Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Water-mediated influence of a crowded environment on internal vibrations of a protein molecule

Anna Kuffel and Jan Zielkiewicz*

Faculty of Chemistry, Gdansk University of Technology

Narutowicza 11/12, 80–233 Gdansk, Poland

e-mail: anna.kuffel@pg.gda.pl (A.K.), jaz@chem.pg.gda.pl (J.Z.)

Details of the simulation procedure

The summed length of the analyzed trajectory was equal to 48 ns (PCA analysis) or 96 ns (cross-correlation function of the collective velocities of the surface protein atoms). However, the trajectory was not collected in one simulation, but in many parallel simulations. The starting conformation was simulated for 4 or 8 ns in NPT conditions, with the backbone atoms of the proteins restrained to prevent the change of the distance between the protein molecules. Every 200 ps, new atom velocities were generated from the Maxwell distribution. Each conformation with randomized atom velocities was a starting point for another simulation. The procedure was repeated for these 20 or 40 simulations – each one of them was a starting point to 20 simulations. This way, we obtained starting conformations for 400 or 800 independent simulation runs in NVE conditions (without any restrains). The simulations were 138 ps long, but the length of the analyzed trajectory was equal to 120 ps – the first 18 ps were treated as an equilibration period and were excluded from the analysis. For each 120-ps-long trajectory, the values of the parameters describing the properties of water and the dynamics of the protein were calculated. The results presented in the article are means obtained from all of these trajectories.

Autocorrelation function of the dipole moment of the protein.

The autocorrelation function of the dipole moment of the protein was calculated. The dipole moment was calculated according to the following equation:

$$\mathbf{M} = \sum_i \mathbf{r}_i q_i$$

where $\mathbf{r}_i$ is the location of charge q_i. This vector is independent of the choice of the reference point used to obtain $\mathbf{r}_i$ only if the sum of all q_i is zero. This is not the case of *Cf*AFP molecule. The overall charge is equal to $+2e$; hence the presence of the chloride counterions.

To overcome this problem, the dipole moment was calculated in the internal coordinate systems of the protein. The beginning of the coordinate system was in the center of mass of the protein. The direction of the axes were the same as the direction of the main moments of inertia. The choice of this coordinate system allowed us to ascribe the changes of the dipole moment to the conformational changes of the molecule. Therefore, it can be analyzed as complementary to the principal components analysis. We calculated the mean dipole moment and analyzed its changes. The equation is as follows:

$$C_M(t) = \frac{\left\langle \left(\mathbf{M}(0) - \overline{\mathbf{M}}\right) \cdot \left(\mathbf{M}(t) - \overline{\mathbf{M}}\right) \right\rangle}{\left\langle \left(\mathbf{M}(0) - \overline{\mathbf{M}}\right) \cdot \left(\mathbf{M}(0) - \overline{\mathbf{M}}\right) \right\rangle}$$

The autocorrelation function of the differential vector (momentary minus mean) is presented in Figure S1.

