

**Cytochrome P450 from *Photobacterium profundum* SS9, a piezophilic bacterium,
exhibits a tightened control of water access to the active site**

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Supporting Information

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P450-SS9  MTESEVVRE-NQLIKRTL--DDLQPQKGWPLLGNFLQ-LQSKNLHQVLEQWCLEYGDTYKVDIAGLLFVVIADPVVVKDILRRRPKSFNRT----ASLERV
P450-3TCK MTESEVVRE-NQLIKRTL--DDLQPQKGWPLLGNFLQ-LQSKKLHQVLEQWCLEYGNTYKID IAGLLFVVIADPVVVKDILRRRPKSFNRT----ASLERV
P450-PE36  MNAKMVKGVNSPVLPTISFDELPGPKQQAILGNFTQ-ISAESFHTHLEQWAKEEYGSAYQMRLLNKPYLVI SDPKIGLEIIKQRPKLFNRTERLEWLFED-
P450-SK209 MTMLPPKPPSRPERVSL-----WQYLRLFRQDILSAQPAKLYRAWMAEF-----RAPRIRSFLINQPELLQRVLKEEPDLFPKSDRIAEGLPK-
P450BM-3   -----TI--KEMPQPKTFGELKNLPL-INTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKEA-CDESRFDKNLSQALKFVRD-
1.....10.....20.....30.....40.....50.....60.....70.....80.....90.....100

P450-SS9  FKELGIHGVLSANGESWKRQRRLIMPAFSKKSLASFFPLLEQTTERLRLRLVKKRGQD-TLAIHDDLRRFTVDITTSSLVFGHDTR--LLEHGDGLQKHL
P450-3TCK  FKELGIHGVLSANGESWKRQRRLIMPAFSKKSLASFFPLLEQTTERLRLRLVKKKGQD-TLAIHDDLRRFTVDITTSSLVFGHDTR--LLEHGDGLQKHL
P450-PE36  ---LGIHGVFSSNGDKWKRQRRLIMPAFSYNTLANFVPQLKSLSINLQVAIDKKIATGEAFNVHKLQHFTIDITTSSLVFGYQTN-MLSGSSDTHLRDNI
P450-SK209 ---LLGNSVFLTNGEAWKRQRRLIDPAFEGGRLKETTFPAMRAAAEAVRRELKQVAENDTVEIEEVTSHAAADVIFRTLSPIE-----HEVAGEVF
P450BM-3   FAGDGLFTSW-THEKNWKAHNILLPSFSQAMKGYHAMMVDIAVQVQKWERLNADE-HIEVPEDMTRLTLDTIGLCGFNYRFNSFYRDQPHPFITSMV
.....110.....120.....130.....140.....150.....160.....170.....180.....190.....200

P450-SS9  EVIFPQLNSRTRMPFPYQYIKFKKDRKLD----QALIEVEKYALKIVEQTRDELQFNPQLADAPETILQAMVAASD--DNR-LTNEELFANILTLLLAG
P450-3TCK  EVIFPQLNSRTRMPFPYKYIKFKKDRKLD----QALIEVEKYALKIVEQTRDELQFNPQLADAPETILQAMVAASD--DNR-LTNEELFANILTLLLAG
P450-PE36  DRLFRALNKRSKYPPFPWRYIRTPETRRID----KAREEVYQLAVSMITKAKVVLAENSALAEPETILQAMIVASDSEENK-LTDELVANILTLLLAG
P450-SK209 SRFRDYQRSQPLLNLAAFLPLPGWMPRRFRPGTLSNAREIRALITRLTETRMSEIKAGT----APDDIATKIMCAKDPETGTGFDEEMVDQVAIFFLAG
P450BM-3   RALDEAMNKLQRANPDDPAYDENKRQFQ-----EDIKVMNDLVDKIIADRKASG-----EQSDLLTHMLNGKDPETGEPLDDENIRYQIITFLIQG
.....210.....220.....230.....240.....250.....260.....270.....280.....290.....300

