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Enantiospecific interactions are key to many biological processes. Commonly, bioaffinity values measured are higher than calculated. Here, the interaction between chiral peptides is probed using an atomic force microscope’s cantilever functionalized with chiral molecule and a monolayer of chiral peptides. A new enantiospecific interaction term attributed to exchange interaction is described and supported by calculations. This short-ranged term is relevant in crowded biological systems. The results shed light on the importance of spin and exchange interactions in biological processes.

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Highlights
Enantiospecific interactions are probed using a functionalized AFM cantilever

A force difference of 70 pN between homo- and heterochiral pairs is obtained

The force is directional resulting from transient spin exchange interactions

Toy model calculations reveal the short range and directionality of the force

Kapon et al., Chem 7, 2787–2799
October 14, 2021 © 2021 Elsevier Inc.
https://doi.org/10.1016/j.chempr.2021.08.002
Evidence for new enantiospecific interaction force in chiral biomolecules

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SUMMARY
Enantiospecific biorecognition interactions are key to many biological events. Commonly, bio-affinity values, measured in these processes, are higher than those calculated by available methods. We report here the first direct measurement of the interaction force between two chiral peptides (right- and left-handed helical polyalanine peptides) and the quantification of difference in the interaction force between homochiral and heterochiral pairs of molecules using atomic force microscope (AFM), together with supportive calculations based on a simple theoretical model. A force difference of 70 pN between same and opposite enantiomer interactions is measured. Additional measurements show spin dependency and fast decay of the interaction term, consistent with spin exchange interactions. This short range enantiospecific interaction term is especially relevant in crowded biological systems. The results shed light on the importance of spin and exchange interactions in biological processes.

INTRODUCTION
Nature is based on chiral molecules, namely molecules that appear in two forms, enantiomers, which are mirror images of each other. Interestingly, chiral biomolecules, such as proteins and sugars, appear in nature mainly as one enantiomer. The origin of “homo chirality” in nature was—and is—discussed very intensively in the literature.1 However, the focus of this work is related to a more fundamental question, i.e., why did nature preserve chirality so persistently over the many millions of years of evolution? In other words, does chirality per se, independent on the specific handedness, provide properties that serve an important role in life? The ability of biological molecules to interact selectively with each other is at the heart of all biological processes and the basis of many pharmaceutical concepts. Two important properties, related to chirality, characterize interactions in nature, i.e., very high enantioselectivity and the relatively fast rates of very complex processes, e.g., the rate of protein folding2–4 and the repair of damage in DNA.5

It has been suggested that an interaction term that involves the electrons’ spin can improve the enantioselectivity in reactions of chiral molecules, due to the symmetry constraints resulting from the dispersion-induced charge reorganization, which is accompanied by transient spin polarization (see Figure 1A).6 However, such spin-related interaction term has never been measured directly before. Herein, we use atomic force spectroscopy, to directly measure the enantioselective interaction between oligopeptides of different handedness. Simple modeling of the spin-related enantiospecific interaction energies shows that the spin constraint also imposes directionality in the interaction.

The bigger picture
Most biological molecules are chiral. Interestingly, in life, they appear in one chirality. This is not trivial as most of non-biological chemistry is achiral. Indeed, the earliest evidence for amino acids on earth suggests that both enantiomers were present. One of the driving forces to preserve homochirality in life may be enantiomer-specific forces.

Here, the interaction between chiral peptides is probed using a chiral molecule functionalized atomic force microscope. The results show that the same-handed (chirality) polypeptides generate large attraction potentials (~10 Kcal/mol), in agreement with simulations. This force, related to the chiral-induced spin selectivity (CISS) effect, is short ranged and directional and is therefore especially relevant to crowded biological systems. This force places symmetry constraints on protein folding, it may provide a reason for preserving chirality in life. For applications, these results may promote simulations enabling to improve chiral synthesis.
The enantioselectivity measured here does not correspond to any of the established biorecognition mechanisms related to structural properties, such as the “lock and key” model, and induced fit and allosteric interactions, and neither can it be explained through differential, enantiomer-specific, long-range electrostatic interactions. Although existing computational methods account for enantioselectivity, none are accounting for the dynamic effect of the spin polarization occurring during the collision between chiral peptides. Conventional calculations are unable to reproduce the high selectivity observed in the present experiments. This suggests that the enantioselective spin interaction is not captured properly by the theories currently employed to describe (bio)molecules. Although additional research will be needed to delineate the exact scope and pervasiveness of this effect, it can be expected to alleviate some of the notable discrepancies between observed versus computed enantiospecificity, interaction energies, and reaction rates. Furthermore, the directionality emerging from our model may help to explain the high efficiency of many complex bioprocesses, such as protein folding and enzymatic reactions, since it significantly reduces the phase space the systems have to explore.

