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On the complementarity of methods in structural biology

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Structural biology has transformed a number of fields of biochemistry and biology. A few examples are the following. The allosteric uptake and release of oxygen from haemoglobin obtained an elegant description from the work of Perutz [Perutz (1970), *Nature (London)*, **227**, 726–739; Perutz *et al.* (1998), *Annu. Rev. Biophys. Biomol. Struct.* **27**, 1–34]. The structure of tRNA highlighted the fact that the two functional facets of the molecule, the anticodon and the 3′-end, which is charged with amino acids, were 75 Å apart [Robertus *et al.* (1974), *Nature (London)*, **250**, 546–551; Kim *et al.* (1974), *Science*, **185**, 435–440]. This had interesting consequences both for charging by tRNA synthetases and for the role of tRNA as the adaptor in protein synthesis on the ribosome. The structure of the ATP synthase illustrated how the enzyme could hydrolyze or synthesize ATP by a rotating mechanism [Abrahams *et al.* (1994), *Nature (London)*, **370**, 621–628]. This transformation is a consequence of the fact that such structural insights on different levels and of different characters can provide a basis for the interpretation or reinterpretation of old observations. In addition, the design of experiments from a structural basis has become more relevant and focused.

1. The translation system, a field in need of complementary methods

The complementarity of methods in structural biology has become particularly clear in the field of protein synthesis on the ribosome. Throughout years of study, the ribosome has demanded the utmost of the established methods as well as the development of new methods. Initially, these methods were at low resolution; for example, bifunctional cross-linking (Traut *et al.*, 1980), neutron scattering of pairs of deuterated proteins in a protonated environment (Capel *et al.*, 1987) and EM studies of ribosomes where antibodies would show the location of different proteins (Stöffler & Stöffler-Meilicke, 1984; Lake, 1985). For a long time, crystallographic structures of ribosomal subunits promised a level of detail that was expected to eliminate most other structural studies (Yonath *et al.*, 1980). Thus, when such structures appeared around the turn of the millennium they were met with great excitement (Ban *et al.*, 2000; Wimberly *et al.*, 2000; Schlünzen *et al.*, 2000; Harms *et al.*, 2001; Yusupov *et al.*, 2001). The great amount of new information satisfied all the demands of rapid scientific development. Unfortunately, it has so far been impossible to crystallize many functional complexes of ribosomes. Here, we depend to a large extent on cryo-EM structures. Despite being

limited to moderate resolution, very exciting findings have been made from these studies.

2. A new conformation of tRNA

The initial cryo-EM studies of ribosomes with the ternary complex of elongation factor Tu (EF-Tu), a GTP analogue and aminoacyl-tRNA (Stark *et al.*, 1997) showed that the tRNA was in an unanticipated orientation (Fig. 1*a*). The aminoacyl residue was far from the peptidyl-transfer site (Stark *et al.*, 1997). Subsequently, crystallography has shown the anticodon stem and loop bound to the decoding site of the small subunit (30S; Ogle *et al.*, 2001) and whole tRNA bound to complete ribosomes (70S; Yusupov *et al.*, 2001). The location of the tRNA has in these cases generally agreed with expectations. The ternary complex bound to the ribosome has so far not been accessible to crystallography. Cryo-EM studies at higher resolution (Stark *et al.*, 2002; Valle *et al.*, 2002; Valle, Zavialov, Li *et al.*, 2003) show that in order for the aminoacyl-tRNA to interact with the codon of the mRNA in the so-called A-site, the tRNA has to adopt a conformation not seen before by crystallography, one in which the anticodon stem and loop make a kink with regard to the D-stem (Fig. 1*b*). This kink is a fundamental step for the decoding of the mRNA (Daviter *et al.*, 2005). If the codon–anticodon interaction is accepted, the GTP of EF-Tu is hydrolyzed and the protein factor dissociates from the tRNA. The tRNA can then adopt a conformation in which the kink is closed, with the anticodon stem and loop now dictating the orientation of the tRNA, thereby placing the aminoacyl residue in the peptidyl-transfer site (Fig. 1*c*). Subsequently, the peptide can be transferred to the incoming aminoacyl residue (Fig. 1*d*).

