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Review

Crystallization of RNA

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Abstract. Even as the number of RNA structures determined and under study multiplies, the critical step in X-ray diffraction analysis, growth of single well-ordered crystals, remains at the boundary between art and science. Recent advances in methods of RNA synthesis, purification, and characterization, as well as empirical and technical improvements in crystallization techniques, the development of cryo-crystallography, and the wider availability of bright, tunable, X-rays from synchrotron sources are improving the chances of obtaining RNA crystals suitable for X-ray structural analysis. In this review, we summarize the current status of the design, preparation, purification, and analysis of RNA for crystallization and describe the latest approaches to obtaining diffraction-quality crystals.

Key words. RNA crystallization; X-ray crystallography; RNA crystal structure; sparse matrix crystallization; X-ray diffraction.

Introduction

The history of RNA crystallization is the story of timing of scientific breakthroughs. Single crystals of transfer RNA (tRNA) were initially reported in the late 1960s [1]. This breakthrough was made possible by the development of purification methods using reverse-phase column chromatography. The discovery that the addition of spermine improved crystal quality soon led to the almost simultaneous crystal structure determination of yeast phenylalanine tRNA by several research groups [2–4]. Following this pioneering breakthrough, in the 1980s, several other tRNAs were crystallized and their structures determined [5, 6]. A quiescent decade in RNA crystallography followed awaiting the next breakthrough. The second wave of RNA crystallography occurred in the late 1980s and early 1990s, fueled by advances in chemical and enzymatic synthesis of RNA oligomers, and improved purification methods. Many crystal structures of RNA duplexes [7] and duplexes incorporating internal loops [8] were determined, as were larger RNA domains including ribozymes [9], the P4–P6 domain of the group I intron [10], and fragments of ribosomal RNA (rRNA) [11–13]. The latest wave of RNA crystallography, the crystallization and structure solution of the ribosome and ribosomal subunits by several research groups [14–16], greatly increases our knowledge of RNA structure and function and will be analyzed and studied for many years to come. Thus, the history of RNA crystallography demonstrates how technological innovations and the dedicated efforts of scientists willing to take risks can be responsible for leaps forward followed by years of steady progress in the field.

The synthesis, purification, and crystallization of RNA have been previously reviewed [17–22]. However, rapid developments in each of these areas, and a better under-

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standing of design principles, are changing our approach to obtaining diffraction-quality crystals for structural analysis. This review will summarize the current approaches and trends in the crystallization of RNA for structural analysis by diffraction methods.

Design of RNA molecules for crystallization

Large, flexible, structurally heterogeneous biological RNAs are often difficult to purify to homogeneity and therefore will not crystallize. However, many questions in RNA structural biology can be answered by knowledge of the structure of a model compound, fragment or derivative of a biological RNA, or other construct amenable to crystallization. Therefore, careful consideration should be given to the length, sequence, and overall design of the RNA to be crystallized. A homogeneous RNA sample will tend to crystallize out of several different conditions, while a heterogeneous preparation will not form crystals under any conditions. Thus, proper RNA design is more important than searching for the correct crystallization condition in sparse-matrix searches.

Crystallization strategies are now employed that utilize sequence/structure variation as a variable in a multiple screening procedure [23]. When the RNA to be crystallized is naturally, or can be designed as, two or more annealed strands, a combinatorial approach is practical in which variants of the two strands are mixed and annealed to form a large number of constructs for crystallization. This approach was used successfully for crystallization of the 10–23 DNA enzyme [24], but should be directly applicable to RNA crystallization.

Several factors affect the ability of RNA to crystallize. Design techniques can be applied for increasing the likelihood of obtaining RNA crystals, for improving the size of those crystals, and for extending the limit of the diffraction maximum [25]. Approaches to design are listed in table 1. The minimal sequence or structure necessary to answer the biological or structural question will often be the easiest to crystallize, and biochemical or biophysical assays may be necessary to determine this starting point. Once the basic structure to be studied is set, the RNA may be prepared as a single-chain or multi-chain annealed molecule and the length and sequence of duplex regions can be varied with the incorporation of either blunt ends or overhangs. Other decisions include whether to incorporate protein-binding sites for co-crystallization, and whether to include modified bases.

