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Article

Role of Electron Spin, Chirality, and Charge Dynamics in Promoting the Persistence of Nascent Nucleic Acid−Peptide Complexes

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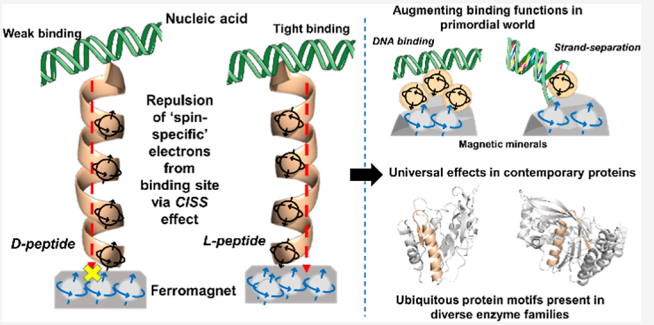
ABSTRACT: Primitive nucleic acids and peptides likely collaborated in early biochemistry. What forces drove their interactions and how did these forces shape the properties of primitive complexes? We investigated how two model primordial polypeptides associate with DNA. When peptides were coupled to a ferromagnetic substrate, DNA binding depended on the substrate's magnetic moment orientation. Reversing the magnetic field nearly abolished binding despite complementary charges. Inverting the peptide chirality or just the cysteine residue reversed this effect. These results are attributed to the chiral-induced spin selectivity (CISS) effect, where molecular chirality and electron spin alter a protein's electric polarizability. The presence of CISS in simple protein−DNA complexes suggests that it played a significant role in ancient biomolecular interactions. A major consequence of CISS is enhancement of the kinetic stability of protein−nucleic acid complexes. These findings reveal how chirality and spin influence bioassociation, offering insights into primitive biochemical evolution and shaping contemporary protein functions.



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INTRODUCTION

A long-standing debate in evolutionary biology revolves around peptide-first or nucleic-acid-first scenarios, questioning whether proteins/peptides (i.e., metabolism) or nucleic acids (i.e., information storage) emerged first in the origins of life. These two elements, however, are not necessarily mutually exclusive—it is likely that they existed in some rudimentary form well before the emergence of the last universal common ancestor.[1−3] In support of this emerging view is the apparent ease with which peptides and nucleic acids can interact.[3−8] Model primordial peptides have been shown to readily bind to and forming coacervates with nucleic acids.[9−12] In some instances, these primordial peptides can even demonstrate elaborate functional interactions, with nucleic acids, that extend from mere binding regimes, such as strand separation and exchange.[10] Unlike contemporary binding sites, however, the earliest peptide−nucleic acid complexes were bound less specifically and with weaker bonds. Here we address the question, *what forces drove these interactions, that are primitive and yet, evolutionarily relevant, and how did they contribute to the emergence of functional molecular complexes?*

Of particular note is the molecular *polarizability*, the capacity for charge reorganization within a biomolecule, which partially dictates both the kinetics and thermodynamics of binding. The significance of polarizability in protein structure and activity is now increasingly recognized as demonstrated by several experimental and theoretical studies.[13−17] In contemporary proteins, charge reorganization can even extend to sites remote from the active site, a process dubbed charge-reorganization allostery.[18] These long-range reorganizations are essential for ligand binding and complex formation.[18,19] It has been demonstrated that the flow of electrons within a biopolymer, and thus its polarizability, is subject to a fundamental phenomenon referred to as the chirality-induced spin selectivity (CISS) effect. The CISS effect refers to the experimental observation that the motion of electrons through or within chiral molecules (and materials) depends on their spin state. Which spin is preferred depends on the handedness of the molecule and on the direction of motion of the electrons.[20] Thus, the CISS effect is a direct consequence of molecular chirality and the intrinsic angular momentum, or spin, of the electron. As of now numerous studies have demonstrated CISS-effect in physical, chemical, and evolved biological systems.[21−23] However, whether CISS exists and, if it does, its significance for weakly interacting primitive peptide−nucleic acid complexes is so far unclear.

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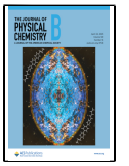

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Whether CISS was plausibly operative and significant to primitive biomolecular complexes has profound implications for their kinetic stability as well as for the prebiotic selection of chiral molecules and the potential roles of ferromagnetic (FM) minerals. As CISS modulates the rates of electron flow within a biomolecule upon binding, the presence or absence of strong CISS effects suggests distinct regimes of polarizability[24] and thus differences in the (dis) association process. Moreover, given that the CISS is a direct consequence of the chiral centers within biomolecules, it stands to reason that these differences could promote or suppress the recruitment of chiral or achiral molecular forms. Finally, strong CISS effects indicate that under certain conditions, FM minerals such as magnetite could readily alter the binding properties of an electronically coupled peptide or nucleic acid. Thus, while the CISS phenomenon is quantum mechanical in nature, its ramifications extend to the origins of life and biochemistry.[25,26]

To explore the role of CISS in primitive protein−nucleic acid complexes, we leveraged two model primordial peptides: a β-(P-loop)-α motif derived from the ancient P-loop NTPase enzyme family and a simplified helix-hairpin-helix (HhH) motif constructed from just 10 amino acid types.[10,11] Both peptides interact with nucleic acids and are less than 50 residues long. Notably, the simplified HhH motif is functionally ambidextrous, with both natural and mirror-image peptides readily binding to and forming coacervates with natural nucleic acids.[27] Upon coupling to a ferromagnet through a reactive cysteine residue, we find that the DNA binding properties of both peptides become highly sensitive to the orientation of the magnetic dipole direction of the substrate, namely, to the direction of electrons' spin that can be injected into the substrate or from the substrate. Despite charge complementarity between the peptide (positive charge) and the DNA (negative charge), binding can be almost completely abolished upon application of a magnetic moment in the nonpreferred orientation. Inverting the chirality of either the entire peptide or just the bridging cysteine residue likewise inverts the effect of the magnetic field. These observations are most reasonably interpreted as evidence for the CISS. Finally, using a simple physical model, we show how the CISS can augment the kinetic stability of primitive peptide-nucleic complexes to support primordial biopolymer collaboration and accretion. The extended reach of the CISS effect, as demonstrated here, suggests an ancient and enduring preference for molecular worlds rich in chiral centers. Finally, given the ubiquitous nature of peptide motifs used in this study, CISS effects are likely to be widespread in the modern-protein world.

