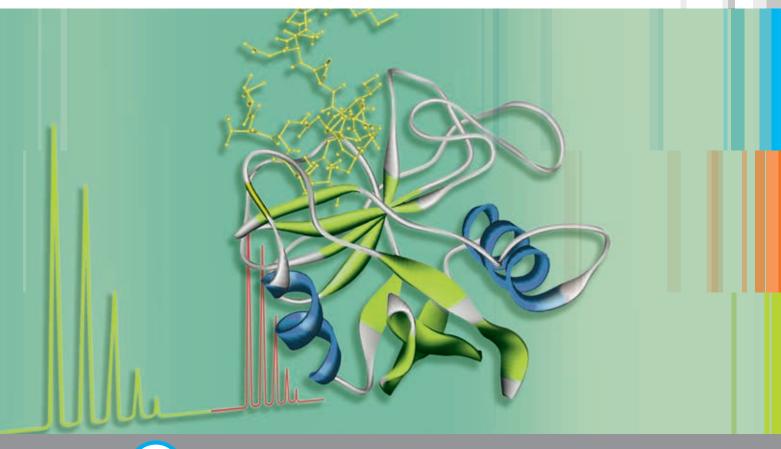
# Innovative Peptide Solutions



# Proteomics

# Peptide Tools & Services

- > Targeted & Quantitative Proteomics
- > Functional Proteomics
- **Biomarker Discovery**
- **Clinical Proteomics**



# History

JPT Peptide Technologies is a service provider located in Berlin, Germany that has achieved worldwide credibility for its commitment to rigorous quality standards and a reputation for developing and implementing innovative peptide-based services and research tools for various applications.

Together with its US-subsidiary JPT serves its clientele in the pharmaceutical and biotechnology industries as well as researchers in universities, governmental and non-profit organizations.

# Technology & Application

Over the past decade, JPT has developed a portfolio of proprietary technologies as well as innovative products and services that have helped to advance the development of new immunotherapies, proteomics and drug discovery.

# Quality Assurance

JPT is DIN EN ISO 9001:2015 certified and GCLP audited.



Management System ISO 9001:2015



www.tuv.com ID 9105022388

#### JPT's key technologies are:

#### **Custom & Specialty Peptides**

We are the peptide experts and offer the largest variety of peptide chemistries, formats and modifications.

#### SpikeTides™

Light or stable isotope-labeled and quantified peptides for mass spectrometry-based proteomics assays.

#### SpikeMix™

Stable isotope-labeled (SIL) peptide pools used as peptide standards in mass spectrometry-based assays.

#### **SPOT**

High-throughput peptide synthesis platform for T-cell epitope and peptide lead discovery on proteome-wide levels.

#### PepStar™

Peptide microarrays for epitope discovery, immune monitoring and enzyme substrate identification or optimization.

#### PepMix™

Defined antigen spanning peptide pools to stimulate CD4+ and CD8+ T-cell responses.

#### PepTrack™

Peptide libraries offering various specifications and optimization for different types of assays.







# **04/Mass Spectrometry-Based Proteomics**

#### 05 / Standardization & Controls

Control kits such as retention time kit, carbamidomethylation check kit and more

#### 06 / SpikeTides™ & SpikeMix™

Low cost SIL peptides for discovery and relative protein quantitation

#### 08 / SpikeTides™\_TQL & SpikeTides™\_TQL PLus

Absolutely quantified peptide standards for targeted and clinical proteomics

#### 10 / SpikeTides™ Kits & SpikeMix™ Kits

Reference peptide kits covering cytokines, kinases, MHC epitopes and more

#### 11 / SpikeTides™ & SpikeMix™ PTM Reference Kits

Proteotypic peptides, kits and pools with many post-translational modifications

#### 12 / The Human Proteome Peptide Catalog

Validated reference peptides for essentially all human proteins

#### 13 / Protein Interaction Screen on Peptide Matrix (PRISMA)

Assay to systematically explore protein interactions by mass spec

#### 14 / NeoSpikeMix™ for Immunopeptidomics

Fast custom reference peptide libraries for immunopeptidomics

#### 15/Functional Proteomics

#### 16 / Phosphatases, Kinases & Proteases

Peptide substrate sets and microarrays for substrate identification and optimization

#### 19 / Acetylases & Deacetylases

Peptide microarrays displaying the human acetylome and a Universal Sirtuin Substrate Kit

#### 20 / Enzyme Profiling Services

We profile enzymatic activities, enzyme specificity and selectivity using a wide range of biological samples

#### 22 / Histone Code Peptide Tools

Largest histone peptide library, available as ELISA, microarray and individual peptides

#### 24 / Histone Code Analysis Service

Mapping of protein-histone interactions, e.g. histone antibodies, enzymatic activity profiling and histone reader profiling







# JPT – Your Partner for Proteomics

# **Fast & Inexpensive Peptide Libraries**

Using our proprietary ultra-high-throughput SPOT synthesis, we produce thousands of peptides within two weeks at very low prices. We can also include post-translational and other modifications.

# All Human Proteins in one Peptide Catalog

We offer validated reference peptides for mass spectrometry-based proteomics, covering essentially all human proteins. The most comprehensive source for light and heavy proteotypic peptides comes with CID, HCD, ETD and EThcD spectra for each peptide.

#### **Low Cost Quantification**

Our proprietary quantification technique by UV is cheaper and faster than conventional techniques such as AAA and reaches equal or better accuracy.



# Post-Translational Modifications

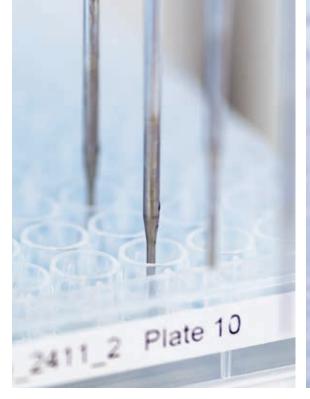
PTMs such as protein phosphorylation, methylation or succinylation play an important role in virtually all biological processes. JPT has extensive experience in the synthesis of a large variety of PTM peptides and we will be happy to discuss your requirements.

# **Assay Standards & Controls**

Standardize and control your mass spectrometry-based proteomics assays such as MRM assays and monitor efficiency of carbamidomethylation and trypsination during proteomic sample preparation.

