

substrate-threading power. The opposing side of the DnaK NBD, IB and IIB, where the substrate-binding domain (SBD) sprouts out, points toward the ClpB NTD, which forms the top ring of the pore in the ClpB hexamer^{7,19}. Despite the absence of the DnaK SBD in the structure, the polypeptide substrate bound to the SBD is presumed to be tethered to the entrance of ClpB pore. Further structural studies that include the DnaK SBD and a polypeptide substrate will provide a more detailed picture.

In addition to determining the structure of the ClpB–DnaK complex, Rosenzweig *et al.*⁸ used simple yet powerful chemical shift–mapping NMR experiments to obtain new insight into the functional mechanisms for the disaggregase machinery. Specifically, by characterizing the binding interactions of ClpB, DnaK and the nucleotide-exchanging factor GrpE⁵, they showed that ClpB and GrpE compete for the same binding surface on DnaK (Fig. 1). Following up on this observation, functional assays indeed showed that the presence of GrpE is detrimental for DnaK–ClpB activity, although it is required for DnaK activity downstream of ClpB in assisting with substrate refolding. On the basis of

these findings as well as several previously reported results²⁰, Rosenzweig *et al.*⁸ proposed a model for how ClpB and DnaK collaborate in aggregate unfolding and refolding (reactivation): First, DnaK along with DnaJ binds the aggregated polypeptide, and DnaK SBD engages the polypeptide substrate. Next, DnaK binds ClpB through the NBD–CCD interaction, followed by substrate incorporation into the ClpB pore. Substrate binding to ClpB is coupled with ATP hydrolysis, which is required for substrate threading through the pore of the hexameric ClpB. In the final step, DnaK receives the substrate emerging from ClpB and, in collaboration with DnaJ and GrpE, acts as a foldase chaperone to refold the substrate. Although still rudimentary, the model provides a major step toward understanding of how the disaggregase machinery works. Future structural work that includes DnaJ and a polypeptide substrate will certainly enrich and further refine the *mode d'emploi* of the disaggregase. It is clear that an integrated approach including NMR spectroscopy and crystallography, such as the one presented by Rosenzweig *et al.*⁸, provides a highly effective avenue for disentangling such dynamic protein machineries.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

- Hartl, F.U., Bracher, A. & Hayer-Hartl, M. *Nature* **475**, 324–332 (2011).
- Hartl, F.U. & Hayer-Hartl, M. *Nat. Struct. Mol. Biol.* **16**, 574–581 (2009).
- Tyedmers, J., Mogk, A. & Bukau, B. *Nat. Rev. Mol. Cell Biol.* **11**, 777–788 (2010).
- Doyle, S.M. & Wickner, S. *Trends Biochem. Sci.* **34**, 40–48 (2009).
- Harrison, C.J., Hayer-Hartl, M., Di Liberto, M., Hartl, F. & Kuriyan, J. *Science* **276**, 431–435 (1997).
- Zhu, X. *et al. Science* **272**, 1606–1614 (1996).
- Lee, S. *et al. Cell* **115**, 229–240 (2003).
- Rosenzweig, R., Moradi, S., Zarrine-Afsar, A., Glover, J.R. & Kay, L.E. *Science* **339**, 1080–1083 (2013).
- Sprangers, R. & Kay, L.E. *Nature* **445**, 618–622 (2007).
- Ruschak, A.M. & Kay, L.E. *Proc. Natl. Acad. Sci. USA* **109**, E3454–E3462 (2012).
- Religa, T.L., Sprangers, R. & Kay, L.E. *Science* **328**, 98–102 (2010).
- Gelis, I. *et al. Cell* **131**, 756–769 (2007).
- Zhuravleva, A., Clérico, E.M. & Gierasch, L.M. *Cell* **151**, 1296–1307 (2012).
- Hamel, D.J. & Dahlquist, F. *J. Am. Chem. Soc.* **127**, 9676–9677 (2005).
- Takahashi, H. *et al. J. Biomol. NMR* **34**, 167–177 (2006).
- Chen, L. *et al. Cell Rep.* doi:10.1016/j.celrep.2013.02.025 (in the press).
- Battiste, J.L. & Wagner, G. *Biochemistry* **39**, 5355–5365 (2000).
- Clare, G.M., Tang, C. & Iwahara, J. *Curr. Opin. Struct. Biol.* **17**, 603–616 (2007).
- Wendler, P. *et al. Cell* **131**, 1366–1377 (2007).
- Saibil, H.R. *Science* **339**, 1040–1041 (2013).

Allostery through DNA

Allostery occurs when the binding of a ligand at one site of a protein causes conformational changes at a distant site. Such effects have been extensively studied for enzymes and other proteins, but there were also indications that allostery could occur in DNA. Now Xie, Sun, Su and colleagues have directly observed and characterized allosteric effects through DNA, using a single-molecule fluorescence microscopy approach (*Science* **339**, 816–819, 2013).

The authors immobilized a DNA fragment containing binding sites for two different proteins, separated by a linker of variable length. The first protein was fluorescently labeled, so that its association with DNA could be visualized; then the second protein was added, and the dissociation of the first one was monitored.

The data revealed that the dissociation rate (k_{off}) of the first protein was increased or decreased by the presence of the second protein (see illustration), in an oscillating manner, as a function of linker length and with a periodicity of 10 bp, which is the helical pitch of B-form DNA. The effect disappeared when the linker was longer than 25 bp, yielding a decay length of ~15 bp. Similar results were obtained for DNA-binding proteins, with different sizes, shapes, surface electrostatic features or affinities for their DNA targets.

The oscillating effect was not sensitive to ionic strength conditions, ruling out contributions from potential protein–protein electrostatic interactions. On the other hand, distorting the structure of the linker region—with a nick or mismatch, or using GC-rich sequences—dampened the oscillation. Moreover, replacing the first protein-binding site with a DNA hairpin similarly affected the k_{off} of a bound protein nearby. Notably, the amplitude of the oscillation was lower with a long-loop hairpin, which should cause a smaller DNA distortion, than with a short-loop hairpin. These observations suggest that DNA mechanical properties may produce the allosteric effect.

The authors then used molecular dynamic simulations to examine the structure of DNA, either free in solution or with its central base pair pulled apart to mimic the effect of a bound protein. In the latter condition, the major groove width was altered, compared to that of free DNA, in an oscillating manner and with a periodicity of ~10 bp. Such deviations in major groove width would affect the stability of a DNA-bound protein nearby, accounting for the allosteric coupling between the two sites.

Finally, the DNA allosteric effect was seen in two biologically relevant systems, in both cases with the characteristic oscillatory pattern described above. The presence of a nearby nucleosome affected the binding of a glucocorticoid receptor DNA-binding domain to its target *in vitro*. The activity of T7 RNA polymerase on a promoter was affected by LacR bound nearby: detailed binding kinetics data were obtained *in vitro* and, importantly, similar effects were seen on transcriptional activity in bacterial cells.

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