Regions that are not of functional interest are good candidates for redesign. Sequence and length variations in the 3′ and 5′ end regions can influence crystallization of RNA oligomers [26]. The sequence of duplex strands may also be varied while still maintaining complementarity within the double helix. Anderson et al. [25] found sequence variation to be the most important factor in obtaining high-quality crystals. The length and sequence of RNA oligomers should therefore be considered as the first parameters to be varied in crystallization trials, and several variants should be tried. Biochemical experiments may also suggest that variation of the identity or number of bulged nucleotides or the number and type of nucleotides in a loop can be varied while maintaining biological activity.

Several RNA and DNA crystal structures have shown that adding overhanging bases to an expected double helix can result in the formation of excellent crystals [27–29]. Such single-strand overhangs of one to two nucleotides from a double helix may allow formation of extended helices and co-axial stacking by intermolecular base pairing.

Table 1. RNA design factors affecting crystallization and crystal structure solution.

Design principle	What do we do?	Goal	Reference
Sequence variation	change the sequence at the ends or, in non-functional double-helical regions, insert non-native mutations	determine the effect of sequence on crystallization	23, 24, 63
Minimal functional	Remove non-relevant regions of the RNA	improve crystallization by reducing flexibility	10, 34
Interhelical stacking	make 5′ and 3′ termini as blunt ends or 1–2 base overhangs	good crystal packing requires intermolecular stacking between helices	8, 26
Remove competing oligomeric forms	make duplexes as two strands rather than one strand with hairpin loop	crystallization requires homogeneous conformation in sample	8, 42
Make protein-RNA complex	engineer a protein-binding site (hairpin) into RNA, crystallize the complex.	improve crystal packing by allowing protein-RNA and protein-protein contacts	40
Selection of stable RNAs	using SELEX methods, identify a stable sequence variant	increased stability and reduced flexibility improves crystallization	64
Incorporation of modified nucleotides	replace U or C with 5-bromo or 5-iodo U or C	collect MAD data for crystallographic phasing	41, 42

MAD, multi-wavelength anomalous diffraction; SELEX, systematic evolution of ligands by exponential enrichment.

Table 2. Sparse matrix screens for the crystallization of RNA.

Reference	Number of trials	pH	Precipitant	Monovalent salt	Multivalent salt	Divalent salt	Notes
65	48	5.4–8.5	MPD, PEG (400, 1K, 4K, 8K), Li_2SO_4, isopropanol, $(NH_4)_2SO_4$, ethanol, hexanediol, acetone	LiCl, NaCl, KCl	$Co(NH_3)_6Cl_3$	$MnCl_2$, $ZnCl_2$, $CoCl_2$, $CdSO_4$, $CaCl_2$, $BaCl_2$, $NiCl_2$, $SrCl_2$, $CuCl_2$	specifically for large RNAs
66	48	6.0–8.0	MPD, isopropanol, PEG (400, 500, 1K, 4K, 8K), $(NH_4)_2SO_4$, sodium acetate, t-butanol, acetone, ethanol, dioxane	NaCl, KCl, NH_4Cl, LiCl, argininamide	$Co(NH_3)_6Cl_3$, spermine, spermidine, putrescine	$MgCl_2$, $MnCl_2$, $BaCl_2$, $CdCl_2$, $ZnCl_2$, $CoCl_2$, $CaCl_2$	8–12 nucleotides in length
67	24	5.5–7.0	MPD	KCl, NaCl, LiCl	spermine, $Co(NH_3)_6Cl_3$	$MgCl_2$, $BaCl_2$, $SrCl_2$	DNA and RNA oligomers
68	44	5.5–8.0	MPD, isopropanol, PEG (8K), $(NH_4)SO_4$, dioxane, 1,6-hexanediol, ethanol, t-butyl alcohol, spermine			$MgCl_2$, $CaCl_2$, $BaCl_2$, $CoCl_2$, $CrCl_2$, $NiCl_2$, $CuCl_2$, $SrCl_2$	4–50 kDa RNAs synthesized in vitro
23	48	5.6–8.5	MPD, PEG (400, 4K, 8K), Li_2SO_4, isopropanol, $(NH_4)_2SO_4$, dioxane, hexanediol	KCl, NH_4Cl	$Co(NH_3)_6Cl_3$, NH_4OAc	$MgCl_2$, $MgSO_4$, $Mg(OAC)_2$, $MnCl_2$, $CdSO_4$, $CaCl_2$, $CoCl_2$	small RNA/hammerhead RNAs
24	48	5.5–8.0	isopropanol, MPD, PEG (400, 1K), ethanol, butanol, heptanediol, dioxane, Li_2SO_4, NaCl, $(NH_4)_2SO_4$, LiCl, Na tartarate, KCl	NaCl	$Co(NH_3)_6Cl_3$, spermine, spermidine, sodium citrate	$MgCl_2$, $CuSO_4$, $CaCl_2$, $CuSO_4$, $Ca(OAC)_2$,	DNA enzyme complexed with its RNA substrate

