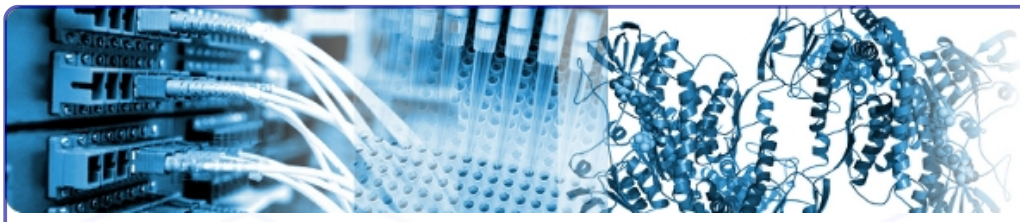

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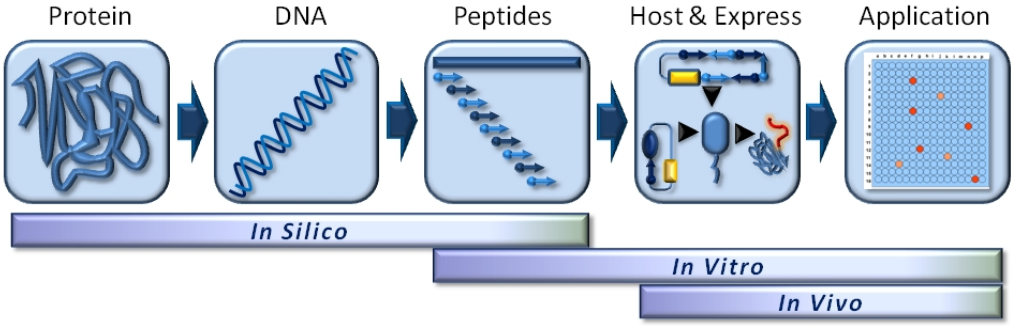
# PepID

**The bio-peptide alternative to chemically synthesized peptides**

## PepID: multi-peptide library construction and expression

PepID is the bio-peptide alternative to chemically synthesized peptides. The libraries are designed in silico, generated in vitro and maintained and expressed in vivo. As the peptides in the library are derived from organismal sources and thus closer to the “real thing“ than random peptides.




Design, build and express pathogen-specific focused peptide libraries from the target protein you study for diagnostic or therapeutic applications

- rational
- flexible
- cost-efficient
- easily regenerated and sustainable

Develop diagnostic tools for viruses and bacteria, test epitope binding of antibodies, study protein-protein interactions and more...

[Learn more about the technology and its applications. ]

Influenza viruses, especially once they go epidemic, cause public health scares almost every year. The same holds true for other (rarer) viruses with significant health and economical impact, e.g. West Nile, Japanese encephalitis, etc.

Thus, research tools that will result in diagnostic procedures and potentially also therapies, are in great demand. **Peptide libraries** have been successfully used in biomedical research for decades, mostly in the field of immunology. Epitope mapping serves to identify (continuous or discontinuous) protein-derived epitopes that mediate or initiate biochemical or phyiological processes, e.g. binding of high-affinity antibodies or stimulation/attenuation of an immune response. Peptide epitopes can also be biological signatures for certain pathogens. Identification of such epitopes is the basis for diagnostic and, ideally, therapeutic applications, e.g. protective epitope-based vaccines.

Finding suitable epitopes becomes harder for closely related pathogens that vary only slightly in their protein sequences. Epitopes undergoing rapid mutation (a prerequisite for immune evasion) and thus leading to variant new strains also make development of vaccines with a broad effeciency spectrum

difficult. In this case, a method for a) discriminating between viruses/strains, and b) detecting conserved, yet still immunogenic or diagnostically relevant epitopes that cover different strains, would benefit research efforts.
Generally, one can either use huge numbers of peptides with random sequences which require little or no background information on the biology of the virus or pathogen and hope to get a lucky punch.

Alternatively, one can use a more **rational approach** that also incorporates knowledge about the system one is studying.
**PepID** is such a rational-design system for generating and expressing a multitude of potentially biologically relevant peptides.

While random approaches jumble strings of DNA whether they are biologically relevant or not, PepID follows a different path.
As scores of microorganismal and viral genomes have become available in public databases, a **rational design approach** is used to partition any protein or protein-coding DNA from a given organisms or virus into fragments of freely definable but uniform length (see fig. 1).

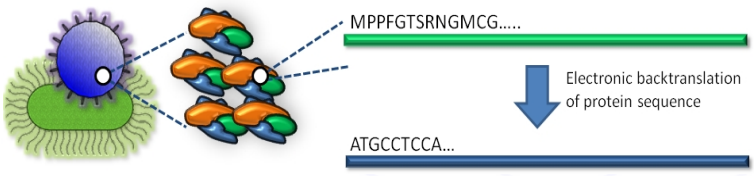



**Figure 1:** Converting a target protein into DNA.

Potentially diagnostically or therapeutically interesting proteins from pathogens (bacteria, viruses, etc.) are either electronically backtranslated into DNA or their protein-coding DNA sequence extracted from sequence databases and directly used for constructing the peptide-CDS library.

Target protein sequences are entered into the PepID submission form and are then electronically backtranslated into DNA sequences. These are then further dissected into peptides of defined length and overlap (see fig. 2).

