Co-Immobilization of SpyTag-Cyclized Enzymes on a γPFD-SpyCatcher Hydrogel to Address Broad Specificity

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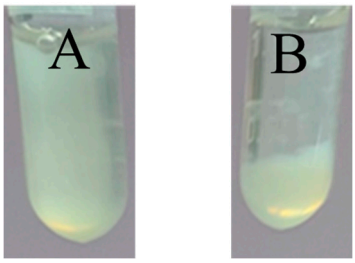
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Supplementary Table S1 Sequence of enzymes used in this study

Name	Sequence
SpyCatcher	gdsathikfskrdedgkelagatmelrdssgktistwisdgqvkdfylypgkytfvetaapdgyevatai tftvneqgqvtvng
SpyTag	mahivmvdaykpt
ʁPFD	mvnevidineavrayiaqieglraeigrldatiatlrqslatlkslktlgegktvlvpvgsiaqvemkvek mdkvvvsvgqnisaeleyeealkyiedeikklltfrlvleqaiaelyakiedliaeaqqtseeekaeeeen eekae
ispA	mdfpqqleacvkqanqalsrfiaplpfqntpvvetmqygallggkrlrpflvyatghmfgvstntldap aaavecihayslihddlpamddddlrrglptchvkfgeanailagdalqtlafsilsdadmpevsdrdris miselasasgiagmcggqaldldaegkhvpldalerihrhktgaliraavrlgalsagdkgrralpvldk yaesiglafqvqddildvvgdtatlgkrqgadqqlgkstypallgleqarkkardliddarqslkqlaeqsl dtsalealadyiiqrnk
PS	gpgslqrnglqqgtsnqrfgfhvgngitvtvlvavvertcnvecvffsadwnvniwlqqfpvqafdyv gdhrvnviqsvffrhtwvvfhitrngrgfftastvrfvatgvatqtqnsarqvvqfrrkvhflqnmirerni hsqdrlqsadtmaarnftvfvvfikgrqipgrnpasllhvrvdvrfpclarigqgvtalslfsltghfihgv vnsniyafhvlwqainarrvpafhsggeqfqfvygaegvvhivqhgdqvtgfceaqtetavlrfksda arkrivfnvtvsaeghfretgflppayqrfqltflkgledvkfqfrqfqeffglnirlsvfcilpenlnivtsfq trqvsvptvfqhiadfaaqrgdrdflqclfqifggknfcfihdffarkcnqrrakqv*natdffvgldvfra gelailtfecfedirghrvamqtqgtqaqsgavqvgydrltttpnavltpvrvyvvqsafdfifemtvda qtfqgiydpqaldqvvqrahqltifqthavehvlyftdkffrtfqvgfflvcgrqrldhvvvpkvrvkitht tthavvghngsgrhgdarsdrfatatrttrsfef

