Additional Experimental Details and Figures

**Protein Labeling.** TAMRA was chosen as the dye for covalent attachment to proteins since rhodamines (which are also common laser dyes) tend to have strong optical properties (e.g. 13). Labeling of standard protein sample was carried out using a slight modification from the manufacturer’s protocol, by using a reduced molar ratio of dye to protein (1:1). Briefly, 600 μg (60 μl) of SERVA IEF standard was diluted into 50 mM borate buffer, pH 8.5 to a final volume of 120 μl, to which was added 0.75 nmol of TAMRA-NHS reagent dissolved in 1 μl of dimethyl formamide, for a final reaction containing 0.8 % DMF. After 1 h incubation in the dark at room temperature, the protein reaction was desalted on a PD-10 column into 50 mM Hepes pH 7.5. The desalted protein was then concentrated and buffer exchanged with 8M urea, 10 mM Hepes pH 7.5, and 0.5 % CHAPS on a 5k mw cutoff microspin filter, to remove any remaining unreacted dye. Protein concentration was determined using the Biorad protein assay. Samples were stored at -80 °C for later analysis.

**2DGE.** All gel electrophoresis reagents were purchased from Biorad. For isoelectric focusing, stated loading amounts of protein were typically <5 μl volume of labeled proteins (in 8 M urea storage solution) or unlabeled proteins (in sample buffer containing 10% glycerol, 0.01% bromphenol blue, and 0.01% methyl red) were diluted into sample rehydration buffer containing 8 M urea, 2% CHAPS, 50 mM DTT, 0.2% Bio-Lyte 3/10 ampholyte, and 0.001% bromphenol blue for rehydration of 11 cm strips (3-10 nonlinear IPG). The IEF strips were focused after the method of Leimgruber, *et al.* 14 over 16-20 h for a total of ~60,000 Vh using a shallow gradient of ‘slow ramp’ voltage steps, reaching a maximum of 8,000V on a Biorad Protean IEF Cell apparatus. After focusing, the strips
were reduced and alkylated in equilibration buffer containing 6 M urea, 0.375 M Tris pH 8.8, 4% SDS, 20% glycerol, and either 2% DTT or 2.5% iodoacetamide for 10 minutes each. For the second dimension, the strips were run on single well, pre-cast, 12.5% acrylamide gels. After separation, the gels were fixed in 10% methanol, 7% acetic acid and, if not TAMRA labeled, they were stained with Sypro Ruby for imaging.
Figure S1. Shown here are gels loaded with 2 µg total SERVA protein sample that was covalently tagged with TAMRA, imaged with a Typhoon scanner using 532 nm excitation, and 580 BP 30 emission filter, PMT 550 V.
Figure S2. Shown here are gels loaded with 0.2 µg total protein sample that was covalently tagged with TAMRA, imaged as described in Figure S1 with PMT at 700 V.
Figure S3. Experiment for evaluation of TAMRA labeling efficiency. The 2 µg protein loaded gel from Figure S1A, was fluorescence imaged for TAMRA signal in panel A (with 532 nm excitation and 580 bandpass 30 emission filter), then stained with Sypro Ruby and imaged in panel B using 532 nm excitation and 610 BP 30 emission filter, PMT 650 V.
Figure S4. Shown here are fluorescence images of two gels loaded with 2 μg total SERVA protein sample, stained with Sypro Ruby, and fluorescence imaged using 532 nm excitation, 610 band pass 30 nm emission filter, and using PMT voltage of 650V. Molecular weight calibration was from protein ladder, pH values were from commercial information for the 3-10 pH non-linear gradient IPG strips from Biorad. Major protein spots identified from the commercial source (SERVA, DE) or mass spectrometric peptide sequencing are 1) amyloglucosidase, 2) glucose oxidase, 3) β-lactoglobulin 4) carbonic anhydrase, and 5) myoglobin.
Figure S5. Shown here are gels loaded with 0.2 μg total protein standard, passively stained with Sypro Ruby then imaged as described in Figure S4.