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Competition between Nucleic Acids and Intrinsically Disordered Regions within Proteins

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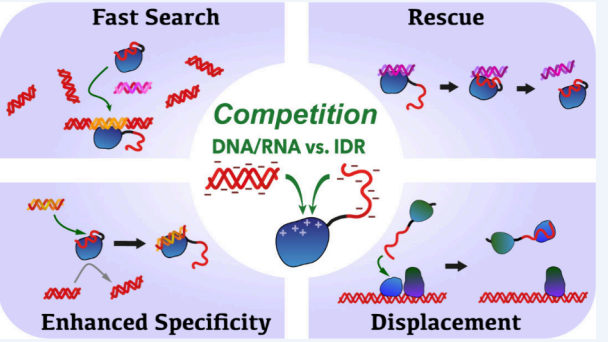
CONSPECTUS: Intrinsically disordered regions (IDRs) are important components of protein functionality, with their charge distribution serving as a key factor in determining their roles. Notably, many proteins possess IDRs that are highly negatively charged, characterized by sequences that are rich in aspartate (D) or glutamate (E) residues. Bioinformatic analyses indicate that negatively charged, low-complexity IDRs are significantly more common than their positively charged counterparts rich in arginine (R) or lysine (K). For instance, sequences of 10 or more consecutive negatively charged residues (D or E) are present in 268 human proteins. In contrast, the corresponding sequences of 10 or more consecutive positively charged residues (K or R) are present in only 12 human proteins. Interestingly, about 50% of proteins containing D/E tracts function as DNA-binding or RNA-binding proteins. Negatively charged IDRs can electrostatically mimic nucleic acids and dynamically compete with them for DNA-binding domains (DBDs) or RNA-binding domains (RBDs) that are positively charged. This leads to a phenomenon known as autoinhibition, in which the negatively charged IDRs inhibit binding to nucleic acids by occupying the binding interfaces within the proteins through intramolecular interactions.



Rather than merely reducing binding activity, negatively charged IDRs offer significant advantages for the function of DNA/RNA-binding proteins. The dynamic competition between negatively charged IDRs and nucleic acids can accelerate the target search processes for these proteins. When a protein encounters DNA or RNA, the electrostatic repulsion force between the nucleic acids and the negatively charged IDRs can trigger conformational changes that allow the nucleic acids to access DBDs or RBDs. Additionally, when proteins are trapped at high-affinity nontarget sites on DNA or RNA ("decoys"), the electrostatic repulsion from the negatively charged IDRs can rescue the proteins from these traps. Negatively charged IDRs act as gatekeepers, rejecting nonspecific ligands while allowing the target to access the molecular interfaces of DBDs or RBDs, which increases binding specificity. These IDRs can also promote proper protein folding, facilitate chromatin remodeling by displacing other proteins bound to DNA, and influence phase separation, affecting local pH. The functions of negatively charged IDRs can be regulated through protein−protein interactions, post-translational modifications, and proteolytic processing. These characteristics can be harnessed as tools for protein engineering. Some frame-shift mutations that convert negatively charged IDRs into positively charged ones are linked to human diseases. Therefore, it is crucial to understand the physicochemical properties and functional roles of negatively charged IDRs that compete with nucleic acids.

KEY REFERENCES

- Yu, B.; Wang, X.; Tan, K. N.; Iwahara, J. Influence of an intrinsically disordered region on protein domains revealed by NMR-based electrostatic potential measurements. *J. Am. Chem. Soc.* **2024**, *146*, 14922−14926.[1] *D/E tracts and DNA show similar electrostatic properties and compete for the DNA-binding domains. The competition promotes the dissociation of the protein−DNA complex and influences the molecular behavior of the HMGB1 protein.*
- Wang, X.; Bigman, L.; Greenblatt, H. M.; Yu, B.; Levy, Y.; Iwahara, J. Negatively charged, intrinsically disordered regions can accelerate target search by DNA-binding proteins. *Nucleic Acids Res.* **2023**, *51*, 4701−4712.[2] *D/E tracts allow DNA-binding proteins to avoid distractions of decoys. This effect, along with an induced-fit mechanism, accelerates the target search process.*

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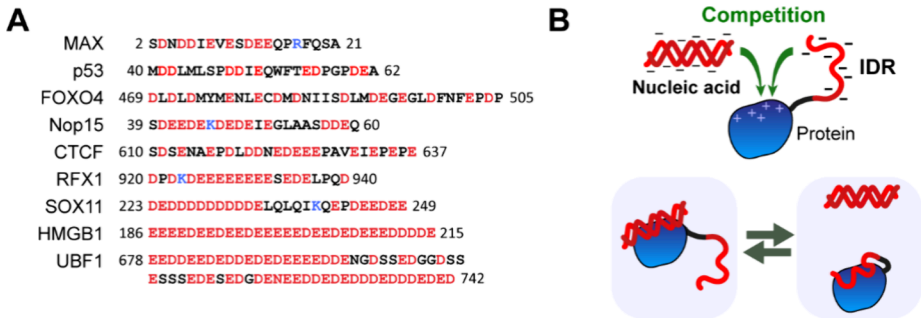

Figure 1. Competition between nucleic acids and negatively charged IDRs within DNA/RNA-binding proteins. (A) Examples of negatively charged IDRs that cause autoinhibition through interactions with DNA-binding domains (DBDs) or RNA-binding domains (RDBs) within the same protein molecules.[9,15−24,26−28,35] Acidic residues are indicated in red. (B) Competition between nucleic acids and negatively charged IDRs causes autoinhibition.

