Stellungnahmen zum Antrag

Projekt: „Die Rolle der Konformationsänderungen und der Dynamik bei molekularer Erkennung und der Funktion von Proteinen“ (FI 841/11-1 | STA 1325/3-1)

Antragstellende Personen: Herr Professor Dr. Jörg Fitter, Aachen und Herr Privatdozent Dr. Andreas Stadler, Jülich

Gutachten 1:
„In their joint research proposal, the applicants Jörg Fitter, Gerald Kneller, Andreas Stadler and Judith Peters plan to investigate native-state conformational dynamics and its role in binding of the maltose binding protein (MaBP), a bi-lobate, fully folded protein domain, and the myelin basic protein (MBP), an intrinsically disordered protein (IDP), using a combination of experimental and theoretical approaches. They propose to apply neutron scattering (NS) techniques and small angle x-ray scattering (SAXS) to obtain information on rapid dynamics and low-resolution structures under various conditions, complemented by isothermal titration calorimetry covering the thermodynamics of binding. Light scattering experiments are planned to be accompanied by single-molecule fluorescence resonance energy transfer (smFRET) and fluorescence correlation spectroscopy (FCS) measurements. Molecular dynamics simulation (MDS) studies will be conducted to provide a deepened understanding of the dynamics and aid the interpretation of experimental results at a molecular/atomic level from the theoretical side. The applicants propose to investigate the two proteins both in dilute solutions as well as under conditions of macromolecular crowding. This is a strong research proposal of high quality. The topic is timely. Protein motion is an integral part of function. The number of high-resolution protein structures deposited in the protein data bank continues to increase at high speed but approaches that investigate their conformational dynamics that are related to function are lagging behind. Such studies are of high interest and desired because they add the fourth dimension, time, to structural biology, quoting from Dorothee Kern’s review in Nature from 2007. The two protein systems to be studied are well chosen. The bi-lobate MaBP is related to ligand binding domains of ionotropic glutamate receptors, which are major players of signal transduction in the central nervous system and neurological disorders. Expected results relating lobe dynamics to binding affinities in a venus fly trap mechanism, proposed to be studied elegantly on homologues of varying binding affinities that can be related to changes in dynamics may translate to insights into glutamate receptor domains. MBP, on the other hand, is a representative of IDPs that lack a regular structure but constitute a high portion of the human proteome, yet their functional mechanisms are elusive but certainly related to dynamics. The objectives and work programme, targeting to different proteins studied by various experimental and theoretical approaches in four laboratories are well-thought-out and appear feasible. A strength of the proposal is the integrated approach that combines experimental and theoretical methods from four different laboratories complementing each other in the exploration of time scales and observation parameters. The picosecond time scale probe by NS is complemented by FCS and smFRET probing the nanosecond to millisecond time scale of motion. Results can be expected to provide new and fundamental insights into venus fly trap domain motions and dynamics of IDPs. MDS computer simulation and modelling of NS experimental data approach the problem from the theoretical side. Experimental results can benchmark methods applied in theory. ITC and SAXS are equilibrium spectroscopic techniques that probe the thermodynamics of conformational change and binding. Since, to date, little is

known about the relation between dynamics and function, the combination of techniques probing both dynamics and binding is a particular strength of the proposal. An added value are the proposed studies applying macromolecular crowding agents. Macromolecular crowding confines the space for diffusion of proteins inside the cell and influences their activities, and in particular their dynamics, significantly. These are interesting experiments of valuable expected outcome because there is little literature on these effects on functional protein dynamics. The use of crowding agents instead of in vivo studies bypasses complications from background signals from complex cytoplasmic content that would prevent application of the proposed combination of spectroscopy. The applicants are experts in their respective fields, which is documented by very good track record of publications, respectively, and already existing collaborations between them. The work and research environments are well suited to conduct the proposed project. Jörg Fitter is professor at the Biophysics department at RWTH Aachen providing single-molecule fluorescence, ITC and FCS setups dedicated to the project. Andreas Stadler is senior scientific investigator at the Jülich Centre for Neutron Science with dedicated setups and expertise in neutron and x-ray scattering in soft matter. Gerald Kneller is professor of physics at CNRS/University of Orleans and expert in theoretical biophysics, covering the theoretical methodology of the project. Judith Peters is senior scientific investigator at University of Grenoble with background and expertise in theoretical and experimental biophysics, covering the fields of macromolecular crowding, neutron scattering and calorimetry. The project team is certainly qualified to conduct the proposed research. The requested funds are well-balanced and justified by the proposed work plan. I recommend approval of the project proposal with highest funding priority.“

Gutachten 2:
1. How would you assess the quality of the project, especially with regard to originality and the anticipated contribution to knowledge?
The work will certainly provide some new insights into the connection between structure and dynamics and thermodynamics in the chosen proteins/ligand systems. First IDP’s are a class of protein that is still not fully understood of for sure, the role of dynamics in processes such as molecular recognition and binding are far from being understood. Second, a challenge comes in setting up the molecular dynamics simulations from IDP’s where initial crystal structures are not available, and are purely predicted.
2. To what extent do the objectives and work programme convincingly reflect clear working hypotheses and an appropriately distinct topic? Please comment on the strengths and weaknesses of the planned investigations, the suitability of the methods and the appropriateness of the schedule. How do you rate the explanations on the handling of research data?
The work proposed has 3 main objectives. First is the characterisation of the dynamics in maltose binding protein, MaBP. Motions explored will range from large inter-domain movements to fast local fluctuations, and how the smaller motions relate or even drive the larger collective domain motions. MaBP is chosen as it is not too big, has important functions, represents the tightest protein-carbohydrate interactions and as a 2-domain protein, the hinge forms a key part of the dynamical behaviour and its ligand binding properties. Local fluctuations contribute to conformational entropy, so the dynamical data will be correlated to the thermodynamics parameters. Finally, they will take this part of the work one step further by exploring the role of crowding on structure, dynamics and ultimately ligand binding ability of the protein.

