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
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Funding Information:	National Science Fund of Hungary (OTKA K K81180) — Dr György Váró
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Elasto-mechanical properties of living cells

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

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

Data Analysis

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

Results

High resolution topographies were made on living cells grown in a Petri dish and in each case three cells were chosen with proper shape. On each cell two different locations were selected, one over the nucleus, the other at the cell periphery (figure 1). At these selected six points elasticity measurements were effectuated cyclically. Duration of one cycle was about 30s. The whole experiment of 60 to 80 minutes, resulting 120-150 measurement at each point. During the experiment in each selected place a classical force curves were taken (figure 2) and the elasticity of the measured point was calculated. In this way the time dependence of the cell elasticity in the selected points could be followed (figure 3) simultaneously.

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

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

Browsing through the elasticity series three different kind could be distinguished based on their oscillating amplitude: large amplitude (figures 5 a, b, d,) small amplitude (figures 5 e, f) and transitional traces (figure 5c). The FFT signal contains several sinusoidal components with well determined time period. In order to estimate how many oscillating components describe the time dependent elasticity traces, a multi sinusoidal fit was applied ranging from one to ten components. Similar set of experiment was measured on MDCK epithelial cells and the data treated in a similar mode. The tendency of the elasticity signal was similar to that measured on D3 cells, but the noise was commensurable with the signal (figure 6 dots). The data analysis yielded apparently faster FFT components (figure 7), with an almost constant amplitude for large time intervals.

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

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

Discussion

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

The amplitude of the fluctuation should be related to the activity of the studied part of the cell. If the measured point is close to an active part its elasticity is varying due to molecular structural changes either in the cytoskeleton or in the organelle in the cytosol or in the glycocalyx. This elasticity change produces a “pressure shock” which propagates in the cytoplasm and in the extracellular media, producing a signal for the neighboring active part. The signal can influence an active part positively by more activation or negatively, by inhibiting it, depending in the earlier state and other signal arriving in the same time.

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

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

References

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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.

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

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

Figures

Figure 1.The image of the endothelial D3 cells (panel A) before and (panel B) after the force measurements. The dots with letters (a-b-c-d-e-f) show the locations where the forces were measured cyclically .

Figure 2. The force signal approaching trace (blue) and retrace (red) curves. For elasticity calculation the trace is used.

Figure 3. The time dependency of the D3 cell elasticity measured over the nucleus (a, c, e) and at the periphery (b, d, f). The curve gel is the control measured on acrilamide gel. The sequence of the measurement is similar to that measured on cells but only noise could be detected

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

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

Figure 6. The time dependency of the epithelial MDCK cells elasticity. The measuring and calculation protocol was similar as used for the D3 cells. The dots are the measured signal while the continuous line is the fit with 8 sinus curve.

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

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

Figure 9 The correlation coefficient calculated for the elasticity of the D3 and MDCK cells.

