forum
Technical Notes and Applications for Laboratory Work
No. 13, 2012

Content

CrystalQuick™ X: Optimised platform for in-situ analysis of protein crystals

1. Introduction: In-situ analysis of protein crystals

X-Ray analysis of protein crystals plays an important role in structure determination of biological macromolecules. This technique provides important data for both basic science and structure-based drug discovery. Automation of most working steps involved improve the success rate and reduced the required time for structure determination significantly.

In automated systems set-up and storage are done in a high-throughput manner in 96 well plates. Analysis of crystallisation drops takes place in automated imaging systems. These systems are using visible as well as polarised and UV-light for crystal detection. Polarised light allows the detection of crystalline structures in ambiguous samples. UV-light allows, if suitable plates are used, a definite discrimination between salt and protein crystals. Nevertheless, no visual method can provide information about diffraction properties of crystals and therefore about their suitability for X-ray analysis. In contrast to most other working steps in protein crystallisation harvesting of the crystals out of the wells of the microplates as preparation for X-ray diffraction analyses is still done manually and remains therefore one of the major bottlenecks in structure determination.

Jacquamet et al. [1] described for the first time in 2004 an in-situ approach for X-ray analysis directly in microplates –

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without any manipulation of the crystals. This method can be fully automated with the help of suitable robotic systems. It enables a definite identification of protein crystals, an estimation of diffraction properties and - depended on crystal properties - structure determination directly in the microplate.

With state-of-the-art crystallisation plates the full potential of in-situ analysis cannot be utilised. The plastic material of the bottom of the crystallisation wells, directly located in the path of the X-ray beam, generates significant background scattering and impacts quality of data collection *in situ*. The geometry of the commonly used crystallisation plates is an additional disadvantage: it allows data collection only within an angular range of up to 30°. With angles above 30° the beam path will hit the side walls of the reservoirs. Because of the resulting background scattering data quality is impaired significantly.

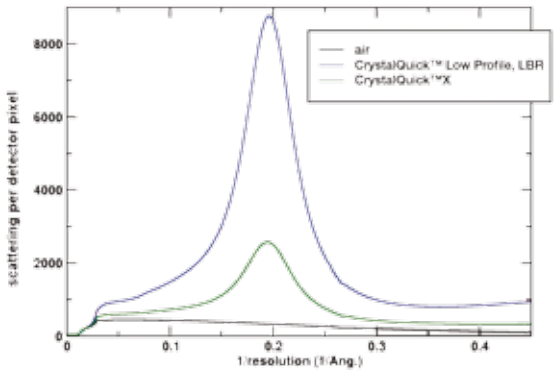
2. Features of CrystalQuick™X

CrystalQuick™X has been developed in collaboration with Beamline FIP-BM30A at the ESRF in Grenoble, France, and is especially designed for in-situ X-ray screening and data collection:

- An ultra-thin well bottom reduces background scattering considerably.
- The open geometry of the crystallisation wells and the inclined side wall of the reservoir allow data collection within an angular range of up to 80°.

The combination of both features definitely improves the quality of the data obtained by in-situ X-ray analysis.

1A



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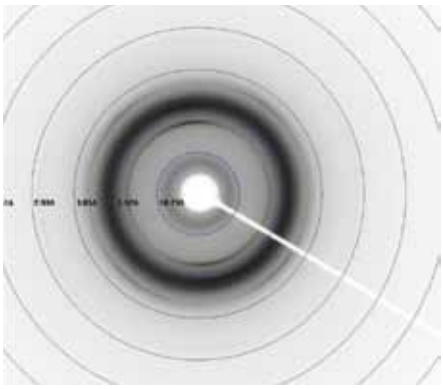
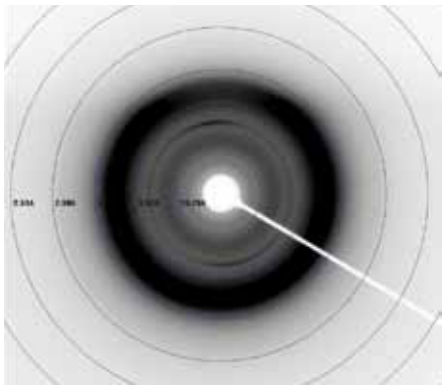


CrystalQuick™ X



CrystalQuick™ Square Well

Figure 1: CrystalQuick™X reduces background scattering significantly in comparison to state-of-the-art plates.
A: Comparison of CrystalQuick™X with CrystalQuick™Low Profile LBR (image is courtesy of Jean-Luc Ferrer, IBS, Grenoble, France).
B: Comparison of CrystalQuick™X and CrystalQuick™Square Well (images are courtesy of Karl Harlos, The Wellcome Trust Centre of Human Genetics, University of Oxford, UK).

