Published in final edited form as:

Phys Rev E Stat Nonlin Soft Matter Phys. 2011 June ; 83(6 0 1): 061918.

DYNAMICS OF THE CYTOSKELETON: HOW MUCH DOES WATER MATTER?

Guillaume Lenormand¹, Emil Millet¹, Chan Young Park¹, C. Corey Hardin², James P. Butler¹, Nicanor I. Moldovan², and Jeffrey J. Fredberg¹

¹School of Public Health, Harvard University, 665 Huntington Ave., Boston, MA.

²Biomedical Engineering Department, Ohio State University, Columbus, OH.

Abstract

The principal constituent of the living cell is water. The role of the hydration shell and bulk H₂O solvent is well recognized in dynamics of isolated proteins, but the role of water in the dynamics of the integrated living cytoskeleton (CSK) remains obscure. Here we report a direct connection of dynamics of water to dynamics of the integrated CSK. The latter are known to be scale-free and to hinge upon a frequency, f_0 , that is roughly invariant across cell types. Although f_0 is comparable in magnitude to the rotational relaxation frequency of water (gigahertz range), the physical basis of f_0 remains unknown. Using the human airway smooth muscle cell as a model system, we show here that replacing water acutely with deuterium oxide impacts CSK dynamics in major ways, slowing CSK remodeling dynamics appreciably and lowering f_0 by up to 4 orders of magnitude. Although these observations do not distinguish contributions of bulk solvent versus hydration shell, they suggest a novel and unifying hypothesis, namely, that dynamics of integrated CSK network are slaved in a direct fashion to fluctuations arising in intracellular water.

INTRODUCTION

The cytoskeleton of the living cell comprises a crowded micro-environment in which the average distance between macromolecules is only 2 nanometers, which is a distance corresponding to about 10 water molecules [1–3]. Within these interstitial spaces, water is present in two forms: as bulk solvent and as protein hydration shell [3–9]. The effects of water in such a system are complex because proteins dynamics evolve on a rugged energy landscape with multiple conformational sub-states separated by large energy barriers [10, 11]. On such an energy landscape, dynamics can be drastically slowed, anomalous, and even glassy [12, 13]. Furthermore, the dependence of some protein motions upon temperature follows that of the solvent, in which case protein structural fluctuations are slaved to the fluctuations of the solvent [6, 14]. Fluctuations within the bulk solvent (α fluctuations) are thought to control large scale protein motions, whereas fluctuations within the protein hydration shell (β fluctuations) are thought to control internal protein motions [6].

The integrated cytoskeleton of the adherent living cell displays manifestations of glassy dynamics [15–24], where fluctuations within the cytoskeletal network are not just thermal (of energy $k_B T$, the Boltzmann constant times the absolute temperature) but also include active conformational changes driven by the hydrolysis of adenosine triphosphate (ATP),

Author Contributions

G.L. designed, performed, and analyzed all experiments, contributed to data interpretations, and wrote the manuscript. E.M. conducted statistical analyses. C.H. and J.P.B. contributed to data interpretations. N.I.M. initiated the project and contributed to data interpretations. J.J.F. oversaw the project and wrote the manuscript.

which releases about 20 $k_B T$ per hydrolysis event [19, 25–28]. For these reasons, the dynamics of the protein studied in isolation might differ rather substantially from its behaviour in the highly crowded and metabolically active microenvironment of the living cell, and a connection between conformational motions of proteins in the integrated cytoskeletal network have yet to be related to thermal fluctuations in the solvent. Previously we have shown that dynamics of the CSK are scale-free and hinge upon a frequency, f_0 , or fixed point, that seems to be the same for all cell types and all cellular interventions [16, 18, 29, 30]. Because f_0 falls in the GHz range, we had speculated that f_0 might be linked to the dielectric relaxation frequency of water molecules interacting with proteins in solution [29]. To test that idea experimentally, here we ask the question: in the dynamics of the living cytoskeleton, how much does water actually matter?

