*Bio*Peptides

A synthetic biology alternative to combinatorial chemistry and new tool in research, diagnostics and therapy

The development of analytical tools for detecting bacterial and viral agents as well as the matching vaccines requires a profound knowledge of the pathogen itself and of the immune processes in the host. Deciphering the genomes of numerous microorganisms and viruses as well as parallel intensive research into the mammalian immune system significantly contributed to understanding the complex, diverse immune processes in the healthy and sick organism. These developments of the Omics era have opened up new avenues in vaccine research. Synthetic and systems biology contribute their share with gene synthesis, pathway engineering, metabolic modeling, etc. ATG:biosynthetics's novel *Bio*Peptide technology is an alternative to chemical peptide synthesis with a wide range of potential applications in immunobiological research.

Immunomics and vaccine development

Immunomics as a field was born about 10 years ago [1, 2]. It set out to develop a complete picture of the mammalian immune system with all its effector, mediator and signaling molecules (peptides, receptors, co-receptors, cytokines, etc), the different participating cell types and of the physiological mechanisms involved (antibody biology, cytotoxic pathways, etc.). Information from fields such as systems biology, genomics, proteomics, etc. would then coalesce into one functionally coherent model.

Researchers expect this holistic understanding of immune processes to propel vaccine development [3]. While the 1st and 2nd generation vaccines were products of a relatively „untargeted" immunization with entire organisms or preparations of their cellular components, the systematic analysis of immune processes allows the development of a 3rd generation of vaccines that are, in parts rationally, designed on the bioinformatical drawing board [3].

Peptides

Peptides, among other things, act as signals that can either elicit or curb processes in the humoral and cellular immune response [4, 5]. Their importance has also been recognized in research and development [6]. To determine their biological function, one can create and test extensive libraries. Randomized peptide fragments have traditionally been chemically synthesized [7, 8] or integrated in phage libraries [9]. Yet, working with these huge libraries equals firing a scattershot as numerous biologically irrelevant amino acid combinations are generated [e.g. ref. 7].

PepID

In some cases it might be more reasonable to build focused peptide libraries using immunologically relevant proteins from genetically well characterized pathogens. ATG: biosynthetics offers a technology that allows researchers to produce peptides in biological systems (outlined in fig. 1). First, a bioinformatic algorithm dissects the targeted protein sequences *in silico* into overlapping or non-overlapping peptide fragments (as desired) of defined but flexibly selectable length. The peptide fragments are codon-optimized at the DNA level and subsequently „cast" into DNA in groups of 8 to 11 peptides using gene synthesis.

The peptides are hosted in a compact library on a plasmid (source vector). This recombinant construct can be propagated in *E. coli*. A simple double restriction digest releases the peptide-coding sequences that are then cloned into a suitable expression vector, e.g. to fuse the peptides to a protein carrier etc. These carriers may be cleavable tags for affinity purification or reporters. The expression library is transformed into *E. coli* to yield individual clones for each peptide. Upon verification of correct identity, these clones can be stored as permanent stocks for future use.

Advantages

One advantage of this technology jumps out: the peptides can be regenerated from the source vector and/or the permanent stocks at any time and in almost unlimited quantity. As the number of peptides and their individual length increases, the process will become cheaper compared to chemical synthesis. The technology is suited to convert coding sequences from entire genomes into peptide

