A Unique Protein Labeling System Based on Melittin and the Non-covalent Binding-induced Pyrene Excimer

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(Electronic Supplementary Information)

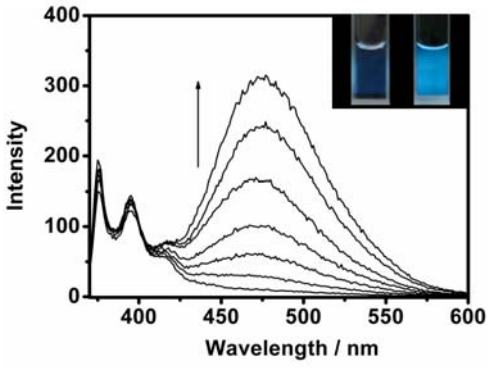

Fig. S1 Fluorescence emission spectra of **1** (5.0 μM) with various concentrations of melittin (0-5.0 μM) in 20 mM Tris-HAc buffer solution (pH 7.2, containing 2% DMSO). The excitation wavelength is 354 nm.

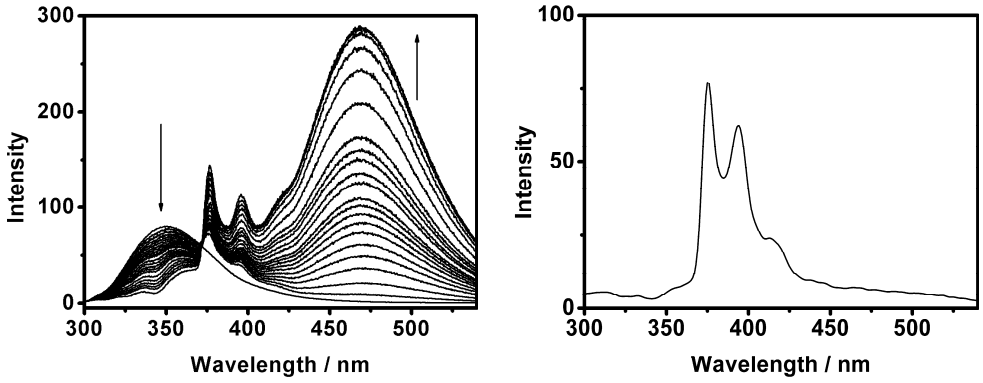

Fig. S2 Left, Fluorescence emission spectra of melittin (5.0 μM) in the absence and presence of **1** with various concentrations of 0-20 μM in 20 mM Tris-HAc buffer solution (pH7.2, containing 2% DMSO). Right, Fluorescence emission spectra of **1** (20 μM) in 20 mM Tris-HAc buffer solution (pH7.2, containing 2% DMSO). The excitation wavelengths in both cases are 280 nm.

When excited at 280 nm, the sole Trp[19] in melittin contributes a broad emission band at around 340 nm, which decreases with the addition of **1** as the involving energy transfer from Trp[19] to pyrene in **1** after binding. In addition, with the gradual addition of **1** typical band at 376 and 396 nm increased and essentially a significant broad band centered at 475 nm was observed for pyrene excimer. Such results indicated that a direct interaction occur between **1** and melittin, and most probably the bind sites (or at least some of the binding sites) are close to the C-terminal of melittin which result in both the energy transfer[1] from Trp[19] to pyrene and the formation of pyrene excimer.

Calculation of the binding constant between melittin and 1

Generally, fluorescent quenching can occur in two different mechanisms, static quenching and dynamic quenching. Based on literatures the later possibility was eliminated for the present case. For the static quenching, the relationship between fluorescence quenching intensity and the concentration of quenchers can be described by equation:

$$\lg(F_0\text{-}F)/F = \lg K + n\lg[Q] \qquad (1)$$

Where K is the binding constant, $[Q]$ is the concentration of **1** and n is the number of binding sites per Melittin. The fitting to plot of $\lg[(F_0\text{-}F)/F]$ *vs* $\log[\mathbf{1}]$ in Fig. S3 supplied a binding constant (K) of 4.6×10^{17} and a n of 3.6.

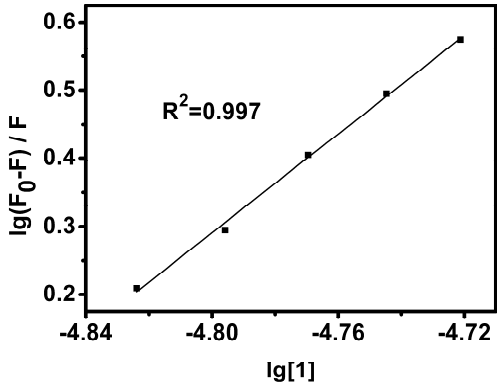

Fig. S3 Plot of $\lg[(F_0\text{-}F)/F]$ *vs* $\log[\mathbf{1}]$ as per equation (1). The concentrations of **1** are 15-19μM, respectively.

