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Electrostatically Accelerated Coupled Binding and Folding of Intrinsically Disordered Proteins

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Abstract

Intrinsically disordered proteins (IDPs) are now recognized to be prevalent in biology, and many potential functional benefits have been discussed. However, the frequent requirement of peptide folding in specific interactions of IDPs could impose a kinetic bottleneck, which could be overcome only by efficient folding upon encounter. Intriguingly, existing kinetic data suggest that specific binding of IDPs is generally no slower than that of globular proteins. Here, we exploited the cell cycle regulator p27^{Kip1} (p27) as a model system to understand how IDPs might achieve efficient folding upon encounter for facile recognition. Combining experiments and coarse-grained modeling, we demonstrate that long-range electrostatic interactions between enriched charges on p27 and near its binding site on cyclin A not only enhance the encounter rate (i.e., electrostatic steering), but also promote folding-competent topologies in the encounter complexes, allowing rapid subsequent formation of short-range native interactions en route to the specific complex. In contrast, nonspecific hydrophobic interactions, while hardly affecting the encounter rate, can significantly reduce the efficiency of folding upon encounter and lead to slower binding kinetics. Further analysis of charge distributions in a set of known IDP complexes reveals that, although IDP binding sites tend to be more hydrophobic compared to the rest of the target surface, their vicinities are frequently enriched with charges to complement those on IDPs. This observation suggests that electrostatically accelerated encounter and induced folding might represent a prevalent mechanism for promoting facile IDP recognition.

Introduction

Intrinsically disordered proteins (IDPs) are functional proteins that lack stable tertiary structures under physiological conditions ^{1,2}. They often, but not always, fold into stable structures upon specific binding ³. IDPs are highly prevalent in proteomes, play crucial roles in cellular signaling and regulation, and are associated with numerous diseases ^{4–7}.

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Substantial progress has been made in prediction, identification and general characterization of IDPs ^{8;9}. However, significant challenges exist in detailed structural studies of disordered protein states, limiting the current understanding of specific conformational properties of unbound IDPs such as transient residual structures ¹⁰. Consequently, the physical basis of how intrinsic disorder mediates function remains poorly understood. Nevertheless, the prevalence of intrinsic disorder strongly supports the notion that protein conformational heterogeneity confers crucial functional advantages. This concept has been discussed extensively ^{11–14}.

For cellular signaling and regulation, it is essential to achieve high association and disassociation rates ¹⁵. Nonspecific binding of unstructured and presumably extended conformations of IDPs could increase the capture radius to enhance the encounter rate, up to 1.6 fold ¹⁶. However, such “fly-casting” effect is offset by slower diffusion ¹⁷. Instead, conformational flexibility itself appears more important for binding kinetics. It could reduce the orientational restraints of forming specific complexes and increase the efficiency of peptide folding after initial encounter ^{17–20}. Nonetheless, specific recognition that requires both binding and folding should be no faster than cases where folding is not required. Indeed, a recent dual-transition-rate theory ²¹ predicts that the (diffusion-limited) encounter rate constant represents the upper bound for that of a coupled binding and folding interaction. More importantly, the upper bound can be achieved only if the peptide readily folds upon encounter, which requires folding timescales beyond the μ s “speed limit” estimated for folding of isolated proteins ²². Therefore, intrinsic disorder, while fulfilling certain functional constraints such as structural plasticity, could lead to a folding kinetic bottleneck that must be resolved for IDPs to viably function in signaling and regulation.

Intriguingly, existing kinetic data suggest that IDP binding is generally no slower, if not slightly faster, than that of globular proteins ¹⁷. The implication is that folding does not usually become rate limiting in IDP recognition, or, more specifically, IDPs can achieve efficient folding upon encounter. This concept is consistent with the observation that interacting domains of regulatory IDPs tend to be small and adopt simple folded topologies (i.e., with low contact order) in specific complexes. It is also consistent with an apparent prevalence of induced folding as the baseline mechanism of coupled binding and folding, despite the frequent presence of pre-folded structures in unbound IDPs ^{7;23}. Nevertheless, it is not clear whether and how IDPs may exploit additional physical properties besides size and topology to achieve efficient folding as required for facile coupled binding and folding interactions.