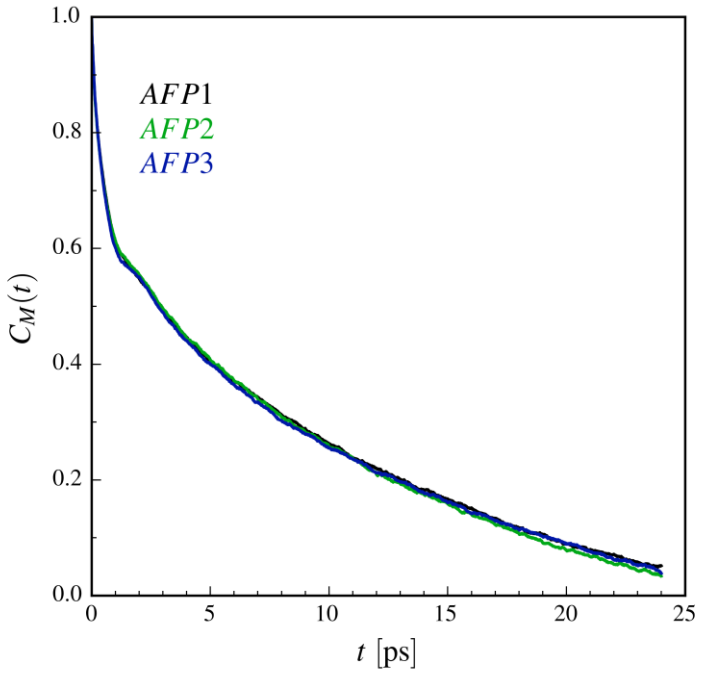

Figure S1. The autocorrelation function of the differential vector of dipole moment (momentary minus mean).

After the Fourier analysis, the amplitude spectrum can be obtained. It characterizes the inner dynamics of the protein as a function of frequency. The spectra for the single AFP molecule (AFP1) and molecules with one (AFP2) and two neighbors (AFP3) were compared. The differential spectra are shown below.

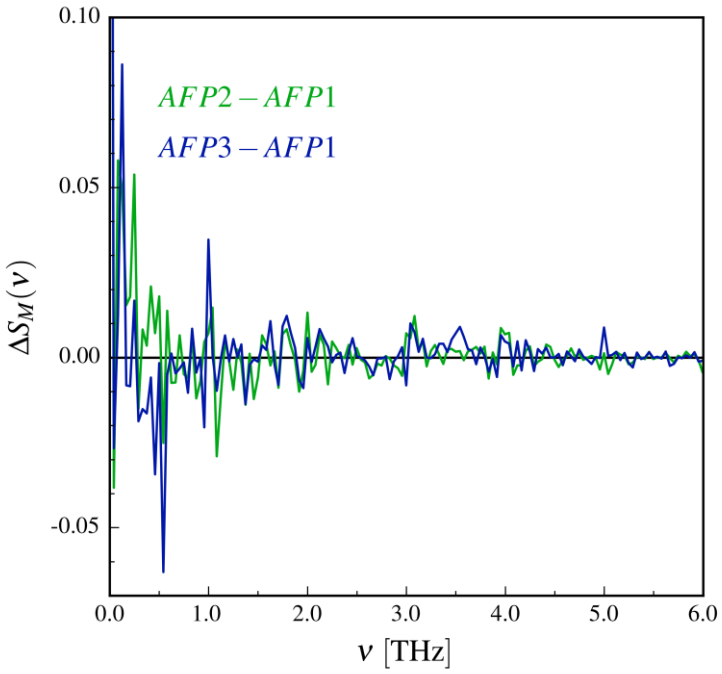

Figure S2. Differential power spectrum of the differential dipole moment. The values $\Delta S_M(\nu)$ are equal to $S_M(\nu)_{AFPX} - S_M(\nu)_{AFP1}$, where X is 2 or 3.

Although the differential spectrum is noisy, the most prominent changes can be seen for low frequencies. This frequency range corresponds to the frequency range of the biggest changes of frequencies estimated after the principal components analysis. We believe that this supports the conclusions drawn from the eigenvalues.

The changes of the amplitudes – influence of the scaling.

