



The PGStE pulse sequence

Use in NMR-Spektroscopy for characterization of biological macromolecules

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Diffusion based signal suppression

The characterization of biological macromolecules poses particular challenges.

They are typically extracted from complex biological matrices and not all of the various other molecules and impurities can be readily removed with available purification methods. Signals of these molecules, as well as solvent signals can hinder the characterization, when overlapping with the signals of the macromolecular analyte.

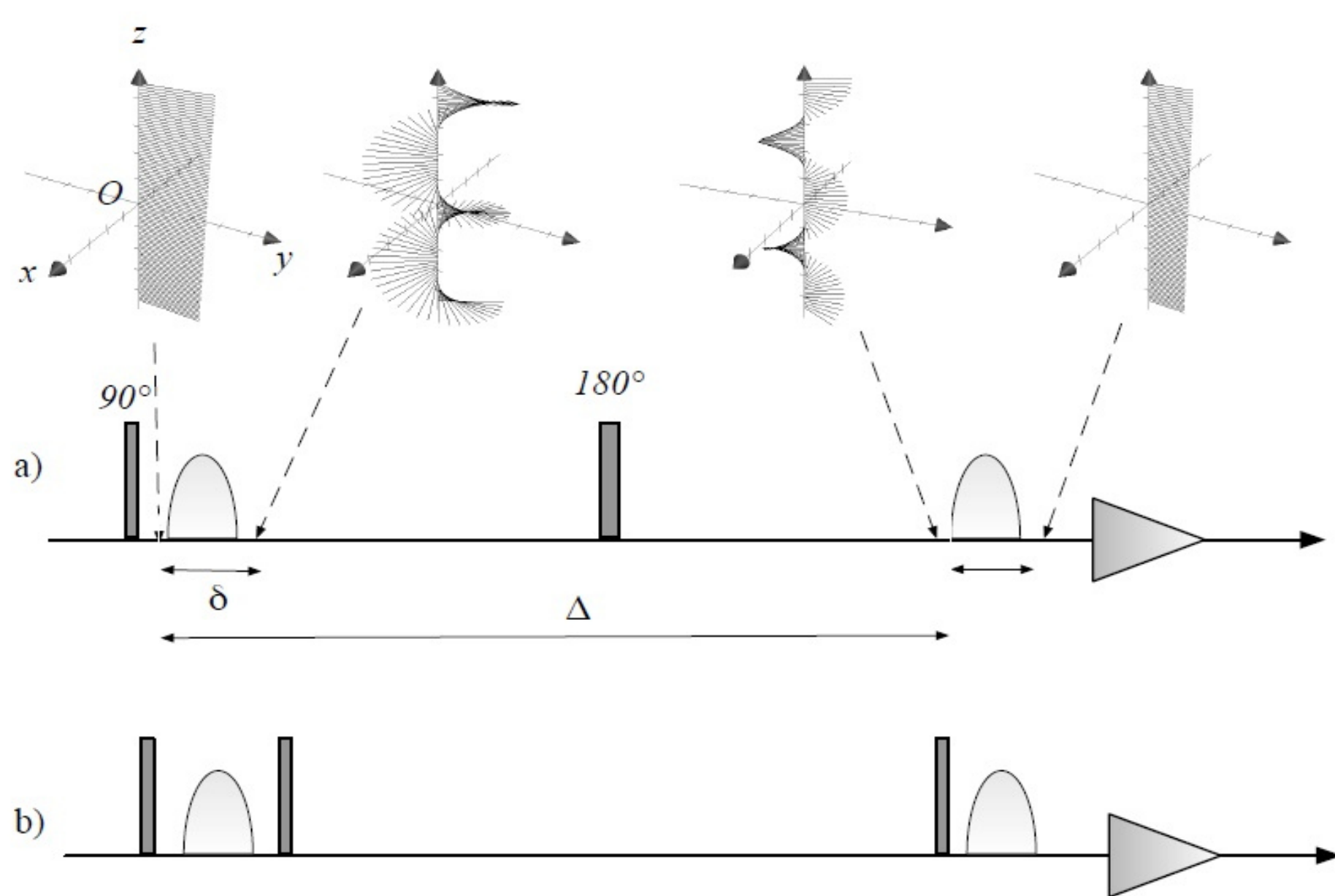
While most regular signal suppression schemes target signals based on their chemical shift, pulsed gradient stimulated echo (PGStE) can suppress unwanted small molecule signals without significantly affecting the signals of the macromolecular analyte in the same shift region. This work focuses on the comparison of PGStE-NMR with regular ¹H-NMR in regard to the analysis of various molecules of the glycosaminoglycan family and their mixtures.

