# Dynamic Properties of Artificial Protein Hydrogels Assembled through Aggregation of Leucine Zipper Peptide Domains

**Wei Shen, Julia A. Kornfield,* and David A. Tirrell***

*Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125*

*Received July 6, 2006; Revised Manuscript Received November 21, 2006*

ABSTRACT: Network relaxation dynamics of hydrogels formed from a genetically engineered multidomain protein ($AC_{10}A$, where A is an associative leucine zipper domain and $C_{10}$ is a random-coil polyelectrolyte domain) were investigated by shear rheometry. Physical gels form by tetrameric association of the leucine zipper end-blocks (A). The longest stress relaxation time ($\tau_r$) of these gels varies strongly with pH, increasing from $\tau_r \approx 80$ s at pH 8.0 to $\tau_r \approx 1000$ s at pH 7.0. The rate of strand exchange of the end-blocks was studied by using fluorescence quenching of the labeled form of the A domain. Fluorescence is quenched in solutions of fluorescein-labeled A; dequenching occurs when labeled A is mixed with a 60-fold excess of the unlabeled peptide. The dequenching transient after mixing reveals the characteristic strand exchange time ($\tau_e$) of the A domain. As pH decreases from 8.0 to 7.0, $\tau_e$ increases from ca. 200 s to ca. 4500 s. Thus, $\tau_r$ of $AC_{10}A$ hydrogels and $\tau_e$ of the A domain vary in parallel with pH. The strong correlation between macroscopic and molecular properties indicates that network relaxation is regulated by the lifetime of associations in the transient network. Because the rate of leucine zipper strand exchange is sensitive to interstrand electrostatic interactions, the relaxation behavior of artificial protein hydrogels can be engineered systematically by genetic programming of the amino acid sequence.

## Introduction

Hydrogels assembled from the genetically engineered multidomain protein $AC_{10}A$,[1] which consists of two associative leucine zipper end-blocks (A) and a random coil midblock ($C_{10}$), introduced a new class of biomaterials for use in a broad range of applications in biology and medicine. The dynamic properties of these physically cross-linked hydrogels are important in biomedical technologies such as controlled drug release and cell immobilization. For example, the ability to control the change of viscosity in response to environmental stimuli is the key to successful use of these materials in cell immobilization. The linear viscoelastic properties are governed by both structure (which determines the storage modulus) and dynamics (which control the stress relaxation time).[2] Understanding the dynamic properties of these hydrogels, and their underlying molecular determinants, will enable materials design on the molecular level.

Such studies are also important for fundamental reasons. It was first proposed by Green and Tobolsky[3] that the stress relaxation time of a physically cross-linked transient network is regulated by the lifetime of the associative group in a junction. But even 60 years later, the relation between these two properties remains controversial.[4−8] Here we examine the dynamic properties of $AC_{10}A$ hydrogels. We independently determined the network stress relaxation time and the leucine zipper strand exchange time as functions of solution pH. Network relaxation behavior was studied using rheological measurements, while the strand exchange time was probed by a fluorescence method. The observed correlation between the macroscopic dynamic behavior of $AC_{10}A$ hydrogels and the strand exchange kinetics of the leucine zipper domains provides a clear mechanistic rationale for control of the dynamic properties of artificial protein hydrogels.

* Corresponding authors. J.A.K.: Tel (626) 395-4138, Fax (626) 568-8743, e-mailjak@cheme.caltech.edu. D.A.T.: Tel (626) 395-3140, Fax (626) 793-8472, e-mail tirrell@caltech.edu.

## Experimental Section

**Protein Synthesis and Purification.** Expression vectors for $AC_{10}A$ and for the leucine zipper domain alone, terminated either with a cysteine residue or a tryptophan residue (Figure 1), were constructed previously by Petka.[9] To enable removal of the hexahistidine sequence from $A_{cys}$ and $A_{trp}$, new vectors containing a thrombin cleavage site were prepared. An oligonucleotide segment (T) encoding the thrombin cleavage site (LVPRGS) was excised from pUC18L2T[10] by digestion with *Nhe*I and *Spe*I and ligated into the *Nhe*I sites of pUC18L2A[9] and pUC18L1A,[9] respectively. The DNA segments encoding $TA_{cys}$ and $TA_{trp}$ were excised from the resulting plasmids by digestion with *Bam*HI and inserted into the *Bam*HI site of pQE9 to yield pQE9$TA_{cys}$ and pQE9$TA_{trp}$. The sequences were verified at the DNA sequencing core facility of the Beckman Institute at the California Institute of Technology.