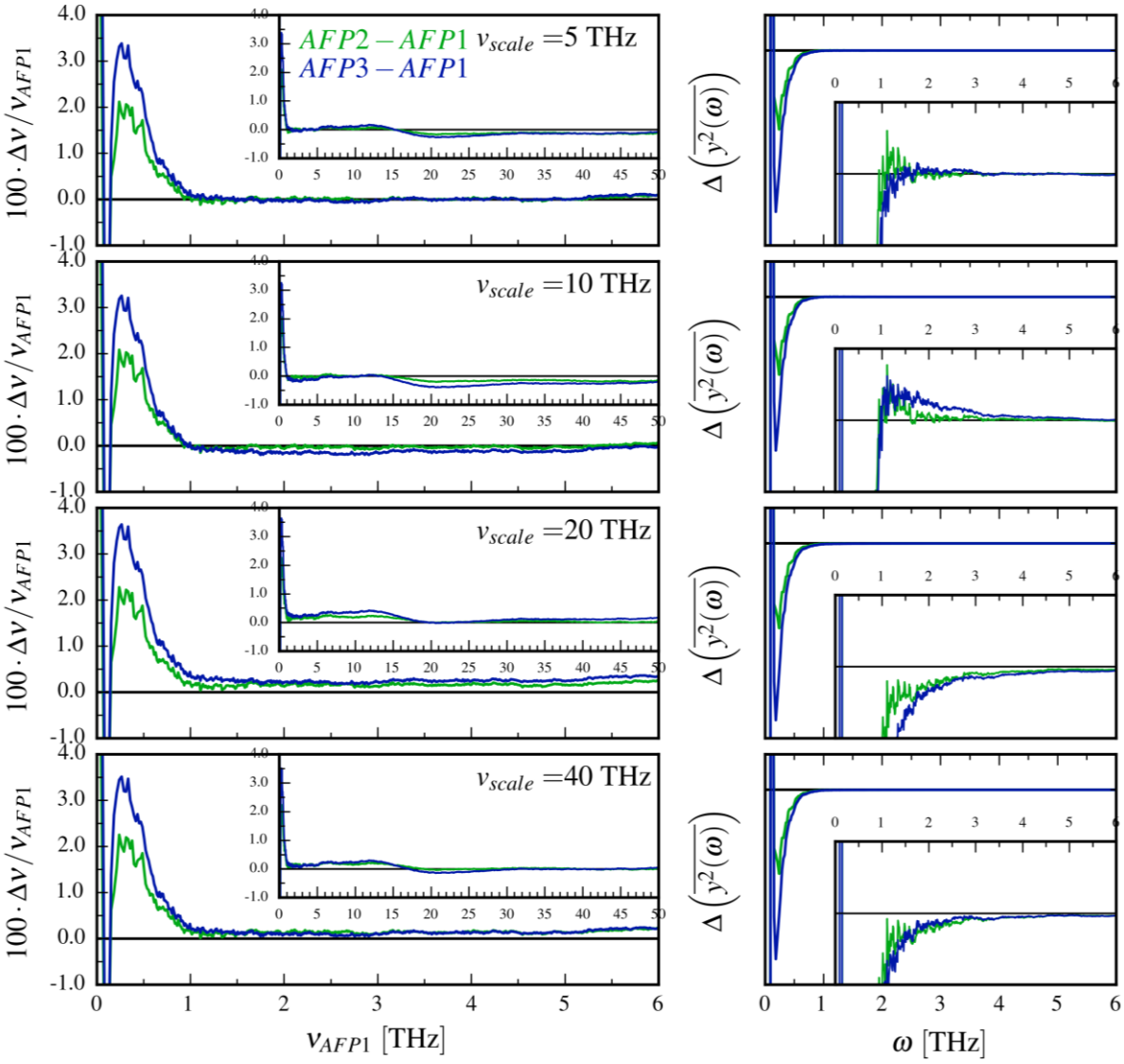

Figure S3. Left: The relative change of the frequencies of the normal modes of the AFP molecule in the presence of one and two neighboring protein molecules. The relative change is plotted as a function of the frequencies of the protein without any neighbors (AFP1). The change $\Delta\nu$ is equal to $\nu_{AFPX} - \nu_{AFP1}$, where X=2 or X=3. The plots are shifted close to zero for high frequencies by scaling the corresponding eigenvalues. The relative changes are not strictly constant for high frequencies, therefore we obtain slightly different results when we shift the plots to be equal to zero for different reference frequencies (as described by the values ν_{scale}).

Right: The comparison of differences of amplitudes $\Delta\left(\overline{y^2(\omega)}\right)$ obtained from the simulations after the scaling of the eigenvalues. The insets display the enlarged fragments of the plots. The ranges of the axes of these plots are the same here.

The changes of the amplitudes – influence of the friction coefficient.

