Springer

Journal of Mechanical Science and Technology 22 (2008) 2203~2212

Journal of Mechanical Science and Technology

www.springerlink.com/content/1738-494x
DOI 10.1007/s12206-008-0706-0

AFM-based identification of the dynamic properties of globular proteins: simulation study †

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(Manuscript Received May 14, 2008; Revised July 10, 2008; Accepted July 11, 2008)

Abstract

Nowadays a mathematical model-based computational approach is getting more attention as an effective tool for understanding the mechanical behaviors of biological systems. To find the mechanical properties of the proteins required to build such a model, this paper investigates a real-time identification method based on an AFM nanomanipulation system. First, an AFM-based bio-characterization system is introduced. Second, a second-order time-varying linear model representing the interaction between an AFM cantilever and globular proteins in a solvent is presented. Finally, we address a real-time estimation method in which the results of AFM experiments are designed to be inputs of the state estimator proposed here. Our attention is restricted to a theoretical feasibility analysis of the proposed methodology. We simply set the mechanical properties of the particular protein such as mass, stiffness, and damping coefficient in the system model prior to running the simulation. Simulation results show very good agreement with the preset properties. We anticipate that the realization of the AFM-based bio-characterization system will also provide an experimental validation of the proposed identification procedure in the future. This methodology can be used to determine a model of protein motion for the purpose of computer simulation and for a real-time modification of protein deformation.

Keywords: Nanomechanics; AFM cantilever; Proteins; Dynamic parameters; System identification

1. Introduction

Recently, the development of model-based analytical approaches to the prediction and dynamic interpretation of biomolecular systems has become a focus in computational and structural biology. In particular, the single-molecule biomechanics of protein domain motion, deformation, and unfolding has become the new frontier in proteomics [1]. Protein deformation and motion are important concepts in the molecular biomechanics. For example, the deformation of protein molecules under mechanical forces or motions may be a key element to mediate the mechano-chemical signal transduction pathways within the cells [2]. As illustrated in Fig. 1, the modes of protein deformation include domain hinge motion, domain shear motion, and unfolding of secondary structures. Conformational changes in a protein make an alteration on its potential energy by either creating or breaking physical and chemical interactions. These interactions are implicitly represented as viscosity and stiffness coefficients. Thus, it is expected that even from the same protein there are various dynamic responses, each of which can be interpreted as a unique signature of protein deformation.

Concerning the applications of identification tech-

† This paper was recommended for publication in revised form by Associate Editor Dae-Eun Kim

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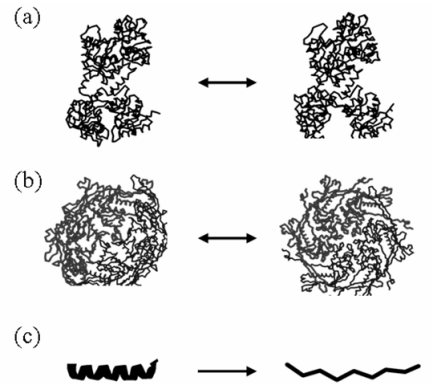


Fig. 1. Modes of protein deformation: (a) domain hinge motion, (b) domain shear motion, (c) unfolding of a secondary structure.

niques to obtain mechanical properties in biosystems, there have been several successful results focused on a macro human body system (human neuromusculoskeleton system). Chizeck et al. [3] investigated a noninvasive *in vivo* identification of the parametric models of the electrically stimulated quadriceps muscles to predict muscle torque outputs. Frequency separation and the exponentially weighted least squares method were used to estimate the time-varying compliance parameters in the elbow joint. Kearney et al. [4] reported the contribution of the intrinsic and reflex portions in the human ankle dynamics through a system identification method in parallel-cascade scheme. However, researches on the modeling of the biomolecules dynamics at cellular and molecular levels are rare. Ikai et al. [5] investigated the intra- and inter-molecular mechanics of the proteins in a static or quasi-static motion, by assuming constant stiffness and neglecting the inertial and viscous drag effects during the motions of proteins. In [5, 6], it is reported that the estimation of the dynamic stiffness in a static or quasi-static motion of proteins might have some limitations. Also, the interaction dynamics between a protein and a mechanical probe in a fluid has been rarely investigated. The identification of protein dynamics still remains as a challenging problem due to its nonlinearity and time variance [7, 8].