Models of Primordial Protein−DNA Complexes. Two families of nucleic-acid binding peptides, both with relevance to early protein evolution, were analyzed (Tables S1 and S2). First, N-$\alpha\beta\alpha$ and N-$\beta\alpha$ (Figure 1A,B) are simplified "prototypes" derived from the core functional fragment of P-loop NTPase enzymes.[10] P-Loop prototypes used in this study have been shown to bind cofactors and nucleic acids, promote strand separation and exchange, and weakly catalyze phosphoryl transfer reactions.[10,28] The profound conservation of the β-(P-loop)-α motif and the broad functional profile of P-loop prototypes support the hypothesis that these fragments are evolutionary nuclei around which contemporary P-loop NTPase and other enzyme domains, with the P-loop motif, accreted.[5,9,29−31] Second is the *precursor* peptide (Figure 1C), which is derived from an ancestor sequence reconstruction and amino acid deconstruction[11] of the ancient and ubiquitous HhH family.[32] This peptide has been shown to form coacervates with RNA, within which the peptide can dimerize to adopt the $(HhH)_2$-fold.[12] More recently, it was demonstrated that both the HhH peptide and the $(HhH)_2$-fold are "functionally ambidextrous"—able to bind RNA and dsDNA of either chirality.[27] These primordial peptides can be produced either biologically using *E. coli* expression and purification systems or chemically synthesized according to the requirements (Supporting Information Methods).

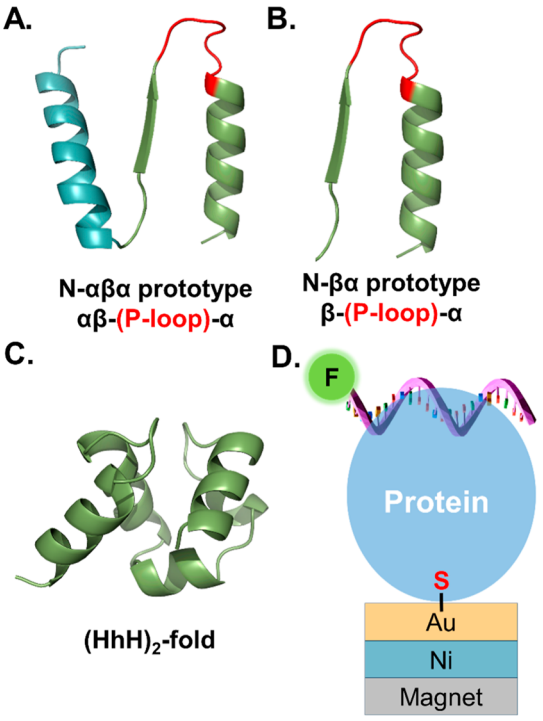

Figure 1. Representative primordial peptide models and experimental setup. (A) N-$\alpha\beta\alpha$ prototype is composed of the ancestral β-(P-loop)-α motif and one additional α-helix at the N-terminus. (B) N-$\beta\alpha$ prototype is composed of only the ancestral β-(P-loop)-α motif. The structural models in panels A and B were generated using PyMOL and depict the ancestrally inferred β1 and α1 in green and the connecting P-loop in red. The additional α-helix in the N-$\alpha\beta\alpha$ prototype is shown in cyan. The descriptor below each prototype indicates the order of secondary structure elements (from N- to C-terminus). The prototypes have a propensity to form dynamic oligomers in solution;[9,10,28] therefore, the monomeric models shown here are only schematic depictions. (C) AlphaFold2 model of the $(HhH)_2$-fold, which is recapitulated upon dimerization of two HhH peptides.[12] (D) Experimental setup. Proteins carrying a C-terminal cysteine with a free thiol (-SH) group are adsorbed onto a gold-coated FM nickel layer via an S−Au bond. The ferromagnetic substrates (FM-substrates) with the adsorbed proteins are then placed onto a 0.42 T permanent magnet. The entire assembly (magnet + FM-substrates) is transferred to a microscope, and binding to fluorescent DNA is monitored. The experiments were carried out with both orientations of the magnet (north or south) facing the substrate. Models for P-loop prototypes are adapted with permission from ref[10]. Copyright 2021, PNAS.

Experimental Setup to Detect CISS Effects. All molecular binding events induce a dynamic charge redistribution within the participating molecules. Due to the different electrochemical potentials of the molecules that interact, the interaction is equivalent to applying an electric field.[17] The binding process is mediated in part by the electrical polarizability of the interacting partners, and greater

