Supporting Information

for

Thermodynamics of Various F420 Coenzyme Models as Sources of Electrons, Hydride Ions, Hydrogen Atoms and Protons in Acetonitrile

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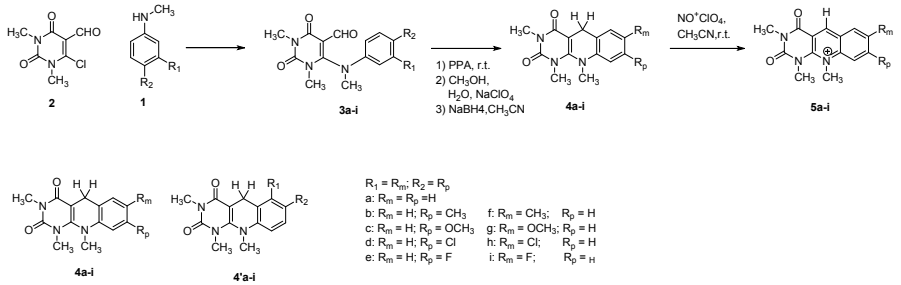
SI–1. General Methods

Solvents and reagents were obtained from commercial sources and used as received. ^{1}HNMR spectra were recorded in $CDCl_3$ and CD_3CN on 400 MHz or 300MHz NMR spectrometer. The chemical shifts (δ) were described in parts per million (ppm) downfield from tetramethylsilane (TMS, 0.00 ppm) as an internal standard.

All reagents of commercial quality were from freshly opened containers or were purified according to the standard methods before use. Reagent grade acetonitrile was refluxed over $KMnO_4$ and K_2CO_3 for several hours and was distilled over P_2O_5 under argon twice before use. The commercial tetrabutylammonium hexafluorophosphate (Bu_4NPF_6, Aldrich) was recrystallized from CH_2Cl_2/Et_2O and dried in vacuo at 110 ^oC overnight before preparation of a supporting electrolyte solution.

Acetonitrile containing 0.1 M tetra-n-butylammonium hexafluorophosphate (Bu_4NPF_6) was used as solvent in electrochemical measurements. Ferrocene/ferrocenium redox couple (Fc/Fc^+) was used as an internal reference for all measurements. All electrochemical measurements were performed under dry nitrogen atmosphere using 0.1 V/s scan rate, unless otherwise specified, the concentration of samples was 10^{-3} M.

SI-2. Synthesis Route of $6FH^-$ and 6F:



Solvents and reagents used here are all commercial available and were used as received. ^{1}H NMR spectra were recorded on 400 MHz or 300 MHz spectrometer using CD_3Cl as a solvent for XFH_2 and CD_3CN as a solvent for XF respectively. The chemical shifts (δ) are given relative to

internal $SiMe_4$ standard.

Substituented N-methylanilines (**1**) and 6-chloro-5-formyl-1,3-dimethyluracil (**2**) can be purchased from the Alfa Company or be synthesized easily according to literature.[S1-S3]

Preparation of 3a-i:

5 Mmol 6-chloro-5-formyl-1,3-dimethyluracil (**2**) was solved in CH_2Cl_2 in a round-bottomed flask, add 5 mmol substituented N-methylanilines (**1**) into the flask, then 0.7ml of dried Et_3N was added, stirred the solution for 10 h at room temperature. Then the reaction mixture was evaporated in vacuo and will get yellow products. The yellow residues are pure enough for the next step, so they do not need any purification, although they can be purified easily through column chromatography by using a mixture of petroleum ether and ethyl acetate (PE : EA = 5 :1) as eluate, for example, the intermediate **3c**.

6-((4-methoxyphenyl)(methyl)amino)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carbaldehyde (**3c**): ^{1}H NMR ($CDCl_3$, 300M): δ 10.05 (s, 1H), 6.85 (d, 4H), 3.79 (s, 3H), 3.41 (s, 3H), 3.37 (s, 3H), 3.06 (s, 3H).