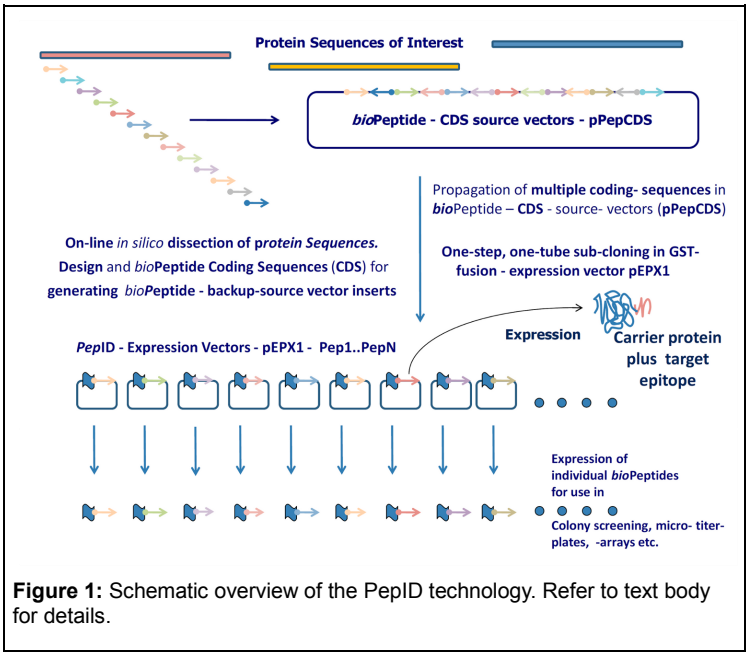

Figure 1: Schematic overview of the PepID technology. Refer to text body for details.

libraries. Various display technologies (phage, *E. coli*, yeast display etc.) can be used for analysis but microtiter plate assays or array systems can be established as well. Such high-throughput methods are already known for randomized libraries [10] and should thus be easily realized for biopeptides as well.

The PepID system was originally developed to screen patient sera for viral load using phage-displayed randomized peptide fragments from the human papilloma virus. A first diagnostic application is currently under development in Dr. Sebastian Ulbert's group in Leipzig who investigate immune reactions to infection with the West Nile virus [11]. He sees the advantage of peptide libraries that are rationally designed from the complete proteome of viral pathogens consists in enabling quick pre-screens of suitable epitope candidates. As the epitopes can span longer amino acids stretches, this allows to analyze not only linear B cell epitopes but also simple structural epitopes encompassing anywhere from 20 to 30 amino acids. Moreover, the system facilitates the identification of motifs that allow to exactly differentiate between infections with closely related pathogens. This cannot be achieved sufficiently with currently existing methods. Further research should enable development of rapid test kits for the differential diagnosis of patient sera.

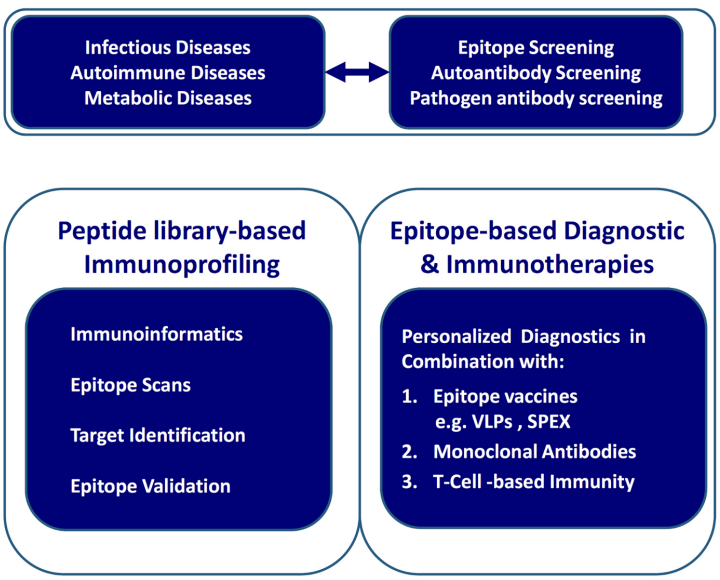

Figure 2: (Potential) applications of the biopeptide technology in biomedical areas. These overlap widely with those for chemically synthesized peptides.

