SUPPORTING INFORMATION

MODELING OF THE ENDOSOMOLYTIC ACTIVITY OF HA2-TAT PEPTIDES WITH RED BLOOD CELLS AND GHOSTS†

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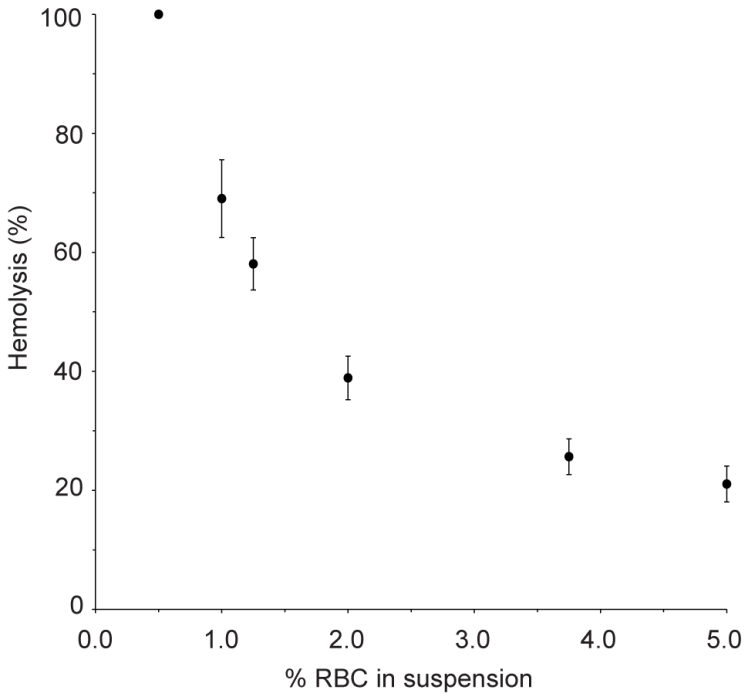

Figure S1. Effect of the number of RBCs present in suspension on the hemolytic activity of E5-TAT. RBCs were diluted in PBS at pH 4.0 to different % suspensions but the concentration of E5-TAT was kept constant in each sample (0.5 μM). The hemolysis assay was performed as described in Figure 2. For each RBC suspension, % lysis was determined by addition of Triton X. The data represent is the average of four experiments and the error bars correspond to the standard deviation.

