Electronic Supplementary Material (ESI) for ChemComm.

Supercharging SpyCatcher toward an Intrinsically Disordered Protein with Stimuli-Responsive Chemical Reactivity

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Electronic Supplementary Information

Experimental Section

DNA Construction. All oligonucleotide primers were ordered from Invitrogen. Sequence encoding SpyTag-ELP-SpyCatcher (AB) is as reported before.[1] All mutants toward SpyTag-ELP-SpyCatcher(-) were obtained by QuickChange Site-directed mutagenesis.[2] The SpyCatcher and SpyTag-YFP were sub-cloned into pET-28a for expression. The SpyCatcher(-) was amplified by PCR directly from the plasmid pQE-80L containing SpyTag-ELP-SpyCatcher(-) and inserted into pET-28a for expression. All DNA sequences were confirmed by direct sequencing.

Protein Expression and Purification. Single colony was inoculated into 5 mL of LB medium with certain antibiotics (100 μg/mL ampicillin for pQE-80L vectors or 50 μg/mL kanamycin for pET-28a vectors) and grown in a shaker (37 ^{o}C, 250 rpm) overnight. The cultures were inoculated to 200 mL of 2xYT medium containing certain antibiotics following the procedures reported.[3, 4] Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM to induce target protein expression. Then, SpyTag-ELP-SpyCatcher(-) cultures were shaken at 16 ^{o}C for 20 hours before cells were harvested via centrifugation (20 min x 6000 g at 4 ^{o}C), while SpyTag-YFP, SpyCatcher and SpyCatcher(-) were shaken at 37 ^{o}C for 5~6 hours. Cell pellets were lysed by ultrasonication in lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, 5% glycerol, pH=8.0). Supernatant was collected after centrifugation (20 min x 25000 g at 4 ^{o}C) and then mixed with Ni-NTA resin (GE Healthcare, Inc.) in lysis buffer to capture target proteins. Resins were washed by lysis buffer for 4~5 column volumes, wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, 5% glycerol, pH=8.0) for 4~5 column volumes and eluted by elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 300 mM imidazole, 5% glycerol, pH=8.0). The eluate was collected and further purified by Superdex 200 increase

10/300 GL column in an ÄKTA FPLC system (GE Healthcare, Inc.), using PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH = 7.4) as the mobile phase at a flow rate of 0.5 mL/min. The target peak was collected, rapidly frozen by liquid nitrogen, and stored at -80 ^oC.

Protein Characterization. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed to analyze the topology of SpyTag-ELP-SpyCatcher mutants and quantify the product of SpyCatcher(-) reacted with SpyTag-YFP. Samples were mixed with 5x SDS-PAGE loading buffer (250 mM Tris-HCl, 50% glycerol, 10% SDS, 250 mM β-mercaptoethanol, 0.05% bromophenol blue). Then, they were heated at 98 ^oC for 10 min before loading into the gel wells. Relative protein quantification was performed using Typhoon FLA 9500 (GE Healthcare, Inc.) and ImageQuant TL software (GE Healthcare, Inc.). Molecular weight of SpyCatcher(-) was detected by using Ultra-performance liquid chromatography-electrospray ionization mass spectrometry with quadrupole rods SQ Detector 2 mass spectrometer (Waters Corp.). MassLynx V 4.1 (Waters Corp.) was used to convert the *m/z* spectrum to the mass spectrum using MaxEnt1 algorithm (Waters Corp.). Matrix assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF MS) was conducted on a MALDI TOF/TOF 5800 mass spectrometer (AB Sciex, USA) with sinapinic acid as the matrix.

Reaction Between SpyTag-YFP and SpyCatcher(-). Protein concentrations were measured by UV-absorbance with NanoPhotometer P330 (Implen, Inc.). The pH of buffers was adjusted and confirmed by pH Meter FE20-FiveEasy™ (Mettler Toledo International, Inc.). From pH 5.8 to pH 7.5, 20 mM NaH_2PO_4-Na_2HPO_4 buffering pair was used, while from pH 8.0 to pH 9.5, 20 mM Tris-HCl was used. Proteins were added into the buffers in stoichiometric amounts and incubated at certain temperatures in a thermal cycler (Bio-Rad Inc.).

CD Spectroscopy. Concentrations of the stock samples were determined by NanoPhotometer

P330 (Implen, Inc.) and confirmed by Bradford method.[5] Before measurement, samples were diluted into 0.01x PBS until a final concentration of 0.02 mg/mL. The CD spectra were recorded on a MOS-500 spectrometer (Bio-Logic., France). Each sample and its buffer were scanned every 0.5 nm from 190 nm to 250 nm with 10 mm path length in triplicate. Time per point was set as 1 s.

2D ^{15}N-^{1}H HSQC-NMR. The ^{15}N-labelled SpyCatcher(-) was expressed in M9 medium (6.8 g Na_2HPO_4, 3.0 g KH_2PO_4, 0.5 g NaCl, 0.49 g $MgSO_4 \cdot 7\ H_2O$, 1g $^{15}NH_4Cl$ and 1L dd-H_2O) and handled with the same procedure as described earlier in the section of "Protein Expression and Purification". The samples for NMR spectroscopy were prepared in PBS buffer (pH 7.4) or 200 mM NaAc-HAc buffer (pH 6.0), containing 10% D_2O. Reactions were set at 4 ^{o}C with SpyTag (sequence: AHIVMVDAYKPTKGSGSC). NMR experiments were performed at 37 °C or 15 °C on a Bruker-500 UltraShield spectrometer (Bruker Corp.), equipped with a CryoProbe. All spectra were processed and analyzed with TopSpin 3.5 pl7.

