

The study of Azide probes : Comparison of primary, secondary and aromatic series

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❖ Introduction

As IR probe of side chain dynamics, the azide group has been used as local environmental probe. It is useful study of dynamics and structure. It has been found that azide probe has large extinction coefficient. It means that it has the potential to make low concentration protein samples. However, It has weak point like fermi resonance. Also, Azide probe has short lifetime. This points make less attractive in IR probe study.

To overcome this, we knew from the label that Fermi resonance was gone from FTIR in 3-azidopyridine. In CN probe case, The isotopic effect caused the vibrational lifetime to be twice as long. We applied this strategy to primary azide, secondary azide, and aromatic azide to investigate the effect of vibrational lifetime as well as fermi resonance peaks.

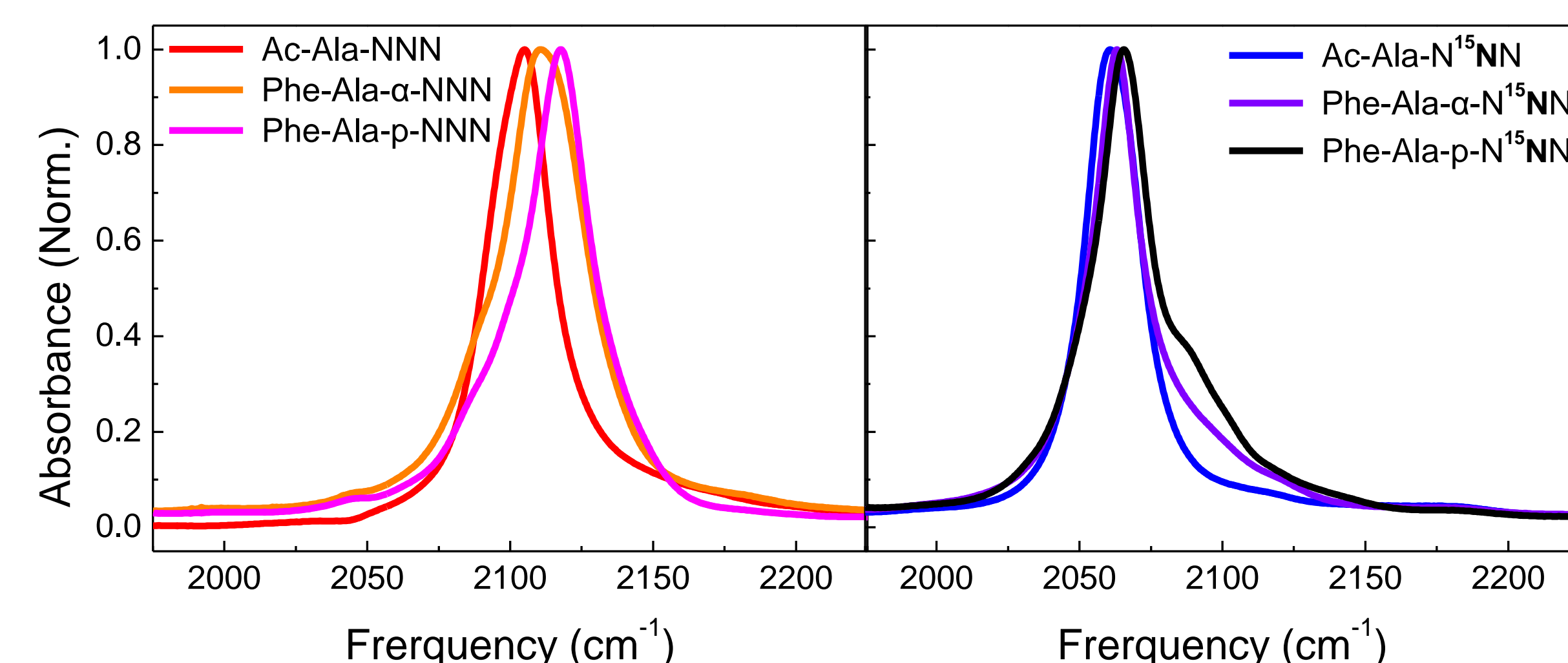


Fig 1) FTIR spectra of azide probes in DMF

❖ Azide Probes

X = NNN or N¹⁵NN