In this paper, an AFM-based real-time identification method is conceptually investigated in order to determine the dynamic properties of globular proteins. The attempt of using an AFM for nanoscale sensing and/or manipulation at a cellular or molecular level has been reported in several areas including biomaterial detection [6, 9, 10], characterizations of nanomaterials and devices [9, 11, 12], and manipulation of biological structures [13-15], to name a few. For various mechanical manipulations of biomolecules like DNA extension, bending and twisting, protein domain motion, deformation, and unfolding, we need to measure the interactive force between a single molecule and the AFM tip or at least to establish an analytical model for predicting the motions involved.

The contributions of this paper are as follows: First, a new AFM-based characterization system for identifying the dynamic properties of proteins is proposed. Second, a dynamic model of the AFM-protein interaction system including a piezoresistive AFM cantilever in fluid is presented. Finally, the use of a recursive least squares identification method using the oscillatory motion of the piezo oscillator as an input and the deflection of the piezoresistive cantilever tip as an output is proposed for system identification.

2. AFM-based system configuration

2.1 Self-sensing AFM cantilever: A bioforce measuring device

Two types of measurement technique, the contact mode and the tapping mode, are used in the current AFM technology. The tapping mode AFM has been introduced to overcome the drawbacks of the contact mode, in which the tapping mode minimizes the continuous contact with the surface. The tip excitation vibration, using a piezo oscillator, is provided in an aqueous environment by oscillating the cantilever at or near the resonance frequency of the cantilever beam. The oscillating tip is then moved toward the surface until it begins to lightly touch or tap the surface. The lateral force therefore can be much reduced because of the short duration of contact. Moreover, unlike the contact mode, the tip vibration provides sufficient amplitude to overcome the tip-sample adhesion force. There are some review papers that deal with the principles of AFM systems and their applications to biological samples [16, 17].

However, the optical beam deflection sensor in most AFM systems is externally located, which may prohibit the movement of the AFM cantilever in the z-direction. Hence, such a system may not be adequate for manipulating biomolecules. On the other hand, Arai et al. [18] and Dargahi et al. [19] have proposed the use of piezoresistive or piezoelectric materials for "self-sensing" effect. Kim et al. [20] also

presented a self-sensing piezoresistive AFM cantilever for measuring and characterizing the dynamic force between a protein and an AFM cantilever. The piezoresistive cantilever enables the sensing of the interactive forces in nanoscale by the measurement of the stress-induced electrical resistance.

2.2 System configuration

Fig. 2 shows the system configuration proposed for AFM-based nanobiological measurement and characterization. It consists of a self-sensing AFM cantilever and a 3-DOF nanopositioner under the confocal laser-scanning microscope. It allows the movement of the sample stage in the z-direction, especially for a sinusoidal movement, which is used in measuring the dynamic responses of a single molecule through recording both the cantilever deflection and the piezo movement. This configuration is the advanced model that we had proposed previously [21] and dynamic identification and control using microgripper was introduced by our group [22]. The dynamic measurements are necessary to study mechanical properties such as the viscoelastic properties of biomolecules.

In order to observe both the nano- and macroscopic environments, the system is equipped with two types of sensors: a visual sensor for macroscopic observation of surrounding environment and a piezoresistive force sensor for nanoscopic movement. The visual sensing data acquired from the confocal laser-scanning microscope provide a global monitoring of the cantilever movement and the target biomolecules. The lateral and vertical resolutions of the confocal laser microscope are about 200 nm and 100 nm, respectively. In addition, the self-sensing cantilever enables the sensing of the tip-sample interactive forces in sub-nanonewton resolution by measuring the changes of the stress-induced electrical resistances.

