Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Halo-tag mediated self-labeling of fluorescent proteins to molecular beacons for nucleic acid detection

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Materials and methods

Construction of expression vectors

The HaloTag vector was purchased from Promega. The HaloTag sequence was PCR amplified, double digested, and then inserted into pET24a-ELP[$KV_8F]_{40}$ [1] using the SacII and EcoR1 sites to form ELP-Halo. The CFP and YFP sequences were PCR amplified using pUC19.1D2A-FRET [2] as a template, then double digested with Not1 and Blp1 and inserted into separate ELP-Halo constructs to form ELP-Halo-CFP and ELP-Halo-YFP. The tat peptide was inserted via primers during PCR amplification of the T7 promoter and LacO. This T7-LacO-Tat amplified product was inserted on the N-terminus of ELP-Halo-YFP using sites MluI and Nde1 to form Tat-ELP-Halo-YFP.

Protein expression and purification

All constructs were transformed into *E. coli* BLR [F- ompT hsdSB (r-B m-B) gal dcm(DE3) Δ (srl-recA)306::Tn10(TetR); Novagen, Madison,WI] cells for protein expression. Starting cultures were grown to OD 0.7 at 37^{o}C, induced with 250μM isopropyl-thiogalactopyranoside (IPTG), and grown at 20^{o}C overnight. Cells were gathered by centrifugation, resuspended in NiNTA column buffer, and then sonicated. The soluble fraction was isolated by centrifugation at 20,000xg for 20 min and transferred to a Ni-NTA His Bind (Novagen, Madison,WI) column for purification via the C-terminal his6 tag. Purified proteins were eluted with 250 μM imidazole and the residual imidazole was removed by precipitation of the proteins with 1M $NaSO_4$ at 37^{o}C. The pellet was recovered at 15,000xg for 15 min and washed with 37^{o}C PBS (1x) to remove any residual salt. The

proteins were then re-solubilized in cold PBS (1x) and centrifuged again to remove any insoluble matter. The cell lysates and purified proteins were loaded onto a 10% SDS-PAGE gel and stained with coomassie blue for analysis.

Molecular Beacons and Protein Attachment

The molecular beacons were ordered from Integrated DNA Technologies (Coralville, IA). The Kras MB sequences were identical to those from Santangelo et al.[3] The donor MB ordered was modified with a 5' amine group with a sequence of CCTACGCCACCAGCTCCGTAGG. The acceptor MB was modified with a 3' amine group with a sequence of AGTGCGCTGTATCGTCAAGGCACT. Upon arrival, each MB was modified with the Chlorohexane (CH) ligand for HaloTag attachment. The CH ligand was mixed with the MB at a molar ratio of 30:1 and incubated at room temperature for 4 hours. The mixture was then purified, to remove excess CH ligand, using a 3,000 Da ultrafiltration column (Vivaspin 500, Sartorius Stedim Biotech). The purified MB was then mixed with the purified Halo fusion, using a 3x molar excess MB, and incubated overnight at 4 ^{o}C. Then, utilizing the ELP tag, the excess/unbound MB was removed by pelleting the protein-MB and washing away any residual MB. The protein only, protein plus excess MB, and purified protein-MB samples were loaded onto a 10% SDS-PAGE and stained with coomassie blue for analysis of labeling efficiency.

Native PAGE mobility shift

Mobility shift experiments were carried out in a 4.5% non-denaturing acrylamide gel. The protein only, protein plus excess MB, and purified protein-MB samples were loaded and run for 45 min at a constant 90V. The gel was then stained with ethidium bromide for 5 min before imaging.

Fluorescence measurements and target sequences

All fluorescence measurements were taken using a Synergy plate reader. All samples contained approximately 400nM of Halo-CFP:MB and Halo-YFP:MB. The samples were excited at a wavelength of 434 nm and the fluorescence emission spectra were taken from 460 nm to 600 nm. Dose response data was gathered by measuring the emission at 478 nm and 524 nm using a 434 nm excitation. The specific target used for all characteristic binding experiments was 5'- T AC G GAG CTG GTG GCG TAG G tagatgc **AGT GCC TTG ACG ATA CAG C** GC A -3'. The donor MB binding sequence is underlined, the 7 nucleotide spacer is in lower case, and the acceptor MB binding sequence is in bold. The target sequences used for the spacing characterization were as follows: 0sp- 5'- TAC GGAGCTGGTGGCGTAGG **AGTGCCTTGACGATACAGC** GCA -3', 2sp- 5'- TAC GGAGCTGGTGGCGTAGG ta **AGTGCCTTGACGATACAGC** GCA -3', 4sp- 5'- TAC GGAGCTGGTGGCGTAGG taga **AGTGCCTTGACGATACAGC** GCA -3', 10sp- 5'- TAC GGAGCTGGTGGCGTAGG tagatgctta **AGTGCCTTGACGATACAGC** GCA -3', 15sp- 5'- TAC GGAGCTGGTGGCGTAGG tagatgcttaatata **AGTGCCTTGACGATACAGC** GCA -3', and 20sp- 5'- TAC GGAGCTGGTGGCGTAGG tagatgcttaatatattaat **AGTGCCTTGACGATACAGC** GCA -3'.

