Electronic Supplementary Material (ESI) for Metallomics
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Distinct cellular fates for KP1019 and NAMI-A determined by X-ray fluorescence imaging of single cells.

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Table S1: Elemental area densities (μg cm^{-2}) within cultured SH-SY5Y cells (control) and cells treated with either NAMI-A or KP1019 for 4 h. The data for P-Zn were collected at 10 keV, the data for Ru were collected at 22.5 keV.

	Whole Cell			**Nucleus**		
	Control (n=6)	**NAMI-A** (n=4)	**KP1019** (n=4)	**Control** (n=6)	**NAMI-A** (n=4)	**KP1019** (n=4)
P	1.3(3)	0.9(2)	1.8(3)	2.7(8)	1.7(3)	3.4(5)
S	0.5(1)	0.4(1)	0.7(1)	1(3)	0.7(2)	1.3(3)
Cl	2(1)	0.8(3)	0.69(7)*	2(2)	1.0(5)	0.8(1)
K	0.15(10)	0.2(1)	0.4(1)	0.3(2)	0.3(2)	0.7(2)
Ca	0.04(7)	0.02(1)	0.012(4)	0.07(10)	0.03(2)	0.019(6)
Fe	0.006(1)	0.005(1)	0.007(2)	0.009(3)	0.008(2)	0.011(3)
Cu	0.0010(2)	0.0026(2)*	0.0020(5)*	0.0013(3)	0.0034(5)*	0.0027(8)*
Zn	0.05(4)	0.03(1)	0.028(4)	0.09(7)	0.05(2)	0.046(8)
Ru	BDL	0.0006(2)*	0.054(6)*	BDL	0.0005(4)*	0.08(1)*
ROI Area (10 keV)	333(111)	323(100)	212(65)	48(23)	71(38)	63(29)
ROI Area (22.5 keV)	299(108)	327(78)	208(55)	49(24)	71(38)	64(30)

The values are presented as an average of n technical replicates (i.e. data collected from n distinct single cells grown and treated within one culture well) with the standard deviation in the last decimal place provided in brackets. Statistical significance was accepted at the 99% confidence interval with *P*-values determined by a two-tailed Mann Whitney test (* - $P \leq 0.01$). BDL: Below Detection Limit

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Instrumentation

Low-resolution electrospray mass spectra were obtained with a Micromass Platform II Quadrupole Mass Spectrometer fitted with an electrospray source. The capillary voltage was at 3.5 eV, and the cone voltage was at 15 V. UV−vis−NIR spectra were recorded on 0.1−2 mM solutions in quartz cuvettes using Varian Cary spectrophotometer.

Accurate high-resolution mass spectra were obtained with a Bruker BioApex II 47e FT-ICR MS instrument fitted with an Analytical Electrospray Source. Samples were introduced by a syringe pump at a rate of 1 μL min^{-1} and the capillary voltage was 120 V.