The cyclin-dependent kinase (Cdk) regulators (CKRs) are among the first regulatory IDPs identified ²⁴. They regulate the cell division cycle through direct interaction with Cdk/cyclin complexes ²⁵, and play key roles in transcriptional regulation, cell differentiation, and apoptosis ²⁶. The CKR kinase inhibitory domains (KIDs) fold upon specific binding to Cdk/cyclin complexes ²⁴. A crystal structure of CKR p27^{Kip1} (p27) in complex with Cdk2/cyclin A is available ²⁷ (Fig. 1B), which shows that subdomains 1 and 2 (D1, D2) of p27-KID interact with cyclin A and Cdk2, respectively. A linker helix (LH) connects D1 and D2. This subdomain exhibits partial helicity in the unbound state ²⁸, and provides structural adaptability that is crucial for binding diverse Cdk/cyclin complexes ²⁹. Structural, thermodynamic and kinetic studies together demonstrated that p27 recognized Cdk2/cyclin A via a sequential mechanism, initiated by rapid cyclin binding with an association rate constant of $k_a \sim 10^6 \text{ s}^{-1}$ ³⁰. Subsequent binding to Cdk2 involves significant remodeling of the kinase structure, and is ~ 1000 -fold slower ³⁰. Nonetheless, much remains to be understood on the specific molecular steps involved in binding and folding of p27. Extensive biochemical and biophysical data exist to suggest intriguing complexities ²⁵, and both nascent structures and conformational flexibility have been emphasized for promoting

quick binding^{31–33}. Here, we exploit the CKRs as a model system and combine computation and experiment to investigate how the interplay of residual structures, conformational flexibility and electrostatic interactions of IDPs might support facile recognition and regulation.

Results

Surface electrostatic potential: potential roles of electrostatic forces

Analysis of the surface electrostatic potential of the Cdk2/cyclin A complex (Fig. 1B) shows that several prominent features exist to complement a large number of conserved charges on p27 (Fig. 1A). For example, two large negative patches anchor two pairs of conserved charges on the p27-KID N- and C-termini; a positive patch on cyclin A complements five largely conserved negative charges within the LH N-terminus (Fig. 1C). In contrast, the Cdk2/cyclin A surface opposite to the p27 binding interface lacks any significant electrostatic features (data not shown). The highly evolved nature of the surface electrostatic characteristics strongly suggests key functional roles. Besides neutralizing enriched p27 charges to stabilize the folded state, long-range electrostatic interactions could directly mediate coupled binding and folding. For example, electrostatic force is known to be a dominant long-range force for accelerating protein binding³⁴, and can guide protein orientation in protein-DNA interactions^{35,36}. More importantly, electrostatic force can modulate protein folding³⁷. Well-located complementary charges can enhance the folding rate by promoting productive topologies early in the folding process³⁸ and/or by preventing the protein from accessing (folding) inefficient regions of the free energy landscape³⁹. Conversely, salt-induced premature formation of compact intermediates can slow down folding, over 10,000 fold for the ribosomal protein S6⁴⁰. The conformational ensembles of free IDPs are highly fluctuating and susceptible to weak electrostatic forces⁴¹. It is conceivable that electrostatic forces exerted on enriched p27 charges modulate its coupled binding and folding and promote efficient folding upon encounter as required for facile recognition.

Salt dependence of p27/Cdk2/Cyclin A association kinetics: beyond electrostatic steering

The salt dependence of p27-Cdk2/cyclin A binding thermodynamics and kinetics was probed using isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR) (Fig. S1 and Table 1). The result show that k_a displayed the expected strong salt dependence, decreasing ~12 fold when the ionic strength was increased from 0.075 to 0.6 M. However, binding thermodynamics showed surprisingly small salt dependence. This is likely due to the complex multi-step nature of p27 coupled folding and binding³⁰. Indeed, inspection of the interaction of p27-D1 alone with Cdk2/cyclin A revealed the expected salt-dependence of binding thermodynamics (Table S1). The transient complex theory of protein binding kinetics predicts that the salt dependence of k_a for a simple binding interaction (i.e., without significant protein conformational transitions) can be well described by a Debye-Huckel-like approximation, $\ln k_a = \ln k_{a0} - U_0 / kT / (1 + a \kappa)$. Here, κ is the Debye-Huckel parameter, k_{a0} is the basal rate constant without electrostatic steering, U_0 corresponds to the electrostatic interaction energy of the transient complex, and a is a fitting parameter³⁴. Interestingly, fit of k_a from Table 1 to this empirical linear relation appears problematic, yielding unphysical parameters with a positive U_0 (which suggests an electrostatically destabilized transient complex) (Fig. S2A). Restricting U_0 to be negative leads to a much poorer fit (Fig. S2B). The implication is that the observed electrostatic acceleration of p27 binding to Cdk2/cyclin A arises from a more complex mechanism than simple electrostatic steering.