#### Our Service is the Best!

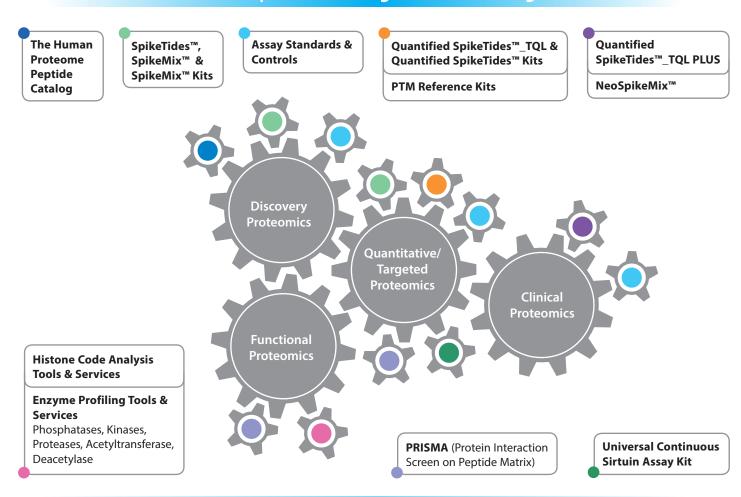
We offer quick and personal consultation with experienced scientists and help you with the selection of peptide specifications, provide tips for storage, solvents and more. Take advantage of our rush order service for urgent projects.





# Our Technologies & Products

# Mass Spectrometry-Based Assays



# Functional Assays

# Mass Spectrometry-Based Proteomics

Proteomics is the study of a cell's protein inventory at different times by protein identification and quantification. The application of LC-MS/MS has tremendously facilitated this process. Generally, samples containing relevant proteins are digested into peptides and analyzed by LC-MS/MS as a surrogate measurement of protein levels. To increase accuracy of detection and to enable protein quantitation, chemically synthesized stable isotope-labeled (SIL) peptides are added to samples. As classical assembly and quantitation methods for such peptides are tedious and expensive, JPT developed unique and highly efficient procedures for both fast and low cost synthesis as well as absolute quantitation of SIL peptides.

Ouantification of Proteins with JPT's Proteomics Tools. Proteins of interest or whole cell proteome Tryptic digestion SpikeMix™ Peptide Pools · Customized or off-the shelf Stable isotope-labeled Proteotypic peptides • PTMs & alkylation • Inexpensive & fast • e.g. TAAs, cytokines and more A) Shotgun experiments or B) SRM/MRM assay setup SpikeTides<sup>™</sup> Peptides Customized • Stable isotope-labeled or light • PTMs & alkylation Identification and relative Inexpensive & fast quantification of proteins Quantified SpikeTides\_TQL™ SRM/MRM assay with · Customized or off-the-shelf absolutely quantified · Stable isotope-labeled reference peptide(s) · Absolute quantification • PTMs & alkylation • Inexpensive & fast Absolute quantification of proteins of interest

#### **Standardization & Controls**

Comprehensive control and standardization kits for MS-based proteomics assays ensure the quality of your assays and help to achieve successful experiments.

#### **Applications**

- Normalize HPLC-MS retention time
- Compare collision energy settings
- Monitor efficacy of carbamidomethylation
- Check efficacy of trypsination
- Standardize MRM assays across species
- Measure organelle marker peptides

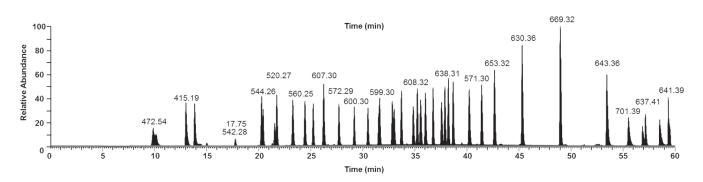
#### **Selected References & Application Notes**

- "PROCAL: A Set of 40 Peptide Standards for Retention Time Indexing, Column Performance Monitoring, and Collision Energy Calibration" Zolg et al., Proteomics (2017)
- "Building ProteomeTools Based on a Complete Synthetic Human Proteome" Zolg et al., Nature Methods (2017)
- "Fast and Accurate Determination of Cysteine Reduction and Alkylation Efficacy in Proteomics Workflows" Schnatbaum et al. Application Note (2016)

#### Standardization Kits & Controls

Kit	Application	
Retention Time Standardization Kit (PROCAL)	Normalization of retention times in HPLC-MS proteomics experiments, evaluation of HPLC column performance and optimization of HPLC gradients, collision energy normalization	
CAMCheck	In situ determination of disulfide reduction and cyste- ine alkylation conditions and reproducibility, including potential over-alkylation	
SpikeMix™ ABRF (cross-species standard)	1000 stable isotope-labeled proteotypic peptides from human, mouse and rat proteins as MS references across species	
TrypCheck Kit Fluorescence	Easy to use fluorescent peptide kit to estimate efficiency and reproducibility of tryptic sample preparation	
PTM Reference Kits	→ See page 11	

Representative LC-MS chromatogram of the Retention Time Standardization Kit (PROCAL).



# SpikeTides™ & SpikeMix™

Our proprietary peptide synthesis technology enables ultra fast and inexpensive provision of light or heavy reference peptides for proteome-wide profiling by mass spectrometry. Our SpikeTides™ peptides and SpikeMix™ reference peptide pools enable robust and multiplexed identification and relative quantification of protein expression levels.

#### SpikeTides™

- Individual synthetic light or heavy peptides
- Flexible scales and attractive pricing
- Many PTMs available

#### **Applications**

- Mass spectrometry-based assays (MRM, SRM)
- Relative quantification of proteins
- Selection of proteotypic peptides
- Identification of predominant peptide fragments specific for your proteotypic peptide to be used in MRM transition
- Development of kits for quantitation of entire biologic pathways

#### **Benefits**

- Unmatched turnaround times (10 000 peptides/week)
- Aliquotation and mixing service available
- Fully compatible with downstream analyses like MS and HPLC
- Also available as larger scale or highly purified Maxi SpikeTides™

#### SpikeMix™

- Pools of synthetic light or heavy peptides
- Ultra fast at lowest costs (i.e. 10 000 peptides/week)
- Mixes of 250 to thousands of peptides

#### **Selected References**

- "Targeted Proteomics for Multiplexed Verification of Markers of Colorectal Tumorigenesis"

  Uzozie et al., Mol Cell Proteomics (2017)
- "Quantitation of 87 Proteins by nLC-MRM/MS in Human Plasma: Workflow for Large-scale Analysis of Biobank Samples" Rezeli et al., J Proteome Res. (2017)
- "Identification of Host Proteins Predictive of Early Stage Mycobacterium Tuberculosis Infection" Bark et al., EBioMedicine (2017)
- "An MRM-Based Cytokeratin Marker Assay as a Tool for Cancer Studies: Application to Lung-Cancer Pleural Effusions" Perzanowska et al., Proteomics Clin Appl. (2017)
- "A 14-Protein Signature for Rapid Identification of Poor Prognosis Stage III Metastatic Melanoma" Sykes et al., Proteomics Clin Appl. (2017)



SpikeTides™ are delivered freeze-dried in 96- or 384well plates with an excel file that contains all peptide sequences and the plate layout.