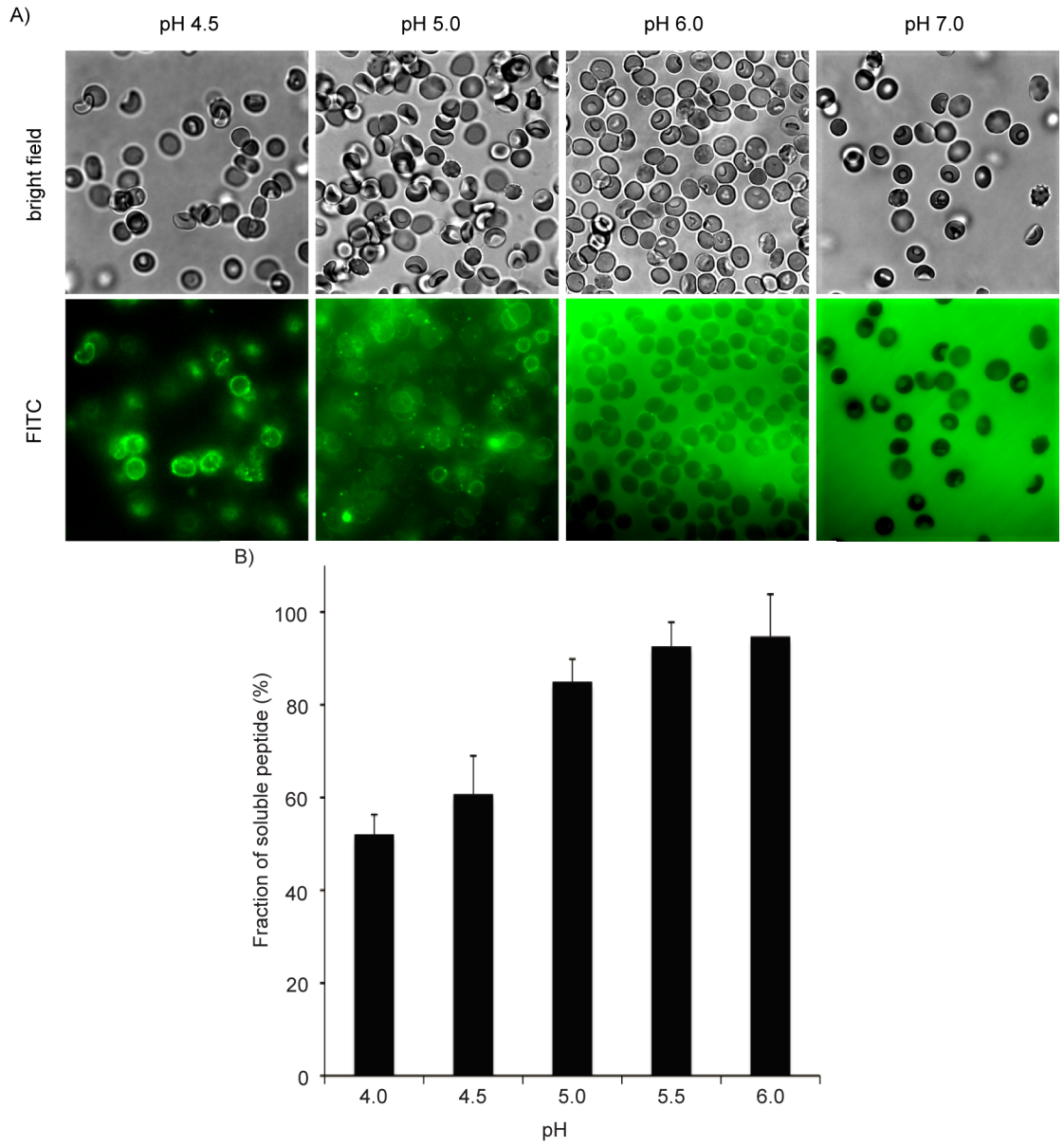

Figure S2. A) Binding of FI-E5-TAT (1.0 μM) to intact RBCs as a function of pH as observed by fluorescence microscopy. The peptide was added to 1.25% RBC suspensions and the samples were imaged after 5 minutes of incubation. At pH 7.0, the fluorescence signal is detected in solution. As the pH is reduced, the peptide accumulates at the membrane as detected by an increase in the contrast between the signal at the membrane and the signal in solution (the methods used are the same as in Figure 3). Note: The samples also contain 5 to 10% of lysed ghosts but intact RBCs can easily be identified in the bright field image because of their darker contrast. B) The samples described in A) were centrifuged to separate RBC-bound peptides from the fraction of peptide remaining in solution. The amount of soluble peptide was determined by SDS-PAGE and densitometric analysis. Each sample was normalized to a pH 7.0 control containing the fluorescent peptide but no RBCs (= 100% peptide in solution).

Binding Assay. The binding of Fl-E5-TAT to intact RBCs as a function of pH was determined by incubating peptide (1 μM, <HD50 to minimize lysis) with 1.25% RBC suspensions in PBS at different pH values (adjusted with HCl or NaOH, 4.5<pH<7.5). After 5 min incubation, the samples were centrifuged for 5 minutes at 1500g. The amount of peptide present in the supernatants was analyzed by SDS-PAGE using 12% SDS-gel (pH 8.8). The gels were imaged with the Typhoon 9410 imager (GE Healthcare) set with an excitation wavelength of 488 nm and a green emission filter at 526 nm to detect the fluorescence of Fl-E5-TAT. Densitometric analysis of the intensity of the Fl-E5-TAT bands was performed with the ImageQuant 5.0 software. The fluorescence of the fluorescein label on E5-TAT is pH dependent. However, because all samples are exposed to the same pH within the gel, the fluorescence intensity of the bands is only proportional to the amount of peptide present in the sample. The data were normalized using a sample containing Fl-E5-TAT at pH 7.0 but with no RBC present as the one hundred percent soluble control.