

Poster Presentation

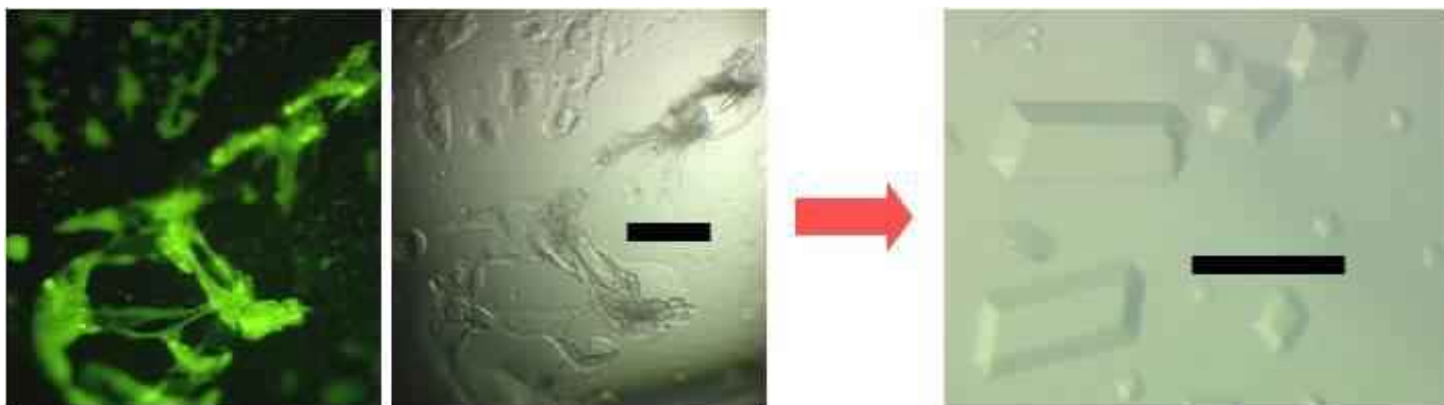
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Optimization of Cryptic Leads from Trace Fluorescent Labeling Screening

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We use trace fluorescent labeling (TFL) as a means of rapidly identifying crystals in the screening plate. The method involves the covalent labeling of between 0.1 to 0.2 % of the protein molecules with a fluorescent probe. Our standard labeling process uses the amine reactive dye 5(6)-carboxyrhodamine 6G succinimidyl ester (Invitrogen, C-6157), with reaction conditions (pH) adjusted to label random side chain amines. Previous results had shown that labeling below 1% does not affect the nucleation rate or diffraction data quality. Identification of crystalline outcomes is based on intensity; (labeled) protein packing density is highest in the crystalline form which will fluoresce more brightly than other precipitated forms. We found that there were many outcomes where the fluorescent images had regions of high intensity, but no corresponding crystalline structures were apparent using white light transmission microscopy. Under the governing paradigm, that intensity = structure, we hypothesized that these were likely lead conditions and tested that hypothesis with optimization screening using capillary counter diffusion (CCD). Four CCD experiments are set up for each lead condition experiment having different ratios of the stock screening cocktail components. The capillaries (40 mm long, 0.3, 0.2, or 0.1 mm ID) are filled with protein at 2.5X the sitting drop screening experiment concentration, sealed at one end using soft wax, then inserted open end down into 40 uL of the precipitant solution in a 1.2 mL titer tube (E&K Scientific, cat.# 684510-R), which is then sealed with a stopper (E&K Scientific, cat.# 64108-P). The capillaries can be examined using a microscope through the walls of the tube, using either white light or fluorescence illumination. We are currently finding ~40% of the identified lead conditions yield crystals upon optimization, representing a significant increase in the success rate for the screening experiment.



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