Project Description – Project Proposals – for ANR DFG 2024 NLE

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Project acronym: DYNPROT

Project title: The role of conformational changes and dynamics in molecular recognition and protein function / Die Rolle der Konformationsänderungen und der Dynamik bei molekularer Erkennung und der Funktion von Proteinen / Le rôle des changements de conformation et de la dynamique dans la reconnaissance moléculaire et la fonction des protéines

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Project Description

1 Starting Point

State of the art

Since the pioneering kinetic studies of ligand rebinding myoglobin by Frauenfelder and his collaborators in the 1970's ample evidence has been provided that protein dynamics plays a fundamental role for protein function and that the three-dimensional structure of a protein alone is not sufficient to explain its function [1]. A characteristic feature of proteins, as of complex systems in general, is that their internal dynamics exhibits *self-similarity*, in the sense that the asymptotic form of the relevant time correlation functions is scale invariant. The phenomenon was first investigated by kinetic experiments [1, 2, 3], later by fluorescence correlation spectroscopy [4], and by Molecular Dynamics simulations [5, 6]. In this context, it is important to emphasize that protein dynamics are relevant for the function of the protein in many ways. Here, we can distinguish between at least three different categories: (i) Translational and rotational diffusive motions of the entire molecule. They are important for intermolecular collisions and binding of biomolecules [7]. (ii) Internal structural equilibrium fluctuations on fast ps to ns time scales play an important role for structural conformational changes on much longer time scales which are often directly linked to the function of the protein (e.g. catalytic activity) [8]. (iii) The fast equilibrium fluctuations also allow the protein to sample and populate a large number of conformational sub-states, at least under physiological conditions (i.e. at room temperature and dissolved in aqueous buffer). Depending on the environmental conditions, for example for ligand bound vs. non-bound states, the conformational sub-states can be populated very differently, which is reflected in the conformational entropy of the protein[9,10]. This parameter has often a direct impact on the thermodynamics of the intermolecular binding affinity, for example to other proteins or to ligands.

A special type of conformational changes in proteins is the relative movement of domains against each other, which in many cases is directly connected with the catalytic function or with

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[1] This is the person who submits the proposal at http://elan.dfg.de and who should be mentioned as 'référent pays' when submitting this document to ANR. Otherwise, this role has no other particular meaning.

[2] This is the person who is coordinator from the ANR point of view. This role may not be assumed more than once (see ANR's AAPG eligibility rules for details).

the establishment of signal transmission states. The character of these large amplitude motions is encoded in the topology of the protein structure, as shown for example by normal mode analysis. In particular, in multi-domain proteins, like in various kinases, domain motions modulate the accessibility of substrate binding sites and thereby the ligand binding affinities [11]. At the same time much faster local motions, typically driven by stochastic collisions between the solvent molecules and the protein surface, can also modulate the binding affinity of ligands [12].
Many aspects of these protein dynamics, see categories (ii)-(iiii), which can typically be observed on time scales ranging from picoseconds to milliseconds, have been studied using different experimental methods [13]. Mainly spectroscopic techniques, for example neutron spectroscopy, fluorescence spectroscopy and NMR spectroscopy (and the combination of these techniques), allow the study of dynamics on these very different time scales. Often a larger gain in knowledge from experiments can be obtained with the aforementioned techniques if the measurement results from different time scales are linked with the help of MD simulations and model building[14, 15, 16, 17, 18]. Here the interpretation of protein dynamics obtained from experimental data can benefit by making use of “minimalistic” models [19, 20, 21, 22] or by using normal mode analysis (NMA) [23] and accompanying coarse-grained simulations [17].
Most features of the dynamics-function relationships described above were identified in the context of case studies with proteins with a well-defined three-dimensional structure. However, in the case of intrinsically disordered proteins (IDPs), the well-established structure-functions paradigm is no longer fully valid[24, 25]. IDPs are defined as proteins that lack a unique fold and therefore exist as an ensemble of structures with rapidly interconverting conformations [24, 26]. Despite the lack of a well-defined structure, IDPs do have distinct functional properties. Importantly, these functional proteins, which exist in a variety of conformations, allow a single IDP to interact with many different partners. Studies have shown that IDPs and proteins with intrinsically disordered regions (IDRs) play central roles in protein networks, in particular, acting as hub proteins for molecular communication via protein-protein interactions (PPI) [27, 28]. Upon binding to target proteins, IDPs become structured to varying degrees, from fully ordered complexes, in which α-helices are usually formed, to those that exhibit a high degree of disorder in the bound state, the so-called fuzzy complexes. Obviously, the process of binding can be coupled with a significant loss in conformational entropy of the IDP/IDR. However, if for example the gain in enthalpy coupled with folding is sufficient to pay the entropic penalty of binding, the overall process of binding is feasible [29]. Although dynamic properties of IDPs have been studied with various experimental techniques, such as NMR [16, 30] , neutron spectroscopy [21, 31], fluorescence spectroscopy [32, 33] and with simulations [34], the role of these dynamics in molecular recognition and binding is not yet fully understood.

Preliminary work

In the past all participants of the project group have been working intensively on various aspects of protein dynamics. An important methodological focus was and still is on experimental methods using neutron scattering and the modelling as well as the specific analysis of the data. In the most frequently used approaches the measured neutron scattering of biological samples is dominated by incoherent scattering from hydrogen nuclei for most of the angular range (i.e., when Bragg reflections are absent and the coherent scattering contribution can be neglected in the observed q-space and when protonated biomolecules are dissolved in D_2O). The high density of hydrogen nuclei in all biological macromolecules results in a total scattering function $S(\mathbf{q},\omega)$ which is dominated by the incoherent scattering function. The Fourier-transform in space and time of the latter provides a direct interpretation of dynamical properties by means of the so-called Van Hove self-correlation function. Because the bound hydrogens are distributed

more or less homogeneously in the protein structure, they serve perfectly as local probes to monitor internal structural fluctuations of the protein (see for example ref. [35]). By making use of quasi-elastic incoherent neutron scattering (QENS), in the past we analyzed the dynamics in the range of a few picoseconds to nanoseconds of folded vs. unfolded proteins[36, 37, 38], folded proteins in ligand bound vs. unbound state[39, 40, 41, 42], as well as folded proteins and IDPs under crowding conditions[43]. In some of these studies, changes in the internal dynamics upon ligand binding could be extracted from the quasi-elastic neutron scattering and were investigated by employing a non-exponential relaxation model for the intermediate scattering function (see for example ref. [19]). On the basis of the analyzed data and by employing an associated energy landscape picture of protein dynamics a view emerged that ligand binding leads to a widening of the distribution of energy barriers separating conformational substates of the protein[20, 42]. In other studies we made already use of the concept, to derive a key thermodynamic parameter, namely the conformational entropy change ΔS_{conf} between unfolded and unfolded states[36, 37, 38] as well as between ligand bound and unbound states[41] (**Fig 1**).

