

relaxGUI: a new software for fast and simple NMR relaxation data analysis and calculation of ps-ns and μ s motion of proteins

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Abstract Investigation of protein dynamics on the ps-ns and μ s-ms timeframes provides detailed insight into the mechanisms of enzymes and the binding properties of proteins. Nuclear magnetic resonance (NMR) is an excellent tool for studying protein dynamics at atomic resolution. Analysis of relaxation data using model-free analysis can be a tedious and time consuming process, which requires good knowledge of scripting procedures. The software relaxGUI was developed for fast and simple model-free analysis and is fully integrated into the software package relax. It is written in Python and uses wxPython to build the graphical user interface (GUI) for maximum performance and multi-platform use. This software allows the analysis of NMR relaxation data with ease and the generation of publication quality graphs as well as color coded images of molecular structures. The interface is designed for simple data analysis and management. The software was tested and validated against the command line version of relax.

Keywords Graphical user interface · GUI · Lipari and Szabo model-free analysis · NMR spin relaxation · Protein dynamics · Relax software

Abbreviations

CSA	Chemical shift anisotropy
GUI	Graphical user interface
HSQC	Heteronuclear single quantum coherence
OMP	Olfactory marker protein
NOE	Nuclear overhauser effect
R_{ex}	Chemical exchange relaxation

Introduction

Proteins and enzymes are responsible for a wide variety of biochemical functions. Highly specialized, they are able to recognize specific signals and initiate a cascade of events, catalyze chemical reactions or transport selected components from one cellular compartment to another. All these mechanisms rely on the internal motion and dynamics of the proteins involved in molecular recognition and enzyme catalysis (Teilum et al. 2009; Grant et al. 2010). Therefore, it is of interest to understand protein dynamics and how it relates to function. Nuclear magnetic resonance (NMR) has been shown to be an ideal tool for investigating protein motion at an atomic resolution (Jarymowycz and Stone 2006; Igumenova et al. 2006; Sapienza and Lee 2010). One of the most common methods is to analyze ^{15}N relaxation data, such as the steady-state nuclear Overhauser effect (NOE), longitudinal relaxation (R_1) and transverse relaxation (R_2), using the model-free formalism first described by Lipari and Szabo (1982a) and extended to include motions

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on two distinct time scales (Clare et al. 1990). This approach, together with the identification of chemical exchange relaxation, enables the investigation of protein dynamics on the ps-ns and μ s-ms time scales. From model-free analysis a number of parameters that describe motion can be extracted including: the generalized order parameter (S^2) that essentially describes how restricted the motion of a residue is within the molecular frame; the effective internal correlation time (τ_e) which describes motion on a time scale usually faster than the overall molecular tumbling (τ_m) of the molecule; and conformational exchange (R_{ex}) characterized by chemical exchange phenomena that contribute to transverse relaxation rates and occur on the μ s-ms timescale. Determination of these parameters has been fruitful for understanding the functional dynamics of proteins in different states and under different conditions, such as free, ligand bound, mutated, temperature and pH [as reviewed in (Jarymowycz and Stone 2006)].

There are several software packages for performing model-free analysis. The most widely used include: the highly cited Modelfree program (Palmer et al. 1991; Mandel et al. 1995); the automated FAST-Modelfree Graphical User Interface (GUI) to Modelfree (Cole and Loria 2003), the Matlab program DYNAMICS (Fushman et al. 1997; Hall et al. 2003) and Tensor2 which also includes a GUI (Dosset et al. 2000). Recently we published the new program relax (<http://nmr-relax.com>) which performs highly precise model-free analyses (d'Auvergne and Gooley 2008b). We also introduced an automated protocol for solving the chicken-and-egg problem of determining the intrinsically linked diffusion tensor and model-free parameters. This protocol avoids the initial diffusion tensor estimate by first optimizing the model-free parameters independent of a global diffusion tensor, and aims not to simply find an optimization minimum but a combined solution of a single universe optimization minimum and the multi-universe modeling minimum (d'Auvergne and Gooley 2007). The relaxGUI software presented in this article has a GUI that runs equally on GNU/Linux, Mac OS X, and MS Windows. It is fully integrated into the program relax (d'Auvergne and Gooley 2008a, b) and requires no scripting knowledge. The GUI provides a simplified interface to the calculation of the steady-state NOE, to the exponential curve-fitting of the R_1 and R_2 relaxation rates and to full model-free analysis. The model-free analysis component of relaxGUI involves a simple and rapid setup for the user to perform an automated analysis of spin relaxation data using the new model-free protocol (d'Auvergne and Gooley 2007, 2008b) without any intervention and includes the advances in model selection (Akaike 1973; d'Auvergne and Gooley 2003) and the elimination of failed model-free models (Bellomo and Preziosi 1994; d'Auvergne and Gooley 2006). relaxGUI

automatically creates PyMOL macros for molecular representations of the dynamics results, csv files (comma separated files; readable in MS Excel or any text editor) to create tables and grace plots. Peak lists of NMR relaxation spectra and protein structures are selected and imported using file selection dialogs provided by the operating system. As a means of validation, a number of published systems were reanalyzed through the GUI. These include the olfactory marker protein (OMP) relaxation data (Gitti et al. 2005) that was reanalyzed using relaxGUI and compared to that obtained from relax scripting interface (d'Auvergne and Gooley 2008b). The antibiotic hydrolyzing class A β -lactamases PSE-4 (Morin and Gagne 2009) and TEM-1 (Fisette et al. 2010) were also used to compare the results of the automated analysis via the new model-free protocol to that of the original analysis. All these results demonstrate the robustness of the GUI and the underlying protocols and analyses.