The impact of water in a living cell might be manipulated in two different ways: by changing water amount and by changing water kind. As regards water amount, we have recently used osmotic shock to show that acute dehydration causes a dramatic stiffening of the cytoplasm and a slowing of its remodeling dynamics [21]. These changes correspond well to the notion of a strong glass in general, and to a colloidal glass comprising soft particles in particular [21, 31]. As regards water kind, here we report results in the living cell in which we have replaced H_2O acutely with D_2O (deuterium oxide or heavy water). In D_2O , the hydrogen nucleus contains an additional neutron. Compared with H_2O , this isotopic addition increases bulk density by about 11%, increases viscosity by about 25%, and increases the energy of the hydrogen bond by about 20% [32, 33]. Although these changes in physical properties are small, we show here that D_2O has a large impact on cytoskeleton dynamics, and that this impact is not a consequence of D_2O toxicity.

METHODS

Deuteriated media preparation

Nutrient mixture Ham's F-12 medium (Sigma, N6760) or phosphate buffer saline (PBS), both in powder form, were complemented with different amount of H_2O and D_2O (99.9% D_2O purchased from Cambridge Isotope Laboratory, Inc). In contact with water, deuterium oxide will exchange deuterium to form deuterium protium oxide, DHO. For example, a solution composed initially of 70% D_2O and 30% H_2O will be composed, at equilibrium, of 9% H_2O , 49% D_2O , and 42% HOD; for clarity purpose, we will still refer to it as a 70% D_2O solution. In contact with living cells, deuterium oxide will also exchange deuterium with the exchangeable H atoms of organic molecules, leading over time to a progressive deuteration of proteins.

Viability assays

To confirm cell viability in the presence of D_2O , we measured reactive oxygen species with dihydrorhodamine 123 (DHR). DHR is a cell-permeant nonfluorescent agent oxidized by peroxynitrite to the fluorescent dye rhodamine. The formation of rhodamine was monitored by fluorescence spectroscopy in HASM cells incubated overnight in 0% or 70% D_2O media. As a positive control, phorbol myristate acetate (PMA) and antimycin-A were used to modify the oxidative activity of the HASM cells.

Immunofluorescence of actin filaments and microtubules

To evaluate the influence of D_2O medium on the cytoskeleton of HASM cells, actin filaments and microtubules were visualized by immunostaining with TRITC-phalloidin and FITC-conjugated monoclonal anti- α -tubulin (Sigma) respectively. First, serum deprived HASM cells were plated overnight at low density on collagen-coated coverslips. After overnight incubation in media composed of 0% or 70% D_2O , the HASM cells were fixed

with 4% paraformaldehyde for 7 min. After washing twice in phosphate buffer saline (PBS), the cells were permeabilized with 0.3% Triton $\times 100$ for 5 min, and then incubated for 30 min in 1% bovine serum albumin in PBS. After another wash in PBS, the samples were incubated with rhodamine phalloidin or FITC conjugated monoclonal anti- α -tubulin for 20 min, followed by two washes with PBS. The samples were finally washed once with de-ionized water and mounted with coverslips in a mounting medium. The actin filaments and microtubules were visualized by confocal microscopy.

Mechanical properties

Mechanical properties of HASM cells were assessed using optical magnetic twisting cytometry (OMTC), as described in Fabry et al. [16, 18]. Briefly, the OMTC uses a microbead, coated with a peptide containing the sequence RGD and firmly attached to the cytoskeleton of the adherent HASM cells. By mean of an external twisting magnetic field, a mechanical torque was applied to the cell. The torque caused the bead to rotate, and, therefore, the cell to deform. The deformation was measured from the bead displacement. The displacements of a hundred microbeads attached on a similar number of cells are recorded simultaneously. Within a couple of minutes, measurements were performed at twisting frequencies ranging from 10^{-1} to 10^3 Hz. At a given twisting frequency, f , the complex elastic modulus, $g^*(f)$, was defined as the ratio of the complex applied torque per unit bead volume to the resulting complex displacement, and could be decomposed in a real part, $g'(f)$, which is called the stiffness modulus, and an imaginary part, which is called the loss modulus, $g''(f)$.