Second is the exploring how IDP conformational dynamics adapt to environmental conditions, by looking at myelin basic protein (MBP), a key protein in the sheath around axons in the human brain. MBP has interesting folding characteristics depending on its environment, specifically the effect of crowding which is its natural environment in the cytoplasmic membrane leaflet. In addition, they propose to looks ta the ligand and non-bound states of this IDP.
Thirdly, relating specifically QENS data to the conformation entropy of proteins. The authors propose a method which they would like to validate with further systems – already tested with one. Further, they aim at characterising in more detail which segments contribute to the measured QENS spectra and thus to the conformational entropy. Finally, evaluating the effective mass probed by QENS, by really checking the scattering cross-section of the protons in the system.
The proposed work is very well presented and structured. Main strengths are the complete and well defined set of objectives and that the work is a mixture of fundamental insights into proteins, but it is a nice structure-dynamics-function pipeline. The work plans to use a wide range of complementary techniques that are ideal to characterise the systems proposed both in terms of structure and dynamics. SANS, SAXS and other standard lab techniques will be used for structural studies, while the large range of timescales that are covered by the dynamical motions found in proteins, will be probed by a toolset ranging from QENS for the fast local motions in the picoseconds to the longer timescales probed with NSE and smFRET. Further insights into the thermodynamics will be probed via ITC and FCSS. All the experimental data will be further complemented with theoretical and modelling calculations. The research project has a duration of 3 years which is reasonable for the work schedule presented, which requests 4 PhD students and 2 postdocs. Finally, the project aims to publish all results in peer-reviewed journals.
3. How would you evaluate the soundness of the preliminary work, the quality of publications (please refer to the Guidelines for Publication Lists) and the qualifications of the applicant – both in general and in terms of the proposed project? Is the applicant qualified to independently lead the proposed project?
The preliminary work presented follows on from several works from the authors. The authors have published those works. In general, the publication record of the authors is very good, with plenty of publications in areas directly relevant to the proposed work, a few in high impact journals. The applicants bring a good mix of expertise and experience to guide the project, in terms of science, experimental techniques and simulation and modelling. The authors have clearly identified the different work packages and who will lead the work. The main PI is well suited to lead the work proposed based on his background and expertise.
4. How would you assess the work and research environment at the institution where the project is to be carried out?
The students will form part of a team of experts in the field, who are well-established professors with plenty of experience in both supervision and guidance of PhD students. They will have access to a variety of environments (neutron facility versus academia, experimental group versus computational group) but also get exposed to a plethora of characterisation tools. The authors range from medium career to highly established researchers, with very good international standing. Their networks amongst other things will be essential for providing for opportunities to the PhD/postdocs as they think of the next step in their career. 5. Please provide a clear recommendation as to whether the proposal should be approved. If so, comment on whether the requested funds are justified and reasonable in relation to the proposed project, making

recommendations for adjustments to the budget as necessary. I recommend this proposal and rate it excellent and agree that the funds requested are reasonable.
6. Other aspects
The proposal is a Franco-German project bringing together researchers that have some scientific history behind them.

Stellungnahme des Fachkollegiums:
The applicants aim to elucidate protein dynamics relevant for protein functioning, focusing on both whole molecule diffusive motions and fast internal structural fluctuations that influence longer-term conformational changes. The project, part of the ANR-DFG cooperation program, integrates complementary methodologies, including QENS, NSE for fast dynamics, and smFRET for slower changes, as well as SANS, SAXS, FCS, and ITC for structural and thermodynamic characterization and molecular dynamics simulations.
Reviewer #1 recommends approval of the project with the highest funding priority, praising its high quality and timeliness. The integrated approach, combining experimental and theoretical methods, is seen as a particular strength. The proposed studies in macromolecular crowding environments are noted for their potential to provide valuable insights into protein dynamics. The applicants are seen as well-qualified, with strong publication records and suitable research environments. Reviewer #2 also recommends the proposal for funding, rating it excellent and considering the requested funds reasonable. The proposal is seen as well-structured, with clearly defined objectives and a comprehensive set of complementary techniques. The preliminary work is seen as sound, and the applicants as a strong mix of expertise and experience. The research environment is seen as well-suited, offering a variety of characterization tools and strong supervisory support.
The Review Board 2.11, Section 1, concurs with many aspects of the positive evaluations provided by the reviewers. The project effectively combines a great array of complementary techniques, including neutron scattering, SAXS, smFRET, FCS, and ITC, which together offer a comprehensive approach to studying protein dynamics on various time scales. The chosen model systems, maltose-binding protein (MaBP) and myelin basic protein (MBP), are well selected to address the fundamental questions of protein dynamics and function. However, there are some concerns. The study of macromolecular crowding using deuterated PEG raises questions about how well it mimics actual cellular conditions. Additionally, the planned molecular dynamics simulations of intrinsically disordered proteins (IDPs) are somewhat vague and lack necessary detail. Lastly, the personnel requested for the project is not fully justified, and a more detailed rationale for the staffing requirements would strengthen the proposal. Thus, in comparison to all proposals at the meeting, the proposal could not reach high enough funding priority and the recommendation is therefore a rejection.