# Supplemental Information

**for**

**Compound I is the reactive intermediate in the first monooxygenation step during conversion of cholesterol to pregnenolone by cytochrome P450scc (CYP 11A1): EPR / ENDOR/cryoreduction-annealing studies**

Roman Davydov,[1] Andrey A. Gilep,[2] Natallia V. Strushkevich,[3]

Sergey A.Usanov,[2*] and Brian M. Hoffman[1*]

[1]Northwestern University, Department of Chemistry, 2145 Sheridan Road, Evanston, IL 60208

[2]Institute of Bioorganic Chemistry National Academy of Sciences of Belarus, 220141 Minsk, Kuprevicha 5, Belarus

[3]Structural Genomics Consortium, University of Toronto, 101 College Street, Toronto, Ontario, M5G 1L7, Canada

Table S1**:** Spin-Hamiltonian parameters for cryogenerated iron(III) hydroperoxy intermediates as annealed to a temperature just below the decay temperature

| Protein | $g_1$ | $g_2$ | $g_3$ | $A_{max}$ (MHz)[a] | Ref |
|---|---|---|---|---|---|
| P450scc-CH | 2.366<br>2.34 | 2.182<br>2.182 | 1.949<br>1.949 | <br>8.5 | this |
| P4502B4- BHT | 2.32 | 2.156 | nd | 10.4 | 2 |
| gsNOS-arginine | 2.31 | 2.182 | 1.95 | 8.4 | 3 |
| P450cam-Cam | 2.30 | 2.14 | nd` | 11.2 | |
| CPO | 2.28 | 2.254 | 1.918 | 12 | 5 |
| | | | | | |
| Heme oxygenase | 2.37 | 2.257 | 1.908 | 12.9 | 4 |
| HRP | 2.32 | 2.254 | 1.907 | 8.2 | 5 |
| β Chains | 2.303 | 2.18 | 1.946 | 12 | 5 |

[a]Maximum superhyperfine coupling for proton of hydroperoxide ligand

References

1. Davydov, R.; Makris, T. M.; Kofman, V.; Werst, D. W.; Sligar, S. G.; Hoffman, B. M. *J. Am. Chem. Soc.* **2001**, *123*, 1403.
2. Davydov, R.; Razeghifard, R.; Im, S.-C.; Waskell, L.; Hoffman, B. M. *Biochemistry* **2008**, *47*, 9661.
3. Davydov, R.; Sudhamsu, J.; Lees, N. S.; Crane, B. R.; Hoffman, B. M. *J. Am. Chem. Soc.* **2009**, *131*, 14493.
4. Davydov, R.; Kofman, V.; Fujii, H.; Yoshida, T.; Ikeda-Saito, M.; Hoffman, B. *J. Am. Chem. Soc.* **2002**, *124*, 1798.
5. Unpublishede data

