# HYDRATED FULLERENE HELPS TO OPEN THE SECRETS OF THE CLUSTER NATURE OF WATER (UNIFIED MODEL OF BEHAVIOR OF THE ORDERED WATER)

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# ГИДРАТИРОВАННЫЙ ФУЛЛЕРЕН ПОМОГАЕТ РАСКРЫВАТЬ ТАЙНЫ КЛАСТЕРНОЙ ПРИРОДЫ ВОДЫ (ОБЪЕДИНЕННАЯ МОДЕЛЬ ПОВЕДЕНИЯ УПОРЯДОЧЕННОЙ ВОДЫ)

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# *MAIN TERMS:*

**- $C_{60}$HyFn**

*HYDRATED $C_{60}$ FULLERENES*

**- FWS**

*WATER SOLUTIONS of HyFn*
*(Fullerene-Water-Solutions)*

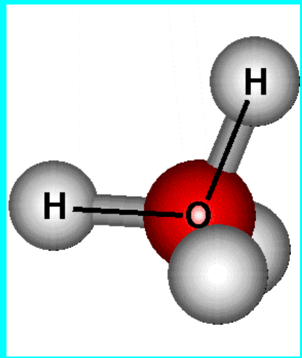
***- CLOSE BOUNDED WATER***

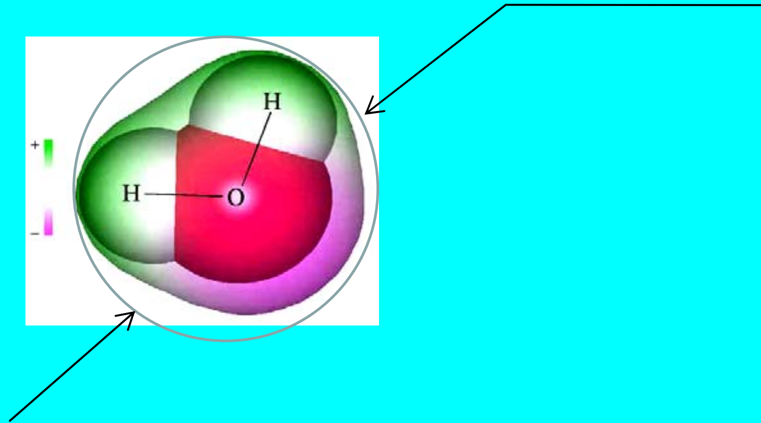
- ***ORDERED WATER***

- **WATER CLUSTERS**

# WATER MOLECULE - $H_2O$

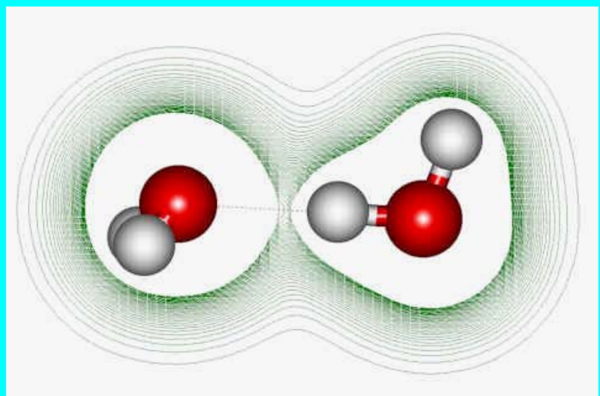
**2.8 (3.0) Å**

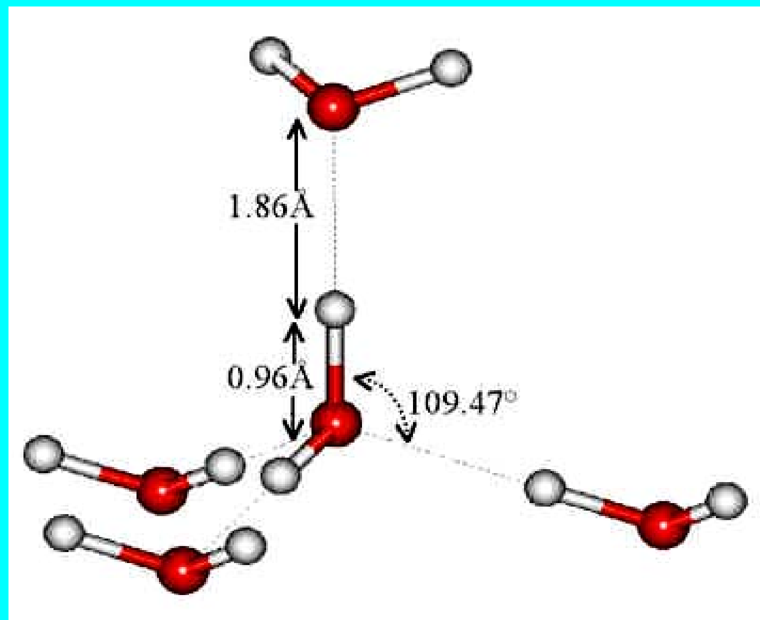



## Hydrogen Bonded Two Water Molecules

## Hydrogen Bonded Water Pentamer

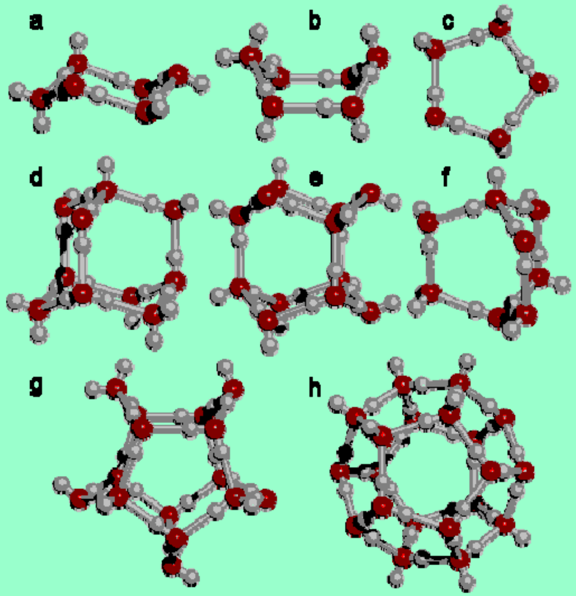


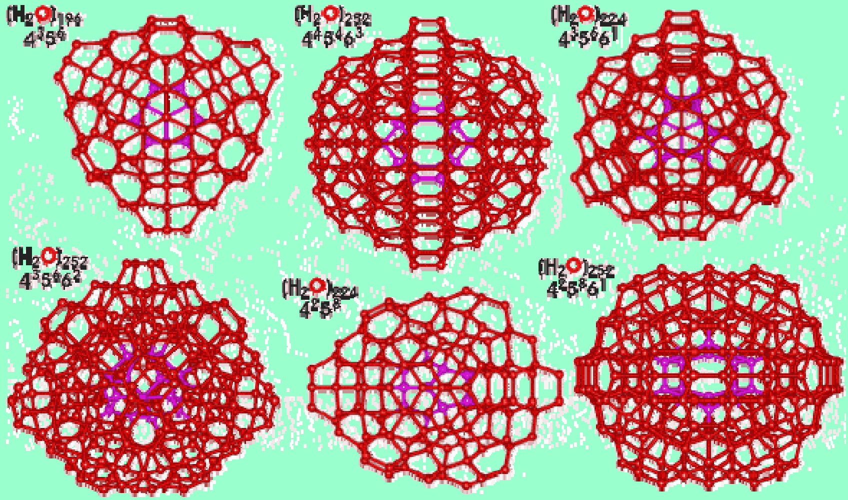


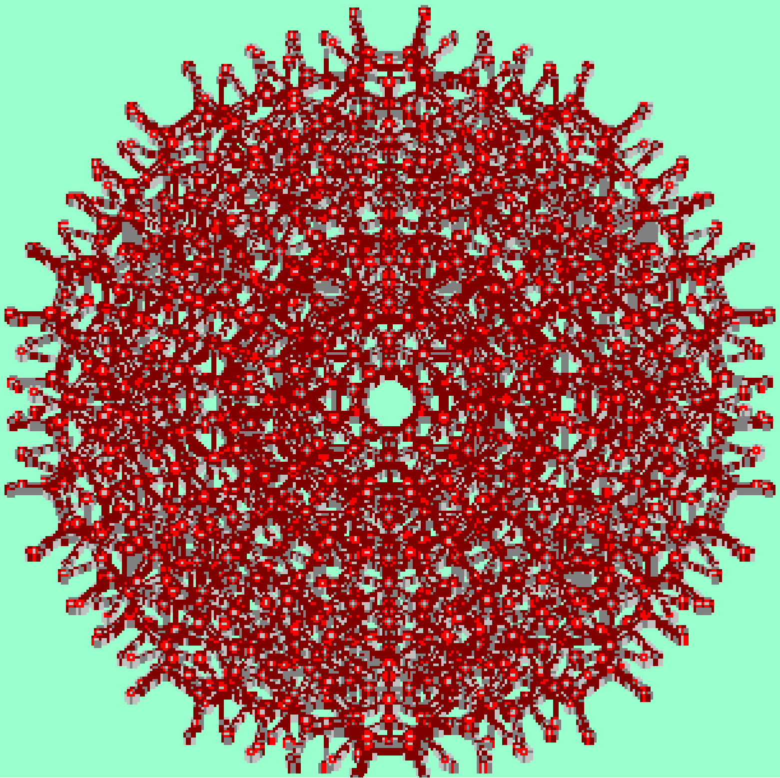

In accordance with:
M. Chaplin, *Water Structure and Science*,
South Bank University, London, UK

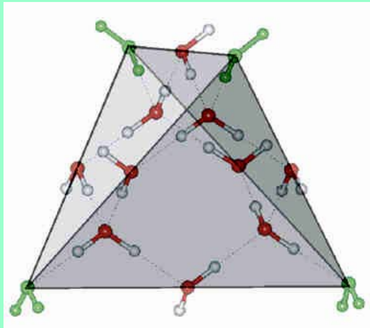
# Various Water Clusters and $C_{60}$ Fullerene

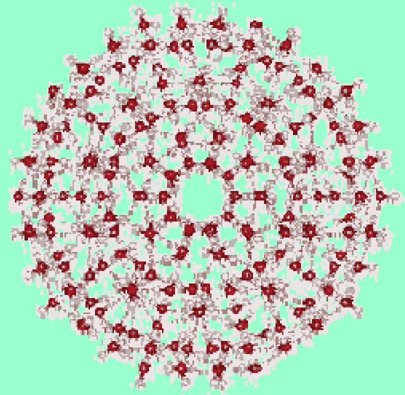
(by M. Chaplin, http://www1.lsbu.ac.uk/water/)

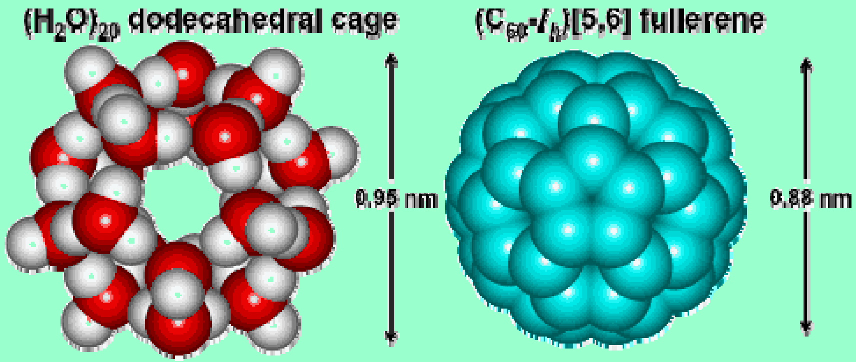










# About observations of ordered water structure with size app. 13-15 Å (www.pnas.org/cgi/doi/10.1073/pnas.0904743106 **PNAS,** Early Edition 1 of 5)

PNAS PNAS PNAS PNAS

## The inhomogeneous structure of water at ambient conditions

C. Huang[a], K. T. Wikfeldt[b], T. Tokushima[c], D. Nordlund[a], Y. Harada[c,d], U. Bergmann[a], M. Niebuhr[a], T. M. Weiss[a], Y. Horikawa[c,e], M. Leetmaa[b], M. P. Ljungberg[b], O. Takahashi[f], A. Lenz[g], L. Ojamäe[g], A. P. Lyubartsev[b], S. Shin[c,i], L. G. M. Pettersson[b], and A. Nilsson[a,b,1]

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Edited by H. Eugene Stanley, Boston University, Boston, MA, and approved July 7, 2009 (received for review May 7, 2009)

**Small-angle X-ray scattering (SAXS) is used to demonstrate the presence of density fluctuations in ambient water on a physical length-scale of ≈1 nm; this is retained with decreasing temperature while the magnitude is enhanced. In contrast, the magnitude of fluctuations in a normal liquid, such as $CCl_4$, exhibits no enhancement with decreasing temperature, as is also the case for water from molecular dynamics simulations under ambient conditions. Based on X-ray emission spectroscopy and X-ray Raman scattering data we propose that the density difference contrast in SAXS is due to fluctuations between tetrahedral-like and hydrogen-bond distorted structures related to, respectively, low and high density water. We combine our experimental observations to propose a model of water as a temperature-dependent, fluctuating equilibrium between the two types of local structures driven by incommensurate requirements for minimizing enthalpy (strong near-tetrahedral hydrogen-bonds) and maximizing entropy (nondirectional H-bonds and disorder). The present results provide experimental evidence that the extreme differences anticipated in the hydrogen-bonding environment in the deeply supercooled regime surprisingly remain in bulk water even at conditions ranging from ambient up to close to the boiling point.**

density fluctuations | liquid–liquid hypothesis | small angle X-ray scattering | water structure | X-ray spectroscopy

Liquid water shows many anomalies in its thermodynamic properties such as compressibility, density variation and heat capacity (1–4). In the low-temperature regime, below the freezing point, these properties deviate strongly from normal and theories, related to a liquid–liquid phase transition between high and low density water, have been proposed to account for these anomalies (5). Although the anomalies are extreme in the supercooled region they are also present at ambient conditions where most of waters' physical, chemical and biological processes of importance occur. In contrast, water at ambient conditions has traditionally been considered as a homogeneous distribution of near-tetrahedral hydrogen-bonded (H-bonded) structures with thermal fluctuations increasing with temperature. This picture has been challenged by recent studies based on X-ray Raman (XRS) and conventional X-ray absorption spectroscopy (XAS) (6), and X-ray emission spectroscopy (XES) (7), suggesting two distinct local structures with tetrahedral as a minority and a highly hydrogen-bond (H-bond) distorted asymmetrical as the majority. In particular the proposed predominant asymmetrical structure has caused intense debate in the last years (see refs. 7 and 8 for detailed discussion).

SAXS and small-angle neutron scattering (SANS) provide the most direct probes of density variations or fluctuations on large length scales in a liquid. Through an enhancement of the structure factor at low momentum transfer, $Q$, small deviations from the average electron density at different length scales can be reliably identified (9). Previous SAXS studies of water have mostly focused on the supercooled region and given contradictory results where both positive (10–12) and zero enhancement (13, 14) at low $Q$ have been reported. With the development of third-generation synchrotron light sources the ability to perform SAXS has been greatly advanced and measurements can now be performed in a large $Q$-range with high accuracy and reproducibility (15).

APPLIED PHYSICAL SCIENCES

### Results

Fig. 1*A* shows the normalized structure factor, $S(Q)$, derived from the SAXS intensity in ambient water ($H_2O$) as function of $Q$ for temperatures from 7 to 74 °C in the full $Q$-range of interest, 0.04–0.78 $Å^{-1}$ (see *SI Methods*). All scattering curves show an enhancement approaching $Q = 0$ after experiencing a minimum ≈0.4–0.5 $Å^{-1}$, which to first approximation directly indicates the presence of density heterogeneities. In particular, the enhancement becomes *smaller* with increasing temperature in strong contrast to expectation from simple thermal density fluctuations.

To address whether the enhancement at low $Q$ can be related to and reproduced by thermal fluctuations in common water models, we have performed molecular dynamics (MD) simulations using the extended simple point charge (SPC/E) potential (see *SI Methods*). The SAXS signal at low $Q$ is given by the Fourier transform (FT) of the longer intermolecular correlations in real space from the simulation. To model SAXS data it is thus essential to use large simulation boxes (here 40,000 molecules) and also to average over long simulation runs (here longer than 0.3 ns) to reduce artificial oscillations in $Q$ space. Fig. 1*B* shows the SPC/E oxygen–oxygen partial structure factor, $S(Q)$ (see *SI Methods*). The finite size of the simulation box causes a sharp artificial increase at $Q < 0.13$ $Å^{-1}$ in the FT. The *Inset* shows the results for smaller simulation boxes with 5,000 molecules, where it was possible to average over significantly longer simulation times (2 ns) resulting in smoother curves, but with the FT artificial increase occurring already at $Q = 0.25$ $Å^{-1}$ due to the smaller box size. The most important scattering enhancement observed at small $Q$ in the experiment is completely missing from the SPC/E data even down to $Q = 0.13$ $Å^{-1}$. For comparison, Fig. 1*C* shows $S(Q)$ of $CCl_4$ measured at temperatures from 6 to 30 °C (see *SI Methods*) and regarded as representing a "normal" liquid. It is clear that SAXS of $CCl_4$ shows no temperature-dependent

Author contributions: A.N. designed research; C.H., K.T.W., T.T., D.N., Y.Harada, U.B., M.N., T.M.W., Y. Horikawa, M.L., M.P.L., O.T., A.L., L.O., A.P.L., S.S., and L.G.M.P. performed research; C.H., T.T., M.N., and D.N. analyzed data; and C.H., K.T.W., L.G.M.P., and A.N. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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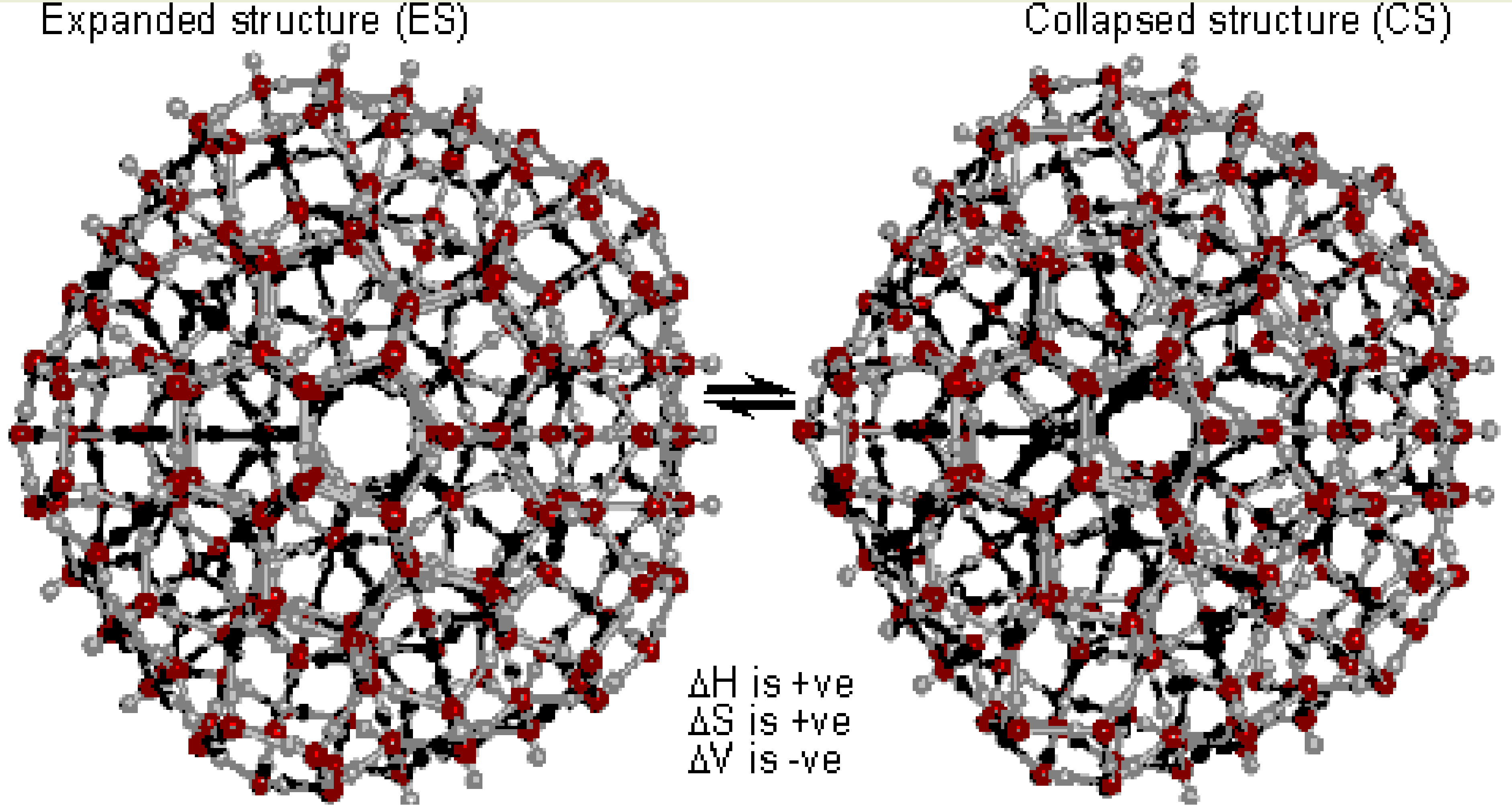
This article contains supporting information online at www.pnas.org/cgi/content/full/0904743106/DCSupplemental.

