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ABSTRACT

Photosynthetic light-harvesting complexes have a remarkable capacity to perform robust photo-physics at ambient temperatures and in fluctuating environments. Protein conformational dynamics and membrane mobility are processes that contribute to the light-harvesting efficiencies and control photoprotective responses. This short review describes the application of magic angle spinning nuclear magnetic resonance (NMR) spectroscopy for characterizing the structural dynamics of pigment, protein, and thylakoid membrane components related to light harvesting and photoprotection. I will discuss the use of dynamics-based spectral editing solid-state NMR for distinguishing rigid and mobile components and assessing protein, pigment, and lipid dynamics on sub-nanosecond to millisecond timescales. Dynamic spectral editing NMR has been applied to investigate light-harvesting complex II protein conformational dynamics inside lipid bilayers and in native membranes. Furthermore, we used the NMR approach to assess thylakoid membrane dynamics. Finally, it is shown that dynamics-based spectral editing NMR for reducing spectral complexity by filtering motion-dependent signals enabled us to follow processes in live photosynthetic cells.

INTRODUCTION

Photosynthetic light-harvesting and energy conversion processes take place in the photosynthetic membrane, the thylakoid, and encompass the interplay between pigments and protein complexes in a dynamic membrane environment.[1–3] Inside the thylakoid membrane, robust photophysical and photochemical processes are carried out at ambient temperatures. Thermal fluctuations, protein conformational dynamics, and membrane transitions contribute to the efficiency of light harvesting and excitation transfer and allow for flexibility of these processes to respond to fluctuating environmental conditions.[3–8] The assessment of photosynthetic protein, pigment, and membrane dynamics on nano- to millisecond timescales provides insight into the molecular and nanoscale mechanisms of photosynthesis regulation. This short review describes the application of Magic Angle Spinning solid-state (MAS ss) nuclear magnetic resonance (NMR) spectroscopy in combination with dynamics-based spectral editing to gain insight into the structural dynamics of light-harvesting pigment–protein complexes and for characterizing protein and lipid dynamics in thylakoid membranes. MAS ssNMR is widely used as a tool for structural characterization of membrane proteins and biomembranes at the atomic level.[9–12] In solution-state NMR spectroscopy, rapid tumbling of molecules averages out anisotropic interactions, such as dipolar coupling and chemical shift anisotropy, which results in long relaxation times for the magnetization and narrow peaks. Large structures, such as membrane proteins in lipid bilayers, polymers, crystals, and molecular assemblies, experience slow tumbling that is usually too slow for averaging out anisotropic interactions, resulting in broad peaks or NMR invisibility. In the solid state, NMR spectroscopy on solids or semi-solids is combined with MAS. Under MAS, the sample inside a small rotor is held under an angle of 54.7° with respect to the static magnetic field (Z-axis) and the XY plane and spun around its axis with frequencies typically ranging from 5 to 50 kHz depending on the rotor size and instrumentation (Fig. 1). Fast rotation of the sample at this fixed angle with respect to the magnetic field reduces

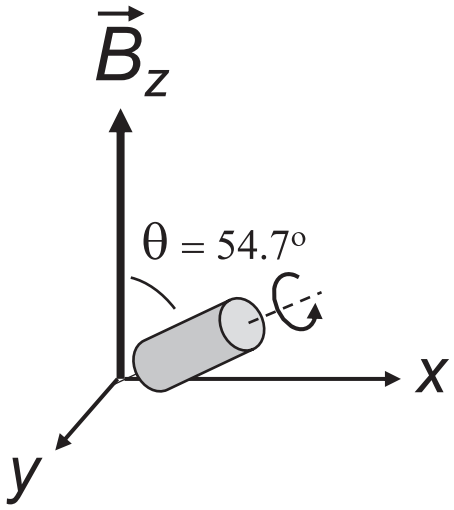


FIG. 1. The concept of MAS NMR. The sample inside an NMR rotor is held at an angle θ of 54.7° (magic angle) with respect to the magnetic field B_Z and spun fast around its axis.

contributions of anisotropic interactions by averaging those, similar to rapid tumbling in the solution state.[13] Due to strong ^{1}H–^{1}H dipolar couplings and magnetic field inhomogeneities caused by structural disorder, ^{1}H line widths are broadened in the solid state, even with MAS applied. Solid-state NMR on biological samples typically involves ^{13}C or $^{13}C/^{15}N$ detection of isotope-labeled samples, sometimes requiring dedicated labeling strategies.[14]

