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Activation of tubulin assembly into microtubules upon a series of repeated femtosecond laser impulses

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Tubulin, a globular protein, mostly distributed in nature in the dimeric α , β form, can polymerize *in vivo* and *in vitro* into microtubules—longitudinal dynamic assemblies, involved in numerous cellular functions, including cell division and signaling. Tubulin polymerization starts upon binding Mg^{2+} with the tubulin guanosine triphosphate (GTP) site. In the current study we show that a series of repeated femtosecond laser impulses activate the same site without adding Mg^{2+} . GTP site activation (without GTP no polymerization occurs) produces hydrated electrons (they are detected by the UV spectra), which are trapped in the shell of biological water, surrounding the tubulin. These electrons generate an additional, nonlinear by nature, polarization effect, responsible for the second harmonic generation at $\lambda=365$ nm (the first harmonic is centered at $\lambda=730$ nm) and manyfold increase in strength of the initial electric field. The results are supported by model calculations, based on the assumption of positive (negative) feedback, appearing on interaction of charge transfer exciton dipoles with the applied electromagnetic field. © 2004 American Institute of Physics. [DOI: 10.1063/1.1814056]

I. INTRODUCTION

Tubulin^{1–5} is a ubiquitous cytoskeletal protein, composed mainly of α (450 amino acid residues) and β (445 amino acid residues) subunits. Each subunit assumes a shape of a compact ellipsoid, measuring, respectively, about 46, 40, and 65 Å in width, height, and depth. The subunits form $\alpha\beta$ heterodimers of dimensions $46 \times 80 \times 65$ Å³. Of crucial biological importance is the capacity of tubulin heterodimers to self-assemble into MTs (microtubules)—hollow cylinder-shaped structures with a length, varying in the μ range, and outer and inner diameters about 25 and 12 nm, respectively.^{4–6} α and β tubulins, as well as minor isoforms (γ , δ , ϵ , ζ , η tubulins), are characterized by about 40% homology of their amino acid sequences. Crystallography analysis of $\alpha\beta$ heterodimers revealed almost identical three-dimensional (3D) structure of α and β tubulins.^{2–4} Three domains are clearly identified in the refined structure of $\alpha\beta$ -tubulin at 3.5 Å resolution. The shortest and least conservative one, containing the C-terminal fragment, participates neither in GTP (guanosine triphosphate) binding nor in formation of intermonomeric contacts. The longest domain, containing the N-terminal part, has a structure, resembling classical GTPases. This domain is responsible for GTP binding. The central domain is involved in formation of intermonomeric contacts and hydrolytic reaction. It also has a site for binding taxol—an agent stabilizing individual MTs. Despite the observed similarity in the spatial structure and high homology of the amino acid sequences, the two GTP-binding

sites of the heterodimer, referred to as *E* site (exchangeable) and *N* site (nonexchangeable) in α and β tubulin subunits, respectively, essentially differ in their behavior.^{1–5} The *N* site (α subunit) tightly binds GTP, whereas the *E* site allows free exchange with GTP in solution. On the basis of the 3D model it was suggested⁷ that position 254 in *E* site is occupied by lysine and in *N* site by glutamate, involved in catalyzing GTP hydrolysis in the *E* site. Binding of Mg^{2+} GTP complex in the *E* site and its subsequent hydrolysis, releasing GDP, is connected with formation of MTs. Intrinsic electrostatic properties of tubulin heterodimers were shown to have relatedness to assembly/disassembly of MT, information processing and signaling.^{6,8} Most physiologically important functions of $\alpha\beta$ tubulin (maintaining cell shape; transport of cellular organelles, secretory vesicles, and neurotransmitters; mitotic and meiotic division; etc.) largely depend on its Mg^{2+} GTP-supported capacity to polymerize, yielding MT-based dynamic self-organized multichannel network for signal transmission and reception.^{2,5,8–10} A breach in the complex mechanism of MT functioning, which apart from tubulin, includes motor proteins (dynein and kinesin) and MAPs (microtubule associated proteins, interconnecting microtubules), may cause drastic events in the living cell and provoke different health disorders, e.g., Alzheimer disease. Recently, we showed that cytostatic effect of platinum complexes, used in antitumor therapy, can be realized not only via producing intrastrand N7(G)-Pt-N7(G) kink structure in DNA, as generally accepted,^{11,12} but possibly also on the level of MT formation, via interaction with GTP.¹³ These observations point to the biomedical impact of studies on the mechanism of MT functioning. Besides that, neuronal and brain tissue,

