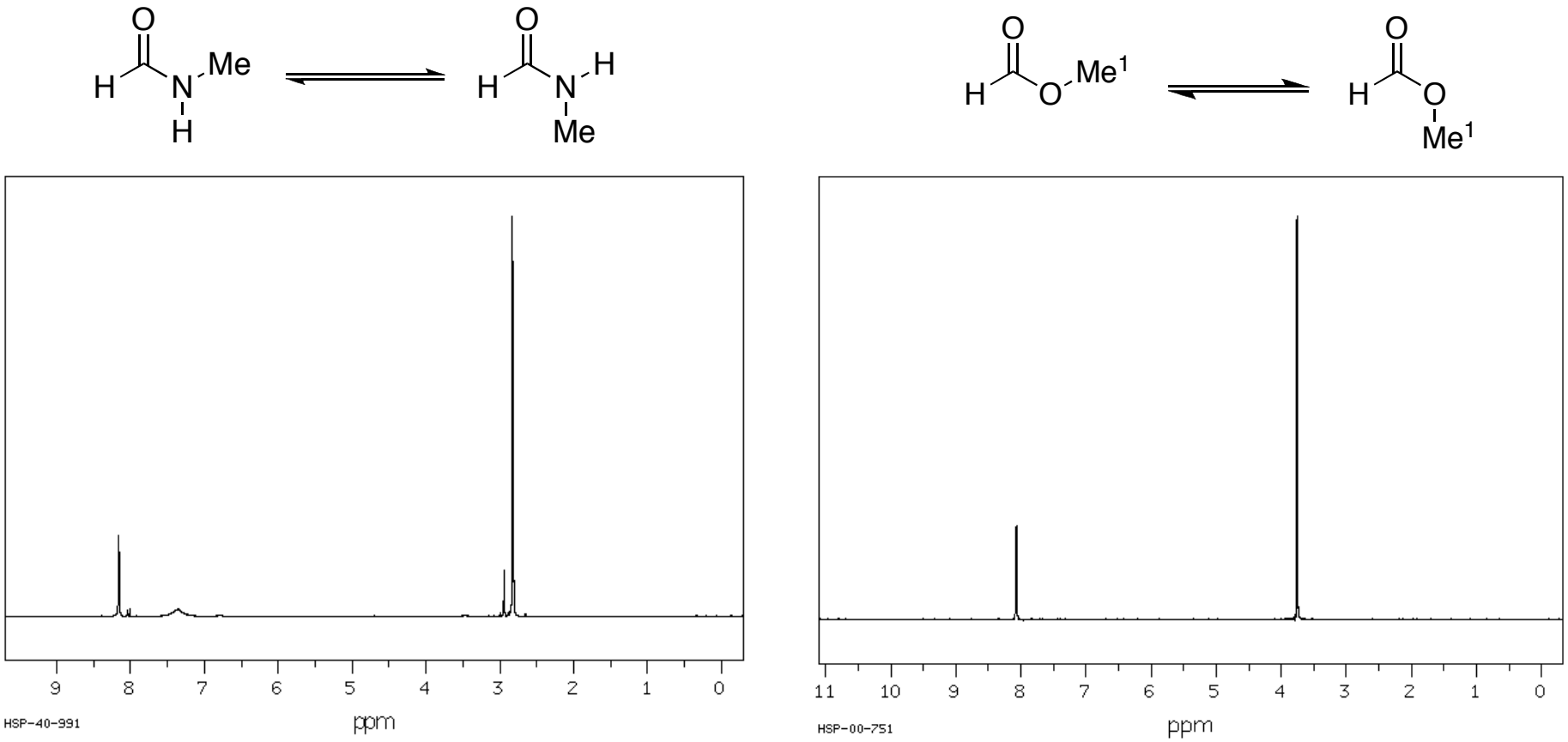
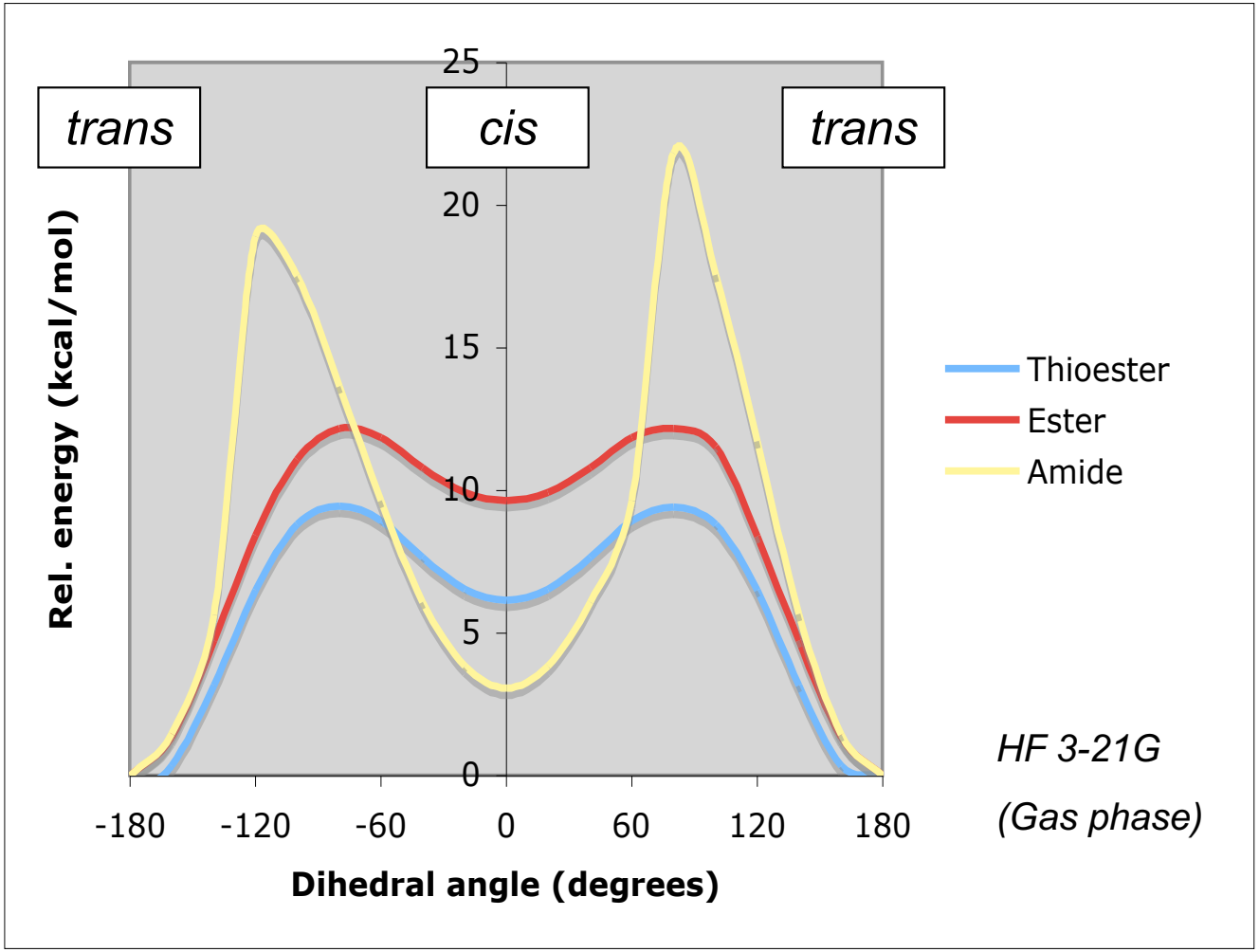
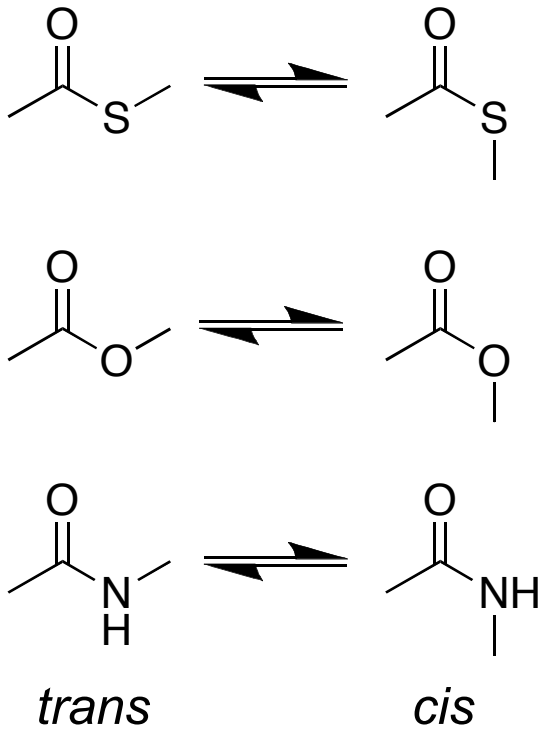
Rates of exchange and NMR



