Electronic Supplementary Material (ESI) for Polymer Chemistry.

Redox-Responsive Nanogels for Drug-Delivery: Thiol-Maleimide and Disulfide-Exchange Chemistry as Orthogonal Tools for Fabrication and Degradation

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Instrumentation

Ultra-pure water was obtained via MilliQ Water Purification System (Merck Millipore, USA). FTIR spectroscopy measurements were done on a Thermo Scientific Nicolet 380 FTIR spectrometer. The size of nanogels was measured using a Zetasizer Nano particle analyzer (Malvern). NMR analysis was carried out using a 400 MHz Bruker spectrometer. Molecular weights of the polymers were monitored using a gel permeation chromatography using a PSS-SDV (gram linear, length/ID 8 × 300 mm, 10 µm particle size) column calibrated with poly(methyl methacrylate) standards (1−175 kDa) using a refractive index detector. Dimethylacetamide with 0.05% lithium bromide was used as an eluent at a flow rate of 1.0 mL/min at 30 °C. Side products of reactions were quantified by liquid chromatography-mass spectrometry (LC-MS) analysis using an LCMS-2020-mass spectrometer system (Shimadzu, Japan) equipped with a C-18, 5 µm, 150 × 4.6 mm column. The mobile phase consisted of deionized water and HPLC grade acetonitrile (ACN) using the following gradient: LC: 0–14 min, 50% ACN; 18.01 min, 95% ACN; 25 min, 95% ACN; 25.01– 30 min, 50% ACN. Drug concentrations were quantified by liquid chromatography-mass spectrometry (LC-MS) analysis using an LCMS-8030-mass spectrometer system (Shimadzu, Japan) equipped with a C-18, 2.7 µm, 100 × 3 mm column. The mobile phase consisted of deionized water and HPLC grade acetonitrile (ACN) using the following gradient: LC: 0.5 min, 10% ACN; 2.0 min, 40% ACN, 7.0 min, 95% ACN; 9.5 min, 95% ACN. Transmittance measurements were carried out using a UV-Vis spectrophotometer (Varian Cary Eclipse, Agilent, USA). Nile Red concentrations were quantified using a fluorescence spectrophotometer (Varian Cary Eclipse, Agilent, USA). During the internalization experiments, cells were visualized using Zeiss Axio Observer inverted fluorescence microscope (Carl Zeiss Canada Ltd., Canada). The dry micellar constructs were

visualized on a holey carbon film-coated 300 mesh Cu-grid (Pacific Grid Tech, USA) using an LVEM5 electron microscope on transmission electron microscopy (TEM) mode (Delong Instruments, USA).

Cell lines

MDA-MB-231 was purchased from ATCC (LGC Standards, Germany) and grown according to the cell culture methods requirements of the manufacturer. Cells were kept in the logarithmic phase of cell growth for the duration of experiments and incubated in a humidified atmosphere of 5% CO2 at 37 °C. Cytotoxicity experiments were done with a plate reader (Multiscan FC, Thermo Scientific, USA), and Cell Counting Kit8 (CCK-8, Fluka) was obtained from Sigma-Aldrich.

