2801-Pos

Twin-FRET: A New Molecular Ruler for Biomolecules

Sankar Jana[1], Marta Diez-Castellnou[2], Euan R. Kay[2], Carlos Penedo[3].
[1]School of Biology, University of St Andrews, St Andrews, United Kingdom, [2]EaStCHEM School of Chemistry, University of St Andrews, St Andrews, United Kingdom, [3]School of Biology, School of Physics and Astronomy, University of St Andrews, St Andrews, United Kingdom.

Conformational changes in biomolecules underpin all biological processes and being able to quantify these structural changes in solution is crucial to understand biological function. By carefully positioning two fluorophores within the biomolecule, it is possible to use fluorescence resonance energy transfer (FRET) as a molecular ruler to measure the desired distance. In current FRET assays, the biomolecule needs to be labelled with two different chemical fluorophores acting as a donor-acceptor FRET pair. Incorporation of these two chemically different species at specific positions within the biomolecule is challenging due to limited chemical labelling strategies. Here, we present a radically different strategy for measuring distances in biomolecules. We have developed the concept of TWIN-FRET which removes the need for two different fluorophores attached to the biomolecule by chemically encoding the FRET pair within the structure of the fluorophore itself. We have designed and synthesised a fluorescent molecule to prove this concept. The fluorophore (FH) has an acid-base equilibrium with a ground state pKa∼8.9. We have derivatized the fluorophore to its succinimide ester, and used this derivative, to label a duplex DNA with two molecules of the same fluorophore at specific positions. Our results demonstrate the transfer of non-radiative energy from the neutral (FH) to the anionic (F^-) state of the fluorescent molecule. We further demonstrate the use of TWIN-FRET to measure nanometer-size distances within the DNA duplex, and we obtained distance values similar to those obtained using a conventional FRET pair (Alexa488-Cy3). By removing the need to introduce two different chemical structures within the biomolecule, we greatly simplified the methodology to measure nanometer-size distances in biomolecules. We expect this technique to be widely used in structural and biophysical studies of nucleic acids, proteins and interactions between them.

2802-Pos

Evaluation of Cell Culture Media using Absorption and Transmission Fluorescence Excitation Emission Matrix (A-Teem)Spectroscopy

Marinella Sandros[1], Boqian Yang[1], Karoly Csatorday[1], Adam Gilmore[1], Alvin Togonon[1], John Bobiak[2].
[1]Horiba Scientific, Piscataway, NJ, USA, [2]Bristol-Myers Squibb, Devens, MA, USA.

With regards to protein production using mammalian cell culture, it is important to utilize the correct cell culture media for use in production processes. Cell culture media is usually prepared as an aqueous solution and should provide everything cells need for optimal growth as well as product yield and quality. This could include amino acids, glucose, vitamins and various other nutrients. The exact composition and concentrations of components found in a given type of cell culture media vary depending on the unique needs of a given cell line and are usually considered proprietary information by their manufacturers. In any given bioreactor process, it is important to identify the proper type of cell culture media and its quality because even subtle variations in composition could have a noticeable impact in the growth rate of the cell culture and its yield. Therefore, the composition and quality of cell culture media in bioreactors must be tightly screened in order to maintain an optimal bioreactor process. As a result, methods of identifying and analyzing the quality of cell culture media has become an important focus. Existing solutions include methods such as chromatographic separations and mass spectrometry. However, these methods are generally considered to be too expensive and time consuming for routine analysis of cell culture media samples. As a result, the industry has begun to turn to spectroscopic methods such as fluorescence for cell culture media analysis due to rapid testing, minimal sample handling, and relatively lower cost when compared to other methods. One method of particular interest is Absorption and Transmission Fluorescence Excitation Emission Matrix (A-TEEM) Spectroscopy. Here we evaluated if we can differentiate different classes of commercially available cell culture media and assess their stability over time.

2803-Pos

Characterization of Lipids in Leishmania Infected Cells by SERS Microscopy

Vesna Zivanovic[1], Geo Semini[2], Michael Laue[2], Daniela Drescher[1], Toni Aebischer[2], Janina Kneipp[1].
[1]Dept Chemistry, Humboldt-Univ zu Berlin, Berlin, Germany, [2]Robert Koch-Institut, Berlin, Germany.

