

Enantioselective Molecular Recognition between β -Sheets

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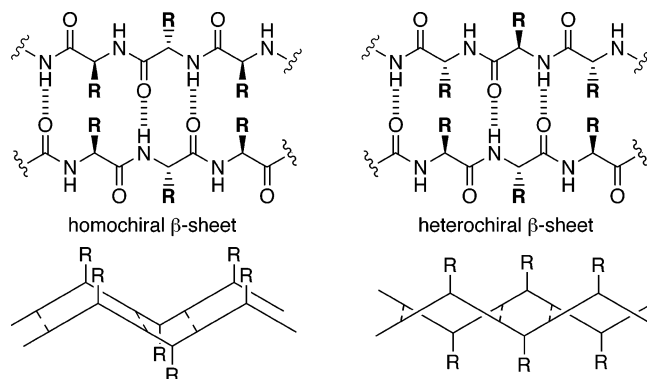
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The coming together of two hands in prayer and the coming together of two hands in a handshake represent fundamentally different interactions among chiral objects. Heterochiral and homochiral interactions of this sort can also occur among biomolecular structures. This communication asks which type of interaction is preferred between β -sheets and finds that homochiral pairing is strongly preferred to heterochiral pairing.

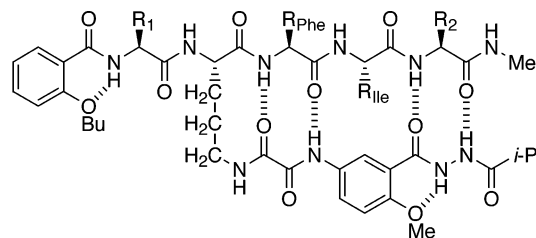
Interactions between β -sheets occur widely among proteins in biologically important processes as diverse as the dimerization of HIV-1 protease, the interaction between Ras oncoproteins and kinase enzymes, and the aggregation of β -amyloid.¹ These interactions are of course homochiral, because nature produces proteins of only one chirality. Whether homochiral interactions between β -sheets are preferred, however, is not clear from various reports scattered throughout the literature: Furhop and co-workers reported the precipitation of heterochiral β -sheets upon mixing aqueous solution of poly(D-lysine) and poly(L-lysine).² Maggio and co-workers have demonstrated that the aggregation of β -amyloid occurs with *homochiral* selectivity, while Gervais and co-workers have found that the inhibition of β -amyloid aggregation by small peptides derived from β -amyloid occurs with *heterochiral* selectivity.^{3,4} Although enantiomeric HIV-1 protease has been synthesized, the formation of heterochiral HIV-1 protease dimers has not been reported.^{5,6}

Although the edges of both L- and D- β -sheets put forth the same pattern of hydrogen-bond donor and acceptor groups, the side chains point in opposite directions. Homochiral pairing of β -sheets generates structures in which the pleats and side chains of adjacent β -strands are parallel to each other, while heterochiral pairing of β -sheets generates structures in which the pleats and side chains are antiparallel (Chart 1).

Chart 1



To test which pairing is preferred, we have prepared and studied β -sheets **1**, which comprise all L-amino acids, and β -sheets **2**, which comprise all D-amino acids.⁷ Variants of each of these compounds were prepared that display different amino acids at two of the non-hydrogen-bonded β -sheet interaction sites (R_1 and R_2). These variants are referred to by their chirality and the residues they display as follows: L-Leu-Leu (**1a**), L-Val-Val (**1b**), L-Val-Ala (**1c**), L-Ala-Val (**1d**), D-Leu-Leu (**2a**), D-Val-Val (**2b**), and D-Val-Ala (**2c**).

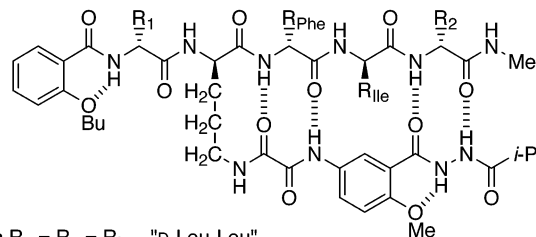


1a $R_1 = R_2 = R_{Leu}$ "L-Leu-Leu"

1b $R_1 = R_2 = R_{Val}$ "L-Val-Val"

1c $R_1 = R_{Val}$ $R_2 = R_{Ala}$ "L-Val-Ala"

1d $R_1 = R_{Ala}$ $R_2 = R_{Val}$ "L-Ala-Val"