#### SpikeTides™ & SpikeMix™

	SpikeTides™_L	SpikeMix™_L Peptide Pools
Individual peptides	х	
Peptide mix		х
Quantification		
Purified		
Stable isotope-labeled*	Х	Х
Amount	10-30 nmol per peptide	2-3 nmol per peptide
Isotopical purity	> 99 %	> 99 %
Incoming material inspection	X	Х
Vendor qualification	X	х
Batch documentation & CoA	X	X

<sup>\*</sup>light versions are also available

(CAt MSKCC, we have been using JPT's SpikeTides™\_L isotopically labeled peptide libraries for developing pathway- and genome-scale atlases for quantitation of cellular signaling. This has been invaluable for the Quantitative Cell Proteomics Atlas (QCPA), a research tool for precise and sensitivity quantitation of cellular signaling, as well as clinical diagnostic assays for medical use.)

Alex Kentsis, MD, PhD, Memorial Sloan Kettering Institue, Functional Proteomics and Genomic Plasticity of Pediatric Cancers, New York, USA





# SpikeTides™\_TQL & SpikeTides™\_TQL PLUS

Our proprietary peptide quantitation method overcomes the limitations of traditional methods such as limited accuracy and high costs. We use our QTag, a small chemical tag attached to each peptide, for robust and reproducible quantitation via HPLC-UV or HPLC-MS and UV. The QTag is readily released by digestion with trypsin and does not interfere with subsequent measurements. The method is very accurate and has been validated in numerous peer-reviewed papers.

#### SpikeTides™\_TQL

- Individual synthetic SIL peptides
- Absolutely quantified
- Very precise quantification method

#### SpikeTides™\_TQL PLUS

- For clinical applications
- Purified peptides (HPLC > 95 %)
- Up-to-date quantification immediately before experiment

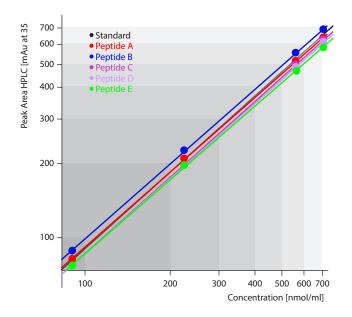
#### **Applications**

- Mass spectrometry-based assays (MRM, SRM)
- Absolute protein quantitation
- Development of kits for quantitation of entire biologic pathways

#### **Benefits**

- Accurate and robust quantitation method
- Cost efficient and fast
- Aliquotation and mixing services available
- In situ LC-MS quantitation feasible

Calibration lines for quantification of 5 different SpikeTides™\_TQL vs. the corresponding peptides quantified by amino acid analysis. X-Axis displays concentration of peptides determined by AAA, Y-Axis shows quantification results of JPT's Quanti-Tag measured by HPLC. JPT's quantification technique is as robust as classical quantification by AAA.



CMy group studies the proteomic composition of distinct chromatin domains, the mechanisms that operate to maintain the composition of histone modifications and the associated proteins. For precise and accurate identification and quantification of histone peptides that carry multiple post-translational modifications directly from biological sample JPT's SpikeTides™\_TQL peptide standards proved to be of excellent value for our research in various projects. ▶

Prof. Dr. Axel Imhof, Adolf-Butenandt Institute, University of Munich, Germany

#### Quantified SpikeTides™\_TQL & SpikeTides™\_TQL PLUS

	RuO SpikeTides™ _TQL	Clinical SpikeTides™_TQL PLUS
Quantification	х	х
Quantification by end-user possible		Х
Quantification method	UV/QTag	UV/QTag
Purified	х	х
Stable isotope-labeled*	x	Х
Amount	5x1 nmol	5 x 10 nmol
Isotopical purity	>99%	>99%
Incoming material inspection	х	Х
Vendor qualification	х	X
Batch documentation & CoA	X	X
Optional: certified vials		X
Optional: additional QC methods		Х

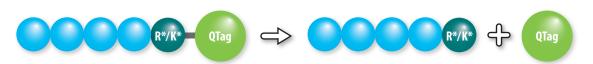
<sup>\*</sup>light versions are also available

#### **Selected References**

- "Quantitative Multiple Reaction Monitoring Proteomic Analysis of Gβ and Gγ Subunits in C57Bl6/J Brain Synaptosomes" Yim et al., Biochemistry (2017)
- "Preclinical Evaluation of a TEX101 Protein ELISA

  Test for the Differential Diagnosis of Male Infertility"

  Korbakis et al., BMC Medicine (2017)
- "A New Mass Spectrometry-Based Method for the Quantification of Histones in Plasma from Septic Shock Patients" García-Giménez et al., Scientific Reports (2017)
- "A New Enzyme-linked Immunosorbent Assay (ELISA) for Human Free and Bound Kallikrein 9" Filippou et al., Clinical Proteomics (2017)
- "Stereocilia-Staircase Spacing is Influenced by Myosin
  III Motors and their Cargos Espin-1 and Espin-Like"
  Ebrahim et al., Nat Commun (2016)
- "Absolute Quantification of Myosin Heavy Chain Isoforms by Selected Reaction Monitoring Can Underscore Skeletal Muscle Changes in a Mouse Model of Amyotrophic Lateral Sclerosis" Peggion et al., Anal Bioanal Chem (2017)



SpikeTides™\_TQL

Tryptic digest

Proteotypic peptide

Quantification tag

# SpikeMix™ & SpikeTides™ Kits

Our ready to use kits feature large numbers of stable isotope-labeled non-quantified or quantified peptide standards for mass spectrometry-based proteomics (i.e. MRM/ SRM assays) at unmatched pricing. All heavy peptides are stable isotope-labeled using heavy arginine (U-13C6; U-15N4) or lysine (U-13C6; U-15N2).