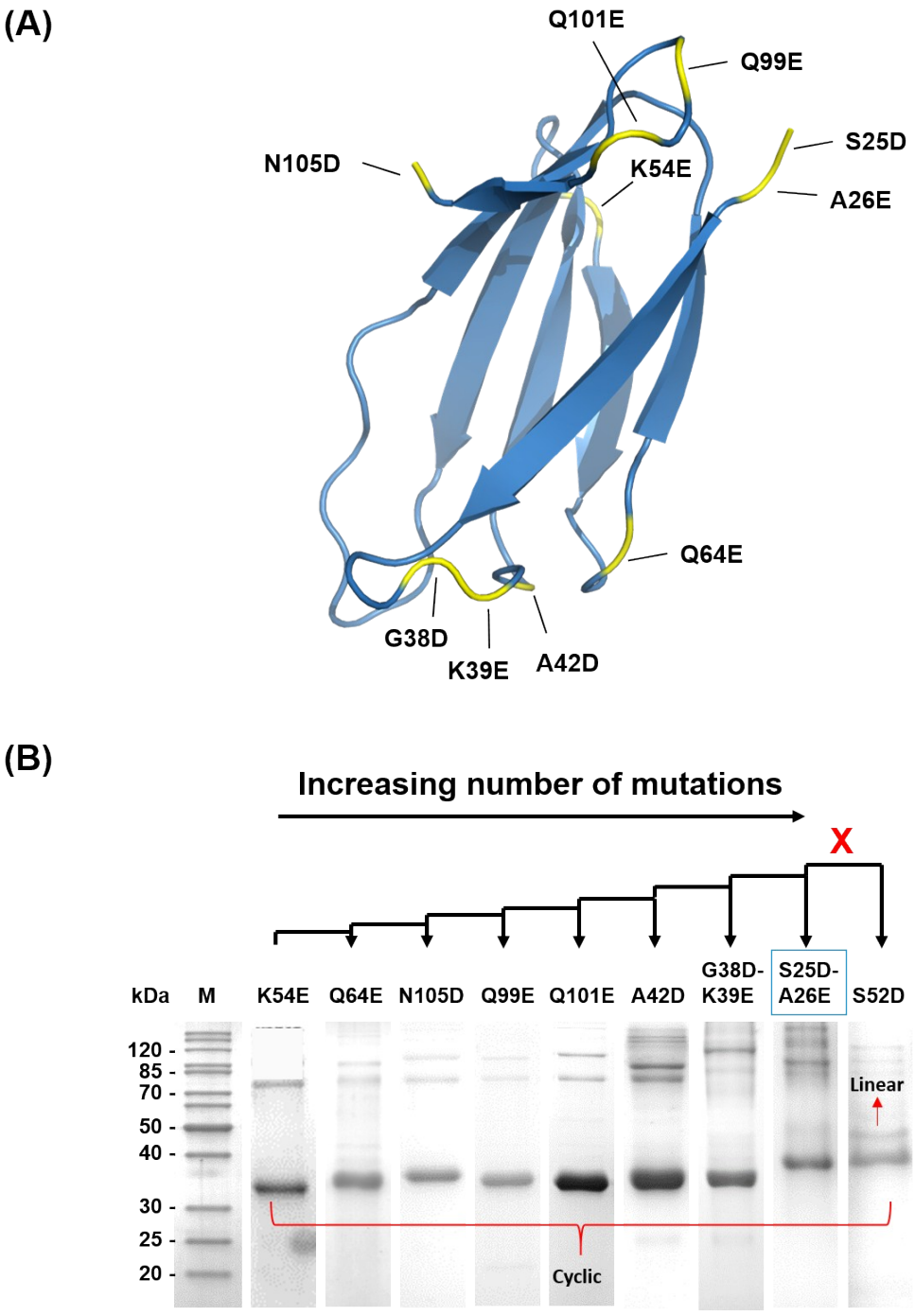

Figure S1. Directed evolution toward supercharging: (A) amino acid residues picked for mutation on SpyCatcher (PDB: 4MLS); (B) SDS-PAGE analysis of SpyTag-ELP-SpyCatcher mutants toward supercharging. Mutations were accumulated from left to right and the mutants except the last one (S52D) show well-preserved reactivity such that the cyclic monomer was the only monomeric product. The blue-square-circled mutant is the final construct SpyTag-ELP-SpyCatcher(-).

(A)

SpyTag-ELP-SpyCatcher(-) 348 a.a. MW=33695

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  1 MKGSSHHHHHHVDAHIVMVDAYKPTKLDGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVP
 61 GVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGELYAVTGRGDSP
121 ASSAPIATSVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGV
181 PGVGVPGVGVPGEGVPGVGVPGVGVPGGLLDIPTTENLYFQGAMVDTLSGLSSEQGQSGD
241 MTIEEDSATHIKFSKRDEDDEELDGATMELRDSDGETISTWISDGEVKDFYLYPGKYTFV
301 ETAAPDGYEVATAITFTVNEEGEVTVDGKATKGDAHIDGPQGIWGQLE*
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(B)