The expression vectors were each transformed into *Escherichia coli* strain SG13009, which carries the repressor plasmid pREP4 (Qiagen, Chatsworth, CA). Proteins $AC_{10}A_{trp}$, $A_{cys}$, $A_{trp}$, $TA_{cys}$, and $TA_{trp}$ were expressed as described previously[11] and purified by affinity chromatography on a nickel nitrilotriacetic acid resin (Qiagen, Chatsworth, CA) through a hexahistidine tag encoded in the pQE9 vectors. The sequences of the proteins examined in this work are shown in Figure 1.

**Enzymatic Cleavage of the N-Terminal Hexahistidine Tag.** Solutions of $TA_{trp}$ and $TA_{cys}$ (100 $\mu$M) were prepared in thrombin cleavage buffer (50 mM Tris·Cl, 150 mM NaCl, 2.5 mM $CaCl_2$, pH 7.5). A 0.5 unit/$\mu$L thrombin (from human plasma, Sigma-Aldrich, St. Louis, MO) stock solution was added to a final concentration of 0.002 units/$\mu$L. Each sample was incubated at room temperature for 4 h and loaded on a column packed with nickel nitrilotriacetic acid resin (Qiagen, Chatsworth, CA). The column was washed with 8 M urea (pH 8.0), and the flow-through was collected, dialyzed against sterile deionized water, and lyophilized. Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) (sinapinic acid matrix) on an Applied Biosystems Voyager mass spectrometer revealed single peaks at 6822 Da for cleaved $TA_{trp}$ and 6985 Da for cleaved $TA_{cys}$ (expected values 6811 and 6972 Da, respectively), suggesting that the segments upstream from the thrombin cleavage site were completely cleaved.

10.1021/ma0615194 CCC: $37.00 
Published on Web 01/12/2007

$AC_{10}A_{trp}(AC_{10}A)$: MR**[6H]**DDDDKA**[A]**IGDHVAPRDTSYRDPMG**[$C_{10}$]**ARMPT**[A]**IGDHVAPRDTS**W**

$A_{cys}$: MR**[6H]**DDDDKWA**[A]**IGDHVAPRDTSMGG**C**

$A_{trp}$: MR**[6H]**DDDDKASYR**[A]**IGDPRMPTS**W**

$TA_{cys}$: MR**[6H]**DDDDKWASLVPRGST**[A]**IGDHVAPRDTSMGG**C**

$TA_{cys}$*: GST**[A]**IGDHVAPRDTSMGG**C**

$TA_{trp}$: MR**[6H]**DDDDKASLVPRGSTSYR**[A]**IGDPRMPTS**W**

$TA_{trp}$*: GSTSYR**[A]**IGDPRMPTS**W**

Abbreviation for domains:
**[6H]**: GSHHHHHHGS
**[A]**: S(or D)GDLENEVAQLEREVRSLEDEAAELEQKVSRLKNEIEDLKAE
**[$C_{10}$]**: $[AGAGAGPEG]_{10}$