Coarse-grained modeling of p27 coupled binding and folding

We exploited topology-based coarse-grained modeling⁴² to further understand the mechanism of folding and binding of p27 to Cdk2/cyclin A and how it is modulated by nonspecific electrostatic and hydrophobic interactions. It has been shown previously that the measured fast association kinetics arises from cyclin binding³⁰, which involves both subdomain D1 and the N-terminal segment of LH. LH exhibits a helix break at Met52, which naturally separates the cyclin A and Cdk2 interacting segments (Fig. 1B). Therefore, we included only D1 and the LH N-terminal segment of p27-KID (residues 25–51) and focused on its interaction with cyclin A in the current modeling study. Such a reduced model also avoids unnecessary complications that might arise from the need to properly model the Cdk2 binding step, which involves p27-induced Cdk2 remodeling but does not contribute to the rapid association phase. Starting from the p27/Cdk2/cyclin A structure, two sets of G - like models were constructed (Table 2). These models were carefully calibrated (see Methods), such that they yielded similar dissociation constants (K_D) (Table 2) and residual structures in unbound p27 (Fig. S3). Such calibration is critical⁴³ for ensuring that the model does not inherently favor particular types of interactions, e.g., intra- vs. inter-molecular or native vs. nonspecific electrostatic. Note that the calculated K_D value can be quite sensitive to small changes in the scaling of intermolecular interaction strengths (e.g., a few percent). Coupled with slow binding/folding (especially for uncharged models 1–7), it is computationally challenging to reproduce experimental K_D values even with replica exchange sampling. Nonetheless, by simulating at corresponding melting temperature (T_m), remaining imperfections in the balance of various interactions are further suppressed, allowing reliable investigation of the effects of different native and non-native interactions on p27 binding and folding.

Mechanism of electrostatic acceleration

Kinetic parameters for binding derived from Langevin simulations using the calibrated G - like models recapitulate the salt dependence of k_a from SPR. As shown in Table 2, uncharged models (Models 1–7), which mimic the high-salt condition with fully screened long-range electrostatic interactions, yield p27 binding/folding transition rates of $k_{TS} \sim 2.3$ – $3.6 \mu s^{-1}$, compared with $k_{TS} \sim 20$ – $30 \mu s^{-1}$ for models with explicit charges (Models 8–14), which mimic the low salt condition with unscreened electrostatic interactions. The predicted ~ 10 fold electrostatic acceleration, as reflected in the salt dependence, is in quantitative agreement with SPR. With 0.15 M salt (Model 15), the calculated transition rate is ~ 5 fold slower than that in the no salt case (Model 8). This also compares well with SPR results, even though there appears to be an over-prediction of salt-induced rate reduction. The over prediction is likely due the use of C_α -only models, and might be corrected with more detailed models where charges are placed near the side chain tips³⁷.

Extensive free energy and kinetic analysis was performed to obtain a mechanistic understanding of the observed electrostatic acceleration. As shown in Fig. 2, electrostatic forces substantially reduce the free energy barrier for coupled binding and folding of p27. The predicted ~ 2 kT barrier reduction is apparently consistent with ~ 10 fold rate acceleration derived from Langevin simulations, suggesting that Q_{inter} is a good reaction coordinate. Analysis of free energy surfaces along combinations of various order parameters supports that p27 recognition follows an induced folding-like baseline mechanism, which is arguably necessary for achieving fast binding with $k_a \sim 10^6 M^{-1}s^{-1}$. For example, Fig. 3A shows that Q_{p27} does not increase until $Q_{inter} \sim 0.4$ (dashed line). That is, binding precedes folding. Further examination of the free energy surface along Q_{inter}^{D1} and Q_{inter}^{LH} supports the existence of two parallel pathways (Fig. 3C). Either subdomain D1 or LH can initiate binding through a key intermediate state, marked as I_{D1} and I_{LH} , respectively. Coincidentally, when projected along Q_{inter} and Q_{p27} , I_{D1} and I_{LH} overlap to give rise to a single apparent

intermediate basin centered at $Q_{\text{inter}} \sim 0.4$, $Q_{\text{p27}} \sim 0.3$ (Fig. 3A). Importantly, long-range electrostatic interactions do not alter these basic mechanistic features of p27 coupled binding and folding, except to lower the free energy barriers separating various states (Fig. 3B and D).