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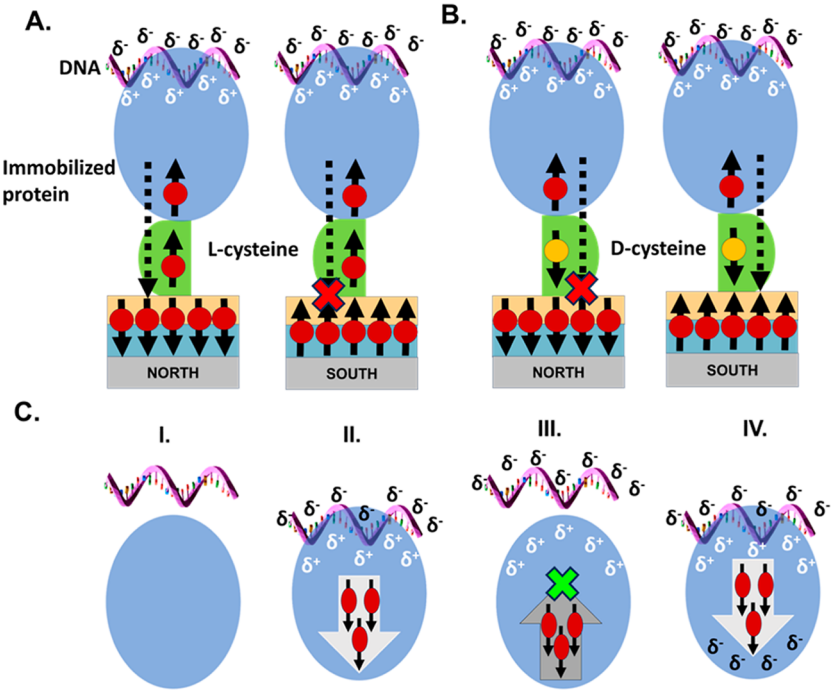
Scheme 1. (A) Schematic Representation of the Mechanism of the Effect of Spin on the Protein−DNA Interaction; When the Spin on the Ferromagnet Is Pointing Opposite to the Momentary Spin of the Charge at the Interface of the Adsorbed Proteins (Representative Spins and Electrons Migrating through the Protein Are Shown as Black Arrow and Red Ball, Respectively), Charge Flows More Efficiently between the Protein and FM-Substrate (Shown as Dotted Arrow Penetrating the Substrate); This Scenario Manifests When the North Magnet Is Facing the FM-Substrate; When the South Magnet is Facing the FM-Substrate, the Spins on the Protein and the Substrate are Pointing the Same Direction Resulting in Inefficient Electron-Flow; (B) Effect of the D-cysteine Linker on Protein−DNA Interaction; The Terminal D-Cysteine Linker, Shown in Green, Which Has the Opposing Chirality to the Rest of the Protein, Flips the Direction of Spins Transmitted through the Protein (Black Arrow, Yellow Ball); Consequently, the Opposite Magnet Polarity is Required to Achieve Electron-Flow, Relative to the Schematic in (A); (C) Model Describing the Role of Electrons Spin-Related Polarizability in the Docking of DNA on the Protein; Reaction Scheme Describes the Dynamic Interaction of a Protein Molecule with a DNA Molecule to form Transient (Protein-DNA)' Complex (Charge-Reorganization Step) and the Final Energetically Favored (Protein-DNA) Configuration (Binding Step); (I) System before DNA−Protein Interaction; (II) Approaching DNA Repels Electrons with Specific Spins away from the Binding Zone; (III) Upon Retraction of the DNA from the Protein, the Electrons that Were Withdrawn Are Not Able to Flow Back because They Have the "Wrong" Spin Direction; (IV) Final Docking that Results in Binding of the DNA to the Protein



electronic polarizability is generally associated with increased affinity. The electronic polarizability (due to the movement of electrons), in turn, is influenced by the chirality and the spin-state of the migrating electrons, i.e., the CISS effect. Which spin is preferred depends on the handedness of the molecule and on the direction of motion of the electrons.[20] While for one handedness, the preferred spin will always point parallel to the electron velocity, for the opposite handedness, the preferred spin will always point antiparallel to the electron velocity. A consequence of this effect is that the movement of electrons, in a chiral molecule, generates both a charge-polarization, i.e., a separation of positive and negative charges within the molecule, and spin-polarization (i.e., a preference for electrons with one specific spin, either "up" or "down") such that one spin has higher density at one electric pole and the other at the opposite pole.[24] The observed spin polarization typically ranges from 20% to nearly 100% of the moving electrons.[21]

We measured the CISS effect by coupling one of the binding partners to a reservoir of spin-polarized electrons (Figure 1D), as has been done previously.[18,19,21,33] Briefly, peptides were adsorbed onto a FM substrate that served as a source for the spin-polarized charge. To facilitate peptide adsorption, a sole cysteine residue was incorporated at the C-terminus, away from the nucleic-acid binding site. The peptides were then coupled to the surface via the formation of a sulfur−gold bond. Finally, the FM-substrates were attached to one pole of a magnet. The CISS effect predicts that binding affinity and kinetics will strongly depend on the orientation of the magnet because the efficiency of electron flow into or out of the coupled ferromagnet depends on the spin states of the electrons, namely, on the direction of magnetization of the substrate. Thus, if the charge reorganization within the protein is spin-dependent, and only electrons with a specific spin are repelled away from the binding site, one magnet orientation will promote nucleic acid binding, while the other orientation will reduce it. This effect occurs because the preferred orientation allows electrons to flow into the substrate, increasing the polarizability of the protein and promoting binding. Binding was measured via monitoring of an increase in fluorescence intensity using a fluorescence microscope. The detailed description of the FM-substrate and the binding assay is described in the Supporting Information Methods. We will refer to magnet orientations as either UP (north magnet pole

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facing the FM-substrates) or DOWN (south magnet pole facing the substrates).