MPD, 2-methyl, 2,4-pentanediol; PEG, polyethyleneglycol.

Existing crystal structures of RNA have shown that intermolecular stacking is strongly preferred for crystallization. Either blunt ends or complementary overhangs (one to two bases) favor this type of crystal packing contact. The sequence of the double-helical regions appears to be a secondary factor. The length of the duplex may be important in making the repetitive contacts of the crystal lattice (for example, RNA duplexes of length equal to the repeat unit of one turn of RNA, 12 base pairs, may be favorable for crystallization).

The high concentration of RNA in crystals favors the formation of duplexes over hairpins for small RNAs. Thus, attempts to crystallize tetraloops such as UNCG and GNRA that exist as hairpin loops in solution, have resulted in crystals of double-stranded RNAs with internal loops (self-complementary) containing non-canonical pairs [8, 30–33]. Crystallization of RNA hairpins has, in fact, only been successful in large RNAs (tRNA, P4–P6, HDV, 5S rRNA domain E) in which the tertiary folding maintains the secondary structure [10, 34, 35], and in protein-RNA complexes [36–38]. Thus, unlike nuclear magnetic resonance (NMR) RNA samples that are routinely made as single-strand hairpins with a tetraloop at the end, samples of small RNAs intended for crystallization should be designed as two (or more) annealed strands. In general, alternate foldings of RNA of similar or greater stability to the desired fold must be considered in the design process. A secondary-structure prediction program such as MFOLD [39] may be helpful in this process.

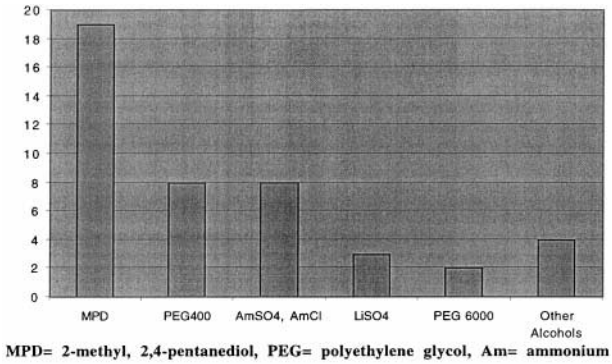


Figure 1. A histogram of precipitants used in RNA crystallization. The vertical axis is the number of crystal structures reported to use a specific precipitant (horizontal axis) in the crystallization. Taken from RNA crystal structures deposited in the Nucleic Acid Database.

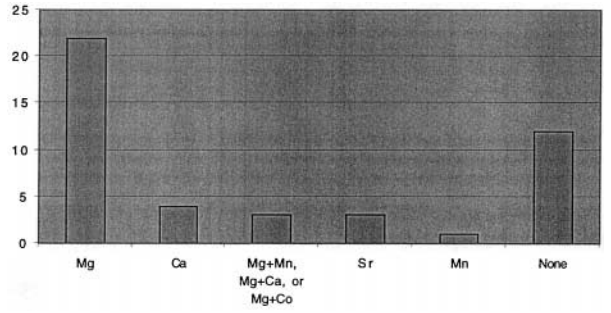
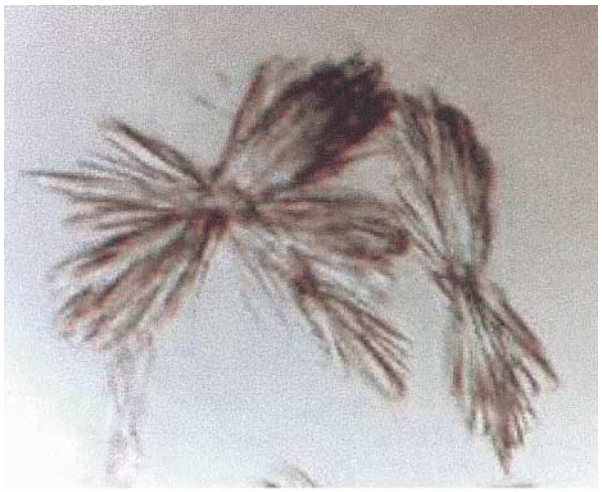


