Kinetics

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Model Studies of the Kinetics of Ester Hydrolysis under Stretching Force**

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Biodegradable polyesters are increasingly important for tissue engineering, drug delivery, nerve regeneration, cardiovascular and orthopedic applications.[1] In many such uses the polymers operate under mechanical loads. Considerable empirical evidence exists that loads on polymers change reactivities of their monomers.[2] Since polyesters often biodegrade by hydrolysis, understanding how loads affect the kinetics and mechanism of ester hydrolysis is needed to improve their performance in existing application, broaden the range of such applications, and develop new stress-responsive materials with the ester group.

Effects of mechanical loads on the susceptibility of the ester group to hydrolysis have not been reported.[3] Here we describe computational and experimental evidence that stretching a polymer containing the ester group does not change its hydrolysis kinetics, potentially making it a mechanochemically inert component of stress-responsive materials subjected to loads in the presence of nucleophiles.

Basic ester hydrolysis follows a $B_{AC}2$ mechanism[4] (Figure 1) with nearly isoenergetic transition states for formation (TS1) and decomposition (TS2) of the tetrahedral intermediate (Int). Reaction path calculations at B3LYP/6-311 + G* in H_2O-parameterized SMD[5] as a solvent model with and without explicit solvation reproduced the key features of this mechanism (see Figure S20 in the Supporting Information). To determine the suitable microsolvation level, we calculated $\Delta G^{\ddagger\circ}$ of basic hydrolysis of AcOMe with up to 5 H_2O molecules (Tables S9–10) and obtained the best agreement with the measured value, $\Delta G^{\ddagger\circ}_{exp}$, with the $H_3O_2^-$ ion as the model nucleophile[6] and no microsolvation of the other states. The relative energies and key parameters of TS1, Int, and TS2 depended weakly on microsolvation (Table S11, Figures S18 and S19). Performance of B1B95 but not MPW1KCIS or MPW1K, functionals (Table S12) was comparable, albeit at greater computational costs. Excellent agreement with $\Delta G^{\ddagger\circ}_{exp}$ was achieved for homologous esters, RCO_2R (R = Et, *n*Pr, *n*Bu) using complete conformational ensembles of TS1, Int, and TS2 states without microsolvation and the $H_3O_2^-$ ion as the nucleophile (Table S14).

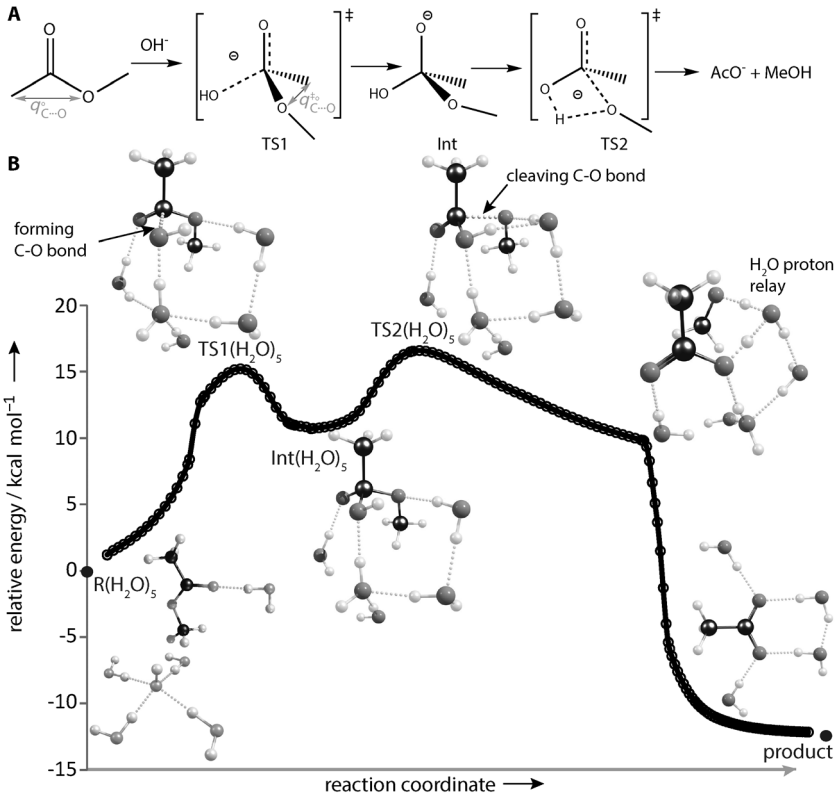

Figure 1. A) The $B_{AC}2$ mechanism of ester hydrolysis. B) A calculated reaction path with 5 H_2O molecules of microsolvation (see Figure S20 for other microsolvation schemes). The forming (TS1) and scissile (TS2) bonds are shown by dotted lines. C, O and H atoms are black, dark gray, and light gray, respectively.