We have previously shown that the complex elastic modulus of living cell, $g^*(f)$ goes as:

$$g^*(f) = g_0 \left(\frac{if}{f_0} \right)^{\alpha_C} + 2\pi if \mu_C, \quad (1)$$

where f_0 and g_0 represent a common intersection at high frequency, α_C is the power law dependence, $i^2 = -1$, and μ_C is an additive Newtonian viscosity; the subscript c stands for each experimental conditions [16, 18]. Remarkably, when the living cell is manipulated by a wide variety of pharmaceutical interventions, the exponent α_C and the viscosity μ_C change, but the power law form and (f_0, g_0) do not. Therefore, the first right-hand side term in Eq. 1 form a family of weak power law curves which, when extrapolated to high frequencies, define a common intersection, (f_0, g_0) (Fig. 1). As such, changes in cell stiffness within the physiological range hinge on a fixed frequency, f_0 , in the gigahertz (GHz) range. This fixed point is not different across cell type, and suggests, therefore, that it is set by a physical phenomenon that does not differ between cell types [16, 18, 29, 30]. Because f_0 falls in the GHz range, we had speculated previously that f_0 might be linked to the dielectric relaxation frequency, f_c , of water molecules interacting with proteins [29].

Cytoskeleton remodeling

Cytoskeleton remodeling was assessed from spontaneous motions of microbeads, as described in An et al. [34]. A RGD-coated microbead, firmly connected to the cytoskeleton, can move only if the cytoskeleton remodels [19, 23, 26, 34]. Briefly, the spontaneous motion of a hundred microbeads attached on a likewise number of HASM cells was recorded for 5 min. For each single bead trajectory, the mean square displacement (MSD) as a function of the time lag between two positions was calculated. At a given time lag, a high MSD reflects a fast rate of cytoskeletal remodeling; a low MSD reflects a slow rate of cytoskeletal remodeling.

Response to a transient stretch

Response to a transient stretch was obtained following a protocol developed in Trepatt et al. [15]. Briefly, adherent HASM cells plated at confluence on a collagen-coated flexible membrane (Flexcell[®] International Corporation, Bioflex plates collagen I, Hillsborough, NC) were subject to a transient isotropic biaxial stretch–unstretch maneuver of 4 seconds duration. Changes in stiffness were monitored before and after stretch using OMTc.

Experimental protocols

HASM cells, incubated in a serum free Ham's F-12 medium supplemented with streptomycin, penicillin, insulin, and transferrin for 36 hr prior to measurements, were plated to confluence in collagen-coated wells. For mechanical assays, cells were incubated for either 1 hr or overnight in a medium consisting of either 100% distilled water, 70% heavy water, or 100% D₂O. The pH (pD) was adjusted to 7.4. The HASM cells were challenged with the contractile agonist histamine (10^{-4} M), with the relaxing agonist N₆,2'-O-dibutyryl-adenosine 3',5'-cyclic monophosphate (DBcAMP, 10^{-3} M), and the actin-filament disruptor, cytochalasin D (2×10^{-6} M). For cytoskeletal remodeling assays, the MSD measurements were performed after 10 min or 2 hr incubation in phosphate buffer saline (PBS), at a pH (pD) of 7.4. PBS media were initially constituted with 0%, 33%, 67%, or 100% D₂O. Incubation times were chosen in two different ways: to be as short as possible to study the direct effect of deuterium (10 min for MSD measurements and 1 hr for stiffness measurements), and to be long enough to study the effect of deuteration of proteins but still short enough to be nontoxic (hence overnight incubation).

Data analysis

Under a specific exposure to H₂O or D₂O media, a group of stiffness measurements was acquired. Each group was composed of four stiffness data sets: under baseline condition, and after treatment with histamine, cytochalasin D, and DBcAMP. For a given group, the median values across all microbeads were fitted using Eq. (1). As reported previously, although α_C and μ_C depended on the experimental interventions, the value of g_0 and f_0 was shown to be invariant [16, 29]. To evaluate the parameters to fit a data set, a least-squares minimization of the squared differences between the logarithm of the complex data and the logarithm of the complex model, summed over all frequencies and all treatment conditions, was performed using the R software [35].