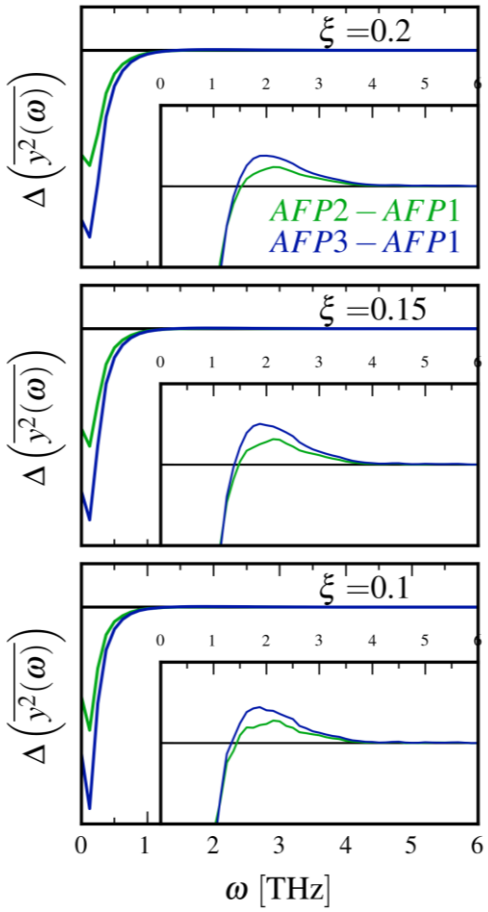

Figure S4. The comparison of differences of amplitudes $\Delta\left(\overline{y^2(\omega)}\right)$ calculated from the equation (7) in the main text. The insets display the enlarged fragments of the plots. The units of $\Delta\left(\overline{y^2(\omega)}\right)$ are arbitrary, therefore the values on the axes are not displayed. Different friction coefficients are denoted by ξ. The plot for ξ=0.05 is in the main text.

The influence of the stiffness of the protein

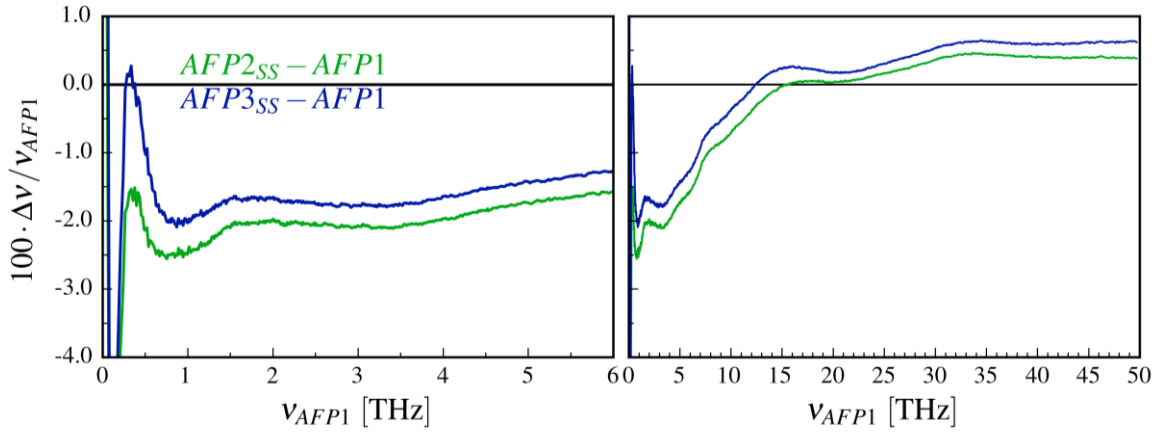

Figure S5. The relative change of the frequencies of the normal modes of the AFP molecule in the presence of one and two neighboring protein molecules. The single molecule (AFP1) was in its native form. In case of the systems containing two ($AFP2_{SS}$) and three ($AFP3_{SS}$) protein molecules, the disulfide bonds were artificially removed. The relative change is plotted as a function of the frequencies of the protein without any neighbors (AFP1). The change $\Delta\nu$ is equal to $\nu_{AFPX} - \nu_{AFP1}$, where X=2_{SS} or X=3_{SS}.