View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Repository of the Academy's Library

European Biophysics Journal

Elasto-mechanical properties of living cells

--Manuscript Draft--

Manuscript Number:	
Full Title:	Elasto-mechanical properties of living cells
Article Type:	Original Paper
Keywords:	cell elasticity; oscillation; atomic force microscope; fast Fourier transform; correlation
Corresponding Author:	György Váró, DSC HUNGARY
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	
Corresponding Author's Secondary Institution:	
First Author:	Béla Varga
First Author Secondary Information:	
Order of Authors:	Béla Varga
	Csilla Fazakas
	Imola Wilhelm
	István A Krizbai
	Zsolt Szegletes
	György Váró, DSC
	Attila G Végh
Order of Authors Secondary Information:	
Funding Information:	National Science Fund of Hungary (OTKA K K81180) — Dr György Váró
Abstract:	Abstract The possibility to directly measure the elasticity of living cell has emerged only recently. In the present study the elastic properties of two cell lines were followed. Both types are widely used as barrier models. During time resolved measurement of the living cell elasticity a continuous quasi-periodic oscillation of the elastic modulus was observed. Fast Fourier transformation of the signals revealed that a very limited number of three to five Fourier terms fitted the signal in the case of human cerebral endothelial cells. In the case of canine kidney epithelial cells more than 8 Fourier terms did not result a good fit. Calculating the correlation of the signals revealed a higher correlation factor for the endothelial cells compared to the epithelial cells.
Suggested Reviewers:	Manfred Radmacher radmacher@uni-bremen.de Expert in the AFM field
	Jean-Luc Pellequer jlpellequer@cea.fr Expert in the field of AFM
Opposed Reviewers:	

Powered by Editorial Manager® and ProduXion Manager® from Aries Systems Corporation

Manuscript
Click here to download Manuscript: Elastomechfn3.doc
Click here to view linked References

Elasto-mechanical properties of living cells

Béla Varga, Csilla Fazakas, Imola Wilhelm, István A. Krizbai, Zsolt Szegletes, György Váró and Attila G. Végh

Institute of Biophysics, Biological Research Centre of the Hungarian Academy of Science, Szeged, Hungary

Corresponding author: G. Váró, BRC, Temesvári krt 62, Szeged, Hungary, H-6726 E-mail: varo.gyorgy@brc.mta.hu

Abstract

The possibility to directly measure the elasticity of living cell has emerged only recently. In the present study the elastic properties of two cell lines were followed. Both types are widely used as barrier models. During time resolved measurement of the living cell elasticity a continuous quasi-periodic oscillation of the elastic modulus was observed. Fast Fourier transformation of the signals revealed that a very limited number of three to five Fourier terms fitted the signal in the case of human cerebral endothelial cells. In the case of canine kidney epithelial cells more than 8 Fourier terms did not result a good fit. Calculating the correlation of the signals revealed a higher correlation factor for the endothelial cells compared to the epithelial cells.

Introduction

All living organisms are dynamic systems, driven by well defined and synchronized mechanical processes between different parts of the whole (Julicher, 2001;Jin et al., 2005;Aon et al., 2003). The smallest living unit, with a complex function, is the cell. Uncountable types of cells, each with different well determined functions can form an organ or organism. To understand the function of the highly complex organisms first we have to know the cell. Regarding the proper homeostasis of the Central Nervous System (CNS) the importance of the cerebral endothelial cells (CEC) cannot be questioned. They constitute the structural basis of blood-brain-barrier (BBB), having crucial role in the control of trafficking substances across their membrane (Wilhelm et al., 2014;Abbott et al., 2010) to and from the CNS. While cerebral endothelial cells have a principal role in the maintenance of the homeostasis

of the CNS, epithelium of the renal distal tubule contributes to the ion homeostasis of the organism. Although they are intensively studied, still limited information is available about their function, or how their internal molecular alterations manifest in mechanical properties

A rather new tool for determining mechanical properties, such as the elasticity or adhesion, of a microscopic object is the atomic force microscope (AFM) (Binnig et al., 1986;Haberle et al., 1991). Besides the imaging of the cell surface with atomic resolution the AFM can provide the value of its micro-mechanical parameters. A great advantage of it is that the measurements can be performed not only in vacuum, but in air or in liquid environment on living cells (Santos and Castanho, 2004;Klenerman et al., 2011;2012) at human body temperature.

Mechanical properties of individual cells are strongly connected to biological functions, dynamically linked to both internal and external stimuli. Measuring the time dependence of some mechanical properties of the biological system a spontaneous quasi periodical oscillation was observed. Oscillation can appear in open nonlinear dynamic system. Biological systems fulfill these conditions (Julicher, 2001;Kruse and Julicher, 2005).The first documented biological oscillation was described by Luigi Galvani in 1780. Just to name few examples when oscillation was observed: mechanical and electrical oscillation in cardiac muscle of the turtle (Bozler and Delahayes, 1973) drosophila tissue motion (Solon et al., 2014), oscillation of the elasticity and adhesion of vascular smooth muscle cell (Zhu et al., 2012), shape oscillations of human neutrophil leukocytes (Ehrengruber et al., 1996), bronchial epithelial cells (Schillers et al., 2010) elasticity oscillation of the cerebral endothelial cells (Végh et al., 2011).

The period of these oscillations show large scattering, spanning from seconds to hours. Although more and more type of cells are intensively studied and several oscillating cells were investigated, the conditions when and why they are produced is mostly undecyphered.

In the present study the elastic oscillation measured on human brain microvasvular endothelial and canine kidney epithelial cells were investigated and compared. Both are used as a barrier model. While the vascular endothelial cells are constantly exposed to mechanical forces from the blood stream the epithelial cells do not have to withstand shear forces. All these information can help to understand the origin of it.

Materials and methods

Cell culture

The human cerebral microvascular endothelial cells (hCMEC/D3 - shortly D3) were grown on rat tail collagen-coated dishes in EBM-2 medium (Lonza) supplemented with EGM-2 Bullet Kit (Lonza) and 2.5% Fetal Bovine Serum (FBS) from Sigma (Wilhelm et al., 2007;Wilhelm et al., 2008). MDCK (Madin-Darby canine kidney) cells were maintained in DMEM/F-12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12) (Lonza) supplemented with 5% FBS.

Cells were cultured at 37°C , in 5% CO_2 atmosphere, seeded at $1.5*10^4$ cell/cm^2 in Falcon petri dish (lid) with 3.5 cm diameter. MDCK monolayer were fed with fresh medium first after 24 hours (post-seeding) than every seconnd day until they reached confluence (3rd day).