We calculated free-energies of R, TS1, Int, and TS2 states of RCO_2R (R = Et, *n*Pr, *n*Bu) as a function of the stretching force applied across the terminal C atoms of each conformer to estimate the kinetic stability of the ester group in a stretched polymer. The relative free-energy of each state increases with stretching force up to about 0.2 nN and decreases thereafter (Figure 2 A). Destabilization of TS1, Int, and TS2 at $f < 0.2$ nN is primarily entropic, as the densities of thermally accessible conformations of these states decrease faster with f than that of R. Such affects were noted in other reactions.[7] At $f > 0.2$ nN the relative

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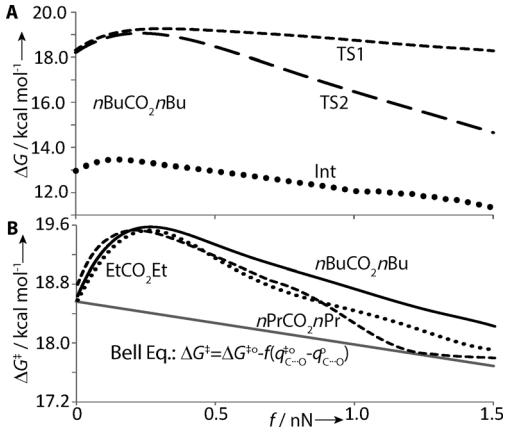
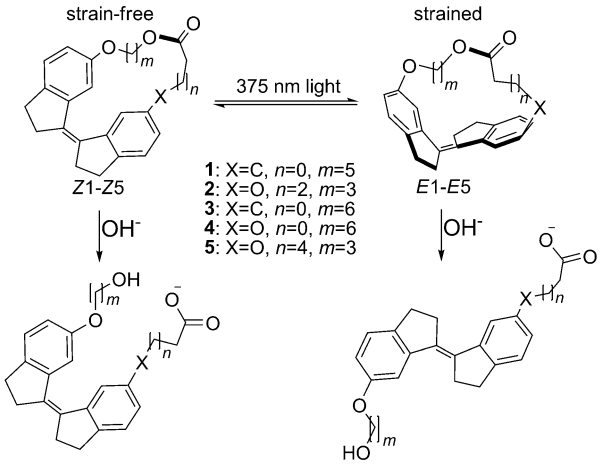


Figure 2. Free-energies of A) TS1, Int, and TS2 relative to R and B) activation, $\Delta G^{\ddagger}$, as a function of the stretching force, f. $\Delta G^{\ddagger} = \Delta G_{TS1} + \Delta G_{TS2} - \Delta G_{int} + RT \ln\{\exp[-(\Delta G_{TS2} - \Delta G_{int})/RT] + \exp[-(\Delta G_{TS1} - \Delta G_{int})/RT]\}$. Table S13 lists the data shown here.



Scheme 1. Macrocyclic esters **1**–**5**; the scissile bond is in bold.

free-energies of TS1, Int or TS2 change mostly because of distortions of bond angles and torsions (i.e., enthalpically). Because the relative energy of TS1 is least sensitive to force, the near-degeneracy of TS1 and TS2 at $f = 0$ is lifted at f of about 0.2 nN, making TS1 rate-determining and resulting in a weak dependence of $\Delta G^{\ddagger}$ on f (Figure 2B). The similar $\Delta G^{\ddagger}(f)$ profiles of the three homologues suggest that ester hydrolysis in a stretched polymer of an arbitrary length or composition follows the same pattern.[7b] The Bell equation (gray line, Figure 2B) yields $\Delta G^{\ddagger}(f)$ within 1 kcal mol^{-1} of the explicitly computed trend when the proportionality coefficient is the difference of the ensemble-average C···O distances (arrows, Figure 1A) of unstrained R and TS1 states of $EtCO_2Et$, while requiring negligible computational resources.

