Structural characterization of the intrinsically disordered domain of \textit{Mycobacterium tuberculosis} protein tyrosine kinase A

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Intrinsically disordered proteins (IDPs) are characterized by a lack of stable structure and exist as an ensemble of heterogeneous conformers rapidly fluctuating in solution \[1–4\]. IDPs can adopt a variety of conformational states ranging from collapsed IDPs (molten globule, MG) to extended IDPs (native coil or native pre-molten globule, preMG) \[5,6\]. IDPs are highly abundant in various organisms and they play an important role during cellular regulation \[7\]. Approximately, 3\% of proteins in bacteria are completely disordered. With regards to long disordered segments (> 41 residues) this value reaches 20\%, which is comparatively lower to the 43\% observed in eukaryotes \[8,9\]. In this report, we have investigated a pathogenic protein containing a 27\% disordered N-terminal stretch. Since this protein has to function both in the bacterium and in the human host, the functional role of the disordered domain might be exerted within the host eukaryotes. Many IDPs are related to human diseases and are therefore particular interesting targets for drug-development \[8,10,11\]. Interactions of IDPs with ligands or binding proteins, post-translational modifications (PTMs, phosphorylation or glycosylation) or coordination of various counterions or osmolytes can promote conformational changes including disorder-to-order transitions.

Keywords: intrinsically disordered protein; \textit{Mycobacterium tuberculosis}; nuclear magnetic resonance spectroscopy; protein denaturation; protein phosphorylation; protein tyrosine kinase

Abbreviations
CSP, chemical shift perturbation; eSTPK, eukaryotic-like serine/threonine protein kinase; IDD, intrinsically disordered domain; IDP, intrinsically disordered protein; KCD, kinase core domain; MG, molten globule; MptpA, low molecular weight protein tyrosine phosphatase A; PreMG, pre-molten globule; PtkA, protein tyrosine kinase A; PTM, post-translational modification; TEV, tobacco etch virus.
The high structural flexibility, the plasticity, and the dynamic behavior of IDPs play a central role in their biological function. These properties allow a combination of low affinity and high specificity during binding, potentially promoting interactions with more than one biological partner [12,15,16]. The evolutionary conserved features of IDPs are, however, a challenge for the probing of their structural, dynamical, and functional propensities; all the characteristics which are essential for establishing a structure-function-relationship and for rational drug design. NMR spectroscopy offers a wide range of methodologies for the extensive structural characterization of IDPs [15–19]. Despite the recent improvements of NMR techniques, allowing for better resolution and higher sensitivity, defining the conformational ensemble of IDPs sampled in the solution is still a challenge.

The *Mycobacterium tuberculosis* (*Mtb*) protein tyrosine kinase A (PtkA, 30.6 kDa) contains an N-terminal disordered region of 80 amino acids (intrinsically disordered domain, IDD) connected to the rigidly folded catalytic domain (kinase core domain, KCD). This structural arrangement is unique for bacterial kinases and is supposed to have a potential functional importance in the context of *Mtb* virulence. PtkA which is exclusively found in *Mtb* is a nonconventional tyrosine kinase with autophosphorylation activity [20]. PtkA interacts with the low molecular weight protein tyrosine phosphatase A (MptpA, 17.5 kDa), the critical virulence factor of *Mtb* [21–23]. The exact role of PtkA alone or in combination with its cognate phosphatase MptpA in promoting virulence or pathogenicity of *Mtb* is poorly understood. PtkA undergoes PTM (phosphorylation) in *Mtb* by presenting itself as a substrate for endogenous eukaryotic-like serine/threonine protein kinases (eSTPKs) [24]. In general, PTMs are known to play essential roles in the regulation of IDPs and may influence significantly the modulation of conformational transitions of IDDs.

We have previously determined the NMR solution structure of MptpA [25]. In this successive study, we characterize the conformational behavior of the IDD from the complementary kinase, PtkA (IDD_PtKA), under various conditions and describe the structural changes induced by phosphorylation. Our results indicate that IDD_PtKA exists as an unstructured state under native-like conditions. Moreover, we observe that the conformational behavior of the IDD in isolation is nearly identical to the full-length protein. Interestingly, denaturation studies of IDD_PtKA by pH and GdmCl delineate regions of nonrandom behavior in a consistent manner. Furthermore, we show that phosphorylation of the IDD has a regulatory effect on the enzymatic activity of PtkA.

