BSTR521 Biocrystallography

Crystallography of RNA and RNA-Protein Complexes

Winter Quarter 2011

Wim Hol
Preparing RNA

Options:

Chemical synthesis
AND THEN ALSO SPECIAL BASES CAN BE INCORPORATED – SUCH AS BROMINATED BASES

Biochemical synthesis
NEEDS GREAT CARE IN PLANNING AND IN EXECUTION
Example of RNA preparation by *in vitro* transcription:
Design of 76nt tRNA^{Mete}

DNA

<table>
<thead>
<tr>
<th>HindIII</th>
<th>T7 promoter</th>
<th>tRNA sequence</th>
<th>BstNI</th>
<th>XbaI</th>
</tr>
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<tbody>
<tr>
<td>GCCAGTAAGCTAAATACGACTCATAAAGCGAGTAGCGAGTGCGAGCGCGTGGTGACTCATATCCCAAAGGTCGTGAGTCGATCTCACCCGGTCGACCA</td>
<td>GCCAGTAAGCTAAATACGACTCATAAAGCGAGTAGCGAGTGCGAGCGCGTGGTGACTCATATCCCAAAGGTCGTGAGTCGATCTCACCCGGTCGACCA</td>
<td>GCCAGTAAGCTAAATACGACTCATAAAGCGAGTAGCGAGTGCGAGCGCGTGGTGACTCATATCCCAAAGGTCGTGAGTCGATCTCACCCGGTCGACCA</td>
<td>GCCAGTAAGCTAAATACGACTCATAAAGCGAGTAGCGAGTGCGAGCGCGTGGTGACTCATATCCCAAAGGTCGTGAGTCGATCTCACCCGGTCGACCA</td>
<td>GCCAGTAAGCTAAATACGACTCATAAAGCGAGTAGCGAGTGCGAGCGCGTGGTGACTCATATCCCAAAGGTCGTGAGTCGATCTCACCCGGTCGACCA</td>
</tr>
</tbody>
</table>

PCR insert from overlapping primers

HindIII  

<table>
<thead>
<tr>
<th>pUC19</th>
<th>XbaI</th>
</tr>
</thead>
</table>

Transform into DH5α, check sequence

Upscale plasmid, maxiprep

Cut plasmid with BstNI and purify linearized plasmid

Small scale transcription, optimize reaction

Large scale transcription

Desalt, store dry at -20°C, refold before use

Electroelute, collect fractions and test concentration at A260

Purify on large denaturing gel, cut out bands

Courtesy of Jessica Kim
Section I: RNA crystallization

Barbara L. Golden and Craig E. Kundrot

Sequence
The sequence of the RNA molecule can have a profound effect on the crystallization.

RNA production
  1. Chemical
  2. In vitro transcription

Checking purity
  PAGE & HPLC & thin-layer chromatography

Purification
  Denaturing PAGE & HPLC

Annealing
  Crystallization
    Not too sensitive to pH
    Cations: mono, di, poly (spermine, spermidine)

Recheck annealing

Cryo-cooling
Tertiary Contacts Distant from the Active Site Prime a Ribozyme for catalysis

Figure 1. The Sequence and the Structure.

(C) The sequence used for structural determination, and its derivation from the *Schistosoma* hammerhead ribozyme.

The conserved nucleotides are numbered using the standard convention and are shown on a gray background. The large arrow points to the scissile bond.

Base-pair switches from the wild-type sequence, introduced to aid crystallization, are boxed, and the base substitutions employed are indicated adjacent to the boxes.

Two 5-bromo-uridine substitutions, used for MAD phasing a single crystal, are indicated by asterisks.

The lowercase m signifies a 2’-O-methyl modification at C-17 to prevent cleavage.

The lowercase d designates a deoxynucleotide used at the 30 end of the substrate to increase synthetic yields.
RNA Synthesis

The sequence was divided into separate enzyme and substrate strands similar to those previously published (Khvorova et al., 2003).

Five nonessential base-pair switches were introduced into the full-length hammerhead construct in order to enrich the heterodimer population and thus facilitate its crystallization without compromising its activity. These changes were introduced outside of the loop, bulge, and invariant regions, aided by an RNA secondary structure prediction algorithm (Mathews et al., 1999, 2004).