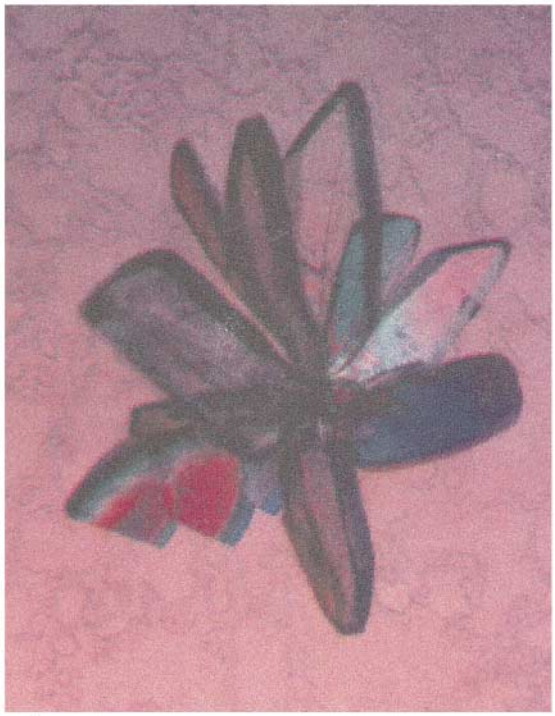
Figure 2. A histogram illustrating the use of divalent cations in RNA crystallization. The vertical axis is the number of crystal structures reported to contain a specific divalent cation (horizontal axis) in the crystallization solution. Taken from RNA crystal structures deposited in the Nucleic Acid Database.

Crystallographic structure solution requires a method of obtaining phases, such as molecular replacement, single or multiple isomorphous replacement (SIR/MIR), or multi-wavelength anomalous diffraction (MAD). Given the current general availability of tunable synchrotron radiation, the most general and facile approach is the use of MAD phasing. Traditional crystal-soaking experiments with heavy metals, particularly osmium hexammine and lanthanides, can result in excellent derivatives for MAD phasing if the metals are specifically and tightly bound [10, 33]. Two general and rational approaches to obtaining derivatives for MAD are increasing in use for phasing RNA diffraction data. The first is engineering of a specific hairpin loop into the RNA that functions as a protein-binding motif and crystallization of the protein-RNA complex in which the protein contains selenomethionine instead of methionine [34, 40]. The second approach is to synthesize the RNA either by transcription or chemical synthesis with either uridine or cytidine replaced by 5-bromouridine or 5-bromocytidine [41, 42], usually in double-helical regions. MAD diffraction data from crystals containing a single bromine atom per asymmetric unit can be used to phase RNAs of up to around 10,000 Da or about 30 residues. Modern crystallographic software can identify many (>50) anomalous scatterers in a crystal; however, incorporation of too many modified bases may interfere with folding or with crystal packing, thus replacement of all uridines or cytidines in a long RNA may not be useful. Design of the RNA with more than one chain will allow incorporation of modifications in only a single chain as has been successful in the hammerhead ribozyme [9, 43]. Finally, the site-specific incorporation of phosphorothioates as potential binding sites for mercury has been proposed as a method for obtaining MAD phases [13].

