Protocol

Protein Interaction Screen on Peptide Matrix (PRISMA)

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1 Introduction and experimental Basics

Protein/Protein interactions are important for signal transduction and cell function. Peptides can act as surrogates for proteins and are frequently used for that purpose. The **PR**otein **I**nteraction **S**creen on peptide **M**atrices (PRISMA) allows the detailed characterisation of peptide/protein interactions. A peptide library is presented as spots on a cellulose membrane. The library format is flexible. Peptides can be of a length up to 25 amino acids and may represent sequence diversity and contain posttranslational modifications (PTM; e.g. Phosphoserine, Phosphothreonine, Phosphotyrosine, Arg(Me, Me2a, Me2s, Cit), Lys(Ac, Me, Me2, Me3), Lys(Mal, Suc, Glu, Biotin, Cro, Hib, For, Prop, But, Lys(GlyGly)) or similar). The cellulose membrane can be incubated with a biological sample which contains potential interaction partners. The peptide spots with the bound interaction partners are then punched out into individual wells of a microtiter plate where a tryptic digest is performed. The proteotypic peptides in each well are subsequently analysed by LC-MS based proteomics experiments.

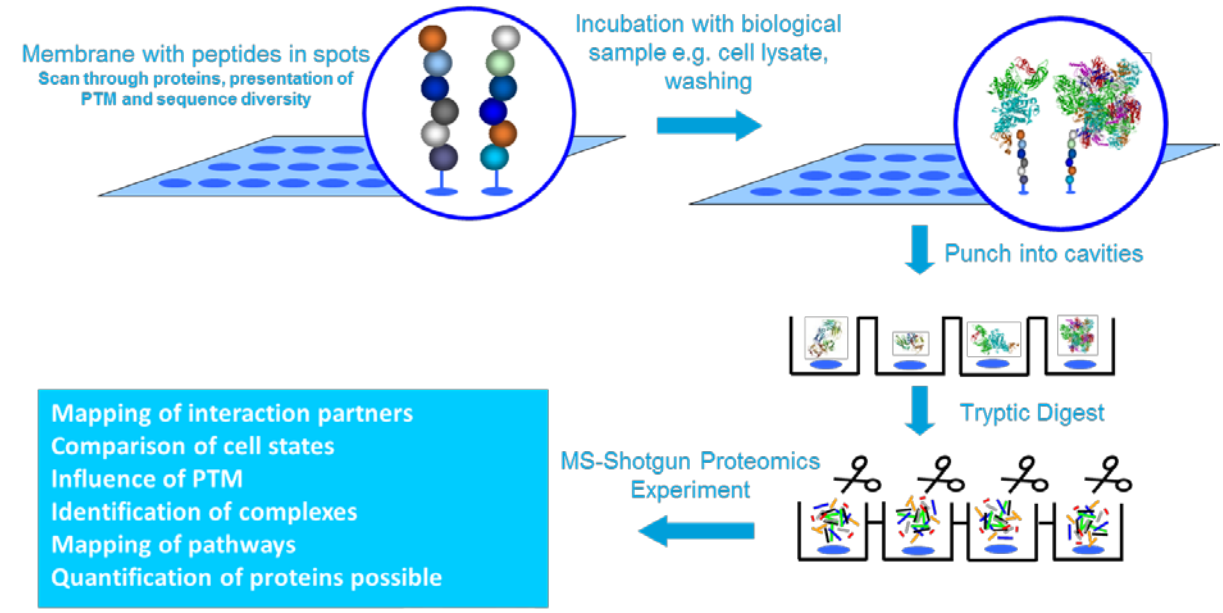

Figure 1: Schematic presentation of PRISMA-workflow.

2 List of components

Component	Quantity
PRISMA Membrane	Quantity according to order
Datasheet including membrane layout and sequence information	One datasheet per batch
Single hole punch plier	One

3 Storage and Handling

- Unused membranes should be stored at –20°C until use.

