



Figure S2 FRET assay to detect thermolysin activity in the wash solution and for the enzyme immobilized on the surface of a glass cover slip modified with tannic acid (a) and pyrogallol (b). In the first wash solution (black) the fluorescence intensity rapidly increased over time, reaching a plateau when the enzyme had converted all the substrate. In the second wash solution (blue), the recorded data did not show any significant change in the fluorescence intensity overnight. The “glass surface” data shows the fluorescence detected when a sample subjected to the more thorough procedure (i.e. after Wash 2) was placed in a FRET substrate solution, thus measuring the irreversibly immobilized enzyme activity (green).