DYNAMICS-BASED SPECTRAL EDITING NMR

A basic ^{13}C NMR experiment encompasses single pulse excitation, also termed direct polarization (DP), of the ^{13}C nuclei (90° pulse) followed by ^{13}C detection. A drawback of this type of NMR experiment is that ^{13}C longitudinal T_1 relaxation times for molecules with low internal motions are very long, requiring long recycle delay intervals in between repeated scans. ^{13}C ssNMR experiments commonly employ ^{1}H–^{13}C heteronuclear cross-polarization (CP), where magnetization is transferred from protons to carbons via dipolar interactions, and ^{13}C is detected. Compared to DP, CP NMR provides enhancement of signal proportional to $\gamma^{1}H/\gamma^{13}C$ and shortens the relaxation times because of the faster longitudinal relaxation of excited protons compared to ^{13}C. In contrast to DP, signal intensities of CP NMR are affected by internal motions that reduce the efficiency of dipolar polarization transfer, leading to the invisibility of molecules with sub-microsecond dynamics.[15] Highly mobile residues can be detected by an alternative polarization transfer NMR scheme, Insensitive Nuclei Enhanced by Polarization Transfer (INEPT), where magnetization is transferred through bond via scalar couplings (*J* couplings).[16,17] INEPT-based polarization transfer makes use of echo pulses and renders inefficient for rigid molecules of which the transverse T_2 relaxation times are very fast. As a consequence, INEPT-based NMR only detects signals of molecules with dynamical motions in the (sub)nanosecond range. The combination of CP and INEPT experiments provides a way to selectively detect rigid molecules (via CP) or very mobile molecules (via INEPT).[15,18] Changes in the structural dynamics are detectable if they move signals in or out the CP or INEPT NMR detection window. DP signal intensities are motion-independent and DP spectra detect signals within a wide dynamics range. Figure 2 summarizes the dynamics ranges for CP, INEPT, and DP NMR detection under the experimental conditions that we used in 1D MAS NMR experiments for analyzing membrane and cell dynamics.

Dynamics-based spectral editing ssNMR has found a wide range of applications. Conventional CP-based ssNMR spectroscopy on membrane proteins or protein assemblies does not detect protein sites with fast or intermediate dynamics that are typical for unstructured loop and tail segments. The combination of ssNMR with spectral editing has been applied by van der Wel and co-workers to address the rigid and flexible regions of amyloid fibrils and their polymorphs and gain molecular insight into their structures and dynamic disorder.[19,20] In addition, they could reveal the structure and dynamics of peripherally bound membrane proteins that contain flexible, water-exposed sites.[21] Furthermore, *J*-coupling-based INEPT ssNMR spectroscopy has been applied to determine the unfolded extra-membrane structures of integral membrane proteins, in addition to the folded, rigid protein sites that are embedded in the membrane and accessible with CP-based ssNMR. The NMR dynamics filtering technique is not only limited to resolving protein structural dynamics but also could resolve aspects of hydration and water behavior in hydrogels and detect phase transitions in lipid membranes and surfactants.[22–24] A novel technique based on the principles of dynamics-based spectral editing is comprehensive multi-phase (CMP) NMR.[25] The CMP NMR method involves a specialized NMR probe for simultaneous detection of solid, semi-solid, and liquid phases. CMP NMR has been applied in *in situ*

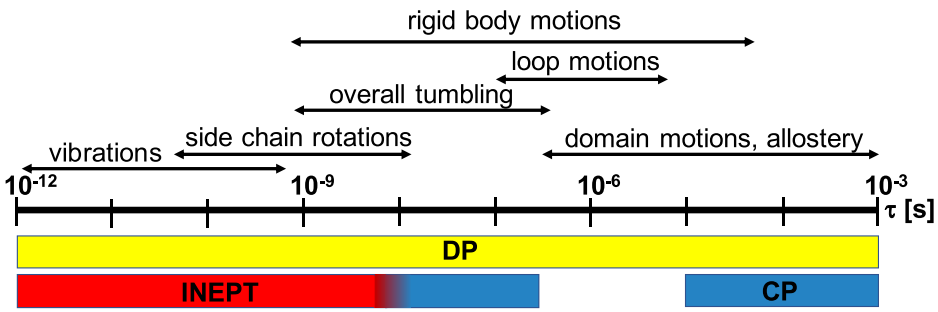


FIG. 2. Dynamics time scales for NMR detection via DP- (yellow), CP- (blue), or INEPT-based (red) MAS NMR experiments and processes occurring on the respective time scales.

NMR experiments on intact micro-organisms or heterogeneous biomaterials for distinction and analysis of rigid, semi-solid, and fluid constituents.