P450-SS9  EDTTSNLIAWMLYFISQRPDIQCKINEEAEQIRLKHGQINVQGLDELTYLEAVARETLRLKSTAFMISAETADQVTLLDGTELPAGTGLFLMTRLGGLN
P450-3TCK  EDTTSNLIAWMLYFISQRPDIQCKINEEAEQIRLKHGQINVQGLDELTYLEAVARETLRLKSTAFMISAETVDQVTLLDGTELPAGTGLFLMTRLGGLN
P450-PE36  EDTTSNMLAWTLFYLAQNPSLQQVVIDEVSRCDGDIENVDLTALEQEFIDAILREGLRLKGTAPLISAEPTEDTVLSNGIKLPKGTAIFILTRPGGLD
P450-SK209 HETSASALAWALYLMALYPEWQEKLEEEAAIL-----GDESFAAVSKLRLSRAVFREALRLYPPVFMVREASCPVRF-RNRDVPKGSQIVLSPWHLHRH
P450BM-3   HETSGLLSFALYFLVKNPHVLQAAEEAAARVLD--PVPSYKVQVKLKYGMVLNEALRRLWPTAFSLYAKEDTVLGGEYPLEKGDELMVLIPQLHRD
.....310.....320.....330.....340.....350.....360.....370.....380.....390.....400

P450-SS9  KEHF-KDAEQFRPERWQEEAVAAEACPHKATSHFPFGGGARHCPGETLAFMETKMVIAMLCQQFDISQPESPPVV-EEYAITMRPKNLQICLRLKPVGR-
P450-3TCK  PEHF-KDAEQFRPERWQEEAVAAEACPHKATSHFPFGGGARHCPGETLAFMETKMVIAMLCQQFDISQPESPPVV-EEYAITMRPKNLQICLQLKTVGK-
P450-PE36  EKVV-ACPEKFNPERWLSTPEKP-VCPHLQSSHIPFGAGARHCPGERLAMEGKAVIARLCWYVVISQPEQAPEVGEEFATMRPTNLALTLTPRK----
P450-SK209 ERLW-NNPDGEDPMRWQSENGKT-----CQREAYIPSAGPRVCTGAGFAMVEGPLILSMILRAFQLERIEGDDPVPVAHLTVRAKNGIRLLRARRR----
P450BM-3   KTIWGDDVEEFRPERFENPSA-----IPQHAFKPFGNQRACIGQQFALHEATLVLGMMLKHFDFED-HTNYELDIKETTLLKPEGFVVKAKSKKIPLG
.....410.....420.....430.....440.....450.....460.....470.....480.....490.....500

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Fig. S1. Multiple alignment of protein sequences of cytochromes P450 from *Photobacterium profundum* SS9 (P450-SS9), *P. profundum* 3TCK (P450-3TCK), *Moritella* sp. PE32 (P450-PE32), *Roseobacter* sp. SK209-2-6 (P450-SK209) and cytochrome P450BM-3 (CYP102) from *Bacillus megaterium* (P450BM-3). The alignment was prepared with COBALT program¹ (<http://www.ncbi.nlm.nih.gov/tools/cobalt/>) with a minor manual correction. Identical residues in all 5 sequences are shown with red letters on yellow background. Blue background symbolizes the residues identical to those found in P450-SS9. Green background indicates the substitutions, which are conservative relative to the sequence of P450-SS9.

