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Supporting Information for

Temperature-Dependent ESR and Computational Studies on Antiferromagnetic Electron Transfer in the Yeast NADH Dehydrogenase Ndi1

Kaiqi Wu,†a Wenfei Li,†b Lu Yu,†c Wei Tong,c Yue Feng,b Shenglong Ling,a Longhua Zhang,a Xiao Zheng,*a Maojun Yang,*b and Changlin Tian,*ac

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[a] *Hefei National Laboratory for Physical Sciences at Microscale, School of Life Sciences, University of Science and Technology of China, Hefei, 230026, P. R. China.*

[b] *State Key Laboratory of Biomembrane and Membrane Biotechnology,Tsinghua-Peking Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing, 100084, P. R. China.*

[c] *High Magnetic Field Laboratory, Chinese Academy of Sciences, Hefei, 230031, P. R. China.*

†. These authors contributed equally.

*. Corresponding authors: xz58@ustc.edu.cn (X.Z.), maojunyang@tsinghua.edu.cn (M. Y.) and cltian@ustc.edu.cn(C.T.)

Materials and Methods.

Over-Expression and Purification of Ndi1 and GadC.

Wide typeNdi1 and mutants H397F and H397A were over-expressed in *E. coli* strain C43 (DE3) at 35°C with shaking at 80 rpm. When the cell density reached an absorbance of 1 at 600 nm, expression was induced with 0.5 mM IPTG and cells were cultured for a further 10–12 h. After harvesting by centrifugation, cells were homogenized in 50 mMTris (pH 8.0) and 1 mM EDTA. After disruption by sonication, low-speed centrifugation was performed for 15 min to remove cell debris, and the supernatant was ultracentrifuged at 150,000 *g* for 1 h. The membrane pellet was resuspended in 50 mMTris (pH 7.6), 200 mMNaCl, 0.1 mM EDTA and 10% glycerol (buffer A). After adding DDM to a final concentration of 0.5% (w/v) and 1 mM PMSF, the sample was slowly stirred for 2 h at 4°C. Ultracentrifugation was repeated at 150,000 *g* for 30 min, and the supernatant was loaded onto Ni^{2+}-nitrilotriacetate affinity resin (Ni-NTA, QIAGEN, Germany) which was pre-equilibrated with buffer A containing 0.2% DDM. The resin was washed three times with 5 ml of buffer A containing 0.2% DDM and 8 mM histidine, and bound proteins were eluted with buffer A containing 0.02% DDM and 200 mM histidine. Eluted Ndi1 was concentrated and further purified by gel filtration chromatography on a Superdex-200 10/30 column (GE Healthcare) equilibrated in 25 mMTris (pH 7.6), 300 mMNaCl, 5 mM DTT, 0.1 mM EDTA and 0.02% DDM. Peak fractions were collected and used in ESR experiments.

Two Ndi1 samples were prepared for ESR measurements: Ndi1(DDM)-NADH was prepared by addition of excess NADH to purified Ndi1 in the presence of DDM that already contained NADH, FAD and UQ_I; Ndi1(DDM)-NADH-UQ was prepared by addition of excess NADH and UQ_6 to purified Ndi1 protein in the presence of DDM that already contained NADH, FAD, UQ_I and UQ_{II}.

GadC (PDB #: 4DJI) was over-expressed in *E. coli* strain BL21 (DE3) at 37°C with shaking at 225 rpm and expression was induced with 0.4 mM IPTG when the cell density reached an absorbance at 600 nm of 1.1. Following culturing for a further 6 h, cells were harvested by centrifugation at cell density of 1.6, resuspended in 70 mMTris (pH 8.0) and 100 mMNaCl, sonicated at 4°Cand centrifuged at low-speed for 30 min to remove cell debris. The supernatant was then centrifuged at 17,000 *g*, 4°C for 2 h, and the resultant membrane pellet was resuspended in Empigen at a final concentration of 3.5% (v/v) and stirred at 4°Cfor 2 h. Following a second high-speed centrifugation step, the supernatant was loaded onto Ni2+-nitrilotriacetate affinity resin (Ni-NTA, Qiagen) that was pre-equilibrated in 20 mMTris (pH 8.0), 200 mMNaCl (binding buffer) and 0.5% (v/v) Empigen. The resin was washed with binding buffer containing 0.5% (v/v) Empigen, then with binding buffer containing 0.2% (v/v) DPC, and finally with and binding buffer containing 0.2% (v/v) DPCand 30 mM imidazole. The GadC protein was eluted with binding buffer containing 0.5% (v/v) DPC and 250 mM imidazole, and peak fractions were used immediately in ESR experiments.