**Figure 1.** Amino acid sequences of proteins.

**Fluorescent Labeling.** Fluorescein-5-maleimide was site-specifically ligated to the cysteine residues engineered at the C-termini of $A_{cys}$ and $TA_{cys}$*, respectively. Protein solutions were prepared in phosphate buffer (10 mM $NaH_2PO_4$, 90 mM NaCl) at a concentration of 100 $\mu$M. A tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (Pierce, Rockford, IL) stock solution (100 mM) was added to each protein solution to a final concentration of 2 mM. The pH was adjusted to 4.5, and the mixture was incubated at room temperature for half an hour to allow reduction of disulfide bonds. The pH was then adjusted to 5.5. A fluorescein-5-maleimide (Molecular Probes, Eugene, OR) stock solution (100 mM) was freshly prepared in DMSO and added to each reduced protein solution to a final concentration of 1 mM. The mixture was incubated in the dark at room temperature for half an hour. The sample was concentrated from 10 to 1 mL in a Centricon YM-3 centrifugal filter unit (molecular weight cutoff 3000, Millipore, Billerica, MA) and subjected to gel filtration on a Sephadex G-25 (Amersham Biosciences, Piscataway, NJ) column (1.5 cm diameter $\times$ 30 cm height) to remove unreacted dye. The protein fraction was collected, dialyzed against sterile deionized water, and lyophilized in the dark. The absorbance at 515 nm (measured on a Cary 50 Bio UV−vis spectrophotometer, Varian, Palo Alto, CA) indicated an extent of labeling of 20−40% with some batch-to-batch variation, on the basis of an extinction coefficient of 83 000 $M^{-1}$ $cm^{-1}$ for fluorescein maleimide.[12] The labeled leucine zipper peptides are designated as $A_{cys}$-FM and $TA_{cys}$*-FM.

**Rheological Measurements.** Protein solutions were prepared in phosphate buffer (13 mM $NaH_2PO_4 \cdot H_2O$, 87 mM $Na_2HPO_4 \cdot 7H_2O$) with 0.5 wt % sodium azide. The pH value was adjusted as needed. Each solution was centrifuged to remove entrapped bubbles and then loaded to a 25 mm diameter cone-and-plate shear cell. The temperature was controlled at 22.0 $\pm$ 0.1 °C by a Peltier thermoelectric device for each measurement. Frequency sweep measurements were carried out under 1% strain on an RFS III rheometer (TA Instruments, New Castle, DE) using a 0.04 rad cone angle. The edge of the sample was covered with mineral oil to minimize solvent evaporation. Creep tests were conducted under 100 Pa stress on an SR-5000 stress rheometer (Rheometric Scientific, Piscataway, NJ) using a 0.1 rad cone angle and a solvent trap.

**Strand Exchange.** Solutions of labeled leucine zipper peptides ($A_{cys}$-FM or $TA_{cys}$*-FM) and unlabeled peptides ($A_{trp}$ or $TA_{trp}$*) were prepared in phosphate buffer (13 mM $NaH_2PO_4 \cdot H_2O$, 87 mM $Na_2HPO_4 \cdot 7H_2O$) at a concentration of 100 $\mu$M. The unlabeled peptide solution (2 mL) was placed in a cuvette and stirred with a magnetic stirring bar at maximum speed in the sample compartment of a Photon Technology fluorometer (Lawrenceville, NJ) at 22 °C. The sample was excited at 495 nm, and the baseline emission at 515 nm was recorded before addition of the labeled peptide. A solution of the labeled peptide (35 $\mu$L) was then injected rapidly, and emission at 515 nm was monitored as a function of time for 2 h at an acquisition rate of 25 data points per second. The time course of fluorescence dequenching was fit by using Origin 6.1 software (OriginLab Corp., Northampton, MA).

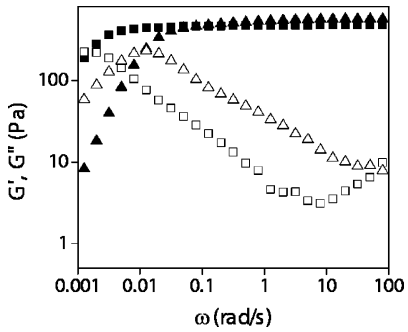


**Figure 2.** Dynamic moduli ($G'$, closed symbols; $G''$, open symbols) of $AC_{10}A$ hydrogels at pH 7.0 and 8.0. (7% w/v, 100 mM phosphate buffer, 22 °C). (▲, △) pH 8.0; (■, □) pH 7.0.

