The relation between dynamics and activity of phospholipase A/acyltransferase homologs

Chatterjee, S.D.

1

Chapter

Introduction

The PLAAT family

Phospholipase A/acyltransferases (PLAATs) are a five-membered family of enzymes. They were first identified as H-Ras-like class II tumor suppressors (HRASLS).[1] Numerous research studies in different scientific domains led to them being named in a variety of ways. These names are summarized in Table1.1. The five members are a part of a larger, diverse superfamily NlpC/P60 thiol proteases or papain-like proteases because of their sequence similarities with lecithin: retinol acyltransferases (LRAT), a member of the NlpC/P60 family.[2] PLAATs share a highly conserved sequence NCEHFV, which contains cysteine that acts as the nucleophile and is part of the catalytic triad.[2–5] This triad consists next to the cysteine of two histidines, one acting as the base that deprotonates the sulfhydryl group of the nucleophile and the other stabilizes the imidazole ring of the basic histidine in PLAAT2-5. In PLAAT1, the latter histidine is replaced by asparagine. This cysteine-histidine-histidine catalytic triad is a hallmark signature of NlpC/P60 superfamily of proteins.[6] Four of the PLAAT enzymes are membrane anchored proteins having a C-terminal trans-membrane domain, while PLAAT5, the largest of all PLAATs, lacks this anchor.[7]

Table 1.1. Alternative names for PLAAT proteins found in literature

PLAAT1	**PLAAT2**	**PLAAT3**	**PLAAT4**	**PLAAT5**
A-C1	PLA/AT-2	HREV107	TIG3	HRASLS-5
HRASLS-1	$PLA_{1/2}$-2	HREV107-1	RIG1	RLP-1
PLA/AT-1	HRASLS-2	H-REV107	PLA/AT-4	HRSL5
HRSL1		PLA/AT-3	HRASLS-4	HRLP5
		PLA2G16	RARRES3	iNAT
		MCG118754	$PLA_{1/2}$-3	PLA/AT-5
		RLP-3		
		adPLA		
		HRASLS-3		

Only the three-dimensional structures of three PLAATs (PLAAT2, PLAAT3 and PLAAT4) are known. The PLAATs share similar secondary structure motifs and have an active site similar to those found in NlpC/P60 superfamily.[2,8,9] The crystal structures of PLAAT2 (PDB

entry 4DPZ) and PLAAT3 (4DOT) were first determined by Golczak *et al.*[2] and the NMR structures of PLAAT3 (2KYT) and PLAAT4 (2MY9) were later solved by Xia *et al.* (Figure 1.1).[8,9] The secondary structure comprises three α-helices (crystal structures and NMR structure of PLAAT3) or four α-helices (NMR structure of PLAAT4) and antiparallel β-sheet containing four strands (crystal structures) or six strands (NMR structures), similar to classic segregated α+β-folds of papain-like proteases.[2,6]

Functions of PLAATs

The PLAATs catalyze the first step of reactions leading to the formation of N-acylphosphatidylethanolamines (NAPE), which undergoes further steps to form N-acylethanolamines (NAEs), an important class of bioactive lipids that play roles in a variety of processes, such as anti-inflammation (N-palmitoyethanolamine),[10–12] catabolism of fat (N-oleoylethanolamine),[13] anti-apoptotic activity (N-stearoylethanolamine),[13] and ligands for endocannabinoid receptors (anandamide), see Figure 1.2.[14–16]

PLAATs, as the name suggests, demonstrate phospholipase $A_{1/2}$ ($PLA_{1/2}$) activities, in which both phosphatidylcholine (PC) and phosphatidylethanolamine (PE) act as substrates.[3,7,17–20] All PLAATs except PLAAT3 show specificity for the PLA_1 position, whereas for PLAAT3 contradicting evidence from various studies suggest that the protein may prefer either the A1 or the A2 position, depending on the type of substrate and assay conditions.[21–23] The second part of the name comes from their ability to transfer the acyl-chain of a phosphoglyceride (for example PC) to the amino group of a phosphatidylethanolamine (PE), leading to the formation of NAPEs, as mentioned above. Furthermore, apart from being an N-acyltransferase, PLAATs can also act as O-acyltransferases, transferring an acyl-chain to the sn-1 or sn-2 position of a lysophospholipid, for example lysophosphatidylcholine.[2,3,17,19,24] See Figure 1.3 for all the reactions catalyzed by PLAATs.

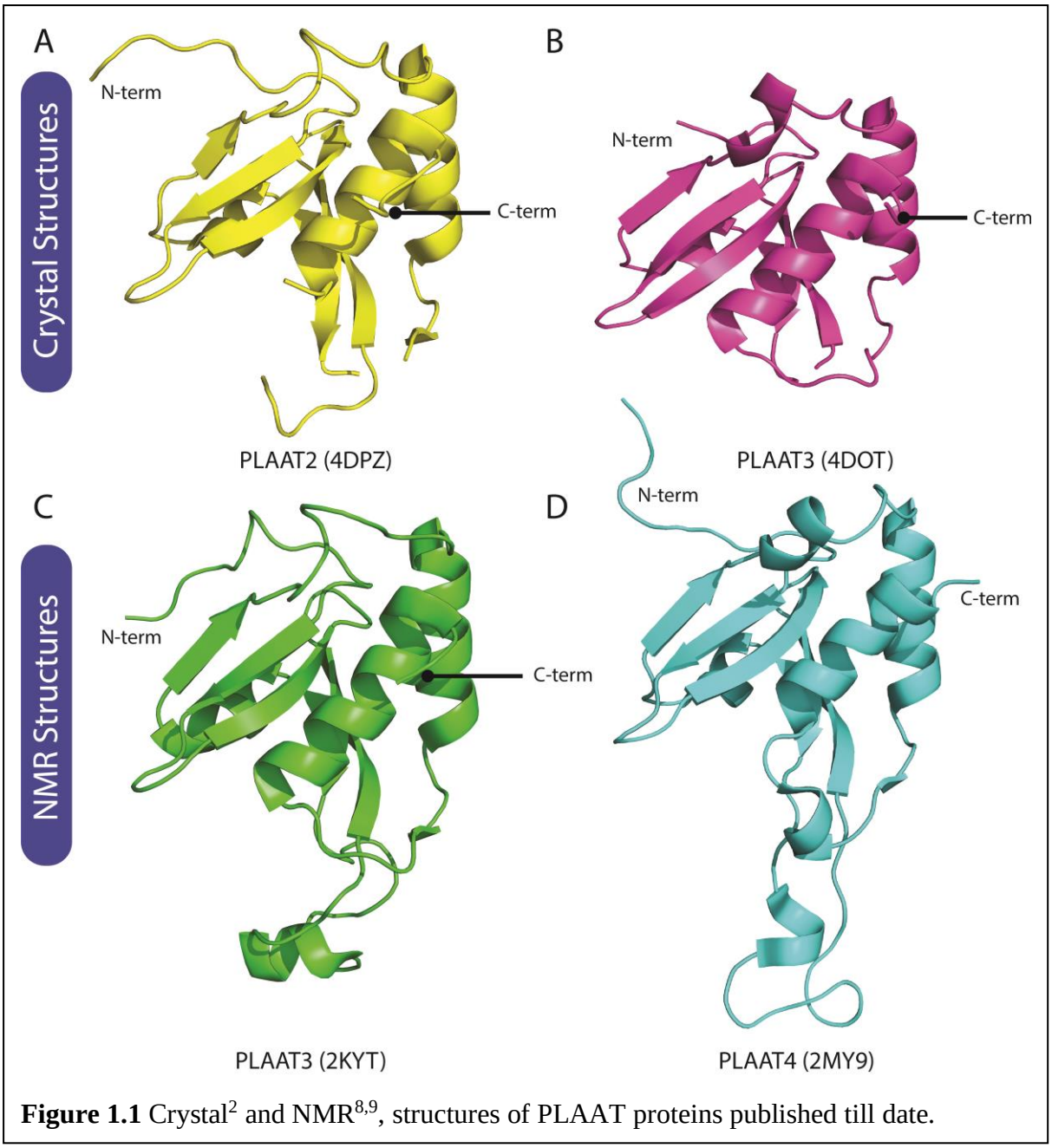

Figure 1.1 Crystal[2] and NMR[8,9], structures of PLAAT proteins published till date.