Using this experimental framework, in this work, we demonstrate that the interaction between the DNA and the protein is essentially electrostatic and that this electrostatic interaction is directly influenced by the chirality-induced spin polarization. Specifically, approaching charged DNA induces charge polarization in the protein, which means that electrons are repelled away from the DNA−protein binding site on the protein. Because of the chirality of the protein, the efficiency of the electron motion away from the binding site depends on their spin. When the protein is attached to a ferromagnet substrate, the substrate can be magnetized so that it can accept electrons from the protein only if they have specific spin. Hence, the ability of the electrons in the protein to be repelled from the DNA−protein binding site depends on the magnetic substrate being magnetized so that electrons whose motion is preferred in the chiral system can indeed penetrate into the substrate. Finally, we provide a simple model describing this phenomenon (Scheme 1).

METHODS

P-Loop prototypes were cloned into pET29(+) vectors by restriction-free cloning, then expressed and purified as described earlier.[10] Synthetic peptides were obtained from commercial sources or synthesized in-house. FM substrates (Ti/Ni/Au on silicon) were cleaned, drop cast with cysteine-containing proteins/peptides (in the presence of TCEP), and subsequently incubated with fluorescently labeled dsDNA or ssDNA to monitor binding kinetics via fluorescence microscopy as described above (see the Experimental Setup to Detect CISS Effects section). Contact potential difference (CPD) values were recorded by using a Kelvin probe setup under controlled magnet orientations. Full details are provided in the Supporting Information.

RESULTS

Nucleic Acid Binding of Adsorbed P-Loop Prototypes Is Sensitive to Magnetic Field Orientation. The biologically produced N-$\alpha\beta\alpha$ prototype binds to fluorescently labeled ssDNA that is rich in thymine and cytosine nucleotides (TC-ssDNA) with a dissociation constant K_D = 0.67 ± 0.16 μM[10] (Figure S6, Tables S3 and S4). Adsorption isotherms of TC-ssDNA binding to FM-adsorbed N-$\alpha\beta\alpha$ prototype exhibit a dependence on the orientation of the magnetic field, with both the rate of binding and the extent of binding increased when the ferromagnet is aligned UP (Figure 2A and Table 1). Remarkably, the effect of the magnetic orientation is so great that binding is nearly abolished when the magnetic field is in the DOWN orientation. However, for ssDNA that is rich in guanines and adenines (GA-ssDNA), which P-loop prototypes bind more weakly with a dissociation constant of K_D = 1.70 ± 0.68 μM[10] (Figure S6, Tables S3 and S4), adsorption profiles are similar for both magnetic field orientations (Figure 2B).

The behavior of the N-$\beta\alpha$ prototype, which lacks the N-terminal α-helix (Figure 1B), is similar to that of N-$\alpha\beta\alpha$: binding to TC-ssDNA is favored when the magnet is in the UP orientation, and binding to GA-ssDNA is weak and insensitive to magnetic field orientation (Figure 2C,D). However, the magnetic field effect is notably smaller for the N-$\beta\alpha$ prototype than it is for the N-$\beta\alpha$ prototype, likely owing to the overall weaker binding and possibly due to the reduced overall

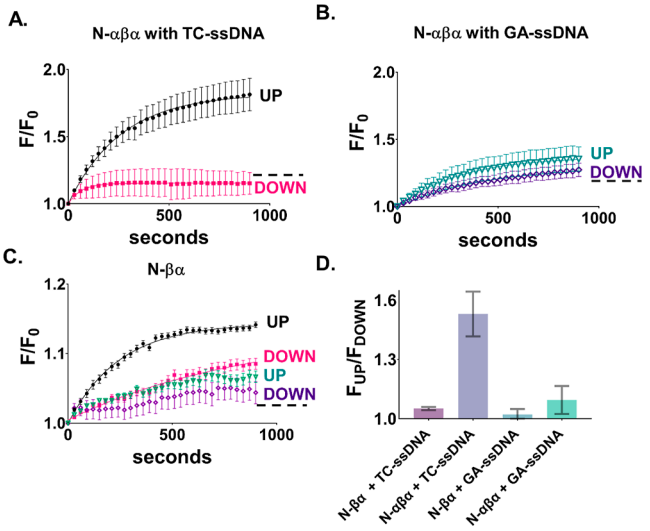

Figure 2. DNA binding to N-$\alpha\beta\alpha$ and N-$\beta\alpha$ prototypes is influenced by the orientation of the magnet. Adsorption isotherms showing binding of (A) TC-ssDNA to the N-$\alpha\beta\alpha$ prototype for UP (black trace) and DOWN (pink trace) orientations and (B) GA-ssDNA to the N-$\alpha\beta\alpha$ prototype for UP (green trace) and DOWN (purple trace) orientations. (C) Adsorption isotherms showing binding of N-$\beta\alpha$ prototype to TC-ssDNA for UP (black trace) and DOWN (pink trace) orientations and to GA-ssDNA for UP (green trace) and DOWN (purple trace) orientations. Fits to data in panels A, B, and C were from a standard *one phase association* equation using GraphPad Prism, and error bars represent standard deviation from three or four independent measurements. Dotted lines in A, B, and C depict background binding to free fluorescein after nuclease digestion of ssDNA (Figure S7) and (D). Relative fluorescence increases. The bar graph displays the relative difference in the end point-F/F_0 values corresponding to the binding of each prototype (N-$\alpha\beta\alpha$ or N-$\beta\alpha$) to each DNA sequence (TC-ssDNA or GA-ssDNA). Here, the end point fluorescence value of the magnet in the UP orientation was divided by the end point fluorescence value of the magnet in the DOWN orientation, for the same DNA−protein pair (F_{UP}/F_{DOWN}). Error bars represent the standard deviation of three or four independent replicates.

polarizability of this construct (Figure 2D). To verify that the observed increase in fluorescence is not an artifact of the N-$\alpha\beta\alpha$ prototype interacting with the fluorophore on the DNA, but rather a consequence of prototype-DNA binding, the adsorption of nuclease-digested TC-ssDNA to the N-$\alpha\beta\alpha$ prototype-coated substrate was measured. As this sample contains only the free fluorophore and nucleotides, a time-dependent increase in fluorescence would reflect binding to the fluorophore. The fluorescence amplitude of the binding isotherms and the effect of the magnetic field, however, are both small, consistent with minimal background binding (Figure S7).

