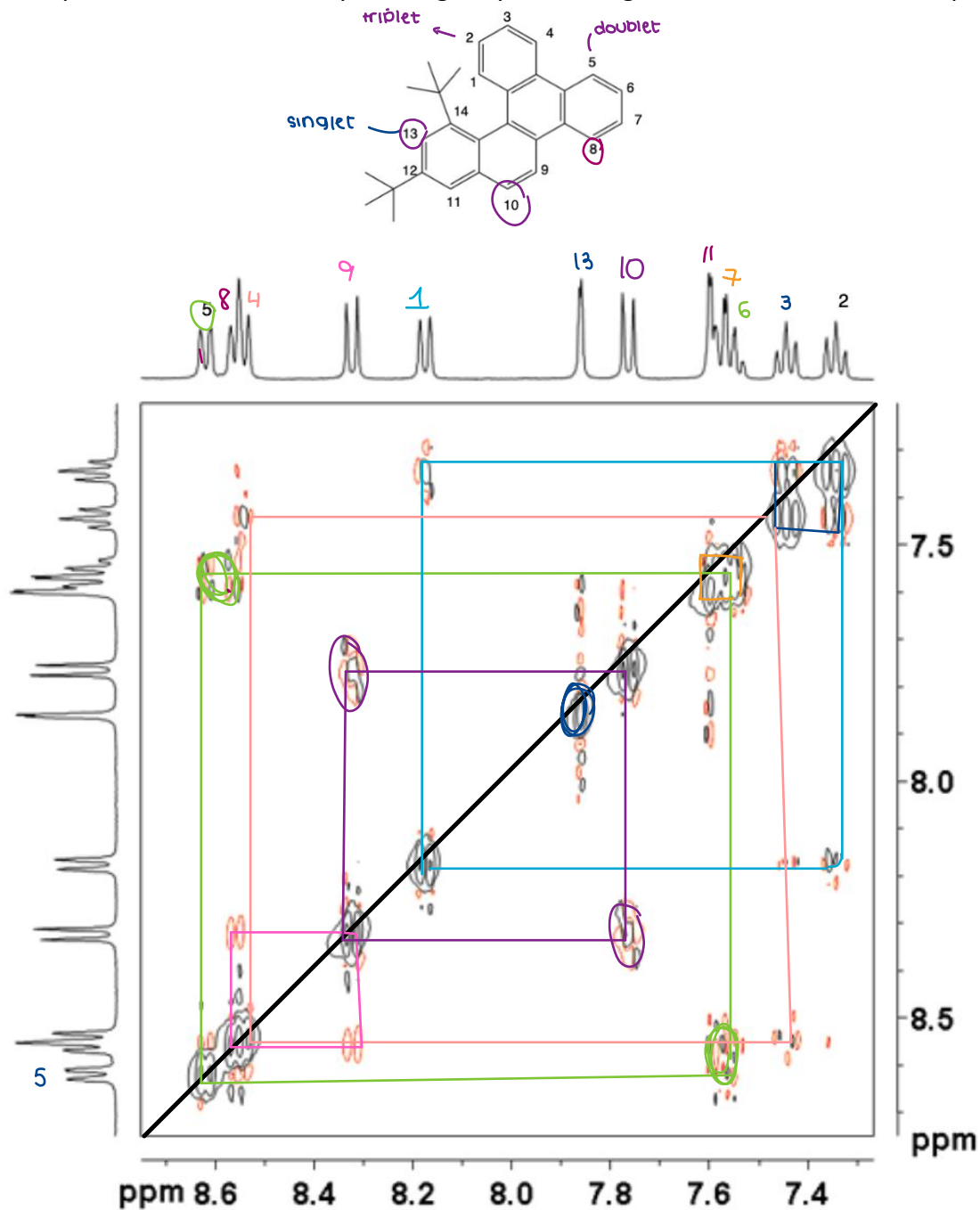


Jigsaw 4B

2D NMR: NOESY and EXSY

1. The 2D NOESY (Nuclear Overhauser Effect Spectroscopy) experiment gives information on through-space interactions, which can be used to identify protons which are close in space. Below, you have the aromatic region of the ^1H 2D NOESY spectrum of 12,14-di t -butylbenzo[g]chrysenes. Assign the different aromatic ^1H peaks.