#### SpikeMix™ & SpikeTides™ Kits

Kit	Content
SpikeMix™ Cytokines for 13 different species	Between 54 and 461 stable isotope-labeled peptides for cytokines from human, mouse, different primates and others
SpikeMix™ Tumor Associated Antigens (Human)	252 stable isotope-labeled peptides for 61 TAAs
SpikeMix™ Peptide Hormones (Human)	More than 500 stable isotope-labeled peptides for 108 peptide hormones and hormone precursors
SpikeMix™ Kinase Activation Loops	Over 400 phosphorylated and non-phosphorylated stable isotope-labeled peptides from human kinase activation loops
SpikeMix™ CEF (extended)	32 HLA restricted T-cell epitopes of CMV, EBV and Influenza represented as stable isotope-labeled peptides
SpikeMix™ Plant Organelle Marker (Arabidopsis thaliana)	67 stable isotope-labeled marker peptides for 14 major subcellular locations
SpikeMix™ Wnt Signaling Pathway (Human)	343 stable isotope-labeled proteotypic peptides for 65 proteins of the human Wnt pathway
SpikeTides™ Set Metabolic Enzymes (quantified)	51 quantified and stable isotope-labeled standard peptides for 24 enzymes involved in central energy metabolism
SpikeTides™ Set Histone H3 (quantified)	37 quantified and stable isotope-labeled standard peptides for Histone H3 with and w/o modifications

Have a look at our full list of SpikeMixes™ and SpikeTides™ Sets in our online catalog at www.jpt.com

#### **Selected References & Application Notes**

- "Multiple Marker Abundance Profiling: Combining SRM and Data-Dependent Acquisition for Rapid Estimation of Organelle Abundance in Subcellular Samples" Hooper et al., Plant J (2017)
- "Quantitative Proteomics of Bronchoalveolar Lavage Fluid in Idiopathic Pulmonary Fibrosis" Foster et al., J Proteome Res (2015)
- "Development of a Multiplexed Targeted SRM Assay for NCI's Top Tumor Associated Antigens" Soderblom et al., Application Note (2015)
- "Development & Characterization of SpikeMix™ ABRF (cross-species standard) Consisting of 1000 Stable Isotope-Labeled Peptides" Colangelo, Application Note (2014)

# SpikeTides™ & SpikeMix™ PTM Reference Kits

Our PTM Reference Kits and Peptides offer many different proteotypic peptides carrying a large variety of post-translational modifications. Choose between absolutely quantified or non-quantified variants and use them as reference material to support mass spectrometry-based PTM proteomics.

#### **Background**

Despite recent improvements, PTM proteomics is still challenging. Typical problems are low endogenous abundance, low ionization intensity, changed fragmentation, limited stability during proteomic workflows, and/or complex fragmentation spectra interpretation, especially regarding correct PTM site localization. Our defined and carefully selected synthetic PTM reference peptides and kits support PTM proteomic and help to overcome these challenges.

#### **Selected Reference**

"ProteomeTools: Systematic characterization of 21 post-translational protein modifications by LC-MS/ MS using synthetic peptides"

Zolg et al., Mol Cell Proteomics (2018)

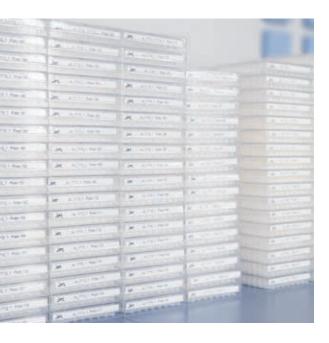
#### **Available PTMs**

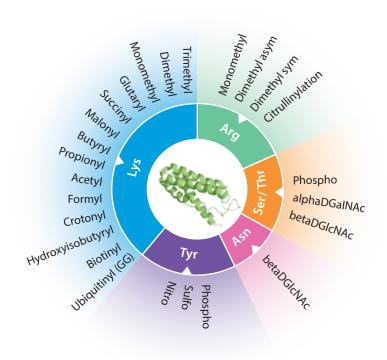
- Phospho (serine, threonine, tyrosine)
- Arginine (Me, Me2a, Me2s)
- Lysine (Ac, Me, Me2, Me3, Cro, Suc, Mal, Biotin, Glu, For, Prop, But, Hib, GG)
- Sulfotyrosine, Nitrotyrosine
- Hydroxyproline
- Glyco-Asn(betaDGlcNAc), Glyco-Ser/ Thr(alphaDGalNAc), Glyco-Ser/Thr(betaDGlcNAc)

#### **Benefits**

- · Large range of PTM reference peptides
- Selected for high recovery in LC-MS
- Available as stable isotope-labeled (SIL) peptides
- Purified and absolutely quantified peptides and kits

A large variety of post-translational modifications is included in our PTM Reference Kits.





# The Human Proteome Peptide Catalog

We offer the most extensive library of validated human reference peptides for mass spectrometry-based proteomics covering essentially all human proteins. The Human Proteome Peptide Catalog is your fast and low cost access to light and heavy labeled proteotypic peptides, allowing development and optimization of MRM assays for multiplexed detection and quantification of proteins. High confidence spectra for each peptide are available through ProteomicsDB.

#### **Applications**

- Multiplexed detection and quantification of proteins
- Development and optimization of MRM assays
- Optimization of proteomics workflows and data analysis tools

#### **Benefits**

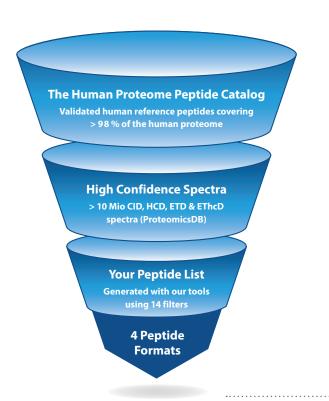
- · Light and heavy validated reference peptides
- Fast and low cost
- CID, HCD, ETD and EThcD spectra available for each peptide (for details see reference)
- Covers vast majority of human proteins
- List will be expanded to include important PTMs
- Currently > 400 000 peptides

#### **Peptide Formats**

- Light SpikeTides<sup>™</sup> peptides
- Stable isotope-labeled SpikeTides<sup>™</sup>\_L peptides
- Light SpikeMix™ peptide pools
- Stable isotope-labeled SpikeMix™\_L peptide pools

#### Selected Reference

"Building ProteomeTools Based on a Complete Synthetic Human Proteome" Zolg et al., Nature Methods (2017)



SPONSORED BY THE



The Human Proteome Peptide Catalog represents the most extensive library of validated human reference peptides with a coverage of over 98% of the human proteome and more than 10 Mio spectra. Generate a peptide list using combinations of 14 different filters (e.g. protein ID, score, peptide sequence, modification) and order directly as individual peptides or peptide pool, stable isotope-labeled or light peptides.