- Bigman, L. S.; Iwahara, J.; Levy, Y. Negatively charged disordered regions are prevalent and functionally important across proteomes. *J. Mol. Biol.* **2022**, *434*, 167660.[3] *This bioinformatics study revealed that D/E tracts are generally longer than K/R tracts and are more common in eukaryotic proteins.*

INTRODUCTION

Protein−nucleic acid interactions are essential for life. Proteins interact dynamically with nucleic acids in processes such as gene regulation, transcription, DNA replication, repair, and recombination. Our understanding of the structural aspects of these processes has significantly advanced in the 21st century. However, the dynamic aspects, particularly those involving intrinsically disordered regions (IDRs), remain largely elusive. Many DNA- and RNA-binding proteins contain IDRs, which are conformationally flexible yet play a crucial role in influencing protein function.[4−7] Some of these IDRs are highly negatively charged, containing a large number of aspartate and glutamate residues (Figure 1A). Because of their electrostatic similarity, these negatively charged IDRs dynamically compete with nucleic acids for binding to DNA-binding domains (DBDs) or RNA-binding domains (RBDs). This competition between nucleic acids and negatively charged IDRs significantly affects the kinetic and thermodynamic properties of DNA/RNA-binding proteins as well as their overall functions. In this Account, we explain the impacts and mechanisms of these effects and highlight the disease relevance of negatively charged IDRs, as well as their potential biotechnological applications.

AUTOINHIBITION VIA NEGATIVELY CHARGED IDRs

Typically, the molecular interfaces of DBDs and RBDs are positively charged, which enhances the electrostatic attractive force to interact with negatively charged nucleic acids. When negatively charged IDRs are present in the same polypeptide chain, they can electrostatically interact with the DBDs or RBDs and block access to nucleic acids. This effect, autoinhibition, causes a decrease in the apparent binding affinity for nucleic acids. Autoinhibition via negatively charged IDRs has been found in many DNA- and RNA-binding proteins.[8] The conformational flexibility of the inhibitory IDRs promotes switching between the inactive autoinhibited and active uninhibited states. Autoinhibition via negatively charged IDRs is particularly prevalent in DNA-binding proteins. For instance, CTCF,[9] FOXO3/O4,[10−13] HMGA2,[14] HMGB1,[15−19] MYC/MAX,[20,21] p53,[22−25] RFX1,[26] Sox11,[27] and UBF[28] undergo autoinhibition via negatively charged IDRs. Some RNA-binding proteins, such as FBF-2,[29] G3BP1,[30,31] Hfq,[32−34] Nop15,[35] and SLBP,[36] regulate RNA binding affinity and specificity in a similar manner. These negatively charged IDRs electrostatically mimic nucleic acids, competing for positively charged DBDs or RBDs within the same polypeptide chain (Figure 1B). Consequently, the apparent binding affinity becomes lower than that of isolated DBDs or RBDs. The impacts of D/E tracts on the binding affinity depend on the lengths of the D/E tracts and their positioning with respect to the DBDs/RBDs.[2,16−18,35]

D/E TRACTS AS PREVALENT INHIBITORY IDRs OF DNA/RNA-BINDING PROTEINS

As seen in Figure 1A, many negatively charged IDRs contain consecutive residues of aspartate (D) or glutamate (E). Hereafter, following the terminology of Pappu and coworkers,[37] we refer to them as D/E tracts, although some other terms such as D/E repeats,[1−3,38] D/E-rich repeats,[39] electronegative clusters,[35,36] and polyD/E[40] were also used in the literature. At physiological pH, the D/E tracts are highly negatively charged. Computational studies show that polyD or polyE tend to adopt an extended conformation due to electrostatic repulsion, depending on pH and the concentrations of salts and divalent cations.[41,42] Several groups have investigated the occurrence of D/E tracts in DNA/RNA-binding proteins and entire proteomes.[3,35,38,39,43,44] These bioinformatic studies across proteomes revealed some unexpected features of the D/E tracts. For example, compared to positively charged K/R tracts, D/E tracts are more prevalent in the proteome and tend to be remarkably longer.[3] As many as ∼50% of the proteins containing D/E tracts are DNA- or RNA-binding proteins.[3] Hundreds of proteins possess D/E tracts in each mammalian proteome. For instance, D/E tracts of 10 or more consecutive residues are found in 268 human proteins. Our current Account covers not only D/E tracts but also D/E-rich regions (as seen in Figure 1A), as these negatively charged IDRs are likely to behave similarly.

