Field-Cycling ^{1}H Relaxometry of biomolecules and paramagnetic proteins

Adam Kubrak[a], Pedro Sebastiao[b], Giacomo Parigi[a]
[a]*Department of Chemistry and Magnetic Resonance Center (CERM), University of Florence, and CIRMMP, via Luigi Sacconi 6, 50019 Sesto Fiorentino, Italy*
[b]*Center of Physics and Engineering of Advanced Materials, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal*

Field-Cycling (FC) NMR relaxometry measures longitudinal relaxation rates (R_1) as a function of the magnetic field, yielding Nuclear Magnetic Relaxation Dispersion (NMRD) profiles. Fitting these profiles with suitable theoretical models provides insights into molecular dynamics across a wide timescale, both in diamagnetic and paramagnetic systems[1]. In paramagnetic systems, the presence of unpaired electron(s) induces Paramagnetic Relaxation Enhancements (PRE)[2]. This effect can be explored through FC relaxometry to dissect the multiple contributions to water-proton relaxation arising from first-, second-, and outer-sphere water molecules, as well as to determine key electronic parameters. Field-cycling relaxometry can be performed either by switching the applied magnetic field generated by an electromagnet or using high-resolution NMR spectrometers equipped with a shuttle system that mechanically moves the sample between different magnetic fields. This latter approach offers high resolution and enhanced sensitivity, enabling the detection of site-specific R_1 values for individual nuclei[3].

To address the need for easily accessible software tools for the analysis of the NMRD profiles, we present fiRelax (fitting interface for Relaxometry), a novel fitting platform equipped with a Graphical User-Interface (GUI), freely accessible as a web application at https://firelax.cerm.unifi.it. The platform is composed of a front-end that allows users to analyze experimental profiles and simulate theoretical relaxation curves with real-time result updates, eliminating the need for page reloads. The application's core is running in Java to ensure stability and longevity. fiRelax is designed to be accessible to users without in-depth knowledge of programming or advanced relaxation theory. On the back-end, all computations and minimization procedures are driven by the One-Fit-Engine[4] (OFE), an open-source algorithm written in Raku and C, designed to serve as the fitting core for external programs. For paramagnetic systems, both the SBM and the Florence models[5] have been implemented by adapting the Fortran code into OFE's C environment, resulting in a faster fitting process.

To show the versatility of the fiRelax platform, selected case studies are presented. These include paramagnetic systems exhibiting static Zero-Field-Splitting (ZFS), analyzed through the (modified) Florence model, as well as macromolecules in biofluids (e.g. human blood serum), where a model-free approach was applied to capture the underlying molecular dynamics.

[1]A. Kubrak, R. Pejanovic, K. Kamau, D. Kruk, F. Ferrage, G.Parigi, *PCCP*, **2025**, 27, 1756–1771
[2]I. Bertini, C. Luchinat, G. Parigi, *Coord. Chem. Rev.,* **2011,** 255, 649
[3]J.A. Villanueva-Garibay et al., *Magn. Reson.*, **2025**, 6, 229–241
[4]github.com/fitteia/OneFit-Engine
[5]I. Bertini, J. Kowalewski, C. Luchinat, T. Nilsson, G. Parigi, *J. Chem. Phys.*, **1999**, 111, 5795