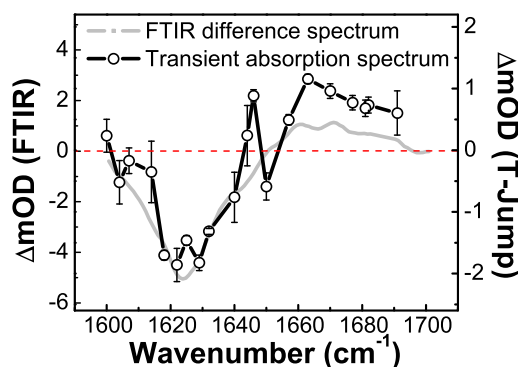


**Figure 5** | The thermal stability analysis of the secondary structure components within the interior of the trimeric units in the DegP hexamer. (a) The SVD-resolved two species-associated spectra centered at 1678  $\text{cm}^{-1}$  and 1683  $\text{cm}^{-1}$ . (b) The population thermal titration curves of the structure components (1678  $\text{cm}^{-1}$ , 1683  $\text{cm}^{-1}$ ) retrieved by SVD analysis.

thermal stability are involved in the disassembly process of the DegP hexamers, we next examined the dynamics for their thermal-induced disruption. For this, we investigated the thermal-induced unfolding dynamics of the DegP hexamers as initiated by temperature-jump from 16°C to 25°C, as followed by using time-resolved IR absorbance difference spectroscopy. The reason to choose this temperature jump range is based on the following observations: (i) It has been reported that the larger oligomeric forms (12- and 24-mers) can be formed from the DegP hexamers even at the room temperatures<sup>21</sup>, suggesting that the DegP hexamers are able to dissociate into their trimeric units at such low temperatures; (ii) our thermal titration results described above indicate that the disruption of the secondary structure components involved in the interaction between the trimeric units of the DegP hexamer apparently starts at least from a temperature as low as 10°C (Fig. 4b).

The transient IR difference spectrum, as recorded two microseconds after the temperature-jump, is presented in Fig. 6. For comparison, the steady-state FTIR difference spectrum between the spectrum recorded at 25°C and that at 16°C (as shown in Fig. 3a) is also displayed here. The reliability of such transient IR spectrum is indicated by its overall correlation with the steady-state difference spectrum described above (Fig. 3a). This transient spectrum reveals the disappearance of three secondary structure components, as indicated by the recording of the bleaching peaks centered at 1622  $\text{cm}^{-1}$ , 1629  $\text{cm}^{-1}$  and 1650  $\text{cm}^{-1}$ , which correlate respectively to the disruption of the fully exposed parts of the interfacial  $\beta$ -sheets, the partially exposed parts of the interfacial  $\beta$ -sheets and the LA loops. It meanwhile reveals the appearance of two major absorption peaks centered at 1646  $\text{cm}^{-1}$  and 1663  $\text{cm}^{-1}$ , which correlate respectively to the formation of random coil and  $\beta$ -turn structures.

We then tried to elucidate the kinetics regarding the temperature-jump induced cooperative conversion of the secondary structure



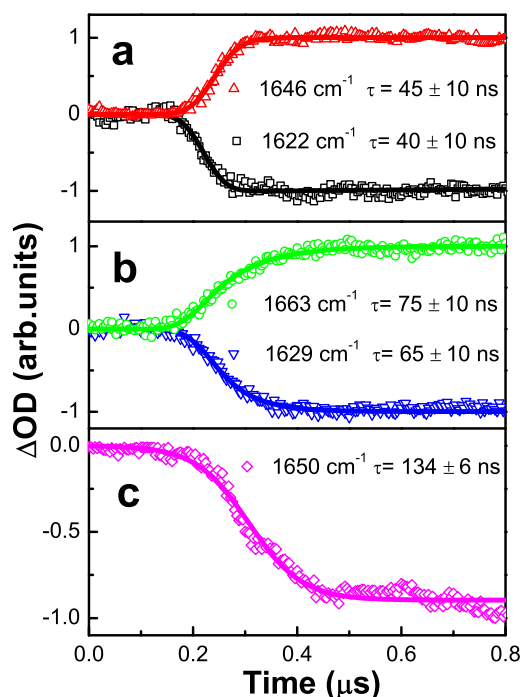
**Figure 6** | The transient IR absorbance difference spectrum of DegP<sub>S210A</sub> recorded at 2  $\mu\text{s}$  after the temperature-jump from 16°C to 25°C. The steady-state FTIR absorbance difference spectrum between 25°C and 16°C is also included for comparison.