Reciprocal Inversion of Chirality and Magnetic Orientation Effects. P-Loop prototypes have a C-terminal cysteine residue that serves as a linker between the protein and the magnetized FM-substrate, serving as the site for electron injection. Because these prototypes are biologically produced, in principle, they should contain only L-amino acids. By contrast, chemically synthesized peptides allow investigation of the role of altered handedness on protein function. To this end, N-$\alpha\beta\alpha$ prototypes that have opposite handedness of the C-terminal cysteine residue but are otherwise composed of L-amino acids were chemically synthesized. These chemically

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Table 1. Apparent Rate Constants (s^{-1}) of DNA Binding to Adsorbed P-Loop Prototypes and HhH Polypeptides[a]

	TC-ssDNA		GA-ssDNA	
	UP	DOWN	UP	DOWN
N-$\alpha\beta\alpha$	4 (0.1) × 10^{-3} R^2 = 0.99		3 (0.8) × 10^{-3} R^2 = 0.98	
N-$\beta\alpha$	4 (0.2) × 10^{-3} R^2 = 0.99	1 (0.2) × 10^{-3} R^2 = 0.98	3 (0.1) × 10^{-3} R^2 = 0.95	0.7 (0.4) × 10^{-3} R^2 = 0.88
N-$\alpha\beta\alpha$_LcysEnd	6 (2) × 10^{-3} R^2 = 0.97			
N-$\alpha\beta\alpha$_DcysEnd		8 (4) × 10^{-3} R^2 = 0.98		

	12-bp dsDNA	
	UP	DOWN
L-precursor		1.9 (0.1) × 10^{-2} R^2 = 0.82
D-precursor	1.8 (0.5) × 10^{-2} R^2 = 0.76	

[a]Rate constants (s^{-1}) were determined by fitting the data to a standard one-phase association equation in GraphPad Prism software (Supporting Information Methods). Numbers in parentheses indicate standard deviation from three or four independent measurements. Amino acid sequences are listed in Table S2. Nucleotide sequences of TC-ssDNA, GA-ssDNA, and 12-bp dsDNA are provided in Table S3. Note that despite a stronger binding for TC-ssDNA, as demonstrated in our previous studies, the association rates observed in the current experiment (which indicates the time taken to reach saturation) are similar due to high concentrations of protein (dictated by the need to have a uniform monolayer on the FM-substrate) relative to DNA (see the "Methods" section). As a result, the binding sites get occupied, and the reaction plateaus at a similar rate for both DNA types. The amplitude however is clearly different and greater for the preferred ligand, TC-ssDNA (Figure 2A,B).

synthesized prototypes N-$\alpha\beta\alpha$_LcysEnd and N-$\alpha\beta\alpha$_DcysEnd bear a L-cys and D-cys residue, respectively, at the C-terminus (Table S2). In principle, the handedness of the terminal cysteine should serve as a valve to filter electrons with a specific spin (in accordance with the CISS effect). While the N-$\alpha\beta\alpha$_LcysEnd peptide favored the UP orientation—like the *E. coli*-expressed N-$\alpha\beta\alpha$ prototype (Figure 3A)—the N-$\alpha\beta\alpha$_DcysEnd peptide favored the DOWN orientation (Figure 3B). As before, the nonpreferred orientation of the magnetic field blocks the binding down to the level of the background (Figure S8).

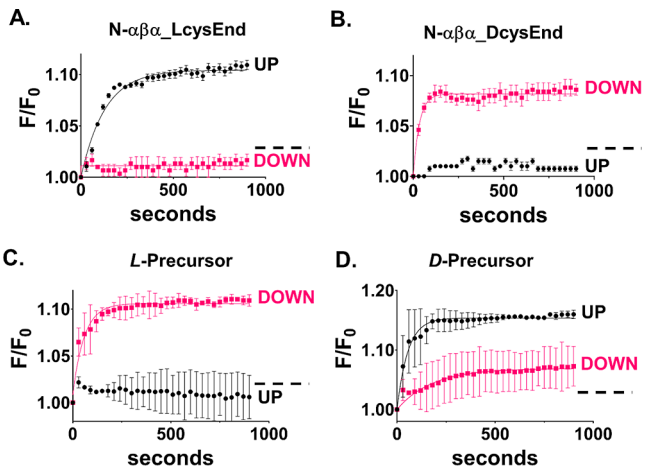

Figure 3. Handedness effect on the magnetism-dependent association. The influence of handedness of synthetic P-loop prototypes and HhH polypeptides on binding to TC-ssDNA or 12-bp dsDNA, respectively, for both magnet orientations. (A) Adsorption isotherms of TC-ssDNA binding to N-$\alpha\beta\alpha$ anchored to a FM surface via L-cysteine (N-$\alpha\beta\alpha$_LcysEnd). (B) Same as (A) but with N-$\alpha\beta\alpha$ anchored via D-cysteine (N-$\alpha\beta\alpha$_DcysEnd). (C) Adsorption isotherms of 12-bp dsDNA binding to the precursor-Arg peptide, which comprises a single HhH motif, and for which all amino acids are in the L-form (L-precursor). (D) Same as (C) but with all amino acids in the D-form (D-precursor). Error bars represent standard deviation from three or four independent measurements. Dotted lines in A, B, C, and D depict background binding of free fluorescein.

