The Seminal Contributions of Gregorio Weber to Modern Fluorescence Spectroscopy

During the last few decades, fluorescence spectroscopy has evolved from a narrow, highly specialized technique into an important discipline widely utilized in the biological, chemical and physical sciences.

As in all scientific disciplines, the development of modern fluorescence spectroscopy has benefited from the contributions of many individuals from many countries.

However, one individual, Gregorio Weber, can be singled out for his outstanding and far-reaching contributions to this field.
Biographical Sketch of Gregorio Weber

1916 Born in Buenos Aires, Argentina (July 4)
1943 M.D. degree from the University of Buenos Aires
   (teaching assistant with Bernardo Houssay)
1943/47 Attended Cambridge University supported by a
   British Council Fellowship.
   Thesis Advisor - Malcolm Dixon
1947 Awarded Ph.D. - Thesis title “Fluorescence of
   Riboflavin, Diaphorase and Related Substances”
1948 /52 Independent investigator at the Sir William Dunn
   Institute of Biochemistry at Cambridge University
1953 Joined Biochemistry Department of Sheffield
   University
1962 Joined the Biochemistry Division of the Chemistry
   Department at the University of Illinois
   at Urbana-Champaign
At the University of Buenos Aires, Bernardo Houssay suggested that his young protégé apply for a prestigious British Council Fellowship to support Ph.D. studies at Cambridge University, UK.

Travel to England in 1943 was an adventure - Weber’s voyage took 44 days in a convoy.
Cambridge

At Cambridge, Weber entered St. John's College where he met Malcolm Dixon, the well-known enzymologist, and talked with him about applying techniques of Physical Chemistry to the study of proteins.

Dixon suggested that Weber consider applying fluorescence techniques to the study of the naturally fluorescent flavin and flavoprotein systems.

The Chapel Tower - St. John's College

At that time, Weber knew little about fluorescence but soon learned that there were a number of low molecular weight flavin compounds, such as riboflavin and FAD, that differed greatly in fluorescence intensity. A few flavoproteins had been purified but only one of them showed fluorescence comparable to the free prosthetic group.

Gregorio Weber was thus given the task of “sorting out” this area.
Fluorescence in 1943

Many of the basic principles of fluorescence had already been developed during the 1920's and 1930's.

- Excited state lifetime (Gaviola)
- Quantum yield (Wavilov)
- Polarization of fluorescence (Weigert, F. Perrin)
- Fluorescence resonance energy transfer (J. and F. Perrin)

The use of fluorescence in biology and biochemistry was, however, descriptive in nature and primarily limited to a role in the isolation, purification and quantification of fluorescent substances such as riboflavin and porphyrins.
Gregorio Weber's introduction to fluorescence polarization started when he read Francis Perrin's classic paper of 1926 in the Journal de Physique.
“Argentine secondary education in the first half of the century included French language and literature so that I could not only understand the scientific content, but also enjoy the literary quality of the writing. It was written in that transparent, terse style of XVIII century France, which I have tried, perhaps unsuccessfully, to imitate from then onwards. The clarity of Perrin’s thought and his ability to do the right experiment were really remarkable.”

Quotes from the 1986 Fluorescent Biomolecules Symposium
“It was from reading Perrin’s papers that I conceived three ideas on the use of polarization: determination of the change in the fluorescence lifetime as one quenches the fluorescence by addition of an appropriate chemical, determination of the molecular volume of proteins by fluorescent conjugates with known dyes and determination of the viscosity of a medium through the polarization of the emission from a known fluorescent probe”
Thesis submitted for the Degree of Ph.D. in the
University of Cambridge

FLUORESCENCE OF RIBOFLAVINE,
DIAPHORASE AND RELATED SUBSTANCES

Gregorio Weber,
St John's College.
“I feel that a knowledge, as deep as possible, of the physical principles concerned is indispensable. Even close collaboration with a physicist cannot spare this task to the biochemist. I am tempted to believe that a biologist having $n$ ideas related to the biological side of the problem and a physicist possessing another $n$ relating to the physical side would result in some $2n$ useful combinations whereas the same ideas collected in one brain would lead to a number of combinations more like $n!$.”
Gregorio Weber's first polarization instrument

The light source was a carbon arc, a source originally developed for use in searchlights during the war.

