Solutions

May 2, 2009

Analytical Cume: Fourier transform pictures.

Whereas NMR spectra of small molecules in solution produce sharp Lorentzian peaks, powders and solids can routinely produce broad peaks from inhomogeneous broadening. In such cases, high resolution spectra are often not required, or even desired because of the long acquisition times necessary for high S/N due to peak broadening and relatively slow spin-lattice relaxation. In such instances, low-resolution spectra can be obtained by first performing a conventional 90° pulse, followed by a series of 180° pulses (see below). Each 180° pulse is followed in time by a spin echo, in which the free induction decay rephases. The entire repeating interferogram is then Fourier transformed to recover the low resolution spectrum. The mechanism by which this operation works can be understood using a Fourier transform basis set toolkit.

(SIC exam)

Preamble.

1. (10) What dictates the frequency resolution (e.g., ppm) in an FT-NMR measurement? Explain.

The time over which the interferogram is recorded. Resonances close in frequency will slowly drift apart in time and enable resolution in the interferogram. In terms of Heisenberg uncertainty, $\Delta\nu\Delta t>1$; decreased uncertainty in frequency resolution is achieved by increased acquisition time.

2. (10) What dictates the time required between sequential NMR measurements for signal averaging? Explain.

The spin-lattice relaxation time. Magnetic dipole must realign in order for the memory of the previous measurements to be lost.

Amble.

3. (10) The answers to the Preamble present a problem, as they are mutually incompatible for the fast acquisition of low-resolution spectra. Let's see if we can unravel this pulse train approach for getting around this fundamental incompatibility. The spin echoes generated by the train of 180° pulses can be interpreted mathematically by the convolution of a comb in time with the even interferogram generated by the first 180° pulse. What is the Fourier transform of a comb in time with amplitude of 1 and a spacing of t_0?

The FT of a comb is a comb. The amplitude of the frequency comb will be 1 and the spacing between frequency maxima will be $f_0 = 1/t_0$.

4. (10) Now, let's consider the influence of this temporal comb on the Fourier transform of the repeating interferogram. If the comb is convolved with the signal in time, what operation will be performed in the frequency domain?

Multiplication.

5. (15) If a low-resolution spectrum with spacing between data points of 0.5 ppm is desired, what value of t_0 should be used in the temporal comb when using an 800 MHz NMR? Explain.

$$f_0 = \frac{0.5}{10^6}\frac{800 \times 10^6}{s} = 400\ Hz$$

$$t_0 = \frac{1}{f_0} = 2.5\ ms$$

6. (15) If the pure dephasing time T_2 is 0.1s, how many times can the comb be repeated for effective signal averaging until the initial coherence from the 90° pulse is lost?

$$400Hz * 0.1s = 40\ cycles$$

7. (15) If the spin-lattice relaxation time T_1 is 1s, how long would it take to obtain comparable signal to noise by conventional signal averaging performed by acquiring numerous spectra (i.e., repeat measurements of the interferogram generated following a 90° pulse)?

$$40cycles * 1s = 40s$$

Postamble.

8. (15) In the spectral domain, the lineshape in a powder is inhomogeneously broadened and can be assumed to be essentially flat over each spike in the low-resolution spectrum. Therefore, the

spectral lineshape that emerges will effectively be dictated solely by the convolution with a temporal comb of finite duration. What functional form for the lineshape is expected, and what is the characteristic bandwidth, f_0 of each spike in the low-resolution spectrum? Please note, the value for f_0 dictating the spectral lineshape is NOT dependent on the value of t_0 from question 6, but arises from a different convolution.

The starting/stopping of the temporal comb represents a multiplication by a rectangle in time, corresponding to a convolution of a comb with a sinc function in frequency. The sinc function lineshape (or sinc squared for intensity) will have a characteristic frequency corresponding to the duration of the comb. Using the notation of F. E. Lytle, $\text{rect}(t_0) \leftrightarrow \text{sinc}(f_0)$. For a rectangle with a duration of 0.1s, the lineshape will be a sinc function with the first node occurring at $\pm$10Hz = $\pm$0.0125 ppm.

Biochemistry Cume
Spring 2009

Quantification of specific proteins is a problem.

a. Why is that? It is easy to quantify amino acids, small organic compounds, inorganic ions, and even DNA species. Why are proteins a problem.
b. What are some of the ways that individual proteins have been quantified and which of the methods you list are best or of greatest utility in specific applications. Give at least three methods of individual protein quantificaiton.

Proteins often exist in multiple forms that range from an unmodified parent to a form containing a specific post-translational medication (PTM), on to splice variants.

c. How would you quantify all forms of a particular protein without regard to modifications? By this I mean the parent protein and all modified forms together.
d. How would you quantify the amount of a protein with a specific PTM? Phosphorylation, proteolysis, methylation, glycosylation, an acetylation are all examples of a PTM you could use.
e. How would you quantify a splice variant form of a protein?