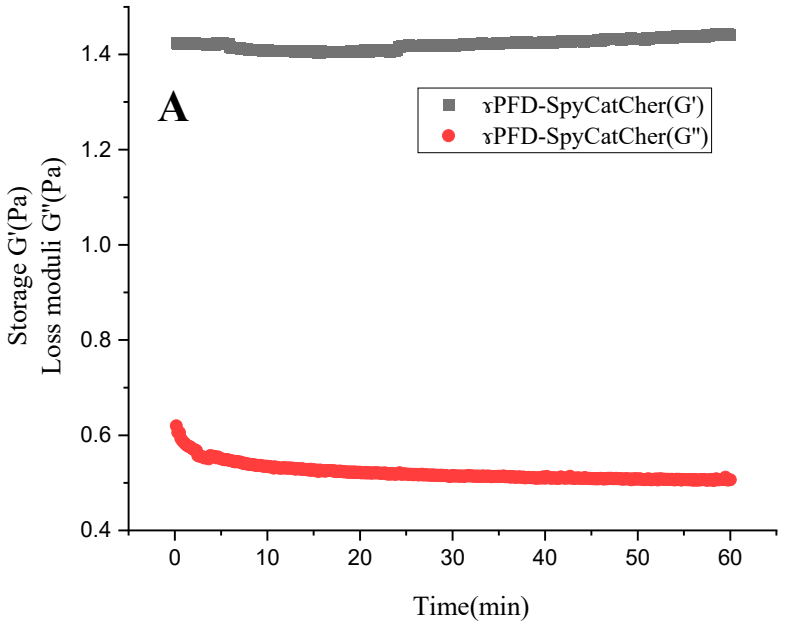
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TAL	Maprptsqnqtrtcpttqvtqvdivekmlaaptdstleldgyslnlgdvvsaarkgrpvrvkdsdeirsk idksveflrsqlsmsvygvttgfggsadtrtedaislqkallehqlcgvlpssfdsfrlgrglenslplevvr gamtirvnsltrghsavrlvvlealtnflnhgitpivplrgtisasgdlsplsyiaaaisghpdskvhvvheg kekilyareamalfnlepvvlgpkeglglvngtavsasmatlalhdahmlsllsqsltamtveamvgh agsfhpflhdvtrphptqievagnirkllegsrfavhheeevkvkddegilrqdryplrtspqwlgplvs dlihahavltieagqsttdnplidvenktshhggnfqaaavantmektrlglaqigklnftqltemlnag mnrglpsclaaedpslsyhckgldiaaaaytselghlanpvtthvqpaemanqavnslalisarrttesn dvlslllathlycvlqaidlraivfefkkqfgpaivslidqhfgsamtgsnlrdelvekvnktlakrleqtns ydlvprwhdafsfaagtvvevlsstslslaavnawkvaaaesaisltrqvretfwsaastsspalsylsprt qilyafvreelgvkarrgdvflgkqevtigsnvskiyeaiksgrinnvllkmla*

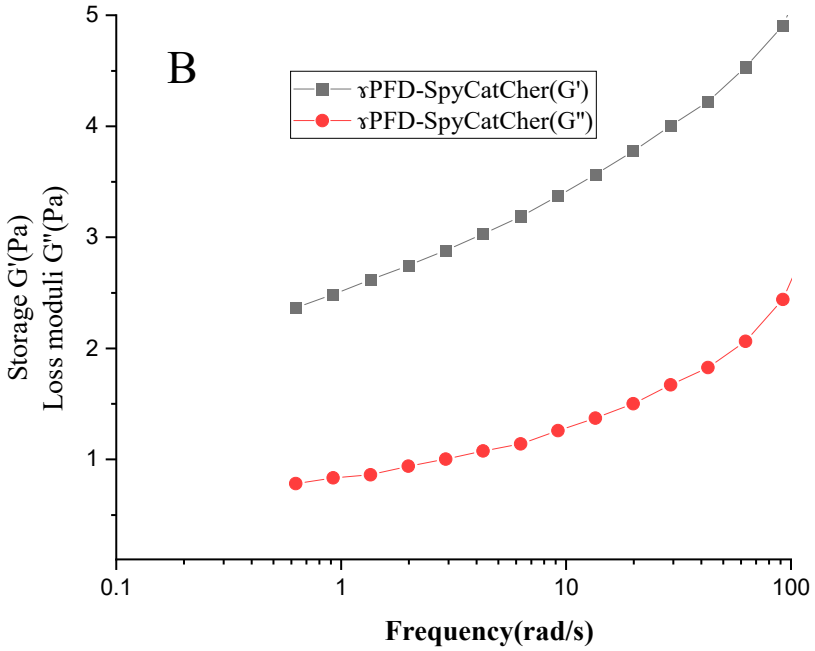
4CL	Mapqeqavsqvmekqsnnnnsdvifrsklpdiyipnhlslhdyifqnisefatkpclingptghvyty sdvhvisrqiaanfhklgvnqndvvmlllpncpefvlsflaasfrgatataanpfftpaeiakqakasntk liitearyvdkikplqnddgvvivciddnesvpipegclrfteltqstteasevidsveispddvvalpyss gttglpkgvmlthkglvtsvaqqvdgenpnlyfhsddvilcvlpmfhiyalnsimlcglrvgaailimp kfeinllleliqrckvtvapmvppivlaiakssetekydlssirvvksgaaplgkeledavnakfpnaklg qgygmteagpvlamslgfakepfpvksgacgtvvrnaemkivdpdtgdslsrnqpgeicirghqim kgylnnpaataetidkdgwlhtgdiglidddelfivdrlkelikykgfqvapaelealighpditdvav vamkeeaagevpvafvvkskdselseddvkqfvskqvvfykrinkvfftesipkapsgkilrkdlrak langl*