SpyCatcher 138 a.a. MW=14799

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  1 MGSSHHHHHHSSGLVPRGSHMASAMVDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDE
 61 DGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFT
121 VNEQGQVTVNGKATKGDAHI*
```

(C)

SpyCatcher(-) 138 a.a. MW=14993

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  1 MGSSHHHHHHSSGLVPRGSHMASAMVDTLSGLSSEQGQSGDMTIEEDDETHIKFSKRDE
 61 DDEELDGATMELRDSSGETISTWISDGEVKDFYLYPGKYTFVETAAPDGYEVATAITFT
121 VNEEGEVTVDGKATKGDAHI*
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(D)

SpyTag-YFP 280 a.a. MW=31121

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  1 MGSSHHHHHHSSGLVPRGSHMASAHIVMVDAYKPTKGGSGGSMSKGEELFTGVVPILVEL
 61 DGDVNGHKFSVSGEGEGDATYGKLTLKLLCTTGKLPVPWPTLVTTLGYGVQCFARYPDHM
121 KQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILG
181 HKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDN
241 HYLSYQSALFKDPNEKRDHMVLLEFLTAAGITEGMNELYK*
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His-tag SpyTag TEV-recognition SpyCatcher(-) SpyCatcher YFP

Figure S2. Amino acid sequences of (A) SpyTag-ELP-SpyCatcher(-); (B) SpyCatcher; (C) SpyCatcher(-) and (D) SpyTag-YFP. The sequences of His-tag, SpyTag, TEV protease recognition site, SpyCatcher(-), SpyCatcher and YFP are colored as red, green, pink, blue, purple and orange, respectively. The lysine and aspartic acid forming isopeptide bond between SpyTag and SpyCatcher are underlined.

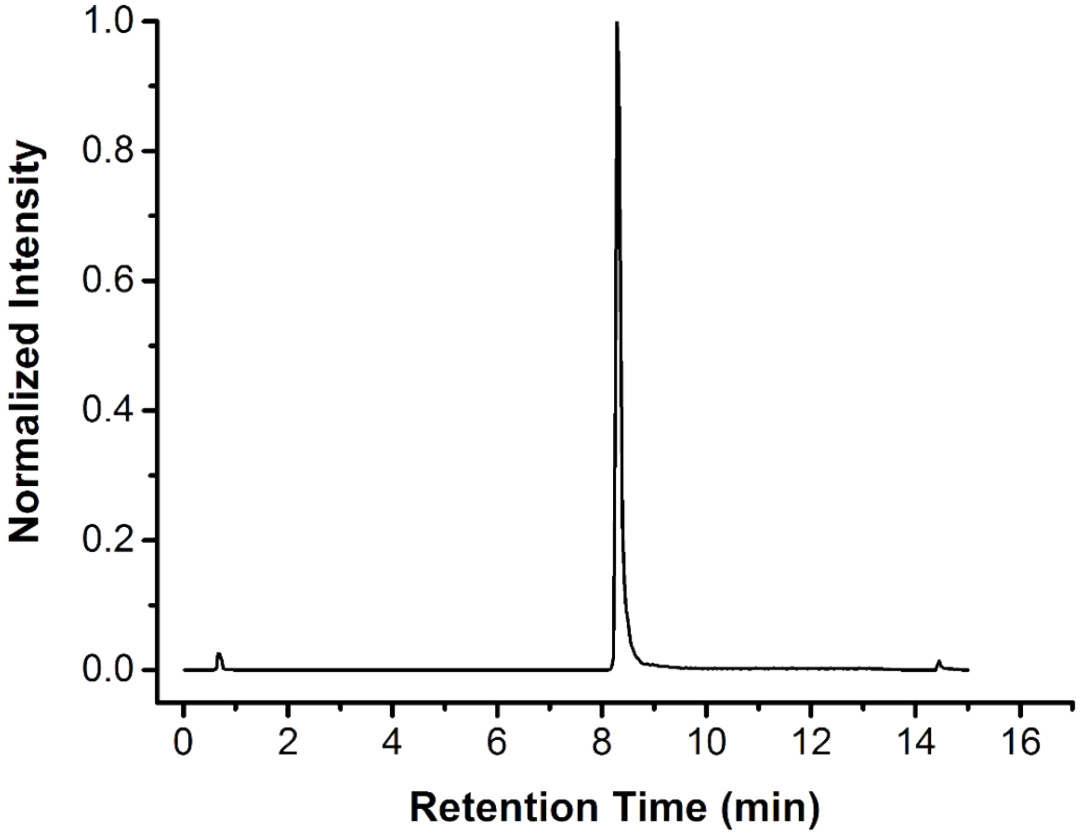

Figure S3. Elution profile of SpyCatcher(-) in LC-MS.

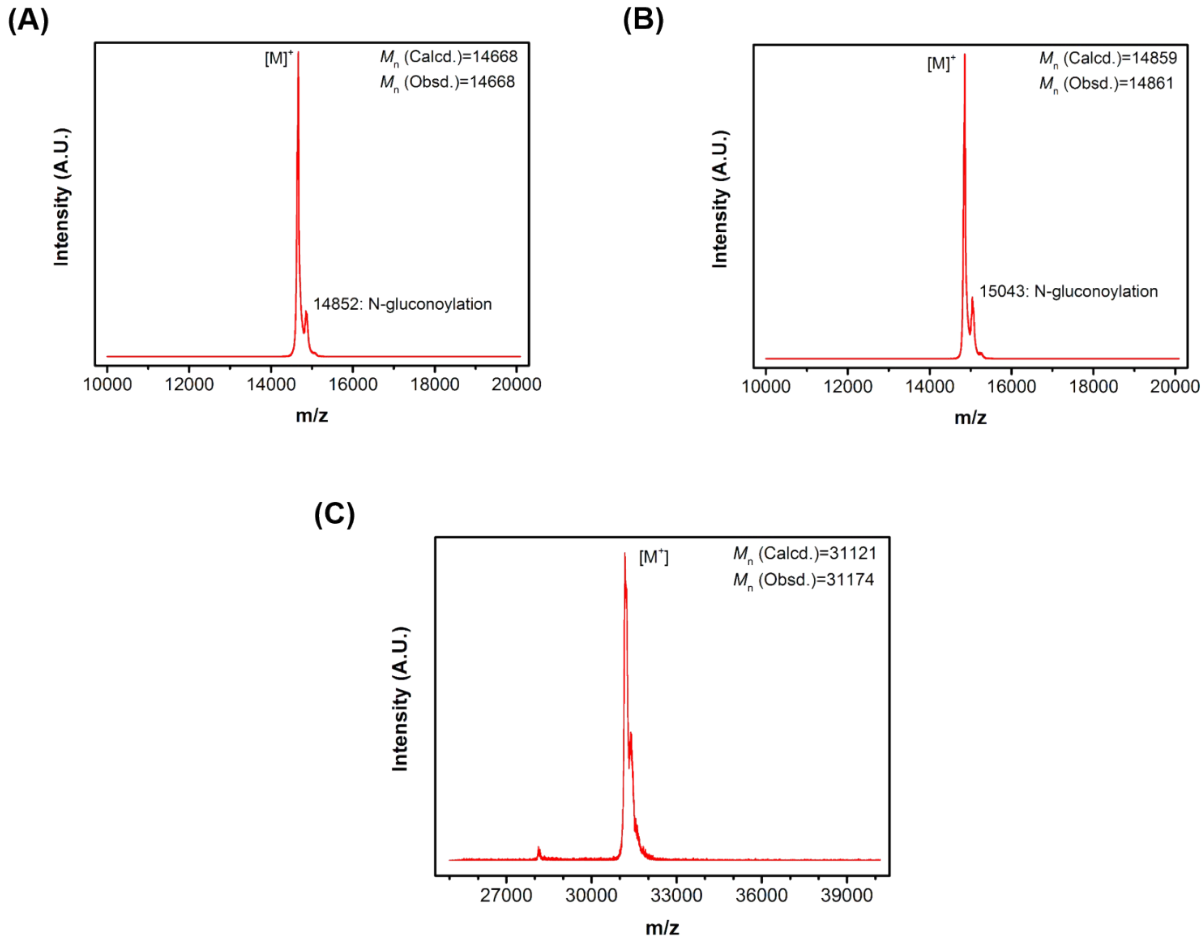