RESULTS

Deuterium and cell viability

Because every protein relies upon hydrogen bonds to stabilize its tertiary structure, the effects of deuterium oxide in the living cell could be potentially numerous and extensive [36–43]. Consequently, we first focused on evaluating the toxicity of D₂O in the HASM cells. We noticed that HASM cells could be kept alive in 70% D₂O medium for several weeks, although we limited exposure during measurements to D₂O to at most, overnight (14 to 18 hr). Cells exposed overnight to 70% D₂O showed no differences compared to H₂O control in oxidative activity monitored using DHR, both at baseline level and after treatments with PMA and antimycin-A, (data not shown).

D₂O is known to promote microtubule assembly, suppress microtubule dynamic instability, and block mitosis [37, 43]. But when microtubules were visualized by immunostaining, no differences in morphology, intensity, and density of microtubules between control and overnight incubation at 70% D₂O groups were evident (data not shown). This lack of difference might be a consequence of serum-deprivation, as used here, which is known to

arrest the cell cycle in the G₁/G₀ phases. Furthermore, when the actin network was visualized, no differences in morphology, intensity, and density of stress fibers could be observed. Taken altogether, these data show that overnight incubation in 70% D₂O did not noticeably modify cellular oxidative activity or actin and microtubule structures.

Influences D₂O on CSK mechanics

We first measured the elastic complex modulus of the HASM cells in control media (0% D₂O) both at baseline conditions and after challenging the cells with histamine (which causes the cells to contract), DBcAMP (which causes the cells to relax), and cytochalasin D (which disrupts actin filaments). These challenges had effects on HASM cells similar to those previously reported [16, 18]. For every challenge, the stiffness modulus measured over 4 decades in frequency followed a weak power law (Fig. 1); the extrapolation of which defined a common intersection, or fixed point, at coordinate (f_0, g_0) . The 95% confidence interval of (f_0, g_0) was evaluated from the fit of the data to Eq. 1 (Fig. 1, close symbols). The loss modulus, $g''(f)$, followed a similar behavior for frequencies below about 30 Hz, and then slowly approached a higher exponent behavior (data not shown). As previously reported [16, 18], the existence of the common intersection (f_0, g_0) implies that differences in responses among the four conditions within the measured frequency range are accounted for by changes in the power law exponent.

We then replicated this set of experiments after overnight incubation in a medium composed of 70% D₂O (Fig. 1, open symbols). With exposure to D₂O, the dependence of cell stiffness upon frequency remained in every case a power law, but the exponent was increased (Fig. 1, triangle symbols). Treatments with histamine, DBcAMP, and cytochalasin D showed similar effects as in H₂O, but the contractile scope between the stiffer condition (histamine) and the softer condition (cytochalasin D) was reduced by almost one order of magnitude. Remarkably, these power law relationships again defined a common intersection (g_0, f_0) . However, with prolonged exposure to D₂O, the coordinate (g_0, f_0) shifted slowly down and to the left, and, after overnight incubation, f_0 was lower by almost 4 orders of magnitude, having shifted from 2×10^8 Hz for H₂O down to 3×10^4 Hz for D₂O (Fig. 1, inset). We further showed that stabilization of microtubules with taxol (10 μ M) in H₂O media did not affect the common intersection (f_0, g_0) , therefore the shift in f_0 observed in D₂O media is not a consequence of microtubule instability suppression.

Cytoskeleton remodeling in D₂O

Cytoskeletal remodeling was measured after 10 min incubation in PBS with 0%, 67%, and 100% D₂O (Fig. 2a), after 2 hr incubation in 70% D₂O (data not shown), and after overnight incubation in 70% D₂O (Fig. 2b). As previously described, all MSD curves were sub-diffusive at short time intervals and super-diffusive at long time intervals [19, 23, 26, 34]. Deuterium oxide had two distinct effects on the MSD curves: in a dose- and time-dependent manner, the amplitude was reduced and the transition time between sub- and super-diffusivity increased (the transition time is defined as the time at which the local slope of the MSD is equal to 1). With a 10 min incubation at progressively higher concentration of D₂O, the transition time between sub-diffusive and super-diffusive behaviors was progressively delayed, from 1.8 s to 3.7 s (Fig. 2a). With 2 hr incubation, the effect was similar to that seen after 10 min incubation (data not shown). But with overnight incubation, the rate of remodeling was even slower and the transition time delayed to 7.9 s (Fig. 2b). In presence of D₂O, remodeling dynamics of the cytoskeleton occurred at a slower rate.