The exciting light was first filtered through a layer of concentrated NaNO$_2$ to remove UV light and then polarized by a Nicol prism. Additional glass filters were used to further delimit the exciting light and to isolate the emission.

The actual measurement of the polarization of the fluorescence was realized using visual compensation techniques involving observation of interference patterns as a “pile-of-plates” polarizer (the compensator of Arago) was rotated.

Using these simple methods and only his eye as the detector, Weber was able to quantify levels of polarized light reaching only 1 or 2%.

From Weber’s Ph.D. thesis
The final chapter of Gregorio Weber’s thesis is devoted to the application of polarization measurements to determine the viscosity of gels.
the low microscopic viscosity necessary for the rapid diffusion of metabolites within it.

Note on the determination of protoplasm viscosity.

The polarization method appears very convenient for the determination of the viscosity of protoplasm.

Both the microscopic and macroscopic viscosity of the protoplasm are of importance; the first in relation to diffusion processes and the second in relation to the organization of the cell. The fluorescent method would allow the determination of the microscopic viscosity only.
This prescient observation anticipated the work he would publish 24 years later which first delineated the application of fluorescence probes to studies of the physical state of lipid systems.

Microviscosity and Order in the Hydrocarbon Region of Micelles and Membranes Determined with Fluorescent Probes.
I. Synthetic Micelles

M. Shinizky,† A.-C. Dianoux,‡ C. Gitler,§ and G. Weber‖

ABSTRACT: The viscosity in micelle interiors, termed here as microviscosity, was derived from an adequate comparison of the degree of fluorescence depolarization of perylene or 2-methylanthracene when dissolved in the tested micelles and in American white oil U. S. P. 35. The latter was used as a reference system of known viscosities. In the series studied, lauryltrimethylammonium bromide, myristyltrimethylammonium bromide, cetyltrimethylammonium bromide (CTABr), and stearyldimethylbenzylammonium bromide, the determined microviscosities at 27°C are all in the range of 17-50 cP. The change in microviscosity with temperature in this series was found to follow a simple exponential form with an activation energy in the range of 6.1-9.6 kcal mol⁻¹. Added salts affected only slightly the microviscosity values. Mixed micelles of perylene-labeled CTABr with cetyl alcohol or cholesterol and with sodium 1-hexadecanesulfonate, were used to test the effect of charge isolation and charge neutralization on the fluidity of the micelle interior. The microviscosity of these mixed micelles was found to increase rapidly with concentration of the admixed component, and at a molar ratio close to 1:1 microviscosities of several poses were obtained. The changes in apparent rotational diffusion with wavelength of excitation indicate that the depolarizing rotations are strongly anisotropic. In-plane rotations in perylene are ten times faster than out-of-plane rotations, independently of the medium (micelles, propylene glycol at -14°C, propylene glycol-glycerol at 4°C). This indicates that the resistance to the motion in the micelles must be close to isotropic. A summary of the findings presented leads to the conclusion that micelle interiors are similar in nature to aliphatic hydrocarbon solvents.

In general, the fluorescence emitted from molecules which are dispersed in a viscous medium is partially polarized. This is customarily expressed in terms of molecular anisotropy, r, or degree of polarization, p, which are measured at right angle to a polarized excitation beam and are defined as

\[ r = \frac{I_1 - I_2}{I_1 + 2I_2} \quad \text{and} \quad p = \frac{I_1 - I_2}{I_1 + I_2} \tag{1} \]

When \( I_1 \) and \( I_2 \) are the fluorescence intensities observed through a polarizer oriented parallel and perpendicular to the plane of polarization of the excitation beam. For a rotating fluorescent sphere the observed r or p values obey the well known Perrin (1926) equation in which \( r_0 \) and \( p_0 \) are the values

\[ r = \frac{1}{r_0} - \frac{1}{r_0} \frac{3}{p_0} = 1 + \frac{6R_s}{\lambda} \tag{2} \]

of r and p when the emitting molecules maintain their orientation excitation and emission (e.g., in a very viscous solvent), \( R_s \) is the rate of rotation of the sphere and \( \lambda \) is the rate of fluorescence emission. The term \( r_0/r \) is defined here as the degree of depolarization.