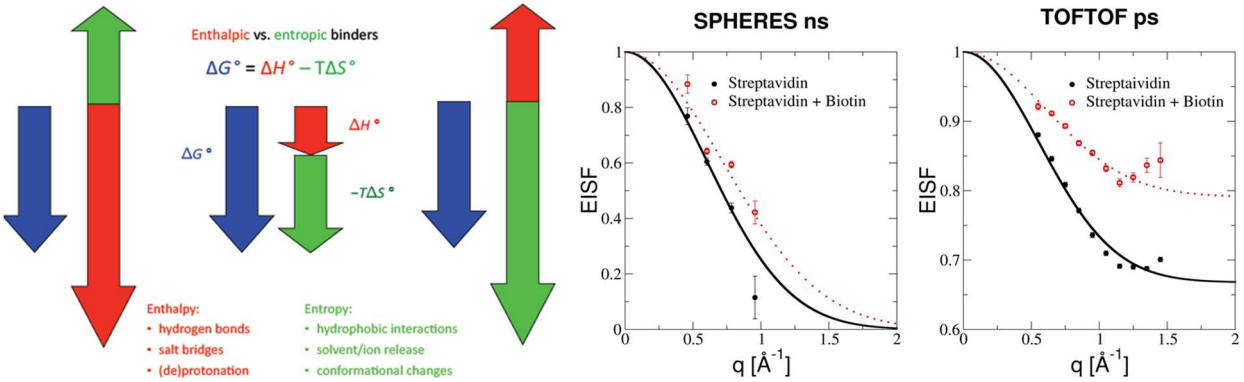

Fig.1: Right: EISF of ligand-free protein (P) and ligand-bound protein (P+L) measured on the ns timescale and on the ps timescale[41]. Symbols represent experimental data and lines are fits which were used to extract the mean square displacement (MSD). The latter were used to calculate the conformational entropy change ΔS_{conf}/residue = 3R ln $(MSD_{P+L}/MSD_P)^{1/2}$. Left: ΔS_{conf} can contribute in an adverse (left scenario) or in a favorable (middle and right scenario) manner (as part of $T\Delta S$) to the free energy change upon binding (ΔG). In the presented case of streptavidin + biotin, it was an adverse contribution.

Due to its extraordinary high effective energy resolution compared to other neutron scattering techniques, neutron-spin echo (NSE) spectroscopy is a method well suited to observe slower dynamical processes up to several hundreds of nanoseconds. We made use of this technique to study slower collective inter-domain motions[44] and overdamped internal dynamic modes (relaxations) and other diffusive processes in IDPs at small scattering angles where the coherent scattering contributions prevail up to Fourier times of around 200 ns[31]. On an even longer time scale single-molecule Förster resonance energy transfer spectroscopy (smFRET) is a powerful approach that complements the methods mentioned above. In the past we investigated conformational states of two-domain proteins with smFRET, but these studies were limited merely to structural characterizations [45] or without having made full use of the methodological possibilities [17]. By combining the NSE & smFRET methods in an integrative approach the observation window can, however, be extended dramatically from the ps up to the ms range, as demonstrated recently[18].

In many studies, knowledge of at least the low-resolution structure of the biological macromolecules under investigation is essential. Therefore, we applied small angle scattering such as SAXS[41, 46] and SANS[31, 47] in numerous cases. Here SANS offers the great advantage of contrast matching, which makes it possible to ensure that only certain proportions in the sample contribute to the measurement signal. For example, deuterated molecules or buffers based on D_2O are effectively "invisible", an approach that is particularly important when studying selected

proteins in highly concentrated crowder environments. Similarly, such a selectivity, often required in many types of measurements, can be achieved using fluorescence-based techniques. We have utilized corresponding approaches in several case studies to investigate translational mobility and conformational properties of proteins in crowded environments by using fluorescence correlation spectroscopy (FCS)[48] and smFRET[49].

2 Objectives and work programme

2.1 Anticipated total duration of the project

36 months

2.2 Objectives

The development and application of methods to study the molecular details of protein dynamics and their specific role in related function is one of the keys to understanding the many vital cellular processes in the cell. We aim to utilize complementary methodical approaches and techniques wherever possible. These include experimental methods that allow **direct access to protein dynamics**: (i) to fast equilibrium fluctuations (QENS, NSE), and (ii) to slower conformational changes with larger amplitudes of motion (smFRET). In addition, further methods for **structural characterization** (SANS, SAXS, smFRET, FCS) of the proteins or protein complexes and for **thermodynamic characterization** of the binding process (ITC, FCCS) will be used. Understanding the role of fast equilibrium dynamics in proteins for protein/protein or protein/ligand binding is still rather limited. Therefore, we would like to elucidate the **modes of conformational entropy contribution in bi-molecular binding** by comparing the corresponding dynamical changes of folded proteins and IDPs upon ligand binding.

Recent advances in the optimization of experimental methods and the corresponding sample preparation have made it possible to obtain precise information on the protein dynamics, for example for a target protein-(complex) in the presence of macromolecular crowders. Finally, mainly for experimental data from neutron scattering experiments, a **comprehensive analysis and modelling of the experimental data accompanied by theoretical simulation tools** will allow a most meaningful interpretation of the experimental results. To obtain the above described in-depth understanding of the specific roles of protein dynamics, we will focus on the following topics:

(A) Dynamics/motions in a two-domain protein (MaBP):

Here we want to elucidate the role of large inter-domain movements and faster local equilibrium fluctuations in a two-domain protein, namely the maltose binding protein (MaBP), for its destined functionality. MaBP (M_w: 41 kDa, from *E. coli*) serves as an initial receptor for active transport and chemotaxis towards malto-oligosaccharides. The related MaBP-ligand complexes represent some of the tightest protein-carbohydrate interactions. MaBP has two distinct globular domains, separated by a two-segment β-strand hinge that facilitates structural dynamics between open and closed states [50]. This allows for MaBP to prefer more closed conformations upon substrate binding, similar to a Venus fly-trap principle. Several studies indicate that inter-domain motions are highly correlated with association and dissociation rates of the ligand [51,52]. In particular, we want to investigate in a comparative study the properties and details of (i) inter-domain movements, mainly hinge-bending and/or collective domain motions by using NSE in the small-angle range (see for example [44,15]) and QENS with polarized neutrons and deuterated MaBP, (focusing on coherent scattering at larger scattering vectors and probing collective

motions at smaller length scales, Nidriche et al., accepted for PRX Life) and (ii) more local dynamics by using classical QENS (focusing on the incoherent scattering which is sensitive to averaged protein motions [22, 41]). We are interested in how or whether the local fluctuations influence the more collective inter-domain movements. We aim to support the analysis of the experimental results obtained on rather different time scales, ranging from picoseconds to tens of nanoseconds, by analyzing and modelling the data with theoretical approaches [15, 22].

Since local structural fluctuations contribute significantly to the conformational entropy, QENS is very well suited to determine this contribution to the thermodynamics of ligand binding[41]. While MaBP wildtype from *E. coli* binds maltose with K_D values of 1.2-3.5 μM[53, 54], some mutants can bind maltose much stronger with values of few 10 nM [51, 53]. Here calorimetric techniques, such as isothermal titration calorimetry (ITC), or fluorescence titration studies allow the direct determination of thermodynamic parameters of the whole system, but they do not allow one to discriminate between contributions from the protein, the ligand and the hydration shell[55]. In contrast, experimental techniques such as NMR or neutron spectroscopy provide specific information on the protein and the hydration water contributions, respectively [12, 41, 55]. Therefore, we want to analyse MaBP in the ligand bound and unbound state using QENS and compare the ΔS_{conf} values obtained with those ΔS values as measured with ITC.

In a further approach, we want to measure the two aspects described above with MaBP under crowding conditions. Numerous studies have shown that proteins adopt a more compact conformation and exhibit reduced translational and rotational mobility under crowding conditions than in dilute solutions [43, 48, 56, 57]. Since it is known that all these factors can potentially change the binding affinity of the ligand [58], we would like to perform QENS, smFRET (see for example [17, 51]) and FCS (see for example [48]) studies under crowding conditions to identify possible correlations.

(B) How IDP conformational dynamics adapt to environmental conditions

IDPs have great similarities to classical macromolecular polymers and their structural and dynamic properties can be partly described with theories from the field of soft matter [21, 31]. As a prominent example of an IDP, the Myelin basic protein (MBP) is an essential component of the myelin sheath that is formed around axons in the human brain [59]. MBP is intercalated between the different membrane layers of the myelin sheaths and ensures an isolating membrane layer that is important for rapid signal transmission along the axons [60]. While MBP is partly folded when it is in contact with negatively charged myelin membranes, it is to a large extent disordered in solution [61]. From a physicochemical point of view, it shows highly unusual properties, e.g. at neutral pH it carries a large positive net charge of +19 e and has a low proportion of hydrophobic residues being typical properties of IDPs (**Fig. 2**).

