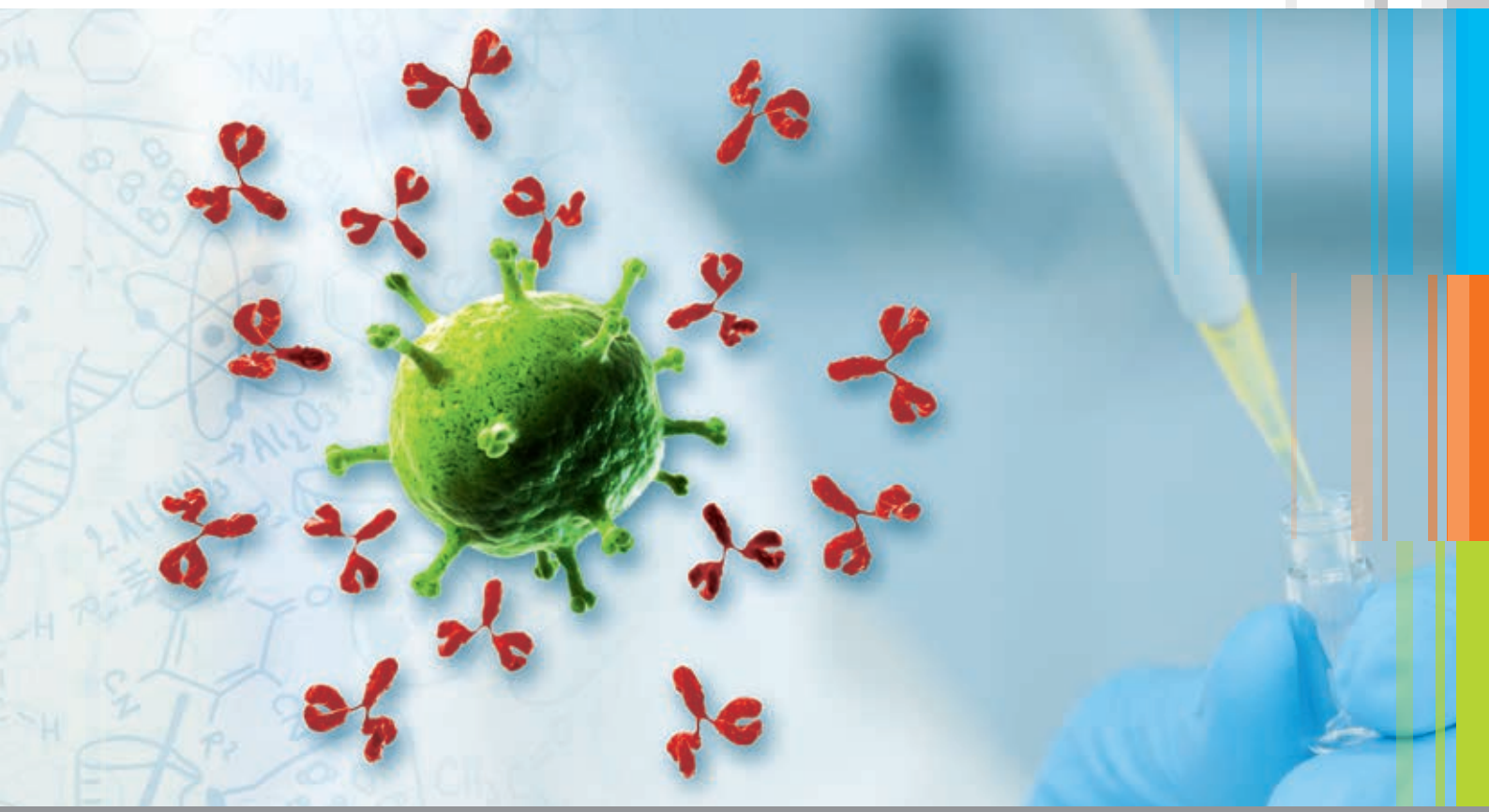
Innovative Peptide Solutions



R&D Collaborations

Peptide-Based Technologies & Know-How for

- Immunotherapy & Vaccines
- Proteomics
- Drug Discovery

JPT
Innovative Peptide Solutions

Why JPT

- *Use our proprietary synthesis and screening technologies*
- *Take advantage of JPT's integrated R&D team for organic and medicinal chemistry, bioinformatics, structural biology, data management, and assay development*
- *Rely on our solid track record, project track record, and ISO 9001 certification*
- *Benefit from flexible business arrangements for collaborative projects*

Technologies & Applications

Over the past decade, JPT has developed a portfolio of proprietary technologies and a series of unique products and services which support research efforts in proteomics, drug discovery and in all developmental phases of novel immunotherapies, and vaccines against infectious and autoimmune diseases as well as against allergies and cancer.

In addition, JPT's technologies find application in all areas where peptide screening is needed. Typical examples are: screening for proteoptypic peptides, enzymatic activities (proteases, kinases, phosphatases, methyltransferases), or systematic optimization of peptide lead structures.

Quality Assurance

JPT is DIN EN ISO 9001:2015 certified.

TÜVRheinland® CERTIFIED
Management System
ISO 9001:2015
www.tuv.com
ID 9105022388

JPT's key technologies are:

PepMix™

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

PepTrack™

Peptide libraries of individual peptides optimized for T-cell and bioactivity assays.

Clinical Peptide Synthesis

Peptide synthesis for the more stringent product requirements of immunotherapy, vaccine, and drug development.

SPOT

High throughput peptide synthesis and screening technology for peptides and peptidomimetics for T-cell epitope and peptide lead discovery and characterization on proteome-wide levels.

PepStar™

Proprietary technology platform for humoral immune response profiling, epitope, and seromarker discovery as well as protein protein interaction studies.

SpikeMix™

Inexpensive source of stable isotope-labeled peptide standard pools for use in mass spectrometry-based proteomics.

SpikeTides™

Light and stable isotope-labeled peptides for proteomics.

MITTELSTAND GLOBAL
HEALTH MADE IN GERMANY

Table of Contents

About JPT

JPT Peptide Technologies is an experienced partner for peptide-based R&D collaborations. Located in Berlin, Germany, JPT has achieved worldwide credibility for its commitment to rigorous quality standards and a reputation for developing and implementing innovative services and research tools for various applications. JPT serves a broad clientele in the pharmaceutical and biotechnology industries as well as researchers in universities and other non-profit organizations.

Flexible Business Arrangements

- Fee-for-service projects
- FTE-based and milestone driven collaboration projects
- Shared risk, partnered development projects

JPT's Background

JPT Peptide Technologies GmbH was established in 2004 as a subsidiary of Jerini AG, a spin-off of Charité in Berlin, focused on the discovery and development of novel peptide-based drugs. In 2009, JPT was acquired to become a part of BioNTech AG, Mainz (Germany), a company that develops novel immune therapy and diagnostic approaches for various cancers.

Why Partner with JPT?

- Proprietary synthesis and screening basis
- Integrated in-house R&D team for organic and medicinal chemistry, bioinformatics, structural biology, data management, and assay development
- Solid track record of successful R&D projects
- ISO 9001:2015 regulated quality assurance system
- Professional project management and communication
- Flexible business arrangements for collaborative projects

JPT's team of skilled staff and knowledgeable scientists

Recent Projects & Partners

Immunotherapy & Vaccines

- Peptide selection and synthesis for individualized neo-epitope based vaccination strategies
- Peptide pool design and synthesis for adoptive cell transfer therapy
- Design of comprehensive peptide libraries for companion diagnostics and treatment
- Identification of serological predictive, prognostic, and surrogate biomarkers
- Proteome-spanning vaccine target discovery
- Deep T-cell epitope discovery

Proteomics

- Development of next generation standards for targeted proteomics
- Characterization of specificity profiles for proteins and protein families
- Design and synthesis of peptide libraries for proteome wide detection and quantification of proteins
- Development of molecular probes for the multiplexed detection and quantification of proteins from biological samples
- Discovery of proteotypic peptide markers for functional proteomics
- Identification of epigenetic states of proteins and characterization of enzymatic alterations

Drug Discovery

- Substrate identification and optimization for several orphan enzymes for screening assays
- Design and production of screening libraries, peptides and peptidomimetics
- Identification and optimization of agonistic and antagonistic ligands
- Identification and optimization of affinity ligands
- Identification and optimization of stabilizing peptides as excipients
- Development of surrogate bioassays for antigen-free detection of therapeutic proteins

Partners include

- *ABRF (Association of Biomolecular Resource Facilities)*
- *Bayer Pharma AG*
- *BioNTech AG*
- *Brighton & Sussex Medical School*
- *Cancer Immunotherapy Trials Network (CITN)*
- *Charité Universitätsmedizin Berlin*
- *CPGR (Centre for Proteomics & Genomics Research)*
- *Epiontis*
- *Friedrich-Loeffeler-Institut*
- *Harvard Medical School*
- *Immudex*
- *Institute for Systems Biology, Seattle*
- *SAP*
- *Technical University Munich (TUM)*
- *Thermo Fisher Scientific*
- *QIMR Berghofer*
- *Yale University*
- *Zellnet*

and various undisclosed partners

Immunotherapy & Vaccines

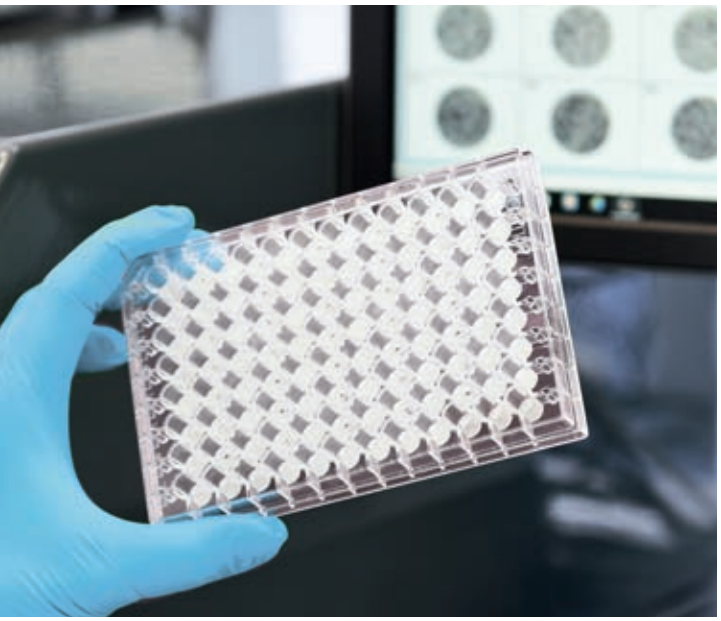
With our technologies and know-how we enabled several successful projects that have led to new targets for therapy, diagnosis, and stratification in indications such as cancer, infections, and autoimmune diseases and allergies.
Furthermore, our comprehensive serological assay platform has been applied in collaborations, developing epitope resolved immune monitoring tools and molecular correlates for protection in various indications.
Finally, our proprietary overlapping peptide pool platform and clinical peptides find widespread application in the development of cellular or immunotherapy and novel diagnostic applications.

