Electronic Supplementary Information

for

Site-specific Protein Labelling and Immobilization Mediated by Microbial Transglutaminase

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Contents

Expression and purification of MTG

Microbial transglutaminase expression and purification from bacterial lysates were performed as previously described[1]. In summary, pDJ1-3 plasmid, containing mTG, was transformed into *Escherichia coli* BL21-Gold (DE3) under 100 mg/ml ampicillin. mTG was expressed using a published auto-induction protocol[2]. The culture was centrifuged at 3000 g for 30 min. Subsequent pellets were resuspended in 50 mM phosphate buffer (pH 8.0) with 300 mM NaCl and placed on ice. Suspended pellets were lysed by sonication (3 cycles of 2 min pulses, at a 20% intensity/1 min pause between cycles) using a Branson sonicator. After lysis, lysate was centrifuged at 18000 g for 30 min, followed by filtration using 0.45-µm filters. Activation of mTG requires cleavage of the pro-sequence at the *N*-terminus of the enzyme with a trypsin digest (1mg/ml), incubated at a 1:10 ratio (w/v) for 75 min at 37 ºC. The activated mTG was purified using a 1-ml His Trap Ni-NTA column (GE Healthcare) equilibrated in 50 mM phosphate buffer (pH 8.0) with 300 mM NaCl and eluted with an imidazole gradient (0–140 mM) on an Åkta FPLC instrument (GE Healthcare). The purified activated mTG was dialyzed against 50 mM phosphate buffer (pH 8.0). Protein concentration was quantified using Bradford protein assay.

Cloning and expression of MBP-Qtag proteins

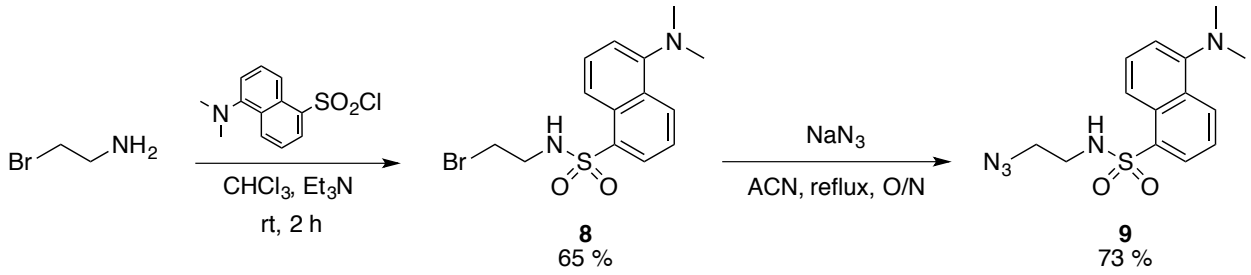
Tagging expression and purification of MBP variants was performed using established molecular biological techniques. In short, for amplification of MBP-7M38/42/48, forward primer (GATCGCTGTTGAAGCGTTATCGCTGATTTATAACAAAGATCTGCTGCCGAACCCGCC AAA) containing Bg1II recognition site and reverse primer (GTCGACGAATTCAAACCA TCCTTGCATTGGATAGATATCGCGGCCGCCCATGGACATATGTGAAATCCTTCCCTC/ GTCGACGAATTCATATGGACGTTGTAATTCCCAGATATCGCGGCCGCCCATGGACAT ATGTGAAATCCTTCCCTC / GTCGACGAATTCATGTGGACGTTGTAATGCCCA GATATCGCGGCCGCCCATGGACATATGTGAAATCCTTCCCTC) containing EcoRI recognition site were used for PCR with a pMAL-c5X as a template. The reaction was performed in a BioRad® thermal cycler. After amplification, digestion and ligation into pMAL-c5X vector were performed. Constructed vector was expressed in *Escherichia coli* BL21-Gold (DE3) under 100 mg/ml ampicillin. Inclusion of the Q-tag was verified through sequencing. Purification of maltose binding protein has been established and the protocol is summarized as followed. A 1-ml sample of MBP pre-culture was used to inoculate a culture of 500 ml LB media + 0.2% glucose. Culture was incubated at 37 ºC until it reached an OD600 of 0.5, followed by the addition of IPTG to a final concentration of 0.1 mM for 2 hours. Cells were harvested through centrifugation (4000 g for 20 min). Cells were resuspended in buffer (20 mM Tris-HCl, 200 mM NaCl 1 mM EDTA, pH 7.4). Suspended pellets were lysed by sonication (3 cycles of 1 min pulses, at a 20% intensity/1 min pause between cycles) using a Branson sonicator. After lysis, lysate was centrifuged at 20000 g for 20 min, followed by filtration using 0.45-µm filters. A 2-ml volume of amylose resin was added to the column, followed by washing with buffer for 5 column volumes. Once the resin was conditioned, the lysate was poured onto the column with resin and incubated

