Distant Solvent-Mediated Interaction Between Different Proteins and Between Proteins and Cells. Virtual replicas of drugs and homeopathic memory.

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During many years our Laboratory of molecular biophysics was involved in study of solvent dependent large-scale dynamics of proteins determined by relative thermal mobility of their domains and subunits. The role of large-scale dynamics in the mechanism of protein function, the signal transmission, allosteric effects and other water dependent effects in protein solutions have been investigated.

The modified for this goal physical methods, like NMR, EPR (spin-label), microcalorimetry, spectroscopy, light scattering, refractometry and others were used. A number of new phenomena in physics of biopolymers have been discovered (Käivärainen, 1985; 1989; 1995, 2001, 2003).

The most important of them are following:

1. The ability of proteins to change the bulk water dynamics and thermodynamic activity, as a result of large-scale pulsations of their big interdomain and intersubunit cavities, accompanied by assembly $\rightleftharpoons$ disassembly (flickering) of water clusters in these cavities and exchange of this water molecules with bulk water;
2. Solvent - mediated remote interaction between different kinds of proteins in the process of their large - scale dynamics (flexibility) change, induced, respectively, by ligand binding to the active sites, by temperature or by variation of solvent composition;
3. Solvent-mediated distant interaction between protein and cells, accompanied by cells swelling or shrinking, correlated with change of protein flexibility and water activity, enhancing or triggering the passive osmos via membranes of cells.

A new kind of interaction of water clusters, containing 30 - 70 molecules, with the open interdomain and intersubunit cavities of macromolecules/proteins, named *clusterphilic interaction*, was introduced (Kaivarainen, 1985, 1995, 2001). Such interaction can be considered, as the intermediate one between the hydrophobic and hydrophilic ones. It follows from our dynamic model of protein behavior in water, that intramolecular *clusterphilic interaction* stands for remote signal transmission, allosteric properties in multidomain and oligomeric proteins. It is a consequence of high sensitivity of clusters stability to perturbation of such protein cavities geometry, induced by the ligand binding. Stabilization or destabilization of water clusters in cavities shifts the dynamic equilibrium $\mathbf{B} \rightleftharpoons \mathbf{A}$ between the open (B) and closed (A) states of protein cavities to the left or right, correspondingly. As far the *assembly* $\rightleftharpoons$ *disassembly* of water clusters in cavities represent mesoscopic 1st order phase transitions, the functionally important changes of proteins configuration, accompanied the shift of $\mathbf{B} \rightleftharpoons \mathbf{A}$ equilibrium need very small change of free energy: $\Delta G = \Delta H - T\Delta S \simeq 0$. This change easily can be provided by binding of ligands to the active sites of proteins (Kaivarainen, 1985, 2001).

The intermolecular *clusterphilic interactions* are crucial in the interfacial effects and

thixotropic structure formation in colloid systems (Kaivarainen, 1995; 2003).

The part of this research activity was summarized in book of this author: "Solvent - dependent flexibility of proteins and principles of their function", D Reidel Pub Co., 1985, ISBN: 9027715343.

The development of new Hierarchic theory of condensed matter was started by this author in 1986. This work was stimulated by the understanding that the progress in biophysics is not possible without the detailed and quantitative description of water physical properties on mesoscopic and macroscopic level. The existing theories of liquid state was not enough deep and general for this end.

One of the results of application of created computer program (pCAMP, copyrighted in USA in 1997), based on our Hierarchic theory of matter (Kaivarainen, 1995, 2001, 2003), was the discovering of molecular *mesoscopic Bose condensation (mBC)* in the ice and in liquid water at the ambient temperatures (even around 36^0C) in form of coherent molecular clusters, named the *primary librational effectons.*

The evidences where obtained, using computer simulations, that just the dimensions and dynamics of these water clusters represent the crucial factors in evolution of biopolymer's spatial and dynamic structure.

Our Hierarchic theory of condensed matter got a lot of convincing computerized verifications on examples of water and ice from comparison of calculated and experimental physical parameters, like heat capacity, thermal conductivity, surface tension, vapor pressure, viscosity and self-diffusion. The new quantitative theories of refraction index, Brillouin light scattering, Mössbauer effect and others, based on the same hierarchical model, are also in good correspondence with experiment.

Because of numerous anomalies, water is a good system for testing of new theories of condensed matter. One may anticipate, that if the theory works well quantitatively for such complicated systems, as water and ice, it must be valid for the other liquids, glasses or crystals also.