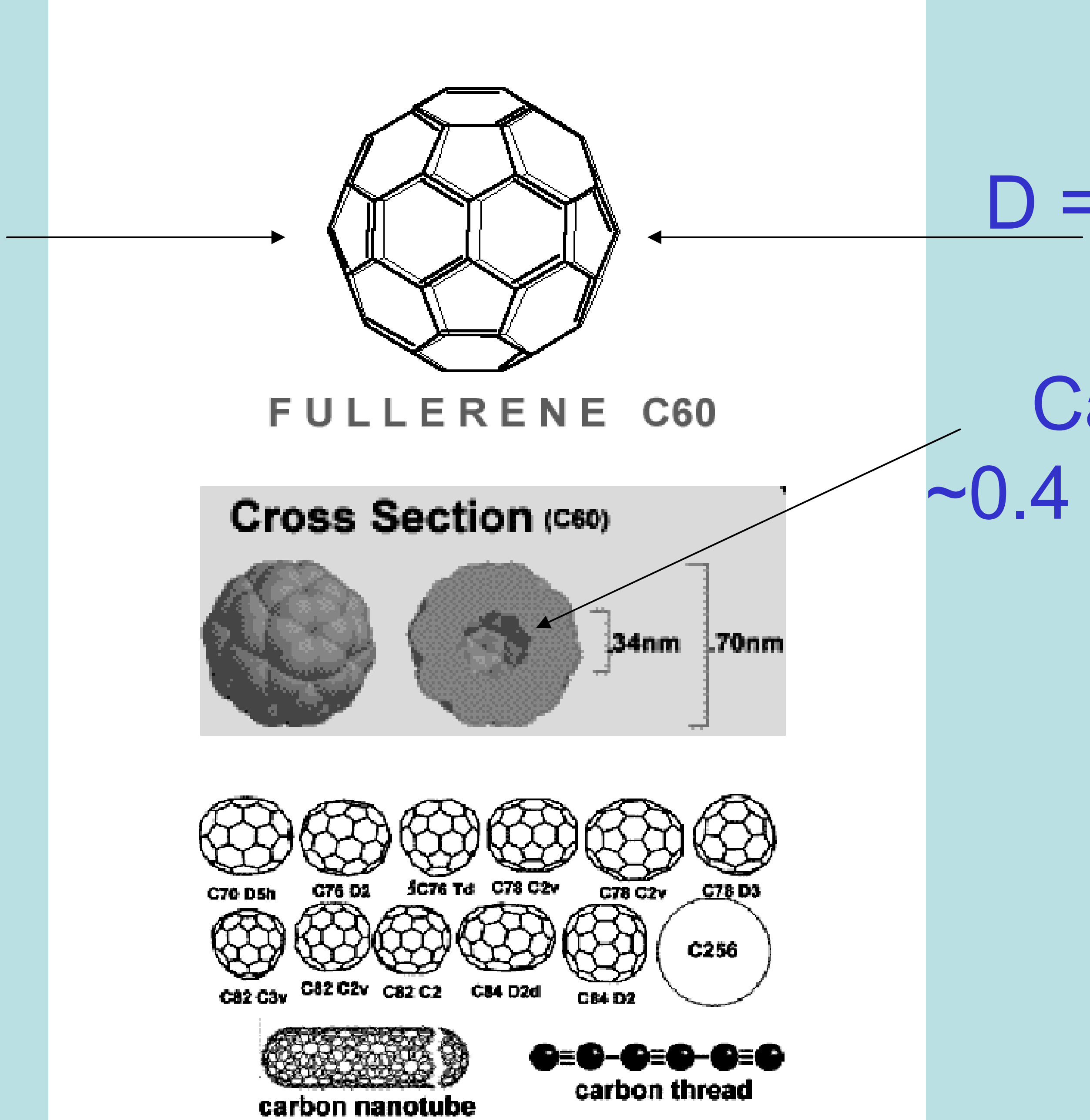
# Icosahedral Water Clusters

*by Professor Martin Chaplin (December 20, 2001,*
*School of Applied Science South Bank University*
*London SE1 0AA (http://www.lsbu.ac.uk/water/icosahedral.html),*



# FULLERENES – VACUUM BUBBLES

# WITH ABSOLUTE PHYSICAL VACUUM INSIDE



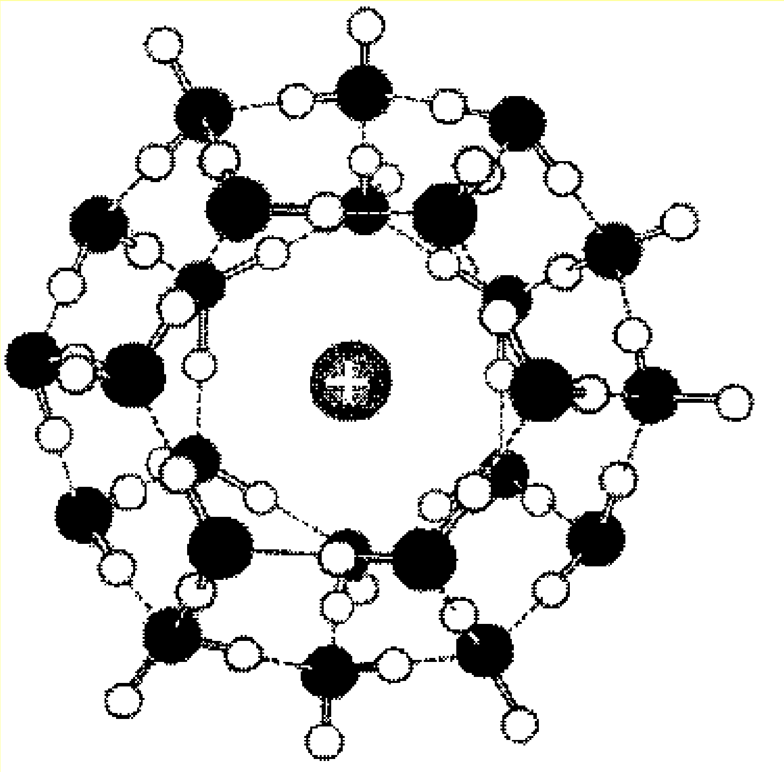

D = 1 nm

Cavity
~0.4 - 0.5 nm

**MONUMENT FOR $C_{60}$ FULLEREN IN KHARKOV, UKRAINE**

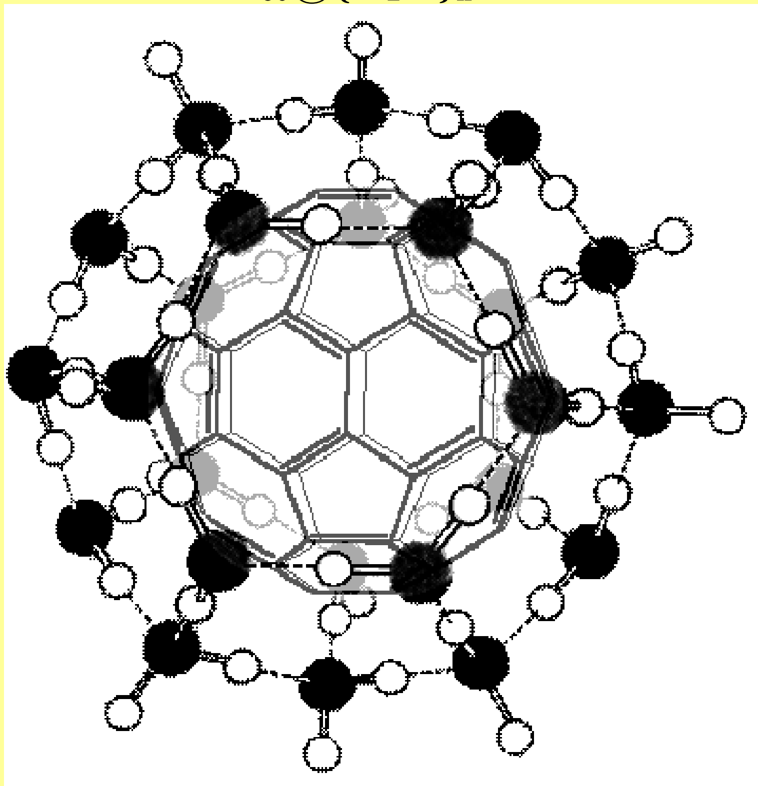
# $C_{60}$FWS – WATER SOLUTION of HYDRATED $C_{60}$ FULLERENE ($C_{60}$HyFn)

## Water Clusters with $H_3O^+$ or Metal Ions (+) Encaged Inside the Clathrate of $(H_2O)_{24}$



[S. Wei, A.W. Castleman Jr., *(Using Reflectron Time-of-Flight Mass Spectrometer Techniques to Investigate Cluster Dynamics and Bonding)* Int. J. Mass Spectrom. Ion Proc. 131 (1994) 233.]

## Hypothetical Model of Hydrated Fullerene - Supramolecular, Donor-Acceptor Complex of H-bonded Water Molecules with $C_{60}$ - $C_{60}@\{H_2O\}_n$

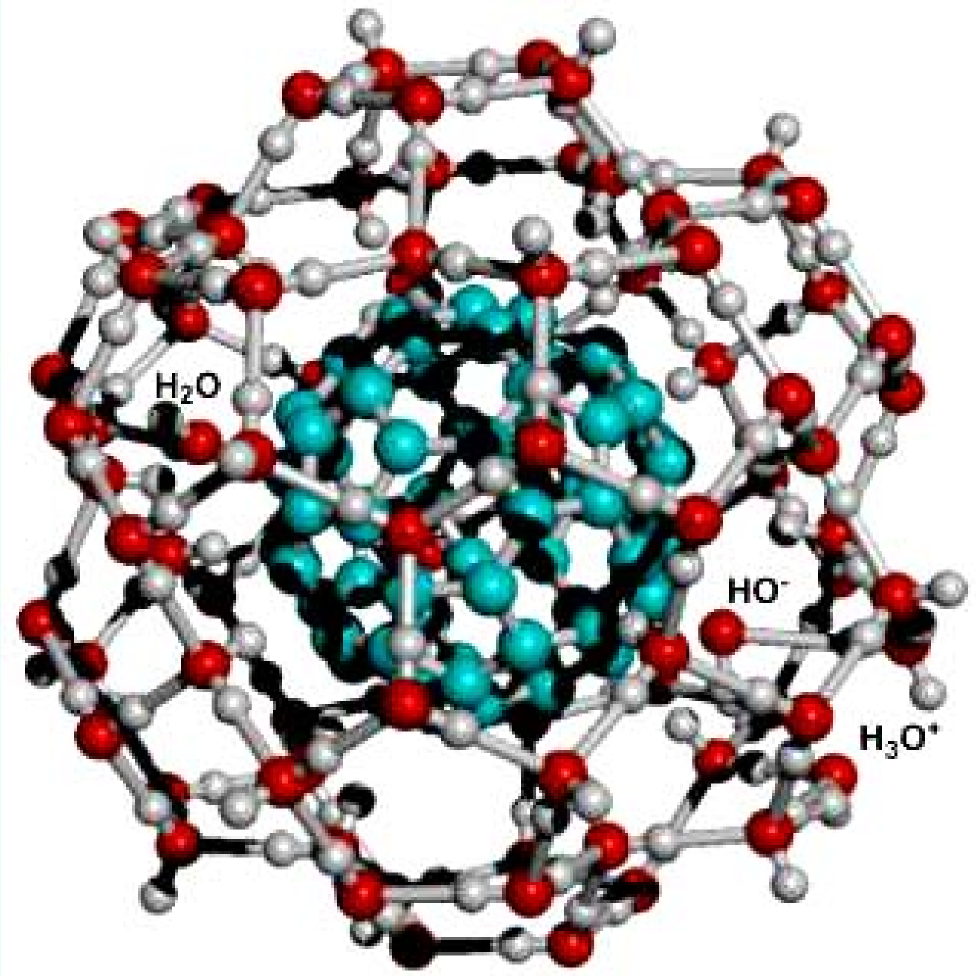


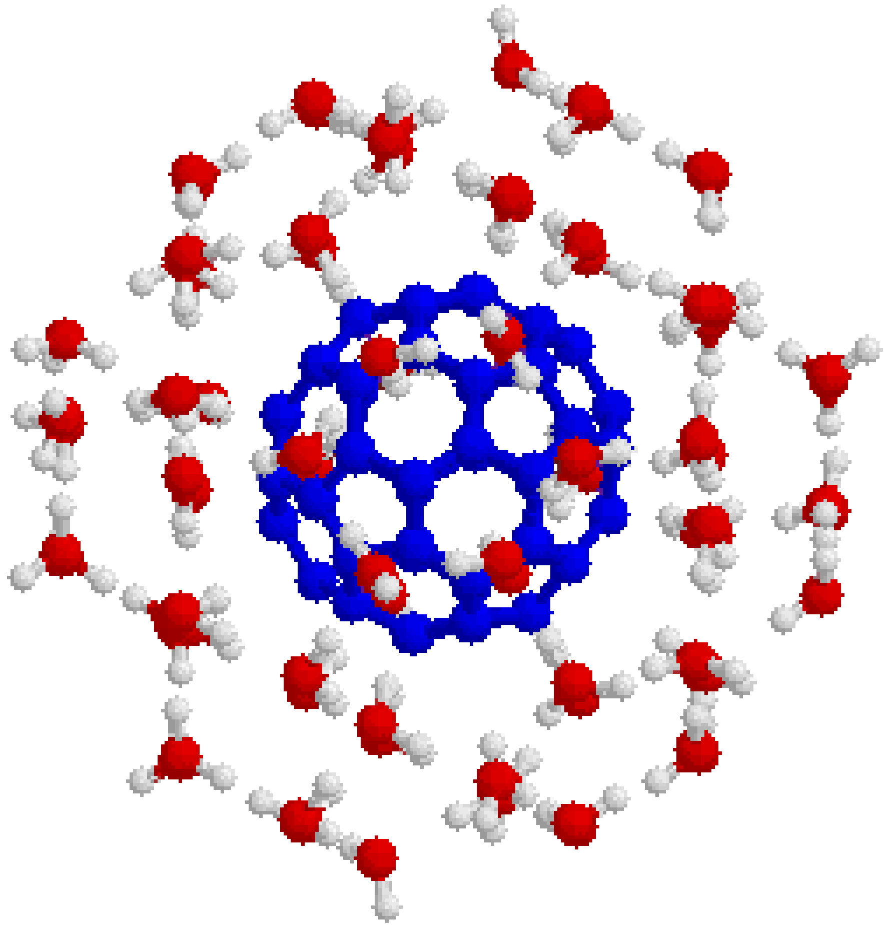
$C_{60}@\{xH_2O \cdot yOH^-\}^{y-} \cdot yH^+$,

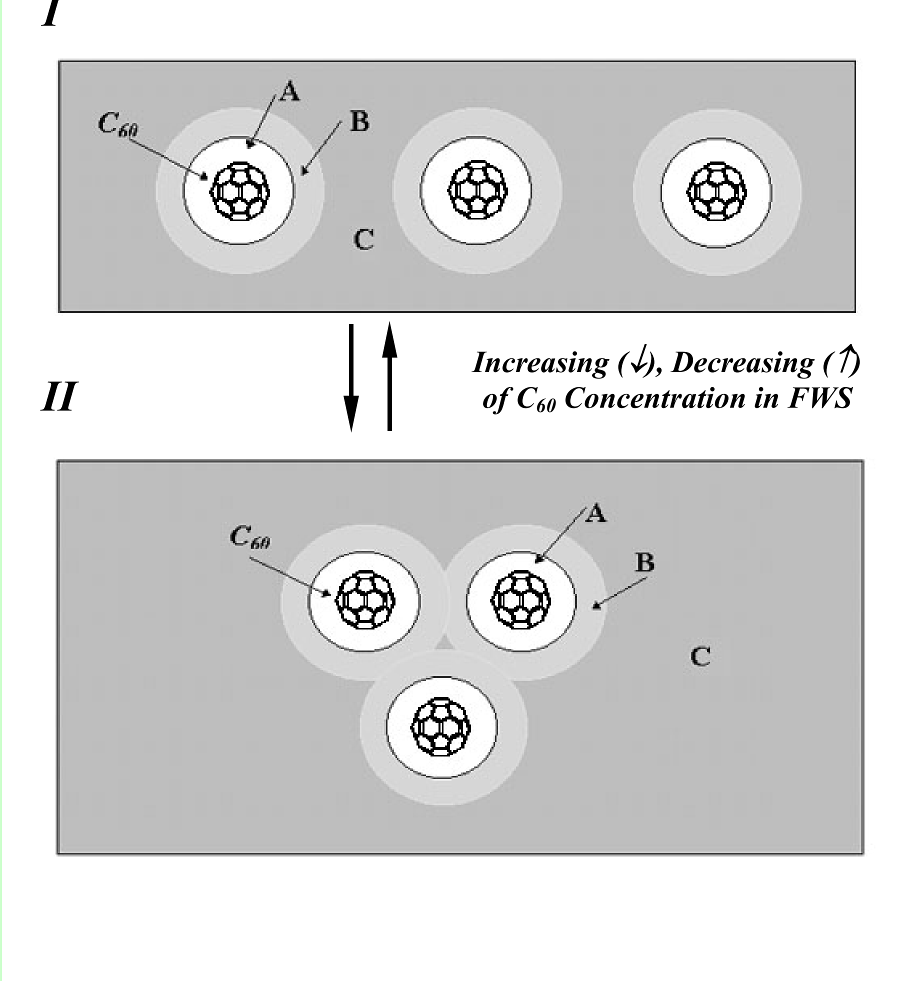
*in which the part of counter-ions ($H^+$) is substituted by metal ions ($Me^{x+}$).*

# Fullerene $C_{60}$ Encaged Inside the Icosahedral Water Cluster of $(H_2O)_{80}$ = = Hydrated $C_{60}$ Fullerene (HyFn).

Designed by Professor Martin Chaplin in December 20, 2001,
School of Applied Science
South Bank University
London SE1 0AA
**(http://www.sbu.ac.uk/water/buckmin.html),**
in accordance with recommendations of Andrievsky G.V.
and Chem.Phys.Lett., 300 (1999) 392-396.