Mammalian Cell Culture and FP-MB delivery

Buffalo green monkey kidney (BGMK) cells were obtained from American Type Culture Collection (ATCC). The cells were grown and cultured at 37°C in a 5% CO_2 atmosphere with 1x autoclavable minimum essential medium (Life technologies: 11700-077) containing 1 vol% of 7.5% $NaHCO_3$, 2 vol% of 1M Hepes, 1 vol% of non-essential amino acids (Fisher: SH3023801), 2.5 vol% of Pen/Strep (Fisher: SV30010), 2 vol% of L-glutamine (Fisher: SH3003401) and 10 vol% of FBS (Sigma-Aldrich: F6178).

BGMK cells were seeded into 96 well plates and grown at 37°C in 5% CO_2 overnight, until the well cell density reached around 90% confluency. The growth media was then removed and the cells were washed twice with 1x TBS. The Tat-YFP:MB was then added to the cells at concentration of 750µM in 1x Leibovitz L-15 medium (Invitrogen). The Leibovitz medium contains no phenol red, resulting in less autofluorescence and easier viewing of intracellular uptake. At 2 hrs incubation, the cell began to fluoresce, so the Tat-YFP:MB media was removed and the cells were washed twice with 1x TBS and images were taken. All imaging was performed on a Zeiss AxioObserver Z1 inverted fluorescence microscope.

Cell Lysate Target Detection

HeLa cells were grown to confluency in a T-150 flask. The cells were trypsinized and then decactivated with serum. The cells were gathered by centrifugation and the total cell count was estimated to be around $1.9*10^7$ cells. The cells were resuspended in 300µL of (1x) PBS and lysed

with glass beads. The glass bead lysis included vortexing for 30s then cooling for 30s (ice bath) for a total of 6 cycles. The lysate was gathered by centrifugation and then diluted 40x with PBS for each 100µL reaction sample. Each RNA detection reaction contained the lysate from ~$1.6x10^5$ cells with 400nM of each FP-MB and various concentrations of the RNA target.

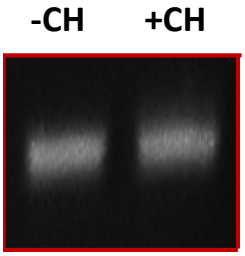

Figure S1: CH-ligand modified MBs. MBs before and after CH modification were analyzed on a 20% Native PAGE gel and subsequently stained with ethidium bromide. Successful modification was indicated with a slight increase in size by ~509 Da or equivalent to ~1 base pair. The entire band shifts up slightly indicating 100% labeling.

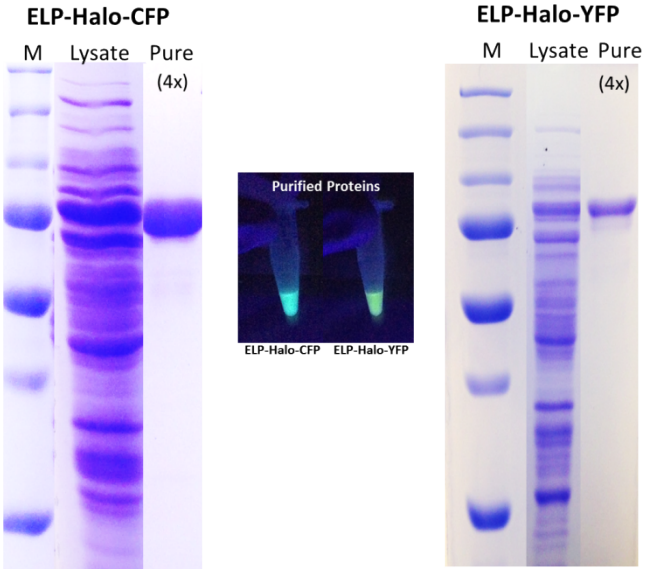

Figure S2: Cell lysates and purified Halo-FP fusions. Halo-FP fusion proteins were purified by two cycles of precipitation and solubilization before visualized by SDS-PAGE. Purified proteins retained the fluorescent property.

References

1. Q. Sun, B. Madan, S.-L. Tsai, M. P. DeLisa, and W. Chen, *Chem. Commun.*, 2014, **50**, 1423–5.

2. Y. Hwang, W. Chen, and M. Yates, *Appl. Environ. Microbiol.*, 2006, **72**, 3710–3715.

3. P. J. Santangelo, B. Nix, A. Tsourkas, and G. Bao, *Nucleic Acids Res.*, 2004, **32**, e57.