Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Bead Based Facile Assay for Sensitive Quantification of Native State Green Fluorescent Protein

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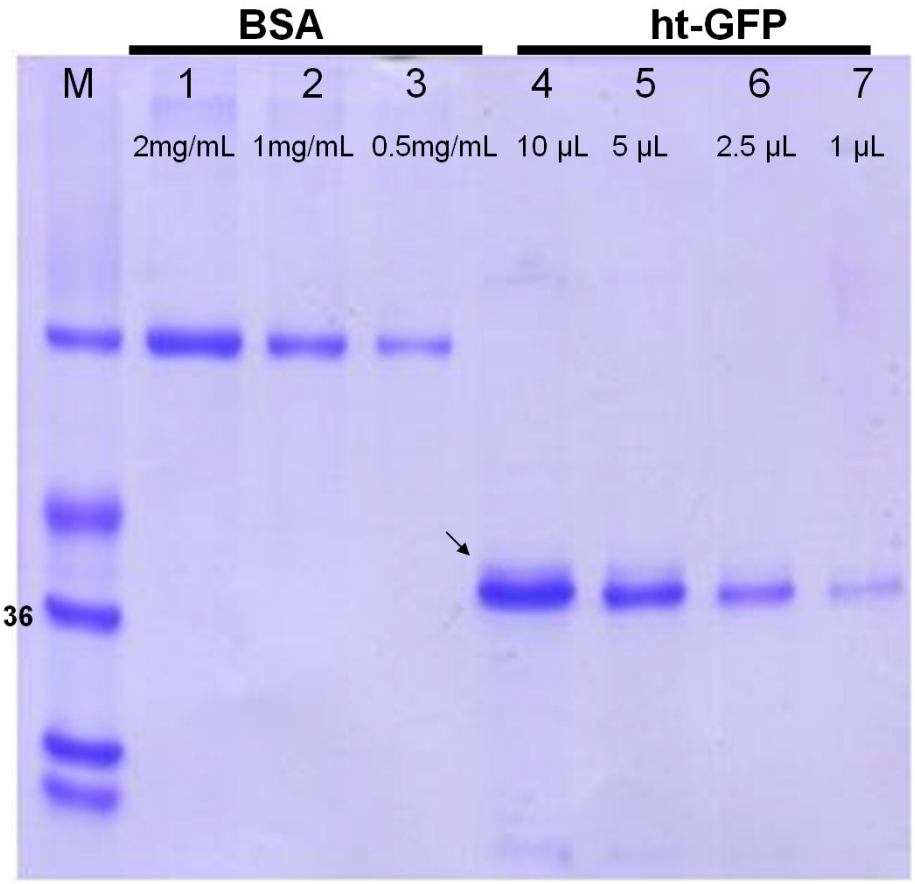

Fig. S1. The results of gel electrophoresis for BSA and serially diluted purified ht-GFPs. M represents the molecular weight marker. Lanes 1, 2, and 3 represent BSA at various concentration (2, 1, and 0.5 mg/mL), respectively. Lanes 4, 5, 6 and 7 represent purified ht-GFP (10, 5, 2.5, and 1 µL), respectively.