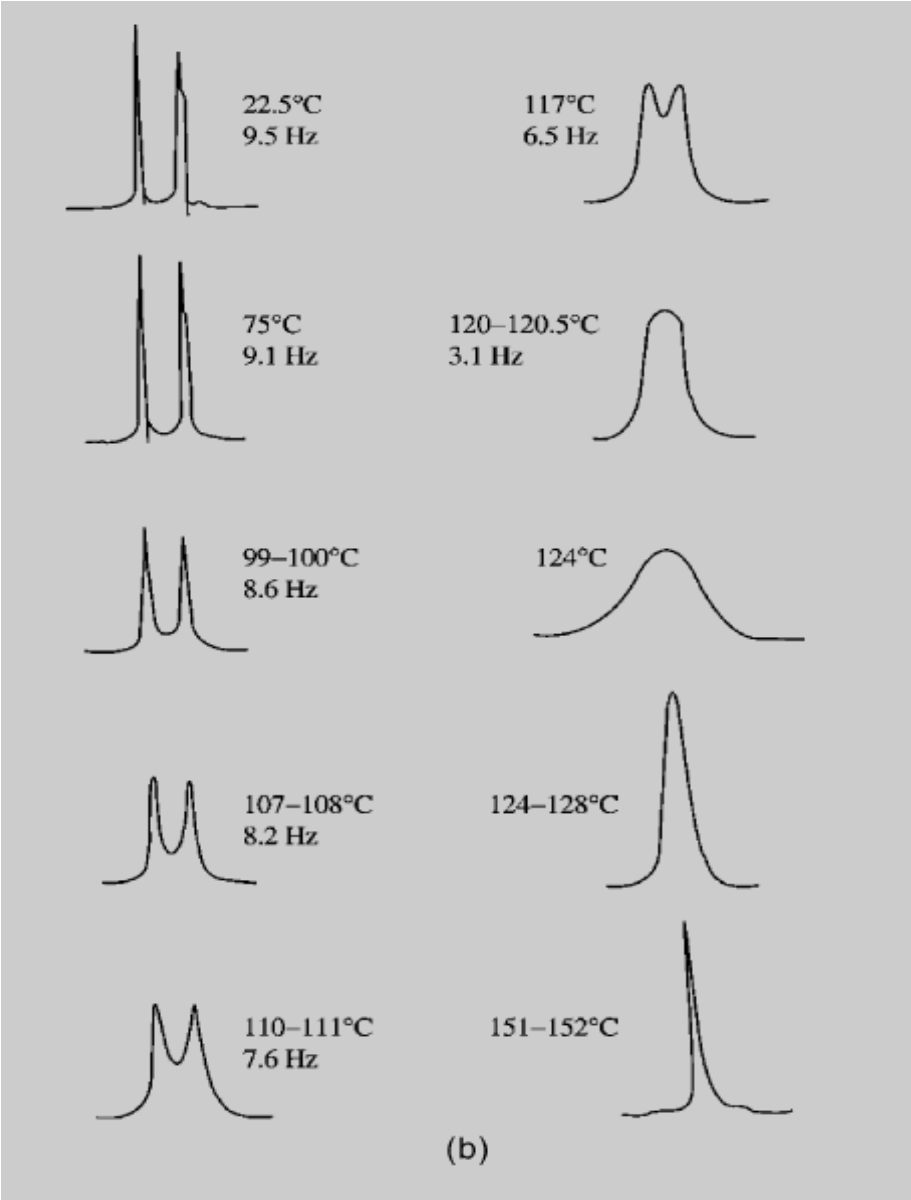
N-methylformamide (left) and methyl formate (right) in $CDCl_3$ at 90 MHz

Calculated barriers to rotation



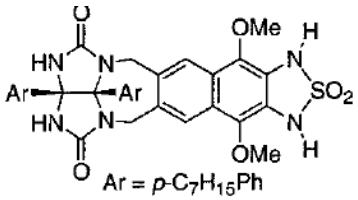


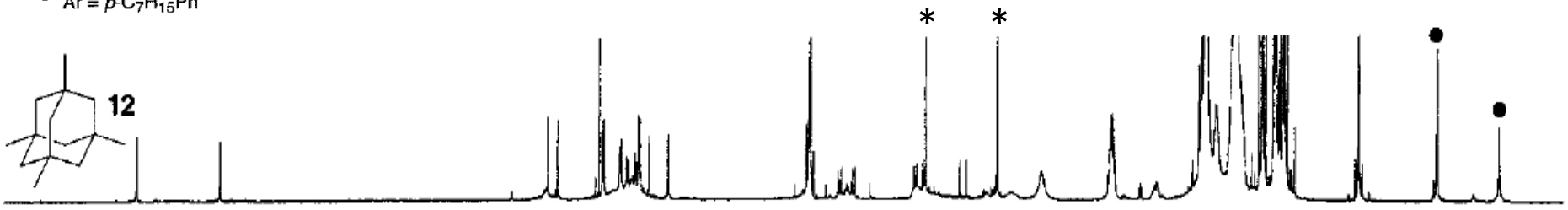
Rates change as a function of temperature



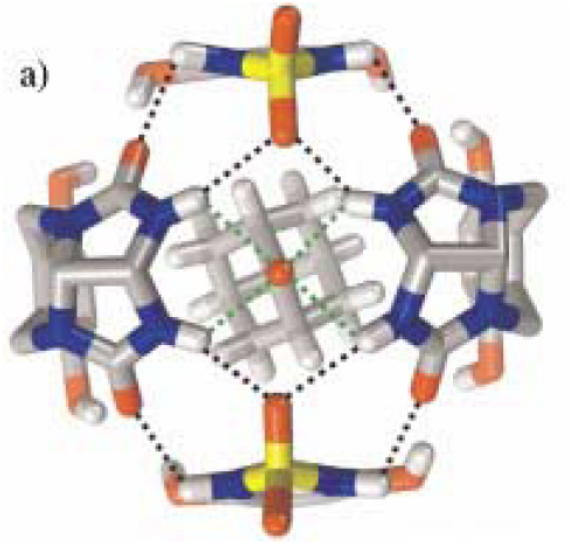
Variable temperature NMR of DMF

Slow exchange: integration to determine complex stoichiometry





Integration of methoxy (*) and adamantane (•) signals gave a 4:1 molar ratio.



(Later confirmed by X-ray)

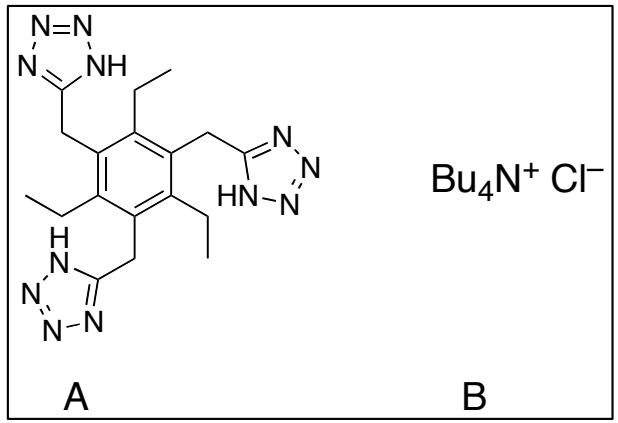
A Practical Guide — Job plot sample prep

