Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary materials

Creating locally crowded environment with nanoclay hydrogel for cell-free biosynthesis

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Supplementary Table S1. Composition of plasmid labeling system

Components	Reaction system（50 μL）
10×Labeling Buffer A	5 μL
1 mg/mL DNA template	5 μL
Label IT®Tracker Reagent	2.5 μL
ddH2O	37.5 μL

Supplementary Table S2. Quantitative real-time PCR reaction components

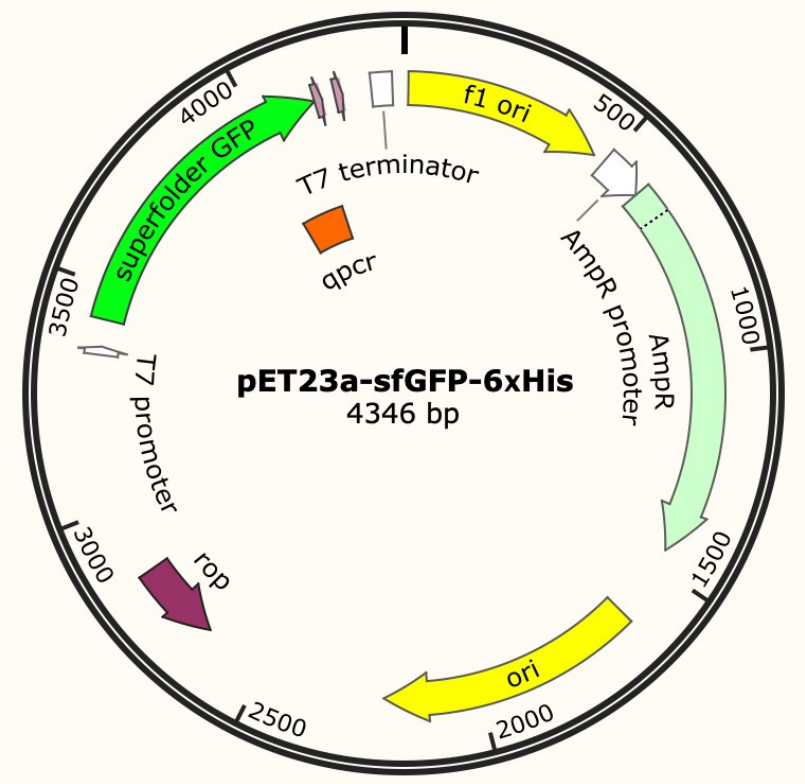
Components	Reaction system（20 μL）
Forward primer (10 μM)	0.4 μL
Reverse primer (10 μM)	0.4 μL
2×TransStart Top Green QPCR SuperMix	10 μL
Passive Reference Dye（50×）	0.4 μL
Nuclease-free Water	4 μL
DNA template	4.8 μL

Supplementary Table S3. Quantitative real-time PCR reaction system

Steps	Temperature	Time
Stage1	94℃	30 s
Stage2（40×）	94℃	5 s
	57℃	15 s
	72℃	31 s
Stage3	95℃	15 s
	60℃	60 s
	95℃	15 s

Supplementary Table S4. List of nanoclay solution concentration and $CaCl_2$ solution concentration

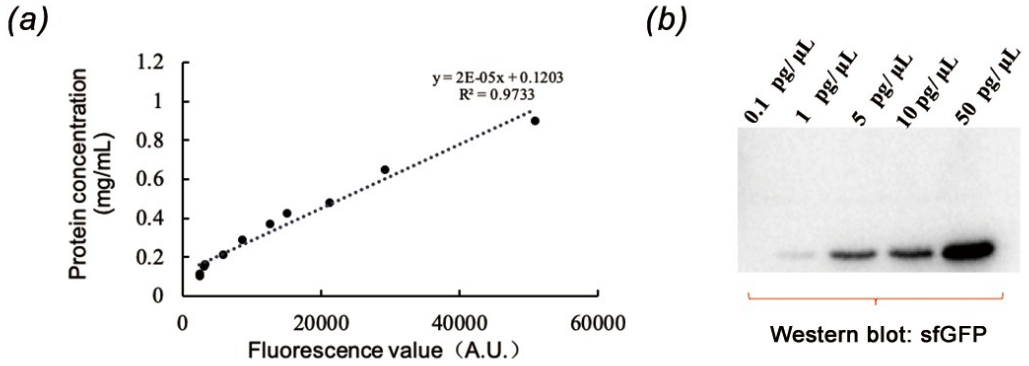
Nanoclay solution（mg/mL）	$CaCl_2$ solution（mM）
2	10
4	20
6	30
8	40
10	50
12	60
14	70
16	80
18	90
20	100



