SINGLE-PARTICLE IMAGING OF MACROMOLECULES BY CRYO-ELECTRON MICROSCOPY

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Key Words molecular machines, ribosome, spliceosome, three-dimensional reconstruction, transcription complex

Abstract Cryo-electron microscopy (cryo-EM) of biological molecules in single-particle (i.e., unordered, nonaggregated) form is a new approach to the study of molecular assemblies, which are often too large and flexible to be amenable to X-ray crystallography. New insights into biological function on the molecular level are expected from cryo-EM applied to the study of such complexes “trapped” at different stages of their conformational changes and dynamical interactions. Important molecular machines involved in the fundamental processes of transcription, mRNA splicing, and translation are examples for successful applications of the new technique, combined with structural knowledge gained by conventional techniques of structure determination, such as X-ray crystallography and NMR.

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PERSPECTIVES AND OVERVIEW

As more and more structures of proteins are being solved by X-ray crystallography and NMR, and the availability of genomic information is starting to facilitate a systematic exploration of sequence and structure data, the focus of structural biology increasingly moves to the interactions of macromolecules in the cell. The complexes formed in these interactions have been termed macromolecular machines [see (5)], a term that attempts to capture the dynamic nature, processivity, and high precision of these localized interactions, as well as the involvement of multiple ligands.

X-ray crystallography has been extremely successful as an approach to solve the structure of large individual proteins, but this technique can run into several limitations when it comes to large (Megadalton range) multi-component complexes: (a) Those large complexes often resist attempts at crystallization, or they may form crystals only after flexible parts have been removed; (b) conversely, successful crystallization may interfere with the study of macromolecular interactions because crystal packing favors particular modes of interaction while disfavoring or entirely prohibiting others; and (c) the large size of a complex increases the volume of data collection and often poses problems in finding multiple isomorphous derivatives with sufficient phasing power. [The recent achievement by several groups in solving the structure of the ribosome (7, 59, 81, 86) is an exception that rather proves the rule: it has followed decades of unsuccessful attempts at crystallization].

Cryo-electron microscopy (cryo-EM) of molecules in single-particle form does not suffer these restrictions, presenting an approach that does not require crystals. The complex being studied is free to assume all functional states without any steric constraints, allowing, in principle, the visualization of the entire dynamic course of the macromolecular interactions. Time resolution can be achieved by taking “snapshots” of the complex at different points in time that are experimentally “trapped” by chemical or physical means. The current drawback of this approach, which might be gradually overcome with time, is that its spatial resolution is limited. However, fortuitously the new methodology has come along at a time when the X-ray structures of many of the players in the interactions are known or have at least become inferable by homology modeling based on sequence comparisons. When all the components of a macromolecular complex are known to atomic resolution, then the low-resolution map of the complex will allow the components to be placed and their atomic interactions to be inferred. Thus, to fully exploit the potential of cryo-EM, it is necessary to find tools that allow the fitting and docking of X-ray coordinates into the lower-resolution cryo-EM maps.

To be sure, the approach of cryo-EM to look at isolated in vitro systems is still a big step away from the solution to the real challenge posed by the need to study macromolecular interactions under conditions closely approaching those in vivo inside the cell [see (38)]. However, there is no doubt that in the long run, three-dimensional (3D) descriptions of the system in isolation will greatly facilitate the task of locating its manifestations in the cellular context [see for instance (8)].
CRYO-EM OF ORDERED ARRAYS VERSUS SINGLE MOLECULES

As a technique of specimen preparation and visualization, cryo-EM has been in existence for almost 20 years (16, 34), not counting the decade before, during which the technique was basically dormant following Taylor & Glaeser’s (69) initial invention of the method. The basic technique involves an apparatus that plunges the grid into liquid ethane (Figure 1): the EM grid, on which the specimen is suspended within a thin water layer, is held at the tip of tweezers, which are in turn mounted on a rod that is held in a position to fall under gravity. (The thickness of the water layer is controlled by blotting.) As the rod is released, the grid is rapidly plunged into a bath of liquid ethane at liquid-nitrogen temperature. Owing to the rapid decrease in temperature, the water turns into vitreous ice, which has properties akin to those of liquid water. Most importantly, no crystals are formed and a disruption of the molecule is avoided.

