

PepSlide® Designer 1.3

User's Quick Start Guide

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PepSlide® Designer is only intended for research and not intended or approved for diagnosis of disease in humans or animals.

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PepSlide® Designer (PSD) facilitates flexible design of peptide arrays and peptide microarrays. It supports interactive generation of peptide libraries and controls. The graphical array editor enables visual layout, edit, and reuse of array designs. Completed array designs can then be saved to array files used by the peptide array synthesizer and analysis software.

Please note that you can customize PSD to chemical compound libraries simply by replacing supported amino acids with chemical compounds.

1. Introduction

Installation

PSD works on Windows XP, Windows 7, and Windows 8. The software requires Microsoft .NET Framework 3.5 or a later version. Usually this framework has already been installed on a Windows computer. Otherwise, it can be freely downloaded from a Microsoft website, e.g. [here](#).

Installation of PSD requires administrator's rights. Simply run the PSD installer. If the current Windows account is not an administrator, the installer will prompt you to input an administrator's account and password.

Product Activation

After installing PSD on Windows, you need to *activate* the software with a *trial serial number* obtained from the software provider or its distributors. This enables the use of PSD with full functionality for *30 days* at no cost.

When the free trial time has expired, you can continue using PSD by purchasing a *perpetual license* or a *term license* from the software provider or its distributors. Upon the purchase, you receive a *serial number* and use it to *activate* the license.

You can refer to the [product activation steps](#) on the product website.

Software Concepts

In the remaining of this manual, the term *array* is used to mean both *array* and *microarray*, unless otherwise stated.

Figure 1 outlines the diagram of a typical peptide array designed and analyzed by PepSlide®.

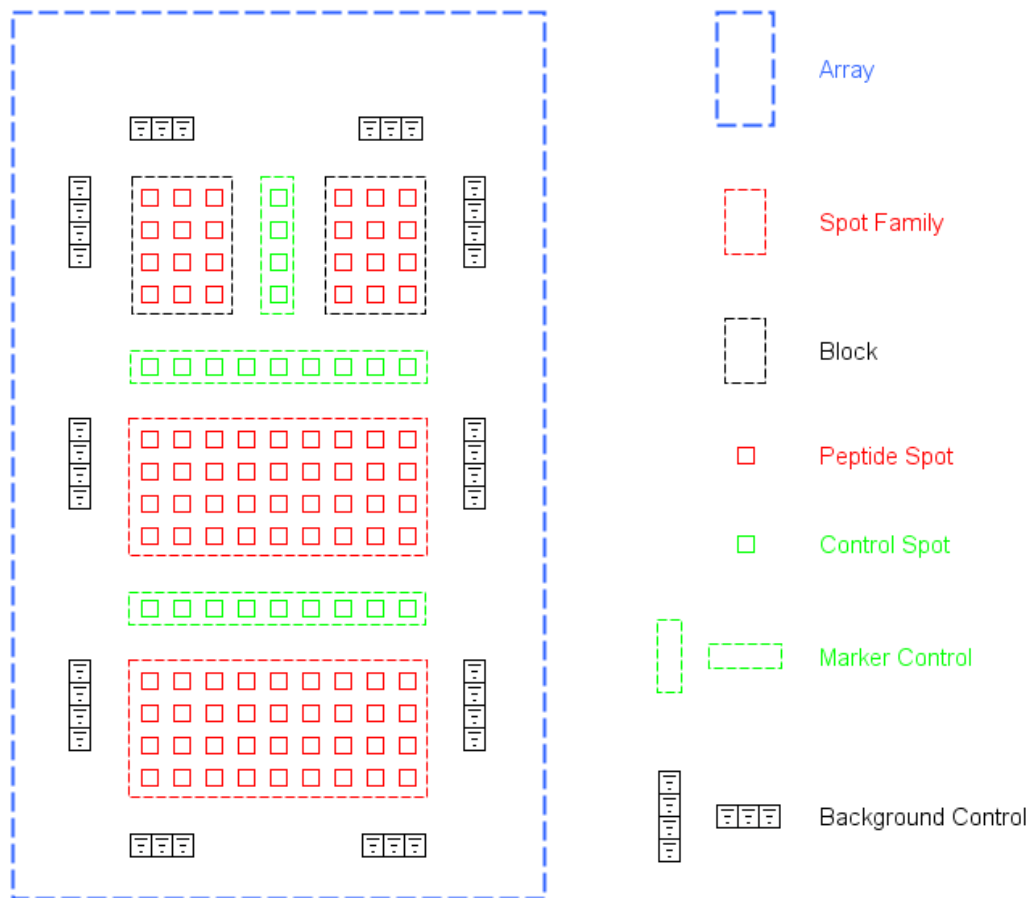


Figure 1: Peptide Array Diagram.

