SpyCatcher-Multiplicity Tunes Nanoscaffold Hydrogels for Enhanced Catalysis of Regulated Enzymes

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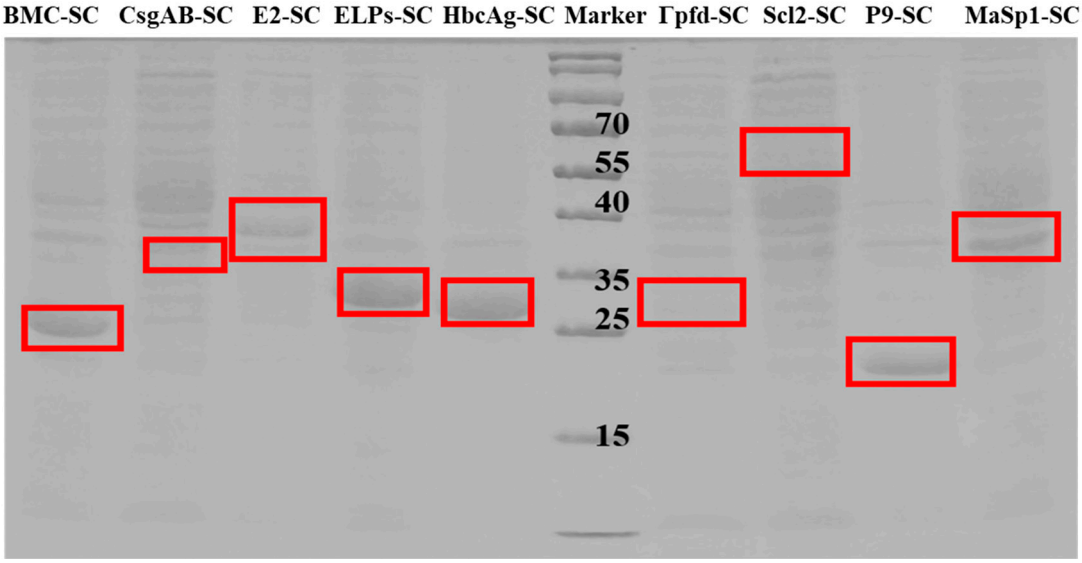
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Supplementary Table S1 Nano-scaffold proteins used in this study

Protein	Sequence
BMC	MSAQSAVGSIETIGFPGILAAADAMVKAGRITIVGYIRAGSARFTLNIRGDVQEVKTA- MAAGIDAINRTEGADVKTWVIIPRPHENVVAVLPIDFSPEVEPFREAAEGLNRR
CsgAB	MKLLKVAAIAAIVFSGSALAGVVPQYGGGGNHGGGGNNSGPNSELNIYQYGGGNSALALQT- DARNSDLTITQHGGGNGADVGQGSDDSSIDLTQRGFGNSATLDQWNGKNSEMTVKQFGGGNGAAV DQTASNSSVNVTQVGFGNNATAHQYGSGGSGMKNKLLFMMLTILGAPGIAAAA- GYDLANSEYNFAVNELSKSSFNQAAIIGQAGTNNSAQLRQGGSKLLAVVAQEGSSNRAKIDQTGDYN LAYIDQAGSANDASISQGAYGNTAMIIQKGSGNKANITQYGTQKTAIVVQRQSQMAIRVTQR
E2	MAAAKPATTEGEFPETREKMSGIRRAIAKAMVHSKHTAPHVTLMDEADVTKLVAHRKKFKAI- AAEKGIKLTFLPYVVKALVSALREYPVLNTSIDDETEEIIQKHYYNIGIAADTDRGLLVPVIKHADRKPI FALAQEINELAEKARDGKLTPGEMKGASCTITNIGSAGGQWFTPVINHPEVAILGIGRIAEK- PIVRDGEIVAAPMLALSLSFDHRMIDGATAQKALNHIKRLLSDPELLLM
ELPs	MSKGPGVGVPGKGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGFGVPGVGVPGKGV PGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGVGVPGFGVPGVGVPGKGVPGVGVPGVGVPGVG VPGVGVPGVGVPGVGVPGVGVPGFGVPGVGVPGKGVPGVGVPGVGVPGVGVPGVGVPGV GVPGVGVPGFGVPGWP
HBcAg	MDIDPYKEFGATVELLSFLPSDFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAIL- CWGELMTLATWVGNNLEDPASRDLVVNYVNTNMGLKIRQLLWFHISCLTFGRETVLEYLVSFGVWI RTPPAYRPPNAPILSTLPETTVV
γPFD	MVNEVIDINEAVRAYIAQIEGLRAEIGRLDATIATLRQSLATLKSLKTLGEGKTVLVPVG- SIAQVEMKVEKMDKVVVSVGQNISAELEYEEALKYIEDEIKKLLTFRLVLEQAIAELYAKIEDLIAEAQ QTSEEEKAEEEENEEKAE
Scl2	MVNKTKTKHSLLCRYGLTSAAALLLTFGGASAVKADEQEEKAKVRTELIQELAQGLG- GIEKKNFPTLGDEDLDHTYMTKLLTYLQEREQAENSWRKRLLKGIQDHALDGQDGRNGERGEQGPT GPTGPAGPRGLQGLQGLQGERGEQGPTGPAGPR- GLQGERGEQGPTGLAGKAGEAGAKGETGPAGPQGPRGEQGPQGLPGKDGEAGAQGPAGPMGPAGE RGEKGEPGTQGAKGDRGETGPVGPRGER- GEAGPAGKDGERGPVGPAGKDGQNGQDGLPGKDGKDGQNGKDGLPGKDGKDGQNGKDGLPGKD GKDGQDGKDGLPGKDGKDGLPGKDGKDGQPGKPAPKTPEVPQKPDTAPH- TPKTPQIPGQSKDVTPAPQNPSNRGLNKPQTQGGNQLAKTPAAHDTHRQLPATGETTNPFFTAAAVAI MTTAGVVAVAKRQENN
P9	MPFPLVKQDPTSKAFTEASERSTGTQILDVVKAPIGLFGDDAKHE- FVTRQEQAVSVVSWAVAAGLIGELIGYRGARSGRKAILANIPFLA
MaSp1	MAASGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAGGAGQG- GYG- GLGSQGTSGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAGGAGQG GYGGLGSQGTSGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGA- GAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGTSGRGGLGG QGAGAAAAAGGAGQGGYGGLGSQGTSGRGGLGGQGAGAAAAAGGAGQGGYGGLGSQGTSGRG- GLGGQGAGAAAAAGGAGQGGYGGLGSQGTS
Spy- Catcher	GDSATHIKFSKRDEDGKELAGATMELRDSSGKTIST- WISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFVNEQGQVTVNG
SpyTag	AHIVMVDAYKPTK

