# Post-Translational Modification of Bio-Nanoparticles as a Modular Platform for Biosensor Assembly

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## Experimental procedures

1. Construction of expression vectors for sensor components

*Escherichia coli* strain NEB 5-alpha (*fhuA2 Δ(argF-lacZ)U169 phoA glnV44 Φ80Δ (lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17* ) was used as the host for genetic manipulations. E2 monomer was expressed using plasmid pGGGE2, constructed by amplifying E2(158) [E2 encoding amino acids 158-427] fragment using the E2-Forward and E2-Reverse primer. The PCR product was digested and ligated into NdeI and BamHI digested pET11(a).

ELP-LPETG-His6 was constructed by first overlapping 5 oligonucleotides: Oligo 1, Oligo 2, Oligo 3, Oligo 4 and Oligo 5 to form the fragment of (G4S)3-LPETG-His6 with BamHI and XhoI overhangs. The overlapping oligos was ligated into BamHI and XhoI digested pET24(a)-ELP[KV8F-40] (Liu, Tsai, Madan, & Chen, 2012) to form pET24(a)-ELP-LPETG.

For HaloTag-LPETG and Nluc-LPETG, HaloTag and Nluc were first individually PCR amplified with HaloTag-Forward, HaloTag-Reverse and Nluc-Forward, Nluc-Reverse. These two PCR amplified fragments were digested with Nde1 and BamH1 and inserted into Nde1 and BamH1 digested pET24(a)-ELP-LPETG to form HaloTag-LPETG and Nluc-LPETG.

Z-Ct-LPETG was done by three-step cloning. GST (Glutathione S-transferase) was first PCR amplified with primers GST-Forward and GST-Reverse. The PCR product was digested with Nde1 and BamH1 and inserted into Nde1 and BamH1 digested ELP-LPETG to form GST-LPETG. Z-domain was PCR amplified with Z-Forwad and Z-Reverse, digested with Nde1 and inserted into Nde1 digested GST-LPETG to form Z-GST-LPETG. Cohesin Ct was then PCR amplified with Ct-Forward and Ct-Reverse, digested with HindIII and BamH1 and inserted into same restriction sites digested Z-GST-LPETG to form Z-Ct-LPETG.

The control fusion protein Z-ELP-Nluc was constructed by first inserting PCR amplified Z-domain into Nde1 digested pET24(a)-ELP-LPETG to form Z-ELP-LPETG. Then Nluc was PCR amplified with Nluc-Forward and Nluc-Reverse, digested with BamH1 and inserted into BamH1 digested Z-ELP-LPETG to form Z-ELP-Nluc.

**Table S1.** Primers used for recombinant gene construction

| | |
|---|---|
| E2-Forward | 5’-GCGCCATATGGGCGGTGGAGCTAGCGTGCTGAAAGAAGAC-3’ |
| E2-Reverse | 5’- GCGCGGATCCTTAAGCTTCCATCAGC-3’ |
| Oligo 1 | 5'- GAT CCC CGC GGG GCG GCG GAG GGT CTG GAG GAG GCG GTT CAG GTG GTG GGG GCT -3' |
| Oligo 2 | 5'- CCC TAC CAG AAA CCG GAG GAT CTG GCC ACC ATC ACC ATC ACC ACT GAT AAC -3' |
| Oligo 3 | 5'- TCG AGT TAT CAG TGG TGA TGG TGA TGG -3' |
| Oligo 4 | 5'- TGG CCA GAT CCT CCG GTT TCT GGT AGG GAG CCC CCA CCA CCT GAA CCG CCT -3' |
| Oligo 5 | 5'- CCT CCA GAC CCT CCG CCG CCC CGC GGG -3' |