Response to a transient stretch

After serum deprivation for 36 hrs, HASM cells were plated on a flexible membrane overnight in 70% D₂O or control H₂O media. RGD-coated microbeads were added, and

stiffness was measured before and after application of a 10% transients stretch (Fig. 3). Before stretch, HASM cells appeared slightly stiffer in 70% D₂O (gray curve) than in control media (black curve). After cessation of the transient stretch, both groups showed a prompt decrease in stiffness, followed by a slow recovery, as previously reported [15]. Interestingly, the recovery phase was much faster in 70% D₂O than in control media; when fitted by an exponential function $g'(t)=a-be^{-t/\tau}$, the characteristic time of recovery was ~25 s for D₂O media versus ~70 s for H₂O media.

DISCUSSION

Regardless of cell type, the rheology of the intact cell always follows a weak power law, and with multiple drug manipulations the resulting family of power laws defines a common intersection at some high frequency f_0 [16, 18, 29]. As such, the power law exponent and its variations are sufficient to define cell rheology [17, 44]. However, a mechanism to explain the power law behavior, its exponent, and the existence of the common intersection at f_0 remains unknown. Because f_0 falls in the GHz range, it coincides roughly with the rotational relaxation frequency of water near globular proteins [7, 8]. And because f_0 is conserved across cell types, we had speculated previously that it might be linked to the dynamics of the protein hydration shell [29]. To test that hypothesis, here we used D₂O to modify the water network within the living cell. Over the course of weeks, D₂O exposure is known to be toxic because it inhibits spindle formation [37, 43], but over the course of hours, corresponding to the results reported here, no such toxic effects were observed.

Probing the water network

Our three main results are as follow. First, water had a tremendous impact on cytoskeleton dynamics. Second, in D₂O media, the power law relationships and the common intersection continued to prevail (Fig. 1). Rather than being inferred by extrapolation over many decades in frequency, as previously, the common intersection is shifted nearby the measurement range. The existence of the common intersection under different water conditions, and its extreme sensitivity to water conditions, supports the hypothesis that it is linked to water dynamics. Third, the magnitude of the shift in f_0 was unanticipated. Compared to H₂O, the physical properties of D₂O change only modestly [32, 33], whereas f_0 shifted by almost 4 frequency decades. If this shift were explained by the glassy behavior of water in the hydration shell [10, 11], then D₂O would be understood to move the hydration shell slightly towards the glass transition, and as a result water dynamics would be dramatically slowed. Moreover, the possible contribution to the shift of f_0 and g_0 over time from progressive deuteration of proteins (Fig. 1, inset) points to a direct effect on the protein conformational energy landscape. Alternatively, to the extent that cytoskeletal rearrangements involve large-scale conformational changes, it is plausible to suspect a role of the α fluctuations in the bulk solvent. Because the MSD measurements involve rearrangements on the nanometer scale, one could expect as well changes in the internal protein dynamics and the β fluctuations of the hydration shell to which they are slaved. Therefore D₂O is seen to have effect on the bulk water, on the hydration shell, and on the protein.

Cytoskeletal dynamics, water, and fragility

Cytoskeletal dynamics show strong similarities with dynamics of a class of materials that includes pastes, foams, clays, or colloids, often termed fragile matter [15–24]. When seen as a fragile system, the cytoskeleton is imagined to be composed of trapped structural elements, where trapping could arise from molecular crowding, acto-myosin interactions, steric constraints, or hydrogen bonding (Fig. 4a). The trapping energy is sufficiently weak – hence the term “fragile” – to allow structural elements to escape their cages. To escape its cage, an element must be “kicked” over an energy barrier that is large compared with thermal