The human HMGB1 protein contains a long (30-residue) D/E tract at the C-terminus. This protein could serve as a general model for the study of the autoinhibitory effect of D/E tracts. Using NMR spectroscopy, our group and others investigated the electrostatic aspects of autoinhibition involving the D/E tract for the HMGB1 protein.[1,16−19] At physiological ionic strength, the D/E tract electrostatically interacts with the DNA-binding domains (i.e., the A-box and B-box). Due to this effect, the full-

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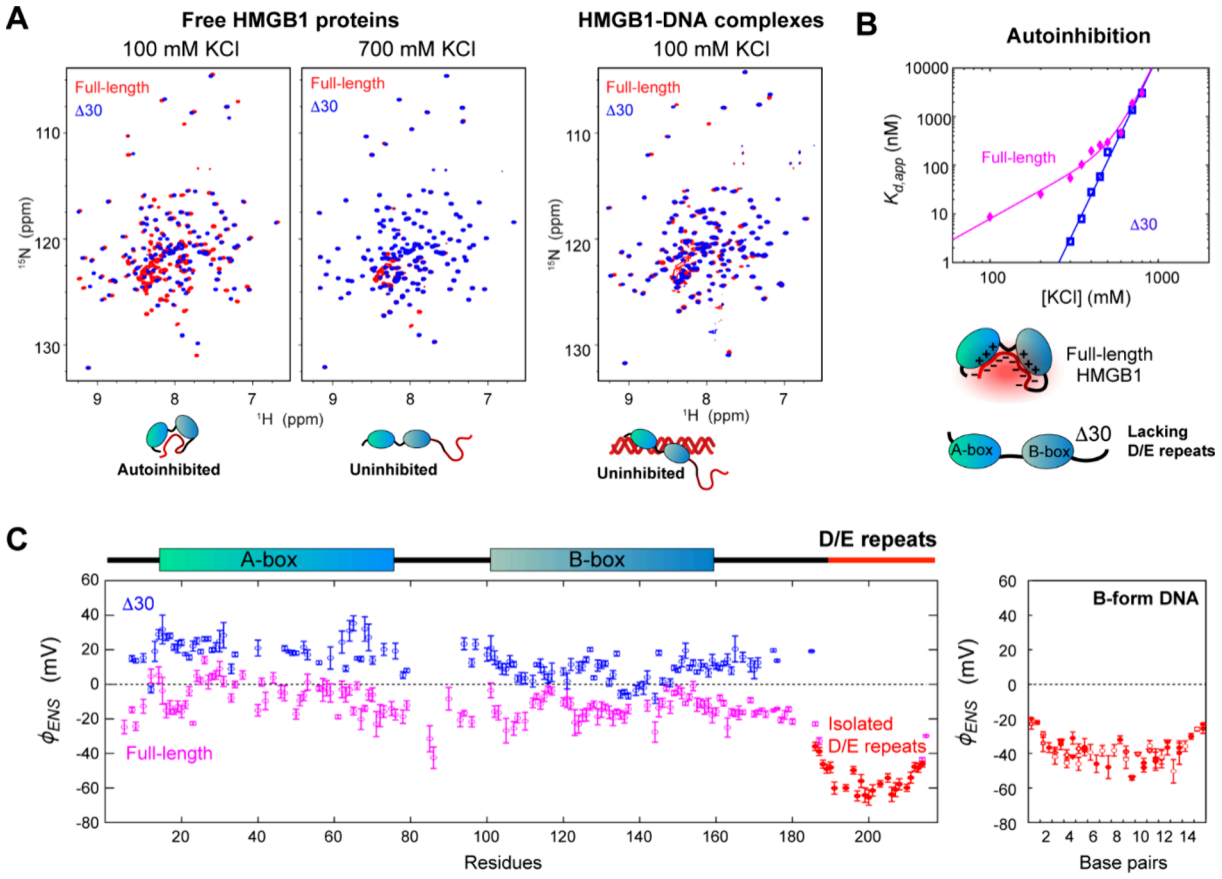

Figure 2. Electrostatic influence of a D/E tract on DNA-binding domains. (A) NMR spectra recorded for the full-length HMGB1 (containing the D/E tract at the C-terminus) and its Δ30 variant lacking the D/E tract in the free and DNA-bound states.[19] Due to the electrostatic interactions between the D/E tract and the DNA-binding domains, the spectra for the free state are significantly different between the full-length and Δ30 variant proteins in 100 mM KCl. However, such differences are not observed in 700 mM KCl, where the electrostatic interactions are weak. For the complexes with DNA, the two proteins are both in the uninhibited state and therefore the spectra are similar even in 100 mM KCl. (B) The dissociate constants K_d measured for the complexes of cisplatin-modified DNA with the full-length HMGB1 protein or the Δ30 variant.[19] Note that at physiological ionic strength, the affinity is remarkably weaker for the full-length HMGB1 protein due to the electrostatic interactions between the D/E tract and the DNA-binding domains. (C) Effective near-surface electrostatic potentials (ϕ_{ENS}) measured for the full-length HMGB1 protein, the Δ30 variant, the isolated D/E tract, and 15-bp DNA in 100 mM NaCl through paramagnetic NMR experiments.[1,45]

length HMGB1 protein and its Δ30 variant lacking the D/E tract exhibit significantly different NMR spectra in the free state (Figure 2A). These intramolecular interactions cause autoinhibition, making the binding affinity for DNA significantly weaker (Figure 2B).[19] Using a paramagnetic NMR method that allows for direct measurements of local electrostatic potentials around biomolecules,[45−48] we also measured effective near-surface electrostatic potentials (ϕ_{ENS}) for the full-length HMGB1 protein, the Δ30 variant (lacking the D/E tract), and the isolated D/E tract (Figure 2C).[1] Due to the interactions between the D/E tract and the DNA-binding domains, local electrostatic potentials of the DNA-binding domains within the full-length HMGB1 protein were largely negative, despite the presence of many positively charged residues. Interestingly, the near-surface electrostatic potentials around the D/E tract were even more negative than those around DNA.[1] This may be qualitatively understandable by considering charge per molecular weight (D/E tract, ca. −0.008e per Da; DNA, ca. −0.003e per Da). The D/E tract of HMGB1 clearly exerts electrostatic influences on the positively charged DNA-binding domains, causing autoinhibition.