**Materials and methods**

**Protein expression and purification**

The expression vector pET151/D-TOPO (Invitrogen, Carlsbad, CA, USA) including the His6-tagged tobacco etch virus (TEV) PtkA sequence was provided from the laboratory of Y. Av-Gay (University of British Columbia). The DNA plasmid representing N-terminal PtkA domain the PtkA 1–81_opt_pET-166 was derived from the Gen Script manufacture. The plasmids were transformed into *Escherichia coli* BL21 (DE3) cells for the expression. The unlabelled protein was expressed using LB growth medium and the uniformly 15N- or 13C,15N-labelled protein was expressed in M9 minimal medium using 15NH4Cl (1 g L⁻¹) and 13C-glucose (1 g L⁻¹) as the sole nitrogen and carbon source, respectively. The growth media were supplemented with 1 mM ampicillin and cells were inoculated at 37 °C until an OD600 of 0.6 was reached. The protein expression was induced with 1 mM IPTG after 15 min of incubation on ice-water. The recombinant PtkA was expressed for over 16 h with aeration (120 r.p.m.) at 16 °C. The cells were harvested by centrifugation (4000 g, 45 min, 4 °C). The cell pellet was either flash frozen and stored at −80 °C for later use or resuspended in lysis buffer (PBS-buffer, pH 7.5) supplemented with one EDTA-free protease inhibitor tablet (Roche, Mannheim, Germany). The cells were disrupted for 15 min using M-110P Microfluidizer (15 000 PSI) and the cell lysate was centrifuged (16 000 g, 40 min, 4 °C) to separate the soluble fraction from the cell debris. The supernatant was loaded using loading buffer (pH 8.0) to a 5 mL Ni-NTA HisTrapHP column (GE Healthcare, Uppsala, Sweden). The purification was performed according to the manufacturer’s guidelines. The His6-tag was cleaved by addition of TEV protease overnight during the dialysis at 4 °C in dialysis buffer (pH 8.0) and separated on the Ni-NTA column using elution buffer (pH 8.0). Subsequently, preparative size exclusion chromatography was performed on a HiLoad 26/60 Superdex 75 column (GE Healthcare, Uppsala, Sweden) in NMR buffer (pH 7.5). The presence of protein was confirmed by SDS/PAGE analysis. The fractions containing pure protein were pooled, flash frozen and stored at −80 °C or immediately used for further experimental procedures.

**Luciferase assay**

The autophosphorylation activity of PtkA was determined using a Kinase-Glo® luminescent kinase assay (Promega, Madison, WI, USA); assay protocol see Materials and
Methods, Appendix S1). The luminescence was measured using Veritas™ Microplate Luminometer.

Phosphorylation of PtkA

Phosphorylation of the $^{15}$N-labeled protein (full-length PtkA or KCD$_{PtkA}$ or IDD$_{PtkA}$ construct) was monitored at 298 K on a 600 MHz spectrometer. PtkA (300 μl) resolved in 180 μl of 50 mM HEPES/NaOH buffer (pH 7.5) containing 300 mM NaCl, 10 mM MgCl$_2$, 10 mM DTT, 10 mM ATP and 10% D$_2$O/90% H$_2$O was transferred to the 3 mm NMR-tube. After addition of PKA (4 μl) to the NMR-tube the PtkA phosphorylation was monitored using 2D-(1$^H$,15$^N$)-HSQC experiments recorded every 40 min within 1 day. 30 μl aliquot of the sample were taken for the MS analysis.