Temperature Dependent ESR Experiments.

Samples of Ndi1-NADH-FAD-UQ_I and Ndi1-NADH-FAD-UQ_I-UQ_II for ESR experiments were prepared in an anaerobic environment. After concentrating the sample to 50 μM, Ndi1 was incubated with or without 5 mM UQ for 1 min, and NADH was added to a final concentration of 2 mM and incubated for a further 1 min. Samples were transferred to a quartz ESR tube and frozen in liquid helium at a temperature of 2 K. ESR spectra were collected using a Bruker X-band EMSplus10/12 spectrometer equipped with a cylindrical resonator (ER 4119hs, TE011), and cryogenic temperatures were maintained throughout the experiment with a liquid-helium continuous-flow cryostat (Oxford Instrument ESR 910). ESR spectra were collected at temperatures of 2, 10, 20, 30, 40, 50, 60, 70, 80, 100 and 200 K. ESR parameters were as follows: center field = 3350 G; modulation amplitude = 5.0 G; modulation frequency = 100.0 kHz; microwave power = 7.96 μW; sweep time = 32.0 s; time constant = 81.92 ms.

GadC mutants were immediately reacted with a tenfold molar excess of spin radical 1-oxyl-2,2,5,5-tetramethyl-Δ3-pyrr-oline-3-methyl methanethiosulfonate (MTSL; Toronto Research Chemicals, Ontario, Canada) at room temperature for 30 min and then at 4°C overnight. Excess spin reagent was removed by gel filtration chromatography in binding buffer containing 0.2% DPC. Spin-labeled samples in DPC micelles were subsequently applied for temperature dependent CW-EPR measurement.

Heisenberg Exchange J Coupling Constant Fitting.

Since the ESR peak intensity is proportional to spin susceptibility, temperature-dependent ESR spectra represent the magnetic susceptibilities of charge-transfer complexes at different temperatures. The *van Vleck* formula was applied to derive the magnetic exchange interaction (Heisenberg exchange J coupling) constants. The Lorentzian line-shape was applied during J constant fitting against temperature-dependent ESR peak intensities. For a one dimensional Heisenberg spin chain, the temperature dependence of χ can be described by the *van Vleck* formula as follows:

$$\chi = \frac{N\sum_n (-E_n^{(1)2}/kT - 2E_n^{(2)})\exp(-E_n^{(0)}/kT)}{\sum_n \exp(-E_n^{(0)}/kT)}$$

Here, $En^{(0)}$ is the energy of the system in zero field while $En^{(1)}$ and $En^{(2)}$ account for the first- and second-order Zeeman interactions, respectively. The latter also involves the exchange interaction between two electron spins.

Quantum Chemical Calculation of J Constants, Molecular Orbitals and Spin Densities.

Quantum chemical calculations were performed using the Our N-layered Integrated molecular Orbital+ molecular Mechanics method with the apo-protein structure (without the cofactors) set as the low level and the FAD-UQ$_I$ and UQ$_I$-UQ$_{II}$ charge transfer complexes set as the high level. For the low level, amber field mechanical dynamics were applied for ground state energy calculation and geometry optimization. For the high level electron transfer complexes, CASSCF(4,4)/aug-cc-pVDZ theory was applied for calculation of ground state energy and geometry optimization. J constants and the stacking effects on J constants (from plots of J constants against different rotational angles) were calculated based on each optimized geometry. Both molecular orbital and spin densities were calculated from Ndi1 crystal structure coordinates (PDB# 4G73 for Ndi1 (DDM)-NADH; PDB# 4G74 for Ndi1 (DDM)-NADH-UQ) using DFT based on ub3lyp-6311G++(d,p) by mixing HOMOs and LUMOs. J constants, molecular orbitals and spin densities were all calculated using the Gaussian09W suite.