ssNMR SPECTROSCOPY APPLIED TO PHOTOSYNTHETIC ANTENNA SYSTEMS

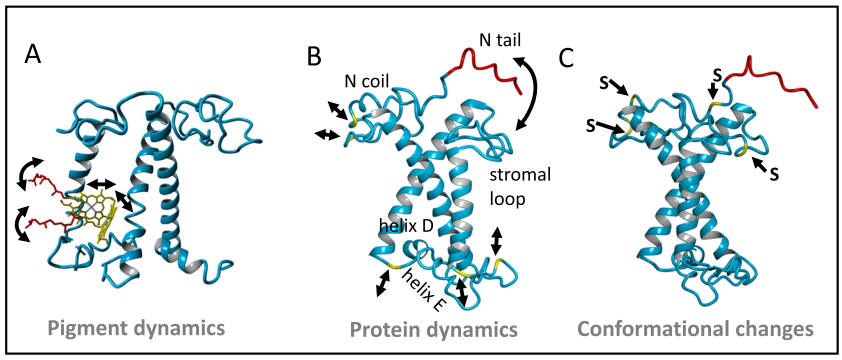
In the past few decades, various structures of photosynthetic light-harvesting proteins have been resolved at high resolution by crystallography and cryo-electron microscopy (cryo-EM).[26–30] Yet, open questions remain concerning the pigment structural and electronic environments and dynamics of light-harvesting protein and pigments that may control light-harvesting and photoprotective processes. ssNMR is a technique that has compelling advantages when it comes to resolving atomic details of pigment–protein interactions and dynamics in a flexible protein environment. Moreover, as crystallization is not a requirement, the molecular structure can be resolved within mesoscopic self-assemblies with substantial dynamics or inhomogeneity. ssNMR was applied to determine the ground-state electronic structures of chlorophylls (Chls) in purple non-sulfur bacterial antenna systems[31–33] revealing molecular mechanisms by which mutual interactions with the protein matrix stabilize the pigment–protein quaternary structures. Alia, de Groot, and co-workers found that the BChls in LH2 antenna complexes modify the electronic structures of the ligating histidines with mutual charge transfer between the histidines and BChls.[34,35] Furthermore, the group of Matysik has applied photochemically induced dynamic nuclear polarization (photo-CIDNP) to uncover the electronic structure and special properties of the reaction center special pair Chls in intact plants, algae, and diatoms.[36–38] de Groot and Buda combined ssNMR data and further optimization by density functional theory (DFT) calculations for elucidation of the molecular structures (unit cells) of chlorosomes and man-made artificial light-harvesting tubular assemblies.[39–41] In recent work from the lab of de Groot, Sevink, and co-workers, molecular dynamics simulations were applied to show how dynamic disorder drives exciton transfer in chlorosome assemblies.[42,43] They created a protocol for *in silico* generation of atomically resolved plastic, tubular structures with defined dihedral angles, and used unit cells derived from ssNMR data on chlorosomes to generate MD models of chlorosome tubular assemblies, which now can be used as a platform to investigate the links between chlorosome structural mechanics, plasticity, and optical properties.[42]

Light-Harvesting Complex II (LHCII) is the major light-harvesting protein in plants and green algae, which is responsible for capturing light and guiding the excitation energy to Photosystem II (PSII) and under high-light conditions also to PSI. An unresolved intriguing property of LHC-type light-harvesting antenna complexes of plants and algae is their capacity to switch their function between fluorescent, light-harvesting, photoprotective, and excitation-quenched states via reversible conformational transitions.[6,44,45] X-ray structures of several LHC complexes have been obtained,[29,30,46,47] which formed the basis for atomistic and coarse-grained MD simulations.[48–54] ssNMR spectroscopy may bridge the gap between *in silico* protein dynamic models and crystallographic pigment–protein structures and highlight specific pigment and protein sites with high plasticity. Moreover, ssNMR spectroscopy is applicable to membrane-embedded proteins and biological membranes and could shed light on protein–lipid interactions that may have importance for the dual function of LHC complexes.

This short review describes the application of dynamics-based spectral editing ssNMR for gaining insight into the conformational dynamics of the most abundant LHC complex, LHCII, in native-like membrane environments and inside native membranes. Furthermore, it is shown that dynamics-based spectral editing ssNMR is applicable for determining lipid membrane dynamics in intact photosynthetic cells and holds promise for simultaneous detection of structural dynamics and metabolic processes in live photosynthetic cells.

