

# New Tools for Nucleic Acid X-ray Structure Determination

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## Introduction

The growing number of biologically important nucleic acid sequences (DNA and RNA) demands a fast and reliable method for their *de novo* 3D structure determination.

To overcome the limitations of the current nucleic acid phasing techniques, we propose a new strategy based on the incorporation of **Selenium** in the sequence (Part I) and advocate the use of the **Phosphorus** anomalous signal for phasing (Part II).

Indeed, current nucleic acids phasing techniques have limitations:

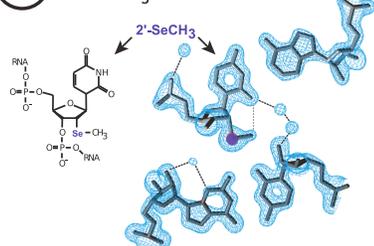
- **Direct methods** → need atomic resolution
- **Molecular replacement (MR)** → need a model
- **Halogenation** of pyrimidines → high radiation damage sensitivity of the anomalous sites
- Crystals soaking in **heavy atom** salt solutions → problem of non-isomorphism

## Part I

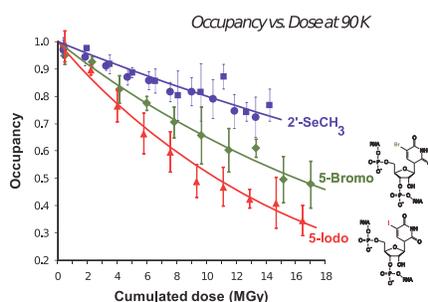
On the use of Selenium for phasing

In the early 1990s, 2'-SeCH<sub>3</sub>-modification (see A) was introduced as a new approach to facilitate crystal structure determination of nucleic acid. However, due to a cumbersome and expensive synthesis, only few structures have been determined and halogen-modification was till now favored (Bromo- or Iodo-modification, although extremely sensitive to radiation damage, see B). Olieric *et al.* (see ref.) described a fast and inexpensive strategy for crystallization and structure determination of nucleic acids and nucleic acid/protein complexes by exploiting the similar crystallization properties of 2'-SeCH<sub>3</sub>- and 2'-OCH<sub>3</sub>-modification (see C).

### A Chemical structure and electron density of 2'-SeCH<sub>3</sub>-uridine-modified RNA



### B Site-specific radiation damage studies of modified RNA



→ 2'-SeCH<sub>3</sub>-modification has an increased resistance to X-ray radiolysis in comparison with commonly used halogen-modification

### C Schematic representation of the strategy

- Initial crystallization screening  
Determination of the best construct suitable for diffraction experiments using unmodified RNA
- Identification of 2'-OH position(s) suitable for Se-labeling  
Inexpensive crystallization and diffraction screening with 2'-OCH<sub>3</sub>-modified RNA
- 2'-SeCH<sub>3</sub> RNA labeling  
Synthesis and crystallization of one Se-labeled RNA sequence only
- MAD or SAD data collection at the Se K-edge for phasing

## Part II

On the use of Phosphorus for phasing

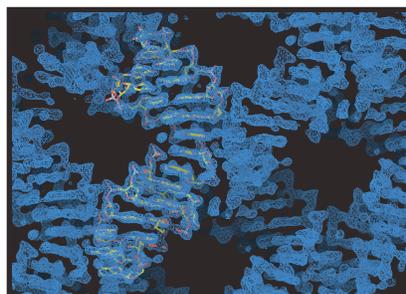
Phosphorus-Single-wavelength Anomalous Diffraction (P-SAD) method is not yet commonly used among crystallographers, owing to the weak anomalous signal of Phosphorus atoms and to the difficulty of locating the large number of P sites for successful phasing. Nevertheless, Dauter *et al.* (see ref.) first showed in 2001 that this method can be successfully applied to solve a Z-DNA hexamer duplex (10 P sites, see A). Here, we show that the phasing of medium-size RNA molecules (26 P sites in this case) by P-SAD is also possible if great care is taken during data collection (see A and B). Data were collected at beamline X06DA at the Swiss Light Source.

### A Diffraction data statistics

Molecule	Z-DNA (10 P-sites)	SRL RNA (26 P-sites)
Bearline	XBC (INLS)	X06DA (SLS PSI)
Wavelength	1.54 Å	1.6 Å
Space group	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	P4 <sub>3</sub>
Unit cell	17.81 x 31.42 x 44	29.40 x 29.40 x 75.52 Å
Resolution	1.5 Å (1.55 - 1.5 Å)	1.9 Å (2 - 1.9 Å)
Oscillation range	360°	720°
Completeness	99.9% (100%)	99.6% (98.6%)
Redundancy	18.9	28.1 (26.2)
I/σ	66.9 (23.1)	76.2 (56.2)
R <sub>meas</sub>	0.027 (0.06)	0.041 (0.058)

Values in parentheses refer to the highest resolution shell.

### B SRL RNA (26 P sites) experimental solvent-flattened electron-density map (AutoSharp)



→ P-SAD can be used successfully with good data and if care is taken during data collection (mainly for redundancy and radiation damage).

## Conclusion

- 2'-SeCH<sub>3</sub>-modified nucleosides are strongly resistant to site-specific radiation damage and are therefore more suitable for experimental phasing of nucleic acids.
- A fast and inexpensive selenium derivatization strategy for both crystallization and phasing of nucleic acid structures has been described.
- Use of the weak anomalous signal of Phosphorus can be successfully applied to solve small to intermediate nucleic acid structures by P-SAD.
- It is foreseen that structure resolution of larger nucleic acid structures would be easier if MR (with a partial model) and P-SAD are combined.

## References

Vincent Olieric, Ulrike Rieder, Kathrin Lang, Alexander Serganov, Clemens Schulze-Briese, Ronald Micura Philippe Dumas and Eric Ennifar. (2009) A fast selenium derivatization strategy for crystallization and phasing of nucleic acid structures. RNA. Apr 15(4):707-15. Faculty of 1000 recommended article

Zbigniew Dauter and Dorota A. Adamiak (2001) Anomalous signal of phosphorus used for phasing DNA oligomer: importance of data redundancy Acta Cryst. D57, 990-995