**_I_ - Model of Aqueous Solution of Hydrated $C_{60}$ Fullerene (HyFn).**

**_II_ - Model of Clusters Formation of Hydrated $C_{60}$ Fullerene in Water Solution (FWS).**

*Were:*

**_A_ - First Water Layer of Close Bonded Water Molecules (~20-24 $H_2O$);**

**_B_ - Transitional Layers of Water Molecules;**

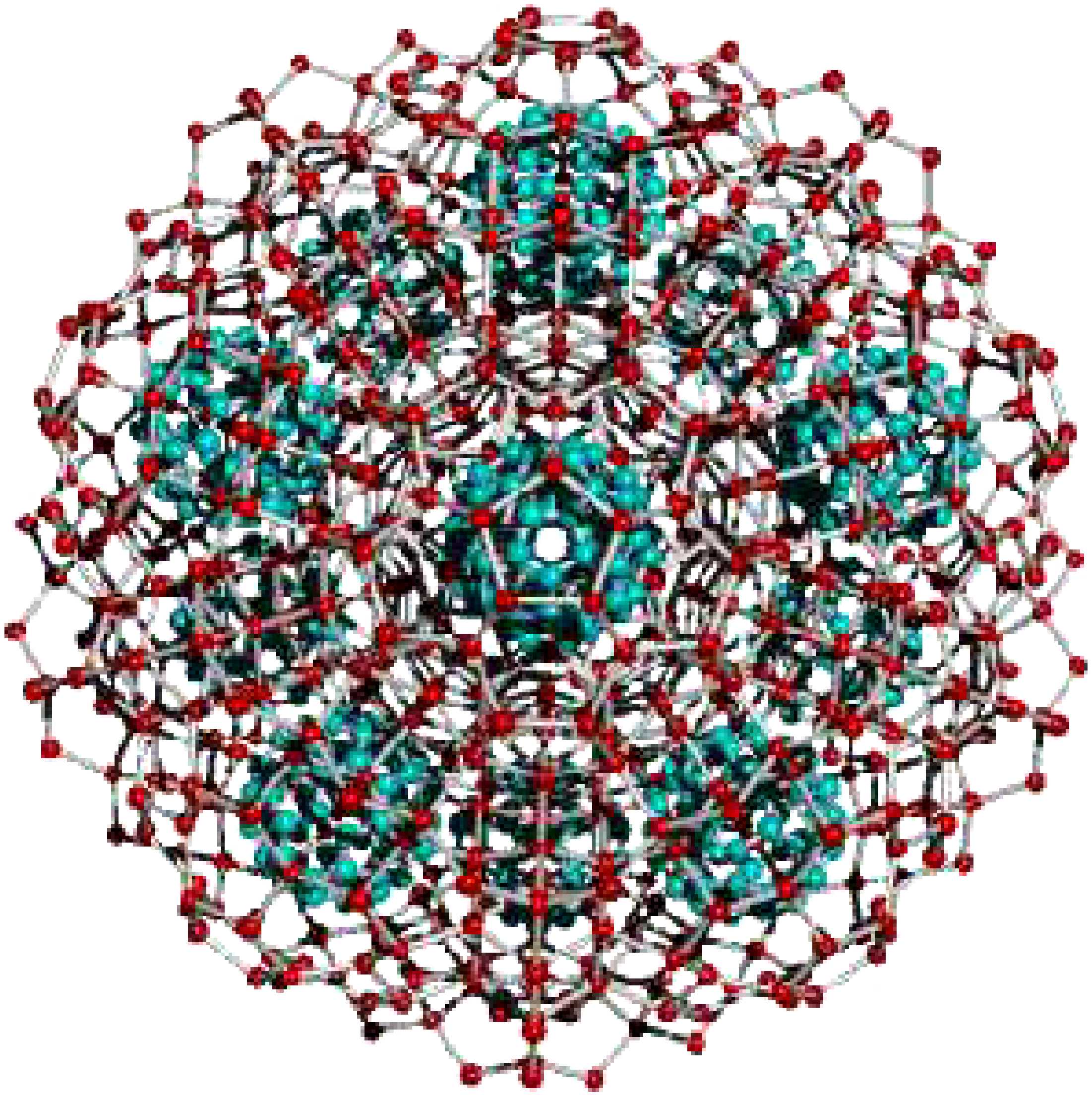
**_C_ - Bulk («Free») Water**

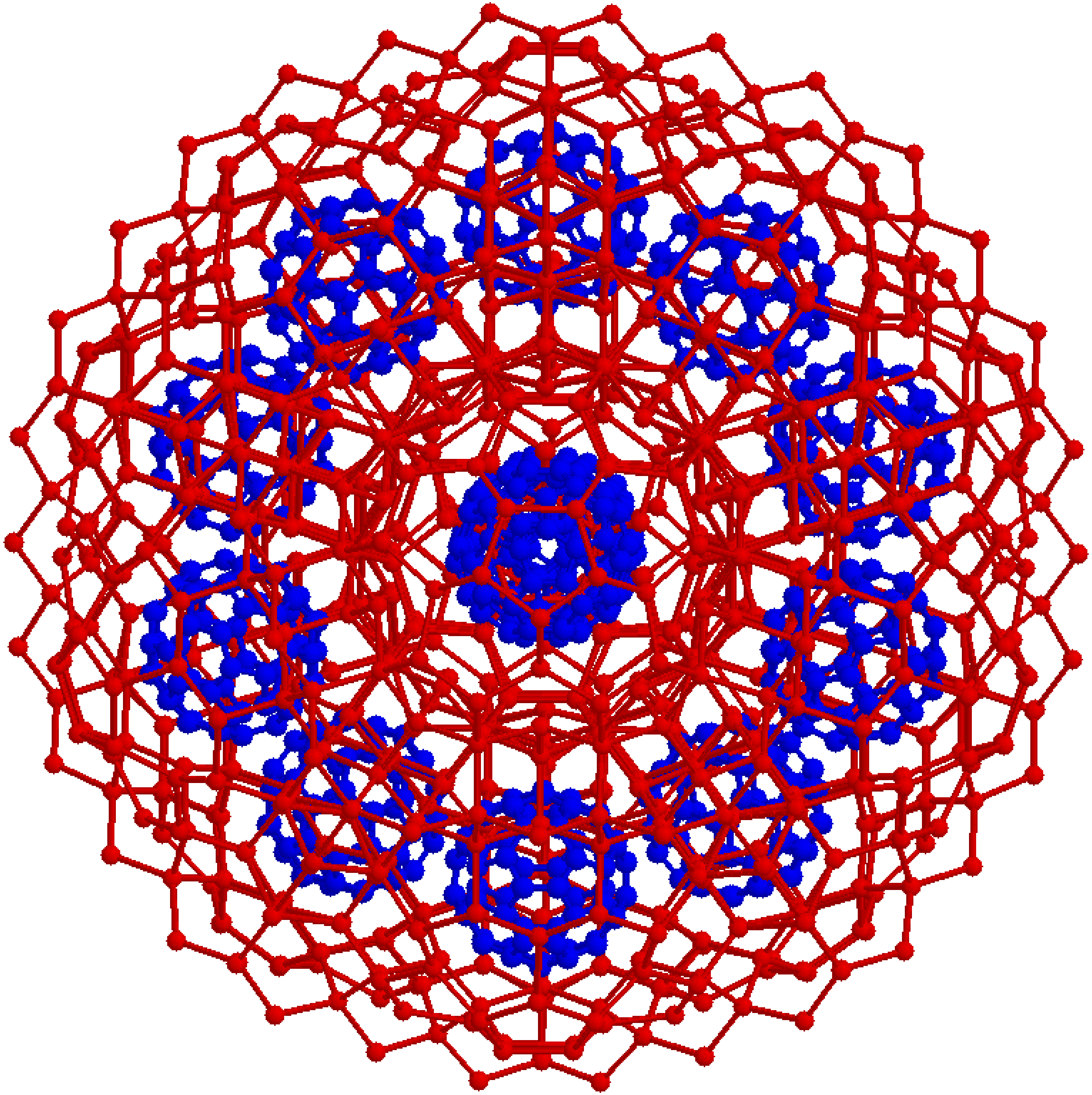
**Designed by Professor Martin Chaplin in December 20, 2001, School of Applied Science South Bank University, London SE1 0AA**

(http://www.sbu.ac.uk/water/buckmin.html),

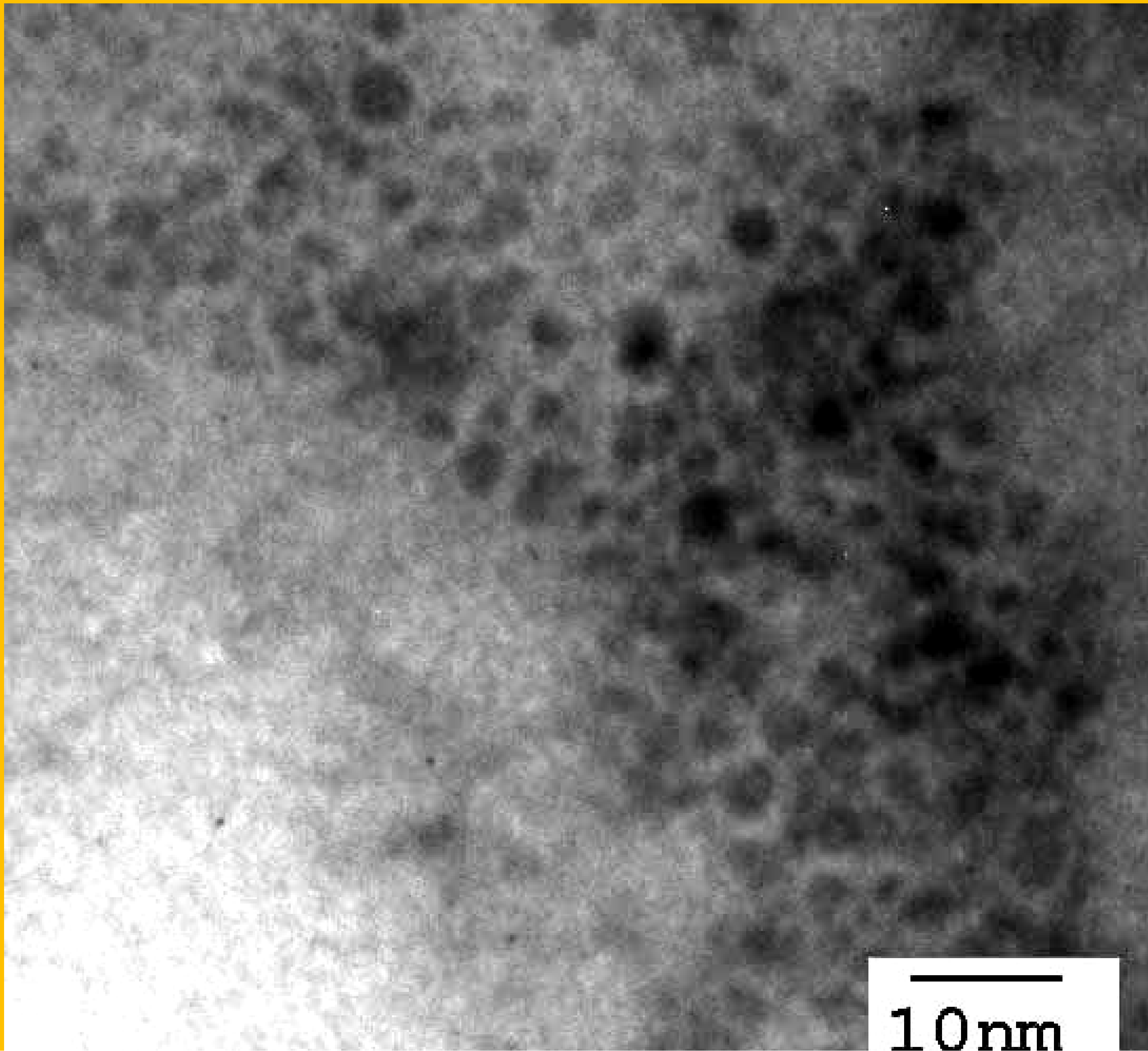
in accordance with recommendations of Andrievsky G.V.

and Chem.Phys.Lett., 300 (1999) 392-396.

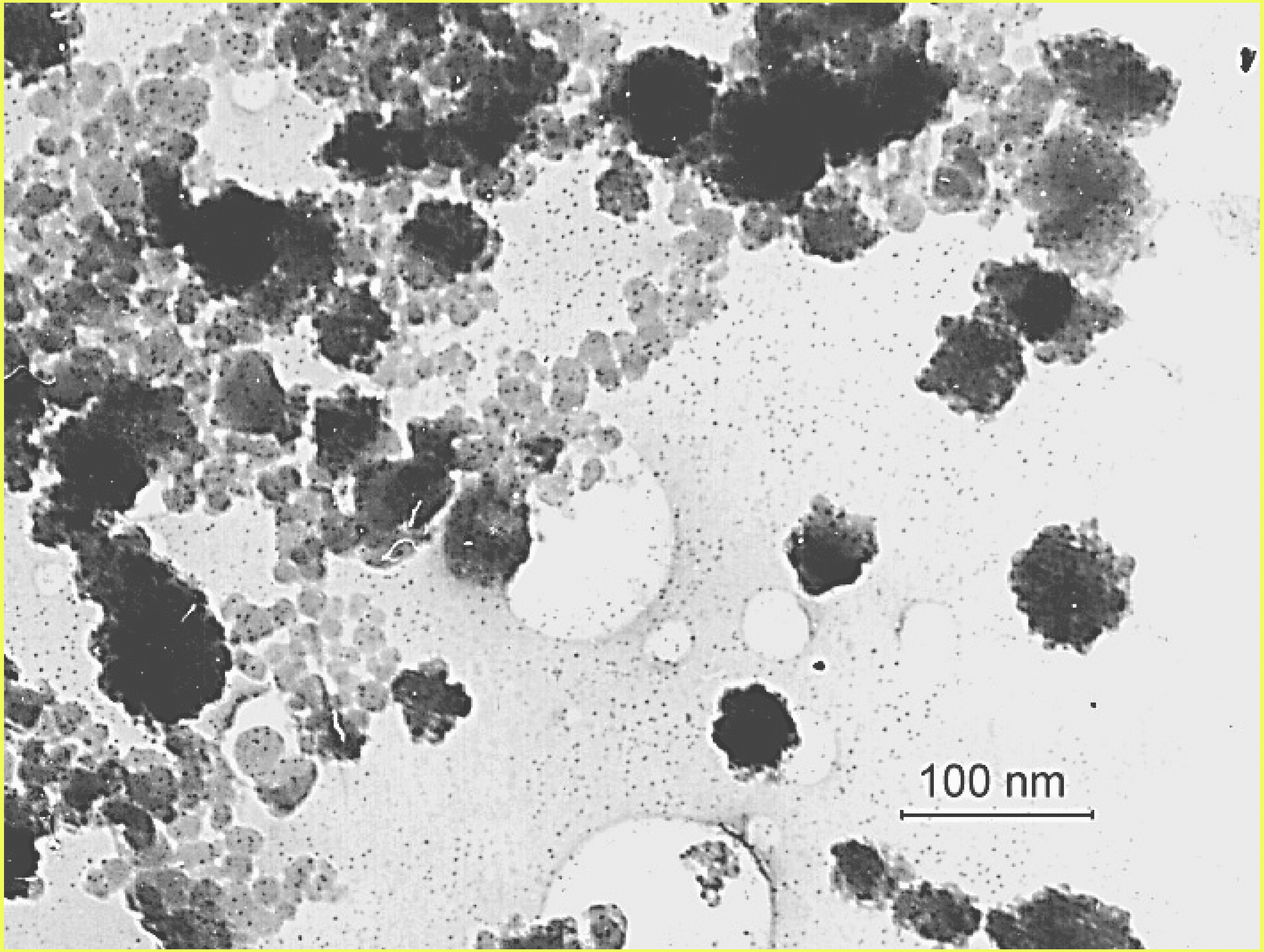




# Transmission Electronic Micrographs (TEM) $C_{60}$ particles sorbed from $C_{60}$FWS on graphite thin film



## The electron micrograph of $C_{60}$ fullerene aggregates after coagulation $C_{60}$FWS with NaCl




**"...The $C_{60}$FWS are shown to be the ultramicroheterogeneous and polydisperse systems containing spherical structures (from ~1.5 to 72 nm). By analyzing the sizes of these structures, it was revealed that their diameters regularly rise within the range from 3.4 to 36.0 nm and are equal to 3.4, 7.1, 10.9, 14.5, 18.1, 21.8, 25.4, 28.8, 32.4, 36.0 nm....."**

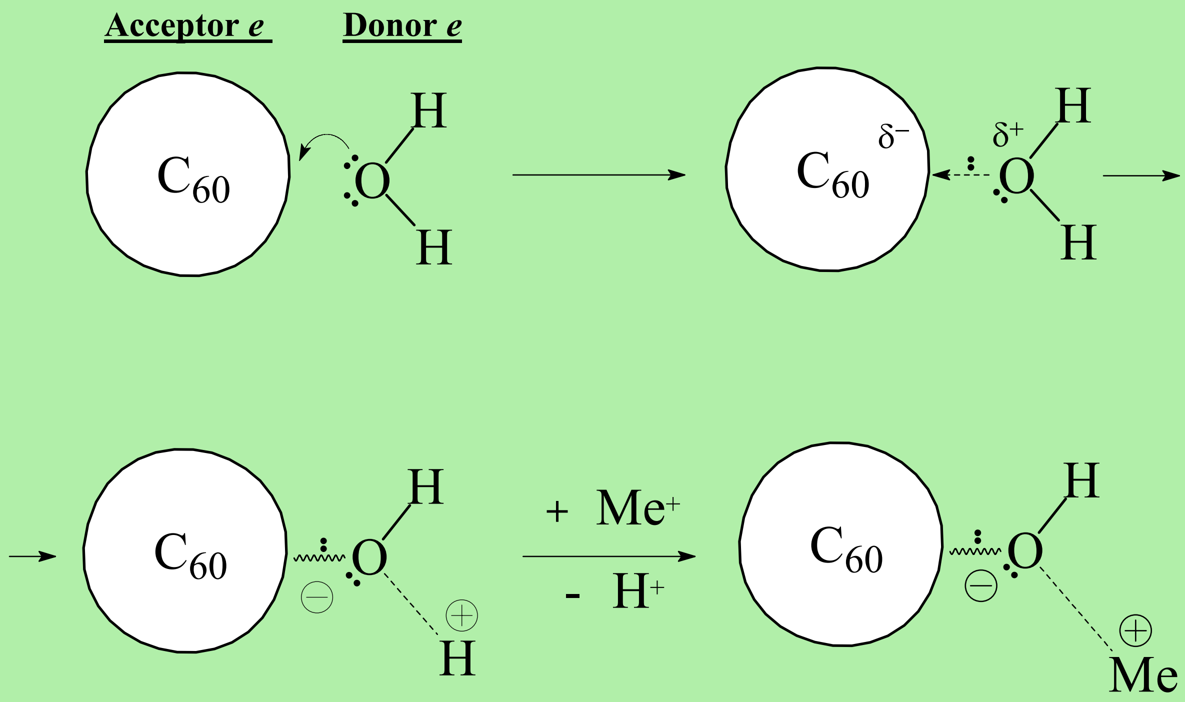
## Diameters of $C_{60}$HyFn Associates Rise Regularly Within the Range from 1.6 to 36.0 nm and are Equal to

**1.6 3.4 7.1 10.9 14.5 18.1 21.8 25.4 28.8 32.4 36.0 nm**

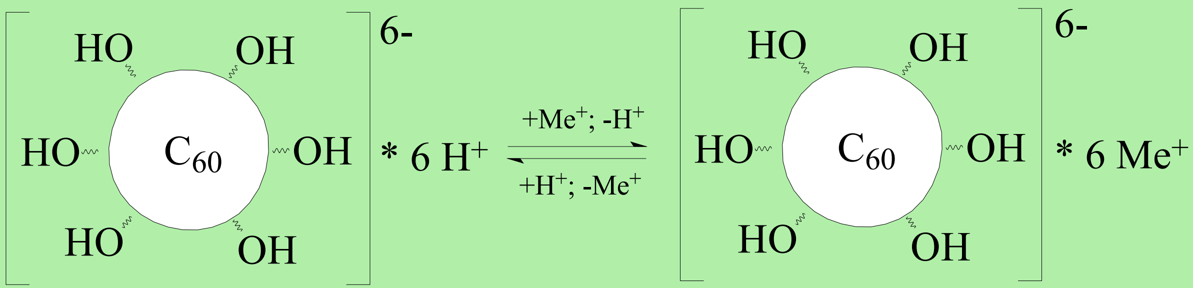
## Hydrated $C_{60}$ Fullerene:

**Localized Hydrolysis of Water Molecules on $C_{60}$ Surface and**

**Cation Exchange of *$H^+$ / $Me^+$* in the First Nearest Aqueous Shell**




at pH < 4



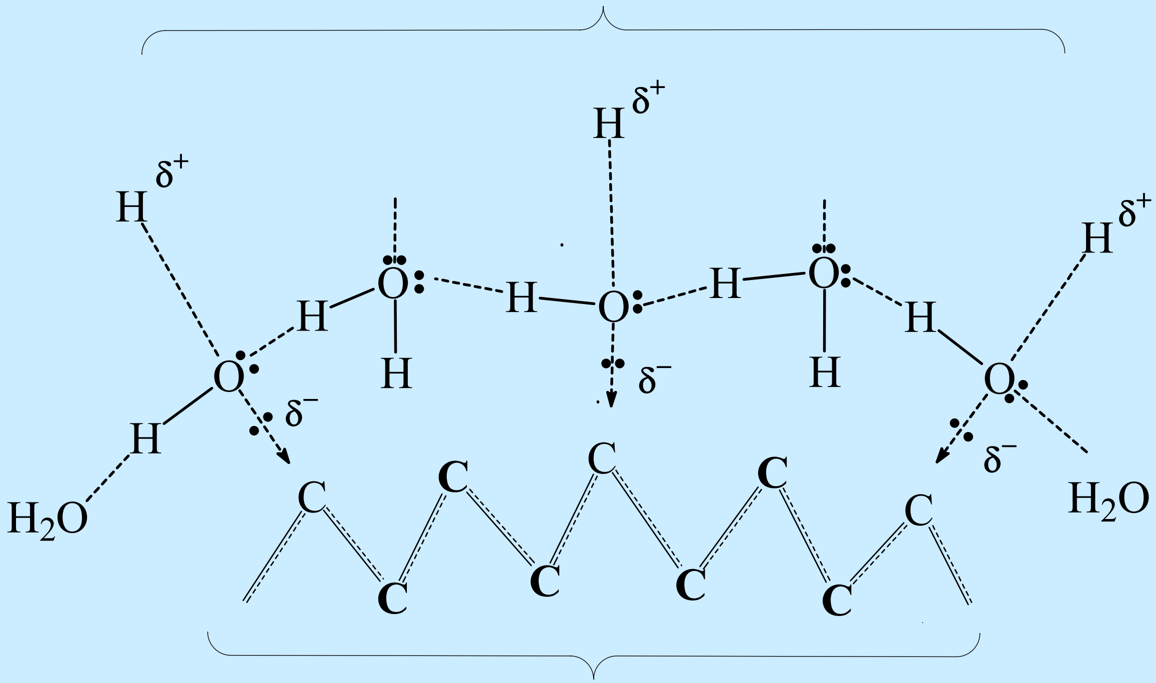
## *Hypothetical Model of Hydrated Fullerene - $C_{60}@\{H_2O\}_n$*

