**COURSE NOTES Analysis of dynamic, equilibrating systems**

*i. Intro and syllabus*

*- Course Delivery*

 *Discussion of student presentations*

*- Evaluation*

 *Presentation*

 *Participation*

 *Final exam*

*- Reference material, online material*

**SLIDES INTRO and LEARNING AIMS**

***A. Introduction to dynamic systems and weak interactions***

**SLIDE SUPRAMOLECULAR CHEMISTRY**

**SLIDE WEAK INTERACTIONS** (see also Chem 537 Jan. 2015)

What does it amount to? **Reversible binding. Equilibrium binding. Supramolecular interactions.**

What is that good for? *Ask class* *and note examples*

* **Enzyme inhibition**
* **Self-assembly = self-organization**
* **Error correction** **in synthesis** *(in contrast to covalent synthesis)*
* **Organized materials from small building blocks**
* **Specific sensing and detection**
* **Specific removal (purifications)**
* **…**

**Characterizing dynamic systems**

**DIAGRAM THE THREE-FOOTED STOOL**

**Thermodynamics, Kinetics, Structure**

We will discuss these in that order of emphasis.

**Quantities that describe dynamic systems:**

**How strong? How fast? How selective?** All of these quantities are meant to be used to compare different systems in order to characterize them and learn about them.

**1. The association constant: Kassoc** (**not Ka** – that’s an acid dissociation constant)

**A + B 🡨🡪 AB**

*Class participation: what’s the expression for Keq?*

**Kassoc = [AB]/[A]•[B] = K11 (to indicate 1:1 stoichiometry)**

**Higher Kassoc, stronger association**

**AB + B 🡨 🡪 AB2**

**Kassoc = K12 = [AB2]/[AB]•[B] = a stepwise association constant**

**Overall association constant is ß12 = [AB2]/[A]•[B]2 (overall A + 2B 🡨🡪 AB2)**

**Generally, ßmn = [AmBn]/[A]m•[B]n**

**Note: for 1:1 stoichiometry, ß11 = K11**

**Note:** This course will focus mainly on 1:1 complexes. Note that the units of stepwise constants are M–1, while the units of overall constants depend on stoichiometry (ß12 --- M-2). Quantities are determined in order to be compared. **Don’t compare constants of different units to compare strengths.** (Which is longer, 1 km or 3 km2? Get it?)

**2. Thermodynamic parameters: ∆G, ∆H, ∆S**

**2a. ∆G free energy of association**

**∆G = –RT ln K**. Just another measure of where the equilibrium lies.

*Class exercise: Determine ∆G for K values of 10, 100, 1000, and 10000 at 298 K. (R = 1.986 cal/K•mol)*

**Rule of thumb: ∆G = –1.4 kcal/mol for each order of magnitude of K.**

Other parameters:

**∆G = ∆H – T∆S**

**2b. ∆H enthalpy of association**

**∆H is a measure of the strength of all interactions before and after binding.** (draw H on both sides of diagram)

**DIAGRAM A + B 🡨 🡪 AB**

**In gas phase**, only A-B interactions occur. **∆H is strength of weak interactions** that bind them.

**In solvent, ALL A-S and B-S and AB-S and S-S interactions are in the ∆H of association.** (DRAW IN S MOLECULES ON ABOVE DIAGRAM)

Hard to tease apart the strength of A-B interactions.

**2c. ∆S entropy of association**

**∆S is a measure of the changes in disorder before and after binding**

translational freedom

rotational freedom

vibrational freedom

*Class participation: What sign is the ∆S of the AB equilibrium in gas phase?*

*AB will almost certainly have fewer degrees of freedom than A and B had independently. So an increase in order makes an unfavorable contribution to ∆G.* ***∆G = ∆H – T∆S.*** *So ∆S is negative.*

**Same solution complications as for ∆H.** Lots of S molecules and their degrees of freedom.

**∆S is often** dominated by solvent molecules, and so is **considered an important measure of solvation effects**

**3. Rate constants (k1 = kon, k–1 = koff)**

**Recall A + B 🡨 🡪 AB**

At equilibrium, the association and dissociation are still occurring.

