Metabolic Dynamics – Kinetics and Transport Measured by 1 and 2D NMR Spectroscopy, Modeling metabolic flux

Chemical Exchange in NMR

- Chemical exchange in NMR refers to any process in which a nucleus exchanges between two or more environments in which its NMR parameters (chemical shift, scalar coupling, dipolar coupling, relaxation rate) differ.
- Chemical exchange in NMR can be a curse or rewarding – its influence can cause signals to become invisible beyond detection or it can allow one to uncover a large range of motional properties at every site within a molecule ...

Types of Chemical Exchange

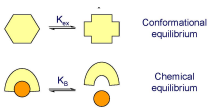
Intramolecular exchange

- Motions of sidechains in proteins
- Helix-coil transitions of nucleic acids
- Unfolding of proteins
- Conformational equilibria

Intermolecular exchange

- Binding of small molecules to macromolecules
- Protonation/deprotonation equilibria
- Isotope exchange processes
- Enzyme catalyzed reactions

A ⇌ B

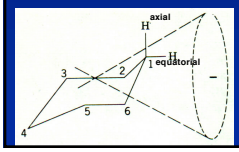


M+L ⇌ ML

Because NMR detects the molecular motion itself, rather the numbers of molecules in different states, NMR is able to detect chemical exchange even when the system is in equilibrium

Conformational Equilibrium

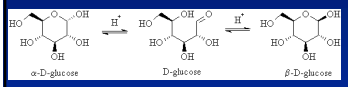
- Chemical shift equivalence is also caused by rapid exchange mechanisms (faster than once in 10^{-3} sec.).
  - For example, the chemical shift of the cyclohexane protons is an average of the shifts of the axial and equatorial protons. At room temperature, the spectrum consists of a singlet at 1.42 ppm, the average of the axial and equatorial chemical shifts.



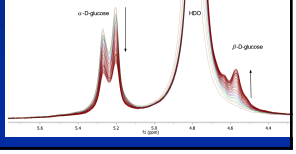
The equatorial protons would be more de-shielded than the axial protons if there was not a rapid exchange of these two positions.

Conformational Equilibrium

- Non-equivalence – E.g. Mutarotation of carbohydrates
- The α- and β- anomers of carbohydrates are typically stable solids. However, in aqueous solution, they quickly equilibrate to an mixture of the two forms.
  - For example, in aqueous solution, glucose exists as a mixture of 36% α- and 64% β- (>99% of the pyranose forms exist in solution).

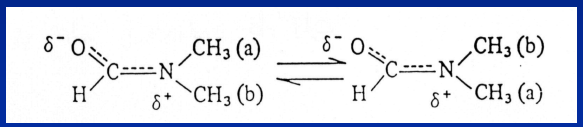


80 mg of α-D-glucose was dissolved in 0.8 mL D2O



Conformational Equilibrium

- At room temperature, N,N-dimethylformamide shows 2 CH_3 peaks, because of the rate of rotation around the hindered C–N bond is slow on the NMR time scale.
- However, as one raises the temperature the barrier to rotation is overcome and the two CH_3 peaks merge into a single peak at ≈ 123° C.



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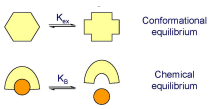
$A \rightleftharpoons B$



$M+L \rightleftharpoons ML$

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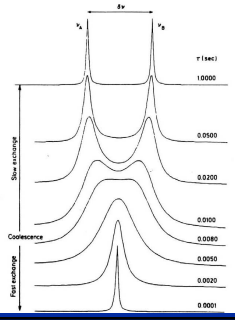
NMR Time Scale

Time Scale	Chem. Shift, δ	Coupling Const., J	T2 relaxation
Slow	$k << \delta_A - \delta_B$	$k << J_A - J_B$	$k << 1/T_{2,A} - 1/T_{2,B}$
Intermediate	$k = \delta_A - \delta_B$	$k = J_A - J_B$	$k = 1/T_{2,A} - 1/T_{2,B}$
Fast	$k >> \delta_A - \delta_B$	$k >> J_A - J_B$	$k >> 1/T_{2,A} - 1/T_{2,B}$
Sec^{-1}	0 – 1000	0 –12	1 - 20

- NMR time-scale refers to the chemical shift timescale.
- The range of the rate can be studied 0.05-5000 s^{-1} for H can be extended to faster rate using ^{19}F, ^{13}C and etc.