All measurements were performed in serum free Leibovitz medium (Sigma) at 37ºC within 3 hours after taking the cells out from the incubator. According to our observations and to literature, within this period cells preserve their viability (Pesen and Hoh, 2005).

AFM

All experiments were carried out with an Asylum Research MFP-3D atomic force microscope (Asylum Research, Santa Barbara, CA; driving software IgorPro 6.32A, Wavemetrics), mounted on a Zeiss Axiovert 200 optical microscope. The experiments were performed with gold coated silicon nitride rectangular cantilevers, nominal spring constant of 0.03 N/m, resonant frequency 37 kHz, with a “V” shaped tip (Olympus, Optical Co. Ltd). The spring constant of the cantilever was determined each time by thermal calibration (Hutter and Bechhoefer, 1993). All images were recorded in Alternate Contact (AC) mode having 256 lines by 256 points, tip velocity of 60 μm/s. Trace and retrace images were both recorded and compared for internal accuracy. Noteworthy differences could not be found which underlines their reliability.

Force Measurements

After taking an image three different cells were selected, with selected points on nuclear and peripheral region respectively. Force curves were effectuated consecutively on each pre-defined spot on cells, having the same time elapsed between two consecutive measurement. Force curves were recorded at constant

112 loading speed (2 μm/s) and sampling frequency (0.5 kHz). Total force distance was
113 kept at 3μm and maximum load below 2nN.

114 Probing any material with a hard indenter (AFM tip) leads to the theory of indenting
115 an elastic half-space with a stiff object. Based on the work of Heinrich Hertz (Hertz,
116 1881) and Ian Sneddon (Sneddon, 1965) later modified for AFM tips (Mathur et al.,
117 2001), which is widely used for indentation tests, regardless of length scale. Elastic
118 characterization was based on calculating the sample's elastic modulus (Végh et al.,
119 2011;Vinckier and Semenza, 1998) from each performed force curve.

120

121 Data Analysis

122 A home made MatLab (Math Works Inc., Natick, Massachusetts) routine was
123 implemented to calculate the frequency spectra of the elastic changes based of Fast
124 Fourier Transform (FFT) method, as well as best fitting sum of sinusoidal functions to
125 raw data. For characterizing the similarities between two data sets, the Pearson's
126 correlation coefficient was calculated.

127

128 **Results**

129 High resolution topographies were made on living cells grown in a Petri dish
130 and in each case three cells were chosen with proper shape. On each cell two
131 different locations were selected, one over the nucleus, the other at the cell periphery
132 (figure 1). At these selected six points elasticity measurements were effectuated
133 cyclically. Duration of one cycle was about 30s. The whole experiment of 60 to 80
134 minutes, resulting 120-150 measurement at each point. During the experiment in

135 each selected place a classical force curves were taken (figure 2) and the elasticity of
136 the measured point was calculated. In this way the time dependence of the cell
137 elasticity in the selected points could be followed (figure 3) simultaneously.

138 The fluctuation of the time traces is larger than the noise. The size of the
139 noise of the whole system was estimated by replacing the cells in the Petri dish with
140 a thin layer of acrylamide gel and the elasticity on six points was measured in similar
141 conditions as with the cells. All six traces were almost straight lines, out of which only
142 one is presented (figure 3, curve gel). No fluctuations can be distinguished at similar
143 scale to those on living cells

144 To eliminate the very slow shift and the fast noise like component a Fast
145 Fourier Transform (FFT) was applied on the time dependent elasticity series (figure
146 4) and the periods below 5 minutes and over 100 minutes were cut. The former was
147 considered too quick to be accurately followed in our system, the latter too slow for
148 proper calculations at this time scale. The truncated curves were converted back to
149 the time space with an Inverse Fast Fourier Transform (IFFT) .(Data not shown)

150 Browsing through the elasticity series three different kind could be
151 distinguished based on their oscillating amplitude: large amplitude (figures 5 a, b, d,)
152 small amplitude (figures 5 e, f) and transitional traces (figure 5c). The FFT signal
153 contains several sinusoidal components with well determined time period. In order to
154 estimate how many oscillating components describe the time dependent elasticity
155 traces, a multi sinusoidal fit was applied ranging from one to ten components. Similar
156 set of experiment was measured on MDCK epithelial cells and the data treated in a
157 similar mode. The tendency of the elasticity signal was similar to that measured on
158 D3 cells, but the noise was commensurable with the signal (figure 6 dots). The data

159 analysis yielded apparently faster FFT components (figure 7), with an almost
160 constant amplitude for large time intervals.

161 The elasticity signals were fitted with increasing number of sinusoidal as well.
162 The fit to the signals belonging to D3 cells resulted a good fit with 4 sinusoidal (Figure
163 5, line), further component not improving considerably the fit, which has saturated
164 after adding four components (Figure 8). Contrary to this the MDCK cells did not
165 saturate even with 8 components and the fit was not improving (Figures 6 and 8).

166 The next step of the analysis was based on the assumption that interaction
167 might exist between different parts of the cell and this is reflected in a cooperative
168 change of several parameter reflecting the function of the cell. Such a parameter is
169 the elasticity of the cell. To get closer in the analysis of the data to observe the
170 cooperative behavior of the elasticity, the correlation between the time dependent
171 series taken above nucleus and periphery were calculated (figure 9).

172

173 Discussion

174

175 Oscillations associated to the cell as a living object could describe fluctuations
176 from the interior of the cell. We try to develop a model, to describe the changes of the
177 cell wall elasticity related to the events happening in the cell. These events are
178 apparently random in time and space. The large number of cells, each receiving and
179 transmitting several signals makes the system too complex.

180 The amplitude of the fluctuation should be related to the activity of the studied
181 part of the cell. If the measured point is close to an active part its elasticity is varying

182 due to molecular structural changes either in the cytoskeleton or in the organelle in
183 the cytosol or in the glycocalyx. This elasticity change produces a “pressure shock”
184 which propagates in the cytoplasm and in the extracellular media, producing a signal
185 for the neighboring active part. The signal can influence an active part positively by
186 more activation or negatively, by inhibiting it, depending in the earlier state and other
187 signal arriving in the same time.

188 The specially chosen sequence of measurement gives possibility of comparing
189 the series recorded in the same time interval at the same different locations (Figure
190 1). The quasi-oscillations show a large variety of amplitude and frequency on the
191 endothelial cell (Figure 3). As a control a thin layer of acrylamide was measured in
192 similar sequential mode. No change in the elasticity could be observed. Another
193 control was published earlier which proved that the oscillation is related to the living
194 cell (Végh et al., 2011). The fixed sample had only noise in the time dependent
195 elasticity signal. Both controls prove that the measured elasticity signal originates
196 from the living cell.