Initially, cryo-EM was exclusively applied to highly ordered specimens: helical fibers, “two-dimensional” crystals (i.e., crystals only one or two unit cells thick),

![Figure 1](image.png)

Figure 1 Schematic diagram of a freeze-plunge apparatus. [From Stewart (66), reproduced with permission].
and viruses with high symmetry. In the then-prevailing view, only such specimens could yield quantitative information. Although significant progress was achieved in these applications, relatively few highly visible results were obtained. For proteins of normal sizes, X-ray crystallography is a formidable competitor, as cryo-EM data collection for two-dimensional (2D) crystals at close-to-atomic resolution faces several obstacles: crystal disorder and experimental difficulties (e.g., charging at high tilts and instabilities of the specimen stage). The most promising niche for cryo-EM has been in the area of membrane proteins, but actually only few atomic structures have been solved in this way to date. For large macromolecular complexes, on the other hand, attainment of crystals with good order is often as difficult in two dimensions (suitable for cryo-EM) as it is in three (suitable for X-ray crystallography). As an example, all attempts to solve the ribosome structure using electron microscopy of 2D crystals [e.g., (39, 85)] were eventually abandoned because of poor crystal order.

A potentially much larger field of application was created owing to the development of the single-particle reconstruction method, which requires the molecule to exist on the specimen grid in multiple isolated copies. This method (48–50, 71) and its 2D precursors (18, 22, 25, 73) were developed initially for molecules prepared by negative staining, but their success there was limited by the artifacts introduced by the preparation technique.

In the negative-staining technique, practiced almost exclusively before the invention of cryo-EM, the aqueous specimen is mixed with a 1–2% solution of a heavy metal salt and then air dried. On the grid, molecules so prepared are surrounded by a cast of stain that outlines their shape, leading to a high contrast in the EM image. However, in addition to the fact that no interior density variations of the molecule are visualized, negative staining has the disadvantage that it results in a distortion of the molecule’s shape. The breakthrough achieved by the marriage of the two methods, cryo-EM and single-particle reconstruction, can be appreciated by comparing density maps of the 70S ribosome of Escherichia coli that were done with negative staining (78) with those by cryo-EM (24). In the first case, the structure appears squashed, without the clearance of the intersubunit space; in the second, the structure is globular, with a clear separation between the 30S and 50S subunits. Another example was provided by comparing density maps of hemocyanin obtained by negative staining with cryo-EM (9) that differ by almost a factor of two in the dimension normal to the electron microscope grid, due to the collapse of the molecule following negative staining and air drying.

**PRINCIPLES OF CRYO-EM OF SINGLE PARTICLES**

The method of 3D cryo-EM of single particles is currently still evolving. Although the experimental protocol for the EM preparation of such specimens (79) is similar to protocols developed earlier for thin crystals, reflecting on the original work by
Dubochet and coworkers (17), the methods of image processing and reconstruction of molecules in single-particle form are quite different in detail. Many of the algorithms and principles, and the relevant literature, have been described at length [e.g., (19)]. Recent outlines of the methodology in its different versions are found in (23, 43). The subject is also covered by Saibil (56) as part of a more general overview article on molecular structure determination by cryo-EM.

In the single-particle reconstruction approach, the particle (molecule) is thought to occur in multiple copies that have (at least to a first approximation) identical structure. These copies have different orientations within the ice layer, so their spatial relationships can be mathematically described by a series of rigid-body movements of a single object. The transmission electron microscope produces parallel projections of such a set of particles (Figure 2). Thus, provided the angular distribution is sufficiently uniform, a series of single micrographs of the untilted specimen (each showing a field with hundreds of particles) will yield all the information necessary to reconstruct the molecule.