at 4 ºC for 2 h while shaking. Using gravity chromatography, the flow-through was collected, followed by 3 washes (using 2 column volumes per wash). MBP-Q was eluted with 1 column elution buffer (20 mM Tris-HCl, 200 mM NaCl 1 mM EDTA, 10 mM Maltose, pH 7.4). Protein concentration was quantified using Bradford protein assay.

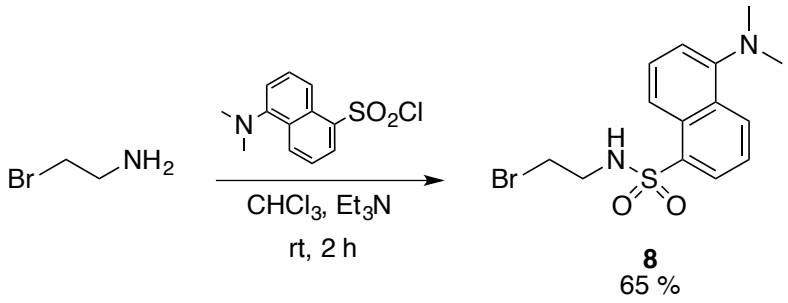
Cloning and expression of RFP-Qtag proteins

Tagging expression and purification of mRuby2-7M48 was performed using established molecular biological techniques. In short, for amplification of mRuby2-7M48, forward primer (AAGGATCCGATGGTGTCTAAGGGCGAAGAGCTG) containing a BamHI recognition site and reverse primer (AGAGAATTCTTACTTGTACAGCTCGTCATGTGGTCTTTGTAATGCCCAACTACTTCC ACTACTTCC) containing a EcoRI recognition site were used for PCR with a pCDNA3-mRuby2 as a template. The reaction was performed in a BioRad® thermal cycler. After amplification, digestion and ligation into pET11a vector were performed. The constructed vector was expressed in *Escherichia coli* BL21-Gold (DE3) under 100 mg/ml ampicillin. Inclusion of the Q-tag was verified through sequencing. Purification of maltose binding protein has been established and the protocol is summarized as followed. 1ml of pET11a-mRuby2-Q preculture was used to inoculate a culture of 500 ml TB media. The culture was incubated at 37 ºC until it reached an OD600 of 0.5, followed by the addition of IPTG to a final concentration of 0.1 mM for 3 hours. Cells were harvested through centrifugation (3000 g for 30 min). Cells were then resuspended in buffer (50 mM phosphate buffer, 250 mM NaCl 1 mM EDTA, pH 8). Suspended pellets were then lysed by sonication (3 cycles of 2 min pulses, at a 20% intensity/1 min pause between cycles) using a Branson sonicator. After lysis, lysate was centrifuged at 18000 g for 30 min, followed by filtration using 0.45-µm filters. The mRuby2 lysate was purified using a 1-ml His Trap Ni-NTA column (GE Healthcare) equilibrated in 50 mM phosphate buffer (pH 8.0) with 300 mM NaCl and eluted with an imidazole gradient (0–140 mM) on an Åkta FPLC instrument (GE Healthcare). The purified activated mTG was dialyzed against 50 mM phosphate buffer (pH 8.0). Protein concentration was quantified using Bradford protein assay.