CONFORMATIONAL DYNAMICS OF LIGHT-HARVESTING COMPLEX II

The conformational dynamics of LHCII has been investigated by various techniques, including single-molecule fluorescence, electron paramagnetic resonance (EPR), and neutron scattering techniques.[6,44,55,56] Single molecule fluorescence has revealed that individual pigment–protein complexes fluctuate between fluorescent and fluorescence-quenched states.[6] Neutron scattering methods including (quasi-) inelastic neutron scattering (QENS) can measure directly protein dynamics extending ps-ns time scales. A combined optical and QENS low-temperature study on LHCII demonstrated that there is a correlation between conformational dynamics and the position of the LHCII Chl excited electronic states due to protein conformational fluctuations.[57] EPR studies revealed that the protein complex possesses a flexible N tail and rigid transmembrane interior.[55] Recently, it was found via neutron scattering techniques that LHCII proteins can adopt different quaternary structures, including small oligomers, depending on the detergent types.[58] We employed dynamics-based spectral editing NMR to assess the structure and dynamics of LHCII in native and native-like membrane environments.[59] ^{13}C-labeled LHCII was obtained from *Chlamydomonas reinhardtii* (*Cr*) cells that were grown on ^{13}C-acetate. The NMR INEPT spectrum of LHCII reconstituted in thylakoid lipid bilayers contained a moderate set of signals that were assigned to the highly flexible N-tail of LHCII and the tails of two chlorophylls [see LHCII structures in Figs. 3(a) and 3(b) and spectrum in Fig. 3(e)]. INEPT NMR detects highly dynamic and unstructured protein segments that are often not accessible for crystallization.[60–63] Consistent with this, the protein N tail and the tails of two Chls (Chl 605 and 606) have not been resolved in the crystal structures of LHCII.[30,46] Sites with intermediate dynamics were identified based on the invisibility of predicted NMR signals in the CP-based spectrum, indicated in the structure in Fig. 3(b). Those sites that undergo motions on microsecond timescales—the timescale for domain motions and protein allostery—are located at the membrane–water interface and part of the stromal loop and lumen helix edges connected to the amphipathic helices D and E. Most of the Chl macrocycle atom signals were invisible with CP detection, indicating that the LHCII Chl rings undergo motions on microsecond timescales. The Chl macrocycles become visible in CP-based NMR at temperatures below 240 K, suggesting strongly reduced pigment and protein dynamics below this temperature, correlating with the onset temperature for conformational motions as indicated in QENS measurements on LHCII.[57]



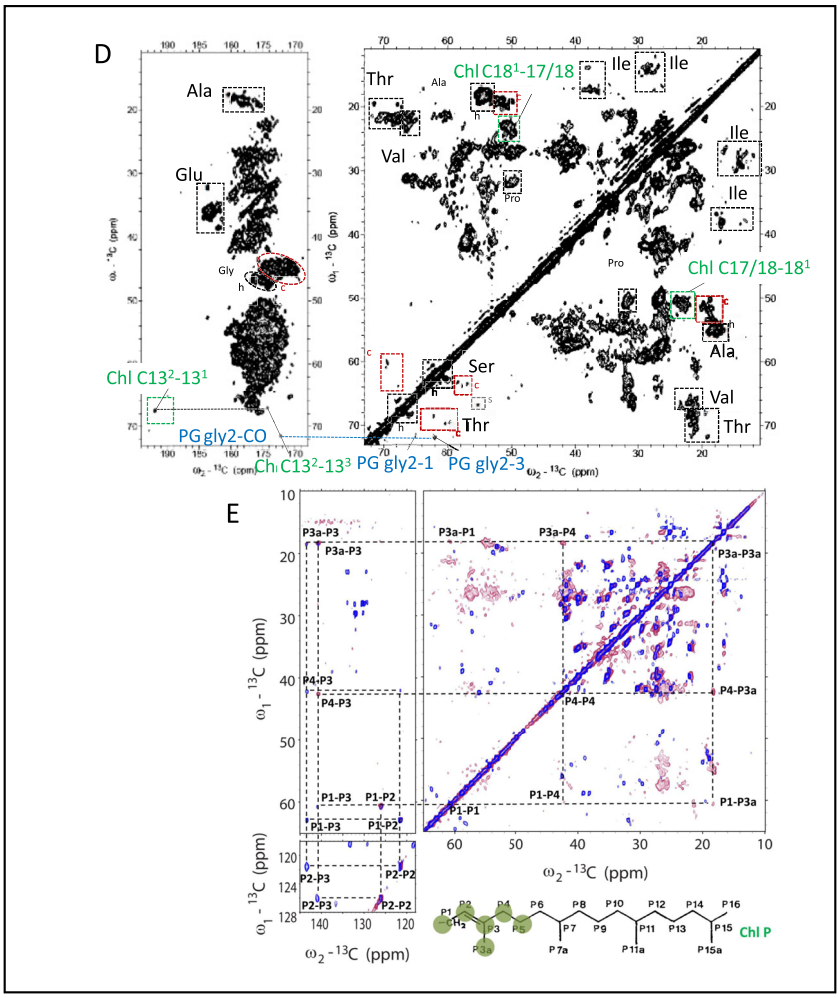


FIG. 3. NMR-detected protein dynamics of LHCII. (a)–(c) LHCII structure with detected dynamic sites indicated. Highly mobile sites are in red and sites with intermediate (μs) dynamics in yellow. (a) Chl dynamics, showing for Chls 605 and 606; (b) protein dynamics, and (c) flexible sites in LHCII. The arrows with an S point to Ser sites that may convert to strand conformations in npq2 LHCII. The LHCII structures were modeled using Lhcbm structures from PDB-IDs 6KAC, 7DZ8, and 7E0H. (d) and (e) D, CP-MAS ^{13}C–^{13}C spectrum with identified protein helix (h) and coil (c) amino acid types, Chl (green) and lipid (blue) signals indicated. (e) Overlaid spectra DP-MAS ^{13}C–^{13}C spectrum (red) and INEPT-TOBSY ^{13}C–^{13}C spectrum (blue) with Chl phytol signals indicated. (d) and (e) are modified with permission from Azadi-Chegeni *et al.*, Biophys. J. **120**(2), 270–283 (2021). Copyright 2021 Cell Press.