A *spot* represents a single peptide spot located in one cell on the array. It can also be a control spot made of e.g. a known epitope. In the diagram and in the graphical array editor of PSD, a spot is depicted as a square. However, the actual spots on glass slides may have rectangular or circular shape depending on the peptide array synthesizer.

A *spot family (SF)* consists of spots generated from the same source as well as the source information. Take an overlapping SF generated from a protein for example. It contains not only the overlapping peptides translated from the protein but also the protein itself, the overlapping shift step, and the peptide length. An SF can be a *peptide library* like the above example or a *control*.

A *marker control* is a group of control spots arranged on either the same row or the same column of the array. You can use marker controls for orientation or positioning. We employ *background controls* during the data quantification for estimating the degree of non-specific binding. These are *virtual spots* and not synthesized on the actual peptide array.

Software User Interfaces

You find the menu at the top of the software. Next to the menu is the toolbar. The left panel of PSD is divided into two sections. The upper one is *Array Structure*. It contains a hierarchical tree of design objects in the current array. The lower section is *Design Information*, displaying the properties of the selected spot or spot family.

The main panel of PSD is the graphical array editor. Section 3 describes the editor in detail.

Array Design Files

An array design of a peptide array is stored in a PepSlide® Designer file (*.psf). You can use PSD to view and edit an array design file. The PSF format is fully supported by PepSlide® Analyzer for peptide array analysis. In addition, PSD can save an array design to a GAL file (*.gal) for using with other analysis software.

You can open and edit up to five array designs at a time. PSD supports copying design objects within an array design and between designs, or copying the whole array, enabling convenient reuse of data.

2. Configuration

Supported Amino Acids

The list of supported amino acids can be viewed and edited with the *Configuration > Supported Amino Acids* menu. PSD automatically uses these amino acids during generation of peptide libraries. By default, this list consists of 20 standard amino acids. You can save, import, or export this list. This enables the software to work with multiple configurations of the peptide array synthesizer.

In the case of chemical compound libraries, instead of amino acids you simply define the list of supported chemical compounds.

Supported Peptide Length

In an array design, peptides from different peptide libraries may have different lengths. The minimum and maximum length of a peptide supported by the synthesizer can be defined with the *Configuration > Supported Peptide Length* menu.

Array Templates

PSD employs the concept of *array template* to define common properties of a peptide array. These include the array's size, the spot's morphology, and distances between spots. Array templates can be managed with the *Configuration > Array Templates* menu.

PSD supports two types of spot's morphology:

- Spot-based: Circular spots generated by the SPOT technology.
- Pixel-based: Rectangular spots generated by the laser printer technology.

You can define an array template for each configuration of the peptide array synthesizer. When create a new array design, simply choose the suitable array template to reuse the predefined properties. Default templates including *Spot-Based on Glass Slide*, *Pixel-Based on Glass Slide*, and *Spot-Based on Membrane*. You can modify these templates or create new ones based on them to suite your synthesizer's configuration.

Configuration for Marker Controls

Marker controls serve for orientation and positioning of the array during analysis. A marker control may be a group of the same control spots. In addition, you can create controls by combining two control spots according to some pattern.

Use the dialog activated by the *Configuration > Control Sequences* menu to edit the list of control spots. The combination patterns between two control spots can be defined with the *Configuration > Control Combinations* menu.

3. Peptide Array Design

Managing Array Designs

To create a new array design, choose the *Array > New* menu. Use the *Array > Save* menu to store the current array design to a PepSlide® Designer file (*.psf). In addition, you can use the *Array > Save As* menu to store an array design to another PSF file or a GAL file.

Use the *Array > Properties* menu to view the array template that the current array design is based on. You can print the layout of an array design using the *Array > Print Preview* menu.

Design Objects

Design objects in PSD are spot families, i.e. either peptide libraries or controls. During creation of a design object, you need to give it a unique name, the size in terms of the number of spots, and the start position. PSD will place the top-left spot of the object at this start position.

