

Structure of a Green Fluorescent Protein Biosensor - Implications for FRET

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Abstract

Förster Resonance Energy Transfer (FRET) between mutants of the Green Fluorescent Protein (GFP) has been widely used in fluorescent biosensors. So far, only low-resolution structural data of FRET biosensors are available, e.g. from small angle X-ray scattering (SAXS). Here we report the crystal structure of a FRET-based Calcium sensor protein constituted by the central Calcium binding domain of Troponin C flanked by a pair of GFP-derived fluorescent proteins undergoing FRET. Our high-resolution structure illustrates the importance of protein contacts and interdomain interactions, including circular permutations of the fluorescent proteins and the choice of linker regions, for the successful design of biosensors. The FRET efficiency predicted from our structure is higher than the experimentally determined value in solution. This discrepancy can be explained by dynamics detectable in solution by NMR.