Understanding the function and metabolism of cellular lipids is crucial for characterization and treating various diseases. Several methods for studying the distribution, dynamics, and function of lipids are developed, e.g., fluorescence resonance energy transfer (FRET), super-resolution and electron microscopy. Nevertheless, a label-free approach for probing lipids in the living, healthy or diseased cells is still lacking. We show here the direct, label-free probing of lipid composition in the Leishmania-infected macrophage cells in vitro by surface-enhanced Raman scattering (SERS).[1] SERS is used as a sensitive tool to study the structure and function of macromolecules inside the infected cells, as it provides comprehensive information on the molecules in the nm-scale proximity of gold nanoparticles that are used as nanoprobes. As revealed by electron microscopy, the gold nanoparticles access parasitophorous vacuoles and parasites inside the infected macrophage cells. Spectra acquired from the parasitophorous vacuoles and the parasite itself provide information on the distribution of the lipid molecules that are important for the virulence of Leishmania, e.g., cholesterol and ergosterol.[2] Spectra of proteophosphoglycans, an important hallmark of the infection, are detected inside the different compartments. In conclusions, SERS enables us to study the interaction and distribution of molecules in Leishmania infected cells and similar disease models with disturbed lipid metabolism. 1. Živanović V et al., Anal. Chem., 2018, 90, 8154-8161. 2. Subha S, PLoS Pathogens, 2011, 7, 9.

2804-Pos

The Fluorescence Lifetime of Bound NADH: Clues from the Phasor Plots

Suman Ranjit, Leonel S. Malacrida, Enrico Gratton.
Dept Biomed Eng, Univ Calif Irvine, Irvine, CA, USA.

Changes in metabolism in cells are often studied by the ratio of NAD+ to NADH. This ratio is correlated to free and enzyme bound NADH, which can be used as a measurement of metabolic states in cells using fluorescence lifetime imaging (FLIM). Binding of NADH to the enzyme causes separation between nicotinamide and adenine moieties of Nicotinamide adenine dinucleotide (NADH) from their collapsed structure in solution and results in increase of fluorescence quantum yield and lifetime. The extent of increase in fluorescence lifetime is dependent on the apoenzyme and presence of auxiliary ions. Reports from past studies show distinctive discrepancies in calculation of the bound NADH lifetime, often related to complications in sample preparation and limitations in data acquisition. In this work, we show that in presence of oxalic ion, proper preparation of lactate dehydrogenase (LDH) bound NADH has a lifetime of 3.4 ns and is positioned on the universal semi-circle of the phasor plot, representing mono-exponential lifetime. Improper preparation results in a mixture of species, with phasor positions inside the universal semi-circle. Measurement in cellular environment show similar trend and a linear trajectory between free NADH and cellular NADH components, which when extrapolated to the universal semi-circle shows a lifetime of 3.4 ns at the crossing point. These results suggests that 3.4 ns can be used as a bound NADH lifetime and phasor approach can correlate lifetime contributions to concentration fractions of free and bound species. The effects of different types of FLIM acquisitions are also discussed in context. This work is supported by NIH grant P41-GM103540.

2805-Pos

Investigation of the Structural Effects of Radiotherapy Dose Rate on Rat Lung Tissue: An FTIR Imaging Study

Ipek Ozyurt[1], Sebnem Garip[1], Fatma Kucuk Baloglu[2], Faruk Zorlu[3], Feride Severcan[1].
[1]Faculty of Medicine, Altinbas University, Istanbul, Turkey, [2]Biology, Giresun University, Giresun, Turkey, [3]Radiation Oncology, Hacettepe University, Ankara, Turkey.

Radiotherapy is widely used to cure cancer. During treatment, ionizing radiation is applied by a linear accelerator to the area that is being treated. During this process normal cells can also be damaged by radiotherapy. The lung is one of the most sensitive organs to ionizing radiation. Radiation-induced lung disease (RILD) is regarded as the result of an abnormal healing response and it can lead to pulmonary fibrosis. Dose amount of radiation plays a key role on healing process and prevalence of side effects on normal tissues. The aim of this study is to evaluate the effect of dose rate on rat lung tissue by using FTIR microspectroscopy. A total of 25 animals were randomly devided into three groups. Group 1: control group, sham irradiated. Group 2: receiving a single dose of 12 Gy in DR of 300 monitor unit (MU) / min. Group 3; receiving a single dose of 12 Gy in DR of 600 MU/min. At the 6th and 16th week of the RT, animals from each group were sacrificed for evaluation. For this purpose FTIR microspectroscopy was used to detect the dose rate-induced changes in the concentration of biomolecules such as lipids and proteins and protein/ lipid and saturated/ unsaturated lipid ratios. These results indicated a remarkable

increase in total lipid amount in both group 2 and 3 depending on dose rate. The current study clearly revealed the power of FTIR microspectroscopy in the precise determination of dose rate induced biomolecular changes in rat lung tissue.

