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Progress in Physics (39)

Bloch meets Alzheimer

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Introduction

Has Felix Bloch ever met Alois Alzheimer? We don't know. In principle it would have been possible. If ever, they would have been open to collaboration. May be even more than researchers today fighting with the administrative hurdles and complicated, sometimes unwritten rules of todays research funding.

In fact not Bloch and Alzheimer met, but two young and enthusiastic scientists, two generations later, met at a conference ready to collaborate and mutually explore their competencies in order to fight against Alzheimer's disease. However, interdisciplinary work is not easy to initiate. In particular it is difficult to get funding without a track record in all disciplines involved. Some years later, and with the goodwill and support of many laboratories [1], the results are extremely promising. A method has been developed to monitor the progress of Alzheimer's disease right at the early beginning, which is not possible with other techniques. This method is not a diagnosis tool, but it allows studying the fundamental biochemical mechanisms involved. In addition, we can test the effect of medicaments on the disease in a short time frame.

In the following, we will summarize the basics of the Alzheimer's disease, the novel Bloch Surface-Wave (BSW) sensing concept and report our results. For further reading we refer to the literature [1].

The Alzheimer's Disease

Alzheimer's Disease (AD) is an aberrant and fatal neurodegenerative disease. As many as 35 million people worldwide are affected, hence it is the most common form of dementia. Alzheimer destroys brain cells, causing cognitive difficulties, memory loss and problems with behavior severe enough to affect work, daily-life routine and relationships even within the patient's families. (from Alzheimer 's Disease International: World Alzheimer Report 2013, http://www.alz.co.uk/research/world-report-2013).

From a pathological point of view, the presence of abnormal structures, formed by the misfolding and subsequent aggregation and deposition of specific proteins is found in the brain of AD patients. One of those structures, called amyloid plaques, are prime suspects in being in correlation with the onset of the disease. Specifically, amyloid plaques are generated by the self-assembling and fibrillization of Aβ peptides, the longest one being the Aβ 1-42, which are naturally formed by the cleavage of a neural transmembrane protein. In the AD, for factors that are still under debate, the concentration of the Aβ 1-42 peptide reaches abnormally high levels, which may be the cause for its aggregation [2].

