subunits in the thylakoid membrane is dictated by the 83 kDa subunits PsaA and PsaB. They form a heterodimer in which the two subunits are related by a pseudo-twofold rotation axis (pseudo-C_2). This heterodimer is surrounded by seven smaller membrane intrinsic subunits and three extrinsic subunits located on the stromal side.

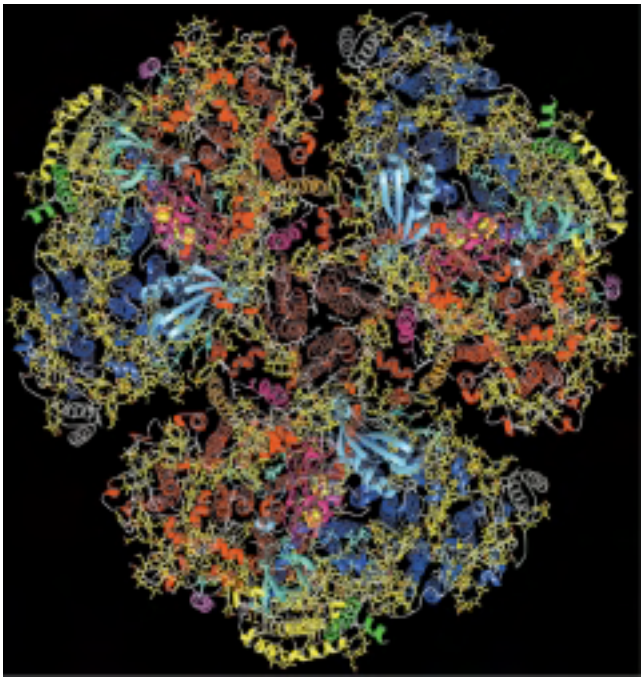

Fig. 8: Complete view of cyanobacterial, trimeric photosystem I from the stromal side of the membrane. The 12 protein subunits are in different colours, chlorophylls in yellow, carotenoids in white.

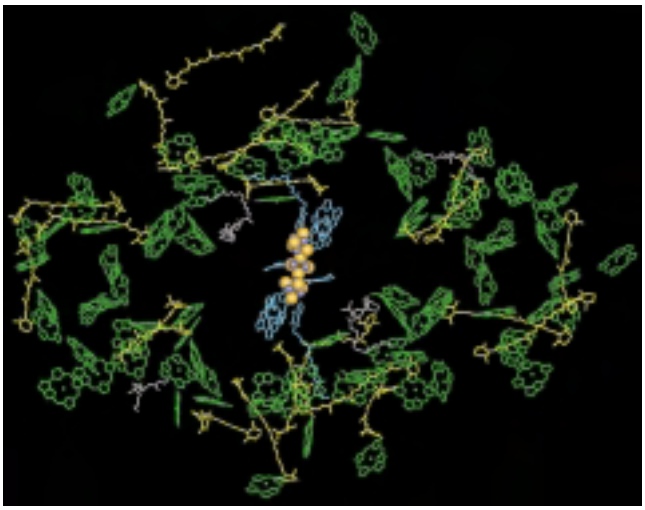

Fig. 9: Cofactors of the antenna system (chlorophylls green, ring substituents omitted for clarity, carotenoids yellow) and of the electron transfer chain (blue and Fe4S4 cluster as yellow and blue spheres) in one monomer of photosystem I.

The charge separation across the membrane is performed by a set of cofactors termed the electron transfer chain (**Figure 9**). It consists of three pairs of chlorophylls and one pair of phylloquinones (Vitamin K_1), positioned along the pseudo-C_2 axis in two branches, followed by the 4Fe4S cluster F_X that is located right on the pseudo-C_2 axis and coordinated to both PsaA and PsaB. The electron is further transferred to the two terminal 4Fe4S clusters F_A and F_B located in the stromal subunit PsaC. The light energy required to drive this process is captured by the integral antenna system containing 90 chlorophyll a and 22 carotenoids (Figure 9). The crystal structure of PSI shows new types of Mg^{2+} axial ligands, not previously observed in the structures of other (bacterio)chlorophyll-protein complexes, the most striking being a phospholipid oxygen and methionine sulphur.

The structure of PSI provides a detailed picture of the architecture of this protein-cofactor complex. Since the locations and orientations of all the cofactors are known, and their chemical environments are visible for the first time, it is now possible to carry out theoretical studies to understand the spectroscopically determined electron and exciton transfer kinetics between the different cofactors. However, an unambiguous assignment of individual spectral and redox properties of the cofactors is not possible at the moment. The new structural data will initiate mutational studies on PSI which will help to unravel structure-function relationships in more detail.