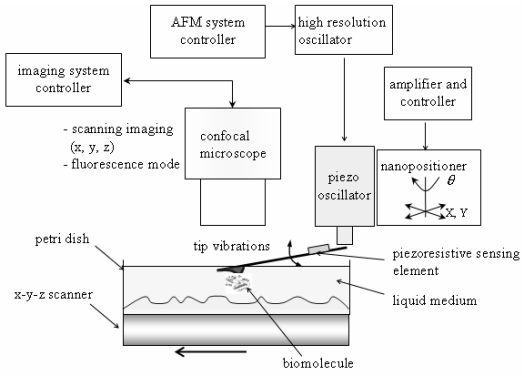


Fig. 2. The system configuration for nanobiological characterization consisting of a confocal laser scanning microscope and a 3-DOF nanopositioner equipped with a piezoresistive AFM cantilever.

3. Dynamic modeling

3.1 AFM cantilever model in a fluid

The interaction force between proteins and a cantilever can be estimated through a modeling of the motion of the cantilever that is vibrating upon the molecules. In molecular manipulation experiments [6, 13], the cantilevers are chemically modified and the measurements are usually done in a fluid. The amount of deflection has shown a significant difference according to the viscosity of the fluid, as shown experimentally in [5].

Fig. 3 shows a schematic of the cantilever oscillating in a fluid. The mass of the cantilever includes the induced mass caused by the fluid being carried along. In this case the Reynolds number is not small, but the amplitude of the oscillations is small compared to the cantilever itself:

$$l^2\omega >> \eta_l/\rho \quad \text{and} \quad A << l\,, \tag{1}$$

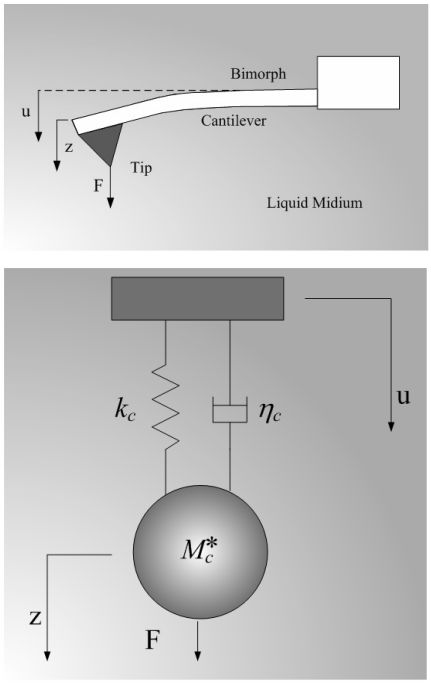


Fig. 3. Schematic of an AFM cantilever vibrating in fluid.

Table 1. Viscosity and density of various liquid mediums.

	η_l [$\times 10^{-3} kg/(m \cdot s)$]	ρ [gcm^{-3}]
Hexane	0.33	0.659
Ethanol	1.2	0.789
Water	1.0	1.0
Hexadecane	3.34	0.773

where l is the characteristic length of the cantilever, ω is the frequency of the cantilever, η_l is the viscosity of the liquid medium, ρ is the density of the liquid medium, and A is the amplitude of the oscillation. For example, a cantilever vibrating in water, which assumes $l = 40$ μm, the resonance frequency $\omega = 3 \times 10^4$ Hz, A=0.1 μm, and the kinematic viscosity of water $\eta_l / \rho = 0.010\ cm^2 s^{-1}$, will satisfy the conditions of (1). Table 1 compares various liquid mediums.

To study the damping effect in a fluid, the cantilever can be modeled as a moving sphere of radius R [23]. For a sphere oscillating in a viscous fluid with a high frequency, the drag force F_d acting on the sphere is given as [24]

$$F_d = 3\pi R^2 \sqrt{2\eta_l \rho \omega} \frac{dz}{dt} + \frac{2}{3}\pi \rho R^3 \frac{d^2 z}{dt^2}, \quad (2)$$

where z is the vertical displacement. In (2), $2\pi\rho R^3/3$ is called the induced mass, which represents the effective mass of the cantilever in the fluid. Because the cantilever is not an exact sphere, the geometric factor R varies with the size and shape of the cantilever. It should be invariant for a given cantilever under any fluid and can be determined by fitting to the experimental resonance peak.