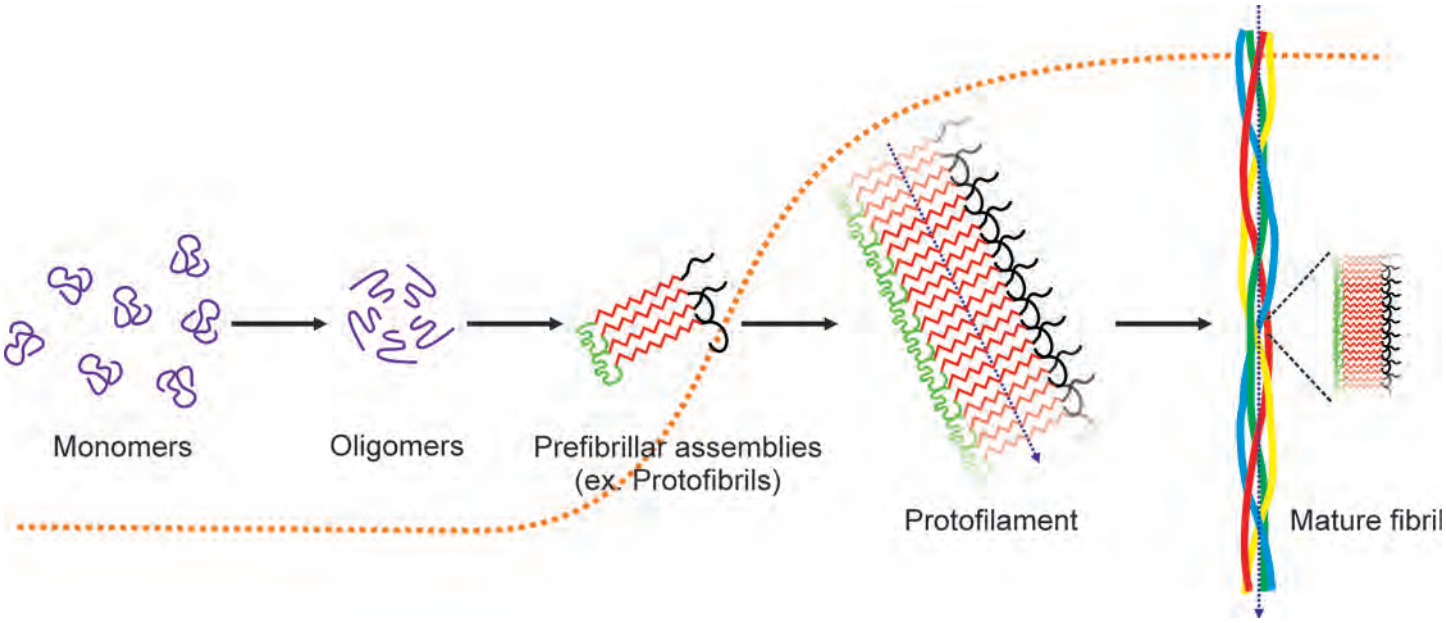

Figure 1: Schematic and simplified amyloid-beta peptide aggregation pathway. At a specific concentration and under pathogenic conditions, monomers can misfold and re-organize to form soluble aggregates commonly known as oligomers. The oligomeric complex rearrange to the prefibrillar assemblies, which are formed by the so-called beta-sheets structures (in red), typical of amyloid aggregates (petkova 2006). Two such cross-beta units comprise the protofilament, here represented in a simplified 2D-single layer. The mature fibrils are made by four protofilaments. The dotted arrow indicates the long axis of the fiber [6]. The dotted orange sigmoidal curve represents the signal obtained while monitoring the time-depending aggregation via the use of a standard amyloid detection technique (ThT binding essay, Congo Red staining, Dynamic Light Scattering, FTIR, etc.). Hence, those are not sensitive to the formation of the small, soluble and toxic oligomers, and the first part of the sigmoidal curve is commonly known as "lag-phase" [7].

Anyhow, the mere presence of this heavily dense Aβ peptide deposition is not enough to explain the neural death linked with the AD. As a matter of fact, recent research has led to the hypothesis that the neural cytotoxicity lies on the first, soluble oligomeric Aβ aggregated species, rather than on the insoluble fibrillar aggregates, forming the mature amyloid plaques [3,4]. The main occurring problem is that there are no available techniques able to reliably detect those oligomeric Aβ aggregates, since the classical amyloid detection techniques are sensitive to the mature-fibril formation and elongation (see Fig. 1), steps which occur when the AD has already started its escalation [5].

The lack of efficient and reliable diagnostic tools have seriously hampered the progress despite the application of increasingly sophisticated instruments and analytical methods. Even our understanding of the process leading from the soluble peptide to the amyloid plaques is full of gaps, sketchy at best. An enormous effort is devoted to diagnosing and treating AD without real success. The statement of Alzheimer cited at the beginning of box 1 clearly circumscribes the problem we are still confronted with almost 100 years after the initial discovery.

The Bloch Surface Wave Phenomenon

Why should we use optical surface waves to monitor the process presented in Fig. 1? Interesting is a concept that measures density variations (refractive index changes) at an interface and that is suitable for integration in a microfluidic system. The interface is the key for the proposed concept. It helps to enhance the local concentration by interactions with the molecules and generates a "clipping effect" once the molecules become too large.

It is well known that most of the bio-sensors used in proteomics are based on the exploitation of Surface Plasmon Resonance (SPR) at the surface of thin gold layers deposited on glass prisms. No other experimental method at the moment provides so much information from a single sensor [8].