Heisernberg Exchange J Coupling Constants Fitting against Temperature Dependent ESR Intensities using van Vleck Formula

The van Vleck formula was used to describe the relation between magnetic susceptibility and temperature of a 1-D Heisenberg spin chain. The deducing process was listed as below:

The magnetization of a substance could be calculated by the relation describing the variation of energy with respect to field.

$$M = -\frac{\partial E}{\partial H}$$

Similar relation holds for the microscopic magnetization of each molecule as:

$$m_n = \frac{-\partial E_n}{\partial H}$$

The macroscopic magnetization is thus obtained by summing the microscopic magnetization, leading to the final formula:

$$M = \frac{N\sum_n (-\partial E_n / \partial H)\exp(-E_n / kT)}{\sum_n \exp(-E_n / kT)}$$

in which the En(n=1,2,3,...) terms is energy term of a quantum mechaincal origin in the presence of a magnetic field. This term could be evaluated after the assumption of Hamiltonian of the system is specified. According to perturbation theory of quantum mechanics, we have the following expansion:

$$E_n = E_n^{(0)} + E_n^{(1)}H + E_n^{(2)}H^2 + \cdots$$

In which En$^{(0)}$ is the energy of the system in zero field. The second term of En$^{(1)}$ is the first-order Zeeman term, and En$^{(2)}$ is the second-order Zeeman term with the following expression:

$$E_n^{(1)} = <\mathrm{n}|\mathcal{H}_{\mathrm{Ze}}|n>, \quad E_n^{(2)} = \sum_{m\neq \mathrm{n}} <n|\mathcal{H}_{\mathrm{Ze}}|m>^2/(E_n^{(0)} - E_m^{(0)})$$

Where H_{Ze} is as described in the spin Hamiltonian. The other nomenclatures are of the common definitions in perturbation theory. From the expansion and the expression of En$^{(1)}$ and En$^{(2)}$, we have:

$$m_n = -E_n^{(1)} - 2E_n^{(2)}H - \cdots$$

When the condition of H/kT<<1 is fulfilled under which the H is not too large and T is not too low, second approximation is the expansion of the exponential. The final form will be:

$$M = \frac{N\sum_n (-E_n^{(1)} - 2E_n^{(2)}H)(1 - E_n^{(1)}H/kT)\exp(-E_n^{(0)}/kT)}{\sum_n (1 - E_n^{(1)}H/kT)\exp(-E_n^{(0)}/kT)}$$

At zero field, the absence of the magnetiztion requires the M = 0, we get:

$$\sum_n E_n^{(1)} \exp(-E_n^{(0)}/kT) = 0$$

Substituting the relation to the above form gives the final form of susceptibility:

$$\chi = \frac{N\sum_n (E_n^{(1)2}/kT - 2E_n^{(2)})\exp(-E_n^{(0)}/kT)}{\sum_n \exp(-E_n^{(0)}/kT)}$$

This the van Vleck formula. When the states are dengenerate, n represents the degeneracy. For a dimer model in applied field,

$$E_1 = (3/2)J$$
$$E_2 = -(1/2)J + g\mu_B H, \quad E_3 = -(1/2)J, \quad E_4 = -(1/2)J - g\mu_B H$$

in which the (3/2)J and -(1/2)J terms represents En(0). En(0) is the first-order Zeeman term. J is the Heisernberg exchange coupling constant.

