The 24th Rudolf Brdička Memorial Lecture

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Seeing, Monitoring, Measuring and Understanding Vesicular Exocytosis of Neurotransmitters with Ultramicroelectrodes

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Vesicular exocytosis is a biologically regulated nanoscale process. The 2013 Nobel Prize in Physiology or Medicine was awarded jointly to J.E. Rothman, R.W. Schekman and T.C. Südhof for investigating and identifying its main stages but the final one, through which chemical messengers (neurotransmitters, hormones, peptides, growth factors, etc.) are ultimately delivered, still resists a thorough understanding after 30 years of intensive works in many excellent research laboratories.

This last stage involves a connection between a nanometric carrier vesicle transported within a cell with the cell membrane where it has been shuttled. This occurs by creation of an initial fusion nanopore (1.2±0.35 nm radius) across the two membranes through which biologically active molecules contained inside the vesicle start to be released into the extracellular environment (synaptic cleft, circulating fluids). Eventually, this initial pore may expand but the amount to which such expansion may occur or its nature and energetics are still strongly debated.

Despite the minute released amounts (attomoles), single exocytotic events can be studied by means of the 'artificial synapse' amperometric method [1], in which a cell is interrogated by a carbon fiber microelectrode collecting and oxidizing released molecules so that the finely-structured electrochemical current tracks quantitatively the exocytotic flux. In the ENS group we investigate essentially chromaffin cells which release adrenaline into the blood stream. Our purpose in this work was to derive topological, energetic and dynamic information about these vesicular exocytotic phenomena.

Such information is obtained by deconvoluting the experimental current by means of simulations involving self-adjustment of time-dependent radius of the fusion nanopore [2-3]. It should be noted, however, that due to the biological variability of the vesicles, the main parameters characterizing a spike (i.e., initial concentration of the neurotransmitter, its diffusion coefficient, vesicle radius, etc.) are not known *a priori*. Nevertheless, reconstruction is possible when at least one of the characteristic dimensions is known as an independent entry. To this "scaling" end, we resorted to initial fusion nanopore radius values, which are well established by patch-clamp measurements [4]. This allowed internal topological calibration of the reconstruction procedure. This resulted in the determination of the average neurotransmitter diffusion rate (D/R_{ves}^2) within the vesicle, which in turn allowed reconstructing the fusion nanopore dynamics from any given spike.

Owing to the large number of spikes in amperometric experiments (several hundred spikes treated) this afforded statistically significant analysis of size distributions of initial fusion pore [4] as well as that in its final stage (full fusion) showing that at the end of the "full" fusion stage the vesicle unmasks only ca 1% of its surface area in contradiction with previous erroneous claims based of TIRF microscopy. In turn this provided for the first time experimental access to the potential energy well governing the thermodynamics of such nanosystems suggesting their pure lipidic nature.

Acknowledgements

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References

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2. C. Amatore, A. I. Oleinick, I. Svir, *ChemPhysChem* **11**, 149-158 (2010).
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Professor Christian Amatore

Christian Amatore, born in 1951 at Sidi-Bel-Abbes (Algeria), was educated at Ecole Normale Supérieure (ENS), the leading French educational and research center. He was Director of the Chemistry Department of ENS, Director of Research in CNRS and is Full Member of the French Académie des Sciences. In 2007 he was appointed as one of the twenty members of the High Council of Science and Technology to advise the President of the French Republic on scientific matters.

His researches involve the development of advanced electrochemical methodologies for investigating extremely complex mechanisms of organic and organometallic chemistry under the very conditions used by synthetic chemists. Amatore's activity in kinetics is best illustrated by the rationalization of electron transfer catalysis, electron transfer activation of molecules and more recently by a thorough series of works relative to the elucidation of the most important mechanistic aspects of catalysis by homogeneous palladium complexes, an extremely active area in today's catalysis for carbon-carbon bond making in fine chemical industry. The well recognized contributions of Amatore's group in this area have opened new views for rationalizing these important processes which have already led to the development of new synthetic strategies.

Amatore's contributions gave electrochemistry new concepts and new tools which are essential in offering electrochemistry new entries in several mechanistic problems in organic, inorganic, and organometallic chemistries and more recently into the biology of living cells. In this respect, one should stress his pioneering work in collaboration with Mark Wightman for the development and promotion of ultramicroelectrodes. This led to the precise detection of extremely minute fluxes of essential messengers emitted by living cells during their interactions within tissues and integrated organisms. These researches involve vesicular release of neurotransmitters, oxidative stress cellular bursts, as well as the intimate cooperative coupling of these basic processes within the brain.

These works correspond to more than 400 primary research publications, altogether cumulating more than 15,000 citations with a "h-index" of 62 (ISI Web of Knowledge, 2012). Amatore received many important French and international prizes and distinctions among which the Silver Medal (CNRS), the Reilley Award (SEAC), the de Broglie Medal (Academia dei Lincei), the Bourke Medal (RSC), the Galvani Medal (SCI) and the Faraday Medal (RSC). He has been distinguished lecturer in many first rank universities (Oxford, Cornell, Caltech, Durham, Modena, Padova, Pittsburgh, Roma (La Sapienza), Japan JSPS, etc.) and is Honorary Professor or Doctor Honoris Causa of several universities in Europe and Asia. He was nominated Knight of the French National Order of Meritus by the President of the French Republic.

