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Uncorrelated Effect of Interdomain Contact on Pin1 Isomerase Activity Reveals Positive Catalytic Cooperativity

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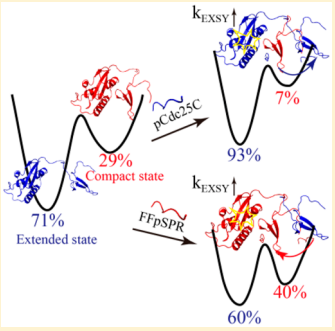
Supporting Information

ABSTRACT: Pin1 is a two-domain peptidyl−prolyl isomerase (PPIase) associated with neurodegeneration and tumorigenesis. The two domains, a WW and a PPIase domain, are connected by a flexible linker, making Pin1 adopt various conformations ranging from compact to extended, wherein Pin1 exhibits different extents of interdomain contact. Previous studies have shown that weakening interdomain contact increases the isomerase activity of Pin1. Here, we propose an NMR chemical shift correlation-analysis-based method that will be general for two-domain proteins to gauge two-state populations of Pin1, and we report a linker-modified mutant of Pin1 with enhanced interdomain contact and increased isomerase activity, with the latter suggesting an uncorrelated effect of interdomain contact on isomerase activity. Thus, although bindings of different substrates in the WW domain impose opposite effects on interdomain contact, in both cases, it may promote isomerization, implying cooperativity between substrate binding in the WW domain and isomerization in the PPIase domain.



Prolyl cis−trans isomerization often acts as a conformational switch between two functional states of a protein and plays a key role in various biological processes.[1,2] The cis−trans isomerization is catalyzed by peptidyl−prolyl isomerases (PPIases).[2] Peptidyl−prolyl cis−trans isomerase NIMA-interacting 1 (Pin1) belongs to the parvulin PPIase family, but unlike other known PPIases, Pin1 exclusively binds to and catalyzes cis−trans isomerization of substrates with phosphorylated Ser/Thr-Pro motifs.[3] In recent years, a number of studies have revealed the roles of Pin1 in diverse biological processes, such as cell cycle,[4,5] neurodegeneration,[6−8] and tumorigenesis,[9,10] and have proposed Pin1 to be a potential therapeutic target for cancer and neurodegenerative disorders.

Human Pin1 consists of a PPIase domain and a WW domain, which are connected by a flexible linker (Figure 1A). The PPIase domain and the WW domain of Pin1 can bind substrates separately, although only the PPIase domain catalyzes cis−trans isomerization of the substrates.[5] Plenty of structures have been solved for Pin1 in both apo and holo forms.[3,11−15] The crystal structures of apo and holo Pin1 display a compact conformation, like a globular protein (Figure 1A), wherein direct interdomain contact between the WW domain and the PPIase domain can be found.[3] On the other hand, the solution NMR structure of Pin1 with Protein Data Bank (PDB) ID of 1NMV shows an extended conformation similar to a dumbbell, as evidenced by the lack of interdomain nuclear Overhauser effects (NOEs) (Figure 1A).[15] Furthermore, NMR spin relaxation and residual dipolar couplings (RDCs) studies have revealed that the WW and the PPIase domain motions are independent to a certain degree and undergo medium- to large-scale interdomain movement,[16] implying that Pin1 in solution exists as an ensemble of different conformations ranging from compact to extended.[15] Although plenty of crystal structures of Pin1 have been determined, it demands more investigation on the interdomain dynamics of Pin1 in solution.

The Pin1 function is regulated by communication between the PPIase domain and the WW domain. The WW domain not only increases the local substrate concentration but also modulates substrate binding and catalysis of the PPIase domain, which relies on the interdomain contact.[5,17,18] As revealed by the crystal structure of Pin1, the interdomain contact involves direct interaction between residues from I28−A31 within the WW domain and residues from D136−P149 within the PPIase domain (Figure 1A). NMR study showed that this interdomain contact is transient in solution and can be affected by substrate binding to the WW domain. For instance, the binding of a 10-residue phosphopeptide pCdc25C from a natural Pin1 substrate, the mitotic phosphatase Cdc25C, weakens the interdomain contact.[18] On the contrary, the binding of FFpSPR, a five-residue artificial substrate from peptide library screening for a minimal sequence with optimal PPIase efficiency, results in enhanced interdomain contact.[17] A molecular dynamics simulation study has suggested that