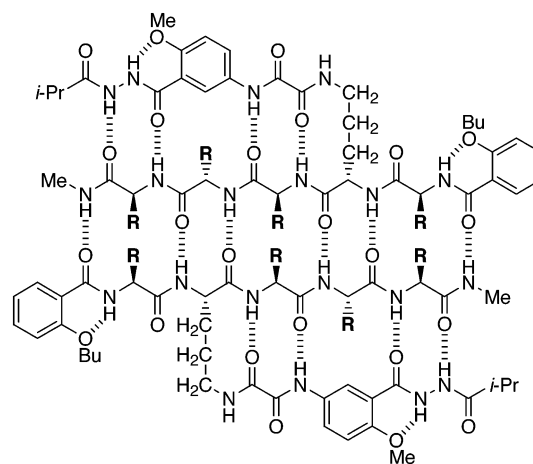
2a $R_1 = R_2 = R_{Leu}$ "D-Leu-Leu"

2b $R_1 = R_2 = R_{Val}$ "D-Val-Val"

2c $R_1 = R_{Val}$ $R_2 = R_{Ala}$ "D-Val-Ala"

Previous studies in our laboratory have established that β -sheets such as L-Leu-Leu (**1a**) and L-Val-Val (**1b**) exist as well-defined dimers in organic solvents (Chart 2).⁷ β -Sheets **1a** and **1b** form

Chart 2. Homochiral β -Sheet Dimer



homodimers (**1a·1a** and **1b·1b**) in $CDCl_3$ solution. When mixed, these compounds equilibrate to form a heterodimer (**1a·1b**). The anilide and hydrazide NH resonances of these species are well-resolved in the 1H NMR spectrum, permitting the identification and quantification of the dimers (Figure 1). β -Sheets **1c** and **1d** also form homo- and heterodimers that exhibit well-resolved anilide and hydrazide NH resonances.

When the L- β -sheets (**1**) are mixed with the enantiomeric D- β -sheets (**2**), homochiral β -sheet dimers predominate, and only small quantities of heterochiral β -sheet dimers form. Thus, mixing of

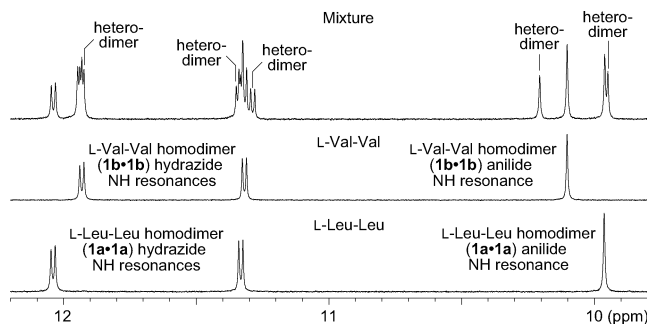


Figure 1. ^1H NMR spectra of the hydrazide and anilide NH groups of L-Leu-Leu peptide **1a** (lower), L-Val-Val peptide **1b** (middle), and a mixture of the two peptides (upper). Spectra were recorded at 500 MHz in CDCl_3 at 253 K at 2.0 mM of each peptide.

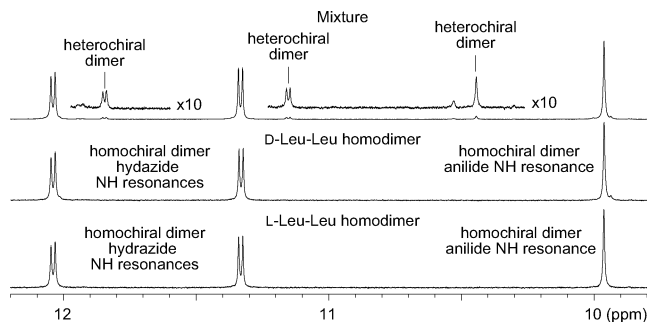


Figure 2. ^1H NMR spectra of the hydrazide and anilide NH groups of L-Leu-Leu peptide **1a** (lower), D-Leu-Leu peptide **2a** (middle), and a mixture of the two peptides (upper). Spectra were recorded at 500 MHz in CDCl_3 at 253 K at 2.0 mM of each peptide. The peak at 10.52 ppm is an impurity present in **2a**.