When considering bio-related chemical processes, the distribution of charges in the reacting species is of major importance. Obviously, these distributions are a direct result of the spatial positions of the charged electrons and nuclei in the molecular systems. However, next to charge, electrons also have another property, their spin, which is their angular momentum and can have two orientation values. In organic molecules, the spin is typically not coupled significantly to the molecular frame; therefore, the orientation of the spin relative to this frame is not defined. Consequently, in such a case, the electron’s spin direction does not affect the interaction between molecules, i.e., the exchange term of the dispersion interaction is spin independent. For chiral molecules, however, this is not the case. In the last 2 decades, it was established that when electrons are displaced in a chiral system, the rate of this displacement depends on their spin. This property was termed the chiral-induced spin selectivity (CISS).

In chiral systems, the spin that results from the charge displacement is strongly coupled to the molecular frame, such that spins of one type are displaced faster than the other, depending on the handedness of the molecule and the direction of motion. Charge displacement occurs whenever two chiral molecules approach each other, resulting in the formation of induced electric dipoles (see Figure 1A). Consequently, the emergence of these dipoles implies that at each electric pole, there is at least a fraction of an unpaired electron, i.e., spin polarization is intrinsically associated with the electric dipole formation in chiral molecules. The concept of charge polarization accompanied by spin polarization was verified in experiments in which the interaction of chiral molecules with ferromagnetic substrates was probed. Hence, when two chiral molecules interact, a spin-dependent interaction term emerges, which is dependent upon the relative handedness of the molecules.

Here, we present experiments in which the force between chiral oligomer attached to the tip of an atomic force microscope (AFM) and a monolayer made from oligomers that possess either the same or opposite handedness, or oligomers that are achiral, are monitored. The energies that associate with the enantiospecific interactions are larger by more than a factor five compared with the thermal energies at room temperature. These experimental results are accompanied by model calculations that show the role of the spin exchange interaction and its effect on the interaction energies and their angle-dependent distribution.
RESULTS

Direct measurements of enantiospecific interaction between helical peptides

Previous work has shown that exchange interactions can be probed using modified atomic force spectroscopy (AFS). This past study probed exchange interactions between ferromagnetic substrates and helical peptides. AFS is widely used to examine biological interactions and functions and is used for the study of binding and unbinding of proteins. This study utilizes the earlier mentioned method to probe spin exchange interactions between helical (hence chiral) peptides and demonstrate the relation between enantiomer selectivity and spin. A chemically modified standard gold-coated AFM cantilever was used. The functionalized cantilever is applied...
to generate a distance-dependent force curve based on short-ranged spin exchange interaction. The gold AFM tip was functionalized with polyethylene glycol (PEG), 60 nm long, bound to a helical peptide, L-AHPA, where AHPA is alpha helix polyalanine (AHPA) [HS-PEG-NH-AAAAAAKAAAAAAKAAAAAAKAAAAAAKAAKAAAAAKAAAAKAAAAAAKAAAAAKOOG] (see Figures 1C and 1D). The AHPA was chosen due to its strong spin separation rates. The AFM tip was functionalized in such a way that the peptide’s carboxyl group is facing the substrate (see experimental procedures section for details). The PEG acts as a spacer to reduce nonspecific interactions and the whole system is immersed in ethanol to eliminate capillary forces as done in Ziv et al. The measured samples consist of self-assembled monolayers of the same helical polypeptide adsorbed on a gold substrate. The adsorption process ensures a peptide alignment such that the carboxylic group is facing up, so that there are no covalent bonds possible between the oligopeptide on the AFM tip and the one adsorbed on the substrate. Over a thousand curves of force versus distance were measured, which were subsequently examined manually. Following previous studies, only curves that showed a clear pulling event (see in Figure S1) were further analyzed as single-molecule rapture events. A worm-like chain (WLC) model was then fitted on the specific interaction’s pulling event, and the pulling force was retrieved. The mean pulling force (MPF) was calculated by averaging over the selected pulling forces. The forces were averaged in order to achieve simple unbiased analysis. The interaction is expected to be transient; however, the time dependence of the tip’s interaction with the surface is not included in this study and is addressed in previous work. The timescale of this transient effect is relatively long since it is controlled by the adsorption and desorption kinetics as discussed in previous works.

