

щуюся в повышении устойчивости этих белков против протеолитической деградации. В совокупности со стабилизирующим действием узла, одновременное ковалентное связывание BV с обоими доменами NIR-биомаркеров с двумя ключевыми остатками цистеина может вносить вклад в устойчивость этих белков к действию различных, в том числе клеточных, протеаз.

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Авторы заявляют, что в проведенных экспериментах люди или животные в качестве объектов не участвовали.

### КОНФЛИКТ ИНТЕРЕСОВ

Авторы заявляют об отсутствии конфликта интересов.

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## Double Covalence Bonding of Biliverdin in Near-Infrared Fluorescent Protein Prevents Their Proteolitic Degradation

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In the present work, we analyze how the double covalent binding of biliverdin ligand (BV) in the near-infrared fluorescent protein iRFP670, containing two key cysteine residues, affects the stability of this biomarker to proteolytic degradation. It has been previously found that the covalent attachment of BV simultaneously with two cysteine residues is the cause of the highest fluorescence quantum yield of BV-containing near-infrared fluorescent proteins (NIR FPs) with two key cysteine residues compared to other BV-containing NIR FPs. Our data indicate that the covalent binding of BV in NIR-FP with two key cysteine residues simultaneously with two regions of the polypeptide chain, which, in addition, forms a figure-of-eight knot, leads to screening of many cleavage sites by the proteolytic enzymes trypsin and chymotrypsin in them. As a result, the covalent binding of BV in NIR FPs simultaneously with two cysteine residues not only stabilizes their structure, but their resistance to proteolytic degradation can also increase, which determines the cellular stability of biomarkers and is important for their use as fluorescent tag in the cell.

**Keywords:** bacterial phytochromes, near-infrared fluorescent proteins, double covalent biliverdin binding