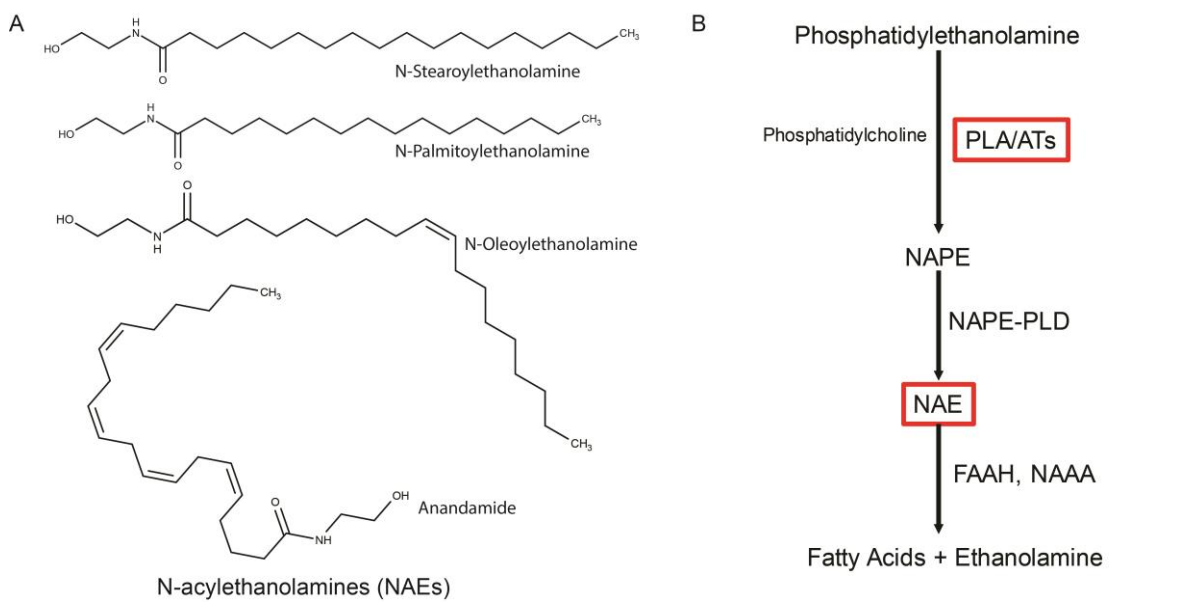

Figure 1.2 A) Structures of several important NAEs. B) Schematic diagram of the reaction catalyzed by PLAATs using phosphatidylcholine (PC) and phosphatidylethanolamine (PE) leading to the formation of N-acylphosphatidylethanolamine (NAPE). NAPE-hydrolyzing phospholipase D (NAPE-PLD) catalyses the conversion of NAPE to important bioactive lipids, N-acylethanolamines (NAEs), which are then further metabolized into fatty acids and ethanolamine by fatty acid amide hydrolase (FAAH) or NAE hydrolyzing acid amidase (NAAA).

In human beings, mice and rats, the gene for PLAAT1 is expressed mostly in skeletal muscle, heart and testes, where its physiological role has not been investigated so far.[17] PLAAT1 shows an N-acyltransferase activity that is higher than the $PLA_{1/2}$ activity.[3,17,24,25] PLAAT2 is only found in human beings and especially in the trachea, stomach, colon and kidneys.[7] It has been known to suppress tumors (class II) in breast cells and cervical cancer cells.[7] This protein also exhibits strong N-acyltransferase activity and less $PLA_{1/2}$ activity and, interestingly, prefers the sn-1 position of PC for the former activity.[19,24] PLAAT3 was also discovered as a tumor suppressor [20,26–32], however contradicting reports showed that PLAAT3 rather increases tumor progression.[29,33] In contrast to PLAAT1 and PLAAT2, PLAAT3 shows a preference for PLA activity over N-acyltransferase activity.[17,24] However, PLAAT1 and PLAAT3 both share common function in organelle degradation in lens.[34] PLAAT3 also plays an important role in viral entry pathways by acting as host factor for enterovirus[35–37]. PLAAT3 is found mostly in white adipose tissue and less in brown adipose tissue[21,38]. In white adipose tissue, it modulates lipolysis and therefore is a critical factor in obesity, as was elegantly demonstrated in mice models by Jaworski *et al.*[38] PLAAT3 inhibitors[39,40], therefore would be potential therapeutic

anti-obesity and anti-viral targets. Like PLAAT3, PLAAT4 was also discovered and shown to work as tumor suppressor.[41–46] PLAAT4, like PLAAT2 is a human-specific ortholog. It is found in skin cells, where it interacts with and activates transglutaminase I (TG1), which in turn produces cornified envelope, necessary for keratinocyte proliferation and survival, and skin to function as a physical and water barrier.[47–50] As PLAAT4 is a class II tumor suppressor, it was shown to be down-regulated in psoriasis and skin cancer in a study by Duvic *et al.*[51]

PLAAT4 is a homolog of PLAAT3[41,42] and it also shows more $PLA_{1/2}$ activity than N-acyltransferase activity.[17,19,24] However, the two enzymes exhibit a contrasting characteristic regarding the $PLA_{1/2}$ activity. The transmembrane C-terminal domain is crucial for PLAAT3 membrane-attachment as well as $PLA_{1/2}$ activity and truncation of this domain has been shown by Uyama *et al*. to result in loss of phospholipase activity.[52] In contrast, Golczak *et al.*[2] demonstrated with truncated N-terminal domains of PLAATs that, unlike truncated PLAAT3, truncated PLAAT4 is capable of phospholipase activity, suggesting that the transmembrane C-terminal domain is not critical for PLAAT4 $PLA_{1/2}$ activity. Furthermore, these authors demonstrated by studying the rate of breakdown of short-chain phospholipids and studying the protein-acyl intermediates that the truncated PLAAT4 is more active as phospholipase than PLAAT3. Although PLAAT3 and PLAAT4 are homologs, they clearly differ in activity and specificity toward substrates. Physiologically, the phospholipase activity of PLAAT3 plays an important role in adipose tissue as it regulates triglyceride metabolism.[21] Phospholipase activity of PLAAT4, on the other hand, plays a crucial role in tumor suppression, particularly in metastasis and invasion.[46] Moreover Wei *et al.* demonstrated that the NTD of PLAAT4 was found to be enhancing the cell death effect of the CTD, whereas the NTD of PLAAT3 was found to be inhibitory.[8] Therefore, the differences in activity between the two enzymes can be studied by studying the roles played by the N-terminal and C-terminal domains on a molecular level. The findings could hold the key to the understanding of the molecular mechanisms and physiological significance of these enzymes that are yet to be studied in detail. Studying the roles of N-terminal and C-terminal domains of the two enzymes at a molecular level can help to obtain an understanding of the molecular mechanisms and physiological significance of these enzymes which may advance the design and discovery of selective PLAAT inhibitors.

The aim of the research presented in this thesis, therefore, was to elucidate the reason for the difference in activities (especially phospholipase activity) between PLAAT3 and PLAAT4, present despite their similar sequences and structures. It was hypothesized that differences in the dynamic properties could be the cause of the differences in activity, so NMR spectroscopy

and MD simulations were used to characterize the enzymes. Both methods are briefly introduced below.