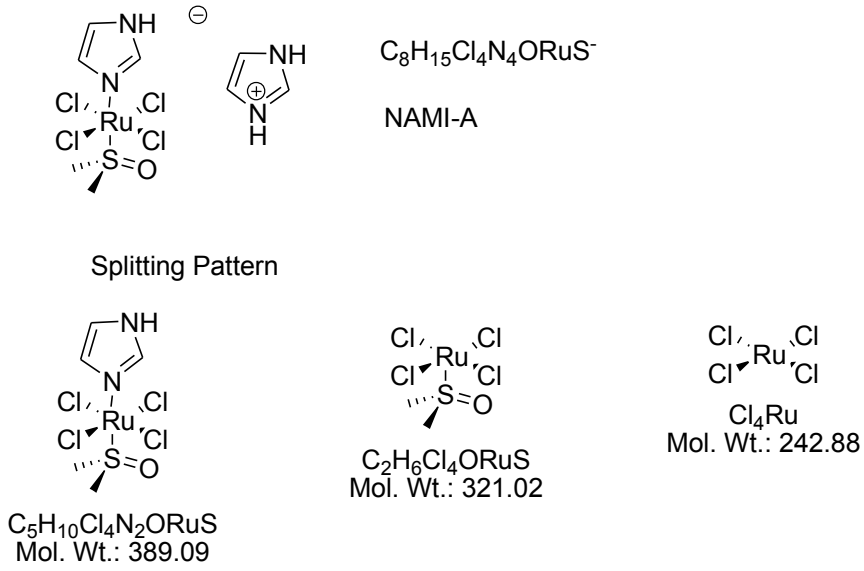

Figure S1: Fragmentation pattern for NAMI-A

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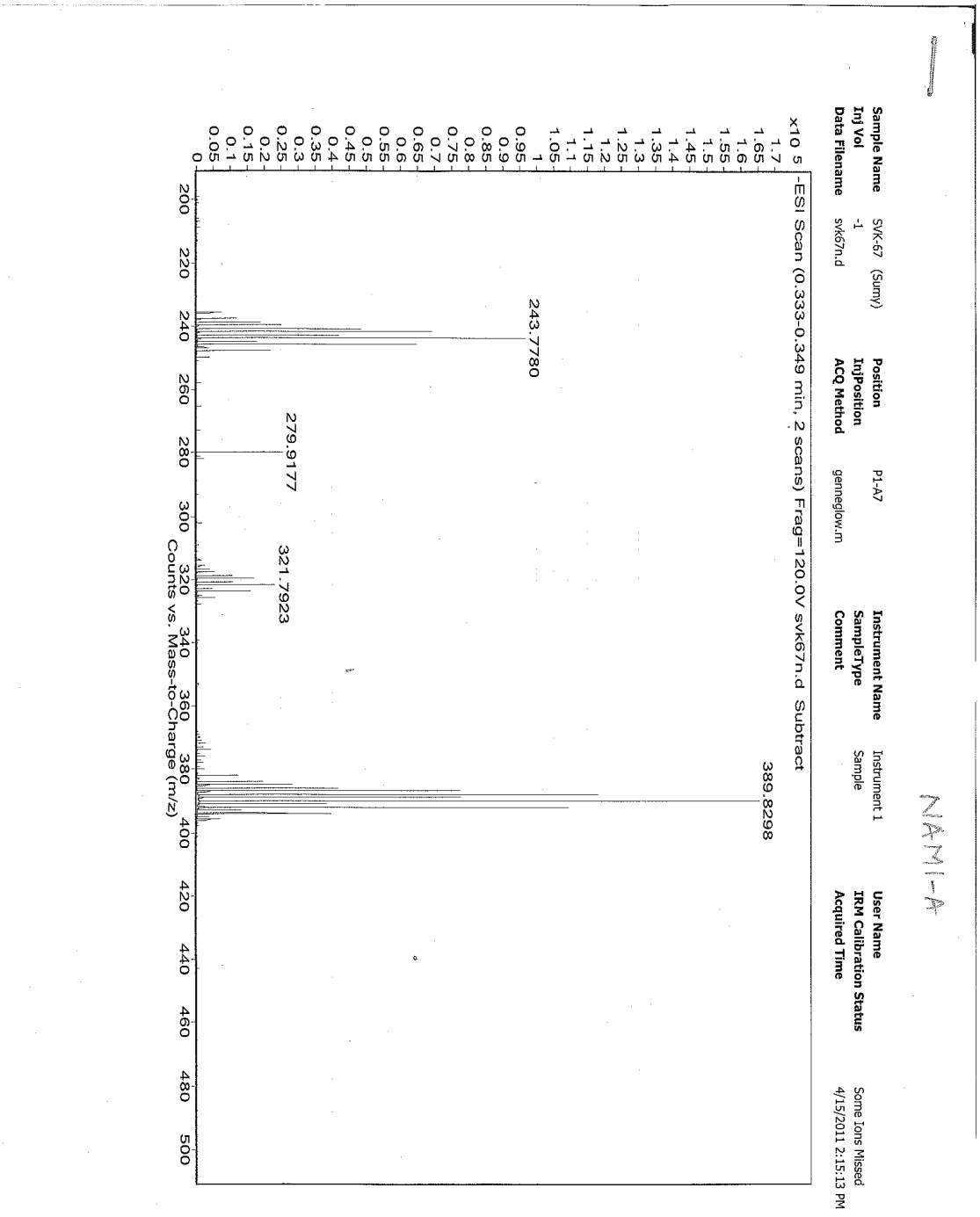