**How fast?**

**von = -d[A]/dt = kon[A][B]** **voff = -d[AB]/dt = koff[AB]**

(v = rates, k = rate constants)

*Class participation: What are the units of kon, koff? Hint: v is always M/s.*

**Since von is in units M/s, kon is in units 1/M•s** (i.e. it is a second-order rate constant)

**Since voff is in units M/s, koff is in units 1/s** (i.e. it is a first-order rate constant)

At equilibrium, concentrations of A, B, and AB are no longer changing so von and voff must be equal.

**von = voff**

**kon[A][B]** = **koff[AB]**

**kon/koff = [AB]/[A][B]** … look familiar? …

**Kassoc = kon/koff**

*Class participation: Check the units: 1/M•s divided by 1/s is M–1… our familiar units for Kassoc.*

You can describe an association constant (and therefore ∆G = thermodynamics!) only by knowing the forward and backward rate constants.

Notice that a Kassoc value of 1000 M–1 might describe a system with kon of 106 (and koff of 103), or a system with kon of 10 (and koff of 10–2). So a complete description of a system at equilibrium also requires an understanding of the rates.

Aside – Some drug companies are now using koff (not Kassoc) as a primary tool for screening leads. It tells them a drug’s **potential for a) long action at target and b) ability to be cleared from the body**.

**4. Weak Interactions**

**Weak interactions are reductionist models**

**SLIDE WEAK INTERACTIONS**

For each interaction

1. Definition: Interacting groups
2. Structural data
3. Energies

**4a) Simple electrostatics**

**Charges, dipoles**

**SLIDE: OPPOSITES ATTRACT**

**Decreases with decreasing charge (z). Consider dipoles as partial charges of strength µ.**

**Epsilon (dielectric constant) is the screening factor of the medium.**

**Decreases with increasing distance (rx).**

**Orientational preferences for dipoles (theta terms).** None for ion-ion interactions. **DIAGRAM** a “bad” theta of 180°. (Cos 0 = 1. Cos 180 = –1).

**4b) Dispersion / vdW**

**Induced dipole–induced dipole** (diagram)

**Ubiquitous** *“Everything touches something else in condensed states – Jack Dunitz”*

**Stronger for more polarizable electrons** *(Xe, S, I, pi electrons)*

**DIAGRAM** Simplified “Lennard Jones Potential”



 *B* is attractive term, *A* is repulsive term

**SLIDE: Potential energy vs. distance**

# Weakly attractive until overlap occurs, then strongly repulsive (steric overlap)

**r is large, both terms 🡪 0**

**r is small, repulsive term 🡪 infinity**

**r is medium, attractive term > repulsive term**

# Translation: if there is any steric overlap at all, the repulsive energy is so strong that the binding will be totally killed.

**SLIDE: Thrombin inhibitors and vdw size matching**

Bigger, just right, TOO BIG!!! Note the steepness of the drop off in activity (~150-fold i-Pr to Ch)

**4c) Hydrogen bonds**

  or 

**Examples: NH---O=C, OH---Cl–**

**Attraction arises from both electrostatic (+ –) attraction and orbital mixing**

*Ask class… how do we know? What’s the Proof?*

**SLIDE: HYDROGEN BONDING ANGLES FROM CSD**

**Distances: Heavy atoms are < vdW radius apart** (H atoms hard to locate in X-ray)

**O–H---O d(O-O) 2.5–3.0 Å (95% criteria)**

**N–H---O d(N-O) 2.6–3.2 Å**

**O–H---N d(N-O) 2.7–3.2 Å**

**S–H---O d(S-O) ~3.5 Å**

**SLIDE VANCOMYCIN’S IMPORTANT HYDORGEN BOND**

Vancomycin is the **antibiotic of last defence** for use against resistant strains

Natural product from a soil bacterium

Mode of action: **binds to D-Ala-D-Ala (**bacterial cell wall precursor) and stops cell wall synthesis

In 1980’s … VRE appeared. The bug uses **D-Ala-D-Lac** and Van efficacy is decreased.