Supplementary Figure S1. SDS-PAGE analysis of SpyCatcher-fused nanoscaffold proteins

Cell lysates containing SpyCatcher (SC)-conjugated nanoscaffold proteins were treated with 4 M ammonium sulfate on ice for 4 h. After centrifugation (8,000 × g, 5 min, 4°C), supernatants were analyzed by 12.5% SDS-PAGE to evaluate residual soluble proteins. Lanes 1-8 correspond to: BMC-SC, CsgAB-SC, E2-SC, ELPs-SC, HBcAg-SC, Scl2-SC, P9-SC, and MaSp1-SC.



The target protein is indicated within the red box.

Supplementary Figure S2 Swelling rate of SpyCatcher (SC)-mediated protein-based hydrogels.

Samples were first freeze-dried under vacuum (FreeZone®; Labconco, USA) and the dry weight recorded as W1. Subsequently, samples were immersed in sterile water at 4°C for 6 h. After removing excess surface water, the swollen weight was measured as W2. The swelling rate was calculated using Equation (S1).

$$\text{Swelling rate } (\%) = \frac{W2-W1}{W1} * 100\% \quad \text{(S1)}$$

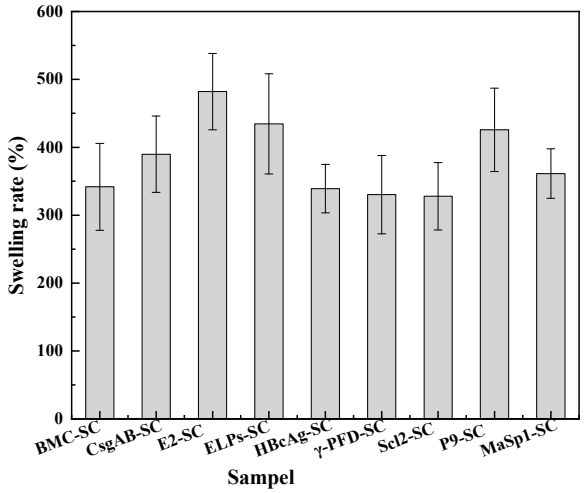
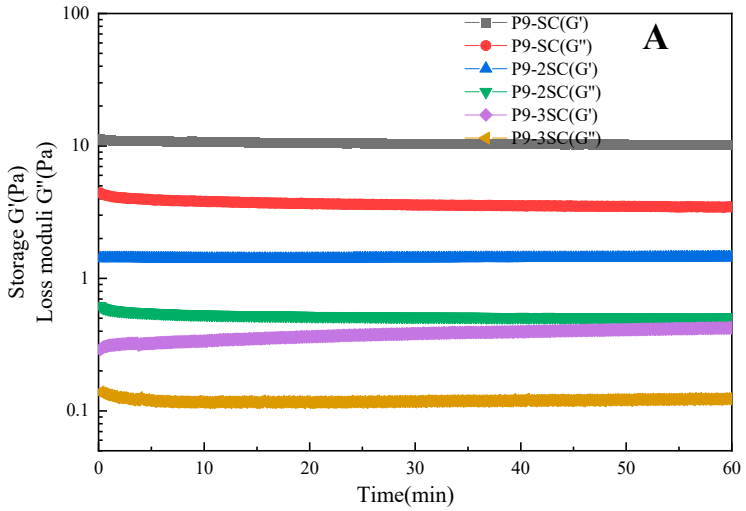
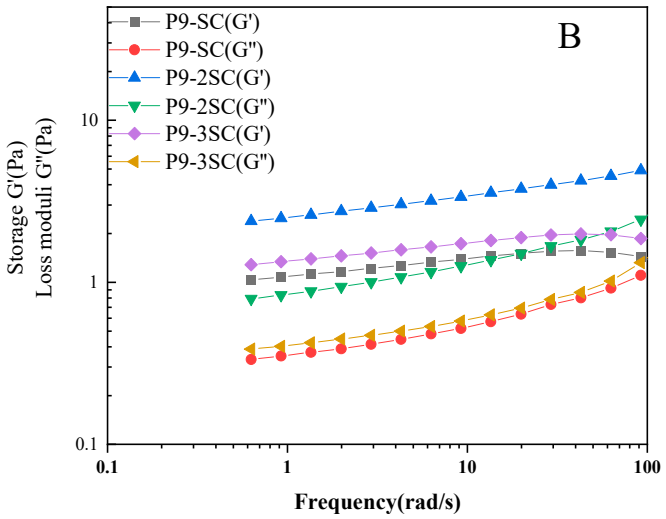