3. The conformational changes of the translocase

The translocation of peptidyl-tRNA from the A-site and the concomitant exposure of a new codon in the decoding site are catalyzed by elongation factor G (EF-G). The structure of EF-G (Ævarsson *et al.*, 1994; Czworkowski *et al.*, 1994; Al-Karadaghi, *et al.*, 1996) was found to mimic that of the ternary complex of EF-Tu, GTP and aminoacyl-tRNA (Nissen *et al.*, 1995). This was not anticipated, despite the fact that they alternate in binding to overlapping sites (Heimark *et al.*, 1976). Since EF-G undergoes a conformational change associated with translocation, numerous attempts have been made to obtain crystal structures of EF-G in different conformations corresponding to different states of its functional cycle. Partial success was obtained with a number of mutants (Laurberg *et al.*, 2000; Hansson *et al.*, 2005*a*,*b*). However, a picture of how EF-G interacts with the ribosome has depended on cryo-EM studies (Agrawal *et al.*, 1998, 1999; Stark *et al.*, 2000; Valle, Zavialov, Sengupta *et al.*, 2003). Two main conformations are seen. One corresponds to a pretranslocation state and the other is after translocation, where the part of EF-G that mimics the anticodon stem and loop has moved into the decoding site. This conformation of EF-G on the ribosome differs in the anticodon-mimicking region by 37 Å (Valle, Zavialov, Sengupta *et al.*, 2003) from crystal structures of EF-G. Similar comple-

