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Measuring Solvent Content of Macromolecular Crystals Using Fluorescence Recovery after Photobleaching MATTHEW SIEWNY, JAN KMETKO, Kenyon College — We work out a novel protocol for measuring the solvent content (the fraction of crystal volume occupied by solvent) in biological crystals by the technique of fluorescence recovery after photobleaching (FRAP). Crystals of proteins with widely varying known solvent content (lysozyme, thaumatin, catalase, and ferritin) were grown in their native solution doped with sodium fluorescein dye and hydroxylamine (to prevent dye from binding to amine groups of the proteins.) The crystals were irradiated by a broadband, high intensity light through knife slits, leaving a rectangular area of bleached dye within the crystals. Measuring the flow of dye out of the bleached area allowed us to construct a curve relating the diffusion coefficient of dye to the channel size within the crystals, by solving the diffusion equation analytically. This curve may be used to measure the solvent content of any biological crystal in its native solution and help determine the number of proteins in the crystallographic asymmetric unit cell in x-ray structure solving procedures.

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