The force between the same and different enantiomers is presented in Figure 2. Right- or left-handed helical oligopeptides (L-AHPA or D-AHPA, respectively) were measured when adsorbed on the gold substrate. As control experiments, we measured a monolayer of an achiral 12-mercaptododecanoic acid with a comparable length and an equivalent carboxylic head group facing up. The MPF of the molecules is shown in Figure 2A. The relatively strong binding forces are attributed to the exchange interaction due to the CISS effect. A force difference of 70 ± 10 pN between the interaction of the homochiral pair of oligopeptides (L-AHPA monolayer [L-AHPA]) and the interaction of the heterochiral pair (D-AHPA monolayer—L-AHPA adsorbed AFM tip) is obtained. The interaction’s energy is retrieved by integrating the force distance curve over the pulling distance. An average energy difference of about 0.3 eV is measured (Figure S2). The force measured here and the interaction energy agree with the splitting between singlet and triplet states (~1 eV).

We attribute the low forces to nonspecific interactions that are the same for all samples (i.e., Coulomb and dispersive forces). The higher forces are attributed to the spin-dependent exchange interaction and is different for homo and heterochiral interactions, i.e., this force is stronger between same enantiomers (L-L) than opposite enantiomers (L-D). The differentiation between high and low forces is discussed in the supplemental experimental procedures (Figure S8; Table S2). As a control experiment, we checked achiral molecules as well. The MPF between the L-AHPA adsorbed on the tip and achiral control was lower than the force for both enantiomers but without statistical significance. It is interesting to note that the MPF of the plain gold sample is lower than the MPF of the chiral L-L interaction. At first sight, this may be surprising since the interaction between a gold substrate and a carboxylic group has a coordinated character, but it can be explained by the gold being coated with organic contamination.
Characterization of the interaction: measurement under magnetic field and interaction range

To verify that the difference in the binding of the two enantiomers results from the spin effect, a sample of gold substrate with adsorbed L-AHPA monolayer was tested (Figure 3 A1). Due to the sulfur-gold bond, the sample becomes paramagnetic.33–36 By adding an external, out-of-plane magnetic field, the spin injection into the chiral monolayer can be controlled. The charge redistribution that happens upon approach of the tip affects differences in the electrochemical potential of the two systems.28 As a result, spin-dependent charge is moving from the substrate through the adsorbed molecule. This charge and its spin can affect the interaction between the molecules. The same functionalized tip, as described earlier, was used and a constant applied magnetic field perpendicular to the surface was applied during the measurement (see Figure 3 A1). The results are presented in Figures 3 A2 and 3A3. The MPF difference between up and down magnetizations is $28 \pm 10 \text{pN}$ (Figure 3 A2). These results support the notion that the interactions strength is spin dependent. A clear difference is also seen in the force distributions histograms (Figure 3 A3).

The effect of the substrate magnetization on the force measured, in the case of chiral molecules, can also be a result of more efficient charge penetration from the substrate into the chiral molecule, when the injected charge has the preferred spin for the given handedness.28 Here, the charge redistribution is caused by the adsorption37 and the pulling of the two molecules.22 These processes are known to change the polarization of the molecule and, therefore, result in an exchange of charge with the substrate. This charge transport from the substrate increases the spin density at the interaction region between the molecule attached to the tip and the adsorbed molecule. To evaluate this

Figure 2. Force spectroscopy measurements of enantioselectivity

(A) Mean pulling force (MPF) between the molecule on the tip (L-AHPA) and an AHPA monolayer adsorbed on a gold substrate. An achiral monolayer (mercaptododecanoic acid) as well as a clean substrate were used as a control experiment. For AHPA monolayers, a difference in the MPF is measured for same (L-monolayer) or opposite (D-monolayer) enantiomer interaction. The same enantiomer interaction is stronger by $70 \pm 10 \text{pN}$, where the error is standard deviation.

(B) The force distributions: the wide range of forces suggests multiple interactions. *Statistical comparison tests (ANOVA followed by post hoc Tukey test) showed a significant difference (p < 0.01) between the marked datasets (see supplemental information, Table S1, for more information).
effect, we investigated the interaction between a tip coated with achiral molecule and oligopeptide adsorbed on magnetized substrates (Figure S3). In this case, the difference in the force measured for the two directions of magnetization is within the noise range of the system. Thus, the main difference in force, under opposite magnetic field, is a result of spin-dependent exchange interactions.

We have shown so far that spin is affecting the biorecognition pulling force. To relate the results to exchange interactions only, and to differentiate them from mechanical and structural-related forces, we probed the interaction range. The spin exchange interaction is characterized by overlap of wave functions and is, therefore, short ranged. In a previous study, the decay length of the spin-dependent interaction was determined to be about 0.7 nm.\textsuperscript{38} To probe the range of the effect observed in this study, a layer of achiral amino-acid (glycine), 0.4 nm long, was added to the respective helical peptide monolayers (L/D-AHPA), which are 5.4 nm high. The adsorption of the glycine was done following a protocol reported

Figure 3. Force spectroscopy results under constant magnetic field

(A1) Schematic of the experimental system where the force is measured under different spin injection conditions. A magnet is placed under the sample with a constant magnetic field. This changes the spin wave function, resulting in different measured pulling force.