Spin Densities Calculation using DFT

To further explicate the origin and direction of charge transfer, the Mulliken spin densities of the ground states for the charge transfer complexes of FAD-UQ_I and UQ_I–UQ_{II} were computed through DFT at UB3LYP/6-311G++(d,p) level of theory, which is believed to obtain reliable spin densities.

Table S1.Spin density values on individual atoms in singlet ground state of FAD-UQ_I complex in Ndi1-NADH at ub3lyp-6311G++(d,p) level of theory (absolute spin density values less than 0.001 were not shown in table S1 and Fig. 2(a)).

Number	Atom	spin density values
8	N	-0.17493
9	C	-0.09732
10	C	0.06553
11	C	-0.14347
12	C	0.01002
13	C	0.06743
14	C	-0.00426
15	C	0.11417
16	C	-0.16065
17	C	-0.12542
18	C	0.05123
19	C	-0.00271
20	O	-0.01891
21	N	0.00505
22	C	0.00697

23	O	-0.03513
24	N	-0.01182
71	N	-0.54423
25	C	0.23696
26	C	-0.10076
27	C	0.18932
28	C	-0.10908
29	C	0.18519
30	C	-0.0898
31	C	-0.01616
32	C	-0.00224
33	C	-0.00134
34	C	0.00536
35	O	0.52359
36	O	0.09118
37	O	-0.01749
38	O	0.08813

Table S2.Spin density values on individual atoms in singlet ground state of UQ_{I}-UQ_{II} complex in Ndi1-NADH-UQ at ub3lyp-6311G++(d,p) level of theory (absolute spin density values less than 0.001 were not shown in table S2 and Figure 2(B)).

Number	Atom	spin density values
1	C	0.10449
2	C	-0.20149
3	C	0.11262
4	C	-0.19261
5	C	0.12009
6	C	-0.25698
8	C	0.00438
9	C	0.00341

10	C	0.01631
11	O	-0.07805
12	O	0.02131
13	O	-0.08055
14	O	-0.53951
15	c	0.02907
16	C	-0.09832
17	C	0.20344
18	C	-0.11099
19	C	0.20307
20	C	-0.1002
21	C	-0.01549
22	C	-0.00551
23	C	-0.00135
24	C	0.00626
25	O	0.51323
26	O	0.12212
27	O	-0.0194
28	O	0.02907