## Results and Discussion

**Network Relaxation Behavior of $AC_{10}A$ Hydrogels.** The linear viscoelastic behavior of $AC_{10}A$ networks was characterized by oscillatory frequency sweep measurements. The dynamic moduli show a prominent loss peak (maximum in the loss modulus $G''$) that coincides with the frequency $\omega_x$ at which $G'$ and $G''$ cross (Figure 2). At pH 8.0, the dominant loss peak separates a high-frequency elastic plateau and a low-frequency regime in which $G' \sim \omega^{1.7}$ and $G'' \sim \omega^{0.89}$, approaching terminal behavior ($G' \sim \omega^2$ and $G'' \sim \omega^1$).[2] Thus, the characteristic frequency of the dominant loss peak corresponds to the longest stress relaxation time of the network ($\tau_r \sim 1/\omega_x$), which dominates the relaxation behavior of $AC_{10}A$ networks and controls their viscosity ($\eta_{(\dot{\gamma}\to 0)} = G'_\infty \tau_r$).

$AC_{10}A$ networks are similar to conventional transient networks (such as those formed from hydrophobically modified urethane−ethoxylate (HEUR) polymers) in exhibiting an elastic plateau at $\omega > \omega_x$ and terminal behavior at $\omega < \omega_x$. However, the relaxation time scale of $AC_{10}A$ networks is much longer that of HEUR networks. The $\tau_r$ of an $AC_{10}A$ network at pH 8.0 is close to 100 s (Figure 2), while the typical $\tau_r$ of a HEUR network is on the order of 0.001−1 s.[4] At lower pH, the relaxation time becomes even longer (Figure 2). Indeed, $\tau_r$ becomes too great to quantify by frequency sweep measurements. (Time−temperature superposition cannot be applied because of the strong temperature dependence of protein secondary structure, and solvent evaporation precludes measurements at extremely low frequencies.) Therefore, creep tests were exploited to quantitatively determine the pH dependence of $\tau_r$ of $AC_{10}A$ hydrogels. In each creep test, a constant stress of 100 Pa was applied, and the strain was recorded as a function of time until a steady rate of straining was reached (i.e., the strain recovery upon removal of the stress equals the strain intercept $\gamma_e^0$ of the creep curve extrapolated to zero time[13]). With decreasing pH from 8.0 to 7.0, the steady shear rate $\dot{\gamma}_\infty$ decreases strongly, while $\gamma_e^0$ changes slightly (Figure 3). The longest stress relaxation time, $\tau_r = \gamma_e^0/\dot{\gamma}_\infty$,[13] increases from ca. 80 s at pH 8.0 to ca. 1000 s at pH 7.0.

**Strand Exchange Kinetics.** The strand exchange kinetics of the associative leucine zipper domain were studied by a method based on fluorescence dequenching. Peptides containing only the leucine zipper domain were used, some labeled with fluorescein ($A_{cys}$-FM) and some unlabeled ($A_{trp}$). A solution containing $A_{cys}$-FM (extent of labeling ca. 30%) was mixed with an $A_{trp}$ solution of the same protein concentration in a volumetric ratio of 1:60. The time course of the increase in fluorescence

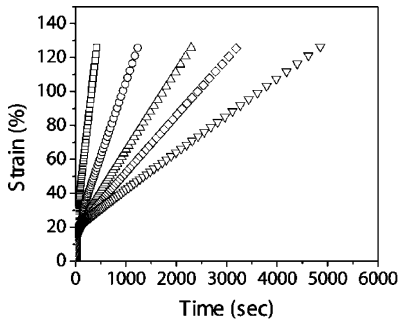


**Figure 3.** Creep measurements for $AC_{10}A$ hydrogels at different pH. Steady rate of straining was confirmed for each test: □, pH 8.0; ○, pH 7.6; △, pH 7.4; ◇, pH 7.2; ▽, pH 7.0 (100 mM phosphate buffer, 7% w/v, $T = 22$ °C, stress = 100 Pa).

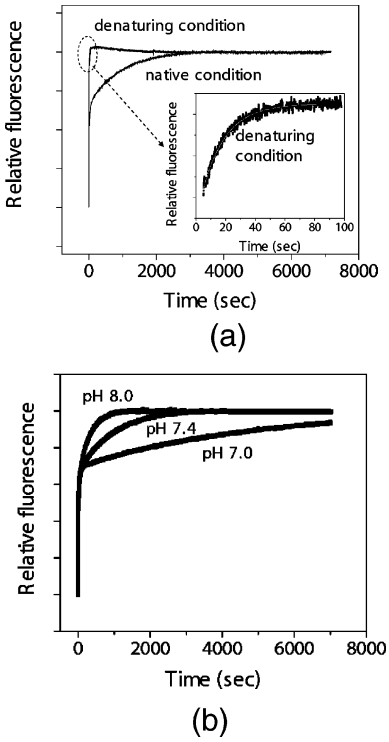


**Figure 4.** Time course of the increase in fluorescence emission intensity following the mixing of labeled and unlabeled leucine zipper solutions: (a) comparison of native (in 100 mM phosphate buffer) and denaturing conditions (in 8 M urea) at pH 7.4; (b) at different pH in 100 mM phosphate buffer (100 $\mu$M leucine zipper solutions, 22 °C).