Preparation of 4a-i

Compounds **3(a-i)** without purification are placed in a 50ml flask, then add polyphosphoric acid (PPA) to cover the yellow compounds, the mixture was stirring at room temperature for 12h by using a mechanical stirrer. Then, 30 ml of cold methanol was added into the flask slowly to prevent violent exothermic. When the mixture became liquid from viscous, the mixture was poured into a 500 ml flask which contains 200ml water in it. Add sodium hydroxide to adjust pH to 7 under ice bath, adding sodium perchlorate ($NaClO_4$) slowly while adjusting the pH, and precipitate of the models' salt will appear, add sodium perchlorate until the precipitate stop to increase. The precipitate was filtered. The pale yellow powder collected was then dissolved in acetonitrile directly. Monitored by TLC, the salt has a bright fluorescence under UV lamp. Then add $NaBH_4$ into the solution, monitored by TLC until the bright fluorescence disappeared. The solution was removed by a rotary evaporator, and the white reduced F420 model will be got. Purification through column chromatography will get pure target products (**4a-i**). The eluate used for **4b-e** is PE:EA = 20:1 to separate the by-products **4'b-e**, and for **4f-i**, the eluate can be pure EA

to remove the inorganic compounds produced during reduction.

1,3,10-Trimethyl-5,10-dihydropyrimido[4,5-b]quinoline-2,4(1H,3H)-dione (**4a**): ^{1}H NMR (CD_3Cl, 400M): δ 7.03～7.26 (m, 4H), 3.76 (s, 2H), 3.47 (s, 3H), 3.37(s, 3H), 3.34(s, 3H); ESI-MS/M^+ = 257.

1,3,8,10-Tetramethyl-5,10-dihydropyrimido[4,5-b]quinoline-2,4(1H,3H)-dione (**4b**): ^{1}H NMR (CD_3Cl,400M): δ 7.05 (d, 1H), 6.90 (d, 1H), 6.86 (s, 1H), 3.71 (s, 2H), 3.49 (s, 3H), 3.37(s, 3H), 3.33(s, 3H),2.34(s, 3H); ESI-MS/M^+ = 272.27.

8-Methoxy-1,3,10-trimethyl-5,10-dihydropyrimido[4,5-b]quinoline-2,4(1H,3H)-dione (**4c**): ^{1}H NMR (CD_3Cl, 400M): δ 7.05 (d, 1H), 6.90 (d, 1H), 6.86 (s, 1H), 3.71 (s, 2H), 3.49 (s, 3H), 3.37(s, 3H), 3.33(s, 3H),2.34(s, 3H); ESI-MS/M^+ = 288.33.

8-Chloro-1,3,10-trimethyl-5,10-dihydropyrimido[4,5-b]quinoline-2,4(1H,3H)-dione (**4d**): ^{1}H NMR (CD_3Cl, 400M): δ 7.02~6.96 (m, 3H), 3.64 (s, 2H), 3.39 (s, 3H), 3.30(s, 3H), 3.26(s, 3H); ESI-MS/M^+ = 292.20.

8-Fluoro-1,3,10-trimethyl-5,10-dihydropyrimido[4,5-b]quinoline-2,4(1H,3H)-dione (**4e**): ^{1}H NMR (CD_3Cl, 400M): δ 7.10 (t, 1H), 6.84~6.73 (m, 2H), 3.71 (s, 2H), 3.46 (s, 3H), 3.37 (s, 3H), 3.33 (s, 3H); ESI-MS/M^+ = 276.27.

1,3,7,10-Tetramethyl-5,10-dihydropyrimido[4,5-b]quinoline-2,4(1H,3H)-dione (**4f**): ^{1}H NMR (CD_3Cl, 400M): δ 7.72 (d, 1H), 7.01～6.91 (m, 2H), 3.71 (s, 2H), 3.49 (s, 3H), 3.37 (s, 3H), 3.30 (s, 3H), 2.32 (s, 3H); ESI-MS/M^+ = 272.27.

7-Methoxy-1,3,10-trimethyl-5,10-dihydropyrimido[4,5-b]quinoline-2,4(1H,3H)-dione (**4g**): ^{1}H NMR (CD_3Cl, 400M): δ 6.91 (d, 1H), 6.71～6.68 (m, 1H), 6.62 (d, 1H), 3.72 (s, 3H), 3.66 (s, 2H), 3.40 (s, 3H), 3.30 (s, 3H), 3.21 (s, 3H); ESI-MS/M^+ = 288.20.

