# Complex conformations and crystal contacts

**To the editor:**

In the October 2002 issue of *Nature Structural Biology*, Kuglstatter *et al.*[1] presented a crystal structure of the signal-binding domain (also called 'M-domain') of human signal recognition particle (SRP) 54-kDa subunit (SRP54) in complex with 7SL SRP RNA and SRP19. This structure explains many of the biochemical properties of the signal recognition particle. One of the major conclusions of Kuglstatter *et al.*[1] is that SRP54 induces a conformational change in its binding site on 7SL RNA upon binding to the preformed SRP19–RNA binary complex.

Although this conclusion is supported by comparison with a previous crystal structure of the binary complex between SRP19 and SRP RNA[2] (see **Fig. 1a,b** for a schematic diagram), chemical probing results (**Fig. 1**) from both archaeal[3] and human[4] SRP19–RNA binary complexes are more consistent with the RNA conformation observed in the crystal structure of the ternary complex than with the crystal structure of the binary complex. These observations raise questions about the biological relevance of the proposed SRP54-induced conformational change.

Chemical probing clearly supports the formation of the A-minor interactions[5,6] in an SRP19-dependent manner. Simultaneous binding of helix 6 and helix 8 by SRP19 likely stimulates formation of the A-minor motifs, which precipitate reorganization of helix 8 to create the SRP54 binding site. Interestingly, a reversed form of the A-minor interactions, in which adenosines from helix 6 contact pyrimidines on helix 8, was observed in a crystal structure of the binary complex of SRP19 and SRP RNA from *Methanococcus jannaschii*[7].

Why, then, does the crystal structure of the binary SRP19–RNA complex not adopt a conformation more like that illustrated in **Figure 1b**? The answer may lie in crystal packing interactions. In the crystal of the binary complex, the helix 8 SRP54 binding site is intimately involved in packing with a symmetry-related molecule (**Fig. 1c**).

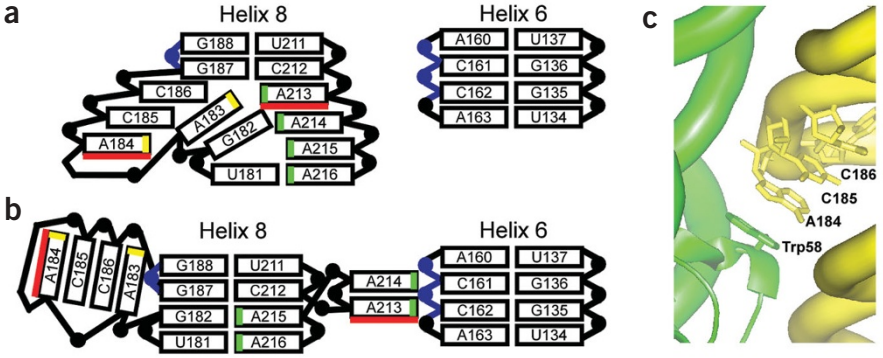


**Figure 1** Schematic representations of the human RNA structure in (**a**) the SRP19–RNA binary and (**b**) the SRP54-M–SRP19–RNA ternary complexes in the region of the SRP54 binding site. SRP19-dependent chemical modifications from both *Archaeoglobus fulgidus*[3] and *Homo sapiens*[4] are indicated on the structures: blue, protection from hydroxyl radicals (probes backbone)[3,4]; red, exposure to diethylpyrocarbonate (indicates base unstacking)[3]; green, protection from; and yellow, exposure to dimethylsulfate respectively (probes Watson-Crick hydrogen bonding)[4]. (**c**) Crystal packing interactions between two symmetry-related molecules in the SRP19–RNA binary complex[2]. SRP19 is green and 7S RNA is yellow (PDB entry 1L9A).

Specifically, Trp58 from SRP19 of one complex in the crystal lattice makes perfect stacking interactions with A184 from the RNA of another complex related by crystallographic symmetry. Thus the structure of the local region of SRP RNA in **Figure 1a,c** is very likely a minor conformation stabilized by crystal contacts. Binding of the M-domain of SRP54 to form the ternary complex leads to a change in the crystal packing, and ultimately in the space group, from $P2_12_12$ to $P6_522$.