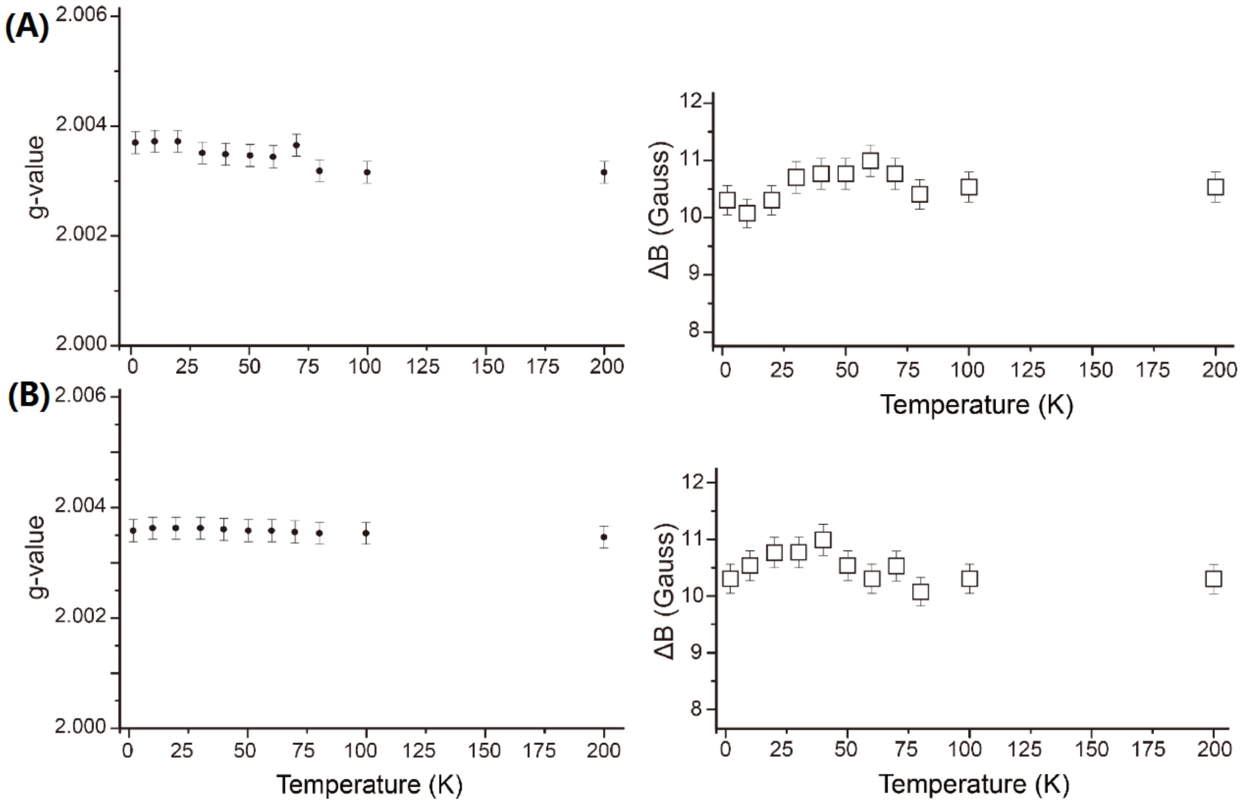


Figure S1. Almost invariant g values and line-width of ESR signals at different temperatures. The g values and line-widths of **(A)** sample Ndi1-NADH-FAD-UQ_I and **(B)** sample Ndi1-NADH-FAD-UQ_I-UQ_{II} were collected at temperatures of 2, 10, 20, 30, 40, 50, 60, 70, 80, 100 and 200 K. There are negligible variations of g values and line-widths of ESR signals under the temperature range from 2 to 200 K.

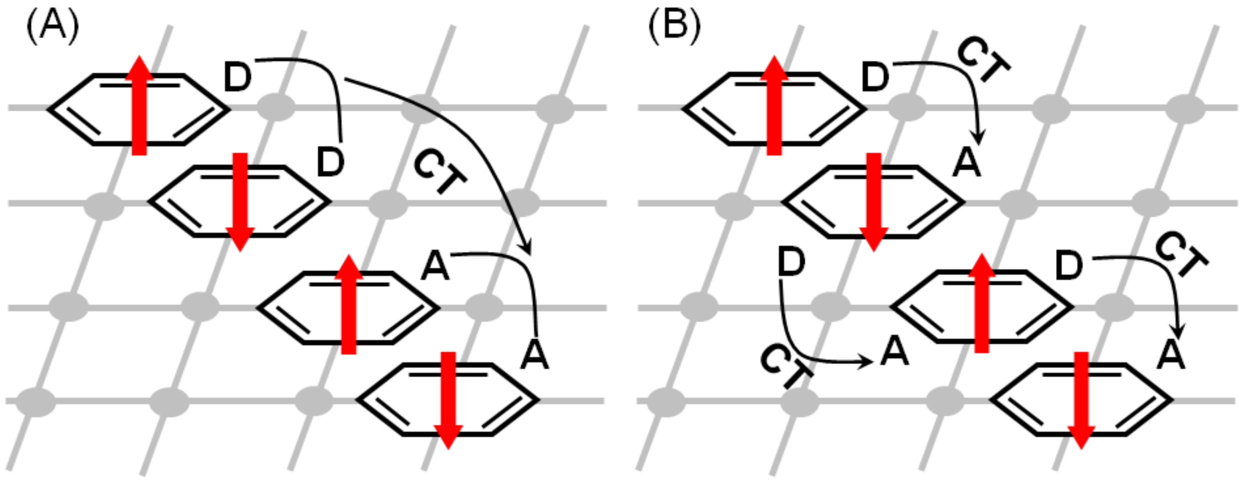


Figure S2. Two different charge-transfer stacks during antiferromagnetic exchange interactions between nearest-neighboring anti-parallel spins in a lattice. **(A)** segregated-stack (...DDD.., …AAA..., D: donor, A: acceptor, CT: charge transfer) in anisotropic lattice.**(B)** mixed-stack (...DADADA...) in anisotropic lattice.