# Protein Interaction Screen on Peptide Matrix (PRISMA)

Protein-protein interactions are a hallmark of signal transmission and key to understanding regulatory mechanisms. The PRISMA assay enables you to systematically explore the interactome.

#### **Applications**

- Identification of protein-protein interaction partners
- Systematic exploration of the interactome
- Decoding the influence of post-translational modifications
- Comparison of cell states
- Mapping of pathways

#### **Benefits**

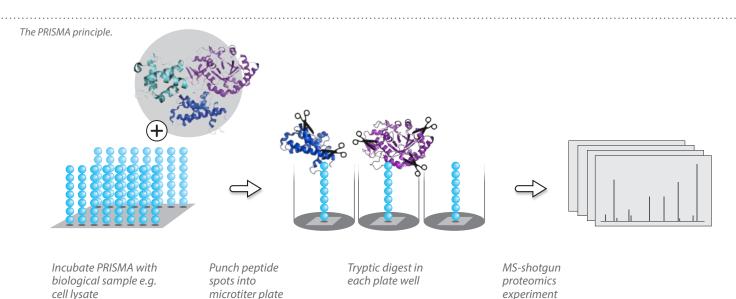
- High-throughput assay with hundreds of peptide sequences
- Validated assay based on comparison with other affinity enrichment approaches, conventional immunoblotting analysis and co-occurrence
- Quantification of proteins possible

#### **Selected References**

- "Protein Interaction Screen On Peptide Matrix (PRISMA) Reveals Interaction Footprints And The PTMDependent Interactome of Intrinsically Disordered C/ΕΒΡβ" Dittmar et al., Biorxiv.org (2017)
- "Mutations In Disordered Regions Cause Disease By Creating Endocytosis Motifs" Meyer et al., Biorxiv.org (2017)

(My research groups at MDC and LIH work on the systematic characterization of protein-protein interactions and the regulation of these interactions by post-translational modifications. Therefore we developed PrISMa, a method for the systematic analysis of these interactions and their regulation which helped us to uncover a complex, interwoven interaction network. JPT's peptide membranes in combination with high-resolution mass spectrometry made the development of PrISMa possible.))

Gunnar Dittmar, PhD, Proteome and Genome Research Unit, Luxembourg Institute of Health, Luxembourg



# NeoSpikeMix<sup>™</sup> for Immunopeptidomics

Immunopeptidomics deals with the detection of MHC I and MHC II binding peptides by mass spectrometry. Despite recent improvements, due to limited sensitivity and specificity, the verification these peptides (and especially of neo-epitopes within the short non-tryptic peptide sequences) is still challenging. LC-MS/MS measurement of ~170 000 synthetic peptides showed, that with standard data analysis routines more than 10% of the assigned spectra were not in agreement with the respective synthetic references. These results highlight the urgent need for reference peptides to be used in immunopeptidomics.

#### > What is NeoSpikeMix™?

NeoSpikeMix<sup>™</sup> are customized peptide libraries for immunopeptidomics, e.g. patient-specific HLA reference libraries or comprehensive iterations to possible epitopes around mutations. They provide fast and cheap access to reference peptides for this clinically relevant and rapidly developing field.

#### **Selected Reference**

"Building ProteomeTools Based on a Complete Synthetic Human Proteome" Zolg et al., Nature Methods (2017)

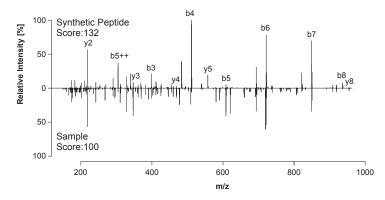
#### **Applications**

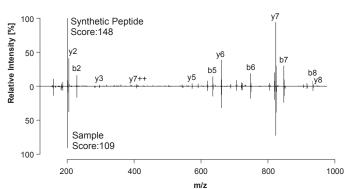
- Mass spectrometry-based neo-epitope prioritization
- Neo-epitope identification
- HLA ligand identification and optimization

#### **Benefits**

- Hundreds or thousands of peptides within one week
- Low price
- Provides high sensitivity as needed for low abundance epitopes
- Light or stable isotope-labeled

Wrong (left) and correct (right) sequence-spectrum assignment proved by synthetic reference peptide measurement.





# **Functional Proteomics**

Many proteins are subject to modifications that are critical to protein function. One very important modification is phosphorylation. In addition, proteins can also be ubiquitinylated, methylated, acetylated, glycosylated and more. Have a look at our corresponding enzyme profiling tools.

#### **Selected References**

- "Nuclear PKC- Facilitates Rapid Transcriptional Responses in Human Memory CD4+ T Cells Through P65 and H2B Phosphorylation" Li et al., Journal of Cell Science (2016)
- "Matrix Metalloproteinase 10 Degradomics in Keratinocytes and Epidermal Tissue Identifies Bioactive Substrates with Pleiotropic Functions" Schlage et al., Mol Cell Proteomics. (2015)
- "Histone H2A and H4 N-terminal Tails Are Positioned by the MEP50 WD Repeat Protein for Efficient Methylation by the PRMT5 Arginine Methyltransferase" Burgos et al., J Biol Chem (2015)

We were very pleased with how easy it was to do the experiment (with Kinase Substrate Sets) using the equipment we had on hand – for example, we just used our 8-channel micropipette to add the enzyme/ATP mix.)

