

A New Crystal Mounting Method for Macromolecular Cryocrystallography

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As macromolecular crystallographers move to solving more and more structures each year, the limitations of current technology for handling and mounting protein crystals are becoming evident. Introduced by T. Y. Teng [1] at the Macromolecular Crystallography facility of the Cornell High-Energy Synchrotron Source (MacCHESS) in the late 1980s, the versatile loop mounting method has had few rivals and surprising longevity. Even so, new methods for the emerging high-throughput environment can improve upon loops in many ways.

New mounting methods should allow easier crystal retrieval and handling with minimal risk of crystal loss or damage. This is especially important for, e.g., membrane proteins and macromolecular complexes, where the cost of crystal growth materials may be high and the number of crystals obtained may be small. They should allow easy handling and mounting of very small crystals (<40 μm), which can now yield complete data sets at high-brilliance beam lines. Data collection from very small crystals is also extremely useful in evaluating crystallization "leads" obtained in screening trials, so that the time spent on molecular modifications, purification and crystallization can be optimized.

New mounting methods should allow excess liquid surrounding a crystal—which increases background X-ray scattering, crystal cooling times during flash cooling and cooling-induced damage—to be easily removed. New mounting methods should both reproducibly position the crystal with respect to the goniometer stage and improve its visibility, thus further simplifying automated crystal alignment. The crystal position should remain fixed during *in situ* annealing [2] and dehydration/hydration [3] procedures sometimes used to improve diffraction properties. Finally, the crystal should not bend or flutter in response to cryostream drag forces.

We have recently reported [4] a very simple crystal mounting technology that appears to improve upon loops in all of these ways. The mount design, shown in Figures 1 and 2, consists of a thin (5–15 μm) microfabricated polyimide film attached to a cylindrical pin. The film has a small hole at its tip for the crystal that is connected via a drainage channel to a larger opening, in a geometry reminiscent of a fountain pen. This allows a wick inserted into the larger opening to remove liquid from around the crystal without touching it. The thin polyimide film is extremely flexible, but the curvature induced by wrapping its base

around the pin provides excellent rigidity and a convenient, scoop-like action in retrieving crystals.

Polyimide is widely used in the microelectronics industry as the base material for flexible circuits, and is familiar at synchrotron beamlines in the form of Kapton tape. It has excellent X-ray transparency properties, and its gold hue provides good optical contrast with most protein crystals. As shown in Figure 3, polyimide crystal mounts produce much less background scatter than loops of 20- μm nylon. The crystal opening can be as small as 5 μm , and can be easily matched to the crystal size and shape, minimizing excess trapped liquid and maximizing heat transfer rates during flash cooling. Film thickness, length

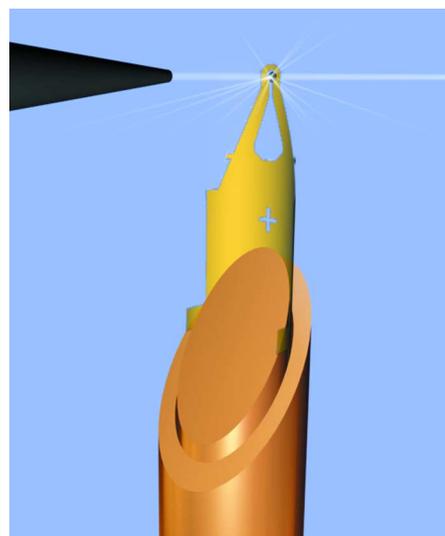


Figure 1: Crystal mounts consisting of a patterned polyimide film attached to a cylindrical rod. The crystal is held in the small hole at the top end of the film, which connects via a channel to a larger opening to facilitate wicking of excess liquid. The film curvature induced by wrapping its base around the rod provides excellent rigidity and a scoop-like action with minimal film thickness.