2806-Pos

The Disruption of Beta Sheets in Amyloidogenic Sequences by Gly-Gly-Ala

Sarah A. Petty[1], Andrew T. Mullin[2], Sam A. Michelhaugh[1], Benjamin R. Fitzgerald[1].
[1]Dept Chemistry, College of the Holy Cross, Worcester, MA, USA, [2]Dept Biology, College of the Holy Cross, Worcester, MA, USA.

Alpha-synuclein is an intrinsically disordered neuronal protein whose misfolding and aggregation is linked to the onset of Parkinson's disease. Deletion of residues 67-71 (GGAVV) has been seen to prevent aggregation of alpha-synuclein, suggesting a potential role of this sequence in nucleating the aggregation of the full protein. We have established that certain short peptides based on this deleted sequence have the ability to aggregate in isolation, whereas others remain monomeric and unstructured as in the full-length protein. In this work, we examine the ability of the disordered peptide, GGA to reverse the aggregation of the beta-sheet forming peptide AVV under various conditions. Further, we will discuss the scope of this structural disruption by GGA based on our experiments with several other beta-sheet peptides derived from alpha-synuclein including the structural similar AVA and other more varied peptide sequences.

Peptides were synthesized using solid phase Fmoc chemistry and were capped at both termini to eliminate charge effects. Fourier transform infrared spectroscopy was used to determine the secondary structure by examining the frequency of the Amide I mode. Combinations of disordered and structured peptides were mixed at different concentrations and in different ratios. The IR spectra of the mixtures were compared to the weighted average of the individual spectra to assess the effect of any interaction between the two peptides.

2807-Pos

Fluorescence Lifetime Imaging of Tetracycline-Stained Retinal Hydroxyapatite: An Early Biomarker for Age-Related Macular Degeneration?

Richard B. Thompson[1], Henryk Szmacinski[1], Kavita Hegde[2], Adam Puche[3], Trevor McGill[4], Martha Neuringer[5], Imre Lengyel[6].
[1]Dept Biochem/Molec Biol, Univ Maryland Sch Med, Baltimore, MD, USA, [2]Coppin State University, Baltimore, MD, USA, [3]Dept Anatomy/Neurobiol, Univ Maryland Sch Med, Baltimore, MD, USA, [4]Dept Ophthalmology, Oregon Health Sciences University, Portland, OR, USA, [5]Oregon Health Sciences University, Portland, OR, USA, [6]Queen's University Belfast, Belfast, United Kingdom.

Age-related macular degeneration (AMD) is the most common cause of loss of vision in the elderly in the developed world, affecting over ten million in the United States alone. It is widely held that the atrophy of the light-sensitive cells and their associated neurons in the retina has its origin in protein- and lipid-rich deposits between the retinal pigment epithelium (RPE) and Bruch's membranes; the best known of these deposits are called drusen. Recently, we found that drusen contained microscopic spherules of hydroxyapatite (HAP; $Ca_5(PO_4)_3OH$), the hard form of calcium phosphate found in bones and teeth, and that these spherules became coated in vivo with proteins characteristic of drusen such as amyloid beta, vitronectin, and complement factor H, a variant of which is the strongest genetic risk factor for AMD (Thompson, et al., PNAS 2015 PMID: 25605911). These findings and others led us to propose that the growth of drusen is nucleated by the HAP spherules, and thus they might be an early biomarker to be used for screening for AMD. We found that the HAP stained by certain tetracyclines exhibited substantial increases in fluorescence lifetime upon binding, significantly longer than the lifetime of the retina background fluorescence imaged by Schweitzer, Zinkernagel, and their colleagues. Thus we were able to image tetracycline-stained drusen in vitro from aged human and macaque retinas using FLIM. Recent results will be shown.

2808-Pos

In Vivo Cell Tracking and Cleared Tissue Imaging with Extended Field of View Selective Plane Illumination Microscopy

Leonardo A. Saunders[1,2], Devin Pace[3], Arianna Gentile[1], Dominik Stich[1], Angeles B. Ribera[4], Douglas P. Shepherd[1].
[1]Pharmacology, University of Colorado Anschutz, Aurora, CO, USA, [2]Integrated Sciences, University of Colorado Denver, Denver, CO, USA, [3]Physics, University of Colorado Denver, Denver, CO, USA, [4]Physiology and Biophysics, University of Colorado Anschutz, Aurora, CO, USA.