Now, the equation of motion of an AFM cantilever, as a second-order linear mass-spring-dashpot system, is derived. As shown in Fig. 3, the position of the bimorph is given by u, the position of the tip is given by z, and the total external force is F. The external force F consists of the external force from globular proteins to the cantilever, F_{ext}, and the hydrodynamic drag force F_d.

Then, the dynamic equation of motion is

$$M_c^* \frac{d^2 z}{dt^2} + \eta_c \frac{dz}{dt} + k_c (z - u) = F, \quad (3)$$

$$F = -F_{ext} - F_d, \quad (4)$$

Table 2. Parameter values of the V-shaped Si_3N_4 cantilever with gold coating.

L [um]	202	$t_{Si_3N_4}$ [um]	0.54
L_1 [um]	116	t_{Au} [um]	0.04
B [um]	205	k_c [N/m]	0.058
W [um]	40.2	n	0.14

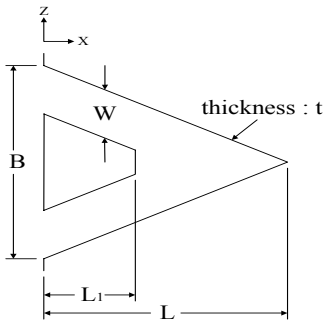


Fig. 4. A schematic of the V-shaped cantilever used.

where $M_c^* = n M_c$ is the effective mass, M_c is the mass of the cantilever, k_c is the spring constant, and η_c is the damping coefficient. For commercially available V-shaped cantilevers, n ranges from 0.137 to 0.2, depending on the geometry. Fig. 4 shows the dimensions of the V-shaped cantilever used in this study. Table 2 gives the parameter values of a V-shaped Si_3N_4 cantilever coated with gold ($E_{Si_3N_4} = 1.5 \times 10^{11}\ N/m$ and $\rho_{Si_3N_4} = 3100\ kg/m^3$ for Si_3N_4; $E_{gold} = 0.8 \times 10^{11}\ N/m$ and $\rho_{gold} = 19300\ kg/m^3$ for gold). The dimensions and calculated spring constants are taken from [23].

3.2 Protein-cantilever interaction

An essential feature of proteins is that biological functions of an individual protein are very closely related to its three-dimensional conformations (i.e., the spatial arrangement of the atoms in its folded structure). It is also well recognized that proteins are not rigid but deformable in static formation. Thus, mechanical forces can alter their conformations leading to changes in downstream biochemical processes that affect cellular behavior and function. Protein molecules are also viscoelastic in nature, similar to all polymeric materials, especially those in aqueous environment [1]. The viscoelastic properties of proteins can be investigated by dynamic measurements and modeling studies [24, 25].

In this paper, the real-time dynamics of globular

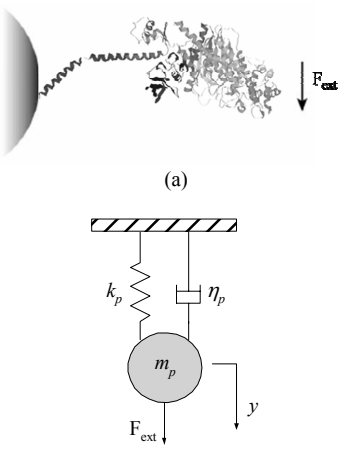


Fig. 5. The motion of a protein under the applied force F_{ext}. (a) A globular protein immobilized on a surface through an α-helix. (b) The mass-spring-dashpot system as a model of protein motion.

proteins is approximated as a second-order linear model. For an illustration, we consider a globular protein immobilized on a surface through a lever-arm (e.g., an α-helix), as shown schematically in Fig. 5(a). Under the applied force F, a small motion of the protein in the vertical direction can be analyzed based on a mass-spring-dashpot system shown in Fig. 5(b). The corresponding governing equation of displacement y is

$$m_p \frac{d^2y}{dt^2} + \eta_p \frac{dy}{dt} + k_p y = F_{ext}\,, \tag{5}$$

where m_p is the mass and η_p is the viscous drag coefficient of the protein, and k_p is the elastic constant of the lever arm.