Images of figure 1

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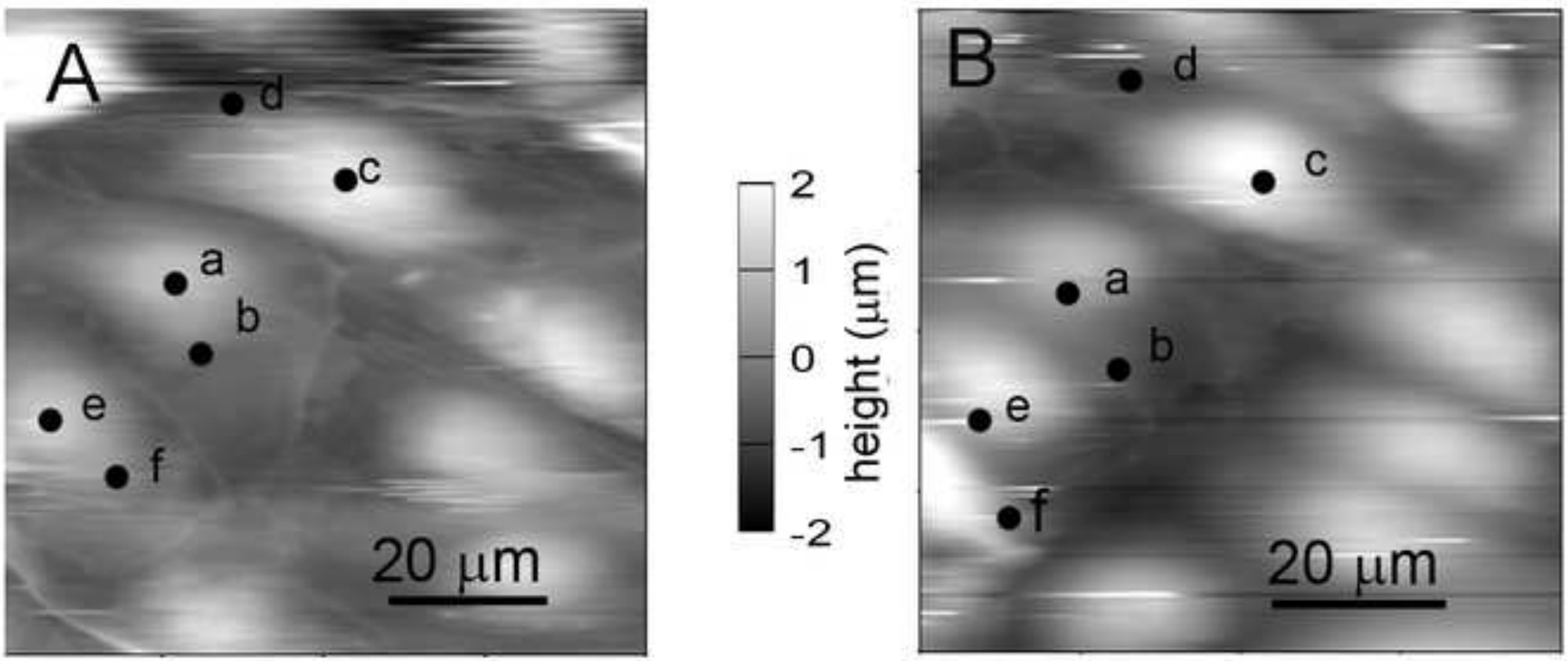

