Improved Phos-tag Biotin and its application toward phosphoproteomics

(改良型 Phos-tag Biotin とリン酸化プロテオミクスへの応用)

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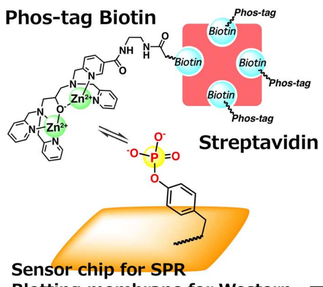
We have developed various analytical methods for phosphoproteomic research that use several derivatives of Phos-tag. Among these, biotin-pendant Zn^{2+}–Phos-tag (Phos-tag Biotin) has been used for surface plasmon resonance analysis of phosphopeptides and for Western blotting analysis of phosphoproteins on a protein-blotting membrane. In this study, our aim is to develop more-advanced applications for the detection of phosphopeptides and phosphoproteins by using several newly synthesized Phos-tag derivatives, including a bisbiotinylated Phos-tag (BTL-108), a tetrakisbiotinylated Phos-tag (BTL-109), and a monobiotinylated Phos-tag with a dodeca(ethylene glycol) spacer (BTL-111), as well as the existent product BTL-104. Among these Phos-tag derivatives, BTL-111 (commercially available form Wako Pure Chemical Industries, Osaka, Japan) showed the best performance in Western blotting by an ECL system using HRP-conjugated streptavidin. In addition, in a quartz-crystal microbalance analysis of a phosphoprotein, the presence of the long hydrophilic dodeca(ethylene glycol) spacer in a novel Phos-tag sensor chip coated with BTL-111 resulted in a greater sensitivity than was achieved with a similar chip coated with BTL-104. Moreover, a peptide microarray chip technique using the ECL system and BTL-111 permitted high-throughput assays for the specific and highly sensitive detection of protein kinase activities in cell lysates. As well as these lab-on-a-chip techniques, Bio-Plex phosphoprotein detection analyses were also carried out successfully by using a complex of BTL-111 and phycoerythrin-conjugated streptavidin. In conclusion, these advanced techniques are expected to be useful for more-sensitive screening to provide information capable of resolving complex kinase/phosphatase-dependent intracellular signaling networks, leading to improved disease diagnosis and drug discovery.

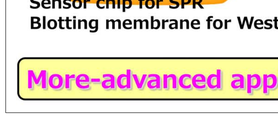
① Background

SPR & Western blotting method using Phos-tag Biotin

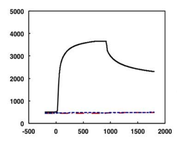
Phos-tag Biotin



Streptavidin

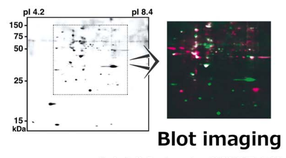


Sensor chip for SPR
Blotting membrane for Western



SPR imaging

Anal. Chem., (2005) 77: 3979



Blot imaging

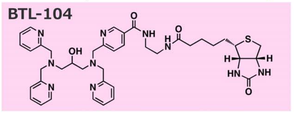
Mol. Cell. Proteomics, (2006) 5: 749

More-advanced application for phosphoproteomics

② Strategy

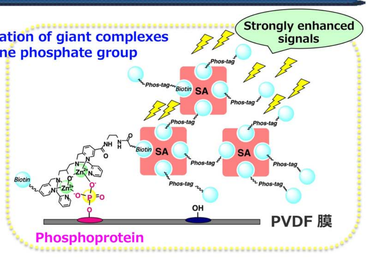
Existent product

BTL-104



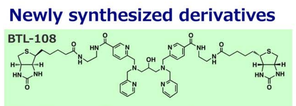
Formation of giant complexes per one phosphate group