Materials and methods

Programming of relaxGUI

The GUI software was written in Python using the wxPython module (<http://www.wxpython.org/>). This allows relaxGUI to run on all platforms at maximal performance. The GUI was tested on an Ubuntu 10.04 Linux box consisting of two 2.26 GHz Intel[®] Core[™] 2 Duo processors and 3 GB working memory as well as on a Fedora 10 Linux box consisting of two 3.0 GHz Intel[®] Core[™] 2 Duo processors and 1.9 GB working memory. relaxGUI was tested by reanalyzing the published relaxation data of OMP (Gitti et al. 2005), PSE-4 (Morin and Gagne 2009) and TEM-1 (Fisette et al. 2010) and the results compared. Graphs for Grace (<http://plasma-gate.weizmann.ac.il/Grace/>) of most relaxation parameters are produced after individual calculations. In addition, color-coded and width-coded structure images are created by macros for PyMOL (<http://www.pymol.org/>) created by relaxGUI. The programs PyMOL and Grace are fully controlled by relaxGUI. Graphs and color-coded and width-coded structures can be readily opened for inspection.

Data preparation

relaxGUI utilizes the model-free formalism first described by Lipari and Szabo (1982a) and extended by Clare et al. (1990). As relaxGUI relies upon the new model-free protocol in relax, it therefore requires NMR relaxation data to be collected at two or more magnetic field strengths (d'Auvergne and Gooley 2008a, b; Korzhnev et al. 2001). R_1 and R_2 relaxation rates and steady-state

NOE of a heteronucleus are typically measured by a set of heteronuclear-edited NMR spectra. relax and relaxGUI are developed for either ^{13}C or ^{15}N relaxation analysis. The equations describing the dependence of R_1 , R_2 and the steady-state NOE on the spectral density function have been fully described (Clare et al. 1990; Lipari and Szabo 1982b) and are restated in the supplementary material. The numerically stabilized forms of these equations were used (d’Auvergne and Gooley 2008a). In addition, the model-free equations and the model-free models that can be extracted are also described in the supplementary material.

Data analysis using relaxGUI

The software relaxGUI performs both model-free relaxation analysis as well as the direct calculation of the steady-state NOE, and R_1 and R_2 relaxation parameters (Fig. 1). The GUI is designed to collect all the required data and pass them to relax for calculation. On the left side of the main window, users are able to select between the analysis of relaxation parameters for up to three different field

strengths and the final model-free calculation. Additionally, results are summarized in the results tab and grouped according to field strength and relaxation parameter (Fig. 1a, b). In each analysis, users have the possibility to exclude certain residues/spin systems from the calculation. Model-free analysis using the new protocols of relax (d’Auvergne and Gooley 2008a, b) is performed in a separate tab to extract dynamics parameters, such as S^2 and R_{ex} , for proteins and other macromolecules (Fig. 1c). Data files (parameter files) created by relaxGUI are automatically listed in the corresponding fields. Alternatively, files obtained from other programs can be loaded and collected in this tab.

Furthermore, relaxGUI is highly customizable and many parameters (including XH bond length, magnitude of CSA, name of heteronucleus, name of proton, size of grid search, minimization algorithm and number of Monte Carlo simulations) can be set in the ‘Global relax Settings’ window (Fig. 1d). Another advantage of relaxGUI is that there is no predefined format for the peak list or intensity information, as numbering of columns consisting of assignments and peak intensities can be set in the ‘Peak File Settings’