Another widely used technique of RNA design is to divide the RNA into separate domains and crystallize each one of them. A typical example is 5S RNA that has been the subject of crystallization efforts for many years. Attempts to crystallize the intact molecule have



(a)



(b)

(c)

Figure 3. A recurring morphology for RNA crystals: plates growing from a common center (RNA flowers). (*a*) Rev-binding element RNA. (*b*) Hammerhead ribozyme. (*c*) Yeast phenylalanine transfer RNA.

resulted in crystals that in the best case diffract to low resolution (< 8 Å) [44, 45]. The Erdmann group has pursued the strategy of 'divide and conquer,' in which they have divided *Thermus flavus* 5S rRNA into five domains (A–E) and attempted to crystallize each one and then reconstruct the intact RNA. They have successfully solved the structure of domain A at 2.4 Å [46] and domain E at 3.0 Å resolution [35]. Recently, they have obtained a 7.8 Å resolution dataset from the intact 5S rRNA crystals grown under both earth and microgravity conditions on the NASA space shuttle [26]. Knowledge of the high-resolution structure of the do-

mains will be critical in building a model using the low-resolution data available for the complete 5S rRNA.

Finally, RNA design can also affect other aspects of the crystallization process, such as the addition of 5′-G(GG) to increase levels of RNA synthesis by transcription.

RNA synthesis

Three methods are commonly used for RNA preparation: purification from the biological source, in vitro enzymatic synthesis by runoff transcription, and chemical synthesis on a nucleic acid synthesizer. Purification from a cellular source may be necessary in cases where modified nucleotides are present, as in tRNAs, but often yields heterogeneous preparations transcribed from different genes or with different modifications.

Techniques for synthesis of RNA by chemical and enzymatic methods have been described in detail [20, 47–50]. State-of-the-art methods of chemical synthesis utilize phosphoramidites with 5′-silyl protecting groups in conjunction with an acid-labile 2′-orthoester protecting group, 2′-bis (acetoxyethoxy)-methyl ether (2′-ACE). The 2′-protecting groups can be rapidly and completely removed under mild conditions in aqueous buffers [51]. Currently, chemical synthesis of RNA oligomers with reasonable yield and purity up to a size of about 35 residues is a routine process. Chemical synthesis of RNA allows site-specific incorporation of a variety of non-standard nucleotides such as 5-bromo- and 5-iodouridine, commonly used as heavy-atom derivatives in MIR or MAD crystallographic phasing [42]. Chemically synthesized RNAs may now be purchased commercially from several vendors.

Larger RNAs must be enzymatically synthesized usually using T7 or SP6 RNA polymerase from a DNA template prepared either biologically or chemically. The template can be a partial duplex chemically synthesized as two single strands [48], a duplex amplified by PCR methods [50], or a linearized plasmid containing the gene for the RNA of interest at the 3′ end. Addition of one or more Gs at the 5′ end of the RNA may be necessary to increase transcription yields.

Synthesis of crystallization-quality RNA by transcription is sometimes hampered by the non-homogeneous nature of the 3′ terminus, adding an additional random nucleotide to form a significant percentage of $n+1$ length transcripts [47] which may be very difficult to separate from the n-length correct transcript. One simple solution to this heterogeneity is to incorporate 2′-O-methoxy nucleotides at the end of the template DNA [52]. Heterogeneity at the 5′ end also occurs in T7 RNA polymerase transcripts, possibly by initiation with abortive di- and tri-nucleotide products [53]. An elegant solution to this problem has been described [21, 54] which utilizes cis- or trans-ribozymes to specifically process the transcript ends to the correct length. The hammerhead or delta ribozymes can be added to the 3′ end for cis cleavage to leave a homogeneous terminus. The VS ribozyme substrate added to the 3′ end can be cleaved by a trans reaction with the VS ribozyme [54]. This trans cleavage reaction does not require specific sequences adjacent to the cleavage site unlike the cis ribozymes.

Although modified nucleotides cannot currently be incorporated site-specifically into enzymatically synthesized RNA, 5-bromouridine can be incorporated during the transcription reaction using modified triphosphates to make a body-labeled RNA [41]. However, incorporation of too many modified nucleotides can interfere with folding, structural stability, and biological activity.

Each source of RNA—cellular, enzymatic, or chemical—has its own intrinsic purification problems. Cellular preparations may be inherently heterogeneous, as slightly different genes or modifications may exist in one cell type. Enzymatic synthesis suffers from length heterogeneity and possibly errors in sequence of the DNA template. Chemical syntheses may be incompletely deblocked and deprotected or may have incorrect internucleotide linkages. Thus, the source of the material must be considered when designing a purification scheme.

RNA purification

RNA molecules are susceptible to chemical and enzymatic hydrolysis reactions, and therefore care should be taken when working with RNA. It is advisable to sterilize all solutions by autoclaving or filtration and carefully avoid the presence of trace metal contamination by including EDTA in solutions.

