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Thursday, March 22, Session I

L1

SPECTRAL WATERMARKING APPROACH TO STIMULATED RAMAN SPECTROSCOPY – BACKGROUND FREE FEMTOSECOND VIBRATION SPECTRA

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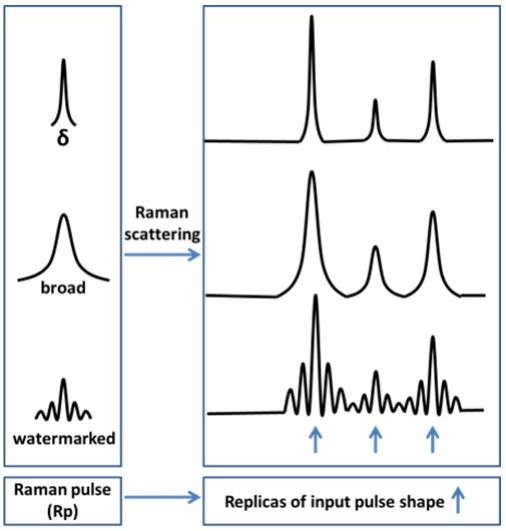
Abstract: Femtosecond stimulated Raman spectroscopy (FSRS) is plagued by serious baseline issues that lead to spurious peaks. Here we present a robust approach to FSRS that finally turns it into a versatile tool in structural biology.

FSRS experiment was introduced already nearly 20 year ago, despite its numerous benefits it is still recognized as a relatively exotic technique with only a handfull of groups worldwide that put it into practice. The reason is in inseparability of FSRS signal from the transient absorption and other non-liear backgrounds that bring numerous adjacent technical dificulties such as fixed patter noise. We developped a "spectra watermarking method" that borows concepts form the field of information procesing but essentialy function as spectral modualtion based lock-in detection. Since Raman signal is a form of hihgly defined spectra shift, such technique can safely extract the Raman signal from the numerous unwanted nonlinear signals.

Digital watermarking is a technique of inscribing a subtle signal (a watermark) on top of another, typically much stronger, signal such as music or video stream (carrier signal). The key problem is in developing a reliable method to



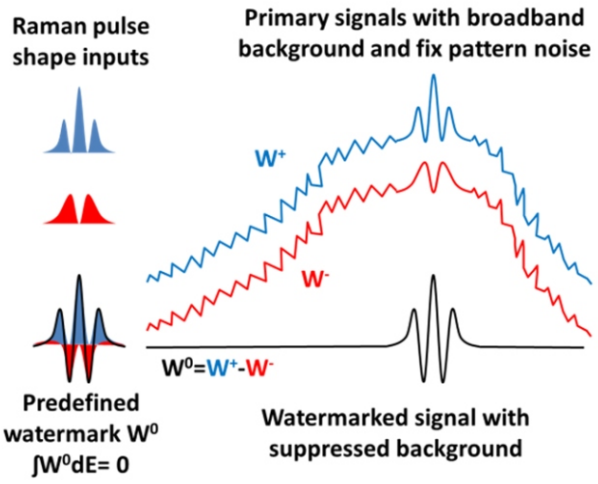


Figure 1. (left) Peaks in the Raman spectra are in fact replicas of spectral shape of the Raman pulse. When a Raman experiment is performed with broad watermarked pulses the Raman peaks are manifested as defined watermarks.

Figure 2. (right) Procedure of differential watermark inscribing: the chosen watermark with zero integral (W0) is divided into positive (W+) and negative (W-) components. Two experiments are performed with Rp pulses W+ and W-. The difference of these experiments results in a signal where Raman peaks carry the W0 watermark while the broadband baseline and fix pattern noise are simultaneously suppressed.

inscribe the watermark in such a way that it does not substantially obstruct the original signal, and at the same time can be retrieved with high fidelity independent of the carrier signal structure, and ideally even sustain signal alteration during transcription to various formats. This can be achieved by inscribing the watermark as pseudorandom wavelets. When data mixed with a watermark are convoluted by a second identical watermark in post-processing, the position of the watermark is manifested as a sharp localized peak due to constructive interference. With this information the separation of the watermark from the carrier signal becomes trivial matrix multiplication.