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‡ Fellow of the Centre National de la Recherche Scientifique (France).

§ Visiting Professor, Department of Biochemistry, Instituto Politecnico Nacional, Mexico.

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The use of fluorescence probes in model and biological membrane systems has become an extremely important and wide-spread technique which, in recent years, has been extended to fluorescence microscopy.
The first paper...
A large portion of Weber's thesis was devoted to measurements on the quenching of fluorescence of riboflavin and on development of a general theory of quenching by complex formation.

These studies led to his first publication:


This paper was the first to demonstrate that fluorescence quenching can take place after formation of molecular complexes of finite duration rather than collisions.
The second paper...

“Fluorescence of riboflavin and flavin-adenine dinucleotide”

This paper gave the first demonstration of an internal complex in FAD.

Subsequent work on FAD and NADH

“Intramolecular transfer of electronic energy in dihydridophosphopyridine nucleotide”

“Transfert d’énergie dans la dihydro-diphosphopyridine nucleotide”


“Emission properties of NADH. Studies of fluorescence lifetimes and quantum efficiencies of NADH, AcPyADH, and simplified synthetic models

Designing fluorescence probes - The beginning...

From 1948 to 1952 Weber carried out independent investigations at the Sir William Dunn Institute of Biochemistry at Cambridge. He began to delve more deeply into the theory of fluorescence polarization and also to develop methods which would allow him to study proteins which did not contain an intrinsic fluorophore.

He invested considerable time and effort in synthesizing a fluorescent probe which could be covalently attached to proteins and which possessed absorption and emission characteristics appropriate for the instrumentation available in post-war England.
The result of two years of effort was dimethylaminonaphthalene sulfonyl chloride or dansyl chloride - a probe which is still utilized today.
Designing fluorescence probes - continued...

Bis-ANS

IAEDANS

LAURDAN  \( R = -(CH_2)_{10}CH_3 \)
PRODAN  \( R = -CH_2CH_3 \)
DANCA  \( R = -\)
Fluorescent Modification of Adenosine-Containing Coenzymes. Biological Activities and Spectroscopic Properties


ABSTRACT: The synthesis of fluorescent derivatives of adenosine and cytidine, by reaction with chloroacetalddehyde in aqueous solution at mild pH and temperature, yielding 1,N6-etheno-adenosine hydrochloride and 3,N4-etheno-cytidine hydrochloride, respectively, is here described. Analogous derivatives of 3'-AMP, 5'-AMP, 3',5'-cyclic AMP, ADP, ATP, and NAD+ were also synthesized. Observation of the spectroscopic properties of the highly fluorescent adenosine derivatives included the emission maximum for 1,N6-etheno-adenosine at ca. 415 nm (corrected) in buffered aqueous solution at pH 7.0, a quantum yield of 0.56, and a fluorescence lifetime of 20 nsec. Variations in fluorescence with respect to changes in the polarity and viscosity of the solvent and with respect to temperature were also examined, as well as fluorescence excitation and fluorescence polarization spectra. All adenosine derivatives had similar fluorescence properties. The 1,N6-etheno-AMP analog showed considerable substrate activity as a replacement of ATP with adenylate kinase, hexokinase, and phosphofructokinase, and exhibited allosteric inhibition of phosphofructokinase. The 1,N6-etheno-ADP analog proved to be an excellent substitute for ADP in the pyruvate kinase system, affording a facile assay for a wide variety of kinases.

