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Transformation and improvement of macromolecular crystal diffraction through accurately controlled humidity changes: Proteros FREE MOUNTING SYSTEM™

Figure 1. The Proteros FMS Head (left) and Humidifier (right).

1. Overview

The Proteros Free Mounting System (FMS) is a humidity control tool (Figure 1) that enables macromolecular crystallographers to optimize individual crystals with respect to their diffraction characteristics. This new approach, to handle and improve protein crystal quality by directed control of humidity, offers substantial benefits for both the synchrotron and home lab environment [1].

Protein crystals are very sensitive to changes in the environment, especially changes in humidity. In a controlled environment, this sensitivity allows for modification of crystalline order by changing the solvent content. Reducing the relative humidity effects a loss of water that forces crystals to shrink. Crystal packing can then be transformed such that diffraction is dramatically improved.

The FMS precisely controls relative humidity (RH) of a gas stream enveloping a crystal while the X-ray diffraction pattern of that crystal is being monitored in real-time. By dehydrating, and possibly rehydrating, a crystal over time, while simultaneously observing the diffraction pattern, it is possible to manipulate a crystal into an optimal state. Flash cooling is then employed to lock the crystal in that state for subsequent high-resolution data collection.

2. Benefits

Manipulation of the hydration state of a crystal has been shown to significantly improve its diffraction characteristics [1, 2]. With a systematic approach to this process, the FMS has demonstrated the following benefits on a repeatable basis:

(1) Optimized resolution,
(2) Optimized mosaicity,
(3) Optimized anisotropy,
(4) Improved cryo-protocols, and
(5) Reduced X-ray background.

Free Mounting System is a trademark of Proteros Biostructures GmbH.

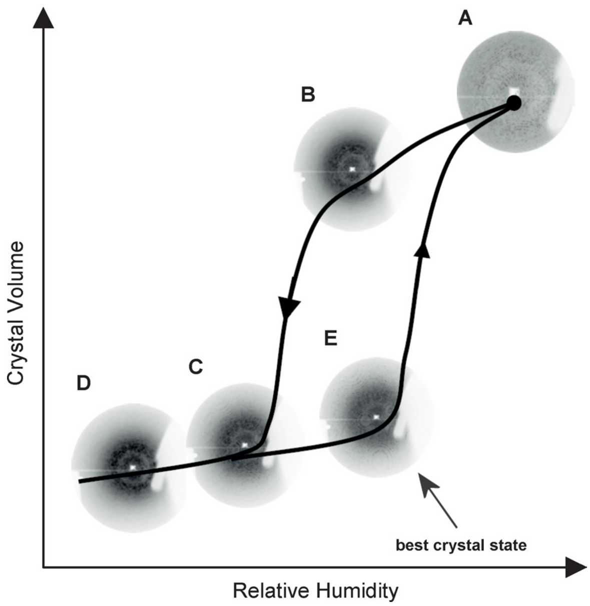

Figure 2. Reversible hydration pattern of CO dehydrogenase (CODH) crystal where dehydration, followed by rehydration, are necessary to achieve optimal diffraction characteristics.

3. Example

As illustrated in Figures 2 and 3, an optimization process often shows hysteresis. In this example, the starting humidity corresponds to point A. Dehydration results in the first optimum state (B). Further dehydration results in a sharp drop in volume, resulting in a second optimum (C) with good resolution, but high mosaicity. Further reducing humidity causes only a linear decrease of the volume (D). Then applying a steeply increasing humidity gradient affords the best crystal state (E), with same resolution (as C) but lower mosaicity.

4. System

The Free Mounting System consists of three components integrated by software running on a personal computer (see Figure 4). Largest of the components is the FMS Humidifier, which produces the precise humid air or gas stream that flows through the FMS Head. Crystals are mounted in the FMS Head, which can be attached to any goniometer, either in a home lab or at a synchrotron source. The FMS Station, equipped with both a microscope and a video system (CCD camera), is used to facilitate mounting crystals and to determine the correct starting humidity.

Crystals, mounted on a standard ACTOR™ pin, reside in an inner axis within the FMS Head (see Figure 5) that can be rotated independently from the casing. The FMS Head is temperature