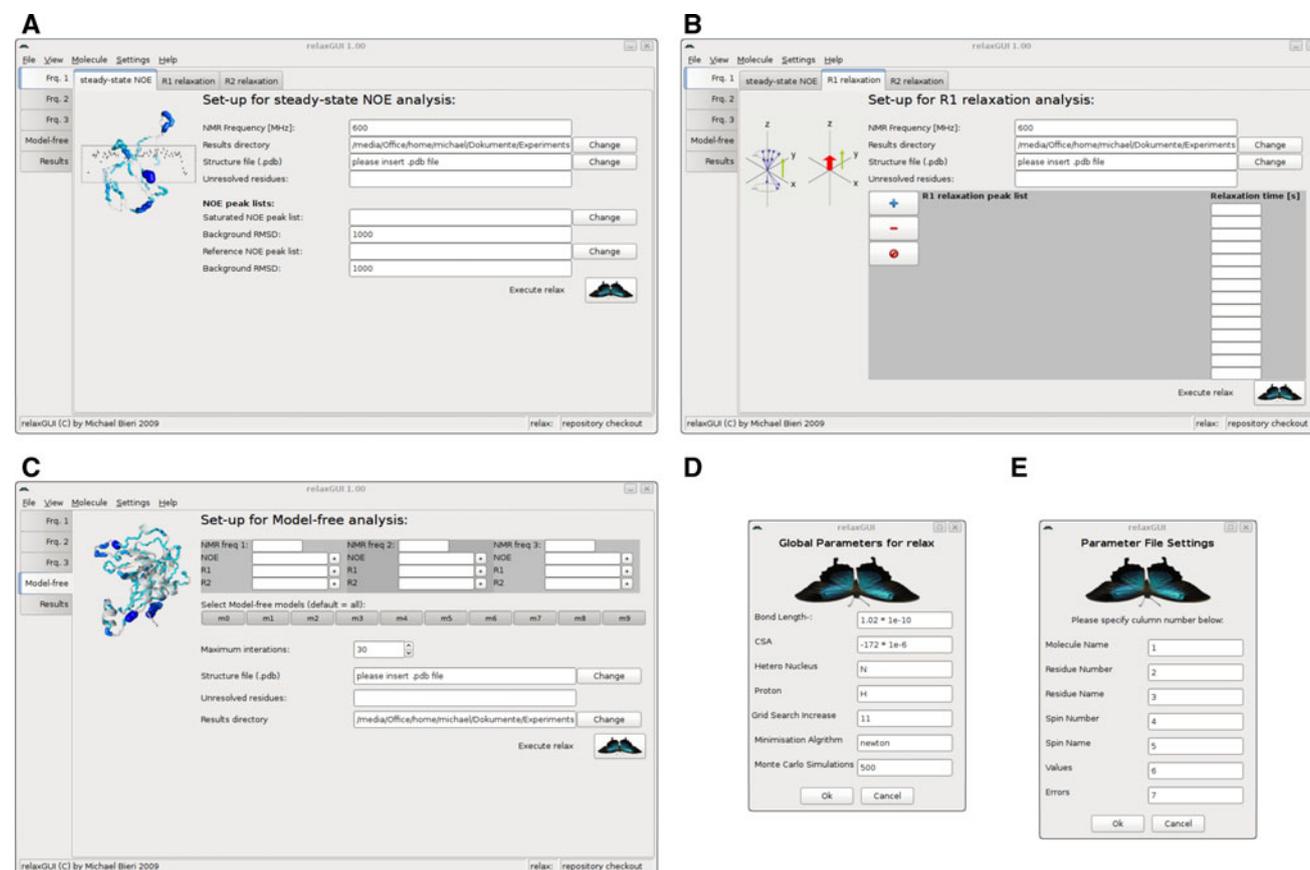


Fig. 1 The graphical interface of relaxGUI. The GUI of relaxGUI is designed for simple and quick data collection and management. Required data for NOE (a), R_1 and R_2 (b, note R_1 and R_2 interfaces

are similar) and model-free analysis (c) are imported and collected in a graphical manner. Furthermore, settings for global parameters (d) and peak files (e) are customized and set in settings dialogs

window (Fig. 1e). Nevertheless, relaxGUI comes with default values that correspond to the analysis presented in this article and settings for Sparky formatted peak lists (Goddard and Kneller 2006).

During the calculation steps, the relaxGUI “relax controller window” will appear which is composed of a text panel showing the calculation output and a progress bar that displays the state of calculation. Projects can be saved and opened at any time for the best management of data. relaxGUI uses the data storage facilities that are part of relax, therefore, the user has the possibility to read and observe the actual state of the project or to switch reversibly between the GUI and the power user prompt mode at any stage.

Workflow

For steady-state NOE calculations, peak files consisting of XH assignments and corresponding peak intensities of a HSQC spectrum for the saturated and the reference NOE experiments are loaded into the corresponding tab (Fig. 1a). The format of the peak file can be specified in the ‘Peak File Settings’ option. The baseplane root mean square deviation (RMSD) of the corresponding spectrum can be added for error estimation. The protein sequence can be imported either by a sequence file or extracted from a PDB structure file. Once all required data are loaded, the calculation can be initiated. relaxGUI will open the relax controller window for real-time monitoring of the calculation (window is closable at any time without interrupting the calculation). After the calculation is complete, relaxGUI will create several graphs including a steady-state NOE representation. In addition, a PyMOL macro to produce a color-coded and width-coded structure representation will be created. Both files can be opened in the results section. The generated NOE parameter file is automatically indexed in the model-free analysis section.

Longitudinal and transverse relaxation parameters R_1 and R_2 are calculated within the corresponding tabs (Fig. 1b). Peak lists (format can be set in the ‘Settings’ menu) are loaded into the data collection tab. The relaxation time for each peak list is set in a graphical manner. If all data are set up and read in, relaxGUI calculates relaxation parameters and Monte Carlo simulated errors and reports the calculation status in the relax controller window. After the calculation has finished, relaxGUI creates graphs for R_1 and R_2 , which can be opened in the results tab.

