

Supporting Information For

**Pulsed nanoelectrospray ionization boosts ion signal in whole protein mass spectrometry**

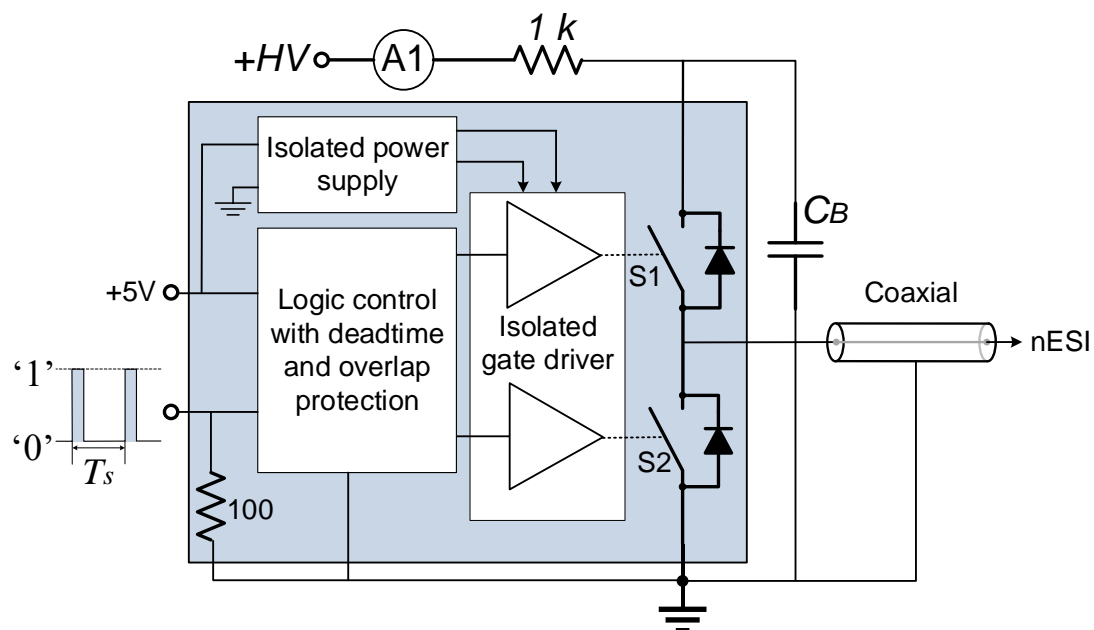
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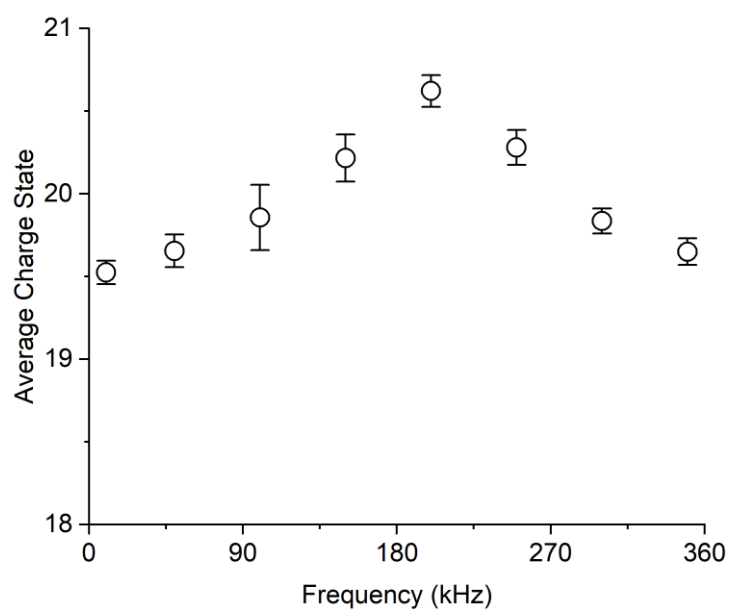
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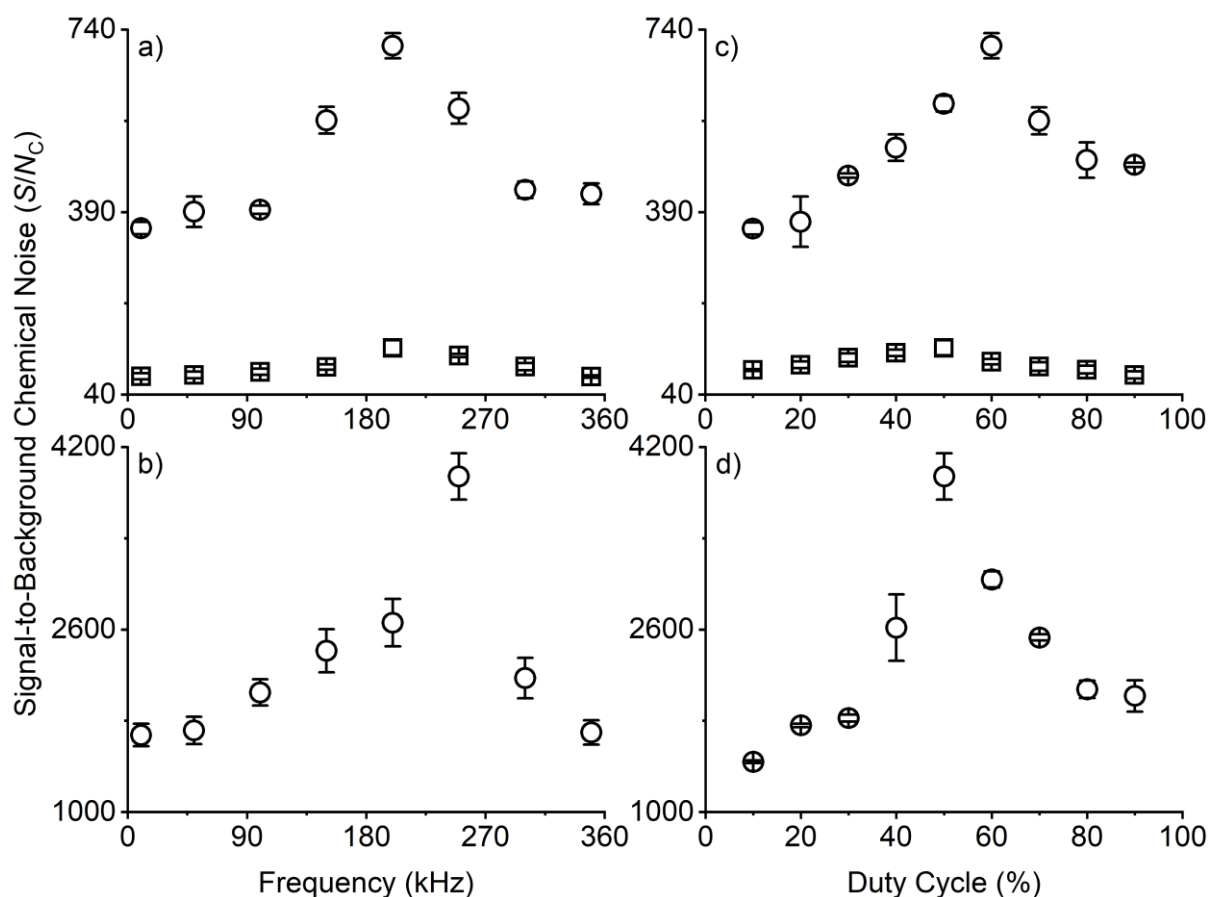
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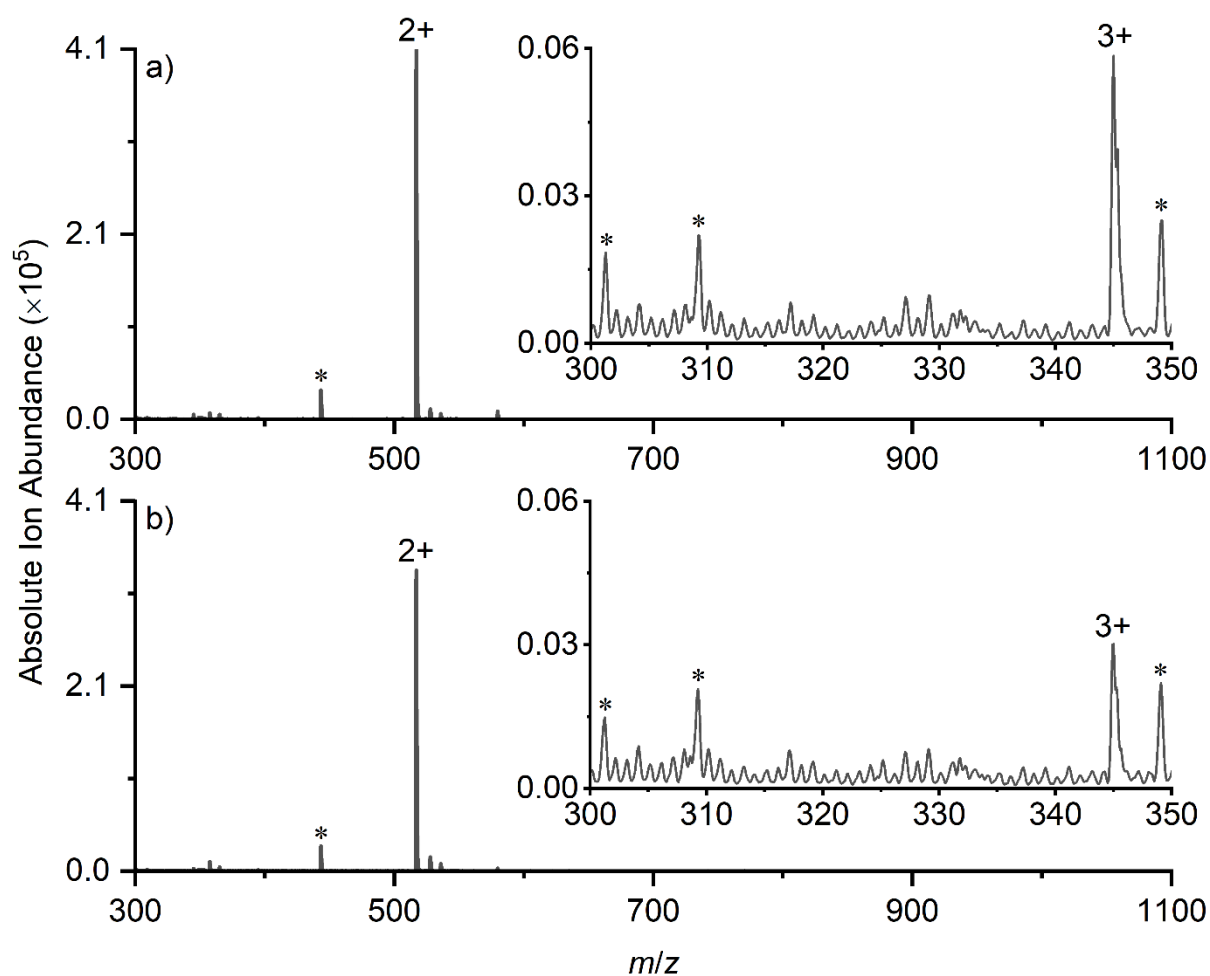
**Figure S1.** Electrical circuit to generate high voltage pulses for pulsed nano-electrospray ionisation for mass spectrometry. *HV*, *A*, *T*, *C* and *R* correspond to high voltage, ampere meter, period of waveform (time), decoupling capacitor, and resistor.



**Figure S2.** Average charge state calculated for myoglobin (5  $\mu$ M) at different frequencies ranging from 10 to 350 kHz by pulsed nESI mass spectrometry.



**Figure S3.** Signal-to-background chemical noise ( $S/N_C$ ) of (a, c) myoglobin (5  $\mu$ M) and (b, d) angiotensin II (1  $\mu$ M) as a function of (a, b) frequency and (c, d) duty cycle of pulsed nESI. The spectra were collected at a frequency ranging from 10 to 350 kHz (duty cycle of 50%) and duty cycle ranging from 10 to 90% (frequency of 250 and 200 kHz for angiotensin II and myoglobin, respectively). The  $S/N_C$  calculated for angiotensin II and myoglobin is presented in circle symbol ( $\circ$ ), whereas  $S/N_C$  of heme group from myoglobin is presented in square symbol ( $\square$ ).



**Figure S4.** Mass spectra of angiotensin II (1  $\mu\text{M}$ ) obtained from pulsed (a) and conventional direct current nESI (b), respectively. Pulsed nESI spectrum was collected at an ‘optimum’ frequency of 250 kHz. The symbol asterisks (\*) indicate peaks corresponding to chemical noise that are present in the solution blank.