Abstract

Peptides are used as synthetically available and easily derivatizable scaffold upon which it is possible to develop ligands targeting broad spectrum of biological targets. A time-tested approach to peptide binder identification is the preparation and screening of combinatorial libraries. Bypassing of this complicated procedure is possible by using biological systems for presentation, identification and selection of peptides based on the principle of *in vitro* evolution – i.e. display techniques.

There are two complementary automated solutions for peptide binder identification described in this work. First is the SPENSER parallel peptide synthesizer, developed as a part of this diploma project, which can be used for peptide ligand discovery and optimization as well as validation of ligands identified using display techniques. Several libraries consisting of a total of 1 052 peptides have been prepared and then used to describe its potential applications. A sample of 154 preparations, representing 14.6 % analytical coverage of the prepared libraries, showed an average purity of 67 ± 19 % according to LC-MS.

The libraries presented illustrate that SPENSER is a suitable tool for the parallel synthesis of linear and disulfide-cyclized peptides with limited variability, or libraries consisting of short peptides. Furthermore, its relatively low reaction scale compared to commercially available synthesizers allows for more economical preparation of libraries from expensive building blocks.

Secondly, the ToRNAdo protocol is presented as an automated variant of the well-established mRNA display method with a minimized number of operations, fully executable by an integrated workcell consisting of commercially available solutions – Agilent Bravo pipetting robot, Biometra T-Gradient PCR cycler and a selective compliance assembly robot arm.

Protocol validation was performed on a model binary library consisting of sequences encoding FLAG and HA epitope tags. Both model sequences were successfully selected with the corresponding antibodies. Necessary modifications of the protocol before its full use outside model libraries are discussed.

Keywords: High-throughput screening; peptide synthesis; *in vitro* evolution; molecular recognition; method development