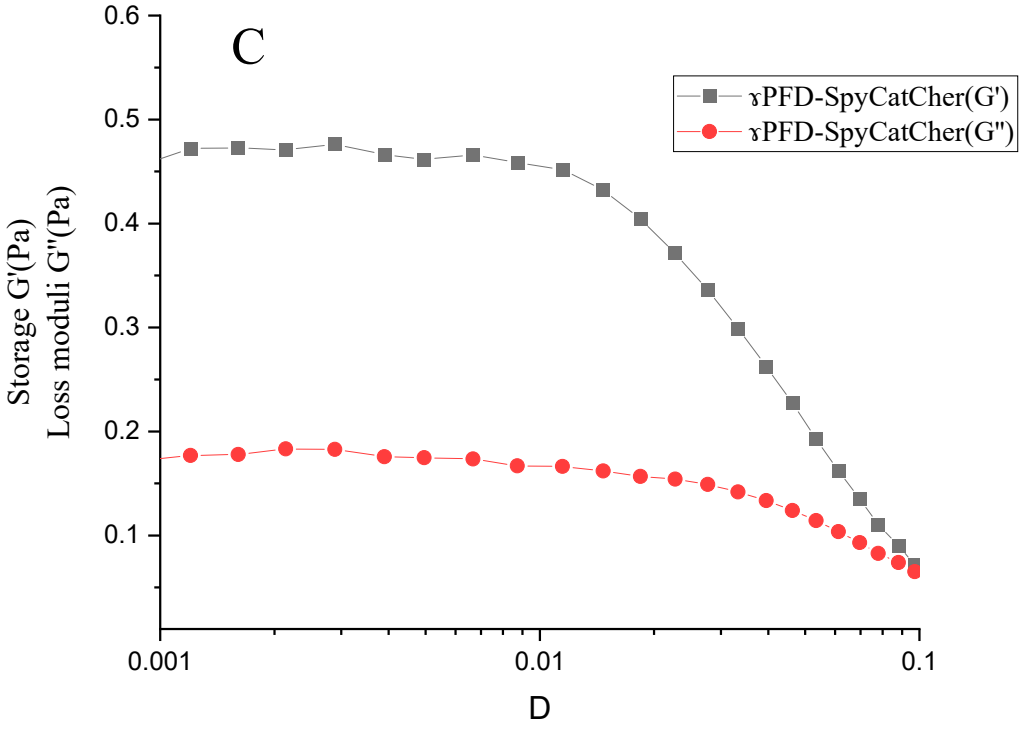


Supplementary Figure S1 SpyCatcher-mediated ɤPFD nanoscaffold hydrogel.

(A) Normal cells rupture without precipitating any water gel. (B) ɤPFD nanoscaffold protein hydrogel.

