



Figure 3. (a) Scheme for the thiol exchange assay. (b) Representative results of the disulfide rearrangement as monitored by RP-HPLC.

Table 2. Stability of Coiled-Coils

coiled-coil	T_m (°C)	ΔG_{unf} (kcal/mol)
Base-L21A/pLEIM	55	10.5
Base-L21A/Acid-p1	42	8.6
Base-p1/Acid-p1	65	11.6
Base-p1/pLEIM	62	11.7

urea denaturations of Base-L21A paired with either pLEIM or Acid-p1 were performed to gain insight into stability and specificity of these coiled-coils (Table 2). Base-L21A/pLEIM is 1.9 kcal/mol more stable than Base-L21A/Acid-p1. This result is consistent with previous host–guest studies involving a different heterodimeric coiled-coil by Vinson et al., who found that *a-a'* Ile-Ala pairing is significantly more stabilizing than a comparable Leu-Ala pairing.⁹ Base-p1/Acid-p1 and Base-p1/pLEIM are nearly identical in stability; thus, the double-mutation of Leu₂₁→Ile and Leu₂₄→Met has little effect on pairing with Base-p1. Table 2 shows that while a single Leu→Ala mutation in Base-p1/Acid-p1 costs ~3 kcal/mol in coiled-coil stability, two-thirds of this energy may be regained via appropriate core mutations. The stability of Base-L21A/pLEIM (30 residues/helix) is comparable to that of GCN4-p1, a 33-residue/helix homodimer (reported ΔG_{unf} of 10.6 kcal/mol).¹⁰

The preference of Base-L21A for pairing with pLEIM vs Acid-p1 was further explored in a thiol-disulfide exchange assay. We prepared variants of these three peptides that contain Cys-Gly-Gly sequence appended to the N-terminus (Base-L21A-SH, pLEIM-SH, and Acid-p1-SH). Oxidation of Base-L21A-SH with Acid-p1-SH gave the disulfide-bonded heterodimer Base-L21A-SS-Acid-p1. Mixing this dimer with an equal amount of pLEIM-SH in degassed PBS at pH 7.0 resulted in disulfide rearrangement to give Base-L21A-SS-pLEIM as the predominant dimer (Figure 3). The phage-derived coiled-coil pairing (Base-L21A-SS-pLEIM) is preferred over the mismatch dimer by ~3:1 under these equilibrium conditions. Similar results were obtained by performing the assay in the opposite direction, beginning with Base-L21A-SS-pLEIM and Acid-p1-SH.⁸

Several groups have explored “steric-match” strategies to generate coiled-coils that contain alanine in the hydrophobic core. However, such approaches have produced stable structures only in systems with trimeric or higher oligomerization states^{11a,b} or greater peptide length (six heptads).^{11c} Furthermore, these studies have

focused entirely on interactions between residues at lateral positions (e.g., Leu₂₁ on Base-p1 with Leu₂₁ on Acid-p1). The apparent ability of a modification in a neighboring side chain layer, Leu₂₄→Met in pLEIM, to participate in accommodating the Leu₂₁→Ala mutation on Base-L21A suggests that analysis of natural sequences and design efforts should not focus exclusively on lateral contacts.

Our results show that heterodimeric coiled-coil pairing selectivity can be profoundly influenced by nonobvious side-chain interactions in the nonpolar core and that phage display is an excellent technique for identifying such interactions. The “compromised” sequence, Base-L21A, appears to be quite discriminating in its pairing preference, but the preferred partner, pLEIM, seems to be promiscuous, as is Base-p1 itself. These observations suggest that insertion of compromising mutations on both partners may be an effective way to generate a coiled-coil in which both partners display high selectivity. Testing of this hypothesis and exploration of other implications of the results described here are underway.