2. [Week 3 Slides 71–80] Consider the EXSY spectrum of a symmetrical 2 spin (A and B) system. The intensity of the four peaks are given by the following equations:

$$I_{AA}(\tau_m) = \frac{1}{2}[1 + \exp(-2k\tau_m)]\exp(-\tau_m/T_1)M_{A0}$$

$$I_{BB}(\tau_m) = \frac{1}{2}[1 + \exp(-2k\tau_m)]\exp(-\tau_m/T_1)M_{B0}$$

$$I_{AB}(\tau_m) = \frac{1}{2}[1 - \exp(-2k\tau_m)]\exp(-\tau_m/T_1)M_{B0}$$

$$I_{BA}(\tau_m) = \frac{1}{2}[1 - \exp(-2k\tau_m)]\exp(-\tau_m/T_1)M_{A0}$$

- a. Draw the 2D EXSY spectrum, taking into account the relative intensity of diagonal and cross peaks, at the following values of $k\tau_m$:

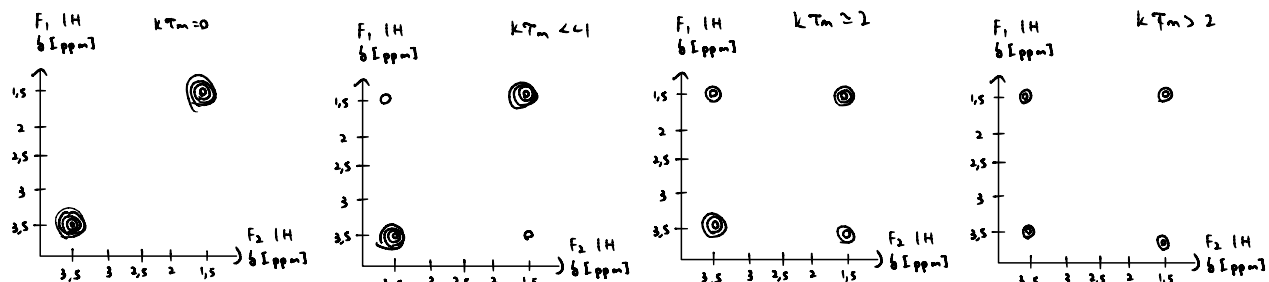
i. $k\tau_m = 0$

ii. $k\tau_m \ll 1$

iii. $k\tau_m \approx 2$

iv. $k\tau_m \gg 2$

Assume that k (exchange rate) is a constant:



- b. For each plot in (a), explain what is happening to the peak intensity and why.

$k\tau_m = 0 \Rightarrow$ No mixing time \Rightarrow No exchange time $\Rightarrow \exp(-2k\tau_m) = \exp(0) = 1 \Rightarrow$ Diagonal peaks are at maximum intensity

$k\tau_m \ll 1 \Rightarrow$ Minimal exchange & mixing time, weak cross peak amplitudes

$k\tau_m \sim 2 \Rightarrow$ Medium exchange

$k\tau_m > 2 \Rightarrow$ Fast exchange

I think I understand what you mean, but this isn't the clearest way to explain it

- c. 2D exchange spectroscopy is used to determine exchange in which motion regime? Explain why.

2D exchange spectroscopy is used for determine exchange in the slow region, the diagonal and cross peaks are too broadened and do not form visible peaks. For fast motion, we only see one peak since the averaged. The slow motion regime is the only motion regime where 2D-NMR is significant.

A bit confusingly worded...correct that EXSY is useful in the slow motion regime because that's the only region where we would see distinct chemical shifts for the sites in exchange. The peak broadening happens as we approach the intermediate exchange regime, and the averaged peak is visible in fast motion. Note that other forms of 2D NMR (COSY, HSQC, etc.) can still be very significant and useful even in systems with fast exchange!

- ii. The mixing time is smaller than the exchange constant, so we only see very minimal evidence of mixing
 iii. The mixing time is on the same scale as the exchange constant, so we see the maximum signal for this system
 iv. When the mixing time is much larger than the exchange constant, we get the effects of relaxation added in, so our overall signal intensity decreases

Laura Gomez
 Eva Guleac
 Justine Serra
 Gillian Dreyfus
 Charlotte Mercier
 Daphné Delacou