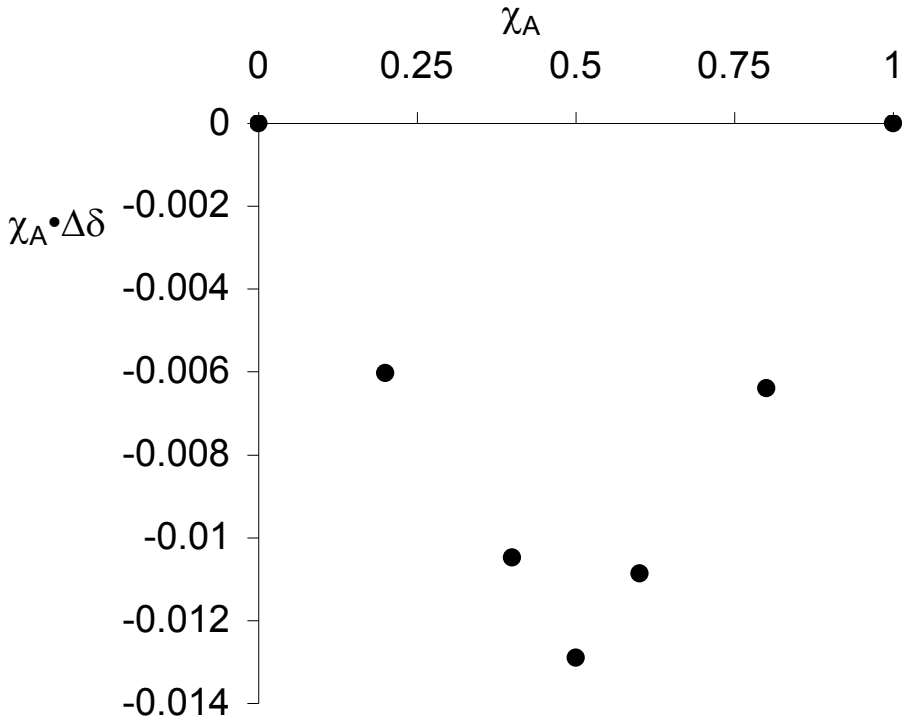
1. Prepare stocks
[A stock] = 5 mM
[B stock] = 5 mM

2. Create samples with fixed [A_t + B_t] as below:

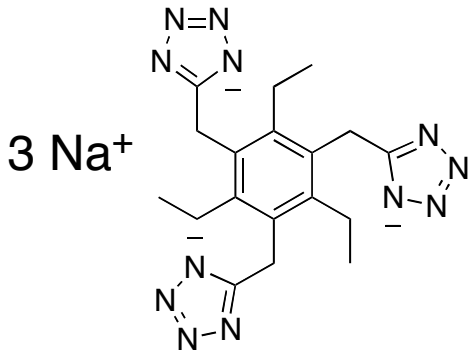
Tube #	Vol. A stock (mL)	Vol. B stock (mL)	χ_A
1	0.5	0	1
2	0.4	0.1	0.8
3	0.3	0.2	0.6
4	0.25	0.25	0.5
5	0.2	0.3	0.4
6	0.1	0.4	0.2
7	0	0.5	0

3. Record $\Delta\delta$, calculate $\Delta\delta \bullet \chi_A$, Plot as shown at right





More Examples of Job Plot Data



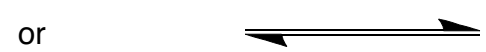
A

$Me_4N^+ I^-$

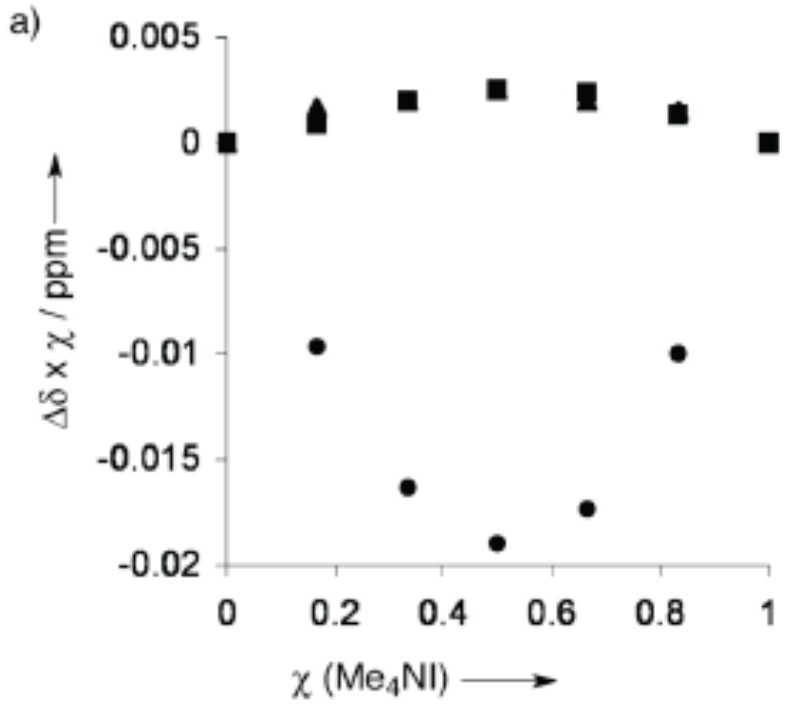
or

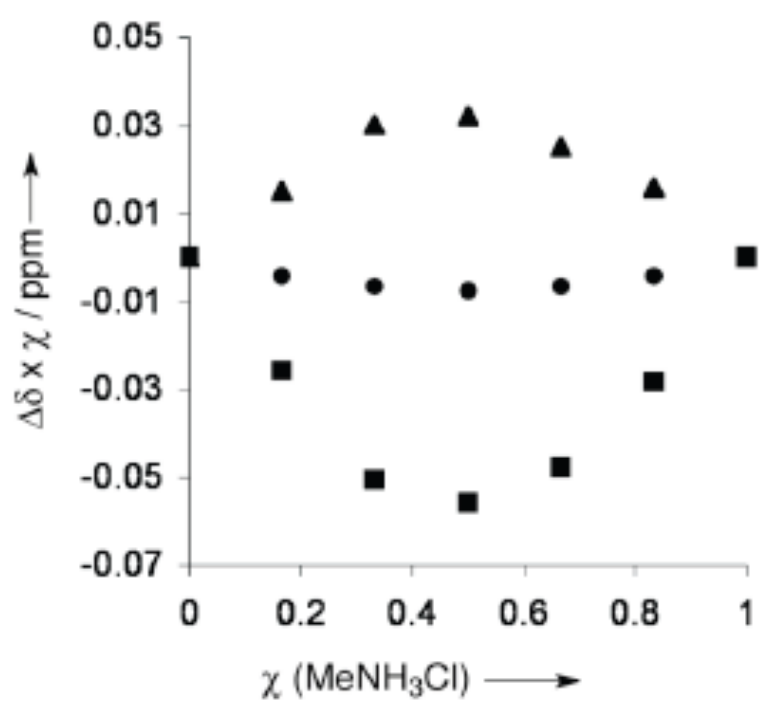
$Me_3NH^+ Cl^-$

B

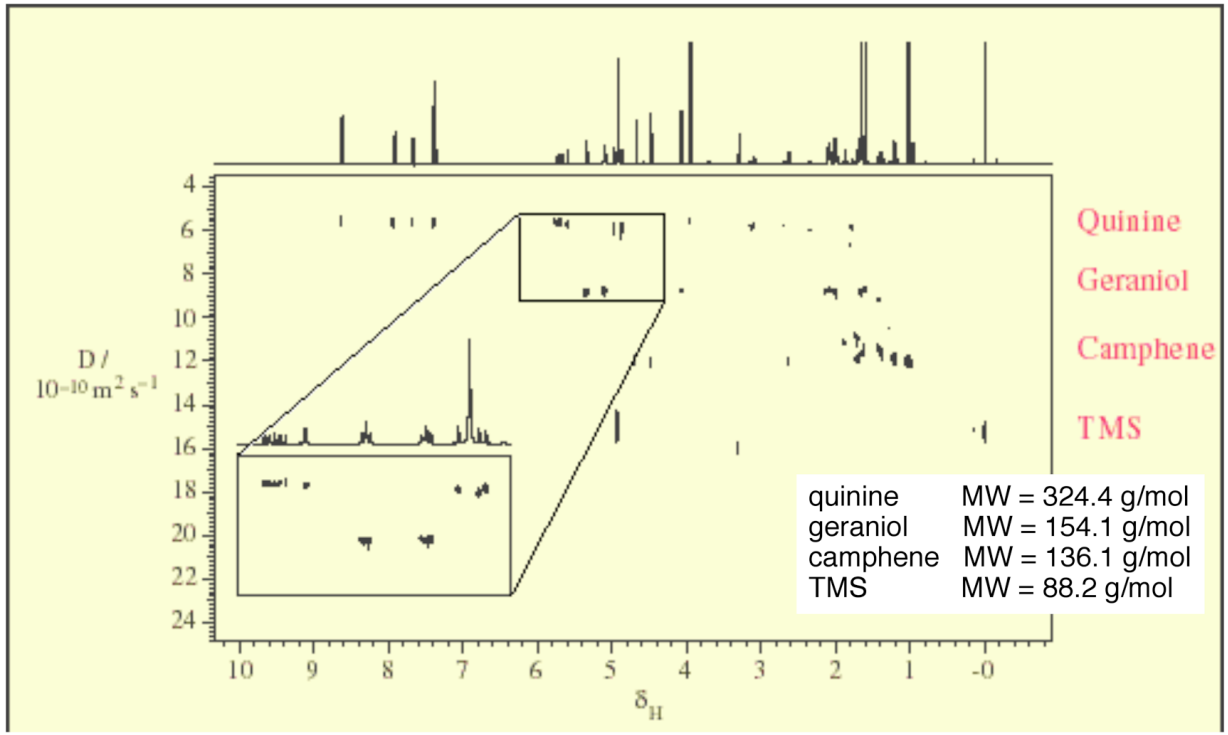


A•B





Diffusion-Ordered SpectroscopY (DOSY)