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Antibiotics Targeting Ribosomes

Analysis of high-resolution structures of complexes of antibiotics with ribosomal particles sheds light on antibiotic selectivity and illuminates various modes of action, from reduction of decoding accuracy via limiting conformational mobility, to interference with substrate binding and hindrance of the progression of growing proteins. Their interactions and the lack of major conformational rearrangements associated with antibiotic binding, support the suggestion that the

ribosome provides a framework for peptide bond formation, rather than enzymatic activity.

Resistance to antibiotics is a significant problem in modern therapeutics. Ribosomes of pathogenic bacteria are major targets for antibiotics. Ribosomes are a cellular organelle catalysing the translation of genetic code into proteins. They are protein/RNA assemblies arranged in two subunits that associate for performing protein biosynthesis. The large subunit (1.5 megaDa, 3000 nucleotides in two RNA chains and ~35 proteins) creates the peptide bonds and provides the path for emerging nascent proteins. The smaller subunit (0.85 megaDa, 1500 nucleotides in one RNA chain and ~20 proteins) has key roles in controlling the fidelity of codon-anti-codon base-pairing and in initiating the biosynthetic process.

The high-resolution structures of ribosomal subunits from two pathogen-models [1], obtained recently by bright synchrotron radiation, were used as a reference that allowed unambiguous localisation of several antibiotics. Among those reported here, six were clinically relevant and one was of no clinical use. All were found to bind primarily to ribosomal RNA and their binding did not cause major conformational changes.

Small subunit antibiotics: Tetracycline was found to be a multi-site antibiotic with inhibitory action that stems from its interference with A-site tRNA binding. Edeine, a universal agent, inhibits the initiation of protein synthesis by linking critical features for tRNA, IF3 and mRNA binding, thus imposing constraints on ribosomal mobility that accompany the translation process (**Figure 10**). Its universality implies conservation of structural elements important for initiation.

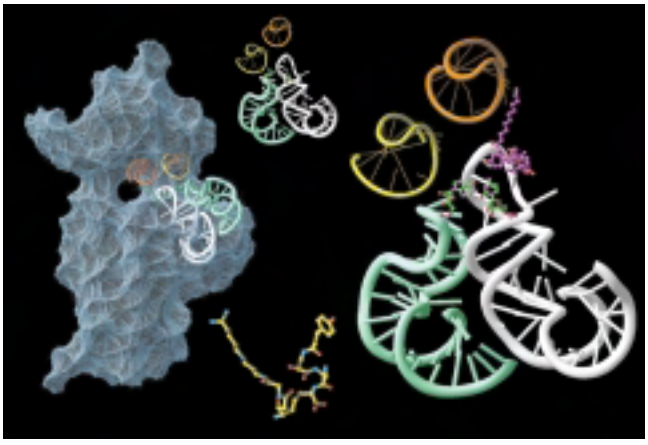

Fig. 10: (Left) The small ribosomal subunit. The mRNA path and the P-(orange) and E-(yellow) sites are shown. The RNA features that are "frozen" by edeine are highlighted in white and cyan. In the assembled ribosome the large subunit will face the left side of the particle.
(Middle) top: the free edeine binding site. Bottom: the structure of edeine. (Right) Detailed view of edeine (purple) binding site. Note the newly formed base pair (green).

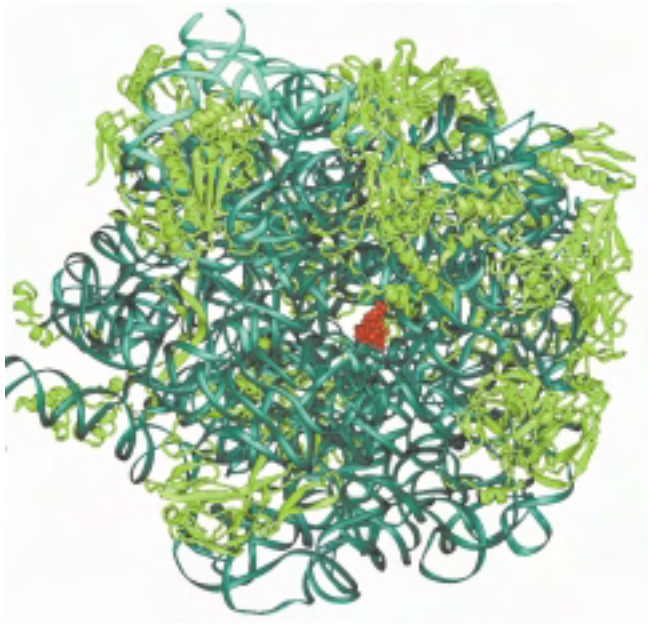

Fig. 11: The position of erythromycin (red) within the large ribosomal subunit - RNA (dark green), the proteins (light green). The view is from the active site into the protein exit tunnel.