2-state First Order Exchange

$A \underset{k_{-1}}{\overset{k1}{\rightleftharpoons}} B$



Lifetime of state A:

$\tau_A = 1/k_{+1}$

Lifetime of state B:

$\tau_B = 1/k_{-1}$

Use a single lifetime

$1/\tau = 1/\tau_A + 1/\tau_B$

$= k_{+1} + k_{-1}$

Slow Exchange k << δ_A -δ_B

A ⇌ B (k1 forward, k_{-1} reverse)

- Separate lines are observed for each state.
- The exchange rate can be readily measured from the line widths of the resonances
- Like the apparent spin-spin relaxation rates, $1/T_{2i,obs}$

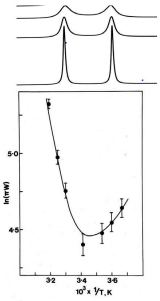
$1/T_{2A,obs} = 1/T_{2A} + 1/\tau_A = 1/T_{2A} + 1/k_1$

$1/T_{2B,obs} = 1/T_{2B} + 1/\tau_B = 1/T_{2B} + 1/k_{-1}$

line width $Lw = 1/\pi T_2 = 1/\pi T_2 + k_1/\pi$

Each resonance is broadened by $\Delta Lw = k/\pi$

Increasing temperature k increases, line width increases



Chemical Exchange Dynamics: 2 site, 1st order case

A ⇌ B (k_{ab}, k_{ba})

$M_a \sim [A]$ Magnetization proportional to concentration

Off Resonance and Relaxation

$$M_a = M_{a,0} e^{i\omega_a t} e^{-t/T_2}$$

$$\dot{M}_a = \left(-k_{ab} + i\omega_a - \frac{1}{T_{2,a}}\right) M_a + k_{ba} M_b$$

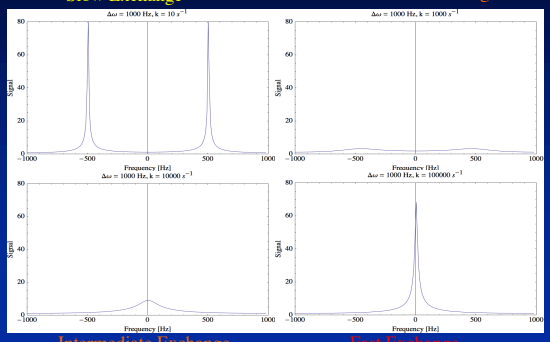
Combined with exchange gives Bloch McConnell Equations

$$\dot{M}_b = \left(-k_{ba} + i\omega_b - \frac{1}{T_{2,b}}\right) M_b + k_{ab} M_a$$

$$\begin{pmatrix} \dot{M}_a \\ \dot{M}_b \end{pmatrix} = \begin{pmatrix} -k_{ab} + i\omega_a - \frac{1}{T_{2,a}} & k_{ba} \\ k_{ab} M_a & -k_{ba} + i\omega_b - \frac{1}{T_{2,b}} \end{pmatrix} \begin{pmatrix} M_a \\ M_b \end{pmatrix}$$