Figure S2: Accurate mass spectrum of NAMI-A; Solvent – DMF/ACN

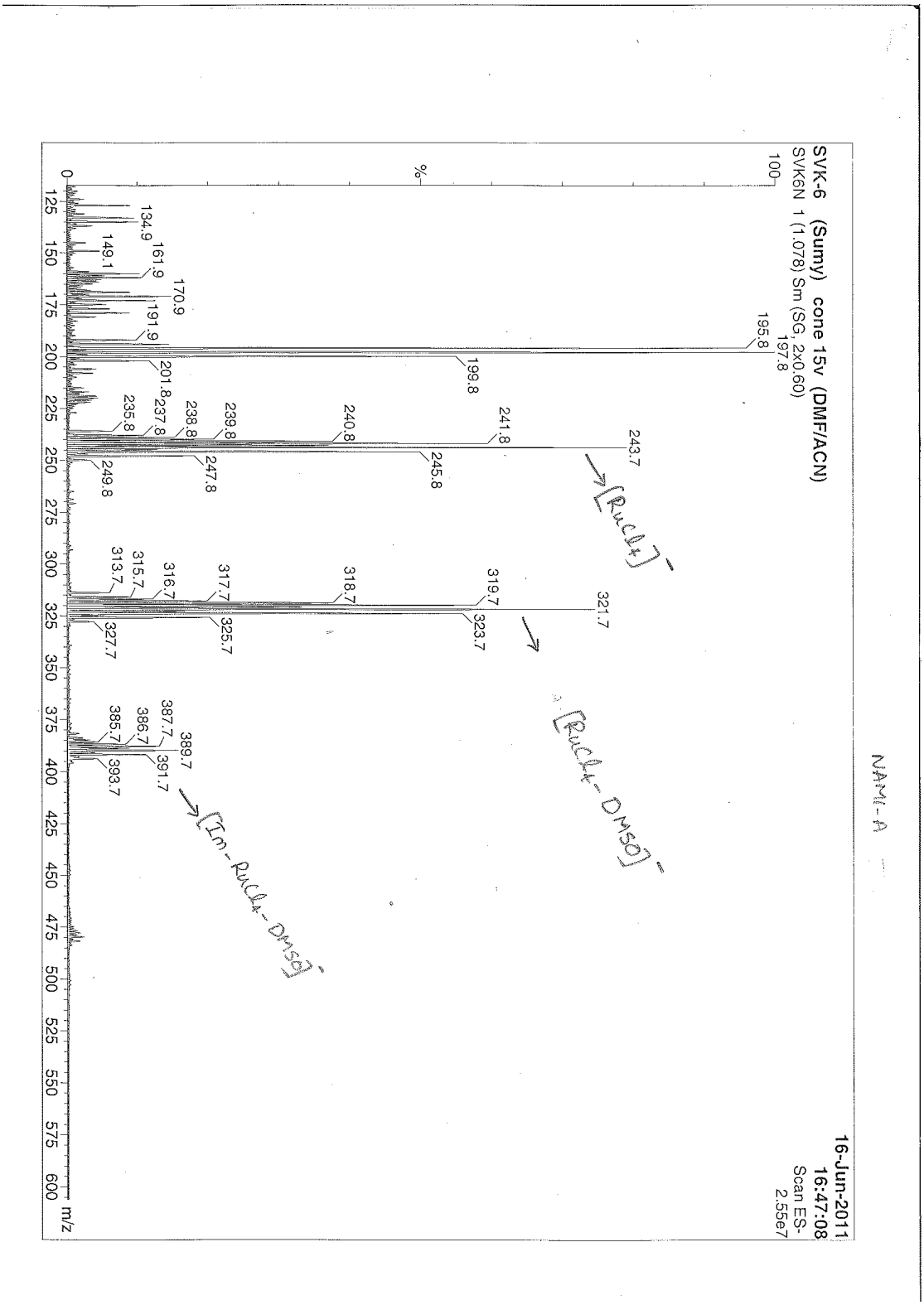

Figure S3: Electrospray mass spectrum for NAMI-A; Cone 15V; Solvent – DMF/ACN

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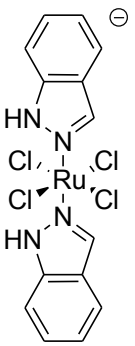
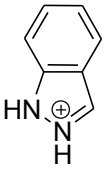
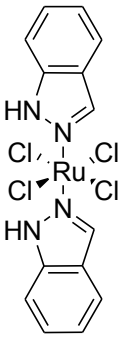