197 The recorded elasticity traces were mathematically processed. The FFT
198 decomposed signal was truncated at the long period end which corresponds to a
199 baseline shift, with still unknown origins. The other end, which contains the noise was
200 also cut (Figure 4). The FFT spectrum of the endothelial cells are dominated by
201 several long lifetime components. The epithelial cells contain more components with
202 almost identical amplitude The result was reconverted back with inverse FFT
203 resulting a filtered signal. The elasticity signal was fitted with increasing number of
204 sinus curves.

The correlation between the series was compared in case of nuclei and their peripheral counterpart. By plotting the calculated correlation factor in function of elasticity ratio, it was obtained an asymmetric arrangement of the points with average value for D3 cells 0.23 while for the MDCK cells this value was only 0.12. A much smaller value as it was predicted by the sinus fit of the signals. The correlation of the elasticity of the D3 cells were larger compared to the MDCK cells.

All these analysis show that a characteristic difference exists between the endothelial and epithelial cell properties. While the average value of the elasticity is almost the same, the oscillation of the two cell types are different in frequency and amplitude.

Acknowledgements

This work was supported by the National Science Fund of Hungary O TKA K81180, K100807 and PD100958. A.G.V and I.W were supported by the Bolyai Fellowship of the Hungarian Academy of Sciences BO/00598/14/8 for A.G.V and BO/00320/12/8

220

221

222

223 References

224

225 2012. Atomic force microscopy. Investigation into biology from cell to protein.

226 Abbott N.J., Patabendige A.A., Dolman D.E., Yusof S.R., and Begley D.J. (2010).
227 Structure and function of the blood-brain-barrier. Neurobiol- 37:13-25.

228 Aon A.M., Cortassa S., Marban E., and O'Rourke B. (2003). Synchronized whole cell
229 oscillations in mitochondrial metabolism triggered by a local release of reactive
230 oxygen species in cardiac myocytes. J. Biol. Chem. 278:44735-44744.

231 Binnig G., Quate C.F., and Gerber C. (1986). Atomic force microscope. Physical
232 Review Letters 56:930-933.

233 Bozler E. and Delahayes F. (1973). Mechanical and electrical oscillations in cardiatic
234 muscle of the turtle. J. General Physiology 62:523-534.

235 Ehrengruber M.U., Deranleau D.A., and Coates T.D. (1996). Shape oscillation of
236 human neutrophil leukocytes: characterization and relationship to cell motility. J.
237 Exp. Biol. 199:741-747.

238 Haberle W., Horber J.K.H., and Binnig G. (1991). Force microscopy on living cells.
239 Journal Of Vacuum Science & Technology B 9:1210-1213.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65

240 Hertz M.G. (1881). Uber die Beruhrung Fester Elastischer Korper. J. Reine Angew.
241 Math. 92:156-171.

242 Hutter J.L. and Bechhoefer J. (1993). Calibration of Atomic-Force Microscope Tips.
243 Review Of Scientific Instruments 64:1868-1873.

244 Jin A.J., Choi E.J., Niu S.L., Smith P.D., and Litman B.J. (2005). Atomic force
245 microscopy studies of rhodopsin (a G protein-coupled receptor) molecules in
246 vision membranes. Biophys. J. 88:253A.

247 Julicher F. (2001). Mechanical oscillations at cellular scale. C. R. Acad. Sci. Paris
248 4:849-860.

249 Klenerman D., Korchev Y.E., and Davis J.D. (2011). Imagin and characterisation of
250 the surface of live cells. Current Opinion In Chemical Biology 15:696-703.

251 Kruse K. and Julicher F. (2005). Oscillations in cell biology. Current Opinion In Cell
252 Biology 17:20-26.

253 Mathur A.B., Collinsworth A.M., Reichert W.M., Kraus W.E., and Truskey G.A.
254 (2001). Endothelial, cardiac muscle and skeletal muscle exhibit different viscous
255 and elastic properties as determined by atomic force microscopy. Journal of
256 Biomechanics 34:1545-1553.

257 Pesen D. and Hoh J.H. (2005). Micromechanical architecture of the endothelial cell
258 cortex. Biophys. J. 88:670-679.

259 Santos N.C. and Castanho M.A.R.B. (2004). An overview of the biophysical
260 applications of atomic force microscopy. Biophysical Chemistry 107:133-149.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65

Schillers H., Walte M., Urbanova K., and Oberleithner H. (2010). Real-time monitoring of cell elasticity reveals oscillating myosin activity. Biophys. J. 99:3639-3646.

Sneddon I.N. (1965). The relation between load and penetration in the axisymmetric Boussinesq problem for a punch of arbitrary profile. Int. J. Engr. Sci. 3:47-57.

Solon J., Kaya- C.A., and CColombelli J.B.D. (2014). Pulsed forces timed by a ratchet-like mechanism drive directed tissue movemen during dorsal closure. Cell 137:1331-1342.

Végh G.A., Fazakas C., Nagy K., Wilhelm I., Krizbai I.A., Nagyőszi P., Szegletes Z., and Váró G. (2011). Spatial and temporal dependence of the cerebral endothelial cells elasticity. Journal of Molecular Recognition l24:422-428.

Vinckier A. and Semenza G. (1998). Measuring elasticity of biological materials by atomic force microscopy. Febs Letters 430:12-16.

Wilhelm I., Farkas A., Nagyőszi P., Váró G., Bálint Z., Végh G.A., Couraud P.O., Romero I., Weksler B., and Krizbai I. (2007). Regulation of cerebral endothelial cell morphology by extracellular calcium. Physics in Medicine and Biology 52:6261-6274.

Wilhelm I., Fazakas C., Molnár J., Haskó J., Végh G.A., Cervenak L., Nagyőszi P., Nyúl-Tóth Á., Farks A.E., Bauer H., Guillemin G., Bauer H.C., Váró G., and Krizbai A.I. (2014). Role of RHO/ROCK signaling in the interactionn of melanoma cells with the blood-brain barrier. Pigment Cell Melanoma Res. 27:113-123.

282 Wilhelm I., Nagyőszi P., Farks A.E., Couraud P.O., Romero I., Weksler B., Fazakas
283 C., Dung N.T.K., Bottka S., Bauer H., and Krizbai I.A. (2008). Hyperosmotic stress
284 induces Axl activation and cleavage in cerebral endothelial cells. Journal of
285 Neurochemistry 107:116-126.

286 Zhu Y., Qiu H., Trzeciakowski J.P., Sun M.Z., Li Z., Hong Z., Hill M.A., Hunter W.C.,
287 Vatner D.E., Vatner S.F., Meininger G.A., and i. (2012). Temporal analylsis of
288 vascular smooth muscle cell elasticity and adhesion reveals oscillation waveform
289 that differ with ageing. Aging Cell 11:741-750.