Why Peptides as Antigens?

- Neo-epitopes enable personalized therapies
- Peptides ensure robust epitope-resolved immune monitoring
- Only peptides allow the study of B- and T-cell epitope spreading
- Peptides can be synthesized in high purities
- Peptides allow proteome-wide target identification
- No expression needed, ADCF policy applicable
- Sequence diversity and post-translational modifications can be addressed

Why Work with JPT?

- We are the quality leader for peptides in immunotherapy
- We develop unique technologies for discovery, therapy and immune monitoring
- We understand your application
- We have a long track record of successful projects and collaborations
- Over 1000 peer reviewed papers with our products
- We are ISO 9001:2015 certified



Benefits

- Broad peptide technology portfolio for all development phases of immunotherapies and vaccines
- Peptide platforms covering cellular and humoral immunity
- Ultra-high content peptide library concepts for deep epitope and target discovery
- Infrastructure and workflows for peptides in clinical applications
- Comprehensive bioinformatics for peptide selection and design

Selected References

- *"Personalized RNA Mutanome Vaccines Mobilize (...) Immunity Against Cancer"*
  Sahin et al., Nature (2017)
- *"An Immunogenic Personal Neoantigen Vaccine for Patients with Melanoma"*
  Ott et al., Nature (2017)
- *"Broadly-specific Cytotoxic T Cells Targeting Multiple HIV Antigens Are Expanded From HIV+ (…)"*
  Lam et al., Molecular Therapy (2015)
- *"A Phase I Trial Combining Decitabine/dendritic Cell Vaccine Targeting (…)"*
  Krishnadas et al., Cancer Immunol Immunother. (2015)

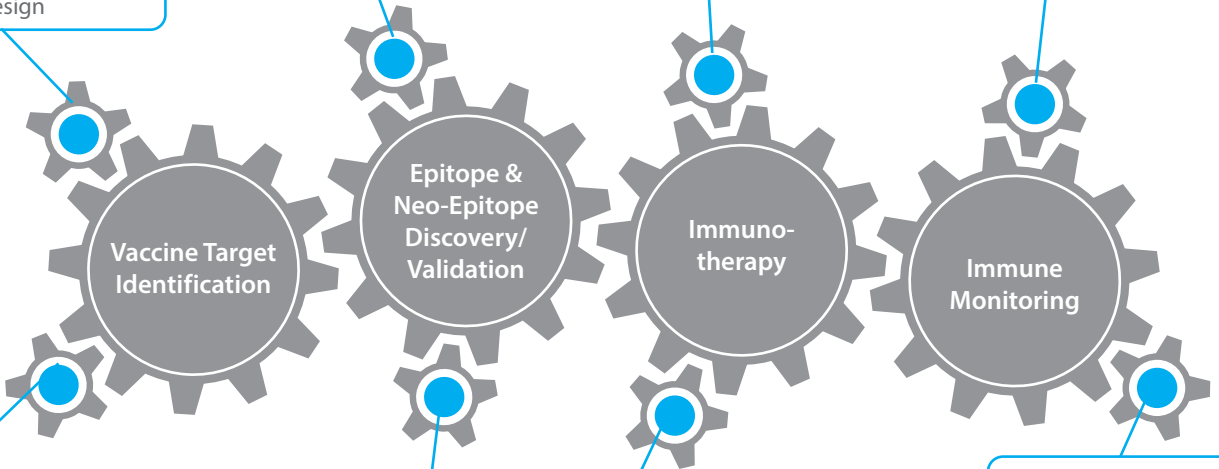
Cellular Immunity

SpotMix™ Peptide Pools & PepTrack™ Fast Track Peptide Libraries
deep, antigen- and proteome spanning target identification
In-Silico Models for Library Design

Matrix Pools & PepTrack™ Research Track Peptide Libraries
fast (neo-) epitope qualification

Clinical Peptides & Pools
individualized therapies i.e. adoptive cell transfer and dendritic cell pulsing
In-Silico Peptide Qualification

PepTrack™ Trial Track Peptide Libraries, PepMix™ Peptide Pools, Dextramer™ Multimers & Universal T-Cell Assay Standard TERS™ Kit
clinical T-cell assays



PepStar™ High Content Peptide Arrays
deep antibody signature profiling
In-Silico Models for Library Design

RepliTope™ Peptide Microarrays & Peptide ELISA
B-cell epitope and seromarker qualification

Antigen Peptides, MAP Peptides & Peptide Conjugates
to proteins, adjuvants, PEG and more for antibody generation

Modular Humoral Immune Profiling
using various platforms, peptide arrays, peptide ELISA and more
Bioinformatic Data Evaluation

Humoral Immunity

Vaccine Target & Epitope Discovery

Specific T- and B-cell epitopes are used for diagnostics, immune monitoring, and patient stratification and as targets for cellular and immunotherapeutic applications. Our peptide-based technologies for systematic, deep and high resolution discovery of epitopes produce outstanding results for every situation. We apply our expertise in bioinformatics to the design of the optimal, assay specific antigen library and use ultra-high throughput peptide synthesis strategies, covering sequence diversity and post-translational modifications where required.

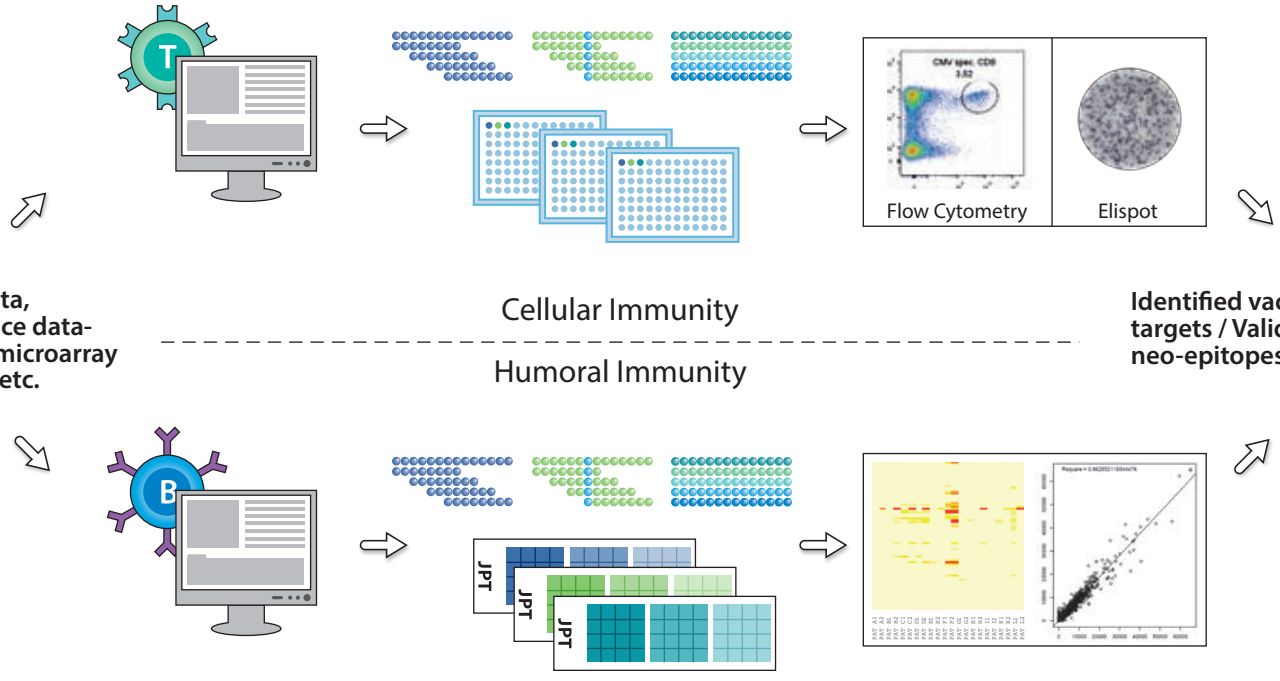
Project Examples

- Deep vaccine target discovery
- Epitope discovery
- Antibody signature profiling
- Neo epitope prioritization
- Biomarker discovery and qualification

Assay Features

- High throughput
- Inexpensive
- Fast
- Bioinformatic support
- Data validation

Exemplaric workflows for discovery projects to detect and validate targets in the cellular and humoral immune system.



