LAB #2: Acquisition and Analysis of a ^{31}P and hyperpolarized ^{13}C spectra from a NMR Compatible Cell Culture Bioreactor

1. What are some of the advantages of using a bioreactor?

2. Why do we encapsulate the cells?

3. What is required to maintain cell viability? How do we achieve this using the bioreactor?

4. Why do we acquire 31P spectra?

5. What information can we obtain from C1 pyruvate? What about C2 pyruvate?

Hyperpolarized NMR Acquisition Protocol:

II.1 Turn on CO2

1. Open Primary ("P") & Reserve ("R") CO2 tanks → turn on monitor → log "P" & "R" pressures
2. Set CO2 flow in NMR room = 1

II.2 Probe Installation

1. Insert 5mm Probe → make relevant connections: VT Temp cable → air hookup → 1H BNC (red) to probe ^{1}H Channel → ^{13}C/^{31}P BNC (green) to probe X Channel
2. Connect X Channel BNC to ^{13}P filter
3. Connect ^{31}P 1/4 wavelength cable
4. From console: Vnmrj → "start" → "spin temp" → "reset VT" → set 37^{o}C → "regulate temperature"

II.3 Sample Loading

1. Convey Bioreactor System to NMR lab
2. Turn on pump + water heater
3. Slide spinner onto NMR tube → measure appropriate depth → tape above spinner
4. Pull sample ejection button
5. Insert NMR tube/Bioreactor Umbilicus into spectrometer → push ejection button
6. Connect CO2 line
7. Check Bioreactor primary line drips → ensure no kinks throughout system

II.4 Probe Tuning

1. From console: open ^{1}H experiment → "**su**"
2. Connect 1H Channel BNC to Tuning Interface → Set Chan=2 → Use (red) match & tune knobs to tune ^{1}H Channel → set Chan=0 → reconnect BNC
3. Use central probe knob to adjust X Channel nucleus selection indicated by sliding bar (set to ^{31}P)
4. From console: open ^{31}P experiment → "**su**"
5. Set **probe= 8** (for ^{31}P) on both indicators using closest and furthest (green) knobs
6. Connect X Channel BNC to Tuning Interface → Set Chan=1 → Use (green) match and tune knobs to tune X Channel → set Chan=0 → reconnect BNC
7. Go back and check/adjust ^{1}H tuning

II.5 Shimming on Proton

1. "Lock" → set z0= 1540 → uncheck "lock" → set power, gain = 0
2. Gradient shim: Tools → "standard calibration experiment" → "setup gradient shim"

→ select 5mm probe → set shim# = 5 (removes 6th order) → “Acquire trial spectra”
3. Select threshold button → set threshold on shim profile → select boundary line button → set boundary on each side of shim profile using left/right clicks
4. “Set window from cursors” → “make shim map from current settings”

II.6 Acquire Pre-Shot ^{31}P Spectrum

1. Check your shim on proton experiment → “**ga**” → “**res**”
2. Run ^{31}P experiment → “**ga**” → use “**wft**” to check progress

II.7 Prep Dynamic ^{13}C Acquisition

1. Connect X Channel BNC to ^{13}C filter
2. Connect ^{13}C 1/4 wavelength cable
3. Use central probe knob to adjust X Channel nucleus selection indicated by sliding bar (set to ^{13}C)
4. From console: open dynamic ^{13}C experiment → “**su**”
5. Set **probe= 13** (for ^{13}C) on both indicators using closest and furthest (green) knobs
6. Connect X Channel BNC to Tuning Interface → Set Chan=1 → Use (green) match and tune knobs to tune X Channel → set Chan=0 → reconnect BNC
7. Make sure sequence is ready to be run with the push of a button

II.8 Acquire Dynamic ^{13}C Spectra

1. Within 10 min. of dissolution, switch bioreactor to reserve supply
2. Within 2 min. of dissolution, open syringe port
3. At time of dissolution, polarizer sample is drawn into syringe
4. Syringe is married to the port without bubbles
5. Simultaneous commencement of injection and acquisition (**ga**[ENTER]) + timing
6. At t=55s, pump is stopped
7. Resume pump at end of acquisition

II.9 Acquire Post-Shot ^{31}P Spectrum

1. Refer to II.4.3-6 & II.6.2

II.10 Clean-up

1. Set CO2 flow in NMR room = 0
2. Turn off monitor/alarm unit → Close Primary (“P”) & Reserve (“R”) CO2 tanks

Lab #2 Questions

Phosphorus NMR

Phosphorus spectrum (with proton decoupling) was obtained from cancer cells encapsulated in alginate and perfused in the bioreactor: 31P_cells.fid. The spectrum is a sum of 2048 transients, each with a delay of 0.1 s and an acquisition time of 2 s.

1. Identify the peaks in the phosphorus spectrum obtained from the cells encapsulated in alginate. Explain the significance of the peaks and the impact of not using proton decoupling.
2. Quantify the amount of ATP (in nanomoles) in the cells using the β-ATP peak. The ERETIC peak corresponds to 430 nanomoles, and the T_1 of the β-ATP ^{31}P nucleus is 0.47 s.
3. Why do we use the β-ATP peak when quantifying ATP? *Hint: What do the spectra for AMP and ADP look like?* How can we be sure we are not quantifying other nucleoside triphosphates?

Hyperpolarized carbon-13 NMR of pyruvic acid

7.5 uL of pyruvic acid were polarized for an hour and dissolved in 5 mL of cell buffer. 750 uL of the dissolution were then injected into the cells at the rate of 0.5ml/min. A series of proton-decoupled spectra was obtained to estimate the dynamic changes: C1Pyr_cells.fid.

1. Identify the major metabolite peaks in the spectra (you should see three singlets and a downstream doublet). What processes contribute to both the increase and decrease of signal we observe over time?
2. Identify the enzymatic pathways that can be interrogated based on the peaks observed.
3. Explain how you could estimate the flux of the enzymes.

All the data sets are on the course website: https://courses.ucsf.edu/course/view.php?id=2748