290

291

292

293

1

2

3 294 **Figures**

4

5

6 295

7

8

9

10 296 Figure 1.The image of the endothelial D3 cells (panel A) before and (panel B) after

11

12 297 the force measurements. The dots with letters (a-b-c-d-e-f) show the locations where

13

14 298 the forces were measured cyclically .

15

16

17

18 299 Figure 2. The force signal approaching trace (blue) and retrace (red) curves. For

19

20 300 elasticity calculation the trace is used.

21

22

23

24 301 Figure 3. The time dependency of the D3 cell elasticity measured over the nucleus

25

26 302 (a, c, e) and at the periphery (b, d, f). The curve gel is the control measured on

27

28 303 acrilamide gel. The sequence of the measurement is similar to that measured on

29

30

31 304 cells but only noise could be detected

32

33 305 Figure 4. Fast Fourier Transform of the signals on figure 3

34

35

36

37 306 Figure 5. The signals from figure 3 (dots) fitted with sum of 4 sinus curves

38

39

40 307 Figure 6. The time dependency of the epithelial MDCK cells elasticity. The measuring

41

42 308 and calculation protocol was similar as used for the D3 cells. The dots are the

43

44

45 309 measured signal while the continuous line is the fit with 8 sinus curve.

46

47

48 310 Figure 7. Fast Fourier Transform of the signals on figure 6

49

50

51

52 311 Figure 8 The change of the goodness of the fit with increasing number of sinusoidal.

53

54

55 312 Figure 9 The correlation coefficient calculated for the elasticity of the D3 and MDCK

56

57 313 cells.