(A2) Mean pulling force (MPF) between the molecule on the tip (L-AHPA) and an L-AHPA monolayer adsorbed on gold substrate under magnetic field. A difference in the MPF is measured up or down magnetization. The interaction under out of plane magnetic field is stronger by 28±9pN demonstrating the dependence of the pulling force strength on the spin wave function. The error is standard deviation.

(A3) Force distributions histograms. Force spectroscopy results for separated chiral monolayer.

(B1) Schematics of the measurement system. A glycine layer was added to an adsorbed AHPA monolayer. The glycine’s carboxyl group facing up.

(B2) Mean pulling force (MPF) between the molecule on the tip (L-AHPA) and the monolayer. In this case, smaller difference is measured in the MPF between the monolayers. To see the effect clearer, the force distributions are analyzed.

(B3) The force distributions. The first correspond to the nonspecific interaction. The second corresponds to the exchange interaction. A different force for L and D monolayers is measured. This is the enantiospecific force. The inset displays the decay of the mean pulling force difference between L and D, for adding zero, one (Figure 3B2), or two glycine layers (see Figure S4). Each glycine layer adds 4Å to the thickness. *statistical comparison tests (ANOVA followed by post hoc Tukey test) showed a significant difference (p < 0.01) between the marked datasets (see supplemental information for more information).
in the literature, and the experimental layout and of the monolayer with glycine is presented in Figure 3B1. The samples were measured with the same functionalized AFM cantilever as before. The results are presented in Figures 3B2 and 3B3. A difference in the MPF of 25 ± 4 pN was measured, suggesting that the effect is still apparent but weaker than without the achiral separation. An additional verification of the range of the force was performed with samples having a thicker bi-layer of glycine (0.8 nm) (see Figure S4), and the results are presented in the inset of Figure 3B3. Note the higher MPF for the longer bi-layer due to possible intertwining of the molecules. In the case of a sample in which the chiral molecules are separated by 0.8 nm, the difference in the force between the enantiomers has completely disappeared, suggesting a length dependence of the force as expected in the case of spin-exchange-related interactions. It is important to note that these distances are relevant to the crowded and high-pressure in vivo environments. These results also suggest that the penetration of the helical peptide into the monolayer is also short ranged, otherwise the structural differences would become apparent in the interaction. It is important to appreciate that when a chiral molecule interacts with achiral one, the spin exchange interaction term vanishes, since the spin direction on the achiral molecule that is associated with the interaction is random and, therefore, on average for ensemble of molecules or when many molecules are studied, this term vanishes.

**Toy model: Two-site system with spin-polarization force**

As mentioned earlier, none of the contemporary computational electronic structure methods can fully describe the spin exchange interaction term. Also, spin-dependent dynamic DFT calculations on molecules have not been implemented in any publicly available quantum chemistry code so far. To obtain an insight into the observed spin-dependent exchange interaction and its relation to enantio-selective interaction between biomolecules, we developed a “toy model” that—despite its simplicity—captures the essential physics in a phenomenological manner. The model does not supply a full theory but is meant to demonstrate that the spin exchange interactions may generate directional forces at short scale, strong enough to match our results and be relevant in vivo. We opted to mimic this phenomenon through inclusion of so-called “spin-polarization sources” (vide infra) within a valence bond (VB) framework. In our toy model, the two chiral helices are represented as 2 two-site systems, i.e., two H-like molecules. For the resulting four-site system, 6 (covalent) VB determinants can be defined, corresponding to the different distributions of 2 σ-electrons and 2 π-electrons in 4 orbitals (see Figure S5). In the dissociation limit, i.e., in the limit of infinite separation between the two molecules, the spins will not interact with each other. To phenomenologically enforce (dispersion-induced) spin polarization, we add a “spin-polarization source” to each of the molecules (Figure S6A). These spin-polarization sources are single sites with a predefined/fixed spin, which induce spin polarization in the adjacent molecules by increasing/decreasing the energy of the respective VB determinants. As can be seen from Figures S6B and S6C, placing the two sources with an opposing spin leads to spin polarization of the same sign in both molecules; placing them with a parallel spin leads to spin polarization of the opposite sign in both molecules. Note that the distance between the source and the actual molecule is an arbitrary parameter, which sets the extent of induced spin polarization; in the calculations performed, a distance of 0.1 nm was selected, which results in net spins of approximately 0.33e on each of the individual sites of the molecules in the dissociation limit.