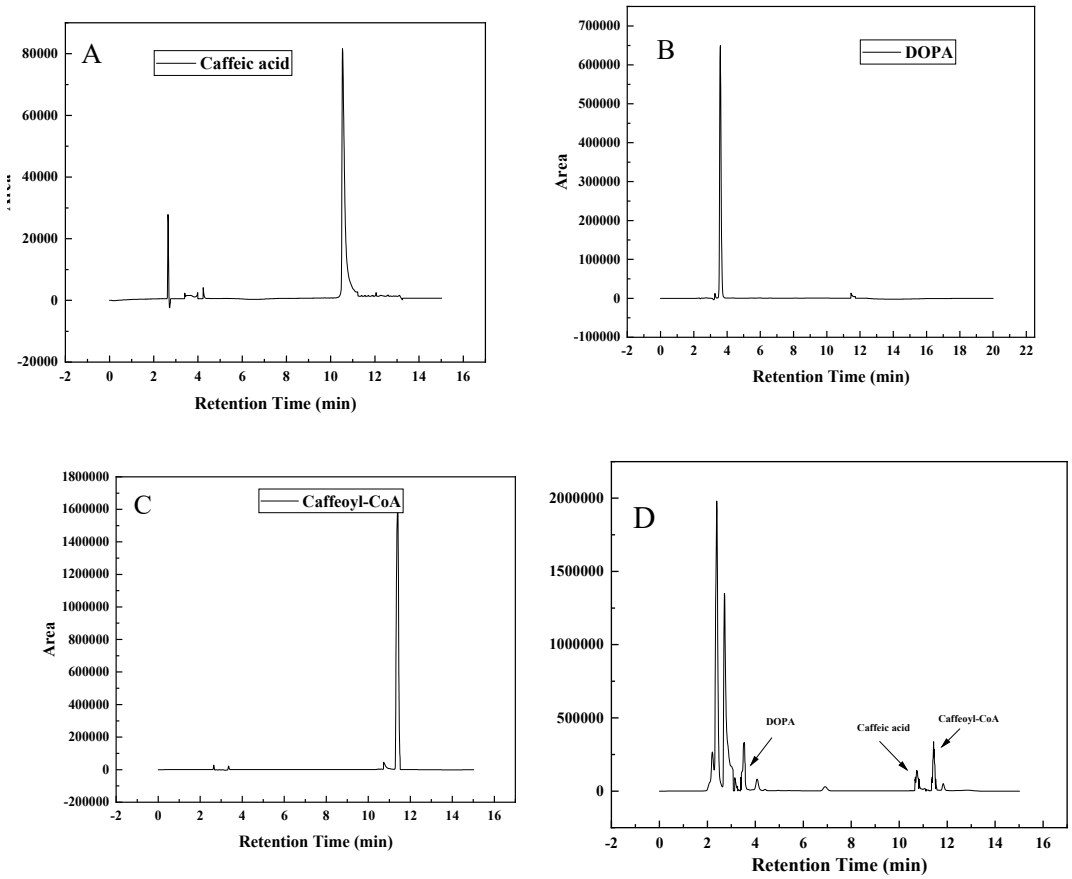

A
ʁPFD-SpyCatCher(G')
ʁPFD-SpyCatCher(G")
Storage G'(Pa)
Loss moduli G"(Pa)
Time(min)
1.4
1.2
1.0
0.8
0.6
0.4
0
10
20
30
40
50
60


B
ʁPFD-SpyCatCher(G')
ʁPFD-SpyCatCher(G")
Storage G'(Pa)
Loss moduli G"(Pa)
Frequency(rad/s)
5
4
3
2
1
0.1
1
10
100



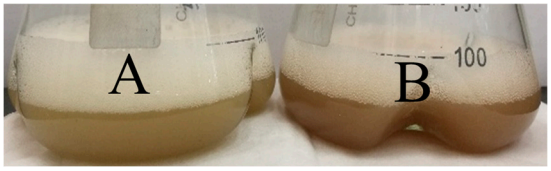
Supplementary Figure S2 SpyCatcher-mediated ɤPFD protein hydrogel viscoelastic properties

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



Supplementary Figure S3 HPLC chromatogram.

(A) The standard sample of caffeic acid. (B) The standard sample of DOPA. (C) The standard sample of Caffeoyl-CoA. (D) SpyTag-cycled HpaBC-TAL-4CL.



Supplementary Figure S4 Color contrast during the fermentation process.

(A) E. coli TYR containing pZEA-SpyTag- TAL- HpaBC-4CL-SpyTag and pZS-ʁPFD-SpyCatcher. (B) E. coli TYR containing pZEA-SpyTag-HpaBC-TAL-4CL-SpyTag and pZS-ʁPFD-SpyCatcher. The color of the fermentation broth can be visually assessed to indicate a significant residual DOPA based Figure 2B.