Rudolf BRDIČKA (1906 - 1970)

Professor of physical chemistry at Charles University, founding member of the Czechoslovak Academy of Sciences, founder and first director of the Institute of Physical Chemistry of the Czechoslovak Academy of Sciences.

An outstanding electrochemist renowned in particular by his pioneering work on kinetic polarographic current and on applications of polarography in medicine. A brilliant university teacher, author of an internationally recognized textbook of physical chemistry. He has crucial merits for development of modern physical chemistry in this country.

To commemorate his work and personality, the Institute of Physical Chemistry of the Academy of Sciences of the Czech Republic has organized since 1991 annually a festive R. Brdička Lecture. Invited speakers have been eminent scientists active in some field relating to the research currently pursued in the Institute.

Rudolf Brdička Memorial Lectures 1991-2013

(1991) *Edgar **HEILBRONNER** (Eidgenossische Technische Hochschule, Zürich)*
"**The old Hűckel formalism**"

(1992) *Kamil **KLIER** (Lehigh University, Bethlehem, Pennsylvania)*
"**Physical chemistry in two dimensions**"

(1993) *Joshua **JORTNER** (Tel Aviv University, Tel Aviv)*
"**Clusters – a bridge between molecular and condensed matter chemical physics"**

(1994) *David J. **SCHIFFRIN** (The University of Liverpool)*
"**Electrochemistry in two-dimensional systems**"

(1995) *Josef **MICHL** (University of Colorado, Boulder, Colorado)*
"**Molecular kit for new materials**"

(1996) *Gerhard **ERTL** (Fritz-Haber-Institut der Max-Planck-Gesellschaft, Berlin)*
"**Self-organization in surface reactions**"

(1997) *Roger **PARSONS** (University of Southampton)*
"**Electrochemistry in the last 50 years: from Tafel plotting to scanning tunnelling**"

(1998) *G. Barney **ELLISON** (JILA and University of Colorado, Boulder, Colorado)*
"**The chemical physics of organic reactive intermediates in combustion and atmospheric processes**"

(1999) *Henry F. **SCHAEFER III** (University of Georgia, Athens, Georgia)*
"**The third age of quantum chemistry**"

(2000) *Alexis T. **BELL** (University of California and Lawrence Berkeley Laboratory, Berkeley, California)*
"**Progress towards the molecular design of catalysts –lessons learned from experiments and theory**"

(2001) *Mario J. **MOLINA** (Massachusetts Institute of Technology, Cambridge, Massachusetts)* "**The Antarctic ozone hole**"

(2002) *Jean-Marie **LEHN** (Université Louis Pasteur, Strasbourg a Collége de France, Paris)*
"**Selforganization of supramolecular nanodevices**"

(2003) *Helmut **SCHWARZ** (Technische Universität Berlin)*
"**Elementary processes in catalysis: looking at and learning from** "**naked**" **transition ion**"

(2004) *Rudolph A. **MARCUS** (California Institute of Technology, Pasadena)*
"Strange isotope effects in stratospheric ozone and in the earliest minerals in the solar system"

(2005) *Avelino **CORMA** (Instituto de Tecnología Química, Valencia)*
" **Supramolecular Entities Based on Molecular Sieves for Catalysis and Synthesis of New Materials**"

(2006) *Paul **CRUTZEN** (Max Planck Institute for Chemistry, Mainz):*
"Atmospheric Chemistry and Climate in the ´Anthropocene´"

(2007) *Harry B. **GRAY** (California Institute of Technology, Pasadena)*
"The Currents of Life: Electron Flow through Metalloproteins"

(2008) *Michael **GRÄTZEL** (Ecole Polytechnique Fédérale de Lausanne)*
"**Mesoscopic Electrodes for Generation and Storage of Electric Power from Sunlight"**

(2009) *Gabor. A. **SAMORJAI** (Department of Chemistry and Lawrence Berkeley Natkional laboratory, University of California, Berkeley)*
"Molecular Foundations of Heterogeneous Catalysis"

(2010) *Pavel **HOBZA** (Institute of Organic Chemistry and Biochemistry of the AS CR)* **"Noncovalent Interactions and their Role in Chemistry and Biochemistry"**

(2011) Klaus ***MÜLLEN** (Max-Planck Institute, Mainz, Germany)*
"Carbon Materials and Graphenes"

(2012) *Enrico **GRATTON** (University of California, Irvine)*
"Nanoimaging technique with high time and spatial resolution: Mechanisms of translocation through the nuclear pore complex"

(2013) *J. Peter **TOENNIES** (Göttingen, Germany)*
"Superfluid Helium Nanodroplets: Very Cold and Extremely Gentle"