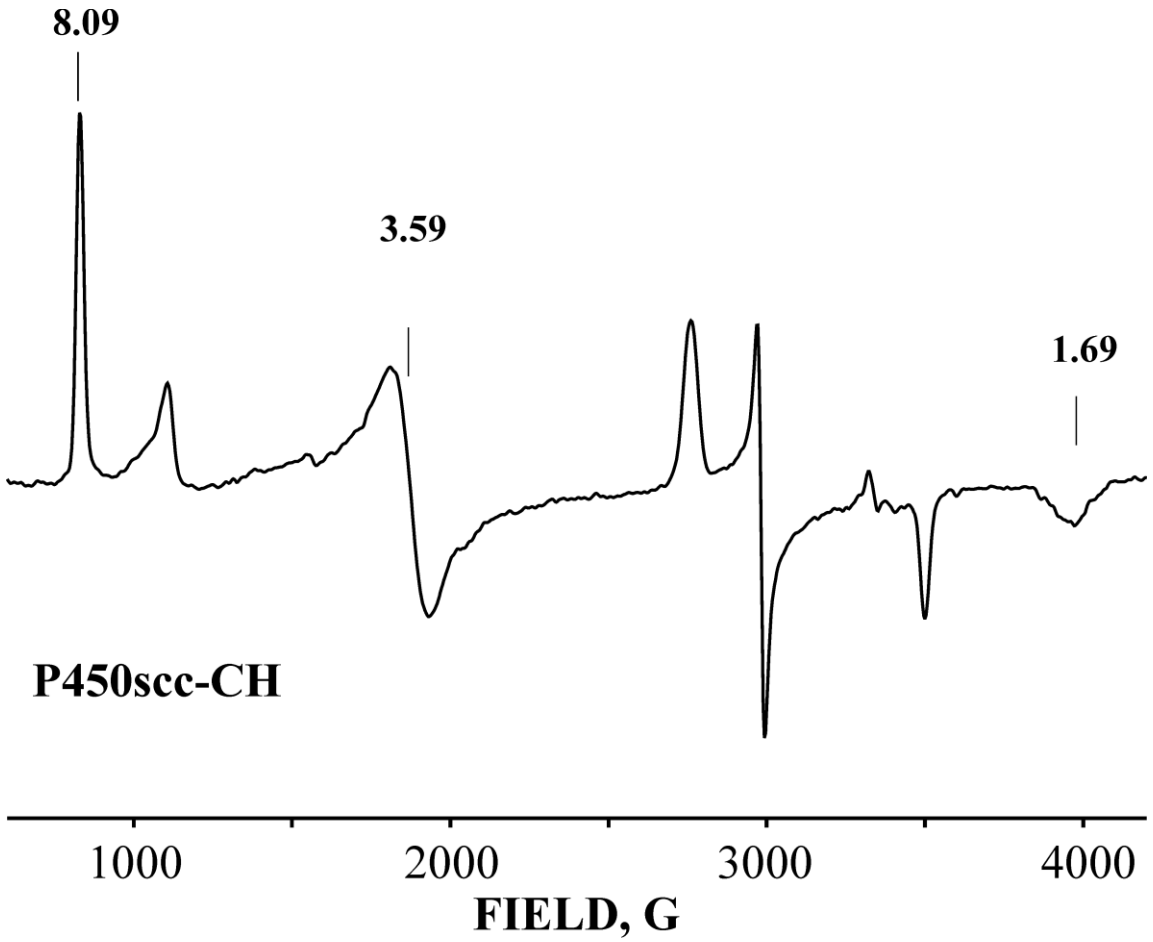


**Fig. S1** X-band EPR spectrum of 0.3 mM ferric P450scc in the presence of 1mM CH in 0.1 M KPi buffer pH 7.4 containing 20% glycerol. Instrument conditions: Am=10G, P=2 mW, f=9.381 GHz, T=10K.

