Supporting Information

Hydrogel particle-based protein display enabled by particle-templated emulsification

Han Wu,* Jiayao Fang, Jiao Chen, Yaoqi Wang, Bo Zheng*

Institute of Chemical Biology, Shenzhen Bay Laboratory, Shenzhen, China

* Corresponding author. Institute of Chemical Biology, Shenzhen Bay Laboratory, Shenzhen, China.
E-mail address: wuhan@szbl.ac.cn (H. Wu); bozheng@szbl.ac.cn (B. Zheng).

1. Hydrogel particle preparation

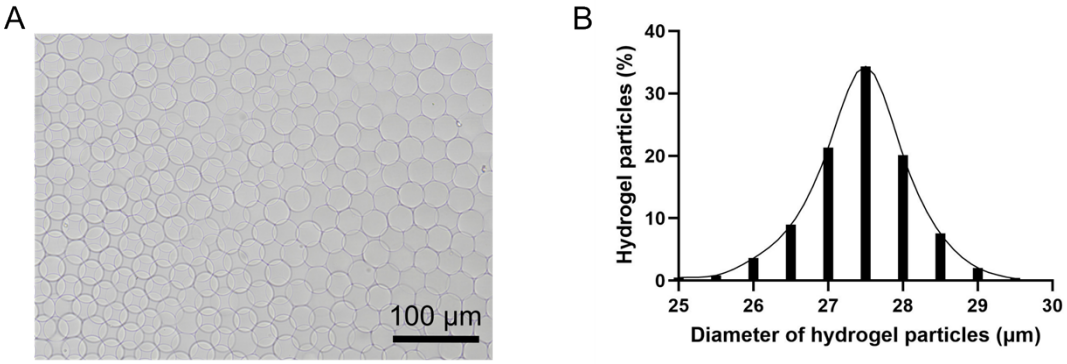

Figure S1. (A) Hydrogel particle preparation by droplet microfluidics. (B) Hydrogel particle size distribution.

2. Droplets generation with particle-templated emulsification

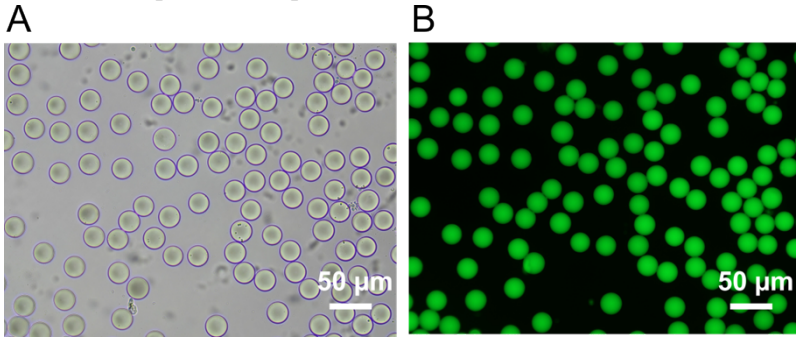

Figure S2. FITC was loaded into PA hydrogel particles, followed by dispersed into the oil phase using particle-templated emulsification. (A) Bright-field microscope image of the particles. (B) Fluorescence microscope image of the particles.

3. Scalable emulsification of hydrogel particles using particle-templated emulsification

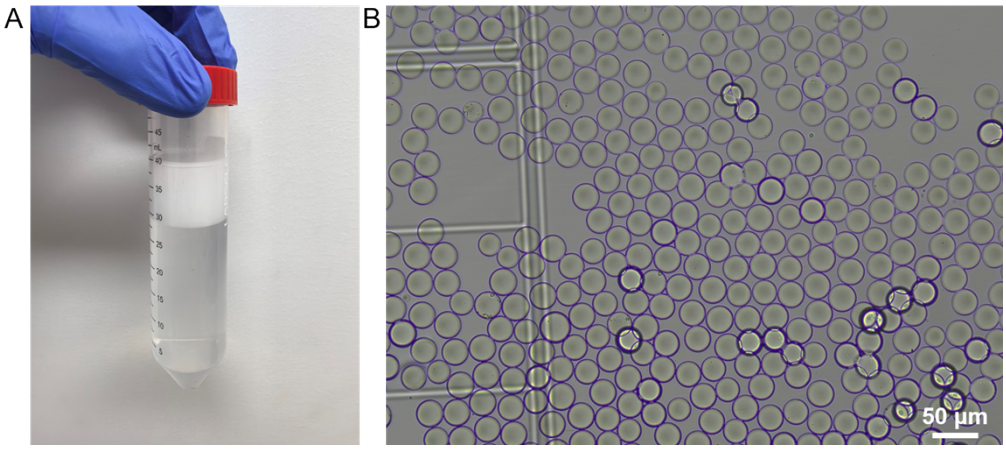

Figure S3. (A) Scalable emulsification of hydrogel particles with particle-templated emulsification in a 50 ml conical tube. (B) Bright-field microscope image of the resulting emulsified particles.

4. Theoretical prediction of single-molecule encapsulation and dual-molecule pairing efficiency in droplets using droplet microfluidics

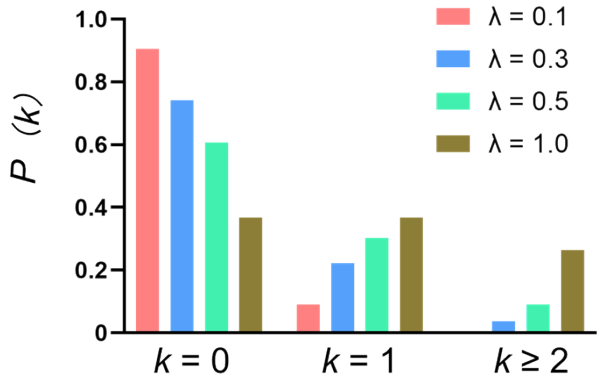

Figure S4. Probability of encapsulating k molecules in a droplet at varying average molecule numbers per droplet (λ), based on Poisson distribution in droplet microfluidics. $P(k)$ represents the probability of encapsulating k molecules in a droplet, and λ is the average number of molecules per droplet.