NMR spectroscopy

The NMR experiments were performed at 298 K on Bruker spectrometers (either 600-, 700-, 800-, 900-, or 950 MHz) equipped with TXI-HCN cryogenic probe. The spectrometers were locked on D$_2$O and 0.3 mm 2, 2-dimethyl-2-silapentane-5-sulfonic acid was used as an internal standard for spectral referencing. The acquisition and processing of the NMR data was carried out with TopSpin version 3.14 (Bruker Biospin) and analyzed using Sparky version 3.114. All experiments were performed on 300 μM protein samples, either 1$^H$ or 15$^N$, $^{13}$C labeled, in NMR buffer (pH 7.5) using 3 mm NMR-tube. The protocols for the backbone assignment, secondary chemical shift analysis, pH- and GdmCl- titration and the temperature series are provided in the Materials and Methods, Appendix S1.

Further protocols on the phosphorylation of unlabeled full-length PtkA, SEC-MALS, CD and buffer compositions are provided in the Materials and Methods, Appendix S1.

Results

Backbone assignment of IDD$_{PtkA}$ (1–81)

The suitability of full-length PtkA for NMR structural characterization was investigated. The 2D-(1$^H$,15$^N$)-HSQC spectrum of PtkA (Fig. 1A) revealed considerable signal overlap characteristic for a random-coil region in addition to signals with a broad chemical shift dispersion indicative for a folded region. These data suggest that PtkA consists of unfolded regions together with a well-folded core domain. Typically, intrinsically disordered regions exhibit a strong bias in their amino acid sequence. By applying IUPred [26], a tool to predict unstructured regions in proteins, we found a good correlation between the NMR observed presence of unstructured regions and the prediction. IUPred predicts the first 80 amino acids in the N-terminal region of PtkA to be unstructured (Fig. S1B). The CD spectrum of IDD$_{PtkA}$ indeed also shows a random-coil behavior (Fig. S2). These characteristics hamper the unambiguous backbone assignment of the N-terminal domain (Met$^1$-Leu$^{81}$) in PtkA (Fig. 1B). Also, the high abundance of proline residues (13 prolines) and the repetition of the same amino acid patterns complicate the resonance assignment of IDD. We decided to focus our investigations of the isolated IDD$_{PtkA}$ (Met$^1$-Leu$^{81}$, 8.5 kDa). At a pH of 7.5 and 298 K, the 2D-(1$^H$,15$^N$)-HSQC spectrum of the IDD$_{PtkA}$ shows 49 broad signals which corresponds to ~70% of the expected amide resonances (Fig. 1C). The signal line broadening and missing amide cross-peaks could arise either due to the pH, temperature, conformational exchange, solvent exchange or protein oligomerization. In order to assess the pH-effect we first performed a pH titration. We varied the pH value from 7.5 to 2.0, which resulted in a narrowing of the amide signals. The 2D-(1$^H$,15$^N$)-HSQC spectrum of IDD$_{PtkA}$ at pH 2.0 shows 69 well-resolved cross-peaks (Fig. 1C) in the chemical shift region typical for a random-coil protein.

Under these conditions, we were able to assign all the backbone atoms using a set of 3D-NMR experiments (HNCO, HNCA,CB, (H)N(CA)NH, HNHA and 1$^H$,15$^N$-NOESY). The backbone assignments from pH 2.0 were transferred to pH 7.5 by using the pH series of 2D-(1$^H$,15$^N$)-HSQC experiments. Forty seven backbone amide signals of IDD$_{PtkA}$ visible at native-like conditions (pH 7.5) were assigned. The above observations indicate that the IDD$_{PtkA}$ is unstructured under these conditions. To rule out the possibility of oligomerization of IDD$_{PtkA}$, we performed a series of 2D-(1$^H$,15$^N$)-HSQC experiment at concentrations ranging between 300 and 35 μM. We used the proton line-width and the peak intensity at each concentration as reporters (Fig. S3). Our analysis shows no concentration-dependent reduction in the line-width. Moreover, the normalized cross-peak intensity in the series of 2D-(1$^H$,15$^N$)-HSQC spectra are essentially the same, indicating no oligomerization of IDD$_{PtkA}$.