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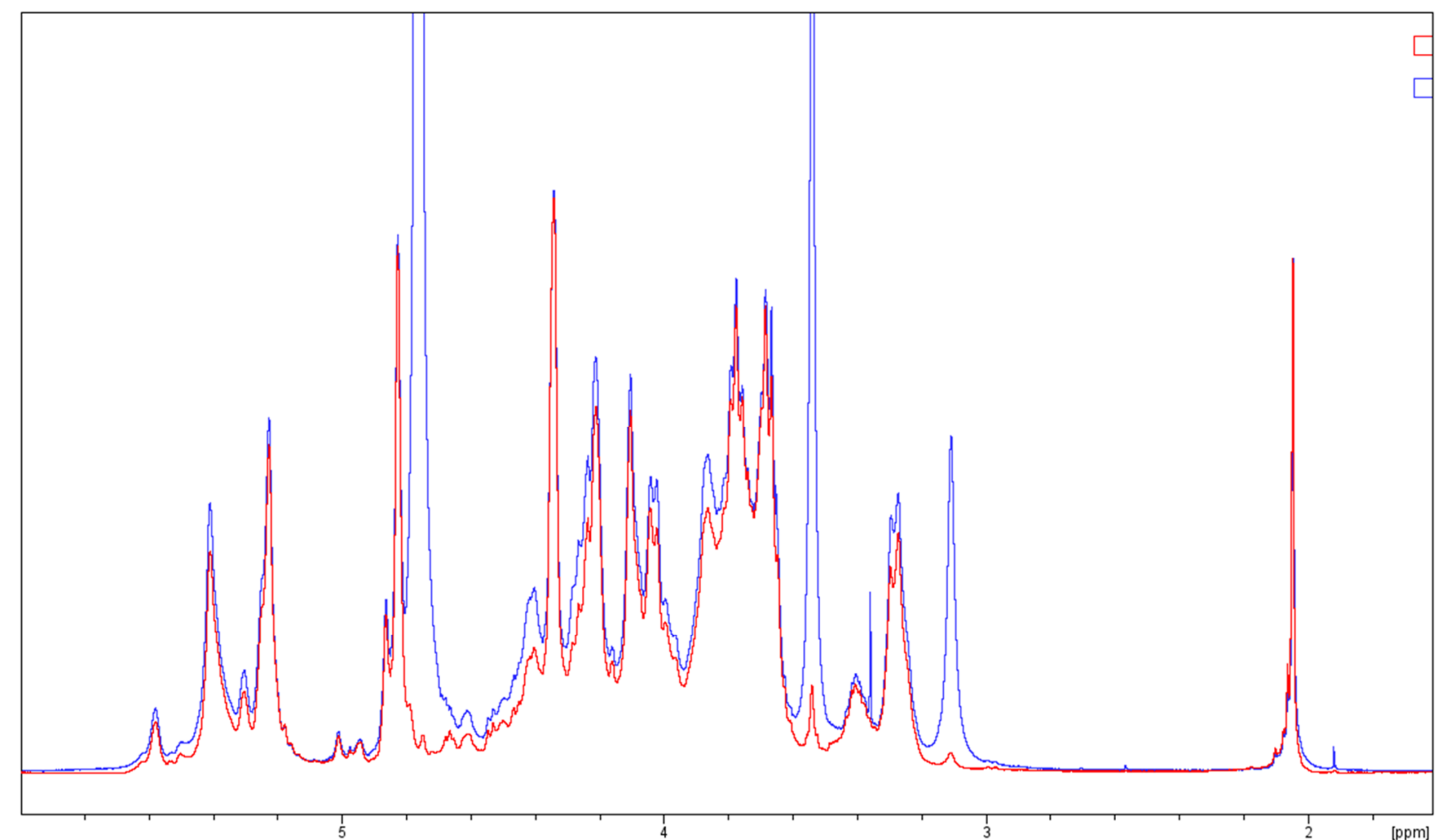
Pulse Sequence