Based on the free energy analysis, the binding process was dissected to include an encounter step followed by multiple steps of p27 folding on the cyclin A surface. Conformations sampled during Langevin simulations were assigned to five states: unbound (U), collision complexes (CC), intermediates I_{D1} and I_{LH} , and bound (B) (see Methods for details). The mean first passage times (MFPTs) and numbers of transitions between these states were then calculated to analyze the effects of electrostatic forces on different stages of p27 binding and folding. Representative results from Models 1 and 8, summarized in Table S2, show that long-range electrostatic force reduces the average encounter time from 1.03 ns to 0.26 ns, a ~ 3.8 fold acceleration. At the same time, the probability of advancing to the intermediate and bound states improves from 0.96% to 2.7%, providing an additional ~ 2.8 fold acceleration. Therefore, electrostatic forces enhance the binding kinetics not only by increasing the encounter rate, but also by enhancing the efficiency of folding upon encounter. Such a mechanistic understanding explains why the measured salt-dependence of k_a is not well described by the Debye-Huckel-like approximation (Fig. S2).

Detailed inspection of the conformational properties of the collision complexes provides further insights into the molecular basis for enhanced efficiency of p27 folding upon encounter. Comparison of Fig. 4 A and B shows that long-range electrostatic forces generate a strong free energy gradient that extends over 10 Å away from the native bound position (see dashed lines), such that p27 is more likely to adopt folded-like orientations as it approaches cyclin A. Without electrostatic guidance, p27 encounters cyclin A in largely random orientations (Fig. 4A), and thus cannot fold efficiently before the collision complex dissociates. Furthermore, the distributions of p27 on the cyclin A surface in the collision complexes (Fig. 5A and B) show that nonspecific electrostatic forces also significantly enhance the probability of capturing p27 near the native binding site. Together, these free energy and structural analyses suggest that long-range electrostatic interactions can promote productive topologies during early stages of coupled binding and folding and thus enhance the efficiency of p27 folding upon encounter.

Effects of nonspecific hydrophobic interactions

Recent modeling studies have suggested that weak nonspecific hydrophobic interactions can enhance IDP binding kinetics^{44,45}. For folding and binding of pKID to KIX, short-range nonspecific hydrophobic interactions minimally affected the encounter rate⁴⁴. However, the escape and folding rates of the “encounter state” are both reduced, but importantly, to different degrees, which causes the overall association rate to first increase and then dramatically decrease with stronger nonspecific hydrophobic interactions. A qualitatively similar trend was observed for p27 binding to cyclin A when the charges were not explicitly modeled (Models 1–7 in Table 2): k_{TS} first slightly increases from 3.2 to 3.6 μs^{-1} with $\epsilon_{\text{HP}} = 0.05$ kcal/mol, but then decreases significantly with larger values of ϵ_{HP} , to 2.3 μs^{-1} when $\epsilon_{\text{HP}} = 0.5$ kcal/mol. However, in the pKID/KIX study⁴⁴, the binding rate did not decrease until nonspecific hydrophobic interactions became stronger than native ones. Besides technical differences in the G-like models employed, the dramatic difference in predicted optimal strengths of nonspecific hydrophobic interactions might be attributed to the protein size. Specifically, cyclin A is over three times larger than KIX. Only $\sim 17\%$ of cyclin A surface residues are involved in native contacts with p27, whereas $\sim 30\%$ of KIX surface residues contribute to specific pKID binding. The higher “non-reactive” to “reactive” surface ratio of cyclin A should render p27/cyclin A more sensitive to kinetic trapping from non-discriminative stabilization of encounter complexes. Indeed, nonspecific hydrophobic

interactions do not help recruit p27 to the native binding interface at encounter (e.g., see the similar distributions in Fig. 5 A vs. C and B vs. D), and the efficiency of p27 folding upon binding decreases substantially with $\epsilon_{HP} > 0.1$ kcal/mol after a small initial increase (Fig. 6, black trace).

Intriguingly, when applied together with long-range electrostatic forces, nonspecific hydrophobic interactions appear to be always decelerating, even for very weak ones such as $\epsilon_{HP} = 0.05$ kcal/mol (Table 2). This slowing effect can be mainly attributed to decreasing efficiency of folding upon encounter with larger ϵ_{HP} (Fig. 6, red trace). This result highlights the central role of electrostatic forces in modulating coupled binding and folding. Extensive free energy analysis supports that the mechanistic details of p27 binding and folding are robust against nonspecific hydrophobic interactions. The main effects include elevated free energy barriers (Fig. 2) and slightly stabilized intermediate states (e.g., comparing Fig. 3 and S4). In addition, nonspecific hydrophobic interactions can also generate a weak free energy gradient that extends a few Å from the native binding interface (Fig. 4C), which explains small increases in p27 folding efficiency observed for small values of ϵ_{HP} (Fig. 6).