Once multi-milligram quantities of the nucleic acid of interest have been synthesized, the sample must be purified to homogeneity before attempting crystallization. This RNA must all be of one sequence, have no contaminants, and be structurally uniform, i.e., with a single conformation or oligomeric state. Three methods are commonly used to purify RNA oligomers. Short oligomers (less than 20 nucleotides long) can be purified using thin layer chromatography (TLC) on glass plates layered with silica containing a fluorophore [55]. The plate is placed in a tank containing a mixture of organic solvents that carry the oligomers as they ascend the plate. The mixture of denatured oligomers is separated according to size, visualized with ultraviolet light (254 nm), the bands of interest are cut out, and the RNA finally eluted from the silica into water.

The second and most widely used method of purification is by denaturing (urea) polyacrylamide gel electrophoresis (PAGE), whereby RNAs are separated according to size through a gel matrix under electric current. Running the gel under high temperature can minimize the folding and intermolecular interactions of RNA encountered during purification under less rigorously denaturing conditions. As with TLC, the separated nucleic acid species are visualized under UV light, cut out from the gel, and eluted into buffer. Denaturing PAGE purification is useful for oligomers larger than eight nucleotides.

The third approach to purification is column chromatography, commonly reverse-phase high-pressure liquid chromatography (HPLC) or anion exchange fast-performance liquid chromatography (FPLC). HPLC often uses denaturing organic solvents such as acetonitrile to elute the oligomer through a hydrophobic matrix. The denatured oligomers will elute according to size. In anion exchange FPLC, the oligomers remain in the native or folded state while migrating through a positively charged matrix. Nucleic acids will elute at different salt concentrations depending on their size, shape, and charge, separating them from contaminating species. This method is appropriate for oligomers longer than ten nucleotides and is particularly useful for separating different conformers or multimers of the same sequence. It has been used to achieve nucleotide resolution of n- and (n + 1)-mer RNA transcripts [50].

Sometimes several methods of purification must be tried before an optimum protocol is adopted. For example, denaturing PAGE can be coupled with anion exchange chromatography to first purify the sequence and then the conformer. Furthermore, in the case of double-helical oligomers composed of non-identical strands, each strand could be purified by TLC, the purified strands annealed, and the duplex purified by anion exchange chromatography.

Each of the methods described above can be used analytically to assay the purity and homogeneity of the nucleic acids. Other methods, such as non-denaturing PAGE, capillary gel electrophoresis [56, 57], dynamic light scattering, [58], and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry [59], are useful as analytical techniques. In addition to their value as analytic tools of molecular homogeneity, dynamic light scattering and gel shift techniques can be used to assay the formation of complexes with drugs, peptides, or proteins.

Following verification that the RNA oligomer is homogeneous in sequence and conformation, it should be dissolved to a concentration of 10–30 mg/ml ($\sim$ 2 mM for a dodecamer duplex) in RNase-free, deionized, sterile, water, often with 0.1 mM EDTA present to chelate stray metal ions. The sample can be concentrated by microfiltration, ethanol precipitation, or by use of a rotary evaporator. The crystallization solution may also include a buffer at low concentration; however, phosphate buffer should be avoided since insoluble salts are formed with the divalent metal ions (such as Mg^{2+}, Ca^{2+}) commonly used in crystallization.

For some RNAs, a cycle of heat denaturation and annealing may be required for the RNA to assume a single conformation. Temperature (40–70 °C), time (1–10 min), methods of cooling (slow cooling in hours vs snap cooling in seconds), buffer, pH, and ionic strength (i.e., 5–20 mM $MgCl_2$) are variables that depend on the type of RNA and on the desired conformation (duplex vs hairpin). The extent of annealing can be assessed by melting curves, binding assays, or activity assays [60].