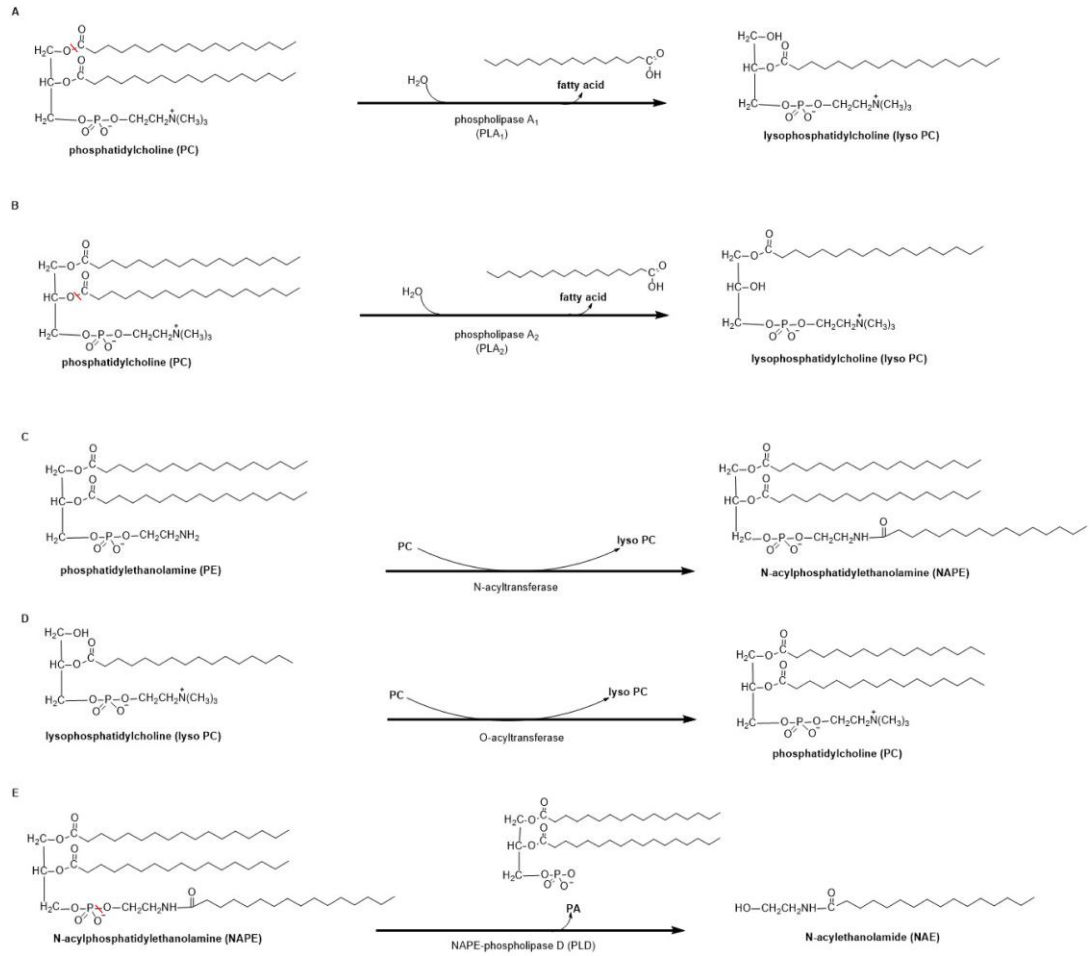

Figure 1.3 Various biochemical reactions catalyzed PLAATs. A, Phospholipase A1 (PLA1) reaction cleaves a fatty acyl chain from the sn-1 position of PC, resulting in the formation of a free fatty acid and lyso PC. B, Phospholipase A2 (PLA2) cleaves at the sn-2 position of PC to form a free fatty acid and lyso PC. C, N-acyltransferase reaction transfers the sn-1 fatty acyl chain from PC to PE to form NAPE. D, O-acyltransferase reaction converts lyso PC to PC, where the acyl donor is the sn-1 acyl chain of PC. E, Hydrolysis of NAPE to N-acylethanolamine (NAE) by NAPE-phospholipase D.

NMR Spectroscopy

Studying protein dynamics with NMR spectroscopy has created a new perspective on what proteins are and how they function. This applies to enzymes in particular. Proteins are no longer seen as static entities but as dynamic ensembles occupying various positions in an free energy landscape.[53–55] Protein dynamics occur at many timescales and different sets of NMR

experiments allow us to probe biologically relevant phenomena occurring at those timescales (Figure 1.4).[56] Protein motions occurring at ps-ns timescale can be studied using longitudinal relaxation (R_1), transverse relaxation (R_2) and heteronuclear nuclear Overhauser effect/enhancement (NOE).[57–61] Several dynamics parameter, such as the order parameter (S^2), global rotational correlation time (τ_c), effective rotational correlation time (τ_e) and the exchange rate (R_{ex}) can be quantified using the model free analysis developed by Lipari and Szabo.[62,63] The order parameter ranges from 0 (highly dynamic) to 1 (rigid), τ_c is an indicator of molecular tumbling time and is highly dependent on the size of the protein. τ_e is in the range of ps-ns ($\tau_e < \tau_c$) and is a measure of local motions, such as loop flexibility. R_{ex} indicates exchange contribution to the linewidth (apparent transverse relaxation rate), due either to motions in the µs-ms timescale that cause chemical shift changes or to chemical exchange phenomena. Generally, data are acquired at two magnetic fields to improve the statistical fitting of the data to one of five "model-free" models, because relaxation rates are field dependent. These five models fit the measured relaxation rates with an increasing number of parameters to obtain S^2, τ_e and R_{ex} for individual nuclei in the protein, usually of backbone ^{15}N atoms. The term model-free refers to the fact that these parameters are defined without a predefined notion of the type of motion in mind, such as rotation in a cone.[64,65] The ps-ns motions are often associated with flexibility of loops and termini, and in many cases such motions may not be related to biological function but rather are a property of protein matter. However, a growing number of studies have shown that protein dynamics at this timescale can also affect function.[66–74]

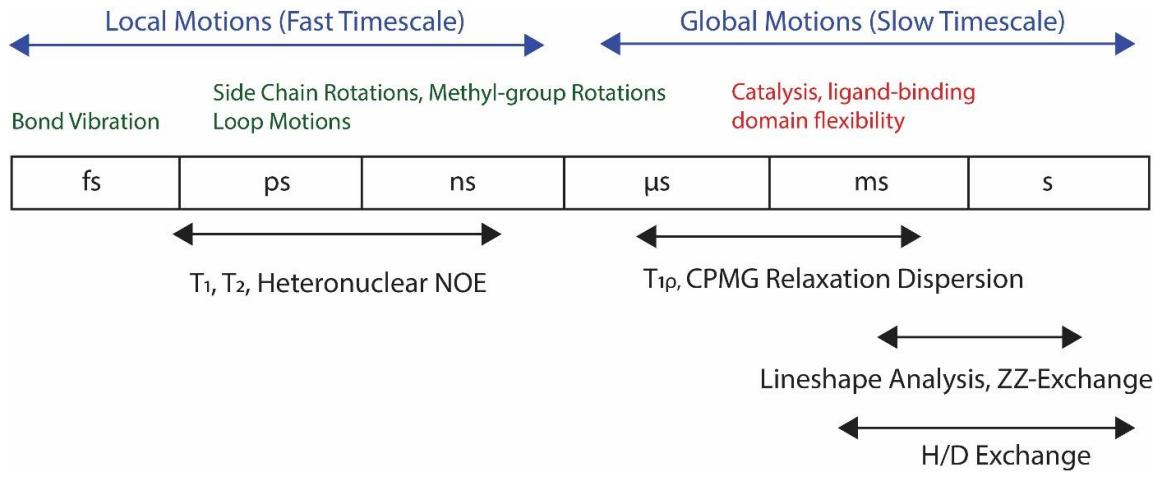

Figure 1.4 A graphical representation of different protein motions occuring at different timescales and NMR experiments to study such motions. In protein dynamics, motions occuring at timescales below µs are termed as "fast timescale motions" whereas those occuring at timescales of µs or above are termed as "slow timescale motions".

