ABSTRACT: Phospholamban (PLN) regulates cardiac contractility by modulation of sarco(endo)plasmic reticulum calcium ATPase (SERCA) activity. While PLN and SERCA1a, an isoform from skeletal muscle, have been structurally characterized in great detail, direct information about the conformation of PLN in complex with SERCA has been limited. We used solid-state NMR (ssNMR) spectroscopy to deduce structural properties of both the A36F41A46 mutant (AFA-PLN) and wild-type PLN (WT-PLN) when bound to SERCA1a after reconstitution in a functional lipid bilayer environment. Chemical-shift assignments in all domains of AFA-PLN provide direct evidence for the presence of two terminal α helices connected by a linker region of reduced structural order that differs from previous findings on free PLN. ssNMR experiments on WT-PLN show no significant difference in binding compared to AFA-PLN and do not support the coexistence of a significantly populated dynamic state of PLN after formation of the PLN/SERCA complex. A combination of our spectroscopic data with biophysical and biochemical data using flexible protein–protein docking simulations provides a structural basis for understanding the interaction between PLN and SERCA1a.

Sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA)\(^1\) is the rate-determining enzyme for Ca\(^{2+}\) removal from muscle cytoplasm, a process that facilitates the relaxation of myofibrils subsequent to contraction. Phospholamban (PLN) regulates cardiac contractility by modulating the activity of SERCA2a, the heart muscle SERCA isoform (1, 2). Regulatory dysfunction of PLN on SERCA2a has been identified as a cause of dilated cardiomyopathy, and alterations of PLN activity can have manifold influence on the progress of heart disease, making the PLN–SERCA complex an important target for therapeutic intervention (3). SERCA is a membrane protein of \(~110\) kDa molecular weight, existing in a number of highly conserved isoforms (4). The skeletal muscle isoform SERCA1a (994 amino acid residues) has been structurally and functionally characterized in great detail, including the structure determination of reaction intermediates (see, e.g., (5–9)). In particular, the structure of the Ca\(^{2+}\)-free E\(_2\) intermediate of SERCA1a, to which PLN can bind, is known at high resolution from X-ray crystallography (5, 10).

PLN is a 52-residue protein, whose structure has been studied by a variety of biophysical techniques. In line with other methods, nuclear magnetic resonance (NMR) spectroscopy revealed a transmembrane helix and a mobile N-terminal domain with some differences in the secondary structure of monomeric PLN mutants (11–14). Wild-type PLN predominantly exists as a symmetric homopentamer with a pore-resembling core spanning the membrane (15, 16), where the cytoplasmic domains can associate with the lipid bilayer (17).

Thus far, detailed structural information obtained directly on PLN in complex with SERCA has been limited. The binding interface of PLN with SERCA1a or SERCA2a, regarding both the transmembrane and cytoplasmic domains of PLN, was described by mutagenesis screening of PLN (18–20) and SERCA (21, 22), as well as cross-linking studies (23–28). Magic-angle-spinning (MAS) ssNMR has been used to selectively probe internuclear distances in pairwise isotope-labeled PLN variants bound to SERCA1a (29), while, for example, EPR and FRET have been employed to study overall conformational changes upon complex formation (30, 31). Current models for the structure of PLN when bound to SERCA1a (E\(_2\) state) were obtained from computational approaches using mutagenesis and cross-linking constraints for all domains of PLN (26, 32) or with a focus on the transmembrane region (27, 28). In these studies, the initial template structure of PLN for molecular modeling was taken from investigations of free PLN (12, 33). Solution-state NMR data obtained for AFA-PLN in the presence...
of SERCA were interpreted in terms of two or more transient conformations that are present during the PLN–SERCA interaction, structurally different from results seen for free PLN in solution (34). Additionally, the existence of a transient WT-PLN intermediate during complex formation with SERCA was hypothesized on the basis of a structural model of the pentamer (16). However, these studies were conducted in micelles where complex formation and protein activity can be compromised (34) and did not provide direct spectroscopic information about PLN in complex with SERCA.