The 43-nucleotide enzyme strand sequence and the 20-nucleotide substrate sequence are shown in Figure 1.

The enzyme strand was in vitro transcribed from a DNA template containing a double-stranded T7 polymerase promoter and 2’-O-methyl modifications on the terminal two nucleotides. The enzyme DNA template, as well as the substrate RNA, was synthesized using an Expedite 8900.

The substrate RNA contained a 2’-O-methyl-cytidine at the cleavage site (C-17), a deoxycytidine at the 3’-terminus, and two 5-bromo-uridines at positions 11 and 18. (See also Figure 1).
The enzyme and substrate were mixed in equimolar amounts in a solution containing 50 mM MES (pH 5.5), 1.5 mM EDTA.

The complex was formed by incubating the mixture at 95 C for 2 min., at 65 C for 2 min., and finally at 27 C for 5 min. One millimolar MgCl₂ was included in the mixture before the final incubation step.

A 10 mg/ml concentration of RNA was used for the crystallization experiments.

The reservoir solution contained 0.5 M (NH₄)₂SO₄, 100 mM MES (pH 6.5), and 35% PEG 3350.

After mixing, the reservoir solution, the salt and PEG phases were allowed to separate, and only the salt phase was used for the drops.

The crystals grew in hanging drops of 2 µl after 12 months of incubation at 28 C.

The crystals were washed in the salt phase of the reservoir solution prior to cryofreezing.

The crystal structure was determined to 2.2 Å resolution using MAD phasing on crystals containing the double-bromine derivative substrate.
A General Module for RNA Crystallization
Ferré-D'Amaré JMB (1998) 279: 621-631

Crystallization of RNA molecules remains a challenging step in structure determination by X-ray crystallography.

A well-folded RNA molecule would typically present a largely undifferentiated molecular surface dominated by the phosphate backbone. During crystal nucleation and growth, this might result in neighboring molecules packing subtly out of register, leading to premature crystal growth cessation and disorder.

To overcome this problem, we have developed a crystallization module consisting of a normally intramolecular RNA-RNA interaction that is recruited to make an intermolecular crystal contact.

The target RNA molecule is engineered to contain this module at sites that do not affect biochemical activity. The presence of the crystallization module appears to drive crystal growth, in the course of which other, non-designed contacts are made.

The GAAA tetraloop/tetraloop receptor interaction was used successfully to crystallize numerous group II intron domain 5-domain 6, and hepatitis delta virus (HDV) ribozyme RNA constructs.

The use of the module allows facile growth of large crystals, making it practical to screen a large number of crystal forms for favorable diffraction properties.
Figure 5. Engineering the hepatitis delta virus ribozyme for facile crystallization with the tetraloop receptor/ tetraloop crystallization module.

(a) Sequence and hypothetical secondary structure of a genomic-strand delta ribozyme (after Tanner et al., 1994). Following selfcleavage, the 50 end of the molecule is at the G residue marked with an arrow. Paired region 4 (P4) can be drastically shortened, or its sequence modified (preserving base-pairing) without affecting ribozyme activity (Been et al., 1992; Tanner et al., 1994).

(b) Sequence and secondary structure of a delta ribozyme engineered for crystallization. P4 has been altered to consist of a tetraloop receptor (TR), and is capped with a GAAA tetraloop. The broken horizontal lines dividing the molecule into three segments are illustrative only; the molecule is a covalently continuous strand of RNA. This is the delta/ TR construct that yielded the crystals.
Preparation of the *T. maritima* RNase P holoenzyme–tRNA$^{\text{Phe}}$ ternary complex.

RNA transcriptions were performed in vitro using purified His6-tagged T7 RNA polymerase using standard protocols.

Sequences from the *T. maritima* RNase P RNA and tRNA$^{\text{Phe}}$ genes were inserted into a pUC19 vector at FokI and BsmAI restriction sites, respectively, allowing for run-off transcription of the DNA plasmid after digestion with the appropriate restriction enzyme (NEB).

