Electronic Supplementary Material (ESI) for Analytical Methods

Supporting Information

Hydrolysis of Lysozyme with an RF-Powered Micro-Reactor

Valerie J. Scott, Peter H. Siegel, and Xenia Amashukeli*

Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California, USA 91109. *Received October 24, 2011.*

*Correspondence should be sent to X. Amashukeli (Xenia.Amashukeli@jpl.nasa.gov)

1. **Experiments run with the same applied head pressures but varied flow rates; in each case, as flow rate was increased, the reactivity decreased.**

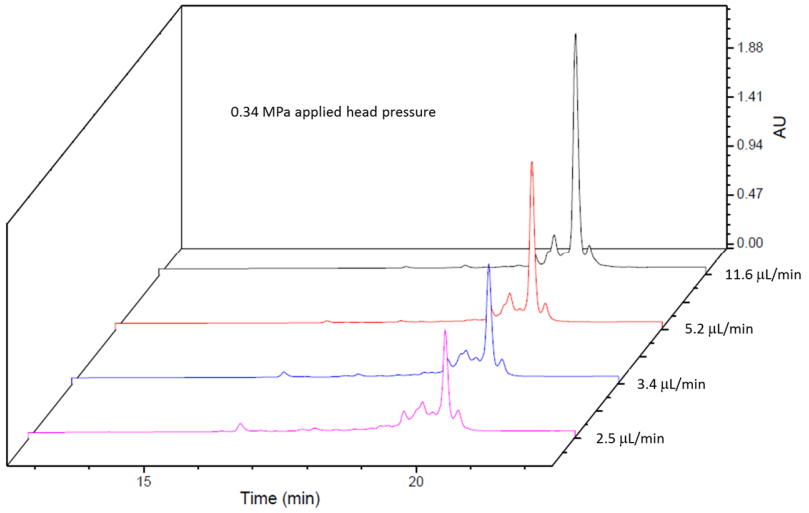

Figure 1 SI. HPLC-UV chromatograph of hydrolyzed lysozyme samples at constant head pressure (0.34 MPa) and different flow rates. An increase in the flow rate results in decreased reactivity.

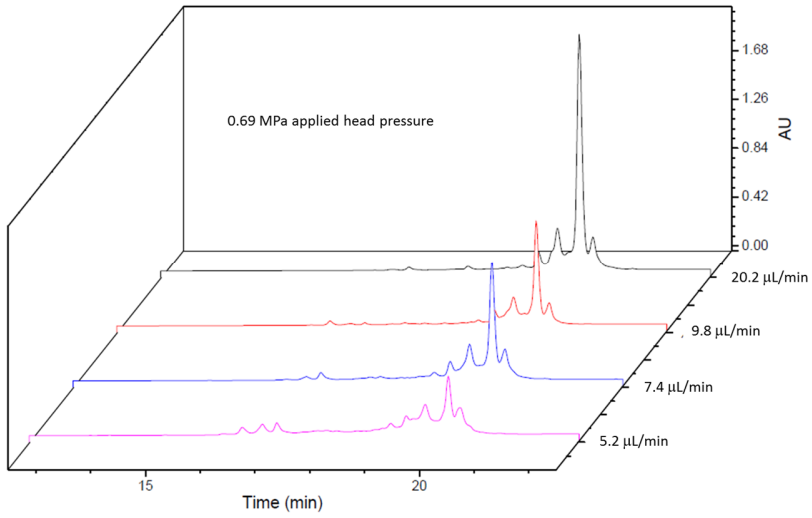

Figure 2 SI. HPLC-UV chromatograph of hydrolyzed lysozyme samples at constant head pressure (0.69 MPa) and different flow rates. An increase in the flow rate results in decreased reactivity.

2. **Experiments where the applied head pressures are different but the flow rates are the same; higher pressure experiments yielded more reactivity.**

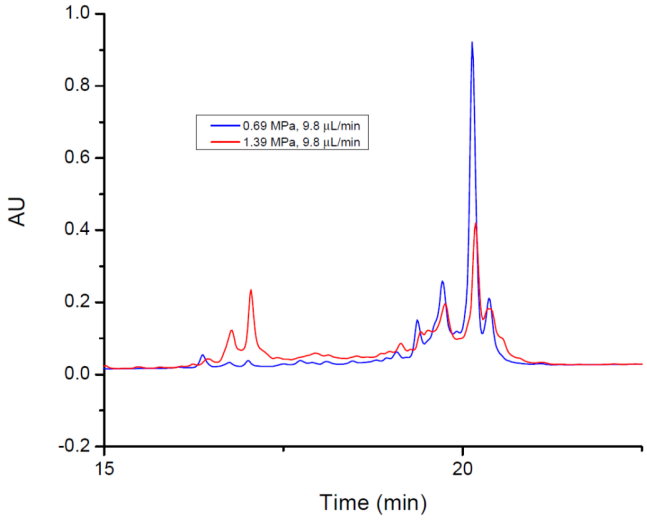

Figure 3 SI. Comparison of HPLC-UV chromatograph for the samples hydrolyzed at a flow rate of 9.8 µL/min