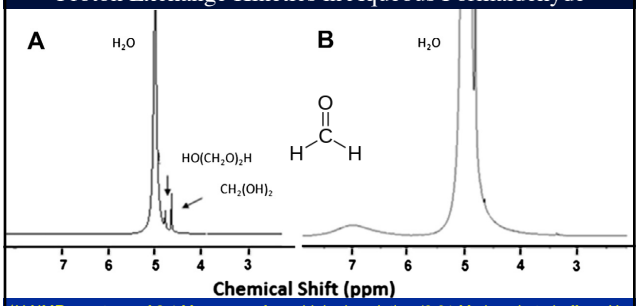
Bloch McConnell Exchange Calculations

Slow Exchange — Intermediate Exchange



Intermediate Exchange — Fast Exchange

Proton Exchange Kinetics in Aqueous Formaldehyde

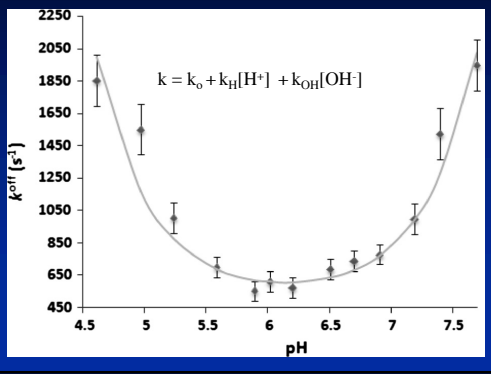


^{1}H NMR spectrum of 2.4 M aqueous formaldehyde solution (0.01 M phosphate buffer, pH = 7.4, T = 1.2 C, 500 MHz). Spectrum B is Spectrum A multiplied by 32.

In The slow exchange regime - exchange rate is related to the linewidth by the expression:

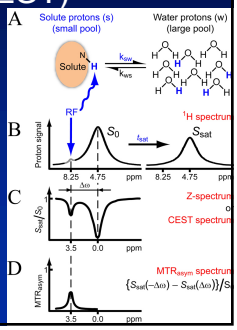
Rivlin, M.et al. *Journal of Magnetic Resonance, 242*, 107–112.

Formaldehyde Proton Exchange – pH Dependence

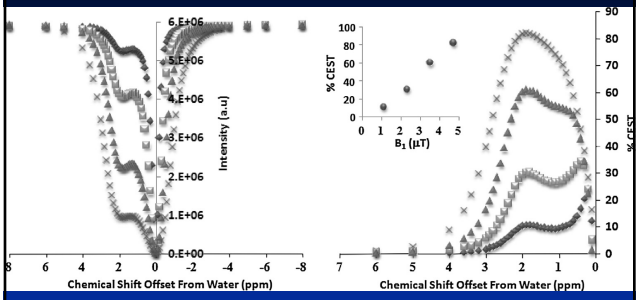


CHEMICAL EXCHANGE SATURATION TRANSFER (CEST)

Chemical Exchange Saturation Transfer (CEST) is a technique where one resonance, in slow exchange with a second resonance, is saturated with a selective low power pulse followed by a hard non-selective 90° pulse. The intensity of the second resonance is then diminished due to the transfer of saturation from the first resonance as the result of chemical exchange.

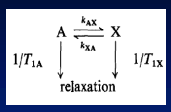


CHEMICAL EXCHANGE SATURATION TRANSFER (CEST) EXPERIMENT



At conditions OH peak cannot be observed directly, it can be detected indirectly through the water signal by the chemical exchange saturation transfer (CEST) experiment. CEST effect of 80% is obtained for methylene glycol concentration of 0.6 M, therefore can use to measure millimole comncentrations of the glycol OH.

Another Exchange Effect: Selective 180° Pulse



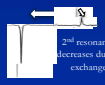
Z-MAGNETIZATIO

① 1ST 180° PULSE

If z-magnetization of each species is inverted relative to the other, exchange will lead to a decrease in total z-magnetization

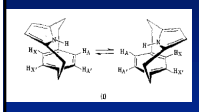
- Inverted spins of one species exchange between pools, depleting z-magnetization of other species
- Subsequent measurement of other species will give lower signal than expected