Recently we have been engaged in a search for reagents to incorporate fluorescence into specific bases in tRNA. While fluorescence techniques (Ward et al., 1969; Beardsley and Cantor, 1970; Wintermeyer and Zachau, 1971) and chemical modifications (see Cramer, 1971; Gauss et al., 1971) have been shown to be extremely valuable in gaining information on tRNA tertiary structure, the two methods have not yet been combined in nucleic acid chemistry, and it was the greatly increased utility of the combined methods which prompted our search. Rather than grapple initially with the difficulties involved in product identification and specificity determination when reactions are carried out directly on tRNAs, we have chosen to study reactions on the bases and nucleosides themselves, employing conditions which could be readily applied to undernated tRNA (Secrist et al., 1971; Barrio et al., 1972a), namely pH 3.5–8.5, temperatures not above 37°, and aqueous solution.

Several approaches to the problem can be readily envisaged. The reagent employed to modify specifically a given nucleoside may have the fluorescence already built into it. Alternatively, a nonfluorescent reagent may be used to cause the production of a fluorescent structure. The first method has the added difficulty of necessitating the careful removal of the fluorescent reagent from the fluorescent product, notwithstanding the fact that the fluorescence properties in the product may be altered, while the second approach does not have this drawback. Additionally, employment of a nonfluorescent reagent permits the use of small molecules, perhaps facilitating reactions in hindered positions, while a fluorescent reagent by its nature necessitates the use of a relatively bulky group. We have used the first method to develop a reagent specific for 4-thiothymidine (Secrist et al., 1971).

The reaction of chloroacetalddehyde with 9-methyladenine and 1-methylcytosine has recently been described (Kochetkov et al., 1971), and it was our feeling that the analogous products with adenosine and cytidine, namely, 1,N6-etheno-adenosine and 3,N4-etheno-cytidine respectively (hydrochlorides 1 and 2 shown in Chart I), would in all probability be fluorescent. We were gratified to find that this prediction was correct and that both exhibited interesting and useful fluorescence properties. The possible ramifications of fluorescent "A" derivatives became immediately obvious to us and we are able to present here the chemical, fluorescence, and enzymatic characteristics.

‡ From the School of Chemical Sciences, University of Illinois, Urbana Illinois 61801. Received April 19, 1972. This work was supported by Research Grants GM-08829 and GM-11223 from the National Institutes of Health, U. S. Public Health Service.
§ Fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina).
The 1952 papers

With Dansyl chloride and with new instrumentation Weber began to investigate several protein systems, publishing his theory and experimental results in two classic papers published in Biochemical Journal in 1952.

“Polarization of the fluorescence of macromolecules. I. Theory and experimental method.”

“Polarization of the fluorescence of macromolecules. II. Fluorescent conjugates of ovalbumin and bovine serum albumin.”

The theory paper - which interestingly contains an acknowledgment to F. Perrin for his suggestions - includes an extension of Perrin’s theory of depolarization to the case of ellipsoidal molecules carrying randomly oriented oscillators of absorption and emission.
Polarization theory continued...

In subsequent years, Weber continued to advance the theory of fluorescence polarization.

Theory of Fluorescence Depolarization by Anisotropic Brownian Rotations. Discontinuous Distribution Approach

Gregorio Weber

Department of Biochemistry, School of Chemical Sciences, University of Illinois, Urbana, Illinois 61801

Dynamics of Fluorescence Polarization in Macromolecules
(rotational diffusion/relaxation/theory/formula)

G. G. BELFORD*, R. L. BELFORD†, AND G. WEBER†

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University of Illinois, Urbana, Ill. 61801

This second paper (1972) is a re-examination of previous treatments by several groups (including Weber) and presents the generally accepted equation for the time-dependence of fluorescence polarization owing to rotational diffusion of fluorophores attached to rigid macromolecules.
In 1953, Hans Krebs recruited Weber for the new Biochemistry Department at Sheffield University. During his years at Sheffield,

Weber continued to lay the foundations of modern fluorescence spectroscopy developing both fluorescence theory and instrumentation.

His pioneering contributions during these early years included his report, with Laurence of the fluorescence properties of anilino-naphthalene sulfonate (ANS).