Biologically relevant and important phenomena occur at µs-ms timescale such as catalysis,[75–78] protein folding,[79–83] protein-protein or protein-ligand interactions.[84–89] NMR experiments such as $R_{1\rho}$ and CPMG relaxation dispersion (RD) and chemical exchange saturation transfer (CEST) are crucial in providing information about dynamics occurring at this timescale.[90–95] The general principle of all these experiments is that different states have different NMR properties (chemical shifts, dipolar couplings or relaxation rates) and that the exchange between states affects the NMR signals, by causing changes in peak intensity or linewidth. Thus, these experiment can extract information about the population, exchange rates and structures of minor conformers that are in exchange with the major ground state conformation.[96–102] Since the minor conformation is sparsely populated, the already low intensity peaks are usually further broadened out due to exchange, which makes them 'invisible'. Therefore, information about the minor conformers is obtained from the effects of exchange on the NMR resonance of the major conformer, or on the signal average in case of fast exchange between the two states. In the CPMG-RD experiment, the chemical exchange is quantified by obtaining the exchange contribution to the apparent transverse relaxation rate (R_2) of the nucleus in the major conformer. This is achieved by using a train of 180° pulses at a frequency of $\nu_c = 1/4\tau_{cp}$ (where ν_c is the CPMG frequency and $2\tau_{cp}$ is time between the 180° pulses in the train) during a period T to reduce the exchange contribution to the linewidth. By performing a series of experiments with varying $\boldsymbol{\nu_c}$, the effective apparent line width ($R_{2,eff}$) can be determined from the peak intensities in each of these experiment (I_{CPMG}) relative to a reference spectrum for which T is set to zero (I_0), equation 1.1.

$$\boldsymbol{R_{2,eff} = \frac{1}{T} ln\left(\frac{I_{CPMG}}{I_0}\right)} \qquad \text{(Equation 1.1)}$$

Nuclei that do not undergo chemical exchange, do not have the exchange contribution and hence, the $R_{2,eff}$ is same at all CPMG frequencies and equals the intrinsic R_2. A plot of $R_{2,eff}$ vs. CPMG frequency shows a dispersion profile (Figure 1.5). A nucleus undergoing exchange will have a profile showing a decreasing $R_{2,eff}$ with increasing CPMG frequency until it reaches a plateau, being the intrinsic R_2. The parameters describing the exchange process, such as the rate of exchange (k_{ex}), population of the minor conformer (p_B) and the absolute chemical shift difference between the major and the minor conformer ($|\Delta\omega|$) can be obtained from the dispersion curves by minimizing the following equation:

$$\boldsymbol{X^2(\zeta) = (R'_{2,eff}(\varphi) - R_{2,eff})/\Delta R_{2,eff}} \qquad \text{(Equation 1.2)}$$

Where $R'_{2,eff}$ is the calculated rate of relaxation obtained by solving the Bloch-McConnel equations,[103,104] $R_{2,eff}$ is the effective transverse relaxation rate, φ is a function of dynamics parameters such as k_{ex}, p_B and Δω. The detailed analysis of the CPMG RD experiments to deduce the structure of the minor conformer as well as the experiments required for calculating the sign of the Δω are outside the scope of this thesis and can be found in many excellent review papers, such as.[105–110]

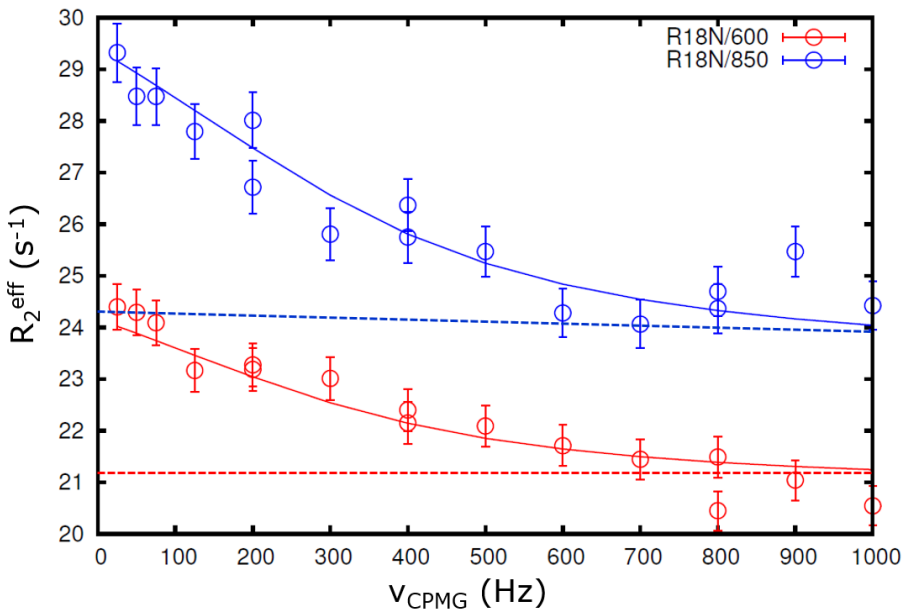

Figure 1.5 A typical relaxation dispersion plot. The dashed lines represent an imaginary amide with no exchange and the dispersion curves for the backbone ^{15}N nucleus of residue R18 in PLAAT4 undergoing chemical exchange, as seen by its decreasing $R_{2,eff}$ with increasing CPMG frequency. The data are obtained at two magnetic fields, red: 14 T (600 MHz), blue: 20 T (850 MHz).

NMR spectroscopy, though a powerful tool to study protein dynamics, is not bereft of limitations.

It is inherently insensitive due to the small energy gaps involved in magnetic resonance transitions, so concentrated samples are required. Furthermore, depending on the rate of the chemical process, NMR will often yield an average observable, making it difficult to determine the properties of the individual components that cause the averaged observable.[111] Exchange dynamics is rich in information but can also hinder observation of nuclei considerably, for example if the resonances broaden beyond detection or due to exchange with solvent hydrogens of which the signals are necessarily suppressed in protein NMR experiments. All these factors can make it impossible to interpret dynamic properties in terms of structural changes.

Molecular Dynamics Simulation

Molecular dynamics (MD) simulation is another powerful tool for studying protein dynamics and can serve to complement the information obtained from NMR spectroscopy. MD simulations allow us to quantify and visualize atomic motions occurring in a protein at fast timescale as well as, due to the growth in modern computational capabilities, slower timescale. An introduction to MD simulations is presented in Chapter 3. For the possibilities of combining NMR spectroscopy on proteins and MD simulations, the reader is referred to two excellent reviews, by Case[112] and Fisette *et al.*[113]

Thesis Outline

At the start of the research in October 2014, NMR assignments of PLAAT3[114] and PLAAT4,[115] the crystal structures of PLAAT2 and PLAAT3[2] and the NMR structure of PLAAT3[9] were available. The structure of PLAAT4 was reported in January 2015.[8] Here, the first protein dynamics data of ^{15}N-labelled PLAAT3 and PLAAT4 obtained with NMR spectroscopy are presented in Chapter 2. To complement our understanding of the dynamics of the two proteins and to get more information on the residues that could not be studied with NMR spectroscopy due to solvent exchange and slow-timescale-exchange-related line broadening, MD simulations were performed on the two proteins, described in chapter 3. The combination of NMR data and MD results enabled us to formulate a hypothesis to explain the differences in phospholipase activity of two proteins. The hypothesis was then tested by performing mutations both *in silico* and *in vitro.* MD simulations as well as activity assays on the variants were performed, leading to the remarkable conclusion that loop exchange from PLAAT4 to PLAAT3 can introduce enzymatic activity in the latter protein, a gain-of-function mutation. These results are described in Chapter 4. In Chapter 5, a study on CPMG RD NMR experiments is described, in which an artifact observed during the slow pulsing regimes is analyzed and the efficacy of different decoupling sequences in its removal was tested. Chapter 6 contains a general discussion on PLAAT3 and PLAAT4 dynamics and activity and the scope for future research on the phospholipase A/acyltransferase family.

References

(1) Ito, H., Akiyama, H., Shigeno, C., and Nakamura, T. (2001) Isolation, characterization, and chromosome mapping of a human A-C1 Ha-Ras suppressor gene (HRASLS). *Cytogenet. Genome Res. 93*, 36–39.