Secondary structure composition of IDD$_{PtkA}$

2D-(1$^H$,15$^N$)-HSQC based backbone analysis suggests similar conformational properties of the IDD in isolation and in full-length PtkA. An overlay of the 2D-(1$^H$,15$^N$)-TROSY/HSQC spectra of full-length PtkA and IDD$_{PtkA}$ measured at pH 7.5 and 298 K shows that the chemical shifts are essentially the same, except for the residues located proximate to the linker region between the IDD and the KCD of PtkA as well as for...
Asn\textsuperscript{48} which could suggest a week interaction between the domains (Fig. S4). Since the backbone amides are prone to chemical exchange with varying pH, we additionally performed a residue specific analysis of the nonexchangeable carbon chemical shifts (C\textalpha{}, C\textbeta{} and CO) for the isolated IDD\textsubscript{PtkA} at pH 2.0 and the full-length PtkA at pH 7.5.

The secondary chemical shift (\Delta\delta) of a certain protein nucleus was calculated as the difference between the observed chemical shift and the corresponding ‘random-coil’ value (Fig. 2). The relationship between the \Delta\delta and the secondary structure elements allows us to distinguish between structured (\alpha{}- helices or \beta{}-strand) and unstructured (random-coil) protein regions [27]. Our results show as expected, a distinct correlation for the large \Delta\delta values of the KCD, indicative for the existence of the secondary structural elements for this core domain. On the other hand, the very small \Delta\delta values for the IDD evidence that there are no clear secondary structural elements, suggesting a random-coil state for this N-terminal domain. In addition, we performed a combined chemical shift analysis using Delta2D [28] and SSP [29]. Both methods clearly illustrate (Fig. S6) the well-defined KCD and the disordered state of the IDD.

**Structural behavior of IDD\textsubscript{PtkA} under different biophysical conditions**

Intrinsically disordered proteins lack defined secondary structural elements under native-like conditions but can adapt a stable fold in a stimulus-dependent manner. Protein-protein interaction, change in pH, temperature or PTMs (phosphorylation) can induce structural changes in the IDPs thereby altering the function of the protein or help during translocation [12,30–32]. We therefore probed the effect of pH, chemical denaturant (guanidine hydrochloride, GdmCl) and phosphorylation on the IDD\textsubscript{PtkA}.

**Effect of pH on IDD\textsubscript{PtkA}**

We monitored the effect of pH (ranging from 7.5 to 2) on the IDD\textsubscript{PtkA} using 2D\textsuperscript{1H,15N}-HSQC spectra. At native-like condition (pH 7.5), 68\% of the amide cross-peaks of the IDD\textsubscript{PtkA} are visible. Missing signals correspond to the amino acid region of Met\textsuperscript{1}-Ser\textsuperscript{3}, Arg\textsuperscript{5}, Ser\textsuperscript{17}, His\textsuperscript{23}-Ser\textsuperscript{29}, Gly\textsuperscript{37}-Gly\textsuperscript{39}, Ser\textsuperscript{57}-Asn\textsuperscript{60}, Gly\textsuperscript{76} and Leu\textsuperscript{81}. When lowering the pH from 7.5 to 5.5, 20 (30\%) additional signals can be observed. A further decrease in the pH to 2.0 resolves all of the 67 (100\%) expected amide cross-peaks (Fig. 3). Beside the (dis)appearance of amide cross-peaks, chemical shift perturbations (CSPs) can be observed due to the change in pH. The strongest CSPs were observed for the residue Asp\textsuperscript{31} and the region Asn\textsuperscript{42}-Ala\textsuperscript{56} of the IDD\textsubscript{PtkA} (Fig. 3B). These residues are adjacent to the regions (His\textsuperscript{23}-Ser\textsuperscript{29}, Gly\textsuperscript{37}-Gly\textsuperscript{39}, Ser\textsuperscript{57}-Asn\textsuperscript{60}) of which the amide cross-peaks are missing at pH 7.5. Chemical shift analysis using the best-fit method shows, that most of the resonances move along a line with changing pH. A slight deviation is observed for...
Asp$^{31}$, Asp$^{47}$, and Asp$^{53}$ in the $^{15}$N-dimension and Ser$^{31}$, His$^{23}$, Asp$^{31}$, Asp$^{47}$, Asp$^{53}$, and Ala$^{56}$ (falling under the line of best-fit) and Thr$^{55}$, Thr$^{33}$, Thr$^{54}$, and Gly$^{49}$ (lying above the best-fit line) in the $^1$H-dimension (Fig. S7). This could be a consequence of different pH-induced effects per residue type or fast exchange between two states, possibly via an intermediate state. In summary, we observe chemical shift changes of the signals together with line-broadening and disappearing peaks during the titration, showing the pH-dependent dynamics of IDD$_{PtkA}$.

