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Architecture of the *Escherichia coli* ribosome as determined by immune electron microscopy

(topography/ribosomal subunits/ribosomal proteins/antibodies)

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ABSTRACT **Binding sites for antibodies specific to nineteen of the twenty-one ribosomal proteins from the 30S subunit of *E. coli* ribosomes have been localized on the surface of the 30S ribosomal subunit by immune electron microscopy. The locations of 13 ribosomal proteins from the 50S subunit were similarily assessed. The arrangement of these proteins is illustrated in three-dimensional models of the 30S and 50S ribosomal subunits and of 70S ribosomes.**

With specific antibodies to six proteins of the 30S subunit we found only one attachment point for each protein. Antibodies against each of nine of the proteins attached at two separate sites that were separated by various distances. Four further proteins were exposed at three or four sites for antibody binding. Altogether eight to ten of the 19 proteins of the 30S subunit have shown antibody attachment sites at remote points on the surface of the ribosome, at distances which are incompatible with globular shapes; these proteins must therefore have elongated or fibrous structures within the ribosome. On the other hand, only two proteins of the 50S subunit, namely L11 and L18, have so far revealed two separated antibody binding sites; proteins L7/L12 occurred, however, at multiple sites.

The 30S ribosomal subunit of *Escherichia coli* is comprised of 21 ribosomal proteins and one 16S rRNA molecule. Substantial information about the primary structure of these components has accumulated during the last several years (reviewed in refs. 1 and 2). Similarily a good deal is known about the contribution of the various components to the function of the ribosome (reviewed in refs. 3–5). Although several approaches have already contributed to the knowledge of the relative topography of ribosomal components, information regarding the absolute location of the ribosomal proteins has only recently emerged, from electron microscopy of antibody-labeled 30S and 50S subunits (5–10). By means of immune electron microscopy, proteins S4, S5, S13, and S14 were mapped on the surface of the 30S subunit (7, 9, 10), and preliminary data on the location of proteins S6, S11, S12, and S19 have also been reported (10).

In this paper we present a summary of our data on the location of 19 of the 21 proteins from the 30S subunit in the three-dimensional structure of both the 30S and 70S ribosome. The location of 13 proteins on the 50S ribosomal subunit is given schematically. A detailed documentation and a critique of the results will be published elsewhere*.

MATERIALS AND METHODS

Preparation of ribosomes and ribosomal subunits from *E. coli* strain K12 (A19), characterization of IgG's purified from monospecific antisera, absorption of antisera, and isolation of 30S subunit–IgG complexes followed methods described previously (6, 7). In brief, incubation mixtures of heat-activated, functionally active ribosomal subunits (11, 12) and specific IgG's were centrifuged through sucrose gradients in a buffer containing 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes)–NaOH, pH 7.8, 5 mM $Mg(OAc)_2$, 200 mM NH_4Cl and 6 mM 2-mercaptoethanol. The fractions containing 30S–IgG complexes ("dimers"), in which a bivalent antibody joined two ribosomal subunits, were used for electron microscopy. 70S ribosomes were isolated by "freeze-thaw-lysozyme lysis," essentially as described by Ron *et al.* (13); incubation with deoxycholate was, however, omitted. 70S·IgG·70S complexes were purified by sucrose gradient centrifugation in a manner similar to that described for 30S and 50S subunits, but at 20 mM $MgCl_2$ (6, 7). Preparation of specimens, negative staining with uranyl acetate, and electron microscopy were carried out as described (6, 7). Between 80 and 300 subunit-dimers joined by any particular antibody were photographically enlarged to ×300,000 magnification and used for the localization of the antibody binding sites.

RESULTS AND DISCUSSION

Structure of 30S subunits

When visualized by negative staining, 30S ribosomal subunits have an elongated and slightly bent prolate shape with approximate dimensions of 180–200 Å × 100 Å × 80 Å. The particle is divided transversely into two globules of unequal size: a smaller head and a larger body. The partition which is generally seen in frontal and lateral views resembles either a hollow, or, in other subunits, a cleft. This hollow or cleft forms the vault of the tunnel observed in 70S ribosomes. In the rear view the partition looks like a dent and is less pronounced. The two lateral edges of the body are elongated and protrude to various extents. We observed several distinct projections of the 30S particles (7), and based on these data, we have recently proposed a three-dimensional model (refs. 7, 10, and Fig. 4). Figuratively, the 30S structure may be compared with an embryo or a telephone receiver. Similar shapes of small ribosomal subunits have been observed independently by several investigators (14–18).