The model-free calculation can be performed in different ways (Fig. 1c). Relaxation parameter files are either generated by relaxGUI and automatically set up for further analysis or parameter files calculated by another software package can be loaded into relaxGUI. The user will have

the option to choose which model-free models can be selected (Eq. 8.0–8.9 of the supplementary material) although it is highly recommended to stay with the default whereby all models are used.

If all required data are collected, the relaxGUI controlled model-free calculation can be initiated. There are different ways in which model-free analysis can be performed. The first is the automatic mode in which relaxGUI fully controls the calculation, starting with the optimization of the local τ_m models for each residue/spin system as by the new model-free protocol described in (d’Auvergne and Gooley 2008b). After this calculation, relaxGUI automatically starts the calculation of the different global models of the spherical, prolate spheroidal, oblate spheroidal and ellipsoidal diffusion tensors. The different diffusion models are then iteratively optimized until convergence (ibid.). Finally, relaxGUI performs model selection for the best global model and creates graphs for relaxation parameters and PyMOL macros for color and width-coded images. These files are summarized and can be inspected in the results tab. The second way a user can perform the analysis is to manually decide which diffusion tensor calculation will be performed by relaxGUI. After a successful local τ_m calculation, users will have the choice of which global diffusion model will be optimized next. After the final calculation and model selection, the same results as in the automatic mode are collected in the results tab.

Results

To demonstrate the robustness of the results generated by relaxGUI, the relaxation data for OMP were reanalyzed (Gitti et al. 2005; d’Auvergne and Gooley 2008b). Note that all the calculations, including data collection, were performed solely through the GUI. OMP relaxation data were collected at 600 and 800 MHz (Gitti et al. 2005). The X-ray structure (1F35) of OMP was used to perform model-free analysis, as it was shown to give a better fit to the NMR data than the solution structure (1JYT). Hydrogen atoms were generated in PyMOL.

After inputting all the required data in the relaxGUI model-free tab, semi-automated (custom selection of the diffusion tensor to calculate) model-free analysis was performed. According to the new model-free analysis protocol (d’Auvergne and Gooley 2008a, b), local τ_m models are calculated first (no diffusion tensor). Subsequently, global models for the sphere (isotropic diffusion), prolate spheroid (axially symmetric diffusion of a prolate spheroid), oblate spheroid (axially symmetric diffusion of an oblate spheroid) or ellipsoid (rhombic or fully anisotropic diffusion of an ellipsoid) diffusion tensors were calculated following the iterative procedure. After

calculation of all model-free models, model selection was performed using Akaike's Information Criteria (AIC) to determine the model with the best global fit (Akaike 1973; d'Auvergne and Gooley 2003). relaxGUI has chosen the ellipsoid diffusion tensor (full anisotropic diffusion, Fig. 2) with a Brownian rotational tumbling time τ_m of 9.096 ns ($D_{iso} = 18.3 \times 10^6 \text{ s}^{-1}$, $D_a = 7.1 \times 10^6 \text{ s}^{-1}$, $D_r = 0.062$, $\alpha = 1.96$, $\beta = 0.99$, $\gamma = 0.65$). Fitting parameters for each individual diffusion tensor is summarized in Table 1. Model selection was performed by comparing AIC values and the lowest value was selected as the global model for the diffusion tensor. Internal motion was calculated using the ellipsoid diffusion tensor model and S^2 and R_{ex} values were extracted. These values are presented in the supplementary material (Table S-01). Two highly mobile regions of OMP with lower S^2 values (Fig. 3a) are located in two separate loops within the protein (Fig. 3b). This result is in accord with the reanalysis using relax (d'Auvergne and Gooley 2008b) and differs to the initial analysis (Gitti et al. 2005). Color and width-coded structures of OMP were generated by relaxGUI. Extraction of R_{ex} from model-free analysis identified two regions of OMP that consist of slow motion (Fig. 3c). Mapping R_{ex} values onto the structure (Fig. 3d) identifies two flexible loops. Figure 4 compares dynamics parameters obtained for the different diffusion tensor models.

The model that was chosen the most for residues within the ellipsoid diffusion tensor was model 2 (Table S-01), which is described by S^2 and τ_e (Eq. 8.2 of supplementary material). Models that include R_{ex} are limited to models 3, 4, 7 and 8 (Eqs. 8.3, 8.4, 8.7 and 8.8 of supplementary material, respectively). The predominant model for residues with R_{ex} was model 4, the second simplest model with R_{ex} .