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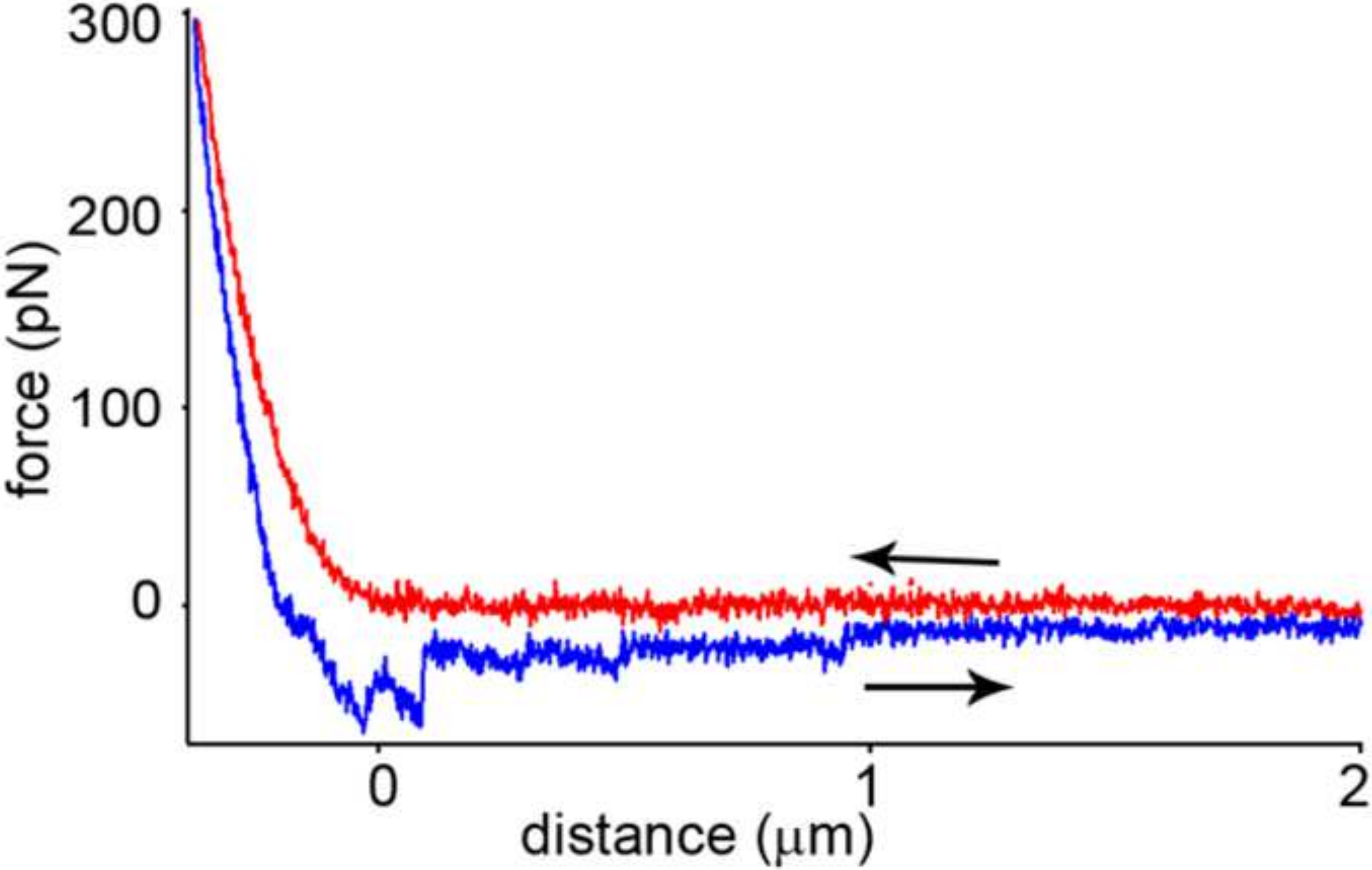