Bioinformatics for peptide library design

Peptide pools, ELISA & microarrays for high- throughput assays

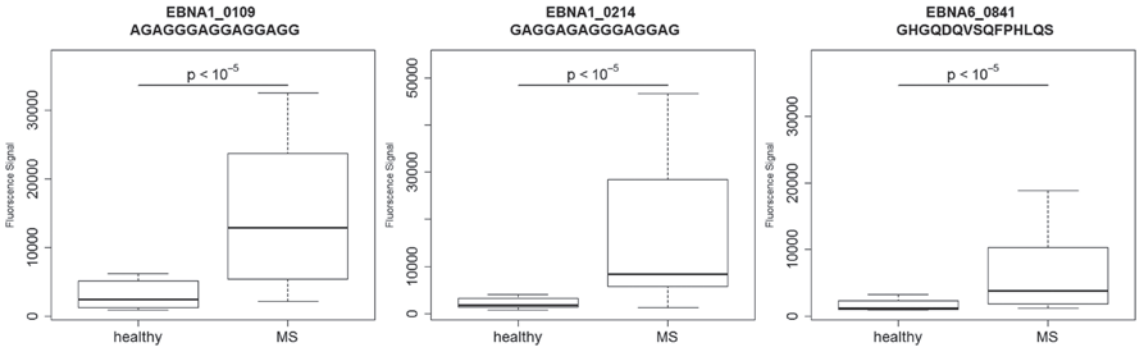
Data evaluation & validation

Connecting Multiple Sclerosis to Epstein-Barr Virus Infection

- Evidence suggests an important role of EBV infection in MS
- The aim of this study was to map EBV antigens that are important in MS patients
- Sera of 29 MS-patients and 22 healthy donors were screened against peptide microarrays displaying 1465 peptides spanning 8 EBV antigens
- Elevated antibody titers towards EBNA-1, -3, -4 and -6 were detected in MS-patients
- Most responses against EBNA-1 were specific for one stretch of the protein, pointing to an important role of this region in the MS-specific immune response

"Serological Profiling of the EBV Immune Response in Chronic Fatigue Syndrome Using a Peptide Microarray."
Loebel et al., PLoS One (2017)

"Multiple Sclerosis: The Elevated Antibody Response to Epstein–Barr Virus Primarily Targets, but is not Confined to, the Glycine–Alanine Repeat of Epstein–Barr Nuclear Antigen-1."
Ruprecht et al., J Neuroimmunol (2014)



The three most significantly (p<10-5) different anti-EBV peptide antibody reactivities between healthy controls (HC, n=22) and patients with multiple sclerosis (MS, n=29). Peptides are designated by the name of the EBV protein followed by the number of the starting amino acid for the 15-meric peptide and the respective amino acid sequence.

Identification of Immunodominant T-Cell Antigens in Mycobacterium Bovis for Vaccine Design

- 119 antigens of M. bovis were produced as peptide pools representing each antigen in overlapping peptides and screened for IFN-γ response in blood from 23 M. bovis-positive cattle
- 59% of the tested antigens induced positive responses
- Proteins of the ESAT-6 family are immunodominant
- Epitope mapping allows for the identification of the most frequently recognized peptides

"Screening of Predicted Secreted Antigens from Mycobacterium Bovis Reveals the Immunodominance of the ESAT-6 Protein Family"
Jones et al., Infect Immun (2010)

Cell- & Immunotherapy

Peptides find increasing application in cellular and immunotherapy. In addition to peptide vaccination, dendritic cell therapy and adoptive cell transfer are among those that have been proven effective. In order to meet requirements of both, individualized immunotherapeutic concepts and cellular therapy approaches requiring hundreds of peptides, JPT has established an enhanced peptide production environment that goes beyond ISO 9001:2015 regulations for more stringent product requirements. The resulting ISO PLUS and Clinical Grade Peptides and Peptide Pools can be applied in collaborations aiming for the development of new therapy concepts targeting indications ranging from cancer and infectious diseases to autoimmune and allergies.

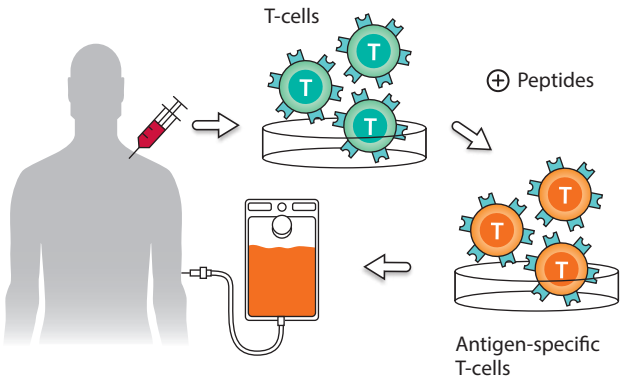
Project Examples

- Development of individualized therapy concepts
- Adoptive cell transfer
- Dendritic cell-based therapies
- Neo-epitope based peptide vaccination strategies

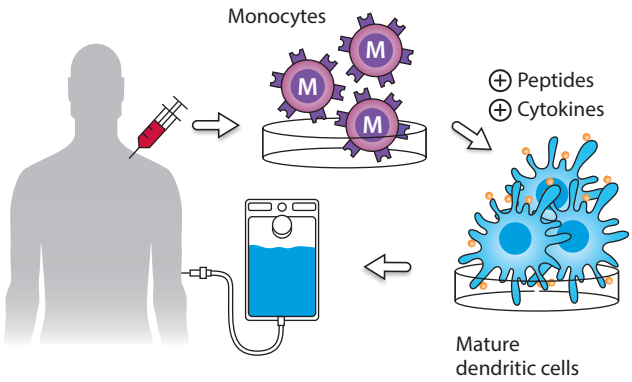
Our Capabilities

- Traceability of workflows
- Comprehensive analytical capabilities
- Documentation acc. to regulations
- Peptide selection and design

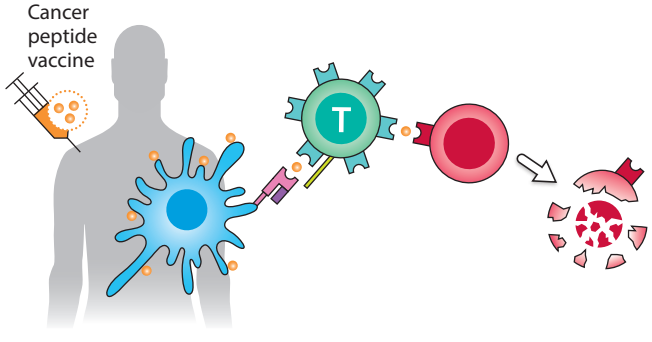
Adoptive Cell Transfer



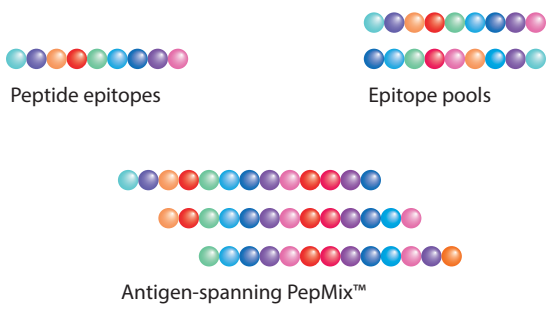
Dendritic Cell (DC) Pulsing



Peptide Vaccination



Peptide Antigen Formats



Examples for cell and immunotherapies that apply peptides or peptide pools as antigens.

A Personalized Genomic Vaccine to Treat Malignancies

- The study tests the safety, tolerability, and immunogenicity of a personalized peptide vaccine that is based on a patient's own tumor sequence
- Tumors of patients with advanced non-hematologic malignancies are sequenced and peptides that correspond to the tumors are made
- These peptides combined with an adjuvant to boost the immune response will expand the patient's immune cells to target cancer

"Responsible Party: Nina Bhardwaj, Director of Immunotherapy Program, Icahn School of Medicine at Mount Sinai"
ClinicalTrials.gov Identifier: NCT02721043

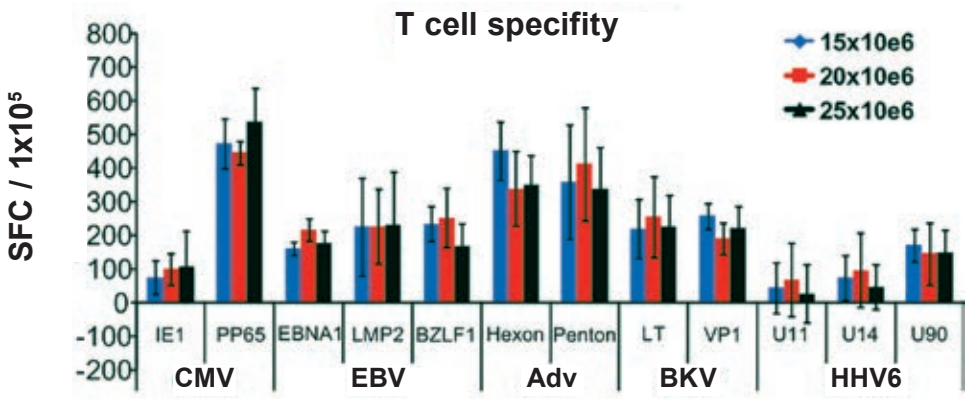
T-Cell Therapy for Viral Infections after Hematopoietic Stem Cell Transplant (HSCT)

- Antigen-specific T-cells are effective for treatment of viral infections after (HSCT)
- The goal was to establish parameters for optimal in vitro CTL expansion and to determine the starting donor blood volumes required
- PBMCs were pulsed with peptide pools spanning antigens from CMV, AdV, EBV, BKV, and HHV6
- IFN-γ ELIspot assays detected actitivity against the stimulating antigens
- 20ml blood from a stem cell donor are adequate to produce sufficient multivirus-specific T-cells for the treatment
- Expansion with synthetic peptide pools was more rapid and simpler than previous strategies

"Rapidly Generated Multivirus-specific Cytotoxic T-Lymphocytes for the Prophylaxis and Treatment of Viral Infections"
Gerdemann et al., Mol. Ther. (2012)

"Long-Term Outcome of EBV-specific T-Cell Infusions to Prevent or Treat EBV-Related Lymphoproliferative Disease in Transplant Recipients"
Heslop et al., Blood (2010)

"Adverse Events Following Infusion of T-Cells for Adoptive iImunotherapy: A 10-Year Experience"
Cruz et al., Cytotherapy (2010)



Specificity of multivirus-directed T-cells. The specificity of the T-cell lines generated were analyzed using IFN-γ Elispot assay as readout and individual PepMixes™ as a stimulus.