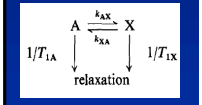
② CHEMICAL EXCHANGE

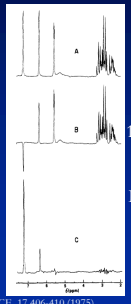


2nd resonance decreases due to exchange

Measuring Exchange with 180° Inversion Pulses



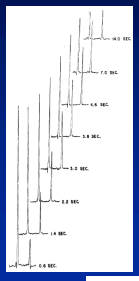




Normal spectrum

After selective 180° delay

Difference spectrum



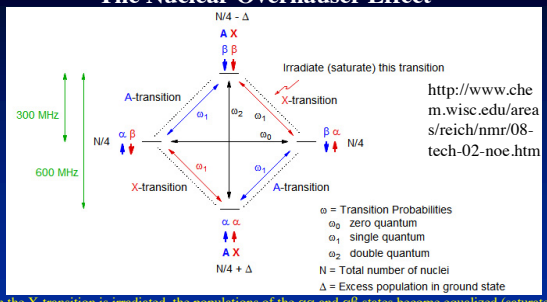
Difference spectra, incremented delay for exchange

$$(A+X) = A_0 e^{-(1/T_{1A})t}$$

$$(A-X) = A_0 e^{-(1/T_{1A}+k_{AX}+k_{XA})t}$$

JOURNAL OF MAGNETIC RESONANCE, 17,406-410 (1975)

The Nuclear Overhauser Effect



http://www.chem.wisc.edu/areas/reich/nmr/08-tech-02-noe.htm

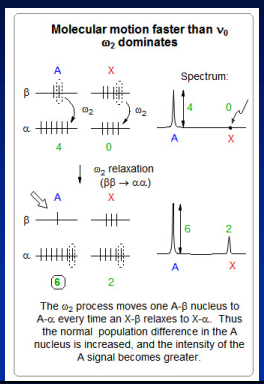
When the X-transition is irradiated, the populations of the αα and αβ states become equalized (saturated), as do the βα and ββ states. As relaxation occurs, the difference in these two populations depends crucially on which of the three relaxation processes dominates.

If ω2 > ω1,ω0 then the βα/ββ population will tend to that of the ββ state, and the αβ/αα states will tend that of the αα state, hence there will be a larger population difference for the A transition (2Δ) than the equilibrium difference (Δ).

The Nuclear Overhauser Effect

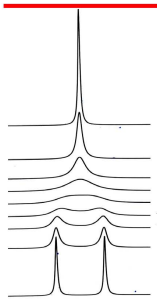
The alteration of normal spin population of a nucleus **X** by irradiation will cause the populations (and hence signal intensities) of other (non-irradiated) nuclei (**A**) to change *provided that **X** is causing T_1 relaxation of **A** by the dipole-dipole mechanism*.

For the ω_2 process, each time an X nucleus relaxes from β to α state, and A nucleus also undergoes a β to α transition (ββ → αα). This has the effect of increasing the population difference of A, i.e. an increase the area of A. **This is a positive NOE.**



Fast Exchange k >> $\delta_A - \delta_B$

$$A \underset{k_{-1}}{\overset{k1}{\rightleftharpoons}} B$$



A single resonance is observed, whose chemical shift is the weight average of the chemical shifts of the two individual states

$$\delta_{obs} = f_A\delta_A + f_B\delta_B, \quad f_A + f_B = 1$$

For very fast limit

$$1/T_{2,obs} = f_A/T_{2A} + f_B/T_{2B}$$

For moderately fast

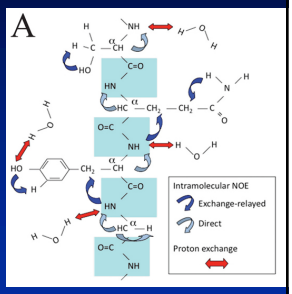
$$1/T_{2,obs} = f_A/T_{2A} + f_B/T_{2B} + f_A f_B{}^2\, 4\pi\,(\Delta\delta_{AB})^2/\, k_{-1}$$

Maximal line broadening is observed when

$$f_A = f_B = 0.5$$

Application to protein dynamics/binding

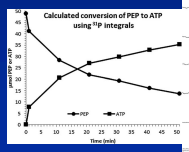
Possible pathways in a mobile protein that can be probed with NMR . Chemical exchange (red) and cross relaxation (blue) occur, the latter either exchange-relayed or through direct excitation of C(α) protons.