Antibody labeling studies on 30S subunits

Pairs of subunits joined by a specific IgG molecule were negatively stained and observed in the microscope. The structure of the 30S subunit provided sufficient orientation markers to allow an accurate localization of the various antibody attachment points.

Proteins which occur at only one site at the ribosomal surface

A single antibody binding site has been observed with anti-S8, -S13, -S14, -S19, -S20, and -S21. Gross locations of the

* G. W. Tischendorf, H. Zeichhardt, and G. Stöffler, to be submitted for publication.

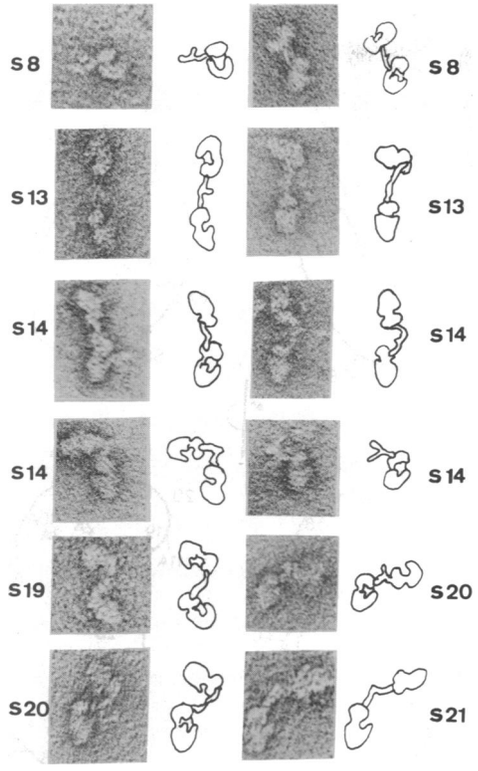

FIG. 1. Selected images and interpretive drawings of 30S·IgG·30S complexes obtained with anti-S8, -S13, -S14, -S19, -S20, and -S21. Antibodies bound to single subunits are shown for anti-S8 and -S14 (see also Fig. 2). Magnification ×234,000.

antibody binding sites are shown in the interpretive drawings (Fig. 1). The refined topography shown in Fig. 4 results from the evaluation of 80 to 300 enlarged dimers examined in the case of each antibody. S8 has been localized in the center of the back within the dent, S13 on the top of the small head, and S14 on the temporal region of the small head (Fig. 1). The attachment sites for anti-S19 and -S21 were found one on each side of the forehead; S20 has been localized in vicinity of S19 (Fig. 1).

Ribosomal subunits joined by specific antibodies were arranged in a rotatory symmetry. Each of the projectional forms has also been observed within dimers, thus greatly facilitating formulation of the refined topography presented in the model (see S14 for an example in Fig. 1; Fig. 4). Several "disrupted dimers" showing an antibody bound to only one subunit eased the localization (Fig. 1).

Proteins which occur at two sites on the ribosomal surface

Nine ribosomal proteins, namely S2, S3, S6, S9, S10, S12, S15, S16, and S18 occur with two antibody binding sites on the surface of the 30S subunit (Fig. 2). The distances between the two sites, however, vary among these proteins. They can therefore be subdivided into three classes: (*i*) Both binding sites of proteins S6, S9, and S16 were found to be in close vicinity (up to 40 Å) of each other (Figs. 2 and 4). Subunits simultaneously connected by two antibodies facilitated the detection of these "double-sites" (see below and Fig. 2). The short distances between "double-sites" do not exclude the possibility that these proteins have globular shapes, since a spherical ribosomal protein with an average diameter of 34 Å (ref. 19) would lie within the dimensions of the Fab arm of the IgG molecules (40 Å × 50 Å × 70 Å, ref. 20; 36 Å × 44 Å × 119 Å, ref. 21). (*ii*) The two sites of S3 and S10 were found to be remote (Fig. 2). The distances between sites S3A and S3B, as well as S10A and S10B, were approximately 50–60 Å. Although the two sites of each of these proteins are well separated, the distances between their respective antibody binding sites would be still compatible with globular or only slightly elongated protein shapes, e.g., by assuming an uneven and fissured ribosomal surface. (*iii*) The third group, comprising proteins S2, S12, S15, and S18, revealed antigenic determinants exposed at well-separated sites (80–190 Å apart). We can positively conclude that these proteins must have elongated or fibrous conformations in the 30S particle.