Influence of zeaxanthin

Notable changes in structure and dynamics were observed for LHCII from the xanthophyll-cycle impaired mutant npq2 that constitutively accumulates zeaxanthin (Zea) in its thylakoid membranes.[64] The npq2 mutant contained LHCII complexes binding Zea instead of Vio and had destabilized LHCII trimers so that the LHCII complexes were isolated as monomers. Monomeric Zea-LHCII complexes from the npq2 mutant display a larger static disorder than wild-type LHCII trimers, shown as broader lines in CP-based NMR spectra, and lacked sites with intermediate dynamics. Distinctive spectral changes were observed in the stromal loop regions where Ser residues in strand conformation were identified [see Fig. 3(c)]. Protein signals in the INEPT spectrum were conserved, indicating

that the npq2 LHCII also contained a highly flexible N tail. Strong signals of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) lipids in the CP-based NMR spectrum of npq2 LHCII indicated that multiple lipids were associated with npq2 LHCII, which were strongly protein bound. In contrast, the CP spectrum of wild-type LHCII trimers only contained a lipid signal of phosphatidyl glycerol (PG), which is a structural lipid that is bound to each monomer in LHCII.[30] Additional coarse-grain MD simulations supported the NMR data of npq2 LHCII and demonstrated that monomerization of LHCII increases structural displacements and promotes lipid association, while substitution of Vio by Zea has an additional effect on the protein–lipid affinities.[64]

Dynamic picture of LHCII and photoprotective states

From the NMR studies, a dynamic picture of LHCII emerges in which the protein has (1) a highly dynamical, disordered N tail, (2) flexible loops at the stromal and luminal membrane–water interface, which undergo microsecond local motions, and (3) stromal loop and N-terminal coil that can undergo conformational transitions that are stabilized by monomerization or Zea. The NMR-determined LHCII dynamic sites agree well with atomistic MD simulation studies that have shown that the N terminus is highly disordered and that the most flexible domains are exposed to the membrane–water interface.[49,53] Reversible transitions have been proposed to occur in two LHCII pigment domains: one formed by lutein (Lut1) and the Chl *a* 610, 611, and 612 and another by Lut2 and Chls 602 and 603.[6,49,65] Protein dynamics has been associated with transitions from active light-harvesting to excitation-quenched states: motions in the N terminus of LHCII correlated with fluctuations in Chl611-612 excitonic coupling[49] and LHC quenching mechanisms may involve short-range carotenoid interactions with multiple Chls that are fine-tuned by protein motions and modest conformational rearrangements.[66–68] Similar effects were found for LHCII using QENS, where it was found that a variety of conformational substates with different spectral positions may be thermally accessible, with a special focus on Chl612, implying that dynamic pigment–protein interactions fine-tune electronic energy levels and electron–phonon coupling of LHC II.[57] An NMR comparison between LHCII in fully active and strongly quenched states showed characteristic changes in signals of Chl*a* macrocycle rings and heterogeneity of the stromal coil connected to the N terminus, while the protein core of intercrossing helices containing the binding sites of Chl610 and Chl602 was unchanged.[69,70] Heterogeneity of the LHCII N-terminus has also been reported in electron paramagnetic resonance (EPR) spin-label studies.[55] The combined NMR results would be consistent with a hypothetical mechanism in which conformational transitions at the stromal loop and N terminus, together with local displacements at the lumen membrane–water interface, propagate into the LHC pigment domains where they are able to control quencher states.

LHCII in native membranes

How dynamic is LHCII in a native thylakoid membrane? To address this question, we performed an NMR analysis on purified ^{13}C-isotope labeled thylakoid membranes from *Chlamydomonas reinhardtii* (*Cr*) cells that were grown in ^{13}C-labeled media.[64] The results revealed that in the native membranes, LHCII does not contain a highly dynamic N-tail, and both large fluctuations and microsecond local dynamics were reduced. Structures of photosynthetic Photosystem (PS)I- and PSII-LHCII supercomplexes have provided detailed information on LHCII protein–protein, protein–pigment, and protein–lipid interactions in thylakoid membranes.[27,28,71–73] The stromal and luminal loops are involved in LHCII–LHCII interactions[27] and the N terminus of LHCII can interact with PSI core subunits.[73] In stacked membranes, LHCII stromal loops can be involved in interactions between adjacent membranes.[74] Such interactions are likely to reduce local motions of LHCII in native membranes and stabilize its N terminus. The absence of highly dynamic protein signals in the thylakoid spectrum, however, implies that also unbound LHCII complexes have stabilized N tails. To conclude, the internal dynamics of LHCII is significantly lower in native membranes than in *in vitro* systems demonstrating that the conformational dynamics of LHCs is tightly coupled with their local environments.