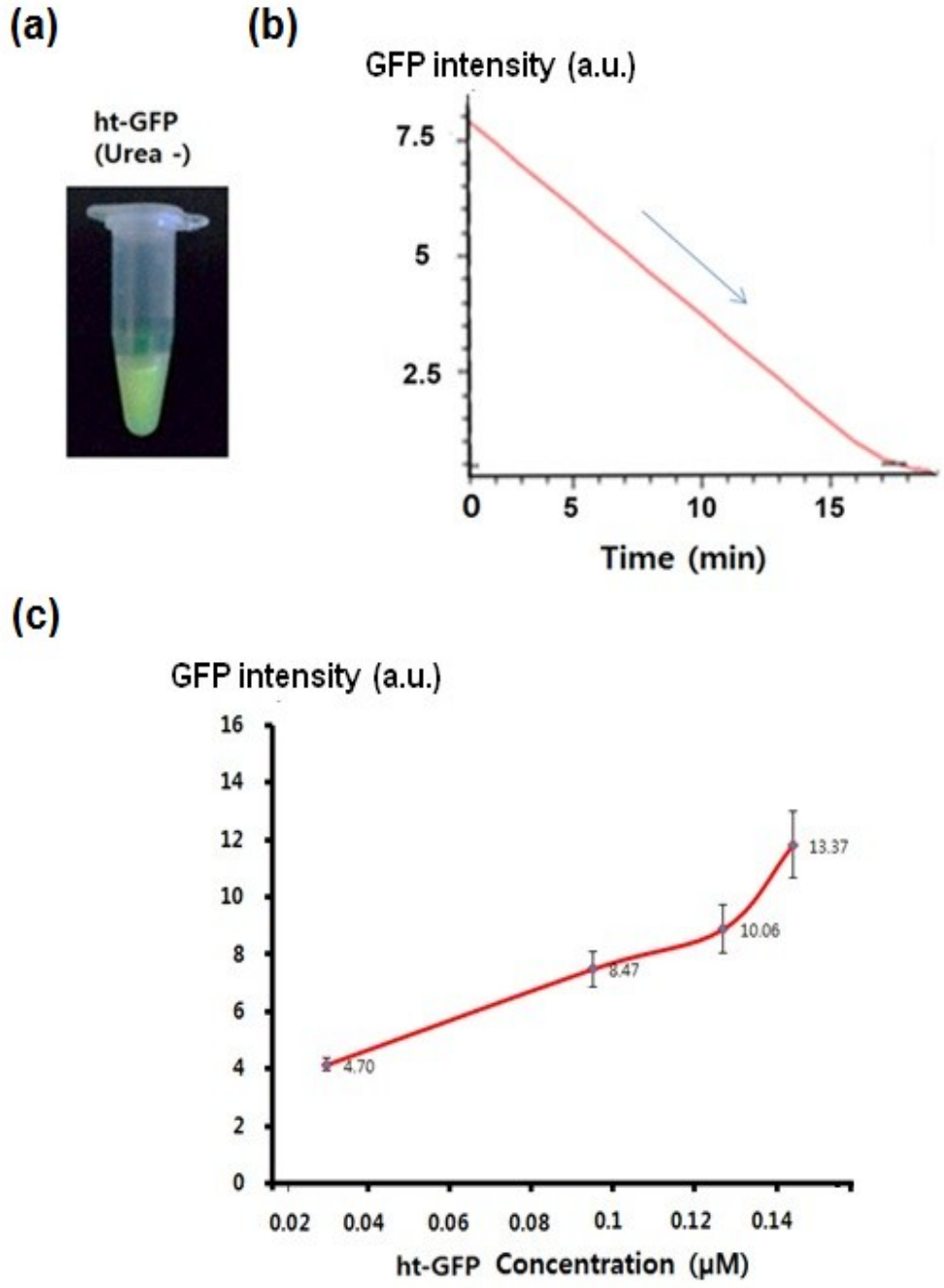

Fig. S2. The changes in the fluorescent intensity of ht-GFPs with time after urea treatment and the concentration dependent fluorescence intensity. (a) The photo of ht-GFP (2 mg/mL) before urea treatment, (b) the changes of fluorescence intensity with time /min after urea treatment, (c) the ht-GFP concentration (0.02 – 0.14 μM) dependent fluorescence intensity. (Ex; 471nm, Em; 512nm).

Table S1. Fluorescent intensity of agarose bead with varying amount of ht-GFPs.

ht-GFP	Fluorescence intensity (a. u.)*
2.5 ng	20
500 pg	< 20
200 pg	> 1.5
50 pg	1.5
0	0

* Fluorescence intensity was a mean value measured from 50 individual bead particles.

