MS40- Crystallization for small and large molecules

Chairs: Prof. Bernhard Spingler, Prof. Terese Bergfors

MS40-P01

The co-crystallization of the DNA sequences – 5'-CGTGAATTCACG-3'(S1) and 5'-CGCGAATTCGCG-3'(S2) with fluorescent markers and ligands

Hristina Sbirkova-Dimitrova[1], Rosica Nikolova[1], Louiza Dimowa[1], Rusi Rusev[1], Boris Shivachev[1]

1. Institute of Mineralogy and Crystallography "Acad. Ivan Kostov", Bulgarian Academy of Sciences, Acad. G. Bonchev str., bl. 107, Sofia, Bulgaria

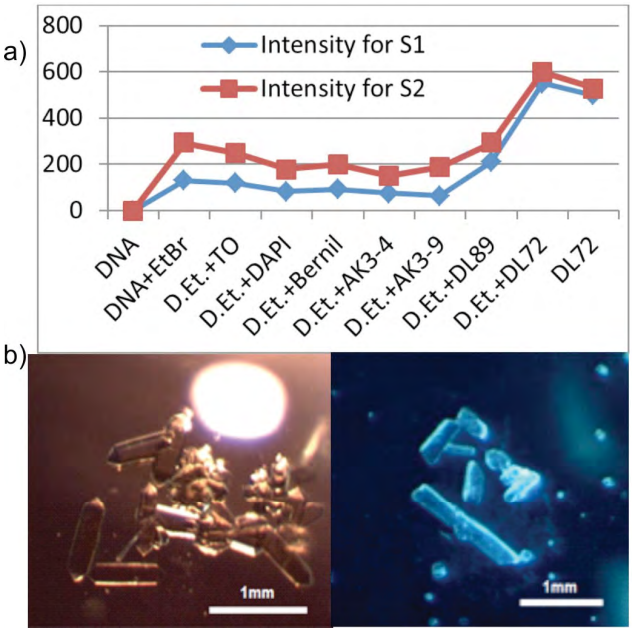
email: sbirkova@mail.bg

With the DNA sequences such as 5'-CGTGAATTCACG-3'(S1) and 5'-CGCGAATTCGCG-3'(S2), for which are characteristic regions rich in AATT bases, bind many drug forms for treatment of oncology diseases and marker dyes used in fluorescence microscopy. These DNA sequences are also interesting because of the presence of a DNA binding site with the restriction enzyme *EcoR*I. [1]

Our aim is to co-crystalize DNA with DAPI and other fluorescent molecules. The co-crystallization include DAPI**,** TO, Berenil and some new fluorescent ligands (AK3-4, AK3-9, DL72 and DL89). DAPI and Berenil lie in the narrow minor groove near the center of the B-DNA fragment positioned over the A-T base pairs. They are bound to the DNA by hydrogen interactions. DAPI inserts itself in the minor groove, displacing the ordered spine waters [2]. DAPI, TO and Berenil show strong fluorescence when bound to DNA, which led to the rapid adoption of these stains for fluorescent labeling in fluorescence microscopy [3]. For verification of DNA ligand interaction, was used Fluorescence Intercalation Displacement (FID) method (Figure 1A). Fluorescence spectroscopy is an effective methodology (nM quantities) suitable for checking and pre-analyzing the expected result of the co-crystallization.

The oligonucleotides of S1 and S2 were crystallized by the vapor diffusion method. The crystallization conditions contained cacodylate buffer (pH 6.5-7.5), alcohol (2-propanol or methylpentanediol (MPD)), cations (Mg^{2+}, Ba^{2+}) and polyamines (Spermine). Finding the right conditions for obtaining good single crystals of the particular DNA sequences has required a lot of careful testing. As found in the process of optimization of the crystallization conditions, for the tested sequences S1 and S2 crystal growth was observed only in the Spermine plates. Dry (lyophilized) oligonucleotide sequences were dissolved in buffer to 2mM concentration. The buffer solution consists of 60 mM NaCaCo (pH 7.0), 17 mM $MgCl^2$, 2 mM Spermine. Ligands were dissolved in the same buffer to 2mM concentration. Crystals were grown by the "hanging drop" method and 1.5µl (2 mM) ligand (3 µl total drop volume) was added to 1.5 µl DNA (2mM) at room temperature equilibrated against 50% MPD. Crystallization plates were stored in controlled temperature rooms (16-20°C). Large crystals (0.4 x 0.3 x 0.25 mm^3) suitable for single crystal X-ray analysis, crystals formed within a month (Figure 1B).

Figure 1. a) Graphical representation of FID data for the impact of DAPI, Berenil, TO, AK3-4, AK3-9, DL89 and DL72; **b)** Obtained crystals.



References:

[1] Sbirkova, H. I., & Shivachev, B. L. (2016). Bul. Chem. Comm. 48(4), 589-592.

[2] Sbirkova-Dimitrova, H. I., & Shivachev B. L. (2017). Acta Cryst. F73, 500-504.

[3] Russell, W., Newman, C. & Williamson, D. (1975). Nature 253, 461-462.

Keywords: DNA, FID, Ligands