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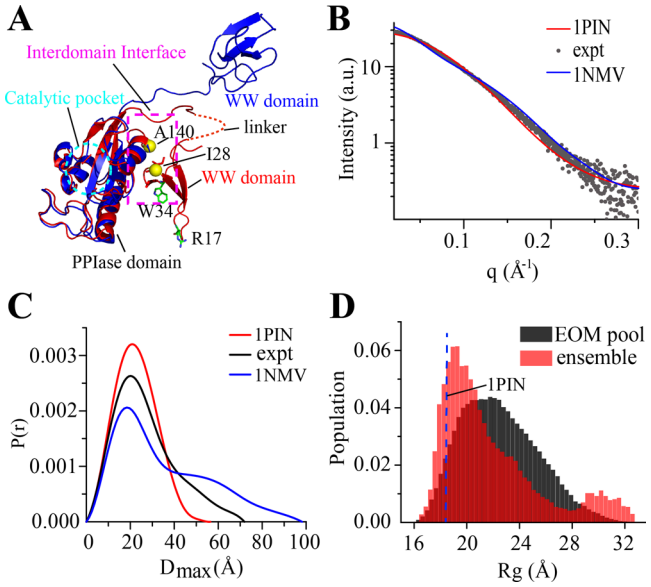

Figure 1. Pin1 adopts a series of conformations ranging from compact to extended. (A) Crystal structure (red, PDB ID: 1PIN) and NMR solution structure (blue, PDB ID: 1NMV). Only the first structure of the NMR structure ensemble is shown for clarity. The interdomain interface and the catalytic pocket are highlighted. I28 and A140, which are key for interdomain interaction, are shown by spheres; R17 and W34, which are essential for substrate binding in the WW domain, are shown with sticks. (B–D) Small-angle X-ray scattering (SAXS) studies of Pin1. (B) Experimental scattering data (gray dots) and theoretical scattering curves of Pin1 derived from 1PIN (red line) and the average structure of the 1NMV ensemble (blue line). (C) Particle distance distribution curves transformed from the experimental scattering data (black line) and theoretical scattering curves in (B). (D) Histograms of the gyration radius (R_g) for the pool generated by the ensemble optimization method (EOM) (black column) and for the selected ensemble (red column). The R_g value of 1PIN is 18.29 Å and is shown with a blue dashed line.

FFpSPR binding to the WW domain results in closure and rigidification of the catalytic loops in the PPIase domain and may thereby affect the isomerase activity.[19] Two pathways have been proposed to mediate this interdomain allosteric regulation, including direct contact between the PPIase domain and the WW domain and indirect communication between the two domains through the bound substrate in the WW domain. Supporting this regulation of isomerase activity through dynamic allostery, a mutation of I28A in the WW domain with weakened interdomain contact shows increased isomerase activity.[18,20] However, whether enhancing interdomain contact will lead to decreased isomerase activity is still not clear. Moreover, considering that Pin1 exists as an ensemble of different conformations that may have different activities, it is important to characterize the ensemble distribution of Pin1, which can reflect the overall extent of interdomain contact and benefit quantitative analysis of the correlation between interdomain contact and isomerase activity. Nevertheless, no method has been reported so far to quantitatively characterize the ensemble distribution of Pin1.

The peak positions from residues in the interdomain interface display a linear pattern in $^{1}H-^{15}N$ HSQC spectra of the isolated PPIase domain and full-length Pin1 in the apo and holo forms, indicating a two-state fast exchange.[18,21] Under the assumption of the two-state fast exchange, the observed chemical shifts are population-averaged chemical shifts of two pure states (e.g., compact and extended states), and state populations can be calculated through chemical shift correlation analysis, which relies on the defined chemical shifts of the two pure states. The chemical shifts of the extended state can be estimated using an isolated domain, such as the isolated PPIase domain of Pin1, because there is no effect from interdomain contact on chemical shifts.[18] However, stabilizing Pin1 in the pure compact state remains a challenge. In this study, we designed two Pin1 constructs that exhibit different extents of compact conformation in solution; these were subsequently employed in quantitatively characterizing the two-state populations of Pin1 through chemical shift correlation analysis and in investigating the effect of enhanced interdomain contact on Pin1 isomerase activity.