(2) Golczak, M., Kiser, P. D., Sears, A. E., Lodowski, D. T., Blaner, W. S., and Palczewski, K. (2012) Structural basis for the acyltransferase activity of lecithin:retinol acyltransferase-like proteins. *J. Biol. Chem. 287*, 23790–23807.

(3) Jin, X.-H., Uyama, T., Wang, J., Okamoto, Y., Tonai, T., and Ueda, N. (2009) cDNA cloning and characterization of human and mouse Ca2+-independent phosphatidylethanolamine N-acyltransferases. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids 1791*, 32–38.

(4) Ruiz, A., Winston, A., Lim, Y.-H., Gilbert, B. A., Rando, R. R., and Bok, D. (1999) Molecular and biochemical characterization of lecithin retinol acyltransferase. *J. Biol. Chem. 274*, 3834–3841.

(5) Kiser, P. D., Golczak, M., Maeda, A., and Palczewski, K. (2012) Key enzymes of the retinoid (visual) cycle in vertebrate retina. *Biochim. Biophys. Acta 1821*, 137–151.

(6) Anantharaman, V., and Aravind, L. (2003) Evolutionary history, structural features and biochemical diversity of the NlpC/P60 superfamily of enzymes. *Genome Biol. 4*, R11–R11.

(7) Shyu, R.-Y., Hsieh, Y.-C., Tsai, F.-M., Wu, C.-C., and Jiang, S.-Y. (2008) Cloning and functional characterization of the HRASLS2 gene. *Amino Acids 35*, 129–137.

(8) Wei, H., Wang, L., Ren, X., Yu, W., Lin, J., Jin, C., and Xia, B. (2015) Structural and functional characterization of tumor suppressors TIG3 and H-REV107. *FEBS Lett. 589*, 1179–1186.

(9) Ren, X., Lin, J., Jin, C., and Xia, B. (2010) Solution structure of the N-terminal catalytic domain of human H-REV107--a novel circular permutated NlpC/P60 domain. *FEBS Lett. 584*, 4222–4226.

(10) Calignano, A., Rana, G. La, Giuffrida, A., and Piomelli, D. (1998) Control of pain initiation by endogenous cannabinoids. *Nature 394*, 277–281.

(11) Lambert, D. M., Vandevoorde, S., and Fowler, K.-O. J. and C. J. (2002) The palmitoylethanolamide family: a new class of anti-inflammatory agents? *Curr. Med. Chem. 9*, 663–674.

(12) Ara, I., Rahman, S., Tsuboi, K., Uyama, T., and Ueda, N. (2014) New players in the fatty acyl ethanolamide metabolism OH OH OH. *Pharmacol. Res. 86*, 1–10.

(13) Rodríguez de Fonseca, F., Navarro, M., Gómez, R., Escuredo, L., Nava, F., Fu, J., Murillo-Rodríguez, E., Giuffrida, A., LoVerme, J., Gaetani, S., Kathuria, S., Gall, C., and Piomelli, D. (2001) An anorexic lipid mediator regulated by feeding. *Nature 414*, 209–212.

(14) Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., and Mechoulam, R. (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science (80-.). 258*, 1946 LP – 1949.

(15) Di Marzo, V. (1998) 'Endocannabinoids' and other fatty acid derivatives with cannabimimetic properties: biochemistry and possible physiopathological relevance. *Biochim. Biophys. Acta - Lipids Lipid Metab. 1392*, 153–175.

(16) Di Marzo, V., De Petrocellis, L., Fezza, F., Ligresti, A., and Bisogno, T. (2002) Anandamide receptors. *Prostaglandins, Leukot. Essent. Fat. Acids 66*, 377–391.

(17) Shinohara, N., Uyama, T., Jin, X., Tsuboi, K., Tonai, T., Houchi, H., and Ueda, N. (2011) Enzymological analysis of the tumor suppressor A-C1 reveals a novel group of phospholipid-metabolizing enzymes. *J. Lipid Res. 52*, 1927–1935.

(18) Jin, X.-H., Okamoto, Y., Morishita, J., Tsuboi, K., Tonai, T., and Ueda, N. (2007) Discovery and characterization of a Ca2+-independent phosphatidylethanolamine N-acyltransferase generating the anandamide precursor and its congeners. *J. Biol. Chem. 282*, 3614–3623.

(19) Uyama, T., Jin, X.-H., Tsuboi, K., Tonai, T., and Ueda, N. (2009) Characterization of the human tumor suppressors TIG3 and HRASLS2 as phospholipid-metabolizing enzymes. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids 1791*, 1114–1124.

(20) Hajnal, A., Klemenz, R., and Schäfer, R. (1994) Subtraction cloning of H-rev107, a gene

specifically expressed in H-ras resistant fibroblasts. *Oncogene 9*, 479–490.

(21) Duncan, R. E., Sarkadi-nagy, E., Jaworski, K., Ahmadian, M., and Sul, H. S. (2008) Identification and functional characterization of adipose-specific phospholipase A2 (AdPLA). *J. Biol. Chem. 283*, 25428–25436.

(22) Uyama, T., Morishita, J., Jin, X.-H., Okamoto, Y., Tsuboi, K., and Ueda, N. (2009) The tumor suppressor gene H-Rev107 functions as a novel Ca2+-independent cytosolic phospholipase A1/2 of the thiol hydrolase type. *J. Lipid Res. 50*, 685–693.

(23) Pang, X.-Y., Cao, J., Addington, L., Lovell, S., Battaile, K. P., Zhang, N., Rao, J. L. U. M., Dennis, E. A., and Moise, A. R. (2012) Structure/function relationships of adipose phospholipase A2 containing a Cys-His-His catalytic triad. *J. Biol. Chem. 287*, 35260–35274.

(24) Uyama, T., Ikematsu, N., Shinohara, N., Jin, X., Tsuboi, K., and Tonai, T. (2012) Generation of N -acylphosphatidylethanolamine by members of the phospholipase A / acyltransferase (PLA / AT) family. *J. Biol. Chem. 287*, 31905–31919.

(25) Uyama, T., Inoue, M., Okamoto, Y., Shinohara, N., Tai, T., Tsuboi, K., Inoue, T., Tokumura, A., and Ueda, N. (2013) Involvement of phospholipase A / acyltransferase-1 in N -acylphosphatidylethanolamine generation. *BBA - Mol. Cell Biol. Lipids 1831*, 1690–1701.

(26) Sers, C., Emmenegger, U., Husmann, K., Bucher, K., Andres, A. C., and Schäfer, R. (1997) Growth-inhibitory activity and downregulation of the class II tumor-suppressor gene H-rev107 in tumor cell lines and experimental tumors. *J. Cell Biol. 136*, 935–944.

(27) Roder, K., Latasa, M.-J., and Sul, H. S. (2002) Silencing of the mouse H-rev107 gene encoding a class II tumor suppressor by CpG methylation. *J. Biol. Chem. 277*, 30543–30550.

(28) Yanatatsaneejit, P., Chalermchai, T., Kerekhanjanarong, V., Shotelersuk, K., Supiyaphun, P., Mutirangura, A., and Sriuranpong, V. (2008) Promoter hypermethylation of CCNA1, RARRES1, and HRASLS3 in nasopharyngeal carcinoma. *Oral Oncol. 44*, 400–406.

(29) Nazarenko, I., Kristiansen, G., Fonfara, S., Guenther, R., Gieseler, C., Kemmner, W., Schafer, R., Petersen, I., and Sers, C. (2006) H-REV107-1 stimulates growth in non-small cell lung carcinomas via the activation of mitogenic signaling. *Am. J. Pathol. 169*, 1427–1439.

(30) Nazarenko, I., Schäfer, R., and Sers, C. (2007) Mechanisms of the HRSL3 tumor suppressor function in ovarian carcinoma cells. *J. Cell Sci. 120*, 1393 LP – 1404.

