Supplemental Material for “Real-time monitoring of enzyme activity in a mesoporous silicon double layer,” by Manuel M. Orosco, Claudia Pacholski, and Michael J. Sailor
Calculation of Mass Loading of Pepsin.
The mass loading of pepsin was determined from the optical reflectivity spectrum by application of the Bruggeman effective medium model. Table S1 contains the assumptions and measurements that went into the calculation:

TABLE S1: Parameters used to determine pepsin loading in nanoreactor

| Parameter                        | Value  | Comment                                                                 |
|----------------------------------|--------|-------------------------------------------------------------------------|
| Pepsin molecular weight          | 34.5 kDa | Fujinaga, M., Cherniaia, M. M., Tarasova, N. I., Mosimann, S. C. & James, M. N. Crystal structure of human pepsin and its complex with pepstatin. Protein science : a publication of the Protein Society 4, 960-72 (1995) |
| Pepsin refractive index          | 1.43    |                                                                         |
| Buffer refractive index          | 1.3365  |                                                                         |
| Porous SiO$_2$ refractive index  | 1.9     |                                                                         |
| Pepsin density                   | 1.42 g/mL | Fischer, H., Polikarpov, I. & Craievich, A.F. Average protein density is a molecular-weight-dependent function. Prot. Sci. 13, 2825-2828 (2004). |
| Optical probe spot size (dia)    | 0.15 cm |                                                                         |
| Layer 1 thickness                | 3104 nm |                                                                         |
| Layer 1 porosity                 | 75.5%   |                                                                         |
| 2nL value in buffer, before loading pepsin | 9104 nm |                                                                         |
| 2nL value in buffer, after loading pepsin | 9119 nm |                                                                         |

A three-component Bruggeman approximation was applied to model the refractive index of the layer (see: Pickering, C., Beale, M.I.J., Robbins, D.J., Pearson, P.J. & Greef, R. Optical studies of the structure of porous silicon films formed in p-type degenerate and non-degenerate silicon. J. Phys. C 17, 6535-6552 (1984)), and the resulting % loading of protein is 3%, the mass loading is 192 ng in the volume element in the path of the 0.15 cm-dia optical beam, or a total loading of 13 µg of pepsin in the (1.2 cm$^2$) chip.
Supplemental Figure S1. Time course of the optical response of both porous Si layers during enzymatic loading and subsequent digestion. In the beginning of the experiment, the porous Si double layer sample is flushed with pH 2.0 buffer. A 28 μM solution of pepsin in pH 2.0 buffer is then introduced, and the optical thickness of Layer 1 is observed to increase, corresponding to ~3 % loading of pepsin (see calculations above). The enzyme is effectively excluded from Layer 2 due to its much smaller pore dimensions. The system is flushed with pH 2.0 buffer and the rest of the experiment proceeds as described in Fig 3 of the main text. The y-axis records the value of nL in the 1st (top, large pore) or the 2nd (bottom, small pore) layers, as indicated. The slight increase in optical thickness of the 2nd layer upon addition of pepsin indicates accumulation of pepsin at the interface between the two layers.
Supplemental Figure S2. Time course of the optical response of both porous Si layers during enzymatic loading and subsequent digestion, enzyme loaded from a complex mixture. Experiment similar to that described in Supplemental Figure S1, but the enzyme is loaded from a complex mixture. An array of amino acids, sugars and vitamins were mixed in a pH 2.0 buffer (closely resembling Eagle’s Medium). Pepsin was then spiked into the media and the enzyme loading and substrate digestion experiment similar to that shown in Supplemental Fig. 1 was performed. The y-axis records the value of nL in the 1\textsuperscript{st} (top, large pore) or the 2\textsuperscript{nd} (bottom, small pore) layers, as indicated. The slight increase in optical thickness of the 2\textsuperscript{nd} layer upon addition of pepsin indicates accumulation of pepsin at the interface between the two layers.
Supplemental Figure S3. Non-linear least-squares fit of reactor data to a Michaelis-Menten Kinetics Model. Higher concentrations of α-casein produce larger changes in the quantity 2nL measured from Layer 2 because a greater mass of substrate is digested per unit time. The reaction velocity ($V_o$) is taken as the slope of the initial, linear region of the curves shown in Fig 4a of the main text (first 30 min). The initial reaction velocity ($V_o$) is then plotted versus the concentration of α-casein substrate. The data are then fit (non-linear least-squares) to the Michaelis-Menten relationship:

$$V = \frac{V_{\text{max}}[S]}{K_m + [S]}$$

where ($V$) is the initial velocity, $K_m$ is the Michaelis constant, $V_{\text{max}}$ is the maximum velocity, and [S] is the concentration of substrate α-casein. Data represent triplicate measurements.
Supplemental Figure S4. Inhibition of the Activity of Pepsin in the Nano-Reactor using Pepstatin A. As in Fig. 4a, data are presented in terms of the response of the size exclusion Layer 2 as a function of time. The quantity $\Delta 2nL$ represents the change in 2nL relative to its value immediately before introduction of the $\alpha$-casein substrate and inhibitor. The initial slope of each curve is indicated by a dashed line. Each curve corresponds to a fixed concentration of inhibitor (1 $\mu$M) and a different concentration of substrate as indicated in the legend.
Supplemental Figure S5. Non-linear least-squares fit of reactor data with inhibitor to a Michaelis-Menten Kinetics Model. The reaction velocity ($V_o$) is taken as the slope of the initial, linear region of the curves at the point of inflection shown in Fig S4. The initial reaction ($V_o$) is then plotted versus the concentration of $\alpha$-casein substrate. The data are then fitted to a non-linear least-squares fit using the following Michaelis-Menton relationship:

$$V = \frac{V_{\max \, app} [S]}{K_{m \, app} + [S]}$$

where ($V$) is the initial velocity, $K_{m \, app}$ is the apparent Michaelis constant, $V_{\max \, app}$ is the apparent maximum reaction velocity, and [S] is the concentration of substrate $\alpha$-casein. Data represent triplicate measurements.

The fit yields values of $K_m$ (apparent) of 0.30 mM and $V_{\max}$ (apparent) of 0.40 $\mu$M/min. In a competitive inhibitor model, the value of $V_{\max}$ (apparent) should be equal to $V_{\max}$ ($= 0.35$ $\mu$M/min), and $K_m$ (apparent) should be larger than $K_m$ ($= 18$ $\mu$M). Thus the values obtained from the nano-reactor are consistent with a competitive inhibition model.
**Conversion of Δ2nL into units of nM/min**

Analysis of the data involves an assumption that the change in the optical measurement 2nL relates to mass of material entering or leaving the porous layer. The relationship is established by Fig. 4b. These data are used to convert Δ2nL into concentration of casein by the following:

Δ2nL from the 2\(^{nd}\) Layer were converted using the linear fit in the plot 1/Δ2nL vs 1/(casein concentration), Figure 4b:

\[ y = mx + b \]
\[ y = 1.0609e^{-7}x + 0.0081304 \]

The value of x is the inverse of the substrate concentration and can be solved by rearranging the equation:

\[ x = \frac{m}{y - b}; \quad y = \frac{1}{\Delta 2nL} \]

The converted concentration values were then plotted versus time in minutes.

**Determination of the value of Ki**

For pepstatin A inhibition, a competitive inhibition model was used to find the inhibition constant, Ki:

\[ K_{m \text{ app}} = K_m (1 + [I]/K_i) \]

Where \( K_{m \text{ app}} \) is the Michaelis-Menten constant in the presence of an inhibitor I, \( K_m \) is the Michaelis-Menten constant, \([I]\) is the concentration of inhibitor, and \( K_i \) is the inhibition constant.

\[ 0.2279\text{mM}=17.59\text{mM} \times \frac{1+(1\text{mM})}{K_i} \]
\[ 0.2279\text{mM}=17.59\text{mM} + 17.59\text{pM}/K_i \]
\[ 0.21031\text{mM}=17.59\text{pM}/K_i \]

\[ K_i = 84 \text{ nM} \]