A complication arises due to the presence of lens aberrations: The projection images produced in the electron microscope are blurred in a complicated way that depends on the distance (“defocus”) of the specimen from the true focus of the objective lens. Removal of the resultant blur, which is described by a “point spread function” [or described alternatively, in Fourier space, by a contrast transfer function (CTF)], necessitates the collection of images with different defocus and the application of CTF correction in the image-processing procedures.

Radiation damage is a major concern, so it is important that data used for the reconstruction originate exclusively from molecules that have “seen” the electron beam only once. Dose levels used are in the range of 10 electrons per A². Lower levels pose some problems in locating particles. On the other hand, the classical work by Unwin & Henderson (70) on 2D crystals of bacteriorhodopsin (unstained and embedded in glucose) seemed to indicate that a much lower dose (∼1 electron/A²) may be required to preserve features in the range below 10 Å. The subsequent years indicated that the maximum dose sustainable by the specimen depends on a variety of factors, including the type of specimen (e.g., fraction of RNA, and compactness), embedding medium, electron voltage, and specimen temperature. Also, newer microscopes with helium cooling may offer a “radiation protection factor” compared to liquid nitrogen (12a).

The most difficult task in the whole procedure, when the molecule’s structure is unknown, is to infer the orientations of the particles from these images. The problem is exacerbated by the great amount of noise in a micrograph, which is a consequence of the low dose needed to minimize radiation damage. This problem of ab initio orientation determination has received a variety of answers: In the “random-conical” data collection method (48), which involves the use of a tilt, geometric relationships are established among a subset of particles that face the grid in the same orientation. In the “method of common lines” (13, 46, 71), particle images are first classified, then class averages presenting different views of the particle are related to one another according to the common lines principle first
formulated by Crowther (13). The latter method cannot establish the handedness, however, and requires a tilt for this purpose.

After the initial determination of orientations, the angles are further refined following an iterative cycle (30, 45), in which 3D projection matching alternates with 3D reconstruction. With the angles having been determined (and provided that the angular range is sufficiently covered), the molecule can be reconstructed according to mathematical principles that were formulated by Radon (51), computationally first realized in electron microscopy (14, 32), and subsequently exploited in many applications of medical tomography.

The importance of the geometry of the angular coverage becomes clear from the mathematical formulation of the reconstruction problem as data collection in Fourier space: Here each projection is represented by a central plane (14). The objective is to fill 3D Fourier space with data up to a radius that corresponds to the limiting resolution. As more and more projections having different angles are being added, the manifold of the corresponding central planes starts to cover Fourier space. If an entire range of orientations is not represented in the data set, then a large gap will remain in Fourier space, and the reconstruction (obtained by Fourier synthesis) will show artifacts, such as elongation of features in the direction of the missing angular range. Although large gaps, entirely devoid of projections, are encountered in some studies, as in the case of the calcium release channel (47), it is more common to find regions of relative sparsity. This implies that these regions will be filled eventually, provided that the data set is large enough. An example of this statistical behavior is provided by Figure 3, which shows the observed orientational preferences of the ubiquinone oxireductase molecule that can be explained as the result of the molecule’s interaction with the air-water interface (28). Although there are two clear preferences of orientation (related by a flipping of the molecule in the ice), other views also do occur, albeit with lower frequency.

So far, the specimen has been described in terms of a rigid molecule occurring in many identical copies in different orientations. A more realistic model of the experiment must account for the coexistence of different ligand occupancies and conformations of the molecule. These are often linked: The ribosome, for example, undergoes large conformational changes in response to the binding of elongation factor G (EF-G) (1, 20). Conformational changes of a macromolecule are also often associated with the hydrolysis of a nucleotide triphosphate [e.g., ribosome (1); groEL (56); AAA ATPase (55, 87)]. If in such a case only part of a molecule population is bound with the ligand, then the reconstruction from the mixed data set will result in an incorrect density map in which the two states are superposed. Preferably, such mixtures should be sorted out prior to EM imaging by biochemical means, but this is not always possible. Sorting the images in the computer is possible by means of multivariate statistical analysis and classification (10, 19, 72, 73), but the inherently small signal-to-noise (S/N) ratio of the raw images makes it often difficult to detect conformational changes or the presence of small ligands.
Figure 3  Angular statistics showing the orientational preferences of bovine NADH:ubiquinone oxireductase molecules in the water layer. Each data point on the theta-versus-phi graph represents the orientation of a molecule. In the lower panel, the molecule is shown in the two most frequently found orientations within the ice layer. [Reproduced from (28)].

