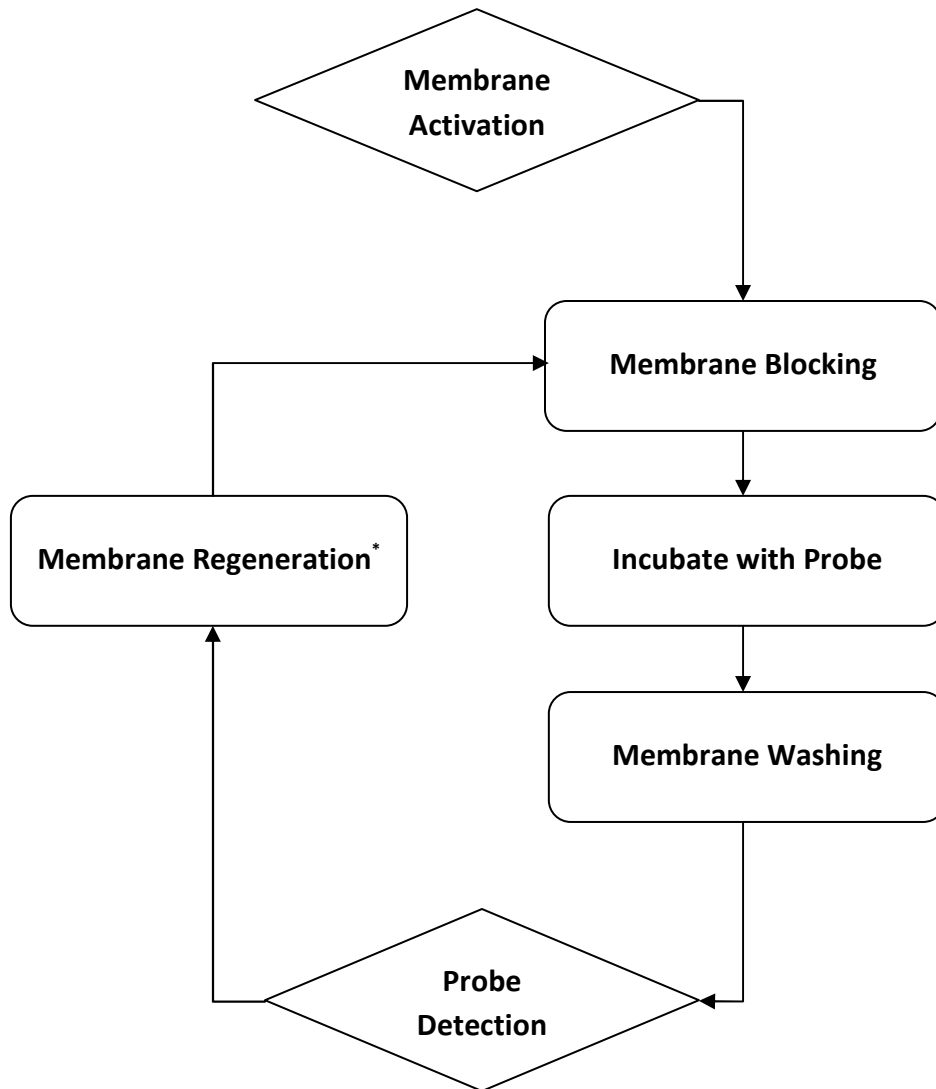


Flowchart of Peptide Array Experiment



* Membrane Regeneration: 1) In general, the membrane can be regenerated for up to 10 times, without loss of signal intensity; however, in some cases, the binding of probe with peptides is too strong to be removed, thus the membrane can be used once only. 2) Before reuse, regenerated membranes must be tested in order to verify the complete stripping of bound probe.

Other Materials Required but not Provided with Peptide Array

1. Methanol or ethanol (95%, technical grade)
2. TBS: dissolve 8.0 g of NaCl, 0.2 g of KCl and 6.1 g of Tris Base into 900 ml H₂O, and adjust pH to 7.0 with HCl, add H₂O to 1000ml, autoclave and store at 4°C
3. T-TBS: TBS buffer containing 0.05% Tween 20

4. Membrane Blocking Buffer (MBS)
 - a. 20 ml Casein-based blocking buffer concentrate (No. C7594, Sigma-Aldrich)
 - b. 80 ml T-TBS (tris-buffered saline [TBS] without phenol red, containing 0.05% Tween-20) (pH 8.0)
 - c. 5 g Sucrose
 - d. Mix the ingredients listed above, the resulting pH will be 7.0. Store at 4°C.
5. Regeneration/Stripping buffer:
 - a. Stripping buffer A: 8M Urea and 0.1% SDS in PBS, stored at room temperature. Before use, add 0.5% 2-mercaptoethanol prior and adjust pH to 7.0 with acetic acid.
 - b. Stripping Buffer B: a solution of 10% acetic acid, 50% ethanol and 40% H₂O, stored at room temperature
6. The probe of your interest: could be an antibody, purified or partial purified protein (receptor, ligand, etc), or cell lysate containing your probe.
7. N,N-dimethyl formamide (DMF): DMF is only used for removing stains during chromogenic detection, and is not mandatory for other detection methods. DMF should be of highest affordable purity, free of contaminating amine. Purity can be checked by adding 10 ul of phenol blue into 1 ml of DMF. If the color is yellow, the batch is good for experimental use. (Phenol blue indicator solution: Prepare 10 mg/ml bromophenol blue in DMF. This stock should have an intense orange color and should be discarded if the color has turned to green)
8. Kit or materials used to detect the probe of your interest (choose the best method for your experiment).