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# ナカライテスク株式会社 テクニカルセミナー

## Olink社 PLA技術について

免疫組織化学分野に革命を起こす、Olink社が誇るPLA技術！
Duolink™ システムをご紹介します。

**Date** : Wednesday, July 2, 2008 14:30-15:30

**Venue** : Seminar Room 7F, Building A (RIKEN CDB)

**Title** : **Duolink™ を用いたタンパク相互作用と翻訳後修飾の可視化**

**Visualization of protein interactions and post translational modifications using Duolink™**

**Speaker** : Erik Nyström, Application Specialist,Olink Bioscience

## Summary:


The *in situ* Proximity Ligation Assay (*n situ* PLA) offers the means to study protein modifications and interactions in unmodified cells and tissue, avoiding both fusion proteins and over expression strategies, making it ideal for studying signaling pathways and complex formations. Furthermore, *n situ* PLA enables detection, visualization and quantification of single molecule events.

Here we present *n situ* PLA using secondary antibodies, conjugated with a DNA strand, specific for immunoglobins from different species. An amplifiable DNA strand is generated when two secondary probes are in proximity due to the binding to a pair of corresponding primary antibodies.

By combining the requirement of dual antibody recognition of the target (e.g. protein complex or post translational modification) with signal amplification, *n situ* PLA exhibits sensitivity and specificity. The signal amplification of a detected event is generated by an amplifiable DNA strand that serves as a template for multiple fluorescent molecules and the targets are visualized with a standard fluorescent microscope.

Individual post translational modifications, in this case phosphorylation events of the PDGF receptor β in response to stimulation, were detected by in situ PLA and quantified. Quantification data showed more than a tenfold relative average increase of modifications per cell after treatment with PDGF-bb. Cells were in addition analyzed individually to investigate the spread of modifications within the cell population.

We also present data on the complex formation of SMAD protein transcription factors using *n situ* PLA. Cells were treated with TGF-β to induce the signaling pathway. The formation and localization of interactions between SMAD4 and SMAD1/2/3 were visualized and quantified. A relative increase of endogenous SMAD complexes in TGF-β stimulated cells were observed compared to untreated cells. The cytoplasmic or nuclear localization was established for each detected complex.
The generic approach to *n situ* PLA is developed by Olink Bioscience as a commercial product marketed as Duolink™.


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