PARALLEL SCREENING OF *IN SITU* CLICK CHEMICAL LIBRARIES IN INTEGRATED MICROFLUIDIC DEVICES

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ABSTRACT

An integrated microfluidic device [1] has been developed with high-throughput (HT) capability to perform 1024 *in situ* click chemistry [2] reactions in parallel for screening high-affinity inhibitors of bCAII [3]. A new rapid hit identification approach using the ZipTip based sample processing for removing phosphate salts and DMSO and ESI-MS with multiple reaction monitoring (MRM) analysis which can greatly improve sensitivity and throughput of the downstream mass spectrometric analysis. The integration of the HT hit identification method with microfluidics not only shortens screening time but also reduces sample consumption, i.e., the amount of target protein and the ligands used with four-order magnitude lower that of the conventional 96-well formats.

KEYWORD: Click chemistry, Microfluidic, High-throughput screening, Parallel syntheses

INTRODUCTION

In situ-click chemistry is a target-guided synthesis to discover high-affinity bi-ligands for protein targets. By assembling azide- and acetylene- derivatized motifs as individual building blocks inside the target's binding pockets, a triazole linkage will form through a Huisgen 1,3-dipolar cycloaddition. If the distance between them is appropriate, a novel compound produces with higher affinity than each mono-ligand alone. However, due to considerable amount (nearly stiochometric) of target protein vs. two binding fragments is required, the broad application using *in situ* click chemistry is severely hampered. Before realizing the large-scale screening of an *in situ* click chemistry library, it is imperative to develop a miniaturized platform to significantly reduce protein/reagent consumption, and enable quick identification of high-affinity ligand in a HT matter. With the aid of speedy MS-MRM analyses, our microfluidic platform promises shorter screening time, sample/reagent economy and improved fidelity.

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RESULT AND DICUSSION

An integrated microfluidic chemical reaction circuit that provides an automated platform for HT screening of an *in situ*-click chemistry library is shown in Figure 1. The microfluidic chip comprises of (i) two parallel multiplexers for controlling 16 x 16 reagent inlets individually, (ii) a miniature rotary mixer (volume: *ca* 150 nL) together with a serpentine channel for efficient mixing and precise metering of reagents (total reaction volume: 400 nL), (iii) an on-chip vacuum channel for increasing sampling loading rate (each reaction cycle: < 5 seconds). Teflon tubing was served as a reservoir for accommodating each droplet come out of chip. To preventing cross contamination, an air and PBS buffer plug were implemented in-between to separate each reaction mixture.

To identify high-affinity bi-ligands for protein target, bCAII, 16 acetylenes and 16 azides were employed to set up an *in situ* click chemistry library which composed of 1024 independent reactions.

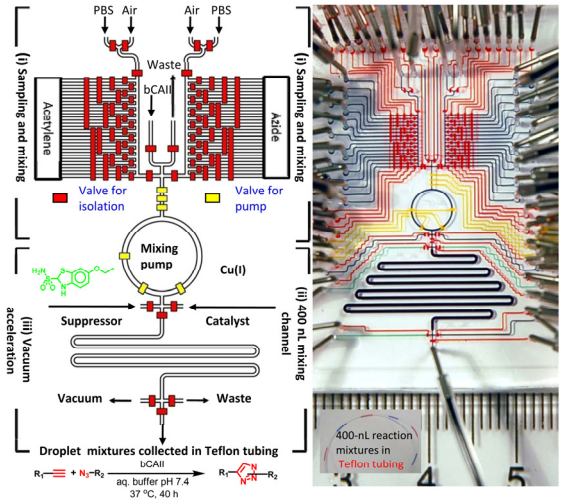

Figure 1. Schematic representation of a microfluidic chemical reaction circuit used for the parallel screening of an in situ-chemistry library. The responsibilities of different valves are illustrated by their colors: red for regular isolation valves and yellow for pump valves. The various channels were load with food dyes to help visualized the different components of the microfluidic chip

The final reaction mixtures in the Teflon tubing were collected and then concentrated/pre-purified through ZipTip-based sample process. The removal of ion-retarding DMSO and PBS salt is crucial for enhancing ionization process in the LC-MS thus it improves sensitivity and lowers detection limit. Furthermore, the utilization of MRM using an ESI-MS enabled one to identify the hit from the non-hit in less than one minute per sample. For proof-of-principle, Figure 2 shows some of MRM results after conducting parallel reactions on a microfluidic chip. Again, it is very important to note that each screening only consume few hundred nanograms of

enzyme and picomoles of both reagents in an automated fashion. Further integrated microfluidic devices capable of wider exploration beyond single application in monolithic devices are currently under development.

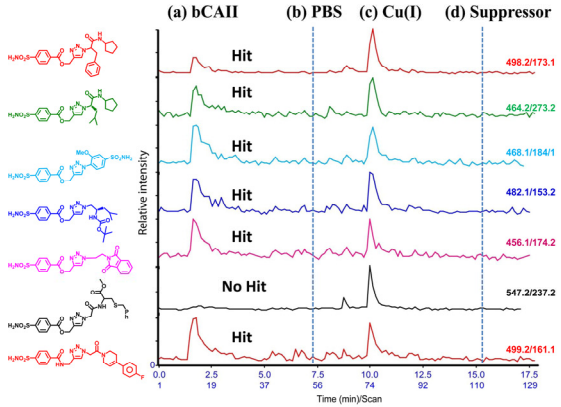

Figure 2. Microfluidic chip-based reactions preformed in situ click chemistry between acetylenes and azides were analyzed by ESI-MS-MRM method: (a) with bCAII (positive control); (b) without bCAII (negative control); (c) Cu[I]-catalyzed reaction (positive hit reference); (d) in the presence of both bCAII and its inhibitor (competition reaction).

CONCLUSIONS

In summary, an integrated microfluidic platform which can perform 1024 *in situ* parallel click chemistry reactions on the nanogram scale has been developed. In addition to fundamental advantages such as low reagent/sample consumption, precise control over reaction conditions, faster reaction kinetics, cost efficiency, easy design/fabrication processes and fast turnover/improvement cycles, the integrated microreactors provide a whole new arena for chemists to test chemical reactions beyond the conventional setting.

ACKNOWLEDGEMENTS

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