Immune Monitoring

Robust and efficient monitoring of the humoral and cellular immune status is a prerequisite for the diagnosis and treatment of many diseases, to follow efficacy of vaccination and immunotherapy studies, organ transplantation and to develop novel therapeutic approaches. JPT possesses the widest portfolio of peptide-based technologies to profile antigen-specific immune responses. Our proprietary high content and high performance techniques allow epitope-resolved monitoring of individual epitope patterns, as well as epitope spreading and addressing natural sequence diversity, mutations and post-translational modifications that occur in cancer cells and different types of viruses. Our high quality standards in combination with technical and bioinformatics knowledge allow application for clinical trial immune monitoring.

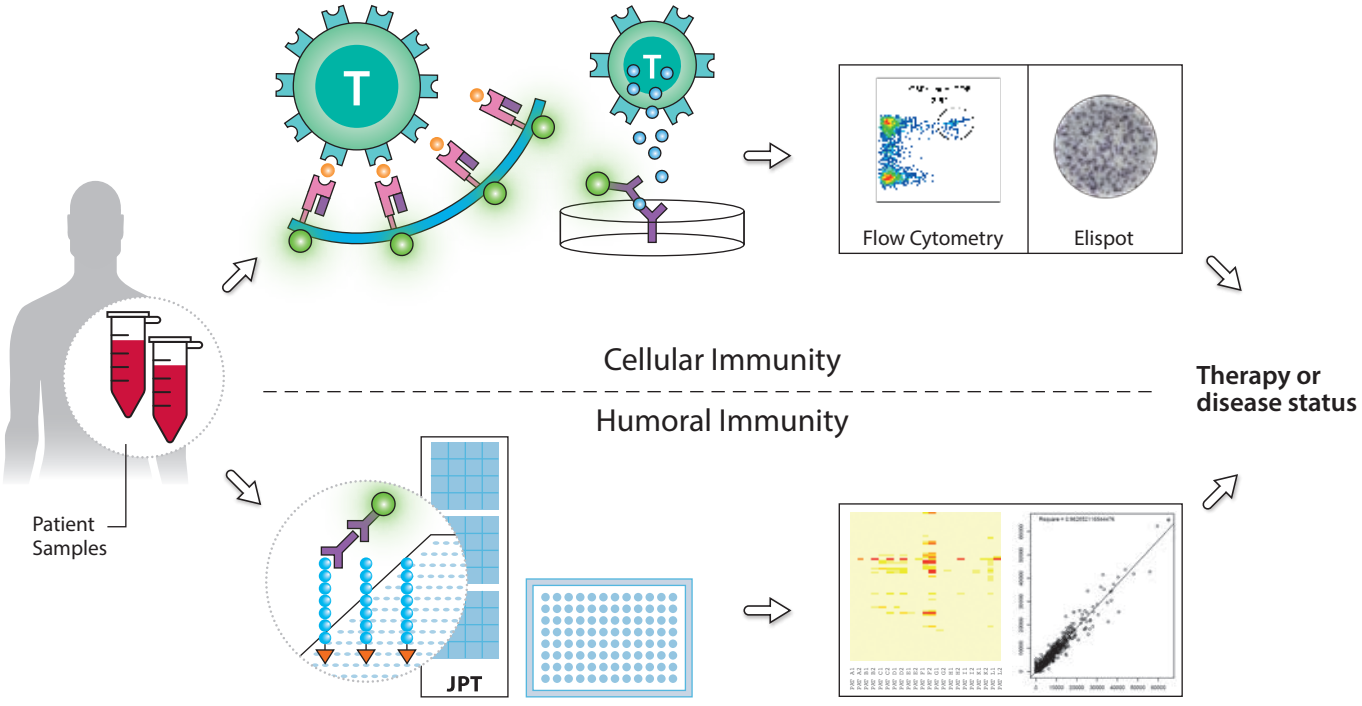
Project Examples

- Clinical immune monitoring
- Development of diagnostic and stratification markers
- Target validation
- Cellular and humoral immune profiling

Our Capabilities

- Technologies to profile humoral and cellular immune responses
- Chemically synthesized tools enable monitoring independent from the biologicals used for treatment
- Measurement of epitope-resolved and PTM-related immune status
- Line clearance for clinical immune monitoring

Various options to monitor cellular and humoral immune responses during trials or therapies.



Elispot, flow cytometry, multimer assays and peptide microarray, ELISA

Data evaluation

Neo-Epitope Based Immune Monitoring

- Monitoring of individual neo-antigen-specific T-cell responses requires large numbers of peptides. Standard synthesis lacks the capacity and speed required for large trials
- ELISPOT data on neo-antigen-specific immune responses using high-throughput, low-cost FastTrack peptides are compared with those obtained with peptides generated by standard methods
- FastTrack peptides show sufficient quality for immune monitoring providing fast, inexpensive access to thousands of peptides

"A Fast, Flexible and Low Cost Process for Neo-Epitope Based Immune Monitoring"
Castro et al., AAI Annual Meeting (2017)

"A Fast & Low Cost Process for Neo-Epitope Based Immune Monitoring"
Derhovanessian et al., Application Note (2017)

"Systemic RNA Delivery to Dendritic Cells Exploits Antiviral Defence for Cancer Immunotherapy"
Sahin et al., Nature (2016)

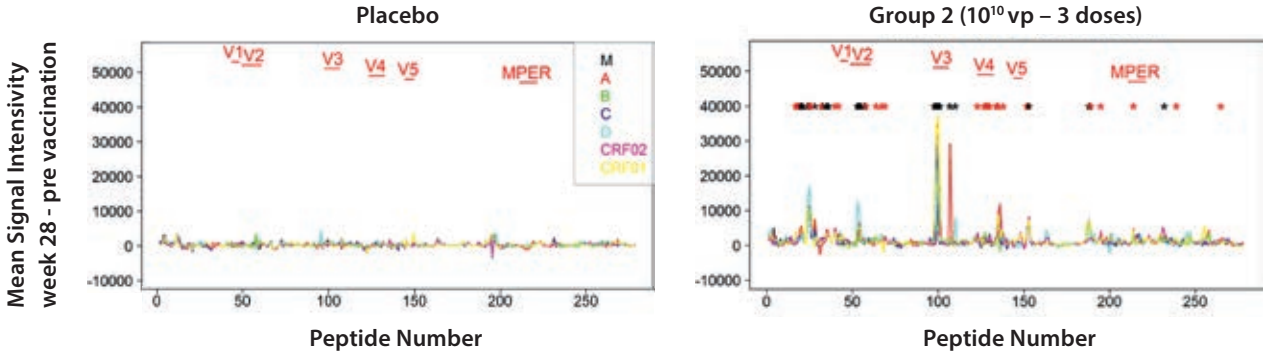
Experimental HIV Vaccines in Animal & Phase I Clinical Trials

- HIV-peptide microarrays were used in different HIV vaccination studies
- Phase I: patient samples from IPCAVD 001 were evaluated on peptide microarrays for antibody reactivities. Dose dependent expansion of the magnitude, breadth, and epitopic diversity was measured and a critical dose for the stimulation of a Env-V2 specific B-cell immune response defined
- Preclinical: rhesus monkeys were primed with Ad26 vectors expressing SIVsmE543 Env, Gag, and Pol and boosted with Env gp140. Complete protection was observed for 50% of vaccinated animals against a repeated, intrarectal SIVmac251 challenge that infected all control animals

"Antibody Responses After Analytic Treatment Interruption in Human Immunodeficiency Virus-1-Infected Individuals on Early Initiated Antiretroviral Therapy"
Stephenson et al., Open Forum Infect Dis. (2016)

"Protective Efficacy of Adenovirus/Protein Vaccines Against SIV Challenges in Rhesus Monkeys"
Barouch et al., Science (2015)

"Characterization of Humoral and Cellular Immune Responses Elicited by a Recombinant Adenovirus Serotype 26 HIV-1 Env Vaccine in Healthy Adults (IPCAVD 001)"
Barouch et al., JID (2013)



Antigen-specific binding antibody profiles. Sera were tested on peptide arrays to monitor linear antibody responses to peptides spanning Env from clades M, A, B, C, D, CRF02, and CRF01. Mean signal intensity (in week 28 with baselines subtracted) from all subjects in one group is depicted. Black asterisks denote peptides with significantly elevated mean signal intensity as compared to placebos (p<0.05, Wilcoxon Mann-Whitney tests), and red asterisks denote trends (p<0.10).

Bio- & Cheminformatics

With our long term experience in bioinformatics, computational chemistry and modeling we are able to support research projects at all stages.

Our Capabilities

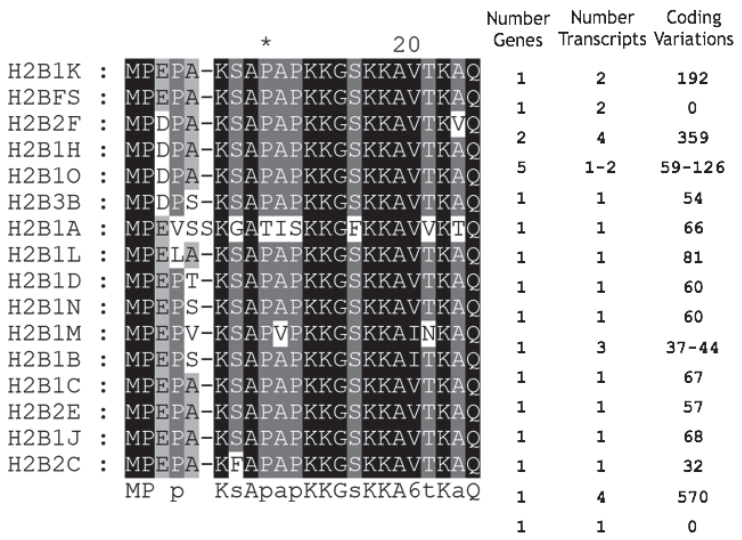
- Library design based on all available and relevant data sources (sequence, structure, function, homology, literature, ligands, and databases)
- Evaluation of experimental data (medium and high throughput assays)
- Management of complex data sets
- Presentation of complex data sets
- Conversion of structure, sequence, and other data to different formats
- Support for compound logistics
- Supply of compound data in any format (sequence or structure)
- Generation of homology models for peptide selection
- Prediction and modelling of data
- Scaffold design for native-like presentation of peptides
- Management and integration of data from different sources
- Customized data presentation

Meeting Sequence Diversity

Sequence diversity is a hallmark of many pathogenic viruses including HIV and Influenza, rendering the control of such infections difficult. Additionally, certain diseases, in particular cancer, are linked to increased numbers of somatic mutations. More complexity is added by post-translational modifications which further alter proteins and give rise to potential neo-epitopes.