(31) Shyu, R.-Y., Wu, C.-C., Wang, C.-H., Tsai, T.-C., Wang, L.-K., Chen, M.-L., Jiang, S.-Y., and Tsai, F.-M. (2013) H-rev107 regulates prostaglandin D2 synthase-mediated suppression of cellular invasion in testicular cancer cells. *J. Biomed. Sci. 20*, 30.

(32) Wang, C.-H., Shyu, R.-Y., Wu, C.-C., Tsai, T.-C., Wang, L.-K., Chen, M.-L., Jiang, S.-Y., and Tsai, F.-M. (2014) Phospholipase A/Acyltransferase enzyme activity of H-rev107 inhibits the H-RAS signaling pathway. *J. Biomed. Sci. 21*, 36.

(33) Xiong, S., Tu, H., Kollareddy, M., Pant, V., Li, Q., Zhang, Y., Jackson, J. G., Suh, Y.-A., Elizondo-Fraire, A. C., Yang, P., Chau, G., Tashakori, M., Wasylishen, A. R., Ju, Z., Solomon, H., Rotter, V., Liu, B., El-Naggar, A. K., Donehower, L. A., Martinez, L. A., and Lozano, G. (2014) Pla2g16 phospholipase mediates gain-of-function activities of mutant p53. *Proc. Natl. Acad. Sci. U. S. A. 111*, 11145–11150.

(34) Morishita, H., Eguchi, T., Tsukamoto, S., Sakamaki, Y., Takahashi, S., Saito, C., Koyama-Honda, I., and Mizushima, N. (2021) Organelle degradation in the lens by PLAAT phospholipases. *Nature 592*, 634–638.

(35) Baggen, J., Liu, Y., Lyoo, H., van Vliet, A. L. W., Wahedi, M., de Bruin, J. W., Roberts, R. W., Overduin, P., Meijer, A., Rossmann, M. G., Thibaut, H. J., and van Kuppeveld, F. J. M. (2019) Bypassing pan-enterovirus host factor PLA2G16. *Nat. Commun. 10*, 3171.

(36) Elling, U., Wimmer, R. A., Leibbrandt, A., Burkard, T., Michlits, G., Leopoldi, A., Micheler, T., Abdeen, D., Zhuk, S., Aspalter, I. M., Handl, C., Liebergesell, J., Hubmann, M., Husa, A.-M., Kinzer, M., Schuller, N., Wetzel, E., van de Loo, N., Martinez, J. A. Z., Estoppey, D., Riedl, R., Yang, F., Fu, B., Dechat, T., Ivics, Z., Agu, C. A., Bell, O., Blaas, D., Gerhardt, H., Hoepfner, D., Stark, A., and Penninger, J. M. (2017) A reversible haploid mouse embryonic stem cell biobank resource for functional genomics. *Nature 550*, 114–118.

(37) Staring, J., von Castelmur, E., Blomen, V. A., van den Hengel, L. G., Brockmann, M., Baggen, J., Thibaut, H. J., Nieuwenhuis, J., Janssen, H., van Kuppeveld, F. J. M., Perrakis, A., Carette, J. E., and Brummelkamp, T. R. (2017) PLA2G16 represents a switch between entry and clearance of Picornaviridae. *Nature 541*, 412–416.

(38) Jaworski, K., Ahmadian, M., Duncan, R. E., Sarkadi-nagy, E., Varady, K. A., Hellerstein, M. K., Lee, H.-Y., Samuel, V. T., Shulman, G. I., Kim, K., de Val, S., Kang, C., Sul, H. S., Val, S. De, Kang, C., and Sul, H. S. (2009) AdPLA ablation increases lipolysis and prevents obesity induced by high-fat feeding or leptin deficiency. *Nat. Med. 15*, 159–168.

(39) Zhou, J., Mock, E. D., Al Ayed, K., Di, X., Kantae, V., Burggraaff, L., Stevens, A. F., Martella, A., Mohr, F., Jiang, M., van der Wel, T., Wendel, T. J., Ofman, T. P., Tran, Y., de Koster, N., van Westen, G. J. P., Hankemeier, T., and van der Stelt, M. (2020) Structure–activity relationship studies of α-ketoamides as inhibitors of the phospholipase A and acyltransferase enzyme family. *J. Med. Chem. 63*, 9340–9359.

(40) Jiang, B., Yu, B., Zhang, X., Liu, M., and Yang, D. (2015) A 15N CPMG relaxation dispersion experiment more resistant to resonance offset and pulse imperfection. *J. Magn. Reson. 257*, 1–7.

(41) DiSepio, D., Ghosn, C., Eckert, R. L., Deucher, A., Robinson, N., Duvic, M., Chandraratna, R. A., and Nagpal, S. (1998) Identification and characterization of a retinoid-induced class II tumor suppressor/growth regulatory gene. *Proc. Natl. Acad. Sci. U. S. A. 95*, 14811–14815.

(42) Huang, S.-L., Shyu, R.-Y., Yeh, M.-Y., and Jiang, S.-Y. (2000) Cloning and characterization of a novel retinoid-inducible gene 1(RIG1) deriving from human gastric cancer cells. *Mol. Cell. Endocrinol. 159*, 15–24.

(43) Casanova, B., de la Fuente, M. T., Garcia-Gila, M., Sanz, L., Silva, A., Garcia-Marco, J. A., and Garcia-Pardo, A. (2001) The class II tumor-suppressor gene RARRES3 is expressed in B cell lymphocytic leukemias and down-regulated with disease progression. *Leukemia 15*, 1521–1526.

(44) Tsai, F.-M., Shyu, R.-Y., and Jiang, S.-Y. (2007) RIG1 suppresses Ras activation and induces cellular apoptosis at the Golgi apparatus. *Cell. Signal. 19*, 989–999.

(45) Huang, S.-L., Shyu, R.-Y., Yeh, M.-Y., and Jiang, S.-Y. (2002) The retinoid-inducible gene I: effect on apoptosis and mitogen-activated kinase signal pathways. *Anticancer Res. 22*, 799–804.

(46) Morales, M., Arenas, E. J., Urosevic, J., Guiu, M., Fernández, E., Planet, E., Fenwick, R. B., Fernández-Ruiz, S., Salvatella, X., Reverter, D., Carracedo, A., Massagué, J., and Gomis, R. R. (2014) RARRES3 suppresses breast cancer lung metastasis by regulating adhesion and differentiation. *EMBO Mol. Med. 6*, 865–881.

(47) Scharadin, T. M., and Eckert, R. L. (2014) TIG3: an important regulator of keratinocyte proliferation and survival. *J. Invest. Dermatol. 134*, 1811–1816.

(48) Jans, R., Sturniolo, M. T., and Eckert, R. L. (2008) Localization of the TIG3 transglutaminase interaction domain and demonstration that the amino-terminal region is required for TIG3 function as a keratinocyte differentiation regulator. *J. Invest. Dermatol. 128*, 517–529.

(49) Sturniolo, M. T., Dashti, S. R., Deucher, A., Rorke, E. A., Broome, A.-M., Chandraratna, R. A. S., Keepers, T., and Eckert, R. L. (2003) A novel tumor suppressor protein promotes keratinocyte terminal differentiation via activation of type I transglutaminase. *J. Biol. Chem. 278*, 48066–48073.

(50) Sturniolo, M. T., Chandraratna, R. A. S., and Eckert, R. L. (2005) A novel transglutaminase activator forms a complex with type 1 transglutaminase. *Oncogene 24*, 2963–2972.

(51) Duvic, M., Helekar, B., Schulz, C., Cho, M., DiSepio, D., Hager, C., DiMao, D., Hazarika, P., Jackson, B., Breuer-McHam, J., Young, J., Clayman, G., Lippman, S. M., Chandraratna, R. A. S., Robinson, N. A., Deucher, A., Eckert, R. L., and Nagpal, S. (2000) Expression of a retinoid-inducible tumor suppressor, tazarotene-inducible gene-3, is decreased in psoriasis and skin cancer. *Clin. Cancer Res. 6*, 3249 LP – 3259.