| | |
|---|---|
| HaloTag-Forward | 5'- CCT CCA GAC CCT CCG CCG CCC CGC GGG -3' |
| HaloTag-Reverse | 5'- TGG CGC GGA TCC GCC GGA AAT CTC GAG CGT CG -3' |
| Nluc-Forward | 5'- GGG AAT TCC ATA TGG TCT TCA CAC TCG AA -3' |
| Nluc-Reverse | 5'- TTC CGC GGG GAT CCG G -3' |
| GST-Forward | 5'- GGG AAT TCC ATA TGA TGC ATG GCA AAA CCC AGG CGA CCA GCG GCA CCA TTC AGA GCA TGC ATG GCA AAA CCC AGG CGA CCA GCG GCA CCA TTC AGA GCA TGC ATG GCA AAA CCC AGG CGA CCA GCG GCA CCA TTC AGA GCA AGC TTG GCG GCG GTG GTA GCT CCC CTA TAC TAG GTT ATT GGA AAA TTA AGG GCC -3' |
| GST-Reverse | 5'- TAT TGG CGC GGA TCC CAG GGG CCC C -3' |
| Z-Forward | 5'- GGG AAT TCC ATA TGA GCG GCA GCG GCA GC -3' |
| Z-Reverse | 5'- TCC CAA GCT TAC TGC CAC CGC TCC CGC CTC CGC TAC CGC CTC CTT TCG GCG CCT GAG CAT -3' |
| Ct-Forward | 5'- GGG AAT TCC ATA TGC CAT CAA CAC AGC CTG TAA CAA CAC C -3' |
| Ct-Reverse | 5'- TAT TGG CGC GGA TCC TAT ATC TCC AAC ATT TAC TCC ACC GTC AAA GAA C -3' |

2. Protein expression

Plasmid pMR5-His6-Sortase A was a gift from Dr. Eric T. Boder, University of Tennessee, Knoxville and used as a source of sortase A. SrtA, GGG-E2, Nluc-LPETG, HaloTag-LPETG, Z-Ct-LPETG were expressed in BL21(DE3) (*F2 ompT gal dcm lon hsdSB(rB2 mB2) kDE3*).

For SrtA expression, overnight SrtA cultures were sub-inoculated into 25 mL Luria-Bertani (LB) medium supplemented with 50 μg/mL kanamycin. The culture was grown at 37℃ until OD600 reached 1. Protein expression was induced at 37 $^{o}$C for 4 hours with 1 mM IPTG.

GGG-E2 was inoculated with LB medium supplemented with 100ug/mL ampicillin, induced with 0.2 mM IPTG when $OD_{600}$ reached 0.5 and grown at 20 $^{o}$C overnight for protein expression.

HaloTag-LPETG was inoculated in LB medium supplemented with 50 μg/mL kanamycin, induced at 30$^{o}$C for 4 hours by 0.25 mM IPTG at OD 0.5. Z-Ct-LPETG was cultured in LB medium supplemented with 1.5% glycerol and 50 μg/mL kanamycin at 37 $^{o}$C until OD600 reached 1. Protein expression was induced by 0.2mM IPTG at 25 $^{o}$C for overnight. Nluc-LPETG was cultured in Terrific Broth (TB) medium until OD reached 1. 0.2mM IPTG was used to induce protein expression for overnight at 25 $^{o}$C.

ELP and Z-ELP-Nluc was expressed in E. coli BLR [*F- ompT hsdSB (r-B m-B) gal dcm(DE3) Δ (srl-recA)306::Tn10(TetR)*; Novagen, Madison,WI]in TB medium.

After protein expression, cells were harvested by centrifugation at 4200 g, resuspended in SrtA ligation buffer (50 mM Tris, 150 mM NaCl, pH 8) and lysed by ultrasonic disruption using a sonicator. The cell debris was removed by centrifugation at 16.1 k rcf for 10 min at 4 $^{o}$C.

Nluc-LPETG, Z-Ct-LPETG and GGG-E2 were partially purified based on their thermal-stable character. Nluc-LPETG was incubated at 55 $^{o}$C for 10 min, while Z-Ct-LPETG, GGG-E2 was incubated at 70 $^{o}$C for 10 min to denature most of the *E. coli* contaminant proteins. The aggregated contaminants were removed by centrifugation at 15,000 rpm at 4 $^{o}$C for 10 min. Purification of the ELP fusion protein was achieved by two cycles of inverse phase transitions. NaCl was added to the cell lysates to a final concentration of 1 M and the mixture was incubated at 37 °C for 10 min before centrifugation for 30 min at 15,000 rpm at 37 °C. The pellet was resuspended in ice-cold buffer and centrifuged for 30 min at 15,000 rpm at 4 °C to remove the insoluble cellular proteins. This precipitation and resolubilization process was repeated for a second time. The purity of the purified protein was determined by 10% SDS-PAGE electrophoresis followed by coomassie blue staining. The molar concentration of SrtA, GGG-E2, Nluc-LPETG, HaloTag-LPETG, Z-Ct-LPETG and ELP-E2 cage were checked by Bradford protein assay from Bio-Rad (Hercules, CA). The molar concentration of ELP was calculated according to measurement at 215 nm.(Liu et al., 2012).