As expected, in the dissociation limit, the spin polarized wave functions obtained for the two spin-polarization-source alignments are degenerate in energy. When the
The spacing between the two molecules is reduced; however, this degeneracy is broken: at a distance of 2–3 Å between the two molecules, the spin interaction starts to become significant.

Next to the (spin-dependent) exchange term, a Lennard-Jones potential with parameters $\sigma = 0.16$ nm and $\varepsilon = 3.0$ kcal/mol was added to the model to collectively account for all the spin-independent interaction terms. The resulting potential energy profile is shown in Figure 4A for the case of collinear approach of the two spin polarized species. The energy one samples with the AFM measurements corresponds to the difference between the bottom of the well in each potential (namely for the two spin configurations, parallel and antiparallel spins). It should be clear that the toy model provides qualitative result that are consistent with the experimental measurements. The interaction energy calculated from the model also fit the order of magnitude interactions, which were estimated using DFT calculations of L polyglycine with opposite magnetize surfaces. In that case also a change in the density of electrons in the molecule was predicted for experiment with similar measurement setup. Figure 4B shows the angle and distance dependence of the energy difference for the two spin configurations. This plot reveals a clear preference for a collinear configuration due to the spin exchange interaction. For 90° orientation between chiral molecules the forces become small and almost cancel out (see Figure S7).

The preference for a narrow range of approach angles emerging from our model can be expected to be relevant to many complex biological processes, since it may reduce the phase space that a system has to explore before reacting, thus enhancing reaction rates. As such this relatively subtle, yet probably ubiquitous, effect may be responsible for the remarkable speed of biorecognition effects in protein folding and in searching DNA by enzymes.

To summarize, three experiments using chiral atomic force microscopy were presented. The first, directly measured the interaction force difference between same and opposite enantiomers. The second and third experiments show interactions between helical
peptides and present two main characteristics of the exchange interaction: its spin dependency and its short range (≈7 Å). The third experiment also suggests that the peptide adsorbed on the tip of the AFM does not penetrate more than 8 Å into the monolayer. Our findings suggest that spin-dependent exchange interaction may play a pivotal role in biorecognition processes. The energy scale for L-AHPA-L-AHPA interactions is on the order of 0.5 eV (45 KJ/mol). When the chiral molecules interact, symmetry constrains, that arise from the chirality create a different spin distribution for homochiral and heterochiral interaction. A toy model for the enantiomer-specific interaction shows that the interaction is controlled by the exchange interaction of electron spin pairs and exhibits a significant radial dependence.

Hence, the experimental results and the model calculations suggest an additional interaction with a new term, not considered so far, that may explain enhanced enantiospecificity and various important processes occurring in biology. The new interaction term introduces short range force that is especially relevant to biological systems, where the interacting systems are typically in crowded environment.

**EXPERIMENTAL PROCEDURES**

**Resource availability**

**Lead contact**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Yossi Paltiel (paltiel@mail.huji.ac.il).

**Materials availability**

The thiol functionalized PEGylated chiral (and helical) polypeptide, Alpha Helix Polyalanine (AHPA) [HS-PEG-NH-AAAAAKAAAAAKAAAAAKAAAAAKAAAAAKAAAAKAAAAKCOOH], and the thiol functionalized PEGylated non-helical reference compound [HS-PEG-NH-6-Ahx-K-COOH] were synthesized by us for this study. There are restrictions to the availability of these molecules because of the lack of an external centralized repository for its distribution and our need to maintain the stock. We are glad to share the molecules with reasonable compensation by requestor for its processing and shipping.

**Data and code availability**

Force curve data reported in this paper will be shared by the lead contact upon request. No original code is reported in this paper.

**Materials**

Fmoc-L-Ala-OH, Fmoc-L-Lys (Boc)-OH, and Fmoc-L-Lys (Boc)-Wang resin (0.343 meq/gm and 100–200 mesh) were purchased from Chem-Impex. NHS-PEG-S-Trt were purchased from Iris Biotech. N, N’-dimethylformamide (DMF), dichloromethane (DCM), piperidine, methanol, trifluoroacetic acid (TFA), diethyl ether, methanol (MeOH), and ethanol (EtOH) were purchased from Bio-Lab (Jerusalem, Israel). Tri isopropyl silane (TIPS), Oxyma pure, N, N’-disopropylcarbodiimide (DIC), 1,2-ethanediol (EDT), ninhydrin, phenol, pyridine, and Fmoc-6-Ahx-OH were purchased from Sigma Aldrich.