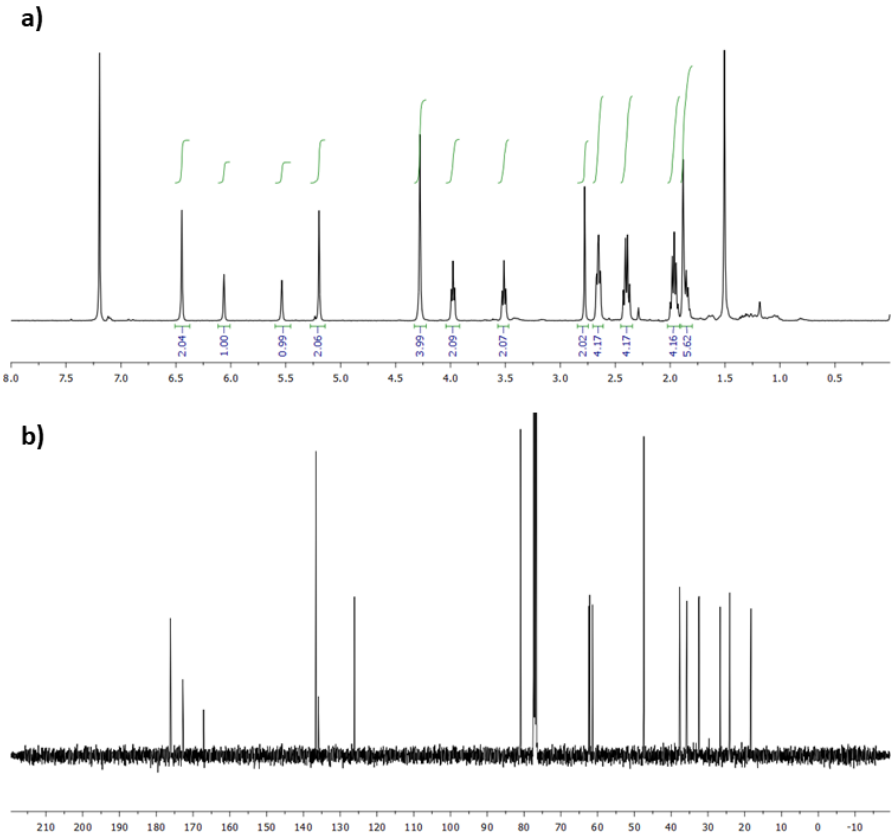

Fig. S1 (a) ^{1}H NMR spectrum of the masked maleimide-disulfide-containing monomer with integrals. (b) ^{13}C NMR spectrum of the masked maleimide-disulfide-containing monomer.

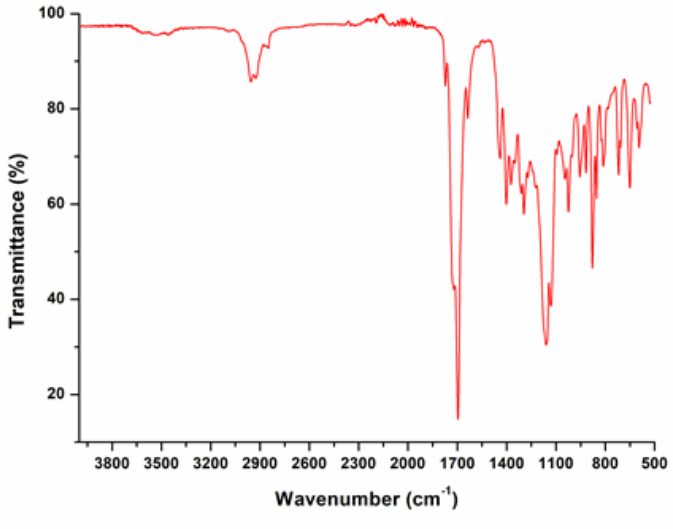

Fig. S2 FTIR spectra of masked maleimide-disulfide-containing monomer.

