Redox characterization of the FeS protein mitoNEET and impact of thiazolidinedione drug binding

Daniel Bak,[a] John Zuris,[b] Mark L. Paddock,[b] Patricia A. Jennings,[b] Sean J. Elliott[a,*]

[a]Department of Chemistry, Boston University, 590 Commonwealth Ave., Boston, MA 02215, [b]Department of Chemistry and Biochemistry, University of California at San Diego, La Jolla, CA 92093.

Supporting Information

Materials and Methods

Protein Expression, Purification and Redox Titrations – Over-expression and purification of mitoNEET proteins was performed as outlined.[S1] Redox titrations were conducted as described.[S4]

Electrochemistry – Protein Film Voltammetry experiments were performed using a PGSTAT 12 AutoLab (Ecochemie) potentiostat, equipped with FRA and ECD modules. A three eletrode configuration was used, containing a platinum wire counter electrode and a saturated Calomel reference electrode. Potentials were corrected by +242 mV in order to be reported vs standard hydrogen electrode (SHE). Room temperature cell solutions were used containing 0.1 M NaCl and either 5 mM Sodium Acetate, MES, MOPS, and TAPS or 5 mM TAPS, CHES, CAPS, and Sodium Phosphate for either low (5-8) or high (8-12) pH measurements respectively.

Pyrolytic graphite electrodes (PGE) with a surface area of 1.4 mm^2 were used to generate protein films of mitoNEET. Electrodes were sanded down and polished with 1 μm alumina. Sonication was used to remove alumina after which the electrodes were rinsed with clean water. Protein films were grown on electrodes by directly depositing 10 μL of 250 μM mitoNEET in 50 mM Tris, 100 mM NaCl at pH 8 (storage buffer) for approximately 5 minutes, followed by rinsing away excess protein with distilled water. Pioglitazone and Rosiglitazone bound protein films of mitoNEET were generated by first combining equal parts 250 μM mitoNEET in storage buffer and 5.6 mM pioglitazone or 140 mM rosiglitazone in DMSO. After a 5 minute incubation these were then applied as described previously for unbound mitoNEET. (Higher concentrations of rosiglitazone were used in the stock solution, due to the high insolubility of the compound in water.)

Non-turnover electrochemical signals were generated on the benchtop with argon bubbled through the cell solution to remove excess oxygen. Data were collected at a scan rate of 200 mV/sec between potentials of -0.8 V and 0.1 V with a current range of 10 μA using the GPES software package (Ecochemie). Non-turnover signals were analyzed by subtraction of baseline electrochemical response of the electrode surface from the raw data using the SOAS package, courtesy of Dr. Christophe Léger.[S2]

For mitoNEET wild-type and TZD-bound states, either a two-proton/one-electron or a one-proton/one-electron model was used to fit the pH dependent midpoint potentials, according to equations 1[S3] or 2,[S5] respectively.

(1) $E_{m,obs} = E_{alk} - 2.303(RT/nF)*\log\{(1+([H^+]/K_{ox2})+([H^+]^2/K_{ox1}K_{ox2}))/(1+([H^+]/K_{red2})+ ([H^+]^2/K_{red1}K_{red2}))\}$

(2) $E_{m,obs} = E_{acid} + 2.303(RT/nF)*\log\{([H^+] + K_{red})/([H^+] + K_{ox})\}$

Supporting References
(S1)   Wiley, S. E.; Murphy, A. N.; Ross, S. A.; van der Geer, P.; Dixon, J. E. *Proc Natl Acad Sci U S A* **2007**, *104*, 5318-23.
(S2)   Fourmond, V.; Hoke, K.; Heering, H.A.; Baffert, C.; Leroux, F.; Bertrand, P.; Léger, C. *Bioelectrochemistry,* **2009**, 141-147
(S3) Zu, Y.; Couture, M. M.; Kolling, D. R.; Crofts, A. R.; Eltis, L. D.; Fee, J. A.; Hirst, J. *Biochemistry* **2003**, *42*, 12400-8
(S4) Conlan, A.R., Axelrod, H.L., Cohen, A.E., Abresch, E.C., Zuris, J., Yee, D., Nechushtai, R., Jennings, P.A. & Paddock, M.L., , *Journal of Molecular Biology* **2009**, *392*(1), 143-153
(S5) Clark, W. M. In *Oxidation-reduction Potentials of Organic Systems*; Williams and Wilkins: Baltimore, 1960.