RNA samples were purified by 6% denaturing polyacrylamide gel electrophoresis (PAGE), identified by ultraviolet absorbance, recovered by diffusion into 50 mM potassium acetate (pH 7) and 0.2 M potassium chloride, and precipitated with ethanol.

tRNA was further purified by anion exchange (MonoQ (5/50 GL)) and gel filtration (HiPrep 26/60, Sephacryl S-200) chromatography (GE Health Sciences).

Overexpression and purification of the RNase P protein from *T. maritima* was performed as described previously.

To form the RNase P holoenzyme–tRNA complex, unfolded P RNA, unfolded tRNA and P protein molecules were mixed at a 1:1:1:1 molar ratio in 66 mM HEPES, 33 mM Tris (pH 7.4), 0.1 mM EDTA (1x THE) and 100 mM CH$_3$COONH$_4$ (Ref. 8). The ternary mix, at a final concentration of 45 mM, was incubated at 94 °C for 2 min and then cooled to 4 °C over 2 min. After addition of MgCl$_2$ to a final 10 mM concentration, the reaction mixture was incubated at 50 °C for 10 min, followed by incubation at 37 °C for 40 min, and finally cooled to 4 °C over 30 s.
Rational design of an RNA tertiary module to build a crystal lattice.
To promote formation of a crystal lattice, intermolecular interactions were facilitated by introducing a tertiary structure interaction module. Based on previous studies, constructs were designed where a tetraloop was inserted into the P12 loop (L12) of the P RNA and a tetraloop-receptor into the anticodon stem of tRNA (Supplementary Fig. 1). These two RNA regions were chosen as they were deemed to be far from the active site or other regions involved in specific interactions. In addition, the P12 stem of P RNA has a highly variable helix length across all organisms, lacks sequence conservation, and is non-essential or absent in several organisms. The P12 and the anticodon loop of tRNA are not known to form any functional contacts.

The length of the anticodon and the P12 stems were systematically varied by single base pair insertions adjacent to the tetraloop and tetraloop receptor module, thus altering the position (~2.7 Å per base pair added) and orientation (~36 degrees per base pair added) of the tetraloop receptor and the tetraloop.

Forty two combinations of molecules were screened for crystallization conditions using a sparse matrix approach employing a set of crystallization conditions developed locally.

A few combinations of RNA molecules produced crystals, with most of them diffracting poorly. The best crystals were obtained from a construct where the P12 and anti-codon stems were elongated by five and three base pairs respectively.

Insertion of two G-U wobble pairs adjacent to the tetraloop-tetraloop receptor module further improved diffraction, and also created a binding site for an iridium hexamine cation.
**Supp Fig 1c, Regions of the P RNA sequence (top) at the P12 helix (P12) are replaced with the tetraloop** (bold grey) and have increasing base pairs.

The anticodon loop (ACL) of tRNA (bottom) was also changed to enable tetraloop recognition (bold grey) with P RNA and contains an altered tetraloop (bold black) to replace the natural GAAA sequence present in this tRNAPhe and that could serve as a GNRA tetraloop.

Forty-two combinations were tested. Dotted boxes are paired combinations which yielded crystals (black and red), and an asterisk denotes the paired combination which gave diffraction to 3.8 Å.
Structure of a bacterial Ribonuclease P holoenzyme in complex with tRNA
Reiter Nature 468: 784 (2010)

Supp Fig 1a, Sequence and secondary structure of P RNA showing major intramolecular interactions observed in the crystal of the RNase P holoenzyme/tRNA complex.

A thick, dashed line between residues 130 and 136 distinguishes the *T. maritima* P RNA sequence from the engineered tetraloop (T)-tetraloop receptor (TR) RNA module (light grey).
Figure S3 | Lattice contacts in the crystals of the *T. maritima* RNase P holoenzyme and tRNA.

**a,** Engineered crystal contacts between the P12 stem of P RNA and the anticodon loop of tRNA. A tetraloop-tetraloop receptor was introduced to favor crystal lattice formation. The tetraloop was placed in the loop capping the P12 stem and the tetraloop receptor was placed in the anticodon stem of tRNA.