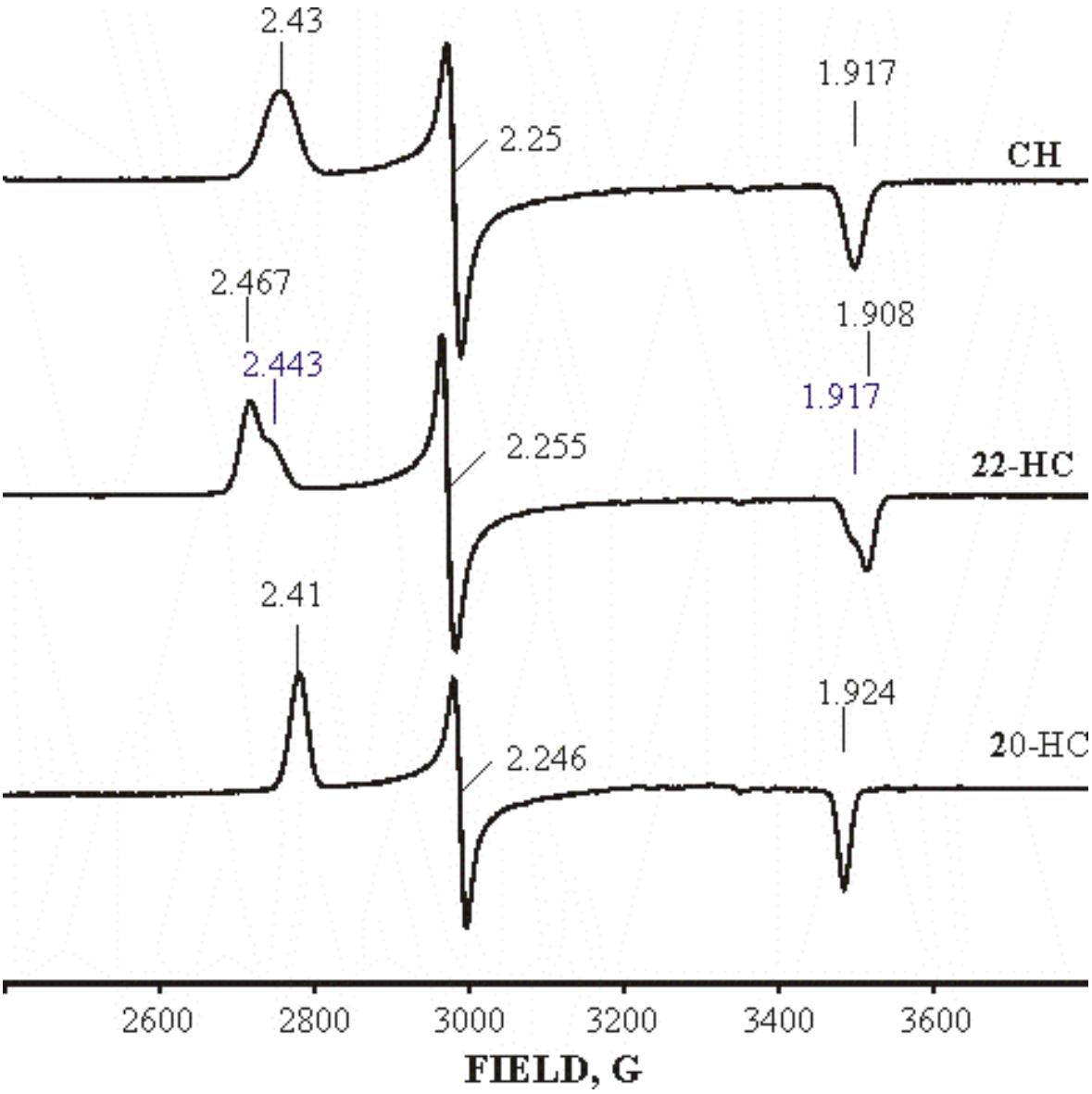


**Fig. S2** X-band EPR spectra of low-spin forms of complexes of Fe(III) P450scc with cholesterol, 22-HC and 20-HC. Instrument conditions: Am=10G, P=10 mW, f=9.381 GHz, T=28K.