Strongly enhanced signals

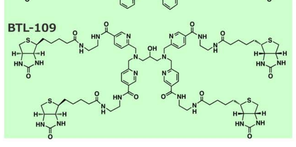


Newly synthesized derivatives

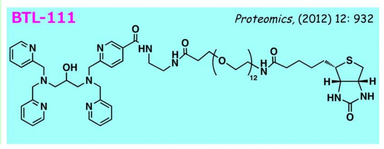
BTL-108



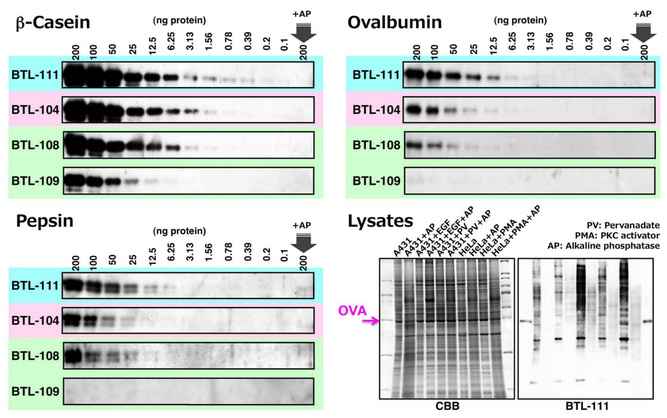
BTL-109



BTL-111 *Proteomics*, (2012) 12: 932

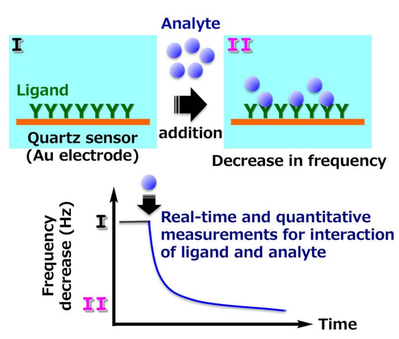


③ Western blotting

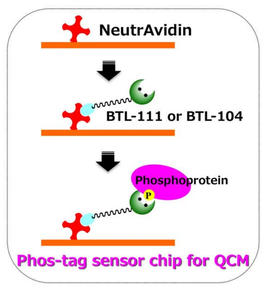


④ QCM analysis 1

Quartz-crystal microbalance using BTL-111 & BTL-104

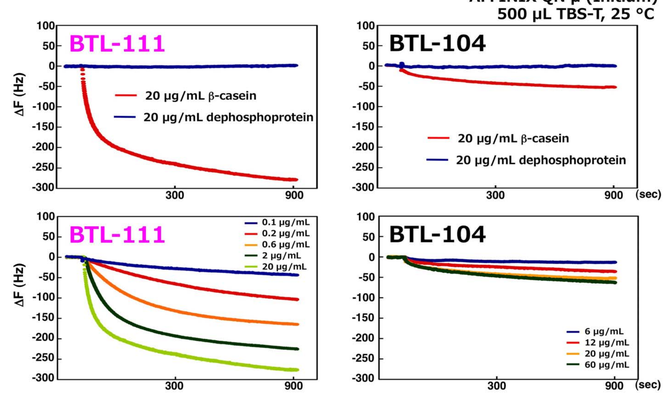


Experimental procedures



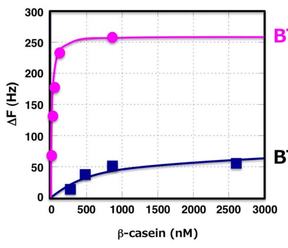
⑤ QCM analysis 2

AFFINIX QN μ (Initium)
500 μL TBS-T, 25 °C



⑥ QCM analysis 3

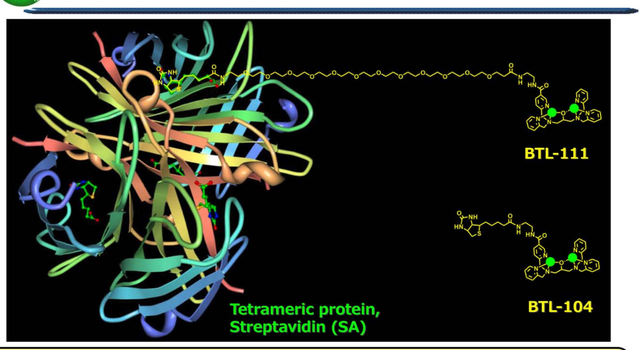
Association & dissociation constants obtained from dose-dependent analysis of β-casein



	K_A (10^6 M^{-1})	K_D (nM)
BTL-111	100	10
BTL-104	1.3	750

More effective by using a long hydrophilic spacer

⑦ Complex of BTL-111 & SA

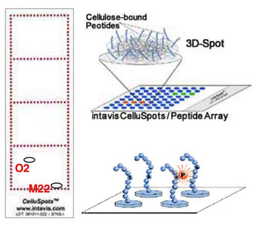


Introduction of the long hydrophilic spacer [dodeca(ethylene glycol)]

High sensitivity in the detection of phosphoprotein

⑧ Peptide microarray

Peptide microarray analysis using Intavis CelluSpots



Tyr kinase substrate-I
(two types of phosphopeptide as controls)

M22: X-X-X-pS/T-X-X-S/T-X-X-X-X-X-X CK1_group

O2 : X-X-X-X-X-X-T-P-pY-X-X-X-X JNK_group

384-peptides spots printed in duplicate on a glass slide (76 x 26 mm)

HRP-SA only

Many false-positive detection (nonspecific ECL signals)

Procedures of blocking treatment

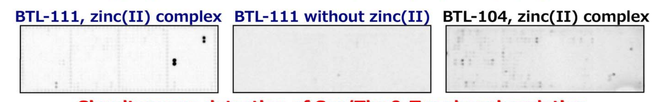
① Blocking with a TBS-T solution containing 10% (w/v) BSA (4 -16 h)

② Washing with a TBS-T solution containing 1% (w/v) BSA + 1 M sodium acetate (5 min, twice)