To further validate the operation of the GUI, the relaxation data of both the class A β -lactamases PSE-4 (Morin and Gagne 2009) and TEM-1 (Fisette et al. 2010) were reanalyzed. For this analysis the PDB files 1G68 and 1XPB for PSE-4 and TEM-1 respectively with protons added

Table 1 Model selection statistics

Model	Number of parameters (k)	Number of data sets (n)	χ^2	AIC
Local τ_m	378	810	191.26414	947.26414
Sphere	299	810	281.15295	879.15295
Prolate	296	810	250.56219	842.56219
Oblate	301	810	262.79794	864.79794
Ellipsoid	293	810	255.85582	841.85582

were used, and the relaxation data files were provided by the authors. The bond length was set to 1.02 Å and the CSA value to -172 ppm as in the original analysis. To demonstrate that relaxGUI obtains the same results as relax in prompt/script mode, the TEM-1 data sets were reanalyzed in script and GUI mode using exactly the same standardized protocol. A comparison of this reanalysis shows that both analyses ended with the identical values and models for all residues (Fig. 5). The final combined χ^2 values for the global model were 1,129.3289791948112 and 1,129.3289791948116 for the GUI and script respectively, only showing a slight difference in the last decimal place.

Discussion

The knowledge of dynamics of proteins is essential for a complete understanding of the relationship of enzyme/protein structure and function. Recent advances in pulse sequences and the increased capacity of data analysis by faster computers has made NMR an ideal tool for studying protein dynamics at an atomic resolution. relaxGUI is offered as a tool for the analysis of protein dynamics for users of all levels, thus enabling simple and fast analysis of NMR relaxation data on all operating systems with minimal user intervention. For a full analysis, no scripting is needed. The addition of relaxGUI to the parent program

Fig. 2 Representation of the Brownian rotational diffusion tensor of OMP as seen from the *side-face* (a) and *top-face* (b). Model-free analysis of relaxation data and the X-ray structure 1F35 fit best with an ellipsoid diffusion tensor. The geometric tensor is positioned at the center of the mass of OMP. The diffusion tensor is saved as a PDB file and can be handled as any PDB file. Representation is displayed using PyMOL via relaxGUI

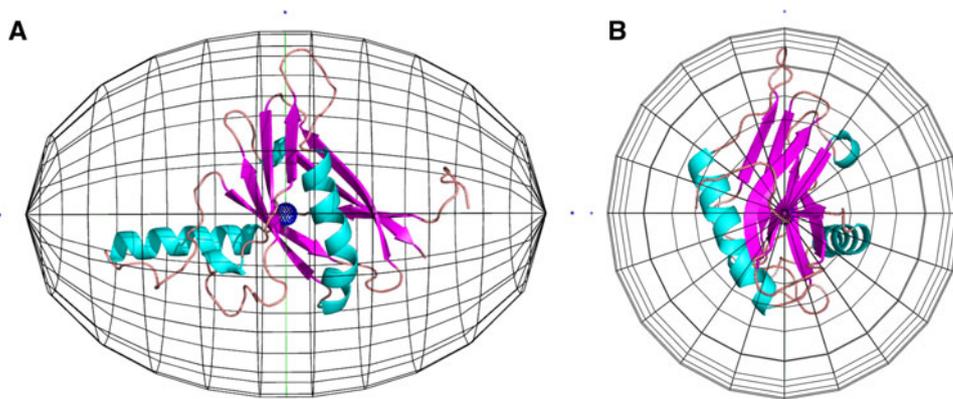
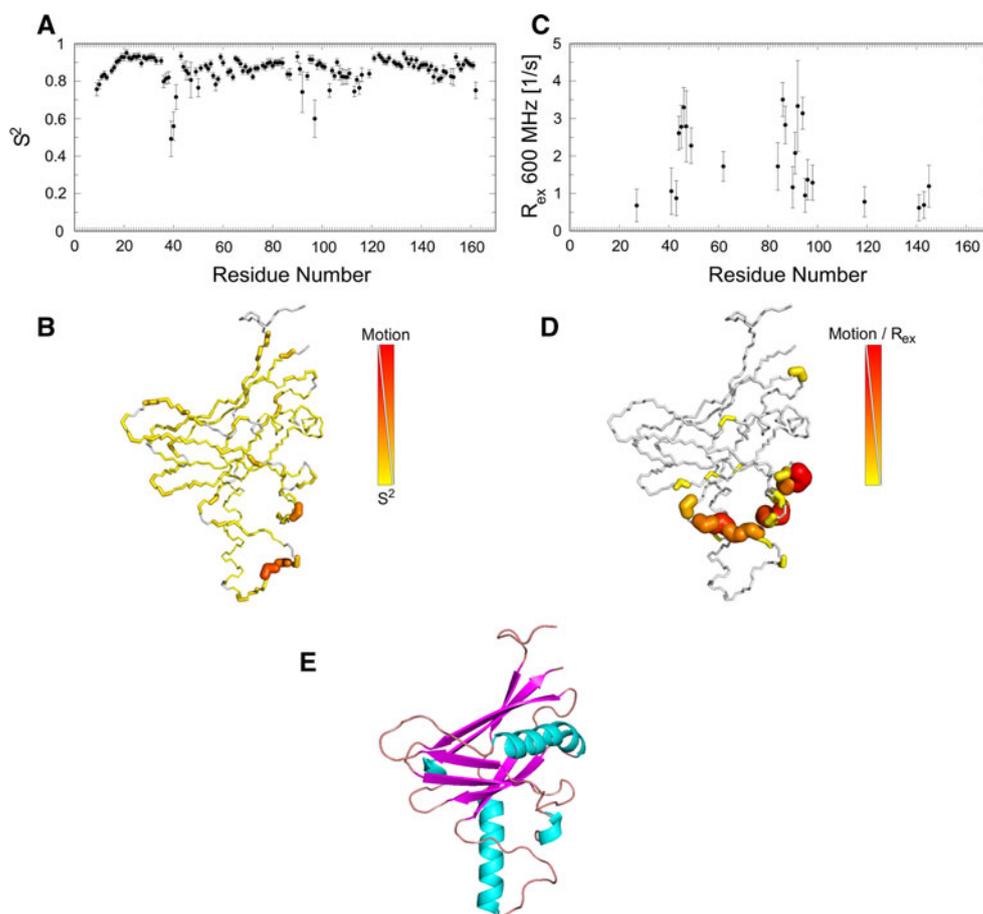