Kinetics of force-accelerated dissociations of backbone bonds in stretched polymers are typically measured in single-molecule force (SMF) experiments,[8] but reactions that are unaffected or even inhibited by stretching polymers remain mostly beyond their scope.

Varying the restoring force[9] of a reactive site using a series of increasingly strained macrocycles of *E* stiff stilbene (Scheme 1) eliminates this limitation.[2b,10] Considerable evidence[11] supports using such macrocycles as quantitative atomically tractable models of reactive sites in stretched polymers. For example, the force–rate correlation of dibromocyclopropane isomerization from macrocycles and SMF experiments is identical.[10,12] The close conceptual and quantitative analogy[13] in chemical response of reactive sites to strain generated molecularly or by stretching a polymer means that molecular design complements micromanipulation techniques for controlling local restoring forces, and enables experimental validation of computed force-dependent reactivity of moieties that cannot be studied in stretched polymers.

To validate the computed results in Figure 2B, we measured the kinetics of hydrolysis of macrocyclic esters **1**–**5** (Scheme 1), calculated at the DFT level the restoring force of the scissile bond in each macrocycle, $\langle f_s \rangle$, and compared $\Delta G^{\ddagger\circ}_{exp}(\langle f_s \rangle)$ in **1**–**5** with $\Delta G^{\ddagger}(\langle f_s \rangle)$ calculated for RCO_2R (R = Et, *n*Pr, *n*Bu).

We synthesized strain-free *Z* isomers of **1**–**5** in three steps and up to 60% yield (Scheme S1). Irradiation of *Z***1**–*Z***5** in hexane at 375 nm yielded photostationary states containing up to 44% of the *E* isomers which were isolated by preparative HPLC. The *E* analogs are strained because the ester-group-containing molecular strap that constrains the separation of two aromatic C atoms of stiff stilbene is too short to accommodate the *E* geometry of the central C=C bond. The identity and purity of esters **1**–**5** were confirmed by ^{1}H and ^{13}C NMR spectroscopy, HRMS, and HPLC.

We measured the hydrolysis kinetics of *Z* and *E* isomers of **1**–**5** in H_2O:1,4-dioxane (5:1 mol) containing 0.8 mM NaOH and 0.18 mM thymolphthalein as acid/base indicator for spectrophotometric monitoring of the reaction progress. $\Delta G^{\ddagger\circ}_{exp}$ of AcOEt hydrolysis, measured by our method was identical to the literature value. For the *Z* isomers $\Delta G^{\ddagger\circ}_{exp}$ (Table 1) agreed with the literature values for hydrolysis of medium and large size cyclic esters in H_2O:1,4-dioxane.[14] Hydrolysis of *E* esters is inhibited relative to the *Z* analogs.

Calculations at B3LYP/6-311 + G* with H_2O-parameterized SMD[5] reproduced $\Delta G^{\ddagger\circ}_{exp}$ with mean absolute deviation of 1.6 kcal mol^{-1} (Table S17). We estimated the restoring force of the scissile bond, $\langle f_s \rangle$, in *E***1**–*E***5** by Equation (1),

$$\langle f_s \rangle = 2\frac{\langle q_s \rangle_E - \langle q_s \rangle_Z}{\langle \lambda_s \rangle_E + \langle \lambda_s \rangle_Z} \quad (1)$$

Table 1: Measured activation free-energies, $\Delta G^{\ddagger\circ}_{exp}$, of hydrolysis of esters **1**–**5** at 298 K in H_2O:1,4-dioxane (5:1 mol) and calculated forces of the scissile bond, $\langle f_s \rangle$ [Eq. (1)].

	$\Delta G^{\ddagger\circ}_{exp}$ [kcal mol^{-1}]		$\langle f_s \rangle_R$ [pN]
	Z	*E*	
1	19.23 ± 0.09	20.52 ± 0.16	308
2	19.82 ± 0.02	20.02 ± 0.18	30
3	19.96 ± 0.09	21.06 ± 0.13	196
4	18.16 ± 0.05	19.08 ± 0.07	381
5	20.04 ± 0.07	20.80 ± 0.06	122

where $\langle q_s \rangle$ and $\langle \lambda_s \rangle$ are the ensemble-averaged length and compliance of the scissile bond. We calculated f_s for each conformer of RCO_2R (R = Et, *n*Pr, *n*Bu) using two independent methods based on the molecular compliance matrix, as previously described[2b,7] (detailed further in the Supporting Information). The two methods yielded similar $\langle f_s \rangle$ after Boltzmann-weighted averaging of individual conformer values.