Backbone $^{1}D_{NH}$ RDCs and ^{15}N R_1, R_2 values were measured first for the prepared Pin1 sample. The magnitude of the alignment tensor (Da) and the rotational correlation time (τ_c) determined for the individual domains of Pin1 were dramatically different. The Da values fitted from RDCs were 5.2 and 13.4 Hz (Table S1), and the estimated τ_c values derived from ^{15}N R_2/R_1 ratios were 7.9 and 11.2 ns for the WW and the PPIase domains, respectively (Figure S1). These results are consistent with a previous report,[16] confirming that the WW and PPIase domains of the prepared Pin1 sample underwent interdomain movement. Small-angle X-ray scattering (SAXS) experiments were further carried out to characterize the conformation distribution of Pin1. The theoretical SAXS curves for the crystal structure (PDB ID: 1PIN) and the NMR structure (PDB ID: 1NMV) were computed for comparison with the experimental SAXS data. The experimental data of Pin1 did not agree well with the theoretical curves of either 1PIN or 1NMV (Figure 1B). Also, in terms of the particle distance distribution $P(r)$ curves computed from the experimental data, 1PIN and 1NMV exhibited significant divergences (Figure 1C). The D_{max} value of the experimental data was significantly higher than the theoretical value for 1PIN, indicating that Pin1 adopts a relatively extended conformation in solution. The second shoulder, usually related to interdomain distance, was missing in the experimental $P(r)$ curve, which may arise from interdomain dynamics.[22] Furthermore, the ensemble optimization method (EOM)[23] was used to characterize the conformational flexibility of Pin1. Seven models were selected from a pool of 10 000 models generated by EOM as they fitted well with the experimental data, affording a χ^2 value of 0.96 (Figure S2). The populations of the selected ensemble exhibited a broad distribution (Figure 1D), indicating that Pin1 sampled a series of conformations ranging from compact to extended. The conformations close to that of 1PIN constituted only a small proportion of the pool, suggesting that the extended state occupies a greater proportion than the compact state.

As aforementioned, according to the two-state exchange model, the conformational state population of Pin1 can be calculated through chemical shift correlation analysis, but chemical shifts of the pure compact state were still missing. Disulfide trapping has been proven to be effective for stabilizing the compact state of some other multidomain proteins.[24,25] However, this method relies on mutations of interdomain interfacial residues. In the case of Pin1, few residue pairs in the interdomain interface were found to be possibly able to be employed in forming interdomain disulfide bonds. A trial employing I28 and A140 for forming interdomain disulfide bonds resulted in widespread chemical

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shift perturbations and maybe a distorted conformation (Figure S3). Thus, inspired by the crystal structures of Pin1,[3,11−14] we employed a substrate-based method to stabilize the compact state of Pin1 (Figure 2A). First, a leucine (L86) at a distal site to the interdomain interface in the PPIase domain was mutated to cysteine (C86), and subsequently, a maleimide-terminated substrate peptide (KSPTpSPS)[26] that can bind with the WW domain was conjugated to C86 via a Michael addition reaction, which was expected to pull the PPIase and the WW domain together. The single-conjugation product of the substrate peptide to $Pin1_{L86C}$ was confirmed by mass spectrometry analysis (Figure S4). The $^{1}H-^{15}N$ HSQC spectrum of $Pin1_{L86C-substrate}$ was subsequently collected and compared with that of Pin1 (Figure S5). The overlaid $^{1}H-^{15}N$ HSQC spectra of Pin1 and $Pin1_{L86C-substrate}$ exhibited widespread chemical shift perturbations in both the WW and PPIase domains, hinting at the formation of a compact state. As expected, $Pin1_{L86C-substrate}$ exhibited different interdomain dynamics from Pin1. The rotational correlation times and Da values of $Pin1_{L86C-substrate}$ were similar for the two domains (Figure S1 and Table S1), indicating that $Pin1_{L86C-substrate}$ tumbled as a single entity. Subsequently, the alignment tensor parameters were determined by a singular value decomposition (SVD) fitting of the observed RDCs to the crystal structure 1PIN and then used to predict $^{1}D_{NH}$ RDC values for the structure. The experimental RDCs ($^{1}D_{NH}$) of $Pin1_{L86C-substrate}$ were in good agreement with the predicted values (Figure 2C), indicating that $Pin1_{L86C-substrate}$ adopted the same conformation as 1PIN. Moreover, the experimental SAXS data of $Pin1_{L86C-substrate}$ agreed well with the theoretical scattering curve of 1PIN, affording a χ^2 value of 1.30 (Figure 2D). Taken together, this shows that the obtained $Pin1_{L86C-substrate}$ construct adopts a pure compact conformation, the same as 1PIN.