We note that the amplitude of the fluorescence increase for reactions with biologically purified proteins (Figure 2) is larger than that for chemically synthesized proteins (Figure 3). The greater fluorescence signal for biologically produced proteins is reproducible upon multiple batches of purification (Figure S9) and may relate to differences in the oligomeric state. In addition, the histidine tag used for purification of the biologically produced samples may change the oligomerization state,[29] resulting in greater binding to DNA but also higher nonspecific interactions with the hydrophobic fluorescent dye. Nonetheless, previous studies have shown that the histidine tag does not modulate the function of P-loop prototypes.[9,10] Crucially, the relative preference for the UP orientation for both N-$\alpha\beta\alpha$ prototype samples remain unchanged.

Finally, reciprocal inversion of the magnetic field orientation preference and chirality was tested on the HhH peptide binding to dsDNA (Figure 3C,D). To this end, we test two chemically synthesized HhH peptides that are composed exclusively of either L- or D-amino acids (homochiral peptides),[27] thus inverting the entire handedness of the protein construct (including their DNA-binding sites) and assuming that the binding mode is largely unchanged. Circular dichroism experiments were performed to confirm that the two constructs demonstrate mirror-image symmetry in their *foldedness* (Supporting Information Methods). The L-precursor peptide enjoys increased affinity for dsDNA when the substrate is magnetized DOWN, the D-precursor peptide has an inverted response with increased affinity for dsDNA when the substrate is magnetized UP. Note that while the preferred magnetic orientation is not conserved between the P-loop NTPase and HhH-derived peptides, both types of peptides exhibit a clear orientation preference that can be inverted by inverting the chirality of either the linker (P-loop NTPase) or the entire

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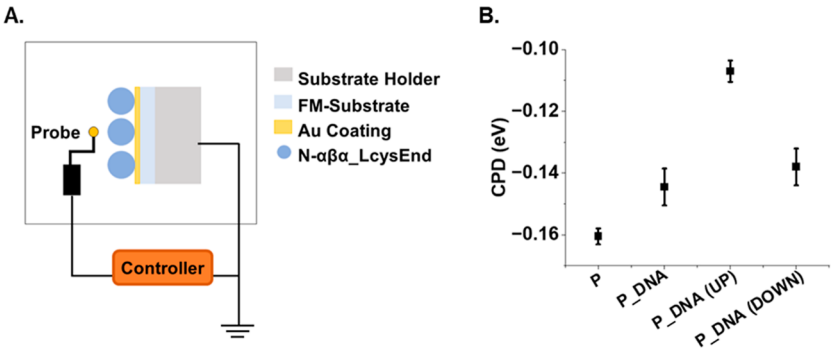

Figure 4. Kelvin probe analysis of N-$\alpha\beta\alpha$ prototype. (A) Schematic of the Kelvin probe setup. (B) Measured CPD values for chemically synthesized N-$\alpha\beta\alpha$. CPD (eV) values were measured for "Protein-only" (P), adsorbed protein without incubation with DNA; for (P_DNA)', adsorbed protein incubated with DNA with the substrate not magnetized; "Protein_DNA (UP)", adsorbed protein incubated with DNA with the north magnetic pole facing toward the protein; and Protein_DNA (DOWN), adsorbed protein incubated with DNA with the north magnetic pole facing away from the protein. Error bars represent the standard deviation from four independent experiments.

peptide (HhH). Importantly, in all cases, the binding rates for L- and D-peptides are similar for the favored magnet orientations (Table 1), suggesting that the actual rate of binding under ideal conditions is the same for the two enantiomers, and the electron spin is not involved in the binding site itself. In other words, different spins are passing through L- and D-peptides (and their binding sites), yet they bind with the same rate constants to dsDNA. Therefore, it is not the spin−spin interaction between the protein and the DNA that mediates binding but rather the positive charge accumulation on the binding site of the protein. The observation that the rate constants for binding are the same for L- and D-peptides, for their respective favored magnet orientations, rules out any contributions from external factors to this effect, other than the alternate handedness.

Lastly, as an independent verification of the results obtained using fluorescence microscopy, we performed CPD studies with a Kelvin probe[34] (Figure 4; refer to Supporting Information Methods for experimental details). Briefly, Kelvin probe experiments allow measurement of the *work-function*, the threshold energy required to remove an electron from a solid surface. These measurements can confirm the extent of ssDNA binding for different magnetization directions by measuring the electric field between the sample and metallic probe. The setup includes a metallic probe electrode (gold grid) placed near the surface of the sample to form a capacitor (Figure 4A). The distance between the probe electrode and the surface of the sample is periodically varied to create a frequency-dependent capacitance. As a result of the CPD, an AC voltage is generated across the gap that is proportional to the difference in the work-function between the sample and the probe electrode. A DC voltage is applied to nullify the AC voltage. A more negative value indicates that the adsorbate reduced the work-function more significantly.

As a representative construct of the proteins described in this study, chemically synthesized N-$\alpha\beta\alpha$_LcysEnd prototype was adsorbed onto FM-substrates and binding was measured as described in the Methods section. The UP orientation exhibited a less negative work-function (i.e., less negative CPD value and higher work function) than the DOWN orientation (Figure 4B), indicating that more ssDNA is adsorbed onto the protein-coated. In comparison, for the surfaces coated with protein only, the CPD values are the most negative, consistent with the smallest amount of material present on the surface. For surfaces covered with protein and ssDNA bound in the absence of an external magnet field, the CPD values are similar to those of the DOWN orientation. Taken together, the Kelvin probe experiment corroborates the results from fluorescence microscopy.