1. **Distant solvent-mediated interaction between macromolecules**

The most of macromolecules, including proteins, can exist in dynamic equilibrium between two conformers (A and B) with different hydration (n_{H_2O}) and flexibility:

$$A + n_{H_2O} \Leftrightarrow B \qquad 1.1$$

Usually the correlation time of more hydrated B - conformer (τ_B), dependent on its *effective* volume (V_B) and solvent viscosity (η) are less, than that of more rigid A-conformer ($\tau_A \sim V_A$) in accordance to Stokes-Einstein law:

$$\tau_{A,B} = \frac{V_{A,B}}{k}(\eta/T)$$

$$k \text{ is the Boltzmann constant} \qquad 1.2$$

$$\tau_A > \tau_B \quad \text{and} \quad V_A > V_B$$

This means that flexibility of more hydrated B-conformer, determined by large-scale dynamics, is higher, than that of A-conformer.

For such a case, the change of the bulk water activity (a_{H_2O}) in solution by addition of other macromolecules or inorganic ions induce the change of the equilibrium constant:

$K_{A\Leftrightarrow B} = (K_{B\Leftrightarrow A})^{-1}$ and the dynamic behavior of macromolecules (Käiväräinen, 1985, 1995) :

$$\Delta \ln K_{B\Leftrightarrow A} = n_{H_2O}\, \Delta \ln a_{H_2O} \qquad 1.3$$

The developed by this author experimental approach, based on quite different dependencies of microdynamics and macrodynamics (Brownian rotation) on viscosity of solvent in solution of spin-labeled macromolecules (see section 3.6 in book by Kaivarainen, 1985) makes it possible to evaluate *separately*:

a) the frequency of spin-label rotation as respect to protein surface ($\nu_R \sim 1/\tau_R$), where τ_R is a correlation time of spin-label itself, depending on microviscosity of protein matrix (small-scale dynamics) and

b) the effective frequency of rotation of protein, as a whole (large-scale dynamics), i.e. the averaged ($\nu_M \sim 1/\tau_M$), where τ_M is the effective correlation time of the mixture of A and B - conformers, depending on $A \Leftrightarrow B$ equilibrium.

The isothermal viscosity $(\eta)_T$ dependences of resulting experimental correlation time of spin-label, conjugated with protein (τ_{R+M}), makes it possible to get a separate information about the protein small-scale and large-scale dynamics:

$$\left(\frac{1}{\tau_{R+M}}\right)_T = \frac{1}{\tau_R} + \frac{1}{\tau_M} \qquad 1.3a$$

or using (1.2) in conditions $T = const$, we get:

$$\left(\frac{1}{\tau_{R+M}}\right)_T = \left(\frac{1}{\tau_R}\right)_T + \frac{(\eta/T)_{st}}{(\tau_M)_{st}}\left(\frac{T}{\eta}\right)_T = \left(\frac{1}{\tau_R}\right)_T + \frac{k}{(V_M)_{st}}\left(\frac{T}{\eta}\right)_T \qquad 1.3b$$

where: $(\tau_M)_{st}$ and $(V_M)_{st}$ are the effective correlation time and effective volume of protein, reduced to standard conditions, when the ratio $(\eta/T)_{st} = 3 \times 10^{-5}$ P/K, corresponding to this value for pure water at $25^0 C$.

The slopes of dependence of $(\frac{1}{\tau_{R+M}})_T$ on $(\frac{T}{\eta})_T$ give us the parameters of large-scale dynamics: $(\tau_M)_{st} \sim (V_M)_{st}$. Usually these parameters are dependent on temperature and ligand binding, shifting the $A \Leftrightarrow B$ equilibrium to the left or right. The interceptions of isotherms with ordinate $(1/\tau_{R+M})$, extrapolated to infinitive solvent viscosity $(T/\eta) \to 0$, give the parameter of small-scale dynamics in spin-label environment $(1/\tau_R)$ (Kaivarainen, 1975, 1985).

Similar viscosity approach for separate investigation of the large scale and small-scale dynamics of proteins has been developed also, using microcalorimetry method (Kaivarainen et.al., 1993).

In mixed systems: [PEG + spin-labeled antibody] the dependence of large-scale (LS) dynamics of antibody on the molecular mass of polyethylenglycol (PEG) is similar to dependence of water activity and freezing temperature (T_f) of PEG solution (see book by Käiväräinen, 1985, Fig. 82).