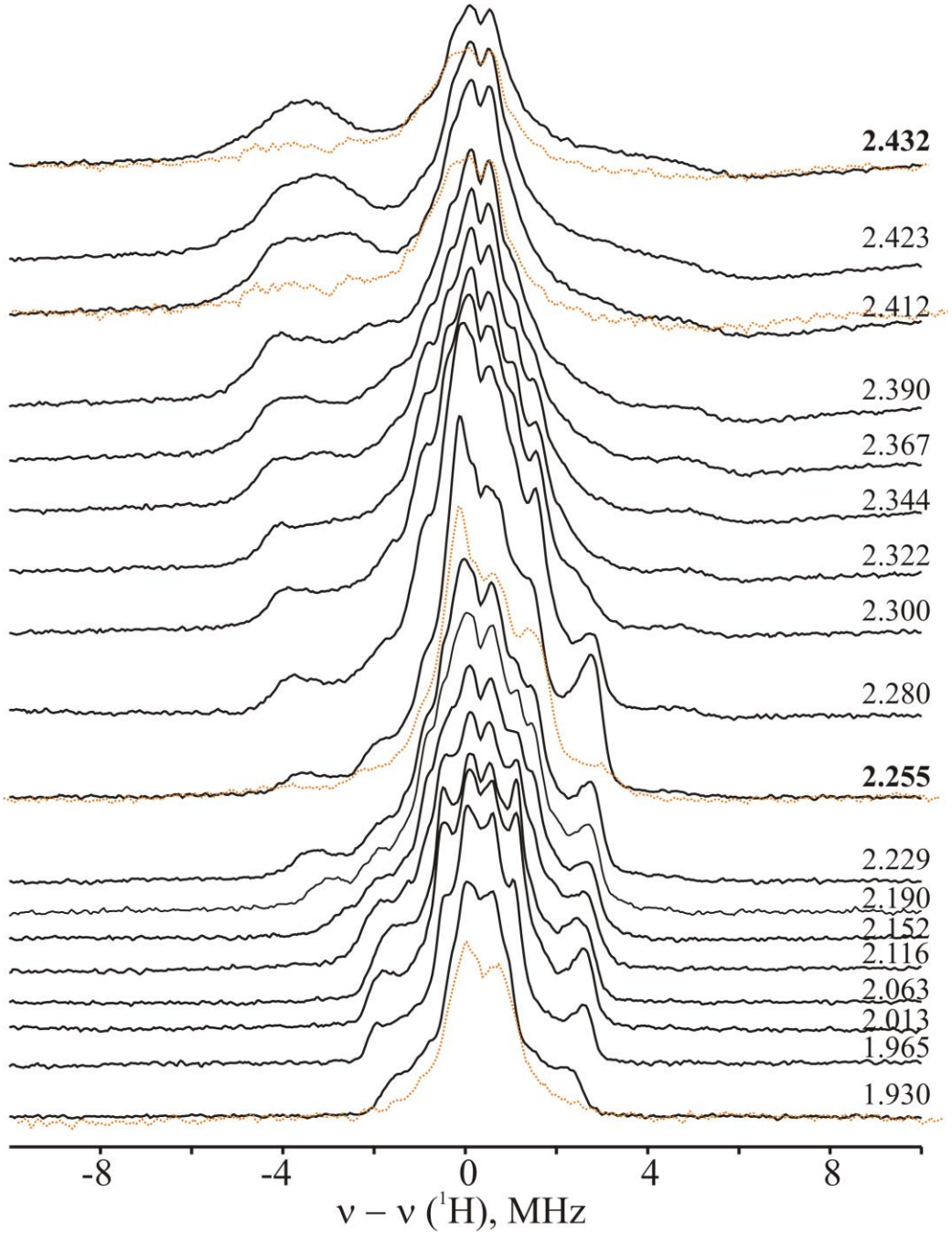


**Fig. S3** Orientation selected $^{1}$H 35 GHz CW ENDOR spectra of Fe(III)P450scc-CH in $H_2O$(black solid line)and in $D_2O$ (red dotted line). Instrument conditions: T=2K, 2G, rf sweep rate= 1Mhz, bandwidth broadening =60kHz, 30scans.