CRIB:

1. Why is it hard to quantify specific proteins?

Answer. Although proteins differ in primary, secondary, tertiary, and often quaternary structure, they all have very similar chemical behavior. This means that in a mixture it is generally not possible with a chemical quantification test to differentiate one protein from another.

2. What are some of the ways that individual proteins have been quantified and which of the methods you list are best or of greatest utility in specific applications. Give at least three methods of individual protein quantification.

Answer. Separation methods have been used in which all other interfering proteins are removed. This can be done by chromatography, electrophoresis, differential precipitation, or some immunological separation method. Having purified the protein it can then be quantified in a number of ways such as absorbance, fluorescence (sometimes), a colorimetric test such as comaisse staining, or silver staining to name a few.

Optical methods such as surface plasmon resonance and interferometry have also been used. These methods are measuring a physical property of the molecule such as refractive index.

Yet another method is to quantify the biological activity of the protein of interest when this activity is directly proportional to it's concentration. Enzyme activity is the most common.

Immunological assays are yet another way to quantify a specific protein although they are another form of separation. After reaction of a specific proteins with a labeled antibody, unbound labeled antibody is separated from the immunological complex and the concentration of antibody in the complex determined.

3. Proteins often exist in multiple forms that range from an unmodified parent to a form containing a specific post-translational medication (PTM), on to splice variants. How would you quantify all forms of a particular protein without regard to modifications? By this I mean the parent protein and all modified forms together.

Answer. You must find some way to isolate all the isforms of the protein from the mixture in which it is contained so all the isoforms can be determined together. This is generally done with an antibody that targets a single epitope common to all the isoforms. Having thus resolved all the isoforms they can be quantified together by all the methods described above in the second answer.

4. How would you quantify the amount of a protein with a specific PTM? Phosphorylation, proteolysis, methylation, glycosylation, an acetylation are all examples of a PTM you could use.

Answer. One way is through an extension of the answer to the third question. Having isolated all the isoforms of a protein one then must determine a specific PTM. This can be done either by using a fluorescent labeled second antibody that targets the PTM or my mass spectrometry of a tryptic peptide carrying the PTM of interest.
0A

A second approach is to do the whole thing by liquid chromatography-mass spectrometry. A proteome would be tryptic digested and the tryptic digest separated by reversed phase chromatography. The PTM bearing peptide would be quantified with a QQQ mass spectrometer in which the molecular weight of the PTM peptide is selected in the first dimension and fragment ions from the PTM peptide are selected in the second dimension.

5. How would you quantify a splice variant form of a protein?

Answer. The solution to this problem can be achieve either through an immunological assay or by MS based proteomics. In the case of the immunological assay you would need an antibody targeting an epitope at the splice junction. Splice junctions are unique to the splice variant. With MS based proteomics you would be qauntifying the tryptic peptide carrying the splice junction. This peptide is unique to the splice variant protein alone. Quantification could be achieved in either the label free mode or by MRM analysis.

No Inorganic crib available
May 2, 2009
Written by Professor Bart

No Organic crib available
May 2, 2009
Written by Professor Chmielewski

Physical Cumulative Exam
May 2, 2009

1.

$$A + FN \rightleftharpoons FNA$$

$$FNA + B \rightleftharpoons NB + F + A$$

$$FNA + B + M \rightleftharpoons FNBM + A$$

$$\frac{d[FNA]}{dt} = k_1[A][FN] - k_{-1}[FNA] - k_2[FNA][B] - k_3[FNA][B][M] + k_{-2}[NB][F][A] + k_{-3}[FNBM][A]$$

$$\frac{d[FN]}{dt} = -k_1[A][FN] + k_{-1}[FNA]$$

$$\frac{d[A]}{dt} = -k_1[A][FN] + k_{-1}[FNA] + k_2[FNA][B] - k_{-2}[NB][F][A] + k_3[FNA][B][M] - k_{-3}[FNBM][A]$$

$$\frac{d[B]}{dt} = -k_2[FNA][B] + k_{-2}[NB][F][A] - k_3[FNA][B][M] + k_{-3}[FNBM][A]$$

$$\frac{d[FNBM]}{dt} = k_3[FNA][B][M] - k_{-3}[FNBM][A]$$

4.