Stokes-Einstein relationship: $D = kT / 6\pi\eta R_H$

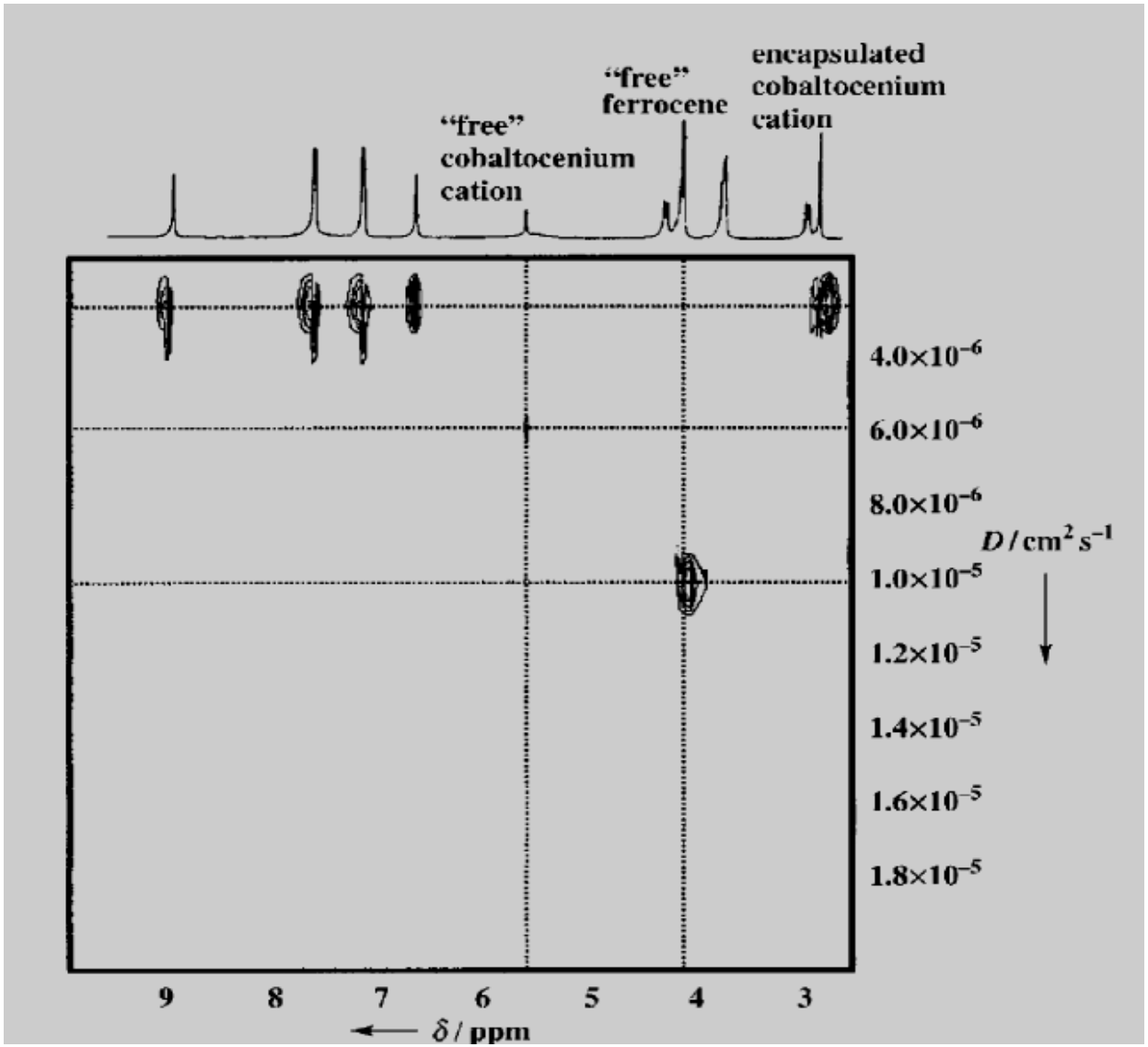
D = diffusion coefficient

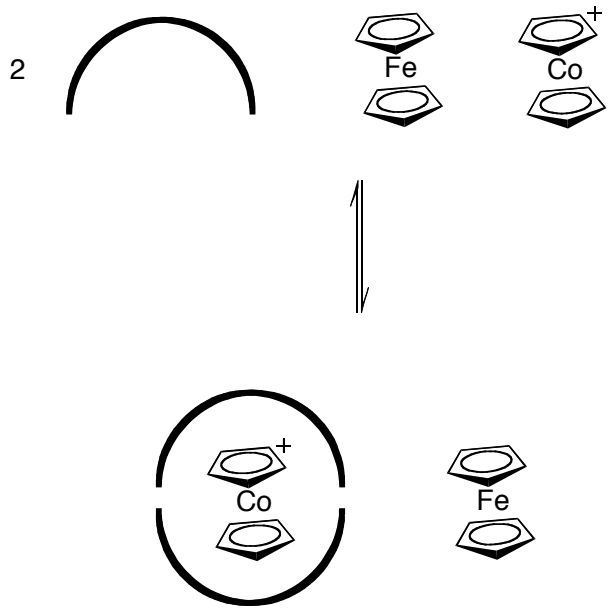
k = Boltzmann constant

η = solvent viscosity

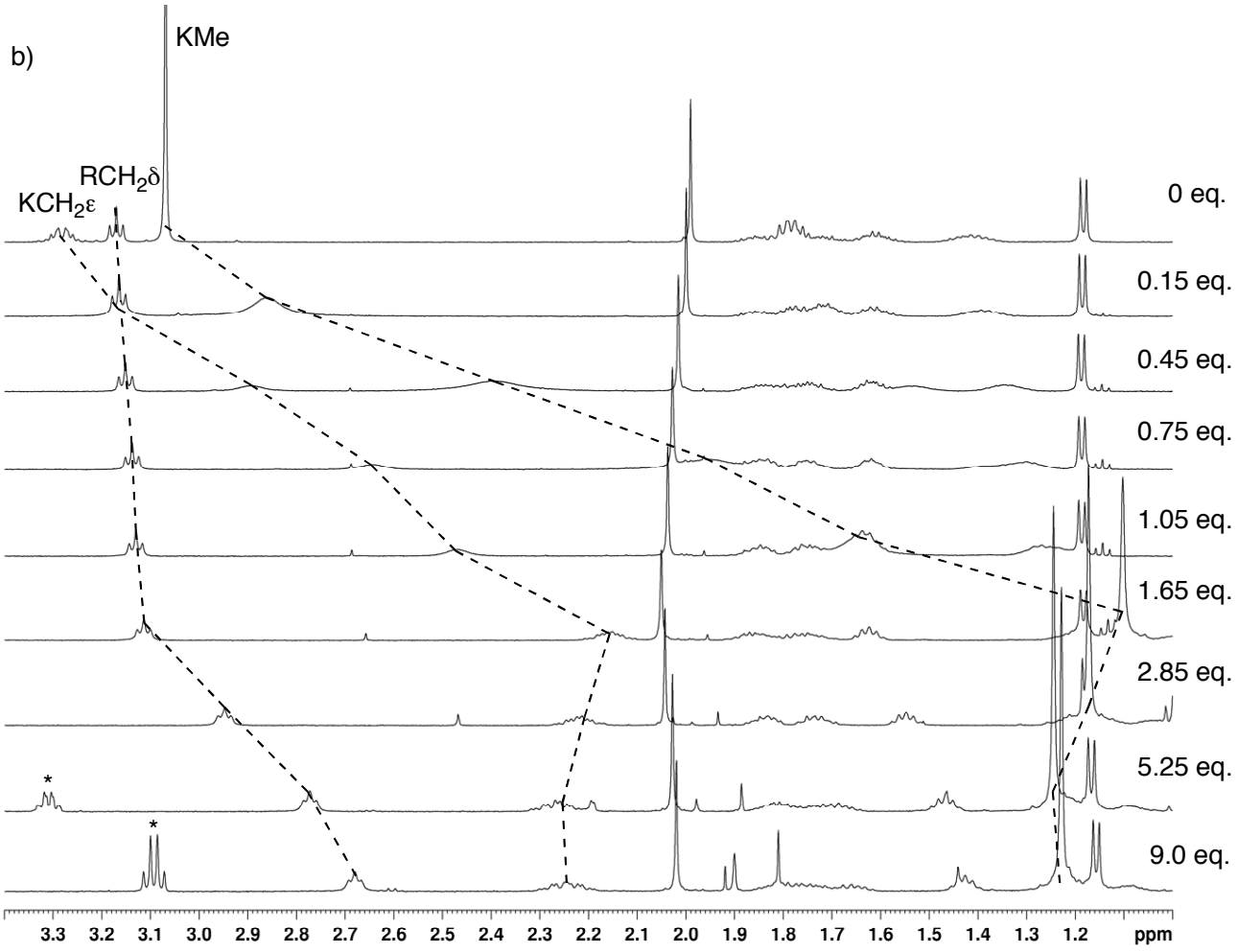
R_H = hydrodynamic radius, which can be related to MW by calibration on related molecules