The presence of PEG with molecular mass and concentration, increasing (a_{H_2O}) and (T_f), stimulate LS-dynamics of proteins decreasing their effective volume V and correlation time τ_M in accordance to eqs.(1.3 and 1.2).

If $\Delta T_f = T_f^0 - T_f$ is the difference between the freezing point of a solvent (T_f^0) and solution (T_f), then the relation between water activity in solution and ΔT_f is given by known relation:

$$\ln a_{H_2O} = -\left[\frac{\Delta H}{R\,(T_f^0)^2} \right] \Delta T_f \qquad 1.4$$

where ΔH is the enthalpy of solvent melting; R is the gas constant.

In our experiments with polymer solutions the 0.1 M phosphate buffer $pH\ 7.3 + 0.3M$ NaCl was used as a solvent (Käiväräinen, 1985, Fig. 82).

One can see from (1.3) that the negative values of ΔT_f in the presence of certain polymers means the increasing of water activity in three component [water - ions - polymer] system ($\Delta \ln a_{H_2O} > 0$). In turn, it follows from (1.1 - 1.3) that the increasing of a_{H_2O} shifts the $[A \Leftrightarrow B]$ equilibrium of proteins in solutions to the right. Consequently, the flexibility of the proteins will increase as far $\tau_B > \tau_A$.

The correlation between freezing temperature T_f, the water activity (a_{H_2O}) and immunoglobulin flexibility (τ_M), corresponding to (1.4 and 1.2) was confirmed in our experiments (Käiväräinen, 1985, Table 13).

It was shown that *protein-protein* distant interaction depends on their LS dynamics and conformational changes induced by ligand binding or temperature (Käiväräinen, 1985).

The temperature dependencies of correlation time of spin labeled human serum albumin (HSA-SL), characterizing the rigidness of macromolecule (the effective volume) in regular solvent and in presence of 3% D_2O is presented at Fig.1.

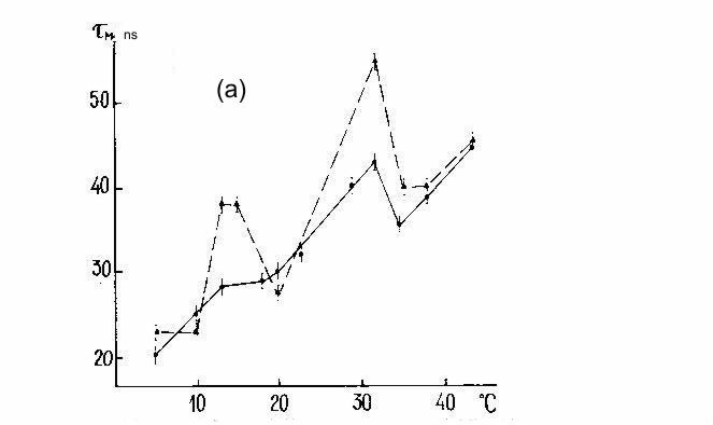

Fig. **1**. The temperature dependence of resulting correlation time (τ_M) of spin-labeled human serum albumin (HSA-SL) and similar dependence in presence of 3% D_2O (dotted line). Concentration of HSA was 25 mg/cm^3 in 0.01 M phosphate buffer (pH 7.3) + 0.15 M NaCl.

Fig.2 demonstrates a distant, solvent-mediated interaction between human serum albumin (HSA) and spin labeled hemoglobin (Hb-SL). We can see, that in presence of HSA its temperature - induced changes of flexibility/rigidity (correlation time, fig. 2) influence the flexibility of Hb-SL. The peak of correlation time of HSA around 15^0C, stimulated by presence of 3% D_2O also induce a corresponding transition in large-scale dynamics of Hb-SL.

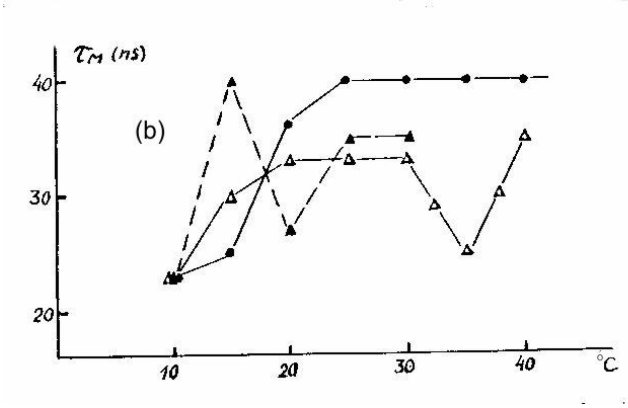

Fig.**2**. The temperature dependence of resulting correlation time (τ_M) of:
a) spin-labeled oxyhemoglobin (Hb-SL) - black dots;
b) similar dependence in presence of human serum albumin (HSA);
c) similar dependence in presence of human serum albumin (HSA) and 3% D_2O (dotted line).
The concentration of oxyhemoglobin was 20 mg/cm^3, the concentration of HSA was 10 mg/cm^3 in solvent: 0.01 M phosphate buffer (pH 7.3) + 0.15 M NaCl.