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Supporting Information Available: Phage display procedures, experimental details for peptide synthesis and acquisition of CD data, thermal denaturation and sedimentation ultracentrifugation data. This material is available free of charge via the Internet at <http://pubs.acs.org>

References

- (1) (a) Crick, F. H. C. *Acta Crystallogr.* **1953**, *6*, 689–697. (b) Pauling, L.; Corey, R. B. *Nature* **1953**, *171*, 59–61. (c) O’Shea, E. K.; Klemm, J. D.; Kim, P. S.; Alber, T. *Science* **1991**, *254*, 539–544. (d) Lupas, A. *Trends Biochem. Sci.* **1996**, *21*, 375–382.
- (2) (a) König, P.; Richmond, T. J. *J. Mol. Biol.* **1993**, *233*, 139–154. (b) Sodek, J.; Hodges, R. S.; Smillie, L. B.; Jurasek, L. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 3800–3804. (c) Chan, D. C.; Fass, D.; Berger, J. M.; Kim, P. S. *Cell* **1997**, *89*, 263–273.
- (3) (a) Havranek, J. J.; Harbury, P. B. *Nat. Struct. Biol.* **2003**, *10*, 45–52. (b) Lee, D. H.; Granja, J. R.; Martinez, J. A.; Severin, K.; Ghadiri, M. R. *Nature* **1996**, *382*, 525–528. (c) Bilgicler, B.; Xing, X.; Kumar, K. *J. Am. Chem. Soc.* **2001**, *123*, 11815–11816. (d) Tang, Y.; Ghirlanda, G.; Vaidehi, N.; Kua, J.; Mainz, D. T.; Goddard, W. A., III; DeGrado, W. F.; Tirrell, D. A. *Biochemistry* **2001**, *40*, 2790–2796. (e) Arndt, K. M.; Pelletier, J. N.; Muller, K. M.; Alber, T.; Michnick, S. W.; Pluckthun, A. *J. Mol. Biol.* **2000**, *295*, 627–639.
- (4) For examples of applications of coiled-coils, see: (a) Petka, W. A.; Harden, J. L.; McGrath, K. P.; Wirtz, D.; Tirrell, D. A. *Science* **1998**, *281*, 389–392. (b) Ryadnov, M. G.; Woolfson, D. N. *Nat. Mater.* **2003**, *2*, 329–332. (c) Severin, K.; Lee, D. H.; Kennan, A. J.; Ghadiri, M. R. *Nature* **1997**, *389*, 706–709.
- (5) (a) Lumb, K. J.; Kim, P. S. *Science* **1995**, *268*, 436–439. (b) Oakley, M. G.; Kim, P. S. *Biochemistry* **1998**, *37*, 12603–12610. (c) Lumb, K. J.; Kim, P. S. *Biochemistry* **1995**, *34*, 8642–8648.
- (6) (a) Kay, B. K.; Winter, J.; McCafferty, J., Eds. *Phage Display of Peptides and Proteins: A Laboratory Manual*; Academic Press: New York, 1996. (b) Smith, G. P.; Patrenko, V. A. *Chem. Rev.* **1997**, *97*, 391–410.
- (7) O’Shea, E. K.; Lumb, K. J.; Kim, P. S. *Curr. Biol.* **1993**, *3*, 658–667.
- (8) See Supporting Information.
- (9) Acharya, A.; Ruvinov, S. B.; Gal, J.; Moll, J. R.; Vinson, C. *Biochemistry* **2002**, *41*, 14122–14131.
- (10) Zitzewitz, J. A.; Ibarra-Molero, B.; Fishel, D. R.; Terry, K. L.; Matthews, C. R. *J. Mol. Biol.* **2000**, *296*, 1105–1116.
- (11) (a) Schnarr, N. A.; Kennan, A. J. *J. Am. Chem. Soc.* **2002**, *124*, 9779–9783. (b) Kashiwada, A.; Hiroaki, H.; Kohda, D.; Nango, M.; Tanaka, T. *J. Am. Chem. Soc.* **2000**, *122*, 212–215. (c) Gurnon, D. G.; Whitaker, J. A.; Oakley, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 7518–7519.

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