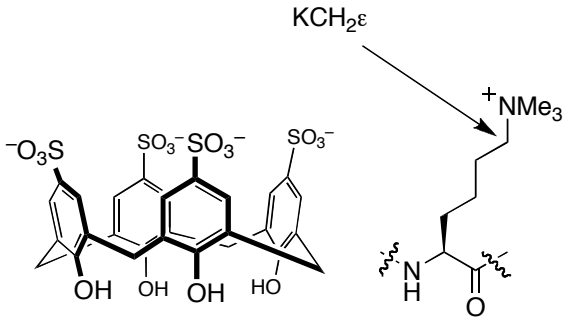
DOSY for a host-guest complex





A system in fast exchange – real data for δ_{free} and δ_{bound}





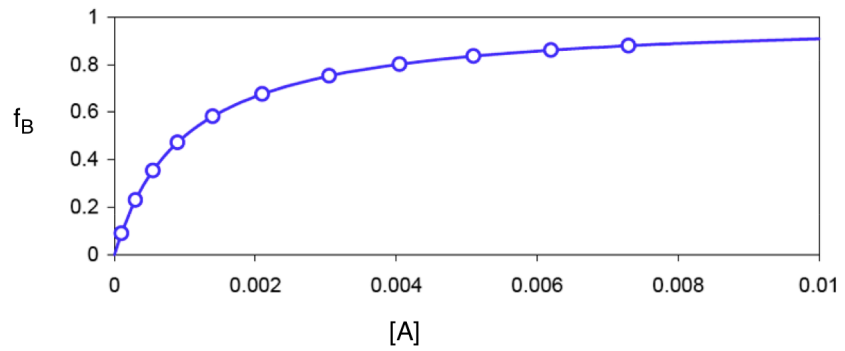
1:1 Complex

$[peptide]_t$ = 1 mM

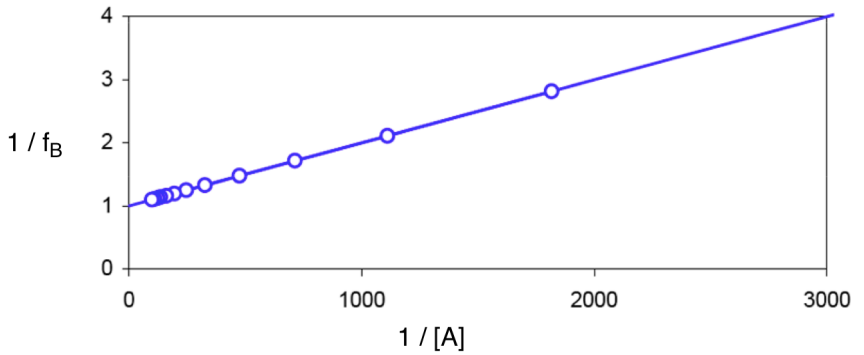
Hypothetical curves for
f_{11} vs. conc. plots based on the generalized 1:1 binding isotherm