Between the clusterphilic interaction, discussed in previous section, and protein flexibility or rigidity, characterized by τ_B, the positive correlation exists. It means that the increasing of dimensions *of librational bulk water effectons is accompanied by enhancement of the water clusters dimensions in protein cavities, following, in turn, by shift of* $A \Leftrightarrow B$ equilibrium of the cavities to the right - to the more flexible conformer ($\tau_B < \tau_A$).

Our interpretation is confirmed by the fact, that lowering of the temperature and corresponding increasing of the dimensions of bulk librational effectons (coherent water clusters) and stabilizing the open states of interdomain and intersubunit cavities - increases the flexibility of protein.

At the *low concentration of macromolecules* (C_M), when the average distance (r) between them, dependent on molar concentration (C_M):

$$r = \frac{11.8}{C_M^{1/3}}\ \text{Å} \qquad 1.5$$

is much bigger, than dimensions of primary librational effecton (cluster in state of mesoscopic Bose condensate) of bulk water ($r \gg \lambda_{lb}$) the large-scale [$A \Leftrightarrow B$] pulsations of proteins, accompanied by exiting of acoustic waves in solvent, induce the enhancement of *water activity* (a_{H_2O}).

Such influence of pulsing proteins on solvent can be responsible for the distant solvent-mediated interaction between macromolecules at low concentration, described above.

The acoustic momentums in protein solutions are the result of the jump-way $B \rightarrow A$ transitions of interdomain or intersubunit cavities with characteristic transition time about 10^{-10} sec. This rapid transition follows the cavitational fluctuation of a water cluster formed by 30 - 70 water molecules in space between domains and subunits of oligomeric proteins. This fluctuation of density is a result of conversion of librational primary effecton to number translational ones (disassembly of coherent molecular cluster).

In concentrated solutions of macromolecules, when the distance between macromolecules

starts to be less than linear dimension of primary librational effecton of water: $r \leq \lambda_{lb}$, the formation of thixotropic structures and trivial aggregation begin dominate. This is a consequence of decreasing of water activity in solutions.

1.1 Possible mechanism of water activity increasing in solutions of macromolecules

Let us analyze in more detail the new effect, discovered in our work: the increasing of water activity (a_{H_2O}) in presence of macromolecules in three component [water - salt - macromolecules] system. The Gibbs-Duhem law for this case can be presented as (Käiväräinen, 1988):

$$X_{H_2O}\Delta \ln a_{H_2O} + X_M \frac{\Delta\mu_M}{RT} + X_i \Delta \ln a_i = 0 \qquad 1.6$$

where X_{H_2O}, X_M, X_i are the molar fractions of water, macromolecules and ions in the system;

$$a_j = y_j X_j = \exp\left(-\frac{\mu_0 - \mu_j}{RT}\right) = \exp\left(-\frac{\Delta\mu_j}{RT}\right) \qquad 1.7$$

is the activity of each component related to its molar fraction (X_j) and coefficients of activity (y_j);

$$\mu_M = f_B G_B + f_A G_A \simeq f_B(G_B - G_A) + G_A \qquad 1.8$$

$$\Delta\mu_M \simeq (G_B - G_A)\Delta f_B \qquad 1.8a$$

is the mean chemical potential (μ_M) of a macromolecule (protein), pulsing between A and B conformers with corresponding partial free energies G_A and G_B and its change ($\Delta\mu_M$), as a result of $A \rightleftharpoons B$ equilibrium shift, taking into account that $f_B + f_A \cong 1$ and $\Delta f_B = -\Delta f_A$ and

$$\Delta \ln a_i = (\Delta a_i / a_i) \simeq -\Delta\kappa_i \qquad 1.9$$

where the fraction of thermodynamically excluded ions (for example, due to ionic pair formation):

$$\kappa_i = 1 - y_i \qquad 1.10$$

One can see from (1.6) that when $a_{H_2O} < 1$, it means that

$$\mu^0_{H_2O} > \mu^S_{H_2O} = H^S_{H_2O} - TS^S_{H_2O} \qquad 1.11$$

It follows from (1.11) that the decreasing of water entropy ($\bar{S}$) in solution related to hydrophobic and clusterphilic interactions may lead to increasing of $\mu^S_{H_2O}$ and water activity.