Figure S4. The MALDI-TOF mass spectra of (A) SpyCatcher, (B) SpyCatcher(-) and (C) SpyTag-YFP, which show that both SpyCatcher and SpyCatcher(-) have been post-translationally modified by cutting off the beginning methionine followed by N-gluconoylation.[6] Molecular weights of all samples were predicted using the ProtParam tool.[7]

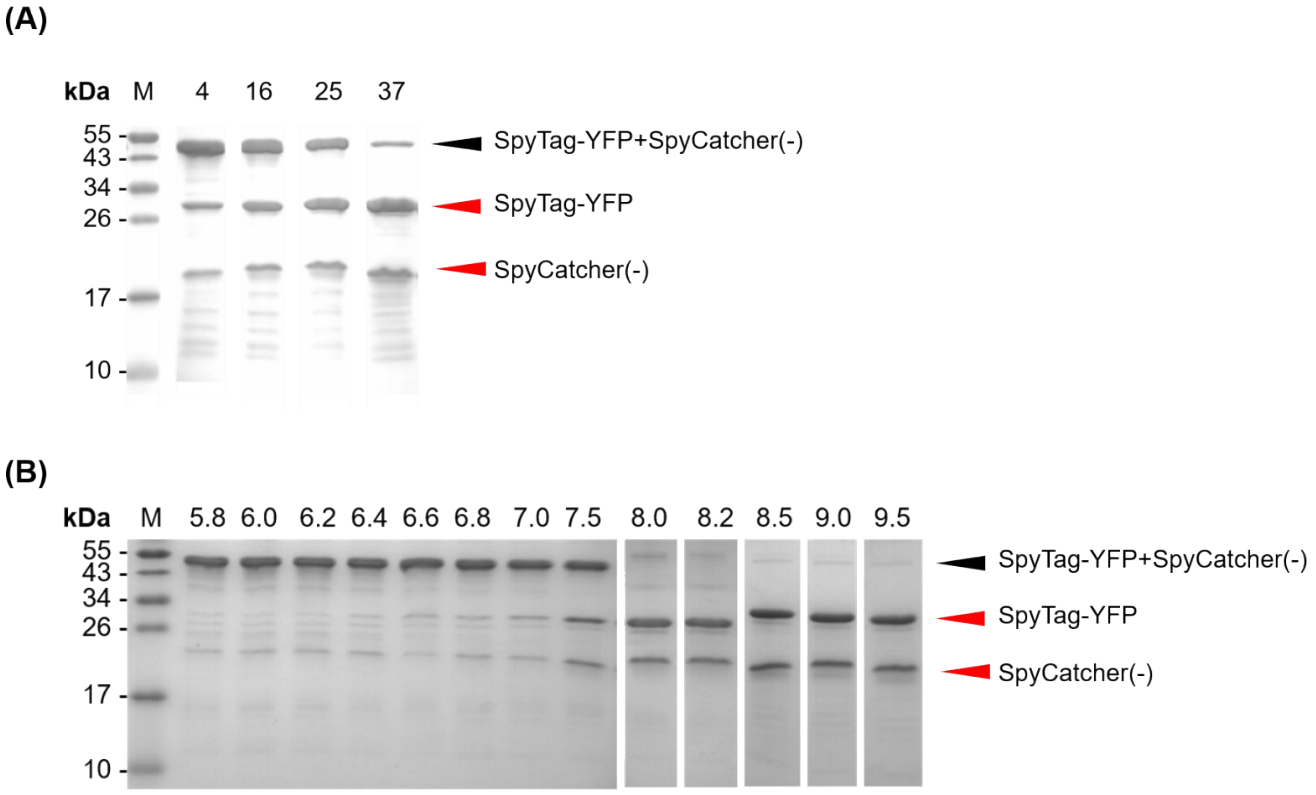

Figure S5. SDS-PAGE analysis of the reaction mixture of SpyTag-YFP and SpyCatcher(-) in PBS buffer: (A) at different temperatures, namely, 4 ^{o}C, 16 ^{o}C, 25 ^{o}C, and 37 ^{o}C at pH 7.4, respectively; and (B) at different pH ranging from 5.8 to 9.5.

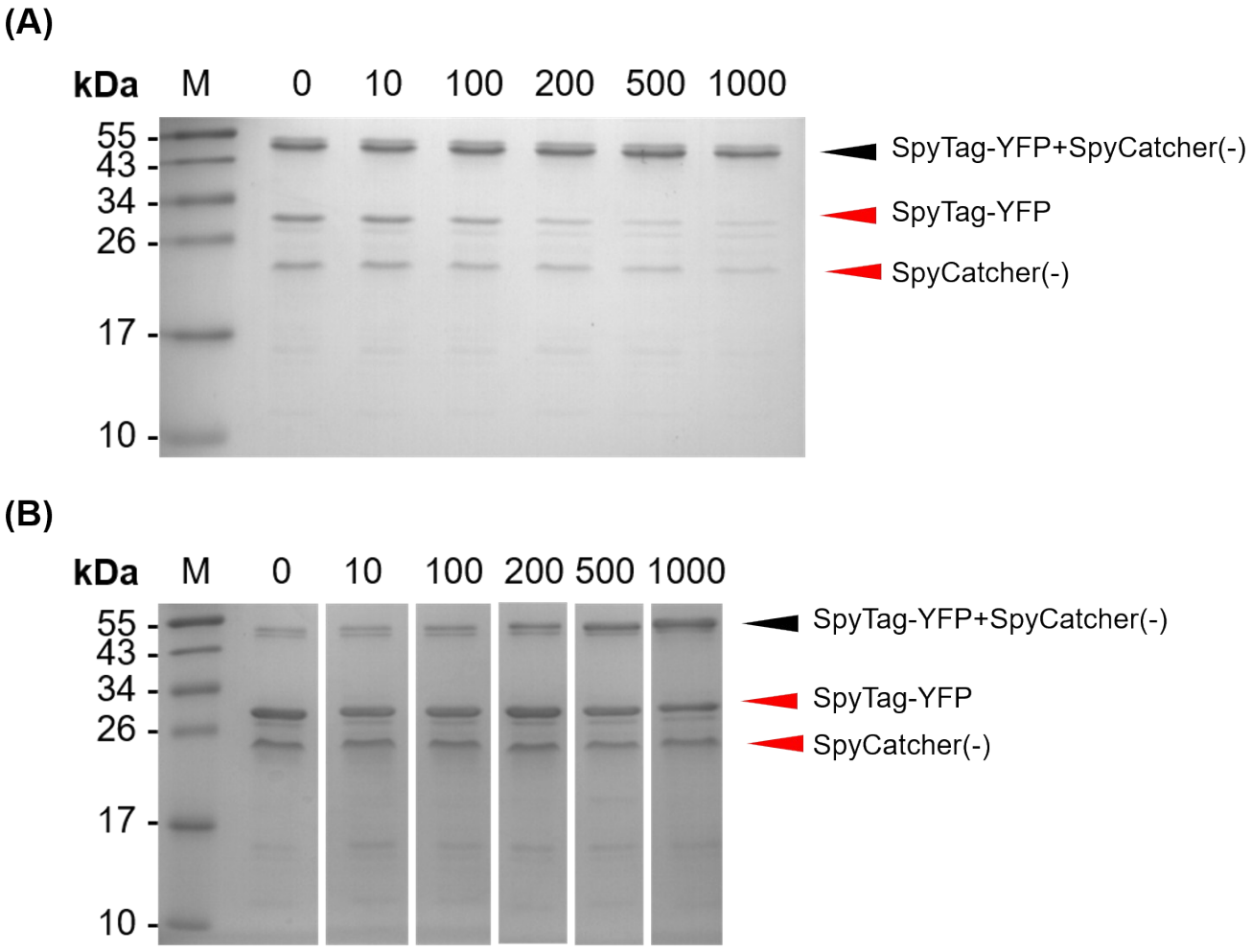

Figure S6. SDS-PAGE analysis of the reaction mixture of SpyTag-YFP and SpyCatcher(-) in PBS buffer with different ionic strengths at (A) 4 °C and (B) 37 °C. The number above each lane indicates the concentration of NaCl in the unit of mM.

