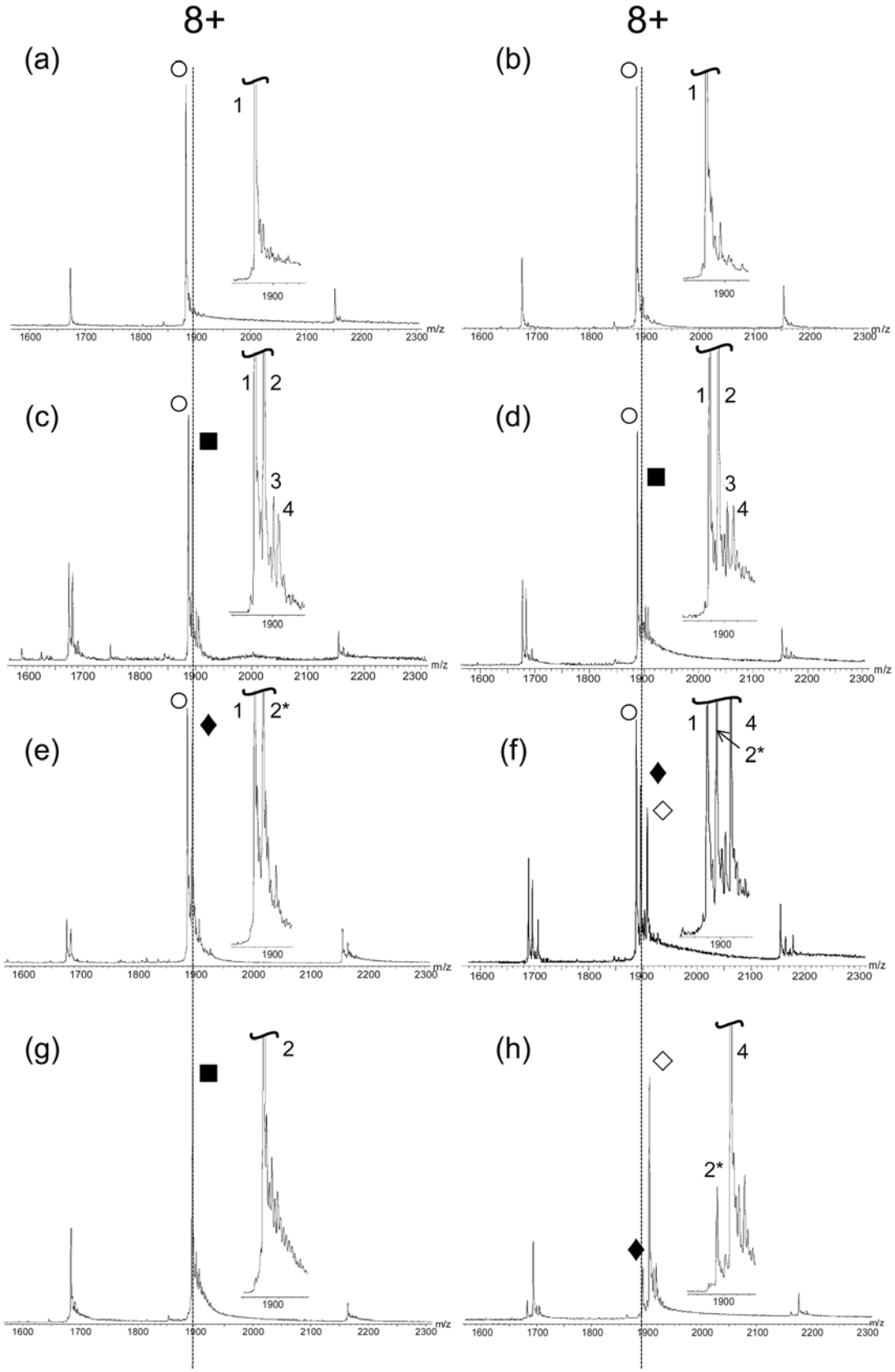

Figure S1. Electrospray ionization mass spectra of the colicin E9 DNase system sprayed under gentle ionization and pseudo-native conditions. Time-of-flight electrospray ionization mass spectra were recorded on a Micromass LC-T mass spectrometer (Manchester, U.K.) operating in the positive ion mode. Prior to analysis a 600-3000 m/z scale was calibrated with CsI (2 mg/ml) in isopropanol/water (1:1). Samples were introduced via a nanoflow electrospray source. All samples were dissolved in 50 mM aqueous ammonium acetate solutions at pH 7.4. In all experiments the protein concentration was 10 μM. The nanospray needle potential was typically set to 1200 V and the cone voltage to 30 V. The mass spectrometer was operated without source heating.

ESI-MS spectra of:

(a) apo E9 Dnase (*i.e.* metal-free).
(b) apo E9 DNase in the presence of 10 μM phosphate (10 μM).
(c) E9 DNase in the presence of 5 μM Ni^{2+}.
(d) E9 DNase in the presence of 5 μM Ni^{2+} and 10μM phosphate.
(e) E9 DNase in the presence of 5 μM Zn^{2+}.
(f) E9 DNase in the presence of 5μM Zn^{2+} and 10μM phosphate.
(g) E9 DNase in the presence of 30 μM Ni^{2+} and 30 μM phosphate.
(h) E9 DNase in the presence of 30 μM Zn^{2+} and 30 μM phosphate.

In all these spectra ions are observed at charge states 9 (at m/z values of approximately 1700), 8 (at m/z values of approximately 1900) and 7 (at m/z values of approximately 2150). Ion peaks indicating the apo E9 DNase are marked by (○). Ion peaks representing E9-Zn^{2+} are indicated by (◆). Ion peaks indicating E9-Ni^{2+} are highlighted by (■). Ion peaks represented by (◇) indicating E9-Zn^{2+}-PO_4^{-3}. In the insets are shown detailed spectra zoomed in on the 8-charged ions. The numberings used in these insets relate to **1**: apo-E9, mass = 15088.3 ± 0.3, **2**: E9-Ni^{2+} mass = 15144.3±1.0, **2***: E9-Zn^{2+} mass = 15151.5±1.2, **3**: E9-Ni^{2+}-acetate, mass = 15202.7 ± 1.2. **4**: E9-Zn^{2+}-phosphate mass = 15248.8±0.9. The non-specific anion binding of the acetate originates from the ammonium acetate buffer, which is present at a concentration of 50 mM, whereas the specific binding of the phosphate anion originates from the phosphate added at a concentration of 10 to 30 μM.