Significant progress has been achieved in monitoring structure, dynamics, and complex formation of multiply labeled proteins in lipid bilayers by ssNMR. Even if only low quantities of the molecule of interest are available, conformation-dependent chemical shifts (35) provide information on secondary structure (see, e.g., ref. 36). We have applied high-resolution MAS ssNMR spectroscopy to multiple (13C,15N)-labeled variants of AFA- and WT-PLN in complex with SERCA1a to (i) directly infer the backbone-binding mode of PLN in complex with SERCA in a functional bilayer environment, (ii) investigate the presence of any additional, dynamic PLN conformation in our preparations, and (iii) generate a structural model that is compatible with biochemical data.

**EXPERIMENTAL PROCEDURES**

**Sample Preparation.** Fully uniformly (U) (13C,15N)-labeled recombinant AFA-PLN was expressed in *Escherichia coli* grown on 13C-glucose and 15N-NH4Cl and purified as described (37). Skeletal muscle vesicles were prepared from rabbit hind leg (38), and the skeletal SR Ca2+-ATPase (SERCA1a) was purified by Reactive-Red Sepharose affinity chromatography (39). Hydrated proteoliposomes were prepared with DOPC as described by Hughes et al. (29). For the reconstitution, the molar ratio of SERCA1a/PLN/DOPC was 1:1.2:160. Typically 22 mg of purified Ca2+-ATPase protein was reconstituted with 1.5 mg of labeled PLN or AFA PLN. ATPase activity of the proteoliposomes was assayed by measuring the absorbance of NADH.

![Figure 1: ATPase activity as a function of the free Ca2+ concentration for Ca2+-ATPase reconstituted in the absence (circles) and presence of recombinant wild-type PLN (triangles) or AFA-PLN (squares). The raw data were normalized to the maximal activity calculated on the basis of fitting the data to the Hill equation (n = 5 for control, n = 4 for wild-type PLN, and n = 3 for AFA-PLN). The data are plotted as normalized ATPase activity versus pCa. Each data point is the mean ± standard error of the mean (SEM).](image)

![Figure 2: (A) Sequence of A36F41A46-PLN used in this study. Labeled and unlabeled residues are given in black and light gray, respectively. (B and C) SDWC experiments conducted on U(13C,15N)-AFA-PLN/SERCA1a (B) and reverse-labeled U(13C,15N)ILRKMYF-AFA-PLN/SERCA1a (C) at a temperature of 266 and 235 K, respectively. See parts A and B of Figure S1 in the Supporting Information for full spectra.](image)
chillers at temperatures ranging from 235 to 275 K. 13C chemical shifts were referenced with adamantane as external secondary standard, calibrating the 13CH2 resonance to 31.47 ppm.

Residue identification and sequential resonance assignments were made from a double-quantum/single-quantum (2Q/1Q) correlation spectrum employing SPCS (41) recoupling and from 2D spectra with mixing units using proton-driven spin diffusion (PDSD) (42, 43) or spin diffusion under weak (C',C") coupling conditions (SDWC) (44). Additionally, NCOCX and NCACX correlation spectra (45) were recorded with SPECIFIC NCO or NCA cross-polarization transfer (46), followed by homonuclear 13C PDSD or DARR (47) mixing. Mobile protein segments were probed by a combination of through-bond (1H,13C) INEPT (48) and (13C,13C) TOBYS (49) mixing, suppressing the signal from residues exhibiting little or no mobility (11). During dipolar and scalar-based correlation experiments, SPINAL64 (50) or GARP (51) proton decoupling was applied at 85 and 10 kHz, respectively. All but through-bond spectra were recorded with 5–7 days of signal averaging, and the spectrum in Figure 2C was recorded over 9 days.

Signal processing was carried out in XWINNMR 3.5 or Topspin 2.0 (Bruker Biospin, Germany), employing zero-filling and exponential line broadening or square-sine-bell apodization functions. Two-dimensional spectral analysis was performed in Sparky 3.110 (Goddard and Kneller, University of California, San Francisco, CA). Secondary chemical shifts ∆δobs = (∆dCα,αp – ∆dCα,αs) – (∆dCa,αp – ∆dCa,αs) (35) were calculated using average chemical shifts from the BioMagResBank (52).

**Modeling Using Flexible Protein–Protein Docking Simulation.** Docking models of PLN in complex with SERCA1a were calculated using the crystallography and NMR system (CNS) (53) with protocols for high-ambiguity-driven biomolecular docking (HADDOCK) (54) on a multinode computer cluster. A linearly stretched, partially prefolded docking template structure for PLN was generated using a standard simulated annealing protocol in CNS and then docked to a structure for SERCA1a in the E2 state, Protein Data Bank (PDB) (55) entry 2AGV (10). Both protein structures were initially separated by 150 Å in random orientations. Docking was driven by intermolecular distance constraints summarized in Table 1. For details, see the Supporting Information.