7-Chloro-1,3,10-trimethyl-5,10-dihydropyrimido[4,5-b]quinoline-2,4(1H,3H)-dione (**7h**): ^{1}H NMR (CD_3Cl,400M): δ 7.11 (d, 1H), 7.09 (s, 1H), 6.90 (d, 1H), 3.64 (s, 2H), 3.39 (s, 3H), 3.29(s, 3H), 3.24(s, 3H); ESI-MS/M^+ = 292.20.

7-Fluoro-1,3,10-trimethyl-5,10-dihydropyrimido[4,5-b]quinoline-2,4(1H,3H)-dione (4i): ^{1}H NMR (CD_3Cl,400M): δ 6.94～6.92 (m, 1H), 6.87～6.78 (m, 2H), 3.66 (s, 2H), 3.40 (s, 3H), 3.29(s, 3H), 3.23(s, 3H); ESI-MS/M^+ = 276.20.

Preparation of 5a-i

1 Mmol of **4a-i** was dissolved in acetonitrile, and 2 mmol of $NO^+ClO_4^-$ was added, the solution became deep red under stirring, and the yellow perchlorate salt precipitated, filter to get pure perchlorate salt of target models $XFH^+ClO_4^-$.

1,3,10-Trimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimido[4,5-b]quinolin-10-ium (5a): ^{1}H NMR (CD_3CN,400M): δ 9.50 (s, 1H), 8.36(d, 1H), 8.20～8.29(m, 2H), 7.93(t, 1H), 4.31(s, 3H), 3.75(s, 3H), 3.41(s, 3H);

1,3,8,10-Tetramethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimido[4,5-b]quinolin-10-ium (5b): ^{1}H NMR (CD_3CN,400M): δ 9.68 (s, 1H), 9.10 (d, 1H), 8.56～8.04(m, 2H), 4.41(s, 3H), 3.88(s, 3H), 3.55(s, 3H) , 2.80(s, 3H);

8-Methoxy-1,3,10-trimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimido[4,5-b]quinolin-10-ium (5c): ^{1}H NMR (CD_3CN,400M): δ 9.45 (s, 1H), 8.54 (d, 1H), 7.86～7.83(m, 2H), 4.24(s, 3H), 3.99(s, 3H), 3.70(s, 3H) , 3.48(s, 3H);

8-Chloro-1,3,10-trimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimido[4,5-b]quinolin-10-ium (5d): ^{1}H NMR (CD_3CN,400M): δ 9.49 (s, 1H), 8.37 (d, 1H), 8.31 (s, 1H), 7.95～7.92(m, 1H), 4.29(s, 3H), 3.77(s, 3H), 3.44(s, 3H);

8-Fluoro-1,3,10-trimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimido[4,5-b]quinolin-10-ium (5e): ^{1}H NMR (CD_3CN,400M): δ 9.60 (s, 1H), 8.58～8.54(m, 1H), 8.10 (d, 1H), 7.88 (d, 1H), 4.37(s, 3H), 3.87(s, 3H), 3.54(s, 3H);

1,3,7,10-Tetramethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimido[4,5-b]quinolin-10-ium (5f): ^{1}H NMR (CD_3CN,400M): δ 9.42 (s, 1H), 8.16(d, 3H), 4.33(s, 3H), 3.77(s, 3H), 3.43(s, 3H) , 2.64 (s, 3H);

7-Methoxy-1,3,10-trimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimido[4,5-b]quinolin-10-ium (5g): ^{1}H NMR (CD_3CN,400M): δ 9.60 (s, 1H), 8.48～8.11(m, 3H), 4.36(s, 3H), 4.18(s, 3H), 3.78(s, 3H) , 3.44(s, 3H);

7-Chloro-1,3,10-trimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimido[4,5-b]quinolin-10-ium (5h): ^{1}H NMR (CD_3CN,400M): δ 9.52 (s, 1H), 8.50 (s, 1H), 8.33 (s, 2H), 4.42(s, 3H), 3.87(s, 3H), 3.53(s, 3H);

7-Fluoro-1,3,10-trimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimido[4,5-b]quinolin-10-ium (5i): ^{1}H NMR (CD_3CN,400M): δ 9.56 (s, 1H), 8.40～8.38(m, 1H), 8.20 (t, 2H), 4.43(s, 3H),

3.87(s, 3H), 3.54(s, 3H);

Preparation of 4j, 4k and 5j

The single and double deuterated **4j** and **4k**, and one deuterated **5j** were achieved by circulations of reduction of 8a by $NaBD_4$ and oxidation again by $NO^+ClO_4^-$. Details are the same as that described above.