To identify the dynamic properties of proteins, the measurement and manipulation of the AFM system is used. Fig. 6(a) illustrates the dynamic motion of a globular protein interacting with an AFM cantilever. The target protein is placed under the AFM tip. The bimorph actuation is controlled by applying a sine wave modulation frequency. Then, the position of the cantilever, that is, equal position of the lever-arm, is detected by piezoresistive sensing element. Fig. 6(b) shows the equivalent dynamic model of the protein-AFM system. The equation of this equivalent model is given by

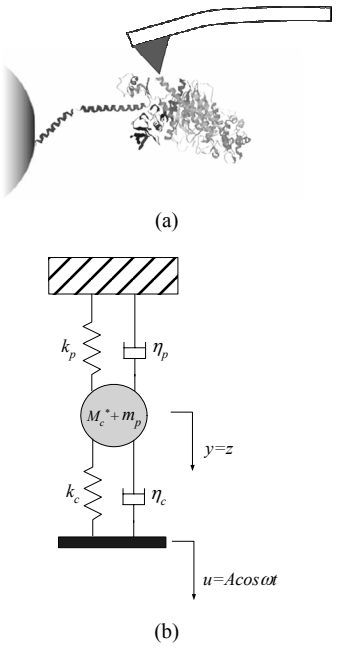


Fig. 6. Modeling of the dynamic motion of proteins using an AFM cantilever. (a) A globular protein interacting with an AFM tip. (b) An equivalent model of the proteins-tip interaction system.

$$(M_c^* + m_p)\frac{d^2z}{dt^2} + (\eta_c + \eta_p)\frac{dz}{dt} + (k_c + k_p)z = k_c u - F_d\,. \tag{6}$$

Combining (2) and (6), the dynamic equation of proteins-cantilever interaction in a fluid is derived as follows:

$$(M_c^* + m_p + \frac{2}{3}\pi R^3)\frac{d^2z}{dt^2} + (\eta_c + \eta_p + 3\pi R^2\sqrt{2\eta_l\rho\omega})\frac{dz}{dt} + (k_c + k_p)z = k_c u\,. \tag{7}$$

4. System identification

4.1 Recursive least squares estimation with Kalman filtering

From the displacement of the deflection of a cantilever, its velocity and acceleration are estimated. In order to apply the Kalman filter technique, the kinematic relationship between displacement, velocity, and acceleration with noises is reformulated in discrete form as follows [27]:

$$\begin{aligned} x_1(t+1) &= x_1(t) + Tx_2(t) + Tw_1(t) \\ x_2(t+1) &= x_2(t) + Tx_3(t) + Tw_2(t) \\ x_3(t+1) &= x_3(t) + Tw_3(t) \end{aligned} \tag{8}$$

where x_1, x_2, and x_3 represent the displacement, velocity, and acceleration, respectively, of the deflection of the cantilever. T is the sampling time. w_1, w_2, and w_3 are possible discrete noise sequences.

The output is the deflection of the cantilever as follows:

$$z(t) = x_1(t) + v(t) \tag{9}$$

where z is the measured variable and v is the measurement noise. It is assumed that $w_i(t)$ and $v(t)$ are stationary white Gaussian noises:

$$E[v(t)] = 0\,, \quad E[w(t)] = 0\,, \text{ t=0,1,2, ...} \tag{10}$$

We can formulate the problem in the standard form.

$$\begin{aligned} x(t+1) &= Ax(t) + Bu(t) + Lw(t) \\ y(t) &= Cx(t) + v(t) \end{aligned} \tag{11}$$

where

$$A = \begin{bmatrix} 1 & T & 0 \\ 0 & 1 & T \\ 0 & 0 & 1 \end{bmatrix} \quad B = \begin{bmatrix} 0 & 0 & 0 \end{bmatrix}^T \quad C = \begin{bmatrix} 1 & 0 & 0 \end{bmatrix} \quad L = \begin{bmatrix} T & 0 & 0 \\ 0 & T & 0 \\ 0 & 0 & T \end{bmatrix}.$$