emission intensity following mixing revealed the characteristic time of leucine zipper strand exchange.

The time course of the fluorescence emission at pH 7.4 involves two widely separated time scales (Figure 4a). A double-exponential fit reveals characteristic times of 17.7 ± 0.2 s for the fast phase and 950.9 ± 0.5 s for the slow phase. Control experiments under denaturing conditions (8 M urea, pH 7.4) show that the rapid process is not regulated by the conformation and association of the leucine zipper (Figure 4a); the transient response of the fluorescence signals under denaturing conditions exhibits a single-exponential relaxation with a characteristic time of 15.8 ± 0.1 s (inset, Figure 4a). This time scale is consistent with the fast component of the relaxation under native conditions, which occurs on a time scale of 10 s under all the conditions examined. Under denaturing conditions, fluorescence is most likely quenched through nonspecific interactions[14] and dequenched when the labeled species are diluted. (Fluorescein is itself hydrophobic and has some tendency to associate in aqueous media.)

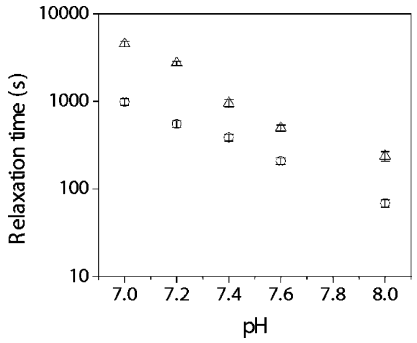


**Figure 5.** Correlation between $\tau_r$ of $AC_{10}A$ hydrogels (7% w/v) (○) and $\tau_e$ of leucine zipper A (in 100 $\mu$M solutions) (△) (100 mM phosphate buffer, 22 °C).

We attribute the slow fluorescence increase which disappears upon denaturation to leucine zipper strand exchange under native conditions. Consistent with this assignment, the time scale of the slow relaxation depends strongly on pH (Figure 4b). The slow relaxation is well described by simple exponential decay (see Supporting Information) and yields characteristic times for leucine zipper strand exchange ($\tau_e$) that range from ca. 200 s at pH 8.0 to ca. 4500 s at pH 7.0 (Figure 5). The value of $\tau_e$ is insensitive to variations in the degree of labeling: each experiment was repeated at least in triplicate with different batches of the labeled peptide, and in all cases consistent results were obtained. Removal of the histidine tag did not affect measurement of the dynamic properties (Supporting Information), suggesting the pH-responsiveness is governed by the electrostatic repulsion among the glutamic acid residues in the leucine zipper domain. Furthermore, the strand exchange time is insensitive to concentration; experiments at 50, 100, and 200 $\mu$M (pH 7.4) yielded similar values of $\tau_e$, suggesting that strand exchange is a first-order process (most likely limited by dissociation of leucine zipper aggregates[15,16]). The pH dependence of the leucine zipper strand exchange time and that of the melting temperature[1] do not parallel each other; this suggests that the leucine zipper domain undergoes strand exchange without going through a denatured intermediate state.

**Link between $AC_{10}A$ Network Relaxation and A-Domain Exchange.** Comparison of the relaxation behavior of $AC_{10}A$ hydrogels and the strand exchange kinetics of the associative leucine zipper domain reveals a strong correlation (Figure 5). The longest stress relaxation time of $AC_{10}A$ hydrogels and the strand exchange time of the leucine zipper domain A are the same order of magnitude at each pH value and vary with pH in parallel. From a fundamental perspective, this result provides direct experimental evidence supporting the close relationship between network relaxation and the lifetime of associative groups in transient networks. From a material design point of view, this result suggests that macroscopic dynamic properties of these artificial protein hydrogels can be tuned by controlling leucine zipper strand exchange kinetics through selection of the amino acid sequence.