(52) Uyama, T., Kawai, K., Kono, N., Watanabe, M., Tsuboi, K., Inoue, T., Araki, N., Arai, H., and Ueda, N. (2015) Interaction of phospholipase A/acyltransferase-3 with Pex19p: a possible involvement in the down-regulation of peroxisomes. *J. Biol. Chem. 290*, 17520–17534.

(53) Frauenfelder, H., Sligar, S. G., and Wolynes, P. G. (1991) The energy landscapes and motions of proteins. *Science (80-.). 254*, 1598 LP – 1603.

(54) Ferreiro, D. U., Hegler, J. A., Komives, E. A., and Wolynes, P. G. (2007) Localizing frustration in native proteins and protein assemblies. *Proc. Natl. Acad. Sci. U. S. A. 104*, 19819–19824.

(55) Jensen, M. R., Ruigrok, R. W. H., and Blackledge, M. (2013) Describing intrinsically disordered proteins at atomic resolution by NMR. *Curr. Opin. Struct. Biol. 23*, 426—435.

(56) Kempf, J. G., and Loria, J. P. (2002) Protein dynamics from solution NMR. *Cell Biochem. Biophys. 37*, 187–211.

(57) Kay, L. E. (1998) Protein dynamics from NMR. *Nat. Struct. Biol. 5 Suppl*, 513—517.

(58) Nirmala, N. R., and Wagner, G. (1988) Measurement of 13C relaxation times in proteins by two-dimensional heteronuclear 1H-13C correlation spectroscopy. *J. Am. Chem. Soc. 110*, 7557–7558.

(59) Nirmala, N. R., and Wagner, G. (1989) Measurement of 13C spin-spin relaxation times by two-dimensional heteronuclear 1H□13C correlation spectroscopy. *J. Magn. Reson. 82*, 659–661.

(60) Palmer, A. G., Rance, M., and Wright, P. E. (1991) Intramolecular motions of a zinc finger DNA-binding domain from Xfin characterized by proton-detected natural abundance carbon-13 heteronuclear NMR spectroscopy. *J. Am. Chem. Soc. 113,* 4371–4380.

(61) Farrow, N. A., Muhandiram, R., Singer, A. U., Pascal, S. M., Kay, C. M., Gish, G., Shoelson, S. E., Pawson, T., Forman-Kay, J. D., and Kay, L. E. (1994) Backbone dynamics of a free and a phosphopeptide-complexed Src homology 2 domain studied by 15N NMR relaxation. *Biochemistry 33*, 5984–6003.

(62) Lipari, G., and Szabo, A. (1982) Model-free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules. 1. Theory and range of validity. *J. Am. Chem. Soc. 104*, 4546–4559.

(63) Lipari, G., and Szabo, A. (1982) Model-free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules. 2. Analysis of experimental results. *J. Am. Chem. Soc. 104*, 4559–4570.

(64) d'Auvergne, E. J., and Gooley, P. R. (2003) The use of model selection in the model-free analysis of protein dynamics. *J. Biomol. NMR 25*, 25—39.

(65) Mandel, A. M., Akke, M., and Palmer Arthur G., I. I. I. (1995) Backbone dynamics of Escherichia coli ribonuclease HI: correlations with structure and function in an active enzyme. *J. Mol. Biol. 246*, 144–163.

(66) Barbato, G., Ikura, M., Kay, L. E., Pastor, R. W., and Bax, A. (1992) Backbone dynamics of calmodulin studied by nitrogen-15 relaxation using inverse detected two-dimensional NMR spectroscopy: the central helix is flexible. *Biochemistry 31*, 5269–5278.

(67) Freedberg, D. I., Ishima, R., Jacob, J., Wang, Y.-X., Kustanovich, I., Louis, J. M., and Torchia, D. A. (2002) Rapid structural fluctuations of the free HIV protease flaps in solution: relationship to crystal structures and comparison with predictions of dynamics calculations.

Protein Sci. 11, 221–232.

(68) Kalodimos, C. G., Biris, N., Bonvin, A. M. J. J., Levandoski, M. M., Guennuegues, M., Boelens, R., and Kaptein, R. (2004) Structure and flexibility adaptation in nonspecific and specific protein-DNA complexes. *Science (80-.). 305*, 386 LP – 389.

(69) Thorpe, I. F., and Brooks 3rd, C. L. (2007) Molecular evolution of affinity and flexibility in the immune system. *Proc. Natl. Acad. Sci. U. S. A. 104*, 8821–8826.

(70) Zimmermann, J., Oakman, E. L., Thorpe, I. F., Shi, X., Abbyad, P., Brooks 3rd, C. L., Boxer, S. G., and Romesberg, F. E. (2006) Antibody evolution constrains conformational heterogeneity by tailoring protein dynamics. *Proc. Natl. Acad. Sci. U. S. A. 103*, 13722–13727.

(71) Das, R., Chowdhury, S., Mazhab-Jafari, M. T., Sildas, S., Selvaratnam, R., and Melacini, G. (2009) Dynamically driven ligand selectivity in cyclic nucleotide binding domains. *J. Biol. Chem. 284*, 23682–23696.

(72) Peng, T., Zintsmaster, J. S., Namanja, A. T., and Peng, J. W. (2007) Sequence-specific dynamics modulate recognition specificity in WW domains. *Nat. Struct. &Amp; Mol. Biol. 14*, 325.

(73) Akke, M., Brueschweiler, R., and Palmer, A. G. (1993) NMR order parameters and free energy: an analytical approach and its application to cooperative calcium(2+) binding by calbindin D9k. *J. Am. Chem. Soc. 115*, 9832–9833.

(74) Yang, D., and Kay, L. E. (1996) Contributions to conformational entropy arising from bond vector fluctuations measured from NMR-derived order parameters: application to protein folding. *J. Mol. Biol. 263*, 369–382.

(75) HAMMES, G. G. (1964) Mechanism of enzyme catalysis. *Nature 204*, 342–343.

(76) Eisenmesser, E. Z., Millet, O., Labeikovsky, W., Korzhnev, D. M., Wolf-Watz, M., Bosco, D. A., Skalicky, J. J., Kay, L. E., and Kern, D. (2005) Intrinsic dynamics of an enzyme underlies catalysis. *Nature 438*, 117–121.

(77) Henzler-Wildman, K. A., Thai, V., Lei, M., Ott, M., Wolf-Watz, M., Fenn, T., Pozharski, E., Wilson, M. A., Petsko, G. A., Karplus, M., Hübner, C. G., and Kern, D. (2007) Intrinsic motions along an enzymatic reaction trajectory. *Nature 450*, 838.

(78) Palmer, A. G. (2015) Enzyme dynamics from NMR spectroscopy. *Acc. Chem. Res. 48*, 457–465.

(79) Korzhnev, D. M., Religa, T. L., Banachewicz, W., Fersht, A. R., and Kay, L. E. (2010) A transient and low-populated protein-folding intermediate at atomic resolution. *Science (80-.). 329*, 1312 LP – 1316.

(80) Neudecker, P., Robustelli, P., Cavalli, A., Walsh, P., Lundström, P., Zarrine-Afsar, A., Sharpe, S., Vendruscolo, M., and Kay, L. E. (2012) Structure of an intermediate state in protein folding and aggregation. *Science (80-.). 336*, 362 LP – 366.

(81) Kimsey, I. J., Petzold, K., Sathyamoorthy, B., Stein, Z. W., and Al-Hashimi, H. M. (2015) Visualizing transient Watson-Crick-like mispairs in DNA and RNA duplexes. *Nature 519*, 315–320.

(82) Libich, D. S., Tugarinov, V., and Clore, G. M. (2015) Intrinsic unfoldase/foldase activity of the chaperonin GroEL directly demonstrated using multinuclear relaxation-based NMR. *Proc. Natl. Acad. Sci. U. S. A. 112*, 8817–8823.