The proposed platform based on Bloch Surface Waves (BSW) is an alternative biocompatible optical sensor to SPR. Exploiting the potential of surface electromagnetic waves at the surface of a one-dimensional photonic crystal (dielectric multilayer), for the first time, a BSW sensor has been applied to the *in vitro* detection of protein aggregation [1]. Such a sensor has a high potential. With the help of the photonic crystal, one can engineer the light distribution in order to generate a strong field enhancement near the interface within a desired observation zone. New performances for selectivity and sensitivity, otherwise impossible with SPR metal based sensors are delivered exploiting the variety of dielectric materials that can be used to produce the multilayer [9].

As the BSWs sustaining structure consists of dielectric materials, their losses can be made very low leading to narrower reflectance dip compared to other surface waves. Another advantage in using BSWs is the possibility of operating within a broad range of wavelengths, by properly designing a suitable multilayered structure. This tunable localized field confinement is particularly attractive for sensing applications [10].

Results

In this study [1], we present what we believe is the first demonstration of the application of a BSW-based approach to the detection of amyloid-beta peptide aggregation.

The BSW sensing detects variations in the refractive index of the media that is contact with the surface wave producer, the multilayer. The measurement consists in incubating a purified Aβ 1-42 sample in this condition, at 37°C. Hence, it is possible to monitor the variation of the Aβ 1-42 monomer concentration. In fact, during aggregation, the monomer concentration decreases, since the sensing chamber is vertically positioned and the Aβ aggregates tend to precipitate away from the sensing surface. The detectable signal is the

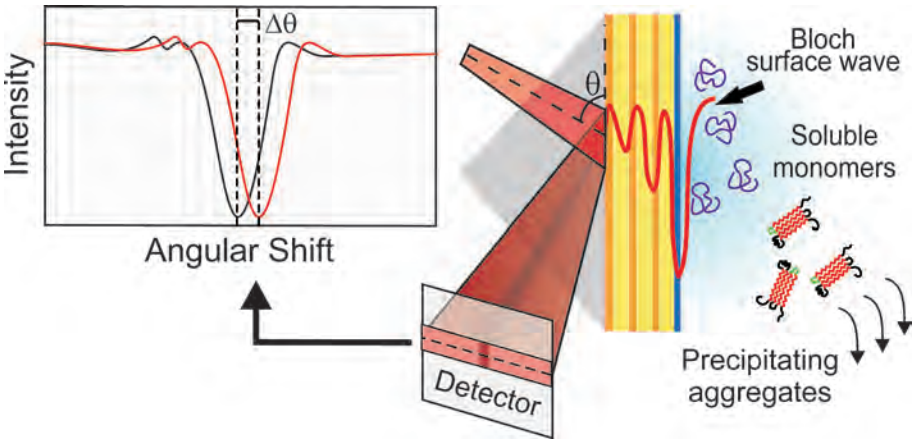

Figure 2: Schematic view of the BSW sensor. The lefts side shows the angular shift of the resonance curve due to the change of aggregation stage of Aβ 1-42 peptide measured on the right side by Bloch surface waves. [#]

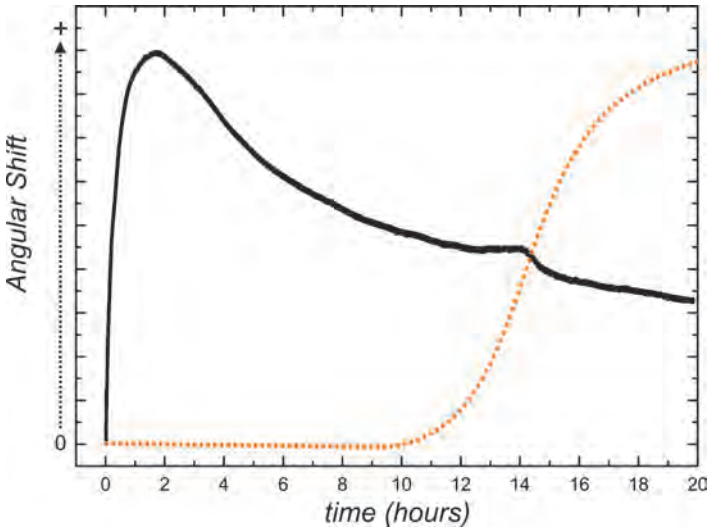

Figure 3: In black, real-time refractive index variation, in angular shift, of an Aβ(1-42) monomer peptide solution incubated at 37°C at the initial concentration of 17,3 μM in 10 mM Tris HCl, pH 7.4, as sensed by the BSW-based sensing approach. The dotted orange sigmoidal curve represents the signal obtained while monitoring the time-depending aggregation via the use of a standard amyloid detection technique, as represented in Figure 1. The two signals are obtained in the same time frame. [#]