The steady-state estimate of the states is given by the Kalman filter equations:

$$\hat{x}(t+1) = A\hat{x}(t) + Bu(t) + H[z(t) - C\hat{x}(t)] \tag{12}$$

where H is the Kalman filter gain vector and is given by

$$H = APC^T[CPC^T + V]^{-1}\,. \tag{13}$$

In (13), P is the steady-state value of the error covariance matrix and V is given by solving the discrete algebraic matrix Riccati equation.

$$-P + APA^T + LWL^T - A^TPC^T[CPC^T + V]^{-1}CPA = 0\,. \tag{14}$$

Now, we apply the recursive least squares (RLS) algorithm to estimate time–varying mechanical parameters in the dynamics of globular proteins. The RLS algorithm is based upon a second-order linear model of the plant. Let the linear regression form be

$$Y(t) = \varphi^T(t)\theta\,, \tag{15}$$

where $Y(t) = k_c u\,,$ (16)

$$\varphi(t) = \left[x_3(t) \quad x_2(t) \quad 3\pi R^2\sqrt{2\eta_l\rho\omega}\,x_2(t) \quad x_1(t) \right]^T, \tag{17}$$

$$\theta(t) = [(M_c^* + m_p + \frac{2}{3}\pi R^3) \quad (\eta_c + \eta_p) \quad 1 \quad (k_c + k_p)]^T\,. \tag{18}$$

Note that Y (t), $\varphi(t)$, and $\theta(t)$ are the output, the regression vector, and the unknown parameter vector, respectively.

The recursive form for identifying the unknown parameter vector $\theta(t)$ is given by [24]:

$$\theta_{est}(t) = \theta_{est}(t-1) + R^{-1}(t)\varphi(t)[Y(t) - \varphi^T(t)\theta_{est}(t-1)]\,, \tag{19}$$

$$R(t) = \lambda(t)R(t-1) + \varphi(t)\varphi^T(t)\,, \tag{20}$$

where $\varphi(t)$ is the regression vector, $\theta_{est}(t)$ is the parameter vector, R is a symmetric and positive definite matrix, and $\lambda(t)$ is a weighting sequence.

4.2 Identification procedure

A block diagram of the proposed identification algorithm is shown in Fig. 7. The steps in the identification procedure are listed as follows.

(1) Design the input excitation $u = A\cos\omega t$ with specified frequency contents.
(2) Apply the input excitation signal to piezo oscillator vibrating with an AFM cantilever.
(3) Collect output data z with 1 millisecond sampling rate by measuring the output voltage signal from the piezoresistive sensing element,

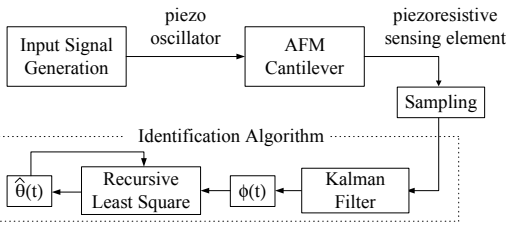


Fig. 7. Block diagram of the proposed identification algorithm.

which is due to the deflection of the cantilever.

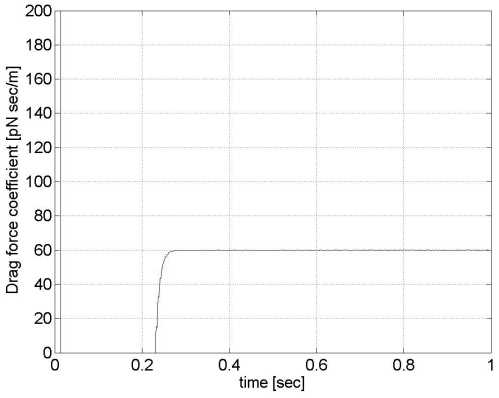
(4) Acquire the regression vector $\varphi(t)$, which is robust to noise, using Kalman filter.
(5) Apply the recursive least square algorithm to perform the identification of dynamic properties of globular proteins.
(6) Determine the drag force coefficient and the spring constant of globular protein.
(7) Verify the result by means of simulation. If the verification result is not satisfactory, go to Step 2) to increase the excitation magnitude, or go to Step 1) to redesign the excitations with a different frequency spectrum.