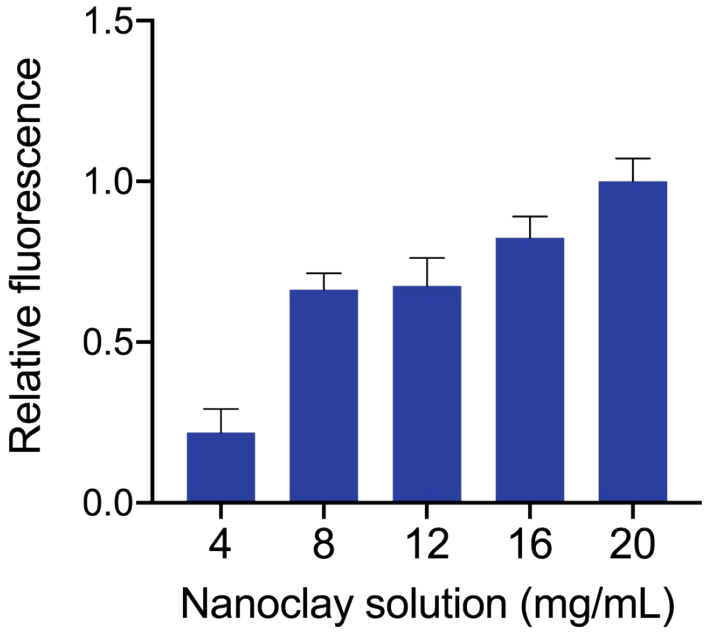
T7 promoter-sfGFP-6\242\252His-T7 terminator

TAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAAT
TTTGTTTAACTTTAAGAAGGAGATATACATATGCGTAAAGGCGAAGAGCT
GTTCACTGGTGTCGTCCCTATTCTGGTGGAACTGGATGGTGATGTCAACGG
TCATAAGTTTTCCGTGCGTGGCGAGGGTGAAGGTGACGCAACTAATGGTA
AACTGACGCTGAAGTTCATCTGTACTACTGGTAAACTGCCGGTACCTTGGC
CGACTCTGGTAACGACGCTGACTTATGGTGTTCAGTGCTTTGCTCGTTATC
CGGACCATATGAAGCAGCATGACTTCTTCAAGTCCGCCATGCCGGAAGGC
TATGTGCAGGAACGCACGATTTCCTTTAAGGATGACGGCACGTACAAAAC
GCGTGCGGAAGTGAAATTTGAAGGCGATACCCTGGTAAACCGCATTGAGC
TGAAAGGCATTGACTTTAAAGAAGACGGCAATATCCTGGGCCATAAGCTG
GAATACAATTTTAACAGCCACAATGTTTACATCACCGCCGATAAACAAAA
AAATGGCATTAAAGCGAATTTTAAAATTCGCCACAACGTGGAGGATGGCA
GCGTGCAGCTGGCTGATCACTACCAGCAAAACACTCCAATCGGTGATGGT
CCTGTTCTGCTGCCAGACAATCACTATCTGAGCACGCAAAGCGTTCTGTCT
AAAGATCCGAACGAGAAACGCGATCATATGGTTCTGCTGGAGTTCGTAAC
CGCAGCGGGCATCACGCATGGTATGGATGAACTGTACAAACATCACCATC
ACCATCATTAAGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCAC
CACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGC
TGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAAC
GGGTCTTGAGGGGTTTTTTG

Supplementary Figure S1. The pET23a-sfGFP-6\242\252His plasmid map and the sequence information. The sfGFP was regulated under the bacteriophage derived T7 promoter and terminator in the pET-23a backbone. The last 150 bps of sfGFP were designed for the qPCR (Real-time Quantitative PCR).



Supplementary Figure S2. Fluorescence value could represent the protein expression level. (a) Standard curve of sfGFP. The fluorescence value of sfGFP increased linearly with the concentration of protein, which indicated that the protein expression level could be characterized by the fluorescence, and the influence of the environment could be ignored. (b) The results of Western blot. The gray-scale value increased linearly with the concentration of protein.



Supplementary Figure S3. The protein expression level with different concentrations of nanoclay solution. When the nanoclay solution concentration was 20 mg/mL, the fluorescence of sfGFP was set to 1.