3. Characterization of protein complex.

The ligation products were analyzed by SDS-PAGE. The ratio of ligated products onto E2 was estimated by densitometry, in which GelQuant (BiochemLabSolutions) was used to quantify the band densities. Five repeats were used to obtain the standard deviation associated with the decoration efficiency of each component. The hydrodynamic diameters of the complexes were measured by dynamic light scattering using Zetasizer Nano ZS ( Malvern Instruments). The samples were in buffer of 50 mM Tris and 150 mM NaCl at pH8 for dynamic light scattering. Full complex assemblies were visualized by transmission electron microscopy. Samples were prepared in DDI water and stained with 2% uranyl acetate on carbon-coated copper grids (Electron Microscopy Science). Zeiss Libra 120 Transmission Electron Microscope was used to visualize the samples with voltage of 120 kV.

**Reference**

Liu, F., Tsai, S.-L., Madan, B., & Chen, W. (2012). Engineering a high-affinity scaffold for non-chromatographic protein purification *via* intein-mediated cleavage. *Biotechnology and Bioengineering*, *109*(11), 2829–35. doi:10.1002/bit.24545

Figure S1. Scheme for stepwise ligation and purification of ELP-E2-Protein No. 1 (Z-domain or HaloTag)-Protein No. 2 (Nluc or HaloTag).

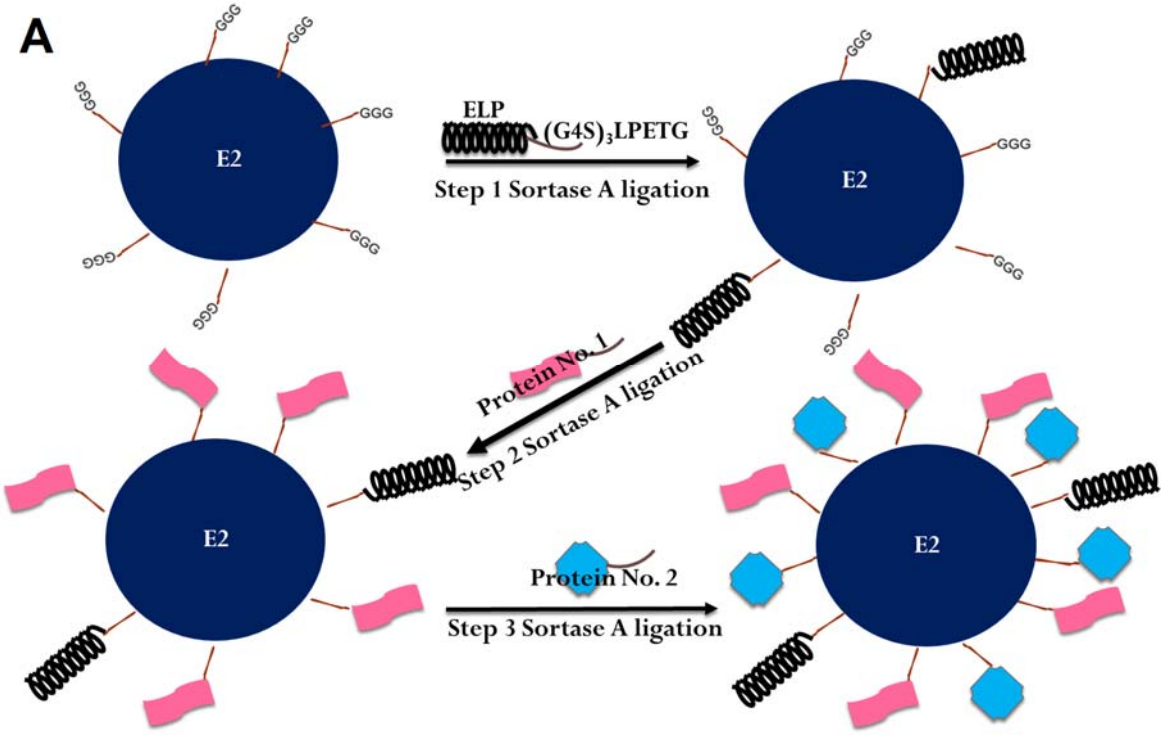

Figure S2. Detailed diagram of the three-step ligation. (A) Step 1: Elastin-like-polypeptide ligation on E2 nanoparticle; (B) Step 2: Protein No. 1 (Z-Ct or HaloTag) ligation on ELP-E2 platform; (C) Step 3: Protein No. 2 (Nluc or HaloTag) ligation on ELP-E2-Protein No. 1.