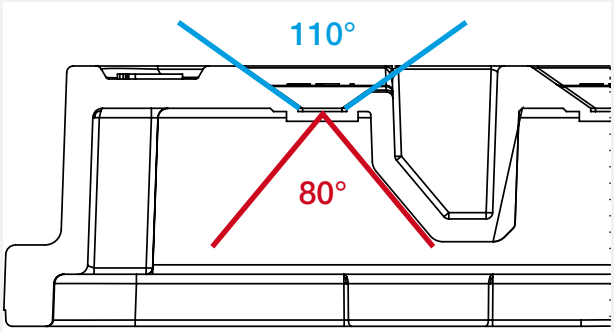


Figure 2: Open geometry of the crystallisation wells and the reservoir of CrystalQuick™X.

- A footprint conforming to the ANSI/SBS 1-2004 standard and a well-to-well distance of 9 mm renders CrystalQuick™X compatible with all conventional automated systems. CrystalQuick™X has a space saving profile height of 8 mm.
- The reservoir volume is approximately 80 µl. The side wall of the reservoir oriented towards the crystallisation wells is sloped. In that way it allows the X-ray beam to pass by within a 40° angle without hitting the reservoir.
- CrystalQuick™X has two square flat bottom crystallisation wells (1.5 x 1.5 mm) per reservoir. The mathematical volume of the wells is about 2.2 µl. With a height of 0.5 mm and a distance of 1.3 mm from the well bottom to the top of the plate the crystallisation wells are relatively shallow. That makes, in combination with the open well geometry, crystal harvesting - if desired - easy. Due to the flat bottom optical properties are very good for bright light microscopy.
- Due to its material properties CrystalQuick™X is optimal for UV-screening and polarised light applications.
- The plate features an alphanumeric well numbering making manual handling steps easier and contains a bar (0.1 × 0.5 mm) for a quick estimation of crystal size (see Figure 3).

