APPENDIX

Derivation of Equations Relating the Fractional Saturation of *N*-Terminal and *C*-Terminal Sites to the Number of Bound Iron Atoms per Molecule of Ovotransferrin

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Suppose that there are A *N*-terminal sites and A *C*-terminal sites. Of these n_N *N*-terminal sites and n_C *C*-terminal sites are occupied by iron. The derivation supposes that an iron atom encounters an unoccupied site at random but that, having encountered such a site, it has a probability p of binding to a *N*-terminal site and a probability q of binding to a *C*-terminal site.

The random encounter implies that the probability of encounter is proportional to the number of unoccupied sites. We also take p and q to be independent of the fractional saturation of either site by iron. Since an iron atom must bind somewhere, $p+q=1$. Hence the probability of an iron atom encountering a *N*-terminal site and binding to it is:

$$(\text{probability})_N \propto p(A - n_N) \quad (1a)$$

and similarly for a *C*-terminal site

$$(\text{probability})_C \propto q(A - n_C) \quad (1b)$$

If, now, a small amount, $A\delta C$, of iron is added, where δC is a small change in C , the number of bound iron atoms per molecule of protein (there are $2A$ sites but only A protein molecules), then the changes in n_N and n_C are:

$$\delta n_N = \frac{p(A - n_N) \cdot A\delta C}{p(A - n_N) + q(A - n_C)} \quad (2a)$$

$$\delta n_C = \frac{q(A - n_C) \cdot A\delta C}{p(A - n_N) + q(A - n_C)} \quad (2b)$$

where the denominators normalize the probabilities.

Write $x = n_N/A$ and $y = n_C/A$ giving $C = x + y$.

Then in the limit as $\delta C \rightarrow 0$ we obtain the differential equations:

$$\frac{dx}{dC} = \frac{p(1-x)}{p(1-x) + q(1+x-C)} \quad (3a)$$

$$\frac{dy}{dC} = \frac{q(1-y)}{p(1+y-C) + q(1-y)} \quad (3b)$$

Take eqn. 3(a). Rearrangement gives:

$$\frac{dC}{dx} + \frac{q}{p} \cdot \frac{C}{1-x} = \left(1 + \frac{q}{p} \cdot \frac{1+x}{1-x}\right) \quad (4)$$

This is a first-order differential equation whose solution is found by a standard procedure. The boundary condition is $x=0$ when $C=0$. Hence:

$$C = [(1+x) - (1-x)^{q/p}] \quad (5a)$$

Eqn. 3(b) gives the same result for y but with p and q interchanged:

$$C = [(1+y) - (1-y)^{p/q}] \quad (5b)$$

Eqns. 5 can be rearranged to give:

$$\begin{aligned} \frac{p}{q} \ln(1-x) &= \ln(1+x-C) \\ \frac{p}{q} \ln(1-y) &= \ln(1+y-C) \end{aligned} \quad (6)$$

Hence plotting $\ln(1+x-C)$ against $\ln(1-x)$ should give a straight line of slope q/p . A similar plot with y gives p/q . Any departures from straight lines would suggest that p and q are not constant but are influenced by the degree of occupation of either site.