A NOTE ON RESOLUTION

It is unfortunate, and quite confusing to nonspecialists, that two different criteria of resolution are in use. These are both based on the same principle of measurement, introduced for the purpose of characterizing the resolution of molecule images formed by single-particle averaging (25): The data are randomly divided in half, and the procedure leading to the average or reconstruction is applied independently to both data sets. The resulting averages or reconstructions are then compared along rings (in 2D applications) or shells in Fourier space [in 3D applications (74)]. [Recently, the entire approach of using such half-set reconstructions in a resolution assessment has been questioned (29), but no clear alternative has been suggested].

The most popular measure, the Fourier ring or shell correlation (FRC or FSC) (58, 75), measures the degree of correlation between the two reconstructions as a function of radius (i.e., spatial frequency) in Fourier space. The FSC is a curve that starts with a value of 1, indicating excellent agreement at low spatial frequencies, and drops more or less gradually to small values indicating poor agreement (Figure 4). Ideally, this curve, which is easy to reproduce as a figure, should
Figure 4 Example for a Fourier shell correlation curve (solid line), which expresses the degree of reproducibility of the reconstruction in Fourier space as a function of shell radius. Dotted line: so-called 3-σ curve, obtained by multiplying the cross-correlation coefficient expected for pure noise by three. The resolution is quoted either as the Fourier radius where FSC falls below 0.5 (dashed line), or as the radius where it falls below the 3-σ curve. It is clear that quotation of a single number (here 11.5 Å for the 0.5 criterion and 8.5 Å for the 3-σ criterion) cannot do justice to the information conveyed by the whole curve. [Adapted from (27)].

accompanies every publication of a single-particle reconstruction because it contains all information allowing the resolution behavior to be assessed. Difficulties only arise because of the attempt to summarize the information contained in the curve by a single value. In one method (11, 36), the cutoff is placed at the spatial frequency where the curve drops below the arbitrary but sensible threshold value of 0.5; in the other method (44), the curve is instead compared with the so-called 3-σ curve, indicating three times the correlation expected for pure noise.

The problem is that what one chooses to call resolution is not inconsequential because it determines the presentation and interpretation of the final results. What choice of value makes sense from this point of view can be gauged by looking at the fundamental relationship between cross-correlation coefficient (CCC) and S/N ratio (21): With decreasing CCC, the S/N ratio decreases quite rapidly. A CCC of 0.5 is already equivalent to a S/N ratio of 1, and any values below that lead to even lower S/N ratios. (Note that an S/N ratio of 1 is comparable to the S/N ratio normally found in raw, unprocessed images.) This means that when using the 3-σ resolution value, one is bound to admit Fourier components that are unreliable.
and polluted by noise [see (36)]. This is not to say that there is no information in this extended range; it is just the fact that the information cannot be reliably distinguished from noise that makes this choice of cutoff questionable.

INTERPRETATION OF CRYO-EM MAPS BY FITTING X-RAY STRUCTURES

Beginning with pioneering work in the virus field (67, 54) and the interpretation of maps showing actin-myosin interaction (52, 60), it has been recognized that cryo-EM and molecular docking can be combined in a powerful way to approach the atomic structure of a large macromolecular complex that cannot be crystallized, provided the X-ray coordinates of the component structures are known. This same approach has proven extremely fruitful in the interpretation of macromolecular complexes visualized by single-particle reconstruction (2, 9, 27, 56, 63).