DISCUSSION

CISS Effect Accounts for the Sensitivity of Nucleic Acid Binding to Magnetic Field Orientation. The magnetization-dependent binding functions suggest that the peptides are spin-selective. Numerous studies have evidenced that the CISS effect underlies various physical, chemical, and biological systems.[21−23] Therefore, prima facie, given its fundamental nature, one might assume that the CISS effect contributed to primitive peptide-nucleic acid systems, as well. However, the fact that CISS exists in ancient biomolecular interactions is far from obvious. Previous studies demonstrating the CISS effect, involved standard and evolved biological (or physical and chemical) systems that demonstrate clear, robust spin-dependent interactions facilitated by well-defined chiral structures and specific binding affinities.[18,19,33] Typically, these systems exhibit strong, highly specific interactions at active sites that enhance the CISS effect. In contrast, primordial proteins are unlikely to have a defined tertiary and quaternary structure as their modern counterparts, possessing weak binding affinities, with promiscuous active sites that may engage in nonspecific interactions, thus potentially hindering the CISS-effect.

The experimental setup shown in Figure 1 allows us to determine spin-selectivity of the peptides and the resulting CISS effect. Since the orientation of ferromagnet dictates the spin orientation that is injected into the adsorbed proteins, the results suggest that P-loop prototypes and HhH peptides demonstrate electron spin-selective DNA binding functions. It is important to note that the role of the FM substrate is only to polarize the spin of the electrons within the Ni/Au substrate and its magnetic field has no direct effect on the protein−DNA binding. Applying an external magnetic field, without having spin-polarized substrate, does not affect the DNA−protein binding. The extent of the spin-selective effect is highly sensitive to the strength of ligand binding—with only an ∼2.5-fold difference in K_D resulting in a near-complete loss of the CISS effect for some protein−ligand pairs. More specifically, in the case of P-loop prototypes binding to the TC-rich sequence or of the HhH peptides to dsDNA, the amount of charge that is reorganized is large, and hence charge has to be spread all

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over the system. However, for GA-rich DNA where the binding to the P-loop prototype is weaker, only a small amount of charge reorganization occurs, and consequently, the charge reorganization is short-range and does not result in a significant charge exchange with the substrate (Figure 2A,B).

The strongest evidence in favor of the CISS effect comes from the experiments involving chiral inversion (Figure 3). Specifically, flipping the handedness of a single terminal residue of P-loop prototypes inverts the spin-preference for nucleic-acid binding, i.e., DOWN orientation is favored for prototypes with terminal D-cysteine, whereas UP orientation is favored for prototypes L-cysteine terminus (Figure 3A,B). Schematically, we suggest that when the linker is L-cys, it shows the same preference as the native protein and thus provides a path for the electrons with the preferred spin to move into the FM substrate when magnetized UP. For D-cys, on the other hand, electrons with the same spin cannot pass efficiently through the linker and instead accumulate between the linker and the binding site to the DNA, reducing the effective polarizability of the protein. However, if the magnet is pointing DOWN, electrons from the D-cys can now be displaced into the substrate, hence the electrons repelled from the binding site can now be inserted (after their spin is flipped) into the D-cys. The fact that the relative spin orientations between the substrate and the linker, and not the linker and the remainder of the protein, dominate the handedness effect suggests a clear separation of time scales that will form the basis of future work along with studies to quantify the spin-polarization effects shown in Figures 2−4.

The observed spin selectivity can, in principle, be a result of the spin-dependent charge polarizability within the proteins or a result of the spin-dependent interaction between the DNA and the protein. The question of which mechanism is the dominant one was resolved by studying the spin-dependent association with HhH peptides that are composed exclusively L- or D-amino acids, thus inverting the entire handedness of the protein construct (Figure 3D). The rates of binding for the L- and D-HhH peptides are similar for the favored spins (Table 1), suggesting that while spins influence the polarizability of the peptides, resulting in inverted spin preferences for the two enantiomers, the actual rate of binding once polarizability is maximized is the same for the two enantiomers. Consequently, the interaction between the DNA and the peptide is not spin dependent, and the observed effect is instead the result of spin-dependent charge polarizability within the protein. This finding supports the conclusion that the interaction is predominantly electrostatic without significant selectivity to the chiral structure.

Overall, inverting the chirality of either the terminal cysteine or the entire peptide construct changes the preference for the magnet orientation, underscoring the role of the peptides in mediating spin-polarized DNA-binding functions. These results also rule out any spurious surface effects, such as magnetostriction, i.e., a change in shape of FM materials when subjected to magnetic field, for the UP vs DOWN orientation. The CISS effect is consistent across two distinct ancient peptides, various sequences of DNA, and types of DNA (single stranded vs double stranded DNA) and is corroborated by an independent Kelvin-probe method (Figure 4).

Evolutionary Implications of Magnetization-Dependent Activity. Mineral−organic interfaces may have played a crucial role in the functionalization of life and biocatalysis in the early stages of the earth.[35−37] Binding to minerals has also been shown to preserve protein sequences and accordingly biominerals have been proposed to be excellent source of ancient protein sequences.[38] Minerals and rock surfaces were also likely to have served as compartments and scaffolds for prebiotic reactions and protocells.[39,40] Nickel deposits (the FM layer used in our studies) abundant on the Archean earth[41] played a key role in biological processes, including enzymatic reactions.[42,43] Iron is the most abundant element in the earth's crust, and it is postulated to be ubiquitous in the primordial world.[44−47] Beyond acting as protective scaffolds, these magnetic minerals may have created unique spin-polarized environments. In a plausible scenario, primordial peptides may have functioned when adsorbed onto mineral interfaces, as previously described, or onto magnetite, which can provide source of spin-polarized electrons upon UV irradiation,[25,26] thereby conferring chiral preference to the adsorbed peptides and, as a consequence, facilitating the transition from heterochiral to homochiral biomolecules. Foremost, given that DNA-binding functions of primordial polypeptides can be promoted or abrogated depending on the substrate magnetization, the functioning of peptides adsorbed to magnetic minerals provides a means to augment binding interactions and on-rates between ancient biomolecular interactors.