THYLAKOID MEMBRANE DYNAMICS

Thylakoid membranes containing the photosynthetic apparatus have evolved remarkable plasticity, forming complex architectures with stacked and unstacked appressed membrane regions and hexagonal phases.[75–77] Changes in thylakoid structure and dynamics have been observed with respect to stacking, protein lateral diffusion, and membrane reorganization in response to light conditions.[78–81] Inelastic neutron scattering has been applied to study the flexibility and motions of photosynthetic membranes inside leaves or whole cells, revealing revealed membrane undulatory motions coupled to photosynthetic activity.[82,83]

Combined INEPT-DP- and CP-based 1D MAS NMR spectroscopy provides a usual means to qualify and quantify lipid dynamics and membrane phase transitions[24,84] that we applied to assess the dynamics of protein and lipid thylakoid components.[85–87] The ^{13}C NMR spectrum of thylakoid membranes contains broad bands of the protein backbone and side chains with superimposed sharp peaks from lipids, where we distinguish the accumulated nCH$_2$ signals of the lipid acyl chain, CH atoms of unsaturated lipid tails, and the head atoms of galactosyl lipids (see Fig. 4). Furthermore, we could distinguish *all-trans* from *trans-gauche* lipid conformations.[88] The thylakoid CP spectrum is selective for signals of the protein backbone and immobilized lipids and the INEPT spectrum mainly contains signals of mobile lipids.

Influence of zeaxanthin

We addressed the influence of Zea on thylakoid membrane dynamics. For membranes of the npq2 mutant, a temperature-dependent increase in protein dynamics within the temperature range 3–25 °C was smaller than for the wild-type, while the increase in dynamic lipids was larger as depicted in Fig. 3.[85] Given the fact that npq2 LHCII monomers strongly bind lipid and have a greater content of immobilized lipids compared to wild-type LHCII trimers, this suggests that the npq2 membranes contain separate regions of immobilized protein–lipid aggregates and lipid pools with high mobility. The overall reduction in membrane fluidity that has been observed for Zea-containing membranes is explained by the fact that the majority of lipids are immobilized so that the overall lipid

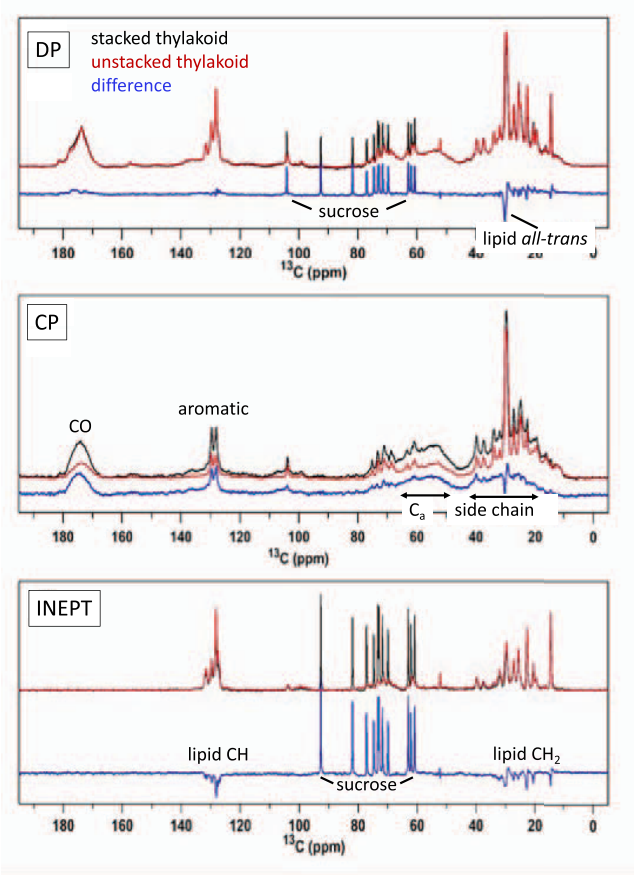


FIG. 4. DP, CP, and INEPT ^{13}C MAS NMR spectra of stacked (black) and unstacked (red) thylakoid membranes and the difference spectrum of stacked minus unstacked (blue). Changes in protein backbone (CO and C_a) and side chain dynamics are observed in CP and changes in lipid dynamics in INEPT. The negative peak in the DP difference spectrum is attributed to lipid *trans-gauche* to *all-trans* transition. Sharp peaks between 60 and 105 ppm are attributed to sucrose present in the stacked membrane sample. The figure is modified with permission from Nami *et al.*, BBA Adv. **1**, 100015 (2021). Copyright 2021 Elsevier.