Assume $k_2 \gg k_3$ that is rates for

and $k_{-3} \gg k_{-2}$

$$\Rightarrow \frac{d[FNA]}{dt} = k_1[A][FN] - k_{-1}[FNA] - k_2[FNA][B] + k_3[FNBm][A]$$

$$\frac{d[FN]}{dt} = -k_1[A][FN] + k_{-1}[FNA]$$

$$\frac{d[A]}{dt} = -k_1[A][FN] + k_{-1}[FNA] + k_2[FNA][B] - k_{-3}[FNBm][A]$$

$$\frac{d[B]}{dt} = -k_2[FNA][B] + k_{-3}[FNBm][A]$$

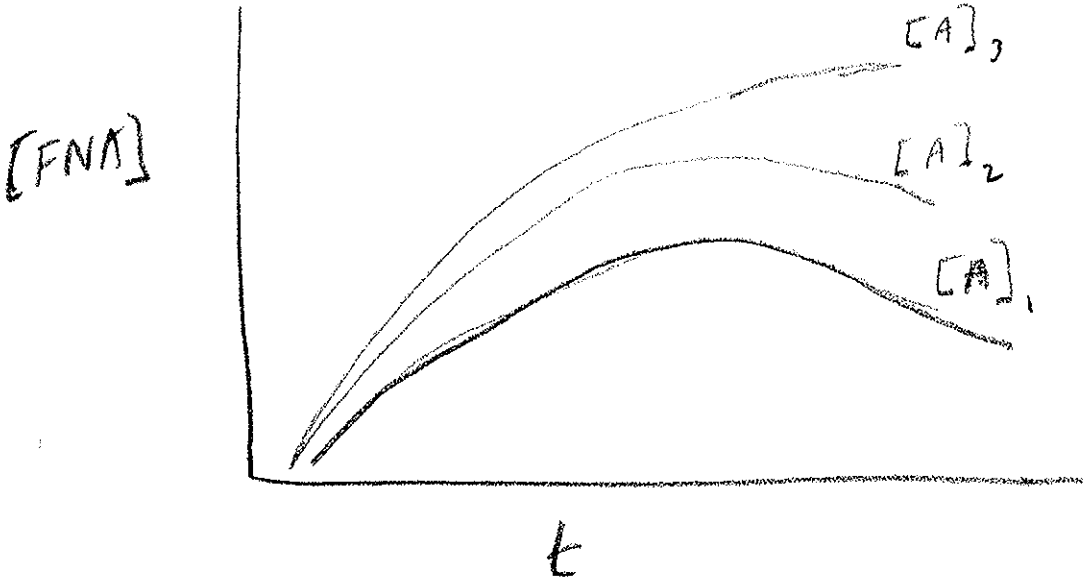
if concentrations of [A] and [B] are large $\Rightarrow \frac{d[A]}{dt} = \frac{d[B]}{dt} = 0$

$$\rightarrow k_1[A][FN] = k_{-1}[FNA]$$

$$\frac{d[FNA]}{dt} = -k_2[FNA][B] + k_3[FNA][A]$$

$$\frac{d[FNA]}{dt} = \left(k_3[A] - k_2[B]\right)[FNA]$$

$$[FNA] = [FNA]_0 \, e^{+\left(k_3[A] - k_2[B]\right)t}$$



when $[A]_1 < [A]_2 < [A]_3$

2.

$$A + X \longrightarrow 2X$$
$$X + X \longrightarrow 2Y$$
$$Y \longrightarrow Z$$

$$\frac{d[A]}{dt} = -k_1[A][X]$$

$$\frac{d[X]}{dt} = -k_1[A][X] - 2k_2[X]^2$$

$$\frac{d[Y]}{dt} = 2k_2[X]^2 - k_3[Y]$$

$$\frac{d[Z]}{dt} = k_3[Y]$$

The overall reaction is

$$A + X \longrightarrow Z$$

so the rate law for the overall reaction

$$-\frac{d[A]}{dt} = -\frac{d[X]}{dt} = +\frac{d[Z]}{dt} = k_{overall}[A][X]$$

However, this is valid under the following conditions

condition (1) $\frac{d[X]}{dt} = \frac{d[Y]}{dt}$ or when $[X]$ and $[Y]$ are under steady state

$$-k_1[A][X] - 2k_2[X]^2 = 2k_2[X]^2 - k_3[Y]$$

$$-k_1[A][X] - 4k_2[X]^2 = -k_3[Y]$$

$$k_1[A][X] + 4k_2[X]^2 = k_3[Y]$$

$$(k_1[A] + 4k_2[X])[X] = k_3[Y]$$

$$\frac{d[Z]}{dt} = (k_1[A] + 4k_2[X])[X]$$

condition (2) if $k_1 \gg k_2$ or if $[A] \gg [X]$

$$\frac{d[Z]}{dt} = k_1[A][X]$$

3.

$$HOBr + OClO^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (HOBrOClO)^-$$

$$(HOBrOClO)^- + HA \xrightarrow{k_{HA}} H_2O + A^- + [BrOClO]$$

$$[BrOClO] + OClO^- \xrightarrow{k_3} Br^- + 2\,ClO_2$$

$$[BrOClO] + OH^- \xrightarrow{k_4} Br^- + ClO_3^- + H^+$$

where $(HOBrOClO)^-$ is in steady state and $[BrOClO]$ is a mech-shake intermediate.