A few subunit dimers were joined simultaneously by two antibodies (Figs. 2 and 3). Although rarely seen, these di-

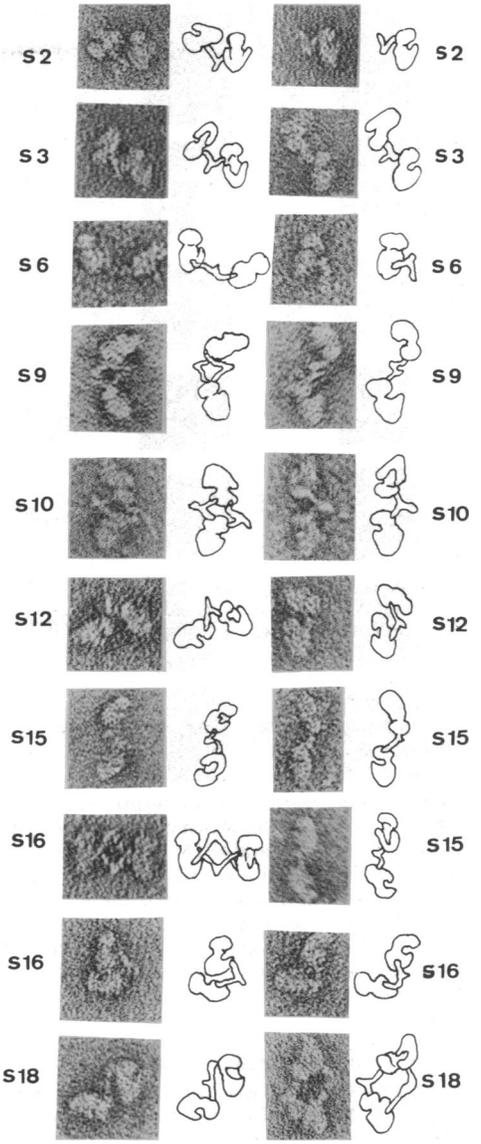

FIG. 2. Electron micrographs and interpretive drawings of small subunits reacted with antibodies which bind at two separate sites. ×234,000. (*i*) Antibody binding sites in close vicinity to each other: S6, S9, and S16 ("double-sites"). (*ii*) Antibody binding sites separated by 50–60 Å: S3, S10. (*iii*) Antibody binding sites remotely separated (80–190 Å): S2, S12, S15, and S18. The two sites obtained with the various antibodies were encountered in ratios between 40% and 60%. Subunit pairs simultaneously connected by two antibody molecules are shown with anti-S9, -S10, -S16, and -S18 (see also Fig. 3).

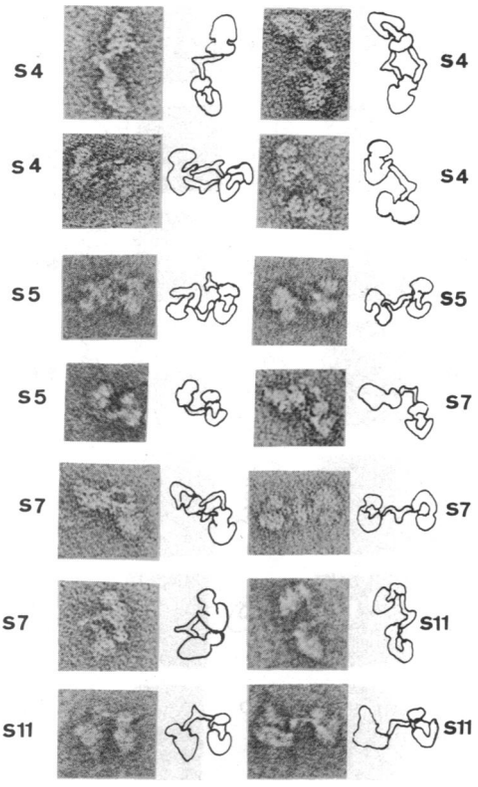

FIG. 3. Electron micrographs and interpretive drawings of 30S subunits reacted with antibodies which attach at three or four separate sites: anti-S4, -S5, -S7, and -S11. ×234,000. The percentage of antibodies bound at each site varied. A site has only been considered when observed in at least 10% of the evaluated dimers. Preabsorption of a serum with its cognate antigen resulted in a loss of all antibody binding sites for the protein concerned; therefore, the occurrence of multiple binding sites cannot be due to contamination of the antisera (7).