WHAT ARE THE ADVANTAGES OF HAVING NEGATIVELY CHARGED IDRs?

A conventional notion of autoinhibition is that this self-imposed inhibition ensures proteins remain inactive under normal conditions and become activated only in response to specific signals.[49,50] Intramolecular interactions can directly or allosterically block binding to the target molecule, thereby regulating enzymatic activity and molecular assembly, which is crucial for protein function. In some cases, post-translational modifications, such as phosphorylation, regulate autoinhibition and alter protein activities through a conformational change between the autoinhibited and uninhibited states. This molecular switch involving autoinhibition is essential for proteins that should become active only when cells receive specific stimuli.

However, highly negatively charged IDRs such as D/E tracts do not appear suitable for molecular switches. Since neither aspartate nor glutamate is typically modified, the autoinhibition via D/E tracts involving multivalent fuzzy electrostatic interactions seems difficult to disrupt through post-translational modifications. During evolution, if a protein's binding affinity for DNA or RNA needs to remain consistently weak, regardless of cellular demands, this could be more easily achieved through mutations in a few key residues within the DBD or RDB rather

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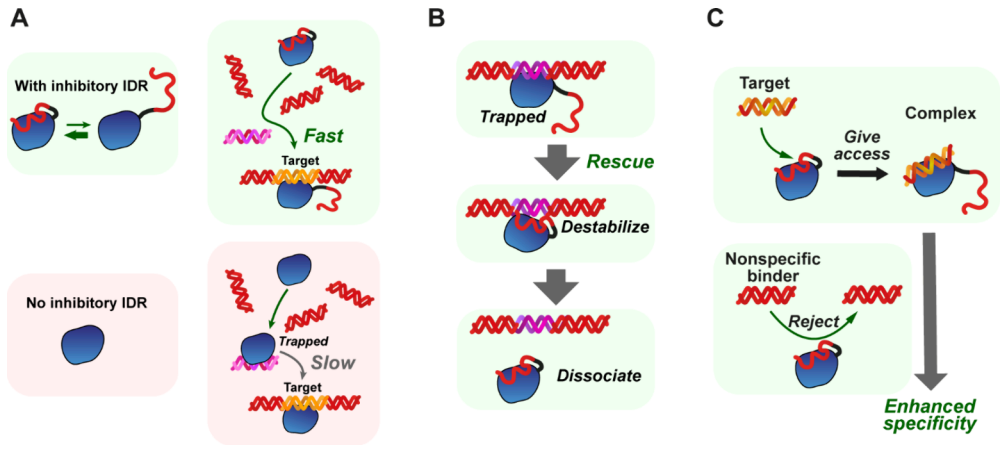

Figure 3. Positive impacts of negatively charged IDRs on the functions of DNA/RNA-binding proteins. (A) Acceleration of target search kinetics in the presence of nonfunctional high-affinity sites ("decoys").[2] This effect can be explained quantitatively by kinetic models incorporating the conformational transitions and the competition between negatively charged IDRs and nucleic acids.[2] (B) Rescue of the DBD or RBD trapped at a wrong site. Through competition with nucleic acid, the negatively charged IDR destabilizes the protein−DNA/RNA complex, promoting dissociation of the protein.[1] (C) Enhancement of specificity in binding. The negatively charged IDRs serve as gatekeepers that reject nonspecific binders but allow the target to access the molecular interface of the DBD or RDB.

than through establishing autoinhibition via the IDR. What advantages do D/E tracts and other negatively charged IDRs provide beyond serving as a device to reduce the binding affinity of DNA/RNA-binding proteins?

As described below, recent studies have provided important insights into the answer to this question. Through competition with nucleic acids, negatively charged IDRs indeed have positive effects on protein functions. In the following sections, the advantages of competition between nucleic acids and negatively charged IDRs are discussed.

Accelerating Target Search Kinetics

Our recent study revealed an unprecedented role of negatively charged IDRs in target search kinetics (Figure 3A).[2] Using the HMGB1 protein as a model system, we demonstrated that D/E tracts can allow DNA-binding proteins to search for their target more efficiently. In principle, DNA binding proteins, such as transcription factors and DNA-repair/modifying enzymes, perform their functions by recognizing specific DNA sequences or structural signatures as their targets. However, locating specific sites among billions of base pairs in the genome may be inefficient. Numerous nonspecific sites are structurally similar to the targets and can act as decoys that effectively sequester the DNA-binding proteins and preclude them from binding to their actual targets.[51] In the overwhelming presence of natural decoys, the intramolecular interactions between DBDs and negatively charged IDRs will significantly reduce the risk of sequestration and thereby accelerate target search. Due to this effect of the D/E tract, the full-length HMGB1 protein can more rapidly associate with the target sites on DNA in the presence of abundant decoys than the HMGB1 variant lacking the 30-residue D/E tract.[2]

It should be noted that avoiding the distraction of decoys is insufficient to accelerate target search kinetics. Our kinetic modeling and coarse-grained simulations highlighted the importance of an induced-fit mechanism that enables the rapid transition from the autoinhibited state to the uninhibited state when the protein encounters its target.[2] The electrostatic repulsion from DNA could be the driving force for such a conformational change. At target sites, DNA interacts specifically with the DNA-binding domains, pushing the negatively charged IDR away from the binding interfaces and thereby forming uninhibited complexes. The kinetic/thermodynamic models, the rate equations, and the analytical expression for approximate solutions are described in ref 2.