Synthesis of dansylethylazide

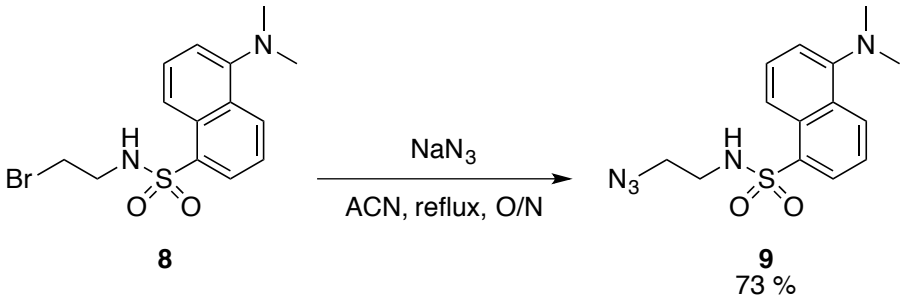


Scheme S1: Synthesis of 2-Azido-1-*N*-dansylethylamine



2-bromo-1-*N*-dansylethylamine (8): A solution of 2-bromoethylamine (0.21 g, 1.04 mmol, 1.1 eq) in $CHCl_3$ (5 mL) was added to Et_3N (0.35 ml, 2.5 mmol, 2.5 eq). While stirring, a solution of dansyl chloride (0.24g, 0.91 mmol, 1.0 eq) in $CHCl_3$ (1 mL) was added dropwise. The reaction mixture was stirred at room temperature for 2.5 h. The solvent was then evaporated and a yellow oil was obtained and dissolved in EtOAc. This was washed three times with a solution of HCl (1M) in water, three times with a saturated solution of $NaHCO_3$ and three times with a saturated solution of NaCl. The organic phase was dried over $MgSO_4$ and evaporated to give **8**, as a yellow oil (231 mg, 0.65 mmol, 65%).

^{1}H NMR ($CDCl_3$, 300 MHz) : δ (ppm) 8.54 (m, 1H); 7.25 (m, 2H); 7.54 (m, 2H); 7.18 (m, 1H); 5.47 (s l, 1H); 3.27 (s, 4H); 2.86 (s, 6H); **^{13}C NMR ($CDCl_3$, 75 MHz)** : δ (ppm) 152.0; 134.5; 130.8; 129.9; 129.8; 128.6; 128.4; 123.1; 118.6; 115.3; 45.5; 44.6; 31.5; 27.9



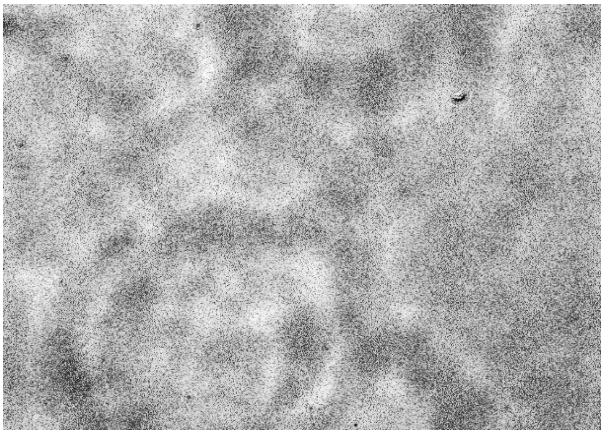
8 **9**
73 %

2-Azido-1-*N*-dansylethylamine (9): The yellow oil **8** (1.14 g, 3.19 mmol, 1.0 eq), was mixed with ACN (50 mL) followed by the addition of NaN_3 (0.84 g, 12.96 mmol, 5.0 eq) while stirring. The reaction mixture was heated to reflux overnight. After evaporation of the solvent, the aqueous phase was extracted with EtOAc. The organic phase was washed three times with water and once with a saturated solution of NaCl. Thereafter, the solution was dried over $MgSO_4$ and evaporated to dryness. The oil obtained was purified by flash chromatography on silica gel with Hex / EtOAc (7:3) to give **9** as a yellow oil (750 mg, 2.34 mmol, 73%).

