# The Pep Genie: a Django Application for Swift Automation of Peptide Array Data Analysis

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## Summary

The Pep Genie is a Django based website application that simplifies and speeds up the data analysis of peptide arrays.

Peptide array screening is a method for analysing peptide binding against target proteins (Amartely et al., 2014; Katz et al., 2011). Peptides arrays are small peptide libraries synthesised as a grid of spots on a membrane. Binding of an overlaid target is determined by immunodetection, resulting in a grid of binding spot signals (Tibbo et al., 2022).

Peptide arrays are analysed by measuring spot intensity and slicing spot images (Figure 1). Done manually, this is a lengthy and painstaking process. The Pep Genie provides a grid drawing method for the quick measurement of peptide array binding spots and slicing of spot images, and prepares the data directly in PowerPoint presentation format, saving considerable of time and work. The Pep Genie is an open-source software that is freely available at the host website and the GitHub repository. Full tutorials and documentation can be found on the website.

http://pepgenie.pythonanywhere.com

https://github.com/thomas-wright-bioinformatics

**Figure 1:** Peptide array analysis provided by The Pep Genie. Peptide array spots are quantified and sliced into figures.

## Statement of need

Peptide array analysis involves three main challenges.

**1. Labour intensive densitometry measurement of tens to hundreds of binding spots**

24   A typical peptide array experiment involves the analysis of 20-800 peptide binding spots. There
25   is a lack of tools that allow for simple and rapid intensity measurement. A researcher would
26   normally rely on freely available image analysis software such as the open-source software
27   ImageJ/FIJI (Schindelin et al., 2012) or ImageStudio^TM Lite (Licor). Measurement would
28   entail drawing selection circles manually over each individual spot in the image. For large
29   arrays consisting of hundreds of spots, this would be tedious and time-consuming. One tool,
30   the full version of ImageStudio^TM (Licor), allows a researcher to draw a grid of spots without
31   having to individually draw each spot. This is a considerable improvement, but the data is
32   arranged in a difficult layout, and the software is available only by special request from Licor for
33   groups who own a Licor Instrument. This is a restrictive requirement. The Pep Genie provides
34   a grid drawing tool, allowing for spots to be quickly selected and analysed automatically.

### 2. Arrangement of binding spot slices into a presentable figure

36   The most time-consuming step in peptide array analysis is the preparation of the strips of spots
37   for the final figure. In peptide array data, figures always include binding images of the columns
38   of peptide spots (figure 2). This is analogous to the images of bands on a western blot figure.
39   Three images are usually included: the test array, the control array, and the UV/Vis control
40   array. These are positioned next to text labels that indicate their amino acid sequence. A bar
41   graph may be included showing the intensity measurements of the spots. In standard peptide
42   array experiments, there are usually three to ten of these figures, one for each peptide series of
43   interest. For example, these can be an alanine scan and N,C-terminal truncations. To make
44   these image slices, a researcher would have to identify the correct spots from an unlabelled
45   grid of binding spots obtained from the array imager and screenshot each series of spots into
46   a PowerPoint file. The strips must be of uniform thickness and perfectly aligned with each
47   other. To add further to the challenge, a series of peptide spots can span more than one
48   column on the peptide array. This means that a researcher must join together two screenshots
49   of the spots, meanwhile making sure to maintain an even image width and proper alignment.
50   Repeated 30 times, this makes for an arduous process. Quite often, these figures must be
51   generated before it can be seen if the experiment was a technical success. If the experiment
52   did not work, this work would have to be repeated. As part of the grid drawing tool of the
53   Pep Genie, not only are the spots quickly measured, but images of the spots are automatically
54   generated and joined together where needed. Equal sizing of height and width and positioning
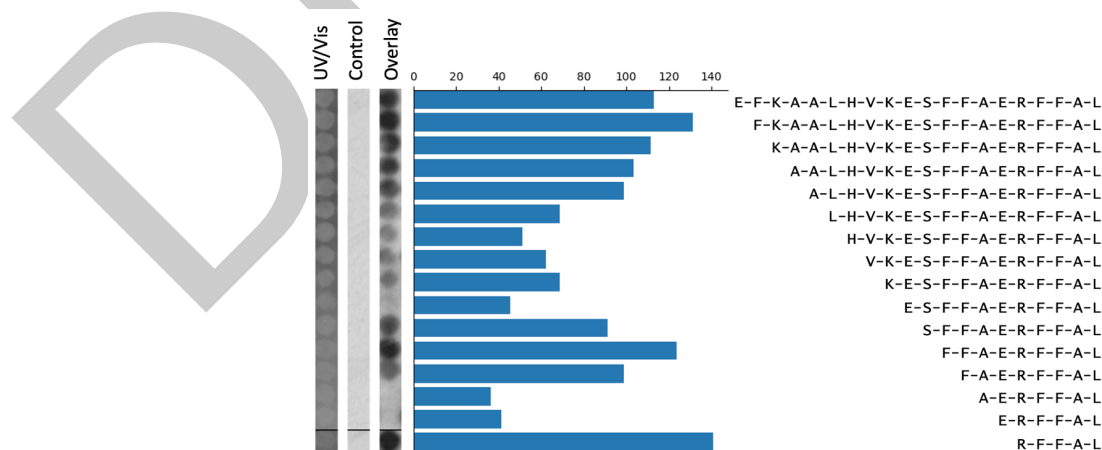55   of strips is all taken care of automatically, saving considerable time and effort.



**Figure 2:** An example peptide array figure. Spot strip images must be perfectly aligned with each other, and with the graph and text.

### 3. Control spots can be too faint to align into a figure

57   In peptide array experiments, a blank image for the control array is desired, as this indicates a

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58 lack of non-specific binding. However, this makes it very difficult to make spot strip images
59 and align them with the test and UV/Vis control images. If using the screenshotting method
60 to capture the rows of spots or drawing circles to measure spots, it would be very hard to find
61 them from a near-blank screen. As part of the grid drawing step in the Pep Genie, the grid for
62 outlining the control array is automatically sized according to the previously aligned test array.
63 With the width and height of the grid preset for the user, it is much easier to align the grid to
64 the control spots.

## Acknowledgements

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