K_{eq} = 1000 M^{-1}

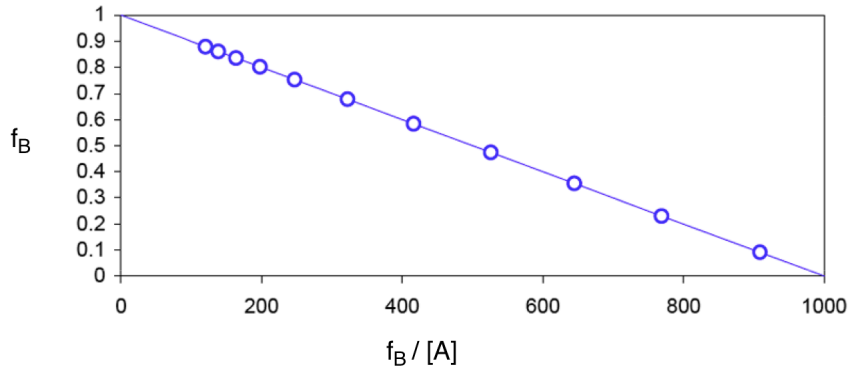
Direct Plot



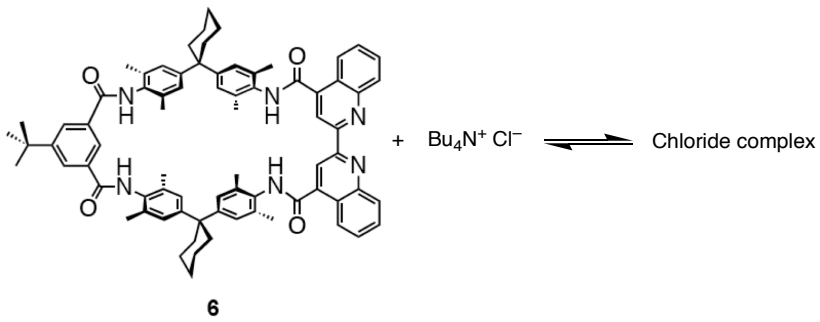
Benesi-Hildebrand Plot



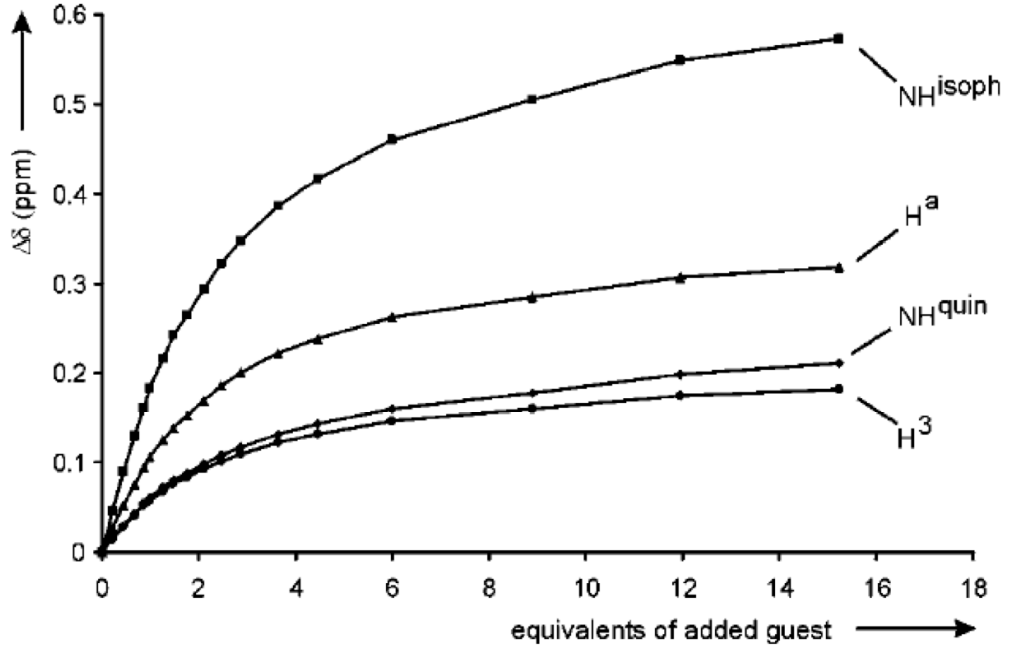
Scatchard Plot

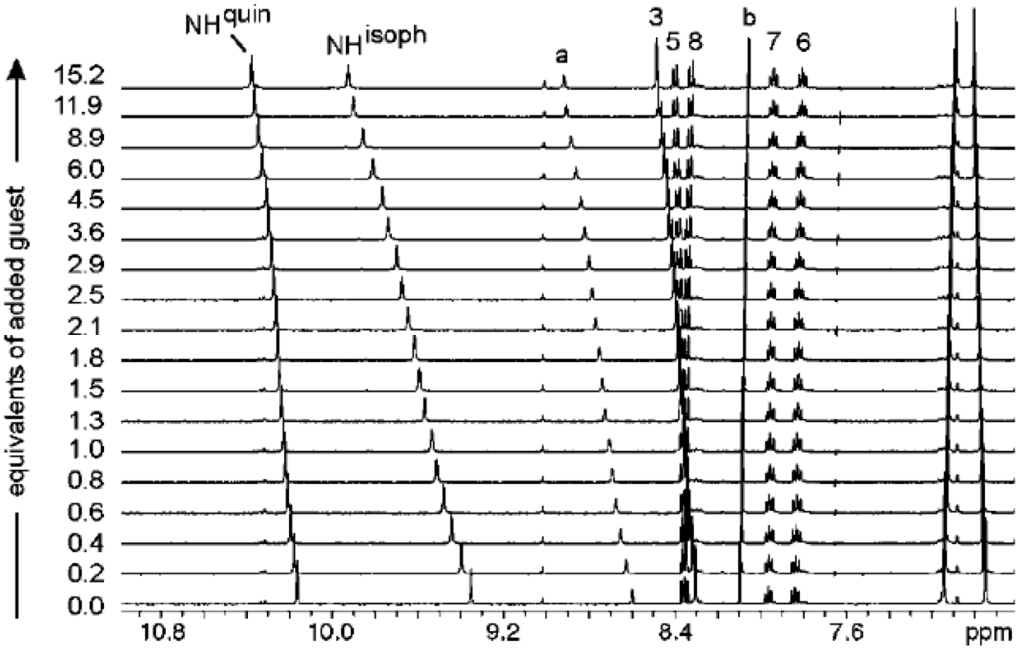


Exemplary NMR Titration Data



Average K_{assoc} = 180 M^{-1}





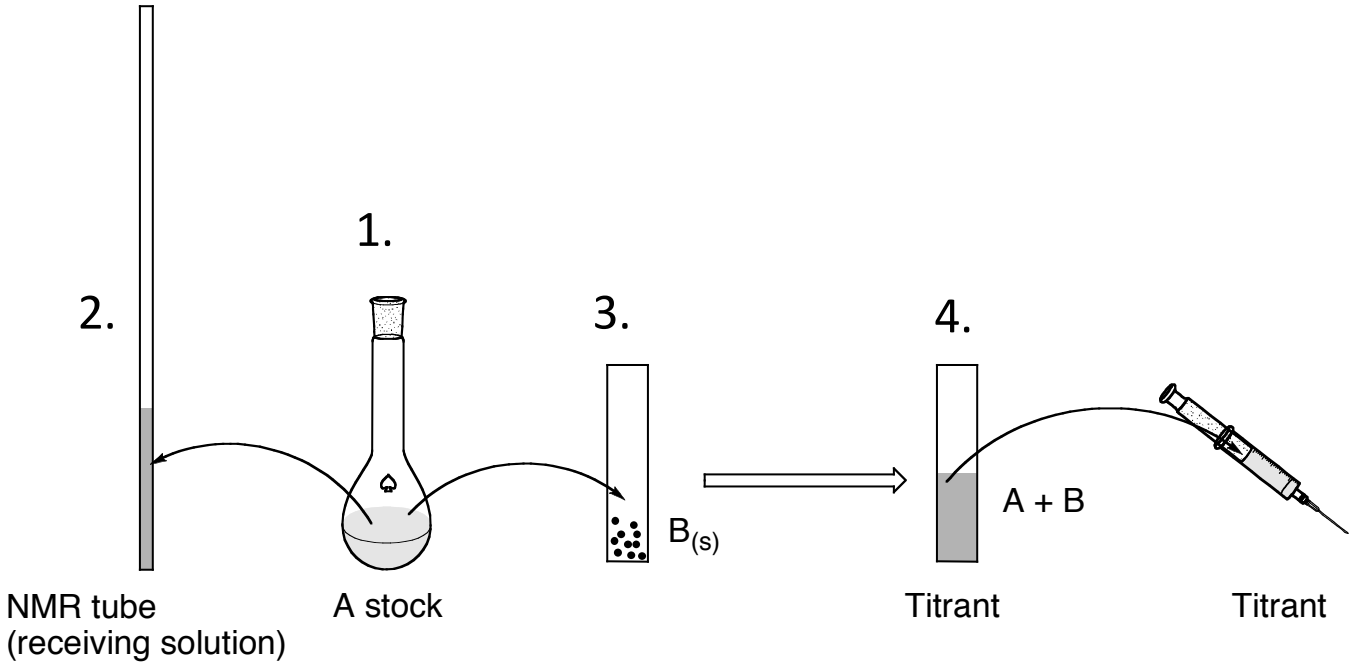
Schalley et al. *Chem. Eur. J.* **2003**, *9*, 1332.

A Practical Guide — Sample preparation

Choose starting concentrations

1. Prepare 5 mL of stock A
2. Remove 0.6 mL of stock and put in NMR tube
3. Calculate amount of B needed to make 4 mL of B at 30x [A]
4. Weigh that amount of B into vial, and dissolve in 4 mL of stock A
5. Transfer that titrant into a gas-tight 100 or 250 uL syringe

All of this ensures that A_t stays constant throughout titration.



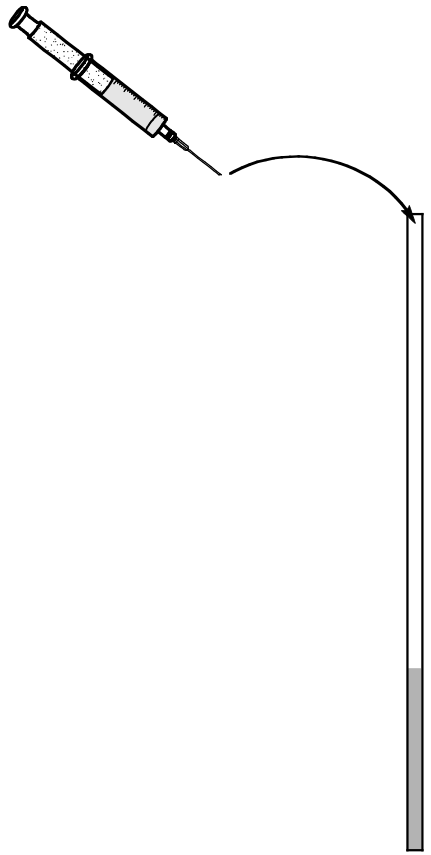
A Practical Guide — Titration

1. Record NMR to determine δ_{free}
2. Add 10 uL of titrant
3. Record NMR again
4. Repeat…

Hints:

-You want to observe a significant $\Delta\delta$ with each addition. You want lots of data points on the curved part of the isotherm. You want to get as close to saturation as possible. This will require making judgments on the fly and increasing the amount you add as you go along. It is not unusual for the increments to start at 10 uL and to be 250 uL by the end of the titration.

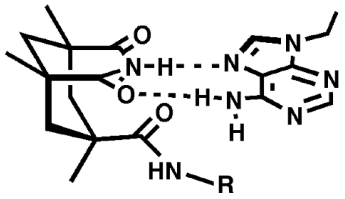
- Mix well at each addition (invert >5 times). Mixing is slow in a narrow NMR tube.



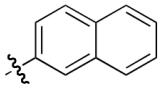
A Practical Guide — Data Analysis

1. In a spreadsheet, record δ_{obs} and total volume of titrant added for each spectrum.
2. Convert to the y and x values needed for plotting, $\Delta\delta_{obs}$ and B_t.
3. Input these two columns of data into Origin.
4. Fit to the 1:1 binding isotherm to determine the parameters $\Delta\delta_{max}$ and K_{assoc}. Be sure to try a few different initial guesses. Be sure to check the quality of fit. If you haven't already done so, confirm stoichiometry by Job plot or other method.