In addition to the tetraloop/tetraloop receptor interactions, the loop capping the anticodon stem interacts with one nucleotide of the L15 loop in a neighboring molecule. The P12 stem is shown in blue with the engineered tetraloop receptor in light blue, the anticodon stem is shown in green with the tetraloop receptor in light green, the neighboring L15 loop is shown in red.
Section II: Crystallization of protein-RNA complexes

- Extensive crystallization screens required
- Comprised of at least two components
- N variants of each component
- $N^2$ complexes to be screened
- Combinatorial crystallization
Lessons from DNA-protein complexes for RNA-protein complexes


Similar approach in other protein-DNA complexes, such as: Aggarwal *et al.*, *Science* 242: 899 (1988) & Schultz JMB 213:159 (1990)
Common Ways to Improve Crystallizibility and Crystals

• Vary length and sequence of double helices
• Introduce overhangs at the ends
• Introduce opportunities for RNA-RNA contacts
• Introduce in RNA binding loop for RNA-binding protein
• Vary the surface of the RNA-binding protein by (multiple) point mutations
Crystallisation of U1A spliceosomal protein bound to a RNA hairpin, its natural binding site on U1snRNA.

RNA oligonucleotides synthesised either chemically or by *in vitro* transcription using T7 RNA polymerase and purified to homogeneity by gel electrophoresis.

Crystallisation trials with the wild-type protein sequence and RNA hairpins containing various stem sequences and overhanging nucleotides only resulted in a cubic crystal form which diffracted to 7-8 Å resolution.

A new crystal form was grown by using a protein variant containing mutations of two surface residues.

The N-terminal sequence of the protein was also varied to reduce heterogeneity which was detected by protein mass spectrometry.

**A further crystallisation search using the double mutant protein and varying the RNA hairpins resulted in crystals diffracting to beyond 1.7 Å.**
Crystallisation of RNA-Protein Complexes II.
The Application of Protein Engineering for Crystallisation of the U1A Protein-RNA Complex

Figure 1. The sequences of RNAs used in the crystallisation search. All molecules have 5' and 3' hydroxyls.

Bases separated with a hyphen are assumed to be base-paired. The "loop" pictogram represents the boxed sequence of 21S1.

Naming reflects the length of sequence, e.g. 21S2 is a 21-nucleotide RNA. 23S1, 23SR and 25S1, 25SR were designed to have complementary three-nucleotide overhangs.

As indicated, deoxyribonucleotides were incorporated in 21S9.
Crystallisation of RNA-Protein Complexes II.
The Application of Protein Engineering for Crystallisation of the U1A Protein-RNA Complex

U1A Protein–RNA Complex Crystallisation

<table>
<thead>
<tr>
<th>Protein construct</th>
<th>Determined mass (daltons)</th>
<th>Consistent sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1-102</td>
<td>Major component (11593)</td>
<td>Ala (2)...Val (102)</td>
</tr>
<tr>
<td></td>
<td>Minor component (11424)</td>
<td>Pro (4)...Val (102)</td>
</tr>
<tr>
<td>A2-98(Y31H,Q36R)</td>
<td>Major component (11253)</td>
<td>Met-Val (3)...Lys (98)</td>
</tr>
<tr>
<td></td>
<td>Minor component (11023)</td>
<td>Pro (4)...Lys (98)</td>
</tr>
<tr>
<td>A4-98(Y31H,Q36R)</td>
<td>Major component (11056)</td>
<td>Met-Glu (5)...Lys (98)</td>
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<tr>
<td>A2-98(V3A,Y31H,Q36R)</td>
<td>Major component (11023)</td>
<td>Pro (4)...Lys (98)</td>
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<td></td>
<td>Minor-1 (11094)</td>
<td>Ala (3)...Lys (98)</td>
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<td></td>
<td>Minor-2 (11226)</td>
<td>Met-Ala (3)...Lys (98)</td>
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<td>A2-98(V3Q,Y31H,Q36R)</td>
<td>Major component (11282)</td>
<td>Met-Gln (3)...Lys (98)</td>
</tr>
</tbody>
</table>

* Refer to Figure 2 for sequences of protein constructs.
* Error in mass is up to ±3 daltons.
* Residue numbers refer to those in Figure 2 rather than those in the protein construct.
Crystallisation of RNA-Protein Complexes II.