**The Fragment of a Structure, *$C_{60}@\{yOH^- \ xH_2O\}^{y-} \ yH^+$*,**

***in which the Part of Counter-Ions ($H^+$)***

***can be Substituted by Metal Ions ($Me^{z+}$).***

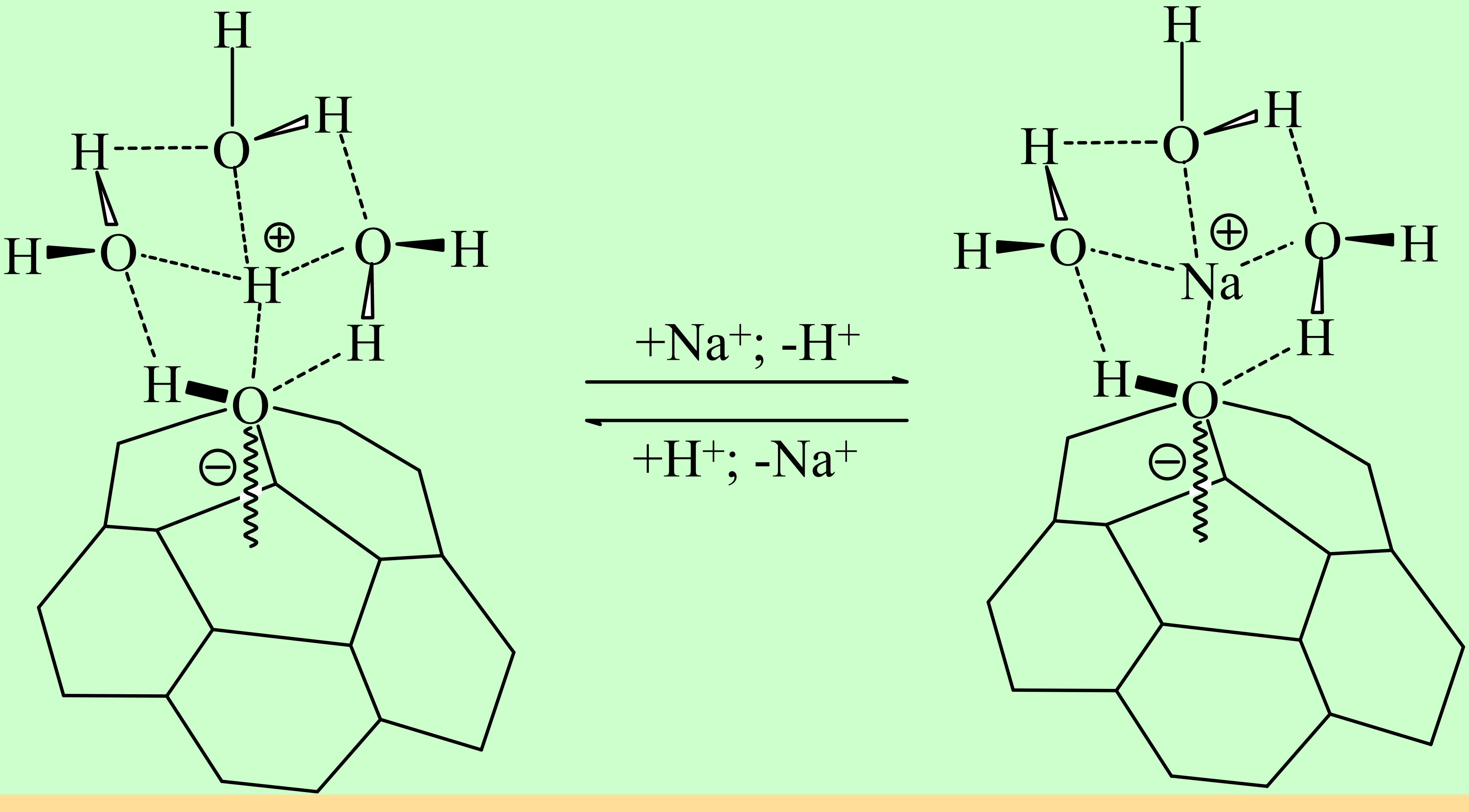
**Close bounded water molecules on $C_{60}$ fullerene surface**




**Carbon atoms of electron-acceptor surface of $C_{60}$ fullerene**

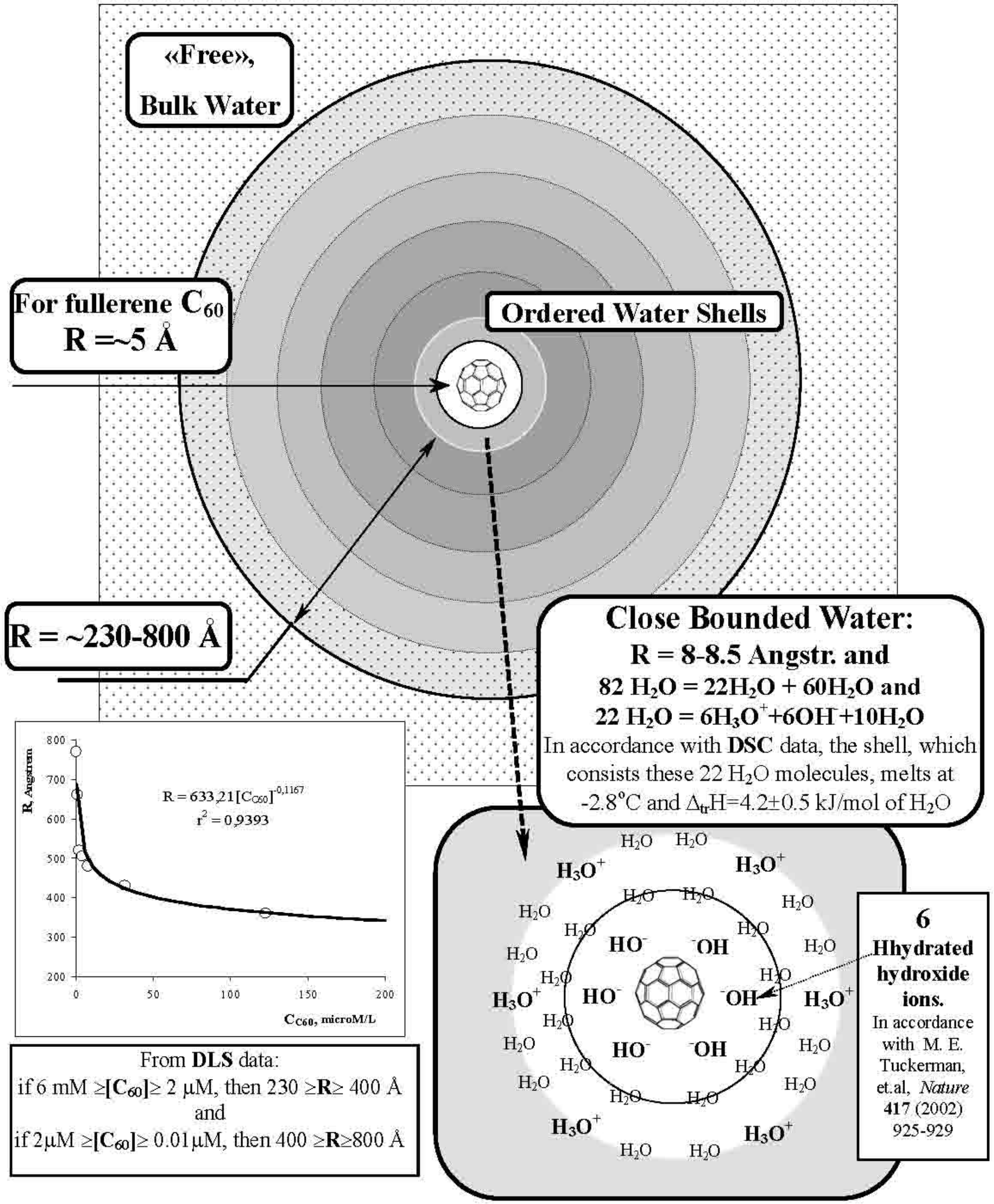
**G.V. Andrievsky**, et.al. COMPARATIVE ANALYSIS OF TWO AQUEOUS-COLLOIDAL SOLUTIONS OF $C_{60}$ FULLERENE WITH HELP OF FT-IR REFLECTANCE AND UV-VIS SPECTROSCOPY. **Chem. Phys. Letters**, **364**,

**If Me$^{+}$ = Na$^{+}$, then:**



**THE HYDRATED $C_{60}$ FULLERENE (HyFn** = DONOR-ACCEPTOR COMPLEX OF $C_{60}@\{H_2O\}_n$ **) SURROUNDED BY ORDERED WATER SHELLS.**

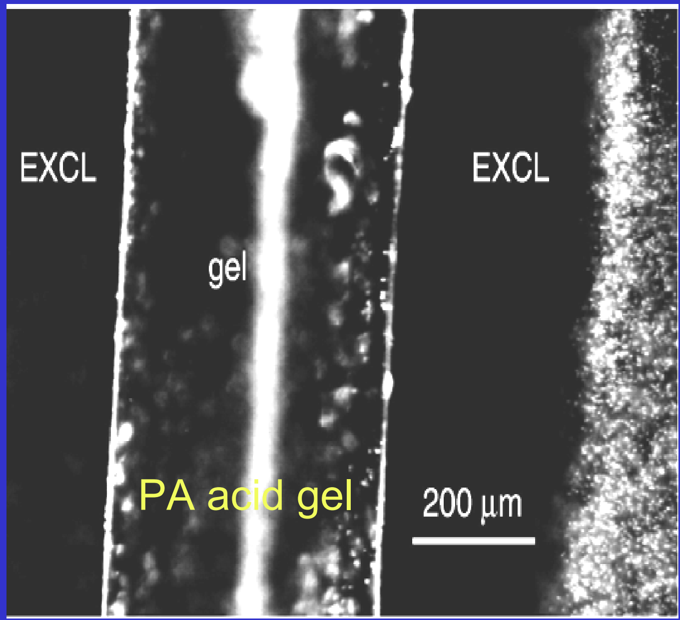
The Probable Model of HyFn Which Based On Data of Dynamic Light Scattering (DLS), Small-Angle Neutron Scattering (SANS), Low Temperature Differential Scanning Calorimetry (DSC) and Molecular Simulation of professor M. Chaplin .

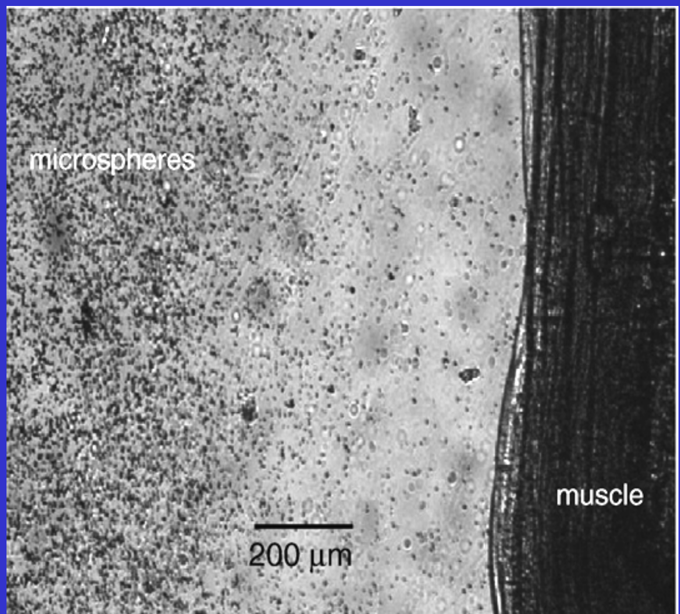


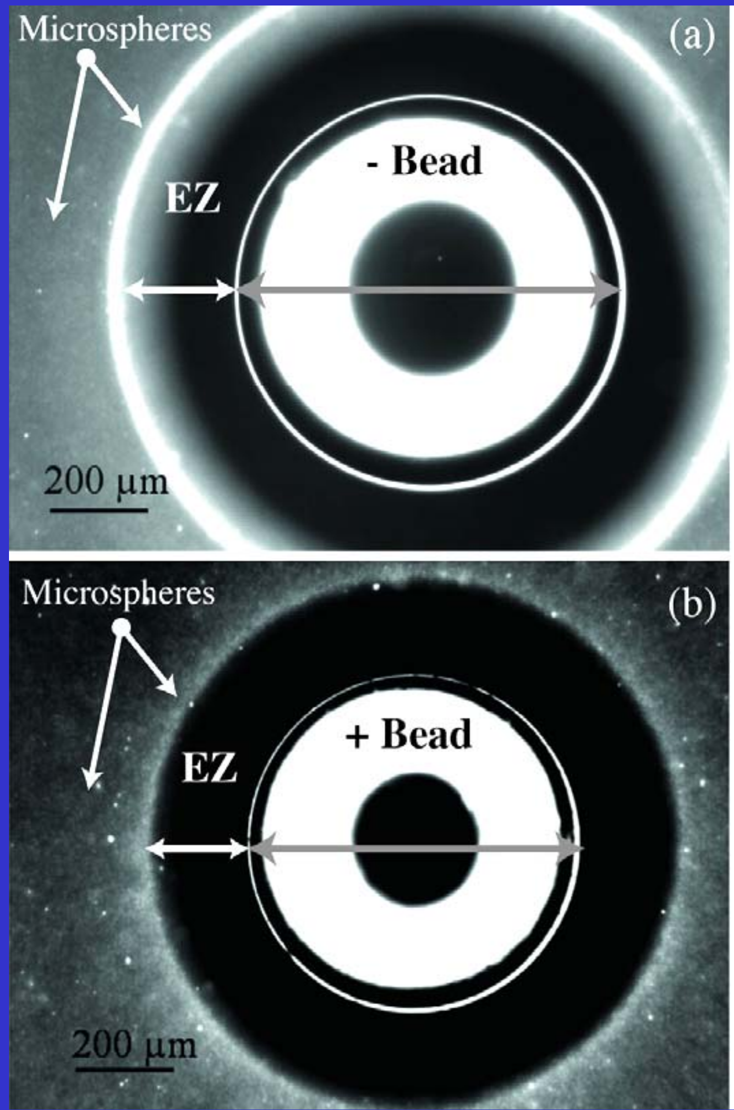
# LET'S COMPARE THE THEORY and FACTS!!!

| Objects / Parameters | Coherence Domain of Water (CDW) by Emilio DelGiudice | Hydrated $C_{60}$ Fullerene ($C_{60}$HyFn) by Grigoriy Andrievsky |
|---|---|---|
| **Size** (Diameter) | **100 nm** | Coherence Domain of Water Ordered by $C_{60}$HyFn (**$C_{60}$CDW**) – **40-160 nm (on the average ~100 nm !!!)** For diluted $C_{60}$HyFn solution. |
| **Another Sizes** | **Is CDW Exists with Size <<100 nm?** | **1.6-1.8 nm** for $C_{60}$ Molecule With First Closely Bounded Water Shell and FracalCluster Nanostructures of $C_{60}$HyFn and Water Clusters of Higher Order (with the size approx. **3.4, 7.1, 10.9, 14.5, 18.1, 21.8, 25.4, 28.8, 32.4, 36.0 nm**) |
| **“0.2 eV”** | **FUNDAMENTAL BIO-PHYSICAL VALUE** | **$\Delta_{tr}H$ - Heat, Enthalpy of $C_{60}$ fullerene HYDRATION** |
| **Energy** | **12.06 eV** **Energy of Excited State of CDW** | **15.5 eV** **Estimated Energy of $C_{60}$HyFn Formation, Stabilization** |
| **For Comparison** | **12.6 eV - Energy of Water Ionization** | |

# EXCLUSION ZONE WATER (EXCL or EZ) by G.Pollack




J. Zheng, W.-C. Chin, E. Khijniak, E. Khijniak Jr., G. H. Pollack. Surfaces and interfacial water: Evidence that hydrophilic surfaces have long-range impact. *Advances in Colloid and Interface Science* 127 (2006) 19–27.
J.-M. Zheng, A. Wexler, G.H. Pollack, Effect of buffers on aqueous solute-exclusion zones around ion-exchange resins, *Journal of Colloid and Interface Science* (2009),

# Особые свойства водной фазы (IV phase of water), прилегающей к поверхностям, индуцирующих ионизацию воды

*(J. H. Pollack, 2003 – 2008, V.L. Voeikov, 2008 )*

Отличия от объемной воды по вязкости, плотности, температуре замерзания, диэлектрической проницаемости

НАИБОЛЕЕ ВАЖНО:

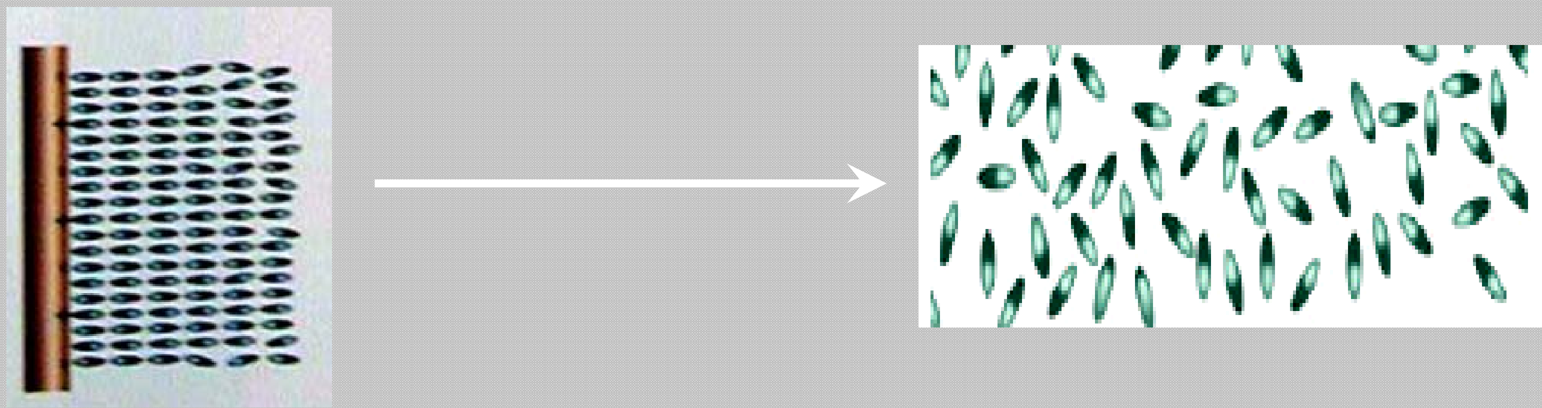
* Пограничная вода заряжена отрицательно относительно объемной воды (до -150 мв),

* Пограничная вода имеет спектр поглощения с максимумом при 270 нм,

* Толщина слоя пограничной воды возрастает при ее освещении светом видимой и особенно ИК-области спектра (пик при 3000 нм).

# IN ACCORDANCE WITH THEORY OF V.L. VOEIKOV

$2H_2O$ (EZ-water) + $O_2 \rightarrow O_2 + 2H_2O$ + $n*h\nu$ (Bulk water)



\+ Энергия электронного возбуждения (свободная энергия)


в этой области. Однако Герни соглашается с Фрэнком и Эвансом относительно разрушающего или укрепляющего действия катионов на структуру воды.

Модель, предложенная Фрэнком и Эвансом [50] для растворов электролитов, наметила некоторые разграничения в гидратации ионов, связанные с наличием двух участков молекул воды, на которые ион воздействует с различной степенью активности.

Более детальные разграничения были сделаны Дж. Бокрисом в 1949 г. [54]. В выполненной им работе показано, что одного

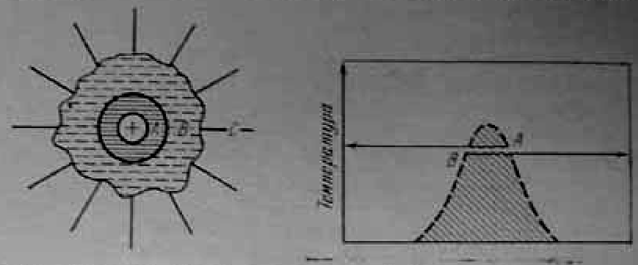



**Рис. IV.1.** Простая модель двухслойной гидратной оболочки иона

*A* — ион с прочно связанными молекулами воды; *B* — участок разрушенной структуры воды; *C* — структурно нормальная вода

**Рис. IV.2.** Схема структурных изменений в системе вода — кристаллогидрат

кулоновского взгляда на сольватацию (гидратацию) простых ионов недостаточно для удовлетворительного объяснения этого явления. Бокрис отмечает, что ион не только связывает определенное количество молекул из своего непосредственного окружения, но в результате ион-дипольного взаимодействия ориентирует молекулы растворителя, не входящие в ближайшее окружение. Такой подход дает возможность качественно характеризовать сольватацию как состоящую из двух частей: «первичная» и «вторичная». Первичная сольватация, согласно Бокрису, определяется прочным связыванием молекул растворителя ионом, который в процессе электролиза движется вместе с ними как одно целое.

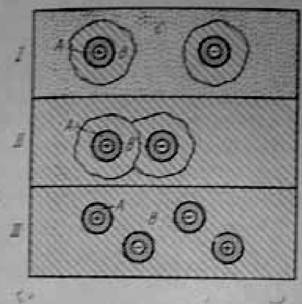
Вторичная сольватация определяется электростатическим взаимодействием иона с молекулами растворителя, что приводит лишь к некоторой упорядоченности в ориентации диполей воды, но отнюдь не к связыванию их. Эти два типа сольватации могут быть объединены понятием «общая сольватация» [54, стр. 174].

О. Я. Самойлов отмечает, что целесообразность выдвинутого Бокрисом разделения сольватации на две части не вызывает сомнения, и предлагает термины: «ближняя» и «дальняя» гидратации.

Н. Е. Хомутов [55] приходит к выводу, что ионы в растворах гидратированы и их движение под действием электрического поля

этом случае, по мнению П. А. Загорца [71], представления о границе полной сольватации значительно более оправданы.

Ю. В. Гуриков [72] считает, что весьма большая степень упорядоченности воды в слое *A* монотонно падает, «достигая предельного значения, соответствующего степени упорядоченности чистой воды, на достаточно большом удалении от иона — в слое *C*» [72, стр. 295]. Он полагает, что в чистой воде в среднем количество молекул, ориентированных по полю и против него, практически совпадает. Поэтому дезорганизующее влияние слоя *C* на слой *B* маловероятно и деструктурированность воды в слое *B* скорее всего



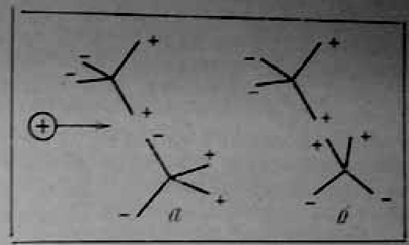




**Рис. IV.3.** Модель водного раствора в широком диапазоне концентраций

*I* — разбавленное состояние; *II* — критическое состояние; *III* — концентрированное состояние

**Рис. IV.4.** Схема взаимодействия катионов с молекулами воды из двух ближайших слоев

вызвана действием электростатического поля ионов на дипольные молекулы воды.

По мнению Гурикова, вблизи иона водородная связь между двумя соседними молекулами воды не только способствует созданию более прочной структуры, а, напротив, участвует в ее разрушении. Если, например, положительный ион окружен двумя соседними молекулами воды из первого и второго слоя его гидратной оболочки (рис. IV. 4, *а*), то между последними возникает водородная связь. В силу того, что протон находится в периферической части молекулы воды, положительный полюс оказывается расположенным ближе к иону по сравнению с отрицательным, и это приводит к некоторому увеличению свободной энергии системы.