Bioinformatics and PepID

In silico analysis that precede and find their way into the actual peptide design play an important role in rational approaches. ATG:biosynthetics closely works together with the bioinformatician Dr. Josef Maier (IStLS, Oberndorf, Germany) in this area. The range of immunologically relevant peptide sequences from a pathogen can be reduced to a reasonable number through metagenomics and analyzing different omics data sets (from the host as well as from the pathogen). The bioinformatic comparison of genomes from closely related pathogenic and non-pathogenics species and strains allows identification of virulence factors with enormous value in diagnostics and therapy. At the same time, cross-genome analyses help researchers avoid conserved potentially antigenic epitopes that are also present in useful intestinal bacteria (commensals) or harmless congenerers [3].

Algorithms for predicting B-cell (antibody production) and T-cell (cellular immune response) specific epitopes identify peptides that are suitable for incorporation into a peptide library. For B cell epitopes, structural aspects of the antigen such as highly accessible surface areas are crucial whereas for T-cell epitopes it is relevant how effectively they are displayed on the highly represented MHC types of a given host population. This will, among other things, determine the strength of the cellular immune response (e.g. against tuberculosis bacteria or viruses). Detailed analysis and integration of the data from metagenomics and immunogenomics studies allows to build optimized libraries with elements that are highly visible to the immune systems of individuals or specific populations.

Other applications

ATG:biosynthetics's biopeptide technology can also be utilized in other areas (see fig. 2), e.g. to probe patients' immune status. The peptides allow to identify characteristic antibody or immune cell reportoires. Such an immunologic fingerprint would be a valuable diagnostic tool for autoimmune diseases or infections as it would contribute to finding the optimum therapy for a given patient (personalized medicine).

Apart from validating new antibodies you can also employ the peptide libraries to build peptide or protein microarrays for research and diagnostics [4, 7, 12, 13, 14, 15], and in cancer therapy [5]. The method lends itself to investigating various protein-protein interactions [16]. Proteins or protein fragments can be trimmed until minimum interaction domains can be defined. These peptides that will vary in length could then be collated into one or a few peptide libraries and subsequently expressed.

Caveats

As any method, this one also has its limits. Modfied amino acids cannot (or only with technical difficulty) be incorporated into the peptides. In such cases, non-ribosomal peptide synthesis in microorganisms could prove an alternative [17]. ATG:biosynthetics is currently collaborating with other researchers to develop such a technology and offers multi-gene expression vectors for such projects, as well as appropriate procedures to assemble such pathways. Biopeptides are no alternative when it comes to mimotopes as these cannot yet be rationally defined.

Peptide production in microorganisms requires separating the peptides from potentially inflammatory endotoxins during purification. While this may seem a disadvantage compared to chemical peptides, this is not a serious technical challange with today's separation technologies and is a routine process in plasmid purification. As is the case with chemically synthesized peptides, biopeptides may be produced with different efficiencies. Yet, the bioinformatics approaches decribed above can assist in e.g. excluding potentially aggregating peptides. This seems legitimate as

they will generally play a subordinate role in immune processes. What takes up a significant amount of time though is experimentally validating the clones, even with robust robotics significantly speeding up analysis. If one wishes to apply similarly rigorous standards as for chemical peptides, experimental verification of peptide identity via mass spectrometry is recommended. This will have to be performed only once as one can assume that the blueprint for the biopeptides that are contained on plasmids remains stable.

Sustainable production

One aspect that should favor biopeptides over their chemically synthesized counterparts in the future is their cleaner ecological fingerprint (if one is looking for more sustainable production, ref. 18). Chemical peptides synthesis consumes enormous amounts of raw materials and, more importantly, water [19]. Substances such as acetonitril, which are profusely used in chemical synthesis, are toxic. While biopeptides also require chemical synthesis of the peptide-coding sequences, the amounts are in the microgram range as microorganisms will take care of the rest of the production process. Scale-up of the entire product process requires larger fermenters and improved process technology.

Conclusion

Rationally designed biopeptides are a legitimate alternative to chemically synthesized peptides. In many areas of research and development they will also complement them. The next few years will certainly tell whether research and development embrace this new technology.