We recognized an important analogy between digital watermarking and the Raman experiment. In both cases the goal is to detect a fine structure on top of a strong, broadband and generally unspecified background. In the Raman experiment, fluorescence or stimulated emission can be treated as the carrier signal while the Raman signal itself can be seen as a watermark. The key problem in current femtosecond frequency domain Raman spectroscopy (FSRS) is that it depends on employing a spectrally-narrow Raman excitation that leads to a single specific manifestation of the Raman signal (upper part of Fig. 1). The advantage of such approach is that the recorded spectrogram represents a direct image of the vibrational spectra. Nowadays, however, in the era of digitized detection this benefit dropped in importance. Implicit data can be automatically converted to an explicit signal provided that the correct routine exists. The traditional approach of improving signal contrast by repeating the same experiment with a fixed narrow Raman excitation leads to improvement of the signal, but the background signal is constantly accumulated as well. If we instead accumulate data by cycling the Raman signal as pseudorandom watermarks (bottom part of Fig. 2) we can in principle recover only the desired signal. This is indeed possible experimentally by watermarking the pulses used to generate Raman signal (Raman pulse-pump: "Rp"), which results in a direct watermarking of the Raman signal as illustrated in Fig. 1-2. Figure 2 show that when watermarkign is generated as a difference of two complementary watermarks, it yields aditional benefit of fixed pattern noise suppression and aditional baseline removal. The method was already proven valid in applications such as investigation of carotenoid S* state origin or inspecting cofactors in proteins (fig 3).

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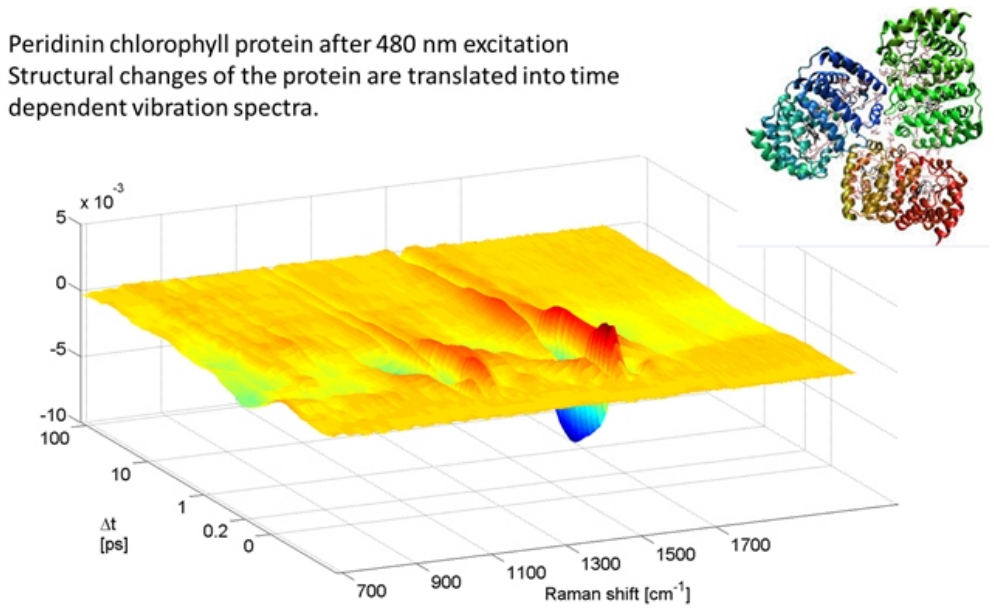

Figure 3. Example of data prcessed via watermarking. Femtosecond Raman spectra evolution of peridinin chlorophysl protein after excitation at 480 nm is displayed above. Note the perfect baseline achieved without any ad hoc corrections.