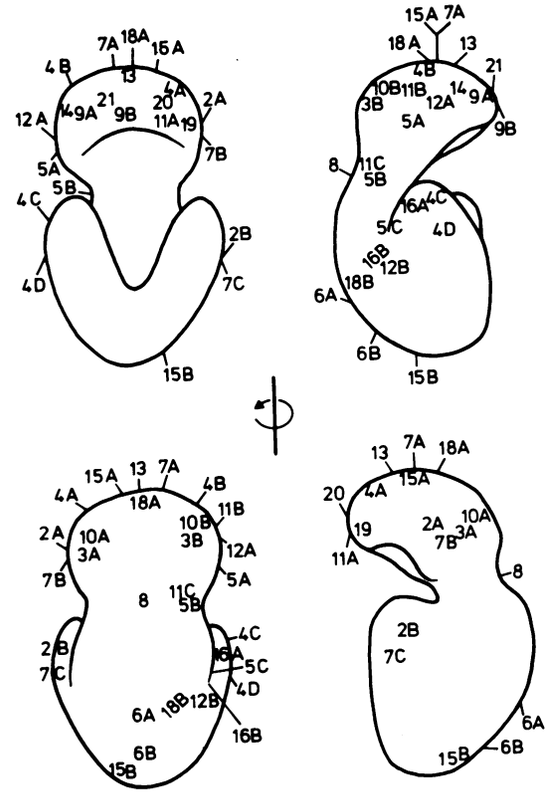

FIG. 4. Three-dimensional model of the 30S subunit with the locations of antibody binding sites for 19 ribosomal proteins. The four forms are related to each other by rotation through an angle of approximately 90° along the long axis. Double or multiple attachment sites of an antibody specific to one protein are designated A, B, C, and D. The binding sites have to be imagined either (*1*) facing the observer (proteins designated within the contours of the model), (*2*) on the "horizon" of the subunits (proteins marked from outside), or (*3*) "behind the paper," in which case the protein is not marked.

mers allowed accurate conclusions to be made concerning the distances between the two sites. A subunit pair which is simultaneously held together by two antibodies, both of them specific for the same protein, also demonstrates that the two antibody binding sites are accessible on one and the same ribosomal subunit. The existence of subunit pairs with two antibodies bound also excludes the possibility that the second site has arisen from movement of the ribosomal protein into a different position on the ribosomal surface during preparation. This conclusion is further supported by the fact that the points of attachment of a single antibody within a dimer were always identical on each subunit.

Proteins occurring at distant and multiple sites on the 30S subunit

Three antibody binding sites have been observed for proteins S5, S7, and S11 (Fig. 3). Protein S4 was even accessible at four separate sites for antibody binding (Fig. 3).

Shape and distribution of ribosomal proteins

The locations of 19 proteins on the 30S ribosomal subunit are summarized in a three-dimensional model (Fig. 4). 37 antibody binding sites have been encountered in all for the 19 ribosomal proteins investigated. An important aspect of our findings is that antibody binding for eight to ten ribosomal proteins occurs at well-separated sites on the 30S ribosomal subunit with distances from 60 Å up to 210 Å. Four of these proteins, S4 (refs. 9 and 10), S5, S11, and S12 (refs. 7 and 10) have previously been shown to have elongated or fibrous shapes *in situ*. We have now observed that a larger number of proteins, namely at least S2, S4, S5, S7, S11, S12, S15, and S18, and possibly also S3 and S10, have elongated conformations within the 30S ribosome (Figs. 2–4). It is probable that additional sites will be detected for some or any of the proteins by improving the methods for the isolation of antibody–subunit complexes and, due to individual specificity in the immune response, by investigating various antisera raised against the same proteins (5, 22).