PGStE (b)) expands on the pulsed gradient spin echo (PGSE) sequence (a)) by replacing the spin echo with a stimulated echo, facilitating longer diffusion times for large molecules.



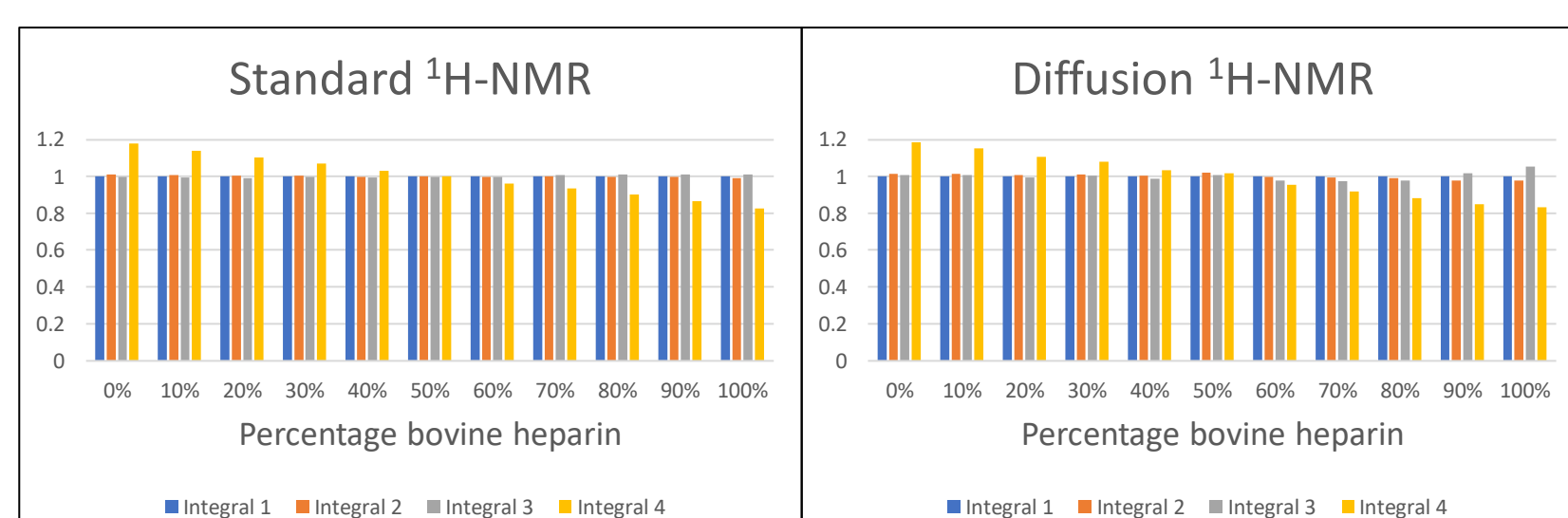