Graphical Array Editor

PSD provides two interfaces to edit a peptide array design. The *Array View* is a graphical array editor. Here you can create peptide libraries and controls. The editor facilitates visual layout of design objects with drag-and-drops and data replication with copy-and-pastes.

For spots on the array, the *Sequence View* provides access to their amino acids, as a whole and separately at each layer. You can regard it as a view to the third dimension of the array. This view is for reading only; you cannot modify the data here.

Selecting a Design Object

To select a design object, activate the *Spot Family* tab in the *Design Information* section and click on the object in the array editor. You then view its information – name, size, and position - in the tab.

You can view the information about a spot - the amino acid sequence and its position – by activating the *Spot* tab in the *Design Information* section and then clicking on the spot in the array editor.

Modification tasks on a design object include creating, moving, copying, and deleting. Please note only design objects are modifiable. Therefore, please activate *Spot Family* tab before performing modification tasks. A spot is part of a design object and is thus not modifiable.

Viewing the Peptide Library Design

If the *selected* design object is a peptide library, you can use the context menu to access further information. Choose the *View Details* menu to switch to the *Sequence View*. This displays the amino acids in different layers of the peptide library's spots.

You can export the peptide library to a *CSV (character-separated values)* file using the *Export to CSV File* menu. You can also review the peptide library design with the *Properties* menu. The next sections will provide further information related to these functions.

Moving a Design Object

Reposition of a design object on the array editor is convenient with drag-and-drop. Simply select the object and then drag it to the new position.

Replicating a Design Object

You can replicate a design object quickly and conveniently by copy-and-paste:

- Select the object and press Ctrl-C to copy it to clipboard.
- Press Ctrl-V and specify the position of the replicated object.

Replicating a design object is possible within an array design or between array designs. In addition to the short cuts, you can use the context menu as follows. First, select the desired object, right-click and choose *Copy*. Then select the array design in which you want to put the copied object, right-click on any point and choose *Paste*.

Deleting a Design Object

Select the design object and press the *Delete* key. Alternatively, right-click on the object and choose *Delete* from the context menu.

Replicating an Array Design

You can conveniently replicate an array design with just a few clicks.

- Select the source array design by clicking on the tab holding its name. Right-click and choose *Copy* from the context menu.
- Select the destination array design. Right-click and choose *Paste*. Specify the start position. PSD will copy all design objects from the source array into the destination array, preserving their layout.

Please note that the destination array may be empty or already contains some design objects. In the latter case, PSD issues a warning if there is not enough space available.

Alternatively, you can select the source array and choose the *Array > Copy* menu. Then select the destination array and choose the *Array > Paste* menu.

4. Control Generation

Marker Control

You can create marker controls with the *Controls > Marker* menu or the corresponding button on the toolbar. You will need to specify how to arrange the control spots, either horizontally or vertically, and the number of control spots.

The next parameters are the two control spots X and Y, and the combination pattern. Take an example with X="0000GGGDYKDDDDK" and Y="00000YPYDVPDYAG". If the combination pattern is XXY, the generated control will consist of the following spots:

spot1 spot1 spot2 spot1 spot1 spot2...,

where spot1 = "0000GGGDYKDDDDK" and spot2 = "00000YPYDVPDYAG".

If you want the control consisting of X spots only, use a combination pattern with one character like X. Please note that a marker control is a design object, thus you can move, delete, and replicate it.

Background Control

During the data quantification, we use background controls as a way to estimate the degree of non-specific binding. The peptide array synthesizer does not synthesize background controls on the actual peptide array. Therefore, we suggest that you skip using background controls in PSD until you start quantifying and analyzing the peptide array data.

5. Peptide Library Design

PSD supports design and generation of the following peptide libraries:

- *Overlapping Library*: Overlapping peptides translated from a protein.
- *Random Library*: Peptides generated stochastically.
- *Substitution (also called permutation or positional scanning) Library*: Variants of a known peptide created by replacing its residues with some amino acids.

In addition, you can import a collection of peptides into an array design with the *Import Library*.

Common Parameters of a Peptide Library Design

PSD handles a peptide library by means of the design object called spot family (SF). When creating a peptide library, PSD arranges its spots in the form of a rectangle. You need to set the *Number of Columns*; it is the SF's number of spots in the horizontal direction. Based on this value, PSD automatically calculates the smallest number of rows that suffices the number of generated spots. Suppose that N is the number of columns. PSD continuously fills the SF's peptides from column 1 to column N in row 1, then again from column 1 to column N in row 2, and so on.