L2

CRITICAL DEFECTS IN CRYOPRESERVED CELL NUCLEI: DNA STRUCTURE CHANGES

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In this work, we shed new light on highly discussed chromatin fragmentation of cryopreserved cells. Moreover, for the first time, we described replicating cell-specific DNA damage and higher-order chromatin alterations after freezing/thawing. We identified DNA structural changes associated with the freeze/thaw process and correlated them with the viability of cells that had been frozen and thawed. We simultaneously evaluated DNA defects and higher-order chromatin structure of frozen and thawed cells with and without cryoprotectant treatment. We found that in replicating (S phase) cells, DNA was preferentially damaged by replication fork collapse, potentially leading to DNA double strand breaks (DSBs), which represent an important source of both genome instability and defects in epigenome maintenance. This induction of DNA defects by the freeze/thaw process was not prevented by any cryoprotectant studied. Both in replicating and non-replicating cells, freezing and thawing altered the chromatin structure in a cryoprotectant-dependent manner. Interestingly, cells with condensed chromatin, which was strongly stimulated by dimethyl sulfoxide (DMSO) prior to freezing and accompanied by the shrinkage of their nuclear envelopes, had the highest rate of survival upon freezing. The results of our work will facilitate the future design of compounds and procedures [1] to decrease injury to cryopreserved cells.

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L3

A DNA STRUCTURAL ALPHABET DISTINGUISHES THE STRUCTURAL FEATURES OF DNA BOUND TO TRANSCRIPTION FACTORS AND HISTONE PROTEINS

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We analyzed the structural behavior of DNA complexed with regulatory proteins (mostly transcription factors) and the nucleosome core particle (NCP). The three-dimensional structures of almost 25 thousand dinucleotide steps from more than 500 sequentially non-redundant crystal structures were classified by using DNA structural alphabet *CANA* (Conformational Alphabet of Nucleic Acids) described in [1] and associations between ten *CANA* letters and sixteen dinucleotide sequences were investigated. The associations showed features discriminating between specific and non-specific binding of DNA to proteins. Important is the specific role of two DNA structural forms, A-DNA, and BII-DNA, represented by the *CANA* letters *AAA* and *BB2*: *AAA* structures are avoided in non-specific NCP complexes, where the wrapping of the DNA duplex is explained by the periodic occurrence of *BB2* every 10.3 steps. In both regulatory and NCP complexes, the extent of bending of the DNA local helical axis does not influence proportional representation of the *CANA* alphabet letters, namely the relative incidences of *AAA* and *BB2* remain

constant in bent and straight duplexes. The analysis is described in detail in [2].

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This work was financially supported by the institutional funding to the Institute of Biotechnology (RVO 86652036), and by two ERDF and MEYS projects: BIOCEV (CZ.1.05/1.1.00/02.0109) and ELIXIR-CZ (CZ.02.1.01/0.0/0.0/16_013/0001777).

L4

MECHANISMS OF G-QUADRUPLEX BIOCHEMICAL SPECIFICITY

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G-quadruplexes are four-stranded nucleic acid structures thought to play widespread biological roles [1]. The growing list of cellular processes thought to be regulated by DNA or RNA G-quadruplexes includes transcription, RNA processing, translation, and mRNA localization. More than 30 proteins have been identified that interact with G-quadruplexes in various ways, and handful of cellular cofactors that bind G-quadruplexes have also been identified. This diversity of biochemical function raises an important question: how does the cellular machinery distinguish the many G-quadruplexes in the genome? We are exploring the hypothesis that this specificity can be achieved by mutations in the primary sequence of the G-quadruplex itself. To test this idea, we generated a 496-member G-quadruplex library, and tested each member for five different biochemical activities associated with

G-quadruplexes: the ability to bind GTP, to promote peroxidase reactions, to form dimers, to form tetramers, and to generate fluorescence [2-4]. This revealed that mutations in both tetrads and loops can significantly alter the specificity of a G-quadruplex to favor a particular biochemical activity. In some cases, changes in specificity are correlated with changes in the multimeric state of the G-quadruplex. We also identified a small-molecule ligand that inhibits multimerization, raising the possibility that G-quadruplex specificity can be modulated by small molecules. We are currently using a combination of NMR and X-ray crystallography to better understand these mutations from a structural perspective, and preliminary results in this area will be discussed. Taken together, these experiments provide new information about the mechanisms of G-quadruplex specificity, and should facilitate analysis of the biochemical roles of these structures in cells.