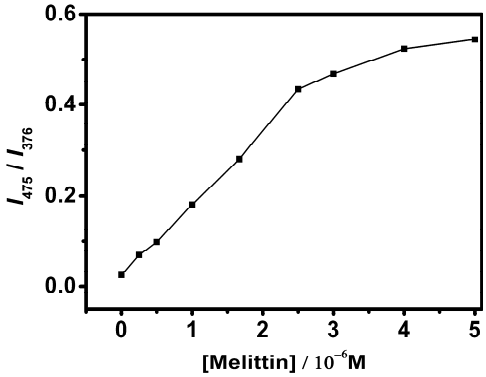

Fig. S4 Fluorescence intensity ratio I_{475}/I_{376} of **1** to melittin with various concentration of 0-5.0 μM in 20 mM Tris-HAc buffer solution (pH7.2, containing 2% DMSO). The excitation wavelength is 340 nm.

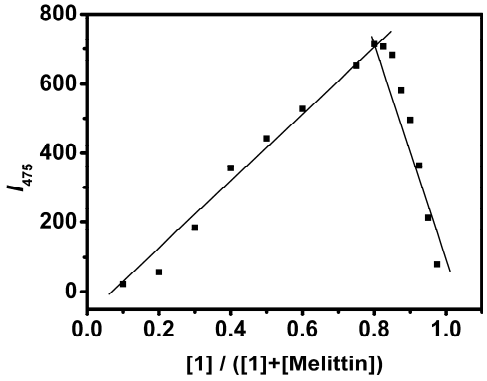

Fig. S5 Fluorescence intensity changes of band at 475 nm of **1** and melittin with a total concentration of 5.0 μM in 20 mM Tris-HAc buffer solution (pH7.2, containing 2% DMSO), indicating a 4:1 stoichiometry for **1** to melittin.

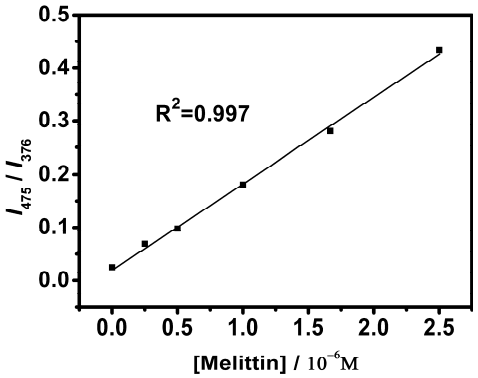

Fig. S6 Straight line fit of I_{475}/I_{376} of **1** (5.0 μM) with the concentrations of melittin between 0 and 2.5 μM (R^2=0.997). Spectra were measured in 20 mM Tris-HAc buffer solution (pH 7.2, containing 2% DMSO) with an excitation wavelength at 340 nm.

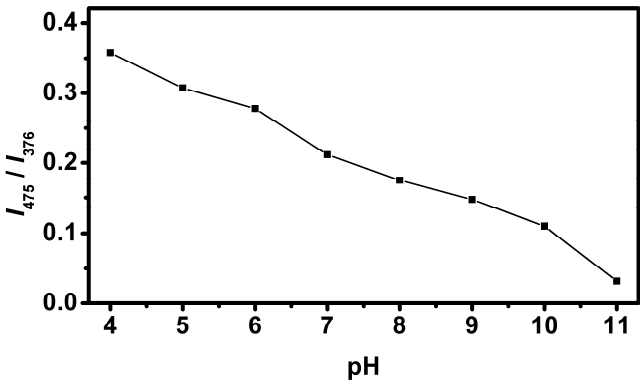

Fig. S7 pH-dependent of the intensity ratio of I_{475}/I_{376} from pH 4 to pH 11. The excitation wavelength is 340 nm. The concentration of **1** is 5.0 μM. The concentration of melittin is 1.0 μM.

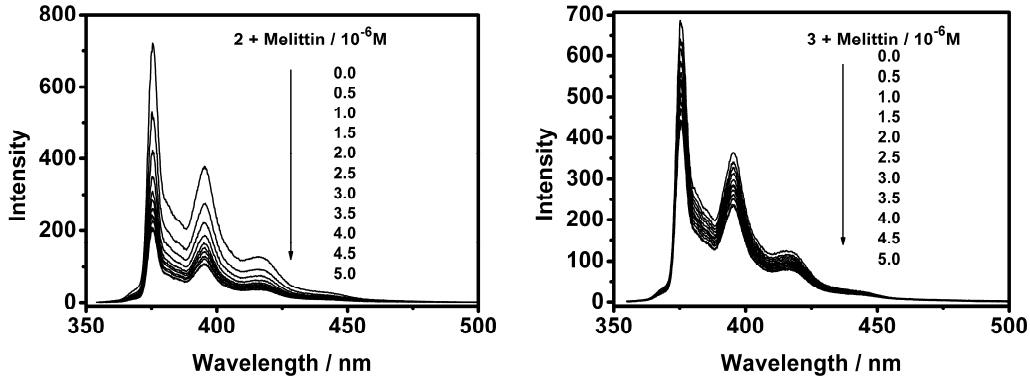

Fig. S8 Fluorescence spectra of **2** and **3** (5.0 μM) with the concentration increases of melittin in 20 mM Tris-HAc buffer solution (pH 7.2, containing 2% DMSO). The excitation wavelength is 340 nm.