Однако термодинамика указывает, что в равновесном состоянии энергия системы должна отвечать минимальным значениям. Поэтому в определенных условиях более выгодным может оказаться расположение, когда молекулы второго слоя переходят в новую конфигурацию (рис. IV.4, *б*). В этом случае вероятность образо-

# $O_2$ and $CO_2$ Solubility in Water (at 18-20 °C):

$O_2$ ~ **1** mM/L

$CO_2$ ~ **45** mM/L

and only **0.2%** of dissolved $CO_2$ converts to $HCO_3^-$ !!!

$$CO_2 + H_2O \longrightarrow HCO_3^-$$

and $[HCO_3^-] = \mathbf{0.09}$ mM/L

---------------------------------

**$CO_2$ Nanobubbles :**

$$(CO_2)_n@(HCO_3^-)_m * m\,H^+$$

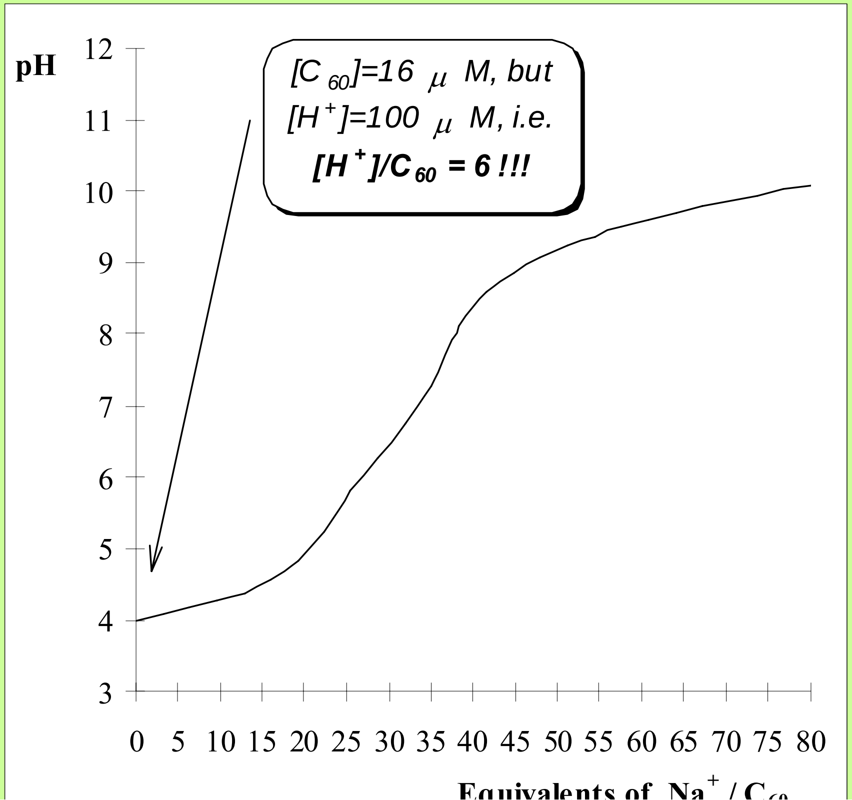
(with size app. **100 nm**)

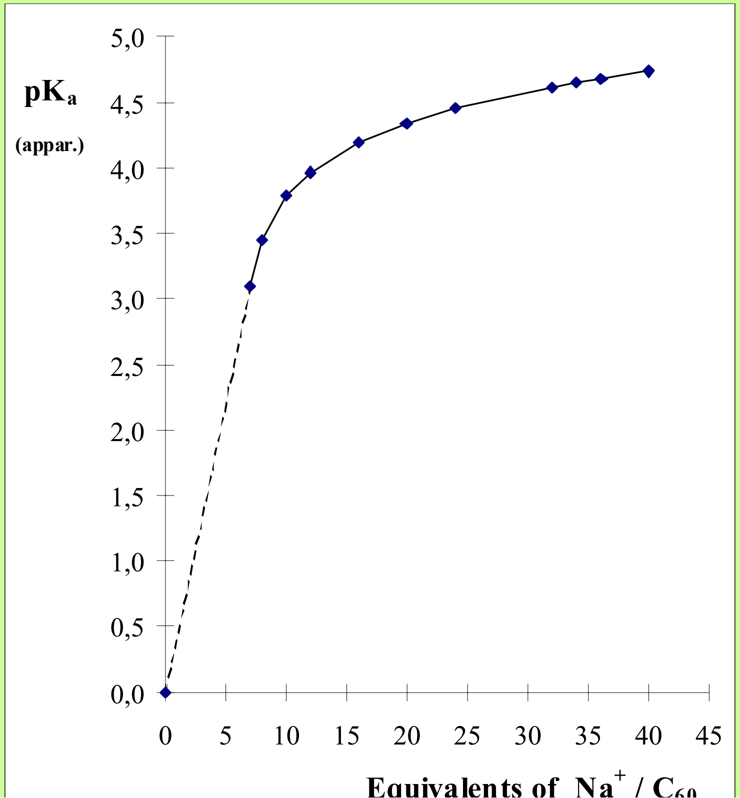
**F. Jin, J. Ye, L. Hong, H. Lam, C. Wu. Slow Relaxation Mode in Mixtures of Water and Organic Molecules: Supramolecular Structures or**

# $CO_2$ Solubility in Water Ordered by $C_{60}$HyFn is More in 5 - 7 Time than in Pure Water

**The changes of acid-basic properties of $C_{60}$ HyFn (in $H^+$- form) at the titration of their aqueous solution by NaOH**

***$[C_{60}]$ = 16 μMol/L; [NaOH] = 10 μMol/L***
***(with the deduction of $CO_2$ titration in deionized water)***

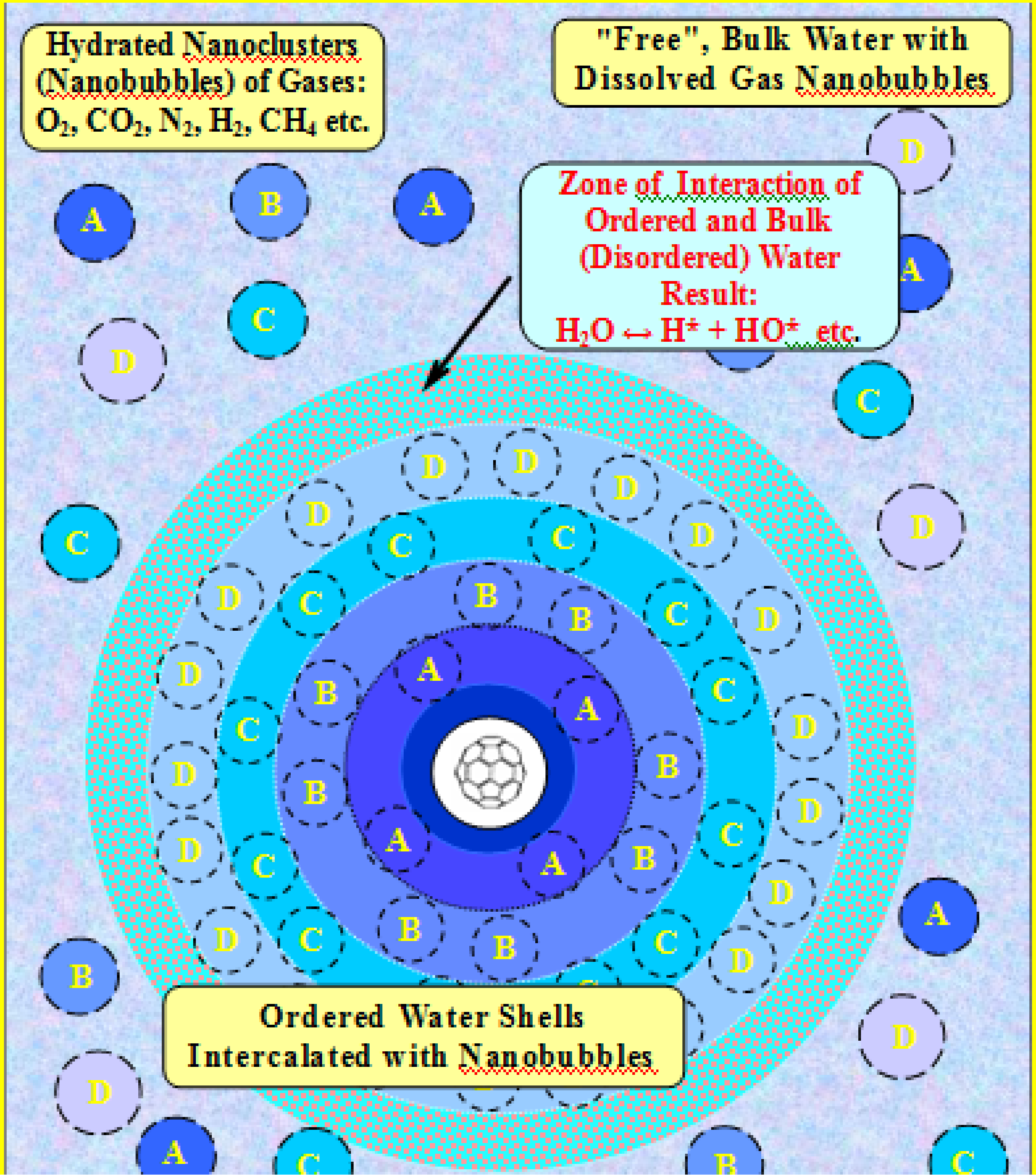


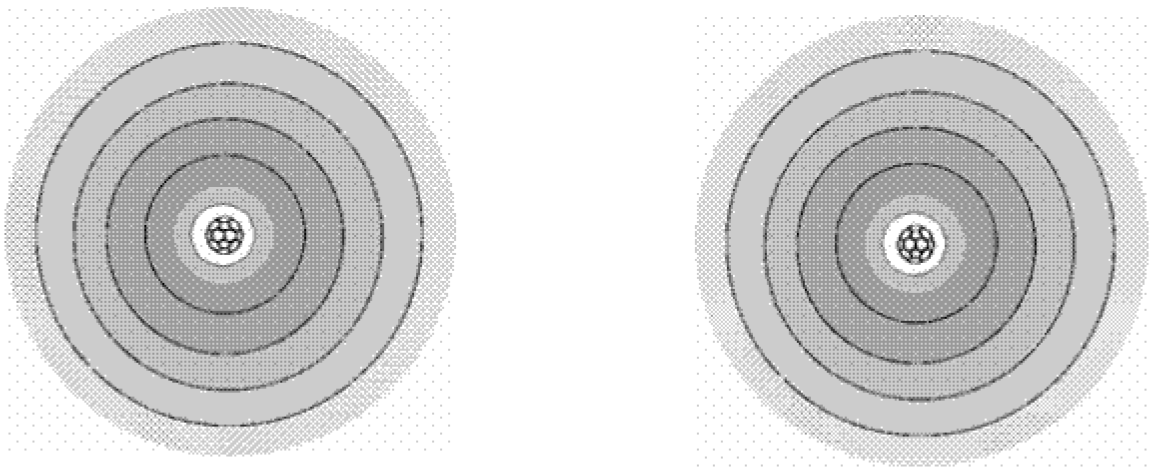
## STRUGGLE of the ORDER AGAINST a DISORDER

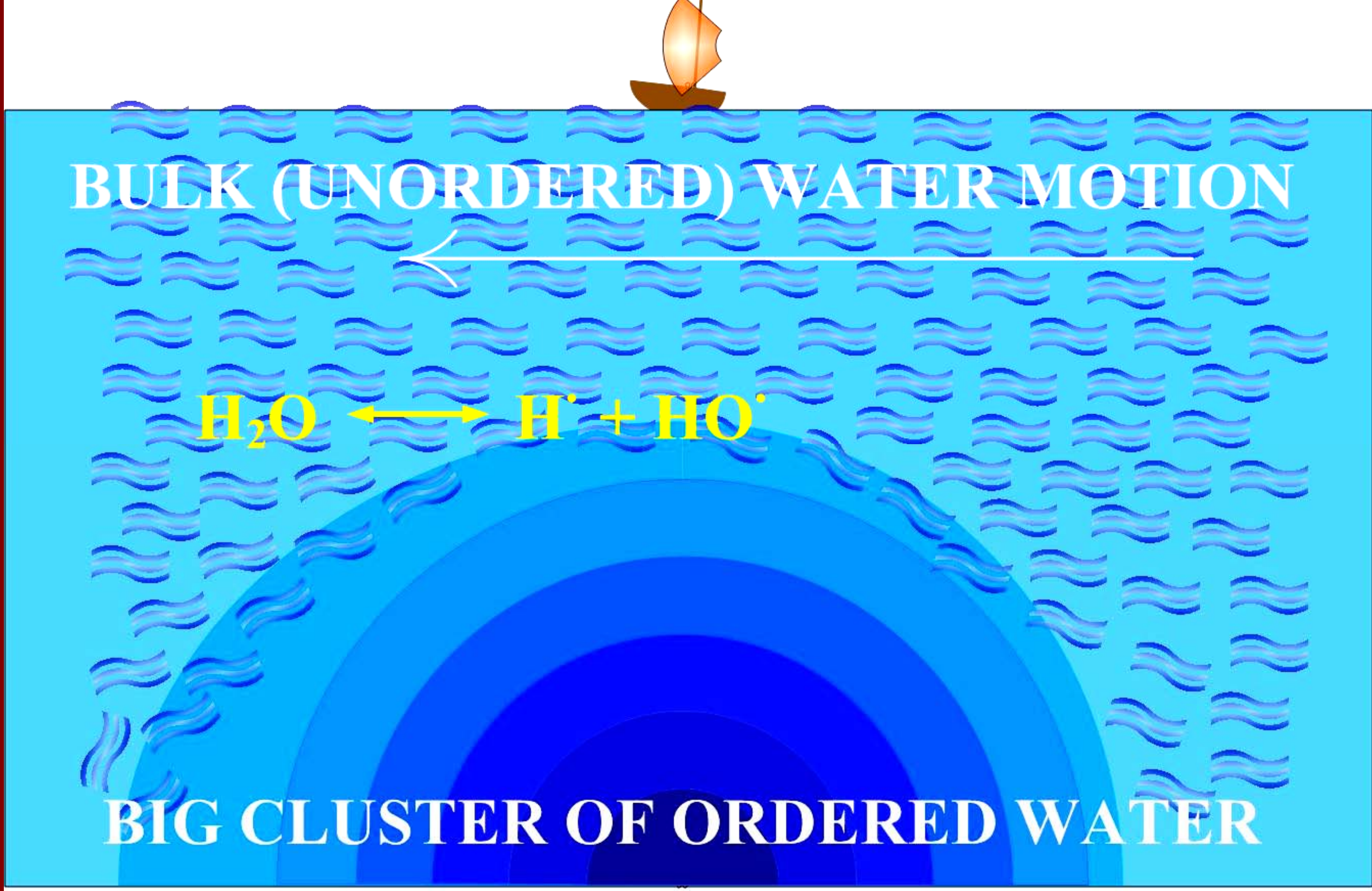
or

## Movements of Ordered Water Cluster in Bulk Water Can Stimulate on It Diffusive Surfaces the Dissociation of Water Molecules with Formation of Quickly Recombining H-, O-, C-, N-Containing Radicals.



# Water Nanoclusters "Collisions" Can Generate On/In Their Diffusive Surface Redox Reactions Between Water Molecules as well as Produce the Free Radical Forms of Oxygen (FR and ROS), $e_{solv}$, etc. …






$$(H_2O)_n(H_2O \leftarrow H\text{--}OH \rightarrow OH_2)(H_2O)_m \quad \rightarrow$$

$$\rightarrow \quad (H_2O)_n(H_2O + H^{\cdot} + {}^{\cdot}OH + OH_2)(H_2O)_m$$

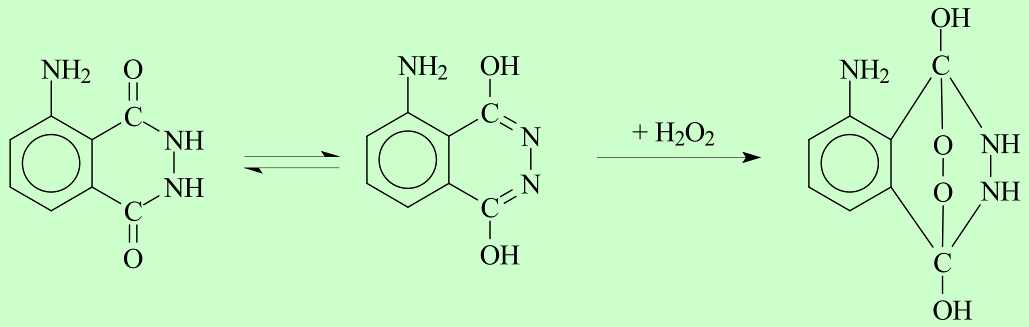
$$CO_2 + {}^{\cdot}OH \rightarrow HCO_3^{\cdot} \qquad O_2 + {}^{\cdot}H \rightarrow HO_2^{\cdot}$$

$$2\,{}^{\cdot}OH \rightarrow H_2O_2 \qquad 2H_2O_2 \rightarrow 2H_2O + O_2 \quad \text{etc.}$$

**Гидратированные фуллерены создают в своем окружении упорядоченную, структурно гетерогенную водную среду, в которой направленность и кинетика химических и биохимических процессов отличается от таковых, происходящих в чистой (неупорядоченной) воде.**

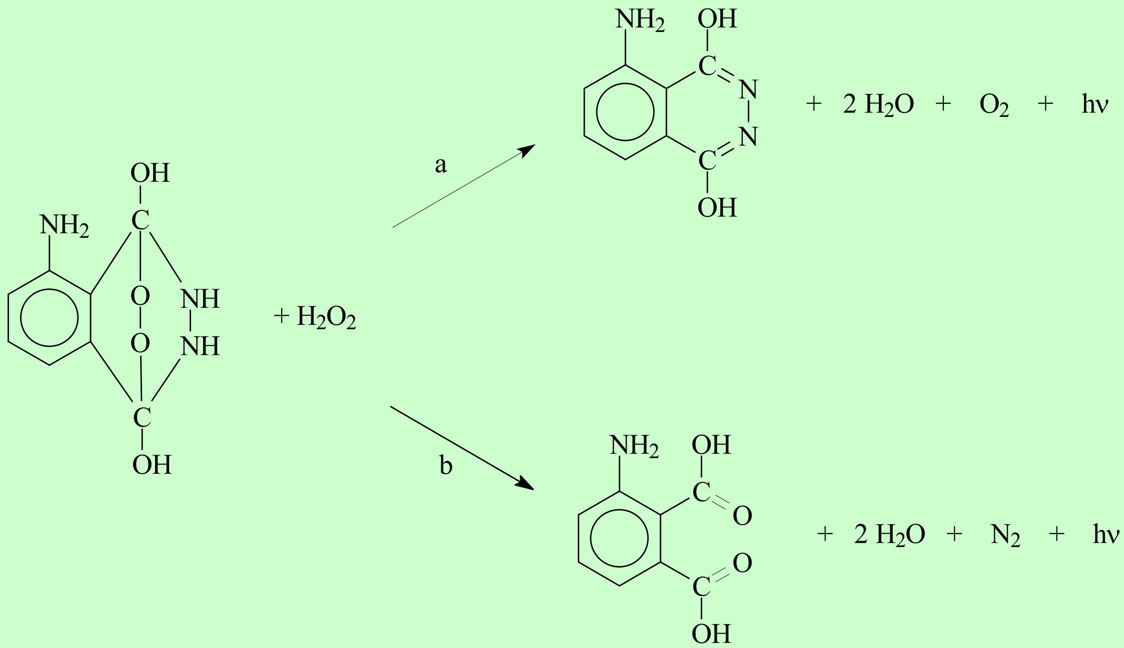
**Hydrated fullerenes create in water medium the ordered, structurally heterogeneous environment in which chemical and biochemical processes go differently than in pure (unordered) water.**