The Application of Protein Engineering for Crystallisation of the U1A Protein-RNA Complex


| Hexagonal I       | $P6_{3}22$   | $a = b = 101$ Å  
|                  |             | $c = 435$ Å     | 21S5   | 'Double' mutations  
|                  |             |                |        | A1-98(Y31H, Q36R)  
| Hexagonal II      | $P6_{3}22$   | $a = b = 111$ Å,  
|                  |             | $c = 135$ Å     | 21S4   | A2-98(V3Q,Y31H, Q36R)  
| Hexagonal III     | $P6_{3}22$   | $a = b = 97$ Å,  
|                  |             | $c = 255.3$ Å   | 21S13  | 'Double' mutations  
|                    |             |                |        | A1-98(Y31H, Q36R)  

Crystal diffraction data obtained from crystals of sufficient quality for determining unit cell parameters and space groups are listed. The data represent clearly distinct crystal forms which were grown from different RNA-protein combinations (except for the cubic form which was grown from a set of RNA-protein combinations). The space group of crystal form Hexagonal I was provisionally assigned as $P6_{3}22$ but is certainly of point group 622. RNA and protein used are as indicated in Figure 1 and Figure 2.
Protein L30 and RNA
### L30-RNA structure determination in: “Combinatorial Crystallization of an RNA-Protein Complex”

<table>
<thead>
<tr>
<th>Top strands</th>
<th>Name</th>
<th>MW (Da)</th>
<th>Extinction coefficients (260 nm) (mM⁻¹ cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GACCGGGAGUGUC</td>
<td>T-1</td>
<td>3922</td>
<td>131.8</td>
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<tr>
<td>CGACCGGGAGUGUC</td>
<td>T-2</td>
<td>4228</td>
<td>139.2</td>
</tr>
<tr>
<td>GACCGGGAGUGUCC</td>
<td>T-3</td>
<td>4228</td>
<td>139.2</td>
</tr>
<tr>
<td>CGACCGGGAGUGUCC</td>
<td>T-4</td>
<td>4534</td>
<td>146.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bottom strands</th>
<th>Name</th>
<th>MW (Da)</th>
<th>Extinction coefficients (260 nm) (mM⁻¹ cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GACGCAGAGAUGGUC</td>
<td>B-1</td>
<td>4928</td>
<td>174.4</td>
</tr>
<tr>
<td>GGACGCAGAGAUGGUC</td>
<td>B-2</td>
<td>5274</td>
<td>186.2</td>
</tr>
<tr>
<td>GACGCAGAGAUGGUCG</td>
<td>B-3</td>
<td>5274</td>
<td>186.2</td>
</tr>
<tr>
<td>GGACGCAGAGAUGGUCG</td>
<td>B-4</td>
<td>5620</td>
<td>198.0</td>
</tr>
</tbody>
</table>

Oligo’s synthesized
Scheme for combinatorial screening of paired oligonucleotides and crystallization strategy for the MBP-L30-RNA complex.

Eight sequences of RNA oligonucleotides were mixed to form 16 unique duplexes containing the secondary structure of the internal loop recognized by L30.

Schematic representation of the positions of overhanging residues and blunt ends in the 16 unique duplexes formed by pairing oligonucleotides T-1 to T-4 with B-1 to B-4. Shaded areas represent variable positions that form complementary base pairs in eight instances.
Protein used quite special:
Maltose-binding protein fused to L30 (MBP-L30)

L30-RNA structure determination in : “Combinatorial Crystallization of an RNA-Protein Complex”

<table>
<thead>
<tr>
<th>Crystallization Screening</th>
<th>Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 screening solution used:</td>
<td>1 = clear</td>
</tr>
<tr>
<td>Hampton Natrix Screen No 2. 1-24</td>
<td>2 = trace precipitate</td>
</tr>
<tr>
<td>Hampton Crystal Screen 2 Nos. 1-24</td>
<td>3 = oil drops</td>
</tr>
<tr>
<td></td>
<td>4 = light precipitate</td>
</tr>
<tr>
<td></td>
<td>5 = precipitate</td>
</tr>
<tr>
<td></td>
<td>6 = spherulites</td>
</tr>
<tr>
<td></td>
<td>7 = microcrystals</td>
</tr>
<tr>
<td></td>
<td>8 = clusters of crystals or many small crystals</td>
</tr>
<tr>
<td></td>
<td>9 = single crystals</td>
</tr>
</tbody>
</table>
Each histogram in the matrix corresponds to a particular MBPL30 - RNA complex comprised of a 'top' (T-1 to T-4) and a 'bottom' (B-1 to B-4) oligonucleotide.