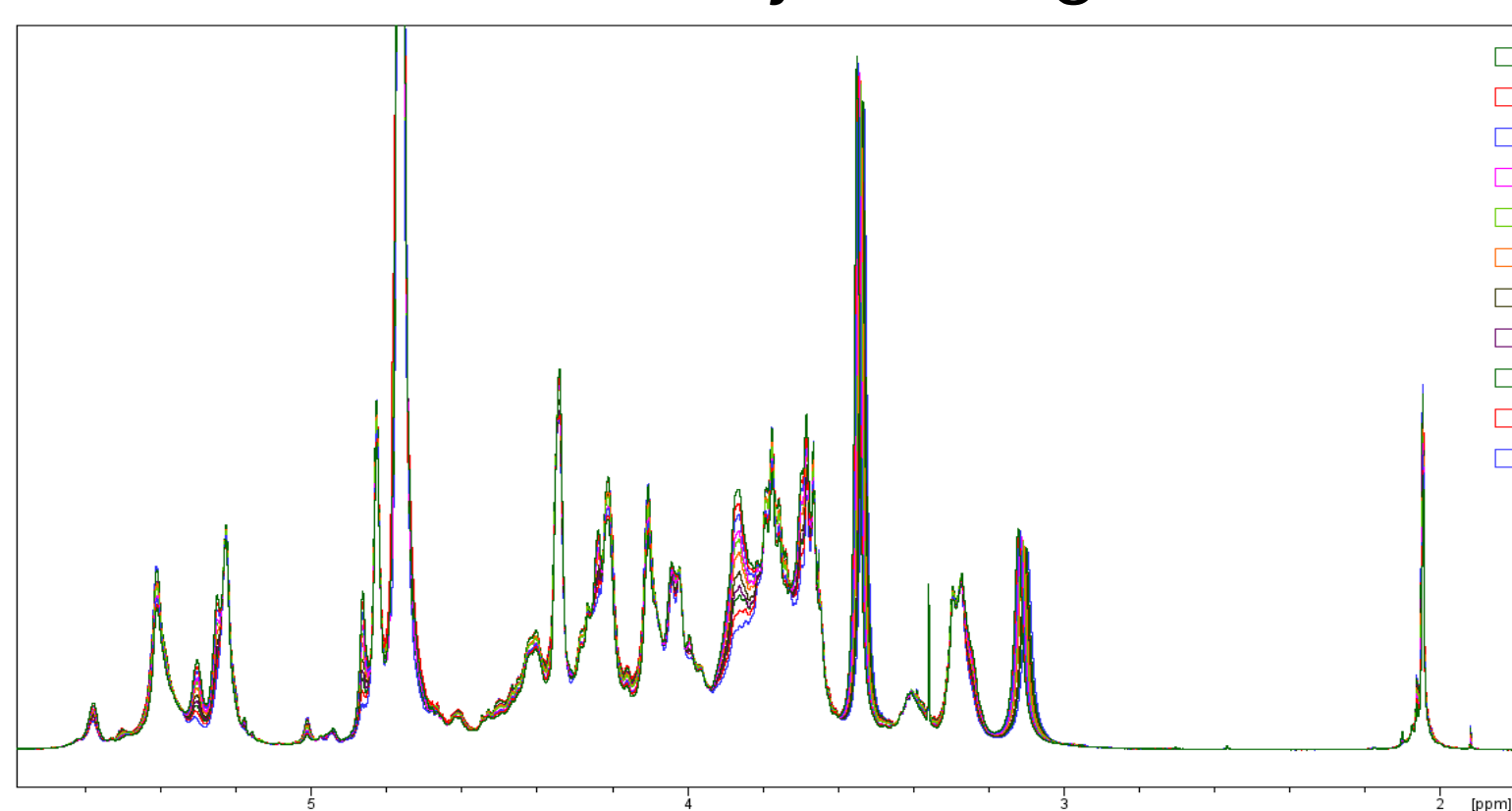
Comparison PGStE and regular ¹H-NMR

In the PGStE-NMR spectrum (red) the watersignal is completely removed and the signals of EDTA are greatly attenuated relative to the regular ¹H-NMR spectrum (blue) of bovine heparin.