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3. Kolesnikova, S., Hubálek, M., Bednárová, L., Cvačka, J. and Curtis, E.A., Nucleic Acids Res., 2017, 45, 8684-8696.

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L5

STRUCTURAL CHARACTERIZATION OF BIOMEMBRANES: THE ROLE AND IMPACT OF COMPOSITION

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The structural characterizaton of lipid bilayers presents fundamental interest both in physics, for the study of thin fluctuating soft layers, and in biology, for the understanding of the function of biological membranes. This represents still a challenge: performing measurements on few nanometer thick, soft, visco-elastic and dynamic systems is close to the limits of the available tools and methods.

Neutron scattering techniques are rapidly developing for these studies. Since many biological processes occur at interfaces, the possibility of using neutron reflection to study structural and kinetic aspects of model as well as real biological systems is of considerable interest.

The most effective use of neutron reflection involves extensive deuterium substitution and this is becoming more

and more an available option in biological systems in general and lipid bilayers in particular [1]. The use of deuterated lipid extracts presents relevant differences both with the hydrogenated counterpart and with synthetic systems [2, 3].

The talk will review some progress made in the last few years by using neutron scattering at the ILL in the structural characterisation of biomembrane systems, efforts to build and characterize more and more complex systems [2-4], the impact in health related studies [4-6], and will provide perspectives for future developments [7]. It will aim at highlighting neutron reflectometry as a versatile method to tackle questions dealing with the understanding and function of biomembranes and their components. The impact of composition on the structure will be highlighted.

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4. V. Rondelli et al., *Sci. Rep. Nat.* **2016**.
5. A. Martel et al., *JACS* **2016**.
6. D. Di Silvio et al., *JCIS* **2017**

L6

STUDY ON ACTIVE SITE OF NUCLEOSIDE N-RIBOHYDROLASES FROM *ZEA MAYS*

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Purine and pyrimidine nucleosides are hydrolyzed by nucleoside N-ribohydrolases (NRHs, E.C. 3.2.2.-). These calcium-dependent enzymes catalyze a cleavage of the N-glycosidic bond in nucleosides to enable the recycling of the nucleobases and ribose. NRHs impose a strict specificity for the ribose moiety while residues interacting with the nucleobase highly vary. There are at least two subclasses of NRHs in plants, which belong to a class of nonspecific inosine/uridine NRHs. They differ in their substrate specificity and preferences towards inosine and xanthosine (subclass I) or uridine and xanthosine (subclass II). We performed a crystallographic study combined with site-directed mutagenesis and kinetic analyses to study nucleoside binding sites in two maize NRHs representing both subclasses, namely ZmNRH2b and ZmNRH3. Crystal structures of ZmNRH2b apoform and its complexes were solved up to 1.75 Å resolution and they were further compared with the structure of ZmNRH3. A role of several active-site residues was deeply studied in both enzymes detail and certain mutations were found to increase the hydrolysis of cytokinin ribosides. We further analyzed spatial and temporal expression of all five ZmNRH genes present in maize as well as a transient expression of ZmNRH-GFP fusion proteins in maize protoplasts. We also constructed and selected homozygous dexamethasone-inducible ZmNRH overexpressor lines (for all five maize NRH genes) in Arabidopsis thaliana to analyze the enzyme function in planta and measured levels of purine, pyrimidine and cytokinin metabolites. Finally, our experiments proved that NRHs metabolize also cytotoxic metabolites like 5-fluorouridine and 2-chloroadenosine.

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