One deuterated 4j: ^{1}H NMR (CD_3Cl,400M): δ 7.21～7.26 (m, 1H), 7.17 (d, 1H), 7.03～7.09 (m, 2H), 3.73 (s, 1H), 3.47 (s, 3H), 3.37(s, 3H), 3.34(s, 3H).

Double deuterated 4k: ^{1}H NMR (CD_3Cl,400M): δ 7.21～7.24 (m, 1H), 7.15(d, 1H), 7.03～7.09 (m, 2H), 3.47 (s, 3H), 3.37(s, 3H), 3.34(s, 3H).

One deuterated 5j: ^{1}H NMR (CD_3CN,400M): δ 8.39～8.41 (d, 1H), 8.24～8.31(m, 2H), 7.95～7.98(t, 1H), 4.35(s, 3H), 3.79(s, 3H), 3.45(s, 3H).

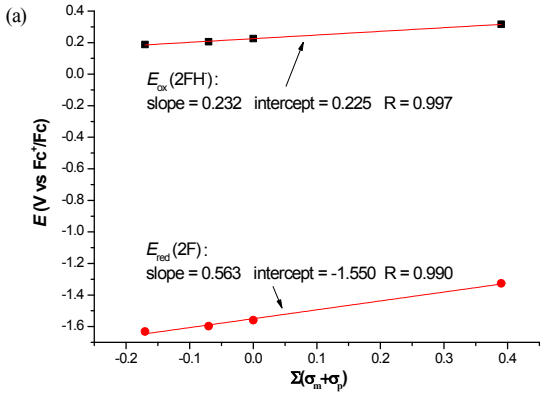
SI-3. References

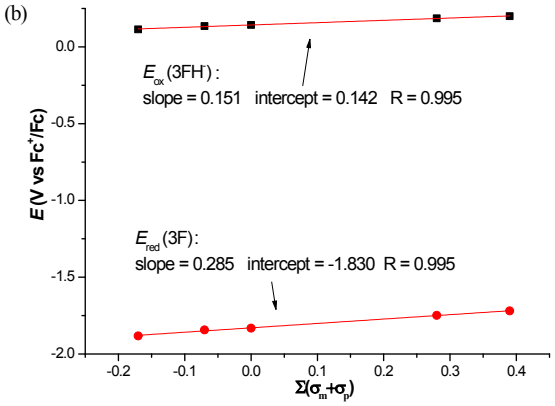
(S1) J. S. Sandhu, D. Prajapati, *Synthesis*, **1988**. *4*, 342-344.

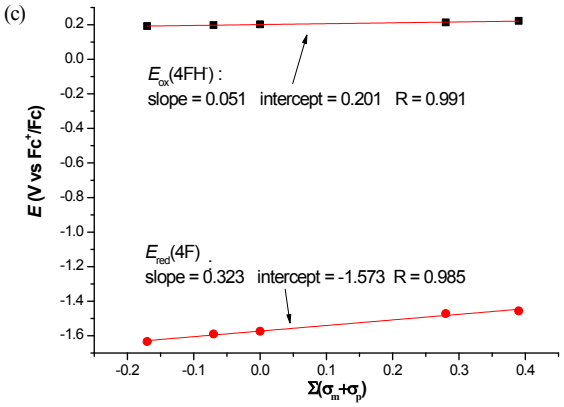
(S2) E. Juaristi, J. D. Reyna, *Tetrahedron Lett*. **1984**. *25(33)*, 3521-3524.

(S3) C. Chiappe, P. Piccioli, D. Pieraccini, *Green Chem.* **2006**. *8(3)*, 277.