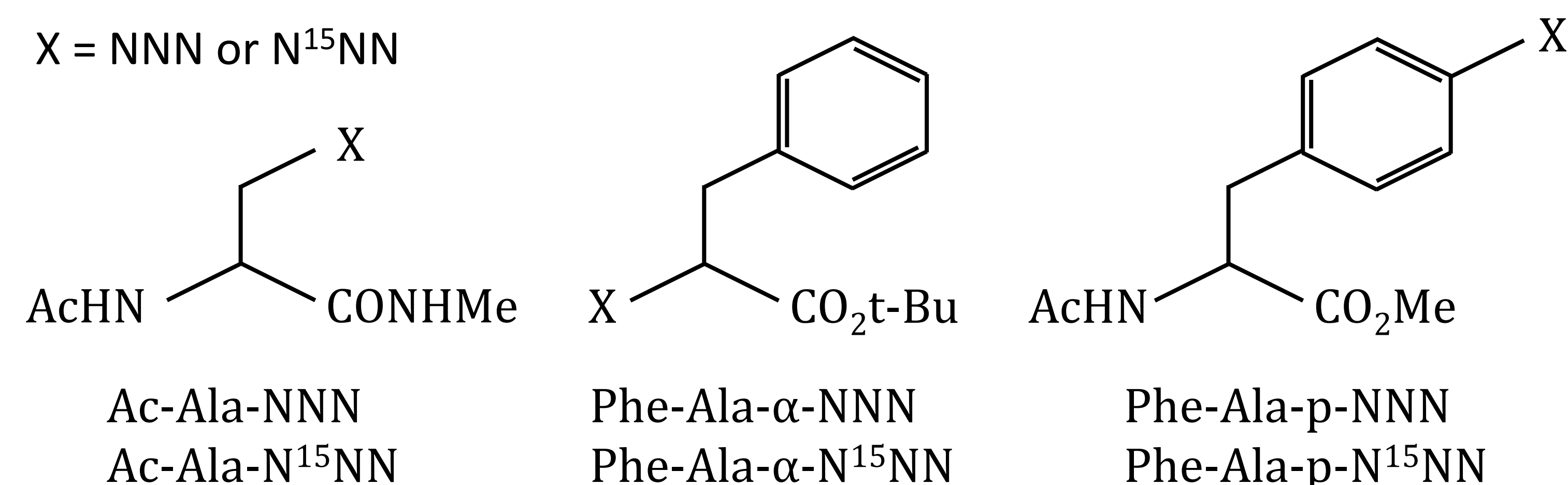


Fig 2) Structure of azide probes used for IR study

❖ Vibrational Dynamics

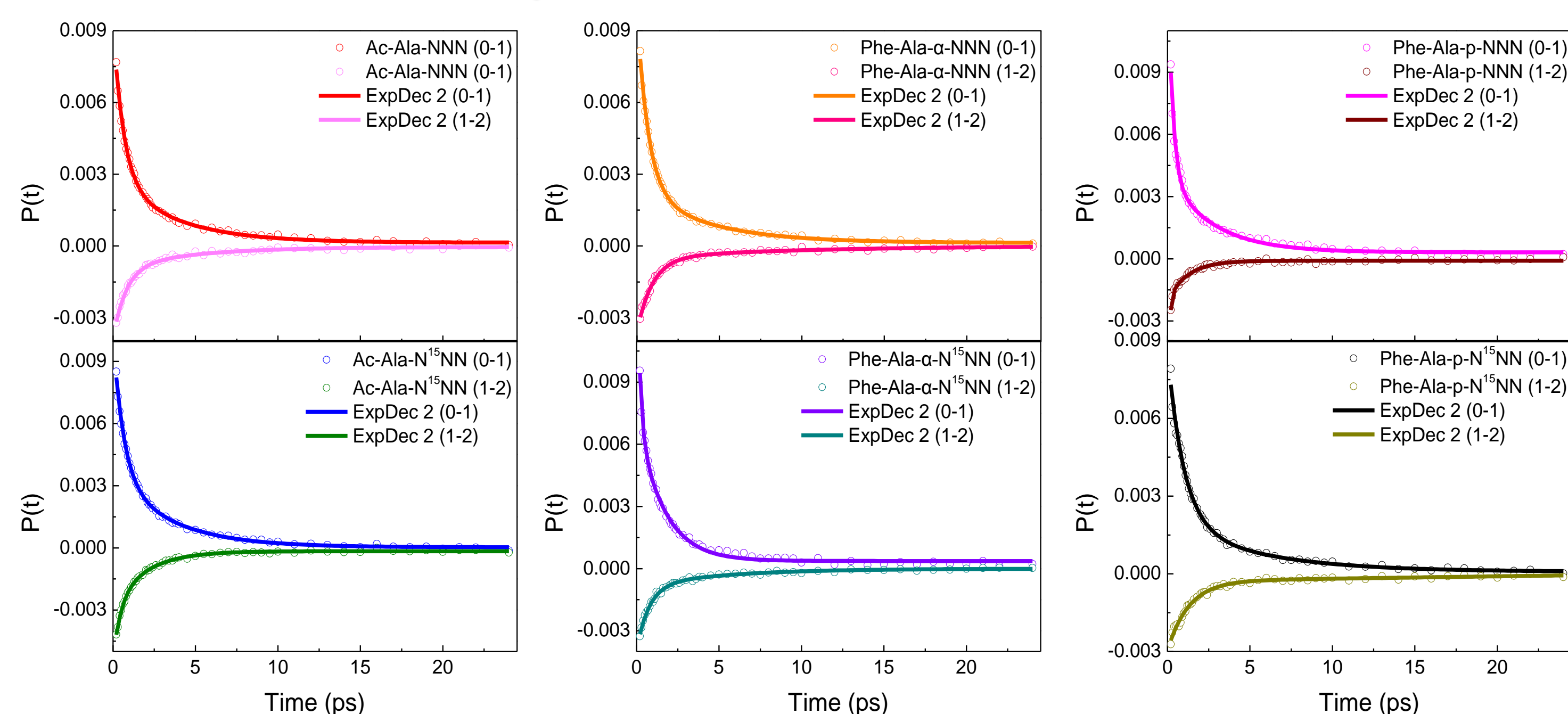


Fig 4) IR pump-probe data of azide probes in DMF at time.

Two selective frequencies (2105.5 and 2074.7 cm⁻¹ in Ac-Ala-NNN, 2063.9 and 2034.4 cm⁻¹ in Ac-Ala-N¹⁵NN, 2112.8 and 2068.5 cm⁻¹ in Phe-Ala-α-NNN, 2064.4 and 2036.7 cm⁻¹ in Phe-Ala-α-N¹⁵NN, 2120.1 and 2068 cm⁻¹ in Phe-Ala-p-NNN, 2068.1 and 2038.5 cm⁻¹ in Phe-Ala-p-N¹⁵NN) for 0-1 and 1-2 transition.