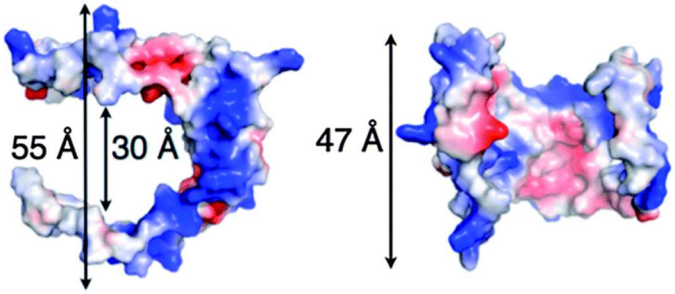

Fig.2: "C"-shape of bovine lipid-bound MBP (M_w: 18.5 kDa) determined by electron microscopy and molecular modelling. Color code: blue: positive charge; red: negative charge; grey: neutral (adapted from ref.[62]).

MBP is produced in the cytoplasma of oligodentrocytes and Schwann cells where it interacts with the cytoplasmic membrane leaflets. The intracellular space is a highly crowded solution having protein concentrations in the range of several hundred mg/mL. Although effects of molecular crowding on the properties of some IDPs were studied already in the past, see for example [33], they are still largely unexplored for MBP. In addition to binding to lipids and membranes, MBP has other binding partners (e.g., actin, tubulin, calmodulin) and acts as a scaffolding protein in cytoskeleton organization [63]. Beyond classical high affinity binding, for example for protein-inhibitor or antigen-antibody pairs[64], it had been demonstrated that also IDPs or IDRs can undergo tight binding interactions with their corresponding targets (with K_D-values even up to few pM) [65,29]. In some cases of IDP-target binding, intrinsically disordered regions become structured and participate with structurally well-defined binding motifs [29], while in other cases the IDPs remain unstructured upon binding [65]. While in "classically structured" proteins we observe typically only a moderate change in conformational entropy upon ligand binding, we would expect a much larger ΔS_{conf} for IDP-target binding.
In the past, we studied MBP already in the interaction with model membrane systems by QENS[62, 66]. Here, we aim now to study the structure and dynamics of MBP using NSE, QENS, SANS and smFRET as a function of (i) different crowding conditions and with (ii) ligand bound vs. nonbound states. The latter will be studied with calmodulin (CaM) as a binding target of MBP (with K_D values of a 10 -100 nM [59, 67]). Furthermore, again computer simulations and theoretical modelling will be applied to gain an unified description of the experimental results. The overriding scientific goal is to study the effect of macromolecular crowding on the properties of MBP to achieve situations that are closer to the crowded environment found in neuronal cells.

(C) Relating QENS data to conformational entropy of proteins

As already mentioned, quasielastic neutron scattering yields an averaged view of protein dynamics, including fast stochastic motions of protein segments[68]. A direct relation between incoherent QENS spectra and conformational entropy can be established via the Franck-Condon picture of incoherent neutron scattering which is presented in Ref. [19]. In this formulation of neutron scattering the dynamic structure factor is the thermally averaged probability for a scattering-induced energy change $\hbar\omega$ of the scattering system for a given momentum transfer $\boldsymbol{q}$, if one considers a quasi-continuous distribution of energy levels,

$$S(\boldsymbol{q},\omega) = \hbar \int dE W_{eq}(E) W(E+\hbar\omega|E;\boldsymbol{q}). \quad (1)$$

Here $W_{eq}(E) \propto exp(-E/k_BT)$ denotes the Boltzmann-weighted equilibrium distribution of energy levels and $W(E'|E;\boldsymbol{q})$ is a Franck-Condon type probability density for a scattering-induced transition from energy E to energy E' of the scattering system for a given momentum transfer $\hbar q$. The details can be found in Ref.[19]. What matters is that the quantum mechanical formulation of the dynamic structure factor as a true probability density enables the definition of a Shannon-type entropy for scattering-induced transitions — and related conformational changes — through,

$$S_{conf}(\boldsymbol{q}) \equiv -k_B \lim_{\epsilon\to 0+} \left\{ \int_{-\infty}^{-\epsilon} d\omega S(\boldsymbol{q},\omega) log(S(\boldsymbol{q},\omega)) + \int_{\epsilon}^{\infty} d\omega S(\boldsymbol{q},\omega) log(S(\boldsymbol{q},\omega)) \right\}, \quad (2)$$

where the Dirac distribution for purely elastic scattering is excluded here. In this picture, the conformational information is encoded in the $\boldsymbol{q} \to \boldsymbol{r}$ Fourier transform of the transition probability densities $W(E'|E;\boldsymbol{q})$ and the neutrons probe the "energy landscape" of the scattering system, which is defined by the ensemble of energies, E, of the scattering system and the corresponding degeneracy factors, $\rho(E)$. This quantum energy landscape can be seen as the analogue of Frauenfelder's classical kinetic energy landscape of proteins[69] where thermally

activated transitions between local minima of the free energy landscape are replaced by neutron ''kicks'' with momentum transfer $\hbar\boldsymbol{q}$. If these kicks are comparable to collisions with the neighboring atoms, a semi-classical approximation of the dynamic structure factor can be applied. This approach has been used in recent work to model QENS spectra from human acetylcholinesterase, myelin basic protein, and phoshoglycerate kinase [20, 21, 42], using the "minimalistic" model for the intermediate scattering function presented in Ref. [19]. This model accounts for non-exponential relaxation with an asymptotic power law relaxation $\propto t^{-\beta}$. Inserting the corresponding dynamic structure factor (the q-dependence of all parameters is omitted and the exponent is here called β, to be compatible with Ref.[19])

$$S(\omega) = EISF\delta(\omega) + (1-EISF)\frac{sin\left(\frac{\pi\beta}{2}\right)}{\pi\omega\left((\tau\omega)^{-\beta}+(\tau\omega)^{\beta}+2cos\,\frac{\pi\beta}{2}\right)} \qquad (3)$$

into Expression (2) leads to the entropy displayed in Fig. 3A (unpublished results). Here $EISF \equiv EISF(\mathbf{q})$ is the elastic incoherent structure factor and the entropy depends only on the exponent β and on the EISF. One recognizes that the conformational entropy increases with decreasing β and decreases with increasing EISF. This can be interpreted in the (semi)classical picture of diffusion in a "rugged" potential, where the roughness increases with decreasing β, since this leads to an increasing number of conformations which are separated by high energy barriers[20, 21, 42]. In the limit $EISF \to 1$, i.e. for purely elastic scattering, the conformational entropy is zero, which is a meaningful result since purely elastic scattering corresponds to a "frozen" protein (strictly speaking, this only applies to an ensemble where all proteins are in only one sub-state, and all in the same sub-state). This is also compatible with a recent comparative QENS analysis of the intrinsically disordered MBP and the well-folded myoglobin (Mb)[22], which shows $EISF_{MBP} \ll EISF_{Mb}$ and $\beta_{MBP} > \beta_{Mb}$, resulting in conformational entropy values with $S_{MBP} > S_{Mb}$, as expected for an IDP. These considerations simply show that the Franck-Condon formulation of incoherent neutron scattering combined with the model (3) for the dynamic structure factor is a good starting point for the characterization of the conformational entropy changes ΔS_{conf} to be observed for proteins, for example in a ligand bound vs. unbound state. Therefore, and one goal is to compare our approach with existing methods [10, 41, 55] and in particular with calorimetric measurements, as mentioned above.