Table S1: Reduction Potentials and pK_a values for mitoNEET[a]   and three Rieske proteins[b]

		pK_{ox1}	pK_{ox2}	pK_{red1}[c]		E_{acid} (mV)
1e-:2H$^+$ model	mN	6.5±0.1	10.1±0.1	9.5±0.1	>12.5	40 ±4
	mN:pio	8.2±0.1	10.0±0.2	9.3±0.2	>12	-80 ±3
	mN:rosi	8.7±0.1	10.6±0.2	9.3±0.2	>12	-100 ±3
	*Rs*Rp[S3]	7.6±0.1	9.6±0.1	12.4±0.4	12.4±0.4	308±3
	*Tt*Rp[S3]	7.85±0.15	9.65±0.12	12.5±0.5	12.5±0.5	161±4
	BphF[S3]	9.8±0.2	11.5±0.4	13.3±0.8	13.3±0.8	-135±5
1e-:1H$^+$ model	mN	6.9±0.1	na	11.4 ±0.2	na	16 ±4
	mN:pio	8.8±0.1	na	11.3 ±0.2	na	-90 ±3
	mN:rosi	9.4±0.1	na	11.1±0.2	na	-110 ±3

[a] mN = mitoNEET, mN:pio = mitoNEET bound to pioglitazone, mN:rosi = mitoNEET bound to rosiglitazone

[b] RsRp = *Rhodobacter sphaeroides* Rieske protein, TtRp = *Thermus thermophilus* Rieske protein, BphF = *Burkholderia* sp. Strain LB400 Rieske ferredoxin

[c] For the three Rieske proteins pK_{red1} and pK_{red2} are the same value and for mitoNEET data, pK_{red2} can only be estimated

Figure S1. Comparison of mitoNEET (mN) pH dependent midpoint potentials, with data for pioglitazone (pio) and rosiglitazone (rosi) bound forms of the protein, as shown in Figure 1. Here, the data are fit to a $1H^+$:1-electron stoichiometry, for the sake of comparison.
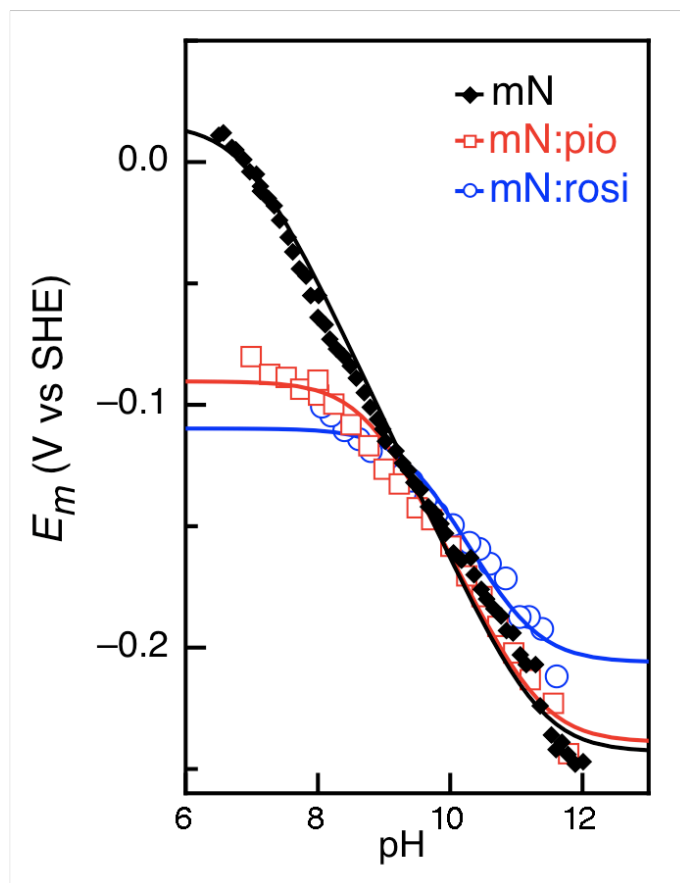
Figure S2. pH dependence of solution redox titrations of wild-type and H87C MitoNEET. The wild-type data are reproduced from reference S4.