

## W0180

**Structural Characterization of Two Variants of the Green Fluorescent Protein.** J.D. Pédelacq, S. Cabantous, T.C. Terwilliger, G.S. Waldo, Los Alamos National Laboratory, Los Alamos, NM 87545 USA.

Current enhanced versions of GFP fold well and are brightly fluorescent only when expressed alone or when fused to very well-folded proteins (1,2). Starting with the traditional folding reporter GFP (FR-GFP), we applied a directed evolution approach for engineering a superfolder GFP (SF-GFP) variant that folds well even when fused to poorly folded proteins (3). We applied a wide array of biophysical techniques to characterize the folding robustness of SF-GFP and several single-point mutants derived from SF-GFP. SF-GFP fusion fluorescence is unaffected by fusion partner misfolding and is directly proportional to total expression. Complete, highly redundant data sets were collected for FR-GFP and SF-GFP to a resolution of 2.5 Å and 1.45 Å, respectively. These studies provide a structural explanation for why the mutations Y39N and S30R confer substantial improvement to SF-GFP folding robustness. SF-GFP should have widespread utility as a less-perturbing fluorescent protein tag for protein localization and trafficking experiments and provides the basis for new fluorescent protein derivatives.

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3. Pédelacq J. D. *et al.* (2006) *Nat. Biotechnology* 24, 79-88