In vitro, Van binds 1000-fold weaker to D-Ala-D-Lac. So this **one H-bond is worth 4.2 kcal/mol!!!**

TOO BIG!!! the new oxygen atom is not an innocent spectator… there are significant **repulsions between the lone pairs** of the incoming carbonyl oxygen and the newly installed ester oxygen.

**4d) Aromatic interactions**

**SLIDE: AROMATIC-AROMATIC INTERACTION GEOMETRIES**

*Describe what drives aromatic-aromatic interactions.*

**Often viewed as ‘special’ pi-stacking but they’re not.**

**Simple combination of electrostatics and vdW forces**

**Electrostatic explanation.** Very popular, but overstated. Pi-stacked benzenes still have a favorable interaction energy, and the calculations show it to be only ~15-20% weaker than other geometries.

**The more strongly polarized the ring, the more electrostatics dictate geometry**

**SLIDE TORSION BALANCES FOR MEASURING EQUILIBRIA**

**High rotation barrier**

**Distinct NMR signals for each conformer**

**Integrate to determine Keq**

**Convert to ∆G**

**Assumption: |∆G| comes from the interaction**

**SLIDES ELECTROSTATICS VS DISPERSION, TWO EXAMPLES**

**4e) Cation-pi interactions**

Regardless of how you consider aromatic-aromatic interactions,

**Arenes have significant pi-electron density and can bind cations**.

**DIAGRAM**



Cation-pi interactions can be as strong as coordination by water (ion-dipole interactions)!

Some cation-pi interactions are important players in biology

**SLIDE: CATION-PI IN A FACTOR Xa INHIBITOR**

* Same polarizability + Same size = same vdW forces
* Hydrophobic effect would make cation binding weaker
* Different energy attributed to electrostatics (cation-pi)

*What is the formula for Kd? Which is a stronger inhibitor? By how much ∆G?* (Factor of 100 = 2.8 kcal/mol)

*This is an estimate of the energy offered by 3 cation-pi interactions. How strong is contribution from the hydrophobic effect? What would the authors need to do?* Test the unsubstituted inhibitor (they didn’t).

**4f) Halogen bonds and other**

**Polarized/charged regions appear in unexpected places**

**Iodine + lots of EWG = ‘sigma hole’ (show slide)**

**SLIDE: halogen bonds**

**4g) The hydrophobic effect**

1. **dH/dS**

**Enthalpy is easy to think about. More attractive interactions, more favorable enthalpy.** All that we’ve discussed until now has been enthalpic.

**Entropy: More degrees of freedom = positive dS = energetically favorable**

**(dG = dH – TdS)**

The **degrees of freedom** that matter most in biology are:

**1) Translational**

**2) Rotational } more particles in system, more entropy, more favorable**

**3) Vibrational** (less so – will not discuss further)

(also Configurational… see below)

**b) Classical hydrophobic effect**

Whatever the explanation… This is not an attractive force, but is actually an indirect consequence of the properties of water and hydrophobic species. Hence the **Hydrophobic “effect” and NEVER Hydrophobic “force”**

**Oil and water don’t mix… but why?**

**Energetics of Hexane (oil) transfer into water: ∆G +7.8 kcal/mol, ∆H = +0.1 kcal/mol, –T∆S = +7.7 kcal/mol. Entropically driven separation! (increasing # of particles)**

And so from olive oil we get the stereotype that the hydrophobic effect is entropically driven.

**SLIDE THE CLATHRATE MODEL OF THE HYDROPHOBIC EFFECT**

**A persistant myth** handed down from generation to generation (Anecdote on academic integrity and textbook publishers… Eohippus “dawn horse” and British Fox Terrier).