It is easy to see from (1.6) that the elevation the concentration and X_M of macromolecules in a system at constant temperature and $\Delta\bar{\mu}_M$ may induce a rise in water activity (a_{H_2O}) only if the activity of ions ($a_i = y_i X_i$) is decreased. The latter could happen due to increasing of fraction of thermodynamically excluded ions (κ) (eqs. 1.9 and 1.10).

There are *two processes* which may lead to increasing the probability of ionic pair formation and fraction κ elevation.

The *first* one is the forcing out of the ions from the ice-like structure of enlarged librational effectons, stimulated by the presence relatively high concentration of macromolecules and

strong interfacial effects (2d and 3d fractions of hydration shell (Kaivarainen 2003). This effect of excluded volume increases the effective concentration of inorganic ions and probability of association, accompanied by their dehydration.

The *second* process dominates at the low concentration of $[A \Leftrightarrow B]$ pulsing macromolecules, when the thixotropic structure fail to form ($r = 11.8/C_M^{1/3} \gg \lambda_{lb}$). The acoustic waves in solvent, generated by pulsing proteins stimulate the fluctuation of ion concentration (Käiväräinen, 1995) also increasing the probability of neutral ionic pairs formation and their dehydration.

In accordance to Gibbs-Duhem law (1.6), the decreasing of ionic activity: $\Delta \ln a_i < 0$, should be accompanied by increasing of water activity: $\Delta \ln a_{H_2O} > 0$ and increasing of $\Delta\mu_M$, meaning shift of $[A \Leftrightarrow B]$ equilibrium to the right - toward more hydrated and flexible B-conformer of proteins, when $\Delta f_B > 0$ (see eq.1.8a).

1.2. The remote interaction between the active sites of proteins and the ligands

The kind of “memory of water”, discovered by Jacques Benveniste team in 1988 (Davenas, et.al. 1988), includes the ability of water to carry information about biologically active guest molecules and possibility to record, transmit and amplify this information. This phenomenon involves the successive diluting and shaking of [water + guest] system to a degree where the final solution contains no guest molecules more at all. However, using hypersensitive biological cells-containing test systems, he observed that this highly diluted solution initiated a reaction in similar way, as if the active guest molecules were still present in water. From the first high dilution experiments in 1984 to the present, thousands of experiments have been made in DigiBio company (Paris), enriching and considerably consolidating the initial knowledge of such kind of water memory. It was demonstrated also by DigiBio team, that low frequency (20 kHz = 2×10^4 s^{-1}) electromagnetic waves are able to activate biological cells. These results indicate that the molecular signal is composed of waveforms in the (10 – 44) kHz range which are specific to each molecular entity. This prompted J. Benveniste to hypothesize that the molecular signal is composed of such low frequency waves and that the ligand coresonates with the cell receptor on these frequencies, stimulating specific attraction between them.

However, we have to note, that just in this frequency range the resonant cavitational fluctuations in water can be excited by EM or acoustic fields (Kaivarainen, 1995, 2001). This effects, following by excitation of acoustic waves, also can be responsible for the membranes perturbations and cells activation.

The perturbation of water properties, induced by haptens and antibodies, concurrent inhibitors and enzymes, viruses and cells in separate and mixed solutions can be studied, using our Hierarchic theory of condensed matter and this theory based Comprehensive Analyzer of Matter Properties (CAMP) (Kaivarainen, 2003). The ways for specific water treatment by EM fields, corresponding to activation or inhibition of concrete biological processes, can be found out. The limiting stages of relaxation of water perturbations, induced by ‘guest’ molecules after successive dilution and shaking, responsible for ‘memory’ of water can be investigated also. Such investigations in case of success could turn the homeopathy from the art to quantitative science.

It can be calculated, that the trivial Brownian collisions between interacting molecules in aqueous medium can not explain a high rate of specific complex formation. One of possible explanation of specific distant interaction/attraction between the ligand and its receptor, is the exchange resonant EM interaction, proposed by DigiBio team.

The another possible explanation of the specific attraction between sterically and dynamically complementary macromolecules and molecules in water, like in system: [antibody + hapten] or [enzyme + substrate] is based on our Unified theory of Bivacuum, particles, duality and theory of Virtual Replicas of material objects (Kaivarainen, 2006; http://arxiv.org/abs/physics/0207027).