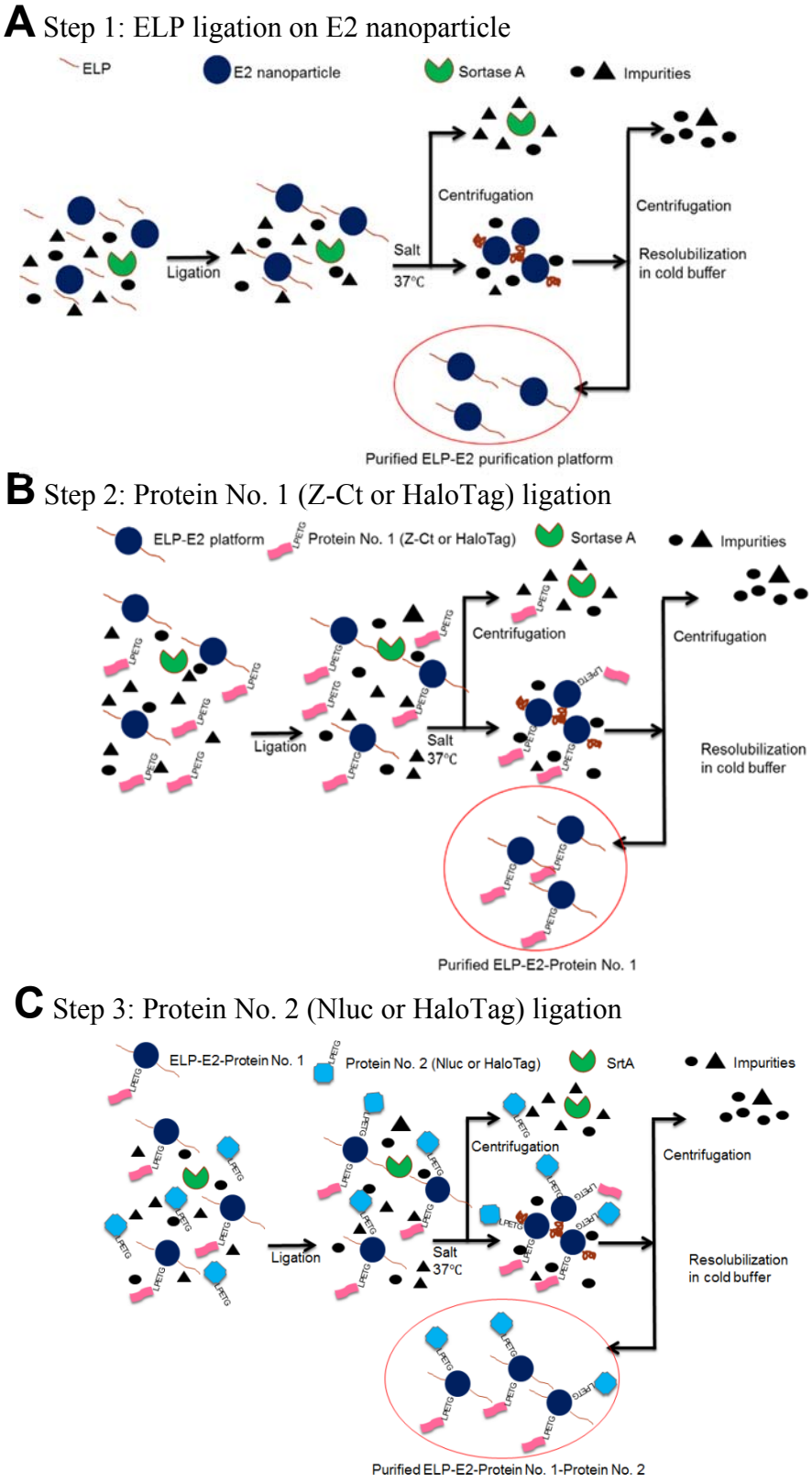

Figure S3. Transmission electron micrograph of (A) unmodified E2 nanoparticles and (B) IgG-bound E2 nanoparticles.