58

59

60

61

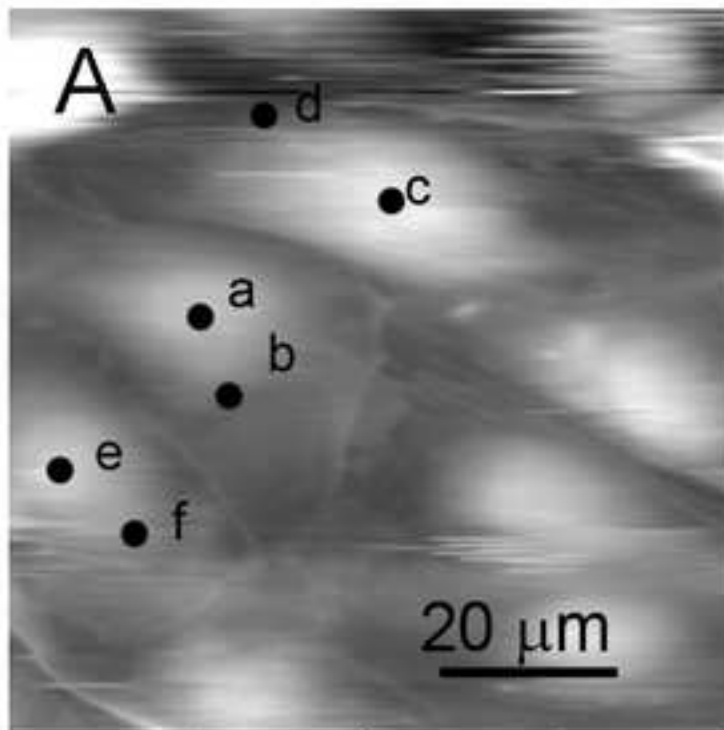
62

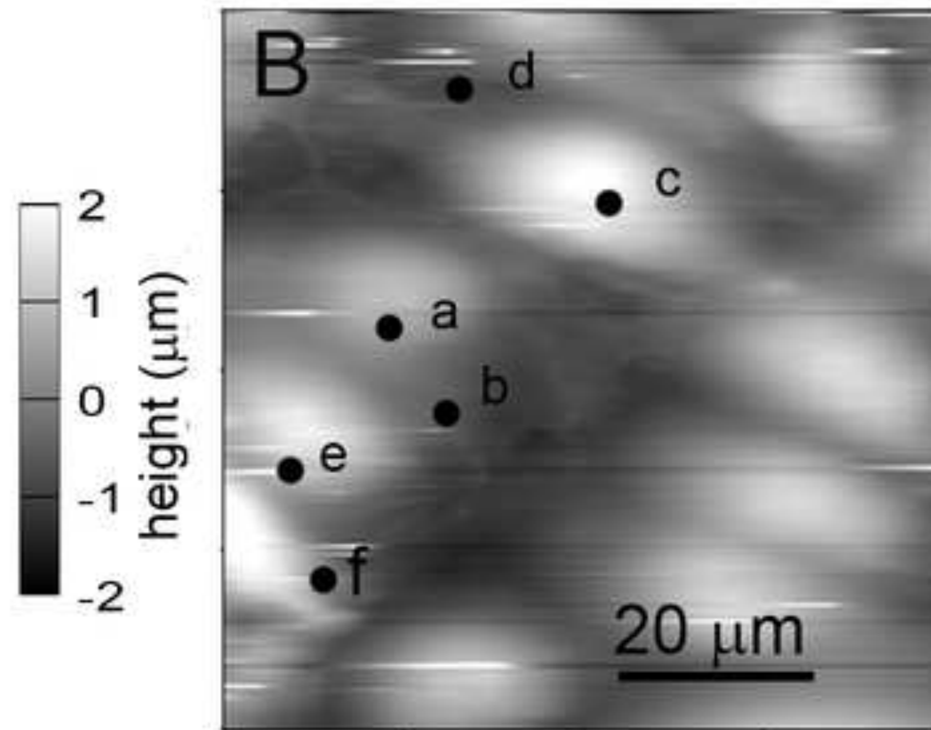
63

64

65

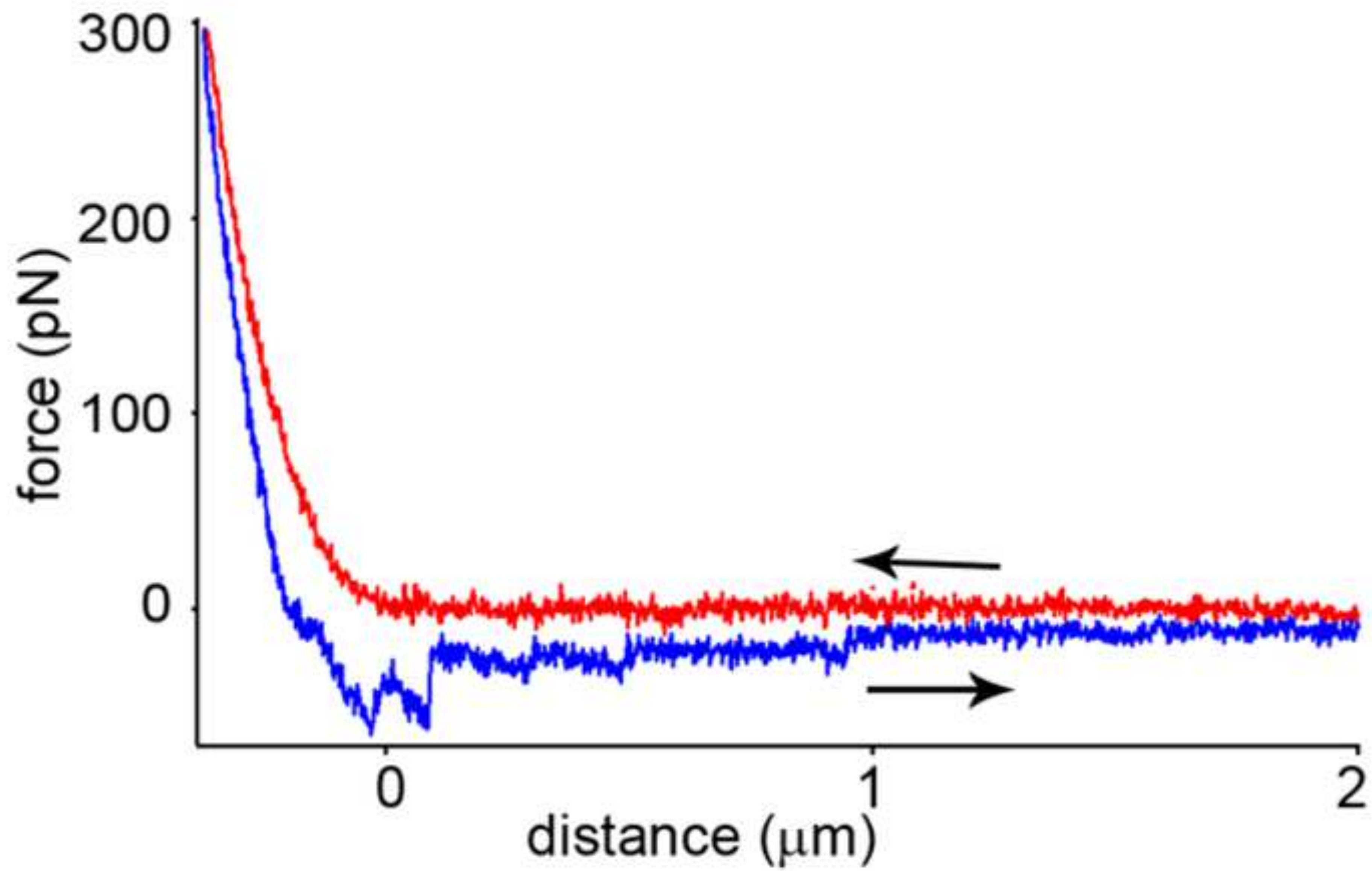
Images of figure 1
Click here to download Images: Fig1.tif