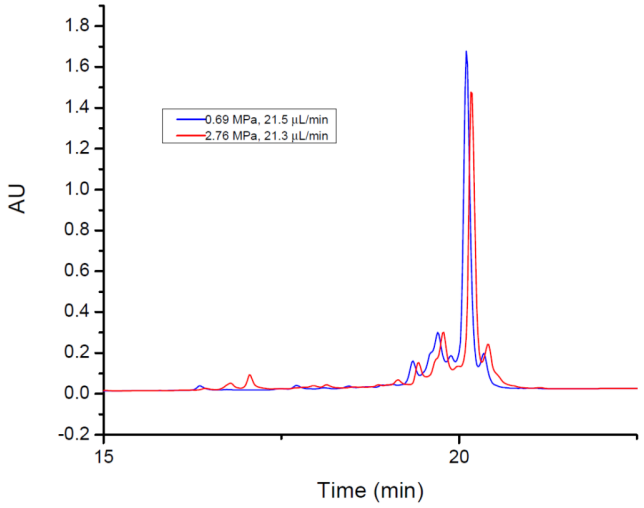

Figure 4 SI. Comparison of HPLC-UV chromatograph for the samples hydrolyzed at fast flow rates.

3. Tis-Tricine SDS-PAGE gels not shown in paper, displaying the RF micro-reactor's ability to break the peptide backbone.

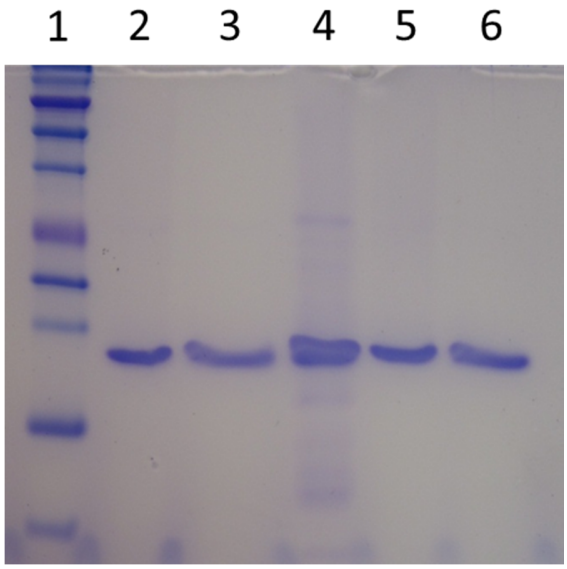

Figure 5 SI. Sample by lane: 1- Rainbow molecular weight marker; 2- Stock Lysozyme solution (6mM HCl) in reducing buffer; 3- Stock Lysozyme solution (6mM HCl) in non-reducing buffer; 4- Heat and pressure, lysozyme (6mM HCl) solution, 130 ^{o}C, 2.07 MPa, 0.92 m flow restrictor, 24 µL/min, ~2.5 inches submerged, in reducing buffer; 5- Stock lysozyme (DI water) in reducing buffer; 6- Stock lysozyme (DI water) in non-reducing buffer.