**Effect of GdmCl on IDD$_{PtkA}$**

In addition to the pH studies, we also investigated the influence on the IDD$_{PtkA}$ due to chemical induced denaturation. A set of titration experiments combining one or more non-native conditions were
performed using the denaturating agent GdmCl. Denaturation of IDD\textsubscript{PtkA} using increasing concentrations of GdmCl was monitored by 2D-(\textsuperscript{1}H,\textsuperscript{15}N)-HSQCs at pH 7.5 at two different temperatures: 298 K and 283 K (Fig. 4).

The spectra reveal that 80\% of the signals observed at pH 7.5 (298 K) disappear in presence of 4 M GdmCl and reappear again almost completely at higher GdmCl concentration (5 M) as sharp signals, with a slightly different chemical shift (Figs 4 and 5). The most affected amide
cross-peaks correspond to the residues glycine, serine and threonine. For a significant number of residues, the signal intensities increase at lower temperature (283 K) and their linewidths become narrower due to reduced exchange with solvent (Fig. 4). Furthermore, the IDD\textsubscript{PtkA} denaturates completely upon titration with GdmCl at non-native pH 2.0, which further reduces the dispersion of the amide signals (Fig. 6). In contrast to the GdmCl-induced denaturation of IDD\textsubscript{PtkA} at pH 7.5, no signal attenuation was observed. The chemical shift analysis using the best-fit method shows that most of the chemical shifts move along the line during the GdmCl-titration. However, slight deviation in the \textsuperscript{1}H-dimension can be observed for Arg\textsuperscript{18}, Thr\textsuperscript{25}, Ser\textsuperscript{31}, Thr\textsuperscript{32}, Asn\textsuperscript{41}, Arg\textsuperscript{43}, Thr\textsuperscript{54}, and Arg\textsuperscript{67} (Fig. 6B). Altogether, these results suggest that in the presence of denaturant the IDD\textsubscript{PtkA} traverses from a native state to denatured state.

**Effect of the temperature modulation on the backbone amide groups of IDD\textsubscript{PtkA}**

The temperature-dependence of the amide chemical shift can be used to identify those groups involved in hydrogen-bonding. Slowly exchanging amide protons, involved in hydrogen bonding, are characterized by a temperature coefficient more positive than $-4.5 \text{ ppb K}^{-1}$ [33]. To analyze whether some residues of IDD\textsubscript{PtkA} are involved in the hydrogen-bonding, we determined the amide temperature coefficients from a series of 2D-(\textsuperscript{1}H,\textsuperscript{15}N)-HSQC spectra recorded at 301 to 280 K (with 3 K increments). The obtained temperature coefficients range between $-3.6$ to $-9.5 \text{ ppb K}^{-1}$ (Fig. S9). For most residues the temperature coefficients are more negative than $-4.5 \text{ ppb K}^{-1}$, indicating fast exchange with the solvent, except for the C-terminal part of the IDD\textsubscript{PtkA} where the temperature coefficients are fluctuating. Noteworthy, significant signal attenuation was observed for the glycine residues with increasing temperature. At lower temperatures (280–283 K) all five glycine signals of the IDD\textsubscript{PtkA} (Gly\textsuperscript{17}, Gly\textsuperscript{31}, Gly\textsuperscript{41}, Gly\textsuperscript{58}, and Gly\textsuperscript{76}) were visible in the 2D-(\textsuperscript{1}H,\textsuperscript{15}N)-HSQC spectrum, whereas at higher temperatures (298–301 K) only glycine (Gly\textsuperscript{41}) could be detected. Similar effects are also observed for the serine and threonine residues.