The computations and experiments show (Figure 3) that ester hydrolysis kinetics is largely insensitive to tensile load. The result may seem counterintuitive. Tensile load weakens and elongates the scissile bond in R (Figure 4 A). This response is similar to that of disubstituted cyclobutenes[7b] and cyclopropanes[10] and of alkyl sulphates[15] (Figure 4 B), where the bond scission is accelerated by force (Scheme 2). The crucial difference between ester hydrolysis and reactions (1) to (3) is the change in the strain energy of the load during the reaction.[16] The latter is proportionally to an elongation of the internuclear separation across which the load acts (e.g., terminal C···C distance),[2b,11] that is, a load stabilizes states, the formation of which increases the constrained distance (i.e., TS2 of ester hydrolysis and the TSs of reactions (1) to (3)) and vice versa. Since the terminal C···C separations are nearly identical in TS1 and R of ester hydrolysis (Table S16), that is, the pulling axis of a polymer containing an ester group is nearly orthogonal to the reaction coordinate up to TS1, force does not affect $\Delta G^{\neq\circ}$. This conclusion is consistent with force distorting TS1 away from the reactant geometry (i.e., it acts along modes with positive force constants), whereas TS2 and the TSs of reaction (1) to (3), which are stabilized by stretching a polymer, are distorted toward the reactant geometry (i.e., force acts along a mode with a negative force constant, Figure 5).

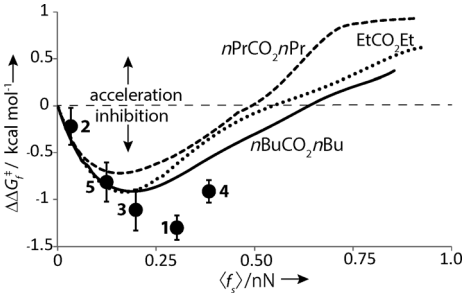
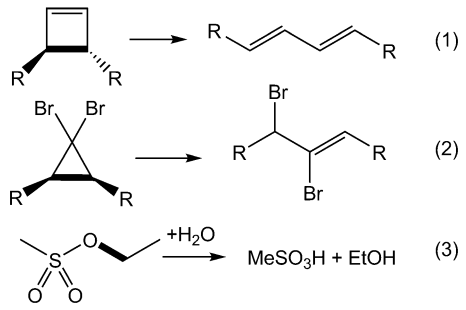


Figure 3. Changes in the activation free-energy of basic ester hydrolysis as a function of the scissile bond force, $\langle f_s \rangle$ in ***E*1**–***E*5** (dots, $\Delta\Delta G_f^{\neq} = \Delta G_Z^{\circ\neq} - \Delta G_E^{\circ\neq}$) and in ester homologs with stretching force applied across the terminal C atoms (solid lines, $\Delta\Delta G_f^{\neq} = \Delta G^{\circ\neq} - \Delta G_f^{\neq}$). The plotted data is from Tables 1 and S15–S17.



Scheme 2. Reactions that are accelerated by force; the scissile bond is in bold.

Stretching a polymer can accelerate a bimolecular reaction by 1) changing the intrinsic kinetic stability of the reactive site and/or 2) increasing the accessibility of such a site to the other reagent (e.g., OH^-). The latter was originally proposed to explain acceleration of S–S bond reduction in proteins under load.[17] A similar mechanism may accelerate ester bond hydrolysis in stretched polymers. However, unlike hydrophobic S–S bonds, which are sequestered inside proteins, the ester group is hydrophilic and stretching a polymer may not affect its accessibility to nucleophiles. These two scenarios may be differentiated by comparing force-dependent fragmentation of polymers containing esters with poor and excellent leaving groups. The latter is thought to hydrolyze by an S_N2-like mechanism, which should be accelerated by