This new kind of Bivacuum mediated interaction between Sender [S] (ligand) and Receiver [R] (protein) can be a result of superposition of two their *virtual replicas*: [$\mathbf{VR}^S \Rightarrow \mathbf{VR}^R$]. The carrying frequency of Virtual Pressure Waves ($VPW^{\pm}$) is equal to basic frequency of [S] and [R] elementary particles [Corpuscle $\rightleftharpoons$ Wave] pulsation ($\omega_0 = m_0c^2/\hbar)^i$, different for electrons and protons and responsible for their rest mass and charge origination. These basic frequencies are modulated by thermal vibrations of atoms and molecules. Just this modulation phenomena explains the nature of de Broglie waves of particles and provide a possibility of resonant distant interaction between molecules with close internal (quantum) and external (thermal) dynamics/frequencies via Virtual guides of spin, momentum and energy ($\mathbf{VirG}_{S,M,E}$). A three-dimensional superposition of modulated by elementary particles of the object standing $\mathbf{VPW}^{\pm}_m$ compose the internal and external Virtual Replicas (VR_{in} and VR_{ext}). The Virtual Replicas (VR) has a properties of 3-dimensinal Virtual Quantum Holograms (Kaivarainen, 2006). The $VR = VR_{in} + VR_{ext}$ of atoms, molecules and macroscopic objects reflect:

a) the internal properties of the object (VR_{in}), including its dynamics, inhomogeneity, asymmetry, etc.;

b) the external surface properties and shape of macroscopic objects (VR_{ext}).

The virtual replicas (VR) and their superpositions may exist in Bivacuum and in any gas or condensed matter. It latter case the properties of matter can be slightly changed.

Our Unified theory, including VR, is in-line with Bohm and Pribram holographic paradigm and make it more detailed and concrete.

The proposed kind of Bivacuum mediated interaction (Kaivarainen, 2006) should be accompanied by the increase of dielectric permittivity between interacting molecules, decreasing the Van-der-Waals interactions between water molecules and enhancing the coefficient of diffusion in selected space between active site of protein and specific to this site ligand. The probability of cavitational fluctuations in water with average frequency of about 10^4 Hz (like revealed by DigiBio group), also should increase in the volume of $\mathbf{VR}^S$ and $\mathbf{VR}^R$ superposition, i.e. in the space between active site and the ligand.

The following three mechanisms of specific complex formation can be provided by:

a) the thermal fluctuations and diffusion in solution of protein and specific ligand;

b) the electromagnetic resonance exchange interaction between oscillating dipoles of protein active site and ligand (Benveniste hypothesis) and

c) the Bivacuum-mediated remote attraction between the ligand and active site, as a result of their Virtual replicas superposition (Kaivarainen, 2003-2006).

It is possible, that all three listed mechanisms of specific complex - formation are interrelated and enhance each other. The elucidation of the role of each of them in specific distant interaction/attraction between ligands and protein's active sites *in vitro* and *in vivo* - is a intriguing subject of future research.

1.3. Virtual replica of drugs and possible mechanism of 'homeopathic memory'

The memory of water, as a long relaxation time from nonequilibrium to equilibrium state, may have few explanations. One of them, for the case of magnetically treated water was suggested by this author in paper: "New Hierarchic Theory of Water & its Application to

Analysis of Water Perturbations by Magnetic Field. Role of Water in Biosystems", placed to the arXiv: http://arxiv.org/abs/physics/0207114.

The another one, described below, may explain the memory of guest molecules properties in solvent even after multiple dilution and shaking, as it is used in homeopathy. The hypothesis of 'homeopathic memory' is based on two consequences of our Unified theory (UT), including theory of Virtual Replicas of the actual objects (Kaivarainen, 2006; http://arxiv.org/abs/physics/0207027):

1. It follows from UT, that between any actual object (**AO**), like guest molecule in water, and its virtual replica (**VR**), - the *direct* and *back* reaction is existing: (**AO** $\rightleftharpoons$ **VR**). For example, when the drug molecule interact with binding site of receptor or substrate with enzyme both of reagents are already 'tuned' by their virtual replicas superposition. We remind, that **VR** may exist in Bivacuum and in any matter in gas, liquid or solid state;

2. It follows from UT, that a stable virtual replica of the guest molecule, as a system of 3D standing virtual pressure waves ($\mathbf{VPW}_{m}^{\pm}$): $VR = VR_{in} + VR_{ext}$ and its ability to infinitive spatial multiplication [$\mathbf{VRM}(\mathbf{r},\mathbf{t})$], may exist even after transferring the object from the primary location to very remote place or even its total destruction/desintegration.