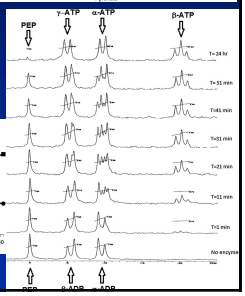


van Zijl et al., Magn. Reson. Med. 2003;49:440–449.

Enzyme kinetics

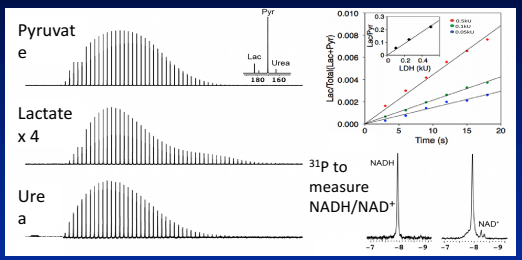
- Simple pulse-acquire
- Magnetization transfer – saturation
  - ^{31}P – Creatine kinase activity *in vivo*





Werner, R. M., & Johnson, A. (2017). *Biochemistry and Molecular Biology Education*, 45(6), 509–514.

Enzyme Kinetics



^{31}P to measure NADH/NAD$^+$

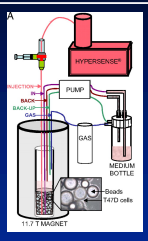
10mm Bioreactor: LDH microspheres: 0.5 kU/mL, 0.1 kU/mL, 0.05 kU/mL 1mL HP [1-^{13}C] pyruvate (7 mM), urea (20mM) injected over 30s. TR=3s FA=10°, 37° C

- Linear characteristics of initial rates of reaction – LDH encapsulated consistently

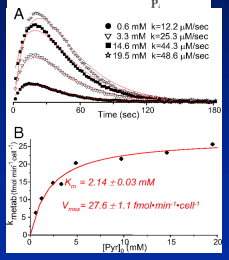
Micro-carrier beads MR bioreactors – reaction

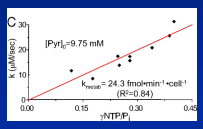
Talia Harris et al. PNAS 2009;106:18131-18136

Scheme of the traditional perfusion system with cells grown on Biosilon polystyrene beads



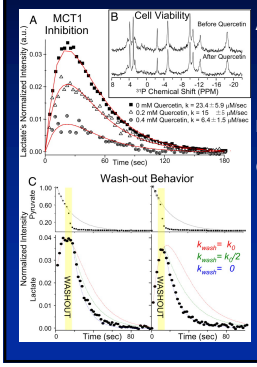
$$k_{metab} = k \cdot \frac{[Pyr]_0}{[P_i]} \cdot \frac{30 \times 10^{-15}\ mol/cell}{\frac{\gamma NTP}{P_i}}$$





Microcarrier beads MR bioreactors – rate limiting factor

Talia Harris et al. PNAS 2009;106:18131-18136



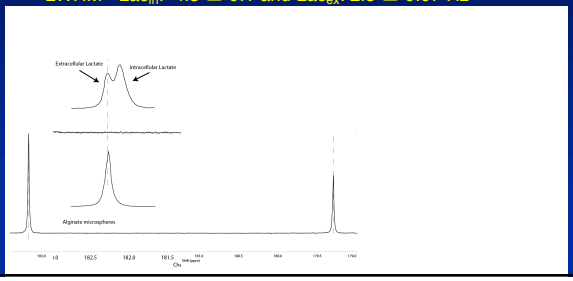
A. MCT1 inhibition - Repeated aliquots of 5.9 mM hyperpolarized $^{13}C_1$-pyruvate into batches of T47D cells, to whose perfusing media the indicated concentrations of inhibitor were coadded

B. ^{31}P NMR spectra showing the constant viability

C. Washout experiments – Simulations of pyruvate-to-lactate conversion whose rate remains unaffected by the washing out ($k_{wash} = k_0$, gray *Upper* and red *Lower*); a conversion whose rate is halved vis-à-vis its original level ($k_{wash} = k_0/2$, green *Lower*); or a conversion whose rate goes suddenly to zero upon restarting the perfusion ($k_{wash} = 0$, blue *Lower*).