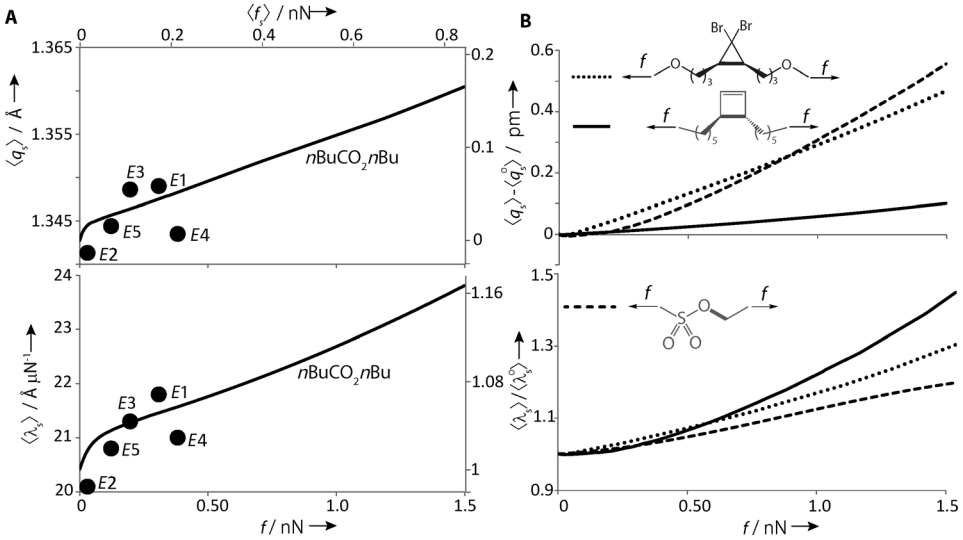


Figure 4. The effect of tensile load on the length, $\langle q_s \rangle$ and the compliance, $\langle \lambda_s \rangle$ of the scissile bond in A) esters and B) reactants in Scheme 2. For *n*BuCO$_2$*n*Bu (lines) the *f* (bottom) and $\langle f_s \rangle$ (top) scales are equivalent; for the macrocycles (dots), only the $\langle f_s \rangle$ (top) scale is meaningful. The deviation of *E*4 from the trend is due to the electronic effect of the α-phenoxy substituent; this influence is eliminated from the data in Figure 3 by considering the difference in activation free-energies ($\Delta G_Z^{\circ\neq} - \Delta G_E^{\circ\neq}$) and calculating the restoring force by Equation (1).

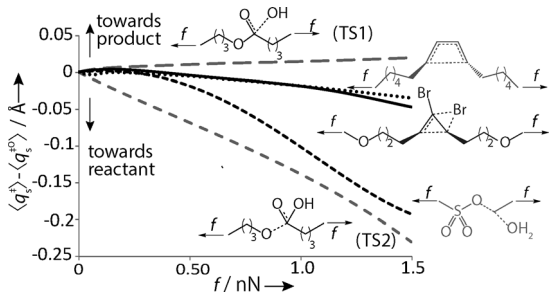


Figure 5. Elongation (TS1) or contraction (TS2) of the scissile bond in the transition states of ester hydrolysis and Reactions (1) to (3) as a function of the applied force, *f*.

force even if the accessibility of the ester group to nucleophiles is unchanged (similar to reaction (3)).

Ester hydrolysis illustrates that while dissociation of a molecule invariably increases its end-to-end distance, such elongation may occur only after the rate-determining transition state. In such a case the fragmentation kinetics is insensitive to tensile loads, which could be exploited in the design and applications of stress-responsive polymeric materials. A polymer chain made up primarily of monomers, the kinetic stability of which is unaffected or even increased by tensile load would effectively transmit force to a few reactive sites, yielding materials that respond to load by undergoing highly localized and selective chemistry.

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[1] a) M. C. Serrano, E. J. Chung, G. A. Ameer, *Adv. Funct. Mater.* **2010**, *20*, 192; b) A. L. Sisson, M. Schroeter, A. Lendlein, *Handbook of Biodegradable Polymers*, Wiley-VCH, Weinheim, **2011**, p. 1.

