¹Anima Cell Metrology, Inc., ²Department of Cell Research and Immunology, Tel Aviv University, ³Department of Chemistry, University of Pennsylvania, Philadelphia

We introduce Protein Synthesis Monitoring (PSM) – a technique to monitor protein synthesis in live cells. In PSM, we transfect cells with tRNA labeled as FRET donors and acceptors. A FRET signal is generated only when a donor- and an acceptor-labeled tRNA come in close contact (< 7nm), as they do on the ribosome during elongation. The intensity of the FRET signal correlates with the number of ribosomes engaged in protein synthesis, providing a real-time, live-cell assay for measuring rates of protein synthesis. PSM can be used to monitor the rate of either total protein synthesis (overall PSM), using bulk tRNAs, or of the synthesis of a specific protein (specific PSM), using specific pairs of tRNA to mark the protein of interest. PSM has sub-micron spatial and sub-second temporal resolutions. Cells continue to live and grow normally, and the synthesized proteins are unchanged since the labeling is on the tRNA itself and not on the amino acid. We have demonstrated specific PSM for monitoring synthesis of a viral protein during viral infection using Isoleucine tRNA, and for monitoring synthesis of collagen during fibrosis in mouse fibroblasts using tRNA-Gly and tRNA-Pro, as collagen is distinguished by many repeats of Gly-Pro dipeptides. We will discuss these results as well as additional applications of PSM in basic research, drug discovery, cell sorting, neurobiology, cancer, biomanufacturing, viral infections, and various protein-synthesis specific diseases.

90

Rapid Protein Analysis: Reagents for Lysate Preparation, Separation, Transfer, and Detection

Hugh White, F. Curtis

Lonza Rockland Inc., Rockland, ME

Protein sample preparation, separation in denaturing gels, detection by staining, and Western blot transfer followed by immunological detection are key elements of basic protein analysis in many lab settings, but can be very time-consuming. Through selection and use of optimized reagents the typical work flow from sample preparation to completion of blot detection may be dramatically reduced from 8 hours-or more, to less than 4 hours in total elapsed time. This process flow uses reagents allowing: lysate preparation in <10 minutes, separations in 20-25 minutes (PAGeR™ EX gels), staining or transfer in 10 minutes (ProSieve™ EX Safe Stain, or ProSieve™ EX Transfer Buffer), and immunological detection in 1-2 hours. This optimized process utilizes reagents that do not require dedicated and costly systems. The reagents used offer further advantages: the PAGeR™ EX gels used for rapid separation are stable for 1 year of refrigerated storage, and the ProSieve™ EX Safe Stain, and ProSieve™ EX Transfer Buffer do not include, or require the use of, any hazardous materials. This optimized workflow has been verified by demonstrating the rapid detection of the widely used control target glyceraldehyde-3-phosphate dehydrogenase (GAPDH), with sensitivities comparable to standard methods.

91

Analysis of Local Dynamics of Human Insulin and a Rapid-acting Insulin Analog by Hydrogen Deuterium Exchange Mass Spectrometry

Joomi Ahn¹, Shiori Nakazawa^{2,3}, Noritaka Hashii³, Kenji Hirose⁴, Nana Kawasaki^{2,3}

¹Waters Corporation, Milford, MA; ²Hokkaido University, Sapporo, Japan; ³National Institute of Health Science, Tokyo, Japan; ⁴Nihon Waters, Tokyo, Japan

Human insulin, used by diabetics to regulate blood sugar, was first introduced as a recombinant therapeutic drug nearly 30 years ago. Human insulin and insulin lispro have identical primary structure, except for the transposition of two amino acids. Lispro is one of the rapid-acting insulin analogs, which has higher tendency to dissociate than human insulin. In this study, we present an analytical workflow to allow us to detect the difference in the oligomeric dynamics using Hydrogen Deuterium Exchange Mass Spectrometry (HDX MS). The HDX analysis on Insulin and Lispro peptides was conducted to identify the location where different deuterium uptakes were observed between human insulin and lispro. The detected areas were illustrated in various formats to help understand their flexibility associated with rapid dissociation of insulin oligomers.

Drug products, human insulin (Humulin R) and lispro (Humalog), were reduced and digested online by pepsin. Deuterium labeling, quenching, and injection to on-line pepsin digestion were prepared using a robotic sample manager. Labeling experiments in 0, 0.5, 5, 10, 60, and 180 min interval were duplicated for both samples. The peptic digests were separated on a UPLC system at 0 °C. Q-TOF MS was used to measure the deuterium incorporation of identified peptides. The amount of deuterium was determined by automated HDX data processing software, DynamX 2.0.

We obtained 98% of sequence coverage for both human insulin and lispro. From peptide HDX determination, two regions were revealed distinctive different values in deuterium uptakes between human insulin and lispro; the N terminus of chain A, and a region adjacent to the C terminus of chain B. We attributed this localized behavior to the relation of hexamerization and dimerization, respectively. Furthermore, characteristic profiles that showed different deuteration margins between two insulins were determined, which was also consistent with their involvement in hexamer and dimer formation.

92

Biochemical Characterization of Human IL-15 Receptor-alpha

Oleg Chertov¹, E. Chertova², R. Sowder², J. Lifson², C. Bergamaschi³, B. K. Felber⁴, G. N. Pavlakis³

¹Protein Chemistry Laboratory, ATP, SAIC-Frederick, Frederick National Laboratory for Cancer Research, Frederick, MD, ²AIDS and Cancer Virus Program, SAIC-Frederick, Frederick National Laboratory for Cancer Research, Frederick, MD, ³Human Retrovirus Section and ⁴Human Retrovirus Pathogenesis Section, Vaccine Branch, CCR, NCI, Frederick, MD

The cytokine IL-15 is a growth, mobilization and activation factor for NK cells, CD8+ T cells and intraepithelial T