Introduction:

The MultiPep 2 instrument is a pipetting robot that can synthesize peptides of up to 25 amino acids. The work area of the MultiPep 2 has exchangeable modules specific to either resin or membrane-based synthesis, amino acid derivatives, reagents and solvent positions for peptide synthesis. It's fully automated in-solution solid phase synthesis operation with an integrated heater and shaker improves the coupling efficiency of longer, more difficult sequences.

Applications of MultiPep 2:

SPOT Membrane Synthesis:

The parallel synthesis of up to 2400 peptides on four cellulose membranes (600 peptides/membrane) for the generation of peptide arrays.

CelluSpot Slide Spot Synthesis:

The synthesis of peptides on four dissolvable cellulose membranes (CelluSpots) at a synthesis scale range from 1-10 µmol per disk.

Create multiple copies of glass slide arrays up to 768 unique peptide per slide.

Create hundreds of identical slides from one synthesis with slide spotting volumes down to 100 nL.

The high throughput slide spotter robot can create 29 slides at the same time.

Filter Plates – Resin Based Synthesis:

The synthesis of up to 384 peptides in four dedicated 96-well filter plates at a synthesis scale ranging from 1-10 µmol.

Multiple Columns – Resin Based Synthesis:

24 to 48 mini-columns (250 and 500 µL) from 1-15 µmol scale.

72 columns (2, 5, 10 and 20 mL) from 10-300 µmol scale.

48 columns (2, 5 and 10 mL) from 10-500 µmol scale.

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Structural & Biophysical

MultiPep 2 – Automated Parallel Peptide Synthesizer

SPOT Membrane Peptide Synthesis - CEM Corporation

- Peptide screening libraries for peptide-protein interaction, epitope mapping and receptor binding.
- High throughput spotting system allows for the synthesis of large scale 24,00 peptides at the same time (600 peptides / membrane).
- CelluSpot Slide Spot Synthesis.
- Solid Phase / In-Solution Synthesis:
  - 4 x 96-well filter plates from 1-10 µmol scale
  - 24 to 48 mini-columns from 1-15 µmol scale
  - 72 columns from 10-300 µmol scale

SPOT Membrane Screening Assay

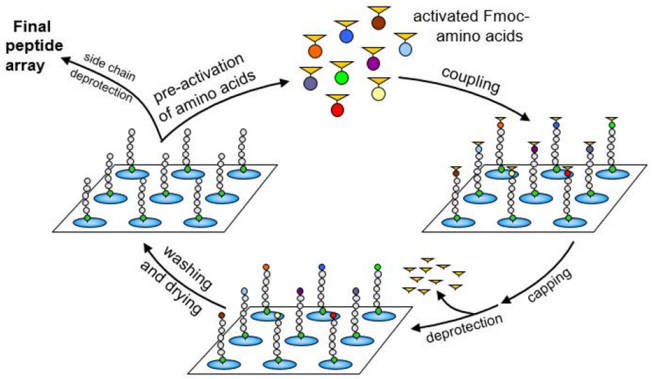
Theoretical Background

Fmoc Chemistry

Amino acids have two reactive groups, an amino group and a carboxyl group, which bind (couple) to form a peptide bond. The amino acid building blocks carry a transient protection group called Fmoc. Fmoc chemistry is used to prevent polymerization and ensures only one molecule binds to the peptide chain at a time. Other reactive groups on the side chains are masked by protection groups during synthesis.

SPOT Membrane Peptide Synthesis

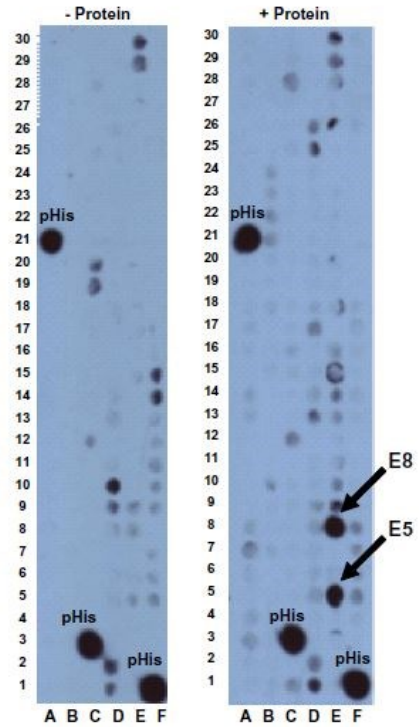
In SPOT membrane synthesis, the peptides are anchored to a cellulose membrane by a polyethylene glycol linker molecule. Peptide synthesis is the step-by-step elongation of a peptide chain by coupling amino acid building blocks. At each step of the synthesis, every chain is reacted with one molecule of the next activated Fmoc amino acid. The peptide is assembled from its carboxyl terminal to the amino terminal end (C to N-terminal direction). After every coupling reaction, the amino-protection group (Fmoc) is removed by a moderately weak acid (Fmoc deprotection reagent), whereas the side chain protection groups remain Intact. The process is repeated until the desired peptide is synthesized.



After the final peptide array has been synthesized, the side chain protection groups are removed from the peptide by treatment with a TFA cleavage cocktail.

Peptide - Protein Binding Assay

A peptide array of 180 peptides with positive controls (poly-histidine) were synthesized on the immobilized cellulose membrane. The peptide array screening was performed to determine which peptides bind to the target protein. The peptide array was immersed in the blocking solution to prevent non-specific binding and then incubated with the His-tagged target protein of interest. The His-tagged protein binds to interacting peptides and the presence of the bound protein is detected by using anti-His HRP-conjugated antibody. An ECL reagent kit was used before exposing the film for visualization.

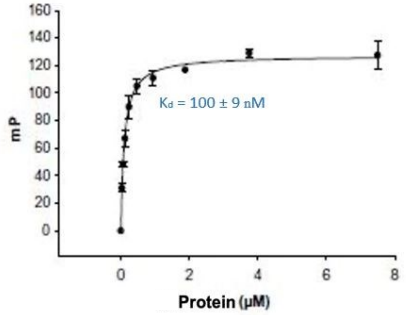


Spot positions E5 and E8 appear on the membrane only when the protein was added and were identified as hits (interaction peptides). The same experiment was performed in the absence of the protein as a negative control indicating that the observed spot intensities for E5 and E8 were not false positives.

Fluorescence Polarization Assay

Fluorescence anisotropy is based on the observation that when a fluorescently labelled molecule is excited by polarized light, it emits light with a degree of polarization that is inversely proportional to the rate of molecular rotation.

For a fluorescence polarization (FP) assay, a fixed concentration of fluorescein-labelled E8 peptide was titrated with increasing concentrations of target protein. The increase in size of the fluorescently labelled peptide due to binding with the protein affects its rate of motion in solution and is detected as an increase in FP signal.



In an FP displacement assay, the binding affinity for compounds (A, B, C) were assessed by quantifying the displacement of the fluorescein-labelled E8 peptide. A decrease in FP signal is observed when the fluorescein-labelled peptide is displaced from the protein by increasing amounts of compound, indicating protein / compound binding.