Scores are along the horizontal axis.

Scores of 8 and 9 are real good.
Some Statistics

- 30 drops (1.32%) out of 2304 drops yielded single crystals with score ‘9’

- For 65 drops scoring ‘8’ or ‘9’, HSC2 yielded 11 drops and Natrix yielded 54 drops.

- For HCS2, 7 conditions yielded crystals, Nos. 7 and 13 yielded crystals in 7 drops.

- For Natrix, 13 conditions yielded crystals, Nos. 12, 13 and 19 yielded crystals in 33 drops.
More Statistics

- RNA with 2 blunt ends yielded on average 12 drops scoring 6 or better.

- RNA with two overhangs yielded on average 6 drops scoring 6 or better.

- Conclusion: blunt ends are favored in lattice formation.

- ‘T-3/B-2’ selected for additional screening

Diffraction Screening

Cryoprotectants prepared from reservoir solutions by addition of suitable cryosolvents
<table>
<thead>
<tr>
<th>MBP-L30 complex</th>
<th>Screen</th>
<th>Condition No.</th>
<th>Drop ratio^ (µl)</th>
<th>Crystal size§ (µm)</th>
<th>Diffraction limit## (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1/B-3</td>
<td>Natrix</td>
<td>6</td>
<td>1:0.5</td>
<td>200</td>
<td>3.8</td>
</tr>
<tr>
<td>T-3/B-2</td>
<td>Natrix</td>
<td>11</td>
<td>1:1</td>
<td>500</td>
<td>3.9</td>
</tr>
<tr>
<td>T-3/B-2</td>
<td>Natrix</td>
<td>23</td>
<td>0.5:0.5</td>
<td>250</td>
<td>3.9</td>
</tr>
<tr>
<td>T-3/B-2</td>
<td>HCS2</td>
<td>1</td>
<td>0.5:0.5</td>
<td>300</td>
<td>6.4</td>
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<tr>
<td>T-3/B-1</td>
<td>Natrix</td>
<td>13</td>
<td>1:0.5</td>
<td>500</td>
<td>7</td>
</tr>
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<td>Natrix</td>
<td>13</td>
<td>1:1</td>
<td>900</td>
<td>7</td>
</tr>
<tr>
<td>T-1/B-3</td>
<td>Natrix</td>
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<td>200</td>
<td>7</td>
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<tr>
<td>T-4/B-2</td>
<td>Natrix</td>
<td>18</td>
<td>0.5:0.5</td>
<td>165</td>
<td>7.5</td>
</tr>
<tr>
<td>T-2/B-3</td>
<td>Natrix</td>
<td>13</td>
<td>0.5:0.5</td>
<td>165</td>
<td>7.5</td>
</tr>
<tr>
<td>T-2/B-1</td>
<td>Natrix</td>
<td>19</td>
<td>0.5:0.5</td>
<td>300</td>
<td>9.6</td>
</tr>
<tr>
<td>T-3/B-1</td>
<td>Natrix</td>
<td>12</td>
<td>1:0.5</td>
<td>370</td>
<td>10</td>
</tr>
<tr>
<td>T-4/B-1</td>
<td>Natrix</td>
<td>19</td>
<td>0.5:1</td>
<td>435</td>
<td>11</td>
</tr>
<tr>
<td>T-4/B-1</td>
<td>Natrix</td>
<td>19</td>
<td>0.5:0.5</td>
<td>Aggregate</td>
<td>None</td>
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<tr>
<td>T-2/B-3</td>
<td>Natrix</td>
<td>12</td>
<td>0.5:1</td>
<td>120</td>
<td>None</td>
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<tr>
<td>T-3/B-1</td>
<td>Natrix</td>
<td>6</td>
<td>1:0.5</td>
<td>60</td>
<td>None</td>
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<tr>
<td>T-3/B-1</td>
<td>Natrix</td>
<td>19</td>
<td>0.5:0.5</td>
<td>75</td>
<td>None</td>
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<tr>
<td>T-1/B-3</td>
<td>HCS2</td>
<td>14</td>
<td>0.5:1</td>
<td>Small</td>
<td>None</td>
</tr>
</tbody>
</table>