Large subunit antibiotics: Chloramphenicol targets the peptidyl transferase cavity close to the amino acceptor group of tRNA. Clindamycin interferes with substrate binding and physically hinders the path of the growing peptide chain. The macrolides erythromycin, clarithromycin and roxithromycin bind to the entrance of the protein exit tunnel and block the progression of nascent proteins (**Figure 11**). Interestingly, none of these antibiotics binds to the nucleotides assigned to be crucial for the catalytic mechanism of the ribosome that was proposed based on the 2.4 Å structure of the *Haloarcula marismortui* large subunit [2].

Comparative studies have helped to identify elements that may confer drug selectivity (*e.g.* **Figure 12**). The antibiotics

Fig. 12: Clindamycin binding site shown on a superposition of the backbone of the peptidyl transfer ring of a eubacterial pathogen model (D50S) and of its archeal counterpart (H50S) which serves as a model for eukaryotes (*E. coli* numbering scheme).

modes of interactions and the preservation of the active-site conformation, favour the suggestion that the peptidyl transferase center serves as a template for proper positioning of tRNAs to allow for spontaneous, rather than enzymatic, creation of peptide bonds. The ribosomal components constructing the frame for accurate positioning of the tRNA molecules may include proteins, CTC, L27 and L16.

Antibiotics targeting ribosomes are excellent tools for studying ribosomal function and for understanding mechanisms of drug action. Analysis of their modes of action should lead to structure-based design of improved antibiotics.

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The Structure of Bacteriophage T7 Endonuclease I: A Holliday Junction Resolving Enzyme

The rearrangement, or recombination, of DNA is an ancient and fundamental biological process. Recombination is central to many diverse biological processes such as the generation of genetic variation (and therefore evolution) and the incorporation of viral DNA into host DNA, resulting in successful viral infection.

The process of DNA recombination occurs in distinct stages (**Figure 13**), with the formation of a four-way (Holliday) junction as a pivotal intermediate. The penultimate step in DNA recombination is regulated by a junction resolving enzyme or 'resolvase'. This enzyme cleaves the Holliday junction resulting in rearranged DNA strands. Bacteriophage

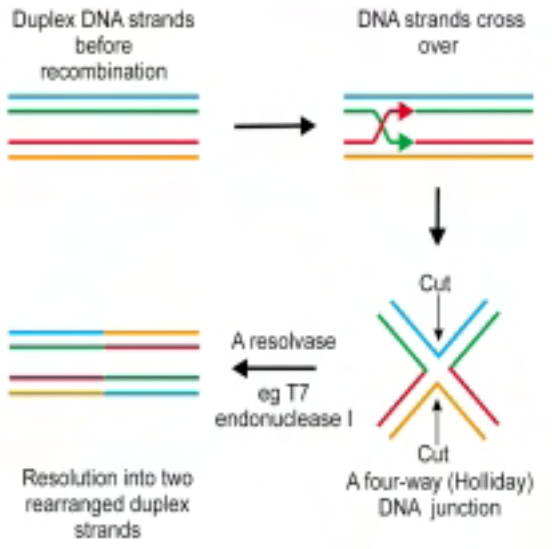

Fig. 13: The process of recombination.

T7 encodes a protein, endonuclease I, which has been shown to act as a four-way DNA junction resolvase.

In order to understand the mechanism by which a four-way DNA junction resolving enzyme cleaves DNA we have solved the structure of an inactive mutant of bacteriophage T7 endonuclease I (E65K), using X-ray crystallography. Extensive crystallisation trials showed that high quality protein crystals could only be obtained when the first 11 N-terminal amino acids were removed from the protein. Crystals of endonuclease I (ΔN11, E65K) diffracted X-rays to 2.1 Å on station ID14-EH3.

The crystal structure was solved by the MAD method using data collected from a selenomethionine-substituted protein. However, endonuclease I (ΔN11, E65K) does not contain any endogenous methionine residues. For this reason a methionione containing mutant (I92M) was generated to allow selenomethionine incorporation (endonuclease I, ΔN11, E65K, I92M). The junction cleavage activity of the protein is unaffected by the introduction of this methionine residue. Selenomethionine-substituted crystals diffracted X-rays rather more weakly than those of the native protein, and three data-sets were collected to 3.0 Å and one to 2.5 Å using station ID14-EH4. All four selenium sites in the asymmetric unit were identified by the SOLVE package, and electron density maps were calculated using the CCP4 suite of programs. The SOLVE derived phases were clear enough to identify protein solvent boundaries and several secondary structure elements, but were significantly improved by density modification and non-crystallographic symmetry averaging. Models were initially built at 2.5 Å using data from the selenomethionine-substituted (ΔN11, E65K, I92MSe) protein. Later in refinement, 2.1 Å data collected from a crystal of endonuclease I (ΔN11, E65K) were introduced.

The structure shows that endonuclease I forms a symmetric homodimer arranged in two well-separated domains