The other common parameter is the *Replication Mode*. PSD supports replication of peptide spots during generation of a peptide library. The default mode is *Double-Spot*; PSD replicates each peptide by assigning it to two spots at the same column in two adjacent rows. The other modes are *Single-Spot* (no replication) and *Triple-Spot*, which replicates a peptide three times.

Overlapping Peptide Library

This peptide library consists of overlapping peptides translated from a protein. Given a peptide length and an overlapping shift step, the protein is broken into peptides of equal length.

The tool is launched from the *Spot Family > Overlapping* menu or by clicking the corresponding button on the toolbar. In addition to common parameters, this peptide library design requires the protein, the overlapping shift step, and the peptide length.

Substitution Peptide Library

This peptide library generates variants of a peptide by substituting residues at specified positions with certain amino acids. Someone may call this tool *Permutation* or *Positional Scanning*. In its simplest form - replacing residues with only Alanine - this library is similar to an *Alanine Scanning*.

PSD supports two modes of generating variants of a peptide, the so-called *parent peptide*:

- *Full Substitution*: Suppose that you configure PSD with 20 amino acids. For each residue of the peptide, the tool generates 20 peptides by replacing the residue with 20 amino acids.
- *Partial Substitution*: You can specify what residue will participate in the substitution and for each residue, an individual list of amino acids for replacing.

You can start this peptide library design with the *Spot Family > Substitution* menu and the toolbar.

Input the parent peptide and choose whether the substitution starts at the C-terminus or the N-terminus. Since the largest possible size of the spot family, which is equal to the number of columns multiplied by the number of rows, is usually larger than the number of generated peptides, you can choose to fill the remaining available spots with the parent peptide or leave them empty.

In the partial mode, you also need to select residues that participate in the substitution and for each, the list of amino acids for creating the variants.

Random Peptide Library

This peptide library generates peptides randomly. The library design tool can be launched with the *Spot Family > Random* menu and the toolbar.

The peptide library design requires the number of peptide spots and the peptide length. In addition, you need to define the list of amino acids constituting the peptides and for each amino acid, its occurring probability in the generated peptides.

Import Library

You can import a collection of peptides into an array design by means of the *Import* library. It can be launched with the *Spot Family > Import* menu and the toolbar.

The peptide collection is stored in a *CSV* file having the following format. The first line of the file is only for annotation. Starting from the second line, in each line you store a peptide and its description in three fields separated by a *comma*. These contain the identifier, the description, and the peptide itself. You can find an example in Figure 2. Here, the collection of three peptides MNFNTQQNKTP, NFNTQQNKTPF, and FNTQQNKTPFS will be imported into the array design.

Peptide Identifier, Description, Peptide
1, first peptide, MNFNTQQNKTP
2, second peptide, NFNTQQNKTPF
3, third peptide, FNTQQNKTPFS

Figure 2: An Example CSV File

To start importing, select the *CSV* file. Define the peptide length that functions as the maximum length of peptides in this library. Suppose that the value is 12. If a peptide's length in the collection is larger than 12, that peptide will be "broken" into overlapping peptides having 12 amino acids.

6. Working with Peptide Array Synthesizers and Analysis Software

Exporting to CSV File

You can export peptides in a spot family (SF), i.e. a peptide library, to a CSV file. PSD can arrange peptides in a list with one peptide per row, or keep them in same layout as in the array design.

To start exporting, select the SF, right-click and choose *Export to CSV*. Alternatively, you can use the *Export > To CSV File* menu.

Working with Analysis Software

If you use PepSlide® Analyzer software for analysis of peptide array data, then simply use the array files generated by PepSlide® Designer (*.psf).

Other microarray analysis software may require array files of GAL format. PSD can save an array design to a GAL file as follows.

- Select the Array > *Save As* menu.
- In the *File Type* box, choose *GAL File (*.gal)*.

Transferring Array Designs to the Peptide Array Synthesizer

PSD can export the data of the array design, including spots and their annotations, to file formats used by the peptide array synthesizer to synthesize the peptide arrays. This module needs customization to the peptide array synthesizer. Please [contact](#) SICASYS Software GmbH for further information of the customization.