#HCS2, Hampton Crystal Screen 2; Natrix, Hampton Natrix Screen.
^The volume of reservoir:sample solutions in the hanging drops.
§Values refer to the maximum dimension.
##Maximum resolution of reflections observed in test exposures of \( \leq 10 \) min and \( \leq 1^\circ \) oscillation.
T-3/B-2/MBPL30 complex crystal from initial screen.

Data collected in-house to 3.9 Å resolution

L30-RNA structure determination in: “Combinatorial Crystallization of an RNA-Protein Complex”
T-3/B-2/MBPL30 Complex Crystal

• Optimal condition contains:
  – 1 M Li$_2$(SO$_4$)
  – 0.05 M Sodium cacodylate pH 6.0
  – 0.01 M MgCl$_2$
  – 0.5% Jeffamine (addition to Natrix # 11)

• Cryoprotectant consisting of 25% glycerol mixed with reservoir solution

• Molecular Replacement with MBP

• Structure refined to 3.28 Å and shown to contain RNA, one of the helices stacks on a symmetry-related copy of itself
Section III: Use a protein to crystallize an RNA
Crystallization and Structure Determination of a Hepatitis Delta Virus Ribozyme: Use of the RNA-Binding Protein U1A as a Crystallization Module
Ferré-D'Amaré and Doudna JMB 295:541-556 (2000)

**Crystallization strategy**

(i) The RNA-binding domain of the protein U1A (U1A-RBD) has a compact globular domain.

(ii) The U1A-RBD binds very tightly to its cognate RNA : $K_d \sim 10^{-11}$ M

(iii) The U1A-RBD – RNA interface is both hydrophylic and hydrophobic, so is expected to be strong in both high and low salt conditions.

(iv) The U1A-RBD binds RNA in two different presentations. These two modes can be used to engineer RNA variants with U1A binding sites in different contexts.

(v) The Nagai group has prepared U1A-RBD variants with changes in solvent-exposed residues (Oubridge JMB 1995) and hence different crystallization properties
Critically important:

Previous studies had shown that the P4-L4 stem-loop of the ribozyme could be freely altered or truncated without affecting adversely the in vitro activity of the RNA.

Fig 1(b) (left) shows the variant RNA molecules made. The introduced nucleotides were expected to form a segment with an A-form double helix rigidly connected to the rest of the ribozyme.
Use what you can get, even if not perfect....

The phage polymerase used for the \textit{in vitro} transcriptions adds a variable number of random nucleotides at the 3’-end of the transcript.

The idea was that constructs incorporating HDV or VS ribozymes would produce homogeneous 3’-termini. This did not happen, however, possibly due to the extraordinary stability of the HDV ribozyme moiety.

\textbf{Therefore:}
\textbf{The RNA used for crystallization had all a well-defined 5’-OH but heterogeneous 3’-termini}

\textbf{SeMet protein} did not give any crystals under wt protein crystallization conditions – but a new screen gave a BETTER crystal form with higher resolution....
Crystal structure of a hepatitis delta virus ribozyme

**Structure of HDV ribozyme plus U1A-RBD**

**b.** Back view of the molecule, tilted to emphasize the snug fit of the J4/2 region between the two helical stacks, and the intricate pseudoknot crossovers.

**c.** Top view of the structure, showing that the two helical stacks are nearly parallel to each other.


Crystal structure of the Ffh andEF-G binding sites in the conserved domain IV of Escherichia coli 4.5S RNA. Jovine Structure 8, 527-540 (2000)


Websites
Crystallization of RNA-Protein Complexes

Kiyoshi Nagai’s website:
http://www2.mrc-lmb.cam.ac.uk/personal/kn/NewFiles/crystal.html
Contains a wealth of crystallization conditions
Up to about 2003….

This is now the spliceosome group page:
http://www2.mrc-lmb.cam.ac.uk/personal/kn/Nagai_Group/Welcome.html