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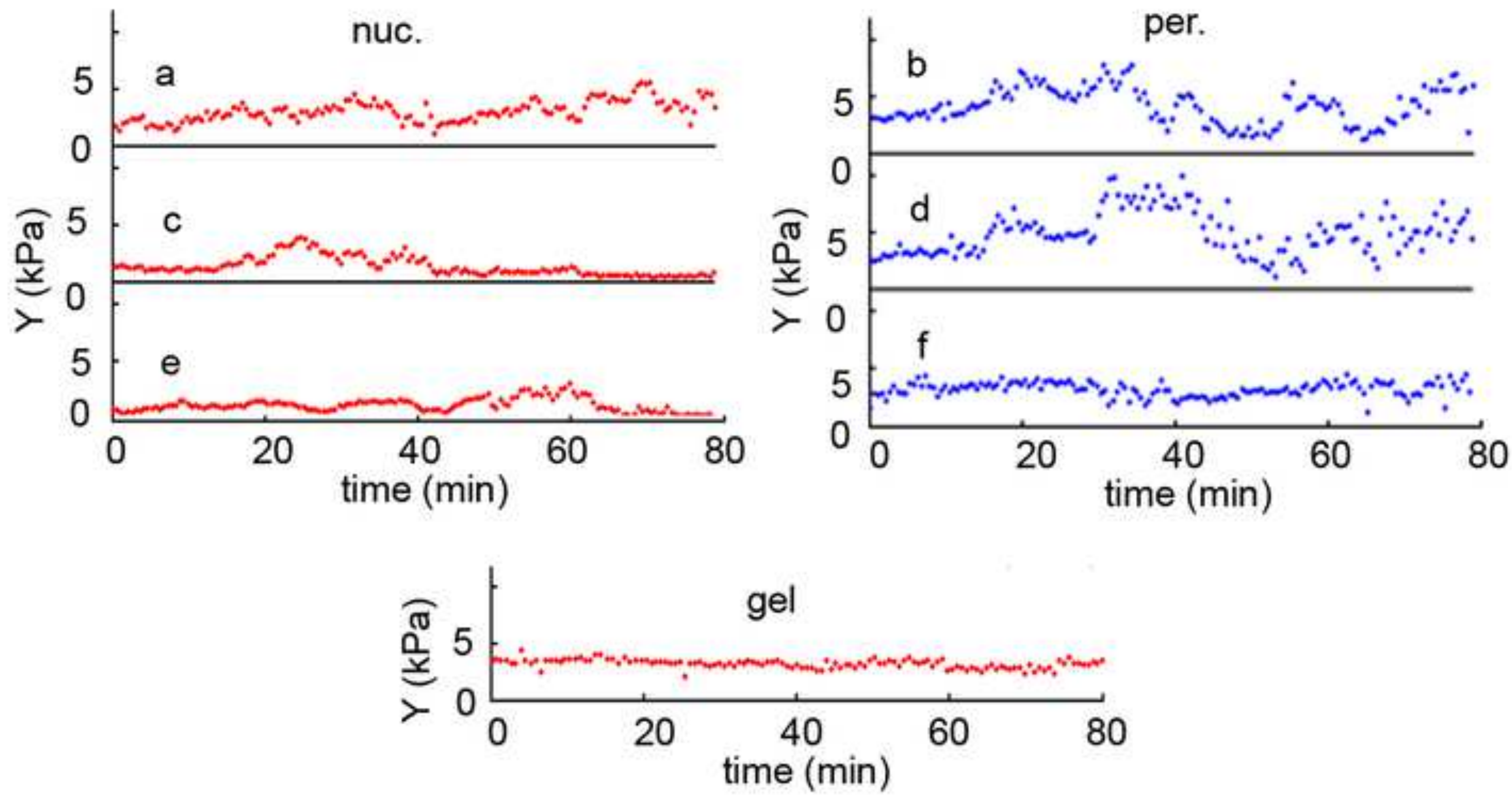

