

seems likely that CP43 is located in an arrangement analogous to that proposed for CP47, but on the opposite side of the reaction centre (so, CP47 and CP43 would be approximately related by a 2-fold rotational axis passing through the centre of the D1/D2 region). Our projection map can be compared directly with the structure of the PSII supercore complex. This complex has been analysed by single-particle averaging^{9–12}, and is composed of a core dimer together with LHC and LHC-like proteins. The best agreement is found when using the dimeric complex from the PSII map outlined in Fig. 2, as this fits well into the region of the PSII supercore complex that is thought to contain the reaction centre and CP47 (Fig. 4). □

Methods

Two-dimensional crystallization. PSII was isolated from grana of spinach thylakoid membranes solubilized with *n*-heptyl- β -D-thiogluconide and crystallized as described in ref. 6 but with a slight modification. The chlorophyll concentration of the PSII OECs used for crystallization was 1 mg ml⁻¹ and the 100- μ l samples were dialysed against buffer containing 30% glycerol. When detergent was removed by dialysis the PSII complex forms well-ordered, large tubular crystals up to 3 μ m long after 1–3 weeks. The crystals are stable at 4 °C for several weeks.

Electron cryo-microscopy. Specimens for electron cryo-microscopy were prepared on carbon film supported by copper grids in the presence of 0.5% tannin solution¹³ in dim light. Specimens were frozen rapidly in liquid nitrogen and mounted in a Gatan cryo specimen holder precooled with liquid nitrogen. Images were recorded at -182 °C using a Philips CM200 field emission gun electron microscope equipped with a cold fork anticontaminator at an electron dose of 5–10 e⁻ Å⁻². Micrographs were recorded on Kodak SO-163 electron emulsion film with an acceleration voltage of 200 kV, a magnification of $\times 50,000$ and 1-s exposure time. Films were developed for 12 min in full-strength Kodak D-19 developer.

Image processing. Well-ordered crystalline areas of 6,000 \times 6,000 pixels of the best four images were selected by optical diffraction and digitized on a Perkin-Elmer 1010 GM microdensitometer with a circular 10- μ m pixel at 7- μ m step size, corresponding to 1.4 Å at the specimen. Images were processed as described^{14,15} using MRC image-processing programs. Distortions of the two-dimensional crystal lattice were corrected by the program CCUNBEND, using a 400 \times 400 pixel reference area in the first pass and 200 \times 200 pixels in the final correction pass. As a result of the unbending procedure, the intensities of reflections increased on average by roughly a factor of two compared with raw data. After the correction of contrast transfer and objective-lens astigmatism, Fourier components from six lattices on four images with IQ value (a numerical grade of the signal-to-noise ratio of spots) ≤ 7 were merged by phase origin refinement. The maximum resolution was examined with the program HALFSTAT (provided by R. Henderson, MRC, Cambridge).

Two-dimensional map comparisons. To prepare Fig. 3a the α -carbon backbone from L and M subunits of *R. viridis* reaction centre was extracted from the Brookhaven protein database PDB file. Two-dimensional projection maps of the structure viewed from the cytoplasmic side were calculated. The resulting map was restricted to a resolution of 8 Å by band-pass filtering of the Fourier transform. Figure 3d was prepared in the same way except that the starting point was a set of three-dimensional coordinates defining the ends of the α -helices identified in a 4-Å map derived from X-ray crystallography⁸. Polyalanine α -helices were constructed between the helix ends and the resulting PDB file was processed as for Fig. 3a. The simulated projection maps were quantitatively aligned by cross-correlation against the experimental PSII data using the image processing system SPIDER¹⁶. The radial distribution of amplitudes in the Fourier transforms of the simulated projection maps was

adjusted to match that of the experimental data. This procedure improved both the discrimination between trial alignments and the absolute values of the correlation coefficients for the best fits.

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addendum

Unaltered susceptibility to BSE in transgenic mice expressing human prion protein

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In this Letter we reported that transgenic mice expressing both human and mouse prion protein are no more susceptible to BSE than their wild-type counterparts. We also included the interim results of challenge of mice expressing only human prion protein (HuPrP^{+/+}Prn-p^{0/0}) with BSE; these mice had remained disease-free 264 days post-inoculation. We have now found that from 489 days onwards, some of these HuPrP^{+/+}Prn-p^{0/0} mice developed a prion disease. More details appear on pages 448–450 of this issue. □