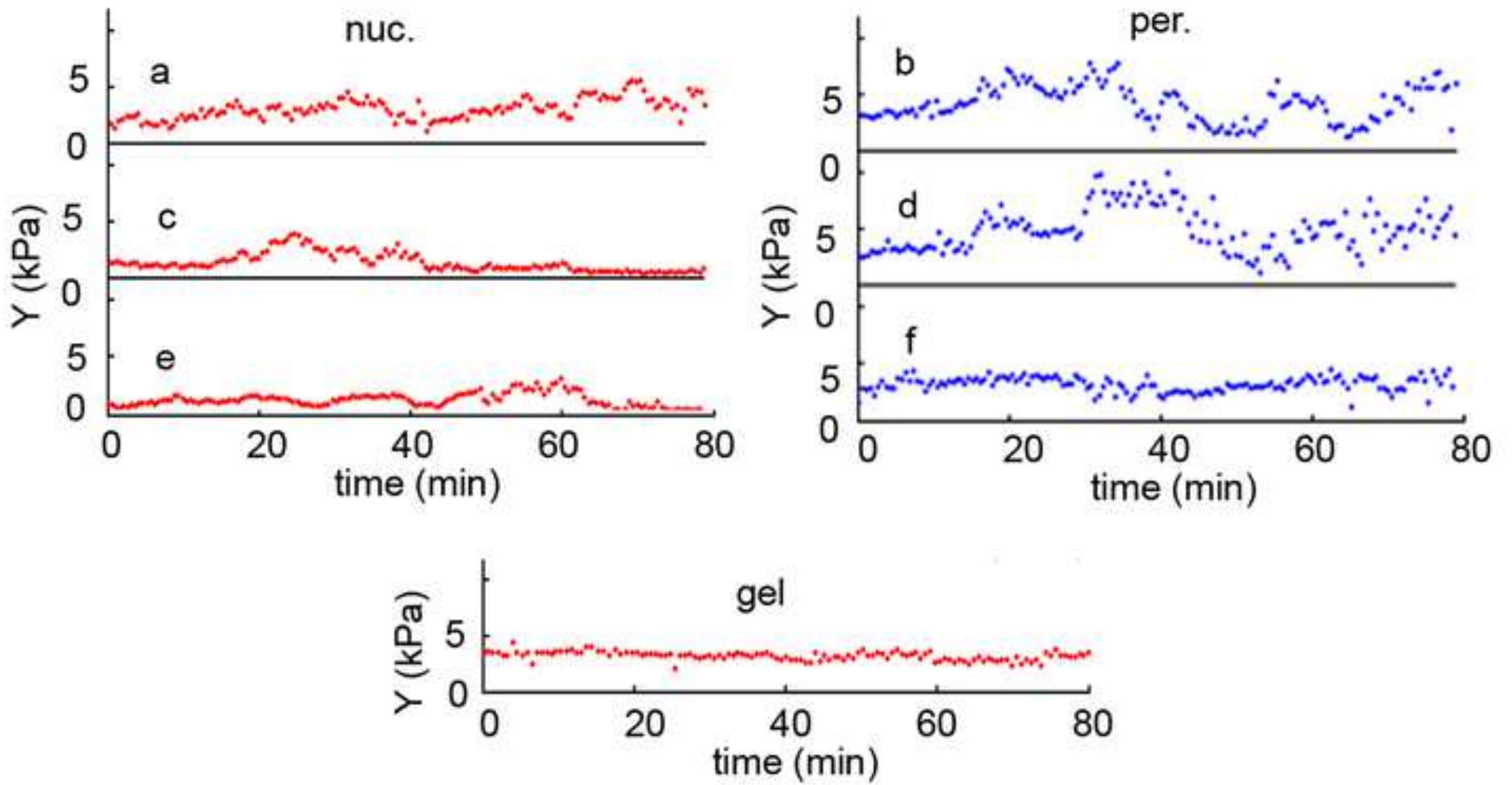


Images of figure 2

Click here to download Images: Fig2.tif

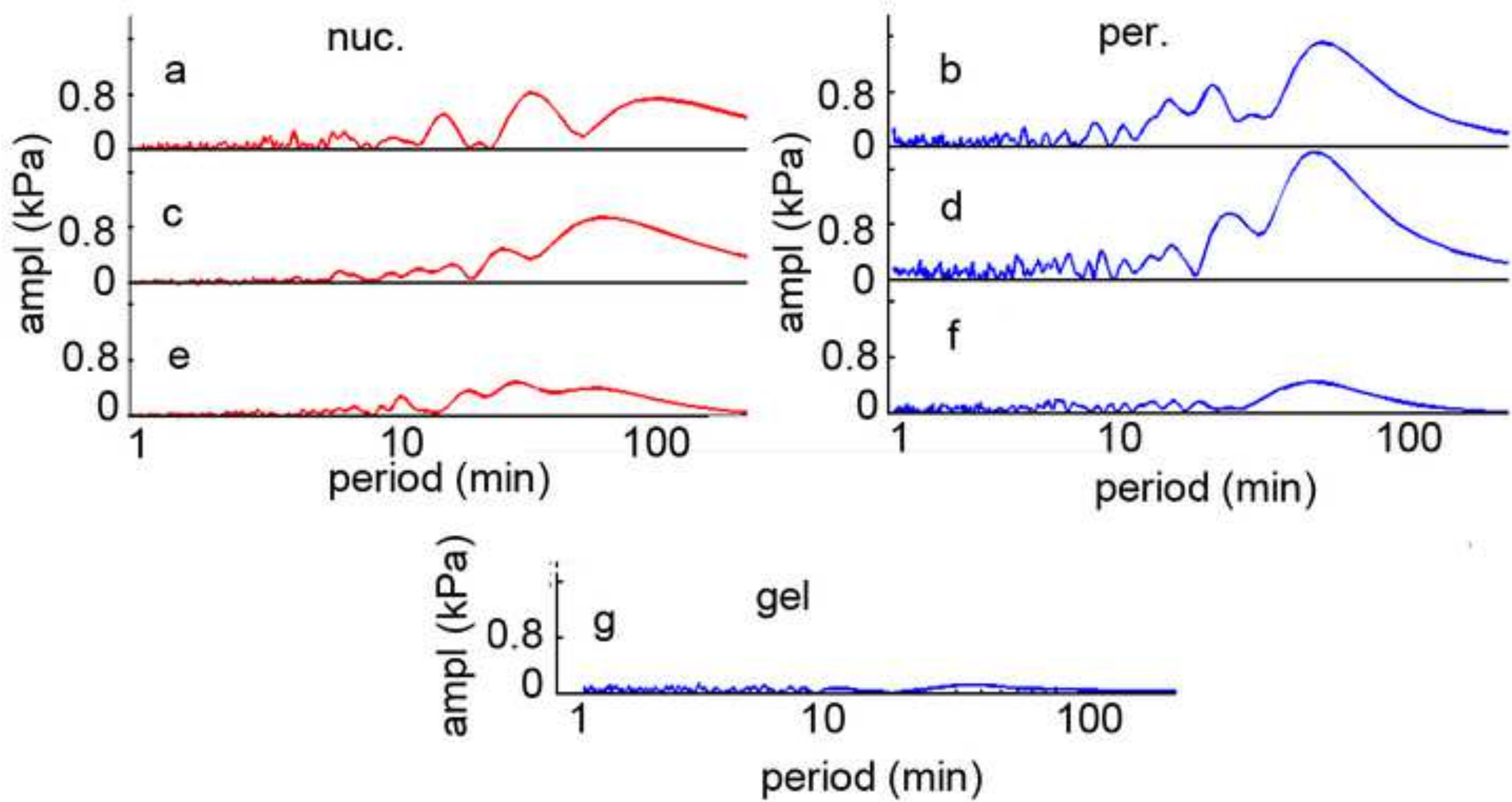


Images of figure 3
Click here to download Images: Fig3.tif