SI-4. Plots of $E_{ox}(XFH^-)$ and $E_{red}(XF)$ as well as $\Delta H_{H-D}(XFH^-)$, $\Delta H_{HD}(XFH^-)$, $\Delta H_{HD}(XFH^\bullet)$, and $\Delta H_{PD}(XFH^\bullet)$ against the Sum of Hammett Substituent Parameters σ_p and σ_m







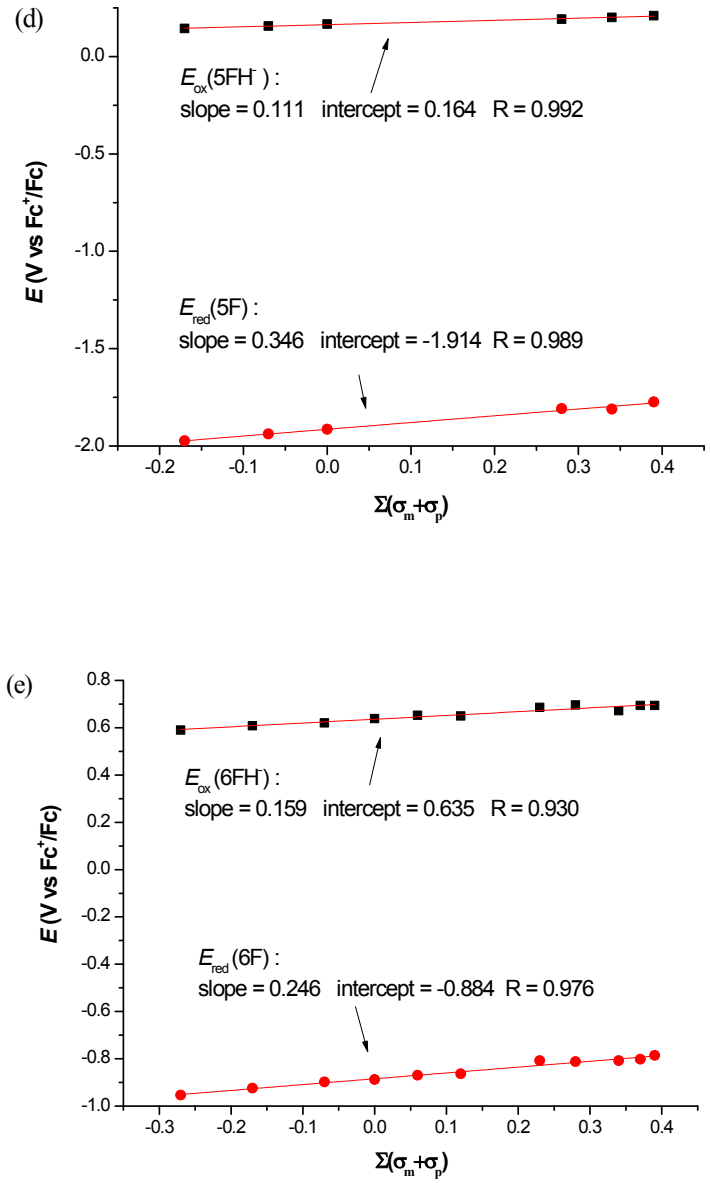
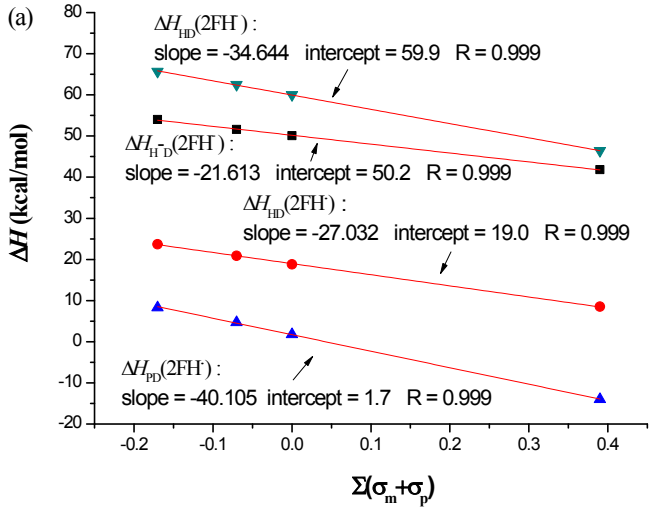
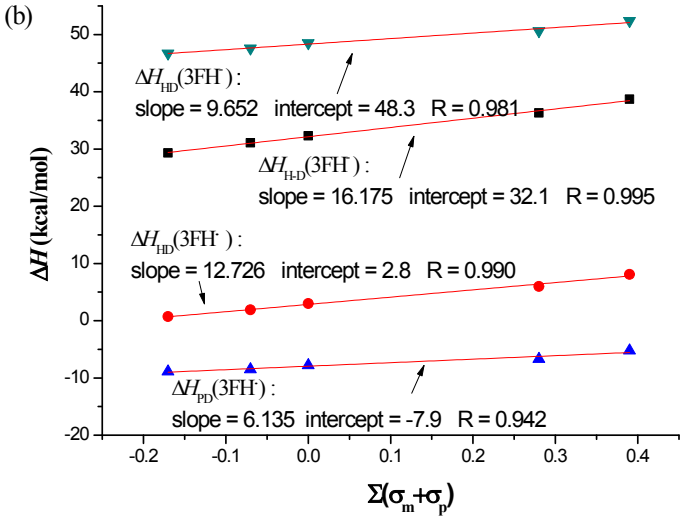
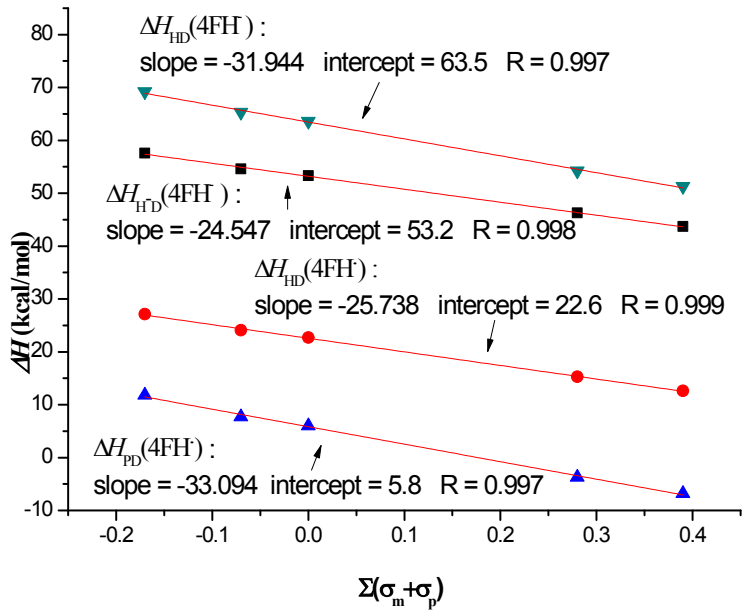