Docking and fitting of X-ray structures into density maps obtained by cryo-EM [see review provided in (53)] is still done mostly by hand. Criteria for fitting are difficult to rationalize because it often involves compromises between boundary conflicts in different parts of the map. Most seriously, the use of contour-only representations for low-density maps leads to a complete disregard for interior variations of density, and the use of different representations—all-atoms or backbone for the X-ray structure versus contours for the low-resolution density map—requires a great amount of intuition. Despite these shortcomings, coordinates derived by such fittings have been instrumental in the interpretation of molecular interactions in some key biological processes, such as the power stroke in muscle (52), virus-antibody interaction (54), the action of molecular chaperones (57), and translocation in the ribosome (2), and the coordinates have found their way into the protein data bank [see recent tabulation in (37)].

As the foregoing account has shown, there is an urgent need for methods of computational fitting according to quantitative criteria. The approaches currently being pursued fall into three classes: (a) those based on cross-correlation [DOCKEM (53), EMFIT (12, 54), COAN (76)], (b) those based on a force field derived from the low-resolution map, in a molecular mechanics approach to the modeling of interaction (R. K. Z. Tan, M. S. Van Loock & S. C. Harvey, manuscript submitted), and (c) those based on vector quantization [SITUS (82–84)]. Both Rossmann (54) and Roseman (53) base the search on the real-space cross-correlation function between the atomic structure (suitably converted into a density function) and the low-resolution cryo-EM map and use the rotation and translation parameters that maximize the CCC. Roseman’s correlation function differs from the standard method in that he uses computation over a local area, along with a local normalization. This method is computationally intensive as it foregoes the computational advantages afforded by the fast Fourier transform algorithm, but it apparently avoids some of the false maxima the global search can run into. Volkmann & Hanein (76) go a step further after the computation of the CCC and define an entire set of possible solutions whose CCC is greater than a certain statistically defined threshold. Next, the atomic structure is placed into all positions of the
solution set and checked for compliance with chemical and steric constraints. The
scoring of violations then leads to the selection of the final position. The method
was recently used in a study of the actin-myosin complex (77).

The force field method by Tan and coworkers (R. K. Z. Tan, M. S. Van Loock &
S. C. Harvey, manuscript submitted) represents a further development of a quan-
titative docking method earlier employed by Malhotra & Harvey (35). Both have
as their goal the modeling of RNA structures and their interactions within the
constraints of molecular mechanics and the additional constraint of a low-density
map. Whereas Malhotra & Harvey (35) devised a penalty only for violation of
the boundary of the density map, the new method takes into account the interior
densities, as well. A force field is derived from the local gradients of the density
map that drives the individual atoms (or pseudo-atoms) of the structure to be fitted
into compliance with local density variations.

An entirely different approach to the problem of docking was introduced by
Wriggers and coworkers (83). Following this approach borrowed from signal pro-
cessing, the low- and high-resolution maps to be compared are represented by a
finite number of vectors (“code book vectors”). The uniqueness of these repre-
sentations, achieved by solving an optimization problem using neural networks,
makes it possible to match the two maps uniquely. Whereas the initial approach
was restricted to rigid body movement of the component structures, the more re-
cent development includes flexible docking, demonstrated on the example of the
docking of the EF-G structure into the EF-G difference density map of an EF-G
ribosome complex (82).

APPLICATION TO SOME MACROMOLECULAR
MACHINES

There has been a recent explosion in the number of applications of cryo-EM single-
particle reconstruction. Some of these applications, especially relating to the study
of molecular machines, have been recently reviewed by Nogales & Grigorieff
(42). In the following, only a few recent areas of application are highlighted, in
particular three that are exemplary for the workings of macromolecular machines:
transcription, mRNA splicing, and translation. In eukaryotes, these three systems
work in tandem, handing products from one to the next. Transcription yields a copy,
in the form of mRNA, of the genetic code residing on the DNA. This product is
called pre-mRNA because it is not yet in the form where it can be translated.
Pre-mRNA leaves the nucleus and enters the cytoplasm, where it is processed
by splicing, i.e., excision of untranslated sequences called introns. The resulting
mRNA is ready to be translated by the ribosome. Each of these processes involves a
large number of proteins in interaction with RNA, and each works to ensure fidelity
in the expression of the genetic intent. (Prokaryotes lack the step of splicing and
have no nucleus, thus translation is not physically divided from transcription by a
boundary.) To different degrees, cryo-EM has made contributions to these areas,
with the ribosome being farthest along and the spliceosome just at the start.
Transcription Complex