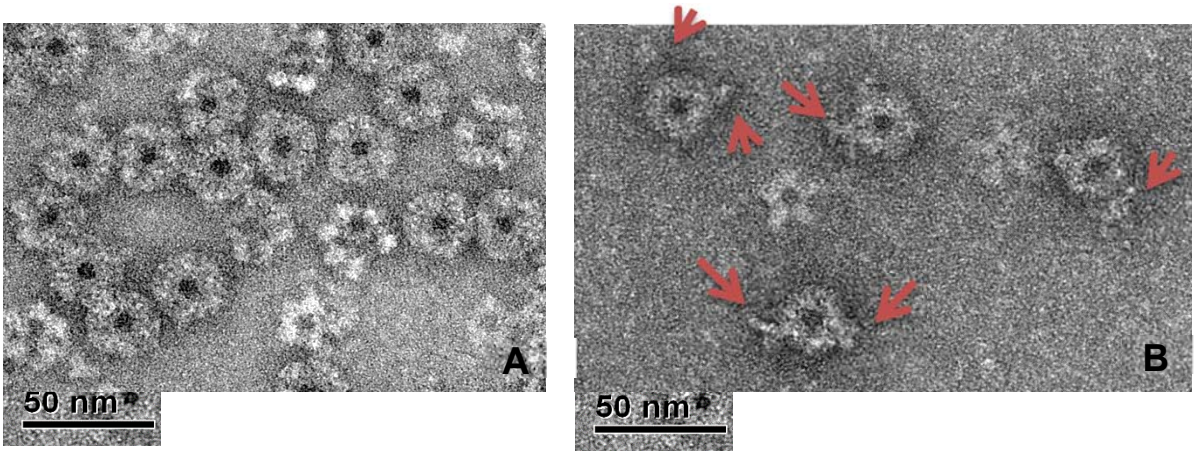

Figure S4. Formation of IgG-binding E2 nanoprobes. (A) Expression and purification of Z-Ct-LPETG. Protein was partially purified by heating whole cell lysates at 70$^{o}$C for 10 min. Most cellular proteins denatured and precipitated and was removed by centrifugation. (B) Binding of rabbit IgG to Z-E2-ELP. Rabbit IgG was mixed with Z-E2-ELP and the bound complex was recovered by one cycle of thermal precipitation and resolubilization. (C) Scheme for three-step ligation and purification of ELP-E2-Protein A-Protein B.