❖ IR Pump-Probe Spectroscopy

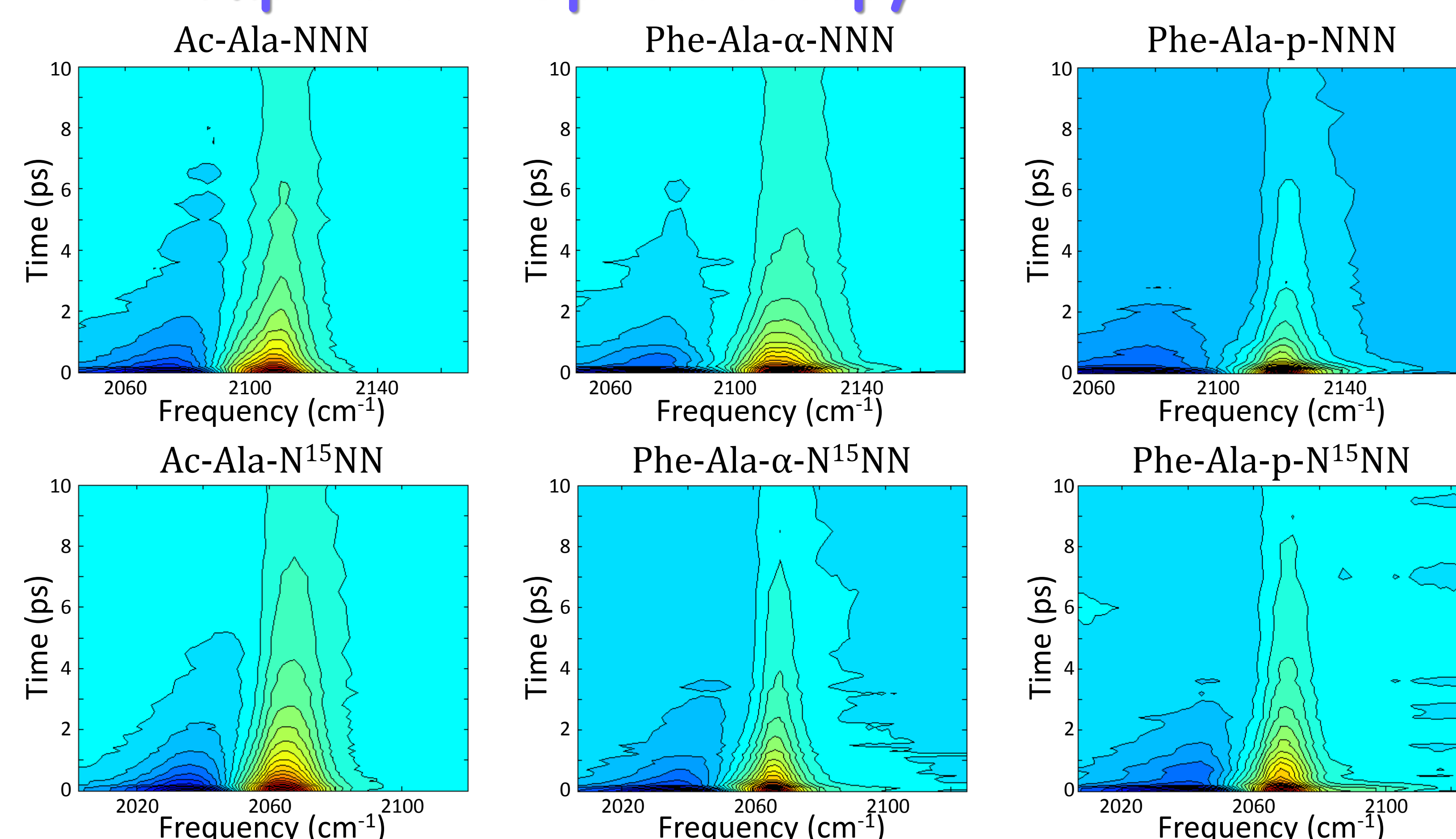