energies. Such an event leads to remodeling dynamics of the cytoskeleton [19, 23, 44]. A power law distribution of the depths of energy wells leads to power law rheology well described by Eq. 1 [16, 18, 45]. In the soft glassy rheology theory [45], the power law exponent, α , of Eq. 1 is linked to an effective temperature of the system, representing the amount of non-thermal molecular agitation. The prefactor, g_0 , represents the stiffness of the living cell when all dynamics are frozen ($\alpha = 0$), and the frequency, f_0 , represents an attempt frequency at which structural elements escape their trap. In this physical picture, the data reported here suggest that the depth of the energy well is affected by the network of water molecules (Fig. 4). In the case of heavy water, the energy well would appear deeper, due to a stronger hydrogen bond, therefore leading to slower cytoskeletal dynamics; it is unexpected though that a small change in energy depth on a broad distribution of energy wells would have such a drastic impact on the attempt frequency f_0 . Although the detailed mechanism of this effect is yet to be revealed, data presented here establish that the dynamics of the integrated cytoskeleton depend directly and importantly upon the dynamics of water.

Acknowledgments

This work was supported by the National Institutes of Health and the Parker B. Francis foundation.

REFERENCES

1. Ellis RJ, Minton AP. *Nature*. 2003; 425:27. [PubMed: 12955122]
2. Dobson CM. *Nature*. 2004; 432:824. [PubMed: 15602547]
3. Pollack, GH. *Cells, Gels and the Engines of Life*. Seattle, WA: Ebner; 2001. p. 305
4. Fulton AB. *Cell*. 1982; 30:345. [PubMed: 6754085]
5. Charras GT, Mitchison TJ, Mahadevan L. *J Cell Sci*. 2009; 122:3233. [PubMed: 19690051]
6. Frauenfelder H, et al. *Proc Natl Acad Sci U S A*. 2009; 106:5129. [PubMed: 19251640]
7. Kabir SR, et al. *Biophys J*. 2003; 85:3154. [PubMed: 14581215]
8. Suzuki M, et al. *Biochem Biophys Res Commun*. 2004; 322:340. [PubMed: 15313212]
9. Stanley HE, et al. *Journal of Physics: Condensed Matter*. 2009; 21:504105.
10. Frauenfelder H, et al. *Proc Natl Acad Sci U S A*. 2001; 98:2370. [PubMed: 11226246]
11. Onuchic JN, Luthey-Schulten Z, Wolynes PG. *Annu Rev Phys Chem*. 1997; 48:545. [PubMed: 9348663]
12. Lubchenko V, Wolynes PG. *Annu Rev Phys Chem*. 2007; 58:235. [PubMed: 17067282]
13. Fenimore PW, et al. *Proc Natl Acad Sci U S A*. 2004; 101:14408. [PubMed: 15448207]
14. Frauenfelder H, et al. *Proc Natl Acad Sci U S A*. 2006; 103:15469. [PubMed: 17030792]
15. Trepat X, et al. *Nature*. 2007; 447:592. [PubMed: 17538621]
16. Fabry B, et al. *Phys. Rev. E*. 2003; 68:041914.
17. Trepat X, Lenormand G, Fredberg JJ. *Soft Matter*. 2008; 4:1750.
18. Fabry B, et al. *Phys. Rev. Lett*. 2001; 87:148102. [PubMed: 11580676]
19. Bursac P, et al. *Nat. Mater*. 2005; 4:557. [PubMed: 15937489]
20. Kollmannsberger P, Fabry B. *Soft Matter*. 2009; 5:1771.
21. Zhou EH, et al. *Proc Natl Acad Sci U S A*. 2009; 106:10632. [PubMed: 19520830]
22. Lenormand G, et al. *J R Soc Interface*. 2004; 1:91. [PubMed: 16849155]
23. Lenormand G, et al. *Biochem Biophys Res Commun*. 2007; 360:797. [PubMed: 17631276]
24. Lenormand G, et al. *Phys. Rev. E*. 2007; 76:041901.
25. An SS, et al. *Biochem Biophys Res Commun*. 2009; 382:697. [PubMed: 19327344]
26. Bursac P, et al. *Biochem Biophys Res Commun*. 2007; 355:324. [PubMed: 17303084]
27. Lau AWC, et al. *Phys. Rev. Lett*. 2003; 91:198101. [PubMed: 14611619]
28. Sunyer R, et al. *Phys Rev E Stat Nonlin Soft Matter Phys*. 2009; 79:051920. [PubMed: 19518493]
29. Laudadio RE, et al. *Am. J. Physiol. Cell. Physiol*. 2005; 289:C1388. [PubMed: 16120653]