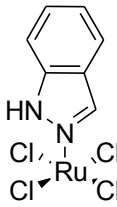


$C_{21}H_{19}Cl_4N_6Ru$
KP1019

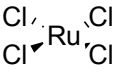
Splitting Pattern



$C_{14}H_{12}Cl_4N_4Ru$
Exact Mass: 477.89



$C_7H_6Cl_4N_2Ru$
Exact Mass: 359.83



Cl_4Ru
Exact Mass: 241.78

Figure S4: Fragmentation pattern for KP1019

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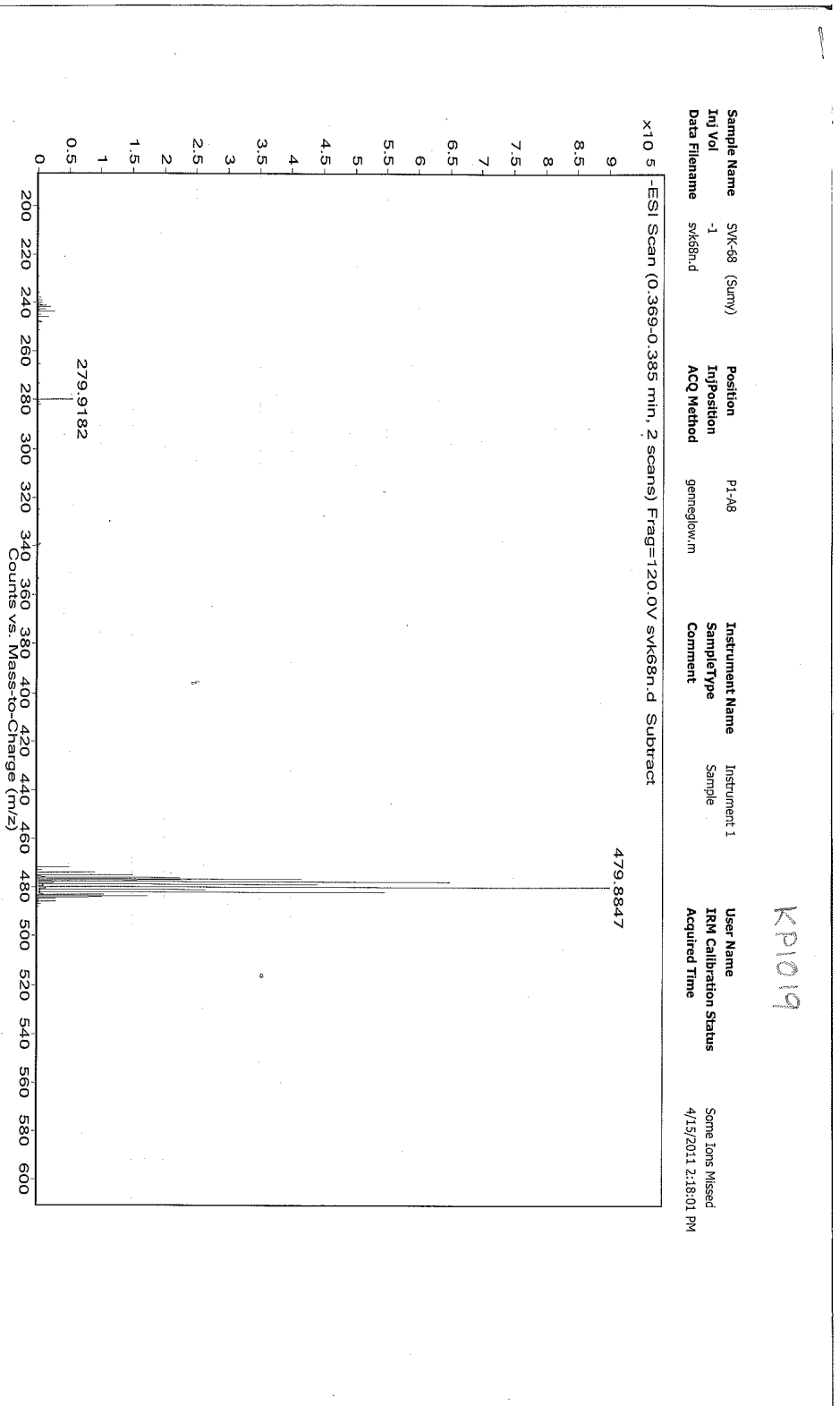