Rescuing Proteins Trapped at Wrong Sites

Dynamic competition between negatively charged IDRs and nucleic acids can also promote the dissociation of a protein trapped at a wrong site on DNA or RNA. Recently, we found that the D/E tract of HMGB1 accelerates the dissociation of the protein from DNA by a factor of ∼5−20.[1] Similar effects that accelerate the dissociation kinetics were also found for transcription factor MYC:MAX[20] and the FBF-2 protein.[29] Due to their conformational flexibility, these negatively charged IDRs may reach and attack the molecular interfaces with DNA and RNA, electrostatically destabilizing protein−DNA/RNA interactions (Figure 3B). By promoting dissociation through competition with nucleic acids, the negatively charged IDRs may help rescue the proteins trapped at the wrong site during the target search process. Such mobilization of the proteins bound to nonspecific DNA/RNA could be important for DNA/RNA-binding proteins, for which the speed of the specific complex formation is more crucial than the stability of the complex.

Enhancing Specificity in Molecular Association

Dynamic competition between nucleic acids and negatively charged IDRs can also enhance the binding specificity of DNA- and RNA-binding proteins (Figure 3C). In general, specific binding to the target DNA or RNA involves hydrogen bonding, base-specific interactions, and shape complementarity in addition to electrostatic interactions, whereas nonspecific binding to DNA or RNA is dominated by electrostatic interactions.[52,53] Due to such differences, nontarget sites are more susceptible to the inhibitory effect of negatively charged IDRs. In the competition between DNA and the inhibitory IDR for a DBD, the high-affinity target can easily defeat the IDR and bind to the DBD, whereas low-affinity nontarget DNA can easily lose to the IDR. In this manner, negatively charged IDRs can serve as gatekeepers that limit access to the DBDs or the RDBs, thereby increasing the binding specificity.

For example, Schütz et al.[20] investigated the binding of the MAX homodimer to its target E-Box DNA and nontarget DNA.

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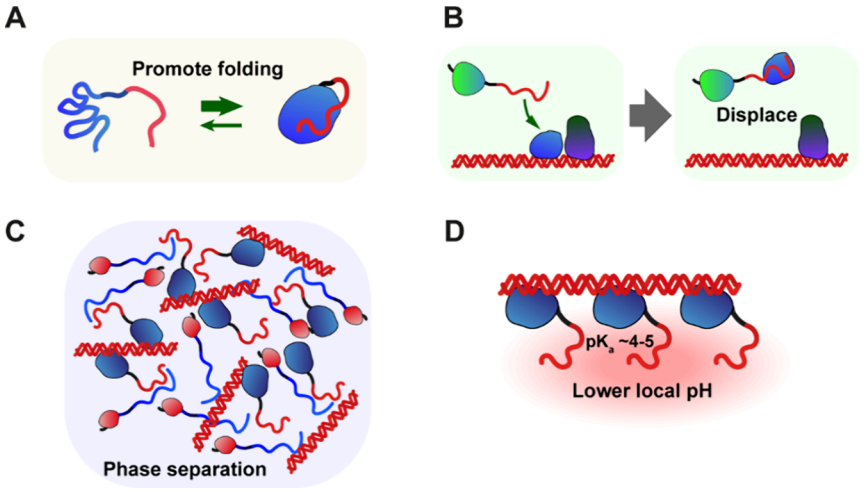

Figure 4. Other roles of negatively charged IDRs. (A) Assisting protein folding.[35,36,40,54] (B) Displacing a protein from a complex with DNA. This activity involves protein−protein interactions. The displacement process is particularly relevant to histone chaperones.[55] (C) Playing a role as charge blocks in phase separation.[37,56−58] (D) Lowering the local pH.[37,59]

For full-length protein, the binding affinity for the target DNA was ∼50-fold stronger than that for nontarget DNA. By contrast, when the same experiments were performed for the variant lacking the inhibitory IDR, the affinities were different between the target and nontarget DNA duplexes only by a factor of ∼4, although both of them were stronger than the affinities of the full-length protein. Similar effects of negatively charged IDRs were also observed for other DNA-binding proteins such as FOXO4,[11] p53,[22,23] and SOX11.[27] For RNA-binding proteins, enhancement of RNA-binding selectivity by negatively charged IDRs was reported for Hfq and Nop15.[34,35] Zhang and co-workers found that D/E tracts can stabilize the neighboring RNA-binding motif in Nop15 and inhibit its nonspecific RNA binding.[31] Through bioinformatics analysis of D/E tracts, they found that halophiles have a smaller number of D/E tracts, possibly due to the high salt concentration that suppresses nonspecific RNA binding. Those studies of DNA/RNA-binding proteins highlighted the general role of negatively charged IDRs in enhancing binding specificity.