Nascent structures, conformational flexibility vs. long-range electrostatic forces

A triple alanine mutant of p27 (E40A/D44A/K47A) was previously designed to investigate the role of nascent helices in binding kinetics³². The mutations increased the residual helicity of subdomain LH from ~30% to ~75%. However, the rate of forming inhibited p27/Cdk2/cyclin A complex was reduced ~2–3 fold. This was interpreted as strong evidence for the binding kinetic advantage of intrinsic disorder³². Interestingly, an un-intended effect of this mutant is removal of three key charges within the LH N-terminus, and potential consequences have not been considered. For this, two additional G-like models were designed to determine the differential contributions of nascent helices, conformational flexibility and electrostatic forces (Table 2). In model 16, the residual helicity of LH remained at ~30% and only charges on E40/D44/K47 were removed, allowing the consequence of charge removal to be selectively determined. In Model 17, the residual helicity of LH was re-calibrated to be ~75%, allowing the effects of both helix stabilization and charge removal to be studied. The results show that the charge removal alone reduces k_{TS} from 30.0 μs^{-1} to 22.4 μs^{-1} . Such a significant rate reduction is notable, as the net charge differs only by –1. Stabilization of the LH helix reduces k_{TS} further to 20.1 μs^{-1} . We note that the predicted overall rate reduction is smaller than 2–3 fold determined by the kinase activity assay³². It is plausible that a flexible LH is important for the second, slower step of Cdk2 binding. Nonetheless, these calculations clearly support that both long-range electrostatic forces and conformational flexibility are important for p27 binding kinetics. We note that concerted involvement of flexibility and electrostatic interactions has also been shown to facilitate protein-DNA binding⁴⁶.

Enrichment of charges near IDP binding sites

IDPs are known to be significantly enriched with charged and polar residues⁴⁷. It is probable that electrostatically accelerated binding and folding observed for p27 might be prevalent in IDPs. To investigate this further, we analyzed the surface charge distributions for 51 complexes that involve a single IDP interacting with one or more structured protein partners (see Fig. S5 for a list of the PDB IDs). The results confirm the importance of hydrophobic contacts for intermolecular interactions involving IDPs⁴⁸, manifested as a depletion of charged residues at the binding interface. On average, 32.4% of substrate surface residues are charged, whereas only 27.6% at the IDP binding interface are charged. At the same time, charges are substantially enriched in the vicinity of the IDP binding site, where 34.2% of surface residues are charged. A continuum of electrostatic characteristics is

also evident even within this modest set of IDP complexes. Some IDP-binding interfaces are heavily charged and surrounded by surfaces that are depleted of charged residues (e.g., complexes listed to the right end of Fig. S5). Nonetheless, a majority of these complexes (~60%) appears to possess electrostatic signatures similar to those observed for p27/Cdk2/cyclin A (Fig. 1). The implication is that electrostatic forces likely play a general role in IDP binding and folding and the surface electrostatic features of IDP-binding proteins have evolved to promote facile recognition.

Interestingly, a correlation between charge signatures and binding mechanisms appears to exist. Formation of complexes with charge enrichment near the binding site tends to follow induced folding-like baseline mechanisms. Previously characterized examples include 1dt7⁴⁹, 1jsu³³, 1dpj^{50;51}, 1kdx^{52;53}, 2rnl⁵⁴ and 1cee⁵⁵ (highlighted in red in Fig. S5). Note that complex 1cee (WASP/Cdc42) is the only (weak) exception, with a slight depletion of charges near the WASP binding site. This correlation is consistent with the notion that a key role of long-range electrostatic forces is to promote efficient folding of IDPs upon encounter (that is, induced folding) to achieve fast binding.