5. Simulations

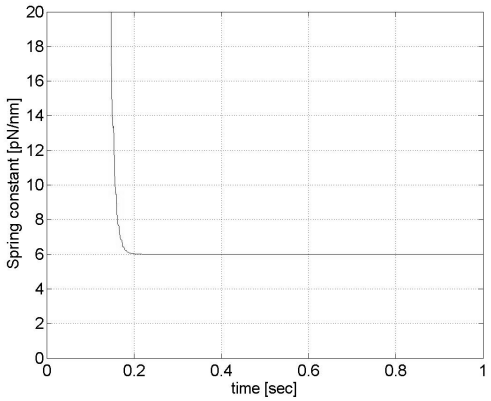
As a baseline of this study, we restrict attention to only the theoretical verification of the feasibility of the proposed recursive estimator. In fact, when we try to implement the experimental system proposed and perform the identification procedure in the preceding section, some questions arise as follows: 1) how can we trap or manipulate a single globular protein in a solvent? 2) Is the volume ratio between the AFM tip and protein reasonably close enough to generate an output signal different from the input excitation? That is, can we expect a significant signal change caused by the dynamics of protein compared to the order of signal noise? These problems are beyond the scope of this paper and will be investigated in the future.

In this section, the real AFM-based bio-characterization system is simply replaced with a second-order linear system model. Thus, the AFM excitation signal is applied to the system model during the simulation. Then this system model computationally generates the output signal corresponding to the measured output of the AFM, provided that the real experiment is performed. Using this output, the system identification is performed by the recursive least squares algorithm, described in (19)-(20). The simulation parameters used are tabulated in Tables 1 and 2.

First, the simulation results of identifying the dynamic properties of globular proteins are shown in Fig. 8, without considering the hydrodynamic drag force, i.e., not in fluid. From Fig. 8, we see that the drag force coefficient estimate converges to 59.99 pN s/m in 0.3 second, and the spring constant estimate converges to 5.99 pN/nm in 0.2 second. The performance of the estimation results depends on the design choice of the excitation signal and the forgetting factor in the recursive least squares algorithm. The input excitation force from the piezo oscillator is applied to vibrate the motion of the cantilever tip in the sinusoidal form, as follows.



(a)



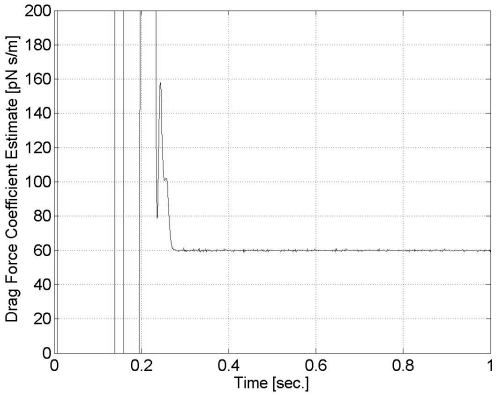
(b)

Fig. 8. The system identification of the protein motion not including the hydrodynamic drag force: (a) Estimation of the drag force coefficient. (b) Estimation of the spring constant.

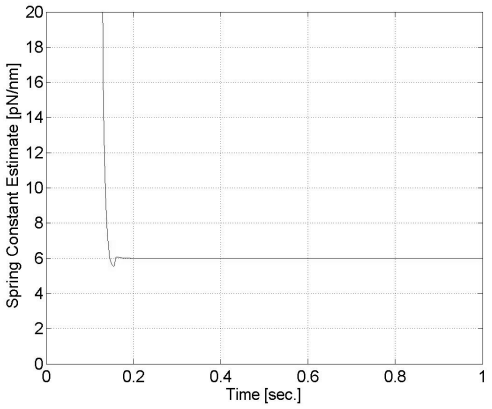
$$u = A\cos\omega t\,, \tag{21}$$

where A is the amplitude of the input signal and ω is the input angular frequency. In our study, the input modulation frequency is applied by bimorph actuation of the cantilever from 1 Hz to 5 KHz. The sampling rate for dynamic measurements is set to 1 millisecond. The maximum amplitude of the piezo actuator by the excitation signal is chosen to be 10 μm.