Betty A. Eipper, Ph.D, University of Connecticut, Farmington, USA

#### > Enzyme Families & Profiling Tools

Enzyme Family	Protein Modification	Biological Role	Profiling Tools
Kinase	Phosphorylation of serine, threonine and tyrosine resi- dues (and rarely histidine)	Cell Signaling Several hundred protein kinases present in mammals Two classes: serine/threonine kinases and tyrosine kinases	Kinase Substrate Microarrays Kinase Substrate Sets Enzyme Profiling Service SpikeMix™ Kinase Activation Loops
Phosphatase	Dephosphorylation of proteins	Cell Signaling Alkaline phosphatase is present in many organisms	Phosphatase Substrate Microarray Phosphatase Substrate Sets Enzyme Profiling Service
Acetyltransferase and Methyltransferase	Acetylation or methylation of lysine residues or N-terminal alpha amine	Important for DNA-binding property of histones (histone acetyl-transferase)	Acetylome – Acetyltransferase and Deacetylase Microarrays Universal Continuous Sirtuin Assay Kit
Histone-Modifying Enzymes	Several enzyme classes modify histones at different sites, e.g. by acetylation, methylation, phosphorylation, ubiquiti- nylation, sumoylation	The combination of histone modifications, the so-called histone code, is thought to play a major role in gene regulation	Histone Code Microarray Histone Code ELISA Histone Peptides and Sets Histone Analysis Services SpikeTides™ Set Histone (quantified)
Protease	Hydrolysis of peptide bonds between amino acids	Signal transduction pathways (e.g. blood-clotting cascade or apoptosis pathways) Food digestion	ProteaseSpots™

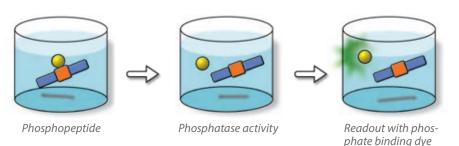
# **Phosphatases**

JPT's ready to use Phosphatase Substrate Sets and Phosphatase Substrate Microarray feature phosphorylated substrate peptides derived from human phosphorylation sites. They can be incubated with a variety of biological samples to identify substrates or to detect enzymatic action.

The detailed sequence lists and plate layouts are available at www.jpt.com.

#### Phosphatase Substrate Sets

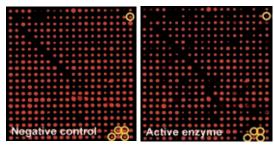
Our Phosphatase Substrate Sets each include a 384-well microtiter plate containing phosphopeptides (250 pmol per well) derived from human phosphorylation sites. You can test your sample in solution under physiological conditions.



Hundreds of substrates can be tested in parallel using our phosphatase substrate sets.

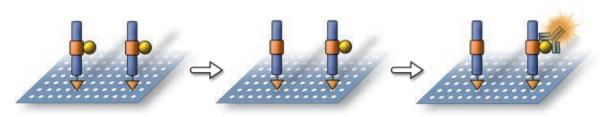
#### Annotated Phosphosites Tyr-Phosphatase Microarray

The Annotated PhosphoSites-Tyrosine Microarray displays over 6 000 human phosphorylation sites as phosphotyrosine containing peptides for high-throughput screening. Incubation with your tyrosine phosphatase containing sample results in identification of both peptidic substrates and potential *in vivo* substrate proteins. Additionally, we offer a comprehensive assay service based on this microarray.



Results of incubation with phosphatase and negative control (inactive enzyme). Yellow circles indicate spots with reduced or eliminated signals (=identfied substrate peptides).

Determination of substrates for your phosphatase using JPT's Annotated Phosphosites Tyr- Phosphatase microarray.



Phosphopeptides on microarrays

Phosphatase activity

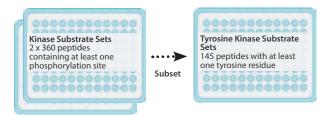
Readout with phosphotyrosine specific antibody

#### **Kinases**

JPT Peptide Technologies has compiled a unique database of human phosphorylation sites and overlapping peptide scan libraries through known kinase substrate proteins. By incubation with cell lysates, purified enzymes, serum or other samples you will receive information on substrate specificity, enzyme activity and reaction kinetics.

#### Kinase Substrate Sets

Kinase substrate peptides for identification and validation of kinase substates *in vitro*.



#### Kinase Substrate Microarrays

Substrate peptides on glass slides for screening of thousands of peptides with your sample.

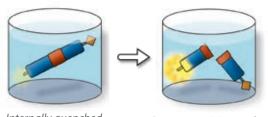
Kinase Microarray	Number of Peptides	Peptide Sequence Source	Application
Annotated Phosphosites-K	720 peptides (on two microarrays)	From annotated human phosphosites	Kinase substrate identifi- cation and consensus sequence determination
Random Libraries-K Tyrosine, Threonine or Serine	1536 peptides each	Potential kinase substrates with either threonine, serine or tyrosine in the center for phosphorylation	Substrate identification or optimization for serine/threonine or tyrosine kinases
Over 50 catalog Phosphorylation Site Detectors	Depending on protein length	Overlapping peptide scans through a variety of proteins (e.g. C-Jun, Histone H1, MBP, p53, Pin1)  For full list please visit our website www.jpt.com!	Identification of phos- phorylation sites within known proteins
Your customized Phosphorylation Site Detector	Depending on protein length	Overlapping peptide scan through your target protein	Identification of phos- phorylation sites in your target protein

#### **Proteases**

JPT offers a variety of protease profiling tools and services serving the aim to identify, validate and optimize cleavage sites, to acquire semi-kinetic data and to monitor proteolytic activity within your biological sample.

#### Protease Substrate Sets

The Protease Substrate Sets are composed of internally quenched (Dabcyl/EDANS) peptides derived from proteolytic cleavage sites (from P4 to P4'position). The purified and lyophilized peptides come in a 384-well microtiter plate (75 pmol per well). Subsequent to incubation with target protease, the evolving fluorescence can be measured using standard fluorescence plate reader systems.

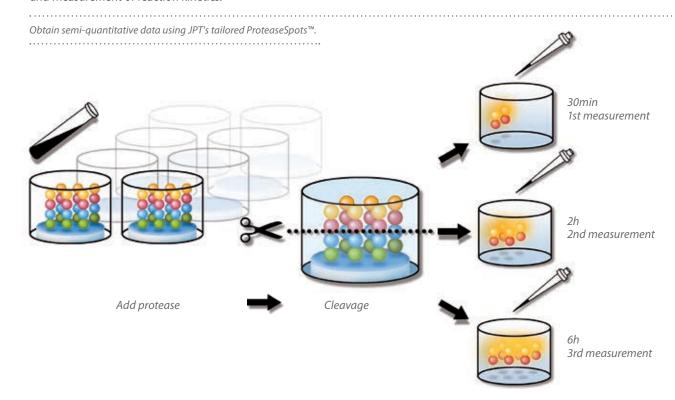


Internally quenched Protease activity results substrate peptides in change of fluorescence

Substrate identification using JPT's Protease Substrate Sets, the tools for economic and efficient substrate screening under physiological conditions.

