PS-04.02.12 IMPROVED METHOD FOR RNA SECONDARY STRUCTURE PREDICTION. by Xuemei Yuan*, Yu Luo, Luhua Lai, Xiaojie Xu, Department of Chemistry, Peking University, Beijing 100871 , CHINA

The database of RNA sequences is now rapidly expanding. However, only the structural information of a few tRNA molecules is known in detail. RNA's main biological functions are determined largely by its tertiary structure. To predict RNA's structure through its sequence information is reasonable and important.
The first step to modeling an RNA molecule is to predict its secondary structure. Many programs have been developed, nevertheless, the problem of RNA secondary structure prediction has not been solved. The main difficulty is that the algorithms may not reflect the real folding process of an RNA molecule and the energy parameters need to be optimized before satisfied results obtained. Our program simulates a stepwise folding process, and the free energy parameters are optimized for each kind of loops. Five possible pseudoknot structures are permitted to occur if they were reasonable.This program has been tested on a number of RNA sequences, and it gives some better results than the published programs.

PS-04.02.13 STRUCTURE OF HIGHLY CONCENTRATED PHASES OF DNA BY X-RAY DIFFRACTION D.Durand(1), J.Doucet*(1), F.Livolant(2),(1)L.U.R.E., Université Paris-Sud, 91405 Orsay Cedex, France; (2)Centre de Biologie Cellulaire, 67 rue Maurice Günsbourg, 94205 Ivry-sur-Seine Cedex, France

In aqueous solution, pure DNA forms multiple liquid crystalline and crystalline phases whose nature depends on the polymer concentration. The following phase sequence is observed when the DNA concentration increases: isotropic —> cholesteric —> columnar hexagonal —> crystalline phases. The aim of this work is to obtain structural information about the highly concentrated phases formed by 500Å long DNA molecules – in particular about the crystalline phases – by means of X-ray diffraction. We show that in the two-dimensional (2D) ordered hexagonal phase a longitudinal order *progressively* appears between neighbouring DNA helices leading continuously to a three-dimensional (3D) ordered hexagonal phase (D.Durand, J.Doucet, F.Livolant, J. Phys. II France 1992, 2, 1769-1783). For higher concentrations the specimens undergo a discontinuous transition towards an orthorhombic phase. The characteristic structural parameters of these different phases have been determined. The most important result is that the number of nucleotides per helix turn decreases *continuously*, when the DNA concentration increases, from 10.3±0.1 at the cholesteric —> hexagonal transition down to 9.±0.1 without any apparent change of the B conformation of the molecules.

PS-04.02.14 ORTHORHOMBIC AND TETRAGONAL STRUCTURES OF THE OCTAMER d(GTACGTAC) By M. Hospital*, B. Langlois d'Estaintot, A. Dautant, C. Courseille, G. Comberton and G. Précigoux, Laboratoire de Cristallographie, Université de Bordeaux I, 33405 Talence, France.

The synthetic self-complementary deoxyoctanucleotide d(GTACGTAC) crystallized as an A type DNA double helix in both space group $P4_32_12$ and $P2_12_12$. The tetragonal structure was refined at 2.4 Å with an R factor of 17%, the orthorhombic one was refined at 2.2 Å with an R factor of 16%. The tetragonal structure is similar to the other octanucleotides crystallizing in the same $P4_32_12$ space group, and displays one single strand and 56 water molecules in the asymmetric unit. The orthorhombic structure presents a double strand solvated by 66 water molecules in the asymmetric unit. It is the first time that such a crystal form has been observed for long oligonucleotides. The molecule adopts a bend with a value 30° sharper in the orthorhombic structure than in the tetragonal one and an unusual packing between symmetry-related molecules generating a pseudo-hexadecaoligonucleotide.

As we observed here the same sequence of DNA crystallized in two different space groups, with local distorsions and a more significant bend for the molecule crystallizing in the orthorhombic space group, we cannot rule out that the overall conformation is totally independent of crystal packing forces.