Fig. 3 Model-free analysis of NMR relaxation experiments of OMP. The order parameter S^2 (a) is color and width-coded onto the structure of OMP (b). Thicker lines and gradient from yellow to red indicate lower S^2 values and higher flexibility on the ps-ns time scale. Slower time scale motion (μ s-ms) is represented by R_{ex} (c). The color and width-coded structure for R_{ex} (d) identifies two mobile loops in this time frame. Comparing the coded structures with the cartoon representation of OMP (e) highlights loop regions with mobility on both time scales. Structure representations were prepared in PyMOL; color coded structures were created using PyMOL macros automatically created by relaxGUI



relax offers a user friendly interface to perform and manage model-free analysis and to help interpret data. As relaxGUI is fully integrated into the core of relax, the time needed for an entire model-free calculation is comparable to the default scripting/prompt interface of relax. In addition, relaxGUI has a fully automatic mode, which runs the whole analysis without any user intervention once the data files have been loaded. Furthermore, the dialog for several settings allows the user to adjust most parameters employed in relax and to set all kinds of input files such as the PDB file and peak lists from different analysis softwares. Finally, relaxGUI generates plots for the main results and summarizes calculated values, such as selected models, S^2 , R_{ex} and other parameters, in a table that can be read by any spreadsheet software (such as Microsoft Excel or OpenOffice Calc). For the best data presentation and interpretation, relaxGUI generates PyMOL macros that color and width code NOE, S^2 and R_{ex} values onto the structure.

Comparison of the reanalysis of relaxation data from OMP using relaxGUI and the results presented in (d'Auvergne and Gooley 2008b) demonstrate the robustness of relaxGUI. Only small differences between S^2 values (<0.01 units) calculated using relaxGUI compared to the initial relax analysis by (d'Auvergne and Gooley

2008b) were observed (Supplementary material Figure S-03). The main difference between these two analyses was the selection of the diffusion tensor model. In this study, an ellipsoid diffusion tensor was chosen, whereas the prolate diffusion tensor was chosen in the previous study (d'Auvergne and Gooley 2008b), although the internal dynamics remains the same. This difference may be due to newer versions of Python or Python modules, such as Numpy or Scipy (<http://numpy.scipy.org/>), or differences in machine precision. Note that many minimization iterations are performed during an analysis that may end up with slightly different minima. By comparing the quality of fits (χ^2 values, Table 1) of prolate and ellipsoid, it becomes clear that both models fit equally, with a slightly better fit for the prolate model. The local τ_m model has even a lower χ^2 value, which means that this model fits the experimental data the best. During model selection, it is important to evaluate the benefit of introducing an additional parameter to a model, as more parameters generally fit the experimental data better. relaxGUI uses Akaike's information criteria (AIC; $AIC = \chi^2 + 2k$, where k is the number of parameters) as a statistical test to evaluate which model best describes the data. AIC values of ellipsoid and prolate models in Table 1 are close (841.86 and 842.56,