Alois (Aloysius) Alzheimer (14.6.1864 – 19.12.1915)

Citation from Alois Alzheimer on Epilepsy: "*If we aim to understand the nature of a disease, to predict its prognosis, to elucidate its course and finally treat it prophylactically or therapeutically, we must have clear, precisely defined disease entities before us.*" [1]

Alois Alzheimer born in the Bavarian town of Marktbreit studied medicine between 1884 and 1888 in Berlin, Tübingen and Würzburg. During his career he has contributed equally to the development of modern clinical services for psychiatry as to the development of neuropathology. In 1888 Alzheimer started his career in the *Städtischen Anstalt für Irre und Epileptische* in Frankfurt am Main "working during the day with the patients and spending the evenings over the microscope" [2]. Alzheimer collaborated during this period with important experts in the developing fields of psychiatry, neuropathology and neurohistology making himself major contributions. In 1901 he met the 51 old patient, Auguste Deter, "who suffered from impaired memory and finally lost most of her higher mental functions". Deter died in Frankfurt in 1906, when Alzheimer was already working in München as scientific assistant (equivalent today to an assistant professor position) first and then as associate Professor of the well-known Professor Kraeplin dedicating the whole of his time to the work in his research laboratory. Alzheimer had kept the contact to the clinic, so that he could study Deter's brain identifying the amyloid plaques and neurofibrillary tangles using silver staining techniques. On the 3rd of November 1906 Alzheimer presented in Tübingen side by side the pathology and the clinical symptoms of presenile dementia [2]. This case marks the beginning of the Alzheimer's disease research. Most of the hallmarks of Alzheimer's disease were already described in this first study. In 1912 he was appointed as Full Professor of Psychiatry at the University of Breslau. On the way to Breslau he fell ill and never completely recovered. Alzheimer died at the age of 51 as a result of heart failure.

[1] R. A. Stelzmann, H. Norman Schnitzlein, and F. Reed Murtagh, An english translation of alzheimer's 1907 paper, "über eine eigenartige erkankung der hirnrinde". Clinical Anatomy, 1995. 8(6): p. 429-431.
[2] K. Maurer and U. Maurer, Alzheimer: The Life of a Physician and Career of a Disease. 2003, New York: Columbia University Press. 256 p.

shift of the resonance angle at which the evanescent wave is generated, as imaged on the detector array (see Fig. 2).

Figure 3 presents the real-time monitoring curve describing the variation of the refractive index, expressed in angular shift, correlated to the aggregation dynamics of the Aβ 1-42 peptide. A positive angular shift corresponds to an increase of the refractive index of the solution, vice versa, a negative angular shift corresponds to a decrease of the refractive index of the peptidic solution. One can notice that the signal decreases while the monomer concentration is depleted during the aggregation. On the other hand, a positive angular shift is reported at the very beginning of the measurement. We explain this event with the adsorption of the Aβ 1-42 peptide onto the sensing multilayer surface, which leads to a local increase of the refractive index, as sensed by the BSW.

