inner core[2,3], with the consequent release of latent heat and with gravitational energy powering the magnetic field.

Terrestrial planets with a liquid core and no solidification of the inner core may lack a dynamo — as proposed for Venus[4,5]. Although vigorous thermal convection in a liquid core could theoretically generate a magnetic field[4], no examples of this have been found in the terrestrial planets so far. Therefore, although an early dynamo driven by the cooling of a hot liquid core is possible, the most likely scenario for a terrestrial-type dynamo is onset after the beginning of inner-core solidification and shut-off when the core is substantially frozen.

The Moon provides support for this hypothesis. Correlation of Apollo subsatellite magnetometer data with lunar geology shows that magnetic fields were stronger over Imbrian age units than pre-Imbrian, consistent with a late dynamo turn-on[6]. Lunar palaeointensity data show that a dynamo turned on relatively abruptly about 4 Gyr ago and that the magnetic field became weaker over 1 billion years[7]. This late onset of the lunar dynamo may mark the beginning of inner-core solidification.

The belief that the Martian dynamo stopped after only several hundred million years has led to theories such as the cessation of a plate-tectonic style of mantle convection more than 4 Gyr ago[8]. But we are not convinced that the early dynamo interpretation is correct, and believe the onset time of the Martian dynamo is uncertain.

Rather than requiring a dynamo that turned on and off over 4 Gyr ago, the evidence suggests that the dynamo did not begin until well after this. Only the weakness of the present Martian magnetic field limits its duration. The absence of magnetic anomalies at the Hellas and Argyre basins implies that the Martian dynamo did not exist until after the bulk of the southern hemisphere's crust had formed. If it was operative then, the crust should have been magnetized. Large impacts that subsequently punched holes in the crust would have produced distinctive magnetic anomalies. As no anomalies associated with impact basins have been observed, the bulk of the crust in the south is not magnetized and there was no Martian dynamo at crustal formation or when the basins formed at about 4 Gyr or earlier. Impacts into the previously unmagnetized crust of a Mars with a magnetic field should have created magnetic anomalies.

Although we cannot rule out the possibility that the dynamo turned on after the southern crust formed and stopped before the major impact basins were formed, it is easier to explain its turn-on after 500 Myr of core evolution than its turn-off before this time. Models of core cooling suggest that a dynamo lasts longer than a few hundred million years, especially as core cooling leads to inner-core solidification that powers the dynamo as long as the inner core continues to grow[4,9]. This dynamo action might await the onset of inner-core growth, which could take 500 Myr or more. So although dynamo action could be shortened by a change in Martian mantle convection from a plate-tectonic regime (efficient core cooling) to a rigid-lid regime (inefficient core cooling)[8], we still believe that the onset did not occur until after 500 Myr of core evolution and the formation of the major impact basins.

If the dynamo turned on after the giant impact basins formed, then the magnetized southern regions must either be later magmatic additions to the crust (after 500 Myr of evolution) or thermal reworking of older crust. Although, on average, the crust has cooled over time, local regions have been heated by upwelling plumes. The non-uniformity of the magnetization in the south suggests that it arose mainly from localized heating and cooling events that postdate the global cooling to below the Curie point of the southern highland crust. As there does not seem to be magmatic activity in most of the magnetized regions, these heating events must have been due to upwellings smaller than those responsible for Tharsis and the Elysium volcanoes.

There is no unambiguous constraint on the dynamo turn-off time other than its absence at present. The remanence of the SNC (Shergotty, Nakhla and Chassigny) meteorites with formation ages of 1.3 Gyr to 180 Myr is consistent with ancient surface fields of 500 to 5,000 nanotesla (ref. 10), but there could be such fields at the surface of Mars even today. Nevertheless, the presence of localized magnetic anomalies in the younger crust of the northern hemisphere[11] indicates that the dynamo could not have turned off too quickly. Strongly magnetized terrain also extends from the southern hemisphere highlands into the Tharsis region, one of the youngest surfaces on Mars, emplaced well after the heavy bombardment[12].

We believe that high spatial resolution magnetometry with balloons or airplanes in both northern and southern hemispheres would resolve the nature and timing of the magnetization, much as shipborne magnetometers improved our understanding of the Earth's dynamo and plate tectonics.