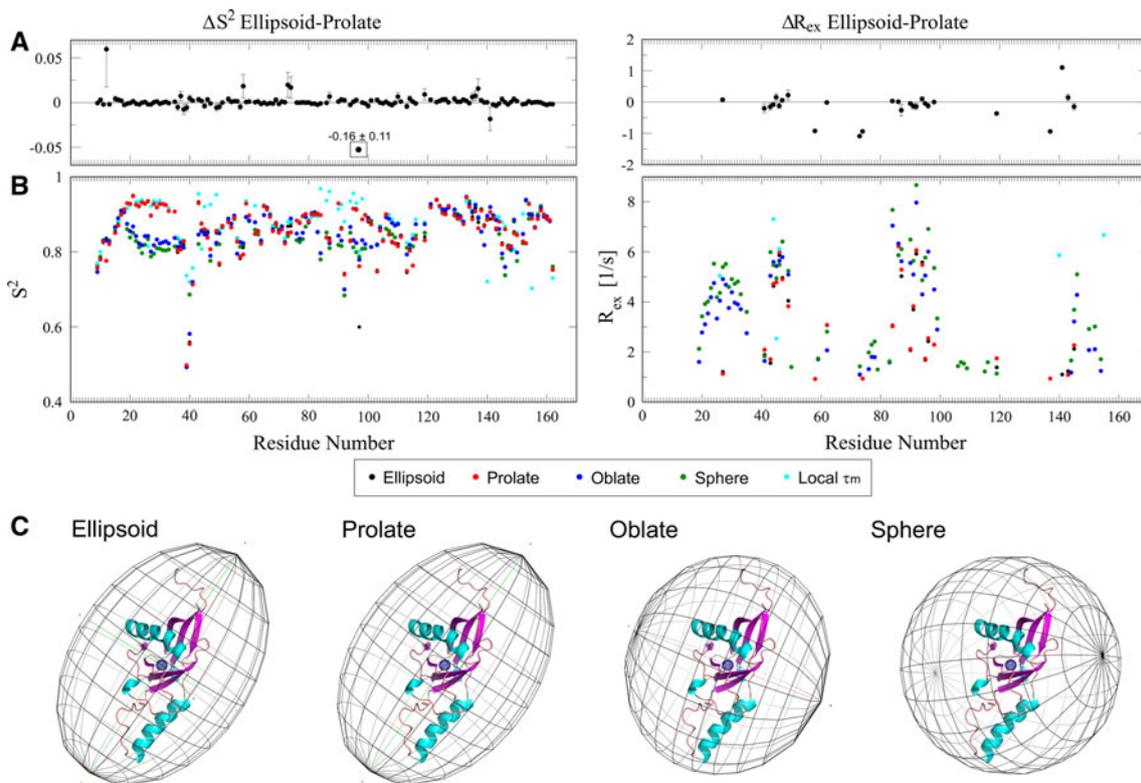
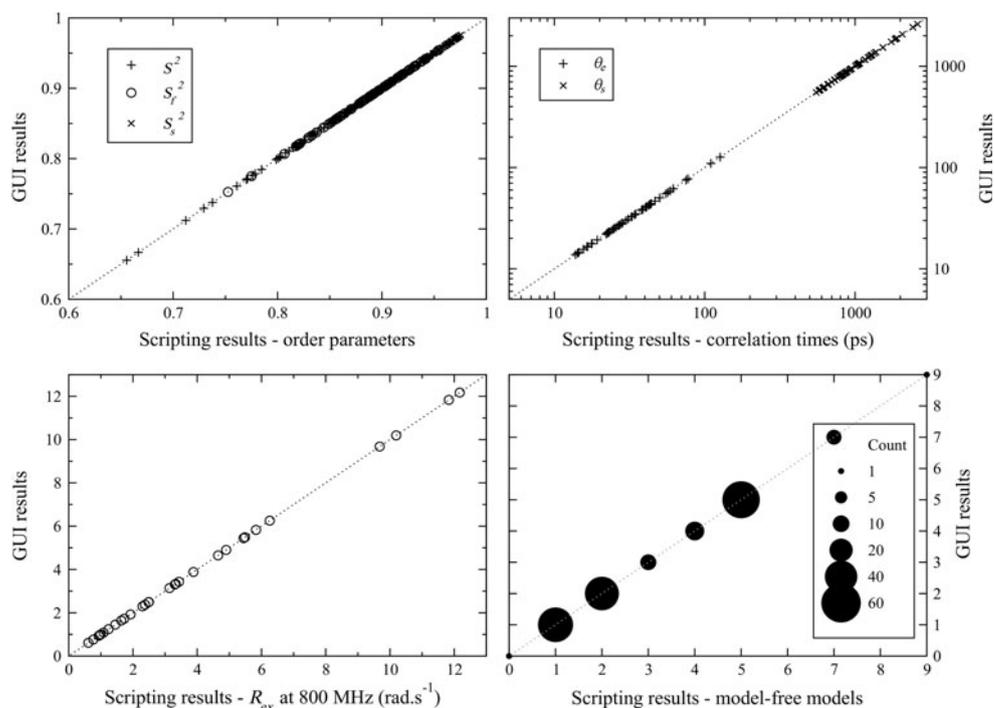


Fig. 4 Comparison of dynamics results for different global diffusion models. **a** The results from the *ellipsoid* and *prolate* diffusion tensor models only slightly differ. **b** The overlay of relaxation parameters S^2 and R_{ex} for each diffusion tensor model shows major aberrations for the *oblate*, *sphere* and local τ_m models compared to ellipsoid and

prolate models for residues 15–35 and 75–115. **c** A comparison of diffusion tensor geometries, overlaid on the OMP structures, shows diverging tensors for oblate and sphere diffusion tensor, and an almost identical fit for the ellipsoid and prolate