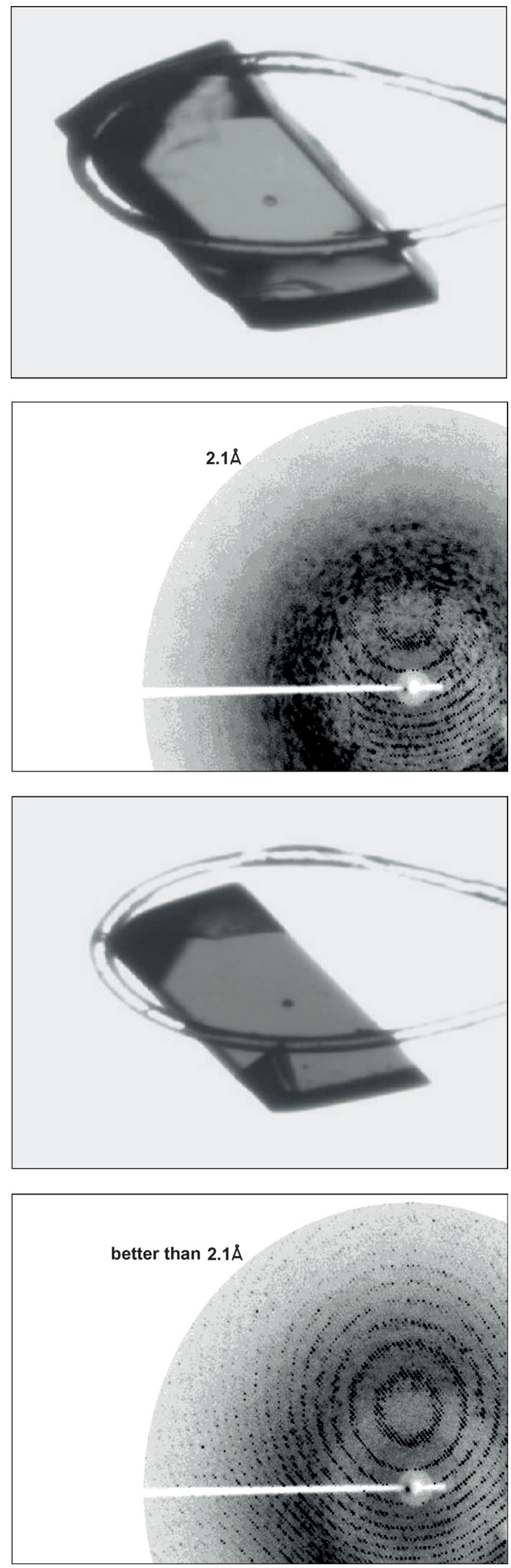

Figure 3. CODH crystal in native state with associated diffraction pattern (top) compared to FMS-processed crystal and optimized pattern (bottom).

controlled to maintain stable humidity. Humid air adopts correct temperature, and thus humidity, in the head before entering the central chamber where the crystal is located. To allow for X-ray exposure, the pin loop protrudes from the outlet by a few millimeters.

Fully adjustable in the range of 50% to 98% RH, the FMS Humidifier allows for very precise control of both humidity and temperature of air or nitrogen. In a supporting role, the FMS Station is used to find the correct starting humidity by examining the area projection of a drop of reservoir buffer (or mother liquor) in a loop mounted in the FMS Head. A stable drop size, as measured by analysis of the video output from the FMS Station, indicates the correct starting humidity.

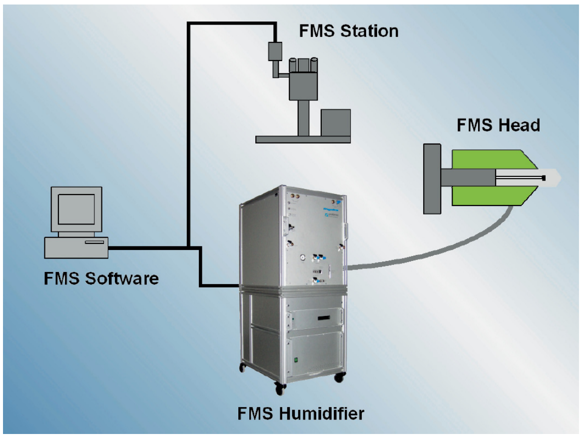

Figure 4. Schematic of the Free Mounting System.

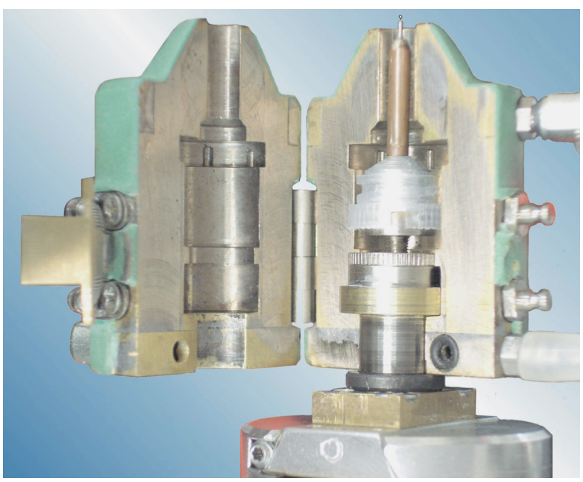

Figure 5. The FMS Head opened to show pin and detail of mounting mechanism.

5. Operation

After the starting humidity has been determined, the FMS Head can be attached to the X-ray system and the crystal mounted. An initial still diffraction pattern is normally acquired. Then a humidity gradient is applied while continuing to collect diffraction data at the same orientation. Once a diffraction optimum is found, the crystal can be flash cooled for further study. Standard procedure for new crystals involves application of a gradient going down to 10% below starting humidity (see Figure 6).