Figure S1. Plots of redox potentials of XFH^- and XF against the sum of Hammett substituent parameters σ_p and σ_m.

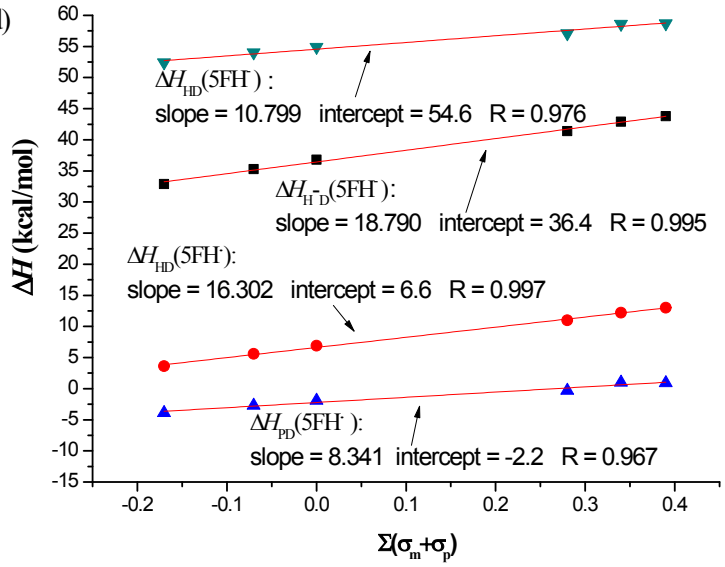

(a)
$\Delta H_{HD}(2FH^-)$:
slope = -34.644 intercept = 59.9 R = 0.999
$\Delta H_{H^-D}(2FH^-)$:
slope = -21.613 intercept = 50.2 R = 0.999
$\Delta H_{HD}(2FH^-)$:
slope = -27.032 intercept = 19.0 R = 0.999
$\Delta H_{PD}(2FH^-)$:
slope = -40.105 intercept = 1.7 R = 0.999
ΔH (kcal/mol)
Σ(σm+σp)