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ATP-dependent proteases

Docking of components in a bacterial complex

Proteases are enzymes that cut up other proteins for the purposes of tailoring or degradation. Some depend on ATP as an energy source for unfolding protein substrates, and these are often organized into rings of ATPase subunits stacked coaxially onto rings of protease subunits[1]. Bochtler *et al.*[2] have reported a crystal structure for the ATP-dependent protease complex HslVU (also known as ClpYQ) from *Escherichia coli*. They claim this consists of a double hexamer of the protease HslV flanked by hexamers of an ATPase, HslU, which mainly lie in a ring of ATPase domains whose I-domains protrude to form a smaller ring that binds HslV. Based on cryo-electron microscopy of HslVU in buffer conditions that support enzymatic activity, we find that the HslU rings bind in the opposite orientation — that is, their I-domains protrude distally instead of making contact with HslV. Redefinition of this interaction has implications for the functional architecture of the complex.

The components of HslVU[2] share features with other protein structures: HslV is similar in sequence and fold to the proteasome β-subunit[3], and the ATPase domain of HslU resembles the D2 domain of the protein NSF (for *N*-ethylmaleimide-sensitive fusion protein)[4], apart from containing an insertion of a 133-residue intermediate (I) domain. In the reported crystal structure of HslVU[2], the I-domain ring is in contact with HslV. This configuration is inverted relative to that suggested by negatively stained electron micrographs, which show the wider, denser ring of HslU in contact with HslV[5]. Bochtler *et al.*[2] attribute these earlier observations to a flattening artefact, and expand on the functional implications of the contacts between the I-domain and the protease.

We observed HslVU preserved virtually in its native state by cryo-electron microscopy. The averaged side view at 30 Å resolution agrees well with our negatively stained rendition of the complex (compare Fig. 1a to b, c): in both cases, the wider, larger ring of HslU is adjacent to HslV. In this respect,

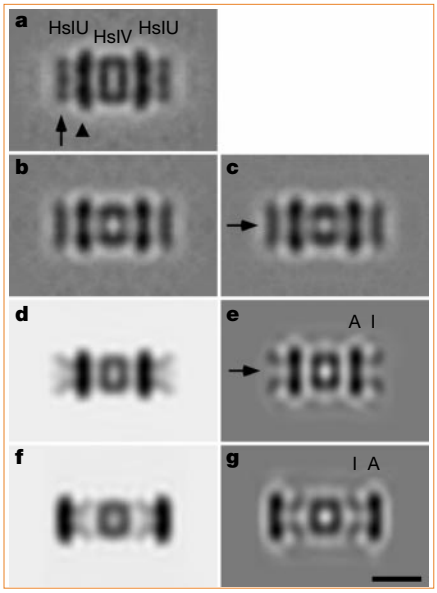

Figure 1 Averaged side-view projections of HslVU complexes. **a–c,** Electron micrographs of complexes formed in 50 mM Tris–HCl, pH 7.5, 0.2 M KCl, 10 mM $MgCl_2$ and 1 mM ATP, representing appropriate conditions for proteolytic activity. **a,** Negatively stained molecules (ATP-γS state; number of particles, $N = 65$; resolution, 32 Å). Note that the proximal ring of HslU (arrowhead) is wider and more dense than the outer ring (arrow); **b,** frozen-hydrated molecules (ATP-γS state; $N = 250$; resolution, 33 Å); **c,** frozen-hydrated molecules in the AMP-PNP state, where AMP-PNP is an inactive ATP analogue ($N = 400$; resolution, 33 Å). **d–g**, Side-view projections calculated[6] from the crystal structure[2] but limited to 30 Å resolution. Projections corresponding to different rotational settings of the complex around the axis were averaged to give a cylindrically averaged side view, as in the electron micrographs (EMs). In **d** and **e**, HslU is in the opposite orientation from the one in the crystal structure, whereas in **f** and **g** this corresponds to the published orientation[2]. Projections shown in **e** and **g** were created by applying a phase-contrast transfer function (CTF; corresponding to 2.0 μm underfocus) to images in **d** and **f**, and so are more comparable to the cryo-EMs. With or without CTF correction, it is evident that the wider, denser ring, corresponding to the ATPase domains of HslU, is adjacent to HslV. Arrows in **e** and **c** mark the axial density that is missing in **e** but present in **b** and **c**, which we attribute to residues 175 to 209. In **e** and **g**, I denotes the I-domain ring, and A denotes the ATPase-domain ring. Scale bar, 100 Å.

the images are inconsistent with a side-view projection (Fig. 1f) generated from the published coordinates of HslVU[2] and limited to the same resolution[6].

