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## **CCEPTED MANUSCRIPT**

Hydrogen Bonds in the Vicinity of the Special Pair of the Bacterial Reaction Center Probed by Hydrostatic High-Pressure Absorption Spectroscopy

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**ABSTRACT** 

Using the native bacteriochlorophyll a pigment cofactors as local probes, we investigated the

response to external hydrostatic high pressure of reaction center membrane protein complexes

from the photosynthetic bacterium Rhodobacter sphaeroides. Wild-type and engineered

complexes were used with varied number (0, 1 or 2) of hydrogen bonds that bind the reaction

center primary donor bacteriochlorophyll cofactors to the surrounding protein scaffold. A

pressure-induced breakage of hydrogen bonds was established for both detergent-purified and

membrane-embedded reaction centers, but at rather different pressures: between 0.2 and 0.3

GPa and at about 0.55 GPa, respectively. The free energy change associated with the rupture

of the single hydrogen bond present in wild-type reaction centers was estimated to be equal to

13- 14 kJ/mol. In the mutant with two symmetrical hydrogen bonds (FM197H) a single

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