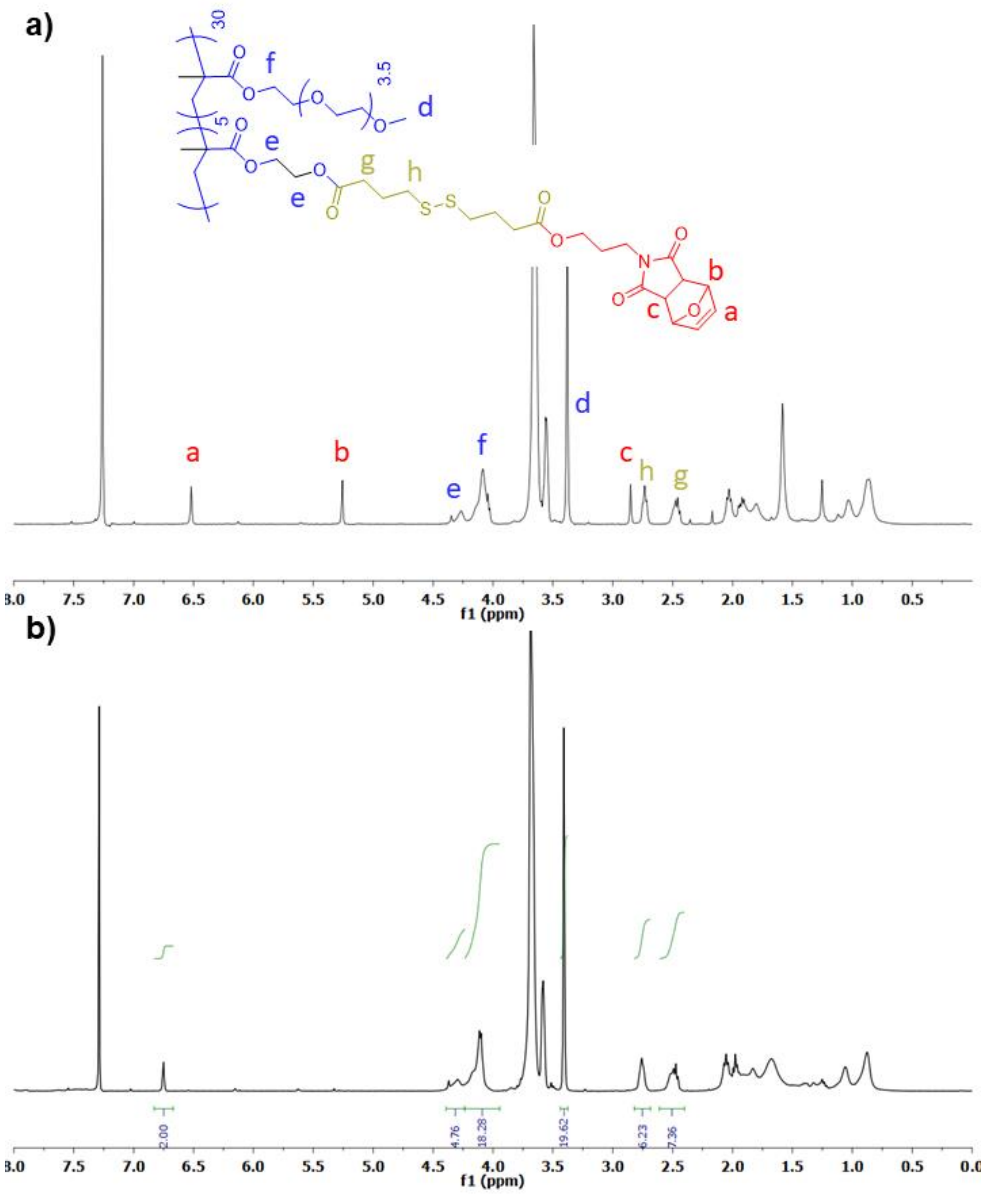

Fig. S3 (a) ^{1}H NMR spectrum of the redox-responsive copolymer before retro-Diels-Alder reaction. (b) ^{1}H NMR spectrum of the redox-responsive copolymer with integrals.

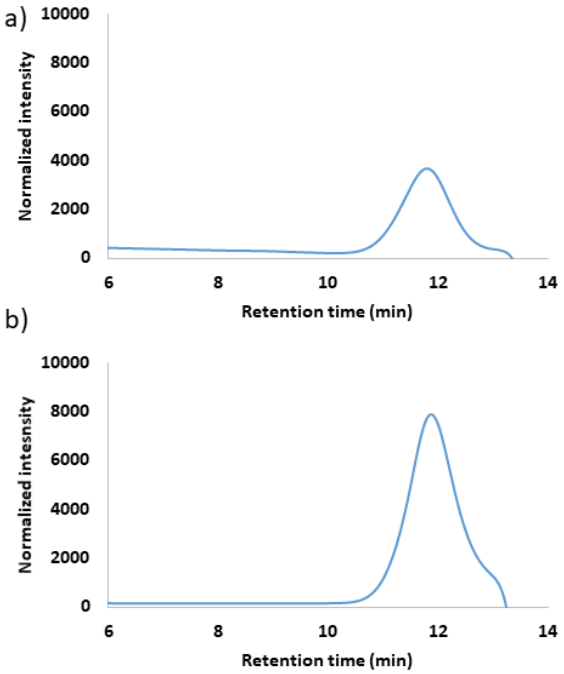

Fig. S4 SEC chromatograms of (a) the redox-responsive masked copolymer, and (b) unmasked redox-responsive maleimide-containing product after rDA reaction.