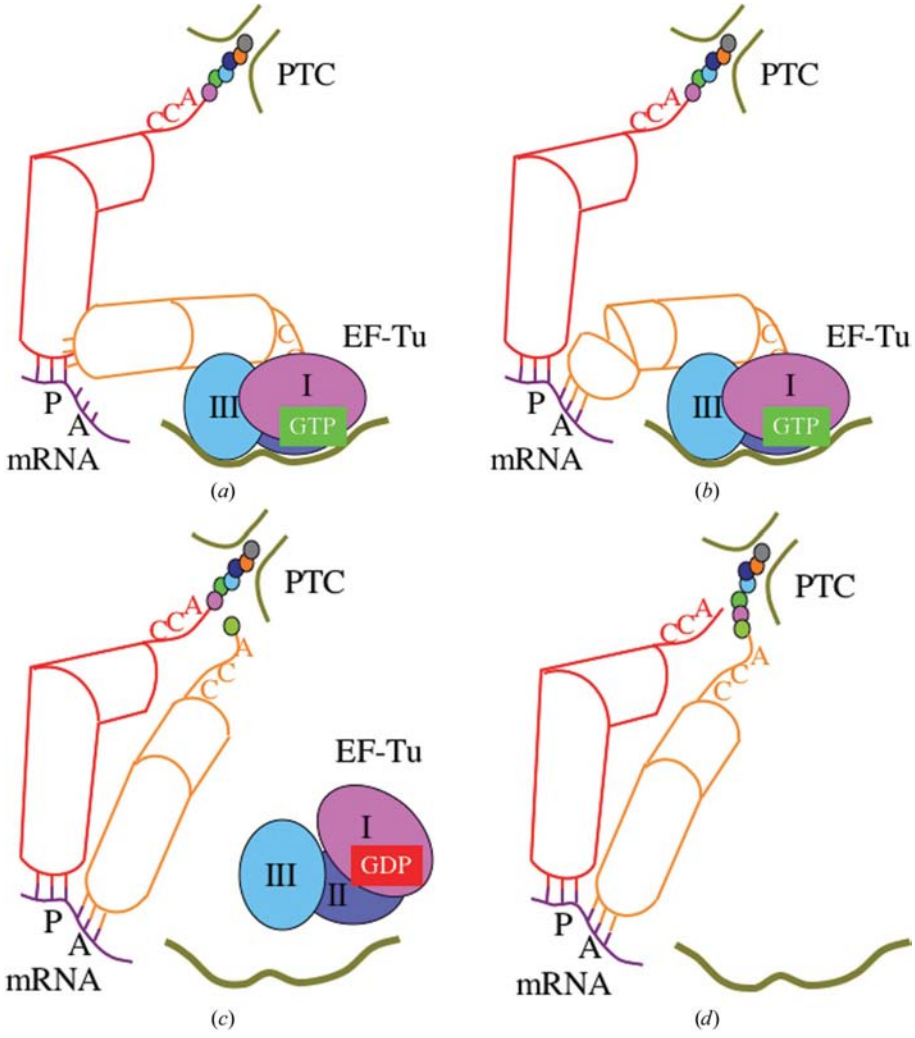

Figure 1
The binding of aminoacyl-tRNA to the ribosomal A-site is illustrated. The peptidyl-tRNA is located in the P-site. (*a*) The aminoacyl-tRNA in complex with EF-Tu–GTP binds to the ribosome. The anticodon of the tRNA is unable to interact with the codon in the A-site without a conformational change. (*b*) A kink between the anticodon stem and loop and the D-stem develops that allows the anticodon to interact with the codon. (*c*) After the identification of a cognate codon, EF-Tu is enabled to hydrolyze its GTP molecule and dissociate from the ribosome. The tRNA can then bind properly into the A-site. (*d*) Peptidyl transfer can occur.

mentary insights have been gained in the studies of the eukaryal translocase eEF2 (Jørgensen *et al.*, 2003; Spahn *et al.*, 2004). Clearly, our present structural understanding of the function of the translocase depends on a combination of crystallography and cryo-EM.

4. The dramatic rearrangement of the release factors

When the decoding arrives at the end of a gene on the mRNA, a termination codon appears in the decoding site. No tRNA can normally bind since they compete unfavourably with the termination or release factors RF1 and RF2. Since these factors interact both with the decoding site and the peptidyl transferase site, they could have some structural similarity to tRNA. Vestergaard *et al.* (2001) published the first crystal structure of a bacterial release factor. It had some similarity in shape to a tRNA; however, the two regions of the molecule known to interact with the decoding site and the peptidyl transfer site were not 75 Å apart, but only around 23 Å. Was there a problem with the crystal packing, a problem with the identification of the functional regions of the molecule or was there a large conformational change associated with its function? The cryo-EM identification of the release factor bound to the ribosome gave a very different picture of the protein structure (Rawat *et al.*, 2003; Klaholz *et al.*, 2003). The release factor interacted with both functional sites of the ribosome and the crystal structure had to be rearranged extensively to fit the cryo-EM data. This has subsequently been confirmed by crystallography (Petry *et al.*, 2005). Low-angle X-ray scattering measurements of the release factor free in solution gave strong support for the conformation identified by cryo-EM (Vestergaard *et al.*, 2005). The dramatic conformational changes seen in the release factors remain poorly understood, but the need for complementary methods is nevertheless quite obvious.

5. How does the ribosome recycling factor bind to the ribosome?

The ribosome recycling factor (RRF) dissociates the translational machinery after the termination of protein synthesis with the aid of EF-G (Hirashima & Kaji, 1972). The structure of RRF was found to mimic a tRNA almost perfectly (Selmer *et al.*, 1999). This led to the thought that RRF might bind to one of the tRNA-binding sites on the ribosome. Using affinity labelling, it could be shown that this hypothesis was not correct, but a different mode of binding was suggested (Lancaster *et al.*, 2002; Brodersen & Ramakrishnan, 2003). Subsequent cryo-EM and crystallography of RRF bound to the ribosome confirmed that the picture obtained by chemical methods was the correct one and that the tRNA-mimicry hypothesis was misleading (Agrawal *et al.*, 2004; Wilson *et al.*, 2005).

6. Has the ribosome been fully characterized structurally?

A complicated object such as the ribosome can of course hardly ever be fully characterized. In the present crystallographic structures, certain flexible regions are not seen (Ban *et al.*, 2000; Yusupov *et al.*, 2001; Harms *et al.*, 2001). One example concerns the N-terminal tail of protein L27. Contrary to the claim that the ribosome is a ribozyme (since there is no protein seen at the peptidyl-transfer centre), the three N-terminal residues of L27 are very important for full peptidyl-transfer activity (Maguire *et al.*, 2005).