**Molecule synthesis**

The thiol functionalized PEGylated chiral (and helical) polypeptide, Alpha Helix Polyalanine (AHPA) [HS-PEG-NH-AAAAAKAAAAAKAAAAAKAAAAKAHAAAAAKAAAAKCOOH], and the thiol functionalized PEGylated non-helical reference compound [HS-PEG-NH-6-Ahx-K-COOH] were synthesized on solid phase following solid
phase peptide synthesis procedure using microwave peptide synthesizer (CEM, Discover Bio). Fmoc-L-Lys (Boc)-Wang resin (0.343 meq/gm and 100–200 mesh) was used as solid support. The resin was swelled overnight in dichloromethane (DCM) and N, N’-dimethyl formamide (DMF) solvent mixture (1:1) prior to the synthesis. The Fmoc deprotection was performed by 20% piperidine in DMF and coupling reaction was performed using Oxyma pure and N, N’-diisopropylcarbodiimide (DIC) in DMF solvent under microwave. Fmoc-L-Ala-OH, Fmoc-L-Lys (Boc)-OH, and NHS-PEG-S-Trt were used to make the chiral alpha helix polyalanine. Fmoc-6-Ahx-OH and NHS-PEG-S-Trt were used to make the non-helical reference compound. Ninhydrin test was performed at each step of coupling and Fmoc deprotection to check whether the reaction was completed or not.

Cleavage of molecule from the resin
The resin was washed five times with each of the following solvents DMF, DCM, methanol, and diethyl ether and kept under vacuum for 4 h to ensure complete dryness. Cleavage cocktail containing 92.5% TFA, 2.5% TIPS, 2.5% EDT, and 2.5% water was used. The molecule attached on the resin was taken with the cocktail mixture and shacked for 4 h at room temperature. The solution was drained and poured into ice cold diethyl ether for precipitation. It was kept at −20°C overnight and centrifuged at 5,000 rpm at 4°C. The residue was dissolved in water and lyophilized. A white solid compound was obtained.

Tip functionalization and AFM measurements
The solid compound was dissolved in triple distilled water to prepare 1-mM solution. The gold tip was washed with EtOH, dried in air, and incubated with the 1-mM solution of the molecule overnight at room temperature. The tip again was washed with water to remove the non-attached molecules and dried in air. AFM measurements were performed in EtOH.

Sample preparation
A gold substrate (Si/Cr [15 nm]/Au [100 nm]) was used for all samples’ preparation. The substrates were cleaned by boiling (70°C) acetone and then ethanol for 10 min each. Then the samples were introduced into a plasma Asher system (Diener PICO UHP) for 10 min at 50% strength. Finally, the samples were soaked in absolute ethanol for 20 min and dried under nitrogen.

1st part
The plain gold substrate reference sample was cleaned as mentioned earlier. The L and D AHPA samples and achiral (12-mercaptopdodecanoic acid) sample were prepared, as described and characterized in previous works, by dipping the gold substrates in a 1 mM solution of: L-AHPA (L-AHPA-36 [1 mM]) | D-AHPA (D-AHPA-36 [1 mM]) | achiral molecule (12-mercaptopdodecanoic acid [1 mM]) overnight. Then, rinsing in absolute ethanol and drying under hydrogen. The whole process is done under nitrogen chamber.

2nd part
L-AHPA molecules were adsorbed on a gold substrate as in the 1st part. The molecules were measured under a constant magnetic field of 3,000 gauss using an external magnet during the measurement.

3rd part
L and D-AHPA samples on gold substrate were prepared as in the 1st part. The monolayers’ carboxylic groups were activated by an EDC-NHS process standard
protocol. Then glycine was added to the samples for 2 h. Finally, the samples were rinsed with water and dried with nitrogen. For the thicker layer of glycine, the process was repeated twice.

**Force spectroscopy measurements**

Force spectroscopy curves were retrieved using JPK AFM (NanoWizard3). A commercially available gold-coated tip was functionalized by immersing the tip for 20 min in ethanol and then by overnight adsorption of the above-mentioned molecules. Over 1,000 curves were taken (1,500–4,500 depending on the sample). Only the curves that showed a significant distinguished pulling event were taken and analyzed by the JPK data analysis software. The WLC model was fitted to the pulling events to find the rupture point and pulling force (see Figure S1). The MPF was retrieved by averaging the pulling forces.

**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.chempr.2021.08.002.

**ACKNOWLEDGMENTS**

Y.P. and R.N. acknowledge the support of the John Templeton Foundation and the MOS Israel. R.N. acknowledges the partial support of the ISF and of the Minerva Foundation. S.S. and T.S. are supported by the ISF grant 520/18. T.S. acknowledges the Research Foundation-Flanders (FWO) for a position as a postdoctoral research fellow (1203419N). A.S. acknowledges the support of the Shunbrun Fellowship.