Figure S5: Accurate mass spectrum of KP1019; Solvent – DMF/ACN

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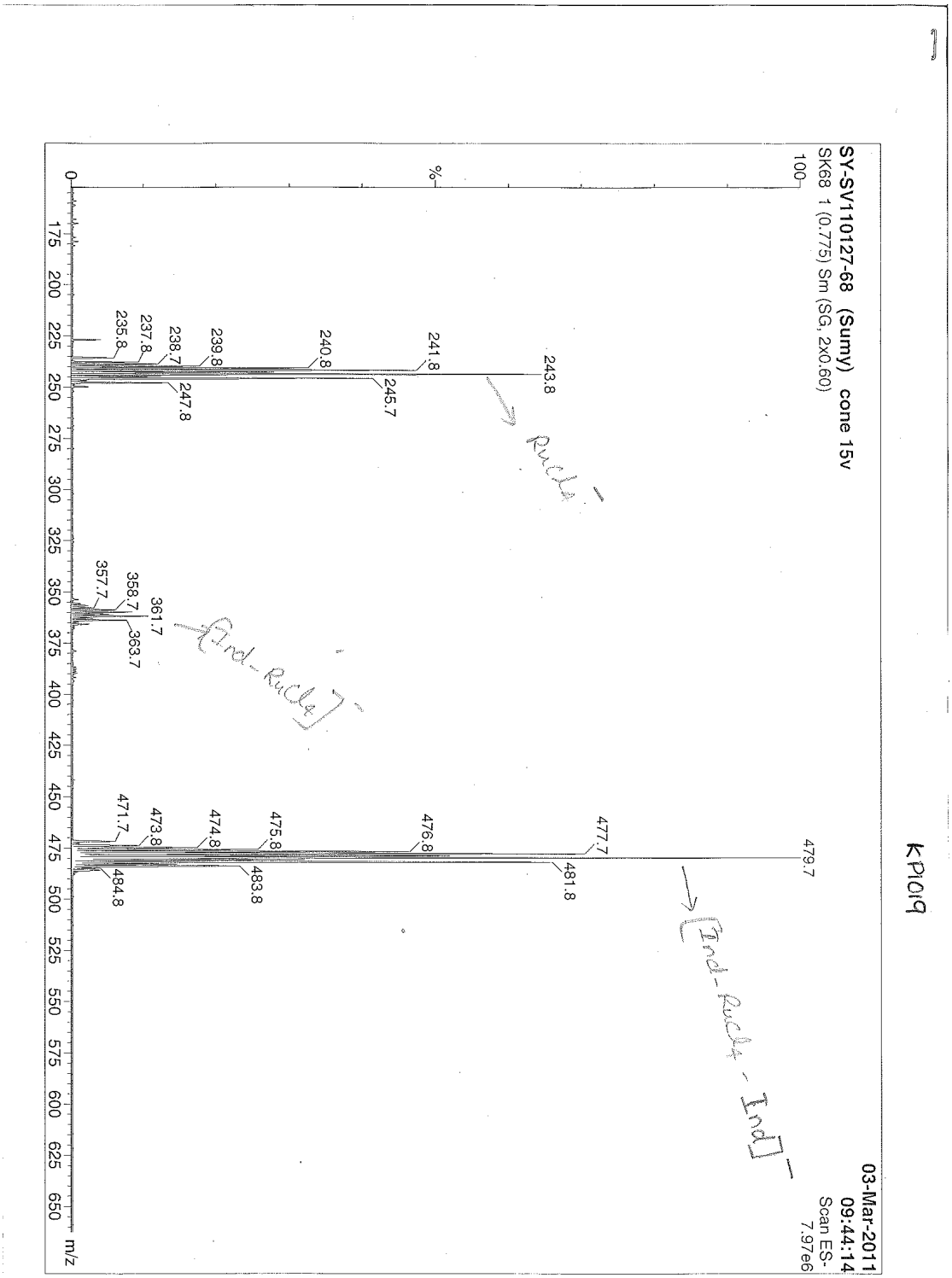