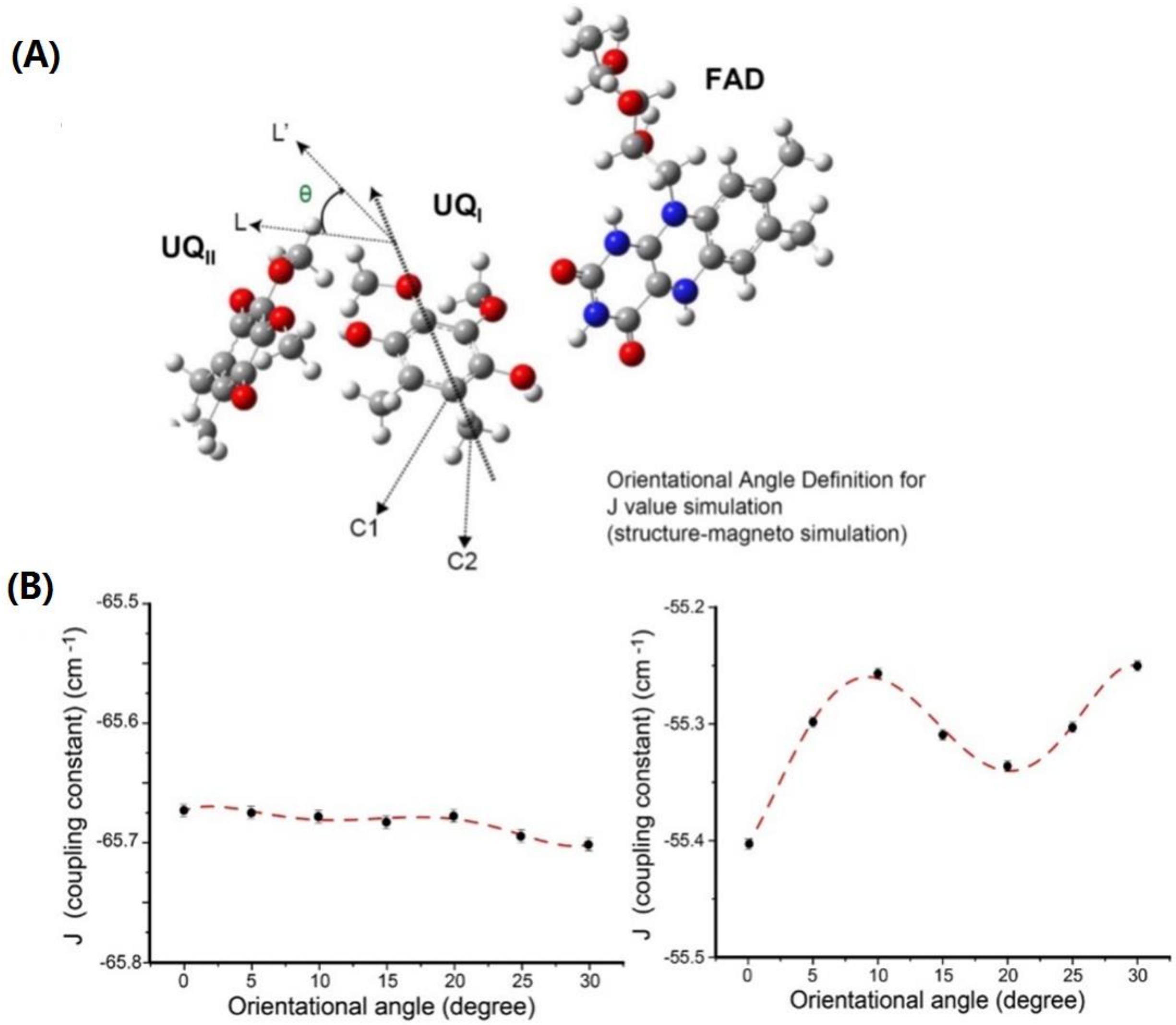


Figure S3. Orientation angle parameters used in the J value simulation. **(A)** The rotation axis passes through atoms C1 and C2 as described. L is in the plane of the aromatic ring of UQ$_{\mathrm{I}}$, perpendicular to the rotation axis. L' is the position of L after a right-handed rotation. The definition is the same for FADH$_2$-UQ$_{\mathrm{I}}$ and UQ$_{\mathrm{I}}$-UQ$_{\mathrm{II}}$. **(B)** J constants were