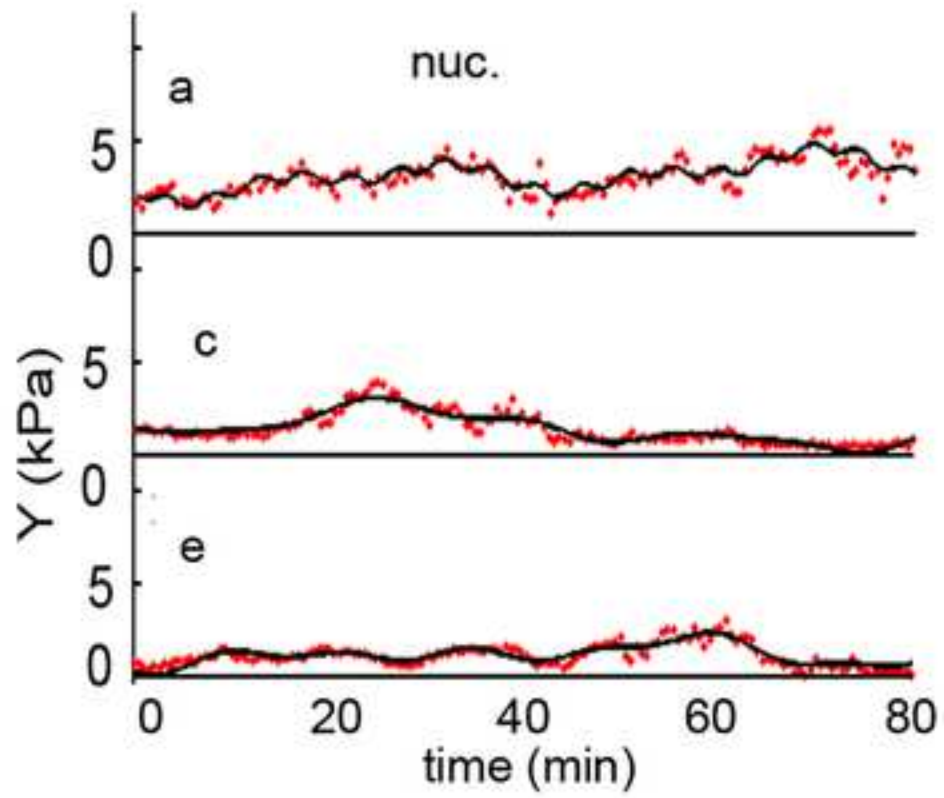


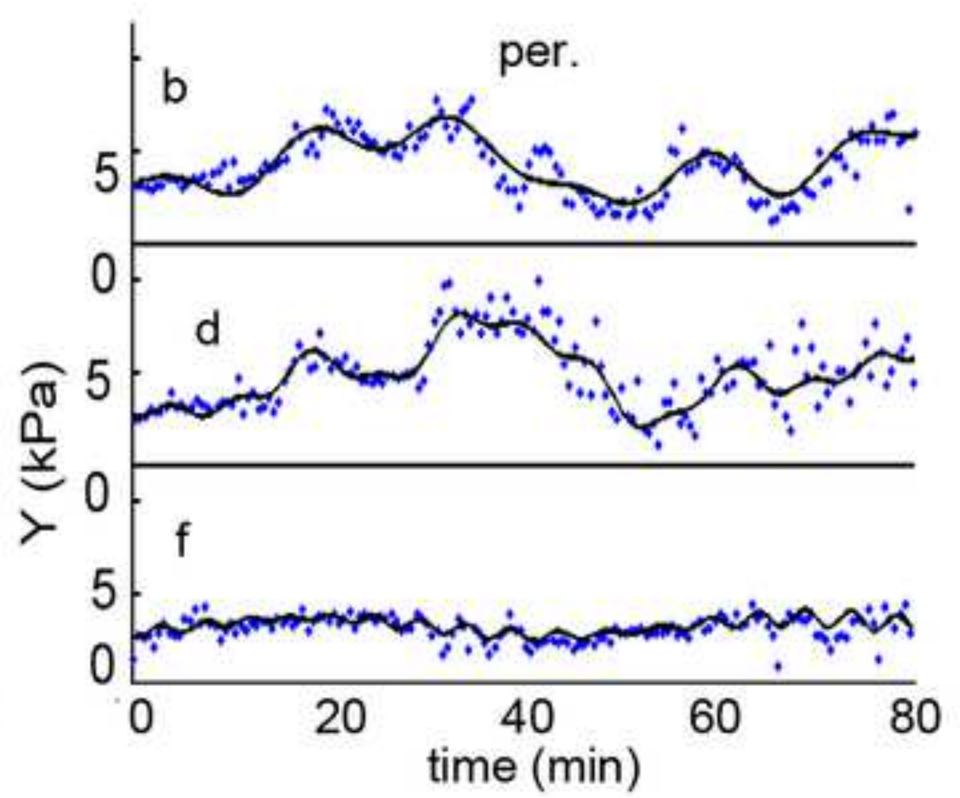
Images of figure 4

Click here to download Images: Fig4.tif



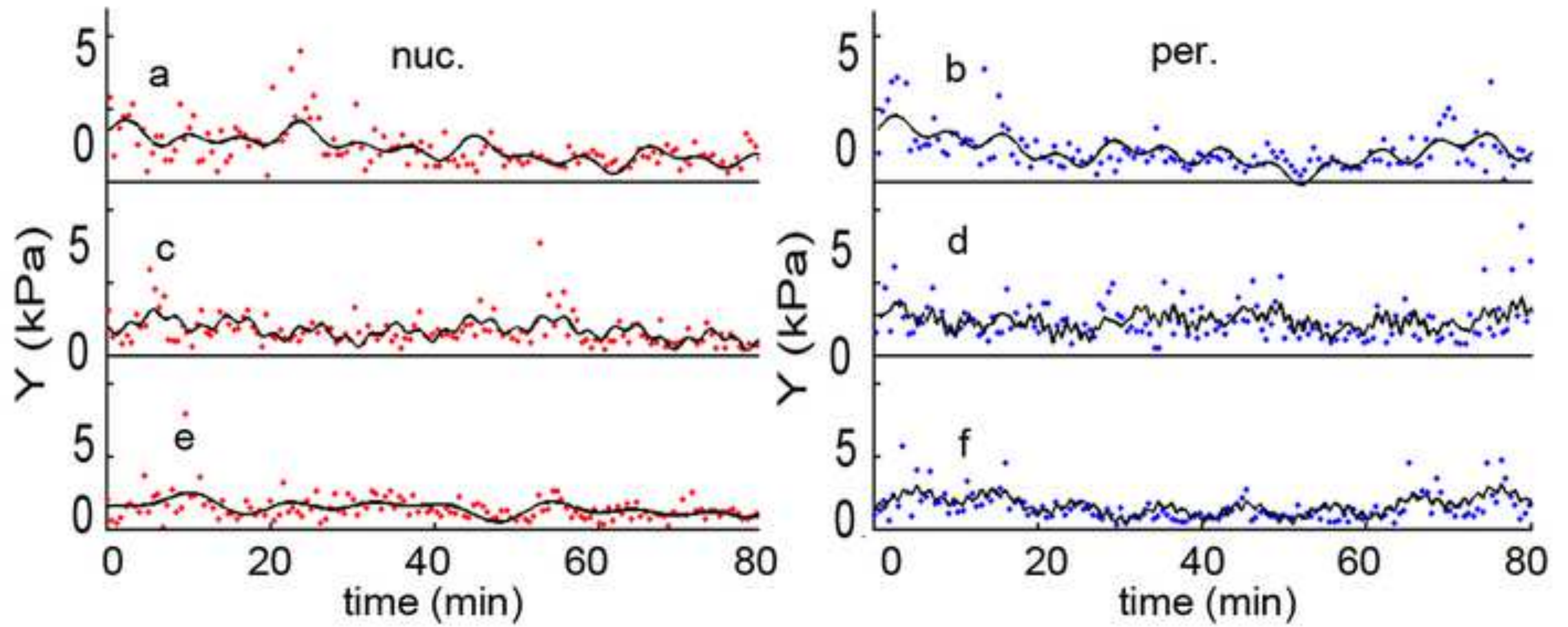
Images of figure 5
Click here to download Images: Fig5.tif