Figure S6: Electrospray mass spectrum for KP1019; Cone 15V; Solvent – DMF/ACN

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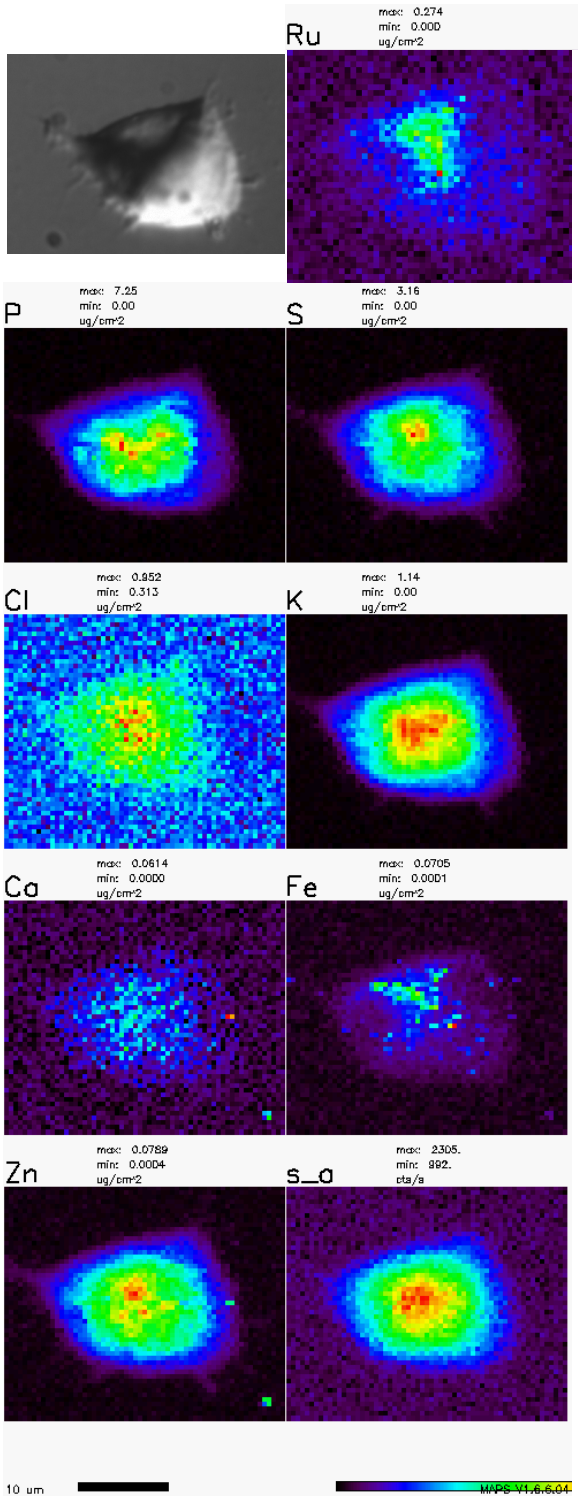

