Electronic supplementary information (ESI) for:

Mechanisms of Chemical Protein ^{19}F-labeling and NMR-Based Biosensor Construction In Vitro and In Cells Using Self-Assembling Ligand-Directed Tosylate Compounds

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Electronic supplementary information (ESI) available: DLS analyses, optical densities, representative HPLC chromatograms, ^{19}F NMR spectra and SDS-PAGE analyses. Fig. S1-S5.

Supplementary Figures

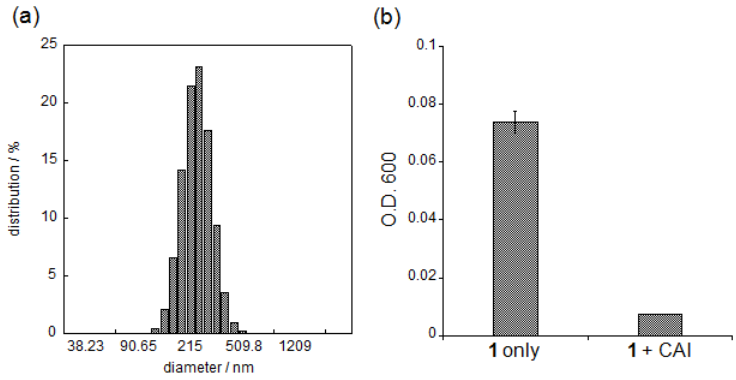

Figure S1. Self-assembly properties of **1**. (a) DLS analysis of particle-size distribution of the self-assembled reagent **1** (25 μM). (b) Optical density (O.D.) at 600 nm of an aqueous solution containing reagent **1** (25 μM) in the absence or presence of CAI (25 μM). Experiments were performed in triplicate to obtain mean and standard deviation values (shown as error bars). All experiments were performed in 50 mM HEPES buffer (pH 7.2, 0.2 mM TFA).

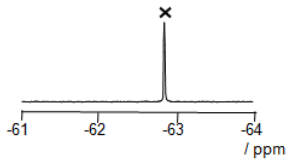

Figure S2. ^{19}F NMR spectrum of $\mathbf{FB_{OH}}$ in buffer solution. The chemically synthesized $\mathbf{FB_{OH}}$[S1] (500 μM) was dissolved in 50 mM HEPES buffer (pH 7.2, 0.2 mM TFA, 10% D_2O (v/v)) and subjected to ^{19}F NMR measurement. ×: −62.9 ppm.

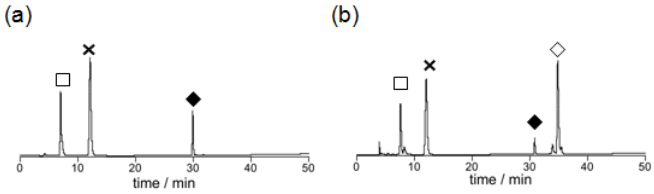

Figure S3. Representative HPLC chromatograms for the quantification of cleaved fragment $\mathbf{SA_{OH}}$. These chromatograms were obtained by incubating **2** (50 μM, ◆) in the absence (a) or presence (b) of CAI (50 μM, ◇) in 50 mM HEPES buffer (pH 7.2, 0.2 mM TFA) for 24 h at 37 ºC. EZA (100 μM, ×) was used as an internal standard. □: $\mathbf{SA_{OH}}$.