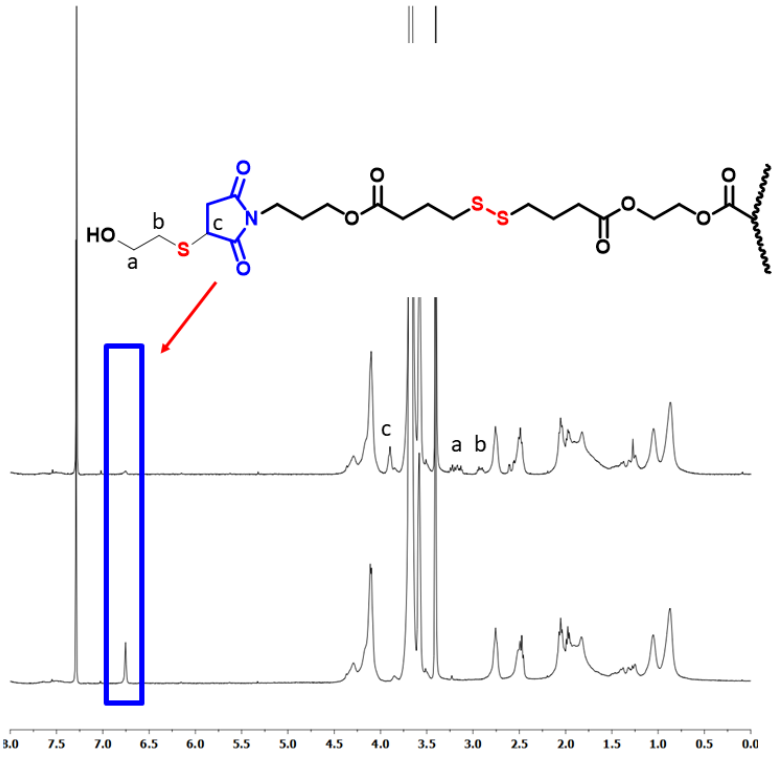

Fig. S5 ^{1}H NMR spectra of the polymer before and after treatment with 2-mercaptoethanol.

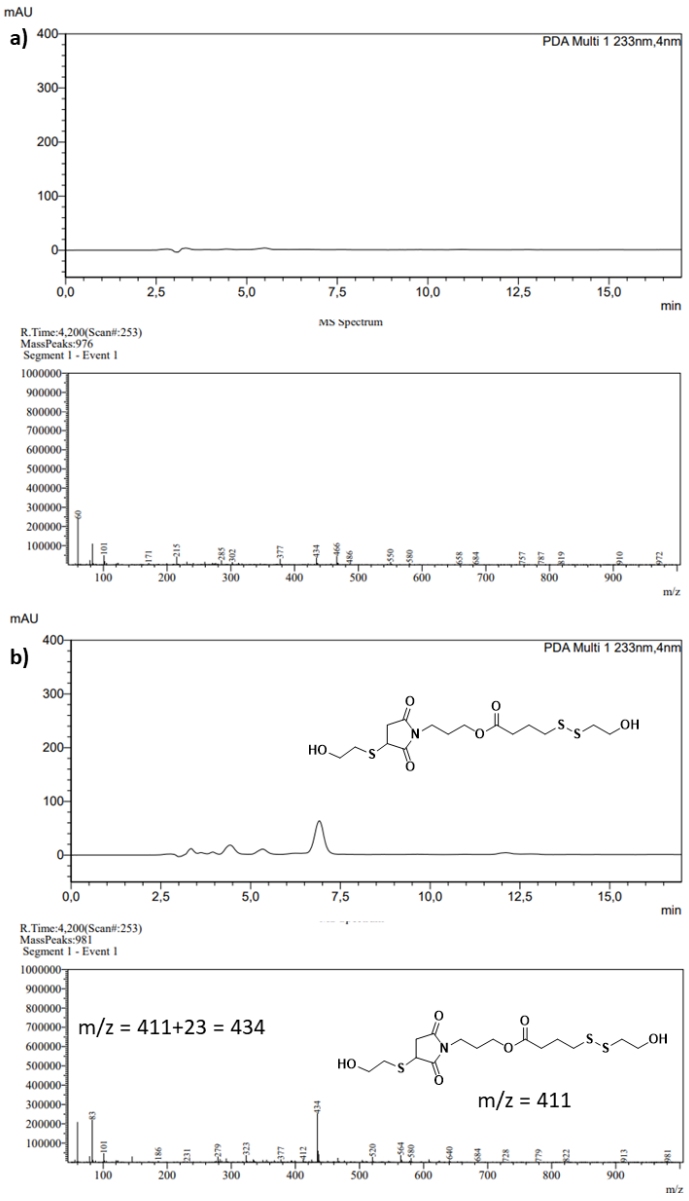

Fig. S6 LCMS characterization of the solution of the reaction between copolymer with (a) the stoichiometric equivalent of 2-mercaptoethanol and (b) four equivalent of 2-mercaptoethanol.

