Derivation of $Y_{reduced}$ and $E_{midpoint(0bs)}$ for redox titrations of deoxy-α ± AHSP
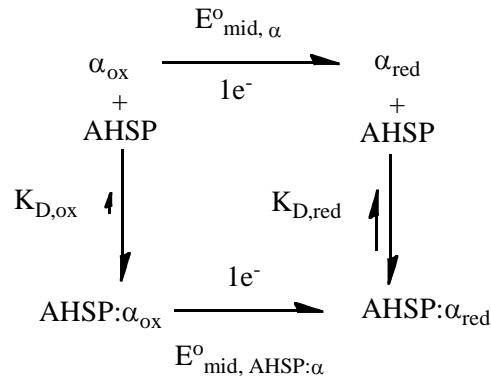
The following derivation is based in large part on Moore and Pettigrew (1):

Thermodynamic Scheme:

$$\alpha_{ox} + AHSP \xrightarrow[1e^-]{E^o_{mid,\,\alpha}} \alpha_{red} + AHSP$$

$$K_{D,ox} \downarrow \qquad\qquad K_{D,red} \updownarrow$$

$$AHSP{:}\alpha_{ox} \xrightarrow[E^o_{mid,\,AHSP:\alpha}]{1e^-} AHSP{:}\alpha_{red}$$

Scheme 1. A thermochemical cycle showing the redox equilibrium of free and AHSP-bound α subunits (horizontal) and the association and dissociation equilibrium (vertical) of AHSP with oxidized and reduced α subunits (α_{Ox} and α_{red}, respectively).
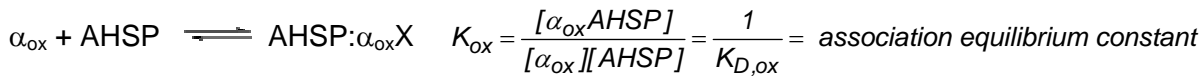
Reactions: Equilibrium equations:

$$E_{halfcell} \quad or \quad E_{obs} = E^0_{mid,Hb} - \frac{RT}{nF} ln\left(\frac{[\alpha_{red}]}{[\alpha_{ox}]}\right) \quad or$$

$$\alpha_{ox} + 1e^- \rightleftharpoons \alpha_{red}$$

$$\frac{[\alpha_{red}]}{[\alpha_{ox}]} = exp\left(\frac{-nF\left(E_{obs} - E^0_{mid,\alpha}\right)}{RT}\right)$$

$$\alpha_{ox} + AHSP \rightleftharpoons AHSP{:}\alpha_{ox}X \qquad K_{ox} = \frac{[\alpha_{ox}AHSP]}{[\alpha_{ox}][AHSP]} = \frac{1}{K_{D,ox}} = \; association\; equilibrium\; constant$$

$$\alpha_{red} + AHSP \rightleftharpoons AHSP{:}\alpha_{red} \qquad K_{red} = \frac{[\alpha_{red}AHSP]}{[\alpha_{red}][AHSP]} = \frac{1}{K_{D,red}}$$

Definition of the Fraction reduced, $Y_{reduced}$:
Define relative concentrations from
equilibria above:

$$Y_{reduced} = \frac{[\alpha_{red}] + [\alpha_{red}AHSP]}{[\alpha_{ox}] + [\alpha_{ox}AHSP] + [\alpha_{red}] + [\alpha_{red}AHSP]}$$

$$Y_{reduced} = \frac{exp\left(\frac{-nF\left(E_{obs} - E^0_{mid,\alpha}\right)}{RT}\right) + K_{red}[AHSP]\,exp\left(\frac{-nF\left(E_{obs} - E^0_{mid,\alpha}\right)}{RT}\right)}{1 + K_{ox}[AHSP] + exp\left(\frac{-nF\left(E_{obs} - E^0_{mid,\alpha}\right)}{RT}\right) + K_{red}[AHSP]\,exp\left(\frac{-nF\left(E_{obs} - E^0_{mid,\alpha}\right)}{RT}\right)}$$

1

$$[\alpha_{ox}] = 1$$

$$[\alpha_{red}] = exp\left(\frac{-nF\left(E_{obs} - E^0_{mid,\alpha}\right)}{RT}\right)$$

$$[\alpha_{ox}ASHP] = K_{ox}[AHSP]$$

$$[\alpha_{red}AHSP] = K_{red}[AHSP]\,exp\left(\frac{-nF\left(E_{obs} - E^0_{mid,\alpha}\right)}{RT}\right)$$

$$Y_{reduced} = \frac{\left(1 + K_{red}[AHSP]\right)exp\left(\dfrac{-nF\left(E_{obs} - E^0_{mid,\alpha}\right)}{RT}\right)}{1 + K_{ox}[ASHP] + \left(1 + K_{red}[AHSP]\right)exp\left(\dfrac{-nF\left(E_{obs} - E^0_{mid,\alpha}\right)}{RT}\right)}$$

$$Y_{reduced} = \frac{\dfrac{\left(1 + K_{red}[AHSP]\right)}{\left(1 + K_{ox}[AHSP]\right)}\,exp\left(\dfrac{-nF\left(E_{obs} - E^0_{mid,\alpha}\right)}{RT}\right)}{1 + \dfrac{\left(1 + K_{red}[ASHP]\right)}{\left(1 + K_{ox}[AHSP]\right)}\,exp\left(\dfrac{-nF\left(E_{obs} - E^0_{mid,\alpha b}\right)}{RT}\right)}$$