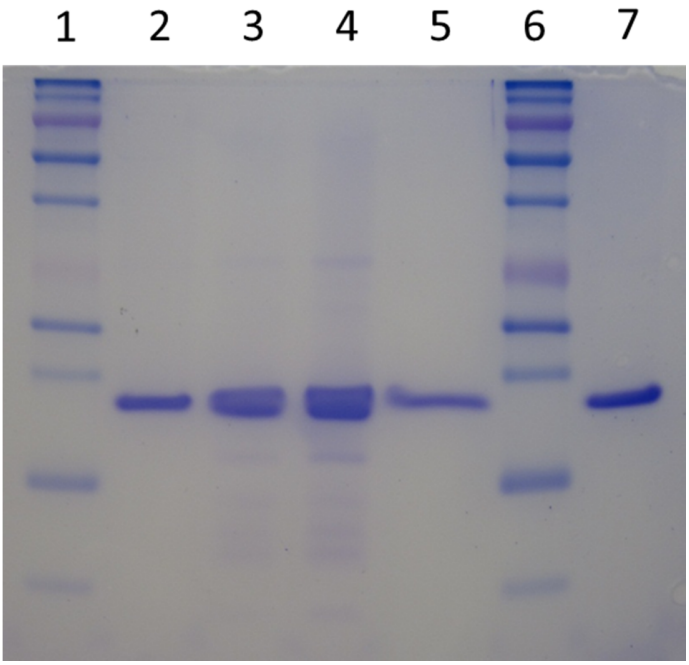

Figure 6 SI. Sample by lane: 1- Rainbow molecular weight marker; 2- Stock Lysozyme solution (6mM HCl) in reducing buffer; 3- Heat and pressure, lysozyme (6mM HCl) solution, 130 ^{o}C, 2.07 MPa, 0.91 m flow restrictor, 24 µL/min, ~2.5 inches submerged, in reducing buffer; 4- Heat and pressure, lysozyme (6mM HCl) solution, 130 ^{o}C, 2.07 MPa, 0.91 m flow restrictor, 24 µL/min, ~2.5 inches submerged, in reducing buffer; 5- Stock Lysozyme solution (6mM HCl) in reducing buffer; 6- Rainbow molecular weight marker; 7- Stock Lysozyme solution (6mM HCl) in reducing buffer.

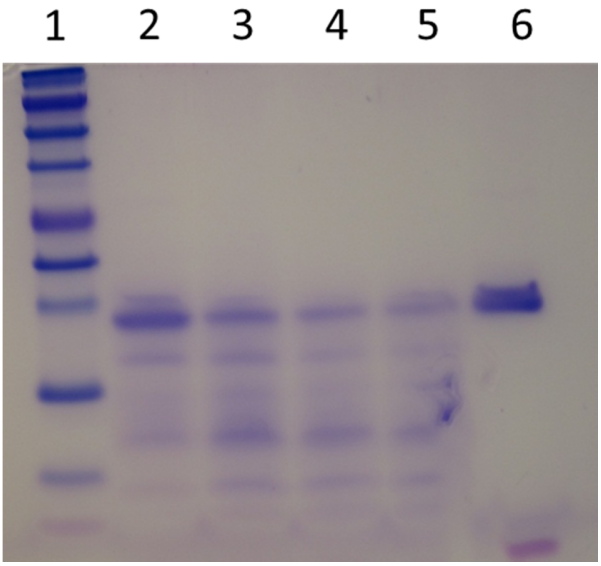

Figure 7 SI. Sample by lane: 1- Rainbow molecular weight marker; 2- RF micro-reactor, lysozyme solution (6mM HCl), 0.34 MPa, 2.5 µL/min, 1.22 m flow restrictor, in reducing buffer; 3- RF micro-reactor, lysozyme solution (6mM HCl), 0.69 MPa, 5.2 µL/min, 1.22 m flow restrictor, in reducing buffer; 4- RF micro-reactor, lysozyme solution (6mM HCl), 1.34 MPa, 9.8 µL/min, 1.22 m flow restrictor, in reducing buffer; 5- RF micro-reactor, lysozyme solution (6mM HCl), 2.07 MPa, 15.6 µL/min, 1.22 m flow restrictor, in reducing buffer; 6- Stock Lysozyme solution (6mM HCl) in reducing buffer.

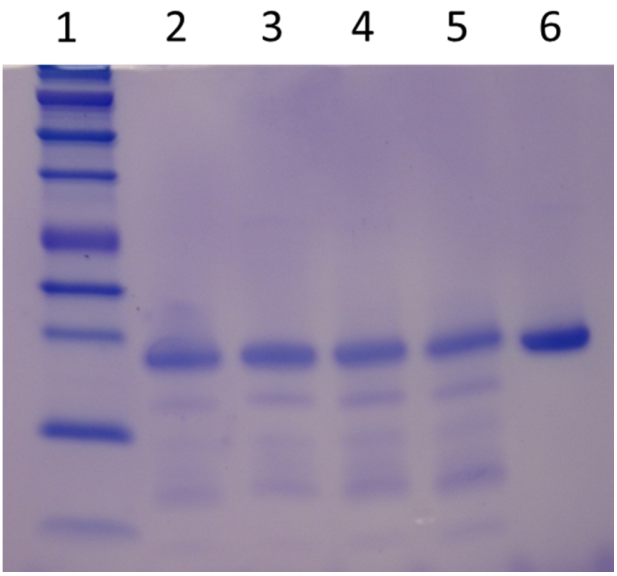

Figure 8 SI. Sample by lane: 1- Rainbow molecular weight marker; 2- RF micro-reactor, lysozyme solution (6mM HCl), 2.76 MPa, 1.22 m flow restrictor, 19.4 µL/min, in reducing buffer; 3- RF micro-reactor, lysozyme solution (6mM HCl), 0.34 MPa, 0.91 m flow restrictor, 3.4 µL/min, in reducing buffer; 4- RF micro-reactor, lysozyme solution (6mM HCl), 0.69 MPa, 0.91 m flow restrictor, 7.4 µL/min, in reducing buffer; 5- RF micro-reactor, lysozyme solution (6mM HCl), 2.07 MPa, 0.91 m flow restrictor, 18.9 µL/min, in reducing buffer; 6- Stock Lysozyme solution (6mM HCl) in reducing buffer.