Methane clathrates do exist (SLIDE??), but…

**No evidence for “frozen” water around hydrophobic surfaces has ever been observed. Ever.**

**SLIDE INTERFACIAL WATER EXPLANATION**

**In REAL systems, both ∆H and ∆S can be favourable.** Depends on local structure.

**c) The nature of the pocket / interface matters**

**SLIDE CYCLODEXTRINS AND THE NON-CLASSICAL H-PHOBIC EFFECT**

Cyclodextrins are naturally occurring glucose macrocycles. Size depends on # of sugars.

OH’s point out (water soluble), CH’s point in to make hydrophobic cavity.

**CD host-guest binding is a good model of enzyme pocket-inhibitor binding.**

**a-CD binding PnP is ∆H driven (∆H = –5 kcal/mol (favourable), –T∆S = +3 (unfavourable)).**

**b-CD binding PnP is stronger overall, but much less driven by ∆H (–2.5 kcal/mol) than –T∆S (–1 FAVOURABLE)/**

**SLIDE NON-CLASSICAL MODEL OF H-PHOBIC EFFECT**

The non-classical (∆H driven) model: Water in a small, confined cavity is unable to form ideal hydrogen bonds to neighbouring water molecules. When released into bulk solvent, water is free to form as many hydrogen bonds as possible. **More (and better) hydrogen bonds = more favourable enthalpy.**

a-CD is smaller = confined water. b-CD is bigger = less confined water.

**Data**

**SLIDE Lysozyme mutants and surface area correlation**

DIAGRAM random coil 🡪 folded lysozyme. Deletions of residues in core. Folding energy

**H-phobic effect is the primary driver of protein folding.** (H-bonds, e-statics are competed by water… H-phobic is strengthened by water)

**H-phobic effect is the primary driver of DNA duplex formation. G-C and A-T H-bonds matter very little** **for energy, but are important for specificity.**

Carefully chosen aromatic bases that have no H-bonding sites have shown equal duplex stabilization and specificity.

**SLIDE H-bonds + hydrophobic effect in Thrombin inhibitors**

**-A(nother) example of non-linear perturbation effects** (recall vancomycin)

**The presence of the H-bond increases the H-phobic contributions of a neighboring group.**

***B. Stoichiometry and Kassoc by NMR***

**1. NMR of free and bound states and the NMR time scale**

**1H chemical shift  depends on environment.** It is a frequency of spin procession (Larmor frequency).

**DIAGRAM Shielding and deshielding environments. Aromatic rings, anions, cations, hydrogen bonds**

So **** of free A and bound AB are often different. Call them **∂free**and ∂bound.

So what does the NMR spectrum of a dynamic system look like?

**DIAGRAM PEAKS FOR A AND AB AT Slow, medium, and fast exchange**

**What’s fast? What’s slow? The NMR time scale**.

**From** ∆E•∆t ~ π/root(2) **(Heisenberg uncertainty principle) we get:**

**kcoalescence = π • ∆ / root(2)**

**where ∆ is the difference in frequency between ∂free and ∂bound in Hz (not ppm)**

**So the “NMR time scale” depends on the ∂ of the species that are exchanging dynamically.**

Back to diagram… clear sharp signals at >100-fold faster or >100-fold slower than kcoalescence

**SLIDE NMR OF DMF (SLOW), methyl formate (FAST)**

**SLIDE CALC. BARRIERS TO ROTATION OF DMF (HIGH), methyl formate (LOW)**

High barrier = slow exchange. Amides spin slower than esters.