Sequence diversity plays an important role in immune recognition. Therefore, it has to be taken into account for development of immune monitoring or -therapy approaches. Peptide libraries are ideally suited for accommodating such diversity.

JPT's ULTRA concept allows the generation of libraries which optimally cover sequence diversity with a minimum number of peptides at minimal cost. This includes both, diversity at the amino acid level as well as post-translational modifications.



Sequence variances: recent advances in DNA sequencing technologies have considerably extended the view on sequence diversity in genomes and proteomes. A protein known under a single name can consist of numerous isoforms, splice variants, transcripts, and sequence variants as shown here for one of the core histones, H2B. Especially, but not exclusively, the picture is even more complicated for histones due to many post-translational modifications. Peptide libraries are perfect tools to cover sequence diversity.

Peptide Prioritization

Chemical peptide accessibility, stability, and solubility vary largely based on their specific amino acid sequence, physicochemical properties and secondary structure propensities and need to be considered for selecting appropriate candidates for immunotherapy and vaccination. Backed by our experience in synthesizing hundreds of thousands of peptides and deducted prediction algorithms, we provide assistance to select peptides with favorable properties regarding synthetic access, solubility, and shelf-life.

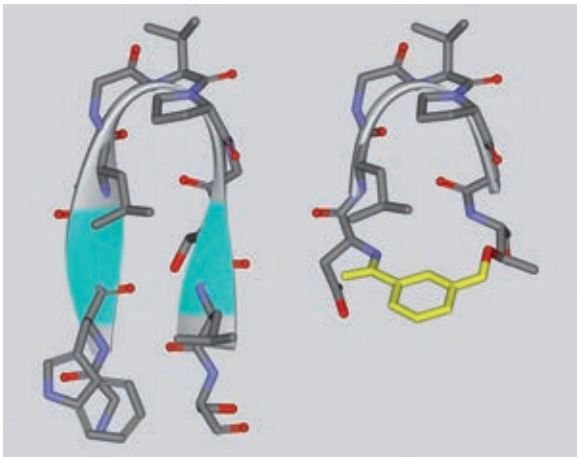
Cheminformatics & Modeling

We aggregate and process all available information using databases (e.g. sequence, mutation, epitope databases), prediction algorithms (e.g. homology structure prediction, epitope prediction, prediction of physicochemical properties for peptides) and modeling tools (protein/ligand complexes, modeling of structural scaffolds and constraints, SAR models) to identify the optimal path forward, fast, and efficiently.

Selected References

- *"Antibody Responses after Analytic Treatment Interruption in Human Immunodeficiency Virus-1-Infected Individuals on Early Initiated Antiretroviral Therapy"*
  Stephenson et al., Open Forum Infect Dis. (2016)
- *"Fine Specificity of the Antibody Response to Epstein-Barr Nuclear Antigen-2 and other Epstein-Barr Virus Proteins in Patients with Clinically Isolated Syndrome: A Peptide Microarray-Based Case-Control Study"*
  Schlemm et al., J Neuroimmunol. (2016)
- *"Protective Efficacy of Adenovirus/Protein Vaccines against SIV Challenges in Rhesus Monkeys"*
  Barouch et al., Science (2015)
- *"Quantification of the Epitope Diversity of HIV-1-Specific Binding Antibodies by Peptide Microarrays for Global HIV-1 Vaccine Development"*
  Stephenson et al., J Immunol Methods (2015)
- *"Mass-Spectrometry-Based Draft of the Human Proteome"*
  Wilhelm et al., Nature (2014)
- *"Multiple Sclerosis: the Elevated Antibody Response to Epstein-Barr Virus Primarily Targets, but is not Confined to the Glycine-Alanine Repeat of Epstein-Barr Nuclear Antigen-1"*
  Ruprecht et al., J Neuroimmunol (2014)

X-ray loop structure (left) and model (right) stabilized by scaffold.



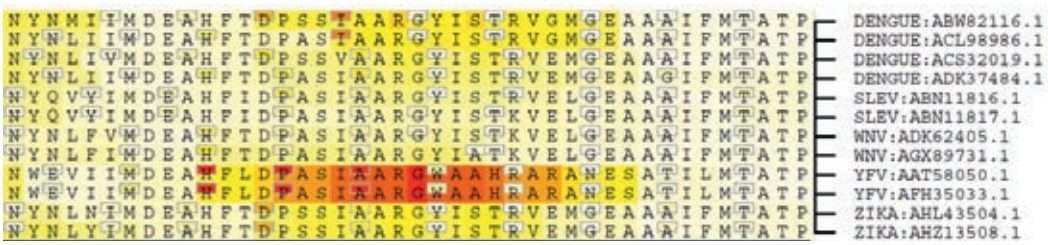
Pan-Flavivirus ULTRA Peptide Library

Flaviviruses are a genus of arthropod-borne viruses including the mosquito-borne: dengue virus (DENV), West Nile virus (WNV), yellow fever virus (YFV), Saint Louis encephalitis virus (SLEV), and zika virus (ZIKV) as well as the Japan encephalitis virus (JEV), tick-borne encephalitis virus (TBEV), and Omsk haemorrhagic fever virus (OHFV). Clinical syndromes following infection are fever-arthralgia-rash, viral haemorrhagic fever, and/or neurological disease. Even though the viruses are similar in sequence, there is considerable sequence diversity between and within single viruses.

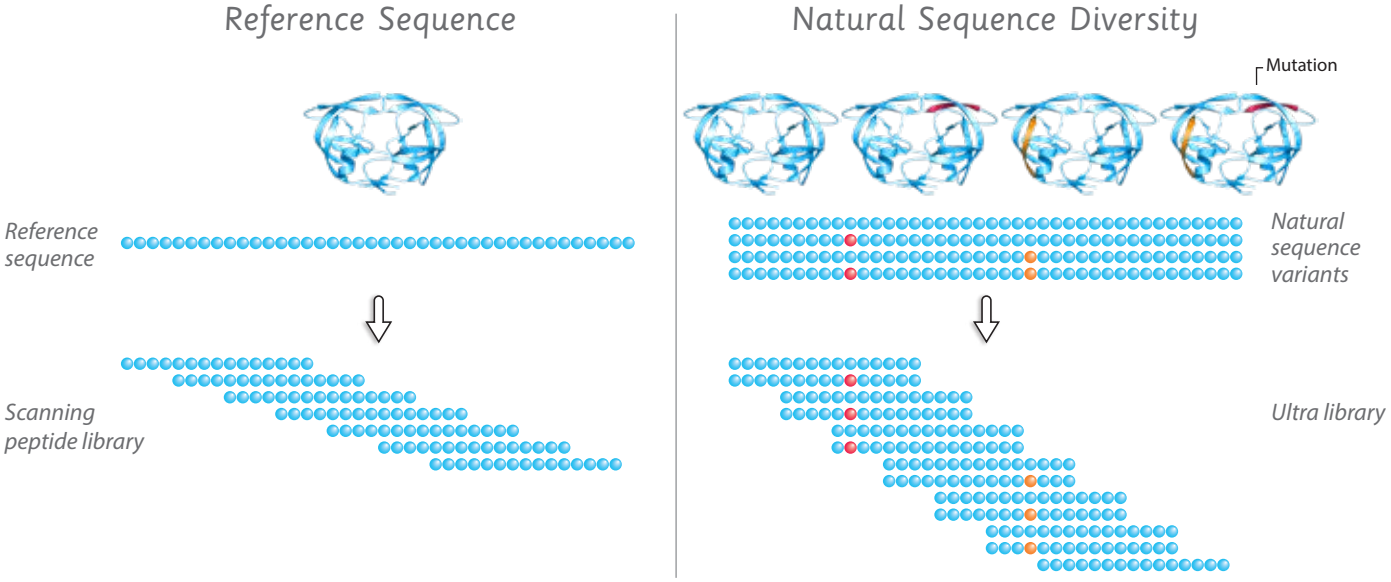
We designed an ULTRA library presenting the sequence spaces of DENV, WNV, YFV, SLEV, and ZIKV and immobilized respective peptides on microarrays. This is a valuable tool for the characterization of specific and cross-virus antibody responses in patients and vaccinees. The arrays were used in a study for the comparison of different vaccination platforms for the development of a ZIKA-vaccine.

→ *"Protective Efficacy of Multiple Vaccine Platforms Against Zika Virus Challenge in Rhesus Monkeys"* Abbink et al., Science (2016)

Yellow Fever Virus (YFV)-specific antibody response in a patient towards the virus protein NS3. The color underlying the alignment indicates the average signal intensity of the residue as calculated from overlapping peptides. The color of the small squares indicate the signal intensity of an actual 15mer peptide starting in this position.



Classical peptide library and ULTRA scanning library.



Proteomics

JPT is providing the Proteomics community with a multitude of innovative research tools. Besides high throughput chemical and analytical approaches to accelerate the discovery of novel biomarkers from biological samples, JPT has developed a new peptide standard technique for absolute quantification of target proteins in clinical and targeted proteomics. Furthermore, JPT has a decade-long track record providing services and project expertise in the area of functional proteomics ranging from elucidation of protein-protein interactions and enzymatic activities to epigenetic events.

