Relationship between dissociation and $[\text{protein}]_{tot}$

Take as a simple example: $H_2 \rightleftharpoons 2H$ (dimer-monomer equilibrium)

- Take as an estimate of the K_d value of the dimeric H structure (H_2) a value of about 10^{-6} M.

- You can calculate the concentration of H subunits [H] at different $[\text{protein}]_{tot}$, by the equation for the K_d value:
  - $K_d = [H]^2/[H_2]$ from the reaction for the dissociation: $H_2 \rightleftharpoons 2H$

  - Therefore, $[H]^2 = K_d \cdot [H_2]$
  - But, to get [H] in one equation with one unknown, you can use the relationship: $[\text{protein}]_{tot} = [H] + [H_2]$; or $[H_2] = [\text{protein}]_{tot} - [H]$
  - Therefore, $[H]^2 = K_d \cdot ([\text{protein}]_{tot} - [H])$
  - And, $[H]^2 = K_d \cdot [\text{protein}]_{tot} - K_d \cdot [H]$;

    $[H]^2 + K_d \cdot [H] = K_d \cdot [\text{protein}]_{tot}$

    $[H]^2 + K_d \cdot [H] - K_d \cdot [\text{protein}]_{tot} = 0$, which is a quadratic equation* where a=1, b= K_d, and c=$-K_d \cdot [\text{protein}]_{tot}$
  - Therefore, $[H] = (-K_d \pm ((K_d)^2 - 4 \cdot 1 \cdot -K_d \cdot [\text{protein}]_{tot})^{0.5})/2 \cdot 1$

- At a concentration of 1 mM ($[\text{protein}]_{tot}$), $[H] = (10^{-6} \pm ((10^{-6})^2 - (4 \cdot -10^{-6} \cdot 10^{-3}))^{0.5})/2$, or $3.1\text{x}10^{-5}$ M, and $[H_2] = 0.001 - 0.000031$ M = 0.00097 M. Ratio of 31:1 (H_2:H)

- At a concentration of 1 μM ($[\text{protein}]_{tot}$), $[H] = (10^{-6} \pm ((10^{-6})^2 - (4 \cdot -10^{-6} \cdot 10^{-6}))^{0.5})/2$, or $[H] = 6.2\ \text{x}10^{-7}$ M, and $[H_2] = 1\text{x}10^{-6} - 6.2\text{x}10^{-7} = 3.8\text{x}10^{-7}$ M. Ratio of 0.6:1 (H_2:H)

Therefore, as you go from 1 mM and dilute 1000x to 1 μM TOTAL [protein], the amount of dimer goes from 31-fold excess of the monomer to less that 1:1 (0.6:1).

*The quadratic equation:
For $ax^2 + bx + c = 0$

$x = (-b \pm (b^2 - 4ac)^{0.5})/2a$