**SLIDE NMR OF DMF AT VARIABLE TEMPERATURES**

*What is the rate of exchange at 124°C?*

**2. Determining complex stoichiometries**

You can’t determine Kassoc unless you know the stoichiometry of assembly

**2a. Heteromeric assemblies: stoichiometry by integration**

A + B 🡨 🡪 AmBn

**If in slow exchange**, A and B might have different NMR signals that are visible. Integrate them!

**SLIDE TETRAMERS AND GUESTS**

**2b. The method of continuous variation (Job plot).**

**Theory.**

**If in fast exchange,**

**For a complex of stoichiometry AmBn, and a fixed total conc. (At + Bt) the max. amount of complexation occurs when A and B are present in the correct ratio (i.e. when At:Bt = m:n).**

Derivation on **Connors page 24.**

**Practice.**

**For NMR, when the total conc. (At + Bt) is held constant and the ratio chi = At/(At + Bt) is varied, the amount of complex AB in solution is proportional to ∆∂•chi (mole fraction A).**

**So. Plot ∆∂•chi vs. chi and observe maximum.**

**DIAGRAMS MAX AT 0.5 = 1:1, MAX AT 0.66 = A2B, MAX AT 0.25 = AB3**

**Example.**

**Make stock solutions of 5 mM A and 5 mM B.**

If you mix always to the same total volume, than the total [At + Bt] will always remain constant while ChiA varies from 1 to 0.

**TABLE OF SAMPLES AND MOLE FRACTIONS**

|  |  |  |  |
| --- | --- | --- | --- |
| **Tube #** | **Vol. A stock (mL)** | **Vol. B stock (mL)** | **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** |

Record NMRs and plot as shown

**SLIDE REAL DATA FROM TETRAZOLE-chloride**

**Max at chiA = 0.5, so a 1:1 complex is formed**

**SLIDE REAL DATA FROM TETRAZOLATE-CATION BINDING**

Note that the binding of a monocation by a trianion has a stoichiometry that is hard to predict.

Note that ∆∂ can be positive or negative.

**2c. Stoichiometry by Diffusion Ordered SpectroscopY (DOSY-NMR)**

**Determines molecular weight.**

Useful for all sorts of equilibria, but **consider especially:**

**mA 🡨 🡪 Am**

*What does the NMR look like in slow exchange? Fast exchange?* Not useful to integrate.

**Diffusion-Ordered SpectroscopY (DOSY)**

Theory not to be discussed here. 2D NMR technique where 1D is 1H NMR spectrum and the 2nd D is the Diffusion coefficient (*D*).

**Larger *D* = lower hydrodynamic radius = lower MW.**

**Can be calibrated to give you exact MW of a complex.**

**SLIDE DOSY DATA FOR SMALL MOLECULES**

**SLIDE DOSY DATA FOR HOST-GUEST COMPLEXES**

Indicate that the *D* value of 3 x 10–6 corresponds to MW of dimer plus cobaltocenium.

**2d. Other methods for determining solution stoichiometry**

**Vapor pressure osmometry (VPO)** – used to determine MW, but not terribly accurate

**Electrospray Mass Spec**

**X-ray crystallography**

(The latter two assume that gas phase and solid-state stoich. Is same as in solution)

**3. Kassoc for systems in slow exchange** (less common, much easier)

**Theory.**

If you can see free A and bound AB distinctly, you can:

**-integrate signals to get free:bound ratio**

**-use mass balance equations to determine actual concentrations.**

**-plug into Kassoc expression**

**Practice/Example.**

**DIAGRAM REPRODUCE HOST+COBALTOCENIUM NMR WITH INTEGRALS FOR FREE (1) AND BOUND (4) SIGNALS**

**Assume 1:1 stoichiometry of H and Co (H + Co 🡨 🡪 H•Co)**

**Cot = 2 mM** (THIS IS HOW WE DENOTE THE **TOTAL AMOUNT PUT IN)**

**Ht = 3 mM**

**What’s the Kassoc ? [HCo]/[H][Co]**

You know Ht and Cot, but Kassoc expressions always include actual, free concentrations

*This is the central problem of determining Kassoc values*

**Cot = [Co]free + [HCo] A MASS BALANCE EQUATION**

**[HCo] = 4[Co]free** from integration

**Cot = [Co]free + 4[Co]free = 2 mM**

**[Co]free = 0.4 mM**

**[HCo] = 1.6 mM**

**Ht = [H]free + [HCo] = 3 mM, so [H]free = 1.4 mM**

**Kassoc = .0016 M/(.0014 M • .0004 M) = 2856 M–1**

**4. Kassoc for systems in fast exchange** (more common, much harder)

**DIAGRAM AB equilibrium and A SINGLE NMR SIGNAL SEEN FOR A AND AB**

Integration useless. All you see for A and AB is a single chemical shift, ∂obs.