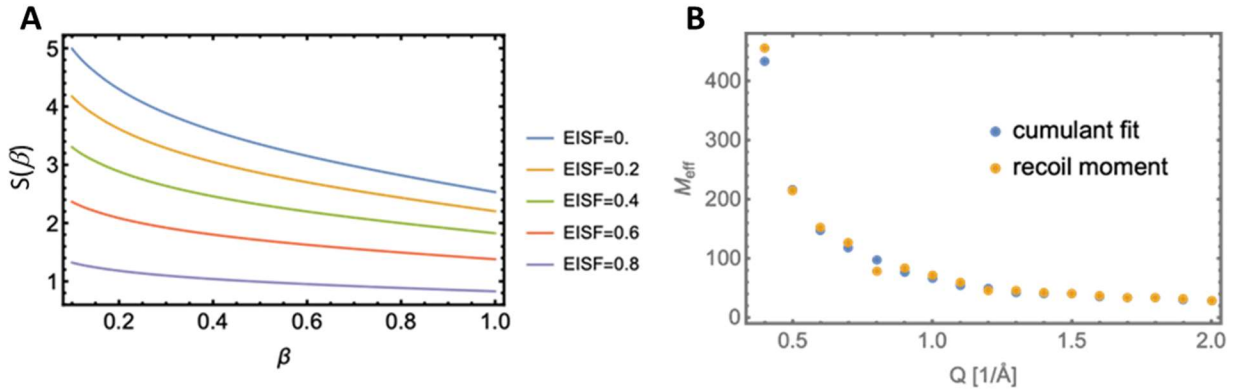

Fig.3: (A) Shannon entropy corresponding to model (2) for the dynamic structure factor. (B): Effective mass in a.m.u. of the hydrogen atoms in myoglobin probed by QENS from IN6 at ILL (unpublished work). Explanations are given in the text.

In the context of the estimation of conformational entropies we wish to characterize the segments whose motion contribute essentially to the measured QENS spectra and thus to the conformational entropy. The importance of segmental motions for the interpretation of QENS spectra from proteins in the native and denatured state has been discussed recently in Ref.[68] In

an older simulation study on myoglobin in solution[70] it has been demonstrated that rigid side-chain motions account practically completely for QENS from this protein. Here we want to take this study a step further by evaluating the effective mass probed by QENS. The effective mass concept has been introduced in the historical article by Sachs and Teller[71] which deals with the computation of scattering cross sections of protons in small molecules, like H_2O and CH_4. The quantum mechanical interpretation is simply that internal vibrations cannot be excited, in contrast to translations and rotations of the whole molecule. In complex macromolecules like proteins, a segment appears as rigid if its internal degrees of freedom cannot be excited by the incoming thermal neutrons. A way to check that, is to probe the effective proton mass of the hydrogen atoms in proteins, which is defined through the "effective recoil moment"

$$\frac{\hbar q^2}{2M_{eff}} = \int_{-\Omega}^{\Omega} d\omega \omega S(\boldsymbol{q}, \omega) \quad (4)$$

where Ω is the maximal accessible energy transfer for a given instrument (in units of $\hbar$) and $S(\boldsymbol{q}, \omega)$ is the measured dynamic structure factor. Using spectrometers like SHARP (formerly IN6) at the Institute Laue-Langevin in Grenoble a small recoil effect should be visible. Fig. 3B shows unpublished results of an analysis of QENS data from a myoglobin solution, which have been recorded on the IN6 time-of-flight spectrometer [72]. The blue dots correspond to a cumulant fit to the short-time imaginary part of the intermediate scattering function and the yellow dots have been obtained from Eq. (4). The results show that the q-averaged effective mass is about 100 a.m.u., which is the mean mass of a protein side-chain, confirming thus the simulation results[70]. Finally, we like to mention that the effective mass can be interpreted in terms of excitable states, using the Franck-Condon formulation of the dynamic structure factor derived in Ref. [19], generalizing thus the historic work by Sachs and Teller for small rigid molecules (work in progress). Our goal is to compute effective masses for all QENS measurements and compare the results from the intrinsically disordered protein MBP with those of the well-folded MaBP.

2.3 Work programme including proposed research methods

The work packages presented here require contributions and close co-operation from all groups involved and are described in detail below.

AG Biophysik, RWTH Aachen (Germany): The principal investigator J. Fitter has established a working group in the Physics department at RWTH Aachen. The focus of the group is single molecule spectroscopy on biological macromolecules, mainly by using confocal and TIRF microscopes. All smFRET measurements described in topic (A) and (B), as well as general sample characterization with respect to thermodynamic parameters (ITC, FCS, TCCD) will be conducted by this group.

Theoretical Biophysics, CNRS/University of Orleans, (France): The research group of Gerald Kneller works on data analysis and model building for experimental data probing the structural dynamics of biomolecular systems. The recent work combines the concepts of non-equilibrium statistical mechanics, energy landscapes, and asymptotic analysis in the development of "minimalistic" few-parameter models for protein dynamics probed by quasielastic neutron scattering experiments. The group has also a long-standing experience with molecular simulation techniques for biomolecular systems and their integration into the analysis of experimental data from neutron scattering, electron microscopy, and NMR spectroscopy.

JCNS-1, Forschungszentrum Jülich (Germany): The research group of A. Stadler uses neutron and X-ray scattering methods to study structure and dynamics of IDPs and their interaction with

biological membranes. A focus of the group is the application of high-resolution neutron scattering such as NSE and neutron backscattering spectroscopy. Structural studies are performed using small-angle neutron and X-ray scattering. High-resolution QENS, NSE and SAXS/ SANS experiments will be performed by the Stadler group.

Univ. Grenoble Alpes (France): The research group of J. Peters employs mainly neutron scattering techniques (spectroscopy, SANS, diffraction) on various protonated and deuterated samples. A focus is on extreme and crowded environments, among them high hydrostatic pressure conditions, low and high temperature and crowding in live cells. Coherent and incoherent scattering studies as well as sample characterizations by calorimetry will be conducted by this group.

2.3.1 Dynamics/motions in a two-domain protein (MaBP):

In this topic we would like to study the effect of binding of different ligands to MaBP from the mesophilic *E. coli* and different MaBP isoforms from the hyperthermophilic *Thermotoga maritima*. MaBP from *E. coli* (ecMaBP) is very promiscuous in its ligand affinity and binds a variety of maltose-based ligands with markedly differing affinities. *Thermotoga maritima* maintains three isoforms of MaBP (tmMaBP1-3) that are high affinity receptors for di-, tri- and tetra-saccharides, respectively (see for example ref.[73] and references therein). Thus, this range of possible MaBP variants offers an interesting opportunity to investigate the mechanisms of the underlying affinity profiles. Several studies indicate that a decreased stability of the open state leads to a decrease in the rate of dissociation between MaBP and its ligand, which results in an increased binding affinity[53, 54]. In this picture, access to and from the binding cleft of MaBP is modulated by hinge-bending movements between the two domains[51, 52]. In contrast to the aforementioned role of conformational dynamics, much less is known about thermodynamic parameters that characterize the binding affinity. While in most cases, mainly K_D-values of various MaBP-ligand bindings were measured with fluorescence titrations, to our knowledge, only one study (using ITC) has derived thermodynamic parameters in terms of ΔH, ΔS and ΔC_p so far [74]. Interestingly, this study showed that ecMaBP-maltose binding is entirely entropy-driven. To better understand the related backgrounds, we would like to perform a sequence of the following measurements and analyses:

(i) We will perform QENS and NSE experiments to study the dynamics of three MaBP isoforms (ecMaBP, tmMaBP2&3) in the ligand-free and ligand-bound states. Since in particular the fast picosecond dynamics (as measured by QENS) samples the vast majority of the conformational space, we will make use of this information to calculate the conformational entropy change ΔS_{conf} upon ligand binding for the selected isoforms. These calculations will be performed with established approaches[41] (see Fig. 1) and with an energy-landscape based approach, see topic C. Finally, we will compare our ΔS_{conf}-values with those obtained from thermodynamic (ITC) measurements which are partly known from the literature[74], or will be determined as part of the project, otherwise. (J. Peters, G. Kneller, J. Fitter)

(ii) In contrast to QENS measurements with protonated proteins and deuterated buffers, as used in subtopic (i), we would like to measure samples with deuterated ecMaBP. This would allow for a polarization analysis of the QENS signal and can be employed to distinguish the collective dynamics (coherent scattering) and the local motions (incoherent scattering). We will analyse the data by using the approach developed by G. Kneller, which was already proven to be successful for the analysis of QENS spectra

from different proteins in solution and powder environments[20, 21 42], including in a recent application to polarization analysed data, see Nidriche et al., PRX Life (J. Peters, G. Kneller), and from water[75]. To allow for a routine application of our QENS model by a broader community, we plan to integrate our analysis software [G.R. Kneller et al. DOI 10.5281/zenodo.7058344] into the Mantid package[76], which is currently the standard for the analysis of QENS data. (J. Peters, G. Kneller)

(iii) Furthermore, we would like to investigate in a comparative study the properties and details of inter-domain movements and potential correlations with the underlying mechanisms which control the on- and off-rates in ligand binding of MaBP[51]. For this purpose, NSE (sensitive for slower inter-domain motions on a several hundred nanosecond time scale) and SAXS (gives low resolution structural information) will be employed. (J. Peters, A. Stadler, G. Kneller)

(iv) Macromolecular crowding is highly expected to have a significant impact on inter-domain motions in MaBP. Therefore, we aim to study the effect of macromolecular crowding by using synthetic polymers (deuterated PEG with different molecular masses) and deuterated protein crowder dMaBP, i.e. self-crowding. MaBP/dMaBP is particularly suitable for self-crowding because it is very soluble in buffers up to high millimolar concentrations[77]. In this respect we will analyse inter-domain motions, structural conformations and local equilibrium fluctuations of the MaBP isoforms using SANS, NSE and QENS, as described in subtopics (i)-(iii). (J. Peters, G. Kneller)

(v) Complementary to the scattering techniques described above (with neutrons and x-rays), we would like to apply fluorescence-based methods, first for one MaBP isoform. For this purpose, we will produce double-cysteine mutants of the selected ecMaBP which can be labelled for smFRET studies[58]. We aim here also for the investigation of dynamics in the ns- μs time regime, to be measured with diffusing molecules in confocal microscope setups. Studies will be performed with ecMaBP in the presence and absence of ligands and crowder molecules, see (i)-(iv). Due to the fact that only molecules (i.e. proteins) of interest are labelled with bright fluorophores we can apply our methods for studying proteins in highly crowded and cytosol-like environments[78 48, 49]. Since we will make use of the confocal setups equipped with pulsed laser excitation, TCSPC and polarized-sensitive detection (which allows multi-parameter fluorescence analysis) we have access to the fluorescence lifetime and anisotropy in addition to the fluorescence intensity[79]. FRET-based conformational dynamics typically appears either as (i) conformational transitions between distinct states separated by an activation barrier or as (ii) conformational fluctuations within states characterized by the shape of the potential wells [80]. In order to obtain information on kinetics and dynamics, fluorescence correlation spectroscopy (FCS) based methods have already been applied in the past for model-independent approaches by combining smFRET with FCS [81 82]. In this approach, FRET dynamics that are faster than the diffusion time scale (a few milliseconds) can be quantified. Structural dynamics within a given conformational state can also be studied by modelling continuous distributions rather than state-dependent distributions. Information regarding this type of dynamics will be retrieved by describing the conformational dynamics in FRET efficiency versus lifetime (E vs. τ) plots [79]. In addition to these approaches we also plan to make use of dynamic photon distribution analysis (PDA) that analyses the width of FRET efficiency distributions beyond photon shot noise[83] and of hidden Markov models on photon-by-photon basis. The latter extends the achievable time resolution into the microsecond time regime [84]. With respect to the other experimental approaches applied in our joint project, the fluorescence based

approaches complement the time regimes covered by neutron spectroscopy (ps-ns) on the side of slower time scales. (J. Fitter, G. Kneller)

(vi) The above studies will be accompanied by molecular dynamics simulation studies of MaBP in solution and subsequent calculations as well as further analyses of “virtual” QENS spectra and NSE data. For this purpose, we will use nMoldyn (for details see Ref. [85]). The code computes in a first step coherent and incoherent intermediate scattering functions, which can be directly compared to NSE data. As for the experimental NSE data, we can in particular extract q-dependent diffusion coefficients from the decay of the coherent intermediate scattering function, which can be also directly computed and validated via appropriate Kubo-formulae from the simulated trajectories. Using the method described in Ref.[86], the results can be compared to the diffusion tensor of MaBP, in order to evaluate the translational and rotational diffusion of the molecule separately, as well as the coupling between them. The obtained results will be compared to those obtained from experimental data (including fluorescence techniques, see (v)) and will help in the data analysis of the NSE and QENS spectra. In particular, the separation of global diffusion from internal motions is crucial to determine the conformational entropy. In the latter context we will also perform a principal component analysis of the motions of MaBP as well as an analysis of the rigid-body dynamics (as for myoglobin in Ref[70]) in order to further decompose the simulated conformational entropy. (G. Kneller)

2.3.2 How IDP conformational dynamics adapt to environmental conditions

In living cells, molecular crowding is a routinely occurring condition with concentrations of macromolecules reaching up to 300-400 mg/ml. Such high concentrations and the presence of potential binding partners can easily alter the behavior and the properties of proteins in the cytosol. As discussed before, the high intrinsic flexibility of IDPs may cause a large entropic cost of binding and would therefore be a considerable obstacle to function. However, this apparent disadvantage can be exploited to create a mechanism to incrementally tune protein function. Since the relatively flat energy landscape of IDPs is quite sensitive to local changes in the environment, incremental changes in the environment allow a stepwise modulation of the structural heterogeneity, or conformational flexibility, of IDPs or IDRs[87]. In this manner, local changes in the environment induced by crowding, can for example vary the binding properties of IDPs to their targets significantly. A prominent example that has attracted a broad interest recently is the ability of certain IDPs to phase-separate into a dense fluid phase and to form biomolecular condensates under crowding conditions. In a similar way, also MBP exhibits structural polymorphism and multifunctionality, with variable conformations that are highly dependent on the environment of the protein[59, 67]. From a biophysical point of view, it can reveal the basic characteristics of disordered proteins in crowded solutions or during binding to target molecules.

(i) First, we plan to study the effect of crowding conditions on bovine MBP by using SANS. We aim to investigate the effect of macromolecular crowding on the structure and self-assembly of MBP by performing contrast variation SANS experiments. For that purpose, we will measure the structure of MBP in the presence of macromolecular crowders at the contrast match point condition of the crowders, see **Fig. 4**, adapted from ref.[77].

