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Structure of the reassembled Venus

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Bimolecular fluorescence complementation (BiFC) assay based on the association of a fluorescent protein fragments is widely employed to study protein-protein interactions in cells. Non-specific interaction is a significant problem, because it interferes with the BiFC assay. To understand the mechanisms of reconstitution, we have attempted to determine the structure of the fluorescent protein, Venus, complemented between the N-terminal and C-terminal fragments. The fragments were co-expressed in *E. coli* and purified by the affinity and ion-exchange chromatographic techniques. The fluorescent fraction was crystallized by the sitting-drop vapor diffusion method. The diffraction data were collected to 2.5 Å resolution at 100 K using an ADSC CCD detector at the NW12 station of PF, Japan. The crystal belongs to $P2_12_12_1$, with unit-cell parameters of a = 58.97, b = 116.02, c = 156.62 Å. Assuming three molecules of the reassembled Venus in the asymmetric unit, the V_M value was calculated to be 2.9 $Å^3$ Da^{-1}. The crystal structure was solved by molecular replacement using the program *MOLREP* with the structure of Venus (PDB: 1MYW) as a search model. Completion of model building and structure refinement are underway.