**∂obs is a weighted average of ∂free and ∂bound**

**∂obs = f10•∂free + f11•∂bound**

**Where f10 is the fraction of A in free state and f11 is the fraction of A in the 1:1 bound state**

**f10 = [A]free/At and f11 = [AB]/At**

**All A is in free or bound state**, **so**

**f10 + f11 = 1, f10 = 1 – f11**

**∂obs = (1 – f11)•∂free + f11•∂bound**

**4a. A special case: when ∂free and ∂bound are known**

**Not to be used.** But a useful warmup.

**Theory.**

**If you know ∂free and ∂bound then**

**-∂ tells you the fraction bound, which tells you ratio of free:bound**

**-use mass balance equations to determine actual concentrations.**

**-plug into Kassoc expression**

**Practice/Example.**

**SLIDE REAL DATA FROM A CALIXARENE-PEPTIDE COMPLEX**

**∂free = 3.3 ppm, ∂bound = 2.2 ppm**

**peptidet = Pt = 1 mM**

**0.75 eq of calixarene (C) Ct = 0.75 mM**

**∂obs = 2.62 ppm**

Recall **∂obs = (1 – f11)•∂free + f11•∂bound**

*Group algebra problem — What is f11?*

*2.62 = 3.3 – 3.3 f11 + 2.2 f11*

*–0.68 = –1.1 f11*

*f11 = 0.62 (and f10 = 0.38)*

**[CP]/[C] = 0.62/0.38**

**[CP] = 1.63[C]free** from ∂obs

**Ct = [C]free + [CP] A MASS BALANCE EQUATION**

**Ct = [C]free + 1.63[C]free = 0.75 mM**

**[C]free = 0.28 mM**

**[CP] = 0.47 mM**

**Pt = [CP] + [P]free = 1 mM**

**[P]free = 0.53 mM**

**Kassoc = [CP]/[C]free[P]free = 0.00047/0.00028•0.00053 = 3167 M–1**

**Errors and assumptions.**

**This Kassoc arises from a single observed data point**

**Errors in ∂free, ∂bound, and ∂obs are relatively large**

**Large error in Kassoc**

**Assumption is 1:1 binding and no other species.**

**Provides no internal check of stoichiometry**

**If you must use this method, you should average over several different data points.**

**4b. The normal case: when ∂bound is unknown — titrations**

**Theory.**

**During a titration of B into A, the concentration of AB increases.** (Equilibrium driven forward)

**DIAGRAM The change in ∂obs as a function of Bt follows an asymptotic path toward saturation**

**Steeper = higher Kassoc, shallower = lower Kassoc** (higher Kassoc is driven to the right more quickly)

**What is the formula for that curve? (f11 in terms of [B]?)**

**f11 = [AB]/At**

**Since At = [AB] + [A]**

**f11 = [AB]/([A] + [AB])**

**Since [AB] = K11[A][B]**

**then f11 = K11[A][B] / ([A] + K11[A][B]) remove [A] from brackets**

**f11 = K11[A][B] / [A](1 + K11[B])**

**f11 = K11[B] / (1 + K11[B]) The generalized 1:1 binding isotherm.**

**f11 (fraction bound) must be related to some experimental observable.**

**NMR (∂ values!):**

**Recall that ∂obs = (1 – f11)•∂free + f11•∂bound**

**∂obs = ∂free – f11•∂free + f11•∂bound** collect f11

**∂obs = ∂free + f11(∂bound – ∂free)**

**Now define:**

**∆∂obs = ∂obs – ∂free**

**∆∂max = ∂bound – ∂free**

**f11 = ∆∂obs / ∆∂max This makes sense!!!