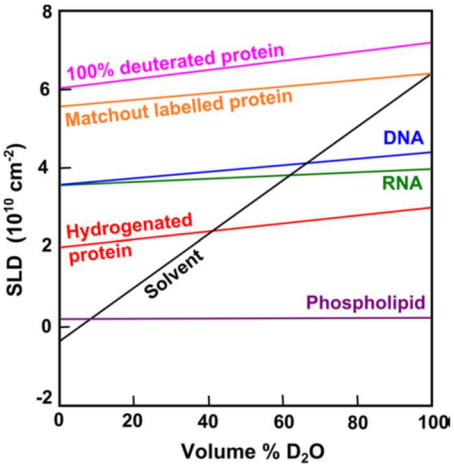

Fig.4: The scattering length densities (SLDs) of the four major biomolecules are depicted as a function of the volume percentage of D_2O, assuming all labile hydrogen atoms are exchanged. The black line represents the variation of the solvent SLD. The match point of each biomolecule corresponds to the intersection of the solvent SLD with that for each biomolecule. Perdeuterated protein, in which all the hydrogen atoms are replaced by deuterium, has an SLD that is higher than that of D_2O and cannot be solvent matched.

We will use the partially deuterated protein MaBP and the synthetic polymer PEG with different molecular masses as crowders. Protein crowders will induce conditions due to hard particles, while polymers represent soft crowders. Additionally, we will study the effect of the polysaccharides dextran and ficoll on MBP structure and self-assembly. For this aspect we will use deuterated MBP and hydrogenated dextran and ficoll and perform the SANS experiments at the contrast match point condition of the crowders. SANS experiments will explore the effect of molecular crowding on MBP in a concentration range of the crowders from 50 mg/mL up to 400 mg/mL The aims are (I.) gaining insights on structural changes of MBP by crowding and (II.) investigating whether self-assembly and liquid-liquid phase separation of MBP occurs under crowding conditions. Previous experiments using PEG as a crowder demonstrated that high concentrations of PEG result in the liquid-liquid phase separation of MBP, Graf von Westarp et al. in review. (A. Stadler)

(ii) In a further subtopic we will study the dynamical properties of MBP as well as the perturbation of MBP dynamics under different crowding conditions using NSE and QENS (MaBP and PEG as crowders as well as self-crowding of MBP at high concentrations). NSE experiments will be performed with MBP in solutions containing partially deuterated PEG and MaBP that are ‘contrast matched’ in 100 % D_2O. QENS experiments will be performed for technical reasons with MBP solutions that contain fully deuterated PEG and MaBP as crowders. In addition, QENS experiments will also be performed of MBP at sufficiently high concentrations which would allow for investigating the impact of inter-molecular interactions under self-crowding conditions, if possible, also after the liquid-liquid phase separation of MBP. (A. Stadler, G. Kneller)

(iii) Neutron scattering of biological samples gives always access to coherent and incoherent parts, the first one is informing on structure and collective motions, the second one on local dynamics. The experimenter can highlight one part over the other by protonating or deuterating the sample itself or its surrounding solution. When preparing a perdeuterated protein in D_2O, one has mainly access to coherent scattering, still this concept was recently challenged (see Nidriche et al., PRX Life). Instruments equipped with devices permitting to perform polarization analysis allow to separate the coherent and incoherent parts from each other. Today, the spectrometer LET at ISIS, UK, allows such an approach for QENS studies. To the best of our knowledge, nobody has tried to undertake such a study on an IDP. As our collaborator P. Kursula can provide us with a fully deuterated MBP protein, we want to investigate it in D_2O on this instrument to see what are the local and collective motions in such a protein (J. Peters).

(iv) Since MBP can bind calmodulin (CaM) with an affinity in the order of a few ten nanomolar[67], we plan to investigate the change of structural and dynamical properties of MBP upon CaM binding. For this purpose, deuterated CaM (partially deuterated CaM that is matched out in D_2O buffer or fully deuterated CaM) will be added to MBP solution samples which will allow to perform SANS, NSE, and QENS measurements and to extract the structural information as well as the dynamics of MBP (deuterated CaM will be produced according to Ref. [88]). An elementary aspect of this subtopic is to compare the parameters determined with regard to the conformational entropy change ΔS_{conf} upon ligand binding from the two systems analyzed in this project, namely MaBP/maltose and MBP/CaM. We expect to gain a deeper understanding of how conformational entropy plays a role in our systems by comparing the "folded protein vs. IDP" scenario. (A. Stadler, G. Kneller)

(v) Like in topic 2.3.1 (subtopic (v)), we also plan to complement the scattering data obtained from MBP by applying high resolution fluorescence spectroscopy. Here, we will start again by producing double-cysteine mutants of bovine MBP which can then be labelled for smFRET studies (feasibility was already demonstrated with a H85C-S159C mutant, Master thesis Harauz-Lab). To our knowledge, nor quantitative FRET neither smFRET studies to characterize MBP conformations have been performed yet. Therefore, amino acid residues in the MBP sequence need to be identified which make it possible to measure changes in energy transfer upon CaM binding in a FRET study. After such an approach has been developed, we would like to characterize the conformational changes induced by ligand binding in detail by using smFRET studies. In addition to details about conformational states of MBP, which should be investigated also as a function of crowding conditions, see subtopic (i), we plan to characterize the translational and rotational mobility (via FCS analysis and time-resolved anisotropy decays, see Ref. [48]) and, if possible, dynamical properties of MBP, for methodical details see subtopic 2.3.1 (v) and ref. [65, 82]. (J. Fitter, G. Kneller)

(vi) Molecular dynamics simulation of IDPs are a challenge since initial crystal structures are not available to start the simulation. In order to be able to set up an initial structure of MBP for subsequent MD simulations, we plan to follow a recent publication[89] where the initial structure of the intrinsically disordered region in MBP is predicted[90]. In a first step, the simulated QENS and NSE spectra/data of MBP will be compared to the experimental ones, ensuring that they agree reasonably well. Next we plan to analyse the data as we did in the case of MaBP, considering in particular the structural changes induced by the binding of CaM. (G. Kneller)

2.4 Handling of research data

Any results obtained within this project will be made available for the scientific community by publications in open access peer-reviewed journals.

2.5 Relevance of sex, gender and/or diversity

The working groups are characterized by an international and gender-balanced composition.

2.6 Added value of the French-German scientific cooperation

This German-French DFG-ANR project in the field of molecular biophysics brings together an international team of specialists in different spectroscopic techniques as well as in theoretical modelling and simulation, who focus on a common subject: understanding protein dynamics

from a physical point of view and the implications of real life conditions in cells for the function of proteins. The experimental techniques are all established in the field of molecular biophysics, and carefully chosen to study protein dynamics in the context of the project. The project combines the use of neutron scattering techniques, which probe the collective and local dynamics of proteins at the atomic level on time scales on the ps – 10 ns time scale, with fluorescence-based spectroscopy, which probes molecule dynamics on the complementary ns – μs time scale on single molecule level. We are planning to carry out experiments on proteins in environments representing different levels of confinement, mimicking cellular environments: in solution, in crowded solution, including the presence and absence of vital functionally relevant binding partners. On the theoretical side we combine long-standing expertise in the theory of neutron scattering and correlation spectroscopy, molecular dynamics simulation, as well as simulation-based analysis of neutron scattering and data analysis.

3 Project- and subject-related list of publications

Works cited from sections 1 and 2, both by the applicant(s) and by third parties. Please include DOI/URL if available.
***A maximum of ten** of your own works cited may be **highlighted**; font at least Arial 9 pt.*

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4 Supplementary information on the research context

4.1 Ethical and/or legal aspects of the project

Not applicable

4.1.1 General ethical aspects

Not applicable

4.1.2 Descriptions of proposed investigations involving humans, human materials or identifiable data

Not applicable

4.1.3 Descriptions of proposed investigations involving experiments on animals

Not applicable

4.1.4 Descriptions of projects involving genetic resources (or associated traditional knowledge) from a foreign country

Not applicable

4.1.5 Explanations regarding any possible safety-related aspects ("Dual Use Research of Concern; foreign trade law)

Not applicable

4.2 Employment status information

Last name	First name	Employment status
Fitter	Jörg	University professor (W2) permanently employed by the RWTH Aachen
Stadler	Andreas	Staff scientist permanently employed by the Research Center Jülich
Kneller	Gerald	University professor (Classe Exceptionnelle 2), permanently employed by the University of Orléans
Peters	Judith	University professor (Classe Exceptionnelle 2) permanently employed by the UGA

4.3 First-time proposal data

Not applicable

4.4 Composition of the project group

List only those individuals who will work on the project but will not be paid out of the project funds. State each person's name, academic title, employment status, and type of funding.