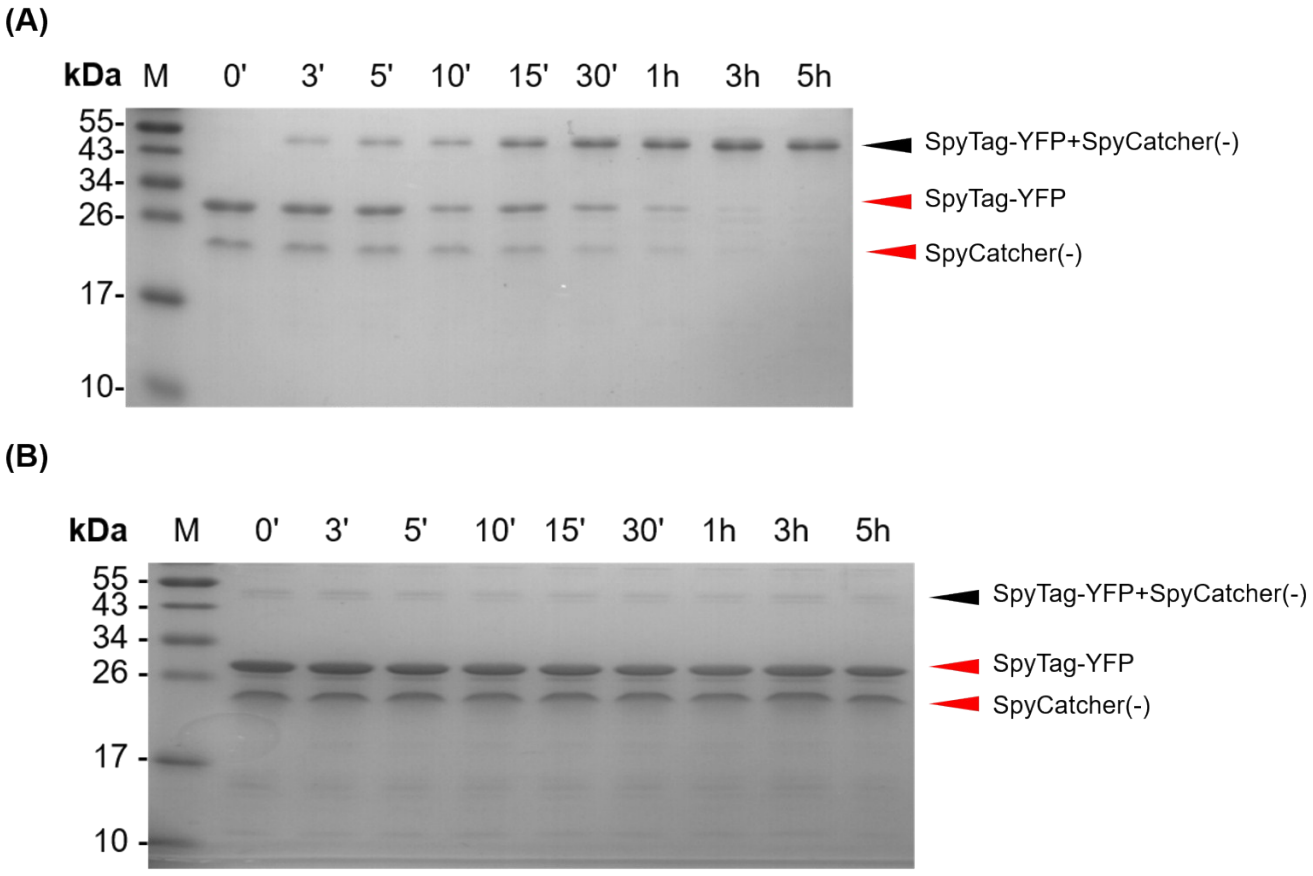

Figure S7. SDS-PAGE analysis of the reaction mixture of SpyTag-YFP and SpyCatcher(-) at 4 ^{o}C at different time points (A) in 20 mM NaH_2PO_4-Na_2HPO_4 buffer, 1M NaCl, pH 5.8 and (B) in 20 mM Tris-HCl buffer, pH 8.5.

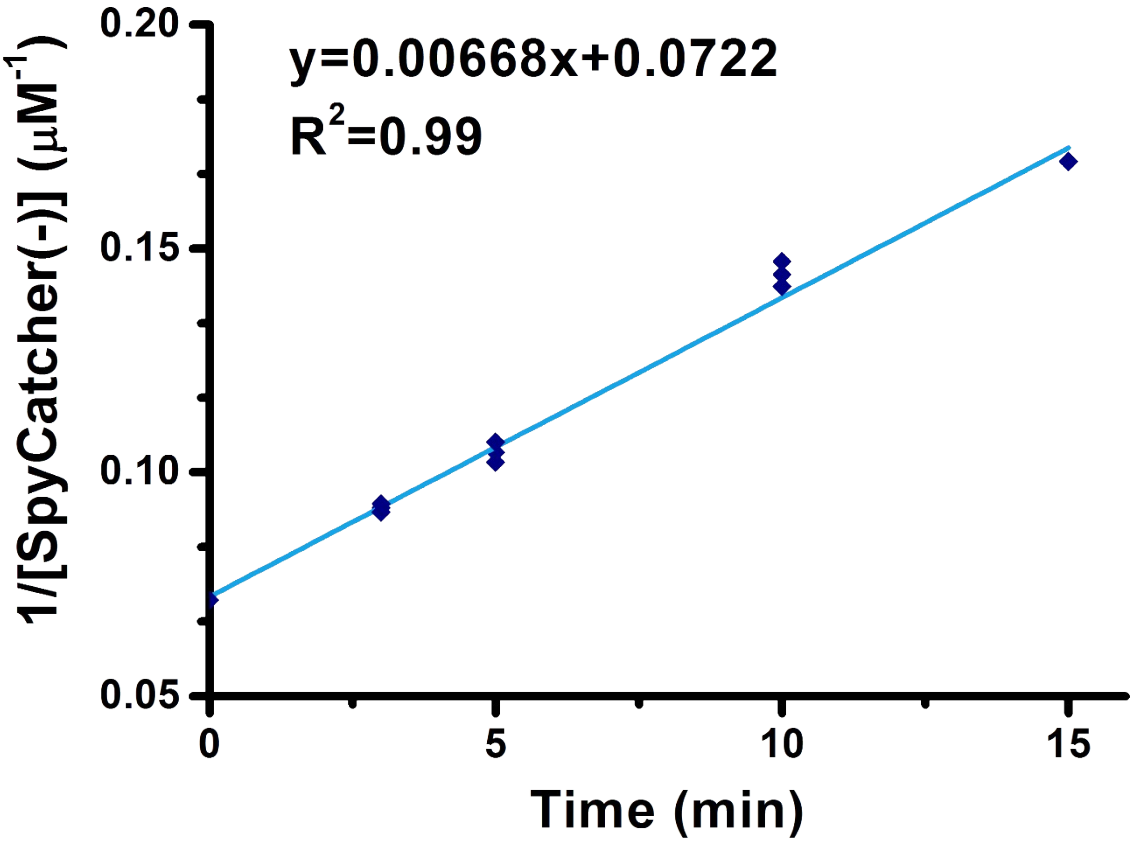

Figure S8. Plot of 1/[SpyCatcher(-)] to determine the rate constant for the reaction between SpyCatcher(-) and SpyTag-YFP. The data are from triplicate measurements (each point shown) of SpyCatcher(-) depletion under conditions as in Figure S7. The equation for the trend-line and the correlation coefficient are shown.

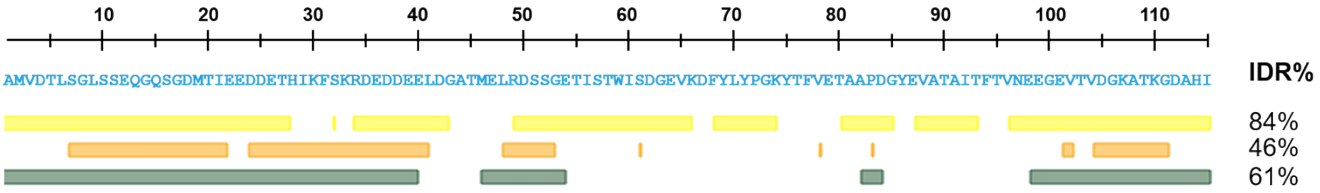