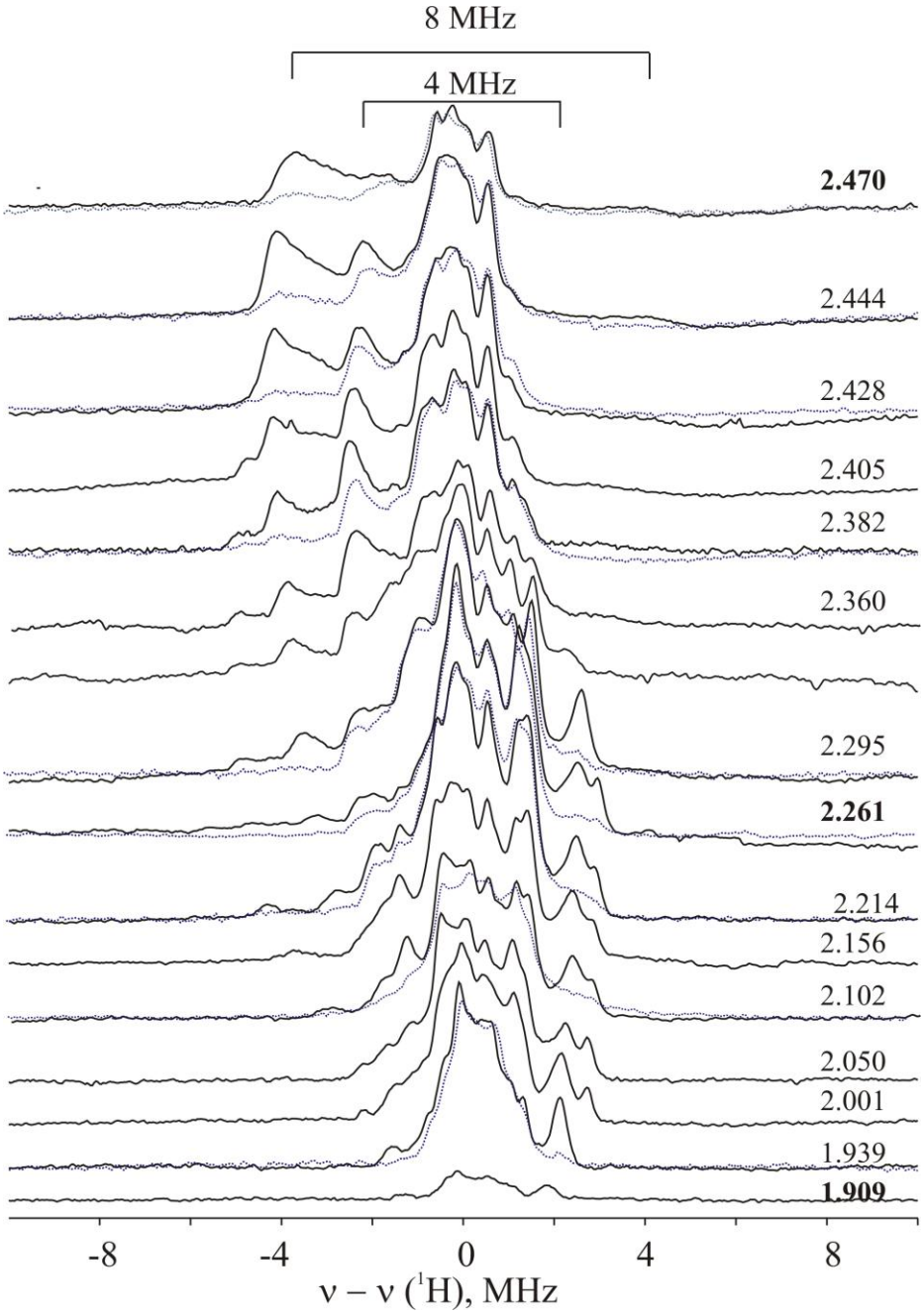


**Fig. S4** Orientation selected $^{1}$H 35 GHz CW ENDOR spectra of Fe(III)P450scc-22-HC complex in $H_2O$(black solid line)and in $D_2O$ (blue dotted line). Instrument conditions: T=2K, 2G, rf sweep rate= 1Mhz, bandwidth broadening =60kHz, 30scans.