(b)
$\Delta H_{HD}(3FH^-)$:
slope = 9.652 intercept = 48.3 R = 0.981
$\Delta H_{H-D}(3FH^-)$:
slope = 16.175 intercept = 32.1 R = 0.995
$\Delta H_{HD}(3FH^-)$:
slope = 12.726 intercept = 2.8 R = 0.990
$\Delta H_{PD}(3FH^-)$:
slope = 6.135 intercept = -7.9 R = 0.942
ΔH (kcal/mol)
Σ(σm+σp)

(c)


ΔH_HD(4FH⁻) :
slope = -31.944 intercept = 63.5 R = 0.997
ΔH_H⁻D(4FH⁻) :
slope = -24.547 intercept = 53.2 R = 0.998
ΔH_HD(4FH⁻) :
slope = -25.738 intercept = 22.6 R = 0.999
ΔH_PD(4FH⁻) :
slope = -33.094 intercept = 5.8 R = 0.997
ΔH (kcal/mol)
Σ(σ_m+σ_p)

(d)


ΔH_HD(5FH⁻) :
slope = 10.799 intercept = 54.6 R = 0.976
ΔH_H⁻D(5FH⁻):
slope = 18.790 intercept = 36.4 R = 0.995
ΔH_HD(5FH⁻):
slope = 16.302 intercept = 6.6 R = 0.997
ΔH_PD(5FH⁻):
slope = 8.341 intercept = -2.2 R = 0.967
ΔH (kcal/mol)
Σ(σ_m+σ_p)

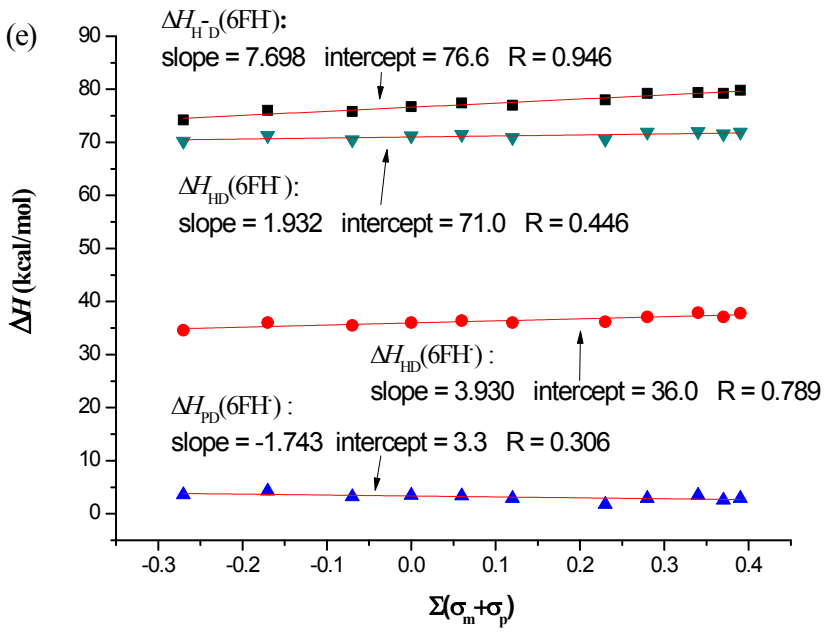


Figure S2. Plots of $\Delta H_{H\text{-}D}(XFH^-)$, $\Delta H_{HD}(XFH^-)$, $\Delta H_{HD}(XFH^{\bullet})$ and $\Delta H_{PD}(XFH^{\bullet})$ against the sum of Hammett substituent parameters σ_p and σ_m