[2] a) M. M. Caruso, D. A. Davis, Q. Shen, S. A. Odom, N. R. Sottos, S. R. White, J. S. Moore, *Chem. Rev.* **2009**, *109*, 5755; b) T. J. Kucharski, R. Boulatov, *J. Mater. Chem.* **2011**, *21*, 8237; c) Y. Chen, A. J. Spiering, S. Karthikeyan, G. W. Peters, E. W. Meijer, R. P. Sijbesma, *Nat. Chem.* **2012**, *4*, 559; d) J. Ribas-Arino, D. Marx, *Chem. Rev.* **2012**, *112*, 5412; e) H. M. Klukovich, T. B. Kouznetsova, Z. S. Kean, J. M. Lenhardt, S. L. Craig, *Nat. Chem.* **2013**, *5*, 110.

[3] An MD simulation of amide hydrolysis under load was reported: F. Xia, A. K. Bronowska, S. Cheng, F. Grater, *J. Phys. Chem. B* **2011**, *115*, 10126.

[4] a) A. J. Kirby in *Comprehensive Chemical Kinetics*, *Vol. 10* (Eds.: C. H. Bamford, C. F. H. Tipper), Elsevier, Amsterdam, **1972**, p. 57; b) J. F. Marlier, *Acc. Chem. Res.* **2001**, *34*, 283.

[5] A. V. Marenich, C. J. Cramer, D. G. Truhlar, *J. Phys. Chem. B* **2009**, *113*, 6378.

[6] In aqueous solution the OH^- ion is quandruply solvated (Figure 1B), e.g., M. E. Tuckerman, D. Marx, M. Parrinello, *Nature* **2002**, *417*, 925, but is often modeled as a singly-solvated $H_3O_2^-$ ion, for example, Y. Kim, J. R. Mohrig, D. G. Truhlar, *J. Am. Chem. Soc.* **2010**, *132*, 11071.

[7] a) M. Hermes, R. Boulatov, *J. Am. Chem. Soc.* **2011**, *133*, 20044; b) Y. Tian, R. Boulatov, *ChemPhysChem* **2012**, *13*, 2277.

[8] A. L. Black, J. M. Lenhardt, S. L. Craig, *J. Mater. Chem.* **2011**, *21*, 1655.

[9] Restoring force[2b] develops in an elastically-deformed object, opposes further deformation and is responsible to the recovery of the original shape after load removal. In the harmonic approximation the molecular compliance matrix relates the restoring force of a local coordinate of a molecule both to the absolute distortion of this coordinate and to the externally imposed force. The restoring force is rigorously defined both at the continuum and atomistic levels, connecting the two descriptions of the response of an object to load. Its primary use in chemistry is to describe the reaction kinetics of stretched polymers, where partial relaxation of nonreactive molecular degrees of freedom away from the reactive site makes large contributions to activation barriers. The restoring force provides a simple estimate of this contribution without treating such molecular coordinates explicitly.[11] Relaxation of nonreactive coordinates is not a primary contributor to increased reactivity of most small strained molecules, making restoring force of limited use in such context.

[10] S. Akbulatov, Y. Tian, R. Boulatov, *J. Am. Chem. Soc.* **2012**, *134*, 7620.

[11] a) R. Boulatov, *Nat. Chem.* **2013**, *5*, 84; b) Y. Tian, R. Boulatov, *Chem. Commun.* **2013**, *49*, 4187.

[12] D. Wu, J. M. Lenhardt, A. L. Black, B. B. Akhremitchev, S. L. Craig, *J. Am. Chem. Soc.* **2010**, *132*, 15936.

[13] S. L. Craig, *Nature* **2012**, *487*, 176.

[14] R. Huisgen, H. Ott, *Tetrahedron* **1959**, *6*, 253.

[15] T. J. Kucharski, Q.-Z. Yang, Y. Tian, R. Boulatov, *J. Phys. Chem. Lett.* **2010**, *1*, 2820.

[16] This is also known as "work potential" and the $f \times q$ term in Marx's "force-transformed" energy surfaces: P. Dopieralski, J. Ribas-Arino, D. Marx, *Angew. Chem.* **2011**, *123*, 7243; *Angew. Chem. Int. Ed.* **2011**, *50*, 7105.

[17] N. Bhasin, P. Carl, S. Harper, G. Feng, H. Lu, D. W. Speicher, D. E. Discher, *J. Biol. Chem.* **2004**, *279*, 45865.