CH-314 Structural Analysis
 ruben.rodriguezmadrid@epfl.ch
 ray.cowen@epfl.ch

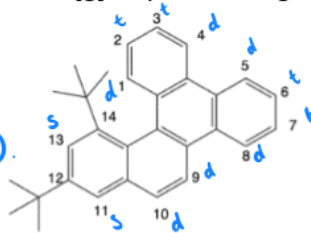
Jigsaw 4B

2D NMR: NOESY and EXSY

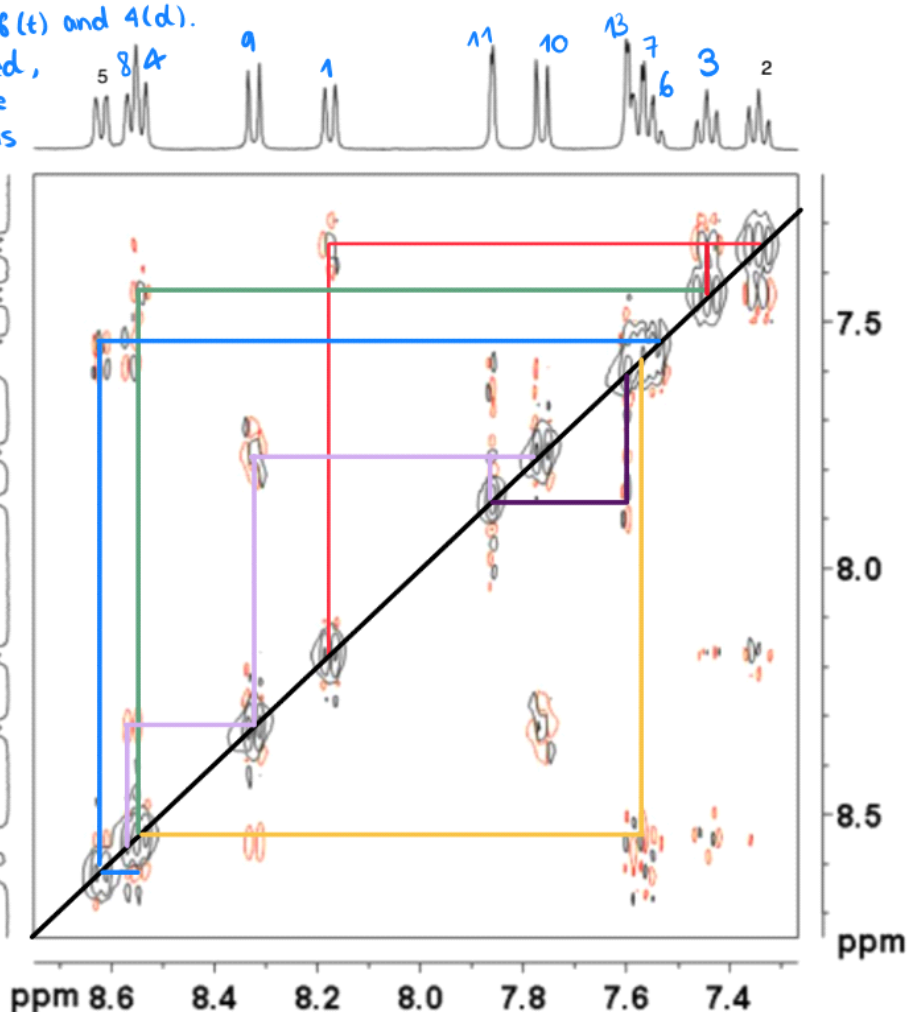
- The 2D NOESY (Nuclear Overhauser Effect Spectroscopy) experiment gives information on through-space interactions, which can be used to identify protons which are close in space. Below, you have the aromatic region of the ^1H 2D NOESY spectrum of 12,14-di t -butylbenzo[g]chrysenes. Assign the different aromatic ^1H peaks.

Peaks on the diagonal are used as a graphical reference.

- 2 is surrounded by 1(d) and 3(t) on short range. \rightarrow strong correlation peaks (—).
- 3 is surrounded by 2(t) and 4(d) \rightarrow strong signal of 3 with a doublet (—).
- 5 is surrounded by 6(t) and 4(d). \rightarrow 5 is already assigned, so 6 should be in the region 7.5-7.6 ppm. (but it is a messy zone) (—).



- (—) should correspond to protons 8(d) to 11(s). The doublet in the middle should be 9 as it interacts both with 8 and 10. \rightarrow 10 is assigned to the peak interacting only with 11 and 9. \rightarrow The last peak is therefore 8. \Rightarrow 4 and 8 (both doublets) are overlapping thus creating a triplet.
- In the messy region, the peak interacting with 8 should be the 7 one. (—). Thus the last peak in the messy region is 13 as it is the only proton interacting with 11. (—).