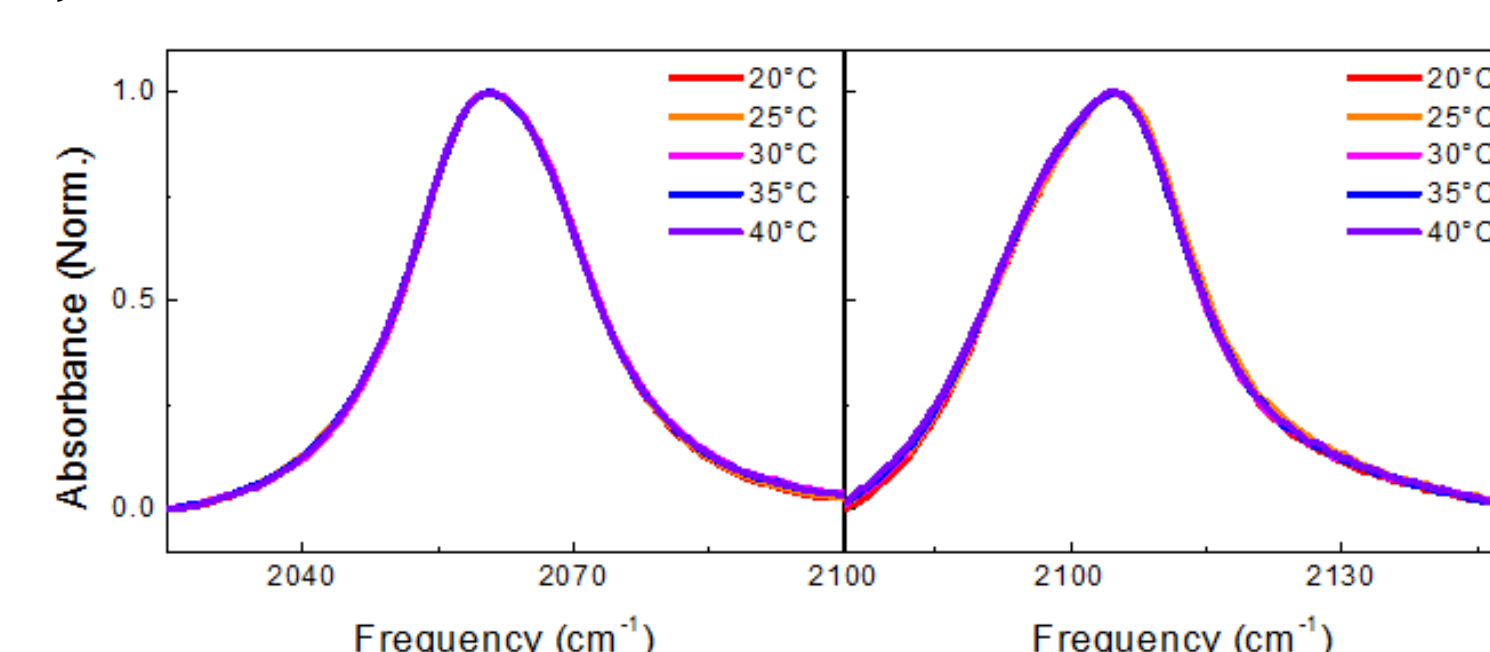