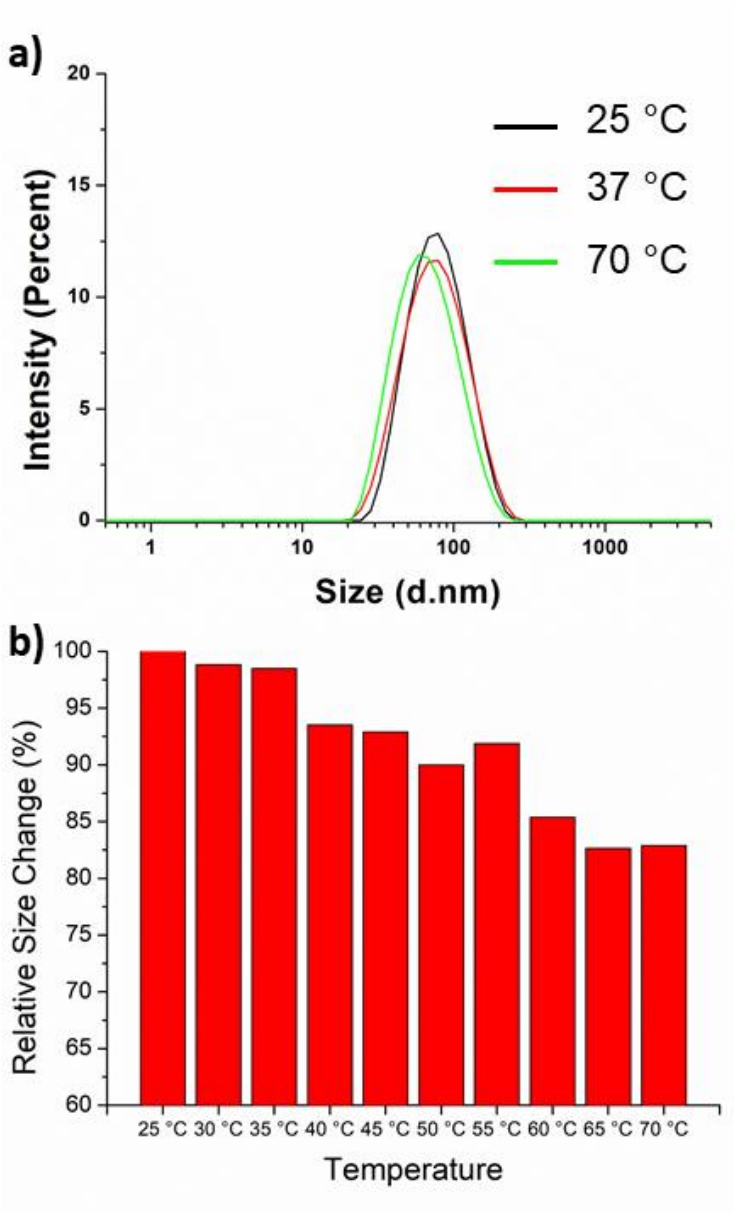

Fig. S7 (a) Hydrodynamic size as determined by DLS of the nanogel solution at 25, 37 and 70 °C. (b) Temperature responsive size change of crosslinked nanogels.

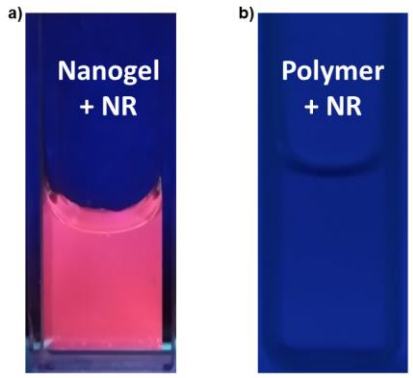

Fig. S8 (a) Loading of NR in nanogel and (b) in polymer using co-solvent evaporation method.