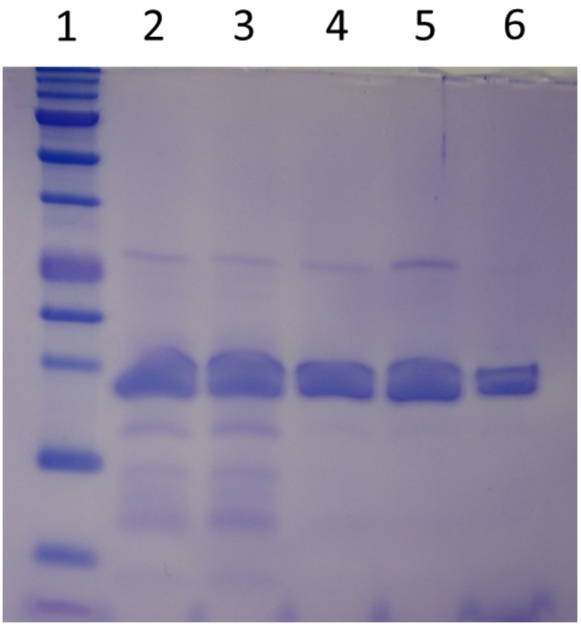

Figure 9 SI. Sample by lane: 1- Rainbow molecular weight marker; 2- RF micro-reactor, lysozyme solution (6mM HCl), 0.34 MPa, 0.30 m flow restrictor, 11.4 µL/min, in reducing buffer; 3- RF micro-reactor, lysozyme solution (6mM HCl), 0.69 MPa, 0.30 m flow restrictor, 20.2 µL/min, in reducing buffer; 4- RF micro-reactor, lysozyme solution (6mM HCl), 1.38 MPa, 0.30 m flow restrictor, 56.4 µL/min, in reducing buffer; 5- RF micro-reactor, lysozyme solution (6mM HCl), 2.07 MPa, 0.30 m flow restrictor, 70.0 µL/min, in reducing buffer; 6- Stock Lysozyme solution (6mM HCl) in reducing buffer.

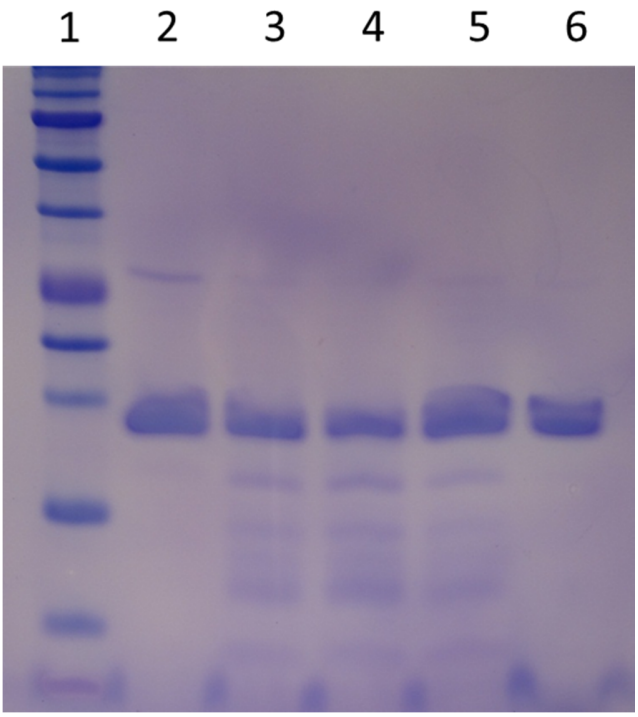

Figure 10 SI. Sample by lane: 1- Rainbow molecular weight marker; 2- RF micro-reactor, lysozyme solution (6mM HCl), 2.76 MPa, 0.30 m flow restrictor, 77.6 µL/min, in reducing buffer; 3- RF micro-reactor, lysozyme solution (6mM HCl), 0.34 MPa, 0.61 m flow restrictor, 5.9 µL/min, in reducing buffer; 4- RF micro-reactor, lysozyme solution (6mM HCl), 0.69 MPa, 0.61 m flow restrictor, 10.2 µL/min, in reducing buffer; 5- RF micro-reactor, lysozyme solution (6mM HCl), 2.07 MPa, 0.61 m flow restrictor, 31.7 µL/min, in reducing buffer; 6- Stock Lysozyme solution (6mM HCl) in reducing buffer.