Name	Academic title	Employment status	Type of funding
Jörg Fitter	Dr. rer. nat. Physics	University professor	Permanently employed RWTH
Benno Schedler	M. Sc. Physics	PhD student	Termed Funding RWTH Aachen
Julia Walter	Dipl. Chem.	Technician (part time: 20hrs/week)	Permanently employed RWTH Aachen
Alida Meyer	M.Sc. Phys./Biol.	PhD student	Termed funding RWTH Aachen

Name	Academic title	Employment status	Type of funding
Andreas Stadler	Priv.-Doz. Dr.	Senior scientist	Permanent contract by FZJ
Julio Pusterla	Dr.	Postdoctoral researcher	Fixed-term
Irina Apanasenko	Dr.	Postdoctoral researcher	Fixed-term
Ralf Biehl	Dr.	Senior scientist	Permanent contract by FZJ
Jürgen Allgeier	Dr.	Senior scientist	Permanent contract by FZJ

Name	Academic title	Employment status	Type of funding
Gerald Kneller	Dr. rer. nat. Phys. Chem	University professor	permanently employed by the University of Orléans
Konrad Hinsen	Dr. rer. nat. Physics	Chargé de recherche CNRS	permanently employed by CRNS

Name	Academic title	Employment status	Type of funding
Judith Peters	Dr. rer. nat. Physics	University professor	Permanently employed by UGA
Shiva Ahsani	M.Sc. Physics	PhD student	Funded by ANR
Antonino Calio	Dr. rer. nat. Physics	Post-doc	Pathfinder grant

4.5 Researchers in Germany with whom you have agreed to cooperate on this project

Not applicable

4.6 Researchers abroad with whom you have agreed to cooperate on this project

Prof. Petri Kursula (Univ. Bergen, Norway), MBP
Dr. Laura Stingaciu (Oak Ridge National Lab, USA), all selected isoforms of MaBP

4.7 Researchers with whom you have collaborated scientifically within the past three years

J. Fitter: M. Pohl (IBG-1, FZ Jülich), A. Stadler (JCNS, FZJ), C. Sachse (ER-C-3, FZ Jülich), A. Schug (J.v. Neumann Inst, FZ Jülich), T. Schrader (JCNS, MLZ), D. Willbold (IBI-7, FZ Jülich), S. Kondrat (Polish Academy of Sciences, Warshaw), K. Alexandrov (QUT, Brisbane).

A. Stadler: T. Schrader (JCNS, MLZ), J. Fitter (RWTH Aachen), C. Seidel (Univ. Düsseldorf), Ch. Herrmann (Univ. Bochum), J. Klare (Univ. Osnabrück), U. Krauss (Univ. Bayreuth), A. Schug (J.v. Neumann Inst, FZ Jülich), A. Thalhammer (Univ. Potsdam), G. Kneller (Univ. Orleans, France), D. Lairez (Institut Polytechnique de Paris, France), L. Stingaciu (Oak Ridge National Lab, USA), A. Stradner and P. Schurtenberger (Lund Univ, Sweden), B. Schuler (Univ. Zürich, Switzerland), R. Best (NIH, USA).

G. Kneller: Andreas Stadler (FZ Jülich / JCNS), Heloisa Bordallo (Univ. Copenhagen / European Spallation Source), Judith Peters (Univ. Grenoble-Alpes / ILL)

J. Peters: P. Oger (INSA Lyon), A. Pastore (King's College London), A. Desfosses (IBS Grenoble), T. Matsuo (National Institutes for Quantum Science and Technology (QST), Chiba, Japan), Y. Suenaga (Chiba Univ., Japan), R. Winter (TU Dortmund), K. Kornmüller and R. Prassl (Med. Uni. Graz), S. Kapoor (Indian Institute of Technology Bombay, India), A. Paciarone (Univ. Perugia), F. Sterpone (LBT/IBPC), V. Arluison (Univ. Paris), F. Wien (Synchr. SOLEIL), G. Kneller (Univ. Orléans).

4.8 Project-relevant cooperation with commercial enterprises

If applicable, please note the EU guidelines on state aid or contact your research institution in this regard.
NOTE: Within the call ANR DFG 2024 NLE, no commercial partners can be directly involved in a project.
Not applicable

4.9 Project-relevant participation in commercial enterprises

Information on connections between the project and the production branch of the enterprise
NOTE: Within the call ANR DFG 2024 NLE, no commercial partners can be directly involved in a project.
Not applicable

4.10 Scientific equipment

List larger instruments that will be available to you for the project. These may include large computer facilities if computing capacity will be needed.

J. Fitter (RWTH Aachen): Absorption- and fluorescence spectrometer and two advanced fluorescence microscopes for single molecule detection are available (including a dual color confocal microscope with pulsed excitation at different wavelengths and two detection channels, incl. an *in situ* temperature control system). In addition, we operate a widefield/TIRF microscope with simultaneous dual color detection. Furthermore, we run a wet-lab with biochemical instrumentation for sample preparation and characterization (e.g. desk ultracentrifuge, chromatography for protein purification, etc.). Protein expression and purification of cysteine double-mutants will be conducted in the FZJ (ER-C-3/IBI-6).

A. Stadler (FZ-Jülich): Access to ITC, DLS, FT-IR, UV/Vis, in-house SAXS, PFG-NMR; Chemistry labs at JCNS allow deuteration of synthetic polymers with all needed equipment.

G. Kneller (Univ. Orleans / CNRS): Access to a regional computer center (CaSciModOT) and the national computer center for Higher Education (CINES).

J. Peters (Univ. Grenoble): Access to neutron scattering instruments at the ILL (spectrometers, SANS, NSE etc.), lab instruments as DSC, DLS, FT-IR, UV/Vis, centrifuges, lyophilizer etc.

4.11 Other submissions
Not applicable

4.12 Other information
Please use this section for any additional information you feel is relevant which has not been provided elsewhere.
Substantial support in terms of sample preparation (deuterated biomolecules for neutron scattering measurements) are provided by two co-operation partners (Prof. P. Kursula and Dr. L. Stingaciu, see attached letters of support).

5 Requested modules/funds
Explain each item for each applicant (stating last name, first name).

German side – requested modules/funds (from DFG)

5.1 Basic Module

5.1.1 Funding for Staff

Fitter, Jörg (AG Biophysik, RWTH Aachen)
1 Doctoral Researcher for 3 years at E 13 TV-L Level (75%): N.N. start at 1.1.2025
The part of the research program proposed in this grant application requires a talented candidate (physicist) who will conduct all fluorescence spectroscopic measurements, the data analysis and contribute to the integrative modelling (combining results from all experimental techniques and simulations).

Stadler, Andreas (FZ Jülich)
1 Doctoral Researcher for 3 years at E 13 TV-L Level (75%): N.N. start at 1.1.2025
The research project requires a very good candidate (physicist or physical chemist) who will perform the neutron scattering experiments (incl. suppl. biophysical studies) and data analysis.