Over the course of the docking simulation, PLN was constrained to a helical backbone conformation for the residues discussed below and fully flexible elsewhere. Interface residues of SERCA were allowed to have flexible side chains or backbone during various stages of the docking. Other parts of SERCA remained rigid during the simulation.

**RESULTS**

**SsNMR Assignments and Secondary Structure of Mutant PLN.** We used one- and two-dimensional (13C,13C) and (13C,15N) solid-state NMR correlation experiments to determine NMR chemical shifts of (monomeric) AFA-PLN in complex with SERCA1a. To reduce spectral overlap and assignment ambiguities, studies were performed with fully and reverse-labeled AFA-PLN, as well as two samples with

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*Additional ambiguous interaction restraints (AIRs), as described for use in HADDOCK (54), were used to enforce interface contacts, such as residues with known loss-of-function mutations (see the Supporting Information). Of these residues, the following were defined as “active”, mandating an ambiguous interface contact: (SERCA1a) 89, 321, 802, 805, and 809 and (AFA-PLN) 2, 4, 7, 9, 12, 27, 31, 34, 35, 38, 41, 42, 45, 48, 49, and 52. Abbreviations: SS, disulfide bond; EMCS, N-(ε-maleimidocaproyloxy)succinimide ester; BMH, 1,6-bismaleimido-hexane; Cu–Phe, Cu–phenanthroline; KMUS, N-(ε-maleimidoundecanoyloxy)sulfosuccinimide ester; bBBBr, dibromobimane.

Amino acid-selective labeling. Isotope-labeling schemes are displayed in Figure 2A.

Residue-type spin systems were identified from 2D (13C,13C) correlation spectra using PDSD mixing and from a homonuclear 13C 2Q/1Q correlation spectrum (see the Supporting Information). Sequence-specific resonance assignments were facilitated by analysis of 2D (13C,13C) correlations with 150 ms SDWC mixing (44), shown in parts B and C of Figure 2 for fully and reversely (ILRKMKYF) labeled AFA-PLN, respectively. Several correlations could be assigned directly from fully labeled AFA-PLN, such as those involving C9 in P21 in all (13C,13C) spectra or M50C′–V49C′ in (13C,13C) SDWC (Figure 2B and Figure S1A in the Supporting Information). The spectrum of fully labeled AFA-PLN, however, is dominated by intra- and inter-residue correlations involving Ile and Leu. Using reverse-labeling, a significant reduction of spectral overlap (Figure 2C) and assignment ambiguities in the primary sequence (Figure 2A) was achieved. For example, Q5Cm–V4Cm, T17C2–S16C9, and N30C9–Q29C9, and their cross-diagonal correlations could now unambiguously be identified (Figure 2C and Figure S1B in the Supporting Information). In addition, a sequential assignment for R13C′ was obtained from a 1D SPECIFIC NCO-edited 13C spectrum for (13C,15N)-Arg-labeled AFA-PLN (Figure S3 in the Supporting Information). Spectroscopy of (13C-Thr,15N-Ile)-AFA-PLN assisted in resolving Thr resonances (Figure S4 in the Supporting Information). Assignments are in-line with 2D NCACX and NCOCX correlation spectra for uniformly labeled (U) AFA-PLN (parts D and E of Figure S2 in the Supporting Information); however, because of lower resolution, dispersion, and sensitivity in these spectra, the assignments were primarily obtained from the homonuclear carbon correlations.

Even without unambiguous sequence-specific residue identifications, many cross-peaks could be assigned to
degenerate residue groups that occur exclusively within the same domain of AFA-PLN. This includes LI/IL pairs between L37 and I44, which give rise to numerous, strong sequential correlations (Figure 2B), or residues F32, F35, and F41 (see the Supporting Information for details). Met C\textsuperscript{\alpha\beta\epsilon} chemical-shift correlations corresponding to a \( \beta \) strand (56) are not visible in the 2Q/1Q correlation spectrum, whereas the signal is detected for random-coil shifts (56), suggesting that there is no well-ordered \( \beta \) strand around M20 (16).