The peptides of Mlt-C-7, Mlt-C-9, Mlt-C-13, and Mlt^{W19A}-C-13 were purchased from GL Biochem (Shanghai, China) Ltd., which were assayed by the reverse phase HPLC (purity >98%).

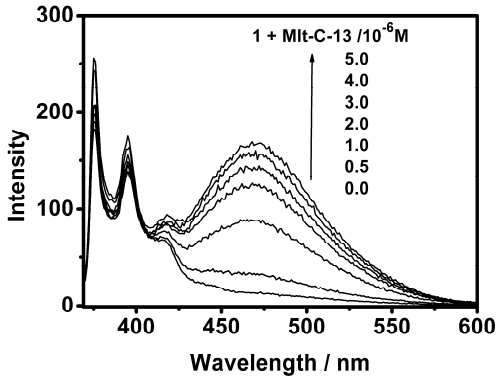

Fig. S9 Fluorescence spectra of **1** (25.0 μM) with the concentration increases of Mlt-C-13 (0-5.0 μM) in 20 mM Tris-HAc buffer solution (pH 7.2, containing 2% DMSO). The excitation wavelength is 354 nm.

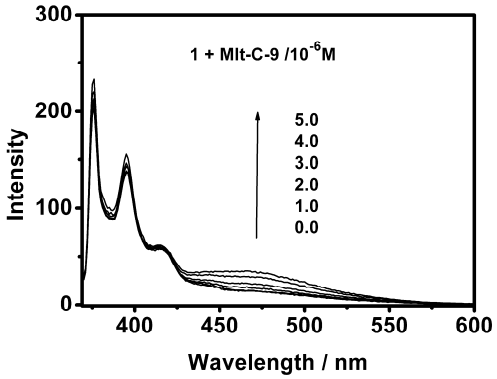

Fig. S10 Fluorescence spectra of **1** (25.0 μM) with the concentration increases of Mlt-C-9 (0-5.0 μM) in 20 mM Tris-HAc buffer solution (pH 7.2, containing 2% DMSO). The excitation wavelength is 354 nm.

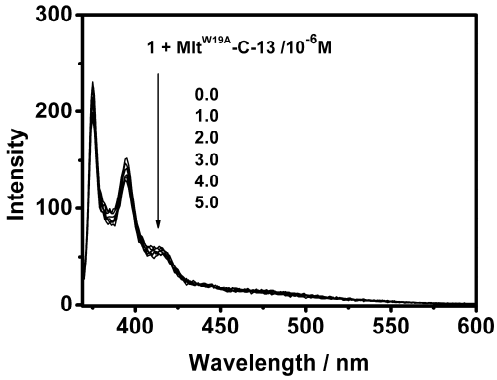

Fig. S11 Fluorescence spectra of **1** (25.0 μM) with the concentration increases of Mlt^{W19A}-C-13 (0-5.0 μM) in 20 mM Tris-HAc buffer solution (pH 7.2, containing 2% DMSO). The excitation wavelength is 354 nm.

Expression and purification of GST and GST-Mlt-C-13

The C-terminal residues of melittin (PALISWIKRKRQQ) was cloned into pGEX-6p1 vector and named *GST-Mlt-C13*, both were expressed in *E. coli* BL21 (DE3). The proteins were purified by affinity chromatography by using glutathione sepharose 4B (GE Healthcare), and concentration-desalting by ultra-filtration.

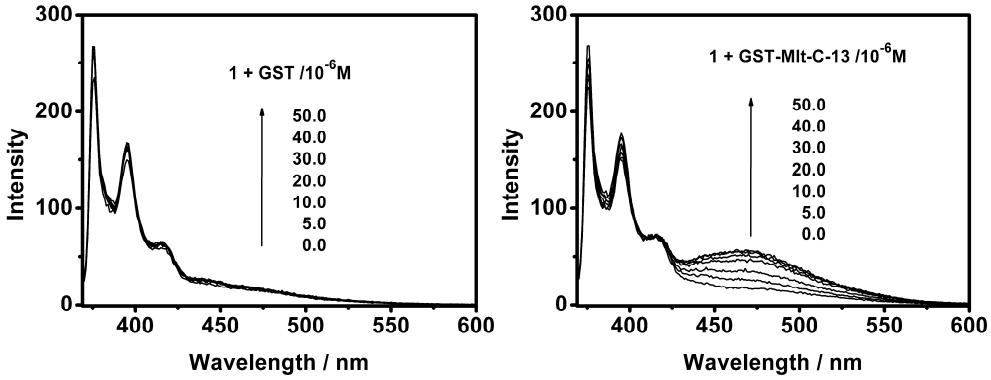

Fig. S12 Fluorescence spectra of **1** (25.0 μM) with the concentration increases of GST (0-50 μM, left) and GST-Mlt-C-13 (0-50 μM, right) in 20 mM Tris-HAc buffer solution (pH 7.2, containing 2% DMSO). The excitation wavelength is 354 nm.

References

1 Sato, T., K. W. Mattison, P. L. Dubin, M. Kamachi, and Y. Morishima, *Langmuir,* 1998, 14, 5430.