components by analyzing the temporal coincidence between the disappearance of the transient bleaching peaks and the appearance of the absorption peaks (shown in Fig. 6). The kinetic curves, displayed in Fig. 7a, indicate a clear correlation between the disappearing bleaching peak centered at 1622  $\text{cm}^{-1}$ , having a time constant of  $40 \pm 10$  ns, and the appearing absorption peak centered at 1646  $\text{cm}^{-1}$ , having a time constant of  $45 \pm 10$  ns. This demonstrates that the disruption of the fully exposed parts of the interfacial  $\beta$ -sheets that absorb at 1622  $\text{cm}^{-1}$  leads to the formation of the random coil structures that absorb at 1646  $\text{cm}^{-1}$ . Similarly, the disappearing bleaching peak centered at 1629  $\text{cm}^{-1}$ , having a time constant of  $65 \pm 10$  ns, correlates to the appearing absorption peak centered at 1663  $\text{cm}^{-1}$ , having a time constant of  $75 \pm 10$  ns (Fig. 7b), indicating a correlation between the disruption of the partially exposed parts of the interfacial  $\beta$ -sheet structures that absorb at 1629  $\text{cm}^{-1}$  and the formation of the turn structures that absorb at 1663  $\text{cm}^{-1}$ . In contrast, the disappearing bleaching peak centered at 1650  $\text{cm}^{-1}$ , having a time constant of  $134 \pm 6$  ns (Fig. 7c), finds no counterpart among the appearing transient absorption peaks, suggesting that the LA loops that absorb at 1650  $\text{cm}^{-1}$  detach from the opposing trimeric units, which in turn leads to the falling apart of the two interacting trimeric units in the DegP hexamer.

Together, these observations suggest the following time course for the sequential opening of the secondary structure components and dissociation of the DegP hexamer. First, the fully exposed parts of the interfacial  $\beta$ -sheets unfold in about 40 ns, then the partially exposed parts of the interfacial  $\beta$ -sheets open in about 70 ns, and finally the LA loops detach from the opposing trimeric units in about 134 ns. In consistent with the results of the thermal stability analysis described above (Fig. 4), these dynamic data suggest an increase of the dynamic stability of the secondary structure components in the following order, the fully exposed parts of the interfacial  $\beta$ -sheets, the partially exposed parts of the interfacial  $\beta$ -sheets and the LA loops.