At the midpoint when $Y_{reduced} = 0.5$ or $\sum$reduced species = $\sum$oxidized species and $E_{obs} = E_{mid,obs}$

$$0.5 = \frac{\dfrac{\left(1 + K_{red}[AHSP]\right)}{\left(1 + K_{ox}[AHSP]\right)}\,exp\left(\dfrac{-nF\left(E_{mid,obs} - E^0_{mid,\alpha}\right)}{RT}\right)}{1 + \dfrac{\left(1 + K_{red}[ASHP]\right)}{\left(1 + K_{ox}[ASHP]\right)}\,exp\left(\dfrac{-nF\left(E_{mid,obs} - E^0_{mid,\alpha}\right)}{RT}\right)} \Rightarrow \frac{\left(1 + K_{red}[AHSP]\right)}{\left(1 + K_{ox}[AHSP]\right)}\,exp\left(\frac{-nF\left(E_{mid,obs} - E^0_{mid,\alpha}\right)}{RT}\right) = 1$$

$$exp\left(\frac{-nF\left(E_{mid,obs} - E^0_{mid,\alpha}\right)}{RT}\right) = \frac{\left(1 + K_{ox}[AHSP]\right)}{\left(1 + K_{red}[AHSP]\right)} \Rightarrow \left(E_{mid,obs} - E^0_{mid,\alpha}\right) = -\frac{RT}{nF}\,ln\left(\frac{\left(1 + K_{ox}[AHSP]\right)}{\left(1 + K_{red}[ASHP]\right)}\right)$$

$$\boxed{E_{mid,obs} = E^0_{mid,\alpha} - \frac{RT}{nF}\,ln\left(\frac{\left(1 + K_{ox}[ASHP]\right)}{\left(1 + K_{red}[AHSP]\right)}\right) = E^0_{mid,\alpha} - \frac{RT}{nF}\,ln\left(\frac{\left(1 + \dfrac{[ASHP]}{K_{D,ox}}\right)}{\left(1 + \dfrac{[AHSP]}{K_{D,red}}\right)}\right)}$$

(This expression assumes [AHSP] = [AHSP]$_{free}$)

B. Estimation of $K_{D,red}$ from change in midpoint potential at a slight excess of AHSP.
1. The ratios of [AHSP]/$K_{D's}$ are >> 1 because [AHSP] is on the order of 1-20 μM and $K_{D,ox} \sim 0.00017$μM and $K_{D,red} \sim 0.020$ μM. Under these conditions:

$$E_{mid,obs} \approx E^0_{mid,\alpha} - \frac{RT}{nF}\,ln\left(\frac{\dfrac{[ASHP]}{K_{D,ox}}}{\dfrac{[AHSP]}{K_{D,red}}}\right) \approx E^0_{mid,\alpha} - \frac{RT}{nF}\,ln\left(\frac{K_{D,red}}{K_{D,ox}}\right) \text{ or } \boxed{\frac{K_{D,red}}{K_{D,ox}} = exp\left(\frac{nF\left(E^0_{mid,\alpha} - E_{mid,obs}\right)}{RT}\right)}$$

Thus: $\boxed{\dfrac{K_{D,red}}{K_{D,ox}} = exp\left(\dfrac{nF\left(0.040 - (-0.078)\right)}{RT}\right) = exp\left(\dfrac{1 * 96,485 * 0.118}{8.314 * 281}\right) = 126}$

This value agrees well with the ratio calculated using kinetic parameters determined by Mollan et al. (2):

$$\frac{K_{D,red}}{K_{D,ox}} = \frac{0.017\mu M}{0.00017\mu M} = 100$$

References:

1. Moore, G. R., and Pettigrew, G. W. (1990) *Cytochromes c: evolutionary, structural, and physicochemical aspects*, Springer-Verlag, Berlin, New York
2. Mollan, T. L., Khandros, E., Weiss, M. J., and Olson, J. S. (2012) The kinetics of alpha-globin binding to alpha hemoglobin stabilizing protein (AHSP) indicate preferential stabilization of a hemichrome folding intermediate. *J. Biol. Chem.* **287**, 11338-11350