Discussion

One of the most important properties of IDPs is arguably that they can readily fluctuate among alternative conformational sub-states, spontaneously or in response to external stimuli such as binding of specific targets¹³. Such conformational flexibility is particularly important for IDPs to overcome the potential kinetic bottleneck due to the requirement of peptide folding for specific recognition^{19;20}. Combining kinetic analysis using SPR, topology-based modeling and statistical analysis, we demonstrate that electrostatic interactions between enriched charges on IDPs and near their binding sites also play a key role in allowing IDPs to overcome the folding bottleneck. Specifically, long-range electrostatic forces do not only accelerate the encounter rate, but could also promote efficient folding upon encounter by modulating the early stages of coupled binding and folding. Importantly, previous studies have suggested that nonspecific (electrostatic) interactions can compete with native ones to destabilize the folded state and/or reduce folding kinetics⁵⁶. These effects could translate into a competition between binding and folding rates as previously predicted in the case of protein-DNA assembly⁵⁷. The observed electrostatic acceleration of folding in the p27/Cyclin A interaction requires sufficient level of self-consistency between charge distribution and folded topology. It is not clear how common IDP complexes possess such self-consistency for nonspecific electrostatic interactions to accelerate both binding and folding, even though our initial charge analysis of known IDP complexes clearly supports a prevalent role of electrostatic forces. Nonetheless, considering that interaction domains of IDPs are often small and adopt simple folded topologies (as required for fast folding), it should not be surprising that strong self-consistency between charge distributions and native folds has been frequently achieved in IDP complexes.

Interestingly, post-translational modifications frequently add or remove charges, and can thus profoundly impact IDP structure and interaction. For example, phosphorylation of p27 Tyr88 and possibly Tyr74^{58;59} can lead to local unbinding and partial activation of p27/Cdk2/cyclin A (see Fig. 1). This allows subsequent intra-complex phosphorylation of p27 Thr187 by Cdk2, to promote p27 proteasomal degradation and allow the cell cycle to advance. An intriguing correlation also can be identified between patterns of charge conservations and multi-specificity of CKRs in Cdk/cyclin binding (Fig. S6). This correlation suggests that a generally conserved, electrostatics-guided binding and folding mechanism exists to mediate interactions of CKR with diverse Cdk/cyclin targets in cell cycle regulation. The mechanistic knowledge on CKR binding-induced folding from the

current study will thus help understand their function and regulation in both normal and malignant cells.

Materials and Methods

Topology-based protein modeling: nonspecific electrostatic and hydrophobic interactions

A C α -based sequenced-flavored G β -like model⁶⁰ was first derived from the p27/Cdk2/cyclin A structure (PDB: 1jsu)²⁷. Details of this model have been described previously²⁷. The model was first reduced to include only cyclin A and the p27 N-terminal segment (residues 25–51; see Fig. 1). The model was then calibrated to balance intrinsic folding and intermolecular interactions using a previously described protocol⁴³. Briefly, the strengths of intra-molecular native contact interaction were first scaled to match the experimental helicity of unbound p27 (~30% in LH)³². The strengths of inter-molecular contacts were then adjusted to reproduce the experimental binding affinity, $K_d \sim 25$ nM³⁰. The final model contains 47 inter-molecular (20 from D1 and 27 from LH) and 17 intra-molecular native contacts. Nonspecific electrostatic interactions are modeled using the Debye-Huckel potential to account for ionic screening³⁷. The dielectric constant is 80. Proper unit charges are assigned to charged residues (Lys, Arg, Glu and Asp) on both p27 and cyclin A. The strengths of native salt-bridge interactions were adjusted to 60% of the original values to avoiding double counting. Nonspecific interactions between all hydrophobic residues (Ala, Cys, Val, Leu, Ile, Met, Phe, Pro, Trp, Tyr) were modeled using 12-6 Lennard-Jones potentials with various strengths (ϵ_{HP} =0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 kcal/mol). Models with different combinations of nonspecific interactions were independently calibrated using the same protocol to reproduce the experimental residual helicity and binding affinity (Table 2).