Fig. 5 Comparison of model-free analyses of TEM-1 using the scripting mode of relax and relaxGUI via the same protocol. The results from both are identical



respectively). Both models equally describe the NMR data, slightly favouring the ellipsoid model due to the lower AIC value. Figure 4 compares the results obtained for the individual diffusion tensors. Extracted S^2 values for the prolate and ellipsoid diffusion tensor models are essentially the same, whereas the other models differ significantly. Only one value differed significantly between the two models, namely S^2 of residue 97 which differed by -0.16 ± 0.11 (ellipsoid—prolate) (Fig. 4). The extracted R_{ex} values are also close between the prolate and ellipsoid tensor. Two mobile regions experiencing chemical exchange contribution are observed in the loops 40–50 and 80–100. Four additional residues (58, 73, 74 and 137) show R_{ex} values for the prolate diffusion tensor model [also chosen in previous the analysis (d’Auvergne and Gooley 2008b)] compared to the ellipsoid model; whereas only one additional residue (141) was extracted in the ellipsoid model compared to the prolate. By comparing the structural representations of the four diffusion tensor models (Fig. 4c), the ellipsoid and prolate models show better and similar fits. The similarity of the prolate and ellipsoid is highlighted by the ellipsoidal D_r parameter, that has a range between 0 and 1 for axially symmetric to fully rhombic diffusion respectively, having a value of 0.062.

A comparison of the reanalysis of the relaxation data of PSE-4 and TEM-1 shows that using the script or GUI mode of relax ended with the identical values and models thus giving confidence that relaxGUI produces identical results in either mode (Fig. 5). However, minor differences were found by comparing these results with the published values. For both systems, the ellipsoidal diffusion tensor was selected. The PSE-4 diffusion tensor values were ($\tau_m = 12.58$ ns, $D_a = 3.66 \times 10^6$ s $^{-1}$, $D_r = 0.09$, $\alpha = 2.63$, $\beta = 2.62$, $\gamma = 2.35$) compared to the published values of ($\tau_m = 12.86$ ns, $D_a = 3.78 \times 10^6$ s $^{-1}$, $D_r = 0.08$, $\alpha = 2.90$, $\beta = 2.56$, $\gamma = 2.30$). The TEM-1 diffusion tensor values were ($\tau_m = 12.51$ ns, $D_a = 3.08 \times 10^6$ s $^{-1}$, $D_r = 0.10$, $\alpha = 1.19$, $\beta = 0.17$, $\gamma = 0.51$) compared to the original values of ($\tau_m = 12.53$ ns, $D_a = 2.78 \times 10^6$ s $^{-1}$, $D_r = 0.10$, $\alpha = 0.85$, $\beta = 0.17$, $\gamma = 0.74$). All model-free results are presented in the supplementary material. The similarities between the original and re-analysis model-free S^2 , S_f^2 , S_s^2 , τ_e , τ_f , τ_s , and R_{ex} parameters and models are compared in Figure S-01 for PSE-4 and Figure S-02 for TEM-1. For the PSE-4 and TEM-1 model-free analyses, all residues were included in the analysis and calculations using the relaxGUI. Therefore the only steps required for a full and complete analysis were to load the relaxation data files, the PDB file, and the sequence file, then to set the bond-length and CSA values, specify in which columns the data is located, and to click on ‘execute relax’. The differences found here compared to the original analyses lies

in not excluding any residues and in a difference in the model-free analysis protocol used in the published analyses (Fisette et al. 2010; Morin and Gagne 2009) whereby AICc model selection was used as a last step (d’Auvergne and Gooley 2003). AICc model selection is designed to under-fit based on the condition of low data quantity, and will result in parameters closer to insignificance being absent in a model-free analysis. This can be seen in Figures S-01 and S-02 whereby the removal of the low level, system wide R_{ex} values is clearly visible. The under-fitting effects of AICc compared to AIC model selection are also visible in the model-free model comparison graphs, wherein AIC will select a more complex model in a number of cases. The effects on the order parameters and correlation times are insignificant, as only a few residues are partly affected. The TEM-1 dynamics is a little more perturbed than PSE-4, but that is due to AICc model selection picking the single time scale motion model m2 rather than the two time scale motion model m5 for a number of residues. The overall dynamic picture for the two β -lactamases however, does not significantly change.

Taken together, the software presented here is a powerful and simple tool to analyze NMR relaxation data at multiple field strengths. relaxGUI is part of relax program and is freely available online (<http://www.nmr-relax.com>, version 1.3.9 and greater). The graphical user interface can be started by the prompt commands:

```
$ relax -g
$ relax - -gui
```

Support is granted by an active mailing list and further development aims to fully automate analysis of protein dynamics with the least amount of user intervention. The greatest benefit is the possibility of analyzing protein dynamics experiments by non-experts, which will greatly lead to a deepened understanding of structure and functional relationships of proteins and enzymes. This will also be of benefit for RNA/DNA work, and for the study of small organic molecules. Another advantage of automated data analysis is to avoid introduction of errors due to manual intervention. relaxGUI minimizes preparation time to start the analysis, while offering the user flexibility to change parameters and to select models for analysis. The relax project, including relaxGUI, is still under development and new features will continue to be added. As this project is open source, users are encouraged to contribute and have the ability to write their own extensions.

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