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4 Additional Materials required

4.1 Additional Reagents

- Blocking reagent e.g. yeast tRNA (1 mg/ml; Invitrogen)
- Buffer for incubation (dependent on experiment: incubation with cell lysates - lysis buffer, incubation with cell extracts – binding buffer)
    - Lysis buffer:
        - 50mM HEPES pH 7.6
        - 150mM NaCl
        - 1mMEGTA
        - 1mM $MgCl_2$
        - 10% Glycerol
        - 0.5% Nonidet P-40
        - 0.05% SDS
        - 0.25% Sodiumdeoxycholate
        - Protease Inhibitor Cocktail
        - Benzonase
    - Binding buffer:
        - 20mM HEPES pH 7.9
        - 0.2 mM EDTA
        - 100mM KCl
        - 20% Glycerol
        - 0.5 mM DTT
        - Protease Inhibitor Cocktail
        - Benzonase
- Denaturation, alkylation and tryptic digestion
    - Denaturation buffer:
        - 6M urea
        - 2M thiourea
        - 10 mM Hepes pH 8

- Alkylation
  - 18.3 mM iodoacetamide
- Digestion
  - Protease of your choice, LysC and Trypsin recommended

4 Experimental Protocols

The cellulose membrane with the bound peptides can be used for the characterisation of interactions between peptides and proteins or protein complexes from any biological sample. The protocol described herein is mainly based on two publications (Dittmar et al., 2017, Meyer et al., 2017).

The protocol is a suggestion and can be modified according to the scientific question and experimental needs. When high background binding is observed, more stringent washing can be performed.

4.1 Preparation of the membrane

- Wetting
  - Use the binding or lysis buffer for wetting the membrane
- Blocking
  - 1mg/ml yeast t-RNA diluted in binding or lysis buffer for 10 min
- Washing
  - Wash twice with binding or lysis buffer

4.2 Sample Incubation

You can incubate the wetted membrane with many different samples. For example HeLa cell nuclear extract can be used at 5 mg/ml and cell lysates are applied at similar concentrations in binding or lysis buffer, respectively. The volume is dependent on the size of the membrane. After 2 hours of incubation the membrane is washed three times in buffer and air dried.

4.3 Spot Processing

Normally, the peptides are hardly visible on the cellulose membrane. Therefore, the position of the peptides is indicated on the membrane using pencil squares (Figure 2 and 3). Peptides can be made visible using UV light. Figure 3 illustrates the process of punching out the spots:

- Hold the membrane with tweezers (3a)
- Position the square, indicating a single peptide spot, in the one-hole puncher
- Punch out the spot (3b) and keep hold of it using tweezers (3c)
- Place the spot in a defined well of a microtiter plate with tweezers (3d)

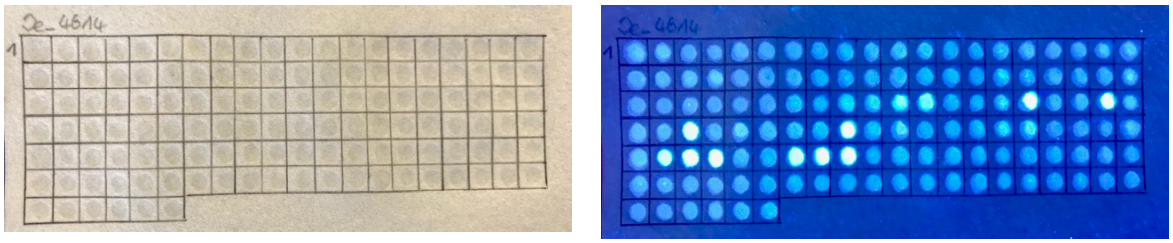

Figure 2: Visualization of peptides on membrane. Left: Squares indicating peptide spot locations. Right: Peptides are visible as bright spots on a UV-table. The production ID of the library indicated in the upper left corner..