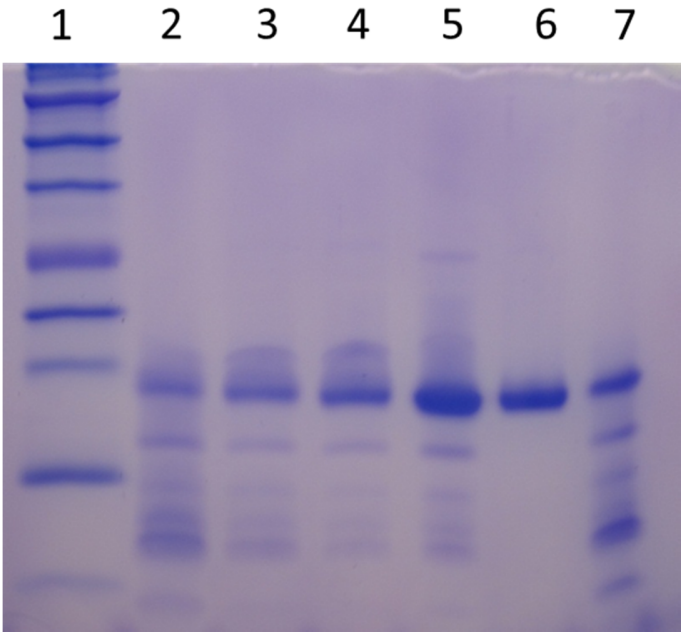

Figure 11 SI. Sample by lane: 1- Rainbow molecular weight marker; 2- Heat and pressure, lysozyme (6mM HCl) solution, 130 ^{o}C, 0.69 MPa, 0.91 m flow restrictor, 6.9 µL/min, ~2.5 inches submerged, in reducing buffer; 3- Heat and pressure, lysozyme (6mM HCl) solution, 130 ^{o}C, 1.38 MPa, 0.91 m flow restrictor, 19.0 µL/min, ~2.5 inches submerged, in reducing buffer; 4- Heat and pressure, lysozyme (6mM HCl) solution, 130 ^{o}C, 2.07 MPa, 0.91 m flow restrictor, 20.8 µL/min, ~2.5 inches submerged, in reducing buffer; 5- Heat and pressure, lysozyme (6mM HCl) solution, 130 ^{o}C, 2.76 MPa, 0.91 m flow restrictor, 24.4 µL/min, ~2.5 inches submerged, in reducing buffer; 6- Stock Lysozyme solution (6mM HCl) in reducing buffer; 7- RF micro-reactor, lysozyme solution (6mM HCl), 1.39 MPa, 1.22 m flow restrictor, 9.8 µL/min, in reducing buffer.

4. Heat and pressure control experiments (without RF)

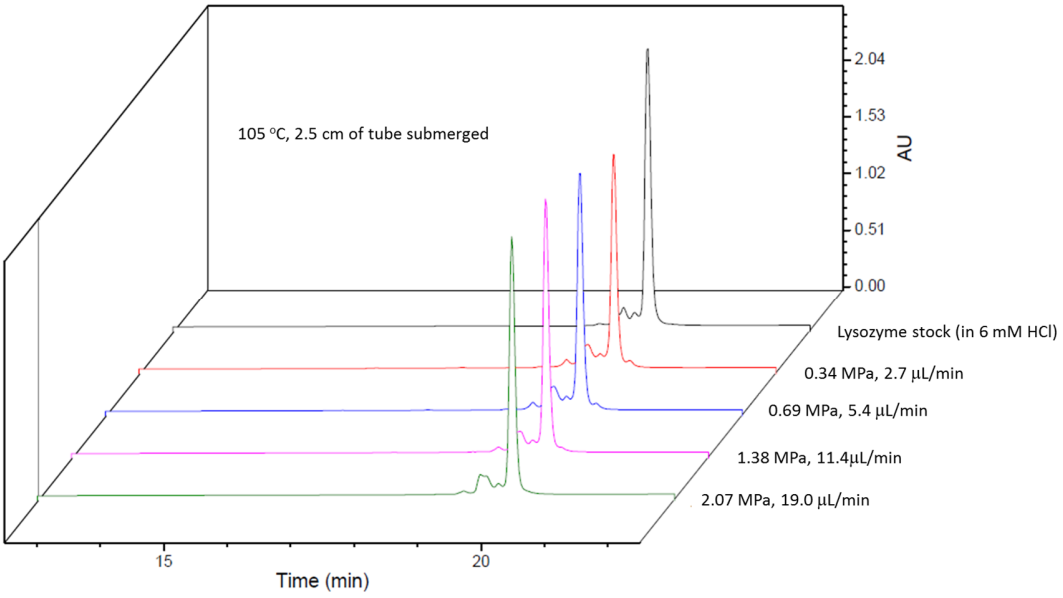

Figure 12 SI. Samples of lysozyme in 6mM HCl run with 2.5 cm of Teflon tubing submerged in a 105 °C oil bath.