A qualitative separation of monomeric PLN segments exhibiting helical, extended, and coil backbone character was subsequently performed using secondary chemical-shift analysis. Backbone carbon resonances for V4, Q5, T8, and S10–R13 as well as Q29, N30, N34, V49, and nonspecifically assigned residues between F32 and I45 have helical character, whereas, for example, T17, Q22, and M50 show nonhelical propensities. In addition, we detected amino-acid-specific resonances of Ala (weak), Glu, Met, Ile, Ser, and Leu, corresponding to coil-like backbone structure (such as E2, A15, I18, and E19) and found Arg in different backbone configurations (Figure 2 and Figures S1–S4 in the Supporting Information). Because of signal overlap, Lys correlations indicative for a specific secondary structure could not be assigned.

When these findings are taken together, they suggest the existence of two helices comprising, at least, V4–R13 and Q29–V49, flanked by several residues of different backbone structure. Additional chemical shifts may be assigned to residues within helices that cannot be distinguished because of resonance overlap, such as Y6. Finally, taking into account earlier ssNMR distance measurements regarding A24\textsuperscript{\alpha\beta\epsilon}–Q26\textsuperscript{\alpha\beta\epsilon} and the chemical shift of A24\textsuperscript{\alpha\beta\epsilon}, indicating a helical backbone in this region (including R25), we could deduce a nonhelical linker range from R14 to Q22. Secondary chemical shifts of P21 and Q22 exclude continuation of the C-terminal \( \alpha \) helix in these two residues. A summary of our secondary chemical-shift analysis for AFA-PLN in complex with SERCA1a is displayed in Figure 3A. Secondary chemical shifts indirectly depending upon previously published data for A24/Q26, as well as R13, depending upon R13\('\) only, are colored gray.

Wild-Type PLN and PLN Mobility. To study whether structural differences previously seen between the AFA mutant and wild-type PLN are present in complex with SERCA, we conducted ssNMR experiments on a U(\( ^{13}\text{C},^{15}\text{N} \)-WT-PLN/SERCA complex. Cysteine residues in WT-PLN gave rise to distinct (C\textsuperscript{\alpha\beta\epsilon},C\textsuperscript{\alpha\beta\epsilon}) correlations not observed in AFA-PLN in a \( ^{1}\text{C},^{12}\text{C} \) correlation spectrum with 15 ms PDS mixing (Figure 4A and Figures S5 in the Supporting Information). Corresponding to \( \alpha \)-helical backbone conformation of the nonoxidized form. Ala (C\textsuperscript{\alpha\beta\epsilon},C\textsuperscript{\alpha\beta\epsilon}) cross-peaks were clearly diminished, owing to the absence of A36 and A46. While overall, the sample yields a lower signal-to-noise ratio, other cross-peaks appear identical at the given signal-to-noise level. In both spectra, recorded without freezing the samples, residues not buried in the membrane (e.g., M, E, Q, and R) give a rather weak signal, indicative of reduced structural order or increased mobility.

Protein segments exhibiting molecular motion in the micro- to nanosecond regime were probed by mobility-filtered INEPT or INEPT–TOBYS \( (^{1}\text{H},^{13}\text{C}) \) correlation experiments (11) (Figure 4B). In contrast to free PLN, these spectra were devoid of typical protein correlations after one-bond H–C and additional \( ^{13}\text{C}–^{12}\text{C} \) transfer (dashed-framed and solid-framed boxes, respectively), as would be found for protein segments showing a high degree of mobility (11).

Docking Model. Next, we performed docking calculations to infer whether our spectroscopic data are compatible with previous biochemical studies. The five models with the best docking score are shown in Figure 5A. Close-up views for the best model, fulfilling all of the experimental constraints.
and K3 to K397 chemical shifts. The proximity of charged side chains E2 isible with Q22 backbone torsion-angle predictions from DISCUSSION is fulfilled (Figure 5D).