This discussion raises two points. First, if crystal packing is in fact stabilizing the observed conformation of the binary SRP19–RNA complex, then it is likely that the conformational change proposed by Kuglstatter *et al.*[1] is biologically irrelevant. The biochemical data suggest that binding of SRP19 to the RNA largely reorganizes the asymmetric loop of helix 8 to create something very much like the RNA structure in **Figure 1b**, thereby greatly enhancing the affinity of SRP54 for a pre-organized SRP RNA. Second, as a general point, if a significant localized conformational change is proposed based on crystallographic structures, then the adjacent crystal contacts should be shown and discussed.

COMPETING INTERESTS STATEMENT
The author declares that he has no competing financial interests.

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**Oubridge *et al.* reply:**

**Recently we reported the crystal structures of two assembly intermediates of human signal recognition particle and proposed a molecular mechanism of its assembly process[1,2]. Diener now claims that part of our structure of the binary complex between 7SL RNA and SRP19 might be influenced by crystal packing. Hence our proposed mechanism might not be biologically relevant.**

**This concern is solely based on results of RNA probing experiments by Diener and Wilson[3] on *Archaeoglobus fulgidus* SRP and by Rose and Weeks[4] on the human binary**

complex alone. The sequence of *A. fulgidus* SRP RNA lacks the asymmetric loop ACC trinucleotide conserved in the majority of species and is significantly different from the human counterpart; the projection of the *A. fulgidus* results onto human RNA is misleading. Unlike in the human system, *A. fulgidus* SRP54 binds to SRP RNA in the absence of SRP19. Hence, the results on the *A. fulgidus* RNA are not directly applicable to the human system. Rose and Weeks studied only free RNA and the binary complex, and results of the ternary complex would be necessary to establish whether there is sufficient evidence against our proposed mechanism. Here we present the results of our RNA chemical probing experiment on human SRP.

We have chosen dimethyl sulfate (DMS) as a probe, as it reacts with the unpaired N1 and N3 positions of adenosine and cytosine respectively and hence serves as the most suitable probe to detect a conformational change of the asymmetric loop within helix 8, which is rich in these nucleotides. Addition of SRP19 causes protection of A199 and A201 in the tetraloop of SRP RNA helix 8 (Fig. 2a,b), in agreement with Rose and Weeks[4] and our crystal structure[2]. A further addition of SRP54 enhances the DMS reactivity of C185, A208 and A215 and reduces the reactivity of A192 (Fig. 2b,c). The N3 atom of C185 and the N1 atom of A215 become completely exposed in the ternary complex, in agreement with enhanced reactivity toward DMS in the ternary complex. A192 is occluded by the M-domain; this observation explains the reduced DMS reactivity toward A192 in the ternary complex. A208 with enhanced reactivity is more exposed in the ternary complex. The change in the DMS reactivity observed here is therefore consistent with our mechanism based on the crystal structure of the binary and ternary complexes[1,2].

Diener claims that SRP19 induces the formation of A-minor motif interactions already in the binary complex, and the structure of the asymmetric loop is similar between the binary and ternary complexes. This might be true in *A. fulgidus* but not necessarily so for the human system. From the structural standpoint, the formation of the A-minor motifs and the RNA platform in the binary complex is unlikely. The phosphate backbone of the long strand of the asymmetric loop comes very close to the phosphate backbone of the symmetric loop in the human ternary complex[1] and the *Escherichia coli* Ffh complex[8]. The RNA platform is stabilized by an intricate network of hydrogen bonds involving RNA, the positively charged M-domain and metal ions. This structure is unlikely to form in the absence of the M-domain. In the absence of such an intricate network of interactions, the asymmetric loop is inherently flexible in the binary complex, as indicated by high *B*-factors. This explains why it can be readily involved in crystal packing. Similarly the asymmetric loop of *E. coli* 4.5S RNA is flexible[9] and is involved in crystal contact but retains high *B*-factors[10]. In the *M. jannaschii* binary complex the bases in the long strand of the asymmetric loop are also splayed out and involved in crystal contact. The high temperature factors[7] of these nucleotides also indicate inherent flexibility of the asymmetric loop in the *M. jannaschii* binary complex.

