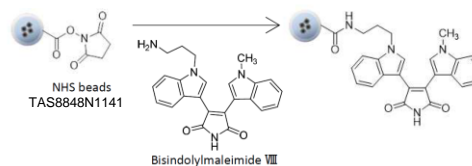


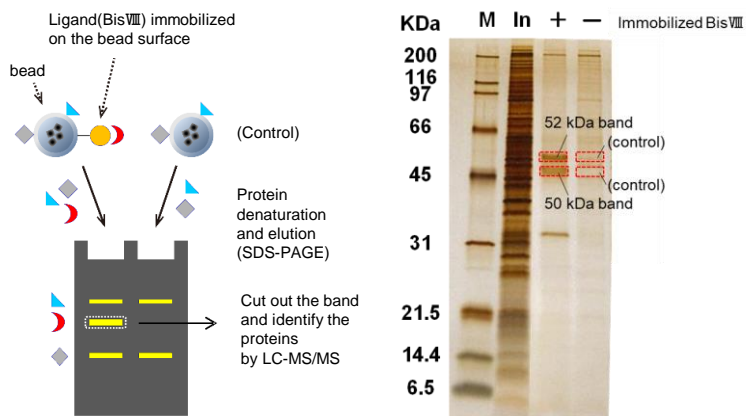
Purification of target proteins for kinase inhibitor Bisindolylmaleimide VIII

Analysis of proteins purified by FG beads[®]

Kinase inhibitor Bisindolylmaleimide VIII (BisVIII) were immobilized on FG beads[®] and the bound proteins were purified from HeLa cell lysate. The bound proteins were analyzed by two methods.



Method for separation the bound proteins by SDS-PAGE (Cut out gel bands)



52 kDa band

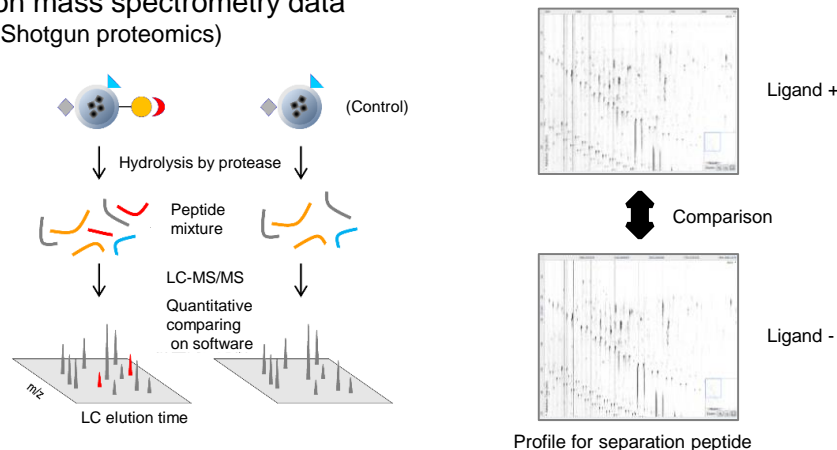
Identified protein	MW (10 ³)	Number of identified peptides	
		Ligand +	Ligand - (Control)
Glycogen synthase kinase-3 alpha (GSK3α)	51	9	0
Glycogen synthase kinase-3 beta (GSK3β)	47	5	0
Tubulin alpha-1B chain	50	3	1
Dermcidin	11	3	3

50 kDa band

Identified protein	MW (10 ³)	Number of identified peptides	
		Ligand +	Ligand - (Control)
Glycogen synthase kinase-3 beta (GSK3β)	47	14	0
Actin, cytoplasmic 1	42	3	2
Dermcidin	11	2	2
Elongation factor 1-alpha 1	50	2	1
Glycogen synthase kinase-3 alpha (GSK3α)	51	1	0
Desmoplakin	332	0	4

Several proteins were identified from the 52 kDa and 50 kDa protein bands. Comparing the number of identified peptides with the control band considers that the major proteins in the bands are GSK3α and GSK3β, respectively.

Method for comparing the detection intensities of peptide fragments on mass spectrometry data (Shotgun proteomics)



Protein	Gene name	MW (10 ³)	Measured value of protein		Multiple value
			Ligand +	Ligand - (Control)	
Glycogen synthase kinase-3 beta	GSK3B	47	8.4 × 10 ⁵	1.6 × 10 ⁸	195.4
Glycogen synthase kinase-3 alpha	GSK3A	51	3.7 × 10 ⁵	5.7 × 10 ⁷	153.2
NAD(P)H dehydrogenase [quinone] 1	NQO1	31	5.9 × 10 ⁴	7.0 × 10 ⁶	117.8
Apoptosis-inducing factor 2	AIFM2	41	6.5 × 10 ³	1.2 × 10 ⁵	18.2
Heme-binding protein 1	HEBP1	21	4.9 × 10 ⁴	7.2 × 10 ⁵	14.7
Ferrochelatase, mitochondrial	FECH	48	1.5 × 10 ⁴	1.9 × 10 ⁵	12.3
RNA-binding protein 25	RBM25	100	1.0 × 10 ³	8.3 × 10 ³	8.0
RNA-binding protein EWS	EWSR1	68	2.1 × 10 ³	1.3 × 10 ⁴	6.3
60S ribosomal protein L17	RPL17	21	4.1 × 10 ⁸	2.4 × 10 ⁴	5.9
Heterogeneous nuclear ribonucleoprotein U-like protein 1	HNRNPUL1	96	8.5 × 10 ¹	4.7 × 10 ²	5.5
Serine/arginine-rich splicing factor 5	SRSF5	31	1.5 × 10 ³	7.9 × 10 ³	5.2
LETM1 domain-containing protein 1	LETMD1	42	2.2 × 10 ³	1.1 × 10 ⁴	5.1

The analysis provided measuring information for 273 proteins. Comparing the detection intensities between the two samples selected 12 binding proteins as target protein candidates. GSK3α and GSK3β are known as BisVIII target proteins, but other proteins may be novel binding proteins.