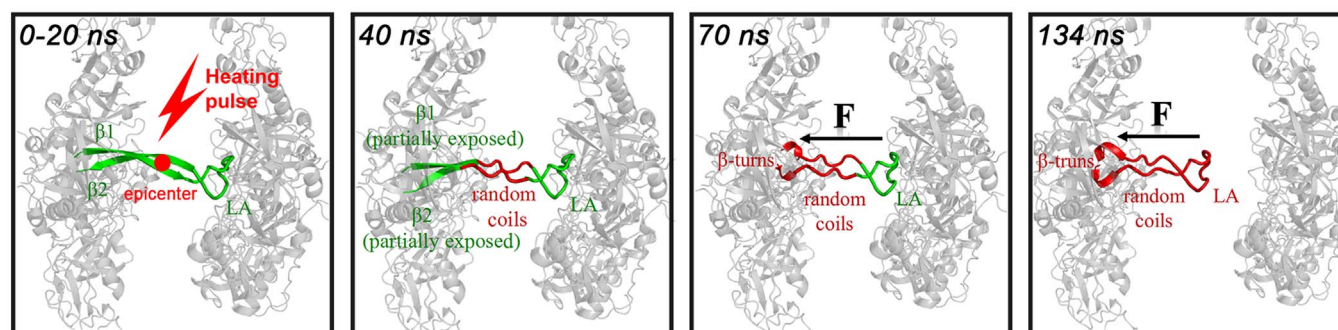
## Discussion

In an attempt to elucidate the structural mechanism for the inactive DegP hexamers to be converted to the active 12- and 24-mers, we hereby examined the process for the DegP hexamer to disassemble into its trimeric units in both thermodynamic and dynamic terms, by using high time-resolved spectroscopic methods to trace the temperature-jump induced structure conversions. Our most remarkable findings include the following. First, our qualitative and quantitative thermal titration analysis reveals that the different secondary structure components of the DegP hexamers possess a degreed thermal stability. Second, our temperature-jump nanosecond time-resolved IR difference spectroscopic results demonstrate also a degreed dynamic stability for the interfacial secondary structure components



**Figure 7** | Unfolding and folding kinetic curves for the interfacial secondary structure components of the DegP hexamer acquired by time-resolved IR absorbance difference spectroscopy at the indicated wavenumbers, after a temperature-jump from 16°C to 25°C. (a) 1622 cm<sup>-1</sup> and 1646 cm<sup>-1</sup>, (b) 1629 cm<sup>-1</sup> and 1663 cm<sup>-1</sup> and (c) 1650 cm<sup>-1</sup>.

of the DegP hexamers. Together these revelations strongly suggest that the interfacial secondary structure components unfold in a sequential manner such that the disassembly of the DegP hexamers occurs via a “proteinquake” mechanism, as illustrated in Fig. 8. Here the term “proteinquake” is defined as such that a perturbation in one part of the protein molecule leads to a successive further perturbations nearby in a quake-like manner, eventually leading to a large conformational change<sup>22–24</sup>. For the DegP hexamer, in particular, the epicenter locates at the fully exposed parts of the interfacial  $\beta$ -sheets which unfold in about 40 ns at 25°C, the perturbation then propagates to the partially exposed parts of the interfacial  $\beta$ -sheets in about 70 ns, and subsequently to the LA loops in about 134 ns, eventually leading to the dissociation of the DegP hexamer to its trimeric units. Owing to the formation of the random coils from the fully exposed part and the formation of  $\beta$ -turns from the partially exposed part of the interfacial  $\beta$ -sheets, the backbone of which is made less extended.



**Figure 8** | Schematic illustration of the proteinquake mechanism for the thermal-induced dissociation of the DegP hexamer with four typical snapshots at the indicated time points. Only one (green colored) of the six  $\beta$ 1-LA- $\beta$ 2 structures at the interface of the trimeric units is displayed. The unfolded structures are red colored. The epicenter (denoted as a red circle) locates at the fully exposed part of the interfacial  $\beta$ -sheet. F denotes the force of the tension. The black arrows denote the direction of the motion of the LA loop.

This creates a tension (denoted as F in Fig. 8) along this backbone, which meanwhile would detach the LA loop away from the opposing trimeric unit, eventually leading to the dissociation of the DegP hexamer into two trimers. The fact that the middle parts of the interfacial links are the most exposed and thus most fragile would make them the ideal sensor for the environmental changes, and thus being the ideal epicenter for the proteinquake, a small perturbation of which would initiate a domino effect that causes a cooperative disruption of the more stable neighboring secondary structures, leading to the effective disassembly of the DegP hexamers. It should be pointed out that the unfolding midpoint temperature for the LA loop, being at  $31.5 \pm 1.5^\circ\text{C}$ , can be taken as that for the DegP hexamer to disassemble into its trimeric units. This midpoint temperature correlates well with the earlier reports, as achieved by size-exclusion chromatography analysis, that the DegP protein exists as hexamers at 25°C but completely dissociates into trimers at 42°C<sup>5,6</sup>, the midpoint temperature of which would thus be 33.5°C.

The fact that the DegP hexamers start to dissociate into trimers even at room temperatures, strongly implicates that this protein can become fully active at the normal growth temperature of 37°C of the bacterial cells. In this regard, the essentiality of DegP for bacterial cell to grow at the heat shock temperatures should be attributed to the increased level of its substrate (i.e., unfolded proteins), instead of the activation of DegP proteins at such temperatures. It follows that the binding of the unfolded protein substrates would help to shift the equilibrium towards the dissociation side by binding to the trimeric units, which leads to the formation of the active and larger oligomers (12- and 24-mers). We envisage that this proteinquake mechanism for oligomeric dissociation might be utilized for other oligomeric proteins that modulate their activities through a similar disassembly and reassembly process.