If we consider a solution of any biologically active guest molecule in water (or in other liquid, in general case), then it follows from the above consequences of Unified theory, that the guest $\mathbf{VR}_{guest}$ may retain its ability to affect the target via its superposition with virtual replica of the target $\mathbf{VR}_{target}$ (i.e. the active site of cell's receptor, antibody, or enzyme). This can be a result of mentioned above back reaction of modulated virtual replica of target on the actual target (object): $[\mathbf{VR}_{guest} \bowtie \mathbf{VR}_{target}] \rightarrow \mathbf{AO}$ even after super-dilution, when no one guest molecule (ligand) is no longer present in solution.

The proposed mechanism of homeopathic memorization of VR in space and time [$\mathbf{VRM}(\mathbf{r},\mathbf{t})$] (Kaivarainen, 2006) may explain the homeopathic drugs action in super-dilute solutions. The luck of 100% reproducibility of results, like presented by Benveniste's team in 1988, can be explained by perturbation of virtual replicas of small objects (drugs, ligands, etc.) by changes in external virtual replicas ($\mathbf{VR}_{ext}$) due to virtual replicas interference. The changes in $\mathbf{VR}_{ext}$, produced, for example, by geophysical conditions fluctuations or by variation of cosmic factors, like Sun activity, spatial disposition in system: [*Earth – Sun – Moon*] and corresponding change of Solar system virtual replica. If we learn in future to take these factors into account, providing the same conditions of experiments, the 100% reproducibility of so-called "subtle" effects, including paranormal phenomena will be achieved.

2. Distant solvent-mediated interaction between proteins and cell

The unknown earlier phenomena, like distant, solvent - mediated interaction between different proteins (fig.2), was revealed in our experiments, using modified method of spin-label (Kaivarainen, 1985, section 8.5). The similar kind of interaction between proteins and cells, modulated by temperature and protein ligand state, was discovered on examples of mixed systems: erythrocyte suspension + human serum albumin (HSA), using turbidimetry (light scattering) method (Fig 3).

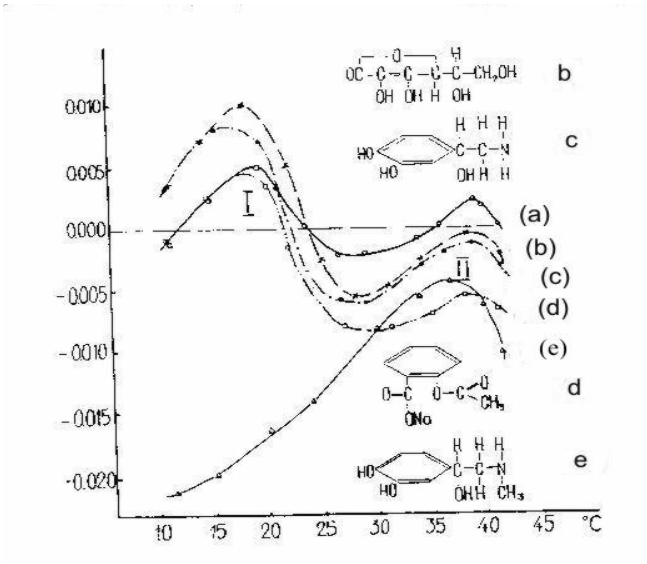

Fig.**3**. The differential temperature dependence of turbidity of human erythrocytes suspension at the light wave length $\lambda = 600$ nm in the presence and absence of human serum albumin (HSA) in different ligand state:

$$\Delta D^* = D^*_{ER+HSA} - D^*_{ER}$$

a) the interaction with intact HSA; b) HSA + ascorbic acid; c) HSA + noradrenaline; d) HSA + sodium acetyl-salicylate; e) HSA + adrenaline. The experiments where performed in modified physiological Henx solution (pH7.3), where the glucose was excluded to avoid possible artefacts on osmotic processes (Kaivarainen, 1985, fig.84).

The concentration of erythrocytes was 2×10^5 cm^{-1} and the concentration of HSA was 15mg/cm^3. All ligands had fivefold molar excess over HSA. The erythrocytes suspension was incubated at least 24 hours before measurements for exhaustion of cellular ATF and elimination of the active osmos. A number of special experimental controls where performed to prove the absence of absorption of HSA on the membranes of cells and direct effect of ligands on turbidity of erythrocyte suspension (Kaivarainen, 1985, section 8.5).