5. Macromolecules loading and immobilization in hydrogel particles via glutaraldehyde conjugation

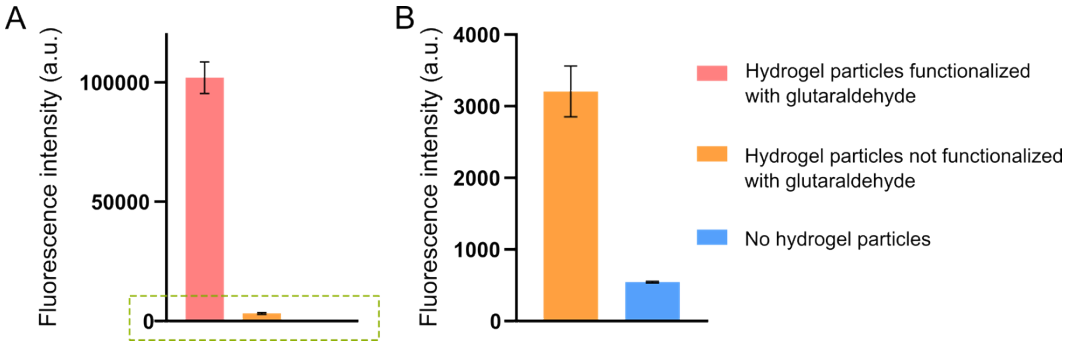

Figure S5. Streptavidin conjugated HRP was incubated with hydrogel particles with and without

glutaraldehyde functionalization, followed by washing. HRP immobilization was assessed by measuring fluorescence intensity after reaction with an Amplex Red-based substrate. (A) Fluorescence intensity of wells containing hydrogel particles with or without glutaraldehyde functionalization, along with a blank control containing no hydrogel particle. (B) Magnified view of the rectangular region in (A).

6. Histidine-tagged protein immobilization on Ni-NTA functionalized hydrogel particles

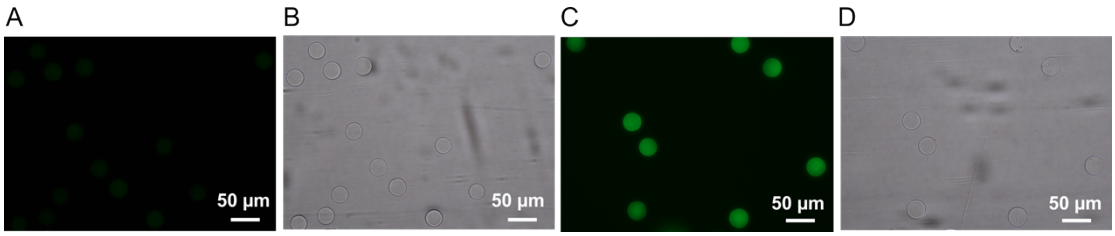

Figure S6. Immobilization of histidine-tagged enhanced green fluorescent protein (EGFP) on hydrogel particles. (A) Fluorescence and (B) bright-field microscope images of hydrogel particles without Ni-NTA functionalization. (C) Fluorescence and (D) Bright-field microscope images of hydrogel particles with Ni-NTA functionalization.

7. Dual display of EGFP and mCherry on hydrogel particles from a mixed plasmid library

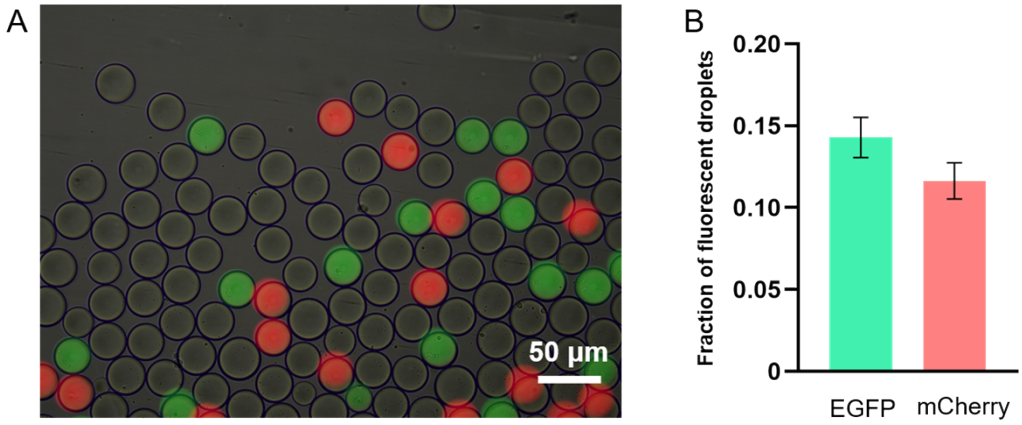

Figure S7. (A) Microscope image of hydrogel particles after protein expression. Bright-field, green fluorescence (EGFP), and red fluorescence (mCherry) channels were overlaid to evaluate the dual display of EGFP and mCherry. (B) Fraction of droplets exhibiting green fluorescence from EGFP expression and red fluorescence from mCherry expression.