Benefits

- Proprietary high throughput peptide technologies for highly multiplexed identification of proteins
- Unique approach for absolute and multiplexed quantification of proteins
- Coverage of post-translational modifications
- Rapid access to hundreds of thousands of peptides covering the entire human proteome

Example Projects

- Synthesis of 100s of thousands of reference peptides for proteomics
- Provision of heavy labeled and/or absolutely quantified peptides for quantification

Selected References

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

"Human SRMAtlas: A Resource of Targeted Assays to Quantify the Complete Human Proteome"
Kusebauch et al., Cell (2016)

"Nuclear PKC-e Facilitates Rapid Transcriptional Responses in Human Memory CD4+ T Cells Through p65 and H2B Phosphorylation"
Hardy et al., J Cell Sci. (2016)

"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)

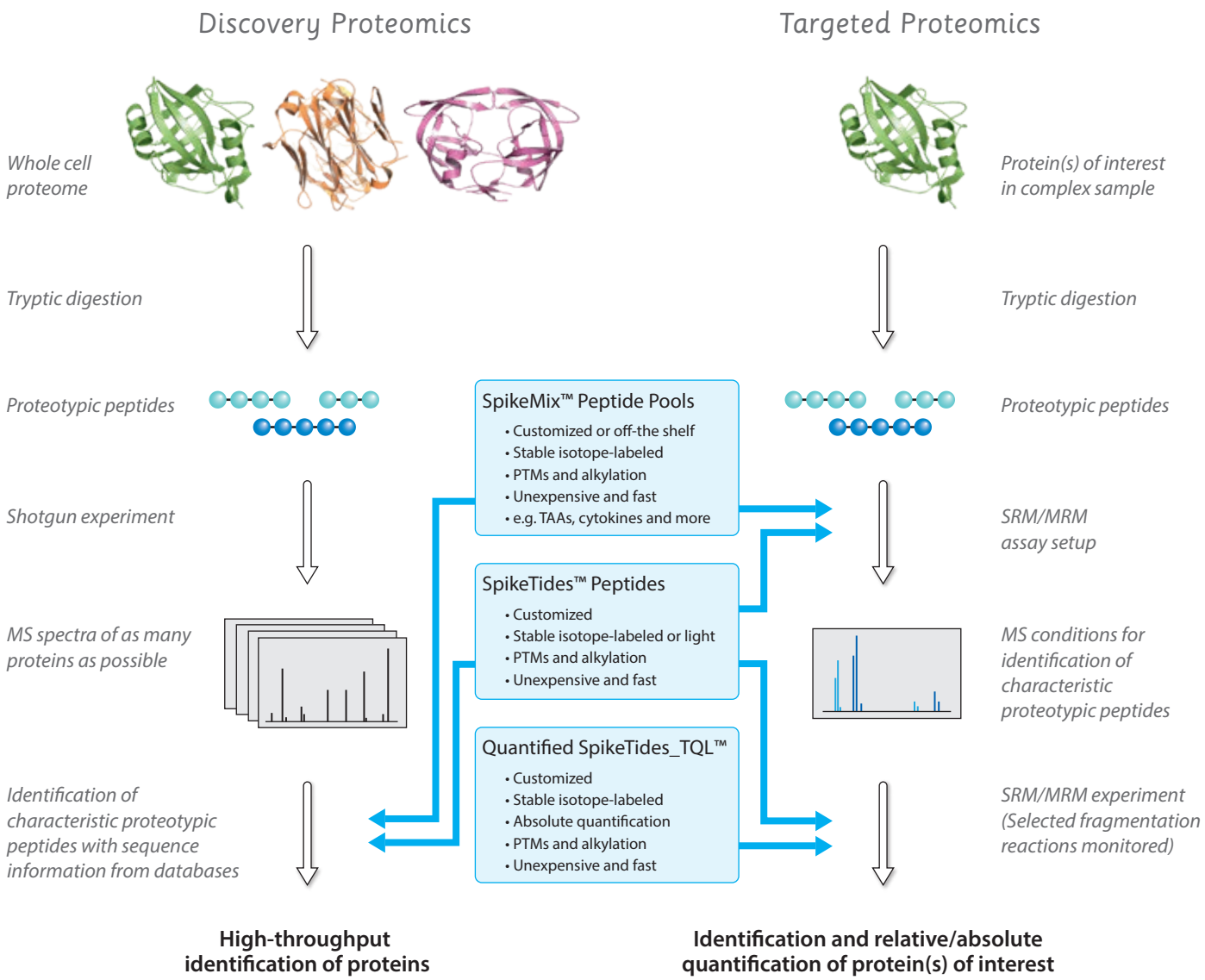
"Mass-Spectrometry-Based Draft of the Human Proteome"
Wilhelm et al., Nature (2014)

"High-Throughput Generation of Selected Reaction Monitoring Assays for Proteins and Proteomes"
Picotti et al., Nature Methods (2009)

Discovery & Targeted Proteomics

JPT has been providing the proteomics community with tools for discovery and targeted proteomics for years. The needs of researchers for inexpensive, heavily labeled or unlabeled and absolutely quantified peptides triggered the development of the SpikeTides™ product family, which finds widespread application in many laboratories. Our Bioinformatics expertise allows for intelligent peptide library design that, in combination with our technologies, helps to solve complex problems in target discovery and validation as well as biomedical research.

Impact of JPT's technologies on the proteomics workflow.



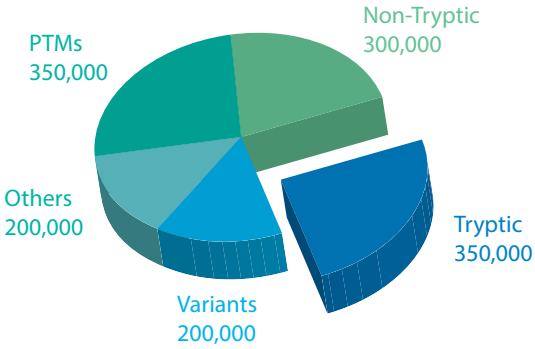
ProteomeTools Project: Synthesis of More than 1 Million Synthetic Reference Peptides

- Joint effort of the Technical University of Munich (TUM), JPT Peptide Technologies, SAP SE, and Thermo Fisher Scientific
- Goal: translating the human proteome into molecular and digital tools for drug discovery, personalized medicine and life science research
- Over the course of the project, ~1.4 million synthetic peptides covering essentially all human gene products including important post translational modifications will be synthesized and analyzed using multimodal LC-MS/MS
- We welcome participation to the project by the scientific community by e.g. suggesting sets of peptides to synthesize, offering measurements using other types of LC-MS/MS, or developing applications and data analysis tools

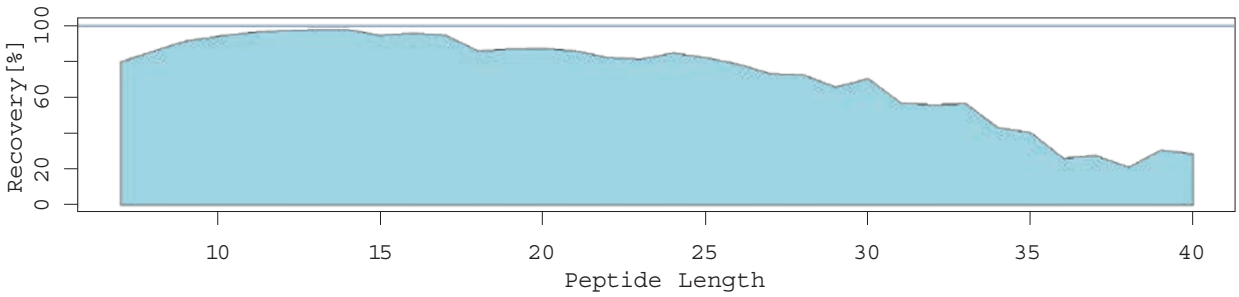
"Building ProteomeTools Based on a Complete Synthetic Human Proteome"
Zolg et al., Nature Methods (2017)

"Mass-Spectrometry-Based Draft of the Human Proteome"
Wilhelm et al., Nature (2014)

ProteomeTools



Planned segmentation of the >1 million peptides that will be selected from the human proteome and synthesized over the course of the project.

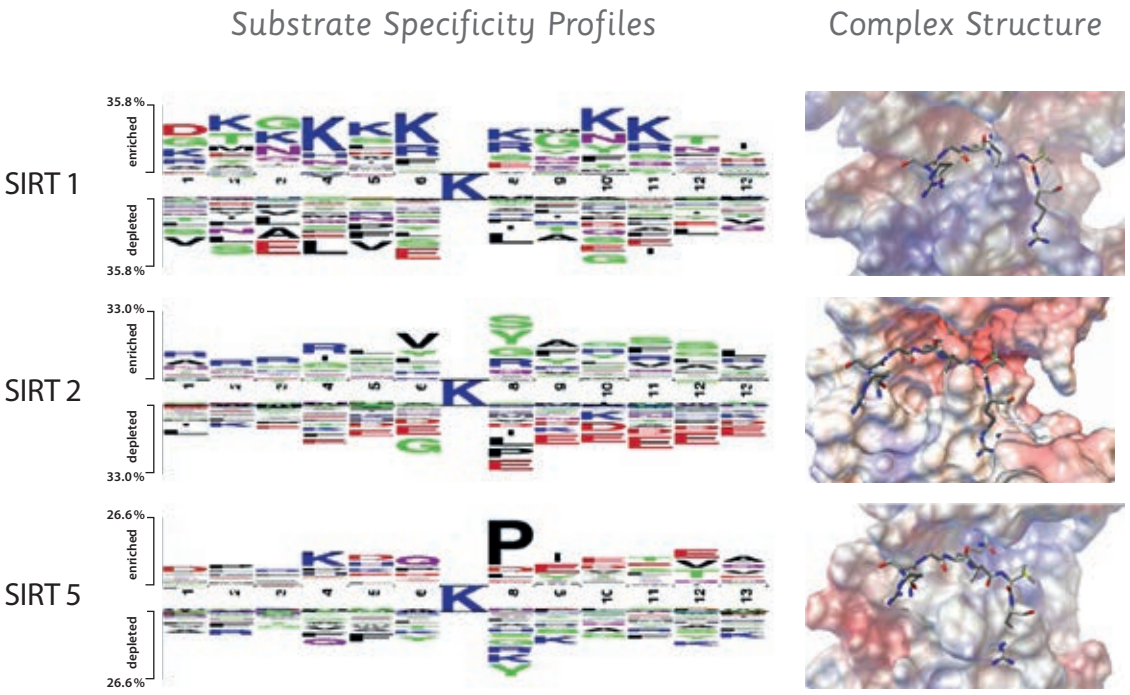


Percentage of unambiguously detected peptides after ultra-high throughput synthesis and analysis by different MS fragmentation methods. The synthesis and analysis of 124,875 peptides is shown. Detection limit is an Andromeda Score >= 80.