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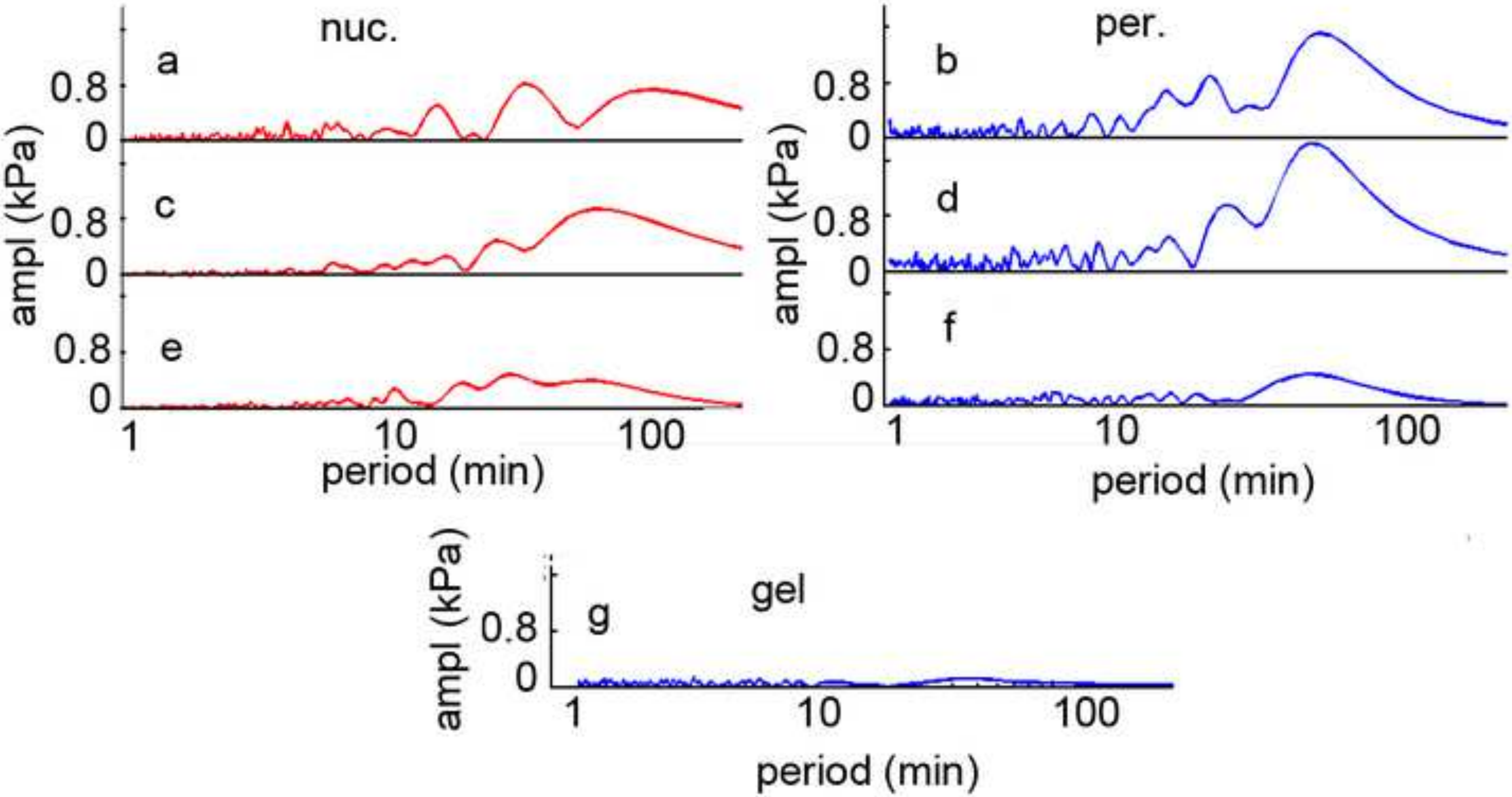

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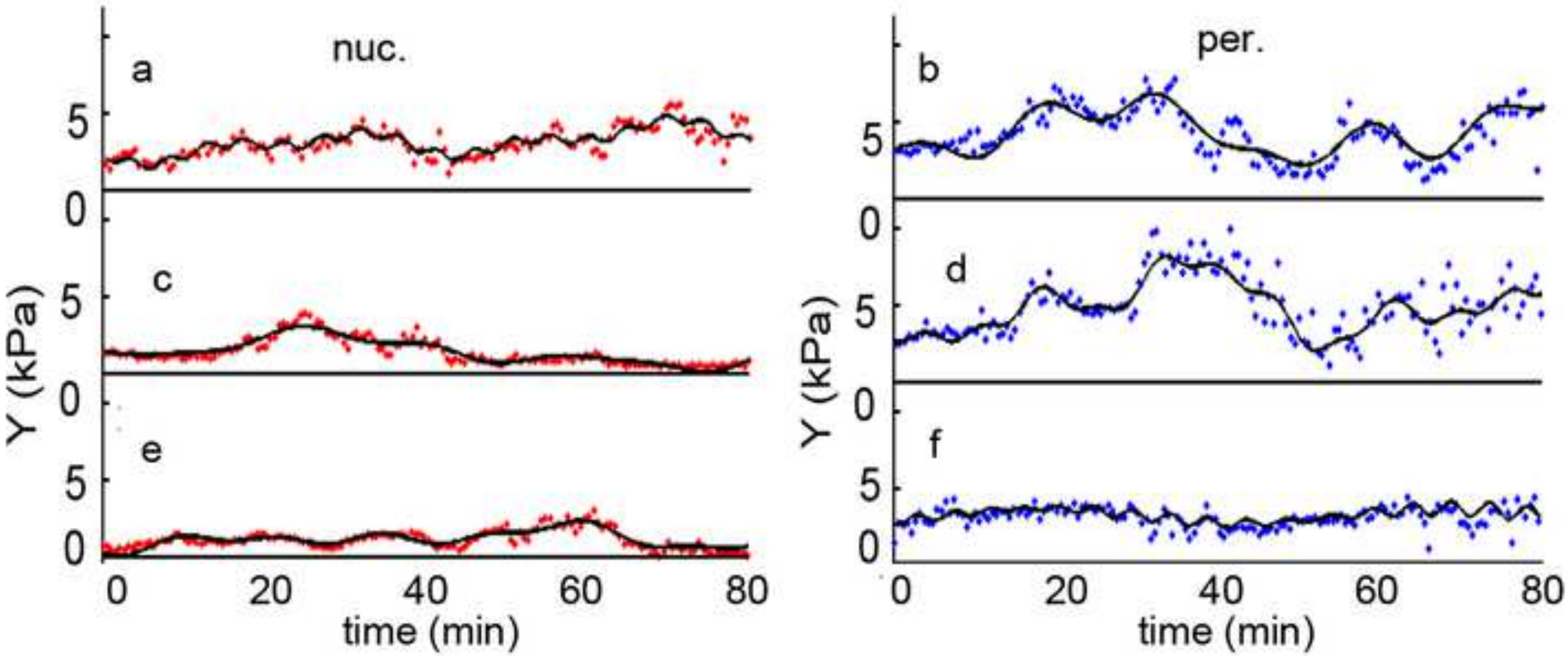

