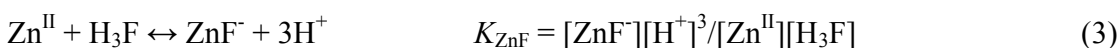
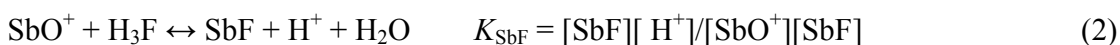
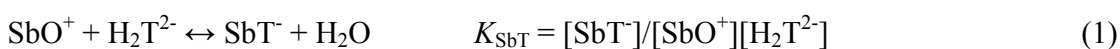


Determination of experimental Sb binding constant

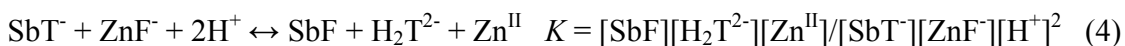
Determination of Sb binding constant was performed through titration of preformed ZF with the trivalent antimony compound potassium Sb^{III} tartrate and taking advantages of the known equilibrium constants for the formation of potassium Sb^{III} tartrate¹⁷ and ZnF peptide.^{16b} Experiment was performed at 298 K in 20 mM potassium phosphate, KCl 0.075 M, pH 6 (*I* = 0.1 M). Initial peptide concentration was 3.3 μM and Sb concentration varied from 3.3 to 95 μM (8 data points).

The following reactions and equilibrium constants were considered:



Where SbO⁺, H₂T²⁻ and H₃F, Zn^{II}, SbT⁻, SbF and ZnF⁻ represent the reactive antimony species in solution, the fully deprotonated form of tartrate, the apo-peptide, the free Zn^{II} cation, the 1:1 Sb^{III}-tartrate complex, the 1:1 Sb^{III}-peptide complex and the 1:1 Zn^{II}-peptide complex, respectively.

From reactions (1), (2) and (3), the following reaction and equilibrium constant were deduced:



Since [SbF] = [H₂T²⁻], [Zn^{II}] = [Zn]_{tot} - [ZnF⁻] and [SbT⁻] = [Sb]_{tot} - [SbF],

where [Zn]_{tot} and [Sb]_{tot} represent the concentrations of total (free and bound) Zn and Sb, respectively, the equilibrium constant *K* could be expressed as follows:

$$K = [\text{SbF}]^2 ([\text{Zn}]_{\text{tot}} - [\text{ZnF}^-])/[\text{ZnF}^-]([\text{Sb}]_{\text{tot}} - [\text{SbF}])[\text{H}^+]^2 \quad (5)$$

From the fluorimetric titration data, [SbF] and [ZnF⁻] were calculated as follows:

$$[\text{SbF}] = [\text{F}]_{\text{tot}}(I - I_{\text{Zn}})/(I_{\text{Sb}} - I_{\text{Zn}})$$

$$[\text{ZnF}^-] = [\text{F}]_{\text{tot}}(I - I_{\text{Sb}})/(I_{\text{Zn}} - I_{\text{Sb}})$$

Where [F]_{tot} represent the concentrations of total (bound to Zn and Sb) peptide,

Where *I* is the fluorescence measured from any added potassium Sb^{III} tartrate concentration and *I*_{Zn} and *I*_{Sb} correspond to the fluorescence intensities of the fully Zn^{II}- and Sb^{III}-saturated peptide, respectively.

The plot of [SbF]² ([Zn]_{tot} - [ZnF⁻])/[ZnF⁻] as a function of ([Sb]_{tot} - [SbF])[H⁺]² followed by linear regression, according to eq 5, allowed the determination of *K*:

$$K = 22820 \pm 1890$$

Since K is related to the equilibrium constants of reactions (1), (2) and (3), according to the equation $K = K_{\text{SbF}}/K_{\text{ZnF}}K_{\text{SbT}}$, $K_{\text{SbT}} = 1.638 \times 10^8$ is given in the literature¹⁷ and Zn binding constant to the peptide at pH 6 and 298 K is: $K'_{\text{ZnF}} = K_{\text{ZnF}}/[\text{H}^+]^3 = 10^{7.5} \text{ M}^{-1}$ according to literature,^{16b} K_{SbF} was calculated as: $K_{\text{SbF}} = 118$.

A binding constant $K'_{\text{SbF}} = K_{\text{SbF}}/[\text{H}^+] = 1.18 \times 10^8 \text{ M}^{-1}$ was determined at pH 6 and 298 K.