**AUTHOR CONTRIBUTIONS**

Y.K. and A.S. performed and analyzed the experimental results. T.D.-A. and A.Z. helped in the AFM measurements. T.M. and S.Y. helped in all the chemical aspects of the paper. T.S. did the modeling and theoretical calculations. S.S. supervised the theoretical part of the manuscript. S.Y., M.R., R.N., and Y.P. conceived the experimental part of the work and participated in planning and analyzing the results. All authors participated in writing the manuscript. All the data are presented in the manuscript and supplemental information.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

**INCLUSION AND DIVERSITY**

One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in science.

One or more of the authors of this paper received support from a program designed to increase minority representation in science.

Received: April 13, 2021
Revised: June 6, 2021
Accepted: August 3, 2021
Published: August 27, 2021
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Supplemental information

Evidence for new enantiospecific interaction force in chiral biomolecules

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**Supplemental information**

**This PDF file includes:**

| Supplemental items: |
|---------------------|
| **Figure S1:** A force vs. distance curve example and explanation on the force curve analysis |
| **Figure S2:** Energy analysis of homo-chiral interaction of AHPA and hetero-chiral interaction of AHPA |
| **Figure S3:** Single molecule force spectroscopy results under a constant magnetic field with a-chiral tip |
| **Figure S4:** Spaced monolayers with 0.8nm glycine spacer |
| **Figure S5:** The different (covalent) distributions of 2 alpha and 2 beta electrons in the 4 orbitals making up the model system. Note that we do not consider ionic structures here explicitly. |
| **Figure S6:** The model system with the “spin-polarization sources” included and the different covalent determinants contributing to the wave-function in the dissociation limit |
| **Figure S7:** Perpendicular approach of molecules for the toy model |

**Supplemental experimental procedures:**

| Statistical comparison of the MPF presented in the paper |
| Differentiating between high and low forces in the force distributions |
| Additional information regarding the addition of the Lennard-Jones potential |
Figure S1: A force vs. distance curve example. (1) The cantilever is in contact with the sample’s substrate. (2) As the cantilever retracts the helical peptide on the tip captures a molecule from the surface and (3) both molecules stretch and align, this is the pulling event. (4) At the breaking point, the molecules break apart and the cantilever sensed force is back to zero.

To extract the pulling force a worm like chain (WLC) extension model is fitted to the relevant pulling event. The breaking point is the point in which the curve diverts from the WLC model. The pulling force is the force at the breaking point.

The pulling energy is extracted by integrating the force curve from the breaking point to the point in which the force is back to zero.
Energy analysis

Figure S2: **Energy analysis** homo-chiral (L-L) interaction of AHPA (blue) and hetero-chiral (L-D) interaction of AHPA (orange). The energy of a single pulling event is retrieved by integrating over the force curve from the rupture point to the end of the pulling event. The energy attributed to a sample is the mean energy of all the pulling events in that sample. **Top:** the mean energy for homo- and hetero-chiral interaction of AHPA. The energy gap between same and opposite enantiomers' interaction is $0.3 \pm 0.2 \text{ eV}$. **Bottom:** the energies' distributions.
A reference non-helical peptide (PEG-6-Ahx-K) was synthesized and attached to a gold coated AFM cantilever. The functionalized cantilever was used in Force spectroscopy to measure the interaction force between the reference peptide and a L-AHPA monolayer under a constant magnetic field applied by an external magnet. There is no difference between the mean pulling force (over the margin of error) measured for opposite magnetic field. Since the reference peptide does not create a spin dipole, there is no specific symmetry for the exchange interaction and therefore there is no difference between the magnetizations. This supports the argument that the strength of the interaction is determined by the exchange interaction.
Figure S4: **spaced monolayers.** homo-chiral (L-L) interaction of AHPA (blue) and hetero-chiral (L-D) interaction of AHPA (orange) with a spacer of 0.8 nm (glycine). The force difference disappears.
Figure S5: The different (covalent) distributions of 2 alpha and 2 beta electrons in the 4 orbitals making up the model system. Note that we do not consider ionic structures here explicitly.
Figure S6: a) The model system with the “spin-polarization sources” included. b) The different covalent determinants contributing to the wave-function in the dissociation limit for opposite spins on the source sites and c) the different determinants for parallel spins on the source sites. The determinants denoted in green are the most favorable under the considered situation; the orange determinants are the least favorable whereas the remaining two determinants remain degenerate. As a result of the increase in the weight of the green determinant in the wavefunction, the overall wavefunction polarizes accordingly (cf. the bottom of panel b and c; the spin densities shown were obtained for a distance of 1Å between the spin-polarization sources and the actual molecules).
Figure S7: perpendicular approach. The toy model considers only head-to-head approaches. The interaction term does not cancel out in perpendicular approach because perfect cancellation can only be expected to occur in a perfectly symmetric situation, i.e., when the second molecule approaches the first one perfectly perpendicular to the middle point of its spin distribution (cf. Fig. below). Here, we exclusively considered head-to-head approach. In other words, we simply rotated the second molecule with respect to the endpoint of the first one, so this symmetry condition is never strictly fulfilled in our case, i.e., the individual repulsive/attractive terms do not cancel each other out entirely.
Supplemental experimental procedures:

Statistical comparison of the MPF presented in the paper

A one-way analysis of variance test (ANOVA) was used to determine whether the four samples measured and presented in Figure 1 (L&L, D&L, non-chiral molecule &L, gold substrate &L) had a significant statistical difference.