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FIG. 1. Structure of the $[\text{Mg}^{2+}(\text{H}_2\text{O})_4\text{GTP}]$ complex within the tubulin GTP site. Gua is guanosine ring.

involved in the processes of learning and memory, is particularly rich in tubulin, which forms there a dense and highly active MT system. This fact accounts for a temptation to compare the MT system with a giant molecular computer, consisting of quantum and classical parts.^{14–16} Despite numerous data,^{4–6,17–19} regarding the process of tubulin self-assembly *in vivo* and *in vitro*, physical mechanisms, lying in its basis, evoke many questions. The role of Mg^{2+} , initiating the process, remains elusive. It is known^{5,17,20} that, as a natural cofactor of this process, Mg^{2+} cannot be efficiently replaced by other cations (Ca^{2+} or Zn^{2+}). Thus, Zn^{2+} is known²¹ to promote formation of antiparallel sheets of tubulin, thereby suppressing normal self-assembly of MTs. An important prerequisite of this study is availability of *in vitro* system of tubulin assembly.

Mammalian α , β tubulins assemble *in vitro* into MTs under definite conditions: 37 °C, presence of GTP buffer, and excess of Mg^{2+} salt.²⁰ It is assumed that GTP, when bound, structurally affects tubulin, thus “preparing” it to further assembly,²² but no assembly occurs. To start the assembly, some external stimulus is required. It may come from Mg^{2+} , which chelates GTP terminal oxygens, O^γ and O^β , Fig. 1. When chelated, Mg^{2+} GTP complex, together with a series of closely linked aromatic amino acid residues (tyrosine, tryptophanes, and others), winding the tubulin,²³ initiates electron current. The current is a response to nonequilibrium redistribution of electrons between two electron zones [I(occupied) and II(conductivity)], initially existed in the tubulin-water complex.²⁴ According to recently conducted quantum chemical/molecular mechanics (QM/MM) computations (quantum water subsystem includes 317 water molecules),²⁵ the energy gap between I and II zones is about 1.20 kcal/mol; so electrons, upon the action of magnesium, can easily cross the gap (the Fermi zone), thus providing the filling of the conductivity zone II and producing nonequilibrium electron concentration between the zones.²⁶ The effect is similar to that we observe in nonlinear optics upon laser excitation of electrons in atomic and molecular systems.²⁷ The current study aims at proving the idea that laser excitation of the tubulin-water system (more specifically, GTP site, embedded into the β -tubulin subunit, surrounded by water shell) can initiate tubulin assembly without the magnesium cofactor. If the laser effect on tubulin assembly is identical to that of

FIG. 2. Experimental setup for femtosecond pump-probe laser spectroscopy; FRPP, frequency resolved pump-probe signals; PP, pump-probe signal, produced by amplification with the subsequent integration; 2PE, two-photon echo signal.

Mg^{2+} , the role of the magnesium cofactor as an initiator of electron redistribution and current in GTP-bound tubulin will be experimentally confirmed. Moreover, variation in intensity and duration of laser impulse exposure on tubulin can open the way to controlled assembly. The experiments are carried out *in vitro* with femtosecond (FMS) laser technique.

