

# A Two-Step Cross-Linked Hydrogel Immobilization Strategy for Diacetylchitobiose Deacetylase

Guoyun Sun <sup>1,2</sup>, Ziyang Huang <sup>2</sup>, Zhiqian Zhang <sup>3</sup>, Yanfeng Liu <sup>1,2</sup>, Jianghua Li <sup>2</sup>, Guocheng Du <sup>1,2</sup>, Xueqin Lv <sup>1,2,\*</sup> and Long Liu <sup>1,2</sup>

<sup>1</sup> Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education, Jiangnan University, Wuxi 214122, China

<sup>2</sup> Science Center for Future Foods, Jiangnan University, Wuxi 214122, China

<sup>3</sup> Tidetron Bioworks Technology (Guangzhou) Co., Ltd., Guangzhou 510000, China

\* Correspondence: lvxueqin@jiangnan.edu.cn; Tel.: +86-510-85918312

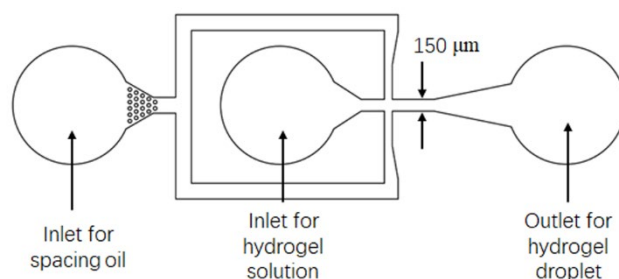
## Table of contents

Supplementary 1 Microfluidic chip design for homogenous hydrogel particle generation ..... S-2

Supplementary 2 Dac activities and leaking Dac activities of hydrogel with different cross-linking durations ..... S-3

### Supplementary 1. Microfluidic chip design for homogenous hydrogel particle generation

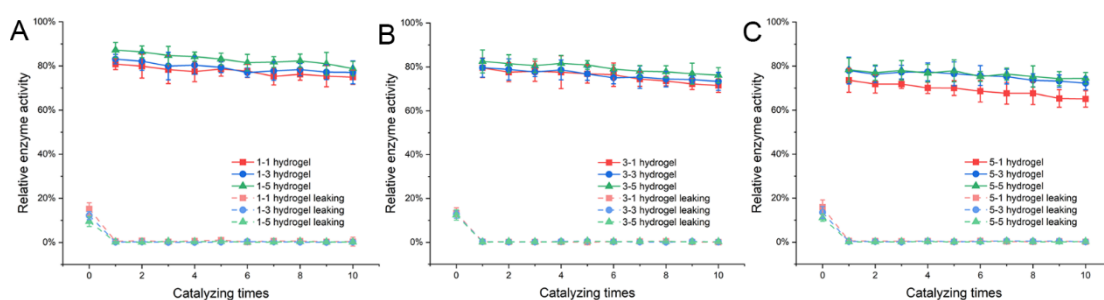
The design of droplet generator was showed in Figure S1. By using this microfluidic chip, the generated droplets were homogenous with the diameter of ~ 200  $\mu\text{m}$ . A heating mat was used to prevent the self-cross-linking of hydrogel solution.



**Figure S1.** Design of droplet generator.

### Supplementary 2. Dac activities and leaking Dac activities of hydrogel with different cross-linking durations

The Dac activities and leaking Dac activities of hydrogel with different cross-linking durations were measured from round 1 to round 10. The Dac leaking activities during the washing step were also measured as round 0. All data were plotted in Figure S2.



**Figure S2.** Relative enzyme activities of hydrogels with different cross-linking times. (A–C) Relative Dac and leaking Dac activities of “1-1”, “1-3”, “1-5” (A); “3-1”, “3-3”, “3-5” (B); and “5-1”, “5-3”, “5-5” (C) hydrogels with 10 rounds of catalysis. .