Simulation and Analysis

The complex was simulated in a 110 Å cubic box with periodic boundary conditions using CHARMM^{61,62}. Langevin dynamics was performed with a dynamic time step of 15 fs and a friction coefficient of 0.1 ps⁻¹. To calibrate intra-molecular interactions, free p27 was simulated at 300 K for 750 ns. Replica exchange (REX) was used to calculate K_d and T_m , performed using the MMTSB Toolset⁶³ with eight replicas spanning 270 to 400 K. The length of calibration REX simulations is 3.15 μ s. For each calibrated model (Table 2), a 22.5 to 30 μ s production simulation was performed at T_m . All free energy (except Fig. 2) and kinetic data shown were calculated from the production simulations. A given native contact is considered formed if the inter-C α distance is no more than 1.0 Å greater than the distance in the native complex. Helicity was calculated as the fraction of 1–5 (backbone) native contacts formed. Temperature weighted histogram analysis method (WHAM)⁶⁴ was used to compute the heat capacity curves and unbiased probability distributions from REX data. The dissociation constants were calculated from the bound and unbound probabilities⁴³, where the unbound state was identified as the one without any native intermolecular contacts formed. To investigate the effects of nonspecific interactions on different steps of coupled binding and folding, five conformational states were defined based on the number of various native and nonspecific contacts formed: U ($N_{inter} < 1$ and $N_{inter}^{ns} < 4$), I_{D1} ($N_{inter}^{D1} > 10$ and $N_{inter}^{LH} < 1$), I_{LH} ($N_{inter}^{D1} < 1$ and $N_{inter}^{LH} > 12$), B ($N_{inter} > 30$), and collision complexes ($N_{inter} < 3$, $N_{inter}^{ns} > 4$ and not assigned to any of the above states). N_{inter}^{ns} is the number of nonspecific inter-molecular contacts, defined when the inter-C α distance is below 10 Å. For charged models, slightly different criteria were used due to small shifts of some free energy basins (e.g., see Fig. 3): I_{D1} ($N_{inter}^{D1} > 9$ and $N_{inter}^{LH} < 1$), I_{LH} ($N_{inter}^{D1} < 1$ and $N_{inter}^{LH} > 10$), B ($N_{inter} > 25$ and $N_{inter}^{D1} > 9$ and $N_{inter}^{LH} > 9$). Using these criteria, <5% of the snapshots were not assigned to

any state and often corresponded to transition paths. All molecular visualization was prepared using VMD⁶⁵.

Sample preparation and procedures for SPR and ITC experiments

Human cyclin A (residues 173–432), full-length Cdk2, p27-KID and p27-D1 were expressed in *E. coli* BL21 (DE3) and purified according to established procedures³⁰. Surface Plasmon resonance (SPR) experiments were performed using a BIACORE 3000 instrument (GE Healthcare, Piscataway, NJ). His-tagged p27-KID was immobilized by direct, chemical capture on a carboxymethyl dextran-coated gold surface (CM4 Chip; GE Healthcare) and the association of Cdk2/cyclin A was monitored in real time. ITC experiments were performed using an Auto-ITC 200 microcalorimeter (GE Healthcare, Piscataway, NJ). His-tagged p27-KID or p27-D1 was titrated into solutions of Cdk2/cyclin A that was previously prepared using gel filtration chromatography. All SPR and ITC experiments were performed in triplicates, and the average values and standard deviations are reported. Details of the SPR and ITC procedures are provided under Supplementary Materials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Efficient folding upon encounter is required for fast IDP association.
- The p27/Cdk2/cyclin A complex contains highly-evolved electrostatics features.
- Long-range electrostatic forces accelerate both encounter and induced folding.
- Nonspecific hydrophobic forces tend to reduce the binding and folding rates.
- IDP binding interfaces are frequently surrounded with enriched charges.

Fig. 1.

A. Sequence alignment of KIDs from three CKRs, The p27 residue numbering is shown, and the locations of three sub-domains (D1, LH and D2) are marked. Conserved residues are highlighted with a pink background. Key charges at the termini and the N-terminal segment are also colored. B. The structure of p27 in complex with Cdk2/cyclin A (PDB: 1jsu²⁷). The sub-domains D1 (residues 25–36), LH (residues 37–60) and D2 (residues 62–90) are colored in blue, yellow and green, respectively. All charged side chains of p27 are shown in sticks. Cdk2/cyclin A is shown in molecular surface and colored based on the surface electrostatic potential calculated without p27 using CHARMM PBEQ⁶⁶ (negative: red; positive: blue). C. A close-up view of the N-terminal half of p27 on cyclin A with key p27 charges labeled.

Fig. 2.

Free energy as a function of the fraction of native inter-molecular contacts formed (Q_{inter}) at 300 K for four representative models: 1 (black), 7 (green), 8 (red), and 14 (blue) (see Table 2). These profiles were calculated from the REX simulations using WHAM. The error bars are not shown for the blue and green traces for clarity.

Fig. 3.

Free energy surfaces as a function of various fractions of native contacts computed using G-like models with (Model 8) and without (Model 1) explicit charges. Q_{inter}^{D1} and Q_{inter}^{LH} are the fractions of native inter-molecular contacts form by sub-domains D1 and LH, respectively. Q_{p27} is the fraction of intra-molecular contacts formed by p27. All surfaces were shifted such that the bound state is at zero. Contours are drawn every kT. Dashed lines in panels A and C indicate the minimal free energy paths. Panel E show two representative ensembles of I_{D1} and I_{LH} with cyclin A, p27-D1 and p27-LH colored grey, cyan and yellow, respectively.