II. EXPERIMENT

Tubulin was extracted from calf brain, purified, and contained in a special freezing camera at -70°C , according to the earlier proposed protocol.²⁸ Physiological solution (without Mg^{2+} salt) of nonpolymerized tubulin, $5 \times 10^{-6} \text{ mol l}^{-1}$, which contained 5-fold excess of GTP buffer ($\text{pH} = 5.65$), was prepared by step-by-step heating of the initially frozen sample to 37°C and was kept at this temperature (without Mg^{2+} salt or other external stimulus, assembly does not occur) in a special thermostatic flask in equilibrium with the preliminary added taxol, $5 \times 10^{-4} \text{ mol l}^{-1}$. Taxol is used *in vitro* experiments as MTs stabilizer and it does not affect the rate and nature of tubulin assembly.²⁹ Before use, the solution was transferred through the embedded in the bottom of the flask injector (Fujika 1-97) into a thin thermostatic $[37 \pm 0.1^\circ\text{C}]$ quartz cell $[25 (\text{height}) \times 10^2 \times 10^2 \mu\text{m}]$, exposed to laser irradiation. Pump-probe laser impulses were generated according to the earlier developed method,³⁰ experimental setup is shown in Fig. 2. Pumping was performed with Nd:YAG (YAG—yttrium aluminum garnet) laser with a tuning frequency of 2 kHz. Each UV impulse had the energy of $5 \mu\text{J}$ and was 50–70 ns long. Impulses, synchronously generated with the Ti-sapphire self-adjusting laser ($\lambda \leq 800 \text{ nm}$), were split into beams of equal duration (15 fs) and equal energy (8 nJ); before mixing with the UV beam, impulses were delayed, according to the previously described method.³¹ The resultant signals were collected with the SC-600 focusing collector, built in the experimental setup, computer processed, and then displayed on the UV-2002 device, allowing collection of signal responses over UV—near IR region. The total exposure ($t = 50\text{--}70 \text{ ns}$) of the tubulin solution to laser beams was divided into a series of repeated in time impacts, separated by 200 fs each, Fig. 3. Tubulin assembly into MTs was evaluated from the scattering/absorption band at $\lambda = 358 \text{ nm}$ (Ref. 32) (UV-2002 device), which was previously resolved (Fumili-01 software³⁰) into two bands, $\lambda = 354 \text{ nm}$ (scattering part of the band, whose intensity is strongly correlated with the length of appearing

FIG. 3. Frequency dependent intensities (integral values, obtained from computer processing of the results of 25 independent measurements) in pump-probe laser experiments with tubulin samples in the femtosecond region of time.

MTs) and 365 nm (absorption part of the band),³⁰ of a log-normal circuit.³³ Along with the spectroscopic studies, the MT growth was monitored microscopically with JEOL-2000 transmission electron microscope. The electron microscopy images were obtained from the computer processing of the results of 25 independent tests (each lasting 20 s, beginning with the start of laser irradiation, $t=0$), using the Microcom-02 software, installed into the built-in microscope processor.

III. RESULTS AND DISCUSSION

Figure 3 shows the frequency signals (790–710 nm), obtained from computer processing of signals, generated in response to irradiation of 25 independent samples, contained tubulin, of identical composition and properties (see Sec. II) by laser impulses in the pump-probe experiments. The root-mean-square deviation does not exceed 3 nm. The observed signals oscillate around the $(n \times 500 \text{ fs}, n \geq 1)$ -delay points between pumping and probing. These oscillations are known in ultrafast laser routines as coherent artifact.³⁴ On a single exposure of the tubulin solution, the recorded signal intensity in 790–740 nm region, counted from the zero-delay point, almost completely vanishes in 140–170 fs. At 730 nm we observe a slight increase in the intensity, and in 720–710 nm region the intensity remains quasiconstant. Periodically repeated $(n \times 500 \text{ fs}, n \geq 1)$ -laser impulses leave the picture practically unchangeable in the 790–740 and 720–710 nm regions. This is not the case for the absorbance bands at 730 and 365 nm, which reveal increase in intensity over 0–50 ns, Fig. 4. On exposure of samples to laser impulses for 50–70 ns, tubulin begins to assemble into MTs. This is unambiguously indicated by the increase in intensity at $\lambda=354 \text{ nm}$ and by the emergence of long MT filaments in electron microscopy images, Fig. 5. The absorption intensity at 730 and 365 nm over 70 ns–20 s period remains at the same level as it was at $t=70 \text{ ns}$. Exposure of tubulin samples, prepared according to the previously mentioned routine (see Sec. II), but

FIG. 4. Increase in the total absorbance A (basic line absorbance excluded) of tubulin sample with time at $\lambda=730 \text{ nm}$ (the first harmonic) and $\lambda=365 \text{ nm}$ (the second harmonic). Sample exposure to laser impacts above 50–70 ns has no remarkable effect on A .

without GTP buffer, has no effect on tubulin assembly: the intensity at $\lambda=354 \text{ nm}$ is equal to zero and no filaments are indicated with electron microscopy.