(83) Franco, R., Gil-Caballero, S., Ayala, I., Favier, A., and Brutscher, B. (2017) Probing conformational exchange dynamics in a short-lived protein folding intermediate by real-time relaxation–dispersion NMR. *J. Am. Chem. Soc. 139*, 1065–1068.

(84) Sugase, K., Dyson, H. J., and Wright, P. E. (2007) Mechanism of coupled folding and binding of an intrinsically disordered protein. *Nature 447*, 1021.

(85) Schneider, R., Maurin, D., Communie, G., Kragelj, J., Hansen, D. F., Ruigrok, R. W. H., Jensen, M. R., and Blackledge, M. (2015) Visualizing the molecular recognition trajectory of an intrinsically disordered protein using multinuclear relaxation dispersion NMR. *J. Am. Chem. Soc. 137*, 1220–1229.

(86) Pratihar, S., Sabo, T. M., Ban, D., Fenwick, R. B., Becker, S., Salvatella, X., Griesinger, C., and Lee, D. (2016) Kinetics of the antibody recognition site in the third IgG-binding domain of protein G. *Angew. Chemie Int. Ed. 55*, 9567–9570.

(87) Xiao, T., Fan, J., Zhou, H., Lin, Q., and Yang, D. (2016) Local unfolding of fatty acid binding protein to allow ligand entry for binding. *Angew. Chemie Int. Ed. 55*, 6869–6872.

(88) Zhao, B., Guffy, S. L., Williams, B., and Zhang, Q. (2017) An excited state underlies gene regulation of a transcriptional riboswitch. *Nat. Chem. Biol. 13*, 968–974.

(89) Delaforge, E., Kragelj, J., Tengo, L., Palencia, A., Milles, S., Bouvignies, G., Salvi, N., Blackledge, M., and Jensen, M. R. (2018) Deciphering the dynamic interaction profile of an intrinsically disordered protein by NMR exchange spectroscopy. *J. Am. Chem. Soc. 140,* 1148–1158.

(90) Akke, M., and Palmer, A. G. (1996) Monitoring macromolecular motions on microsecond to millisecond time scales by R1ρ−R1 constant relaxation time NMR spectroscopy. *J. Am. Chem. Soc. 118,* 911–912.

(91) Massi, F., and Peng, J. W. (2018) Characterizing protein dynamics with NMR R1ρ relaxation experiments, in *Methods in Molecular Biology*, pp 205–221. Humana Press Inc.

(92) Fawzi, N. L., Ying, J., Torchia, D. A., and Clore, G. M. (2010) Kinetics of amyloid beta monomer-to-oligomer exchange by NMR relaxation. *J. Am. Chem. Soc. 132,* 9948–9951.

(93) Kovermann, M., Rogne, P., and Wolf-Watz, M. (2016) Protein dynamics and function from solution state NMR spectroscopy. *Q. Rev. Biophys. 49,* e6.

(94) Carr, H. Y., and Purcell, E. M. (1954) Effects of diffusion on free precession in nuclear magnetic resonance experiments. *Phys. Rev. 94,* 630–638.

(95) Meiboom, S., and Gill, D. (1958) Modified spin-echo method for measuring nuclear relaxation times. *Rev. Sci. Instrum. 29,* 688–691.

(96) Palmer, A. G., Kroenke, C. D., and Patrick Loria, J. (2001) Nuclear magnetic resonance methods for quantifying microsecond-to-millisecond motions in biological macromolecules, in *Nuclear Magnetic Resonance of Biological Macromolecules - Part B* (James, T. L., Dötsch, V., and Schmitz, U. B. T.-M. in E., Eds.), pp 204–238. Academic Press.

(97) Vallurupalli, P., Bouvignies, G., and Kay, L. E. (2012) Studying “invisible” excited protein states in slow exchange with a major state conformation. *J. Am. Chem. Soc. 134,* 8148–8161.

(98) Vallurupalli, P., Sekhar, A., Yuwen, T., and Kay, L. E. (2017) Probing conformational dynamics in biomolecules via chemical exchange saturation transfer: a primer. *J. Biomol. NMR 67,* 243–271.

(99) Gopalan, A. B., Hansen, D. F., and Vallurupalli, P. (2018) CPMG experiments for protein minor conformer structure determination BT - protein NMR: methods and protocols (Ghose, R., Ed.), pp 223–242. Springer New York, New York, NY.

(100) Sauerwein, A. C., and Hansen, D. F. (2015) Relaxation dispersion NMR spectroscopy BT - protein NMR: modern techniques and biomedical applications (Berliner, L., Ed.), pp 75–132. Springer US, Boston, MA.

(101) van Zijl, P. C. M., and Yadav, N. N. (2011) Chemical exchange saturation transfer (CEST): what is in a name and what isn't? *Magn. Reson. Med. 65*, 927–948.

(102) Lokesh, N., Seegerer, A., Hioe, J., and Gschwind, R. M. (2018) Chemical exchange saturation transfer in chemical reactions: a mechanistic tool for NMR detection and characterization of transient intermediates. *J. Am. Chem. Soc. 140*, 1855–1862.

(103) Korzhnev, D. M., Salvatella, X., Vendruscolo, M., Di Nardo, A. A., Davidson, A. R., Dobson, C. M., and Kay, L. E. (2004) Low-populated folding intermediates of Fyn SH3 characterized by relaxation dispersion NMR. *Nature 430*, 586–590.

(104) Vallurupalli, P., Hansen, D. F., Stollar, E., Meirovitch, E., and Kay, L. E. (2007) Measurement of bond vector orientations in invisible excited states of proteins. *Proc. Natl. Acad. Sci. 104*, 18473 LP – 18477.

(105) Neudecker, P., Lundström, P., and Kay, L. E. (2009) Relaxation dispersion NMR spectroscopy as a tool for detailed studies of protein folding. *Biophys. J. 96*, 2045–2054.

(106) Ishima, R. (2014) CPMG relaxation dispersion BT - protein dynamics: methods and protocols (Livesay, D. R., Ed.), pp 29–49. Humana Press, Totowa, NJ.

(107) Hansen, A. L., Lundström, P., Velyvis, A., and Kay, L. E. (2012) Quantifying millisecond exchange dynamics in proteins by CPMG relaxation dispersion NMR using side-chain 1H probes. *J. Am. Chem. Soc. 134*, 3178–3189.

(108) Vallurupalli, P. (2010) Structure and dynamics of protein excited states with millisecond lifetimes. *J. Indian Inst. Sci.*

(109) Hansen, D. F., Vallurupalli, P., Lundström, P., Neudecker, P., and Kay, L. E. (2008) Probing chemical shifts of invisible states of proteins with relaxation dispersion NMR spectroscopy: how well can we do? *J. Am. Chem. Soc. 130*, 2667–2675.

(110) Bouvignies, G., Korzhnev, D. M., Neudecker, P., Hansen, D. F., Cordes, M. H. J., and Kay, L. E. (2010) A simple method for measuring signs of (1)H (N) chemical shift differences between ground and excited protein states. *J. Biomol. NMR 47*, 135–141.

(111) Kruschel, D., and Zagrovic, B. (2009) Conformational averaging in structural biology: issues, challenges and computational solutions. *Mol. Biosyst. 5*, 1606–1616.

(112) Case, D. A. (2002) Molecular dynamics and NMR spin relaxation in proteins. *Acc. Chem. Res. 35*, 325–331.

(113) Fisette, O., Lagüe, P., Gagné, S., and Morin, S. (2012) Synergistic applications of MD and NMR for the study of biological systems. *J. Biomed. Biotechnol. 2012*, 254208.

(114) Ren, X., Lin, J., Jin, C., and Xia, B. (2010) 1H, 13C and 15N resonance assignments of human H-REV107 N-terminal domain. *Biomol. NMR Assign. 4*, 175–178.

(115) Wang, L., Yu, W., Ren, X., Lin, J., Jin, C., and Xia, B. (2012) 1H, 13C, and 15N resonance assignments of the N-terminal domain of human TIG3. *Biomol. NMR Assign. 6*, 201–203.