Promoting Protein Folding

Some D/E tracts are known to promote protein folding (Figure 4A). As demonstrated by Zhang and co-workers, D/E tracts not only stabilize the structure of neighboring RBDs but also promote the folding of unstructured RBDs.[35,36] Schütz et al. proposed a similar effect for the MYC:MAX complex.[20] In the case of HMGB1, the D/E tract enhances the thermal stability of one of its two folded DNA-binding domains.[54] The effects of these nucleic acid-like IDRs on DNA/RNA-binding domains may not be surprising, since folding is often coupled to binding.[60] Interestingly, the impact of D/E tracts on protein folding is not limited to DBDs and RBDs within the same polypeptide chains. Huang et al. demonstrated that DAXX can act as a molecular chaperone, disaggregase, and unfoldase, relying on its D/E tract.[40] They showed that other D/E-tract-containing proteins, such as ANP32A and SET, also exhibit similar activities.

Displacement of Other Proteins Bound to DNA

Negatively charged IDRs can compete with DNA not only for the DBD within the same protein molecule but also for the DBDs of another protein molecule. Such competition can promote the displacement of the protein from a complex with DNA (Figure 4B). This effect was proposed for the interplay between HMGB1 and histone H1.[61,62] The electrostatic interactions between the D/E tract of HMGB1 and the basic tail of histone H1 could destabilize the histone H1−nucleosome complex and facilitate displacement of histone H1, impacting the accessibility of chromatin. The D/E tract of HMGB1 is also required for the HMGB1 protein to facilitate nucleosome remodeling.[63]

Recent studies show that competition between DNA and negatively charged IDRs is also important for the function of histone chaperones, a group of proteins that facilitate chromatin assembly, disassembly, and remodeling.[55] Although histone chaperones are structurally diverse, they commonly contain long, negatively charged IDRs.[55] Highly negatively charged IDRs of histone chaperones can dynamically bind to the highly positively charged histone proteins, shielding them from nonspecific interactions, facilitating their deposition onto DNA, and assisting in their eviction from DNA. Examples of such interactions include ProTα with histone H1,[64−66] Npm2 and Nap1 with H2A/H2B,[67] and the FACT complex subunit SPT16 with H2A/H2B.[68] Negatively charged IDRs of histone chaperones may act on the histone−DNA complexes, electrostatically destabilize them, and help evict the histone molecules from DNA by transiently capturing them.

Roles of D/E Tracts in Phase Separation

Through interplay with nucleic acids, negatively charged IDRs also play a role in phase separation (Figure 4C). The formation of biomolecular condensates is crucial for the spatial and temporal organization of molecules within cells, enhancing efficiency and specificity in biochemical reactions.[69,70] Both experimental and computational studies suggest that “charge blocks” of negatively charged segments and positively charged segments along the polypeptide chain can strongly induce phase separation and play fundamental roles in its spatiotemporal regulation.[37,56−58] Patterned charge blocks facilitate selective partitioning, thereby enabling the functional specificity of a particular condensate. The regulatory roles of negatively charged IDRs in the phase separation of DNA/RNA-binding proteins have also been suggested. For example, a negatively charged IDR of the MYC protein controls its condensation and prevents irreversible aggregation.[71] The D/E tracts of

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nucleophosmin (NPM1) and its phosphorylation regulate the fluidity of heterotypic droplets of NPM1 and RNA.[72]

Recently, Pappu and co-workers proposed the role of D/E tracts as proton carriers, which lower the local pH within nucleolar condensates and create a pH gradient between nucleolus and nucleoplasm (Figure 4D).[37,59] While both DNA/RNA (phosphate diester $pK_a \sim 1$) and D/E tracts ($pK_a \sim 4-5$)[73,74] are negatively charged at physiological pH, only D/E tracts could have significant buffer action due to their pK_a closer to physiological pH. The pH gradient may facilitate specific enzymatic activity and molecular transport and eventually influence nucleolar functions. This effect might also explain why DNA i-motif structures can be formed in vivo despite their stable formation typically requiring pH < 7 in vitro.[75,76]

REGULATION OF NEGATIVELY CHARGED IDRs

Intramolecular interactions involving negatively charged IDRs can be regulated via some distinct mechanisms. For example, interactions with another protein can release autoinhibition, thereby enhancing the association of molecular with nucleic acids (Figure 5A). The autoinhibition of the transcription factor FOXO4 via its negatively charged IDR is released through molecular association with β-catenin, enhancing the transcription activity of FOXO4.[13] In the absence of β-catenin, the negatively charged IDR interacts with the DBD within the FOXO4 polypeptide chain. When this IDR interacts with β-catenin, the DBD becomes uninhibited and increases access to DNA. Similarly, the autoinhibition of the RNA-binding protein FBF-2 is released through molecular association with the LST-1 protein.[29]