The obtained results are of particular interest to understand the nature of tubulin, preceding its assembly. The absorption in the 790–710 nm region (Fig. 3) indicates the appearance of hydrated electrons;^{31,35–37} the fact that this absorption is stipulated by the hydrated electrons and not other particles or molecular groups is supported by the previous study³⁰ in which the tubulin assembly was initiated by the electron beam, directed on the tubulin solution, not containing GTP buffer and Mg^{2+} salt. The femtosecond spectrum, under the discussion, and that, detected from the initially irradiated with electrons tubulin solution, are virtually identical in the 790–710 and 365 nm regions. According to the newest data, the lifetime of the “freshly prepared” electron (zero point) in pure water varies; the hydration appears in 150–200 fs,^{31,35,38} followed by its destruction or stabilization, depending on water composition and external parameters (ionic strength, pH , temperature, etc.). Hydration, in turn, does not mean that the equilibrium between the electron and its water shell is immediately reached.^{39,40} Generally, it is a wide spectrum (1300–705 nm) of states, which could be assigned to absorption of electron in its “water coat.” Mostly, these states are nonequilibrium;⁴¹ the equilibrium, if

FIG. 5. Electron microscopy image of *in vitro* assembled MTs upon laser irradiation for 50 ns.

any, reaches in 450–500 fs and corresponds to absorption in 720–710 nm (pure water). Other states, where absorption appears over 720 nm, are considered as nonequilibrium.³⁹ In our case, the electron absorption (730 nm) is close to that, which conventionally assigned to the equilibrium state. Seemingly, a small redshift in absorption, compared with that at 710 nm (pure water), is a result of a slight perturbation, the hydrated electrons experience in biological water,^{42–44} a shell of three to five layers of water molecules, closely attached to the protein, in our case tubulin, surface.

The appearance of hydrated electrons, which do not vanish in time, but, on the contrary, show the tendency to be accumulated at 730 and 365 nm upon periodically sent laser impulses, is due to a specific character of tubulin-water interactions. According to our previous QM/MM computations,²⁵ slow excitations in tubulin-water system favors transfer of high-energy electrons, initially located in specific “quantum dots” (each dot is considered as a heavy positively charged center with a number of electrons, trapped by it), spreading over the tubulin surface, into the conductivity zone II (see Sec. I). This zone, mostly formed by the electronic states of biological water molecules^{25,26} (the motion of these water molecules is highly restricted to strong interactions with tubulin heterogeneous surface), traps electrons, thus providing their localization within the biological water.⁴⁵ In our case, in the absence of Mg^{2+} , excitation, resulting in the appearance of hydrated electrons, evidently comes from short-run laser impulses. One can assume that these repeated in time impulses, by analogy with action of Mg^{2+} , affect GTP center of tubulin and produce a nonequilibrium state of the tubulin-water complex, accompanied by initiating nuclear dynamics and coincident electron transfer into the conductivity zone II. Special attention must be given to the fact that the absorption intensity at 730 and 365 nm over 50–80 ns remains practically unchangeable, and it is still true up to $t=20$ s, the time indicating the end of tubulin assembly.²⁰ This observation is quite explicable, if assumed that repeated laser excitations, resulting in electron transfer, lead to saturation of the biological water with electrons. Evidently, this saturation is maintained over the tubulin assembly and in the appeared MTs, whose solution often displays for a long time a “blue lighting,”¹⁴ a characteristic feature of most long-trapped solvated electrons.^{14,45–47}

The most intriguing finding, we observe in our experiments, is the second harmonic generation (SHG) at 365 nm; the first harmonic is centered at 730 nm. SHG is strongly coupled with nonlinear polarization effect in medium.^{27,48} In our case, polarization effect can be interpreted in terms of a semiclassical model. By analogy with a semiconductor theory, high-energy electrons, initially located in quantum dots (see above), after excitation transfer into the zone II, thus producing specific dipoles, “hydrated electron-parent dot,” which in further discussion are named charge transfer excitons (CTE). The concentration of CTEs is directly proportional to the number transferred into biological water electrons and seems to be remarkable, according to Fig. 4.

The occurrence of SHG at 365 nm suggests nonlinear effects of the medium on tubulin. With this assumption, on the basis of semiclassical approach, one can show that even a

FIG. 6. Dependence of the resulting electric field $\tilde{E}$ on the value of the initially applied field $\tilde{E}_0$, according to numeric computations of the differential equation system, Eq. (A2), see the text.

very low intensity of the intrinsic electric field of tubulin may undergo a multifold increase. Earlier,⁸ the migration behavior of tubulin MTs connected with electric properties of tubulin was studied in the external electric field. Using video contrast microscopy to follow migration of single MTs in constant electric fields, the authors calculated the electrophoretic mobility of MTs and the average net charge of tubulin. In our case, we consider the electric field, generated around the tubulin molecule by tubulin itself. For the semiclassical analysis of such a system [see the Appendix for the master equation, Eq. (A4), its derivation and relevant notation], an arbitrary value of the field strength $E \cong 10^{-8} - 10^{-2}$ V/cm, corresponding to $(Mg_x-Ge_y-Si_z)$ fiber composite with a similar energy gap between zones I and II, was chosen.⁴⁹ This value has only an auxiliary meaning as a starting point for numeric calculations of nonlinear equations.⁵⁰