It is true that the magnetic field used in this study to observe the spin-polarization is much stronger than the magnetic fields present on early earth. However, it has been shown that similar spin-polarizations can be achieved by superparamagnetic magnetite particles, even when they are formed under weak magnetic fields similar to that of the early earth, due to their remnant magnetizations.[26,48] Furthermore, since the effect we describe results from potential spin-exchange with the substrate, it is the degree of spin-alignment at the protein−substrate (or mineral) interface that is crucial, rather than earth's magnetic field. In other words, the magnetization of naturally occurring minerals is the primary driving force for achieving spin alignment at the protein−substrate (or mineral) interface, rather than the strength of Earth's magnetic field.[26]

Significance of Electron-Spin Polarizability for Contemporary Protein/Enzyme Systems? As described below, the importance of spin selectivity is rationalized by a schematic (Scheme 1). When the DNA approaches the protein adsorbed on the ferromagnet, it induces the polarization of the charge in the protein. Here, we refer not to the charge localized on specific functional groups but rather to charge that can be delocalized. This polarization is related to the high-frequency polarizability of the protein, or in other terms, to the protein's high-frequency dielectric constant. Since the DNA is negatively charged, when it approaches the protein electrons are repelled from the binding site (Scheme 1A,B). Because of the chirality of the protein, the charge rearrangement depends on the electrons' spin.[24] Namely, electrons with one spin orientation move more efficiently through the protein. If the substrate is magnetized so that the electrons with the preferred spin can be injected into it, then the electrons withdrawn from the binding site can move into the ferromagnet, and thereby the effective polarizability of the protein increases. In the specific case of L-proteins, this means that since the preferred spin for the electrons' motion is the spin antiparallel to the electrons' velocity, the ferromagnet should be aligned with its north pole pointing toward the protein (namely its majority spin is aligned away from the protein) and therefore the electrons from the protein can be injected into the minority states of the ferromagnet (Scheme 1A).

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The phosphate-binding motifs used in this study are ubiquitous in present-day protein world.[5,9,11,28,29] The results therefore suggest that CISS is at play in contemporary protein interactions. This spin dependency of the association is surprising, and the question is whether it serves any function in biological systems. In what follows, we will present a possible explanation for the importance of the spin. It is well accepted that the "docking" of substrates/ligands on enzymes/proteins is not an abrupt single-step event but a rather a dynamic process, influenced by the conformational dynamics of the participating biomolecules, involving multiple "binding-unbinding" events, until the minimum-energy configuration is achieved.[49−53] Upon the first encounter of the DNA and the protein, electrostatic interactions induce charge-reorganization in the protein; i.e., due to the approaching negatively charged DNA, electrons are repelled from the binding site in the protein, giving rise to positive charge polarization (Scheme 1C, panel II). As a result of the CISS effect, electrons with spin parallel to their velocity move faster than those with spin aligned antiparallel to their velocity. When the DNA molecules transiently "unbind" from the protein, some of the charge will move back into the binding site, however for these electrons to move back, their spins must flip. This spin-flipping is a relatively slow process that has a lifetime of hundreds of nanoseconds, leading to a delay in the charge returning to its original state (Scheme 1C, panel III). As a result, the internal charge dynamics of the protein is quenched, and the polarization, therefore, remains positive. Thus, the DNA molecules are attracted back toward the protein. This dynamic enhances their binding affinity to the protein and prevents the complete unbinding of DNA before a minimal energy conformation is reached (Scheme 1C, panel IV). It is important to appreciate that interaction of protein with any binding-partner will involve charge-reorganization within the protein due to the different electrochemical potentials of the protein and the binding partner. Hence, the mechanism described above should be relevant to all association processes in which the objects are chiral.

Given the current state of the art methods, it is not possible to simulate a fully realistic, theoretical model to support the schematic shown in Scheme 1. Yet, we have attempted simplified quantum simulations, as described in previous studies,[54,55] to model the dynamics of the charge density using model chiral and achiral systems. The simulations show significant spin polarization in chiral molecules, while almost negligible effect is observed for achiral molecules. Further, for chiral molecules, electrical and spin polarization are related, whereas these are independent properties for achiral molecules (Supporting Information Text: *Quantum mechanical simulations*; Figure S10).

CONCLUSIONS

While it is widely recognized that multiple factors influence protein function, our results highlight a fundamental yet underappreciated consequence of protein chirality, namely, its ability to spin-polarize electrons and thereby directly influence biological function via the CISS effect. We demonstrate that the association between model primordial polypeptides and DNA depends on electron spin, with the effect varying by protein handedness, L-proteins favoring one spin orientation and D-proteins the opposite. Supported by model calculations, our results highlight the role of the CISS effect, where homochirality influences electron polarization, internal charge dynamics, and binding kinetics. We propose that spin selectivity enhances protein−substrate interactions by extending the relaxation time of the chiral-induced electric dipole moment. If true, spin selectivity may have promoted the emergence of homochirality by augmenting and modulating binding kinetics in weakly interacting, ancient protein−nucleic acid systems. Given the ubiquity of these motifs, our findings suggest a widespread role of the CISS effect in biomolecular systems, revealing a fundamental link between spin and biological interactions.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpcb.5c01150.

Quantum mechanical simulations; materials and methods; HPLC chromatograms; CD spectra; polarization modulation-infrared reflection-adsorption spectroscopy spectra; atomic force microscopy (AFM)-based topographical analysis; binding and adsorption profiles of P-loop and HhH peptides; coding DNA sequences; amino acid sequences; oligonucleotide sequences; and binding constants of protein constructs (PDF)

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Notes

The authors declare no competing financial interest.

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