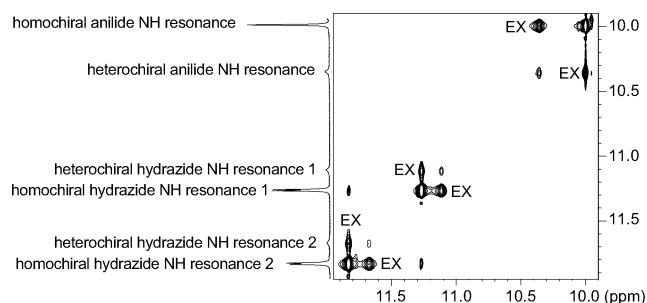


Figure 3. 2D EXSY spectrum of a mixture of L-Leu-Leu peptide **1a** and D-Leu-Leu peptide **2a**. The spectrum was recorded at 800 MHz in CDCl_3 at 308 K at 2.0 mM of each peptide using a 500-ms mixing time. EXSY cross-peaks are marked "EX".

equimolar quantities of the L-Leu-Leu homochiral dimer (**1a•1a**) and D-Leu-Leu homochiral dimer (**2a•2a**) in CDCl_3 solution results in the formation of new anilide and hydrazide NH resonances (Figure 2). A 2D EXSY experiment demonstrates that the new species exchanges with the homochiral species and corroborates that the new species is the heterochiral dimer **1a•2a** (Figure 3).⁸ Quantification of these species by integration or deconvolution of the anilide NH resonances reveals a 95.8:4.2 mixture of homochiral and heterochiral β -sheet dimers at 253 K. This ratio corresponds to a homochiral dimer–heterochiral dimer equilibrium constant of 0.0079 ($K = [\mathbf{1a}\cdot\mathbf{2a}]^2/[\mathbf{1a}\cdot\mathbf{1a}][\mathbf{2a}\cdot\mathbf{2a}]$) and a statistically corrected free-energy difference of 3.1 kcal/mol ($\Delta G = -RT \ln(K/4)$). Small quantities of heterochiral dimer also form upon mixing of the other L- and D- β -sheets (Table 1).

These studies establish that homochiral pairing of β -sheets is preferred to heterochiral pairing, at least within the context of nonpolar side chains and a low-polarity solvent. Since five amino

Table 1. Formation of Homo- and Heterochiral β -Sheet Dimers^a

	1a and 2a	1b and 2b	1c and 2c	1d and 2c
dimer ratio	95.8:4.2 ^b	97.9:2.1 ^b	98.5:1.5 ^b	42.5:53.6:3.9 ^c
K	0.0079	0.0018	0.0009	0.0068
ΔG (kcal/mol) ^d	3.1	3.9	4.2	3.2

^a CDCl_3 , 253 K. ^b $[\mathbf{1}\cdot\mathbf{1}+\mathbf{2}\cdot\mathbf{2}]:[\mathbf{1}\cdot\mathbf{2}]$. ^c $[\mathbf{1d}\cdot\mathbf{1d}]:[\mathbf{2c}\cdot\mathbf{2c}]:[\mathbf{1d}\cdot\mathbf{2c}]$. ^d Statistically corrected free-energy difference ($\Delta G = -RT \ln(K/4)$).

acid residues are involved in the interactions of **1** and **2**, the free-energy differences observed correspond to a thermodynamic preference of 0.6–0.8 kcal/mol per interacting residue.⁹

A number of explanations may be envisioned for the high enantioselectivity of molecular recognition between β -sheets. Favorable nonbonded contacts between the adjacent β -strands may occur when the pleats and side chains point in the same direction. This model might also explain the preferential formation of heterochiral β -sheets in poly(D-lysine) and poly(L-lysine), as heterochiral β -sheet formation should minimize repulsion between the cationic lysine side chains.² Alternatively, the well-known twist of β -sheets might dictate that homochiral β -strands, which should twist in the same direction, fit together better than heterochiral β -strands, which should twist in opposite directions.

The enantioselective recognition between β -sheets described herein differs from the widely studied enantioselective binding of ligands by chiral receptors, because it involves interactions between partners of comparable size and achieves selectivity through the type of shape complementarity that occurs in a handshake, rather than the sort of lock-and-key complementarity that typically characterizes molecular recognition between partners of largely unequal sizes.¹⁰

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Supporting Information Available: Synthetic procedures and extensive 1D and 2D ^1H NMR spectral data for β -sheets **1** and **2** (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- Although the low concentration of heterochiral dimer precludes rigorously establishing the precise mode of heterochiral interaction (e.g., through NOE studies), it is difficult to envision a mode of dimerization other than antiparallel β -sheet formation that would be sufficiently strong to provide slow exchange on the NMR time scale under the conditions of these experiments. Because alternative modes of heterochiral interaction cannot be precluded rigorously, the value of 0.6–0.8 kcal/mol per interacting residue should be taken as a lower limit for the preference of antiparallel homochiral β -sheet formation over antiparallel heterochiral β -sheet formation.
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