At each pH value, the strand exchange time of the leucine zipper domain is 3−4 times greater than the stress relaxation time of an $AC_{10}A$ hydrogel. This difference is consistent with the strong tendency of $AC_{10}A$ to form looped configurations rather than bridges in the network.[17] Looped chains lead to the formation of "superbridges" (Figure 6a). The transient network theory developed by Annable[4] suggests that superbridges accelerate the stress relaxation time of a network because of their increased probability of breaking; the ratio $\tau_r/\tau_e$ varies inversely with the average number of links in a superbridge. In

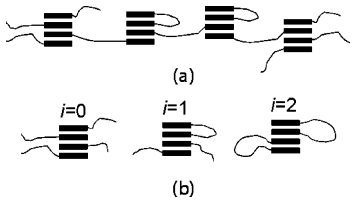


**Figure 6.** (a) A superbridge. (b) Three possible states of tetrameric leucine zipper aggregates designated by the number of loops *i*. The functionalities for $i = 0$, 1, and 2 are 4, 2, and 0, respectively.

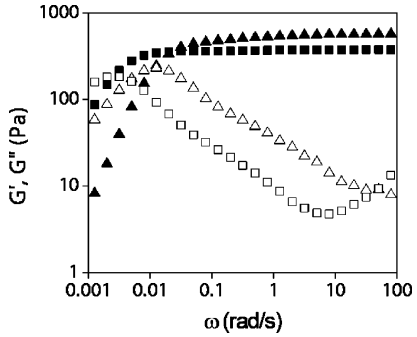


**Figure 7.** Effect of NaCl concentration on dynamic moduli ($G'$, closed symbols; $G''$, open symbols) of $AC_{10}A$ hydrogels at pH 8.0 (7% w/v, 100 mM phosphate buffer, 22 °C). (▲, △) No NaCl; (■, □) 400 mM NaCl.

an $AC_{10}A$ network, there are three possible states of tetrameric leucine zipper aggregates (Figure 6b). Network junctions correspond to aggregates having no loop ($i = 0$). Tetramers participating in the network (possibly as links in a superbridge) include both aggregates with no loop ($i = 0$) and with one loop ($i = 1$). On the basis of the plateau storage modulus determined by rheological measurements and a simple statistical analysis,[18] we estimated the fraction of the aggregates in states $i = 0$, 1, and 2 in 7% $AC_{10}A$ gels to be ca. 0.2, 0.4, and 0.4, respectively. According to Annable's theory, this would give a value of $\tau_r/\tau_e$ of ca. 1/3, which is remarkably close to the ratio of the stress relaxation time to the leucine zipper strand exchange time determined here.

**pH- and Salt-Responsiveness of Dynamic Properties.** One of the unique advantages of artificial proteins as constituents of responsive hydrogels is the potential to use sequence design to precisely control the conditions at which transitions in material properties occur. We observed that both the network stress relaxation time and leucine zipper strand exchange time increased more than 10-fold when the pH value decreased from 8.0 to 7.0. Electrostatic interaction, which governs the pH-responsiveness of the dynamic properties, can be further tuned by choice of amino acid residues in the leucine zipper domain.

Rheological oscillatory shear measurements at different salt concentrations showed that stress relaxation of $AC_{10}A$ hydrogels is slower at higher ionic strength (Figure 7), consistent with the dominant role of electrostatic repulsion among the glutamic acid residues. Screening of electrostatic repulsion at high ionic strength leads to slow strand exchange and network relaxation. These rheological data (Figure 7) also demonstrate that the control of the structure (hence the plateau storage modulus) and the dynamics (hence the stress relaxation time) of a network can be decoupled. The effect of ionic strength on the network topology is imparted through the midblock: the random coil shrinks with increasing ionic strength, which decreases the probability of bridged configurations and reduces the plateau storage modulus.[17] The effect of ionic strength on the dynamics is imparted through the leucine zipper domain: aggregates are stabilized with increasing ionic strength, which leads to a longer stress relaxation time.