#### ▶ ProteaseSpots™

JPT's ProteaseSpots™ are tailored protease substrates that are N-terminally labeled with a fluorescent dye and C-terminally immobilized on cellulose discs (delivered in microtiter plates). The discs can be directly incubated with your protease. Subsequent fluorescent detection of the supernatant can be performed at several time points giving semi-kinetic data. Sequences can be overlapping peptide scans, known substrate peptides, de novo substrates or others offering a tool not only for substrate discovery but also for validation, optimization and measurement of reaction kinetics.



# **Acetylases & Deacetylases**

Acetylation is a frequent post-translational modification. More than 5 500 acetylation sites in human proteins have been identified so far. We created two high-density peptide microarrays, each displaying peptides from reported human acetylation sites. Our Universal Continuous Sirtuin Assay Kit enables systematic discovery of sirtuin 1-6 modulators. By deacylating histones, transcription factors, and metabolic enzymes, sirtuins regulate various biological processes.

#### Universal Continuous Sirtuin Assay Kit

Universal substrate peptide for systematic discovery of sirtuin 1-6 modulators, direct and continuous quantification of deacylase activity of recombinant human sirtuins.

#### Acetylome – Deacetylase & Acetyltransferase Microarrays

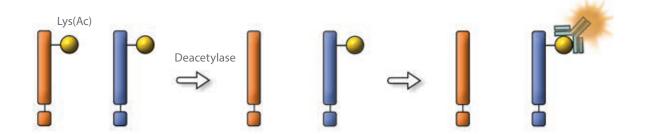
5 599 peptides derived from reported human lysine acetylation sites displayed on peptide microarrays for detection of epigenetic targets for lysine-acetyltransferases, lysine-deactylases and other lysine modifying enzymes, to profile antibodies or study cross-talk of different enzyme modifications.

#### **Selected References & Application Notes**

- "A Continuous Sirtuin Activity Assay Without any Coupling to Enzymatic or Chemical Reactions" Schuster et al., Sci Rep (2016)
- "An Acetylome Peptide Microarray Reveals
  Specificities and Deacetylation Substrates for all
  Human Sirtuin Isoforms"
  Rauh et al., Nature Communications (2013)
- "Exploring the Human Acetylome Using High Density Peptide Microarrays" Masch et al., Application Note (2012)

Download application notes from our webpage: www.jpt.com/application-notes/

Assay principle of deacetylase microarrays: Incubate with your enzyme of interest (e.g. deacetylase) followed by fluorescently labeled acetyl-lysine specific antibody. A signal decrease will be detected differential measurement with a control microarray.



# **Enzyme Profiling Services**

JPT provides extensive services to identify and characterize enzyme substrates using our Enzyme Substrate Microarrays.

Discuss your needs with our scientists and send us a sample of your enzyme. JPT will perform all experiments and will deliver a report including experimental details and statistical analysis of results yielding consensus sequence motifs.

#### **Substrate Identification**

- Select from more than 10 000 peptides available on peptide microarrays or create your customized peptide microarray
- Receive information about your enzyme substrates and consensus sequence
- Learn about potential downstream targets of your enzyme
- Identify enzymatic contaminations in samples
- Minimal sample volume needed

#### **Phosphorylation Site Detection**

- Select from more than 60 ready-to-screen
   Phosphorylation Site Detector Microarrays or
   create your customized Phosphorylation Site
   Detector microarray
- Detect potential phosphosites in substrate proteins
- Determine auto-phosphorylation sites
- Identify upstream kinases for your target protein
- Elucidate signal transduction pathways
- Get information about potential downstream targets of your kinase
- Minimal sample volume needed

#### Kinase Specificity Profiling

- Identify key residues for effective kinase/kinase substrate interaction enabling prediction of kinase substrates
- Optimize your known kinase substrates for stability, selectivity and catalytic constants
- Probe kinase activity by introducing alternative posttranslational modifications into sequences surrounding the phosphorylation site of your substrate

#### Acetyltransferase-Deacetylase Profiling Service

- Screen your enzyme against the whole human acetylome with one experiment
- Identify specific acetylation sites for your enzymes
- Find enzyme substrates in the human proteome
- Minimal sample volume needed



JPT's highly skilled and experienced staff works according to well established protocols using automatic hybridization stations and state-of-the-art equipment.

#### **Benefits**

- Take advantage of our proprietary microarray platform
- Well established and automated assay procedures
- Samples are handled according to ISO 9001:2015 and GCLP regulations
- Strong bioinformatic support for array design and data interpretation

# Send us a short outline of your project and we will:

- Suggest the appropriate array to be used
- Provide bioinformatic support for peptide and microarray design
- Provide project proposal and quotation
- Synthesize peptides and generate peptide arrays
- Incubate arrays with your sample and perform control experiments
- Evaluate and interprete data
- Provide comprehensive and confidential report

#### **Selected References**

- "The Fungal Chimerolectin MOA Inhibits Protein and DNA Synthesis in NIH/3T3 Cells and May Induce BAX-Mediated Apoptosis"

  Cordara et al., Biochem Biophys Res Commun (2014)
- "The Pla Protease of Yersinia pestis Degrades
  Fas Ligand to Manipulate Host Cell Death and
  Inflammation"
  Caulfield et al., Cell Host and Microbe (2014)
- "Insulin Signaling via Akt2 Switches Plakophilin 1 Function From Stabilizing Cell Adhesion to Promoting Cell Proliferation" Wolf et al., J. Cell Sci. (2013)

With JPT's peptide microarray platform, high-throughput kinase profiling using small amounts of cell lysate becomes feasible, providing access to the discovery and monitoring of novel biomarkers in Parkinson's Disease.

Jeremy Nichols, PhD, Parkinsons's Institute, Sunnyvale, USA

# SIRT 1 Substrate Specificity Profiles Complex Structure Proteome-wide screening for Sirtuin substrates by peptide microarrays and structural characterization of enzyme-substrate complexes. Adapted from Rauh et al., Nat Commun (2013) SIRT 5 Ref. Proteome-wide screening for Sirtuin substrates by peptide microarrays and structural characterization of enzyme-substrate complexes. Adapted from Rauh et al., Nat Commun (2013)

# **Histone Code Peptide Tools**

We compiled an extensive library of synthetic histone peptides. It covers the histone tails with various composite histone marks, core sequences with modifications representing the growing number of identified modifications within the histone cores and variant sequences enabling the analysis of preferred non-canonical over canonical histones.