**

**The NMR 1:1 binding isotherm**

**∆∂obs / ∆∂max = K11[B] / (1 + K11[B])**

**∆∂obs = ∆∂max•K11[B] / (1 + K11[B])**

**Practice, Method 1. Linearized plots**

**1a. Benesi-Hildebrand plot (double-reciprocal plot)**

**1/∆∂obs = (1/∆∂maxK11[B]) + 1/∆∂max**

**y axis 1/∆∂obs**

**x axis 1/[B]**

**slope 1/∆∂maxK11**

**y-intercept 1/∆∂max**

**SLIDE BENESI-HILDEBRAND EXAMPLE**

**Errors and assumptions.**

**- At must be kept constant** (it’s not explicitly in the expression)

**- YOU DON’T ACTUALLY KNOW [B]** since that’s the free concentration of B

**- YOU KNOW Bt**

**Assume: If Bt >> At then Bt ~ [B]** (Only At amount of Bt can be bound, the rest is free.)

**- Errors of extrapolation to intercepts are always large**

*If Bt is not >> At, what would this plot look like? Are the errors at the low conc. or high conc. regime?*

 *-At low conc. of Bt, it will deviate more from the statement that Bt ~ [B]. That’s at the right end of the B-H plot of 1/Bt. Actually, [B] < Bt and so you’d expect the actual data to curve upward at the right end.*

*Given what you know about linear regression, which has more influence on the line, a point at high or low concentration?*

 *-move a point at left (high conc.) off the line… DIAGRAM the weighted average.*

 *-move a point at right (low conc.) off the line… DIAGRAM the weighted average.*

 *-Which has the bigger change of* ***slope?***

**- Errors arise from uneven spread of data** Data far from cluster is more heavily weighted

**Practice. Method 1b. Scatchard plot (x-reciprocal plot)**

**∆∂obs/[B] = –K11∆∂obs + ∆∂maxK11**

**y axis ∆∂obs/[B]**

**x axis ∆∂obs**

**slope –K11**

**y-intercept ∆∂maxK11**

**SLIDE SCATCHARD EXAMPLE**

**Errors and assumptions**

**Better spread of data, rest of errors and assumptions are the same as B-H plot.**

**Practice, Method 2. Non-linear plot and curve fitting**

**The problem of Bt ≠ [B] is called generally “ligand depletion.”**

Is there a more complex 1:1 isotherm that includes real values of At and Bt?

**K = [AB]/[A][B]**

**K = [AB]/(At – [AB])(Bt – [AB])**

*Not for the board*

*multiply through*

*K = [AB]/{AtBt – Bt[AB] – At[AB] + [AB]2}*

*then invert*

*1/K = {AtBt – Bt[AB] – At[AB] + [AB]2}/[AB]*

*then move [AB] over*

*[AB](1/K) = AtBt – Bt[AB] – At[AB] + [AB]2*

*then collect [AB] terms on one side*

*0 = AtBt – Bt[AB] – At[AB] – [AB](1/K) + [AB]2*

*then collect factors of [AB]*

**algebra…**

**0 = AtBt – (Bt + At + 1/K)([AB]) + [AB]2**

**This is a quadratic in the unknown [AB] of the form 0 = ax2 + bx + c,**

**The quadratic equation x = –b ± (root(b2 – 4ac))/2a gives us an expression for [AB] as a function of At, Bt, and K**

(where C = AB ; H = A ; G = B)

See **Schalley, page 40**

Remember that

**f11 = [AB]/At = ∆∂obs / ∆∂max**

**∆∂obs = ∆∂max [AB] / At**

**So then the 1:1 isotherm used for NMR titration data is:**

**∆∂obs**  (where a = ∆∂max ; b = K11 ; H = A ; G =B)

**Iterative non-linear regression: initial guesses of parameters are optimized upon iteration, until the curve converges to the data points. DIAGRAM THIS.**