It is interesting to note that even today, more than 50 years after that first report, ANS is still being used in protein studies, quite often as an indicator of the “molten globular” state.
Intrinsic Protein Fluorescence

During his years at Sheffield, Weber and his postdoctoral fellow, John Teale, began their studies on the fluorescence of the aromatic amino acids and proteins.

In the late 1950's and early 1960's, Weber and Teale published a series of important papers including the first description of the excitation and fluorescence spectra of the aromatic amino acids - tryptophan, tyrosine and phenylalanine.

Figure 7 from this paper has been reproduced many times in review articles and books.
Fig. 7. Fluorescence spectra of the aromatic amino acids in water. Abscissa: wavelength (mμ.). Ordinate: relative number of quanta.
More tryptophan ...

In 1960, Weber published the excitation polarization spectra of the aromatic amino acids and numerous proteins.

In these papers he also gave the first demonstration of electronic energy transfer among tyrosines and tryptophans and the critical transfer distance from tyrosine to tryptophan and among tyrosine or tryptophan residues.

Weber continued to make important contributions to this area, including this well-known 1977 collaboration with Bernard Valeur.

RESOLUTION OF THE FLUORESCENCE EXCITATION SPECTRUM OF INDOLE INTO THE $^1L_q$ AND $^1L_b$ EXCITATION BANDS*

BERNARD VALEUR and GREGORIO WEBER
Department of Biochemistry, School of Chemical Sciences, University of Illinois, Urbana, IL 61801, U.S.A.

Figure 2. Corrected excitation spectrum (broken line) and excitation polarization spectrum of indole in propylene glycol at $-58\degree$. The fluorescence is observed through a Corning 7–39 filter.
Red-edge effects...

Electronic energy transfer between identical fluorophores was originally observed by Gaviola and Pringsheim in 1924. In 1960 Weber was the first to report that homotransfer among indole molecules disappeared upon excitation at the red-edge of the absorption band - this phenomenon is now known as the “Weber red-edge effect”.

In 1970, Weber and Shinitzky published a more detailed examination of this phenomenon.
During his stay at Sheffield, Weber conceived of a method to elucidate the number of fluorescing compounds in mixtures of fluorophores by variation of the excitation and emission wavelengths and construction of a matrix of the resulting intensities.

Years later, with the advent of computer-controlled instrumentation and data analysis, Weber’s matrix approach would become widely utilized in Analytical Chemistry and would become known as the Excitation-Emission Matrix (EEM) technique.

(Reprinted from Nature, Vol. 190, No. 4770, pp. 27-29, April 1, 1961)

ENUMERATION OF COMPONENTS IN COMPLEX SYSTEMS BY FLUORESCENCE SPECTROPHOTOMETRY

By Dr. G. Weber
Department of Biochemistry, University of Sheffield

The constancy of the spectral distribution of the fluorescence for a given substance excited with light of different wave-lengths permits the development of a simple quantitative criterion for the determination of the number of independent fluorescent components in a mixture and also results in an interesting extension of the concept of resolution as commonly applied in spectroscopy.

In fluorescence spectrophotometry a number of fluorescence intensity measurements are made, following excitation with different wave-lengths. Both fluorescence observation and excitation cover discrete wave-bands. Continuously recorded fluorescence and fluorescence-excitation spectra are to be considered here as finite series of measurements with effective band-widths determined by the conditions used. In either case the results may be set down as an \( m \times n \) matrix, where the \( m \) columns are determined by the wave-lengths of excitation and the \( n \) rows by the wave-lengths of observation. The matrix elements, \( F_{ij} \), are numbers proportional to the response of the detector, that is, photo-currents in the ordinary case. The \( i,j \) element of this \( EF \) matrix, as I propose to call it, is the fluorescence emitted at wave-length \( j \) on excitation with light of wave-lengths \( i \). When several components are present in a mixture the \( EF \) matrix may be written as:

\[
[EF]_{mn} = \begin{bmatrix}
\sum F_{1k}^k & \sum F_{1k}^k & \cdots & \sum F_{1m}^k \\
\sum F_{2k}^k & \sum F_{2k}^k & \cdots & \sum F_{2m}^k \\
\vdots & \vdots & \ddots & \vdots \\
\sum F_{nk}^k & \sum F_{nk}^k & \cdots & \sum F_{nm}^k
\end{bmatrix}
\]

(1)

The superscript \( k \) refers to the different components. Of the quanta absorbed in the solution from the...
Around 1960, I.C. “Gunny” Gunsalus, who was then the head of the Biochemistry Division of the Department of Chemistry at the University of Illinois at Urbana-Champaign, recruited Weber.