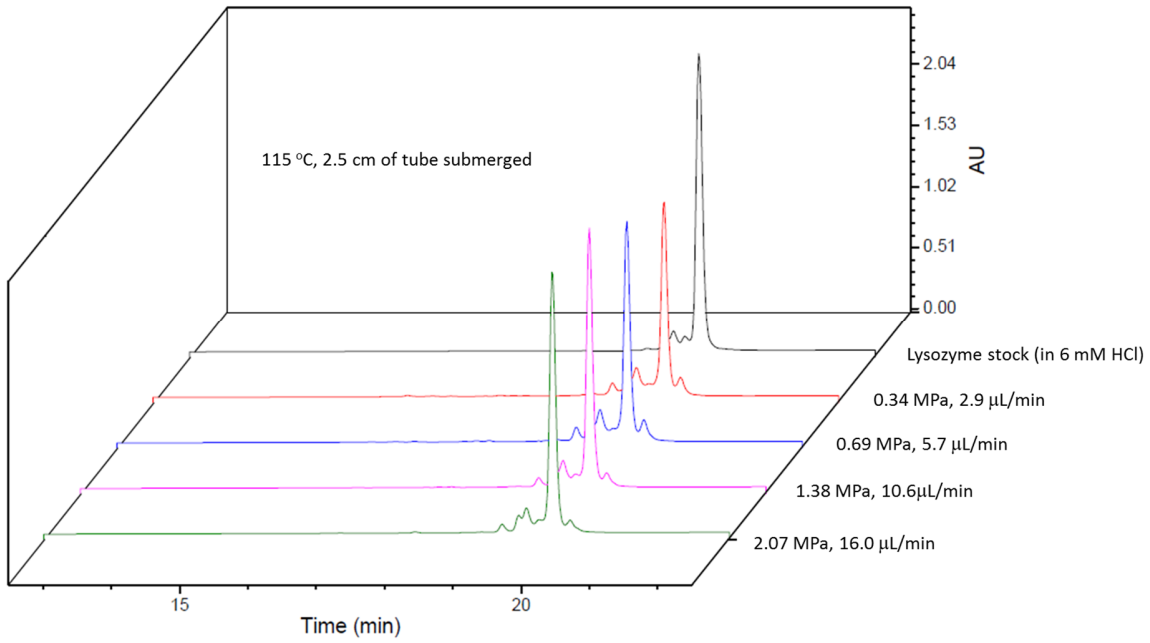

Figure 13 SI. Samples of lysozyme in 6mM HCl run with 2.5 cm of Teflon tubing submerged in a 115 °C oil bath.