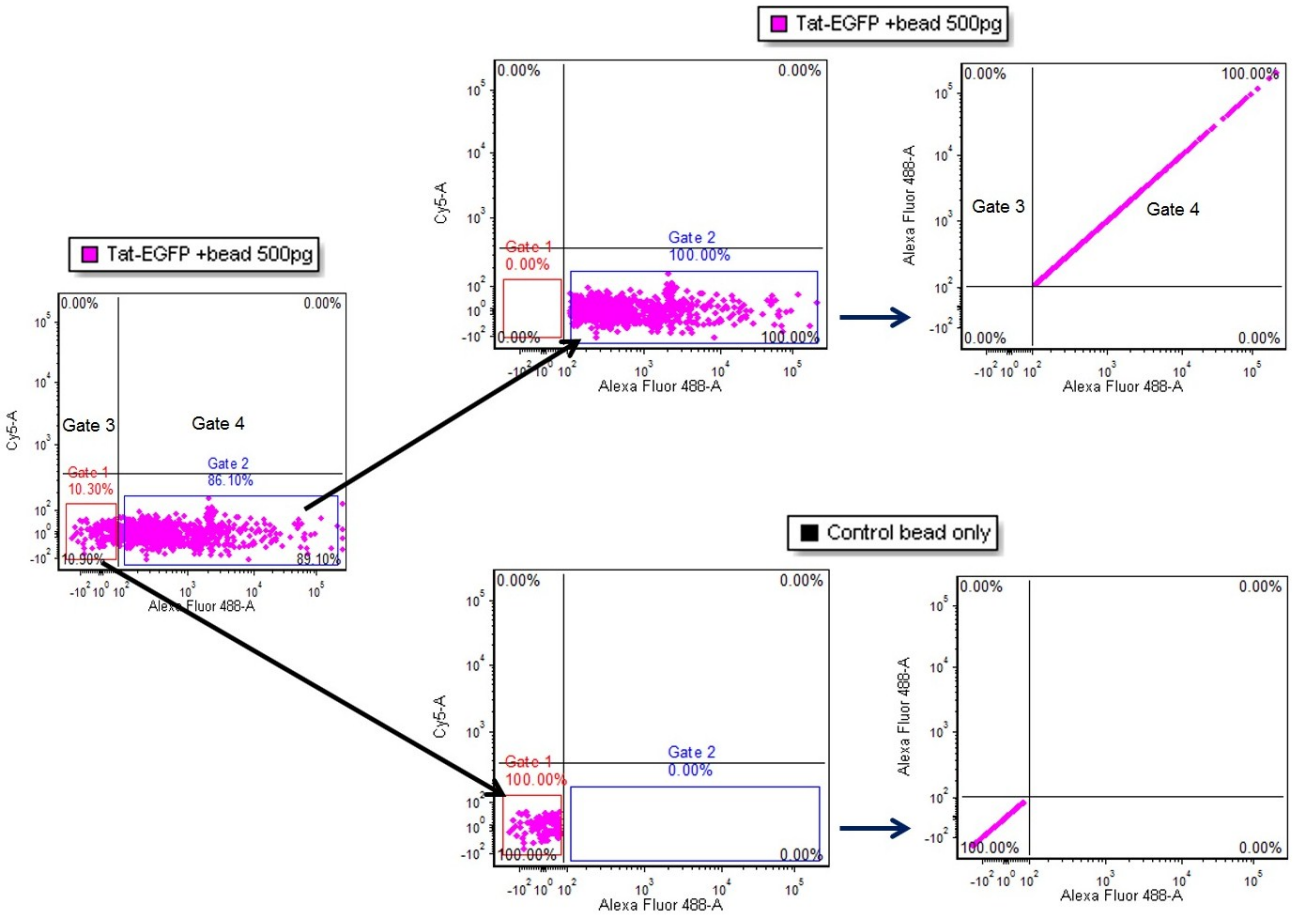

Fig. S3. Quadrant analysis of magnetic bead with ht-GFPs (0.5 ng) or without ht-GFPs. The beads incubated with ht-GFPs (0.5 ng) were identified by Alexa Fluor 488 parameters from subsequent Gate 2 analysis. The NTA beads alone were analyzed as negative controls. One thousand events were recorded for Alexa Fluor 488 with a 525/50 BP filter and a series of hierarchical gates to isolate single beads (Negative; Gate 1, Single positive; Gate 2 and Gate 3, Double positive; Gate 4).

Table S2. Flow cytometry results of magnetic bead binding with ht-GFPs.

ht-GFP	Fluorescence intensity (percent (=%))*
20 μg	96.1
1 μg	> 92
0.5 μg	92
0 μg	0

* Fluorescence intensity was a mean value measured from 10000 individual bead particles by Flow cytometry.

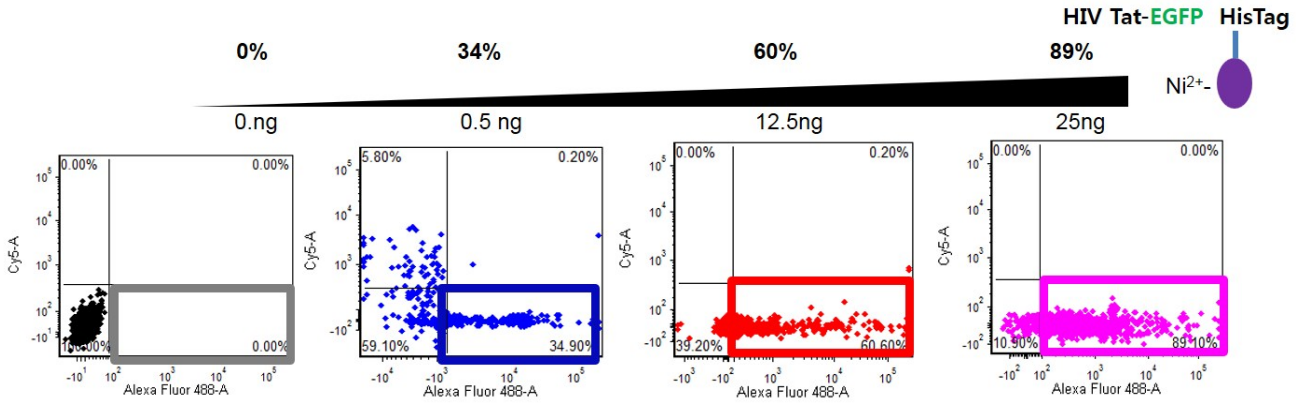

Fig. S4. The flow cytometry quadrant region analysis for the magnetic bead incubated with varying amount of ht-GFPs (0.5 ng ~ 2.0 μg). One thousand particles were recorded from two different channels (Cy5 and Alexa 488).

References

References

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