Another region of the bacterial ribosome where the organization has resisted crystallographic determinations is the so-called stalk of the large subunit. The stalk is composed of one copy of protein L10 and two or three dimers of L12 (Österberg *et al.*, 1977; Pettersson & Liljas, 1979; Ilag *et al.*, 2005). L12, and in particular its C-terminal domain, is important for the GTP hydrolysis that the ribosome induces in the translational GTPases (Kischa *et al.*, 1971; Mohr *et al.*, 2002; Liljas, 2004). The C-terminal domain of L12 was the first ribosomal component for which the structure was determined (Leijonmarck *et al.*, 1980). Much later, the complete molecule was crystallized (Wahl *et al.*, 2000). The organization of the dimers was debated (Sanyal & Liljas, 2000) until an NMR structure (Bocharov *et al.*, 2004) and studies of the L12 dynamics by NMR (Mulder *et al.*, 2004; Christodoulou *et al.*, 2004) were completed. It has long been known that L12 forms strong dimers (Möller *et al.*, 1972). Recently, the crystal structure of a complex between L10 and N-terminal fragments of L12 was determined and modelled into the stalk feature of the large subunit (Diaconu *et al.*, 2005). NMR studies of whole ribosomes suggest that the only two of the four C-terminal domains are mobile (Mulder *et al.*, 2004). The other two must

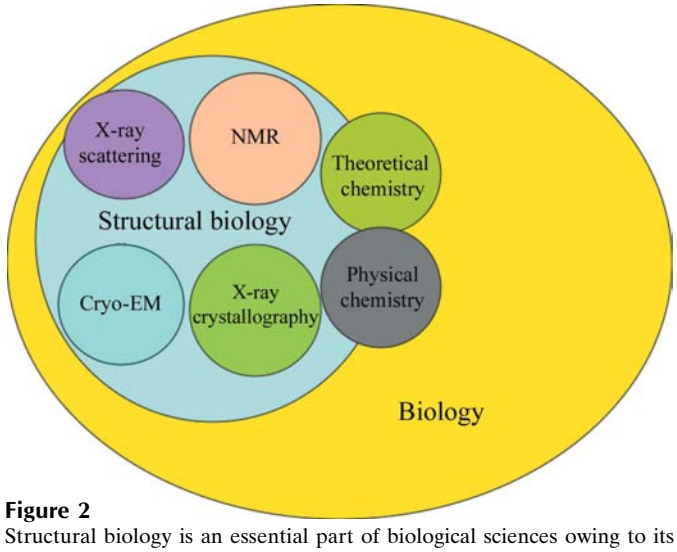

Figure 2
Structural biology is an essential part of biological sciences owing to its ability to characterize the molecules involved and their interactions. Crystallography is a central method in structural biology but needs to be complemented by methods such as cryo-electron microscopy, NMR or X-ray or neutron scattering. In addition, physical and theoretical chemistry are highly relevant for the proper understanding of molecular behaviour.

be immobilized somewhere on the ribosome. Whether the immobilized domains always remain in the same state or whether they exchange with the mobile domains is not known, but is likely. This may be part of the intricate scheme of interactions between the factors that bind to the same site on the ribosome. The transient interactions between L12 and the GTPases, particularly which surfaces are involved, is currently not known, even though the structures involved have been thoroughly characterized. Here, NMR offers unique possibilities for identifying these interacting surfaces.

7. Conclusions

In studies of biomolecular systems, knowledge of the structure is an important ingredient to understand the functional mechanism. Numerous examples have been unravelled over the past decades. Large asymmetric molecular aggregates have become accessible to crystallographic methods only in recent years. Work on such larger problems, among them the translation system, has shown much more than previously the necessity for complementary structural methods. Thus, crystallography complemented by cryo-electron microscopy and NMR methods have jointly provided a picture that provides much for the continued biochemical analysis (Fig. 2). To a certain extent, structural biology is a descriptive science. The level of understanding can be illustrated by the extensive lack of predicative power, particularly of dynamic interactions. Obviously, a complement of physical and theoretical chemistry could provide some further steps towards understanding the functional interplay between biological macromolecules.

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