# PROBABLE REACTIONS OF LUMINOL OXIDATION BY HYDROGEN PEROXIDE




**LUMINOL** **LUMINOL PEROXIDE**

**Non¯luminescent substance** **Stromgly luminescent sudstance**



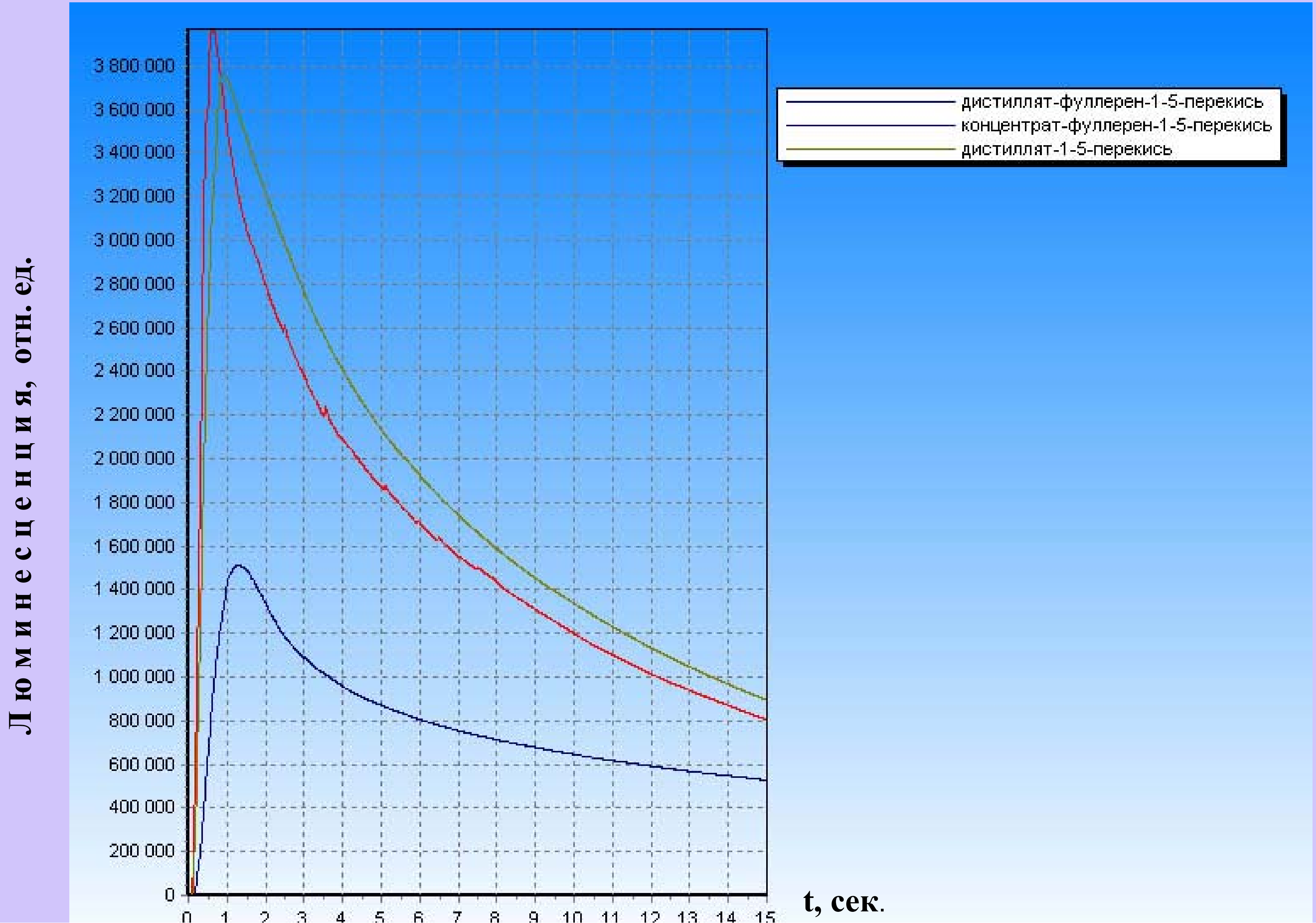

**Weakly luminescent sudstance**

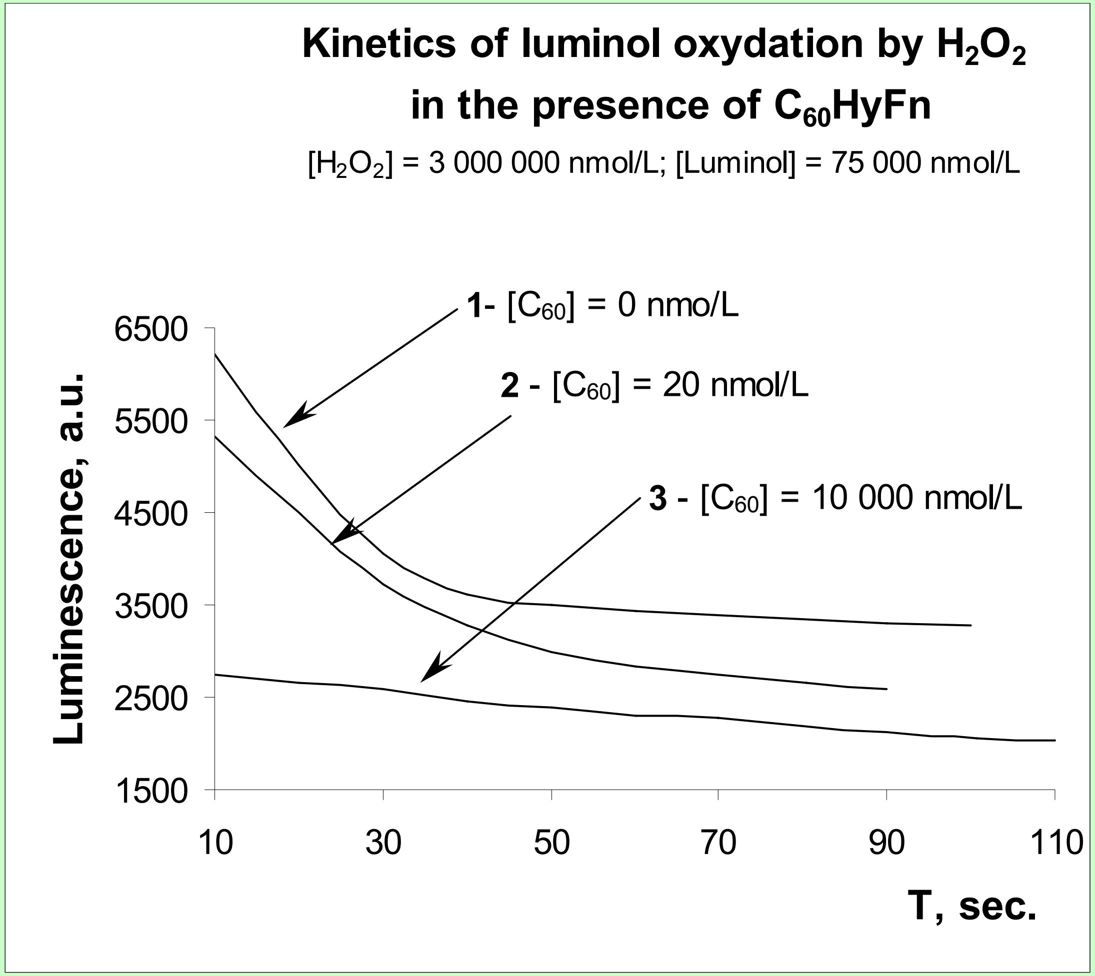
# КИНЕТИКА ЛЮМИНОЛЬНОЙ РЕАКЦИИ В ЧИСТОЙ ВОДЕ И В ПРИСУТСТВИИ ГИДРАТИРОВАННЫХ $C_{60}$ ФУЛЛЕРЕНОВ (HyFn)

(прибор "ЛИК", НИИ "БИНАР», Россия)

**$H_2O_2$ 3x10$^{-3}$ М/л; Luminol 75x10$^{-6}$ М/л**

**HyFn: *1* - 0 М/л; *2* - 5x10$^{-9}$ М/л; *3* - 5x10$^{-7}$ М/л.**





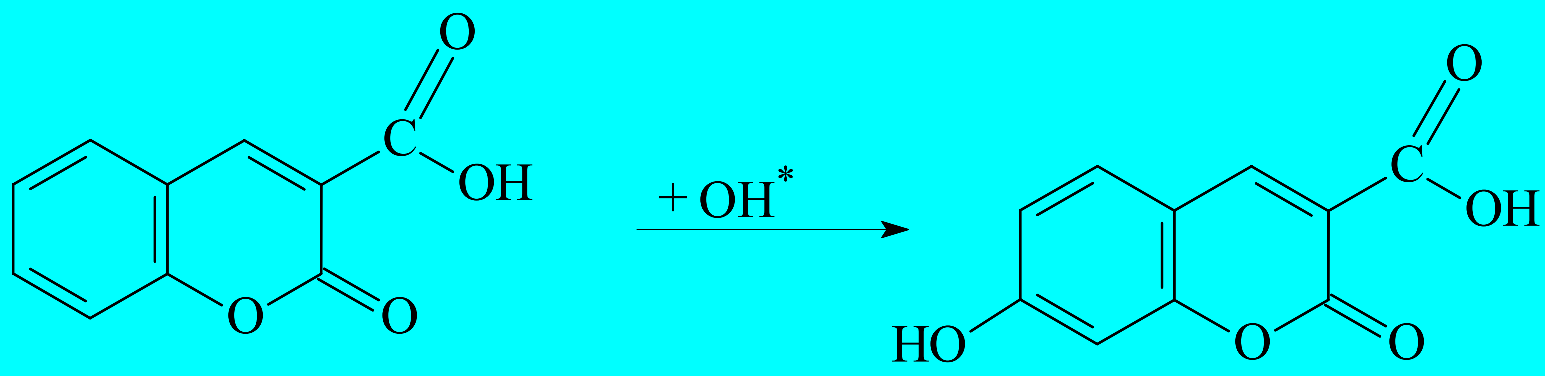

| Curve № | $C_{60}$ concentration, nmol/l | Molar ratio | | % inhibition of reactions |
|---|---|---|---|---|
| | | Luminol / $C_{60}$ | $H_2O_2$ /$C_{60}$ | |
| 1 | 0 | --- | --- | 0% |
| 2 | 20<br>(3) | 3 700<br>(25 000) | 150 000<br>(1 000 000) | 15% in serum and in water |
| 3 | 10 000<br>500 | 7,5<br>150 | 300<br>6000 | 44% in serum<br>61% in water |

# WATER RADIOLYSIS and CCA OXIDATION by MEANS of HYDROXYL RADICALS ($OH^*$)

$$H_2O \xrightarrow{h\nu\ (\gamma)} H_2O^*$$

$H_2O^* \rightarrow OH^*$ (~ 4.8 - 4.9 %)

$H_2O^* \rightarrow H^* + e^-_{hydr}$ + $H_2 + H_2O_2 + H_3O^+$ + **ROS** (Reactive Oxygen Species) (~ 95 %)



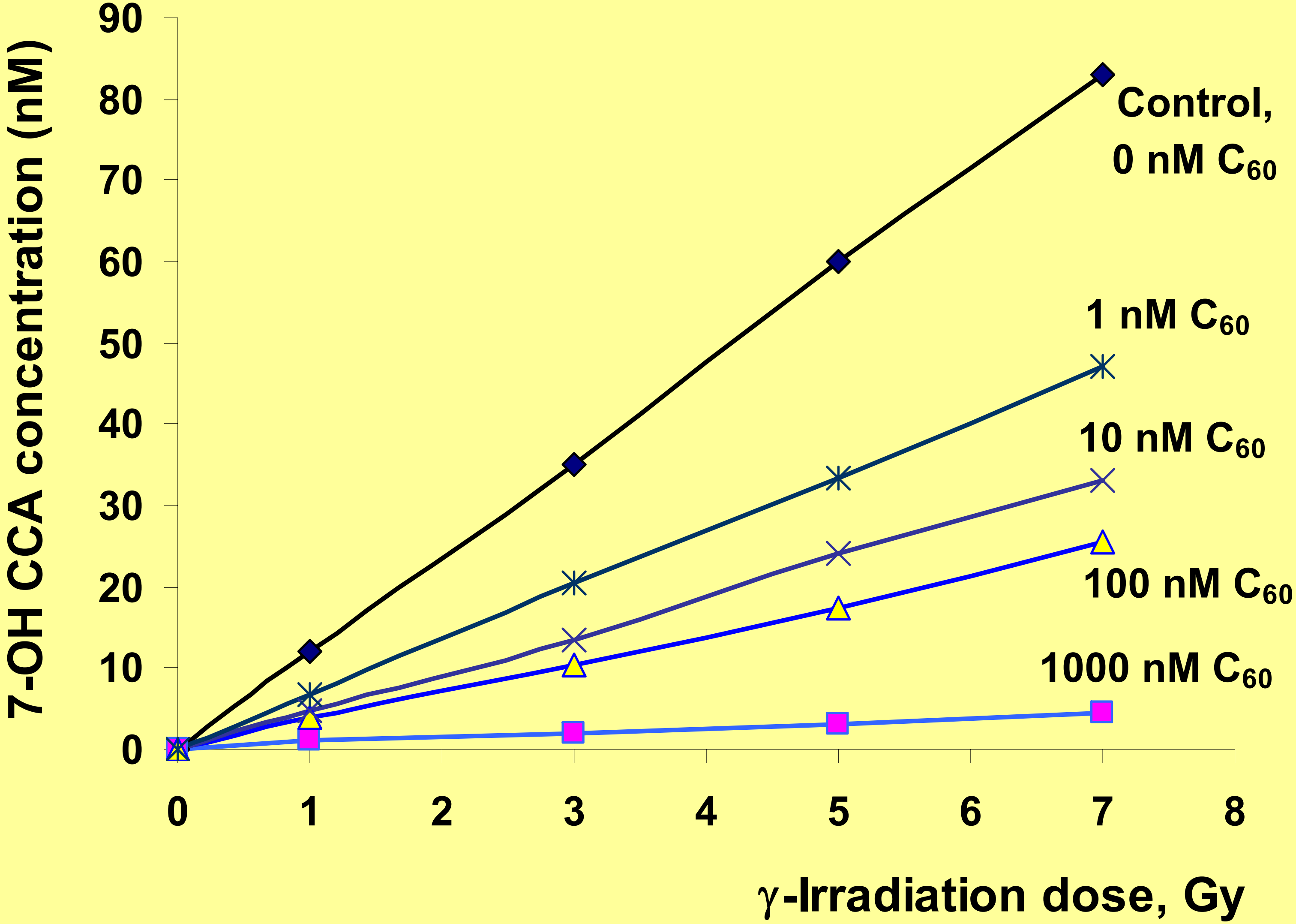

**CCA**

(coumarin-3-carboxylic acid)

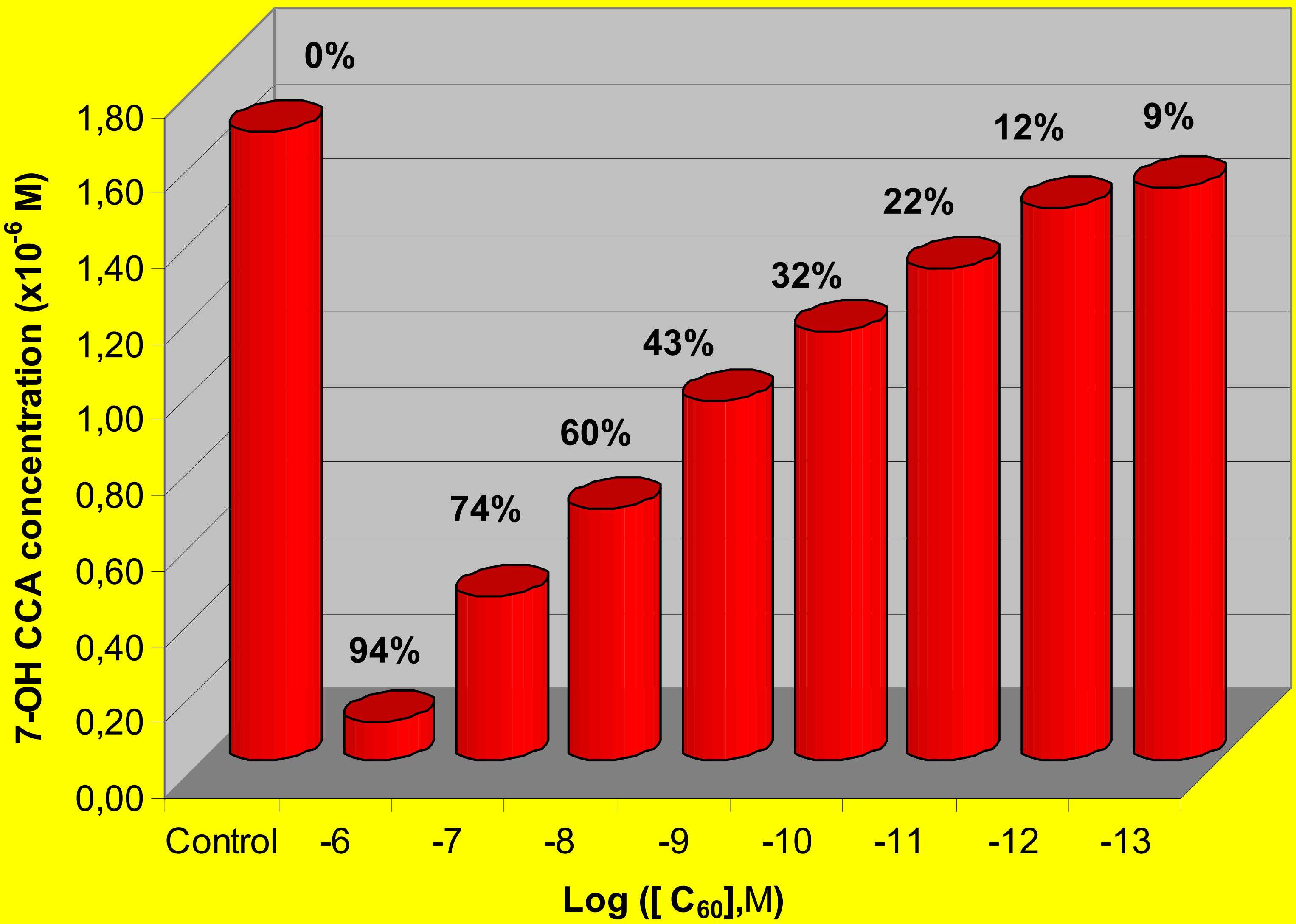
**7-OH CCA**

**Strongly luminescent substance**

# Neutralization of Hydroxyl-Radicals ($OH^*$) Formed by Means of γ-Rays Radiolysis of Water at Presence of Various $C_{60}$HyFn Concentrations



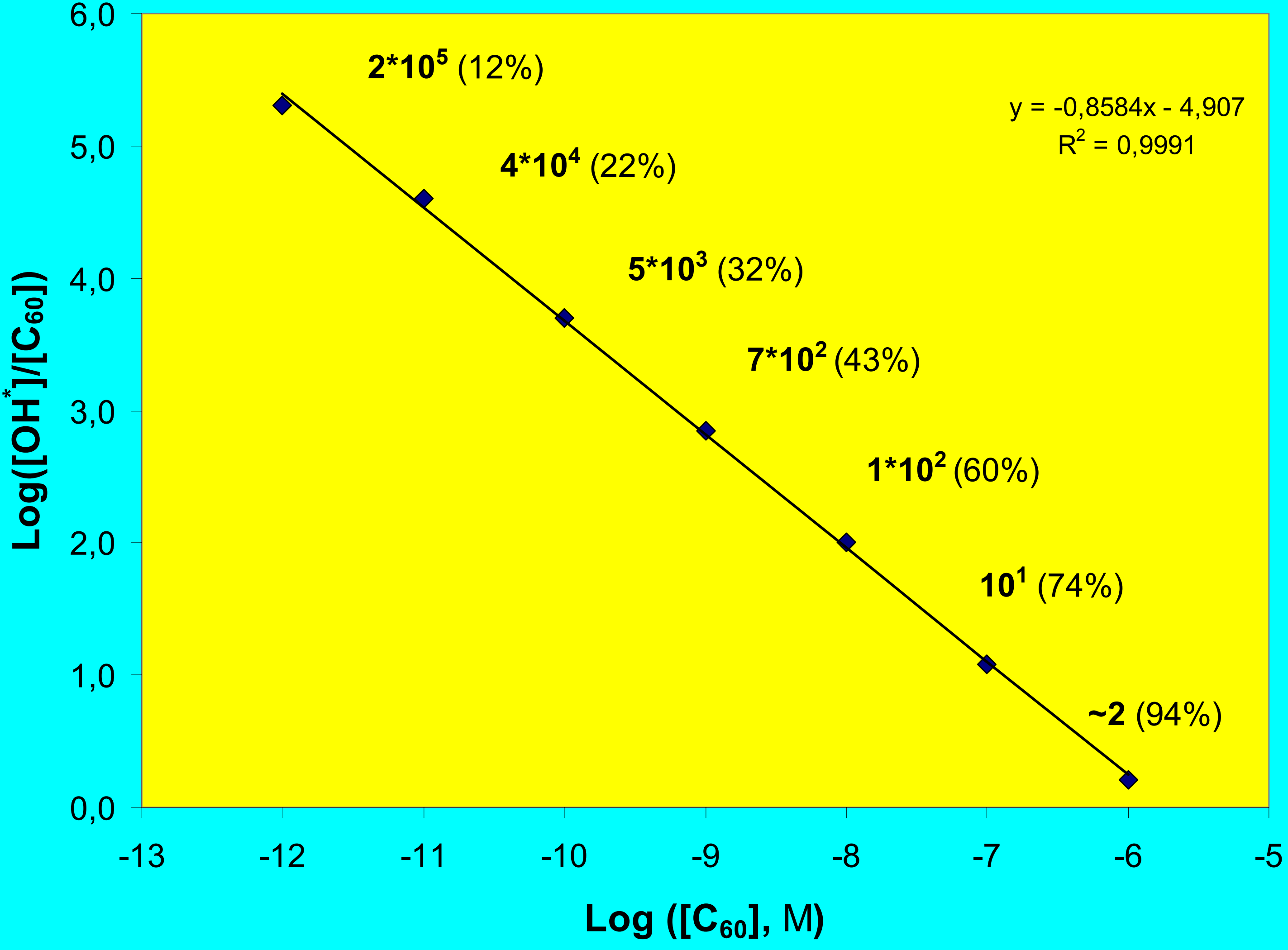
# % Neutralization of Hydroxyl-Radicals (OH*) Formed by Means of 7 Gy Dose of γ-Irradiation of Water at Presence of Various $C_{60}$HyFn Concentrations