5.1.2 Direct Project Costs

5.1.2.1 Equipment up to € 10,000, Software and Consumables

Equipment: Fitter, Jörg (AG Biophysik, RWTH Aachen)

Extension from two to four simultaneous detection channels for the existing confocal fluorescence microscope (for smFRET studies with simultaneous anisotropy measurements and correlation analysis)		
Single Photon Counting Modules	2 x SPADs (for example from Laser Components)	10.500 €
Optical + mechan. components	Dichroic mirror, filters, mirrors	3.500 €
Total		**14.000 €**

Consumables: Fitter, Jörg (AG Biophysik, RWTH Aachen)

Fluorescent probes	Functionalized fluorescent dyes	2.500 €
Optical and optomechanical components	Filters, dichroic mirrors, polarizing beamsplitter, mounts, etc.	1.500 €
Enzymes and kits for molecular biology	Zero Blunt Cloning Kit, In-Fusion kit, restriction enzymes, PCR polymerases, etc.	2.000 €
Biochemicals and Media	Buffers, ultra-pure ficoll/PEG, acrylamide, SDS, TCEP, Tween-20, EDTA, Ingredients for dye photo-protection substances (TROLOX,etc), Ingredients for cover-glass treatment (Sigma_Cote, functionalized PEG, etc.)	2.500 €
Chromatography media	Affinity resins (Ni-NTA, Co-NTA agarose), size exclusion resins (Sephadex G25, Zeba columns), ion exchange resins (Mono-Q)	2.500 €
Total per year		**11.000 €**

Consumables: Stadler, Andreas (JCNS, FZJ)

Chemicals for production of deuterated polymers and neutron scattering experiments	Deuterium oxide (1L from Eurisotop, 99.9% D), chemicals	1.000 €
Lab consumables	Filter units, dialysis vials, consumables	500 €
Proteins for neutron scattering experiments	Bovine myelin basic protein (250 mg from Sigma Aldrich, puritiy > 90%)	9.500 €
Total per year		**11.000 €**

5.1.2.2 Travel Expenses

Fitter, Jörg (AG Biophysik, RWTH Aachen)

Scientific events	One international conference participation per year for the principle investigator and/or the Postdoctoral Researcher	2.000 €
Scientific meetings	All involved collaborators will meet once a year for discussions	1.000 €
Total per year		**3.000 €**

Stadler, Andreas (JCNS, FZJ)

Scientific events		One international conference participation per year for the principle investigator and/or the PhD student	2.000 €
Scientific meetings		All involved collaborators will meet once a year for discussions	1.000 €
Neutron scattering experiments		One experiment per year at the Spallation Neutron Source, Oak Ridge, USA. Travel and guest house costs for the PI and the PhD student.	3.000 €
Total per year			**6.000 €**

5.1.2.3 **Visiting Researchers** (excluding Mercator Fellows)

Not applicable

5.1.2.4 Expenses for Laboratory Animals

Not applicable

5.1.2.5 Other Costs

Not applicable

5.1.2.6 Project-related Publication Expenses

Fitter, Jörg (AG Biophysik, RWTH Aachen)

Publication costs	Page charges and charges for color figures in peer reviewed journals	750 €
Total per year		**750 €**

Stadler, Andreas (JCNS, FZJ)

Publication costs	Page charges and charges for color figures in peer reviewed journals	750 €
Total per year		**750 €**

Total direct project cost (AG Fitter: 58.250 € + AG Stadler: 53.250 €)	**111.500 €**

5.1.3 Instrumentation

5.1.3.1 Equipment exceeding € 10,000

Not applicable

5.1.3.2 Major Instrumentation exceeding € 50,000

Not applicable

5.2 Module Temporary Position for Principal Investigator

Not applicable

5.3 Module Replacement Funding

Not applicable

5.4 Module Temporary Clinician Substitute

Not applicable

5.5 Module Mercator Fellows

Not applicable

5.6 Module Workshop Funding

Not applicable

5.7 Module Public Relations Funding

Not applicable

5.8 Module Standard Allowance for Gender Equality Measures

Not applicable

French side – requested funds (from ANR)

French applicants need to respect the "charte nationale de déontologie des métiers de la recherche"[3] and the "charte de déontologie et d'intégrité scientifique de l'ANR"[4].

5.10 Staff expenses

Costs linked to the researchers, engineers, technicians and other scientific staff affected to the project. Justification in relation to the scientific objectives.

Kneller, Gerald (Univ. Orleans)

1 Postdoctoral Researcher for 3 years (full-time): N.N. start at 1.1.2025 (total costs: 178.512 €)

The postdoctoral researcher should hold a PhD in physics or physical chemistry and have deep interest in linking theoretical concepts with experimental data. Ideally he or she should have some experience in programming, data analysis and molecular simulation.

Peters, Judith (Univ. Grenoble)

1 Doctoral Researcher for 3 years (full-time). N.N. start at 1.1.2025 (total costs: 125.249 €)

The research program requires a talented physicist who will conduct the neutron scattering experiments and data analysis.

5.11 Instruments and material costs

Acquisition, depreciation or rental costs of instruments or material and the scientific consumables specifically used for the achievement of the project. Justification in relation to the scientific objectives.

Peters, Judith (Univ. Grenoble Alpes)

Media to produce deuterated proteins	Deuterated glycerol	10.250 €
Material for neutron scattering experiments	Sample holders, indium, screws in Al	8.000 €
Biochemicals and Media	Buffers, D_2O	8.000 €
Total		**26.250 €**

5.12 Building and ground costs

Rental costs of new premises and lands or the fitting of premises or pre-existing lands for the use of the project. Justification in relation to the scientific objectives.

5.13 Outsourcing / subcontracting

Acquisition costs of (1) Licences, patent, brand, software, database, copyrights etc.; (2) Subcontracting costs; for the achievement of the project. Justification in relation to the scientific objectives.

Peters, Judith (Univ. Grenoble Alpes)

Publication costs	Fees for open-access publications	4.200 €
Total		**4.200 €**

Kneller, Gerald (Univ. Orleans)

Publication costs	Fees for open-access publications	4.200 €
Total		**4.200 €**

5.14 Overhead costs

Missions expenses and travel costs of the permanent and temporary staff affected to the project; conferences organisation costs. Other operating expenses. Justification in relation to the scientific objectives.

[3] https://anr.fr/fileadmin/documents/2019/2015_Charte_fran%C3%A7aise_IS.pdf

[4] https://anr.fr/fileadmin/documents/2019/ANR-Charte-deontologie-et-integrite-scientifique-2019-v2.pdf

Peters, Judith (Univ. Grenoble Alpes)

Scientific events	International conference participation	4.000 €
Scientific meetings	All involved collaborators will meet for discussions	3.350 €
Overheads		23.640 €
Total		**30.990 €**

Kneller, Gerald (Univ. Orleans)

Scientific events	International conference participation	4.000 €
Scientific meetings	All involved collaborators will meet for discussions	6.800 €
Operating expenses	Software	5.000 €
Overheads		28.784 €
Total		**44.584 €**

Overview of requested amounts

ANR items	FRA	**DFG items**	GER
Staff expenses	303.762 €	**Basic module - Staff** **Module Temporary Positions for PIs** **Module Replacement** **Module Mercator-Fellow**	359.100 €
Instruments and material costs	26.250 €	**Basic module** **- Scientific equipment** **- Consumables**	84.500 €
Overhead costs	75.574 €	**Basic module** **- Travel costs** **- Guests**	27.000 €
-	-	**Module Project-specific Workshops**	
Building and ground costs		-	-
Outsourcing / subcontracting	8.400 €	-	-
-	-	**Other items/modules**	
Administrative management costs[5]		**(the usual 'Programmpauschale' will be added automatically if the project is granted)**	-
Total requested from ANR	**413.986 €**	**Total requested from DFG**	**470.600 €**

[5] Please see https://anr.fr/en/funding-regulations/ for the percentage which applies to you.