The *swelling* and *shrinking* of erythrocytes, reflected by increasing and decreasing of their suspension turbidity, is a consequence of increasing and decreasing of the external water activity (Fig.46). The latter factors are responsible for the direction of water diffusion across the membranes of cells (*'in'* or *'out'*) as a result of passive osmos. They are in-phase with corresponding changes of HSA large-scale dynamics (flexibility). The latter was revealed in special experiments with spin-labeled HSA-SL (see fig.1). The same kind of temperature dependence of HSA - SL flexibility was confirmed in modified Henx solution.

We got the modified Boyle van Hoff equation, pointing to direct correlation between the increments of cell's volume and the external water activity (Kaivarainen, 1985): $\Delta \mathbf{V} \sim \Delta \mathbf{a}_{H_2O}$.

Both thermoinduced conformational transitions of serum albumin occur in physiological region of surface tissues of body. Consequently, presented data point to possible role of albumin (the biggest protein fraction in blood) in thermal adaptation of many animals, including human, using the discovered *solvent - mediated mechanism of protein - protein and protein - cell interaction.*

3. Water and selective cancer cells destructor

The hypothesis was proposed by this author (Kaivarainen, 1995; 2001), that one of the reasons of unlimited cancer cell division is related to partial disassembly of cytoskeleton's actin-like filaments and microtubules due to some genetically controlled mistakes in biosynthesis of ionic pumps, water channels and increasing the osmotic diffusion of water into transformed cell.

Decreasing of the intra-cell concentration of any types of ions (Na^+, K^+, H^+, Mg^{2+} etc.), as a result of corresponding ionic pump malfunction, incorporated in biomembranes, also may lead to disassembly of filaments.

The shift of equilibrium: [assembly ⇔ disassembly] of microtubules (MTs) and actin filaments to the right increases the amount of intra-cell water, involved in hydration shells of protein and decreases water activity. As a consequence of concomitant osmotic process, cells tend to swell and acquire a ball-like shape. The number of direct contacts between transformed cells decrease and the water activity in the intercell space increases also.

Certain decline in the external inter-cell water activity, because of dense intercell contacts deterioration leads to the absence of triggering signal for inhibition of cells division/proliferation. The shape of normal cells under control of cell's filament is a specific one, providing good dense intercell contacts with limited amount of water in contacts, in contrast to situation with transformed cells. The activity of water in latter case is simply not low enough to stop the cells division.

In accordance to mechanism of cancer development proposed here, the *absence of contact inhibition* in the case of cancer cells, is a result of loose [cell-cell] contacts and loosing the interaction between cells because of disassembly of microtubules and actin filaments, connecting cells in normal conditions.

If our model of cancer emergency is correct, then the problem of limitless transformed cells proliferation, at least partly, is related to the problem of intercell water activity decreasing by incorporation, for example, of special intercell water soluble macro/poly-ions. The cells membranes should be nontransparent for such macroions.

Another approach to cancer healing, based on mechanism described above, is the IR laser (CO_2) treatment of transformed cells with IR photons frequencies (about 3×10^{13} s^{-1}), stimulating excitation of cavitational fluctuations in water (emergency and collapsing of microbubbles), inducing a collective disassembly of MTs, actin filaments and *gel* → *sol* transition. The corresponding centrioles disintegration will prevent cells division/proliferation and should give a good therapeutic effect. The IR laser based selective cancer cells destructor, proposed by this author, can be combined with ultrasound treatment of tissues and blood. Such treatment with frequency of about 40 kHz, in accordance to calculations, based on our Hierarchic theory of water, also should stimulate cavitational fluctuations in the swallowed cancer cells and their disintegration.

The method of cancer cells destruction proposed here, is based on the assumption that stability of MTs in transformed cells is weaker than that of normal cells fraction of 'free' water higher, than in normal cells. This difference should provide the selectivity of destructive action of the ultrasound and laser beam on the cancer and normal cells, remaining the normal cells undamaged.

Our Hierarchic theory of water (Kaivarainen, 1995, 2001; 2003) predicts besides the mentioned above frequency of ultrasound radiation around 40 kHz, the one more resonant to water fluctuations frequency around 10^7 Hz. The combination of such kind of treatment of blood and organs should increase the selective destruction of cancer cells.

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