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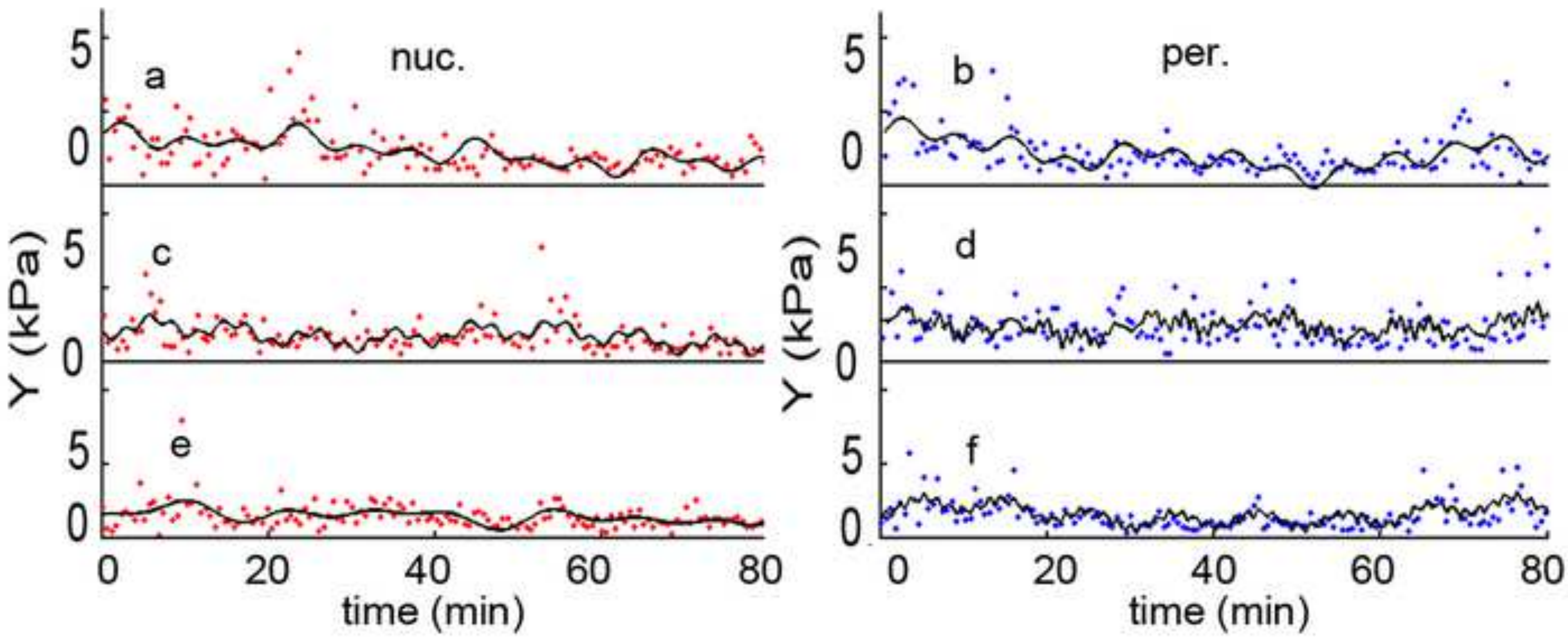