Figure S9. Disorder and flexibility prediction of SpyCatcher(-) by PredictProtein[8]: colored boxes indicate the disordered region in the sequence of SpyCatcher(-).The yellow, orange and green boxes correspond to the calculation methods of PROFbval[9], Ucon[10] and MD[11] respectively. The percentage of intrinsically disordered region (IDR%) of SpyCatcher(-) predicted by these three methods are 84%, 46% and 61%, respectively.

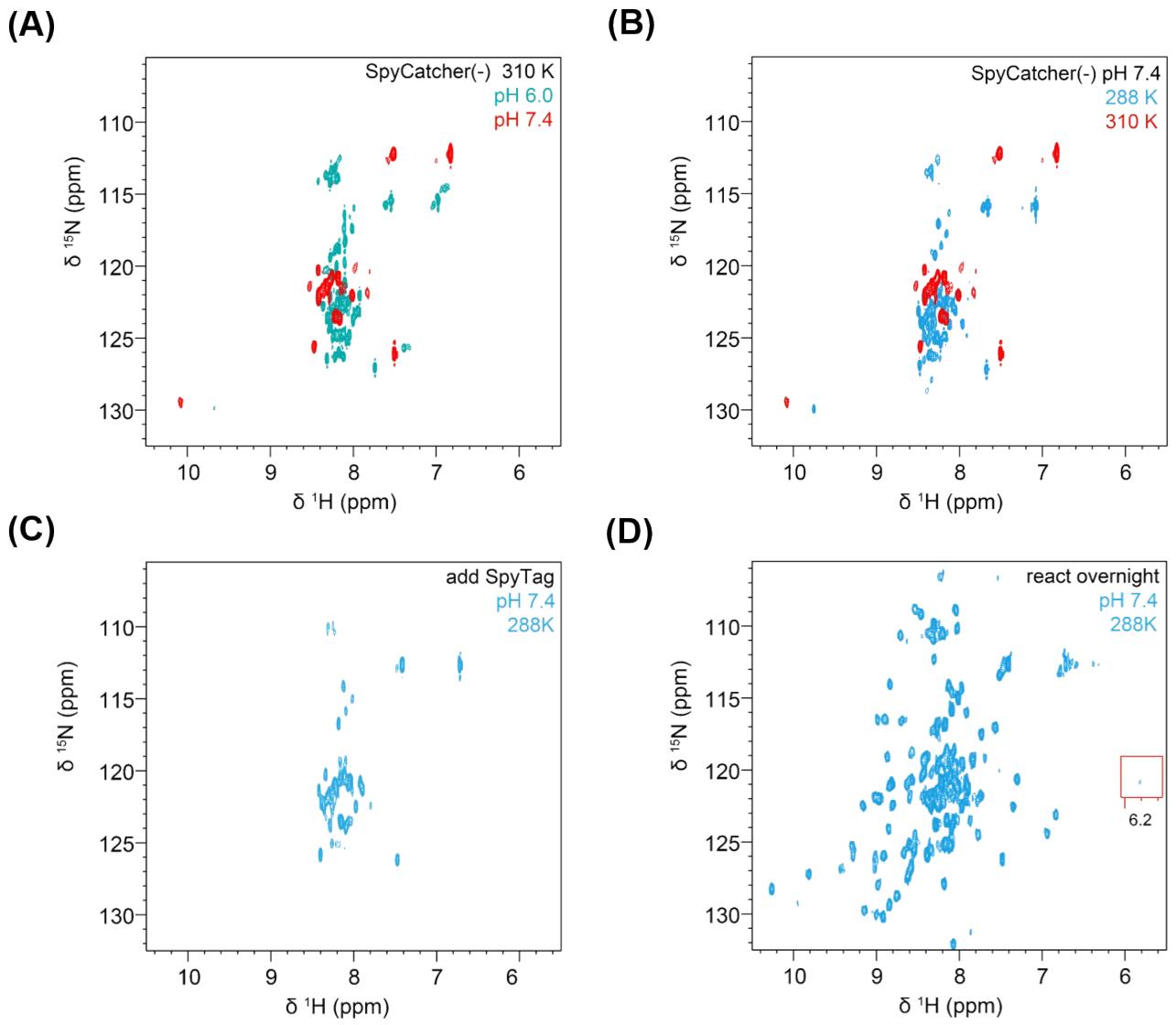

Figure S10. 2D ^{15}N-^{1}H HSQC-NMR spectra of SpyCatcher(-): (A) in PBS buffer (pH 7.4, cyan) and in NaAc-HAc buffer (pH 6.0, red) under 37 ^{o}C; (B) in PBS buffer (pH 7.4) under 37 ^{o}C (red) and under 15 ^{o}C (blue); (C) in PBS buffer (pH 7.4) taken immediately after SpyTag was added; the reconstitution was slow and no folded structure