Fig. 9 shows the simulation results of identifying the dynamic properties of globular proteins in water, considering the hydrodynamic drag force effects. From Fig. 9, we see that the drag force coefficient



(a)



(b)

Fig. 9. Identification of the dynamic properties of globular protein including the hydrodynamic drag force: (a) Estimation of the drag force coefficient. (b) Estimation of the spring constant.

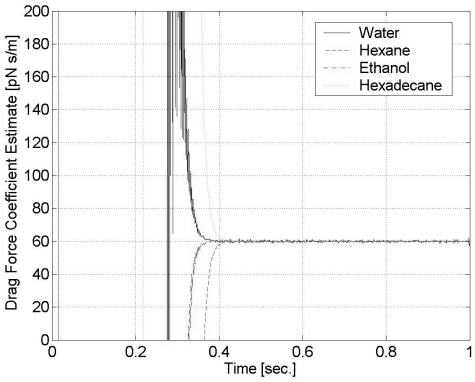
estimate converges to 59.99 pN s/m in 0.26 second, and the spring constant estimate converges to 5.99 pN/nm in 0.18 second.

The effect of different properties of fluids on the accuracy of identifying protein dynamics is illustrated in Fig. 10. It is noticed from Fig. 10(a) that the accuracy of the drag force coefficient estimate is quite good under several different fluidic environments, while the convergence rate of estimation changes with fluids. From Fig. 10(b), we see that the change of accuracy and convergence rate of the stiffness estimate according to different fluids is not significant. This can be explained by the fact that the hydrodynamic drag effect is proportional to the velocity of the cantilever in fluid, and the magnitude of the hydrodynamic drag force as described in Eq. (2) has influence on the drag force coefficient estimate.

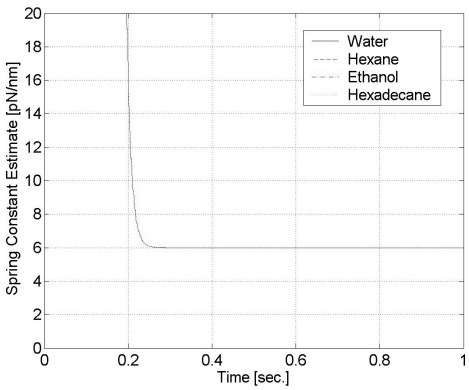
Table 3. Results of the parameter identification of globular proteins.

	Preset Value*	Estimated Value
Elasticity	6pN/nm	5.99 pN/nm
Viscosity	60 pN s/m	59.99 pN s/m

* The measured values in [28] are adopted in our second-order system model.



(a)



(b)

Fig. 10. Simulation results for system identification of protein motion in various fluids: (a) Estimation of the drag force coefficient of globular protein, (b) Estimation of the spring constant of globular protein.

It is assumed that the actual globular protein has a mass of 100 kDa (1 Dalton = 1.66×10^{-24} g) and a drag force coefficient of 60 pN s/m, and the lever arm has a spring constant of 6 pN/nm [28]. Because the actual protein mass is too small, even compared with the mass of the cantilever or the induced mass due to the hydrodynamic drag force, the inertial effect of globular proteins can be neglected, as described in (7). As shown in Table 3, estimation results show a good

agreement with actual values of the mechanical parameters of globular proteins.

6. Conclusions

In this paper, a new AFM-based real-time identification method is proposed for estimating the dynamic properties of proteins. It provides on-line measurement of mechanical properties of proteins as well as identifying protein motions. The system identification has been performed by using a recursive least squares method via computer simulations. The proposed real-time identification technique using an AFM can be applied to investigate mechanics of proteins such as protein domain motion, deformation and folding-unfolding mechanism. Future work includes the experimental validation of the proposed work.

Acknowledgments

This work was supported by the Sogang University Research Grant of 200810027.

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