¹ Papadopoulos, J.S., and Agarwala, R. (2007) COBALT: constraint-based alignment tool for multiple protein sequences *Bioinformatics*, *23*, 1073-1079

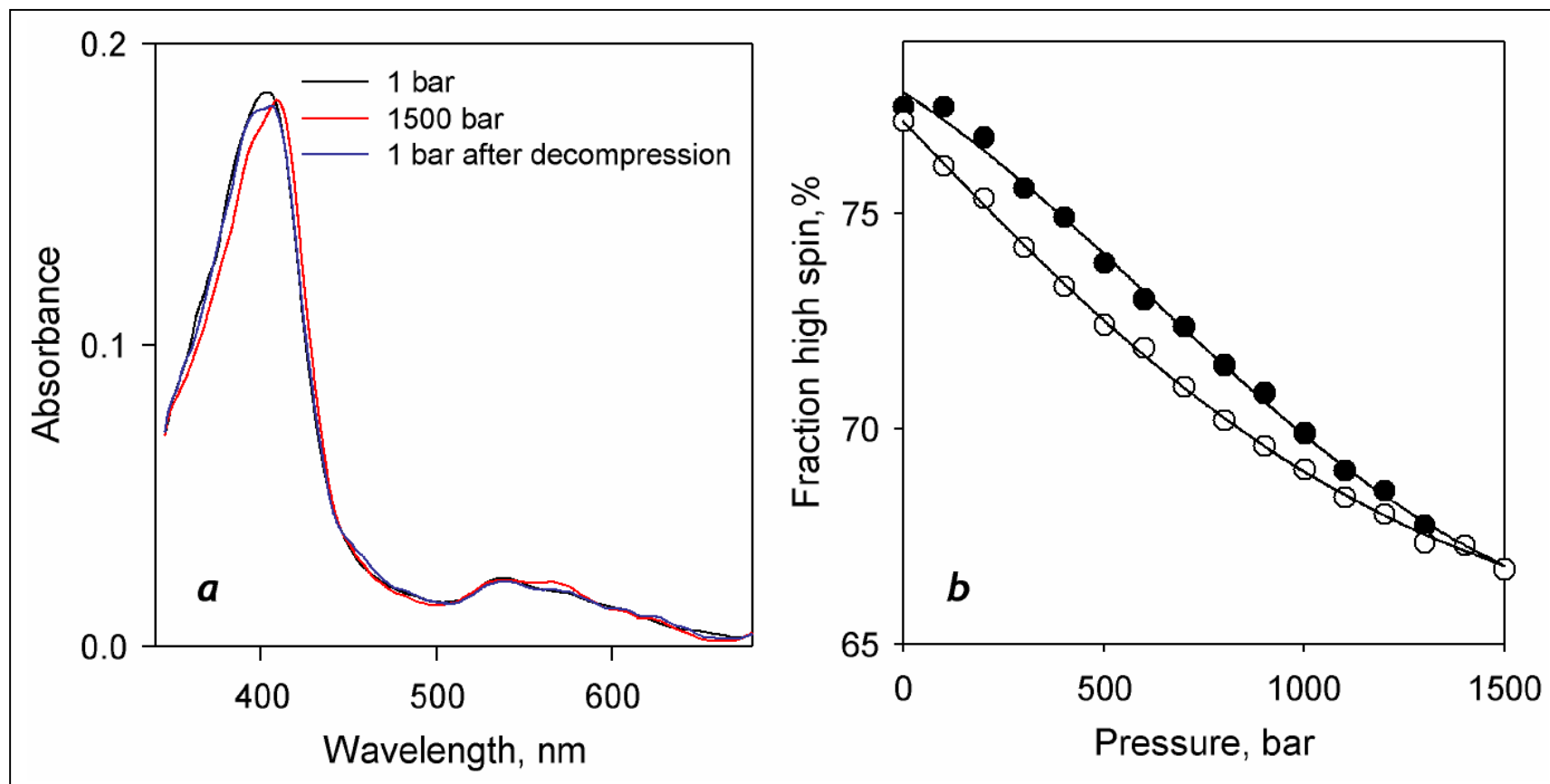


Figure S2. Reversibility of the pressure-induced spin transition in P450-SS9. Panel *a* shows the spectra of P450-SS9 recorded at 1 bar, at 1500 bar and at 1 bar after decompression from 1500 bar. Panel *b* illustrates the changes in the content of the high spin state of P450-SS9 as a function of pressure during pressurization (filled circles) and depressurization (empty circles).