## Methods

**Protein preparation.** The proteolytically inactive and C-terminal His-tagged hexameric DegP<sub>S210A</sub> (in which the active site serine residue was replaced by an alanine) was utilized in this study to avoid the self-cleavage of the wild-type DegP. The broken cage DegP<sub>S210A</sub> and DegP ( $\Delta$ PDZ2) mutants were derived from pET28a-DegP<sub>S210A</sub>-His<sub>6</sub>, which was constructed in our previous work<sup>25</sup>, by using the QuikChange site-directed mutagenesis method<sup>17</sup>.

To express DegP<sub>S210A</sub>, the broken cage DegP<sub>S210A</sub>, and the DegP ( $\Delta$ PDZ2) mutants, as C-terminal His tagged proteins, the expression vector pET28a containing these mutants were transformed into *E. coli* BL21 (DE3) competent cells. The cells were cultured in LB medium at 37°C to an optical density at 600 nm (OD<sub>600</sub>) of 0.6, and protein expression was induced by addition of 0.5 mM IPTG (isopropyl- $\beta$ -D-thiogalactopyranoside). The cells were further incubated for 6 hours at 37°C. After being harvested, washed and resuspended in buffer A (20 mM phosphate buffered saline containing 500 mM NaCl and 20 mM imidazole, pH 7.5), cells were lysed by sonication on ice. After centrifugation at 20,000  $\times$  g for 40 min, the supernatant was loaded onto a pre-equilibrated 5 ml Ni-NTA agarose column (GE Healthcare), washed sequentially with 30 ml buffer A and 30 ml buffer B (20 mM phosphate buffered saline containing 1 M NaCl and 40 mM imidazole, pH 7.5), before the bound proteins were eluted with 25 ml of buffer C (20 mM phosphate buffered saline



containing 500 mM NaCl and 300 mM imidazole, pH 7.5). The eluted samples were then desalted by dialysis. In all the infrared spectroscopy experiments, the freeze-dried protein powder was dissolved in 99.9% D<sub>2</sub>O (pD 7.4) at a concentration of 12.5 mg/ml.

**FTIR spectroscopy.** Temperature-dependent FTIR spectra were collected on a spectrometer (VERTEX 70 V, Bruker Optics, DE). A two-compartment CaF<sub>2</sub> sample cell with a 50- $\mu$ m thick Teflon spacer was used for protein solution and the reference D<sub>2</sub>O, respectively. The measurements were performed in a home-built vacuum chamber with a temperature controlled at an accuracy of  $\pm 0.1^\circ\text{C}$  by water circulation<sup>26</sup>. Each spectrum was recorded in the 1500 cm<sup>-1</sup>–1750 cm<sup>-1</sup> region at a resolution of 4 cm<sup>-1</sup> and 40 scans were signal-averaged. As the presence of salt in protein solution could cause complexity in amide I' IR absorption<sup>27,28</sup>, all the infrared spectroscopy experiments were carried out in D<sub>2</sub>O.

**Temperature-jump time-resolved IR difference absorbance spectra.** The details of temperature-jump time-resolved IR absorbance difference spectrometer have been described previously<sup>20,26,29</sup>. Briefly, the fundamental output of a Nd:YAG laser (Lab 170, repetition rate: 10 Hz, pulse width: 8–12 ns; Spectra Physics, Mountain View, CA) generates the 1.9- $\mu$ m temperature-jump pulse via Raman shifting in a cell that was filled with 750 psi H<sub>2</sub> gas. The temperature-jump induced transient IR absorbance changes were probed by a CO mid-IR laser at a spectral spacing about 4 cm<sup>-1</sup> (Dalian University of Technology, Dalian, CN) in conjunction with a MCT detector (Kolmar, Newburyport, MA) and a digital oscilloscope (Tektronix, TDS520D, Santa Clara, CA). We took the temperature-dependent FTIR spectra of D<sub>2</sub>O as an internal standard for temperature-jump calibration<sup>20,30,31</sup>. The temporal resolution of this system after the deconvolution of the instrumental response is about 30 ns<sup>20,26</sup>. Heating-and-cooling cycled FTIR spectra were collected to determine the highest accessible temperature at which the reversibility of the protein folding/unfolding can still be achieved<sup>20</sup>, which is about 40°C for DegP<sub>S210A</sub> in D<sub>2</sub>O solution.