Fig. 4.

Free energy surfaces of p27 coupled binding and folding as a function of p27-cyclin separation distance, defined as the distance between the centers of mass of p27 and all p27-contacting residues of cyclin A, and p27 orientation, defined using its principal axis with respect to the native bound conformation. Contours are drawn every kT, and dashed lines in panels A and B indicate the minimal free energy paths.

Fig. 5. Distributions of p27 on the cyclin A surface in the collision complexes. Cyclin A is colored based on the probability of each residue in contact with p27 in the collision complex ensembles. The conformation of p27 in the native complex is shown in gray trace as a reference.

Fig. 6.

Efficiency of p27 folding upon binding with increasing strength of nonspecific hydrophobic interactions. The red trace is for models with explicit charges and the black trace is for models without explicit charges. The folding efficiency was calculated as the probability of advancing to the intermediate and bound states (I_{D1} , I_{LH} , and B) from the collision complex (CC).

Table 1

Salt-dependence of p27-Cdk2/cyclin A binding kinetics and thermodynamics.

NaCl (mM)	k_a ($10^6\text{M}^{-1}\text{s}^{-1}$)	K_D (nM)	ΔG (kcal/mol)	ΔH (kcal/mol)	$-T\Delta S$ (kcal/mol)
75	10.6 ± 0.1	1.1 ± 0.6	-12.3 ± 0.3	-50.3 ± 3.6	$+38.0 \pm 3.6$
150	3.21 ± 0.01	1.3 ± 0.8	-12.2 ± 0.4	-49.5 ± 1.6	$+37.3 \pm 1.8$
300	1.26 ± 0.01	0.5 ± 0.6	-12.9 ± 0.7	-51.0 ± 4.2	$+38.0 \pm 4.3$
600	0.894 ± 0.001	0.6 ± 0.3	-12.7 ± 0.5	-48.7 ± 3.3	$+36.0 \pm 3.7$
1000	-	0.6 ± 0.4	-12.6 ± 0.3	-45.4 ± 1.8	$+32.8 \pm 2.1$

The Gibbs free energy of binding ΔG was calculated from the dissociation constant, $\Delta G = RT \ln K_D$, and the binding entropy from the binding free energy and enthalpy as $-T\Delta S = \Delta G - \Delta H$.

Table 2

Calculated dissociation constants, melting temperatures and mean folding transition rates of all coarse-grained models of p27/cyclin A investigated in this study.

Model	Charges	eHP	K_D (nM)	T_m (K)	k_{TS} (μs^{-1})
1	None	0.0	154 ± 192	323	3.2 ± 0.4
2	None	0.05	6.24	327	3.6 ± 0.1
3	None	0.1	16	327	2.9 ± 0.4
4	None	0.2	242	325	2.5 ± 0.3
5	None	0.3	117	325	2.5 ± 0.04
6	None	0.4	20	325	2.6 ± 0.3
7	None	0.5	27	323	2.3 ± 0.4
8	Explicit	0.0	300 ± 148	325	30.0 ± 3.1
9	Explicit	0.05	68	330	28.1 ± 0.9
10	Explicit	0.1	86	327	23.7 ± 0.3
11	Explicit	0.2	174	327	23.4 ± 1.6
12	Explicit	0.3	83	327	23.6 ± 1.9
13	Explicit	0.4	148	325	21.3 ± 2.5
14	Explicit	0.5	153	325	20.9 ± 0.7
15	Explicit; 0.15 M salt	0.0	192	325	5.4 ± 0.6
16	Explicit; (Mutant)	0.0	98	325	22.4 ± 4.9
17	Explicit; (Mutant)	0.0	236	323	20.1 ± 1.1

K_D was calculated from REX simulations (experimental $K_D = 25 \pm 2.7$ nM (29)). k_{TS} was calculated from production Langevin simulations at the corresponding T_m as $k_{TS} = N_{TS}/t_{tot}$, where N_{TS} is the number of reversible p27 binding and folding transitions observed during t_{tot} . Models 16 and 17 were calibrated to yield ~30% and ~75% residual LH helicity in the free state, respectively. All uncertainties were estimated from differences between results calculated from the first and second halves of the data.