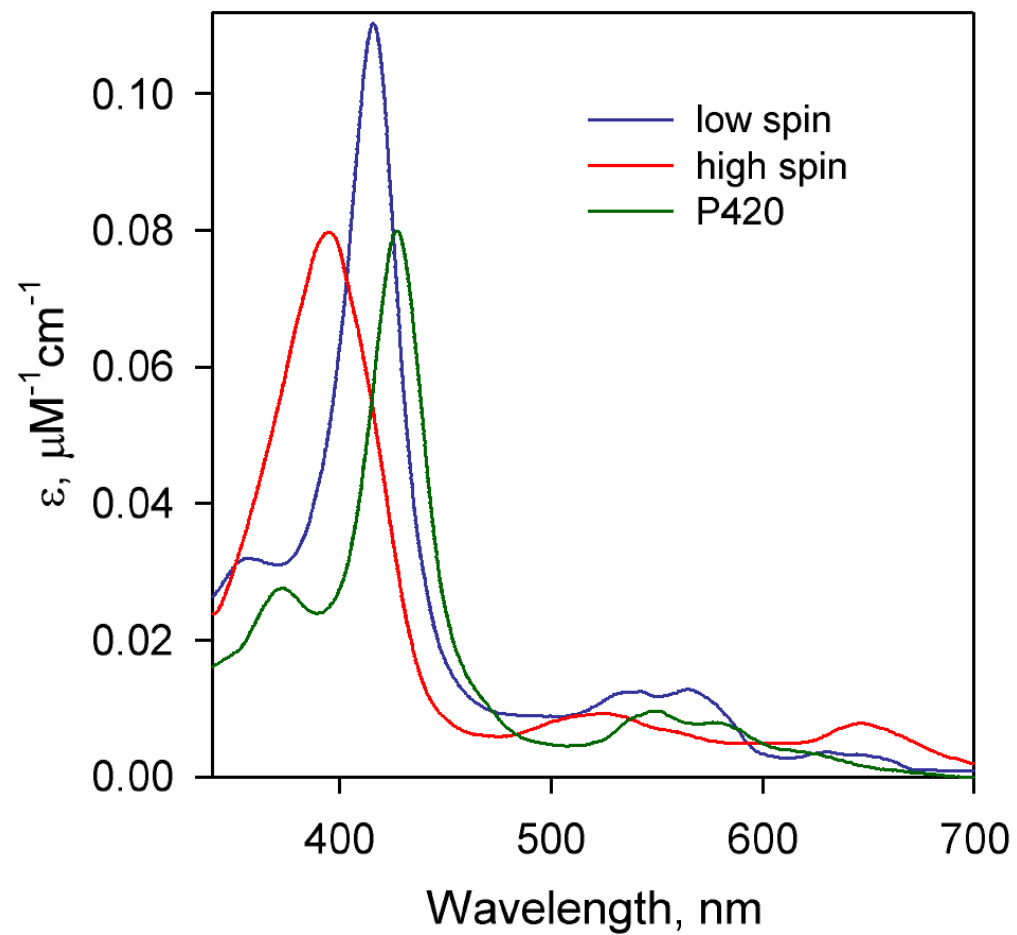


Figure S3. Set of the prototype spectra of the low-spin, high-spin and the apparent ferric P420 state of cytochrome P450-SS9. This set of spectral standards is also available in a tabular form (as a comma-delimited text file found in Supporting Material).