was observed at that point; (D) in PBS buffer (pH 7.4) after reaction with SpyTag at 4 ^{o}C overnight; the signal corresponding to isopeptide bond formation is shown in the red box; the folded structure is clearly demonstrated by the well-dispersed peaks.

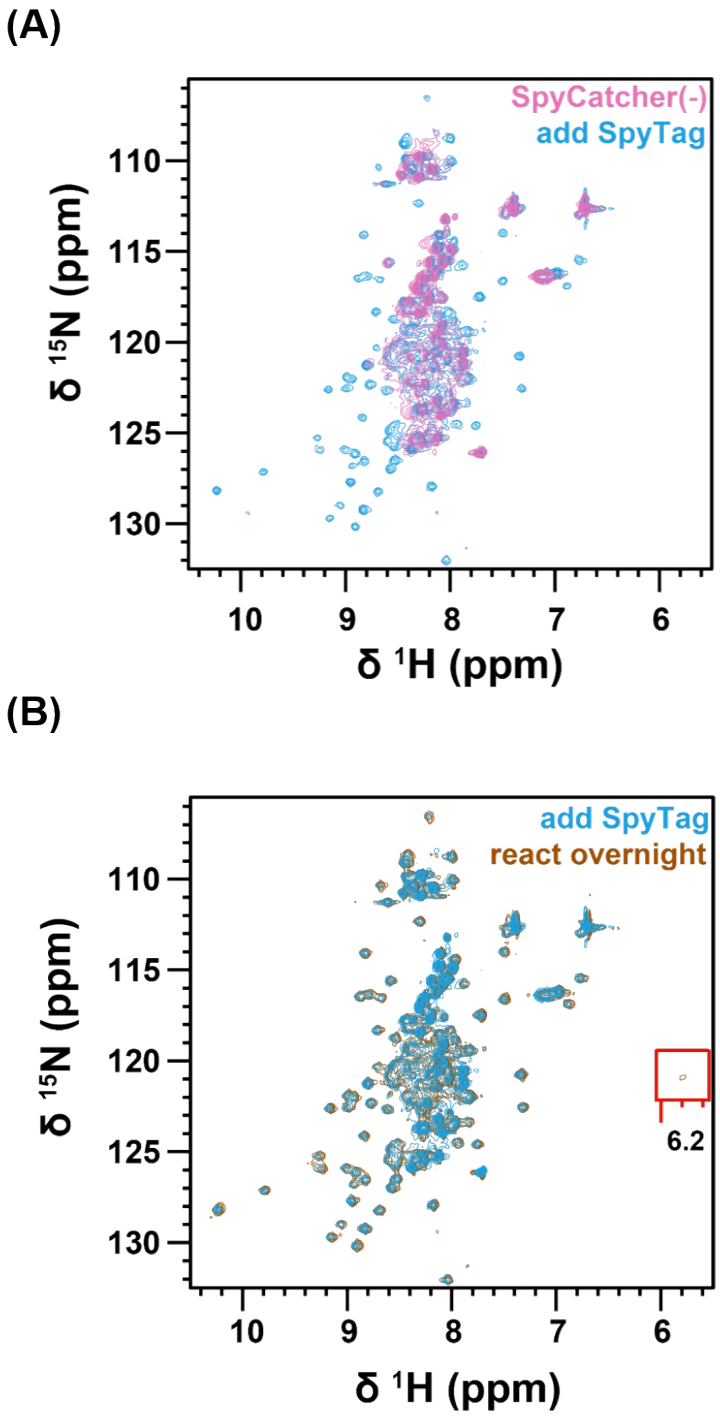

Figure S11. Overlay of 2D ^{15}N-^{1}H HSQC-NMR spectra of SpyCatcher(-) in NaAc-HAc buffer (pH 6.0): (A) before reaction and immediately after the addition of SpyTag; (B) soon after SpyTag addition and that obtained after overnight reaction.

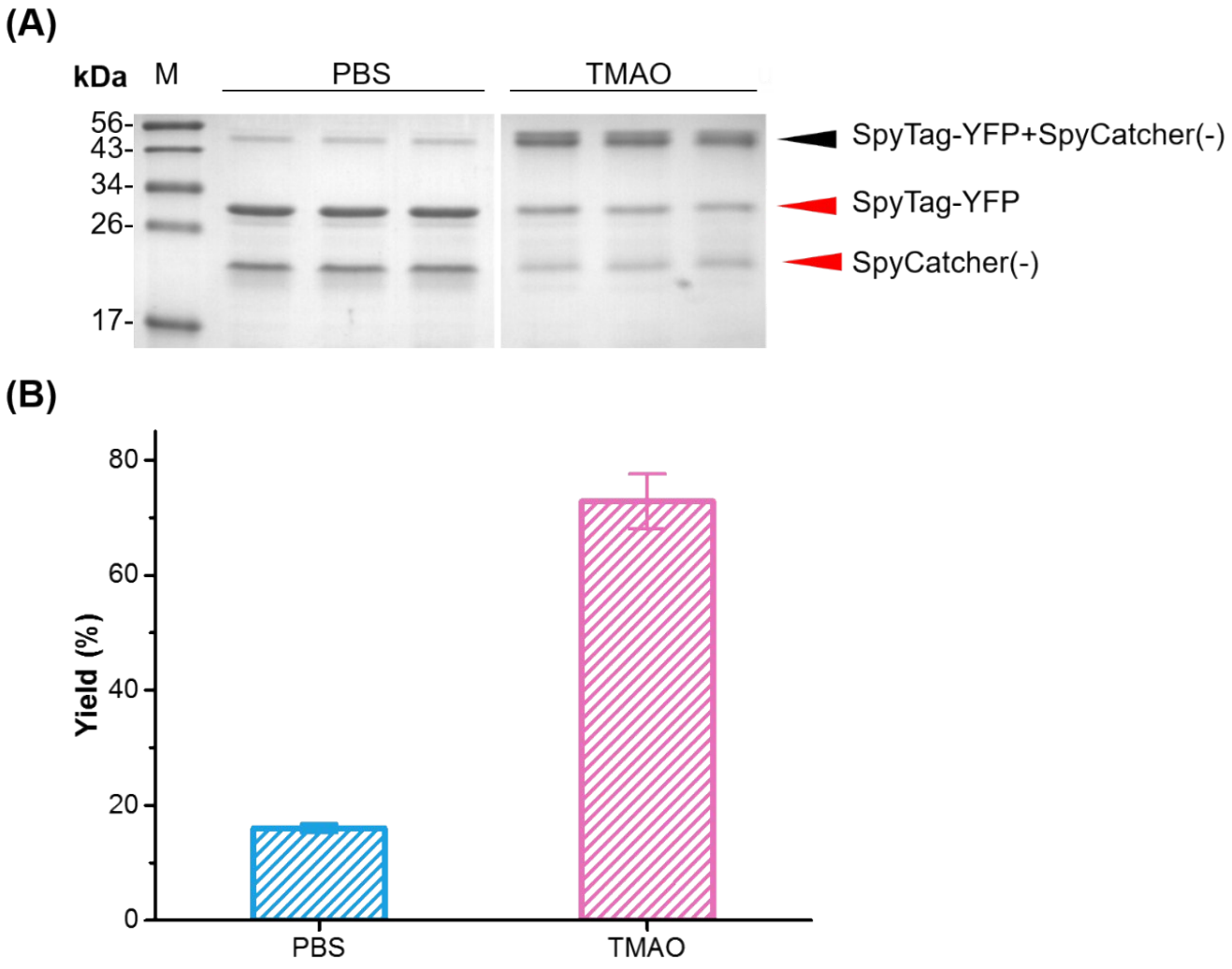

Figure S12. TMAO promotes the reaction between SpyTag and SpyCatcher(-): (A) SDS-PAGE analysis of the reactions; (B) the comparison of the yields.

Reference

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