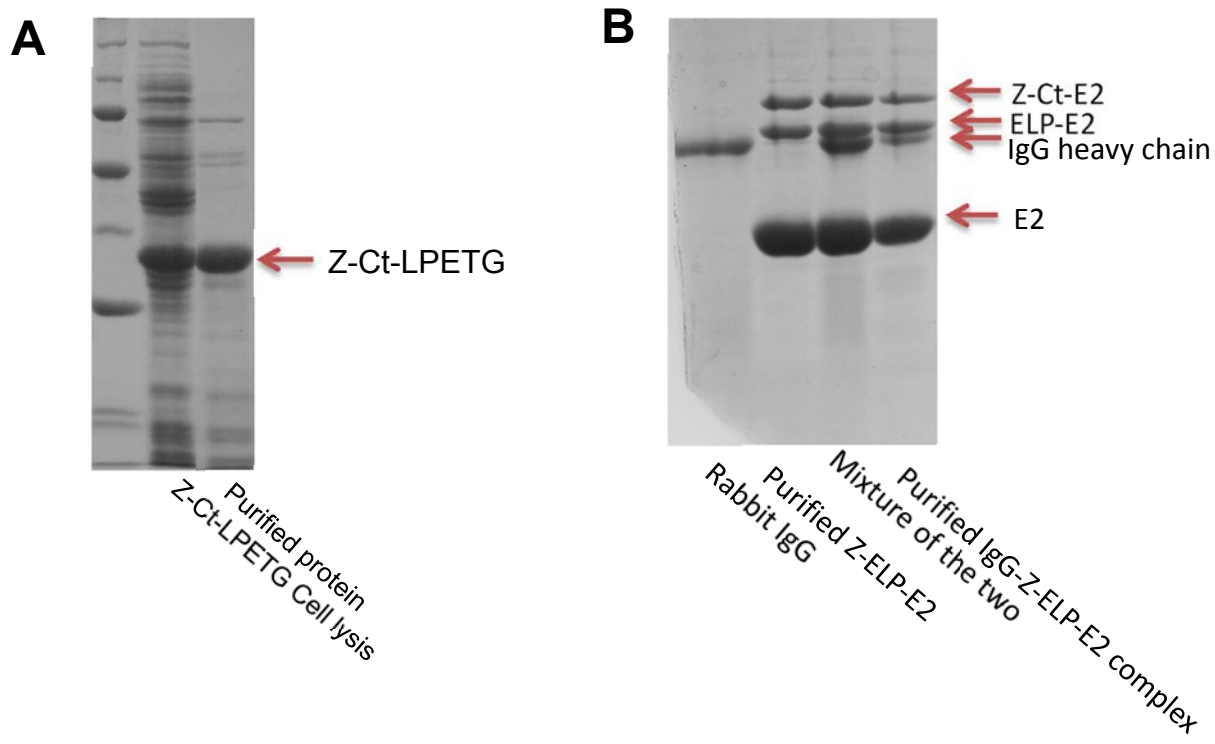

Figure S5. E2 nanoprobe assembly using the Z domain for antibody capture and Nluc for detection. (A) Nluc purification and ligation onto ELP-E2 nanoparticles. (B) Detection of Nluc activity for the purified ELP-E2-Nluc nanoparticles.

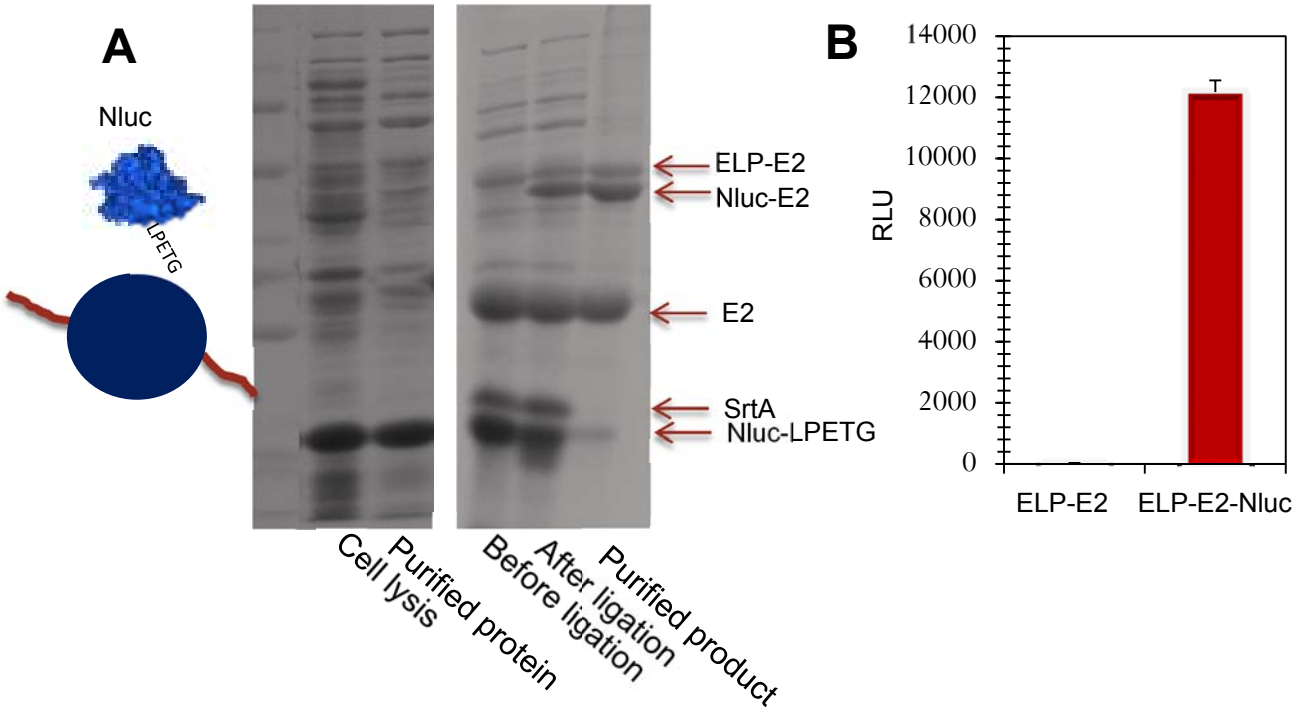

Figure S6. Two-step assembly of Z-ELP-E2-Nluc nanoprobes and their use for thrombin sensing (A) Two-step SrtA-mediated ligation of Z-Ct and Nluc onto ELP-E2 nanoparticles. (B) Dynamic light scattering of ELP-E2 and IgG-Z-ELP-E2-Nluc nanoparticles. (C) Production and purification of Z-ELP-Nluc probes. (D) Thrombin detection using IgG-Z-ELP-E2-Nluc nanoprobes.

