Supplemental Experimental Procedures

Multiple Site Binding

For the interaction of the parkin Ubl (*P*) with each of the three UIM sites in ataxin-3 (L_1, L_2, L_3) the following equilibria were considered.

$$P + L_1 \rightleftharpoons PL_1 \qquad K_1 = \frac{L_1 \cdot P}{PL_1}$$

$$P + L_2 \rightleftharpoons PL_2 \qquad K_2 = \frac{L_2 \cdot P}{PL_2}$$

$$P + L_3 \rightleftharpoons PL_3 \qquad K_1 = \frac{L_3 \cdot P}{PL_3}$$

$$P_T = P + PL_1 + PL_2 + PL_3$$

$$L_T = L + PL_1 + PL_2 + PL_3 \quad \text{where } L = L_1 = L_2 = L_3$$

$$K_1 = \frac{L_1 \cdot P}{PL_1} = \frac{(L_T - PL_1 - PL_2 - PL_3)(P_T - PL_1 - PL_2 - PL_3)}{PL_1}$$

Assuming multiple sites on the ligand protein are not occupied simultaneously (ie. weak binding conditions and relatively low concentrations) and all UIM sites have equal affinity for the Ubl domain then $K_1 = K_2 = K_3 = K_D$ and $PL_1 = PL_2 = PL_3 = PL$

$$K_D = \frac{(L_T - 3 \cdot PL)(P_T - 3 \cdot PL)}{PL}$$

Rearranging this equation and solving the quadratic yields,

$$PL = \frac{(3 \cdot L_T + 3 \cdot P_T + K_D) - \sqrt{(3 \cdot L_T + 3 \cdot P_T + K_D)^2 - 36 \cdot P_T \cdot L_T}}{18}$$

and can be generalized for n equivalent sites on the ligand

$$PL = \frac{(n \cdot L_T + n \cdot P_T + K_D) - \sqrt{(n \cdot L_T + n \cdot P_T + K_D)^2 - 4 \cdot n^2 \cdot P_T \cdot L_T}}{2 \cdot n^2}$$

The observed chemical shift change in the Ubl upon binding of any UIM region in ataxin- 3 is,

$$\delta_{obs} = f_P \cdot \delta_P + f_{PL_1} \cdot \delta_{PL_1} + f_{PL_2} \cdot \delta_{PL_2} + f_{PL_3} \cdot \delta_{PL_3}$$

where (δ_{obs}) was a weighted combination of the chemical shifts and populations for the free (δ_P, f_P) and saturated protein states from binding UIM1, UIM2 or UIM3 (δ_{PL1}, f_{PL1}; δ_{PL2}, f_{PL2}; δ_{PL3}, f_{PL3}) at the same site on the Ubl protein.

If the effect on chemical shift on the Ubl domain is similar regardless of the UIM that binds then, $\delta_{PL}=\delta_{PL1}=\delta_{PL2}=\delta_{PL3}$. This is true to a first approximation since all signals in the Ubl shift in the same direction regardless of which UIM is binding.

Also $f_{PL} = f_{PL1} = f_{PL2} = f_{PL3}$ because $K_1=K_2=K_3=K_D$.

$$\delta_{obs} = f_P \cdot \delta_P + 3 \cdot f_{PL} \cdot \delta_{PL}$$

since $f_P = 1\text{-}3\, f_{PL}$

$$\delta_{obs} - \delta_P = 3 \cdot \frac{PL}{P_T} \cdot \delta_{PL} - \delta_P$$

$$\Delta\delta = 3 \cdot \frac{PL}{P_T} \cdot \delta_{TOT}$$

Combining the above equation with the solution for *PL* (above), the chemical shift ($\Delta\delta$) was plotted as a function of total added ligand (L_t), at a constant total protein concentration (P_t) and fitted for K_D and δ_{TOT}.