calculated with different rotational angles between the aromatic rings of the cofactors. The FADH$_2$-UQ$_{\mathrm{I}}$ and UQ$_{\mathrm{I}}$-UQ$_{\mathrm{II}}$ pairs are shown on the left and right, respectively. The calculated J value of FADH$_2$-UQ$_{\mathrm{I}}$ was less sensitive to variations in the rotational angle than the UQ$_{\mathrm{I}}$-UQ$_{\mathrm{II}}$ pair.

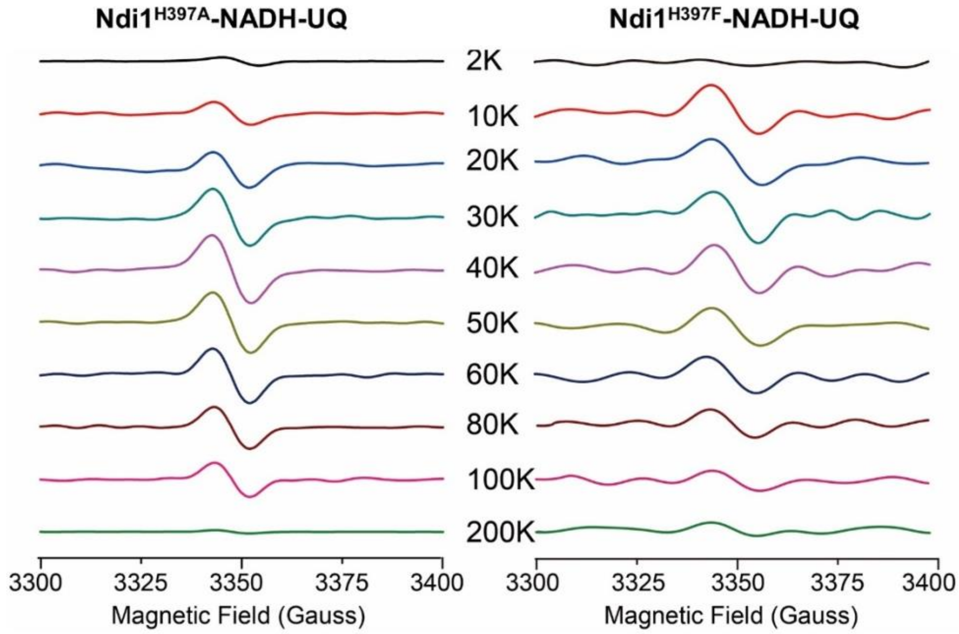
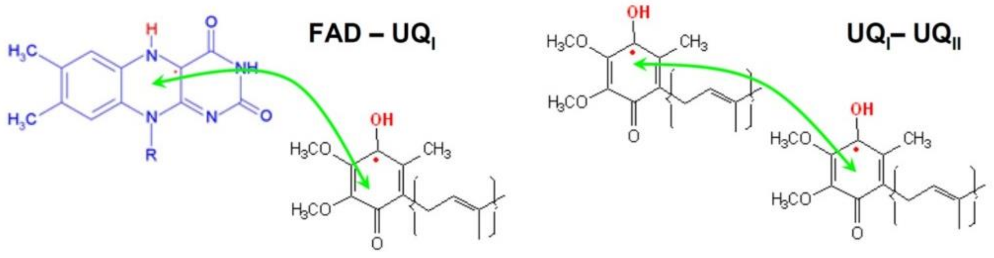


