Influence of humidity on drop evaporation

Friedlander, Tom; Handing, Kasia; Gualtier, Ellen; van Beek, Hans; Ramsey, Lance
Formulatrix

Vapor diffusion is still the most commonly employed technique used to grow protein crystals for X-ray diffraction [1,2]. This method relies on the evaporation of water vapor from the droplet to the reservoir solution, until the two are at equilibrium. The factor that directly impacts the rate of drop evaporation is relative humidity (RH). High RH during setup of the crystallization plate, slows down the evaporation process. RH in the room where plates are set up is often not tightly regulated and can vary significantly between laboratory environments, climates, and across seasons [3]. This may cause discrepancies between crystallization trial outputs, which will negatively impact experimental reproducibility - an important issue within the scientific community [4].

Here, we assess the effects of humidity on droplet size and composition during the course of crystallization plate preparation. We show that droplets setup in ambient RH lost on-average 30-50% of volume after 2.7-6.3 minutes, whereas droplets setup in 85% RH lost only 0-7% volume. Notably, the average time to setup a 96-well plate using a commercially-available drop-setter with 1-3 drops per well was 2-10 minutes, which translates into significant drop loss if RH is not controlled. The decreased drop volumes effectively change the concentration of solutes in the drop, which, in turn, influences the speed of droplet evaporation and, in extreme cases, may cause protein denaturation. Some crystallization robots, including the NT8 drop-setter from Formulatrix, allows for precise humidity control during plate preparation that significantly decreases drop evaporation.

References

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