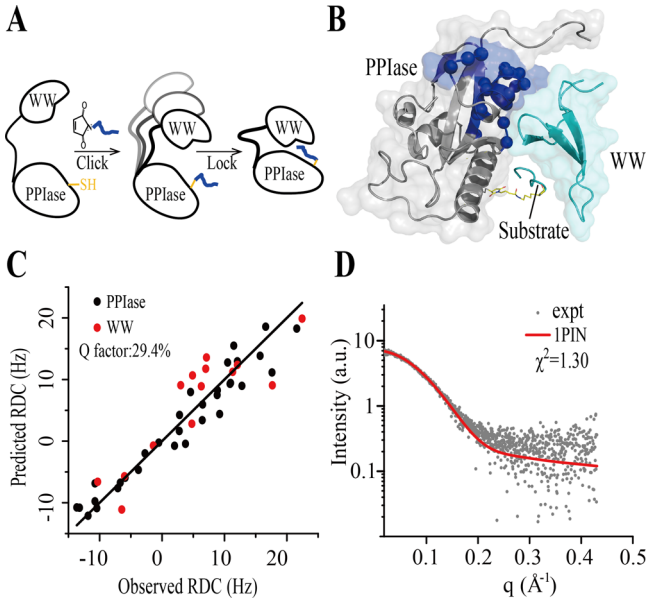

Figure 2. The designed $Pin1_{L86C-substrate}$ construct adopts a compact conformation resembling that of 1PIN. (A) Schematic diagram for stabilizing the pure compact state of Pin1 using a "click and lock" strategy. A solvent-exposed residue (L86) in the PPIase domain away from the interdomain interface was mutated to cysteine (yellow stick); subsequently, the maleimide-terminated substrate peptide interacting with the WW domain would "click" to the PPIase domain via a Michael addition reaction; finally, the binding of the substrate peptide to the WW domain would "lock" Pin1 in a pure compact state. (B) Surface presentation of Pin1 based on the structure 1PIN with residues reporting the equilibrium of two-state exchange highlighted in blue. The covalent linkage between the substrate peptide (cyan loop) and the PPIase domain is depicted by yellow sticks. (C) Fitting of the $^{1}D_{NH}$ RDCs measured for $Pin1_{L86C-substrate}$ to the structure model in (B) with a Q factor of 29.4%. (D) Comparison between the experimental SAXS data of $Pin1_{L86C-substrate}$ (gray points) and theoretical scattering curve computed for the structure 1PIN (red line). Missing loops (N40-G44 and M1-E5) were added and refined by MODELLER,[27] and the substrate peptide was added before calculation.

When comparing the peak positions for the residues of $Pin1_{L86C-substrate}$, Pin1, and the isolated PPIase domain in the interdomain interface, a perfect linearity was found (Figure 3A), indicating that the chemical shifts of $Pin1_{L86C-substrate}$ obey the assumption of two-state fast exchange and can be defined as those of the pure compact state. Chemical shift correlation analysis was then carried out to determine the populations of the two states of Pin1. The plot of $(\delta_{Pin1} - \delta_{PPIase})$ vs $(\delta_{compact} - \delta_{PPIase})$ was expected to be linear with a slope equal to the population of the compact state. Indeed, for residues reporting the equilibrium of the compact vs extended state in the interdomain interface (Figure S6), the plot exhibited good linearity; it afforded a slope of 0.29 ± 0.01 (Figure 3B), indicating a compact state ratio of 29% of Pin1. For cross-validation purposes, a linear combination of SAXS data of