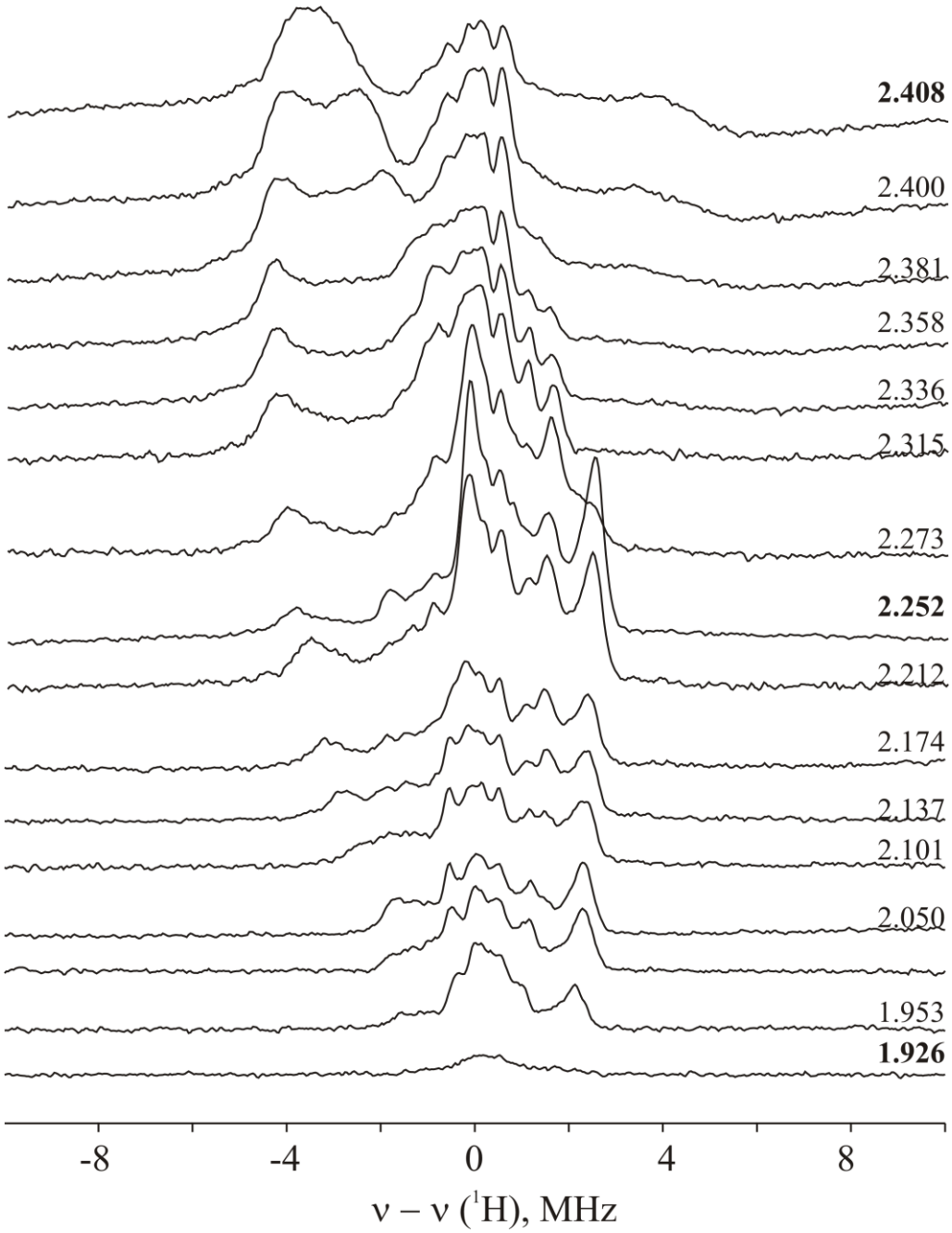


**Fig. S5** Orientation selected $^{1}$H 35 GHz CW ENDOR spectra of Fe(III)P450scc-20-HC complex in $H_2O$. Instrument conditions: T=2K, 2G, rf sweep rate= 1Mhz, bandwidth broadening =60kHz, 30scans.