#### Individual Histone Code Peptides

Several hundred peptides from our histone code library are now available as purified, high quality biotinylated or non-biotinylated peptides. Among other applications they can be used for fast and efficient confirmation of microarray results. All peptides are purified and qualified by HPLC-MS and arrive as freeze-dried aliquots for increased shelf stability.

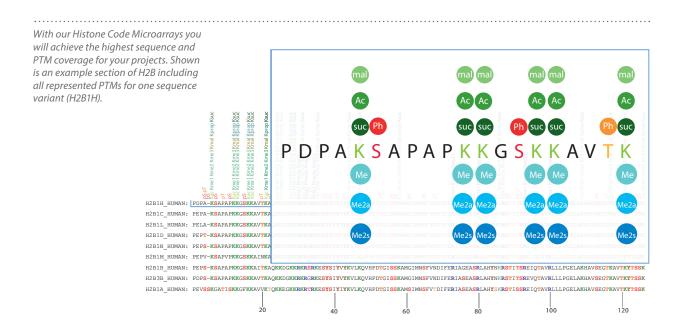
# For a full up-to-date list please visit our webshop at www.jpt.com

#### Histone Code Peptide Microarrays

Histone Code Microarrays are made up of 3 668 peptides derived from our histone code peptide library with and without PTMs. Most potential post-translational modification sites are represented. JPT's comprehensive Histone Code Microarrays enable mapping of protein-histone interactions with unprecedented resolution due to its high coverage of natural sequence variants.

#### **Selected References**

- "Histone H2A and H4 N-Terminal Tails are Positioned by the MEP50 WD-Repeat Protein for Efficient Methylation by the PRMT5 Arginine Methyltransferase" Burgos et al., J. Biol. Chem. (2015)
- "Comprehensive Characterization of Antibodies
  Directed towards Epigenetic Histone-Modifications"
  Masch et al., Application Note (2015)
- "Histone PTM Profiling Reveals Global and Specific Responses to Systematic Enzyme Ablations" Feller et al., Application Note (2015)
- "" "Use of High-Density Histone Peptide Arrays for Parsing the Specificity of a Histone-Modifying Enzyme Complex" Shechter et al., Application Note (2013)
- "Structure of the Arginine Methyltransferase PRMT5-MEP50 Reveals a Mechanism for Substrate Specificity" Ho et al., PLOS ONE (2013)



#### Histone Peptide Sets

Collections of biotinylated peptides with post-translational modifications for Histone H3 tail and core sequences. Delivered freeze dried in individual vials.

#### SpikeTides™ Set Histones (quantified)

37 absolutely quantified and stable isotope-labeled standard peptides for Histone H3 with and w/o modifications. The set is to be used in mass spectrometry-based assays (e.g. MRM assays) for absolute protein quantification.

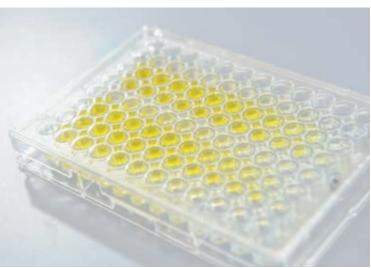
#### Histone Code Peptide ELISA

The Histone Code Peptide ELISA displays 94 20mer peptides from Histones H2A, H2B, H3 and H4. Peptides carry PTMs at reported sites (e.g. acetylation, methylation, phosphorylation). Two control wells are included: 1 x FLAG-Peptide and 1 x empty well. Ready to use with standard ELISA protocols and equipment.

JPT's new extremely high-density histone peptide array is a dramatic leap forward for analysis of the histone code hypothesis. The complete sequence coverage across core histones and histone variants as well as the presence of common and rare histone post-translational modifications — alone and in combination — makes this an unparalleled tool for studying the "writers" and "readers" of the histone code. My laboratory has used these arrays to ask the sequence and PTM dependence of a histone writer enzyme and we have discovered an activity specifying code that will keep us busy for years to come.

David Shechter, PhD, Department of Biochemistry, Albert Einstein College of Medicine, Yeshiva University, New York, NY (USA)





 $Discover\ the\ largest\ variety\ of\ individual\ modified\ histone\ peptides\ and\ our\ ready-to-use\ histone\ code\ ELISA.$ 

# **Histone Code Analysis Service**

Histones are subject to post-translational modifications (epigenetic marks) that influence chromatin structures. The histone code hypothesis suggests that combinations of these modifications play a role in the regulation of gene expression.

Based on our histone code peptide microarray we offer a unique histone code analysis service for mapping and validation of protein-histone interactions including PTM dependencies, e.g. testing of histone antibodies, enzymatic activity profiling and histone reader profiling.

#### **Applications**

- Mapping and validation of protein-histone interactions including PTM dependencies
- Testing of histone antibodies including PTM dependencies
- Enzymatic activity profiling including PTMs
- Histone reader profiling

#### Histone Antibody Specificity Profiling

Test your histone antibody specificity including post-translational modifications. Our comprehensive histone peptide microarray is the tool to validate your anti-histone antibodies.

#### The Service includes:

- Assay with your antibody plus appropriate secondary antibody control (human, mouse, rabbit, rat and others available)
- Raw data analysis
- Summary of results including data table and visualization of results via heatmap and bar plots

#### **Benefits of Histone Analysis Service**

- Coverage of most known natural variants (sequence variants and PTMs)
- All-in-one assay service including incubation and data analysis
- · Tailored peptide microarrays upon request

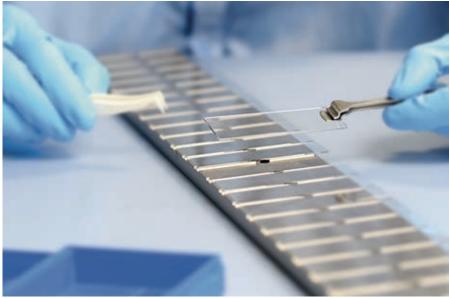
#### Histone Reader Profiling

Identify the preferred epigenetic marks of your histone reader. We characterize the binding profile of your histone binding proteins.

#### The Service includes:

- Fluorescent labeling of your protein or binding domain
- Incubation with histone code microarray and fluorescence read-out
- · Raw data analysis
- Summary of results including data table and visualization of results via heatmap and bar plots







We take pride in our competent service and swift response. Please do not hesitate to contact us for further information. We also very much welcome your feedback and comments.

JPT Peptide Technologies www.jpt.com | peptide@jpt.com

Contact Volmerstraße 5 T +49-30-6392-7878 12489 Berlin F +49-30-6392-7888 Germany