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MICROSCOPIC SCOOPING FOR UNBIASED, SUBCELLULAR, SPATIAL OPTOPROTEOMIC DISCOVERY

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Microscopy-guided proteomics at organelle-level resolution has the power to reveal previously unknown proteins in disease- or function-specific regions.

We present a breakthrough method for spatial protein purification using in situ subcellular photo-biotinylation, enabling precise labeling of proteins within user-defined regions and fully automated replication across thousands of fields of view.

As a compelling example, stress granules have historically been difficult to characterize due to their dynamic and membrane-less nature. Using optoproteomics, two-photon illumination was directed to G3BP1-positive stress granules to trigger localized photo-biotinylation, enabling selective purification and subsequent mass spectrometric analysis. This automated workflow revealed previously unrecognized high-confidence interactors and achieved 96% specificity upon validation.

Syncell's Microscoop enables hypothesis-free, high-resolution mapping of subcellular proteomes within precisely defined regions of interest, advancing our understanding of dynamic cellular structures such as stress granules.

Friday, March 20, Session V

L19

C-TERMINAL DOMAIN OF THE FILAMENTOUS HEMAGGLUTININ FhaB IS CRUCIAL FOR INTERACTION OF *BORDETELLA PERTUSSIS* WITH CILIATED EPITHELIAL CELLS

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Bordetella pertussis, the etiological agent of whooping cough (pertussis), produces a ~370 kDa filamentous hemagglutinin (FhaB) that functions as a key adhesin required for colonization of the respiratory tract. FhaB is secreted via a two-partner secretion (TPS) pathwayas an extended hairpin and, under *in vitro* conditions, it is proteolytically processed to release the ~230 kDa 'mature' FHA antigen used in acellular pertussis vaccines. Here, we show that FhaB remains largely unprocessed in *B. pertussis* adhering to ciliated airway epithelial cells and that its C-terminal domain (CT) is essential for host engagement. Using solution NMR spectroscopy, we determined the structure of the CT and found that it adopts a previously unrecognized, compact protein fold. Genetic ablation of the CT does not impair FhaB folding, secretion, or surface display, but abolishes bacterial adhesion to primary human nasal ciliated epithelial cells and prevents nasal colonization, shedding, and transmission in a murine catarrhal infection model. These findings establish the CT as a critical determinant of upper airway colonization and identify full-length FhaB, rather than processed FHA, as the biologically relevant adhesin during infection. Our results reframe the model of FhaB biogenesis and uncover a unique virulence mechanism with direct implications for pertussis vaccine design.

L20

HIGH-RESOLUTION SOLID-STATE NMR OF PROTEINS: CURRENT STATUS AND PERSPECTIVES

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Solid-state NMR is used for characterization of solid proteins, including supramolecular assemblies, membrane proteins, fibrils, micro-crystalline preparations, and protein sediments. Typical targets are high molecular weight objects that are associated with slow tumbling that compromises solution-state NMR assessment. Recent revolution in NMR hardware with very high magnetic fields (28 Tesla) and ultra-fast sample rotation (frequencies up to 200 kHz) enabled high-resolution in proton detected experiments [1]. At the same time, sample quantities are dramatically reduced, down to about 0.5 mg. Multidimensional approaches are developed to resolve problems with spectral crowding and to facilitate peak assignments, with the current record of (2x)72-kDa tryptophan synthase studied in atomic resolution [2].

Recently, we introduced transverse mixing pulse sequence elements, TROP [3], that systematically enhance sensitivity of multidimensional spectra by a factor of 1.4 for each indirectly sampled dimension. Our work quickly inspired other researchers to look for other pulse sequences mediating the same type of coherence transfer and employ the preservation of equivalent pathways principle to boost sensitivity. SPEPS [4] and TOCSY [5] are the two examples already presented in the literature.

In this talk, I will present the status in the field as well as our own experience with multiply sensitivity-enhanced three-to-five-dimensional spectra of different types, designed to yield backbone as well as side-chain assignments [6]. The results document robustness of TROP sequences and enormous time savings using this approach, unlocking the solid-state NMR methods to study protein assemblies that could escape detection so far.

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L21

HOST, SWEET HOST! A TALE OF GLYCANS, LECTINS, MICROBES, KILLERS

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The constant immune cross-talk of glycans, lectins, microbes, and the various immune cells of our body is one of the essential factors determining our health or disease. Natural killer (NK) cells are one of the early, direct responders to such a challenge. Using their surface receptors, they constantly strive to detect molecular patterns associated with infection, cellular stress, or cancerous transformation.

This contribution will describe recent advances in understanding such molecular dialogues on two specific examples from human NK cells: Siglec-7, a sialic acid-binding immunoglobulin-like lectin inhibitory receptor, and NKp46, an immunoglobulin-like activation receptor. Siglec-7 preferentially binds glycoconjugates with (2,8)-linked disialic acids and branched (2,6)-linked sialic acids [1], as those found in GD3 and GT1b gangliosides [2]. However, its immunosuppressive potential is also exploited by pathogens such as Neisseria meningitidis [3] and Fusobacterium nucleatum [4], causing potentially deadly infections of the brain and spine or supporting tumour transformation, e.g., in colorectal cancer, respectively.

On the other hand, NKp46 has a wide range of known ligands of viral or microbial origin, among them the epithelial adhesin 1 (Epa1), a C-type lectin of pathogenic yeast *Candida glabrata*. While Epa1 lectin activity helps colonize the host, the same activity is used to detect the yeast

through NKp46 [5]. Biochemical and structural details of these recognition processes and their elucidation using a wide range of methods, from eukaryotic recombinant (glyco)protein production and characterization to nuclear magnetic resonance spectroscopy, will be discussed.

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L23

PHOTONIC INTEGRATED CIRCUITS - FUTURE OF IMMIBILIZATION TECHNIQUES

Josef Uskoba

BioTech a.s., Prague

Delta Life Science's pioneering Photonic integrated Circuits (PIC) technology is redefining the landscape of biosensing by integrating advanced photonic circuits with high-sensitivity, multiplexed detection capabilities. At the core of the platform is the innovative inQuiQ® instrument, which utilizes silicon chip-based ring resonators to confine light at the nanoscale, generating evanescent fields that enable real-time detection of biomolecular interactions with exceptional precision. This label-free technique operates on principles similar to Surface Plasmon Resonance (SPR), but surpasses traditional approaches through its compactness, scalability, and ability to conduct multiplex analyses processing multiple targets in parallel without loss of sensitivity.

The PIC system's sensitivity is remarkable, capable of resolving minute changes in refractive index with baseline noise as low as 0.01 RU at a 1Hz read-out, thus facilitating detection down to small molecules and subtle conformational changes in proteins. Its compact photonic chip design not only ensures a cost-effective solution but also enables broad accessibility for life science researchers and pharmaceutical developers, accelerating workflows and enhancing productivity compared to bulky, single-analyte biosensors. The versatility of the PIC platform extends beyond drug discovery; potential applications range from medical diagnostics to food allergen analysis and environmental nutrient monitoring, empowering researchers to study molecular-level phenomena with unprecedented accuracy.

In summary, Delta Life Science's nanophotonic evanescent field sensing technology offers an affordable, highly accurate, and user-friendly biosensing solution, poised to advance scientific research and healthcare diagnostics by delivering rapid, reliable insights into molecular interactions. [1]

1. https://www.deltalifescience.com/faq/.