30. Puig-de-Morales M, et al. Am. J. Physiol. Cell. Physiol. 2004; 287:C643. [PubMed: 15175221]
31. Mattsson J, et al. Nature. 2009; 462:83. [PubMed: 19890327]
32. McQueen-Mason S, Cosgrove DJ. Proc Natl Acad Sci U S A. 1994; 91:6574. [PubMed: 11607483]
33. Lide DR. CRC handbook of chemistry and physics: a ready-reference book of chemical and physical data (CRC Pr I Llc. 2004
34. An SS, et al. J Appl Physiol. 2004; 96:1701. [PubMed: 14729728]
35. Team, RDC. R Foundation for Statistical Computing. Austria: Vienna; 2009.
36. Santra MK, Dasgupta D, Panda D. Proteins. 2005; 61:1101. [PubMed: 16245323]
37. Panda D, et al. Biochemistry. 2000; 39:5075. [PubMed: 10819973]
38. Lamprecht J, Schroeter D, Paweletz N. J Cell Sci. 1991; 98(Pt 4):463. [PubMed: 1650376]
39. Jasnin M, et al. Eur Biophys J. 2008; 37:613. [PubMed: 18286276]
40. Cioni P, Strambini GB. Biophys J. 2002; 82:3246. [PubMed: 12023248]
41. DeCoursey TE, Cherny VV. J Gen Physiol. 1997; 109:415. [PubMed: 9101402]
42. Fisher SJ, Helliwell JR. Acta Crystallogr A. 2008; 64:359. [PubMed: 18421125]
43. Krendel M, Inoue S. Biol Bull. 1995; 189:204. [PubMed: 8541401]
44. Lenormand G, Fredberg JJ. Biorheology. 2006; 43:1. [PubMed: 16627924]
45. Sollich P. Phys. Rev. E. 1998; 58:738.

Figure 1. Cytoskeleton stiffness and common intersection

Mechanical properties were measured after overnight incubation in H_2O media (closed symbols) and after in 70% D_2O media (open symbols), under baseline condition (triangle), after treatment with the contractile agonist histamine (square), the relaxing agonist DBcAMP (circle), and after disruption of actin fibers using cytochalasin D (diamond). From each group of 4 measurements, the common intersection (f_0 , g_0) and its 95% confidence interval were calculated. Each symbol represents the median values over more than 500 measurements. Inset: Common intersection regions and their 95% confidence interval at control (dark grey), after 1 hr incubation with 70% of D_2O (intermediate grey), and after overnight incubation with 70% of D_2O (light grey).

Figure 2. Cytoskeleton remodeling

The mean square displacement of microbeads bound to the cytoskeleton was measured after (a) 10 min incubation in PBS composed of 0% D_2O (black), 67% D_2O (intermediate grey), and 100% D_2O (light grey), and (b) after overnight (14 to 18 hr) incubation in PBS composed of 67% D_2O . As the concentration of D_2O increased, the transition time between sub- and super-diffusion increased, and the rate of cytoskeleton remodeling decreased. With overnight incubation in D_2O , the transition time was even longer, and the rate of remodeling was even slower. Median values over more than 950 microbeads in each group.

Figure 3. Response to a transient stretch

Stiffness of HASM cells was measured before and after application of a 10% transient stretch after overnight incubation in 70% D₂O media (grey curve) and H₂O control media (black curve). After stretch, stiffness promptly decreased and then slowly recovered. The recovery curves were fitted using $g'(t) = a - b e^{-t/\tau}$ (thin solid lines), leading to a characteristic time of recovery of $\tau_{D_2O} = 24.3$ s in D₂O media and of $\tau_{H_2O} = 69.5$ s in H₂O media. Median values over more than 500 beads.

Figure 4. Cytoskeletal dynamics, water, and fragility

Cytoskeletal structural elements find themselves trapped in a cage formed by surrounding neighbors (a and b). This cage is represented by an energy well (c). We hypothesize here that the depth of the energy well is determined by the network of water molecules; in the case of heavy water, the energy well is deeper, therefore explaining the observed slower cytoskeletal dynamics.