Single Plane Illumination Microscopy (SPIM), a type of light sheet fluorescence microscopy (LSFM), is continuously evolving to new demands for rapid and high-resolution volume imaging of biological samples. The OpenSPIM framework [1] was introduced as an open access blueprint to aid wide adoption of light sheet methods. Because of the inherent trade off in field of view (FOV) and resolution when generating the exciting light sheet, the original OpenSPIM has a limited FOV and modest axial resolution. Building on OpenSPIM and existing work on scalable light sheets [2], we introduce an open source Extended Field of View Single Plane Illumination Microscope (eFOV-SPIM). eFOV-SPIM has an adjustable field-of-view that enables both high-resolution imaging of in vivo dynamics and large cleared tissue samples on the same platform. eFOV-SPIM requires minor modification to the OpenSPIM excitation pathway combined with dedicated in vivo and cleared tissue sample chambers and sample mounts. Here we present the optical characterization of eFOV-SPIM system as well as experimental demonstration of single-cell tracking in Danio rerio and near-isotropic imaging of optical cleared tissue.

1. P. G. Pitrone, J. Schindelin, L. Stuyvenberg, S. Preibisch, M. Weber, K. W. Eliceiri, J. Huisken, and P. Tomancak, ''Openspim: an open-access light-sheet microscopy platform,'' nature methods 10, 598 (2013). 2. K. M. Dean and R. Fiolka, ''Uniform and scalable light-sheets generated by extended focusing,'' Opt. express 22, 26141-26152 (2014).

2809-Pos

Rotational and Translational Diffusion in Concentrated Ficoll Solutions

Elton Jhamba[1], **Hacene Boukari**[2].
[1]Health Sciences, University of New Mexico, Albuquerque, NM, USA, [2]Division of Physical and Computational Sciences, Delaware State University, Dover, DE, USA.

We combined fluorescence correlation spectroscopy (FCS), fluorescence anisotropy (FA), and fluorescence imaging microscopy to measure the rotational diffusion and the translational diffusion of different nanoprobes (Alexa488, rhodamine 6G, fluorescein, FITC-Ficoll70) and microbeads in non-fluorescent -hence ''invisible''- aqueous Ficoll (MW$\approx$70 kDa) solutions. We first noticed that the emission spectrum and the lifetime of each fluorophore is not significantly altered by the increase of the Ficoll concentration. Multiple tracking of the microbeads indicated a linear time-dependence of the mean-squared displacements, indicating a diffusion mechanism. Further, changes of the translational diffusion of the microbeads with increase of Ficoll concentration could be readily attributed to changes of the bulk viscosity of the Ficoll solutions, consistent with the Stokes-Einstein relation. However, FCS and FA measurements of the nanoprobes showed that the changes of the translational and rotational diffusion coefficients with increase of Ficoll concentration could not be accounted for by the corresponding changes of the bulk viscosity of the Ficoll solutions as would be suggested by the Stokes-Einstein relations for both diffusions. So, instead, we analyzed the diffusion data -rotation and translation- with the entropic model proposed by de-Gennes and fit the concentration dependence with a stretched exponential [$\exp(-\alpha c^n)$] with n being related to the quality of the solvent and α being proportional to the size of the nanoprobes. Remarkably, for both sets of rotational and translational data, the fits yielded n-value close to one, indicating a theta-like behavior of the host Ficoll-water system. However, the α-value for translation was larger than that of rotation, indicating dissimilar local entropic effects on the rotation and translation.

2810-Pos

Automation of a Laser Tweezers Raman Spectroscopy Apparatus for Biological Investigations

Nathaniel W. Scott, Scott Hancock, Brooke C. Hester, Jennifer L. Burris.
Physics and Astronomy, Appalachian State University, Boone, NC, USA.

The Biophysics and Optical Sciences Facility (BiyOSef) maintains a custom-built and partially automated Laser Tweezers Raman Spectroscopy (LTRS) apparatus. Laser tweezers utilizes optical forces produced through refraction and reflection to trap particles in a laser beam, while Raman spectroscopy measures a unique spectral fingerprint of materials due to unique shifts in vibrational molecules of the materials. Our apparatus combines both methods to increase our resolution of the Raman fingerprint. This method allows for the capture, manipulation, and study of biological cells, microbes, proteins, and other micron-sized particles in a fluid. Through the use of optical trapping of individual particles and confocal methods, we can effectively reduce noise that can interfere with our Raman signal thereby isolating the Raman signal. The alignment of the apparatus takes considerable time, requires months of training, and has the potential to be inconsistent when done manually. Through automation, we have been able to maximize our resolution and minimize the time necessary to achieve