Electronic Supplementary Material (ESI) for Polymer Chemistry.

Electronic Supplementary Information

Site-specific immobilization of proteins on non-conventional substrates via solvent-free initiated chemical vapor deposition (iCVD) process

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S1: Materials and methods

Construction of expression vectors

All *E. coli* strains and plasmids used in this work are listed in Table S1. Schematic view of gene expression systems are shown in Fig. S1. A polymerase chain reaction (PCR) was performed with the C1000™ Thermal Cycler (Bio-Rad, Hercules, CA, USA) using PrimeSTAR HS polymerase. The sequences of the primers used in this work are listed in Table S2. For the expression of autofluorescent proteins (mOrange and mRFP) genes, both genes were amplified by PCR. A mRFP gene was amplified from pRSETB-mRFP by PCR with two primers (F-1 and R-1). The PCR product was digested with two restriction enzymes, *Nde*I and *Hin*dIII, and then ligated into same restriction enzyme sites of pMoPac1 yielding pJGM-01. For the expression of Cys-linked mRFP gene, PCR was performed with two primers (F-1 and R-2) and same template DNA (pRSETB-mRFP). After digestion with *Nde*I and *Hin*dIII, PCR product was ligated into same restriction enzyme sites of pMoPac1 yielding pJGM-02. A mOrange coding gene was amplified from pmOrange by PCR with two primers (F-2 and R-3). For the expression of Cys-linked mOrange, PCR was performed with two primers (F-3 and R-4) and same template DNA (pmOrange). Both PCR products were digested with *Nde*I and *Hin*dIII and, they were ligated into same restriction enzyme sites of pMoPac1 yielding pJGM-03 and pJGM-04, respectively. In pJGM-02 and pJGM-04, Cys residue was linked to C-terminus of mRFP and mOrange via short peptide linker (Glu-Ala-Ala-Ala-Lys).For the M18 scFv gene expression, M18 scFv gene was amplified from pM18-D4 by PCR with primers F-3, F-4, R-5 and R-6. The PCR product was digested with *Nde*I and *Hin*dIII, and then cloned into same sites of pMoPac1 yielding pJGM-05. A KD548 gene was amplified from pPICZa-KD548 by PCR with primers F-5, R-7, and R-8, and after

digestion with *Nde*I and *Hin*dIII, the digested PCR products were cloned into *Nde*I and *Hin*dIII sites of pMoPac1 yielding pJGM-06. In both constructs (pJGM-05 and pJGM-06), Cys residue was linked to C-terminus of M18 scFv and KD548 via short peptide linker (Glu-Ala-Ala-Ala-Lys) which was repeated three times. In pJGM-05, PelB leader sequence was introduced for the secretory production of M18 scFv into periplasm of *E. coli*. All DNA manipulations, including restriction digestion, ligation, and agarose gel electrophoresis, were carried out using standard procedures.[1]