This surface loading effect was further investigated [1]. We verified that the adsorption mechanism does not fully shield the monitoring of the Aβ 1-42 fibrillization that takes place in the bulk. Moreover, the adsorption mechanism itself occurs in the presence of prefibrillar aggregates. Specifically, we reported no appreciable surface loading in the presence of Aβ 1-42 mature fibrils, which were incubated in the sensing chamber under the same conditions as the initially mon-

Felix Bloch (23.10.1905 – 10.09.1983)

Felix Bloch was a Swiss physicist born at the beginning of the 20th century who contributed to the development of NMR, and for which he was awarded the 1952 Nobel Prize for "his development of new ways and methods for nuclear magnetic precision measurements", together with Edward Mills Purcell [1].
Bloch studied physics at the Federal Institute of Technology (ETH) of Zürich, where he had the extraordinary chance to attend courses given by, among others, Debye and Schrödinger. It was thanks to those names that he became aware of the new wave mechanics, a subject that he would never set aside during his whole life. For his PhD, Bloch studied with Heisenberg at the University of Leipzig, gaining his doctoral degree in 1928 with a thesis on the quantum mechanics of electrons in crystals, which established the quantum theory of solids. This thesis defined the well-known Bloch waves as a wavefunction for an electron in a crystal.
Other important names surrounded him during the continuation of his career, influencing Bloch's way to see and describe nature. He was working with Wolfgang Pauli in Zürich, Niels Bohr in Copenhagen and Enrico Fermi in Rome, before he went back to Leipzig to Heisenberg, assuming a position as lecturer.
With the ascent of Hitler in 1933, due to his Jewish origins, Bloch decided to leave Germany for the US, where he started working at Stanford University. Bloch's work in the new continent laid the basis for the development of the modern NMR, which is daily used in the form of MRI in medical diagnosis.
In 1954, almost 10 years after the end of the Second World War, Bloch returned to Europe. He worked as the first general Director of CERN, in Geneva. This European parenthesis did not last long, since he soon returned to Stanford in 1955, where he carried on his investigation on nuclear magnetism.
Felix Bloch died in 1983 in Zürich, at the age of 77, after spending a life devoted to modern (and future) physics.

From: Nobel Lectures, Physics 1942-1962, Elsevier Publishing Company, Amsterdam, 1964.

[1] M. Sohlman, (Ed.) Nobel Foundation directory 2003. Vastervik, Sweden: AB CO Ekblad; 2003.

omeric Aβ 1-42 sample. Moreover, we incubated Aβ 1-42 protofibrils at a concentration around 4 times higher than the initial concentration for the Aβ 1-42 initially monomeric sample, and the surface loading phenomenon extent, as sensed by the BSW, was dramatically minor.

These evidences allowed for the conclusion that the species that are strongly adsorbed onto the multilayer oxi-nitride surface have to be in a precise pre-fibrillar conformation, with a lower molecular weight than the average weight attributed to protofibrils [1]).

Conclusion

Combined with the spread of techniques prone to cure it, this biosensor has great prospective for the future. Our work demonstrates that an integrated approach based on biophysics, biochemistry and use of novel photonic detection methods will decisively contribute to elucidate the molecular processes involved in the early dynamic events of protein aggregation and fibrillogenesis.

Exploiting the use of this innovative real-time biosensing approach, it is possible to monitor the refractive index variation of an Aβ 1-42 peptide solution during its aggregation. We were able to indirectly monitor the formation of Aβ 1-42 fibril and, more interestingly, to cover the first steps of the amyloid aggregation pathway, which comprises the formation of the first toxic oligomeric species.

The precise identification of the Aβ 1-42 species interacting with the surface is yet to come. Nevertheless, we proved that the adsorption/desorption mechanism is specific and occurs during the time lapse corresponding to the so-called lag-phase, which is silent when investigated with the classical amyloid detection techniques.

This consideration opens the application of this BSW based approach to the direct in vitro screening for molecular factors that influence the oligomerization process. We expect a variation in the timing for the adsorption/desorption mechanism, in addition to a variation in the rate of the refractive index diminution during the fibrillization phase.

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[10] P. Rivolo et al., *Real time secondary antibody detection by means of silicon-based multilayers sustaining Bloch surface waves*. Sensors and Actuators B: Chemical, 2012. **161**(1): p. 1046-1052.

[#] The figures are partial and simplified versions of the originals, which can be found as referenced [1].