Figure S4. Temperature-dependent ESR measurements of Ndi1 H397A and H397F. ESR spectra of Ndi1 H397A and H397F were collected at temperature of 2 K, 10 K, 20 K, 30 K, 40 K, 50 K, 60 K, 70 K, 80 K, 100 K and 200 K with the same parameters of Figure 1.



	FAD-UQ$_I$	UQ$_I$-UQ$_{II}$
Center-to-Center Distance (Å)	8.5	7.3
Edge-to-Edge Distance (Å)	4.0	4.9

Figure S5. The center-to-center or edge-to-edge distance of FAD-UQ$_I$ and UQI-UQ$_{II}$ pairs in Ndi1 protein. The center-to-center distance of this two pairs are 8.5 Å and 7.3 Å respectively. The edge-to-edge distance obtained from the coordinates of the Ndi1 crystal structure of this two pairs are 4.0 Å and 4.9 Å respectively. These distances are in the physiological electron tunneling range (14 Å), and in the strong influences of dipolar interactions.

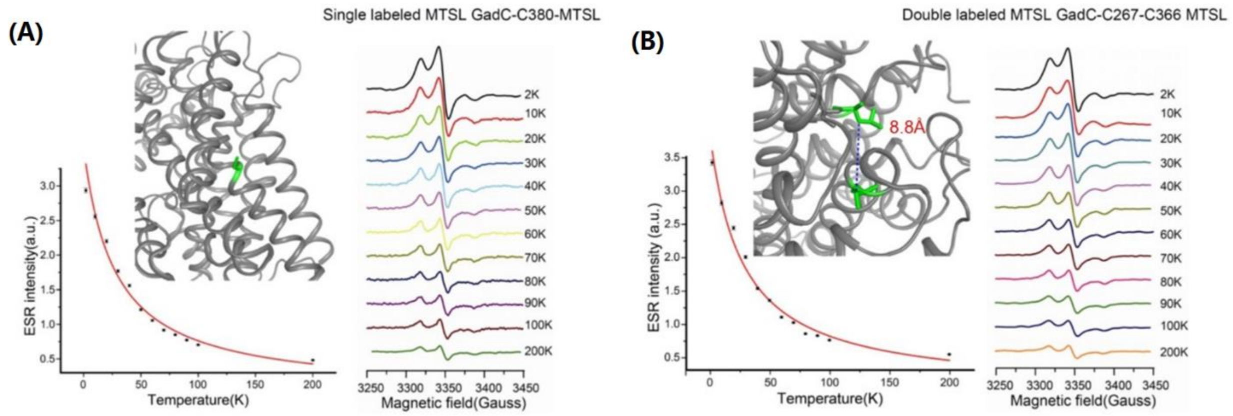


Figure S6. Temperature-dependent ESR spectra of GadC proteins with MTSL radical labeling. **(A)** Ribbon representation of GadC protein is labeled with MTSL radical in the Cys380 (colored green). ESR spectra of GadC-C380-MTSL were collected at temperature of 2 K, 10 K, 20 K, 30 K, 40 K, 50 K, 60 K, 70 K, 80 K, 100 K and 200 K with parameters as follows: center field, 3350 G; modulation amplitude, 5.0 G; modulation frequency, 100.0 KHz; microwave power,7.96 μW; Sweep time, 32.0 s; time constant, 81.92 ms. **(B)** Ribbon representation of GadC protein is labeled by MTSL radicals in the Cys267 and Cys366 (colored green). The distance between the two spins is 8.8 Å. ESR spectra of GadC-C267-C366-MTSL were collected as in Figure S4. Both of the ESR signal intensities were reciprocal to temperature which is the trait of a typical paramagnetic material.