dynamics inside npq2 membranes is lower than that in wild-type membranes [Fig. 5(b)].[89]

Influence of membrane stacking

Light-dependent reorganization of thylakoid membranes involves unstacking of stacked grana in high light, which is controlled by phosphorylation. We analyzed the effect of cation-induced unstacking on thylakoid protein and lipid dynamics, causing loss of long-range ordering and migration of LHCII complexes from PSII to PSI.[87] Unstacking increased the microsecond dynamics of proteins seen as a loss of the protein signal in CP and increased (sub-)nanosecond dynamics of mobile lipids seen as an increase of the lipid signal in INEPT (Fig. 4). The latter was confirmed by additional EPR spin-label experiments performed in the same study that showed increased dynamics of both bulk and annular lipids without changes in their relative fractions. An unexpected result was that, different from thermally induced dynamics, unstacking particularly affected the dynamics of protein backbone helices with relatively high loss of CP signal intensities at the left wing of the protein CO band (Fig. 4). The result suggests that the release of thylakoid stacking interactions and protein long-range ordering enhances microsecond fluctuations of membrane protein helices. The effect may be similar to a phenomenon that has been observed for purple membranes, where the loss of protein ordering increased microsecond rigid body motions of bacteriorhodopsin transmembrane helices.[90] Fluorescence decay-associated spectra of the stacked and unstacked membranes showed remarkable similarities with those of intact *Cr* cells kept in state I and state II,[91] suggesting that changes in lipid dynamics and helix motions may occur during membrane state transitions in intact cells in a similar fashion as depicted in Fig. 5(d).

DYNAMIC PROCESSES IN LIVE *Cr* CELLS

NMR spectroscopy is a non-invasive technique and can be applied to endogenous cell compounds, giving access to monitor *in-cell* processes or determine protein structures inside intact cells.[92] MAS NMR with spectral editing has the advantage that solid-, gel-, and liquid-like phases can be distinguished,[25] allowing simultaneous detection of metabolites, macromolecules, and solids in intact organisms. We demonstrated the application of NMR dynamics spectral editing for the investigation of ^{13}C-labeled *Cr* whole cells.[86,93] Cell viability during NMR experiments was tested,[93] concluding that, at moderate rotor spinning frequencies, the *Cr* cells would survive inside the NMR rotors for several hours.

Membrane dynamics

Membrane dynamics of whole *Cr* cells was assessed at different equilibrated temperatures between 3 and 25 °C. For *Cr* algae, the majority of the lipids reside in the cellular and thylakoidal membranes, with about one-third found in the thylakoid membranes and two-third in the cellular membrane.[94] In contrast to the trend that was observed for isolated thylakoid membranes, in whole cells, the rigidification of lipids was observed at increased temperatures,[86] as illustrated in Fig. 5(c). Inspection of the DP NMR spectra that are insensitive to dynamics discarded any spectral changes associated with lipid conversion or degradation. Further experiments are required to interpret the results, which suggest that cell protective mechanisms against cold stress may rapidly counteract temperature-dependent changes in membrane fluidity.

Metabolic processes and structural changes

In recent work, we performed MAS NMR with dynamics spectral editing on *Cr* cells that were kept under concentrated-induced, dark anoxia conditions, which resulted in fermentation.[93] Whereas previous experiments on thylakoid membranes and cells did not show significant changes in DP spectra over time, here new peaks emerged over a time scale of 0–10 h, depending on the set temperature, which could be assigned to the production of ethanol, CO_2, and glycerol, formation of glucose products, and slow accumulation of triacylglycerol (TAG) and fatty acids (FFA) (Fig. 6). CP and INEPT spectra provided further insight into structural changes,