RNA crystallization

No foolproof method has yet been developed that correlates specific RNA molecules to crystallization conditions. The ability to grow single, well-diffracting crystals is still very much art and experience. The most common way of crystallizing RNA for X-ray diffraction is by using sparse matrix screens with either hanging or sitting drops to obtain initial crystals, followed by optimization of conditions to improve size, order, and to accommodate a cryosolvent. Vapor diffusion methods are used to slowly equilibrate between a drop containing the RNA sample and a low precipitant concentration and a large reservoir with a higher precipitant concentration. As the precipitant concentration increases in the RNA drop and the saturation conditions are approached, RNA crystals may form. Since precipitant, pH, ionic strength, monovalent, divalent, or multivalent cation type, and other additives can all be varied, a full matrix screen of all variables for crystallization is not generally possible. Thus, the sparse matrix screen with variables selected at random attempts to sample the full matrix in such a manner as to identify initial, approximate crystallization conditions. Several sparse matrix screens have been developed for RNA crystallization. A summary of the conditions used in these screens is provided in table 2. Several of these screens are commercially available.

Most crystallization screens consist of approximately 50 conditions (table 2). For hanging-drop vapor diffusion (on coverslips in plastic, multi-well plates), 0.5-ml reservoir solutions are equilibrated against hanging drops consisting of a mixture of typically 1 μl of sample (usually about 2 mM RNA), and 1 μl of reservoir. Thus, approximately 0.5 mg of an RNA oligomer would be needed to conduct one crystallization screen.

A survey of ~50 RNA structures in the Nucleic Acid Database [61] illustrates which precipitants (fig. 1) and divalent cations (fig. 2) are most commonly used in growing well-diffracting crystals. The prevalence of MPD as precipitant and magnesium as a divalent cation is at least partially attributable to the fact that these were components of the first successful RNA crystallizations and are the most frequently tried in attempts to grow new crystals.

RNA crystals can take from days to months to come out of solution. Some crystals grow out of precipitant, others out of clear drops. They can assume a number of different shapes, anything from long thin needles to well-ordered diamonds. A frequently observed morphology of RNA crystals is a cluster of thin plates growing from a common nexus as shown in figure 3. Once a crystal has formed, it will continue to grow until equilibrium of the crystallization drop and reservoir is reached or the supply of RNA in solution is exhausted. At this point, crystal size may be increased either by raising the reservoir precipitant concentration or 'feeding' the drop by addition of RNA in mother liquor.

Occasionally a drop will yield many tiny crystals that can be used to seed other drops. Seeding is a technique of introducing a nucleation center into a new drop in the hope that an artificial nucleation center will promote growth of a single large crystal. Macro-seeding techniques consist of introducing small single crystals into a pre-equilibrated drop as loci for the growth of larger crystals [62].

Another approach is microseeding, in which microscopic crystals are introduced by streaking a drop containing non-usable crystals with a hair, or dipping a pipette tip into the crystalline drop and then dipping the same pipette tip into a new drop to provide nucleation sites in a fresh crystallization drop.

Crystal quality can be assayed crudely using a stereomicroscope equipped with a polarizing lens. If the crystal is birefringent and sharply extinguishes polarized light as it is rotated, it probably consists of a single well-ordered lattice.

Summary

Well-ordered single crystals are required for structural studies by X-ray diffraction methods, but obtaining them is often the most difficult step of the structure determination process. Although we still do not have a solid theoretical basis for crystallization of any macromolecule, experience has provided us with many practical techniques that have been successful in RNA crystallization. To optimize the chances of crystallization of any RNA, carefully considered design, followed by appropriate synthesis and extensive purification to homogeneity are the most important steps. One should also diversify the number of RNA samples for which crystallization is attempted. Perhaps the most standardized step in the process is the actual crystallization by sparse matrix methods. After initial crystals are obtained, they must be optimized by finer screening and appropriate cryosolvents identified. However, since even visually well-formed, large, single crystals that polarize light may have internal disorder, an actual diffraction experiment is necessary to verify crystal quality.

Most RNAs exist naturally as protein-RNA complexes. The crystallization of these complexes has several advantages over crystallization of RNA alone. These include maintenance of the biologically active RNA conformation, the ability to form protein-protein and protein-RNA crystal contacts, and the capacity to use a selenomethionine-derivatized version of the protein for MAD crystallographic phasing. Thus, when available, crystallization of the native protein-RNA complex should be considered.

Given the rapid improvement in methods of RNA crystallization over the last decade, in the near future we can expect the growth of diffraction-quality crystals will become better understood, more straightforward, and that it will enable the rapid structural analysis of many RNA molecules of biological importance.

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