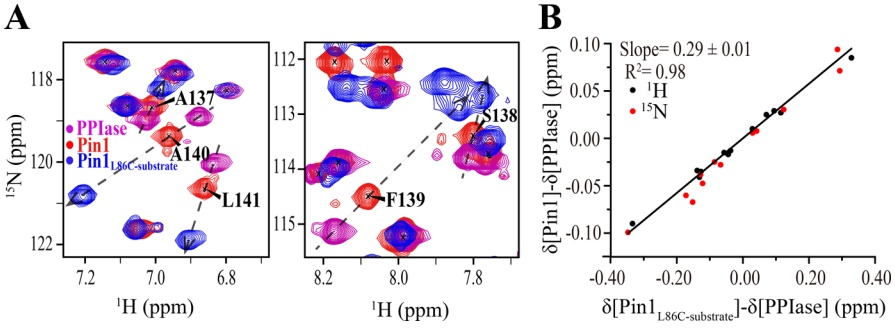

Figure 3. Characterization of the state populations of Pin1 by chemical shift correlation analysis. (A) Overlaid $^{1}H-^{15}N$ HSQC cross-peaks for residues A137−L141 located in the interdomain interface of the isolated PPIase domain (purple), Pin1 (red), and $Pin1_{L86C-substrate}$(blue). (B) Chemical shift correlation plot of $(\delta_{Pin1} - \delta_{PPIase})$ vs $(\delta_{Pin1L86C-substrate} - \delta_{PPIase})$ to gauge the population of the compact state of Pin1, which is equal to the slope of the plot. Black and red circles denote ^{1}H and ^{15}N chemical shifts, respectively, with the latter scaled by 0.154. Residues for correlation analysis were selected from those colored in blue in Figure 2B according to the criterion described in the Supporting Information.

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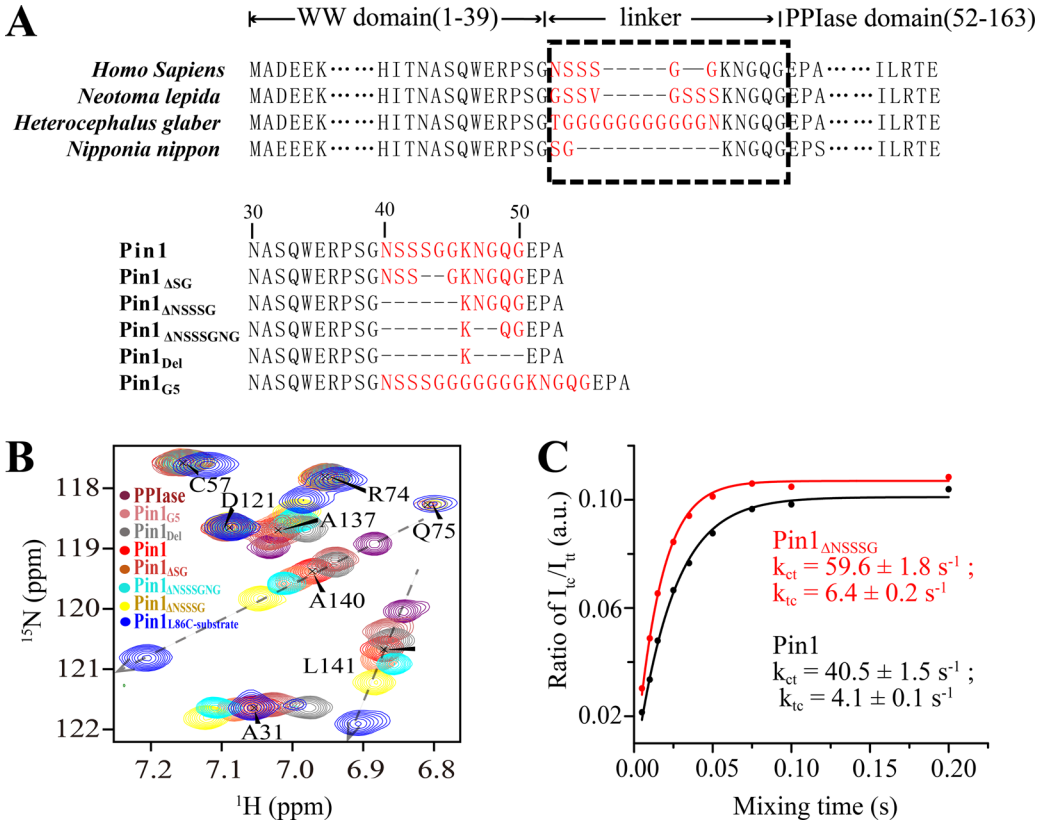

