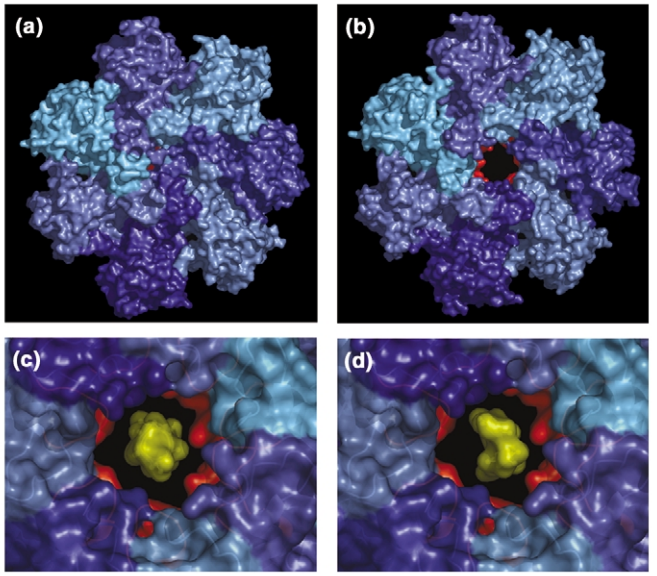

Figure 2. The 20S proteasome entrance. **(a)** Top view of the closed proteasome [7]. N-terminal residues of α-subunits form an ordered structure that covers the α-annulus. **(b)** Top view of the activated proteasome [18,19]. The α-subunit N-terminal tails are restructured to open the gate through the α-annulus. The opening through this gate region is slightly wider than through the α-annulus [19]. The N-terminal residues, up to Tyr8, of each of the proteasome α-subunit do not obstruct the channel in the open conformation [18,19] and have been deleted from this figure to improve clarity. **(c)** Magnified view of the open proteasome conformation showing a model of an α-helix passing the α-annulus. **(d)** This is the same as (c), showing two adjacent β-strands, as seen in a β-sheet, in the α-annulus. The substrate models shown in (c) and (d) are for polyalanine peptides.

annulus does not change conformation, this would be a tight fit requiring sequences with small sidechains.

Substrate-induced gate opening and entry

In addition to their demonstration of endoproteolytic cleavage, Liu *et al.* [12] provide evidence that p21 and α-synuclein facilitate their own degradation, presumably by opening the gate through the 20S proteasome α-subunits. This conclusion follows from the observation of closely similar rates of degradation of the various constructs by the purified 20S and 26S proteasomes, with the 20S proteasome corresponding to the closed form and the 26S proteasome to the open conformation. The 20S proteasomes used in the experiments had closed gates, because they were purified in a latent form that is relatively inactive against model peptide substrates, whereas PA700 appears to open the 20S proteasome gate, because the 26S proteasome shows a higher level of activity against small substrates [20]. Importantly, similar rates of degradation of p21 and α-synuclein substrates were observed using 20S proteasomes prepared in an activated form, in which residues of the gate are thought to be disordered and therefore allow passage of small substrates (G.N. DeMartino, pers. commun.).

The observation that p21 and α-synuclein are degraded at equal rates by 20S and 26S proteasomes differs markedly from the case of another unfolded protein substrate casein, which is degraded rapidly by the 26S proteasome [21,22] but is resistant to degradation by the isolated latent 20S proteasome. Thus, opening of the entrance gate is not a general property of unfolded proteins, but presumably requires specific interactions of defined sequence motifs. An indication that these interactions might include hydrophobic residues is provided by previous reports that some hydrophobic peptides can facilitate peptide hydrolysis by binding to noncatalytic sites of the 20S proteasome, thereby inducing an open conformation for the entrance port [23,24]. It is possible that some unfolded proteins cause residues of the 20S proteasome gate to become disordered because they act as chaotropes (fatty acids and low concentrations of sodium dodecyl sulfate (SDS) appear to activate latent proteasomes *in vitro* by this mechanism). A question for future studies is, 'Do proteins such as p21 and α-synuclein open the 20S proteasome gate exclusively to allow their own entry, or do they also provide a mechanism for degradation of other proteins?'

A clue to how some unfolded substrates might efficiently enter the 20S proteasome is provided by earlier studies that demonstrated a high rate of p21 degradation by purified 20S proteasome, which was dependent upon a specific interaction between p21 and the α7 subunit of the 20S proteasome [25]. Presumably, p21 is able to bind 20S proteasomes in such a way that the closed-gate conformation is destabilized, and p21 is localized to the 'mouth' of the α-annulus and sufficiently unfolded to enter the 20S proteasome. By contrast, α-synuclein has been reported to inhibit proteasome function *in vitro*, perhaps by virtue of its tendency to aggregate and to bind nonspecifically to many proteins [26]. Reconciling these observations and determining the importance of these effects for *in vivo* function will be important challenges for the future.

Concluding remarks

The analysis by Liu *et al.* [12] emphasizes the role of multiple steps in proteasome function. These include targeting of substrate to the proteasome, substrate unfolding, opening of the entrance gate, and high effective concentration of the unfolded substrate at the entrance port. Each of these requirements can be met in more than one way. For example, targeting of substrates can be achieved by polyubiquitylation, association with antizyme (a protein that targets ornithine decarboxylase for degradation) [27] or by direct binding of the substrate to the 20S proteasome. Similarly, some proteasome substrates are actively unfolded by PA700, whereas others are constitutively unfolded. The entrance gate can be opened by binding of activators, such as PA700, by some small hydrophobic peptides or by direct binding of some substrates. In some cases, the high effective concentration of unfolded protein at the α-annulus might result from direct binding of substrate, whereas a more usual mechanism is probably translocation of substrate through the AAA ATPases at the base of PA700 [28] – a process that is more or less coupled to translocation of substrate into the interior of the 20S proteasome.

There are a number of major questions for future studies: how does PA700 bind, unfold and translocate its various substrates? How is the entrance gate opened? To what extent is translocation by PA700 coupled to entry into