# $C_{60}$HyFn Antiradical Efficiency ( *[OH]*/[C$_{60}$]* ) Increases When its Concentration are Decreased

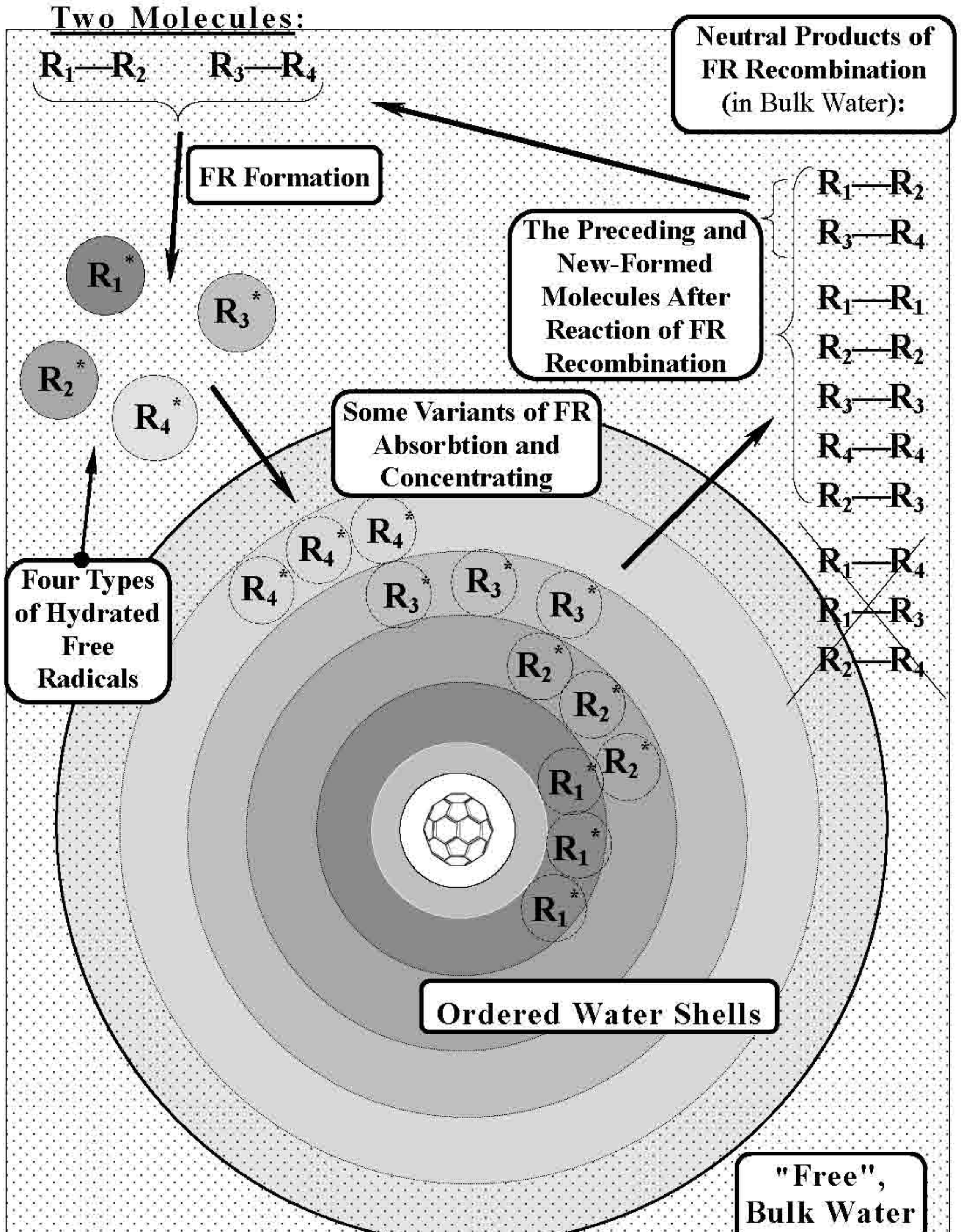
Absolute values of $[OH^*]/[C_{60}]$ ratio and % of ˙OH neutralized are indicated near dots of graph

(by estimation of the 7-OH-CCA formation at 7 Gy Dose of γ-irradiation)




G.V. Andrievsky, V.I. Bruskov, A.A. Tykhomyrov, S.V. Gudkov. PECULIARITIES OF THE ANTIOXIDANT AND RADIOPROTECTIVE EFFECTS OF HYDRATED $C_{60}$ FULLERENE NANOSTUCTURES *IN VITRO* AND *IN VIVO*. *Free Radical Biology & Medicine*, 47 (2009) 786–793

**Probable Scheme of Processes of Free Radical (*FR*) Absorbtion, Concentration and Recombination Under the Influence of the Ordered Water Structures Formed Around the Hydrated $C_{60}$ Fullerenes (*HyFn*).**



**Антиоксидантная Активность Гидратированных Фуллеренов обусловлена их способностью регулировать в водной среде свободно-радикальные процессы (реакции) «разумным» образом.**

**Antioxidat Activity of Hydrated Fullerenes is stipulated their ability to regulate free-radica processes in aqueous medium by ‘wise’ manner.**

**VARIOUS SORT OF HYDRATED NANOPARTICLES WITH THE SIZES FROM 3 UP TO 30 nm CAN POSSESS BIO-ANTIOXIDANT PROPERTIES BUT WHICH, IN VIEW OF THEIR CHEMICAL NATURE, DO NOT ABLE TO PARTICIPATE DIRECTLY IN CHEMICAL REACTIONS WITH FREE RADICALS.**

**SUCH IS CONNECTED WITH THE NATURAL WATER ORDERED STRUCTURES, IN WHICH THE SIMILAR NANOPARTICLES ARE CAPABLE TO BE EMBEDED AND SUPPORT THEM.**

**The similar facts are found out for the following nanoparticles:**

- **- hydrated $C_{60}$ fullerene and it fractal associates with size 3.4, 7.1, 10.9, 14.5, 18.1, 21.8, 25.4, 28.8, 32.4, 36.0 nm** [*1*];
- **- $CeO_2$ with size 3-5 и 7, 20, 30-50** nm [*2, 3*];
- **- $Y_2O_3$ с размерами 6 и 12 нм** [*4*];
- **- Ag with size ~ 15 nm** [*5*];
- **- nanodimond with size 3.4-3.8 (2-10) nm** [*6*].
- **- $BaTiO_3$ (?) with size 10-100 nm** [7]


[1] G.V. Andrievsky et al. *Fullerenes, Nanotubes and Carbon Nanostructures*, 13 (4), (2005) 363.

[2] S. Babu et al. *Chem. Phys.Letters*, 442 (2007) 405.

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[5] J. Tian et al. *ChemMedChem*, 2 (1) (2006) 129.

[6] A.M. Schrand et al. *J. Phys. Chem. B*, 111 (1) (2007) 2.

[7] Y. Katsir et al., *J. Electrochem. Soc.*, 154 (4) (2007) D249.

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# Nanostructures of hydrated $C_{60}$ fullerene ($C_{60}$HyFn) protect rat brain against alcohol impact and attenuate behavioral impairments of alcoholized animals

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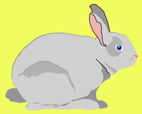
**Abstract**

It is well known that chronic ethyl alcohol (EtOH) consumption is capable to injure brain cells and to cause essential abnormalities in behavioral characteristics of animals addicted to alcohol. In this work we for the first time have shown that administration of aqueous solutions of hydrated $C_{60}$ fullerenes ($C_{60}$HyFn) with $C_{60}$ concentration of 30 nM as a drinking water during chronic alcoholization of rats (a) protects the tissues of central nervous system (CNS) from damage caused by oxidative stress with high efficacy, (b) prevents the pathological loss of both astrocytes (the main cells of CNS) and astrocytic marker, glial fibrillary acidic proteins (GFAP) and, as consequence, (c) due to their adaptogenic effects, $C_{60}$HyFn significantly improves behavioral response and eliminates emotional deficits induced by chronic alcohol uptake. The wide range of beneficial biological effects, zero-toxicity, and efficacy even in super-small doses provide a rationale for the possible application of $C_{60}$HyFn for the treatment of alcohol-induced encephalopathy as well as alcoholism prophylaxis.



*Keywords:* Ethanol; Fullerenes; Hydrated $C_{60}$ fullerene

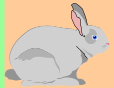
# Basic Biological Properties of Aqueous Solution of the Hydrated $C_{60}$ Fullerene (HyFn) Detected to the Present Time



***In Vivo*** **Experiments reviled that HyFn with $C_{60}$ Doses from Super Small and up to 25 mg/kg of b.w.:**

1. Are non-toxic, not immunogenic, not allergenic;
2. Increase resistance of plasma membranes to damaging factors;
3. Positively influence on antioxidant and energetic systems of organism;
4. Possess radioprotective properties due to suppression of excess level of free radicals;
5. Possess antihistaminic and allergy suppressing abilities, i.e. act as anti-inflammatory agents,
6. Positively influence on activity of adrenergic, GABA-, hystaminic- and, especially, serotoninergic systems and, as a consequence, enhance adaptogenic functions of organisms;
7. Show positive neuroprotecting (e.g., at Alzheimer's disease) and non-specific analgesic effects;
8. Not killing tumor cells, inhibit of the cancer pathologies development;
9. Possess anti-atherosclerotic (anti-atherogenic) properties;
10. Are able to protect against viral infection (e.g., Influenza, etc..): *(HyFn do not kill viruses and microbes, but do not allow them to incorporate into cells).*

***In Vivo* эксперименты** **выявили, что HyFn в широком диапазоне суммарных доз $C_{60}$ (от супермалых и вплоть до 25 мг/кг веса тела):**



1. **Не токсичны, не иммуногенны, не аллергенны;**
2. **Повышают устойчивость плазматических мембран к повреждающим факторам;**
3. **Оказывают положительное влияние на антиоксидантные и энергетические системы;**
4. **Обладают радиопротекторными свойствами за счет подавления избыточного уровня свободных радикалов;**
5. **Обладают сильным и долговременным антигистаминным и противоалергическим действием, т.е. способны работать, как противовоспалительные агенты;**
6. **Оказывают положительное влияние на активность адрено-, ГАМК-, гистамино- и, особенно, серотонин-эргических систем и, как следствие, повышают адаптогенные функции организма;**
7. **Обладают мощной гепатопротекторной активностью;**
8. **Оказывают выраженное нейропротекторное (в т.ч., при болезни Альцгеймера ) и неспецифическое анальгетическое действие;**
9. **Не убивая раковые клетки, тормозят развитие опухолевых патологий;**
10. **Обладают антиатеросклеротическими (антиатерогенными) свойствами;**
11. **Способны защищать организмы от инфицирования вирусами (напр., вирусами гриппа) *(HyFn не убивают вирусы, но не позволяют им эффективно проникать внутрь клетки)*;**
12. **Улучшают и повышают репродуктивные (детородные) функции.**

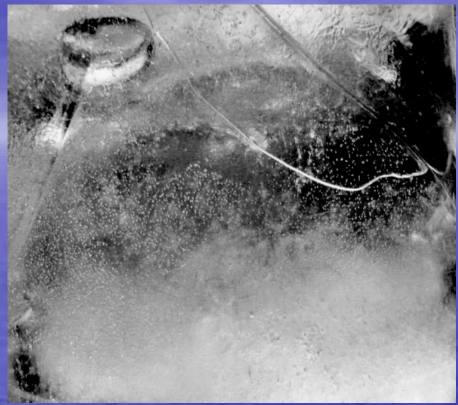
# FULLERENE WATER SOLUTION (FWS) FOR PRECLINICAL TESTING AND CLINICAL TRIALS IN 2009



# IMAGES OF ACI FROM:

## MINERAL WATER:

"ROGANSKAYA", Kharkov, Ukraine
TDS 300 mg/L



ALPINE WATER "EVIAN"
TDS 270 mg/L

"MORSHINSKAYA", Ukraine
TDS 80 mg/L

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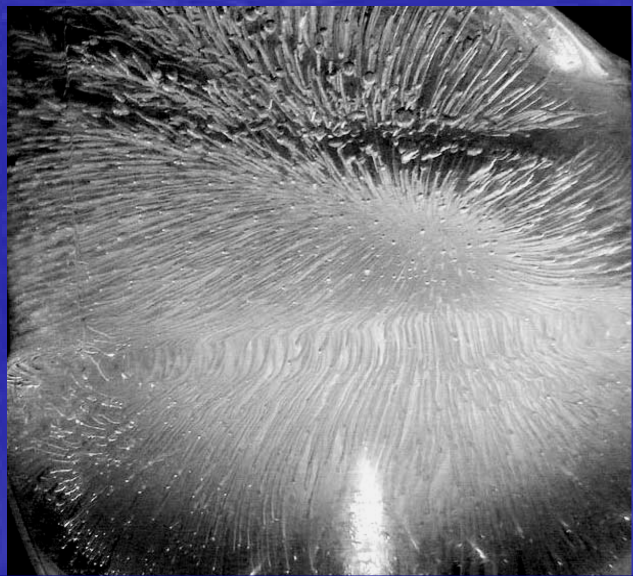
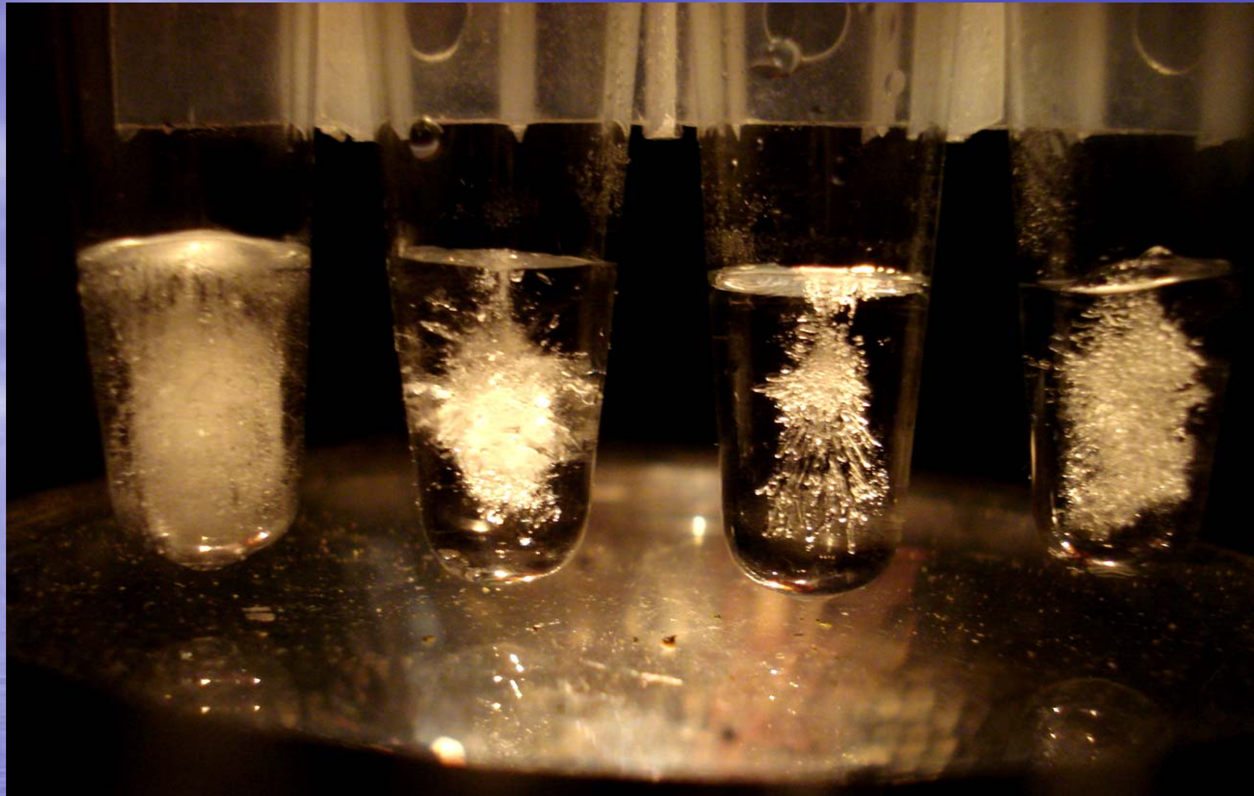
TAP WATER, Kharkov, Ukraine
TDS 450-500 mg/L



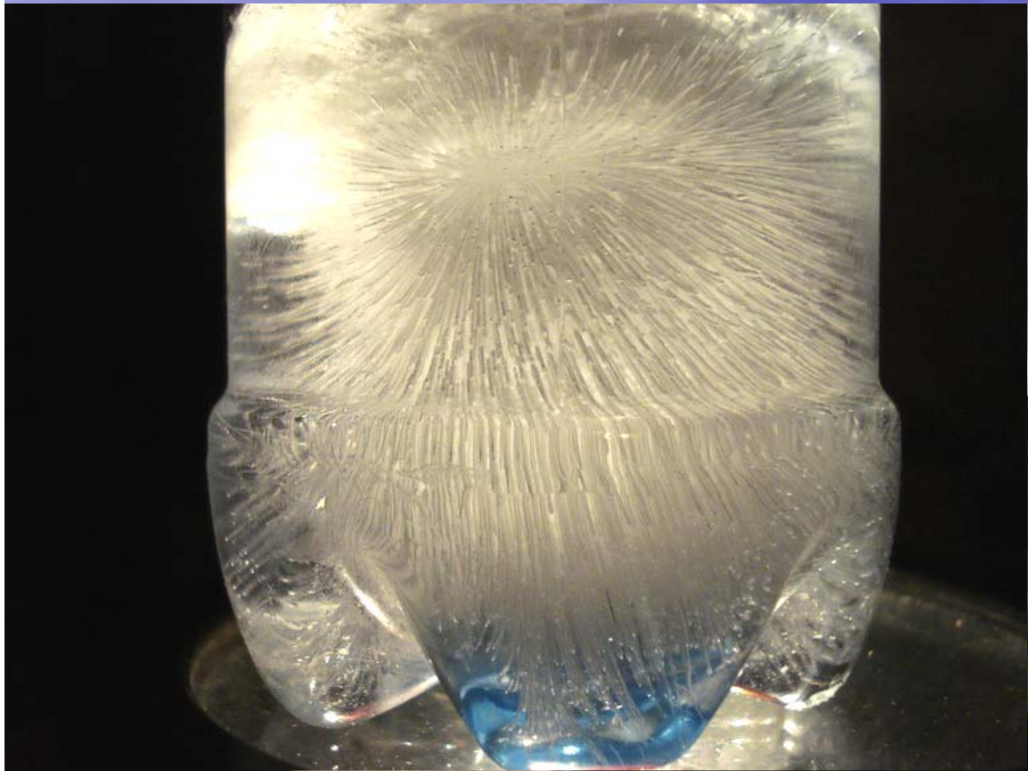
"FULLERENE WATER"

IPAC LLC, Kharkov, Ukraine
TDS 0-2 mg/L

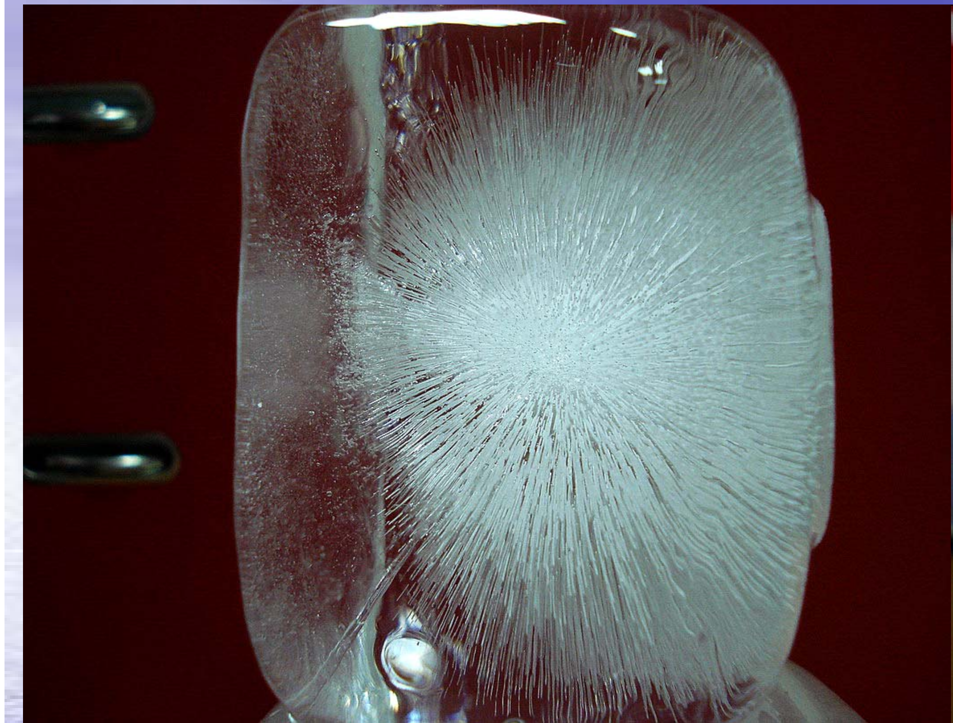


ICE FROM: TAP WATER, DI WATER, FULLERENE WATER

# FULLERENE WATER ICE



# ICE FROM FULLERENE WATER and BENZENE ($C_6H_6$)



In the words of A. Szent-Gyorgyi,

**“Life is water dancing to the tune of solids”.**

**(Жизнь - это есть вода, танцующая под мелодию твердых материй)**

but in the words of G. Andrievsky,

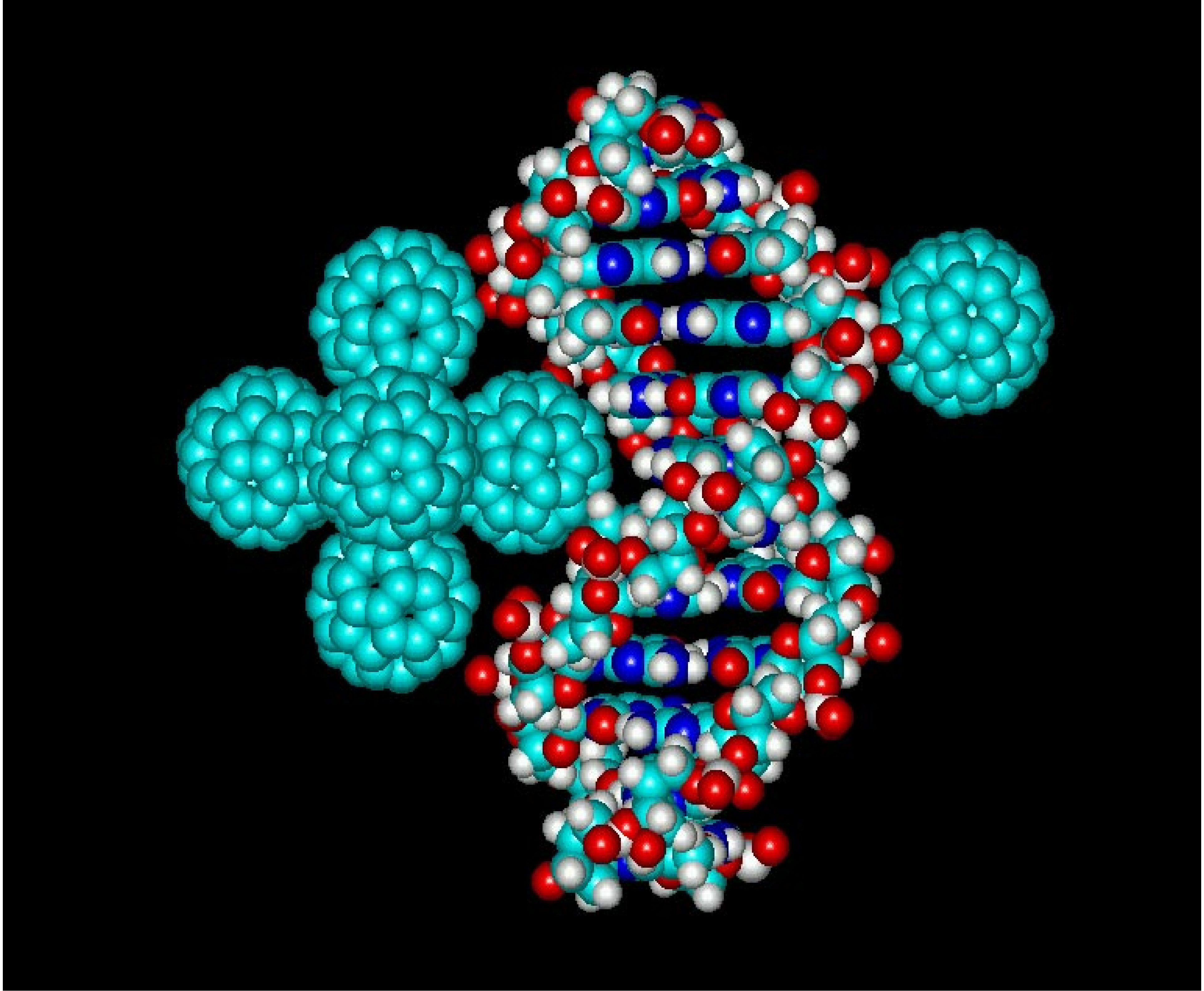
**“Life is the carbon-contained solids dancing to the tune of ordered structures of water”.**