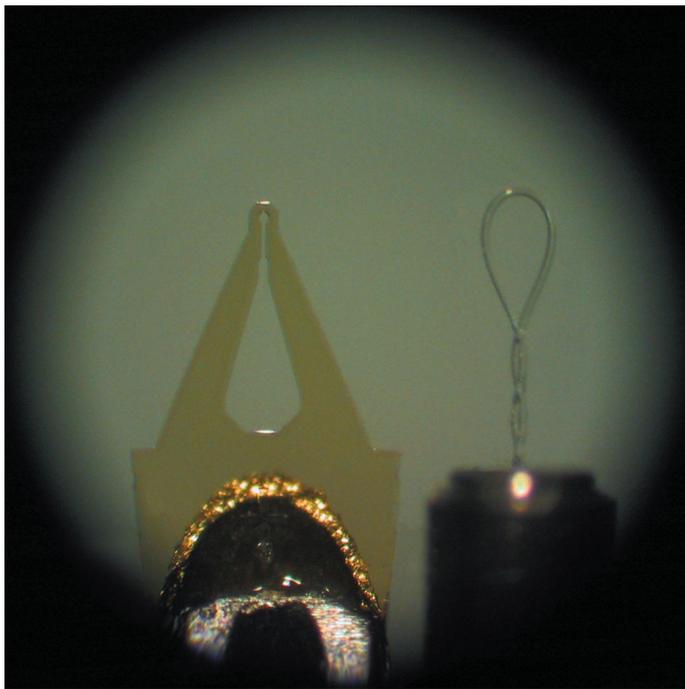


Figure 2: Photograph comparing a polyimide mount with a 30 μm crystal opening (left) and a nylon loop mount with a nominal 100 μm opening (right).

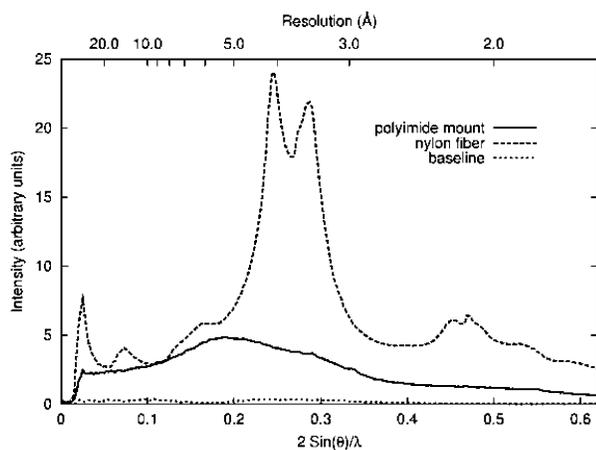


Figure 3: Diffuse scattering intensity versus $2\sin\theta/\lambda$ and resolution for a 20 μm thick nylon loop and a 9 μm thick polyimide mount when illuminated using a 12.7 keV X-ray beam focused to a 10 μm spot. For the nylon loop, the spot was focused on the middle of the nylon line and for the polyimide film the spot was focused normal to the plane of the film. (Reprinted from Ref. 4 with permission of the IUCR.)

and lateral dimensions can be scaled with crystal size to optimize the balance between rigidity and background scatter.

Crystals are mounted by scooping them from a liquid drop into the small hole, carefully inserting a wick into the larger hole to remove excess surrounding liquid, and then flash cooling. The cylindrical pin is compatible with existing magnetic bases for goniometer mounts and with equipment being developed for automated crystal handling. Although designed for protein crystals, mounts without the wicking hole are convenient for any small organic or inorganic crystals, which can be held in place using a small amount of, e.g., glue or ethyl cellulose dissolved in ethyl acetate.

Perhaps the most important feature of this new mounting technology is that it uses standard and inexpensive microelectronics fabrication processes. The mounts are completely reproducible, facilitating automated handling, alignment, and retrieval of crystals. Hundreds of mounts can be fabricated from a single polyimide-coated silicon wafer, and thousands from a single polyimide sheet. Design customization to, e.g., match a given crystal shape, is trivial, requiring only changes in the CAD drawings used to produce the exposure masks, and each wafer/sheet can contain many different designs. Superior performance, design flexibility and inexpensive large-volume production make these new mounts good candidates to meet the burgeoning needs of the high-throughput structural genomics era. Additional information is available at www.ccmr.cornell.edu/~robt/micromounts.html. ■

Acknowledgments

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References

1. T. Y. Teng, *J. Appl. Crystallogr.* **23**, 387–391 (1990).
2. J. M. Harp, D. E. Timm, G. J. Bunick, *Acta Cryst. D* **54**, 622–628 (1998); J. I. Yeh, W. G. J. Hol, *Acta Cryst. D* **54**, 479–480 (1998); S. Kriminski, C. L. Caylor, M. C. Nonato, K. D. Finkelstein, and R. E. Thorne, *Acta Crystallogr. D* **58**, 459–471 (2002).
3. R. Kiefersauer, M. E. Than, H. Dobbek, L. Gremer, M. Melero, S. Strobl, J. M. Dias, T. Souliamane, and R. Huber, et al., *J. Appl. Cryst.* **33**, 1223–1230 (2000).
4. R. E. Thorne, Z. Stum, J. Kmetko, K. O'Neill, R. Gillilan, *J. Appl. Cryst.* **36**, 1455–1460 (2003).