The machinery employed for transcription goes through several stages of initiation and assembly, using a number of shared subunits in a modular way (41) (Figure 5a). Transcription factor TFIID binds to the DNA promoter, triggering the recruitment of the rest of the factors, as well as RNA polymerase II. In the fully assembled machine, RNA polymerase creates a faithful mRNA copy of one of the DNA template strands while the machine ratchets along the DNA. Cryo-EM has been applied to different assembly products: human TFIID-IIA-IIB complex (6) (Figure 5b), human TFIIH (61), and RNA polymerase (F. Asturias, personal communication). Although the density maps obtained are low resolution, antibody mapping allowed the placement of subunits, and further clues were derived from the position of holes that serve as conduits for the DNA or mRNA.

Spliceosomal snRNP U1

Splicing of pre-mRNA involves the spliceosome, a large complex formed during the interaction of small nuclear ribonucleoproteins (snRNPs) with the mRNA. A major difficulty in imaging the spliceosome in its entirety is that it exists in many different processing states in the cell. Three-dimensional imaging with the technique at hand would require some type of synchronization so that all complexes being imaged would be in the same processing state. Because of this difficulty, a more promising approach is to look at individual snRNPs separately. The result obtained by Stark and coworkers (63) for the component U1 is the first step toward a full characterization of the processing spliceosome (Figure 6). As in the case of the transcription complex, the placement of known components into the cryo-EM map amounts to a 3D jigsaw puzzle, which requires clues from immuno-EM and from the topology of the map.

Ribosome

Translation of the genetic message into polypeptide (and from that into protein) is a process that occupies a large fraction of the cell’s resources. The ribosome, the site where protein synthesis occurs, is made up of more than 50 proteins and 3 RNAs, which form 2 subunits of unequal size. The ribosome is a macromolecular machine par excellence, in that it employs many movable parts, engages in interactions with a large number of ligands, and achieves high precision in translating the genetic blueprint. X-ray crystallography of ribosomal subunits from thermophilic bacteria (59, 81, 86) and archaebacteria (7) shows that the proteins and rRNA form a meshwork, especially tight in the large subunit.

Cryo-EM was instrumental in producing the first clear 3D images of this complex organelle (24, 26, 64). The best current maps have resolutions in the range of 10 Å (27, 40), which allow proteins to be docked and rRNA helices to be delineated. The positions of tRNA in its various canonical sites were mapped (3, 4, 64a), and the binding positions of the elongation factors were revealed [EF-Tu ternary complex (65), EF-G (1, 2)]. Figure 7 presents a recent example for
such localizations: the position of tetracycline protection factor Tet(O) on the 70S E. coli ribosome (62).

CURRENT LIMITATIONS AND HOW THEY MIGHT BE OVERCOME

Three-dimensional cryo-EM of single particles, as a method of determining structure and dynamic behavior of macromolecules, is coming of age. Perhaps one of the most telling indications is the title of an article that appeared a few years ago, “Who Needs Crystals Anyway?” (15). One of the most useful features of the technique is the potential for fast turnaround: If everything goes well (high occupancy of binding states; good, random orientational behavior of the particles; flawless electron microscopic data collection), the number of micrographs required to achieve 10–15 Å resolution can be collected within a few days. If a 3D template map exists, a density map for a new data set can be obtained in a matter of days or a couple of weeks.