Figure 4. The $Pin1_{\Delta NSSSG}$ mutant with a shortened interdomain linker exhibits enhanced interdomain contact and increased isomerase activity. (A) Top: alignment of a part of Pin1 sequences in different species, wherein the differences are highlighted in red. Bottom: alignment of a part of the sequences for human Pin1 and its mutations with modified interdomain linkers. The residues N30−G53 are shown, and interdomain linkers are highlighted in red. (B) Overlaid $^{1}H-^{15}N$ HSQC cross-peaks for A140 and L141 of the isolated PPIase domain, Pin1, $Pin1_{L86C-substrate}$, and linker-modified mutants $Pin1_{G5}$, $Pin1_{Del}$, $Pin1_{\Delta SG}$, $Pin1_{\Delta NSSSG}$, and $Pin1_{\Delta NSSSGNG}$. (C) Isomerization rates of Pin1 (black) and $Pin1_{\Delta NSSSG}$(red) for pCdc25C determined using $^{1}H-^{1}H$ exchange spectroscopy (EXSY) experiments.

$Pin1_{I28A}$ (extended state) and $Pin1_{L86C-substrate}$ (compact state) at different ratios was compared with the SAXS data of wild-type Pin1 (Table S2). The chemical shifts of $Pin1_{I28A}$ for the residues in the interdomain interface are very close to those of the isolated PPIase domain,[18] and the compact state population in $Pin1_{I28A}$ was determined to be 6% using chemical shift correlation analysis. Thus, $Pin1_{I28A}$ was used as the reference for the extended state in SAXS analysis with calibration because the isolated PPIase domain behaves like a globular protein and is not suitable for SAXS analysis as the extended state of Pin1. The result showed that a combined scattering curve of $Pin1_{L86C-substrate}$ and $Pin1_{I28A}$ at a ratio of 22:78 (final compact state population of 27%) agreed well with the experimental curve of Pin1, affording a χ^2 value of 1.09, supporting the result of the chemical shift correlation analysis.

Having shown that chemical shift correlation analysis employing $Pin1_{L86C-substrate}$ and the isolated PPIase domain can serve as a "conformational ruler" to quantitatively describe the two-state populations of Pin1 (summarized in Table S3), this method was further used to characterize the conformational change of Pin1 triggered by substrate binding (Figure S7). The results showed that the binding of pCdc25C reduced the population of the compact state from 29 to 7%, while the binding of FFpSPR increased the population of the compact state to 40%. As aforementioned, the compact state population of $Pin1_{I28A}$ with weakened interdomain contact was determined to be 6%; nevertheless, there is no known mutation that enhances the interdomain contact of Pin1. Moreover, although $Pin1_{L86C-substrate}$ shows a pure compact state in solution, it is not suitable for isomerase activity analysis as L86C mutation and the conjugation of the substrate peptide to the PPIase domain may have unpredictable effects on the isomerase activity. Thus, a new mutation or construct was still needed to investigate the effect of increasing interdomain contact on isomerase activity.

Modifying the length of the interdomain linker is effective for modulating the interdomain contact of calmodulin.[28] The interdomain linker of Pin1 is disordered and exhibits no interaction with either the PPIase domain or the WW domain and is not evolutionarily conserved in length and sequence (Figure 4A). Thus, changing the length of the linker was expected to affect the interdomain contact without disrupting the structures of the PPIase domain and the WW domain. To this end, several mutants of Pin1 with modified linkers were designed (Figure 4A). Among these mutants, the cross-peaks of $Pin1_{\Delta SG}$, $Pin1_{\Delta NSSSG}$, and $Pin1_{\Delta NSSSGNG}$ moved toward those of $Pin1_{L86C-substrate}$ and were in a linear pattern with those of $Pin1_{L86C-substrate}$, wild-type Pin1, and the isolated PPIase domain (Figure 4B), suggesting more compact conformations of these three mutants. In contrast, the cross-peaks of $Pin1_{G5}$ and $Pin1_{del}$ shifted toward those of the isolated PPIase domain (Figure 4B), suggesting more extended conformations for these two mutants. Overall, $Pin1_{\Delta NSSSG}$ adopted the most compact conformation among the five mutants.