Fig 3) Time-resolved spectra of azide probes, as indicated.

Blue shifts in the peak position of the 1-2 transition of the azide asymmetric stretching mode were observed.

1) Thermal effect

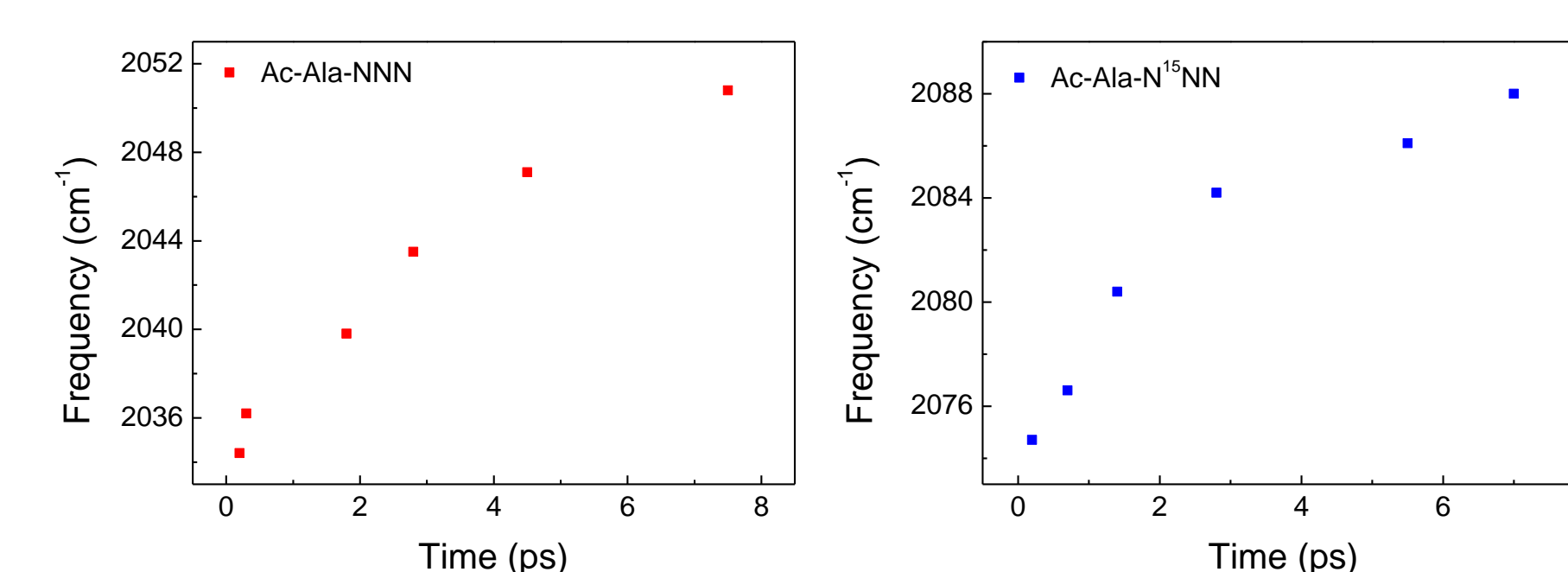


In Ac-Ala-NNN, Ac-Ala-N¹⁵NN case, they did not found notable frequency shift induced by temperature increases.

2) Two different solvation structure

DMF is a strong HB acceptor according to Kamlet-Taft parameters ($\beta = 0.69$, $\alpha = 0$)

3) Solvent fluctuation induced blue-shift



❖ Spectral properties of azide probes

	Ac-Ala-NNN	Ac-Ala-N ¹⁵ NN	Phe-Ala-α-NNN	Phe-Ala-α-N ¹⁵ NN	Phe-Ala-p-NNN	Phe-Ala-p-N ¹⁵ NN
ω_0 (cm ⁻¹)	2104.00	2061.64	2111.53	2062.68	2118.03	2064.98
FWHM (cm ⁻¹)	25.90	21.25	34.27	21.18	25.88	23.86
ϵ (cm ⁻¹ M ⁻¹)	548.7	633.6	416.8	561.2	689.2	661.8
D (10 ⁻² Debye ²)	9.66	9.52	12.7	9.86	9.61	13.9
T_1 (ps)	0.63±0.04 3.75±0.37	0.63±0.03 3.79±0.24	0.69±0.03 3.85±0.38	0.17±0.03 1.60±0.06	0.29±0.03 2.75±0.25	0.97±0.08 5.35±1.42

❖ Reference

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❖ Conclusion

- The isotopic effect doesn't make one peak in FT-IR. It means selectively isotopic labeling did not sufficiently increase the energy difference between the azide asymmetric stretch vibration and the Fermi resonance.
- Selectively isotopic labeling did not significantly affect the increase in vibrational lifetime.
- Time constants indicate that NNN vibration relaxation dynamics of primary azide, secondary azide, aromatic azide in a nonmonotonic manner.