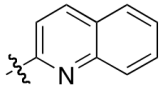
Exemplary Data — van't Hoff Plots

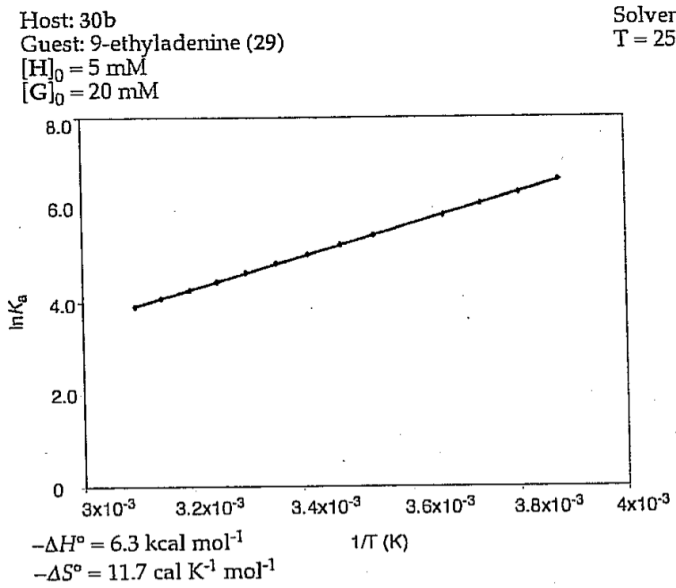


30b

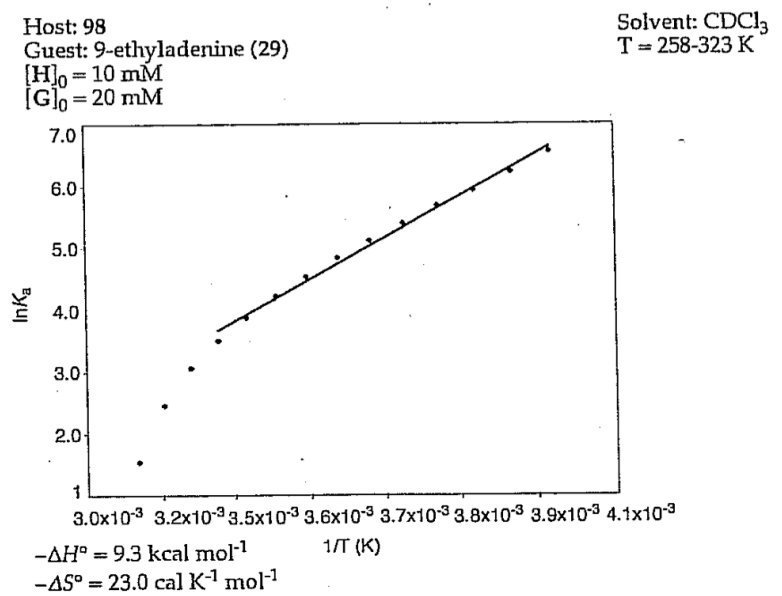


98





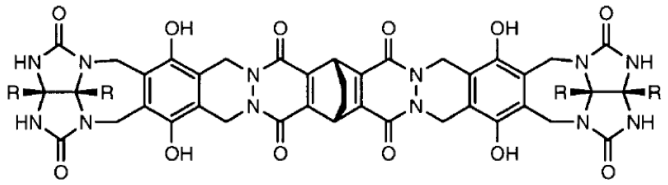
Normally 4-5 values for T are enough



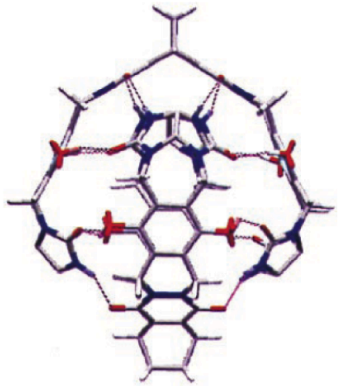
Rafaella Faraoni, PhD thesis, ETH Zurich

Exemplary Data — Very Slow Exchange Kinetics

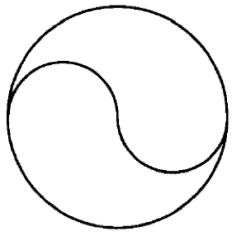
Exchange of guest **P** (complex **1•P•1**; triangles) for guest **A** (complex **1•A•1**; squares) was followed over 4 hours taking a new NMR measurement every 5 minutes.

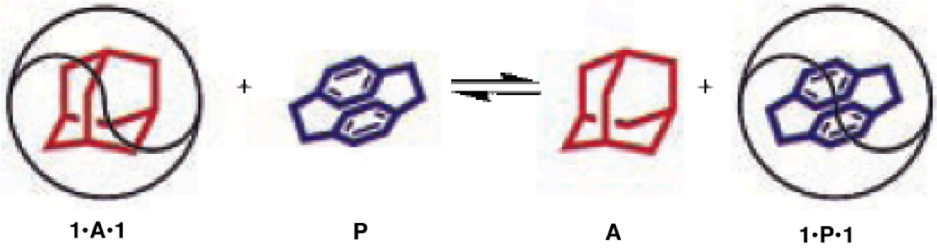


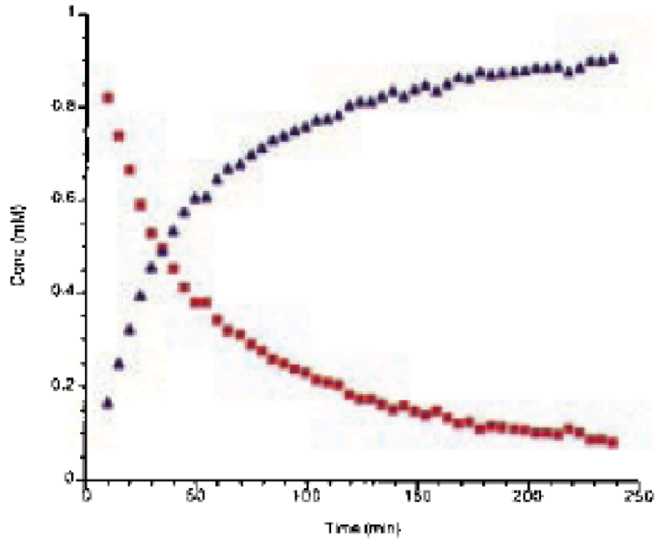
1



1·1







Rebek et al. *Proc. Nat. Acad. Sci. USA* **1999**, *96*, 8344.

NMR Line-Shape Analysis

At a given temperature where intermediate exchange is observed, $k = k_1 + k_{-1}$ can be determined by fitting:

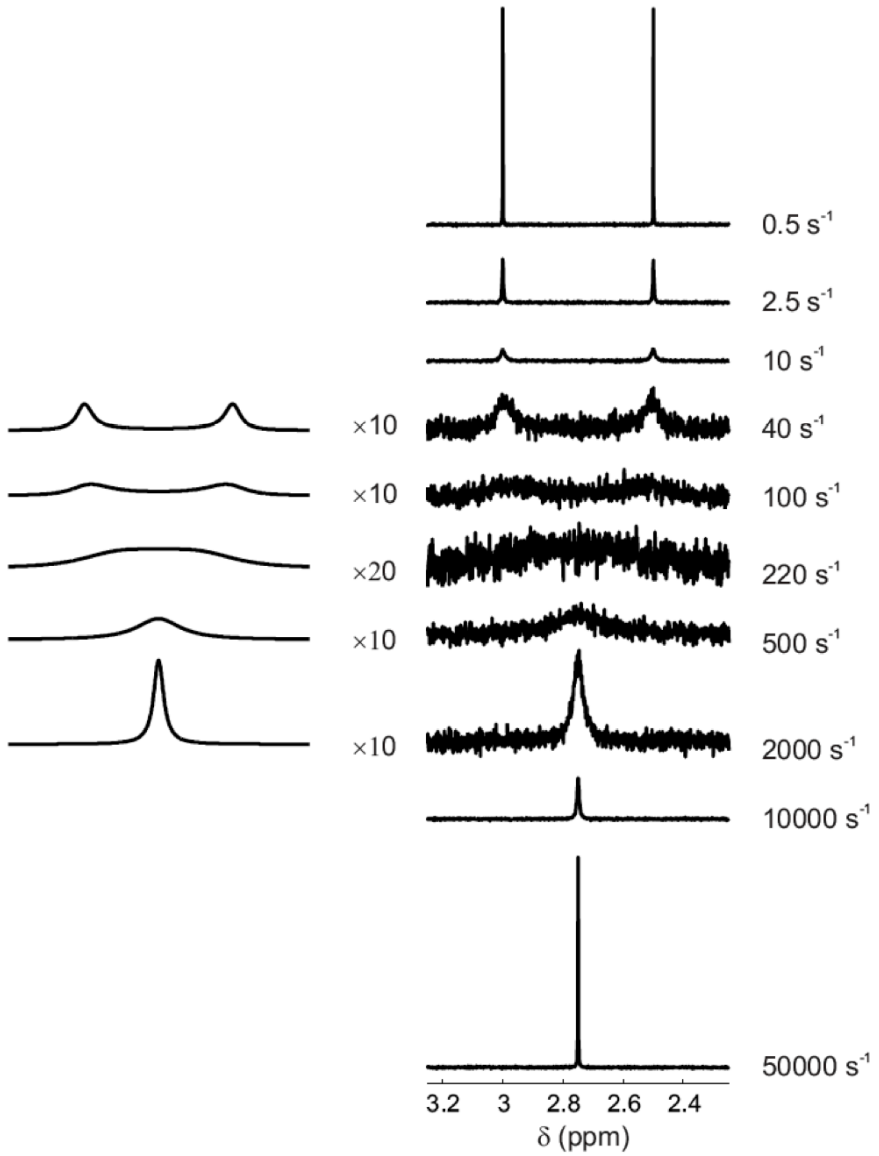
$$A(\omega) = \frac{M_0 k(\Omega_1 - \Omega_2)^2}{(\omega - \Omega_1)^2(\omega - \Omega_2)^2 + 4k^2\left(\omega - \frac{\Omega_1 + \Omega_2}{2}\right)^2}.$$