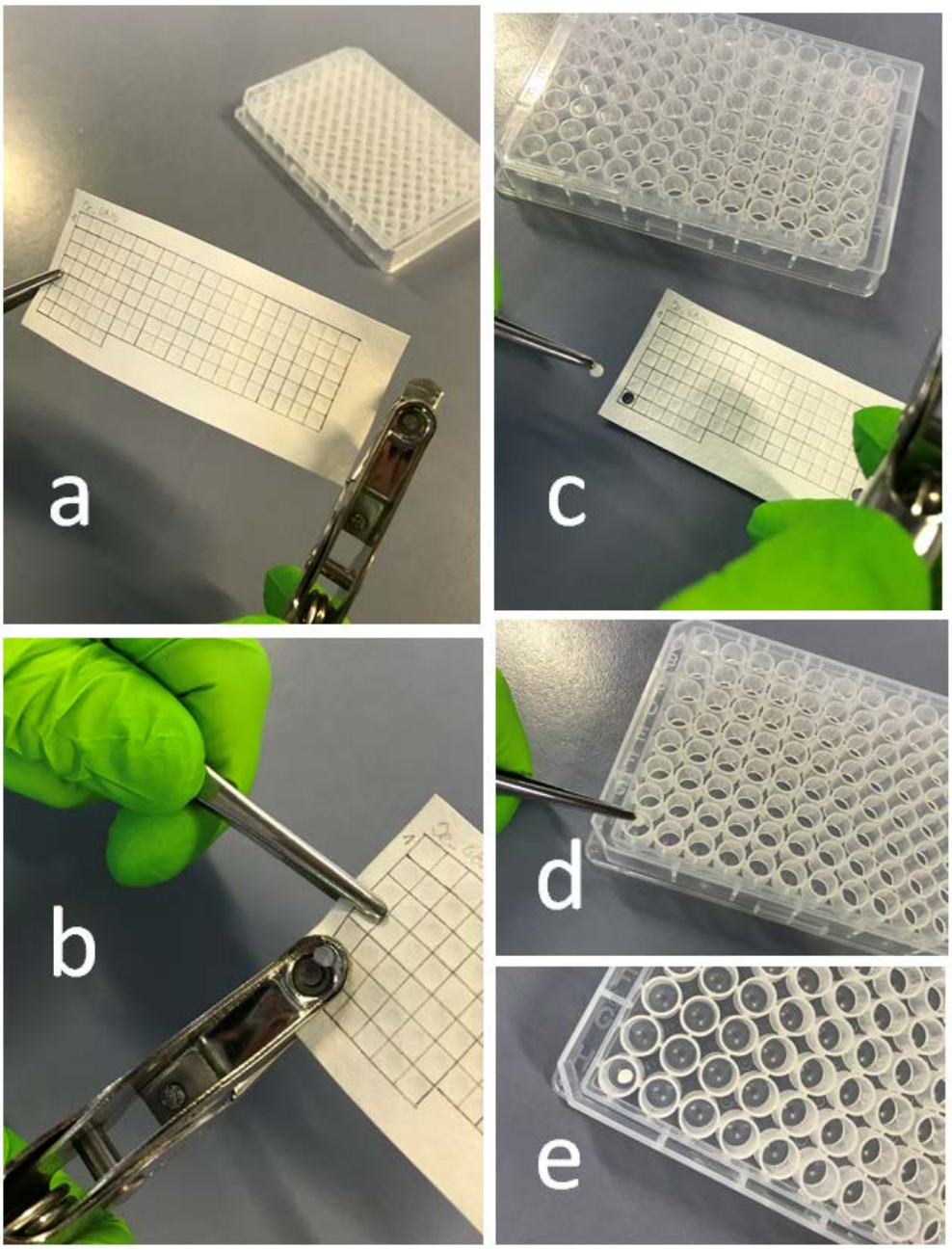

Figure 3: Spot punching: Spots are punched out using the supplied one-hole puncher. Define the wells for each spot before you start the punching process. After punching there is no easy way for the assignment of sequence information to a single spot.

Use your favourite Cys-alkylation and digestion protocol. One example as performed by Meyer et al. is shown here:

- suspend each spot in 30 µl denaturation buffer
- add 10 µl of 3 mM DTT solution for reduction of Cys side chains (30 min, RT)
- add 10 µl of 18.3 mM iodoacetamide for 60 min at RT

- do initial digest by adding 1 µg LysC for 4 h
- add 100 µl 50 mM ammonium bicarbonate (ph 8.5) for dilution
- add 1µg trypsin for 16h
- store final samples on stage tips according to Rappsilber et al., 2003

4.4 Mass spectrometry measurement

Any suitable protocol for LC-MS based peptide identification can be used on the samples generated in chapter 4.3.

5 References

Dittmar et al., 2017. Protein Interaction Screen on Peptide Matrix (PRISMA) reveals interaction footprints and the PTM-dependent interactome of intrinsically disordered C/EBPβ. https://www.biorxiv.org/content/early/2017/12/22/238709

Meyer et al., 2017. Mutations In Disordered Regions Cause Disease By Creating Endocytosis Motifs. https://www.biorxiv.org/content/early/2017/05/24/141622

Rappsilber et al., 2003. Stop and Go Extraction Tips for Matrix-Assisted Laser Desorption/Ionization, Nanoelectrospray, and LC/MS Sample Pretreatment in Proteomics. *Anal. Chem.* **75**, 663-670.

6 Related products

- SpikeTides™ – Proteomics peptide standards
- SpikeMix™ – Stable isotope-labeled proteotypic peptide pools
- Retention Time Standardization Kit
- CAMCheck Kit – Control your alkylation conditions
- TrypCheck – Control your tryptic digestion

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