So far, antibody labeling has not revealed any proteins in two regions of the 30S subunit: (*i*) in the hollow between the two globules and (*ii*) on the anterior side of the body. It appears quite likely that antibody binding to determinants exposed in the hollow is sterically hindered. Such an explanation is, however, not possible for the protein-free region on the large body. There seems to be a protein-rich domain on the small head. Apparently more antibody binding sites are exposed on the side which is in contact with the central protuberance of 50S subunits in 70S monomers.

Although immune electron microscopy only allows an assessment of the protein distribution on the surface of the ribosome, this approach provides in addition a rough idea of the topography of those parts of the protein chain lying between the antibody binding sites. Seven proteins, namely S2, S4, S5, S7, S12, S15, and S18, were shown to occur on the head as well as on the body for antibody binding. Their pro-

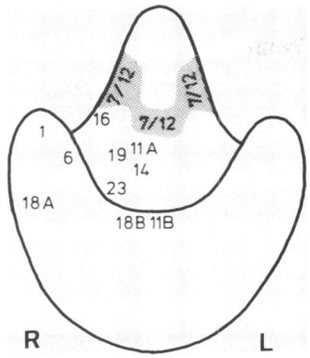

FIG. 5. Three-dimensional model of the 50S ribosomal subunit with the location of 13 ribosomal proteins. Only one view is presented. The stippled areas represent the regions of the various antibody binding sites of anti-L7/L12.

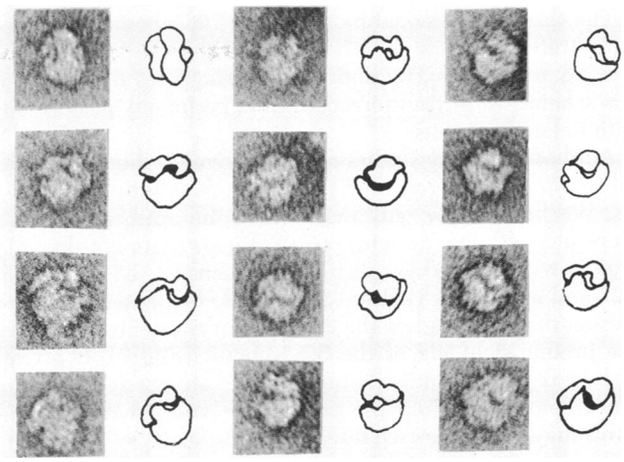

FIG. 6. Electron micrographs and interpretive drawings of 70S monomers seen from various views. ×300,000.

tein chains must, therefore, necessarily extend through the neck, although none of these proteins, except S5, has been found to be exposed in the neck region of the particle. These conclusions have been illustrated by connecting the sites of each protein (10) which then leads to a more homogeneous protein distribution. Our data are, therefore, by no means in conflict with the finding of Moore *et al.* (23) who concluded from neutron scattering studies that there are no separated domains of RNA and protein in the 30S subunit.

Localization of ribosomal proteins on the 50S ribosomal subunit

Electron microscopical studies on the 50S subunit revealed a number of image types: rounded image types, kidney-shaped structures, circular profiles with a "nose," and more angular structures that resemble the profile of a maple leaf or a globule carrying a crown with three crests (6, 8, 10, 18, 24–28). Each of these forms is more or less compatible with one of the projectional forms of the three-dimensional subunit structure proposed recently (6, 10). The binding sites of antibodies specific for 13 proteins, namely L1, L4, L6, L7/L12, L11, L14, L16, L17, L18, L19, L22, and L23, have been mapped on the surface of the 50S subunit by electron microscopy in this laboratory (5, 6, 8, 10). Most of them showed only a single site for antibody binding, and elongated protein conformations are only likely in the case of proteins L7/L12, L11, and L18 (6, 10). The data are summarized schematically in Fig. 5. A more detailed account of these results will be published elsewhere (29).

The 50S ribosomal proteins seem to be asymmetrically arranged and clustered in a domain on the right† side of the subunit (Fig. 5). This finding is consistent with results obtained by neutron scattering (23).