PepID lets you **adjust the length and overlap of the peptides** you wish to scan thus allowing you to **change the coverage of the protein** as required. In addition, if you have extensive knowledge of your protein, you can simply leave out certain biologically irrelevant amino acid stretches and just focus on the important structures and motifs which you can zoom in on and cover in more detail by e.g. increasing the overlap.

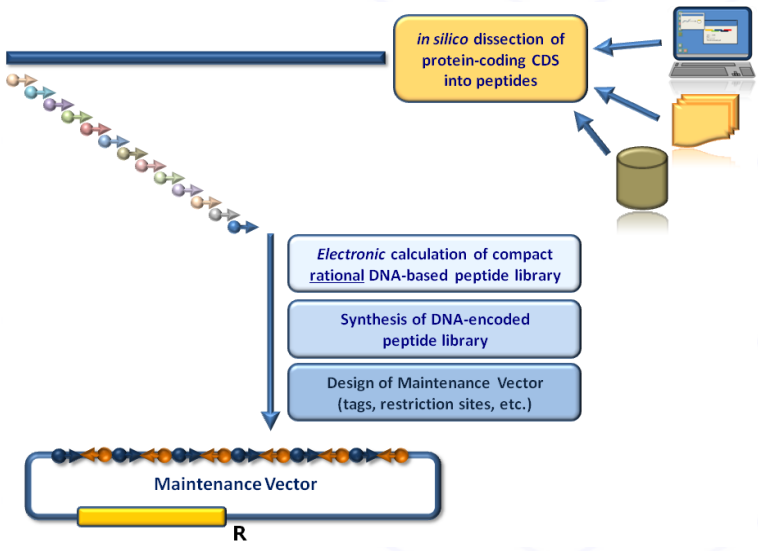



**Figure 2:** Integrating database-derived data and biocomputational modeling into the design of PepID biopeptide libraries.

Peptide sequences are codon-optimized to avoid repeats and to adhere to codon usage in the host organism (usually *E.coli*). In addition, the individual peptides are arranged for a compact design without excess linkers etc.

R: resistance marker

This sets it apart from chemically synthesized random libraries with peptide sequences that are permutated.

In addition, PepID separates hosting and expression into 2 subsystems. It is a ***bio-peptide* system** because the peptides are produced in a biological system, usually a bacterial host organism (but it may be any other microorganism as well), from a plasmid DNA construct.

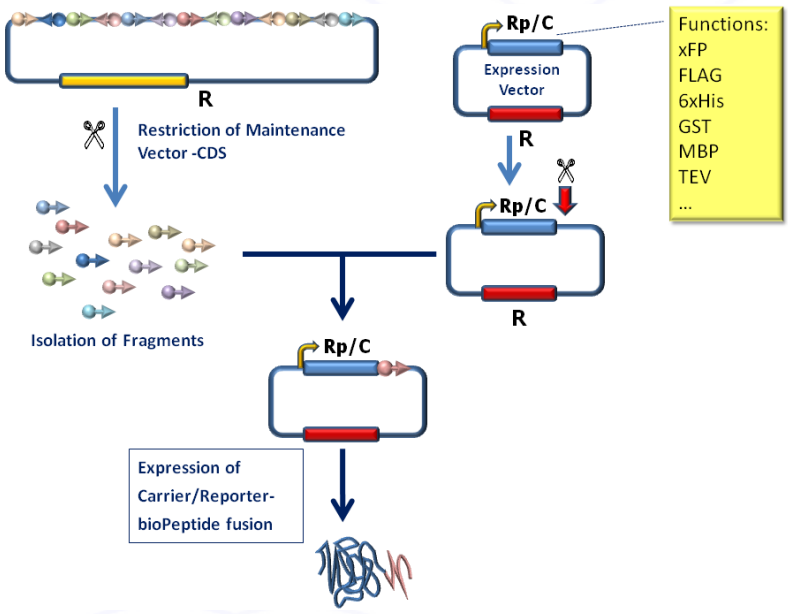



**Figure 3:** Individual peptides are released from their maintenance (host) plasmids by a simple restriction and then cloned into the specifically designed expression vector. This expression vector can carry any custom-tailored functional elements relevant to the research agenda. This is a bulk process and individual clones need to be sequence-verified.

The expression constructs are then hosted in the individual bacterial clones and can be regenerated and retrieved from them.

C: Carrier, R: resistance marker, Rp: reporter (and other) functions.

To make sure you don't miss any potentially interesting antigenic stretches of amino acids, e.g. in known hot spots or variable regions, you can adjust the overlap to allow a higher scanning resolution. The individual peptides are liberated by a simple restriction digest and then cloned into the expression vector. This vector can be designed with functions that meet any individual requirements (see fig. 2). The peptides will be expressed as protein-peptide fusions as peptides usually act as antigens in a protein or functional context (MHC, adjuvants, etc).

These fusion proteins can then be used in various formats (in vitro, phage display, etc.) to probe the peptides with antibodies, immune cells, etc. and assay for any relevant output. Highly reactive/stimulatory peptide epitopes can thus be identified and matched against bioinformatically predicted epitopes. In addition, combinations of functionally diverse epitopes can also be tested.

The technology may also be used to probe differential reactions of patient sera (e.g. infected vs. naive) against certain peptides. This can help identify peptides that can then be used as markers of specific infection, e.g. in a diagnostic tests.

**Advantages of using PepID:**

- more efficient for longer peptides (30 aa and up)
- easily replenished from maintenance / source vectors
- biological system, better ecological fingerprint
- initial rapid screening as prerequisite for chemically synthesized variants, e.g. mimotopes

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