

How new strategies can improve productivity in crystallization and cryoEM

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Abstract

Random Microseed Matrix-Screening (rMMS), where seed crystals are added automatically to random crystallization screens, is a significant recent breakthrough in protein crystallization [1]. During the ten years since the method was published, understanding of the theoretical advantages of the method has increased [2 - 4], and several important practical variations on the basic method have emerged. Important variations that will be discussed include combining seeds from several hits [5], the best methods of selecting hits to optimize [2], and cross-seeding targets with crystals of homologous proteins [6]. I will also present an approach that allows the method to be applied to the crystallization of membrane proteins in LCP [7], and a novel approach to preparing samples for cryoEM using "in situ" dynamic light scattering. This will include discussion of the composition of the ideal screen for cryoEM.

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[2] Shaw Stewart, Patrick D., et al. "Random microseeding: a theoretical and practical exploration of seed stability and seeding techniques for successful protein crystallization." *Crystal Growth & Design* 11.8 (2011): 3432-3441.

[3] D'Arcy, A., Bergfors, T., Cowan-Jacob, S. W., & Marsh, M. (2014). Microseed matrix screening for optimization in protein crystallization: what have we learned?. *Acta Crystallographica Section F: Structural Biology Communications*, 70(9), 1117-1126.

[4] Shaw Stewart, P., & Mueller-Dieckmann, J. (2014). Automation in biological crystallization. *Acta Crystallographica Section F: Structural Biology Communications*, 70(6), 686-696.

[5] Obmolova, G., Malia, T. J., Teplyakov, A., Sweet, R. W., & Gilliland, G. L. (2014). Protein crystallization with microseed matrix screening: application to human germline antibody Fabs. *Structural Biology and Crystallization Communications*, 70(8).

[6] Abuhammad, Areej, et al. "Structure of arylamine N-acetyltransferase from *Mycobacterium tuberculosis* determined by cross-seeding with the homologous protein from *M. marinum*: triumph over adversity." *Acta Crystallographica Section D: Biological Crystallography* 69.8 (2013): 1433-1446.

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