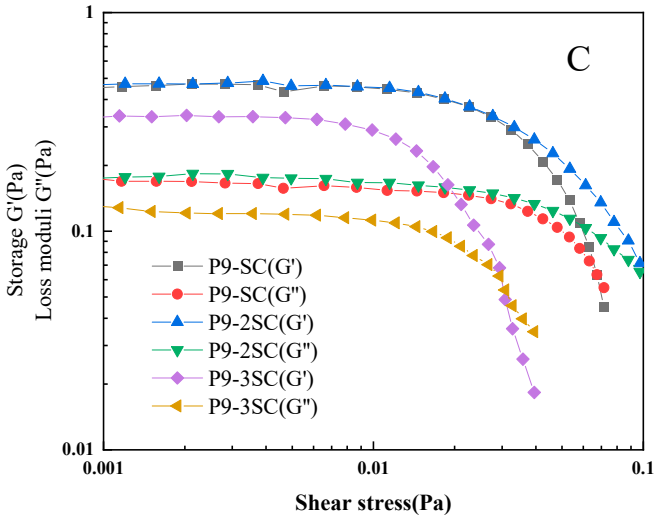
Figure S2

Supplementary Figure S3 SpyCatcher-mediated protein hydrogel viscoelastic properties

(A) Time-dependent viscoelastic moduli (G’ and G’’). (B) Frequency sweep test (G’ and G’’). (C) Shear stress test (G’ and G’’).






C
Storage G'(Pa)
Loss moduli G''(Pa)
1
0.1
0.01
0.001
0.01
0.1
Shear stress(Pa)
P9-SC(G')
P9-SC(G'')
P9-2SC(G')
P9-2SC(G'')
P9-3SC(G')
P9-3SC(G'')

Supplementary Figure S4 Immobilization and catalytic activity of Corynebacterium glutamicum under different models

Cell Culture and Protein Purification

A single colony with plasmid pET28a-GDH (glutamate dehydrogenase) was inoculated into 5 mL of LB medium (5 g/L yeast extract, 10 g/L tryptone, 10 g/L NaCl, pH 7.0) and cultured at 37 °C with shaking (200 rpm). The seed culture was diluted to an OD600 of 0.1 in 50 mL of fresh LB medium and incubated under the same conditions until reaching an OD600 of 0.8. For cultures expressing the pET28a backbone, 1 mM IPTG was added and the cultures were further incubated at 25 °C (200 rpm) for 20 h. Then, Cell lysates were centrifuged at 8000 × *g* for 30 min at 4 °C, and the resulting supernatant was loaded onto a 2-mL Ni-NTA resin column. Protein was collected using a standard HisTrap procedure (binding, elution, collection).

Sample Embedding and Model Preparation

Acrylamide: The ratio of diacrylamide = 29:1, prepare a 8% final concentration PAGE gel. 2 mg/mL purified GDH were mixed with the PAGE gel, and then 0.1% thromboplastic agent (10% $(NH_4)_2S_2O_8$ and 0.5% TEMED) was added and pour it into the pre-prepared model made with candles. A. Uniform cylinder. B. Cylindrical-trapezoidal body.

The oxidative deamination activity of glutamate dehydrogenase (GDH) was determined using a Glutamic Acid Assay Kit (#D799586, Sangon Biotech, China) by measuring NADH formation at 340 nm. Each reaction contained 200 µL of 1 µmol/mL glutamate solution and 10 mg/mL of immobilized enzyme sample, incubated at 37 °C for 30 min. Absorbance was measured at 340 nm using a Synergy Neo2 multimode microplate reader (SynergyNeo2, BioTek, USA).The cylindrical-trapezoidal model showed a 5% increase in oxidative deamination activity compared to the uniform cylindrical model.