In a crystal, the packing forces are strong enough to induce local constraints on the DNA, but an octanucleotide is obviously too short of a sequence and does not constitute a full turn of DNA. Therefore it is impossible to guess how a longer piece of DNA, displaying similar structural parameters, would look like and would pack within the crystal. The present study is a contribution to the analyses emphasizing the very high degree of flexibility of DNA molecules, whether this flexibility is accentuated by base sequence and/or by crystal packing forces.

04.03 – Open Commission Meeting on Small Molecules – New Viewpoints in Structure Analysis

OCM-04.03.01 **NEW *IN SITU* CRYSTALLIZATION TECHNIQUES WITH IR LASER.** By R. Boese* and M. Nussbaumer, Institut für Anorganische Chemie, University of Essen, Universitätstr. 5-7, 4300 Essen 1, Germany.

Single crystal structure investigations of low melting compounds calls for special techniques. Crystallization must be performed *in situ*, e.g. directly in a capillary mounted on a diffractometer which is equipped with a low temperature device. Simple cooling of liquid samples in capillaries could produce single crystals or polycrystalline materials. The most appropriate procedure for growing single crystals is by controlled local heating, producing a fine molten zone. By focusing a computer controlled (position and intensity) IR laser beam to the capillary, the monitoring of the crystallization process is possible and single crystals can be produced easily. The high intensity of the laser allows purifying of the sample by performing a miniature zone melting procedure in the capillary. Sublimation, skipping of intermediate solid phases or even chemical reactions are possible in the capillaries. A complete device (see Figure 1), including a video monitoring of the process has been developed and will be presented.

04-Crystallography of Biological Small Molecules

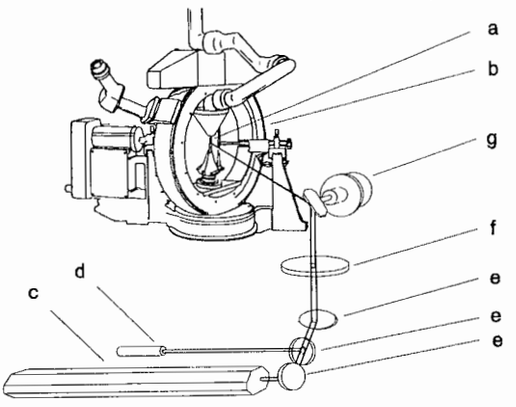

Fig. 1 *in situ*-crystallization device with IR laser: a) capillary b) Eulean cradle c) IR laser d) pilot (visible) laser e) mirror f) lens g) turnable (controlled) mirror

OCM-04.03.02 SINGLE CRYSTAL DATA WITH SYNCHROTRON RADIATION - WHAT CAN IT OFFER ?
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Synchrotron radiation has very high intensity, a continuous range of wavelengths, and very low beam divergence. These can be used with advantage for the study of microcrystals, for time resolved studies, and for a variety of other purposes.
For microcrystals, the practical problems to be faced include the quality of the small crystals (often poor), crystal mounting, radiation damage, and then distinguishing the diffraction peaks from background. The smallest crystal for which we have successfully recorded data with monochromatic synchrotron radiation and determined the structure was 10x10x30 μm, containing a gold cluster of previously unknown constitution, and shown to be $Au_{10}(PPh_3)_7(S_2C_2(CN)_2)_2$ (Cheetham, Harding, Haggitt, and Mingos, 1993, J. Chem. Soc. Chem. Commun. in the press). The structure of an aluminophosphate, $Al_3P_3O_{12}F.C_4H_{10}NO$, was similarly determined from a crystal of dimensions 35x20x15 μm, and in this case comparison could be made with the results of powder diffraction (Harding, Kariuki, McCusker and Simmen, 1993, in preparation for Zeolites).
White beam methods (Laue) now allow unit cell determination (Dodd, Carr and Harding,1993, J. Appl. Cryst.25, in the press) as well as intensity measurement (Helliwell et al, 1989, J. Appl. Cryst. B22, 483-497). Exposure times can be less than 0.1 s for normal sized crystals, and 1-5 min for very small ones; to record all the unique data, 1-10 film packs or image plates, may be required, according to the crystal symmetry.
The constitution and structure of a new organometallic complex, $AuOs_3(CO)_8PPh_3dppm.PF_6$, were determined from six Laue film packs - one for the unit cell and five for the intensitiy measurements - and structure refinement gave R=0.075 for 7163 unique reflections. In crystalline $P_4N_4Cl_8$ the molecule changes from a boat to a chair conformation at ca 65°C, and the crystal symmetry changes. In a preliminary time-resolved study film packs were exposed at 3 min intervals as single crystals were heated (Carr, Cheetham, Harding and Rule, 1992,Phase Transitions, 39, 33-43); from each film pack 200-300 reflections have been measured and used to follow the course of the change.