Within the secondary-structure boundaries given by ss-NMR, the N terminus of the cytoplasmic helix of AFA-PLN is found near the K397′–V402′ loop of SERCA1a (SERCA residues denoted with a prime), while the C terminus of the helix intimately connects to SERCA1a residues at the surface of transmembrane helices M4, M5, M6, and M9 (Figure 5). Residues 802′, 805′, and 809′ which exhibit loss-of-function mutations (21), are in contact with AFA-PLN (parts B and C of Figure 5). The distances between K27C′ (AFA-PLN) and L321′C′ as well as V49C′ and V89′C′ are compatible with the formation of disulphide cross-links of their respective cysteine mutants (26–28) (parts B and C of Figure 5). Furthermore, intramolecular AFA-PLN distances described by Hughes and Middleton (29) for A24C′–Q26N and P21C′–A24C′ (Table 1) are taken into account. Residues P21′–Q23 form a loop allowing for dihedral angles compatible with Q22 backbone torsion-angle predictions from chemical shifts. The proximity of charged side chains E2 and K3 to K397′–V402′, as deduced from charge-altering mutations, leading to the loss of inhibitory function (19, 22), is fulfilled (Figure 5D).

FIGURE 5: (A) Cluster of five conformations of AFA-PLN with the lowest docking score (cartoon) and SERCA1a (PDB ID 2AGV, gray surface). (B–D) Close-up views of the best scoring AFA-PLN conformer from A, for the C-terminal (B and C) and N-terminal (D) regions. SERCA residues that can be cross-linked with PLN by disulphide bonds (V89′ and L321′) are displayed as yellow patches; other cross-linked residues are displayed as magenta; residues 802′, 805′, and 809′ are shown in blue; and residues 397′–402′ are shown in green. Spheres indicate positions of specific isotope labels in an earlier ssNMR study (29). Residues of AFA-PLN and SERCA related to loss-of-function mutants and residues studied by chemical cross-linking are displayed as sticks, while other AFA-PLN residues are displayed as lines.

brought forward by ssNMR, cross-linking, and mutagenesis, are given in parts B–D of Figure 5.

In terms of the three-dimensional PLN/SERCA complex, our model differs substantially from earlier results by Hutter and co-workers (32). In their model, the C-terminal domain is shifted toward the cytosol. Instead, the membrane-spanning helix ends close to the SR luminal side of the lipid bilayer (parts A and B of Figure 5) based on the strong cross-link affinity of V49C to V89′C via a disulphide bridge (26) or Cu–Phe (28). Elsewhere, the position of the C-terminal helix in our model results from constraints derived from cross-links involving N30 and N27 (25–27), as well as the use of mutagenesis-derived AIRs. It is in-line with hydrogen-exchange measurements, which indicate that L44 is buried within the membrane (29). Furthermore, the secondary structure of AFA-PLN in our model was directly obtained from the complex with SERCA1a and not enforced to comply with the experimental NMR constraints on the structure of PLN alone (12), alleviating restraints on almost two full helix turns compared to free PLN.

Our docking model shows a similarity to the model proposed by Toyoshima and MacLennan (26). For the C terminus (Figure 5B), both models reflect the formation of a disulphide bond between V49C/V89′C double mutants and describe the absence of a helical backbone structure for AFA-PLN residues L51 and L52. Unwinding of the helix at V49, however, is not in agreement with our spectroscopic results and the resulting model. Furthermore, the C-terminal helix extends beyond L31, including N30 and Q29 (Figures 3 and 4B). Still, residue positions are in-line with the K27C′–L321′C′ disulphide bond (26) and recently derived cross-linking constraints (24, 25, 27, 28). In addition, our model is compatible with the finding that A24 and Q26 are part of the helix (29) and incorporates the P21C′–A24C′ and A24C′–Q26N distances (29). Despite a longer C-terminal helix, we were also able to dock the cytoplasmic domain of AFA-PLN to residues in a pocket below the K397′–V402′ SERCA1a loop. K3 is found within 15 Å of K397′–K400′ (23), and proximity of E2 and K3 to K397′–V402′ may study are shown in Figure 3B, which points to differences in linker length in comparison to the previous studies.

Comparison to Previous Studies: Models of the PLN–SERCA complex have previously relied on structural data that was obtained for free PLN or deduced from biochemical data, such as mutagenesis and cross-linking. The corresponding secondary structures are given in Figure 3C and relate to (i) a model by Toyoshima and co-workers (26) based on two altered and computationally refined solution-state NMR structures for PLN (12, 33) and (ii) a model by Hutter et al. (32), which, during a docking simulation, enforced NOE constraints in solution for a free PLN monomer (12). As discussed in context of complex formation (16, 28), the secondary structure of WT-PLN pentamers seen by solution-state NMR spectroscopy in detergent micelles (16) is shown in Figure 3C (iii). Helix identifications from our ssNMR
facilitate interactions (Figure 5D). Residues exhibiting loss-of-function mutants are in contact with SERCA1a in both models.

