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Interaction of Monomers in Near-Infrared Fluorescent Biomarkers

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Here, we analyze how the inter-monomeric interaction in the near-infrared fluorescent boimarkers iRFP713 and iRFP713/C15S/V256C is affected by the rearrangement of the hydrogen bond network between the chromophore and the adjacent amino acids and bound water molecules as result of amino acid substitution of threonine at position 204 for alanine (T204A) in its local environment or replacement of natural ligand biliverdin with phycocyanobilin. Previously found allosteric inhibition of covalent binding of the biliverdin to a monomer of iRFP713/C15S/V256C after covalent binding of the chromophore to another monomer is markedly reduced in the protein with T204A substitution. There is no allosteric inhibition of covalent binding of phycocyanobilin to iRFP713/C15S/V256C, in contrast to the binding of biliverdin to this protein. Contrary, the replacement of biliverdin with phycocyanobilin in iRFP713 leads to increased allosteric inhibition of covalent chromophore binding. Our studies indicate that the change in the intramolecular contacts involving the chromophore and its protein environment in biomarkers caused by chromophore replacement or amino acid substitutions influences allosteric communication between monomers the biomarker.

Keywords: bacterial phytochromes, fluorescent biomarkers, allosteric interaction