③ Applying a lysate sample for the kinase reaction

⑨ Kinase profiling 1

Array; Tyr kinase substrate-I, Sample; 1% (w/v) BSA

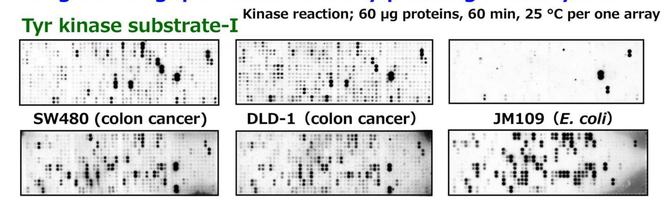


Simultaneous detection of Ser/Thr & Tyr phosphorylation

High-throughput kinase activity profiling in cell lysates

Tyr kinase substrate-I

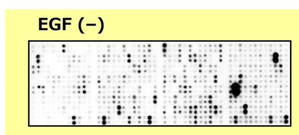
Kinase reaction; 60 μg proteins, 60 min, 25 °C per one array



Ser/Thr kinase substrate-II

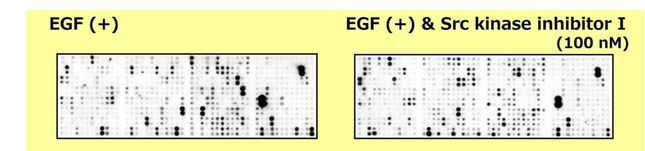
⑩ Kinase profiling 2

Kinase activity profiling using A431 lysates with or without EGF stimulation

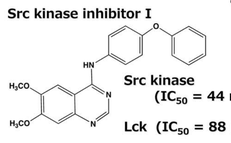


⑪ Kinase profiling 3

Kinase inhibition profiling for drug discovery



Src kinase inhibitor I

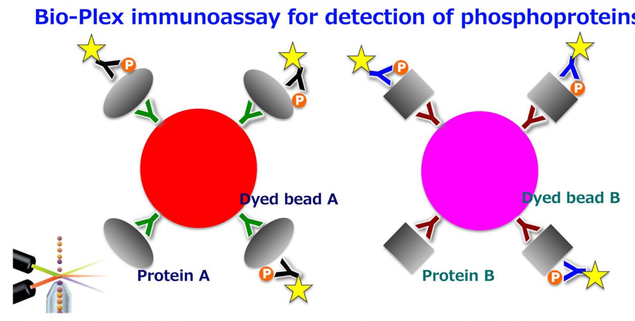


Src kinase (IC_{50} = 44 nM)

Lck (IC_{50} = 88 nM)

⑫ Bio-Plex analysis

Bio-Plex immunoassay for detection of phosphoproteins

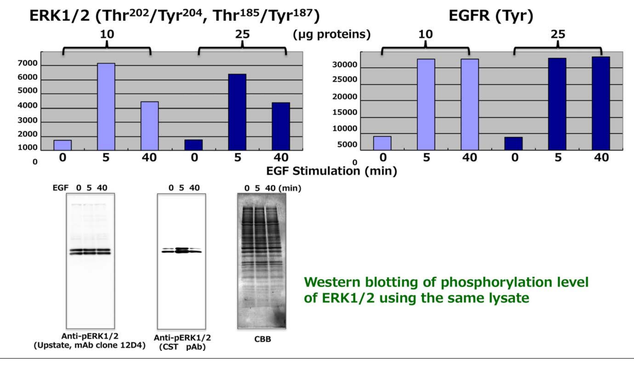


Biotin-labeled anti-phospho antibody + phycoerythrin-conjugated SA

BTL-111 + phycoerythrin-conjugated SA

⑬ Bio-Plex Data 1

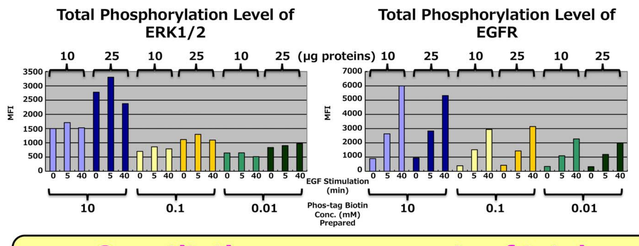
Detection by complex of biotin-labeled anti-phospho antibody with streptavidin-phycoerythrin



Western blotting of phosphorylation level of ERK1/2 using the same lysate

⑭ Bio-Plex Data 2

Detection by complex of Phos-tag Biotin BTL-111 with streptavidin-phycoerythrin



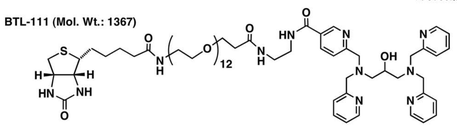
Quantitative measurements of total phosphorylation level per a certain protein

⑮ Conclusions

Highly sensitive detection of protein phosphorylation by using improved Phos-tag Biotin (BTL-111)

Proteomics, (2012) 12: 932

BTL-111 (Mol. Wt.: 1367)



Wako

Commercially available from Wako Pure Chemical Industries

http://www.wako-chem.co.jp/english/labchem/product/life/Phos-tag/index.htm

Acknowledgement

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