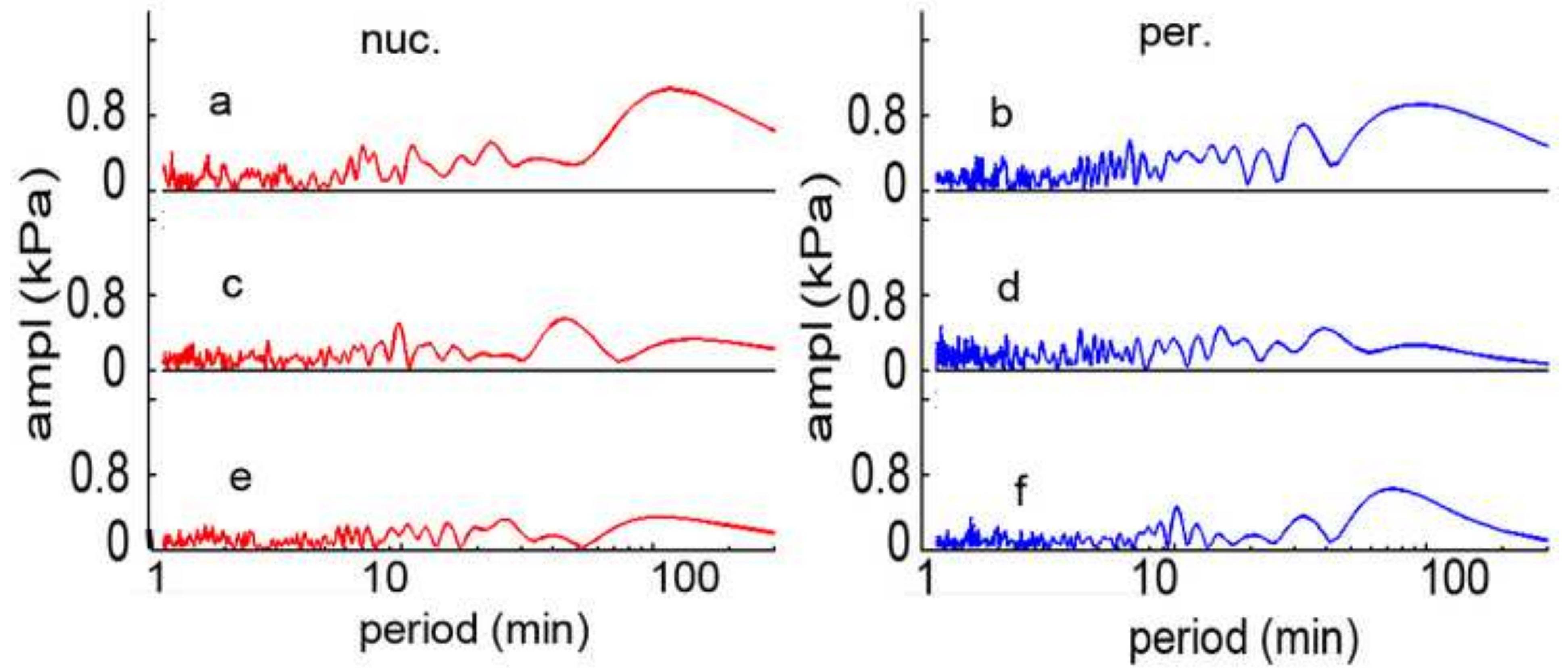


Images of figure 6

Click here to download Images: Fig6.tif

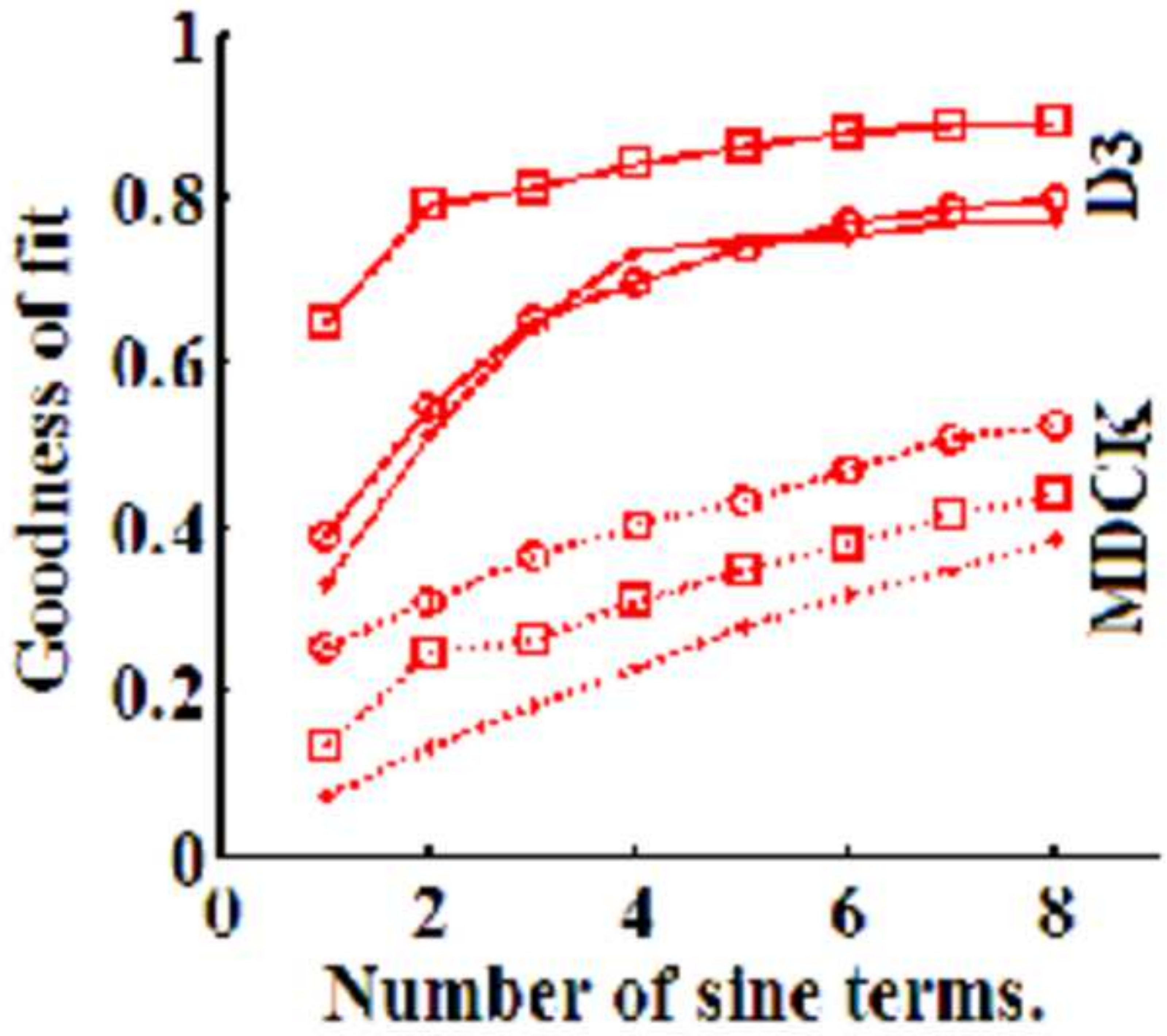


Images of figure 7
Click here to download Images: Fig7.tif



Images of figure 8

Click here to download Images: Fig8.tif



Images of figure 9

Click here to download Images: Fig9.tif