## Conclusions

Hydrogels that respond to environmental stimuli show promise in controlled drug release, cell encapsulation, and tissue engineering. Hydrogels formed from a genetically engineered multidomain protein, $AC_{10}A$, were shown to exhibit a sharp pH-dependent transition in viscosity.[9] However, it was not clear whether this transition should be attributed to the network structure and topology (storage modulus $G'_\infty$) or to the dynamic properties of the system (relaxation time $\tau_r$). Having characterized both the network structure[17,18] and dynamic properties of $AC_{10}A$ gels, we find that the dynamic properties are primarily responsible for the transition. The relaxation time varies strongly with pH, while $G'_\infty$ changes only slightly.[17] Furthermore, $\tau_r$ correlates with the leucine zipper strand exchange time $\tau_e$: both characteristic times are similar in magnitude at each pH value, and both vary strongly with pH. The discrepancy between $\tau_r$ and $\tau_e$ can be explained by effects of network topology that are evident in $G'_\infty$.[17] Finally, the correlation between $\tau_r$ and $\tau_e$ suggests that the relaxation behavior of artificial protein hydrogels can be engineered by genetic programming of the amino acid sequence.

**Acknowledgment.** The authors acknowledge the NSF Center for the Science and Engineering of Materials for financial support.

**Supporting Information Available:** Description of the effect of the histidine tag on leucine zipper strand exchange kinetics. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

(1) Petka, W. A.; Harden, J. L.; McGrath, K. P.; Wirtz, D.; Tirrell, D. A. *Science* **1998**, *281*, 389−392.
(2) Ferry, J. D. *Viscoelastic Properties of Polymers;* Wiley: New York, 1980.
(3) Green, M. S.; Tobolsky, A. V. *J. Chem. Phys.* **1946**, *14*, 80−92.
(4) Annable, T.; Buscall, R.; Ettelaie, R.; Whittlestone, D. *J. Rheol.* **1993**, *37*, 695−726.
(5) Jenkins, R. D. The fundamental thickening mechanism of associating polymers in latex systems: A rheological study. Ph.D. Dissertation, Lehigh University, 1990.
(6) Ng, W. K.; Tam, K. C.; Jenkins, R. D. *J. Rheol.* **2000**, *44*, 137−147.
(7) Petit-Agnely, F.; Iliopoulos, I. *J. Phys. Chem. B* **1999**, *103*, 4803−4808.
(8) Tanaka, F.; Edwards, S. F. *J. Non-Newtonian Fluid Mech.* **1992**, *43*, 247−271.
(9) Petka, W. A. Reversible gelation of genetically engineered macromolecules. Ph.D. Dissertation, University of Massachusetts Amherst, Amherst, MA, 1997.
(10) Kennedy, S. B. Biological activity and dynamic structure in artificial protein hydrogels. Ph.D. Dissertation, University of Massachusetts Amherst, Amherst, MA, 2001.
(11) Shen, W.; Lammertink, R. G. H.; Sakata, J. K.; Kornfield, J. A.; Tirrell, D. A. *Macromolecules* **2005**, *38*, 3909−3916.
(12) Ghanouni, P.; Steenhuis, J. J.; Farrens, D. L.; Kobilka, B. K. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5997−6002.
(13) Macosko, C. W. *Rheology: Principles, Measurements, and Applications;* VCH Publishers: New York, 1993.
(14) Daugherty, D. L.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 4325−4333.
(15) Zitzewitz, J. A.; Bilsel, O.; Luo, J. B.; Jones, B. E.; Matthews, C. R. *Biochemistry* **1995**, *34*, 12812−12819.
(16) Wendt, H.; Berger, C.; Baici, A.; Thomas, R. M.; Bosshard, H. R. *Biochemistry* **1995**, *34*, 4097−4107.
(17) Shen, W.; Kornfield, J. A.; Tirrell, D. A.; Shen, W.; Kornfield, J. A.; Tirrell, D. A. *Soft Matter* **2007**, *3*, 99−107.
(18) Shen, W.; Zhang, K. C.; Kornfield, J. A.; Tirrell, D. A. *Nat. Mater.* **2006**, *5*, 153−158.

MA0615194