A = intensity of NMR signal (y axis)

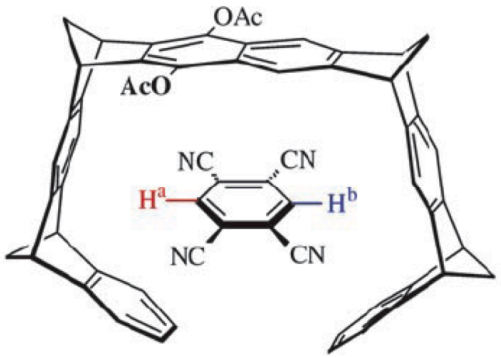
ω= frequency (x axis)

M_0 = some magnetization constant, determined in a separate experiment

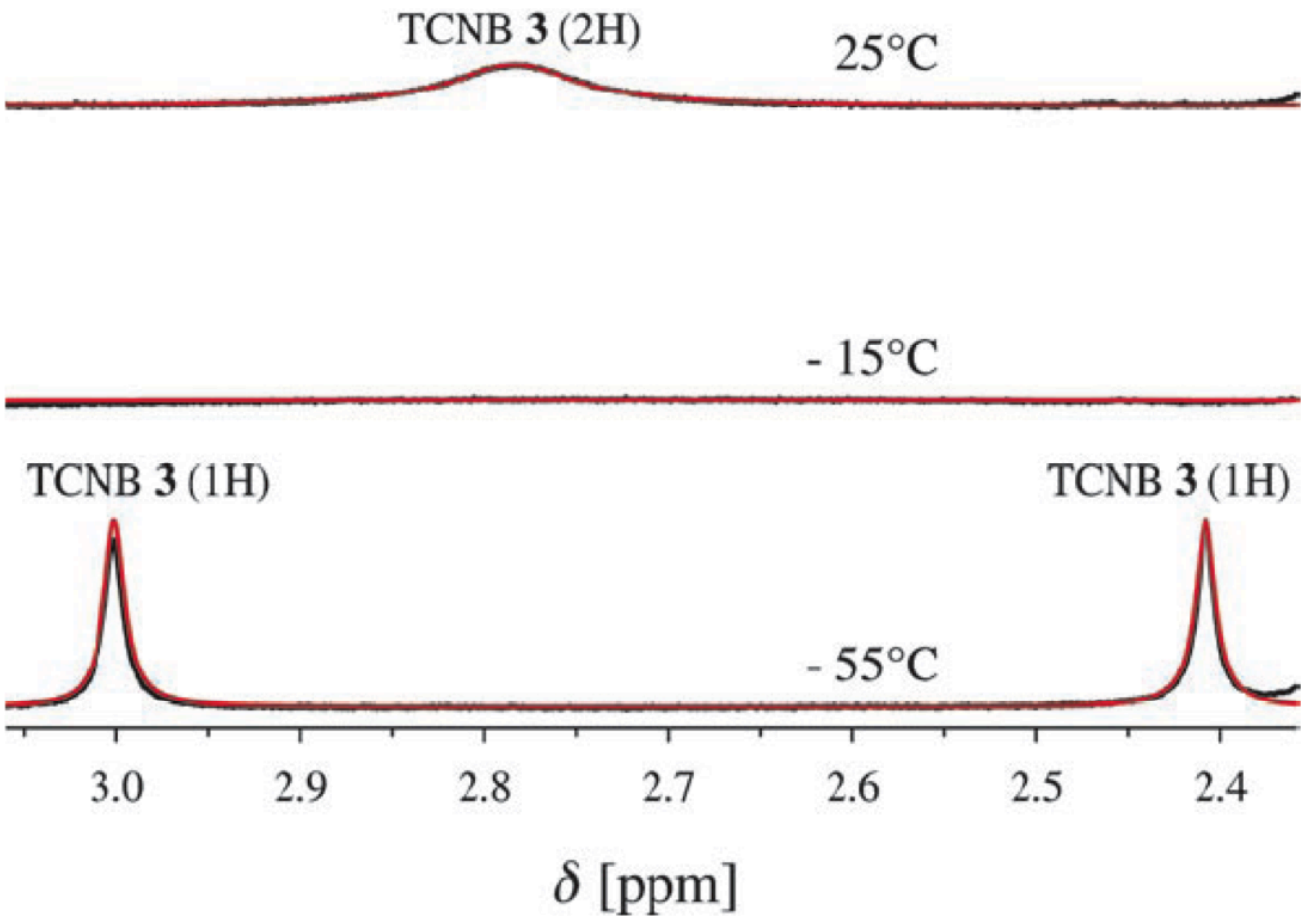
$\Omega_1 = \omega_{obs} - \omega_0$ for signal 1 (offset)
$\Omega_2 = \omega_{obs} - \omega_0$ for signal 2 (offset)



Exemplary Data — NMR Line-Shape Analysis



- Red traces are fitted curves, black traces are actual data.

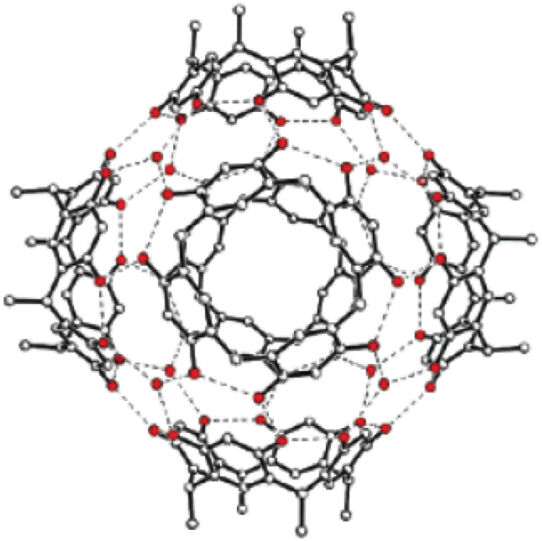


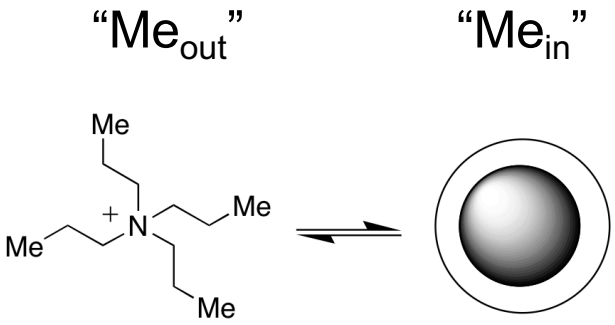
k ~ 3500 s^{-1}

k = 735 s^{-1}

k = 17.5 s^{-1}

Exemplary EXSY data for guest exchange





Integrate 3D on-axis peaks and cross peaks to obtain k_1 and k_{-1}

See Perrin, C. L.; Dwyer, T. J. Chem. Rev. 1990, 90, 935-967 for a review