The paper by Diener and Wilson[3] describes very careful thermodynamic and RNA probing experiments of the *A. fulgidus* SRP. When the structures of the *A. fulgidus* binary and ternary complexes are solved their results can be interpreted in light of the structures and will undoubtedly provide invaluable insight into evolution of SRP and its unique assembly mechanism.

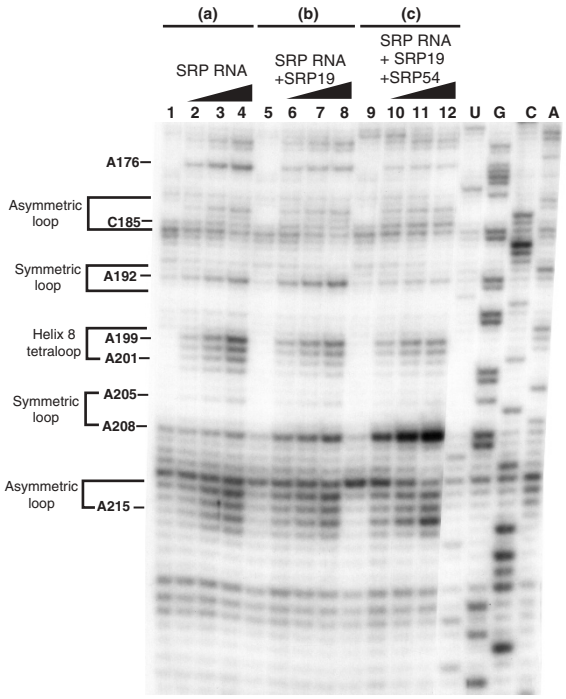


**Figure 2** Chemical modifications of nucleotide bases of SRP RNA. A fragment of human 7SL RNA, termed SRP 151 RNA (nucleotides 101–251) was probed with dimethyl sulfate and modified positions were detected by primer extension using reverse transcriptase[11]. (**a**) Free SRP RNA (3.5 M), (**b**) SRP151 RNA (3.5 M) in complex with 7 M SRP19 or (**c**) SRP151 RNA (3.5 M) in complex with 7 M SRP19 and 7 M SRP54 were incubated with DMS (1 l of DMS diluted 1:15 in ethanol) for 5 min (lanes 2, 6 and 10), 10 min (lanes 3, 7 and 11) or 20 min (lanes 4, 8 and 12) at room temperature. Lanes 1, 5 and 9 were incubation controls in the absence of DMS. BSA was included in **a** and **b** to the same final protein mass as in **c** to compensate for DMS quenching effects. Reactions were stopped with 0.3 M sodium acetate, pH 6.0, phenol extracted and precipitated with ethanol. Primer extensions using a primer complementary to the 18 nucleotides of the 3′ end of the SRP 151 RNA were performed according to standard procedures[11]. Sequencing lanes were run in parallel.

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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1. Kuglstatter, A., Oubridge, C. & Nagai, K. *Nat. Struct. Biol.* **9**, 740–744 (2002).
2. Oubridge, C., Kuglstatter, A., Jovine, L. & Nagai, K. *Mol. Cell* **9**, 1251–1261 (2002).
3. Diener, J.L. & Wilson, C. *Biochemistry* **39**, 12862–12874 (2000).
4. Rose, M.A. & Weeks, K.M. *Nat. Struct. Biol.* **8**, 515–520 (2001).
5. Doherty, E.A., Batey, R.T., Masquida, B & Doudna, J.A. *Nat. Struct. Biol.* **8**, 339–343 (2001).
6. Nissen, P., Ippolito, J.A., Ban, N., Moore, P.B. & Steitz, T.A. *Proc. Natl. Acad. Sci. USA* **98**, 4899–4903 (2001).
7. Hainzl, T., Huang, S. & Sauer-Eriksson, A.E. *Nature* **417**, 767–771 (2002).
8. Batey, R.T., Rambo, R.P., Lucast, L., Rha, B. & Doudna, J.A. *Science* **287**, 1232–1239 (2000).
9. Schmitz U. *et al. RNA* **5**, 1419–1429 (1999).
10. Jovine, L. *et al. Structure* **8**, 527–540 (2000).
11. Brunel, C. & Romby, P. *Methods Enzymol.* **318**, 3–21 (2000).