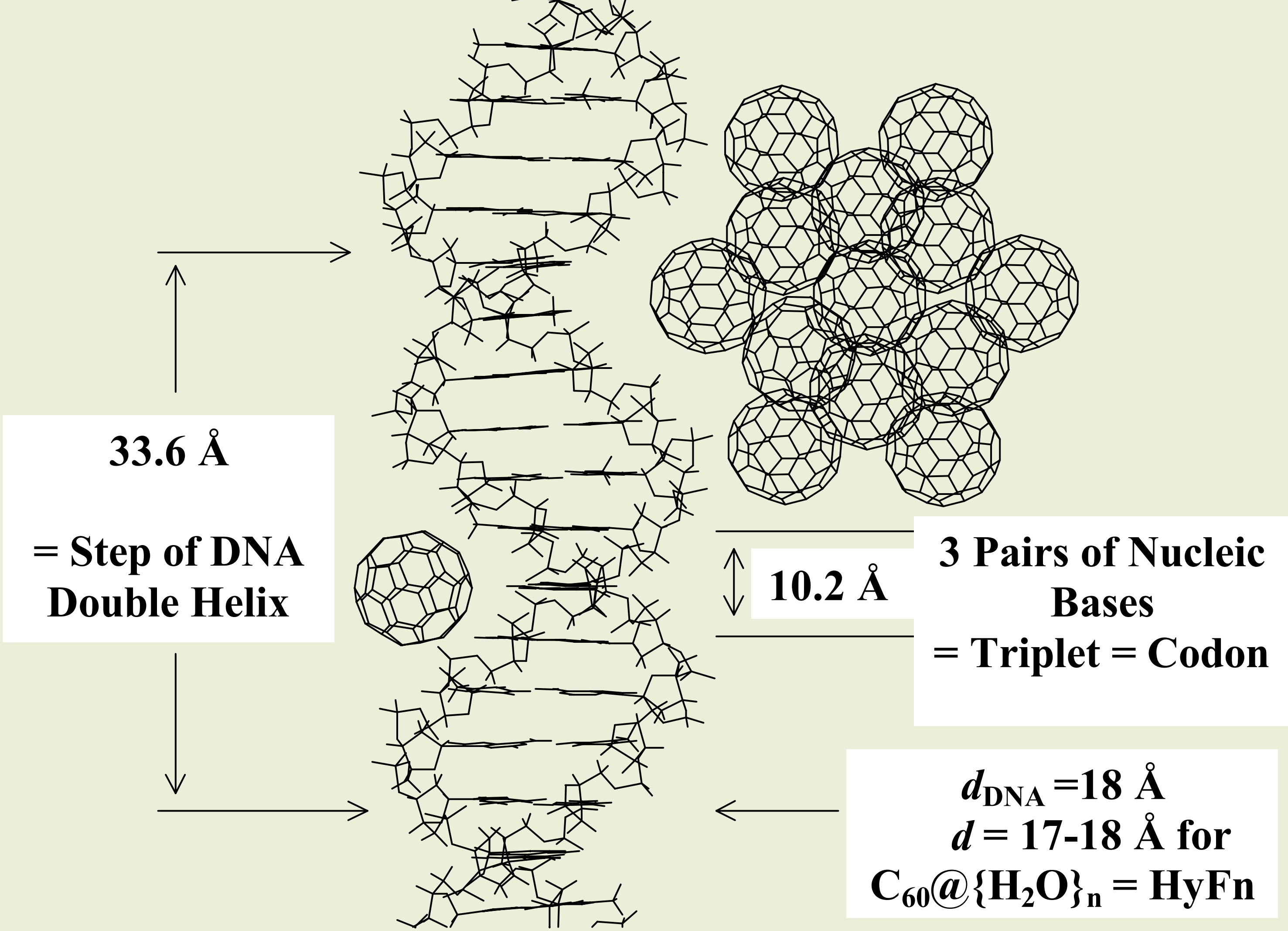
**(Жизнь - это есть углеродсодержащие твердые материи, танцующие под мелодию упорядоченных структур воды)**





THANKS
FOR YOUR
ATTENTION!!!
СПАСИБО
ЗА
ВНИМАНИЕ!!!





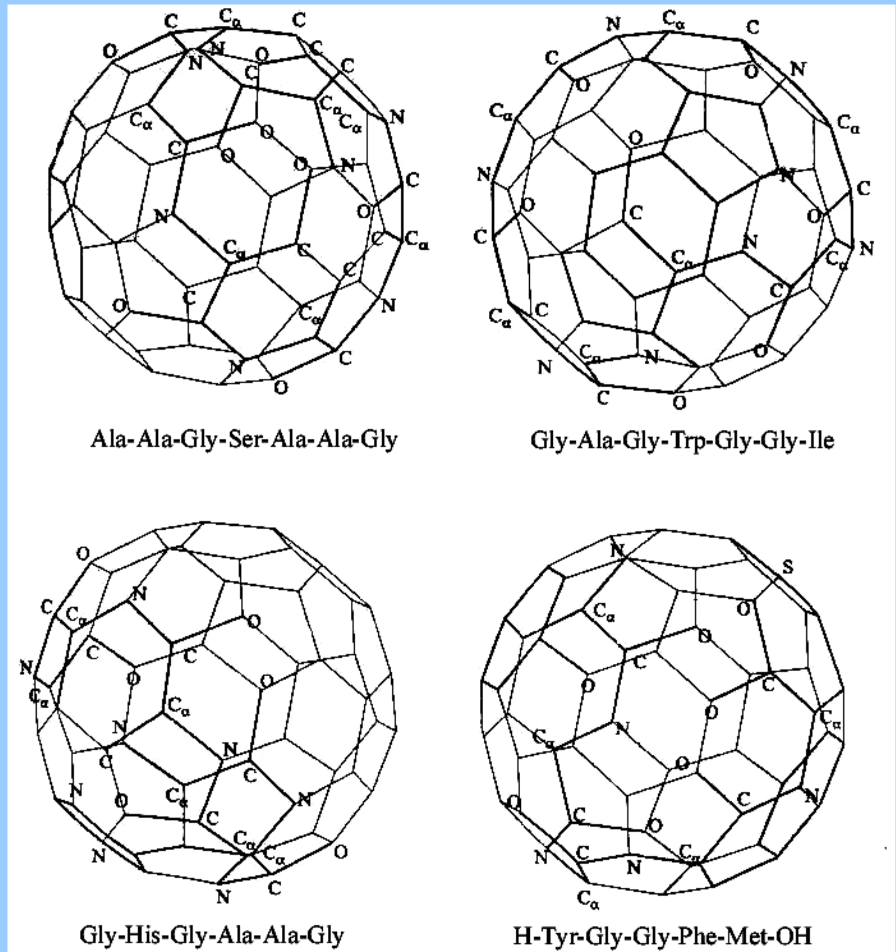

**Correspondence of the DNA Segment Structure in *B*-Form to the Geometry of $C_{60}$ Molecule (10 Å) and First Spherical Cluster of Hydrated $(C_{60})_{13}$ with Diameter of ~34 Å**

**HYDRATED FULLERENES (*HyFn*) PROTECT NATIVE STRUCTURES of HYDRATED BIOMOLECULES (*DNA, PROTEINS, ENZYMES…*) from DENATURING INFLUENCES and INCREASE the TEMPERATURE of their DENATURATION (**on 4-10º C**).**

These Effects are Stipulated by the Native Structure Stabilization of Biomolecules due to Integration (Merging) of their Hydrated Layers with the Similar Water Shells of HyFn.

## Peptides with Matrix of $C_{60}$ Fullerene Structure.

*[by means of Computer Simulation in: "Biocompatibility of Fullerene $C_{60}$ with Oligopeptides Using a Comparative Analysis of their Spatial Form".*
*Baranov A.A., Esipova N.G. Biofizika 45 (2000) 801-808 (Russ)].*



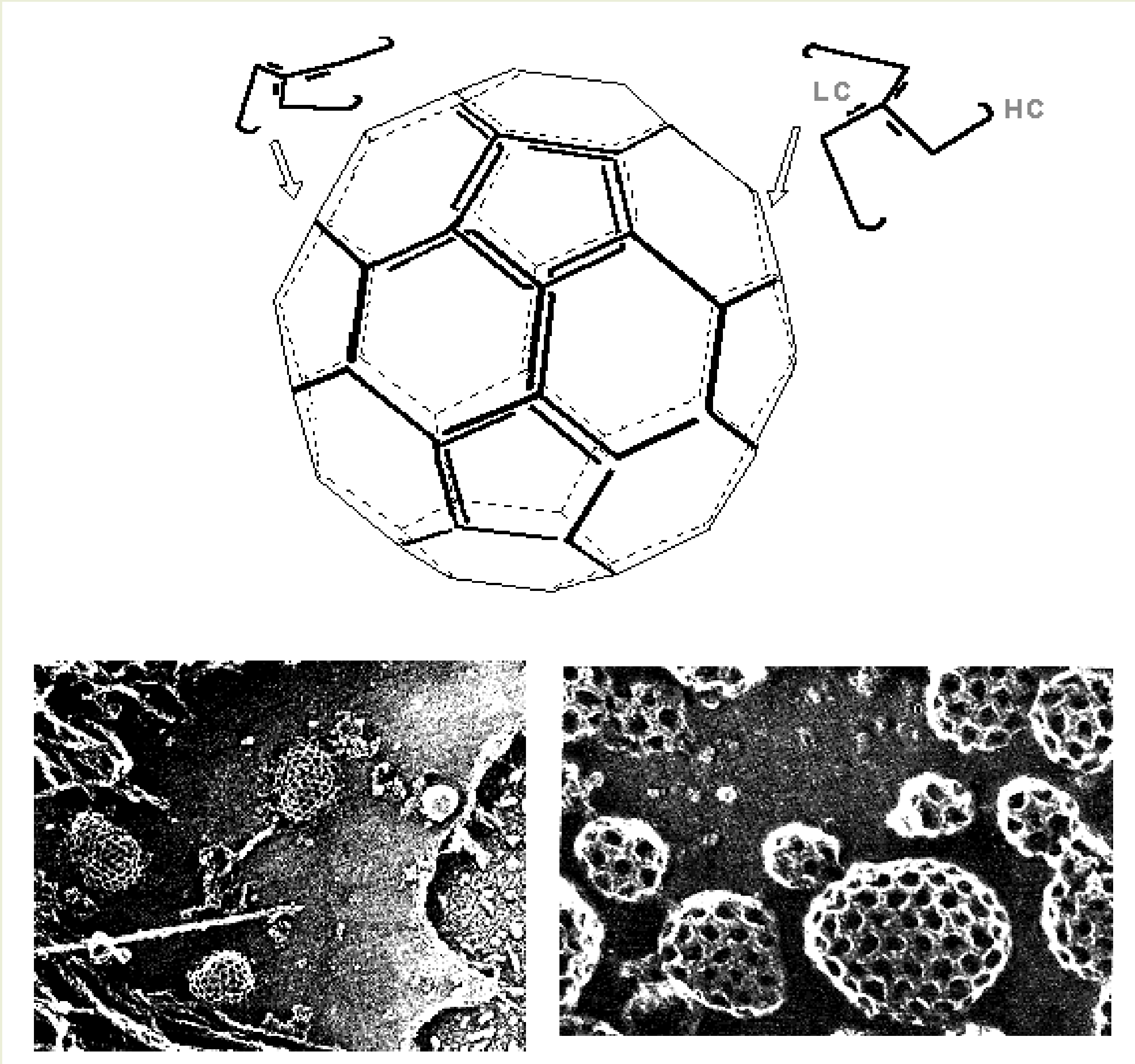

**The Similar Amino Acid Sequences have been Identified in Structures of More than 280 Natural Proteins*, particularly in:**

- *Lethal (1) Discs Large-1 Tumor Suppressor Protein;*
- *Major DNA-Binding Protein;*
- *Glucan Endo-1,3-β-Glucosidase Precursor and Galactokinase (EC);*
- *DNA-Polymerase;*
- *Dehydrin, DHN1;*
- *Met-Enkephalin and Corticotropin-Lipotropin Precursor (Pro-Opiomelanocortin);*

## Their Packing Arrangement in a Cage

***(HC, protein heavy chain; LC, protein light chain).***

Reproduced from Harisson S.C. & Kirchhausen T., Cell, 33 (**1983**) 650 and cited by Wileman T., Harding C., Stahl P.<u>, Receptor-Mediated</u> <u>Endocytosis</u>. *(A Reviw Article)*, Biochem. J., 232 (**1985**) 1-14.




## Intracellular Membranes Surface with Lipid

***"TWO THINGS ARE INFINITE: THE UNIVERSE AND HUMAN STUPIDITY; AND I'M NOT SURE ABOUT THE UNIVERSE"*** **(**Albert Einstein **)**

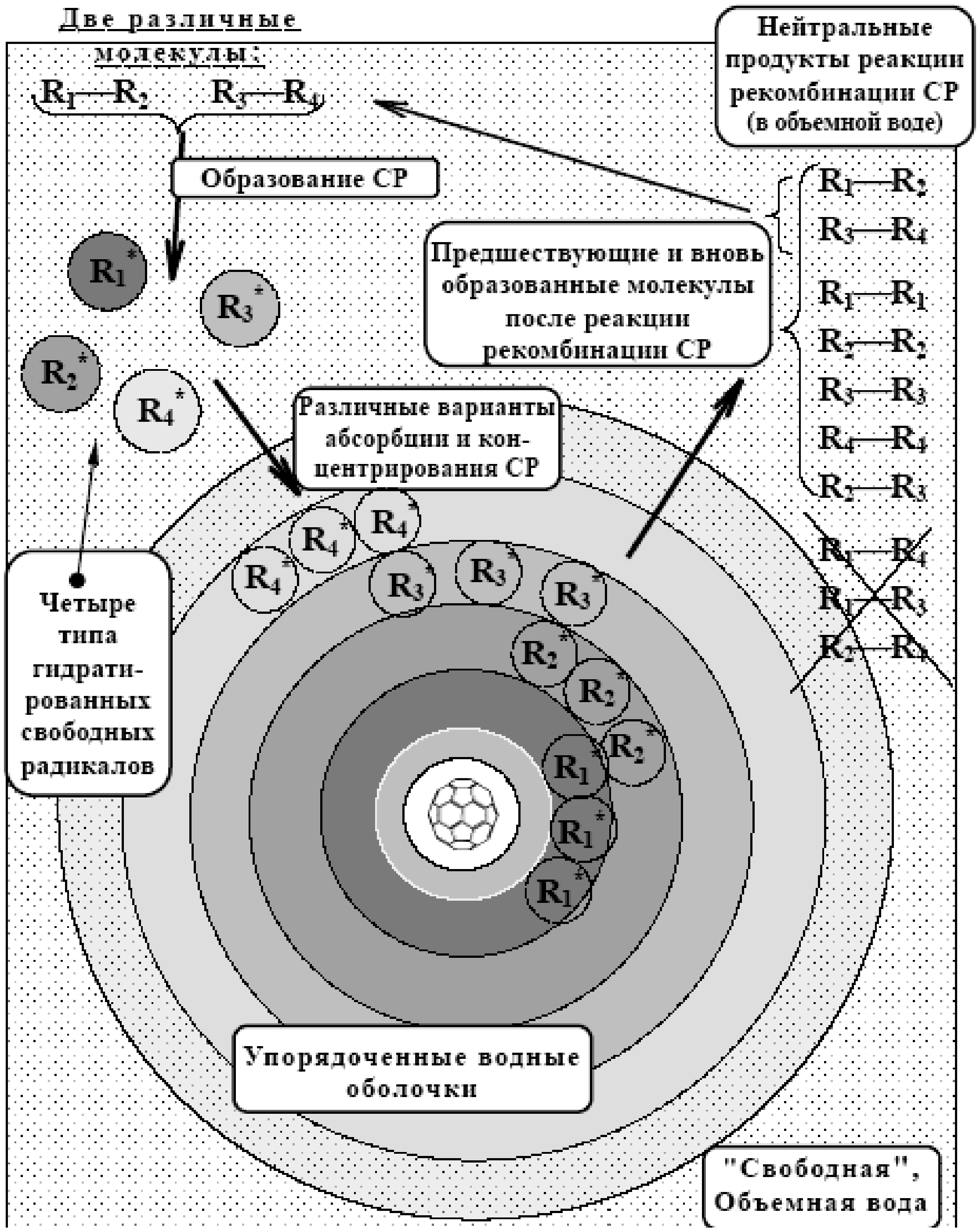
================================================================

**It's Impossible To Find Any Panacea From Human Folly !!!**

***--- OR ---***

**No Panacea Can Be Found Against Human Folly (*Stupidity*) !!!**

***Вероятная схема процесса абсорбции, концентрирования и рекомбинации свободных радикалов (СР) под влиянием упорядоченных водных структур, сформировавшихся вокруг гидратированного $C_{60}$ фуллерена (<u>HyFn</u>).***



**Биоантиоксидатными свойствами обладают различного рода Гидратированные Наночастицы с размерами от 3 до 30 нм, но которые, ввиду их химической природы, не участвуют сами непосредственно в реакциях со свободными радикалами.**

**Такое связано с естественными упорядоченными структурами воды, в которые подобные наночастицы способны «вписываться» и поддерживать их.**

***Подобные факты обнаружены для наночастиц*:**

- **- гидратированных фуллеренов $C_{60}$ и их фрактальных ассоциатов с размерами 3.4, 7.1, 10.9, 14.5, 18.1, 21.8, 25.4, 28.8, 32.4, 36.0 nm** [*1*];
- **- $CeO_2$ с размерами 3-5 и 7, 20, 30-50** nm [*2, 3*];
- **- $Y_2O_3$ с размерами 6 и 12 нм** [*4*];
- **- Ag с размерами около 15 nm** [*5*];
- **- наноалмазов с размерами 3.4-3.8 (2-10) нм** [*6*].
- **- $BaTiO_3$ с размерами 10-100 нм** [7] (***вскоре обнаружат также***)

[1] G.V. Andrievsky et al. *Fullerenes, Nanotubes and Carbon Nanostructures*, 13 (4), (2005) 363.

[2] S. Babu et al. *Chem. Phys.Letters*, 442 (2007) 405.

[3] B.A. Rzigalinski. *Nanomedicine*, 1 (4) (2006) 399.

[4] D. Schubert et al. *Biochem. Biophys. Res. Commun.*, 342 (2006) 86.

[5] J. Tian et al. *ChemMedChem*, 2 (1) (2006) 129.

[6] A.M. Schrand et al. *J. Phys. Chem. B*, 111 (1) (2007) 2.

**In XX century, Nobel laureate Albert Szent-Gyorgyi stated that:**

## "BIOLOGY HAS FORGOTTEN CELL WATER, OR NEVER DISCOVERED IT."

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**В 70-х годах XX века Нобелевский лауреат Альберт Сцент-Дьерди заявил о том, что:**

## "БИОЛОГИЯ ЗАБЫЛА КЛЕТОЧНУЮ ВОДУ, ИЛИ ВООБЩЕ НИКОГДА ЕЕ НЕ ОБНАРУЖИВАЛА."