**Fig 4.** Treatment of IDD\textsubscript{PtkA} with GdmCl at pH 7.5. 2D-(\textsuperscript{1}H,\textsuperscript{15}N)-HSQC spectra of IDD\textsubscript{PtkA} at pH 7.5 and 298 K (top) and 283 K (bottom), recorded during the titration with GdmCl from 1 M up to 5 M, with 1 M increments. The regions which are most affected by GdmCl are highlighted with boxes.

**Fig 6B.** Cross-peaks identified during the temperature titration of IDD\textsubscript{PtkA} at pH 7.5.
Phosphorylation-induced conformational changes

Threonine-specific phosphorylation of PtkA by *Mtb* endogenous eSTPK was recently reported [34]. Previous studies by Husson *et al.* propose serine/threonine-specific phosphorylation as an important regulatory mechanism of *Mtb* virulence [35]. We investigated the effects of the phosphorylation on the structural properties of the PtkA, KCD<sub>PtkA</sub> and IDD<sub>PtkA</sub> by NMR.

**Fig 5.** GdmCl effect on the backbone amide groups of IDD<sub>PtkA</sub> at pH 7.5. (A) <sup>1</sup>H, <sup>15</sup>N combined CSPs obtained from analysis of 2D-<sup>1</sup>H, <sup>15</sup>N-HSQC spectra of IDD<sub>PtkA</sub> with 0 M vs 5 M GdmCl at pH 7.5, 283 K (top) and 298 K (bottom). Dotted lines correspond to the proline residues; regions with significant CSPs are highlighted in beige. (B) Bars chart showing schematically visible amide cross-peaks of IDD<sub>PtkA</sub> pH 7.5 and 283 K (top) and 298 K (bottom), detected in presence of 0, 2, 4 and 5 M GdmCl.
The phosphorylation was performed using a eukaryotic serine/threonine kinase, protein kinase A (PKA). The 2D-(^1H,^15N)-HSQC spectrum of the full-length PtkA after incubation with ATP and PKA showed time-dependent phosphorylation of PtkA, reflected by the appearance of additional signals (Fig. S10A). MS analysis of full-length PtkA incubated for 24 h with ATP and PKA revealed three-fold phosphorylation of PtkA (Fig. S11). We conducted a similar phosphorylation study on the KCD_PtkA and IDD_PtkA. The primary amino acid sequence of the IDD is highly abundant in serine and threonine residues (13 serine/8 threonine) and therefore presents potential sites for PTM. Our data shows that PKA specifically phosphorylates the IDD_PtkA and not the KCD_PtkA. Furthermore, the amide chemical shifts of the newly appearing signals in the 2D-(^1H,^15N)-HSQC of the IDD_PtkA upon phosphorylation overlap with those observed in the full-length PtkA, indicating that the same residues were phosphorylated (Fig. S10B). Typically, phosphorylation of serine or threonine residues in IDPs results in a large downfield chemical shift changes of their backbone amide signals (Δδ ~ 0.5-1.5 p.p.m. (^1H/^15N) at pH ~ 7.0) [36]. Based on the assignment of the nonphosphorylated protein and by following the disappearing signals upon phosphorylation, the substrate sites can be readily identified. The 2D-(^1H,^15N)-HSQC spectra of IDD_PtkA incubated for various time points with PKA and ATP were compared to the 2D-(^1H,^15N)-HSQC spectrum of the nonphosphorylated protein (Fig. 7). The amide cross-peaks of seven (Ser3, Ser11, Ser17, Ser29, Ser73, Ser74, Ser78) of 13 serines, conserved in the sequence of IDD_PtkA, are visible and assigned at pH 7.5. Based on the signal dissappearance and appearance of new cross-peaks, two (Ser11 and Ser74) of four phosphorylated serines could be identified. For the other two serines, we were not able to follow the same trend due to which they remain unassigned. Furthermore, spectra acquired after longer incubation time (5 days) generated an estimated 11 new additional signals. An overlay of the 2D-(^1H,^15N)-HSQC spectra of IDD_PtkA at pH 2.0, 298 K with 0 M vs 5 M GdmCl in the ^1H-dimension (left) and ^15N-dimension (right). Signals showing deviations from the best-fit analysis are labelled to the corresponding residues. (C) ^1H, ^15N combined CSPs obtained after analysis of 2D-(^1H,^15N)-HSQC spectra of IDD_PtkA at pH 2.0, 298 K (top) with 0 M vs 5 M GdmCl. Dotted-lines correspond to the proline residues; blue bars highlight the regions with significant CSPs. Bar chart schematically showing visible amide cross-peaks of IDD_PtkA at pH 2.0 and 298 K without GdmCl (bottom) and with 5 M GdmCl (top).
in the chemical environment and likewise the partial dynamics of this domain.