The one-way ANOVA test gave a p-value of $10^{-14}$, which means there was a significant difference between the four samples. Next, a Tukey test was preformed to determine whether there was a difference between each pair of the four samples. The results are presented in Supplementary table S1.

The results of this test show a clear difference between samples L and D (homo vs. heterochiral interactions) and between samples L, G and N (homochiral vs. non-chiral interactions). The similarities between D and samples N and G can be explained by the suppression of the exchange interaction.

Multiple Comparison of Means - Tukey HSD, FWER=0.05

| group1 | group2 | mean diff | adj  | lower | upper | reject |
|--------|--------|-----------|------|-------|-------|--------|
| D      | G      | 0.0       | 0.366| -0.0  | 0.0   | False  |
| D      | L      | 0.0       | 0.001| 0.0   | 0.0   | True   |
| D      | N      | -0.0      | 0.9  | -0.0  | 0.0   | False  |
| G      | L      | 0.0       | 0.001| -0.0  | 0.0   | True   |
| G      | N      | -0.0      | 0.042| -0.0  | -0.0  | True   |
| L      | N      | -0.0      | 0.001| -0.0  | -0.0  | True   |

Table S1: Multiple Comparison of Means - Tukey HSD. Presented are the results of a Tukey test between the following samples (groups): L-AHPA (L), D-AHPA (D), non-chiral peptide (N), clean gold substrate (G). There is a clear statistical difference between samples L and D (homo vs. heterochiral interactions) and between samples L, G and N (homochiral vs. non-chiral interactions). The similarities between D and N and D and G can be explained by the suppression of the exchange interaction.

For the results in Figure 3 (measurements with magnetic field and samples with spacers) a t-test was performed. For the magnetic field results (Figure 3A) the p-value was 0.0021 and for the separated chiral layer (Figure 3B) a p-value of 2.134e-105 was calculated. Both are very good results showing significant difference.
Differentiating between high and low forces in the force distributions

In the main text, low forces are attributed to non-specific interactions and high forces to the exchange interaction. The difference between interactions is calculated by averaging over all of the force histogram since it is very difficult to differentiate between different forces in the experimental setup. This is due to the multiple molecules on the tip and the variety of approach angles between molecules on the tip and on the substrate. Here we try to quantify how much of the force difference measure is due to high forces by fitting a sum-of-gaussians (2) to the respective force distributions.

**Figure S8: sum of gaussians fit for figure 2's force distributions.**

![Sum of Gaussians Fit](image)

**Table S2: sum of gaussians fit for figure 2.**

|                     | Fit parameters | L-AHPA [pN] | D-AHPA [pN] | Nonchiral [pN] | Au [pN] |
|---------------------|----------------|-------------|-------------|----------------|---------|
| **Non-specific** interaction | center         | 59          | 42          | 45             | 71      |
|                     | width          | 20          | 10          | 19             | 20      |
| **Specific** interaction | center        | 175         | 99          | 104            | 107     |
|                     | width          | 152         | 60          | 80             | 100     |

The main difference in the overall forces stems from the higher forces (for example the force difference between homo and hetero chiral pairs: high forces - 76 ± 163 [pN], low forces -11 ± 22 [pN]). Only in clean gold substrate, larger differences in the low forces were measured. This is expected as the interaction with gold differs from the peptide's carboxylic interactions. It is important to stress that this is still not enough to completely resolve the high and low forces, as apparent from the errors.
Additional information regarding the addition of the Lennard-Jones potential:

The toy model's calculations provide us the spin-exchange splitting between the triplet and singlet orientation. This is the “new” term which is exclusively due to the spin polarization caused by the CISS effect. To account for the conventional Pauli repulsion and dispersion, we add a conventional Lennard-Jones potential, shown below (with $\sigma=0.16$ nm and $\varepsilon=3.0$ kcal/mol):

\[
V_{LJ} = 4\varepsilon \left[ \left( \frac{\sigma}{r} \right)^{12} - \left( \frac{\sigma}{r} \right)^{6} \right]
\]