The structure of 70S monomeric ribosomes

It is essential for an understanding of the structure and function of the ribosome to know how the two subunits interact in order to form the 70S ribosome. Electron microscopy showed that the structure and the dimensions of neither subunit are grossly different in negatively stained 70S monomers as compared to the isolated subunits (27, 30, 31). The selection of various views of 70S ribosomes which is shown in Fig. 6 is in support of these earlier findings, and a three-dimensional model derived from our electron micrographs of 70S monomers is presented in Fig. 7. The long axis of the 30S subunit is oriented horizontally, transverse to the central protuberance of the 50S subunit (Fig. 6). The hollow of the 30S subunit faces the vaulted seat and the large protuberance of the 50S ribosome, leading to the formation of a tunnel (Fig. 6).

The head of the 30S subunit is in the vicinity of the "protein-rich side" of the 50S subunit whereas the body of the 30S subunit is facing the opposite part of the 50S seat (Figs. 6 and 7). Our results are in conflict with a model proposed by Lake *et al.* (31). These authors interpret their pictures of 80S rat liver and 70S *E. coli* ribosomes such that the longitudinal axis of the 30S subunit lies horizontally across the top of the large subunit (31), with an orientation at 90° to that which we propose.

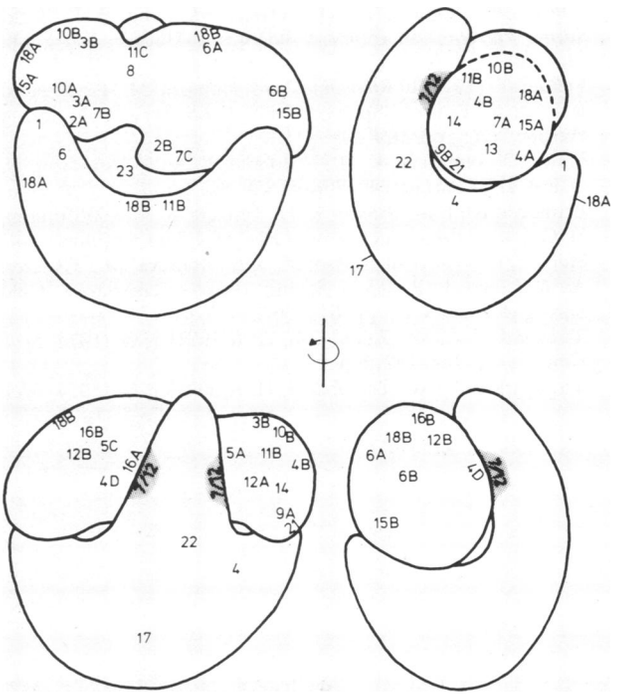

FIG. 7. Three-dimensional model of the 70S ribosome. The four projections are derived as described in legends to Figs. 4 and 6.

† The designation of the left and right side of the 50S subunit is illustrated in Fig. 5. We have previously used the opposite nomenclature which was based on the view of the observer (5, 6, 10).

The three-dimensional model of the 70S ribosome in Fig. 7 illustrates our view as to how the subunits are oriented in the 70S monomer. The antibody binding sites depicted in this scheme were transferred from experiments performed with isolated subunits.

Antibody labeling studies on 70S ribosomes should be useful to discriminate eventually between the alternative models. We have so far only determined the antibody attachment sites of a few proteins on 70S dimers (data not shown). Studies with antibodies specific to proteins L1, L7, L20, and L27 and also to S6, S13, and S14 greatly facilitated our conclusion that the head of the 30S subunit is oriented towards the protein-rich side of the 50S subunit (Fig. 7). It might well be that some antibody binding sites are not accessible in 70S ribosomes, but on the other hand additional determinants may also occur (see ref. 5).

The present data give a first idea about the architecture of the ribosome and the absolute topography of ribosomal proteins. In general our data are in reasonable agreement with results obtained from methods investigating the relative topography of ribosomal components, such as protein cross-linking and affinity labeling, and with data on protein–RNA interaction sites (2–4, 10, 19, 32, 33). Combination of the various data should allow deductions as to the location of functional sites on the ribosome, such as the interaction sites with mRNA, aminoacyl-, and peptidyl-tRNA and factor binding sites. Structural and functional models have so far been proposed under the supposition that ribosomal proteins are globular (19, 33). This notion has to be revised and must be replaced by the consideration of elongated protein structures for future model construction.

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