References:

[1] Klysik J.Concept of immunomics: a new frontier in the battle for gene function? *Acta Biotheor.* 2001;49(3):191-202.

[2] Maecker B, von Bergwelt-Baildon, Anderson KS, Vonderheide RH, Schultze JL. Linking genomics to immunotherapy by reverse immunology -'immunomics' in the new millennium. *Curr Mol Med*. 2001 Nov;1(5):609-19.

[3] Bagnoli F et al. (2011). Designing the Next Generation of Vaccines for Global Public Health. *OMICS: A Journal of Integrative Biology* 15: 545-566.

[4] Smith DJ et al. (1997). Immunogenicity and protective immunity induced by synthetic peptides associated with a catalytic subdomain of mutans group streptococcal glucosyltransferase. *Infection and Immunity* 11: 4424-4430

[5] Kenter GG et al. (2008). Cancer Therapy: Clinical: Phase I Immunotherapeutic Trial with Long Peptides Spanning the E6 and E7 Sequences of High-Risk Human Papillomavirus 16 in End-Stage Cervical Cancer Patients Shows Low Toxicity and Robust Immunogenicity *Clin Cancer Res* 14:169-177; doi:10.1158/1078-0432.CCR-07-1881

[6] Beck A et al. (2007). Peptides as tools and drugs for immunotherapies. *Journal of Peptide Science* 13: 588–602, DOI: 10.1002/psc.852

[7] Grainger D (2004). Immunomics: Principles and Practice. *IRTL Reviews* 2: 1-6.

[8] Shin D-S et al. (2005). Combinatorial Solid Phase Peptide Synthesis and Bioassays *Journal of Biochemistry and Molecular Biology* 38:. 517-525

[9] Ellis SE et al. (2011). Phage display library biopanning as a novel approach to identifying nematode vaccine antigens. *Parasite Immunol.* 2011 Jul 19. doi: 10.1111/j.1365-3024.2011.01317.x. [Epub ahead of print]

[10] Glökler J, Schütze T and Konthur Z (2010). Automation in the High-throughput Selection of Random Combinatorial Libraries-Different Approaches for Select Applications. *Molecules* 15: 2478-2490; doi: 10.3390/molecules15042478

[11] EU-Verbundprojekt »WINGS: West Nile Integrated Shield Project: Epidemiology, Diagnosis and Prevention of West Nile Virus in Europe«. http://west-nile-shield-project.eu/

[12] Scala G et al. (1999). Selection of HIV-Specific Immunogenic Epitopes by Screening Random Peptide Libraries with HIV-1-Positive Sera J Immunol 1999 162:6155-6161

[13] de Oliveira EJ et al. (2008). Synthetic peptides as an antigenic base in an ELISA for laboratory diagnosis of schistosomiasis mansoni. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 102: 360-366

[14] Margarit I et al. (2009) Capturing host-pathogen interactions by protein microarrays: identification of novel streptococcal proteins binding to human fibronectin, fibrinogen, and C4BP. *FASEB J.* 23: 3100–3112

[15] Van Regenmortel MHV (2009). Synthetic Peptide Vaccines and the Search for Neutralization B Cell Epitopes. *The Open Vaccine Journal* 2: 33-44 33

[16] Boxem M et al. (2008). A Protein Domain-Based Interactome Network for C.elegans Early Embryogenesis. *Cell* 134: 534-545.

[17] Stack D, Neville C and Doyle S (2007). Nonribosomal peptide synthesis in Aspergillus fumigatus and other fungi. *Microbiology* 153: 1297-1306.

[18] Meyer H-P (2011). Sustainability and Biotechnology. *Organic Process Research & Development* 15: 180–188

[19] Meyer H-P, Ott L, Quittmann W (2009) "Will synthetic biology lead to radical changes in the manufacturing industry?" Vortrag auf der Biofine 2009, Applied Industrial Synthetic Biology in Europe – Status Quo and Perspectives. April 16 to 17, 2009 - Freiburg, Germany

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