According to the performed calculations, Fig. 6, in some region, $\xi=0.5-2.0$ (see the Appendix), the value of E_0 , due to a nonlinear polarization effect, is increased by several times. Specifically, for $\tilde{E} \cong 1$, a true physical field corresponds to $E \cong 10^5$ V/cm. The intensity of the initial field strength in tubulin taken as a starting $E \cong 10^{-8} - 10^{-2}$ V/cm, the resulting field strength is enhanced by seven to eight orders. Considering that the strengthening polarization part, responsible for the nonlinear effect, is practically independent on the starting field E_0 , the role of the latter reduces to choosing the direction of polarization. In numeric computations A , Δ , σ_0 , γ_0 , χ , and R were treated as variables, with the initial values depending on the specificity of the system:^{25,49} $\Delta = 0.5 \times 10^{-1}$ eV, $A = 10-30$ nm, $\sigma_0 = 10^{-18}$ cm², $\gamma_0 = 10^9$ s⁻¹, $\chi = 5 \times 10^8$ cm⁻¹, $R = 1-3$ nm. Physically, the effect of many-fold enhancement of the initial electric field is of particular interest. After the strength of enhanced electric fields overcomes some critical barrier, electrostatic interaction between initially separated in volume tubulin subunits dominates and makes these subunits attract. Assumed that these enhanced fields are polarized around some nucleation center, the assembly into MTs occurs along the polarization axis.

IV. CONCLUDING REMARKS

The obtained results, with regard to our previous findings^{23,24} suggest that basically the same mechanism governs tubulin assembly into MTs, when two stimuli of different nature are used: Mg^{2+} GTP-complex (natural cofactor) or repeated laser impulses. Electrons play an essential role in initiating tubulin assembly into MTs. In the absence of GTP molecule, embedded into the tubulin matrix, tubulin assembly is forced by intense electron beam, directed on tubulin solution. In presence of GTP, assembly occurs upon repeated laser impulses without any preliminary electron beam. In both series of experiments the size, shape, and time of MTs assembly is identical. The same MTs (no electron beam and no laser impulses are used) appear upon binding Mg^{2+} with GTP molecule in the GTP site. Electrons, trapped in biological water, produce nonlinear polarization effect, responsible for appearing SHG at 365 nm and increasing strength of initial electric field by several orders around every tubulin subunit. These enhanced fields, whose strength reaches maximum in nanosecond interval of time since the start of impact, favors attraction of initially separated tubulin subunits and their assembly into MTs.

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APPENDIX: MASTER NONLINEAR EQUATION FOR THE ELECTRIC FIELD AROUND THE TUBULIN MOLECULE

Excitation cross section of CTE and the rate of recombination on a charged center (hydrated electron+parent dot), according to Ref. 23, can be expressed as follows:

$$\sigma(\omega) = \sigma_0 \exp\left(-\frac{(\omega^* - \epsilon - A)^2}{\Delta^2} - \chi R\right), \quad (\text{A1a})$$

$$\gamma = \gamma_0 \exp(-\chi R). \quad (\text{A1b})$$

Here ω^* is the frequency pumping ($\omega^* = \omega$, main frequency, or $\omega^* = 2\omega$, SH frequency); ϵ the excitation energy of CTE; A Stock's shift; Δ the energy gap between the occupied and free zones; R the distance of electron transfer. Mostly, electrons undergo transitions along and against the electromagnetic field polarization, and the probabilities of these transitions are, on average, equal. Interaction between the $\mathbf{E}$ and CTE dipoles $\boldsymbol{\mu}$ results in splitting ϵ , which appears as a two-level system $\epsilon \rightarrow \epsilon \pm \boldsymbol{\mu} \cdot \mathbf{E}$. The total polarization $\mathbf{P} = \sum \boldsymbol{\mu}$ is directed, by analogy with $\boldsymbol{\mu}$, against $\mathbf{E}$ and results in increasing the applied field, the effect is known as a positive feedback or negative permeability.^{51,52} Let ρ_+ and ρ_- be the probabilities of finding the initial center (hydrated electron+parent dot) in two states, with $\boldsymbol{\mu}$ along and against $\mathbf{E}$. Self-consistent field approximation accepted,³⁰ the rates for $\rho(+)$ and $\rho(-)$ appear as follows:

$$\begin{aligned} \dot{\rho}^*(+) &= I\sigma_0[1 - \rho(+)-\rho(-)] \\ &\times \exp\left(-\frac{(\omega^* - \epsilon(+)-A)^2}{\Delta^2} - \chi R\right) \\ &- \gamma_0 \exp(-\chi R)\rho(+), \end{aligned} \quad (\text{A2a})$$

$$\begin{aligned} \dot{\rho}^*(-) &= I\sigma_0[1 - \rho(+)-\rho(-)] \\ &\times \exp\left(-\frac{(\omega^* - \epsilon(-)-A)^2}{\Delta^2} - \chi R\right) \\ &- \gamma_0 \exp(-\chi R)\rho(-). \end{aligned} \quad (\text{A2b})$$

Here $\epsilon(+)=\epsilon+\boldsymbol{\mu}\cdot\mathbf{E}$; $\epsilon(-)=\epsilon-\boldsymbol{\mu}\cdot\mathbf{E}$; $\mathbf{E}=\mathbf{E}_0-4\pi q\mathbf{P}$; $\mathbf{P}=\alpha\mathbf{E}+n\langle\boldsymbol{\mu}\rangle$; $\langle\boldsymbol{\mu}\rangle=\boldsymbol{\mu}[\rho(-)-\rho(+)]$; I is the pumping intensity; and $\mathbf{E}$ is the macroscopic electric field, consisting of its initial value $\mathbf{E}_0$ and a polarization part $4\pi q\mathbf{P}$, which, in turn, includes a linear part (α indicates the medium permeability) and a nonlinear part $n\langle\boldsymbol{\mu}\rangle$, where n is a concentration of CTE; q is a geometry parameter, which is assumed to be $\sim 1/3$ for a sphere, mimicking, as a first approximation, the form of the hydrated shell of a globular protein, tubulin in our case. A stationary solution, $\dot{\rho}^*=0$, for Eq. (A2) now comes as follows:

$$\rho(+)=\frac{I\sigma_0}{\gamma_0}\exp\left(-\frac{(\omega^*-A-\epsilon-dE)^2}{\Delta^2}\right), \quad (\text{A3a})$$

$$\rho(-)=\frac{I\sigma_0}{\gamma_0}\exp\left(-\frac{(\omega^*-A-\epsilon+dE)^2}{\Delta^2}\right). \quad (\text{A3b})$$

Finally, the equation for $\mathbf{E}$ has a two-exponential form, Eq. (A4) including the positive and negative feedback terms:

$$\begin{aligned} E &= \frac{E_0}{1+4\pi q\alpha} + \frac{4\pi qnI\boldsymbol{\mu}\sigma_0}{(1+4\pi q\alpha)\gamma_0} \\ &\times \left[\exp\left(-\frac{(\omega^*-A-\epsilon-dE)^2}{\Delta^2}\right) \right. \\ &\left. - \exp\left(-\frac{(\omega^*-A-\epsilon+dE)^2}{\Delta^2}\right) \right]. \end{aligned} \quad (\text{A4})$$

Graphically, a solution for Eq. (A4) shows a considerable contribution from the positive feedback term and some bistable region, appearing as a function of $\mathbf{E}(E_0)$, for details see Refs. 24 and 30. Accurate numeric integration of Eq. (A4) (MATLAB 6.5 software) is displayed in Fig. 6. Calculations assumed the choice of two starting points, $\rho^*(+, -) = 0$ (stationary states), and search for asymptotic behavior of the system at big values of t . Such an approach suggests that only stable states are considered. To deal with dimensionless variables, the following parameters are introduced:

$$\begin{aligned} \xi &= \frac{2\omega - \epsilon - A}{\Delta}, \quad f = \frac{I\sigma_0}{\gamma_0}, \quad \bar{\mathbf{E}} = \frac{dE}{\Delta}, \\ \mathbf{E}_0 &= \frac{d\mathbf{E}_0}{(1+4\pi q\alpha)} \times \frac{1}{\Delta}, \end{aligned} \quad (\text{A5})$$

$$g = \frac{4\pi q n \mu^2}{(1 + 4\pi q \alpha)} \times \frac{1}{\Delta}, \quad \tau = \gamma_0 t.$$

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