**Normally**

**- At is known and constant**

**- Bt is the independent variable (x)**

**- ∆∂obs is the dependent variable (y)**

**- ∆∂max and Kassoc­ are parameters to be fitted.**

**SLIDE EXEMPLARY NMR TITRATION DATA**

**Errors and assumptions**

**- You assume no contributions to ∆∂ from 2:1 or 1:2 complexes**

**- The fitted parameters can compensate for each other:**

**the results can depend on initial guesses**

**sizes of errors are linked**

**if >2 parameters are fitted**, **almost any data will fit to the 1:1 curve**, you get farther from real understanding **- Beware the black box**

**5. HOW TO RUN AND ANALYZE A TITRATION**

Overview: sample prep, titration, data analysis by Origin

**SLIDE SAMPLE PREP**

**SLIDE TITRATION**

**SLIDE DATA ANALYSIS**

***C. Thermodynamic parameters by NMR***

Recall that it is often useful to know the enthalpic and entropic contributions to binding

Generally, ∆G = ∆H – T∆S

When this describes an equilibrium association, then

**∆G = –RT ln Kassoc = ∆H – T∆S**

**ln K = ∆H/RT + ∆S/R**

**ln K = (1/T)(∆H/R) + ∆S/R**

So K depends on T!

**van’t Hoff plot** (linearized)

**Determine Kassoc at various T, plot ln K vs. 1/T**

**Slope = ∆H/R**

**y-intercept = ∆S/R**

**SLIDE RAFFA’s VAN’T HOFF PLOT**

**Errors and assumptions**

**-This assumes that ∆H and ∆S are invariant with temperature. Only true over ~30 K ranges, if that**

(often get curved van’t Hoff plots)

**-Extrapolation to y-intercept is very error prone**

***D. Exchange kinetics by NMR***

**I’m not an expert, so:**

The purpose of this section is not to give you step-by-step instructions, but to give you an introduction.

Aim 1 – to be able to understand literature.

Aim 2 – to be able to read further if you want to carry out these experiments.

**Case 1. Very slow kinetics**

**-signals will be resolved on NMR time scale**

-Measure kinetics by taking **many NMRs over time**

**-Integrate to determine concentrations**

**-Fit to 1st-order, 2nd-order kinetics, etc.**

**SLIDE EXAMPLE OF A VERY SLOW GUEST EXCHANGE REACTION**

**Case 2. Intermediate exchange kinetics. Line-shape analysis.**

**For a given broad NMR spectrum, you can calculate the rate of exchange k = k1 + k–1**

This is called **line-shape analysis**

**SLIDE LINE SHAPE ANALYSIS OVERVIEW (DMF)**

**NOTE: you must know: 0 of line 1, 0 of line 2**

**VT-NMR allows you to go below coalescence to determine 0 values**

**SLIDE EXAMPLE OF GUEST EXCHANGE LINE SHAPE ANALYSIS**

**Case 3. Slow-to-intermediate exchange kinetics. EXSY.**

REVIEW: A NOESY experiment.

**DIAGRAM NEARBY PROTONS AND THE NOE**

Pulse – delay – observe.

During delay time, polarization is transferred from one proton to a nearby proton via nOe.

**DIAGRAM A 2D NOE SPECTRUM HAS CROSS PEAKS THAT INDICATE POL. TRANSFER**

**What if the protons change places during delay?**

**DIAGRAM DMF PROTONS EXCHANGING DURING ~1 ms**

**- Polarization will transfer, and cross peak arises**

**EXchange SpectroscopY (EXSY) is a variation on NOESY NMR**

**Same pulse sequence, with variation of delay time between ~10 us and 2 s depending on how fast your system is exchanging.**

**Integration of EXSY cross peaks gives you k1 and k–1**

**SLIDE EXSY DATA FOR HOST-GUEST EXCHANGE**

**Limits:**

**- separate signals must be observed (slow-intermediate exchange).**

**- upper limit for exchange time is set by natural rate of spin-lattice relaxation (T1) of polarization (~0.5-2 s)**