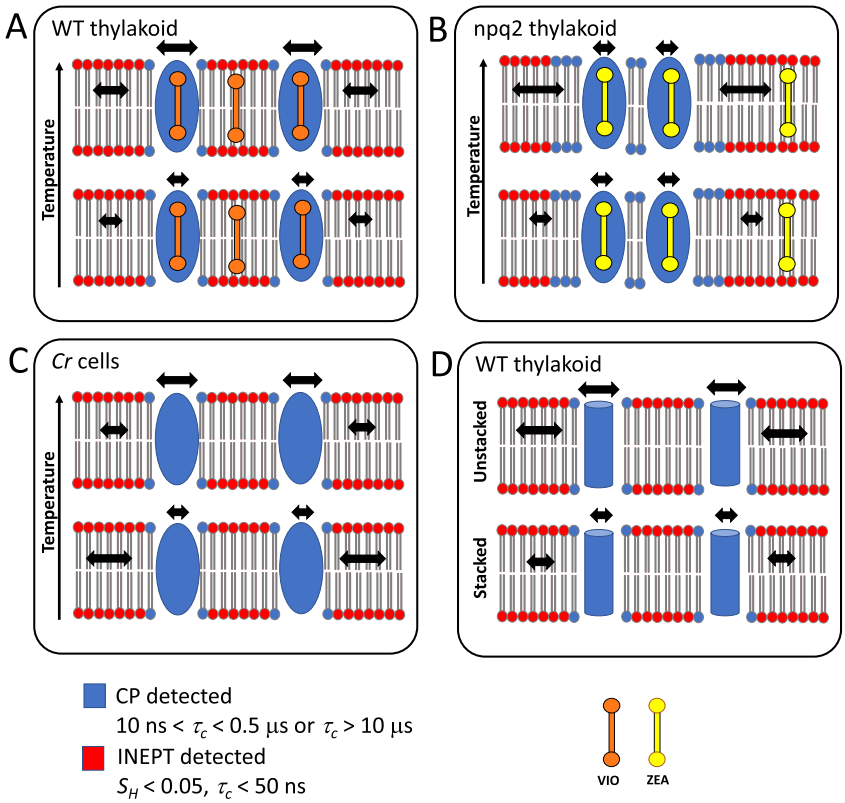


FIG. 5. Influence of temperature and stacking conditions on the dynamics of thylakoid membranes and intact *Cr* membranes, as observed with CP- and INEPT-detected MAS NMR. Relative dynamics is illustrated by the lengths of the black arrows. (a) and (b) Temperature effect on protein (blue ovals) and lipid dynamics in wild-type (a) and npq2 (b) thylakoid membranes. (c) Temperature effect on protein and lipid dynamics in intact *Cr* cells. (d) Thylakoid protein and lipid dynamics in stacked and unstacked membranes. Conditions for INEPT and CP detection as described in Ref. 86 are given with S_H, lipid order parameter, and, τ_c, rotational correlation time.

involving the breakdown of starch and galactolipids and progressive enhancement of lipid dynamics over time that we attributed to destabilization or breakdown of cellular or thylakoidal membranes. After 6–12 h depending on the set temperature, the production of ethanol and CO_2 stopped while glycerol production continued. The time scales correlated with measured cell viability under the same conditions, suggesting that continued glycerol production resulted from non-specific processes occurring in the lysates of death cells. This study demonstrates the proof of principle for the following molecular processes and macromolecular changes in live cells as one

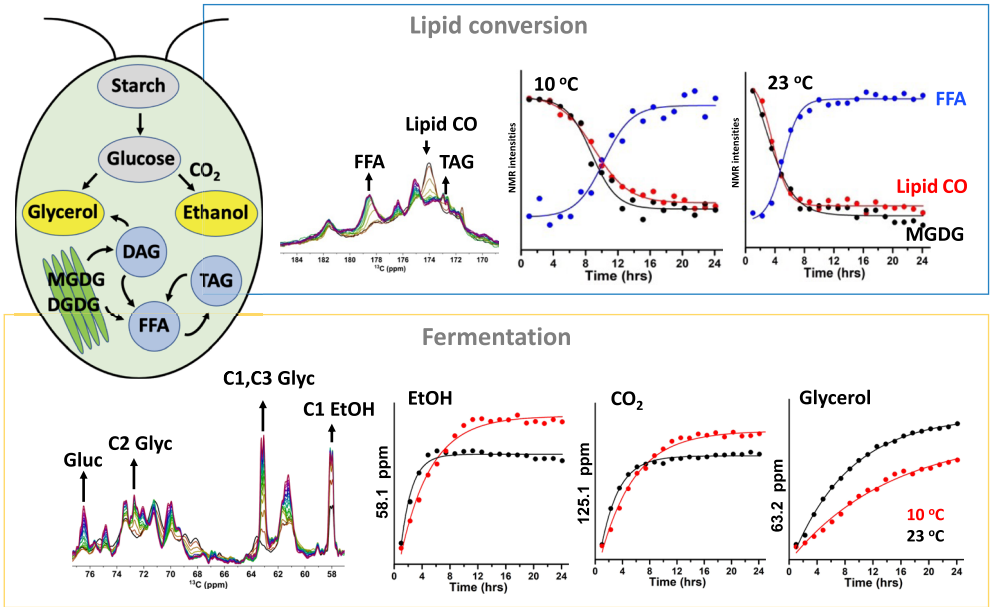


FIG. 6. MAS NMR monitoring of lipid conversion and fermentation in live *Cr cells*. The figure is modified with permission from Nami *et al.*, Angew. Chem., Int. Ed. **61**(14), e202117521 (2022). Copyright 2021 Wiley.

of the exciting potential usages of spectral-editing ssNMR. Further applications will benefit from technical improvements to expand cell lifetimes inside MAS NMR rotors and protect them against mechanistic stresses, which are subject for further research.

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AUTHOR DECLARATIONS

Conflict of Interest

The author has no conflicts to disclose.

Author Contributions

Anjali Pandit: Writing – original draft (lead); Writing – review and editing (lead).

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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