Heparin

Porcine/bovine Heparin mixtures in several 10% increments overlaid in Figure below.



Results

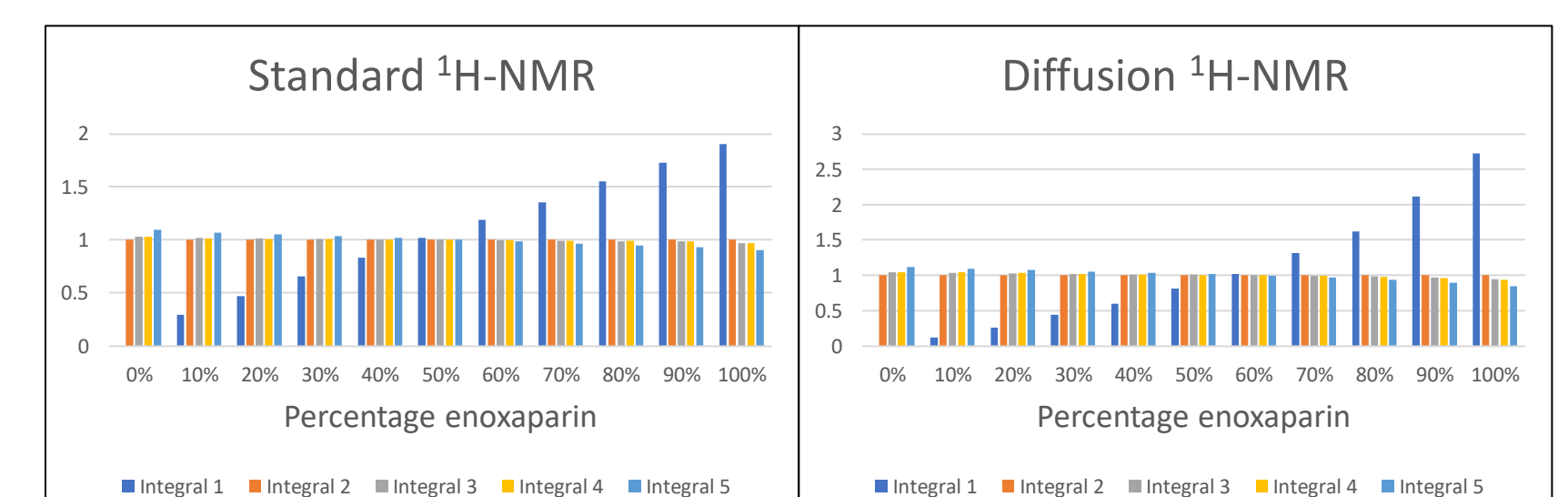
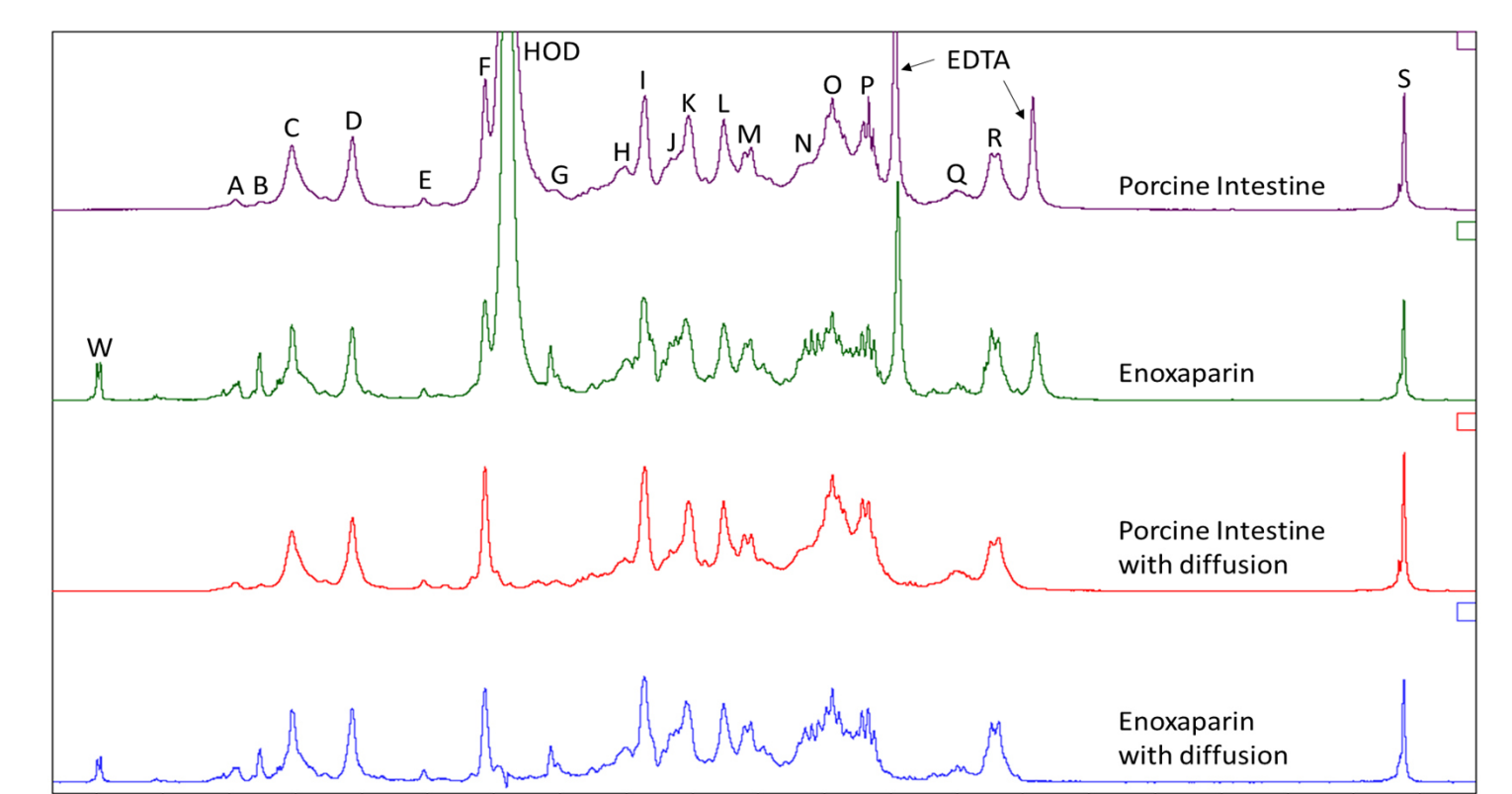
- Good linear correlation of integrals to mixture ratios in regular ¹H-NMR spectra for both types of mixtures.
- Good linear correlation of integrals to mixture ratios for the porcine/bovine heparin mixtures as both have similar molecular weight
- Nonlinear, likely quadratic correlation of integrals to mixture ratios for the heparin/enoxaparin mixtures as the different molecular weights lead to discrepancies in the diffusion related signal attenuation

Integral Nr.	Integral range [ppm]
1	5.70 – 4.92
2	4.64 – 3.59
3	3.45 – 3.19
4	2.19 – 1.98

Integral Nr.	Integral range [ppm]
1	6.04 – 5.97
2	5.70 – 4.92
3	4.64 – 3.62
4	3.45 – 3.21
5	2.19 – 1.98

Heparin/Enoxaparin

Mixtures of Heparin and the low molecular weight heparin Enoxaparin.



Take-Home Message and Outlook

The PGStE sequence is capable of removing small molecule signals from the spectra of biological macromolecules and thus facilitate better overview which signals belong to the macromolecule and which to residual impurities. It also retains linearity when comparing similar signals of similar sized molecules.

Differences in signal attenuation within the same molecule are still under active investigation.