- Wickner, S., Maurizi, M. R. & Gottesman, S. Posttranslational quality control: folding, refolding, and degrading proteins. *Science* **286**, 1888–1893 (1999).
- Spieß, C., Beil, A. & Ehrmann, M. A temperature-dependent switch from chaperone to protease in a widely conserved heat shock protein. *Cell* **97**, 339–347 (1999).
- Strauch, K. L., Johnson, K. & Beckwith, J. Characterization of degp, a gene required for proteolysis in the cell-envelope and essential for growth of escherichia-coli at high-temperature. *J. Bacteriol.* **171**, 2689–2696 (1989).
- Lipinska, B., Zyllicz, M. & Georgopoulos, C. The Htra (Degp) protein, essential for escherichia-coli survival at high-temperatures, is an endopeptidase. *J. Bacteriol.* **172**, 1791–1797 (1990).
- Jiang, J. *et al.* Activation of DegP chaperone-protease via formation of large cage-like oligomers upon binding to substrate proteins. *Proc. Natl. Acad. Sci. USA* **105**, 11939–11944 (2008).
- Krojer, T. *et al.* Structural basis for the regulated protease and chaperone function of DegP. *Nature* **453**, 885–890 (2008).
- Krojer, T., Garrido-Franco, M., Huber, R., Ehrmann, M. & Clausen, T. Crystal structure of DegP (Htra) reveals a new protease-chaperone machine. *Nature* **416**, 455–459 (2002).
- Krojer, T., Sawa, J., Huber, R. & Clausen, T. Htra proteases have a conserved activation mechanism that can be triggered by distinct molecular cues. *Nat. Struct. Mol. Biol.* **17**, 844–852 (2010).
- Ortega, J., Iwanczyk, J. & Jomaa, A. Escherichia coli DegP: a structure-driven functional model. *J. Bacteriol.* **191**, 4705–4713 (2009).
- Haslbeck, M., Franzmann, T., Weinfurter, D. & Buchner, J. Some like it hot: the structure and function of small heat-shock proteins. *Nat. Struct. Mol. Biol.* **12**, 842–846 (2005).
- Skorko-Glonek, J., Krzewski, K., Lipinska, B., Bertoli, E. & Tanfani, F. Comparison of the structure of wild-type HtrA heat shock protease and mutant HtrA proteins. *J. Biol. Chem.* **270**, 11140–11146 (1995).
- Sobiecka-Szkatula, A. *et al.* Temperature-induced conformational changes within the regulatory loops L1-L2-LA of the HtrA heat-shock protease from Escherichia coli. *Biochim. Biophys. Acta* **1794**, 1573–1582 (2009).
- Ismail, A. A., Mantsch, H. H. & Wong, P. T. Aggregation of chymotrypsinogen: portrait by infrared spectroscopy. *Biochim. Biophys. Acta* **1121**, 183–188 (1992).
- Sarroukh, R., Goormaghtigh, E., Ruyschaert, J. M. & Raussens, V. ATR-FTIR: A “rejuvenated” tool to investigate amyloid proteins. *Biochim. Biophys. Acta* **1828**, 2328–2338 (2013).
- Fang, Y. & Dalgleish, D. G. Conformation of beta-lactoglobulin studied by FTIR: Effect of pH, temperature, and adsorption to the oil-water interface. *J. Colloid. Interf. Sci.* **196**, 292–298 (1997).
- Jones, K. C., Peng, C. S. & Tokmakoff, A. Folding of a heterogeneous beta-hairpin peptide from temperature-jump 2D IR spectroscopy. *Proc. Natl. Acad. Sci. USA* **110**, 2828–2833 (2013).
- Jomaa, A. *et al.* The inner cavity of Escherichia coli DegP protein is not essential for molecular chaperone and proteolytic activity. *J. Bacteriol.* **189**, 706–716 (2007).
- Yamaguchi, S. & Hamaguchi, H. Femtosecond ultraviolet-visible absorption study of all-trans  $\rightarrow$  13-cis center dot 9-cis photoisomerization of retinal. *J. Chem. Phys.* **109**, 1397–1408 (1998).