Functional Proteomics

Functional proteomics includes the characterization of enzyme activities as well as protein-protein interactions and the functional state of proteins. Combining our technologies and expertise, we are able to map changes in signaling pathways as a consequence of cell-treatment or changes in environmental conditions. This can either be done by the large scale measurement of enzymatic activities such as kinase, phosphatase, protease, or of other protein modifying enzymes. In addition, valuable information about the epigenetic state of proteins can be collected.
Additionally, we have developed innovative ways to evaluate and present complex data sets that is indispensable to the optimal exploitation of experimental results.

Characterization of signal transduction networks. Proteome wide screening for substrates of Sirtuin class enzymes by peptide microarrays and structural characterization of enzyme-substrate complexes.

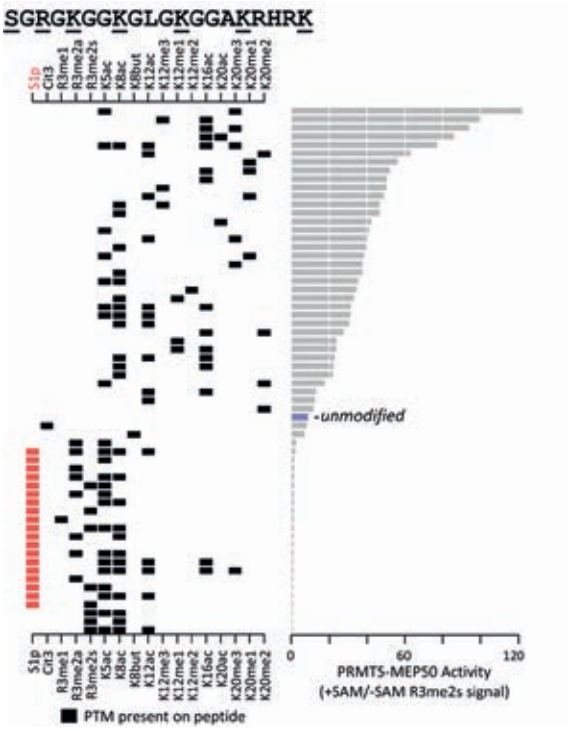


Characterization of PRMT5-MEP50 Histone Methyltransferase in Dependence of Post-Translational Substrate Modifications

- Methylation of arginine and lysine residues is part of the epigenetic code in histones
- PRMT5-MEP50 is known to catalyze the methylation of Arg-residues
- A complex peptide library displayed on peptide microarrays was used to characterize the histone methyltransferase PRMT5-MEP50
- Results show that PRMT5-MEP50 modifies Arg3 in the N-terminal tail of H2A and H4
- This activity is modulated by substrate PTMs: phosphorylation of neighbouring Ser inhibits activity, acetylation of C-terminal Lys residues enhances activity

"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)

"Structure of the Arginine Methyltransferase PRMT5-MEP50 Reveals a Mechanism for Substrate Specificity"
Ho et al., PLoS One (2013)



PRMT5-MEP50 histone methyltransferase activity is modulated by substrate PTMs. High-density histone peptide arrays incubated with PRMT5-MEP50. Data from H4 peptides are shown, each row represents a discrete peptide. The left panel shows individual modifications present on each peptide, the histogram on the right panel shows the relative activity on each peptide. The signal on the unmodified 1-20 peptide is indicated in blue. Inhibition by Ser1 phosphorylation is indicated in red.

Deciphering Substrate-Specificity of Human Sirtuins

- The human class of sirtuins consists of 7 members, only a limited number of substrates is known
- The 7 human sirtuins were tested for enzymatic activity on a library of 6802 human acetylation sites immobilized on peptide microarrays
- Substrates were identified and substrate specificity profiles could be generated (see figure on p. 18)
- These results help to understand the role of single sirtuins in signaling networks

"An Acetylome Peptide Microarray Reveals Specificities and Deacetylation Substrates for all Human Sirtuin Isoforms"
Rauh et al., Nat Commun (2013)

Drug Discovery & Optimization

JPT Peptide Technologies applies its portfolio of proprietary peptide technologies and bioinformatic and medicinal chemistry know-how to manage R&D projects for contract research and R&D collaborations in peptide discovery, hit-to-lead qualification and peptide optimization.
Our experience in drug discovery and optimization finds application in peptide-based drug discovery and vaccine projects, in the discovery of peptidic additives for the cosmetic and nutritional industry, in the development of affinity ligands for purification of biomolecules.

Example Projects

- Identification and optimization of agonistic and antagonistic ligands
- Enzyme substrate identification and optimization for screening assay development
- Design and production of screening libraries of peptides and peptidomimetics
- Identification and optimization of affinity ligands
- Identification and optimization of stabilizing peptides as excipients
- Development of surrogate bioassays for antigen-free detection of therapeutic proteins
- **New:** measurement of important *in vitro* ADME parameters (e.g. plasma/microsomal stability)

Bioinformatic Support

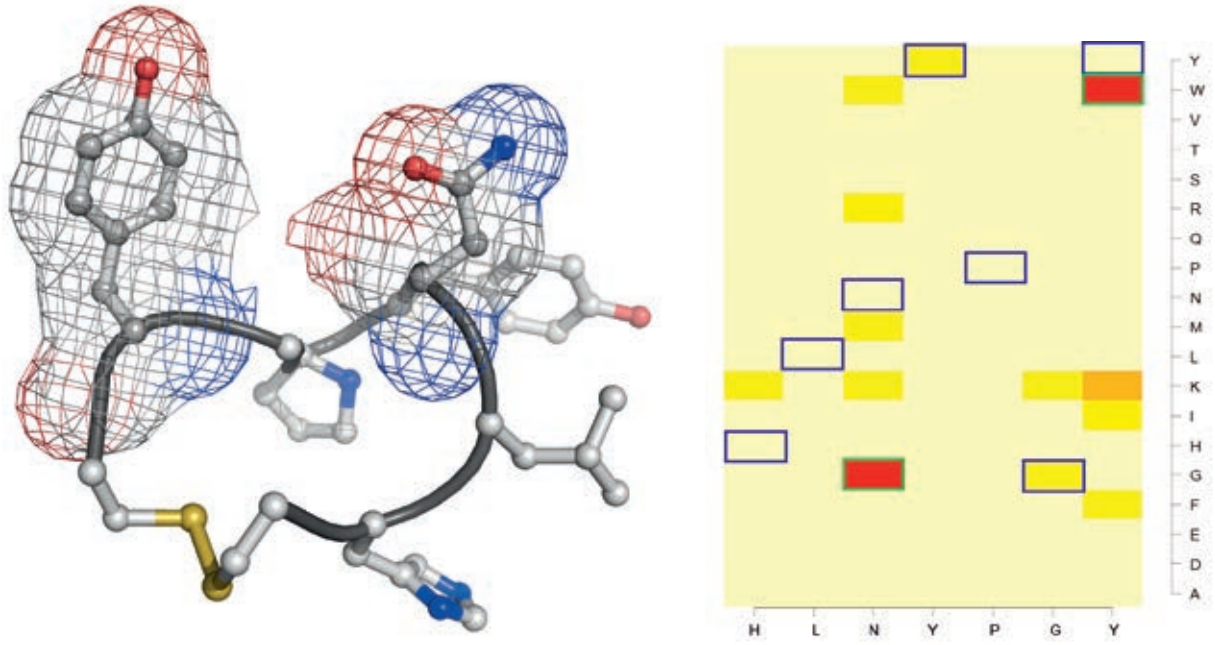
See pages 12 and 13 for details on how JPT's bioinformatics capabilities support peptide optimization projects.

Selected References

- *"Peptide Mimotopes Of The Cd3 T-cell Co-receptor Epsilon Chain and Uses Thereof"*
  Sahin et al., WO 2017/008844 (2017)
- *"Detection of Neurodegenerative Diseases"*
  Moussaoui et al. US#62/304,864 (2016)
- *"Peptide Microarrays Enable Rapid Mimotope Optimization for Pharmacokinetic Analysis of the Novel Therapeutic Antibody IMAB362"*
  Schnatbaum et al., Biotechnol. J. (2014)
- *"High Density Peptide Microarrays for Proteome-Wide Fingerprinting of Kinase Activities in Cell Lysates"*
  Thiele et al., Methods Mol. Biol. (2010)
- *"Novel Small Molecule Bradykinin B1 Receptor Antagonists. Part 1-3"*
  Schnatbaum et al., Bioorg. Med. Chem. Lett. (2010)
- *"Small Molecule Bradykinin B2 Receptor Modulators"*
  Gibson, Schnatbaum et al., WO 2010/031589 (2010)

Characterization and Optimization of a Cyclic Antigen for the Therapeutic Antibody IMAB362

- The key residues of a cyclic peptide for binding to IMAB362 were to be identified
- Substitutional analyses of a lead sequences from phage display was performed using more than 400 cyclic peptides on microarray slides. All peptides were screened for binding to IMAB362
- Key residues and tolerable substitutions were identified. Cyclization was shown to be beneficial for binding affinity
- Binding affinity improvement by factor of 30 leads to very strong binder (KD = 0.15 nM)



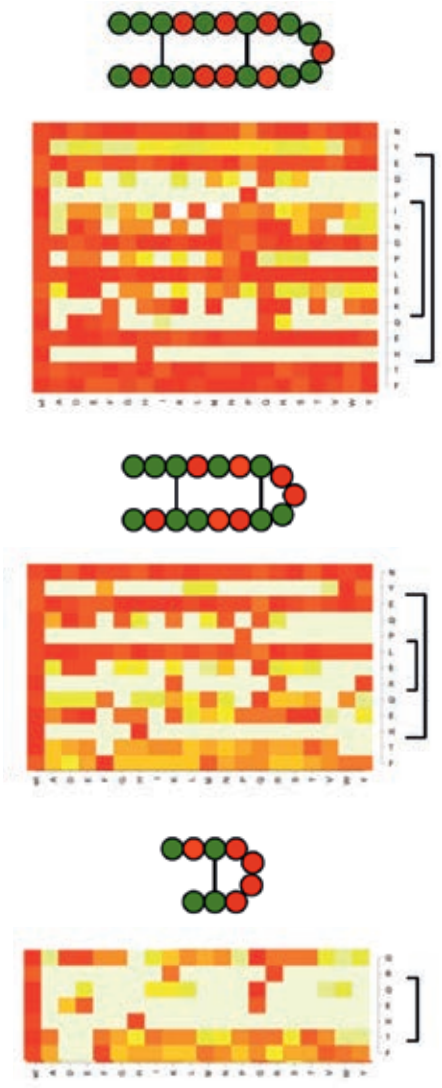
Substrate specificity analysis of a binder to the IMAB362 antibody. The shown seven amino acid sequence is flanked by two cysteines for cyclization.