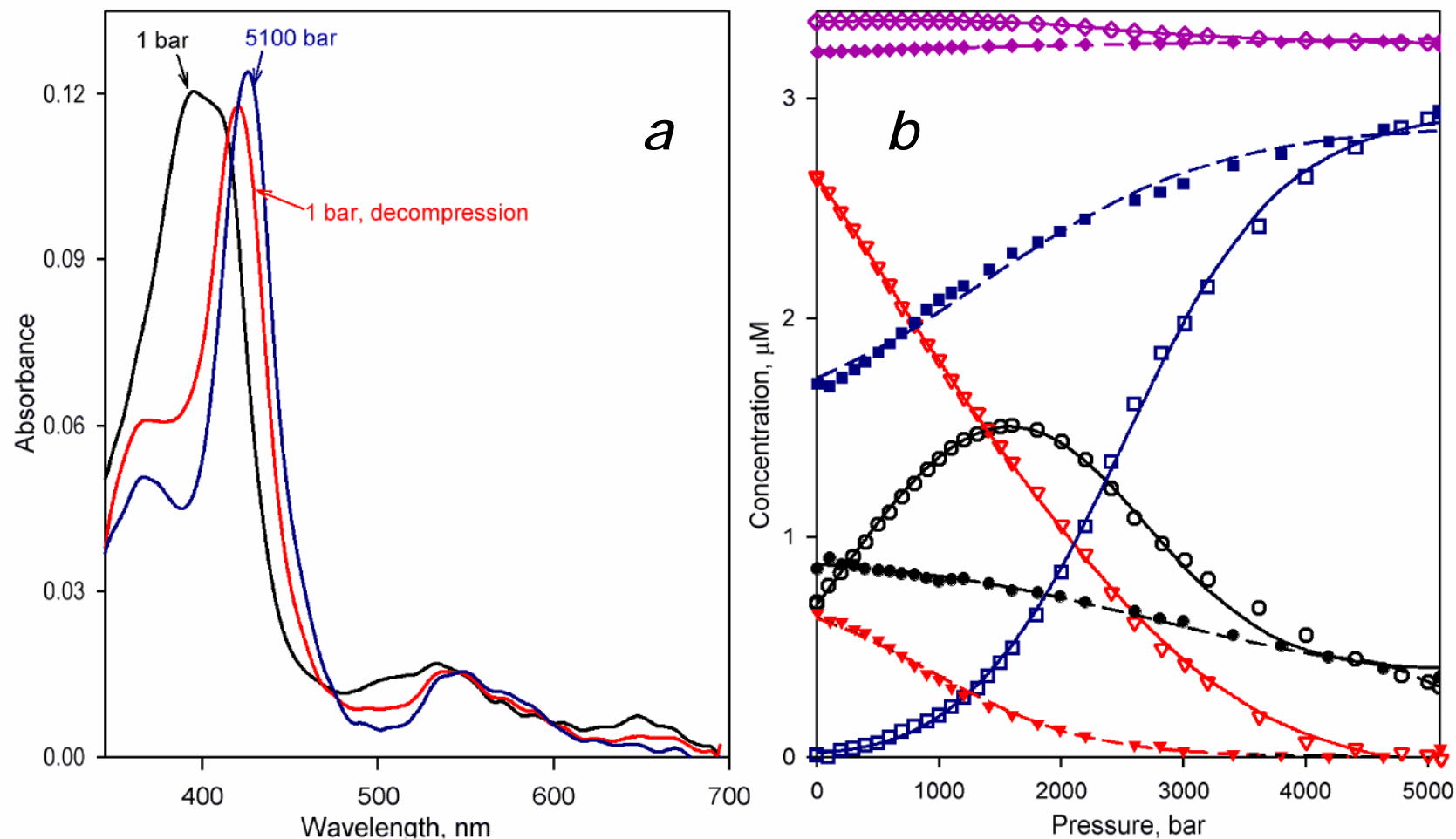


Fig. S4. Partial reversibility of pressure-induced transitions in P450-SS9. The figure represents the results of the same experiment, as shown in Fig. 6 of the manuscript. Panel *a* shows the spectra of P450-SS9 taken at 1 bar before the compression (black), at 5100 bar (blue) and at 1 bar after decompression (red). Panel *b* shows the changes in the concentrations of the low-spin (black), high-spin (red), the apparent P420 heme protein (blue) and the total heme protein content (magenta). The data points obtained upon pressure increase are shown in empty symbols and solid lines, while the filled symbols and dashed lines symbolize the data set obtained upon decompression.