S2: Supporting figures

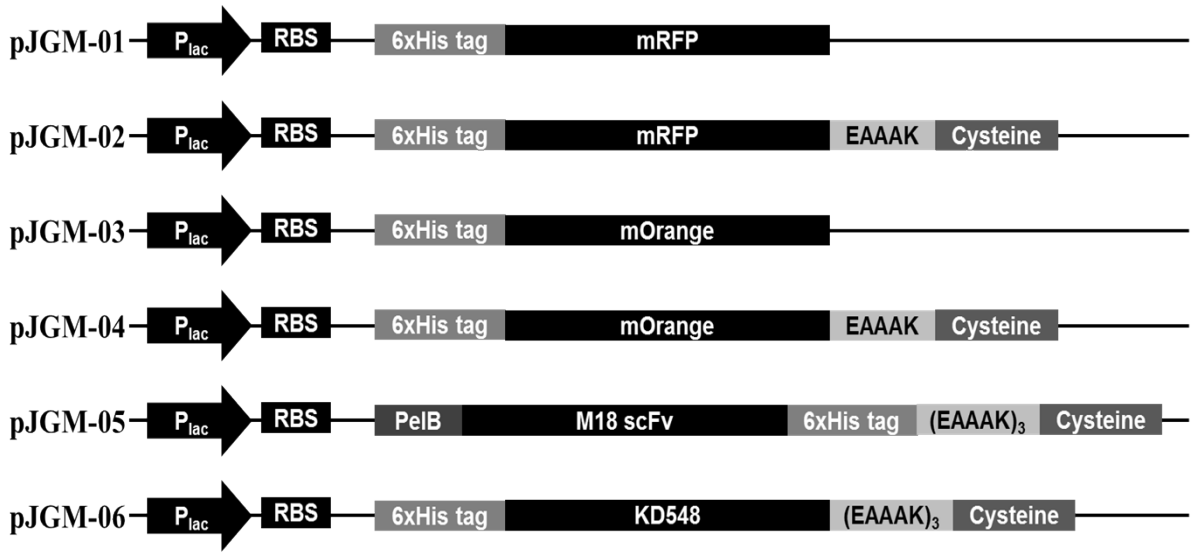

Fig. S1 Schematic view of the gene expression systems. (P_{lac}, *lac* promoter; RBS, ribosome-b inding site, EAAAK, short peptide linker)

S3: Supporting tables

Table S1 *E. coli* strains and plasmids used in this study

Strains/ plasmids	Characteristics	Ref. or sources
E. coli strains		
XL1-Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lacI*^{q}*ZΔM15* Tn*10* (Tet^{r})]	Stratagene[a]
BL21(DE3)	*E. coli* B F– *dcm ompT hsdS*(r_B^- m_B^-) *gal* λ(DE3)	Stratagene[a]
Plasmids		
pMoPac1	ColE1 origin, Cm^{r}, *lac* promoter, lacI^{q}	Hayhurst *et al*.[2]
pRSET$_B$-mRFP	pRSET$_B$ derivative, mRFP	Campbell *et al*.[3]
pmOrange	mOrange expression vector	Shaner et al[4]
pJGM-01	pMoPacI, 6xhistidine tag- mRFP	This study
pJGM-02	pMoPacI, 6xhistidine tag-mRFP-EAAAK-cysteine	This study
pJGM-03	pMoPac1, 6xhistidine tag-mOrange	This study
pJGM-04	pMoPac1, 6xhistidine tag-mOrange-EAAAK-cysteine	This study
pM18-D4	pMoPac1 derivative, Co-expression of M18 scFv and PA-D4-FLAG	Jeong *et al*.[5]
pJGM-05	PelB-M18-6xhitidine tag-(EAAAK)$_3$-cysteine	This study
pJH11	GST-fused PA domain 4, Amp^{r}	Park *et al*.[6]
pPICZa-KD548	pPICZa derivative, KD548 gene	Lee *et al*.[7]
pJGM-06	pMoPacI, 6xhistidine tag-KD548-(EAAAK)$_3$-cysteine	This study
pET21b-DR5	pET21b derivative, DR5 expression vector	Lee *et al*.[7]

[a]Stratagene, Santa Clara, CA

Table S2 Sequences of oligonucleotides used in this study

Primer name	Sequence
F-1	5’- atatatatcatatgcaccaccatcaccatcacgcctcctccgaggacg -3’
F-2	5’- atatatatcatatgcaccaccatcaccatcacgtgagcaagggcgagg -3’
F-3	5’- ggattgttattactcgcggcccagccggccatggcggatattcagatgacacagactacatcc -3’
F-4	5’- atatatatcatatgatgaaatccctattgcctacggcagccgctggattgttattactcgcggccc -3’
F-5	5’- atatatatcatatgcaccatcaccatcaccatgaggaatgtcaccagaccca -3’
R-1	5’- ataaagcttctattaacatttagcagcagcttcggcgccggtggagt -3’
R-2	5’- ataaagcttctattaggcgccggtggagt -3’
R-3	5’- tataagcttctattacttgtacagctcgtccatgc -3’
R-4	5’- tataagcttctattaacatttagcagcagcttccttgtacagctcgtccatgc -3’
R-5	5’- cttggcggcggcctctttagcagcagcttcgtgatggtgatgatgatggtcga -3’
R-6	5’- atatcactaacatttagcagcagcttccttggcggcggcctc -3’
R-7	5’- gcggcttctttggccgcggcttctttcgcggccgcttctggaggtgttgtgcagc -3’
R-8	5’- tataagcttctattagcatttcgccgcggcttctttggccg -3’

4. References

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