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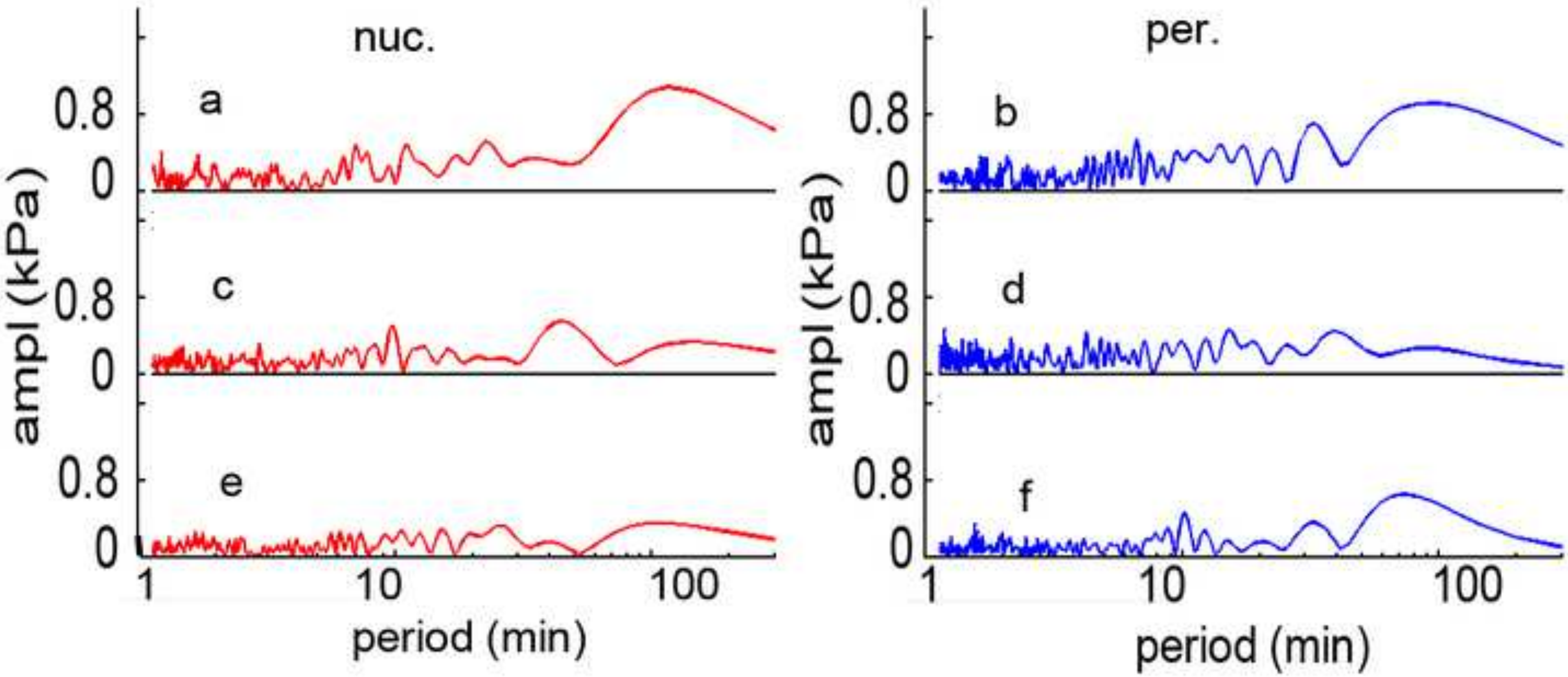