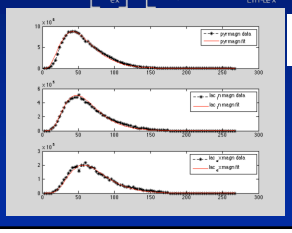
Simultaneous observation of the lactate pools in UOK262 cells

- Lac_{in}: 183.13 ± 0.006 and Lac_{ex}: 183.15 ± 0.006
- LWHM - Lac_{in}: 4.3 ± 0.1 and Lac_{ex}: 2.5 ± 0.07 Hz



Kinetic modeling of bioreactor data

$$\frac{d}{dt}\begin{bmatrix} Pyr \\ Lac_{in} \\ Lac_{ex} \end{bmatrix} = \begin{bmatrix} -1/T_{1P}-k_{PL}-F & k_{LP} & 0 \\ k_{PL} & -1/T_{1Lin}-k_{LP}-k_{Lin\text{-}ex} & k_{Ex\text{-}Lin} \\ 0 & k_{Lin\text{-}ex} & -1/T_{1ex}-k_{Ex\text{-}Lin}-F \end{bmatrix} \times \begin{bmatrix} Pyr \\ Lac_{in} \\ Lac_{ex} \end{bmatrix} + \begin{bmatrix} IF \\ 0 \\ 0 \end{bmatrix}$$

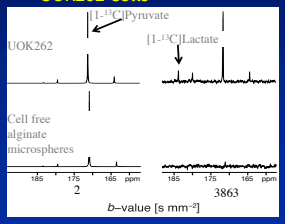


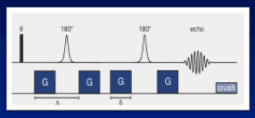
	UOK262	UMRC6
Kpl	0.045±0.0005	0.018

Intracellular lactate T_1 = 26s

Diffusion weighted HP ^{13}C MR in bioreactor

Complete separation of intracellular lactate in UOK262 cells



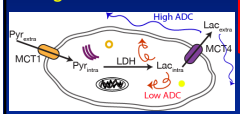


In Bioreactor	Lactate ADC ($\times 10^{-3}$ mm^2/s)
Lactate in solution	1.04 ± 0.08
UOK262 extracellular Lactate	0.57 ± 0.01
UOK262 intracellular Lactate	0.19 ± 0.03

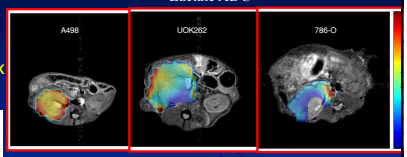
Diffusion weighted hyperpolarized MR *in vivo* to study transport in real time

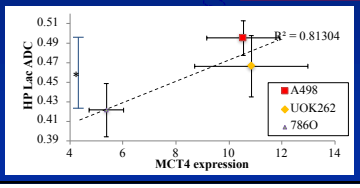
Lactate ADC calculated using 4 b values (R^2>0.7)

High MCT4 -> higher efflux → higher lactate ADC

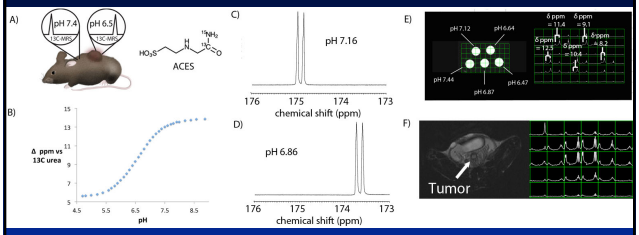


Lactate ADC





Hyperpolarized ^{13}C ACES pH Imaging



- **ACES has a pH dependent chemical shift change over the physiologic range (A-D).**
- **Co-polarize and use 13C Urea as a chemical shift reference**

Hyperpolarized ^{13}C Bicarbonate pH Imaging