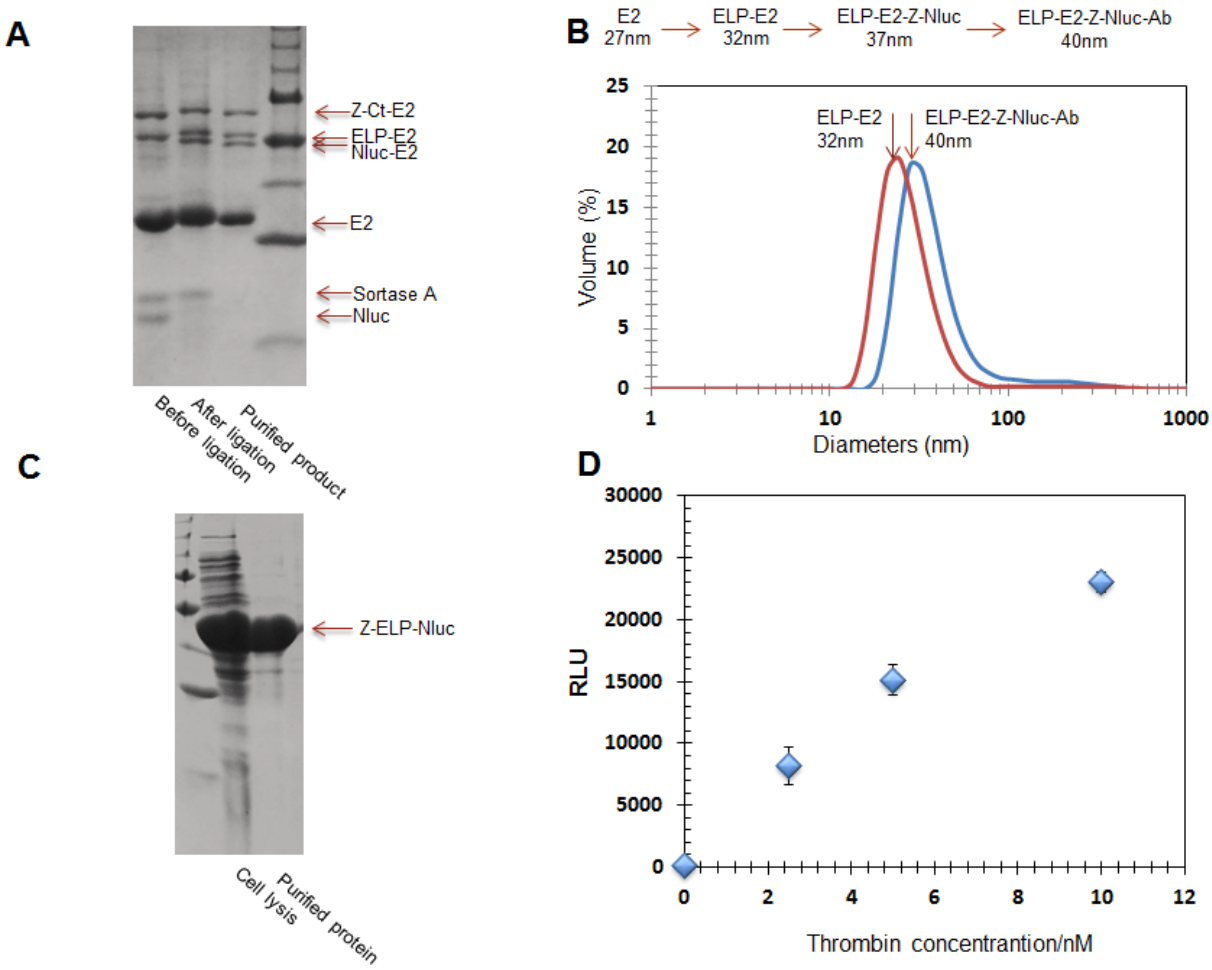

Figure S7. (A) Production and ligaiton of Halo tag onto E2 nanoparticles. (B) Conjugation of CH-Alexa 488 to either (1) Halo-ELP-E2 or (2) Aptamer-Halo-ELP-E2. Successful conjugation was verified by detecting brightly fluorescent bands under UV light. (C) Ligation of Z-Ct and Halo tag onto ELP-E2 nanoparticles.