^{1}H NMR ($CDCl_3$, 300 MHz) : δ (ppm) 8.57 (d, 1H, J=8.49 Hz); 8.26 (d, 2H, J=7.65 Hz); 7.60 (t, 1H, J=7.86 Hz); 7.53 (t, 1H, J=8.49); 7.21 (d, 1H, J=7.32); 4.96 (t, 1H, J=6.66 Hz); 3.31 (t, 2H, J=5.49 Hz); 3.06 (q, 2H, J=5.52, 6.15); 2.89 (s, 6H) **^{13}C NMR ($CDCl_3$, 75 MHz)** : δ (ppm) 130.8; 129.6; 128.7; 123.1; 118.4; 115.4; 50.9; 45.4; 42.4. **IR** : 2104 cm^{-1}.

Imaging of mRuby2-conjugated Nanoparticles

A



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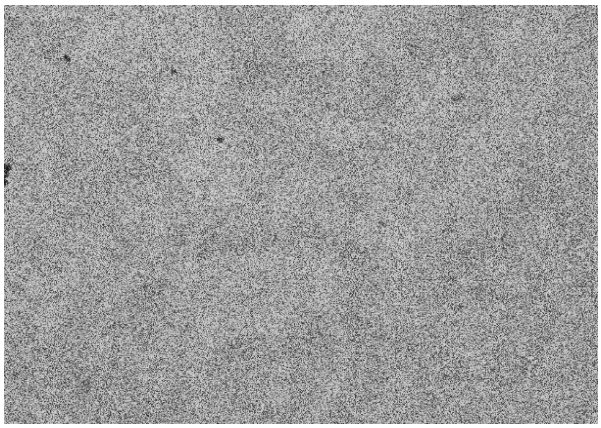

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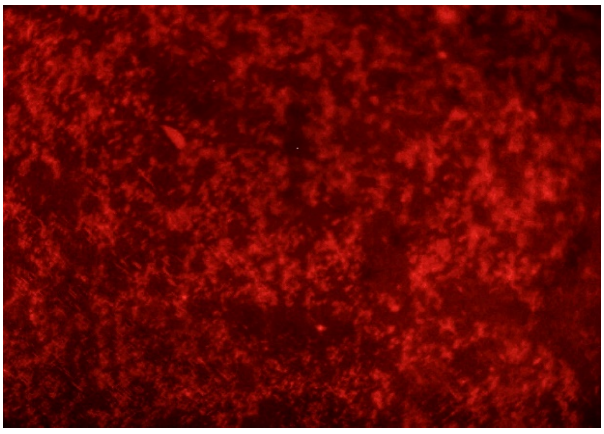
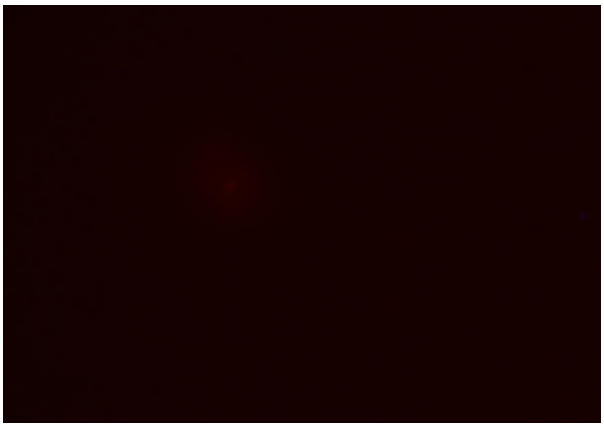


D



Imaging of magnetic, azide-functionalized nanoparticles (30 nm/particle), conjugated through cycoaddition to propargylated mRuby2-Qtag. Images were taken with confocal fluorescence microscope using appropriate filters at a magnification of 40X. A) Bright field imaging of mRuby2-Qtag, conjugated to nanoparticles through mTG-mediated propargylation of the protein and B) control experiment performed in the absence of mTG. C) Dark field imaging of mRuby2-Qtag, immobilized to nanoparticle through mTG-mediated propargylation of the protein and B) control experiment performed in the absence of mTG.

References

(1) Oteng-Pabi, S. K.; Keillor, J. W. *Anal. Biochem.* **2013**, *441*, 169–173.

(2) Studier, F. W. *Protein Expr. Purif.* **2005**, *41*, 207–234.