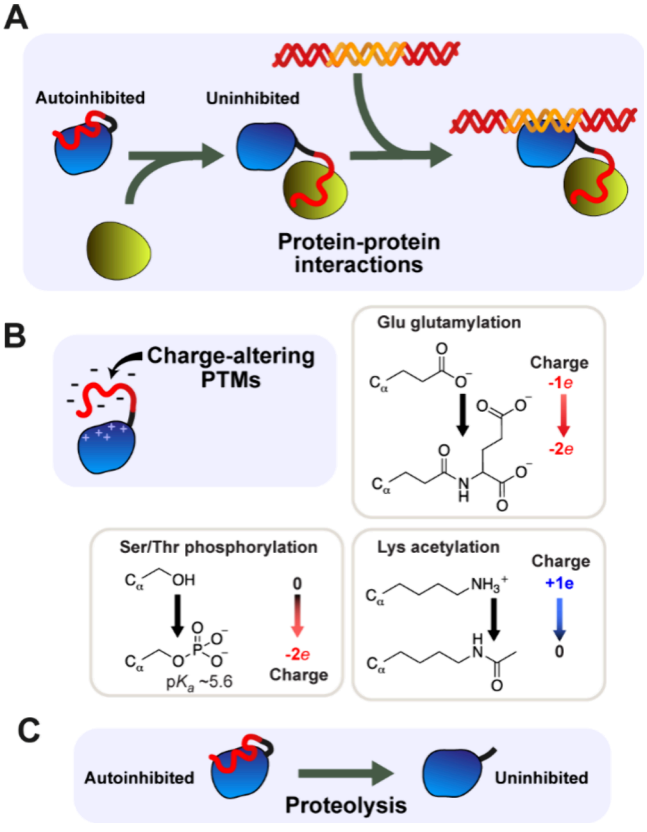

Figure 5. Autoinhibition via negatively charged IDRs can be regulated. (A) Protein−protein interactions can release the autoinhibition.[13,29] (B) PTMs that can impact electrostatically driven autoinhibition involving IDRs.[19,24,67] (C) Proteolytic removal of the inhibitory IDR can eliminate autoinhibition.[77,78]

For some proteins, post-translational modifications (PTMs) can either counteract or reinforce the electrostatic interactions between the negatively charged IDR and the DBD or RBD by altering the charge (Figure 5B). Although glutamate or aspartate PTMs are relatively rare, glutamylation of glutamate residues was reported for the negatively charged IDRs of the histone chaperones Npm2 and Nap1.[67] Lorton et al. showed that this PTM increases the negative charge and enhances the DNA mimicry of these proteins.[67] More canonical PTMs, such as serine/threonine phosphorylation and lysine acetylation, can also modulate autoinhibition involving negatively charged IDRs. For example, phosphorylation of the N-terminal IDR of p53 increases the negative charge and thereby enhances electrostatic interactions between the negatively charged IDR and the positively charged DBD.[24] Acetylation of the cluster of five lysine residues adjacent to the D/E tract in HMGB1 eliminates the positive charge of the lysine cluster and impact the autoinhibition of HMGB1.[19]

Autoinhibition via an IDR could also be regulated via proteolytic processing (Figure 5C).[77,78] Through the removal of the inhibitory IDR, the proteins become uninhibited and exhibit a higher binding affinity. For example, the extracellular HMGB1 protein can be processed via DNA-mediated proteolysis by neutrophil elastase, which cleaves off the C-terminal IDR containing the D/E tract of HMGB1. This proteolytic processing remarkably enhances HMGB1's affinity for DNA and TLR4·MD-2 complex.[78]

RELEVANCE TO HUMAN DISEASES

Given the various effects of D/E tracts on protein functions, one may speculate that abnormalities in these negatively charged IDRs could play a role in the pathogenesis of human diseases. In fact, frameshift mutations of the negatively charged IDR of HMGB1 have been identified as the cause of brachyphalangy, polydactyly, and tibial aplasia syndrome (BPTAS), a rare complex malformation syndrome.[79] The underlying mechanism whereby these mutations cause BPTAS is surprising. The frameshift mutations convert the D/E tracts into highly positively charged segments with clusters of arginine (R) and lysine (K) residues (Figure 6). This charge reversal leads to abnormal phase separation, causing the aberrant partitioning of HMGB1 into the nucleolus.[79] For the HMGB3 and calreticulin (CALR) proteins, the frameshift mutations resulting in charge reversal of their C-terminal D/E tracts also cause aberrant partitioning in the cells, which is associated with microphthalmia and myelofibrosis, respectively.[79]

NEGATIVELY CHARGED IDRs AS A TOOL FOR PROTEIN ENGINEERING

Negatively charged IDRs can potentially serve as a valuable tool for protein engineering, which facilitates the alteration of the kinetic and thermodynamic properties of proteins. We have demonstrated such an application for the Antp homeodomain to which an artificial D/E-repeat tag (DERT) was attached (Figure 7A).[2] The engineered proteins exhibited artificial autoinhibition against DNA binding. The target search kinetics in the presence of decoys was remarkably accelerated when the length of the DERT was in a certain range. If the DERT is too long, autoinhibition may be too strong and may not accelerate the

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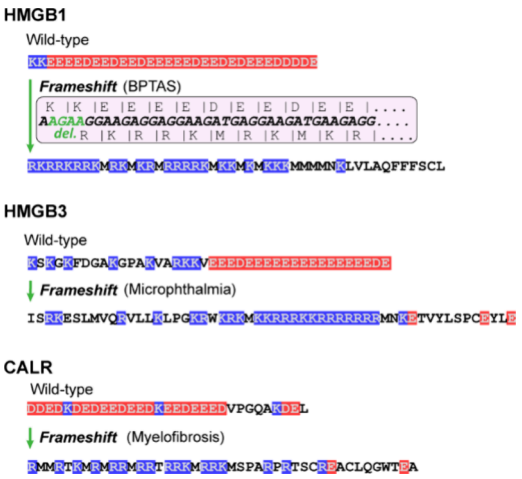