As more powerful and sophisticated electron microscopes are becoming available and the methods of processing and interpretation are increasingly refined, the question is: Where is the limit? Attainment of close-to-atomic resolution is feasible in principle, provided the particle exceeds a certain size (31). To approach atomic resolution, the number of particles has to be greatly increased. The number should go roughly with the cube of 1/d if d is the resolution in Å. For the ribosome, resolutions in the range of ~10 Å can be currently achieved with ~30,000 particles; therefore attainment of ~3 Å would require 27 × 30,000 particles, or close to a million. However, this estimate is optimistic for two reasons: (a) It does not make allowance for the fact that the S/N ratio decreases with increasing spatial frequency, and (b) it does not account for the conformational variability that comes increasingly into plan as the resolution increases. Thus, development of methods for automated data collection and processing will be the key, along with powerful algorithms for sorting data originating from heterogeneous molecule populations.

The work accomplished to date using 3D cryo-EM of single particles has also taught us that many studies of macromolecular interaction already yield significant results at resolutions of 10–20 Å. One of the aims of the development of technology should be to reach the range of resolution routinely, allowing protein subdomain structure and alpha-helical elements to be recognized, i.e., 6–8 Å.

SUMMARY

Understanding molecular interactions is key to understanding the workings of the cell. Molecules engage in dynamic associations (“molecular machines”), forming complexes in this process that are often too large and too flexible for structural studies by X-ray crystallography. As we have seen, cryo-EM is a new, powerful tool that enables us to visualize these complexes despite their unwieldy properties.
Although the resolution is limited, crucial information about the interaction of molecules and their ligands can be gained by fitting atomic structures into the cryo-EM density maps. Each of the examples given, the ribosome, spliceosome, and transcription complex, illustrates the power of this combined approach.

ACKNOWLEDGMENTS

This work was supported by grants from NIH R37GM29169, R01GM55440, P41RR01219, and NSF DBI9871347. I thank Yu Chen for assistance with the preparation of the figures.

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Figure 2  Data collection for single molecule reconstruction in the electron microscope. (a) Schematic diagram of data collection. Arrows symbolize the impact of the parallel electron beam upon the specimen. Many “copies” of the molecule are lying in random orientations in a thin layer of vitreous ice. In the image plane, parallel projections of the particles are obtained at high magnification. Images of particles lying in the same orientation can be found by employing alignment and classification procedures. (b) Example of an actual micrograph, showing 70S ribosomes of *E. coli*. The micrograph was taken on an F30 Tecnai electron microscope (Philips/FEI, Eindhoven) with a helium-cooled specimen stage.
Figure 5  Human transcription factor complex. (a) Cartoon representing the spatial relationships and interactions between DNA, activators, transcription factors, and RNA polymerase. The complex shown in (b–d) is painted green [reproduced from (41)]. (b–d) Single-particle reconstruction of the complex formed by TFIID, TFIIA, and TFIIB in a contour representation, and locations of various components as obtained by difference mapping [reproduced from (6)].
**Figure 6** Cryo-EM map of human spliceosomal U1 small nuclear ribonucleoprotein particle at 15 Å resolution (using the 0.5 FSC criterion). (a) Density map. Labels: A, protein U1-A; 70S, protein U1-70S; IV, location of 3′-terminal stemloop IV. (b) RNA modeled according to known binding interactions between the RNA of U1 and proteins U1-A and U1-70k. Locations of stem loops, heptamer Sm ring (F, E, G, D3, B, D1, D2, based on X-ray structures), U1-70k, U1-A, U1-B, and U1-C are indicated [reproduced from (63)].
Figure 7  Binding of factors to the *E. coli* ribosome. (a) Tetracycline protection protein Tet(O); (b) elongation factor G. In both panels, the factor is colored red, the ribosome blue. In (a), P-site tRNA is colored green. Tet(O) binds in a very similar position as EF-G, but its domain IV is shaped and positioned differently, such that it forces the release of tetracycline bound to 16S RNA of the small ribosomal subunit. Landmarks: h, sh, sp—head, shoulder, and spur of the 30S subunit; CP, SB, and h38—central protuberance, stalk base, and helix 38 of the 50S subunit [reproduced from (62)].