- Zhang, L., Yang, J., Wang, L., Yang, G. & Weng, Y. Direct observation of interfacial charge recombination to the excited-triplet state in all-trans-retinoic acid sensitized TiO<sub>2</sub> nanoparticles by femtosecond time-resolved difference absorption spectroscopy. *J. Phys. Chem. B* **107**, 13688–13697 (2003).
- Ye, M. *et al.* Infrared spectroscopic discrimination between the loop and alpha-helices and determination of the loop diffusion kinetics by temperature-jump time-resolved infrared spectroscopy for cytochrome c. *Biophys. J.* **93**, 2756–2766 (2007).
- Kim, S. & Sauer, R. T. Cage assembly of DegP protease is not required for substrate-dependent regulation of proteolytic activity or high-temperature cell survival. *Proc. Natl. Acad. Sci. USA* **109**, 7263–7268 (2012).
- Ansari, A. *et al.* Protein states and protein quakes. *Proc. Natl. Acad. Sci. USA* **82**, 5000–5004 (1985).
- Xie, A. *et al.* Formation of a new buried charge drives a large-amplitude protein quake in photoreceptor activation. *Biochemistry* **40**, 1510–1517 (2001).
- Zang, C. *et al.* Ultrafast proteinquake dynamics in Cytochrome c. *J. Am. Chem. Soc.* **131**, 2846–2852 (2009).
- Wu, S. *et al.* Interaction between bacterial outer membrane proteins and periplasmic quality control factors: a kinetic partitioning mechanism. *Biochem. J.* **438**, 505–511 (2011).
- Li, H. *et al.* Thermal-induced dissociation and unfolding of homodimeric DsbC revealed by temperature-jump time-resolved infrared spectra. *Biophys. J.* **97**, 2811–2819 (2009).
- Wright, W. W., Laberge, M. & Vanderkooi, J. M. Surface of cytochrome c: infrared spectroscopy of carboxyl groups. *Biochemistry* **36**, 14724–14732 (1997).
- Li, H., Xu, Y. & Weng, Y. Infrared absorption intensity analysis as a new tool for investigation of salt effect on proteins. *Chin. J. Chem. Phys.* **22**, 556–562 (2009).
- Zhang, Q. *et al.* Nanosecond-time-resolved infrared spectroscopic study of fast relaxation kinetics of protein folding by means of laser-induced temperature-jump. *Chin. Phys. Lett.* **14**, 2484–2490 (2005).
- Wang, T. *et al.* Length dependent helix-coil transition kinetics of nine alanine-based peptides. *J. Phys. Chem. B* **108**, 15301–15310 (2004).
- Huang, C. *et al.* Helix formation via conformation diffusion search. *Proc. Natl. Acad. Sci. USA* **99**, 2788–2793 (2002).

## Acknowledgments

This work is supported by the Natural Science Foundation of China (grant 20925313 and 21090342 to Y.W.; grant 31170738 to Z.C.), National Basic Research Program of China (grant 2006CB910302 to Y.W.; grant 2012CB917300 to Z.C.) and Chinese Academy of Sciences Innovation Program (KJCX2-YW-W25).

## Author contributions

Y.W. conceived and designed the research. S.L., R.W., D.L., J.M. and H.L. conducted the experiments. S.L., Y.W., Z.C. and X.H. performed analyses. S.L., Y.W., Z.C. and R.W. wrote the manuscript. S.L. prepared all the figures.

## Additional information

**Supplementary information** accompanies this paper at <http://www.nature.com/scientificreports>

**Competing financial interests:** The authors declare no competing financial interests.

**How to cite this article:** Li, S.S. *et al.* Thermal-triggered Proteinquake Leads to Disassembly of DegP Hexamer as an Imperative Activation Step. *Sci. Rep.* **4**, 4834; DOI:10.1038/srep04834 (2014).



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