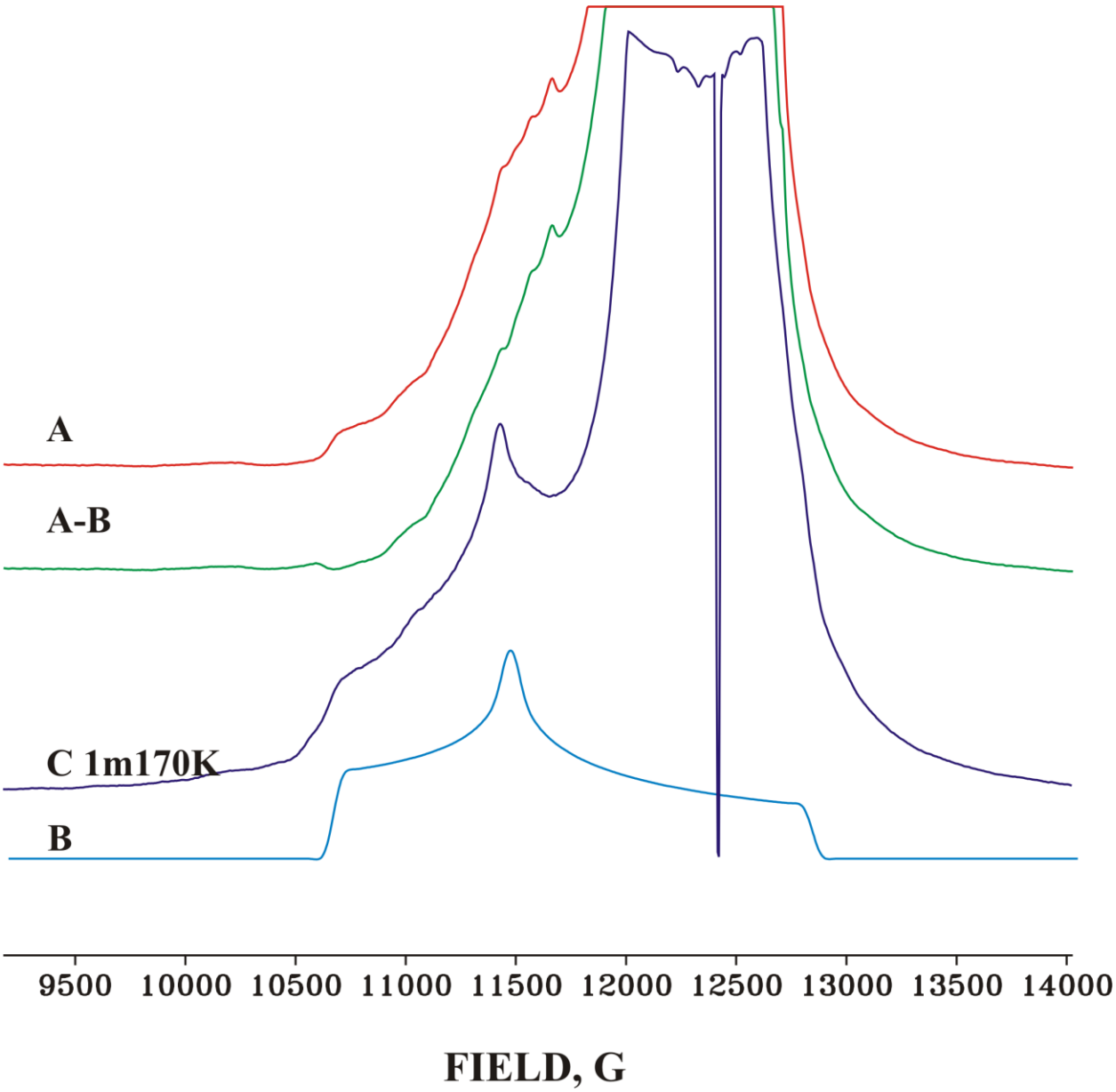


**Fig. S6** 2K 35GHz EPR spectra of cryoreduced oxy-P450scc-CH annealed at 126K (A) and 170K (C) and simulated EPR signal A species (B). The difference signal A-B obtained by subtraction of scaled signal B from signal A. Integration of visible part of difference signal A-B and signal C show that relative contributions of hydroperoxo-feric P450scc species A (70%) and the minor species B and C(`30%) in cryoreduced oxy-P450scc-cholesterol complex.