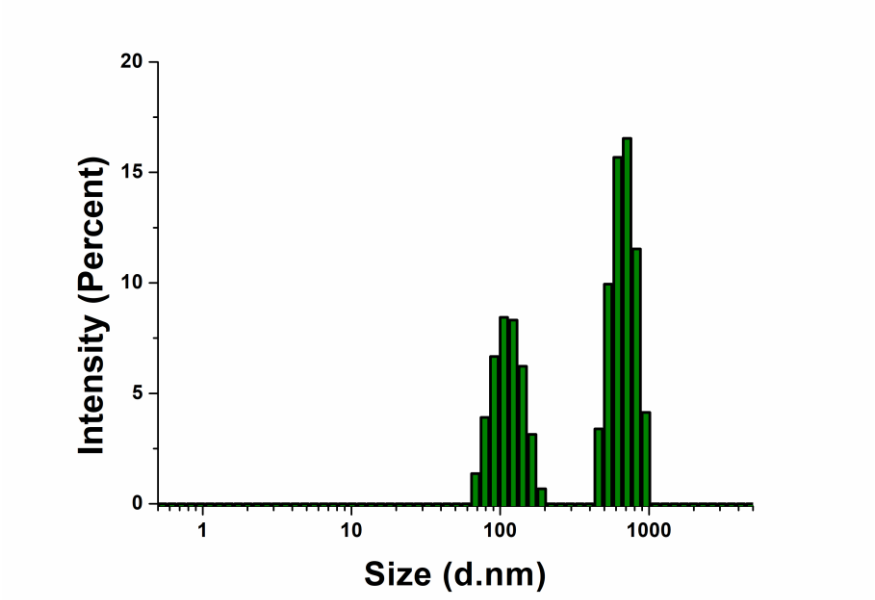

Fig. S9 DLS analysis of NG after 4 hours in pH 5.5 acetate buffer.

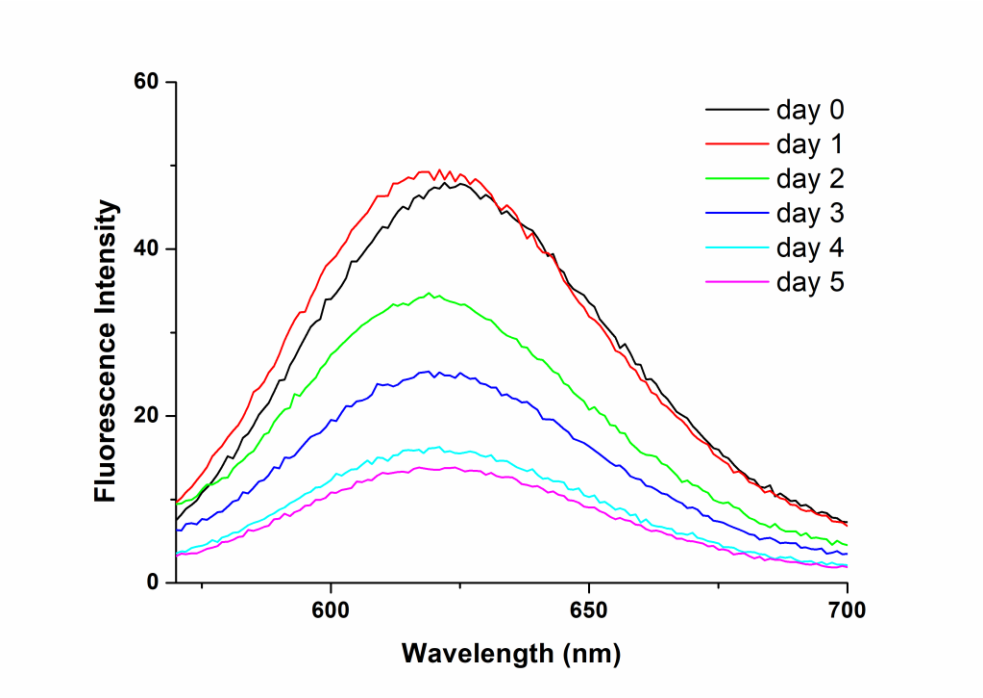

Fig. S10 Fluorescence intensity of NR-containing nanogel solutions incubated in pH 5.5 acetate buffer for five days.