Determination of Binding Constants by NMR Titration

The most simple case: the 1:1 binding isotherm

$$H + G \underset{K_d}{\overset{K_a}{\rightleftharpoons}} C$$

$$K_a = \frac{[C]}{[H]\,[G]} = \frac{[C]}{([H]^0 - [C])\,([G]^0 - [C])}$$

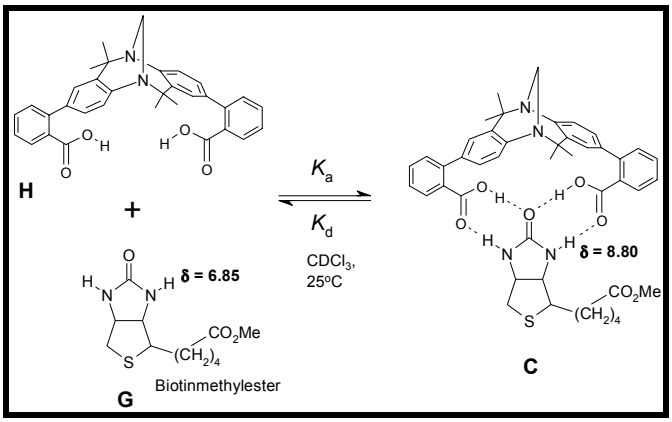
Change in chemical shift (fast exchange on NMR time scale):

$$\delta_{obs} = \frac{[G]^0 - [C]}{[G]^0}\,\delta_0 + \frac{[C]}{[G]^0}\,\delta_{inf}$$

with $x = \frac{[C]}{[G]^0}$: $\quad \delta_{obs} = x\,(\delta_{inf} - \delta_0) + \delta_0$

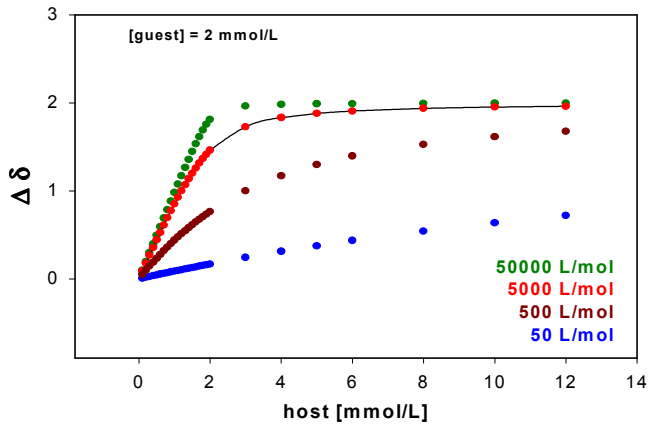
Concentration of the aggregate:

$$[C] = 1/2\,([G]^0 + [H]^0 + 1/K_a) - \sqrt{1/4\,([G]^0 + [H]^0 + 1/K_a)^2 - [G]^0\,[H]^0}$$

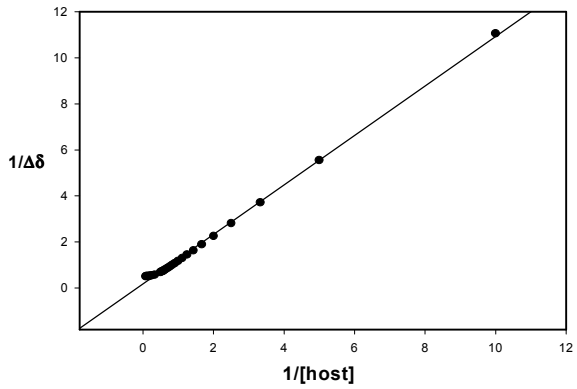


Determination of K_a from titration data:

Non-linear fitting: (recommended)



Double reciprocal plot (Benesi-Hildebrand plot):
WARNING: This treatment requires $[G]^0 >> [H]^0$ or $[H]^0 >> [G]^0$ so that $[G]^0 \cong [G]$ or $[H]^0 \cong [H]$.

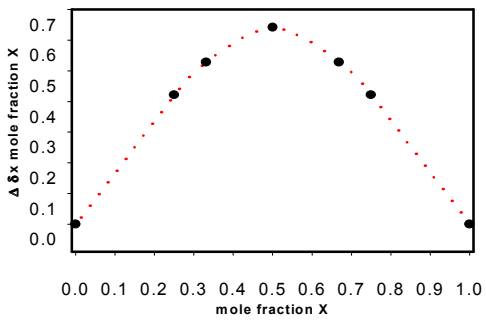


Choosing the right concentration for a titration:

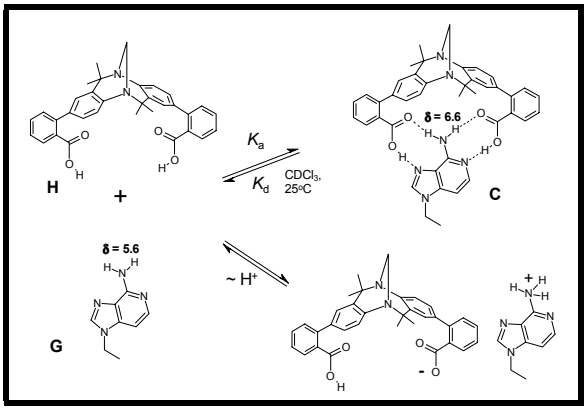
minor compound (fixed concentration): 1/10 K_a; titrant: from 1/10 K_a to 10 K_a
Probability of binding: Weber „p“ values: $p = [C]/[[G]^0$ for $[H]^0 > [G]^0$ and $p = [C]/[[H]^0$ for $[G]^0 > [H]^0$; must be between 0.2 - 0.8 !

Investigation of aggregate stoichiometry:

Job´s plot analysis



Hydrogen bonding vs deprotonation:



A simple dilution experiment of a 1:1 mixture gives the answer: while [C] and therefore Δ δ changes with concentration of $[G]^0$ and $[H]^0$, the proton transfer is independent (no change in Δδ).

Useful references: C. S. Wilcox in *Frontiers in Supramolecular Organic Chemistry and Photochemistry* (Ed.: H.-J. Schneider, H. Dürr), VCH, Weinheim, **1991** (good overview); H.-J. Schneider et al. *J. Am. Chem. Soc.* **1988**, *110*, 6442-6448 (example); I. Horman, B. Dreux, *Anal. Chem.* **1983**, *55*, 1219-1221; C. J. Creswell, A. L. Allred, *J. Phys. Chem.* **1962**, *66*, 1469-1472 (determination of K_a); J. C. Adrian, C. S. Wilcox, *J. Am. Chem. Soc.* **1991**, *113*, 678-680 (influence of water in the solvent); H. A. Benesi, J. H. Hildebrand, *J. Am. Chem. Soc.* **1949**, *71*, 2703-2707 (historical); for questions or calculation routines: B.Koenig@tu-bs.de.