For closer comparison, we subjected this image computationally to phase-contrast effects (Fig. 1g), simulating those of the cryo-electron microscopy images. Conversely, an excellent match was obtained with similarly generated projections (Fig. 1d, e) in which HslU was inverted (compare Fig.1c, e). We conclude that the I-domains are exposed on the distal surfaces of the HslVU complex, and the opposite face of the HslU ring binds to HslV. Despite good overall agreement with the results from cryo-electron microscopy, the calculated reprojection shows the central part of the distal ring of HslU as relatively depleted in density (arrows in Fig.1c, e). We infer that the additional density in the electron microscopy images represents six copies of residues 175–209 which were not seen in Bochtler *et al.*'s crystal structure[2].

HslU belongs to the AAA superfamily[7] of ATPases, as do the ATPases of the 26S proteasome, and ClpA and ClpX of *E. coli*, which both partner the protease ClpP (ref. 1). As demonstrated for ClpA[8] and ClpX[9], all such ATPases are likely to have protein 'unfoldase' activity. Processive degradation is carried out by fully assembled holoenzymes[10] and requires the coordinated activity of multiple sites. The geometry of interaction between the ATPase and proteinase rings is crucial in specifying the positions of the sites at which substrates bind, where they are unfolded, and the path along which they translocate into the digestion chamber inside the protease.

Our model assigns the I-domains to the distal surfaces of the HslVU complex, in an exposed position that would be suitable for substrate binding. This proposition is consistent with data showing protein substrates binding to the distal surfaces of both ClpXP[11] and ClpAP (T.I. *et al.*, manuscript submitted). In this revised model, residues in the ATPase domain of HslU, which includes the carboxy-terminal sensor-2 domain[7], are responsible for binding to HslV.

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Bochtler et al. *reply* — The central issue raised by Ishikawa *et al.* is that of the configuration of the productive HslVU complex. We note that complexes of HslV and HslU in *E. coli* are labile and unstable under many conditions[1]. The original electron microscopy (EM) images of Rohrwild *et al.*[2] appear to show free HslV, free HslU and HslV–HslU complex particles. To explain the discrepancy between the HslV–HslU arrangement in our co-crystals and their negatively stained EM data, we suggested that there might have been a collapse of the fragile I-domain structure in the EM preparations, or a reversal in the orientation of the HslU rings[1]. Ishikawa *et al.* interpret their results from cryo-EM at 30 Å resolution in this latter way, using our crystal data of the components. Although these preparations preserve the native structure better than the negatively stained ones, our HslV–HslU samples are also active under crystallization conditions[3].

We have attempted to distinguish between the two docking modes (I-domains distal or proximal to HslV) in mutagenesis experiments involving more than two dozen mutants[3]. We disrupted putative contact sites to HslV in the I-domain of HslU (Fig. 3b in ref. 1) and on its opposite face and find none of these mutations has any effect on peptide hydrolysis or on casein degradation. This suggests either that no precise complex is required, or that both modes of docking are feasible. In contrast, degradation of the physiological substrate fusion protein MBP–SulA is affected by mutations both in the I-domain as well as those involving the opposite side of HslU. Small-angle X-ray scattering data and a crystal structure of the *Haemophilus influenzae* HslVU complex are also consistent with the EM docking mode[4].

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Addendum

A new model for protein stereospecificity

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Nature **403,** 614–615 (2000)

To clarify possible misunderstanding over the term "new" in this communication, we meant our new model replaces the old Ogston model, as this is incompatible with our X-ray crystallographic data. We did not intend "new" in this context to mean that nobody had ever questioned the Ogston model before (for example, see refs 1–5).

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Editorial note: As Brief Communications do not allow space for conventional introductions, we asked the authors for this addendum to clarify a possible misinterpretation.