Using chemical shift correlation analysis, the population of the compact state of $Pin1_{\Delta NSSSG}$ was estimated to be 56%,

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about twice that of Pin1, indicating that $Pin1_{\Delta NSSSG}$ can be employed in investigating the effect of increasing interdomain contact on isomerase activity. Subsequently, the isomerase activity of $Pin1_{\Delta NSSSG}$ toward pCdc25C was determined by 2D $^1H-^1H$ exchange spectroscopy (EXSY). Surprisingly, $Pin1_{\Delta NSSSG}$ exhibited a k_{EXSY} value ($k_{ct} + k_{tc}$) of 66.0 s^{-1}, approximately 1.5-fold higher than that exhibited by Pin1 (k_{EXSY} value of 44.6 s^{-1}) (Figure 4C), indicating that increasing interdomain contact can enhance the isomerase activity.

It is worth noting that increased interdomain contact can increase the local substrate concentration and elicit allosteric effects via the second pathway mediated by the bound substrate, as previously reported by Zhou and co-workers.[19] Consistent with this, a previously reported mutant $Pin1_{W34A}$ with impaired substrate binding exhibits lower isomerase activity for pCdc25C than wild-type Pin1;[18] also, its isomerase activity for FFpSPR is lower than that of the wild-type (Figure S8). In order to abolish the substrate binding of the WW domain, a second mutation of R17A was introduced,[26] and the resultant $Pin1_{W34A/R17A}$ showed no significant chemical shift perturbations when titrated with pCdc25C (Figure S9A). $Pin1_{W34A/R17A}$, $Pin1_{W34A}$, and $Pin1_{\Delta SG}$ exhibited similar extents of interdomain contact in the presence of pCdc25C (Figure S9B), but the isomerase activity of $Pin1_{\Delta SG}$ for pCdc25C was higher than that of $Pin1_{W34A/R17A}$ (Figure S9C). Thus, in order to evaluate the effect on isomerase activity exclusively contributed by interdomain contact, the two-site mutation of W34A and R17A was introduced into $Pin1_{\Delta NSSSG}$. The resultant $Pin1_{\Delta NSSSG/W34A/R17A}$ mutant exhibited 3.6-fold higher isomerase activity than the control $Pin1_{W34A/R17A}$(Figure S9D), confirming that increasing interdomain contact can enhance the isomerase activity.

No significant chemical shift perturbations were found for the residues in catalytic sites of the PPIase domain between wild-type Pin1 and $Pin1_{\Delta NSSSG}$ (Figure S10), suggesting that no significant conformational change occurs in these sites. Thus, the increased isomerase activity may result from altered dynamic properties of the catalytic sites, similar to that found in $Pin1_{I28A}$.[18,20] Interestingly, Zhou and co-workers found that the interdomain contact affects the flexibility of catalytic loops. The loss of flexibility of catalytic loops can compensate for the entropy cost due to substrate binding and leads to lower K_M but may impede catalysis, resulting in a lower k_{cat}. According to the reversible Michaelis–Menten equation, if k_{cat} and K_M change in the same direction (e.g., increase or decrease simultaneously) but to different extents, the enzymatic activity (k_{EXSY}) can become higher in both cases. In our study, the effect of interdomain contact on isomerase activity manifests as change in turnover rates uncorrelated with the extent of interdomain contact. As a result, both weakening and increasing interdomain contact would lead to enhanced isomerase activity. Accordingly, it can be speculated that, although the binding of pCdc25C weakens interdomain contact while FFpSPR increases interdomain contact,[16] in both cases it can increase the isomerase activity of Pin1; this is supported by the simulation result that the binding affinity for the FFpSPR in the PPIase domain is enhanced upon binding of FFpSPR to the WW domain.[29] Thus, positive regulation of the isomerase activity through substrate binding by the WW domain can be defined.