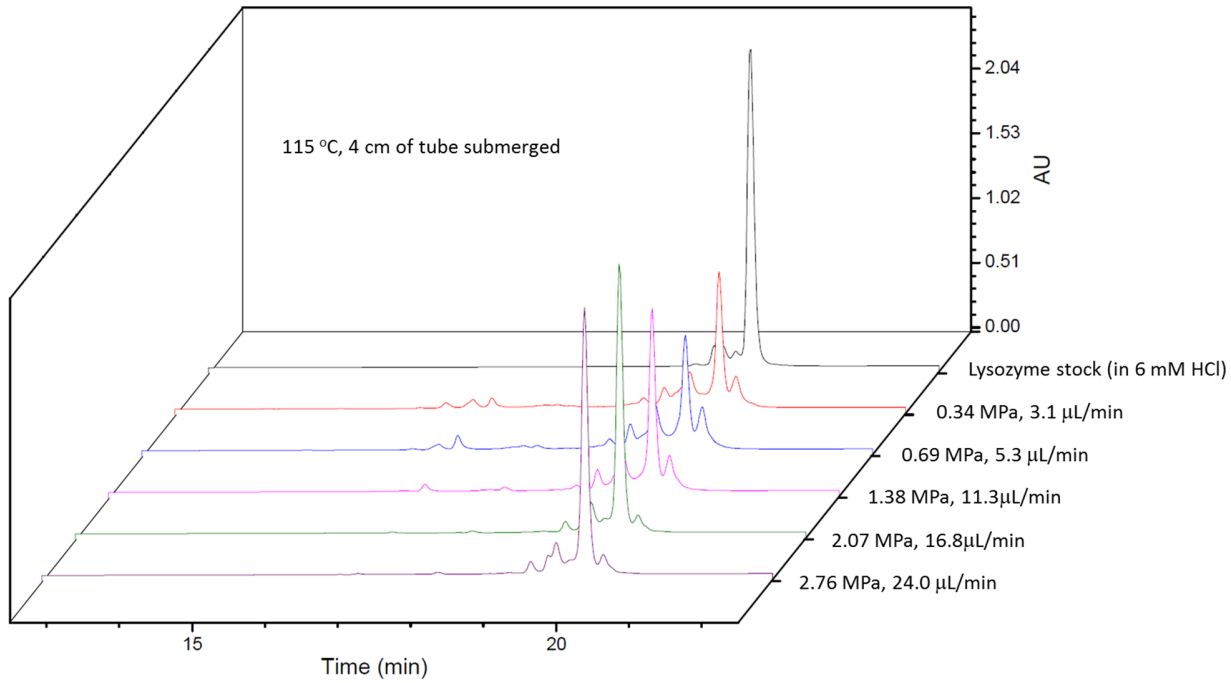

Figure 14 SI. Samples of lysozyme in 6mM HCl run with 4 cm of Teflon tubing submerged in a 115 °C oil bath.

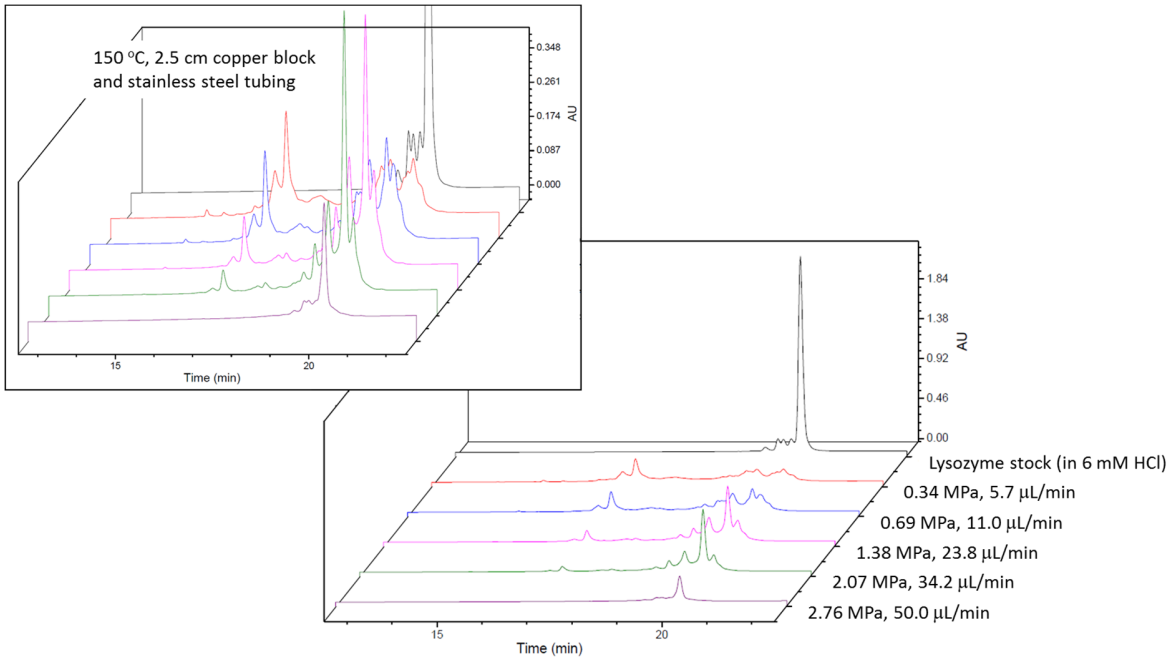

Figure 15 SI. Heat and pressure experiments run at 150 °C using stainless steel tubing and covered with a heated 2.5 cm copper block.

