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Recommendations and instructions for the work with peptide libraries

Background: Peptide libraries typically represent chromatographic separations of body fluids or tissues and usually encompass 50 to 400 individual mother factions.

CFP typically provides aliquots of 1 to 2 % of these mother fractions for screening purposes. The mother fractions remain at CFP and will be used for further purification of bioactive peptides in hit fractions.

Each fraction of a peptide library contains a mixture of peptides in a lyophilized form. Depending on the amount of source material and the individual fractions, the peptide pellets might be easily seen or invisible. CFP provides chromatograms allowing rough estimates of the amount of peptide per fraction. Typically, the provided fractions contain µg to mg amounts of peptide.

Handling: Fractions are frequently insoluble in assay buffers such as PBS. Thus, dissolve the desalted and lyophilized aliquots in about 50 to 100 µl distilled water. Fractions not dissolving immediately under these conditions can be dissolved overnight under shaking at 4 °C, or alternatively using higher volumes, e.g. 400 µl. Note which fractions don't dissolve properly since these fractions may cause misleading results due to unspecific effects of protein/peptide aggregates on e.g. a cell surface.

Store lyophilized or reconstituted peptides at -20°C. Avoid repeated freezing and thawing, e.g. by producing and freezing aliquots.

Testing: The assay to identify bioactive (hit) fractions should be robust, reproducible, with low inter and intra assay variation, and ideally adapted to microtiter format. Typical assay volumes are 100 to 150 µl, with 10 to 15 µl of the resuspended fraction, corresponding to 10% v/v. If possible, perform all assays in triplicate and normalize values to a control (100%) to allow comparison of different screens from various groups.

Considerations: Use serum-free medium when working with cells. Supplemental FCS or human serum may lead to proteolytic degradation of bioactive peptides.

Upon addition of peptide fractions to the cell media or bacterial suspensions, monitor and keep a track record of changes in color of the medium (e.g. pH switch) or formation of precipitates. These fractions might yield misleading results (see above).

If possible, carefully examine all samples by light microscopy and note which fractions cause cytotoxic effects or yield precipitates.

Identification of "hit" fractions: If the primary screen results in the identification of "interesting" hit fractions with bioactivity, CFP will provide additional aliquots of the mother fractions for confirmatory dose-response studies, to rule out possible cell toxicity and to get more information about the specifity of the biological effect. Depending on the outcome, decisions on further purification and prioritization of "hit" fractions will be made.

Further rounds of purification: Preparative amounts of selected "hit" fractions will now be re-purified by consecutive chromatographic steps with the aim to enrich the active peptide to a purity that allows its unambiguous analytical identification. During this process, different kinds of chromatography like ion–exchange, size-exclusion and reverse-phase will be employed to enable an efficient separation. In each round of purification, CFP will provide the user with aliquots to confirm the bioactivity in the different peptide-containing fractions. Step by step the bioactive peptide will be enriched and finally applied to analytical identification.

Analytical identification: After several rounds of purification the bioactivity will be enriched and can be examined by different analytical procedures. These methods include especially mass spectrometry (ESI-MS and MALDI-MS), analytical HPLC and combinations of LC and MS. These techniques are available in the CUMP unit in Ulm. Also N-terminal sequence determination by Edman degradation can be considered.

Resynthesis and proof-of-concept: After sequence analysis of the active fractions, candidate sequences will be evaluated by the involved researchers. Finally, selected candidate peptides will be chemically synthesized by CFP and provided to the user to confirm the biological activity.

Good luck with your experiments. Do not hesitate to ask for further information

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Literature:

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