6. Conclusion

Traditionally, protein crystals were improved by changing salt, PEG/buffer concentration, or manual dehydration. The FMS turns this art into

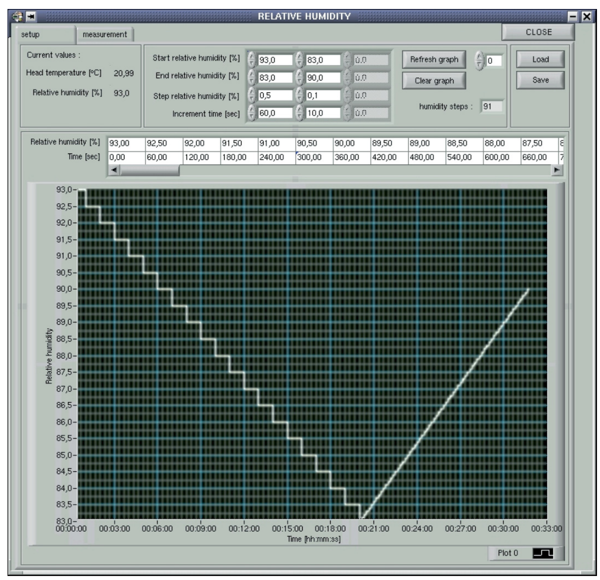

Figure 6. Easy to use control software.

Table 1. Examples of crystal improvement.

Compound	Resolution or Other Benefit	Size	Space Group	Solution
CO-dehydrogenase	3.0 Å → 1.8 Å	277 kDa	$P2_12_12_1$	0.8 M KH_2PO_4
Ba_3-oxidase	3.5 Å → 2.8 Å	85 kDa	$P4_3 2_12$	6% PEG 2000
Dipeptidyl peptidase IV	15.0 Å → 2.8 Å	88 kDa	P1	19% PEG 2000
Transhydroxylase	4.5 Å → 3.0 Å	130 kDa	P1	12% PEG 4000
Nitrate reductase	Increased stability	80 kDa	$P3_121$	PEG 8000
Granzyme B	6.0 Å → 3.0 Å	23 kDa	$P2_12_12_1$	36% PEG 8000
pro MMP1	10.0 Å → 3.5 Å	65 kDa	I4	1.5 M Li_2SO_4
Furin	Lower mosaicity, freezable	39 kDa	$P6_5$	1 M $(NH_4)_2SO_4$

a science by providing a system for reproducible crystal optimization. Table 1 provides a variety of published and unpublished examples illustrating the capability of the FMS to dramatically improve crystallographic data quality for a range of macromolecules of different sizes and space groups. It is believed the Free Mounting System represents a novel and useful technological addition for biotechnology, pharmaceutical or academic labs wishing either to increase their productivity or to rescue difficult crystal specimens.

7. Specifications

Table 2 provides basic specifications for the FMS.

Table 2. The Free Mounting System specifications.

FMS Humidifier:	
Type:	self-contained floor standing unit
Dimensions:	approx. 141 x 56 x 70 cm (H x W x D)
Weight:	approx. 130 kg w/o fluids
Water tank:	3 liters capacity (distilled or deionized)
Humidity range:	50 - 98% RH
RH scale error:	± 0.3 %
Hose length:	1.5 meters to FMS Head
Gas requirement:	air or nitrogen @ >1.5 bar & >1.8 l/min
FMS Station:	
Type:	tabletop microscope workstation
Microscope:	stereo zoom type
Video camera:	CCD type, 752 x 582 pixels
Dimensions:	approx. 57 x 40 x 40 cm (H x W x D)
Weight:	approx. 20 kg
FMS Head:	
Detail:	attaches to goniometer or FMS Station
Dimensions:	approx. 4.2 x 3.2 cm (L x diam.) w/o hoses
Weight:	approx. 360 g (1.7 kg w/ hoses)
Pin type:	ACTOR standard (18 - 21 mm)
FMS Software:	
Type:	LabView based
Platform:	IBM-PC compatible computer
Operating system:	SuSE Linux 8.2+
Frame grabber:	Matrox Meteor-II/MC (PCI)
Other specifications:	
Operating environment:	15 - 25°C, 40 - 70% RH (non-condensing)
Ambient temperature requirement:	less than ± 2°C change per hour
Power requirement:	110 - 260 VAC, 50/60 Hz, 2000 W max.
Warranty:	1 year parts & labor

References

[1] Reiner Kiefersauer, Manuel E. Than, Holger Dobbek, Lothar Gremer, Marcos Melero, Stefan Strobl, João M. Dias, Tewfik Soulimane, and Robert Huber (2000). *J. Appl. Cryst.* **33**, 1223–1230.

[2] R. Kiefersauer, et al. (1996). *J. Appl. Cryst.* **29**, 311–317; M. Weiss, et al. (1999). *Bio. Cryst.* **D55**, 1858–1862; R. E. Thorne, et al. (2001). *Bio. Cryst.* **D57**, 61–68; V. Fülöp, et al. (2004). *Bio. Cryst.* **D60**, 331–333; I. T. Weber, et al. (2001). *Bio. Cryst.* **D57**, 763–765; S. W. Suh, et al. (2002). *Bio. Cryst.* **D58**, 303–305; and A. N. Popov, et al. (2000). *Bio. Cryst.* **D56**, 595–603.