Left:
Cyclic peptide with highlighted key residues. The Cys-Cys bridge is marked in yellow.

Right:
Results of a substitutional analysis of an IMAB362 binding peptide. Red color indicates strong specific binding, light yellow indicates less or no binding. The results for the WT peptide are highlighted by the blue boxes.

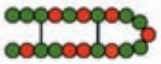
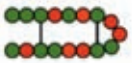
Optimization of a 21-mer Hit to a 9-mer Peptide

- Various cycles of substitutional anlyses, truncation and deletion analyses were used to optimize a peptide in terms of molecular weight, binding characteristics and physicochemical properties
- The 21-mer hit peptide with two internal disulphide bonds was optimized towards a nonapeptide with retained biological activity and superior physicochemical properties



Left:
The figures depict the results of substitutional analyses of the initial peptides, which where used as a basis for convertion into a smaller and more stable peptide. Red indicates strong binding light yellow or white indicate less or no binding. Black brackets show positions of disulfide bonds.

Below:
Table showing various properties measured for intermediate compounds at different stages of peptide optimization.

Parameters			
Peptide Length [aa]	21	17	9
No. of disulfide bonds	2	2	1
IC50 Receptor binding human [nM]	15	28	18
IC50 Receptor binding rat [nM]	265	122	40
IC50 Inhibition of effector mediated Ca-Mobilization [nM]	22	48	23
Plasma stability (human, 1h, 37°C) [%]	80	65	100
Microsomal stability (human, 1h, 37°C) [%]	56	44	86
T1/2 in s.c. rat PK at 5 mg/kg [h]	1	0.8	3.2

Technologies & Know-How

B-Cell Assays & Screening

JPT's experienced scientists will develop the optimal peptide screening strategy for your project. Assays are performed in an S2 laboratory environment using automated incubation stations assuring robust and reproducible performance.

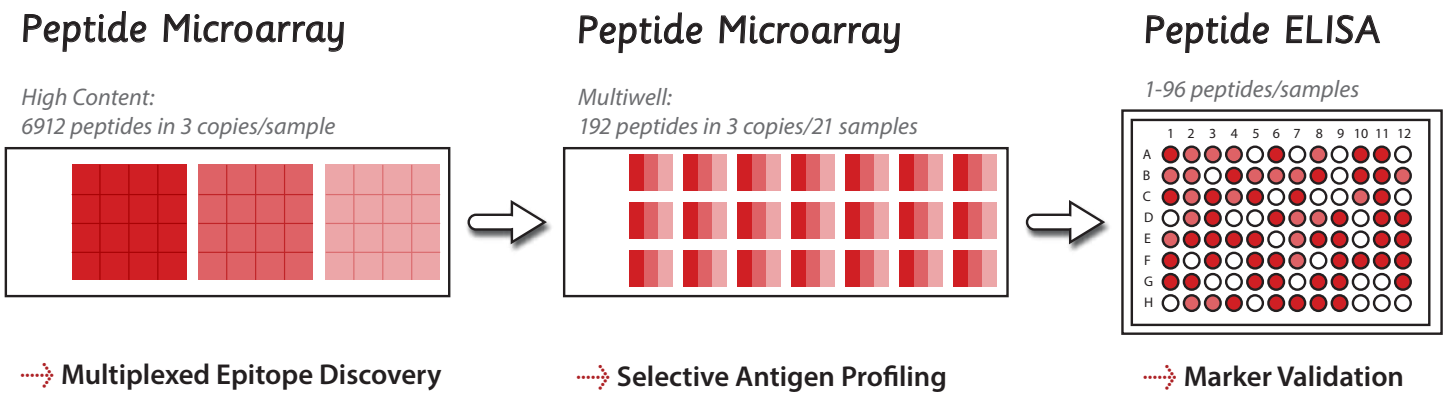
Applications

- Biomarker discovery
- Seroprofiling and antibody epitope mapping
- Development of immune diagnostic and patient stratification tools
- Clinical monitoring and companion diagnostics
- Elucidation of antigen and epitope spreading
- Detection of immunodominant regions in antigens and of novel vaccine targets
- Identification of substrates for orphan enzymes and enzymatic assays
- Characterization of protein-protein interactions
- Investigation of posttranslational modifications and epigenetics

Benefits

- Multiplexed identification and quantification of epitopes for multiple antigens or entire proteomes
- Addressing of natural sequence diversity and posttranslational modifications
- Cost efficient and modular workflow allows efficient and tailored planning of projects (see figure below)
- Minimal consumption of patient material and proteins
- Low background combined with high sensitivity
- Robust assay protocols ensuring reproducible results

Technologies defining JPT's biomarker discovery platform. High Content and Multiwell Microarrays are two manifestations of our unique PepStar™ technology. These formats allow efficient screening of a high number of peptides and samples, respectively. The robust and straightforward Peptide ELISA is applied, as an alternative technology, for the validation and development of diagnostic tests.



T-Cell Assays

JPT is offering a complete T-cell assay portfolio ranging from the early project design to its final execution, including target protein/subunit selection, sequence analysis, bioinformatics, peptide library design and sample processing by Elispot, Flow-cytometry, and other relevant technologies.

Applications

- T-cell vaccine target identification
- Deep T-cell epitope discovery
- Neo-epitope qualification
- Prioritization of neo-epitopes
- T-cell epitope mapping
- Antigen discovery

Benefits

- Reliable and robust workflows
- Design, assays and data evaluation in one hand
- Stringent quality control
- Reduced financial risk
- ISO 9001:2015 certified quality management system

We Provide

- Target protein/subunit selection
- Intelligent sequence analysis/bioinformatics
- Peptide library design including the coverage of known sequence variations
- Peptide synthesis, peptide pooling, matrix pool design, and plating
- Sample processing by Elispot, Flow-cytometry, and other relevant technologies

Bioinformatic Support

See pages 12 and 13 for details on how JPT's bioinformatics capabilities support peptide design and data evaluation.

Chemistry & Technologies

Clinical Peptides & Pools

- Clinical grade peptides
- Cell and immunotherapy
- Vaccine development
- Clinical immune monitoring
- Full analytical documentation
- Stringent quality control

Peptide Synthesis

- Specialty peptides and peptide modifications
- Wide range of analyses
- Substantial, long-standing expertise
- Even complex or unusual peptide sequences
- Stringent quality control

High-Throughput SPOT™ Synthesis

- Screening projects
- Prioritization of neo-epitopes
- Systematic optimization of peptidic lead structures
- Assembly of peptide pools for immune monitoring
- Rapid turnaround at unmatched pricing

High Content PepStar™ Microarrays

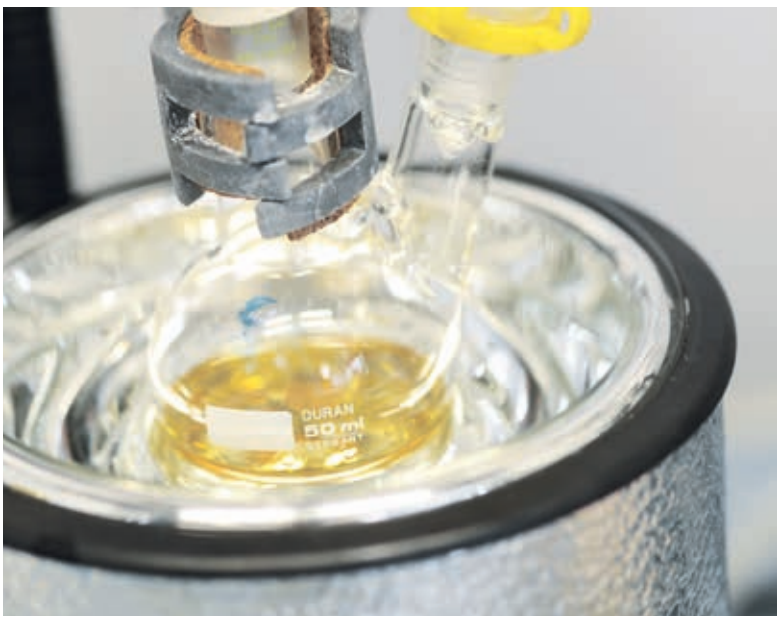
- Antibody epitope mapping and seromarker discovery
- Immune monitoring
- Peptide optimization
- Screening of tens of thousands of peptides in parallel
- Only smallest sample volumes needed
- Low background combined with high sensitivity

Proteotypic SpikeTides™ Peptides

- Isotope labeled and/or absolutely quantified
- Optimization and validation of SRM/MRM assays
- Relative and absolute quantification of proteins
- Unmatched turnaround times
- Quantification less expensive than AAA

Medicinal Chemistry

- Experienced project team
- Long standing record in peptide and small molecule chemistry
- High capacity automated
- Solid and solution phase capabilities
- Extensive building block library



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.

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