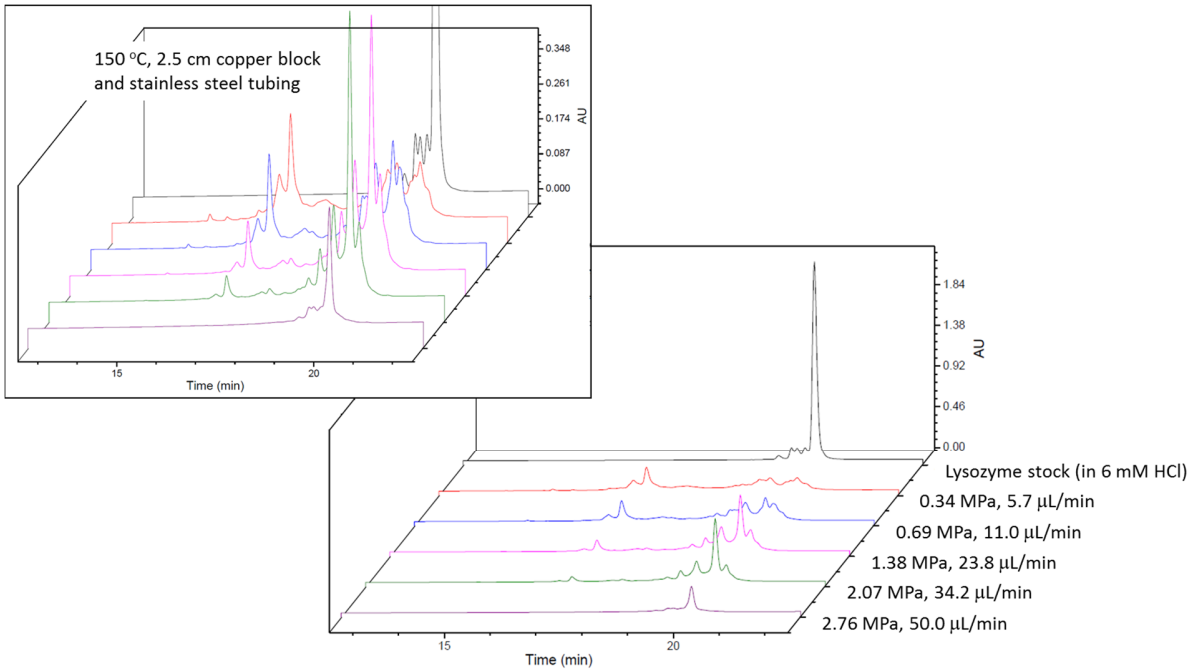

Figure 16 SI. Heat and pressure experiments run at 250 °C using stainless steel tubing and covered with a heated 2.5 cm copper block.

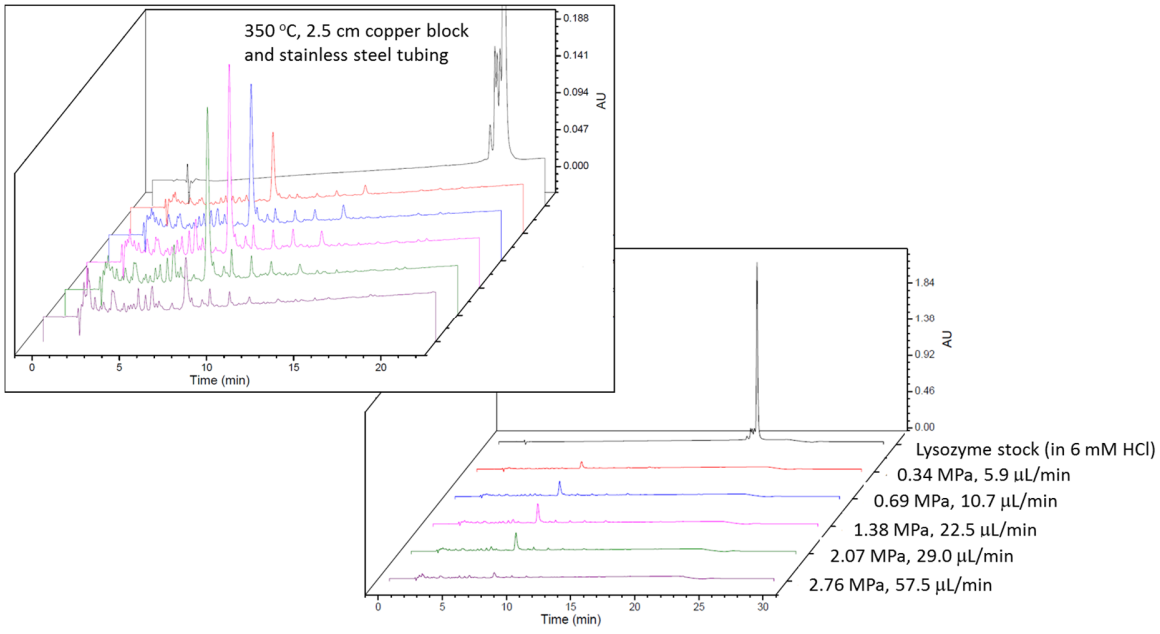

Figure 17 SI. Heat and pressure experiments run at 350 °C using stainless steel tubing and covered with a heated 2.5 cm copper block.

5. Temperature measurements inside waveguide

Applied pressure (boiling point of water at given pressure)	Temperature measured*
0.34 MPa (138 ^{o}C)	167-169 ^{o}C
0.69 MPa (164 ^{o}C)	182-184 ^{o}C
1.38 MPa (194 ^{o}C)	199-203 ^{o}C

*Range of temperatures measured over a period of approximately 30 seconds