Gunny relates the story that while he was convincing his colleagues that Gregorio Weber was an exceptional scientist, someone commented that Weber didn’t have as many publications as one might expect from a senior professor.

Gunny explained that while this was true, Weber’s ratio of outstanding papers to total papers was unity and that this ratio - known thereafter as the Weber Ratio - was certainly the more important consideration.
In his office at the University of Illinois - circa 1980
Phase Fluorometry

During his days at Sheffield, Weber started to think about constructing a fluorescence lifetime instrument. Influenced perhaps by the work of fellow Argentinean Enrique Gaviola, who built the first phase fluorometer in 1927, Weber worked on designing a phase fluorometer. At that time Birks and others had also built several types of phase fluorometers.

At the University of Illinois in the mid-1960's Weber, together with his graduate student Richard Spencer, constructed a highly versatile phase and modulation fluorometer utilizing the principle of cross-correlation (Annals New York Acad. Sci. 158, 361)

The cross-correlation approach proved to be the key to modern phase fluorometry and is still used universally today.
When Enrico Gratton joined Weber’s laboratory as a postdoctoral fellow from 1975-1976, he worked, at the suggestion of Weber, on the development of a phase and modulation fluorometer with continuously variable light modulation frequencies.

Enrico returned to Urbana in 1978 as an Assistant Professor in the Physics Department. By this time he had finished the first true multifrequency phase and modulation instrument, utilizing a Pockels cell as the light modulator, thus completing Weber’s vision.
More Phase Fluorometry...

Weber also greatly extended the theory of phase fluorometry:


He also solved the daunting problem of deriving the analytical solution to resolving multiple lifetimes from multifrequency phase and modulation data:


Phase fluorometry with pulsed sources

Gregorio Weber still had more contributions to make to the development of phase fluorometry as he helped with the establishment of multifrequency phase and modulation instruments at both the Frascati and Wisconsin Synchrotron Radiation Sources.

These instruments utilized the harmonic content of the light pulses to generate the modulation frequencies.

Gratton, Jameson, Rosato and Weber

Gratton, Mantulin, Weber, Royer, Jameson, Reininger and Hansen
Awards

American Academy of Arts and Sciences - 1968
1st National Lecturer of the Biophysical Society - 1969
Guggenheim Foundation Fellow - 1970
Corresponding Member of the National Academy of Exact Sciences of Argentina - 1971
National Academy of Sciences (U.S.) - 1975
Rumford Premium of American Academy of Arts and Science - 1979
ISCO Award for excellence in Biochemical Instrumentation - 1983
First recipient of Repligen Award for the Chemistry of Biological Processes: Awarded by the American Chemical Society -1986
First recipient of the International Jablonski Award for Fluorescence - 1996
Established in 1839, the Rumford Premium is one of the oldest scientific prizes in the US. and recognizes contributions to the fields of heat and light, broadly interpreted. The endowment was created by a bequest to the Academy from Benjamin Thompson, Count Rumford, in 1796.

Previous winners include:

- J. Willard Gibbs
- Thomas Edison
- R.W. Wood
- A.A. Michelson
- Percy Bridgman
- Irving Langmuir
- A.H. Compton
- E.H. Land
- Enrico Fermi
- Lars Onsager
- J. Franck
- S. Chandrasekhar
- C.H. Townes
Commercialization of Fluorescence

Three persons working under Weber's supervision in the late 1960's and early 1970's were Richard Spencer (graduate student and then postdoctoral fellow), George Mitchell (postdoctoral fellow) and Dave Laker (machinist).