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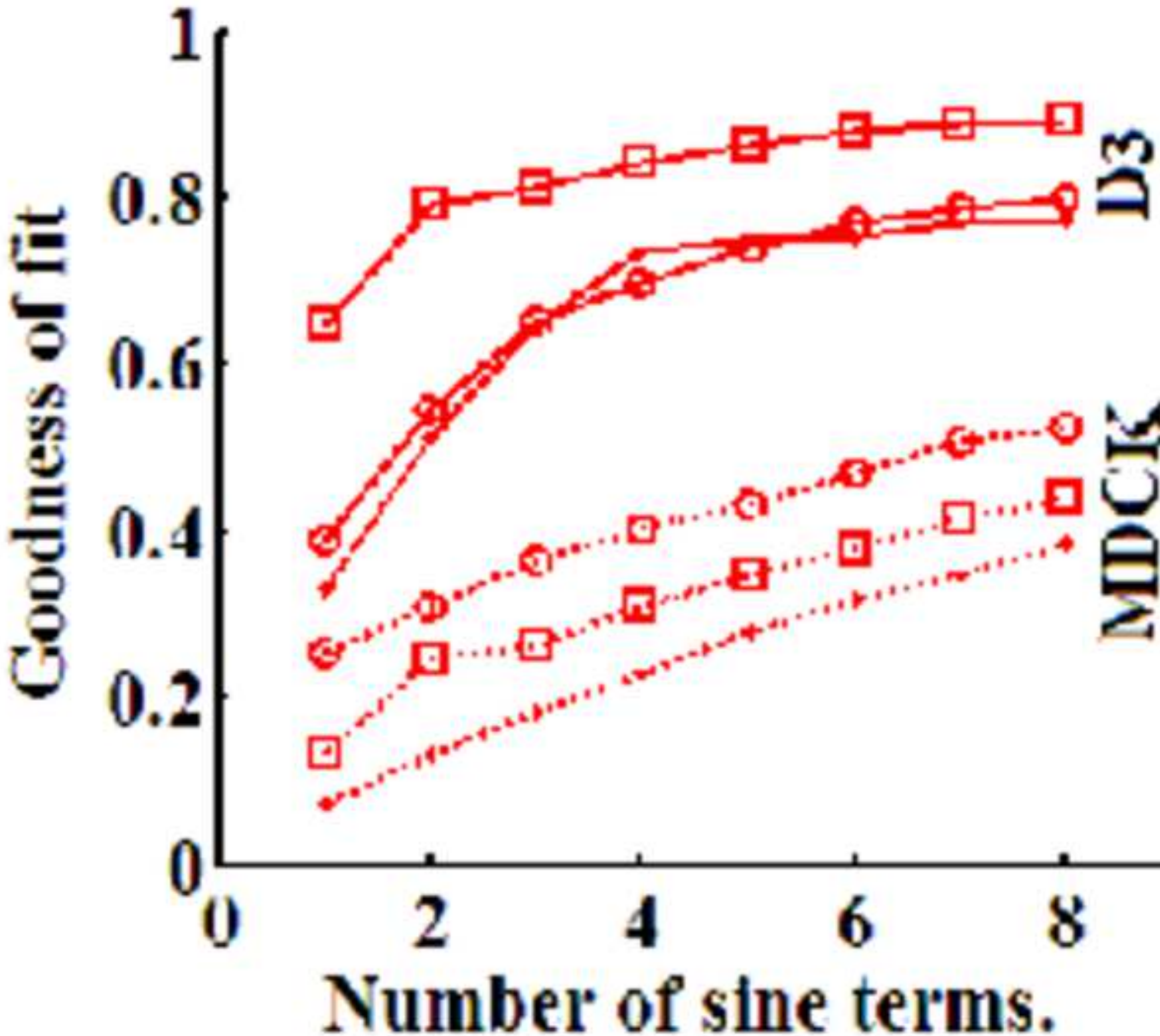

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