(a)

$$\frac{d[HOBr]}{dt} = -k_1[HOBr][OClO] + k_{-1}[HOBrOClO]$$

$$\frac{d[HOBrOClO]}{dt} = k_1[HOBr][OClO^-] - k_{-1}[HOBrOClO] - k_{HA}[HA][HOBrOClO]$$

$$\frac{d[BrOClO]}{dt} = k_{HA}[HA][HOBrOClO] - k_3[BrOClO][OClO^-] - k_4[BrOClO][OH^-]$$

(a) Assume [HOBrOClO] is in steady state

$$\frac{d[HOBrOClO]}{dt} = k_1[HOBr][OClO^-] - k_{-1}[HOBrOClO] - k_{HA}[HA][HOBrOClO]$$

$$0 = k_1[HOBr][OClO^-] - (k_{-1} + k_{HA}[HA])[HOBrOClO]$$

$$[HOBrOClO]_{ss} = \frac{k_1[HOBr][OClO^-]}{(k_{-1} + k_{HA}[HA])}$$

$$\frac{d[HOBr]}{dt} = -k_1[HOBr][OCl^-] + \frac{k_{-1}k_1[HOBr][OCl^-]}{k_{-1} + k_{HA}[HA]}$$

$$= \left(-k_1 + \frac{k_{-1}k_1}{k_{-1} + k_{HA}[HA]}\right)[HOBr][OCl^-]$$

$$\frac{d[HOBr]}{dt} = \left(\frac{-k_1 k_{HA}[HA]}{k_{-1} + k_{HA}[HA]}\right)[HOBr][OCl^-]$$

if $[OCl^-] \gg [HOBr] \Rightarrow [OCl^-]$ should be constant

$$\Rightarrow -\frac{d[HOBr]}{dt} = \left(\frac{k_1 k_{HA}[HA][OCl^-]}{k_{-1} + k_{HA}[HA]}\right)[HOBr]$$

since $[HA]$ is a constant

$$\Rightarrow \frac{k_1 k_{HA}[HA][OCl^-]}{k_{-1} + k_{HA}[HA]}$$ is a constant

$$[HOBr]_t = [HOBr]_0 \, e^{-\left(\frac{k_1 k_{HA}[HA][OCl^-]}{k_{-1} + k_{HA}[HA]}\right)t}$$

b.

$$\frac{d[OCl^-]}{dt} = -k_3[Br_2OCl][OCl^-]$$

$$\frac{d[OH^-]}{dt} = -k_4[Br_2OCl][OH^-]$$

$$\frac{d[OCl^-]}{d[OH^-]} = \frac{k_3}{k_4}\frac{[OCl^-]}{[OH^-]}$$

$$\frac{\frac{d[OCl^-]}{[OCl^-]}}{\frac{d[OH^-]}{[OH^-]}} = \frac{k_3}{k_4}$$

$$\frac{d\ln[OCl^-]}{d\ln[OH^-]} = \frac{k_3}{k_4}$$

Following the $\ln(OCl^-)$ vs $\ln(OH^-)$ concentrations will give the $\frac{k_3}{k_4}$ ratio

4.

$$D + HBr \xrightarrow{k_1} DBr + H$$

$$H + DBr \xrightarrow{k_2} HBr + D$$

$$\frac{d[HBr]}{dt} = -k_1[HBr][D] + k_2[DBr][H]$$

$$\frac{d[DBr]}{dt} = k_1[HBr][D] - k_2[DBr][H]$$

assume $\frac{d[HBr]}{dt} = \frac{d[DBr]}{dt}$

$$-k_1[HBr][D] + k_2[DBr][H] = k_1[HBr][D] - k_2[DBr][H]$$

$$2k_2[DBr][H] = 2k_1[HBr][D]$$

$$\boxed{\frac{k_1}{k_2} = \frac{[DBr][H]}{[HBr][D]}}$$

5.

thermal $A \xrightarrow{\Delta} A^* \longrightarrow$ products

chemical $A \xrightarrow{B^*} A^* \longrightarrow$ products

infrared multiphoton $A + n h\nu \longrightarrow A^* \longrightarrow$ products

Thermal excitation involves the collision of A with wall where by energy is absorbed from the wall to energize A for unimolecular decomposition

Chemical excitation involves the collision of chemical species B which upon collision with A energizes A for unimolecular decomposition

Infrared Multiphoton excitation involves the absorption of multiple infrared photons to energize A for unimolecular decomposition