**Discussion**

In this study, we have characterized the N-terminal IDD\textsubscript{PtkA} of the unique bacterial tyrosine kinase PtkA from \textit{Mtb}, using NMR spectroscopy. Our results describe the conformational behavior of IDD\textsubscript{PtkA} under native-like and various biophysical conditions. Chemical shift analysis reveals no persistent secondary structural elements, indicating that the IDD\textsubscript{PtkA} is unfolded. Furthermore, the IDD undergoes slow structural fluctuations over different conformational states, either in full-length PtkA or when in isolation. This leads to extensive line-broadening of NMR signals. The amide

![Fig. 7. Phosphorylation of IDD\textsubscript{PtkA} by PKA. (A) 2D-\textsuperscript{1}H,\textsuperscript{15}N-HSQC spectra of IDD\textsubscript{PtkA} without PKA (blue) and time resolved spectra after addition of PKA (black). New signals are labeled in red, signals indicating phosphorylation on serine are framed. (B) overlay of the 2D-\textsuperscript{1}H,\textsuperscript{15}N-HSQC spectra of IDD\textsubscript{PtkA} without PKA (blue) and after 5 days incubation with PKA. Regions with new signals are framed. (C) \textsuperscript{1}H,\textsuperscript{15}N combined CSPs obtained after analysis of 2D-\textsuperscript{1}H,\textsuperscript{15}N-HSQC spectra of IDD\textsubscript{PtkA} with and without PKA. Appearing signals (red), disappearing signals (orange), signals which disappear after addition of PKA and appear during the incubation (blue), prolines are indicated with dotted lines. The identified phosphorylated Ser\textsuperscript{11} and Ser\textsuperscript{74} are highlighted in grey.](image)
In summary, we have shown that the N-terminal domain of PtkA is disordered and undergoes conformational exchange on an intermediate timescale upon varying the environmental conditions such as pH, chemical denaturant, temperature and phosphorylation. Such a dynamic property might be tightly linked to pathogenic virulence.

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**Author contributions**

HS designed and supervised the research. AN wrote the manuscript, performed and analyzed the experiments with the support of SS, HJ and HS. MH, CR, RA and CB performed measurements.

**References**


**Supporting information**

Additional Supporting Information may be found online in the supporting information tab for this article:

- **Fig S1.** Sequence-based analysis of PtkA.
- **Fig S2.** Far-UV circular dichroism spectra of PtkA.
- **Fig S3.** Analysis of the width and intensities of IDD AAA selected cross-peaks from 2D-(1H,15N)-HSQC spectra, measured at different concentrations.
- **Fig S4.** 1H,15N combined CSPs and normalized peak intensities of full-length PtkA and the isolated IDD AAA.
- **Fig S5.** Secondary chemical shifts of the IDD for IDD AAA and full-length PtkA.
- **Fig S6.** Chemical shift analysis of IDD AAA and full-length PtkA performed by using Delta2D and SSP.
- **Fig S7.** pH-effect on the backbone amide groups of IDD AAA.
- **Fig S8.** GdmCl-effect on the backbone amide groups of IDD AAA at pH 7.5.
- **Fig S9.** Temperature dependence of the amide proton chemical shift of IDD AAA.
- **Fig S10.** Phosphorylation of PtkA by PKA.
- **Fig S11.** MALDI spectra of IDD AAA and full-length PtkA before and after phosphorylation with PKA.
- **Fig S12.** IDD AAA regions showing nonrandom behavior.
- **Fig S13.** SEC chromatograms and SEC-MALS.
- **Table S1.** Buffer used for purification of PtkA.
- **Appendix S1.** Materials and methods.