OCM-04.03.03 THE BACKGROUND: A NON-EVENT IN SINGLE CRYSTAL DIFFRACTOMETRY
by A.T.H.Lenstra*, S.maes & C.Van Hulle, Dept. of Chemistry, Antwerp University (UIA), Universiteitsplein 1, 2610 Wilrijk, Belgium

A net intensity I is routinely obtained by subtracting the local background B(hkl) from the raw intensity R(hkl):

$$I = R - B \qquad (1)$$

Since the observations B and R are taken as independent, the corresponding variance is:

$$\sigma^2(I) = \sigma^2(R) + \sigma^2(B) \qquad (2)$$

This classical interpretation is correct when one deals with a single observation. However, it is a very poor way to deal with the data in a single crystal analysis. Here one deal with a large set of observations, which in a hidden way includes valuable experience, notably on the background.
Let us only summarize the essential features of the observed background intensities. Within a not too large $\sin\theta/\lambda$ –interval all observed B(hkl) values can be contracted into a normal distribution $N(\langle B\rangle, s^2(B))$,where $\langle B\rangle$ is the averaged background and $s^2(B)$ is the observed spread. In a standard data set one finds $\langle B\rangle s^2(B)$, which connects B(hkl)'s to a counting statistical distribution.
The calculated background averages in different $\sin\theta/\lambda$ – intervals appear to be interrelated by:

$$b = B(ref) \times C \qquad (3)$$

where C is theta dependent correction factor and B(ref) is a reference value characteristic for the whole data set. The correction C includes three component parts, viz:
i) $C1=f^2(O)+(Z-f^2(O)/Z)$ where f(O) is the scattering factor for oxygen at the diffractometer angle θ_o·$F^2(O)$ and $(Z-f^2/Z)$ are the elastic Raileigh and the inelastic Compton scattering produced by the crystal and its emorphous support.
ii) $C2=\cos^2 2\theta_m + \cos^2 2\theta_o$, which is the polarisation of C1.
iii) $C3=(p+qx\mathrm{tg}\theta)(r+sx\mathrm{tg}\theta)$, which connects b to the scan angle and the aperture applied during the data collection.
With C=C1xC2xC3 it is evident that B(ref) is easily calculated using all available background counts in the data set. This suffices to show that the value of B(ref) is virtually error free. At the reciprocal lattice point (hkl) we observed a raw intensity R. Its variance is equal to R. Using (3) the local background b is given by CxB(ref). Now b is an nearly error free estimate of the Bragg intensity of the crystal. An actual observation would reveal a counting statistical variance b.
When we now combine R and b as observation related to (hkl), we get

$$I = R - b \quad \text{and} \quad \sigma^2(I) = R - b \qquad (4)$$

So the background model reduces the variance of I from I+2B to I. This lowers the detection limit of your diffractometer by a factor 10 without any increase in the applied measuring time. One could even save time by skipping almost all background observations without loosing accuracyl.

OCM-04.03.04 WHEN AUTOMATIC STRUCTURE SOLUTION FAILS.
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