Along with chemical cross-linking studies (25, 27, 28), rigid-body docking based on free PLN was to align the transmembrane helix with the structure of SERCA1a, raising the question if PLN K3 could reach near SERCA K397′–V402′ at all. Our results demonstrate that AFA-PLN computationally docked to the E2 state of SERCA1a does satisfy the aforementioned interface constraints, when structural changes upon binding, facilitated by protein flexibility in the simulation, are taken into account. In addition, the SERCA structure in complex with PLN may differ from our docking template (PDB entry 2AGV). However, changes such as seen in crystal structures obtained using different or no inhibitors (57, 58) could be adapted by small structural alterations of PLN in our model.

Implications for Complex Formation and Phosphorylation.

The secondary structure of AFA-PLN in complex with SERCA1a (parts A and B of Figure 3) implies a loss of secondary structure for several residues in the linker region compared to free AFA-PLN (12, 14) and to a lesser extent WT-PLN (16) (Figure 3C). In light of our structural model (Figure 5), this conformational flexibility is required for adaptation to the transmembrane and cytoplasmic SERCA binding sites. The existence of an extended linker already in the free WT form of PLN may be of advantage in complex formation. Indeed, the number of nonhelical linker residues of PLN in the complex is rather similar to WT-PLN (16) (iii in Figure 3C). However, this pentamer model cannot be docked to SERCA1a without violation of interface constraints or a structural rearrangement, as can be seen from manual docking using 3D visualization software (data not shown).

The importance of P21 to sustain unstructured characteristics in the segment preceding the C-terminal helix, providing conformational flexibility, has already been proposed from a structure determination of free PLN (12). An increase of helix propensity by P21A mutagenesis was found to reduce the ability of the N-terminal domain of PLN to efficiently inhibit SERCA1a (59). In fact, P21 does not only show chemical-shift characteristics of a statistical random-coil protein backbone but also bears an increase in ssNMR signal intensity when lowering the sample temperature (parts B and C of Figure 2 and Figure S1 in the Supporting Information), indicative of dynamic disorder in this protein segment. This notion further agrees with difficulties in assigning resonances from the linker segment in our 2D ssNMR data, because of unfavorable signal intensities or broadened resonance lines for these residues. Additional ssNMR experiments at elevated temperature could be used to further delineate the influence of structural and dynamic disorder and to potentially quantify the scale of motion for this segment. As noted earlier (60), this segment hosts the recognition sequence for phosphorylation by protein kinase A, which may be facilitated by local structural flexibility.

In summary, this study provides direct spectroscopic information on structural details of AFA- and WT-PLN in complex with SERCA1a in lipid bilayers. We identified for AFA-PLN in complex with SERCA a short N-terminal (V4–R13) and a long C-terminal (Q29–V49) helix intersected by a linker of reduced structural order from R14 to Q22. The C-terminal helix ends at V49. Our spectra corroborate an earlier site-specific observation that the C-terminal helix comprises residues A24–Q26 (29) but additionally show that P21 and Q22 do not constitute another helix turn. Two-dimensional correlation spectra recorded on WT-PLN in complex with SERCA1a exhibit no significant difference with respect to AFA-PLN. Mobility-filtered (1H, 13C) correlation experiments speak against the existence of a significantly populated PLN conformation with dynamics on the micro- to nanosecond time scale after the formation of the complex. A combination of our spectroscopic results with biophysical and biochemical data using flexible protein–protein docking simulations leads to a structural model for AFA-PLN in complex with SERCA1a. While sharing similarities to the model by Toyoshima and co-workers, our model demonstrates that the extended length of the C-terminal α helix, obtained from experimental data, does not violate N-terminal binding near K397′–V402′ and takes into account recent cross-linking results. Furthermore, structural differences seen in the transmembrane helix and the potential influence of protein flexibility around the phosphorylation sequence may provide an important starting point for a more detailed analysis of protein–protein recognition and the regulatory effect of protein kinase A.

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SUPPORTING INFORMATION AVAILABLE

Detailed description of the docking simulation, supplementary spectra for spectral assignments (Figures S1–S4), full spectra for the comparison of mutant and wild-type PLN (Figure S5), and docking templates and HADDOCK interface residues (Figure S6). This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES