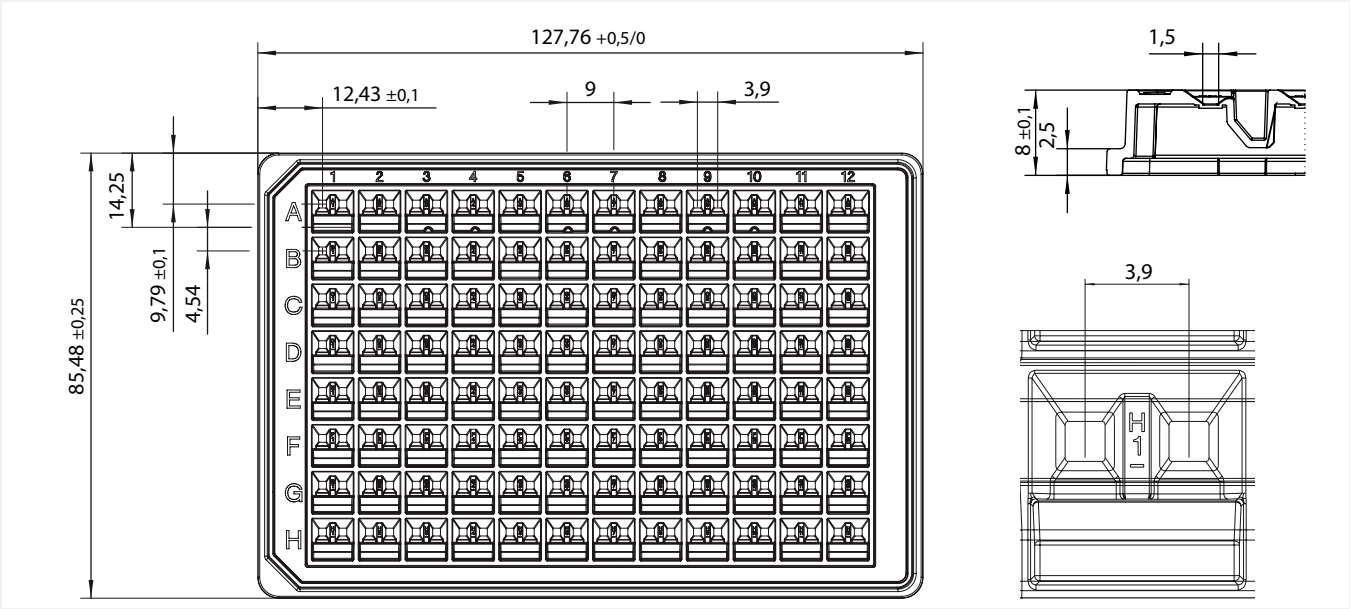


Figure 3: Dimensions of CrystalQuick™X

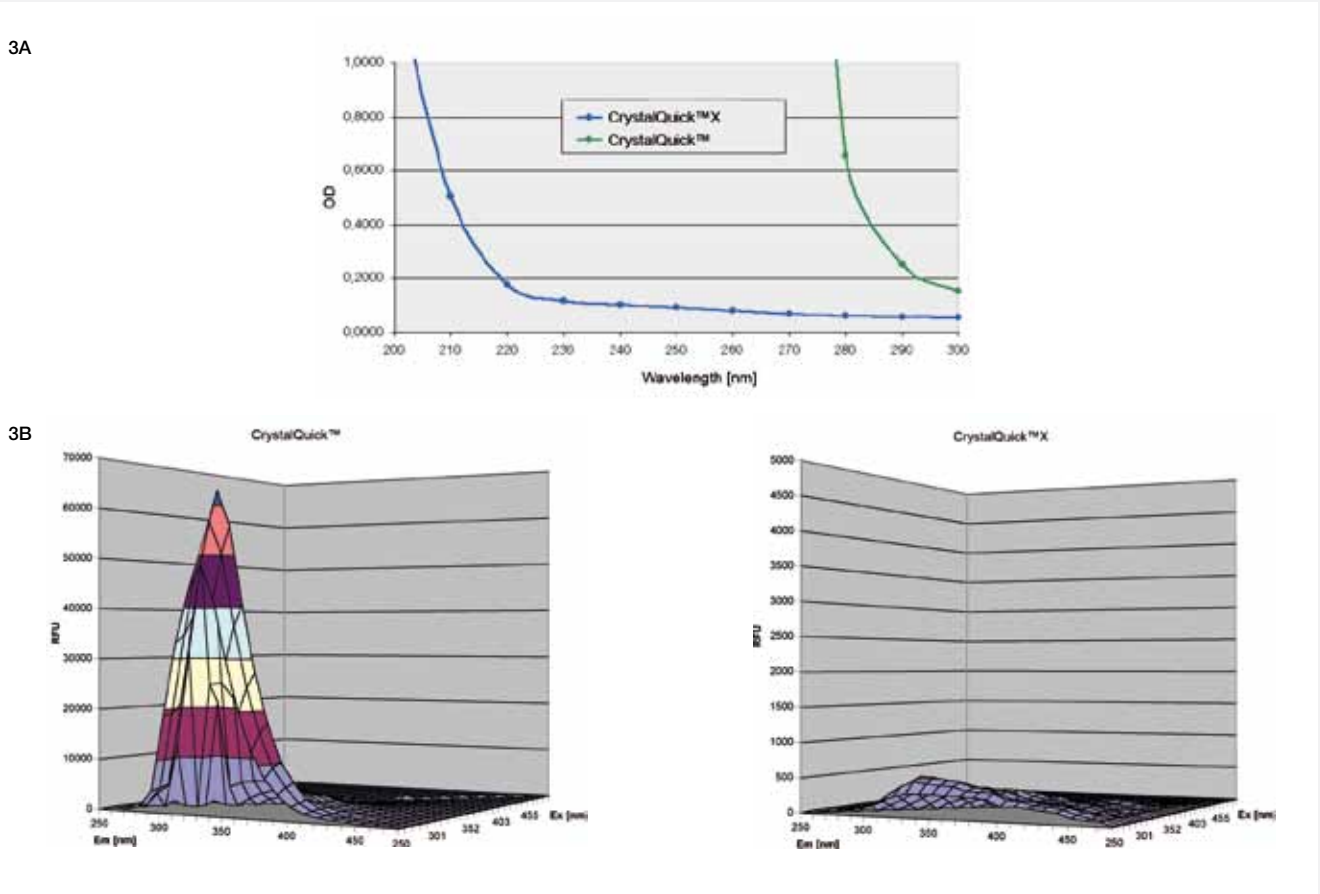


Figure 3: CrystalQuick™ is well suitable for the detection of protein crystals with UV-light.
A: light adsorption in the UV-range. B: Autofluorescence in comparison to CrystalQuick™ plates.

3. Ordering Information

Cat.-No.	Description	Quantity per bag/case
609 890	96 Well, CrystalQuick™ X plate, LBR	20/80
676 070	VIEWseal™ sealing film	100

→ CrystalQuick™ X plate with a hyrophobic surface is available on request.

Distribution of CrystalQuick™ X in USA by NatX-ray (www.natx-ray.com)

4. Literature

[1] Jacquamet L. *et al.* (2004) Automated Analysis of Vapour Diffusion Crystallization Drops with an X-Ray Beam. Structure 12:1219-1225.

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