Because the binding of pCdc25C weakens interdomain contact while FFpSPR increases interdomain contact, would increasing interdomain contact in $Pin1_{\Delta NSSSG}$ affect the substrate binding in return? To answer this question, we measured the respective binding affinities of Pin1, $Pin1_{\Delta NSSSG}$, and the isolated WW domain for pCdc25C and FFpSPR (Table 1). By comparing the affinity of Pin1 and the isolated WW domain to the substrates, it was found that the retained PPIase domain in Pin1 could increase the affinity for FFpSPR but not for pCdc25C, implying that both domains are involved in the binding of FFpSPR but that the PPIase domain contributes little to the binding of pCdc25C, consistent with the previous study.[30] Hence, changing the conformation of Pin1 to a more compact state may increase the binding affinity. Indeed, $Pin1_{\Delta NSSSG}$ exhibited a higher affinity for FFpSPR than both Pin1 and the isolated WW domain (Table 1). Considering the dynamic equilibrium between the compact and extended states of Pin1 in solution, the binding of FFpSPR will lead to the equilibrium moving to the compact state. Thus, the conclusion can be drawn that because of binding preference for the compact state, FFpSPR binding to Pin1 redistributes the relative population of the two interconverting states, increases the extent of interdomain contact, and ultimately modulates isomerase activity. Accordingly, the average affinity for $Pin1_{\Delta NSSSG}$ can be predicted from binding polynomials[25] based on an allosteric cycle (Figure S11A), as described in the Experimental Methods in the Supporting Information, which employed the determined K_d values of Pin1 and the isolated WW domain and the known compact state populations of Pin1, $Pin1_{\Delta NSSSG}$, and the isolated WW domain (Table 1). The K_d value of $Pin1_{\Delta NSSSG}$ for FFpSPR was predicted to be 145 μM, which agreed well with the experimental value of 139 μM (Table 1 and Figure S11B).

Table 1. Compact State Populations of Pin1 Variants and Their K_d Values for FFpSPR and pCdc25C

	K_d WW (μM)[a]		
variant	FFpSPR	pCdc25C	population of compact state (%)
WW	246.8(15.4)	6.2(2.3)	0
Pin1	184.3(2.7)	6.6(2.2)	29
$Pin1_{\Delta NSSSG}$	139.1(2.0)	7.3(1.7)	56

[a]Substrate binding affinity of the WW domain of Pin1 variants measured through NMR titration.

In summary, we proposed two strategies to alter the ensemble distribution of Pin1 in this study. First, we designed a $Pin1_{L86C-substrate}$ construct that resembles the compact conformation of the crystal structure 1PIN using a substrate-based method. The $Pin1_{L86C-substrate}$ construct can be used in chemical shift correlation analysis together with the isolated PPIase domain or in SAXS analysis with the $Pin1_{I28A}$ mutant to serve as a "conformational ruler" for quantitatively characterizing the conformation distribution of Pin1 and its variants, as well as the conformation change of Pin1 induced by substrate binding; this will facilitate further functional, structural, and dynamic studies of Pin1. This substrate-based strategy may also be introduced to studies for other two-domain proteins. Second, we designed a $Pin1_{\Delta NSSSG}$ mutant of Pin1 with twice the population of the compact state as that of wild-type Pin1 by shortening the interdomain linker. Combined with the study of calmodulin, modification of the interdomain linker seems to be a worthy method to use in an attempt to modulate the conformational dynamics of other two-domain proteins. Surprisingly, the $Pin1_{\Delta NSSSG}$ mutant exhibits higher isomerase activity than wild-type Pin1. Given that $Pin1_{I28A}$ adopts a more

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extended conformation compared to wild-type Pin1 and also exhibits higher isomerase activity, the relationship between interdomain contact and isomerase activity should not be simply correlated. Meanwhile, we showed that changing the length of the interdomain linker can alter the interdomain contact and the isomerase activity of Pin1. Thus, the various lengths of the interdomain linker of Pin1 in different species may confer upon them different isomerase activity levels in vivo. It is known that cis phosphorylated Tau is an early driver of neurodegenerative diseases, and cis amyloid precursor protein may favor Aβ production. Pin1 should be more effective to prevent the accumulation of cis substrates.[6,8] On the other hand, it is necessary to turn down/off the isomerase activity of Pin1 in cancers, wherein it is overexpressed.[9,31] Several orthosteric inhibitors have been developed,[32,33] and the allosteric regulation of the PPIase domain by the WW domain suggests a possibility of developing allosteric drugs for regulating Pin1 activity. Our results in this study provide important information for developing allosteric drugs targeting the interdomain interface to regulate the isomerase activity of Pin1, and we argue that the drug design requires being more cautious.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpclett.9b00052.

Details of experimental methods and supporting tables and figures providing Da values, fitting data, chemical shifts and values of the interfacial residues, NMR spin relaxation results, EOM analysis, stabilization results, mass spectrometry analysis, overlaid $^{1}H-^{15}N$ HSQC spectra, chemical shift perturbations, substrate binding changes, isomerization rates, the thermodynamic cycle of substrate binding, and NMR titration curves (PDF)

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Notes

The authors declare no competing financial interest.

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