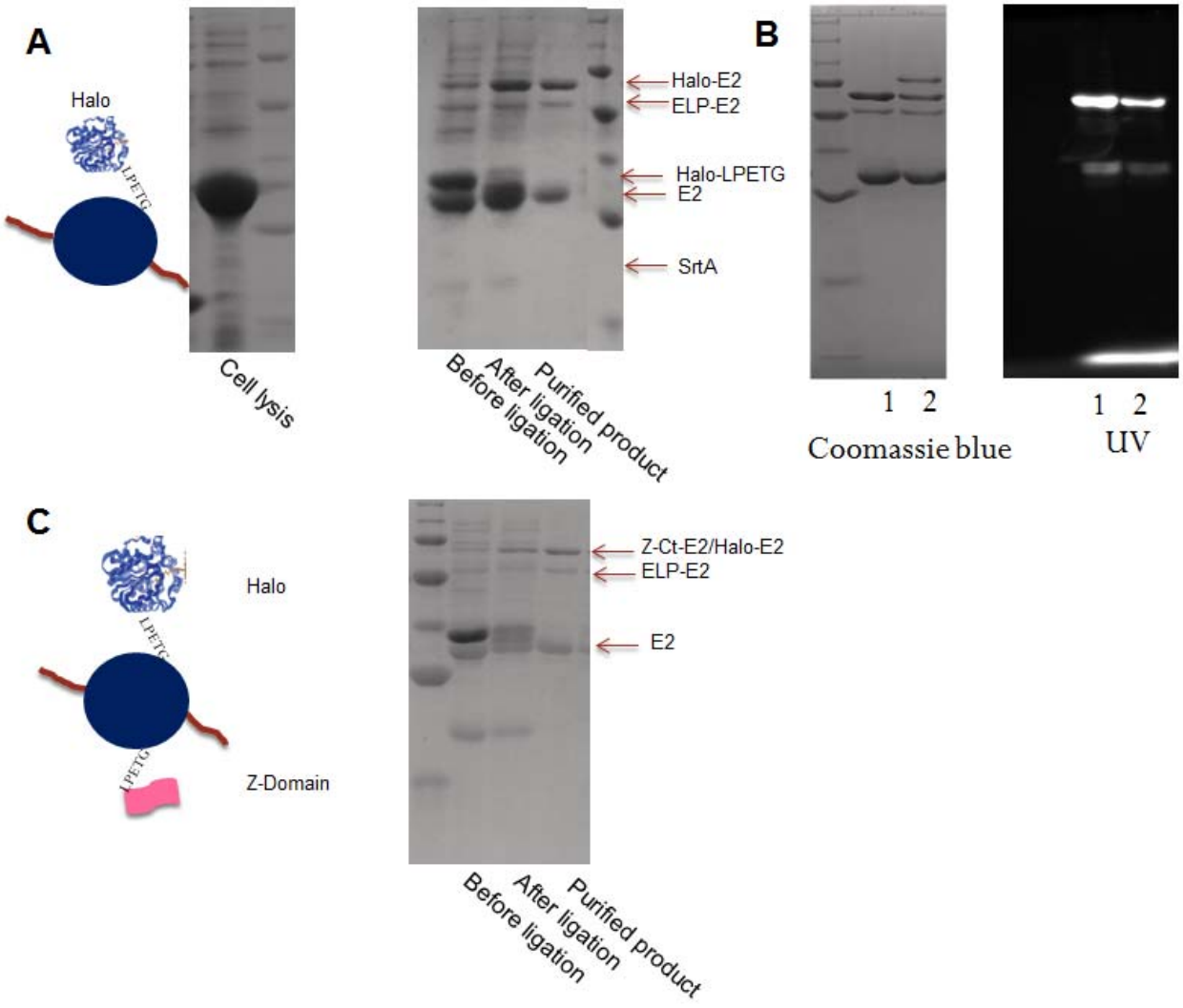

**Table S2.** Nluc decoration efficiency densitometry analysis

| | Nluc decoration quantity estimated by densitometry |
|---|---|
| Lane 1 | 5.33±1.28 |
| Lane 2 | 13.41±1.54 |
| Lane 3 | 16.80±2.11 |
| Lane 4 | 19.28±1.97 |
| Lane 5 | 22.29±2.27 |