Figure 6. Disease-associated frameshift mutations that cause a charge reversal of a highly negatively charged C-terminal IDR into a highly positively charged IDR.[79] These frameshift mutations in the HMGB1, HMGB3, and CALR genes are associated with BPTAS, microphthalmia, and myelofibrosis, respectively.

target search process (Figure 7B). If the DERT is too short, autoinhibition may be insufficient. Thus, optimization may be required to achieve the desired outcome. Coarse-grained molecular dynamics (CGMD) simulations provide helpful guidance. In the case of the artificial constructs of the Antp homeodomain, the CGMD data demonstrated that when the D/E tract is too long, the conformational transition from the autoinhibited state to the uninhibited state becomes too slow (Figure 7C).[2] Since natural D/E tracts as inhibitory segments are also present in various enzymes and signaling proteins (e.g., PLCβ3 and N-WASP),[80,81] applications of D/E tracts could extend beyond DNA-/RNA-binding proteins and may serve as a general approach to alter the kinetic and thermodynamic properties of proteins.

CONCLUSIONS AND FUTURE PERSPECTIVES

Negatively charged IDRs compete with nucleic acids and have various effects on DNA- and RNA-binding proteins. An important kinetic effect is that these nucleic acid-like IDRs allow the proteins to rapidly reach their targets despite the presence of numerous decoys (i.e., high-affinity but nonfunctional sites) in the cellular environment. These IDRs can help rescue proteins that are trapped in decoys by destabilizing the protein−DNA/RNA interfaces and promoting their dissociation. Moreover, negatively charged IDRs can act as gatekeepers that reject weak-affinity ligands while allowing the target to access DBDs/RBDs, thereby enhancing the specificity in the molecular association. The electrostatic interactions involving negatively charged IDRs also promote protein folding as well as the disassembly, reassembly, and remodeling of chromatin. Furthermore, these IDRs are involved in phase separation and can lower the local pH in biomolecular condensates. Autoinhibition through these IDRs can be controlled by other proteins, which may directly interact with the IDRs or cleave the inhibitory regions. Given these characteristics, negatively charged IDRs hold potential for applications in protein engineering. Furthermore, frameshift mutations that convert the negatively charged IDRs into positively charged IDRs are associated with several human diseases. Further biophysical and biochemical research will enhance our understanding of these

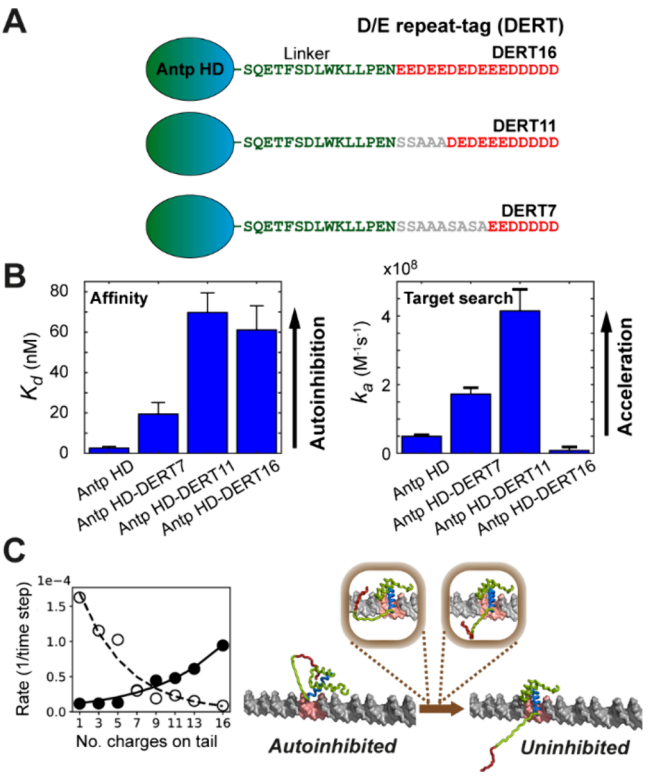

Figure 7. Negatively charged IDRs could be useful for protein engineering. (A) Protein constructs of the Antp homeodomain (HD) with a D/E-repeat tag (DERT). (B) Impacts of D/E tracts on binding affinity and target search kinetics. Note that the impacts depend on the DERT length. (C) Coarse-grained molecular dynamics (CGMD) simulations of the target search process by the Antp HD-DERT proteins. The graph in this panel shows the rates of arrival at the DNA target site by either the autoinhibited or uninhibited states (closed circles) and the rates for the conformational transitions from the autoinhibited state to the uninhibited state at the target (open circles). Note that the conformational transitions become slow when the DERT length is too long. The results from the CGMD simulations explain why the target search kinetics for the Antp HD-DERT11 construct are faster than those for the Antp HD-DERT16 construct. The data were adopted from Wang et al.[2]

nucleic acid-like IDRs and facilitate their biotechnological applications.

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Notes

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