2. [Week 3 Slides 71–80] Consider the EXSY spectrum of a symmetrical 2 spin (A and B) system. The intensity of the four peaks are given by the following equations:

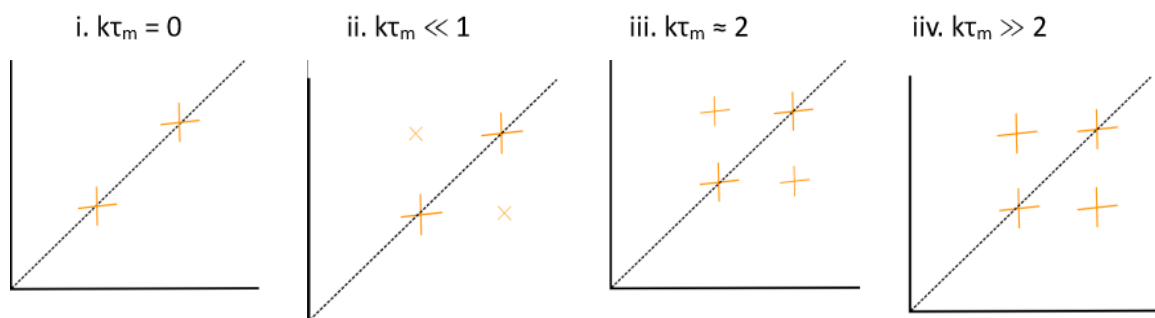
$$I_{AA}(\tau_m) = \frac{1}{2} [1 + \exp(-2k\tau_m)] \exp(-\tau_m/T_1) M_{A0}$$

$$I_{BB}(\tau_m) = \frac{1}{2} [1 + \exp(-2k\tau_m)] \exp(-\tau_m/T_1) M_{B0}$$

$$I_{AB}(\tau_m) = \frac{1}{2} [1 - \exp(-2k\tau_m)] \exp(-\tau_m/T_1) M_{B0}$$

$$I_{BA}(\tau_m) = \frac{1}{2} [1 - \exp(-2k\tau_m)] \exp(-\tau_m/T_1) M_{A0}$$

- a. Draw the 2D EXSY spectrum, taking into account the relative intensity of diagonal and cross peaks, at the following values of $k\tau_m$:



- b. For each plot in (a), explain what is happening to the peak intensity and why.

- c. 2D exchange spectroscopy is used to determine exchange in which motion regime? Explain why.

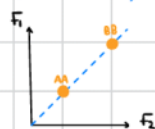
jigsaw 48 - Question 2

(b) symmetric $\rightarrow M_{A0} = M_{B0}$

(taking into account the relative intensities $\rightarrow \exp(-\tau_m/T_1)$ cancels out)

$k\tau_m = 0 \rightarrow$ (relative intensities) $I_{AA} = 1 = I_{BB}$

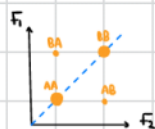
$I_{AB} = 0 = I_{BA}$



\rightarrow no exchange \rightarrow only diagonal peaks

$k\tau_m \ll 1$ (eg 0.1) $I_{AA} \approx 0.909 \approx I_{BB}$

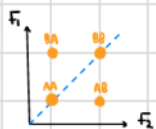
$I_{AB} \approx 0.09 \approx I_{BA}$



\rightarrow small exchange rate, off-diagonal peaks start to show as exchange rate is sufficient for an exchange to be detected during the mixing time (τ_m)

$k\tau_m \approx 2 \rightarrow (I_{AA} = I_{BB}) > 0.5$

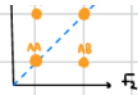
$(I_{AB} = I_{BA}) < 0.5$



\rightarrow exchange rate increases, $\frac{1}{2}(1 \pm \exp(-2k\tau_m))$ approaches (0.5)

$k_{2m} \approx 2 \rightarrow (I_{AA} = I_{BB}) > 0.5$

$(I_{AB} = I_{BA}) < 0.5$



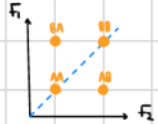
detected during the mixing time (τ_m)

\rightarrow exchange rate increases, $\frac{1}{2}(1 \pm \exp(-2k_{2m}))$ approaches 0.5

$k_{2m} \gg 2 \rightarrow$

$I_{AA} = I_{BB} = 0.5$

$I_{AB} = I_{BA} = 0.5$



\rightarrow equilibrium, fast exchange limit

c) \rightarrow in the slow motion regime, as ω_1 and ω_2 must be distinguishable