These three formed a company:

SLM INSTRUMENTS, INC.  SLM\Aminco®

- at that time one of the most innovative developers of research quality fluorescence instrumentation.
Several of Weber’s former students and post-doctoral fellows went on to prominent positions in other companies manufacturing fluorescence instrumentation, including

- Perkin Elmer Analytical Instruments
- ISA Jobin Yvon-SPEX Horiba Group
- ISS® Fluorescence, Analytical and Biomedical Instrumentation
In the mid-1970's, Abbott Laboratories consulted with Gregorio Weber about the development of a polarization instrument for clinical assays.

The result was the introduction in 1981 of the TD$_x$ instrument which has become one of the leading clinical instruments - more than 30,000 TD$_x$ instruments are presently in the field.
In 1986, the Laboratory for Fluorescence Dynamics (LFD) was formed at the University of Illinois at Urbana-Champaign. The LFD, an N.I.H. National Research Resource, was started by Enrico Gratton and William Mantulin, both of whom had spent a postdoctoral period with Gregorio Weber.

Another NIH National Research Resource - the Center for Fluorescence Spectroscopy - was started at the University of Maryland by Joseph Lakowicz, who had been a graduate student with Gregorio Weber.

More recently, the Gregorio Weber Laboratory for Protein Folding and Virus Assembly was initiated at the Universidad Federal de Rio de Janeiro by Jerson Silva and his colleagues. Jerson had been a postdoctoral fellow with Gregorio Weber.
Pressure

The Brazilian Laboratory honors Gregorio Weber’s important contributions to Protein Chemistry, in particular his application of hydrostatic pressure methods to the study of biomolecules ranging from small complexes to single chain proteins to oligomeric proteins and eventually to viruses.
During the next 3 decades Gregorio Weber published more than 40 papers in the field of high pressure and biomolecules:

Gregorio Weber's final paper:

Virus Inactivation by Anilinonaphthalene Sulfonate Compounds and Comparison with Other Ligands

Carlos F. S. Bonafe,*† Michael Glaser,* Edward W. Voss,† Gregorio Weber,* and Jerson L. Silva†

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Bis (8-anilinonaphthalene-1-sulfonate) (bis-ANS) causes inactivation of vesicular stomatitis virus (VSV) at micromolar concentrations while butyl-ANS and ANS are effective at concentrations one and two orders of magnitude higher, respectively. VSV fully inactivated by the combined effects of 10 μM bis-ANS and 2.5 kbar hydrostatic pressure elicited a high titer of neutralizing antibodies. Incubation of VSV with ≥2 M urea at atmospheric pressure caused very little virus inactivation, whereas at a pressure of 2.5 kbar, 1 M urea caused inactivation that exceeded by more than two orders of magnitude the sum of the inactivating effects produced by urea and pressure separately. Measurements of bis-ANS fluorescence showed that increasing the urea concentration reduces the pressure required to disrupt the structure. We conclude that anilinonaphthalene sulfonate compounds inactivate VSV by a mechanism similar to that produced by pressure. The most effective antiviral compound was bis-ANS which can be used for the preparation of safe viral vaccines or as an antiviral drug eventually.

Key Words: vesicular stomatitis virus; pressure inactivation; bis-ANS.

These processes involve multiple viral protein interactions, making them potential targets for antiviral drugs.

The present study was designed to test the ability of anilinonaphthalene sulfonate compounds to inhibit infection by vesicular stomatitis virus (VSV) and to investigate these compounds' mechanism of action by comparing it with the inactivation caused by high pressure. VSV is a membrane-enveloped rhabdovirus that infects some animal cells and is a good model for studying virus–host interactions (2–5). This virus is composed of a single strand of RNA negative sense, five viral proteins and membrane lipids derived from the host cell. The VSV membrane contains two proteins: an integral glycoprotein (G), and a peripheral matrix protein (M) that aligns in the inner surface of the virion membrane (6). The infectious component of VSV is the ribonucleoprotein core in which the RNA is tightly encased by the nucleocapsid protein (N) and is also associated with two minor proteins (L and NS). Fluorescence microscopy has shown that pressure-inactivated VSV attaches to the plasma membrane but is not internalized by endocytosis (7). High pressure causes VSV inactivation without affecting its antigenicity (8).
Gregorio Weber’s magnum opus on proteins is his 1992 book:

Dedicated to Those Who Put Doubt Above Belief
International Symposia Honoring Gregorio Weber

Fluorescence Symposia

Bocca de Magra - 1986

Frascati - 1991

Maui - 1995

Maui - 1999

Kauai - 2002
International Symposium on Innovative Fluorescence Methodologies in Biochemistry and Medicine

September 23-26, 1991
Villa Mondragone
Frascati (Rome), Italy

Organized by:
Istituto di Medicina Sperimentale, CNR, Rome;
II Universita' di Roma Tor Vergata;
Laboratory for Fluorescence Dynamics at University of Illinois at Urbana-Champaign
Third International Symposium on Innovative Fluorescence Methodologies in Biochemistry and Medicine

Kaanapali
MAUI

July 30 to August 2
1995
Kaanapali
Maui
Hawaii
USA
5th International Weber Symposium on Innovative Fluorescence Methodologies in Biochemistry and Medicine

June 25-29, 2002
Lihue, Kauai
Other Symposia Honoring Gregorio Weber

“Thermodynamics and Kinetics in Biological Systems”

Rio de Janeiro - 1991

This issue of the Brazilian Journal of Medical and Biological Research is dedicated to Professor Gregorio Weber to celebrate his 75th birthday and his appointment as Doctor Honoris Causa of the Federal University of Rio de Janeiro.

Guest Editorial Board

Adalberto Vieyra and Sérgio T. Ferreira

Departamento de Bioquímica Médica
Instituto de Ciências Biomédicas
Universidade Federal do Rio de Janeiro

The papers included in this volume were presented at the Workshop ‘Thermodynamics and Kinetics in Biological Systems: In honor of Gregorio Weber’ during the 43rd Annual Meeting of the Brazilian Society for the Advancement of Science (SBPC) held in Rio de Janeiro, RJ, Brazil, July 14-19, 1991.
INTERNATIONAL SYMPOSIUM
ON PROTEIN CONDENSATION
In Honor of Gregorio Weber
May 21-25, 1997
Rio de Janeiro, Brazil
International Awards Established to Honor the Memory of Gregorio Weber

The Gregorio Weber Fellowship in Biochemistry
This fellowship is intended to recognize Gregorio Weber's contribution to graduate education at the University of Illinois at Urbana-Champaign. It will be awarded annually to a graduate student in the Biochemistry Department at UIUC who has demonstrated exceptional achievement, independence in research and originality of thought. The award will provide financial aid toward completing the recipient's studies.

The Gregorio Weber International Prize for Biological Fluorescence
To commemorate the global scope of Gregorio Weber's scientific career an international award is being established. This Prize will be presented periodically. It is intended to help launch the career of a promising young scientist. This international competition will be open to all recent doctoral graduates who have completed their doctorate within indicated calendar years. The applicant's general topic of research should encompass some realm of biological fluorescence. From materials submitted by the applicant, an international panel will select the awardee.
Gregorio Weber Award for Established Investigators

ISS announces the endowment of the Gregorio Weber Award for Excellence in Fluorescence Theory and Applications

The award is intended to recognize and honor distinguished investigators who gave significant and original contributions to the advancement and applications of fluorescence techniques to biology and biochemistry. Nominees must be senior level researchers with a rank of full professor, lab director or equivalent.

The award is assigned yearly and consists of a plaque and a check in the amount of $2,000, which will be awarded to the honoree at the Fluorescence Biological Subgroup meeting during the annual meeting of the Biophysical Society.
The next slide gives the names of all the students, postdocs and visiting scientists who spent time working in Gregorio Weber's lab.
Final observations... You know David, when I was much younger an older colleague said to me “Gregorio, when you pass the age of 60 you will begin to notice that your students have more ideas than you and better ideas than you”

Gee, really Professor?

...I have not found this to be the case...