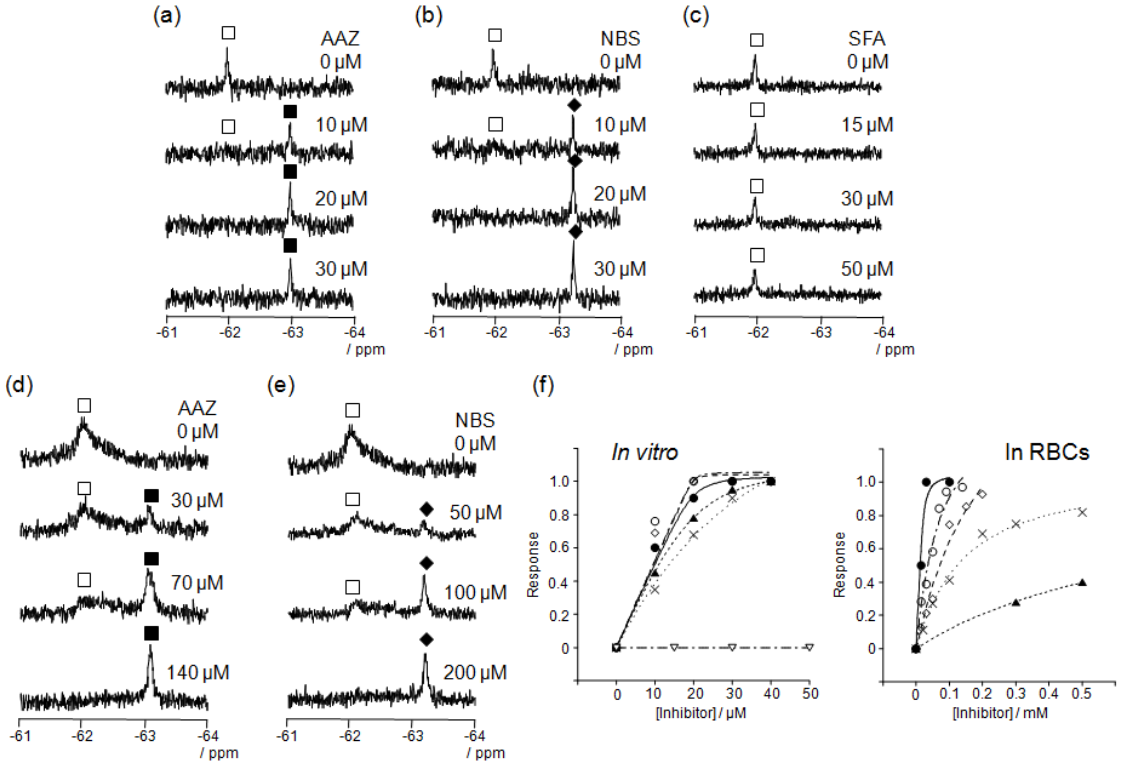

Figure S4. ^{19}F NMR titration of CA inhibitors *in vitro* and in RBCs. (a-c) *In vitro* detection of CA inhibitors (a: AAZ; b: NBS; c: SFA) using **FB**-CAI. (d, e) In RBCs detection of CA inhibitors (d: AAZ; e: NBS). □, −62.0 ppm, **FB**-(e)CAI with a free ligand-binding pocket; ■, −63.0 ppm, **FB**-(e)CAI$_{AAZ}$; ◆, −63.2 ppm, **FB**-(e)CAI$_{NBS}$. (f) Quantitative NMR detection of CA inhibitors with **FB**-(e)CAI. The response shown on the *y* axis is defined as the relative peak area of **FB**-(e)CAI$_{inhibitor}$ divided by the sum of the relative peak areas of **FB**-(e)CAI (□) and **FB**-(e)CAI$_{inhibitor}$ in (a-e), and was plotted against the concentration of inhibitors. ●: EZA; ○: AAZ; ◇: NBS; ▲: SBA; ×: BS; ▽: SFA. Left, *in vitro* system with **FB**-CAI (20 μM); right, in RBCs system with **FB**-eCA (the total concentration of eCA was about 54 μM). The experimental procedure is described in the main text.

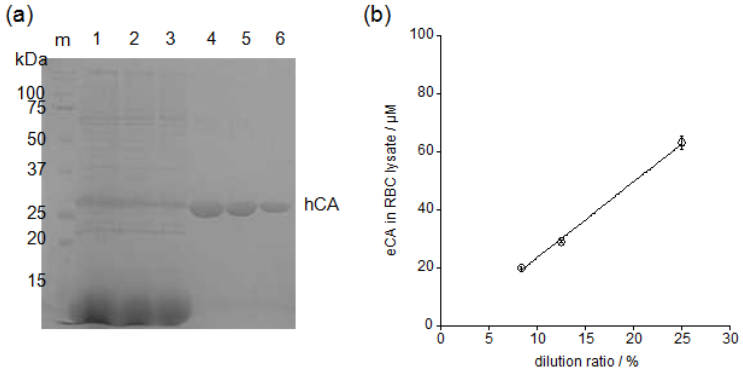

Figure S5. The CBB staining for determining the intracellular concentration of eCA. (a) An image of coomassie brilliant blue (CBB) staining of the RBCs lysate diluted with HBS (diluted by 25%, 12.5% and 8.3% in lanes 1, 2 and 3, respectively) and purified CAI (75 μM, 50 μM and 25 μM in lanes 4, 5 and 6, respectively). Samples were subjected to SDS-PAGE, stained with CBB and detected with white light illuminator. (b) The concentrations of eCA in RBCs calculated from dilution ratio and the concentration of purified CAI. Experiments were performed in duplicate to obtain mean and standard deviation values (shown as error bars).

Reference

S1) S. Tsukiji, M. Miyagawa, Y. Takaoka, T. Tamura, I. Hamachi, *Nat. Chem. Biol.*, 2009, **5**, 341−343.