Figure S7: Optical micrograph (top left), scattered X-ray (SA) and XRF elemental distribution maps of P, S, K, Cl, Fe, Cu, Zn, and Ru of an SH-SY5Y cell treated with 200 μM KP1019 for 4 hr. Maximal area densities are given in μg/cm^2 for each.

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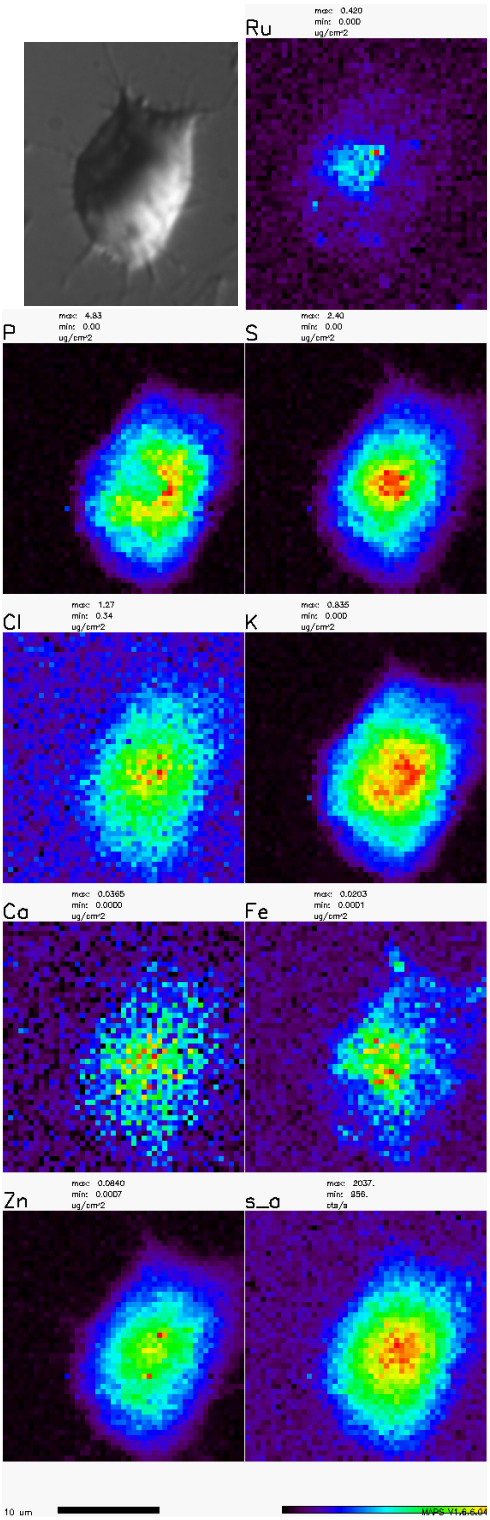

Figure S8: Optical micrograph (top left), scattered X-ray (SA) and XRF elemental distribution maps of P, S, K, Cl, Fe, Cu, Zn, and Ru of an SH-SY5Y cell treated with 200 μM KP1019 for 4 hr. Maximal area densities are given in $\mu g/cm^2$ for each.

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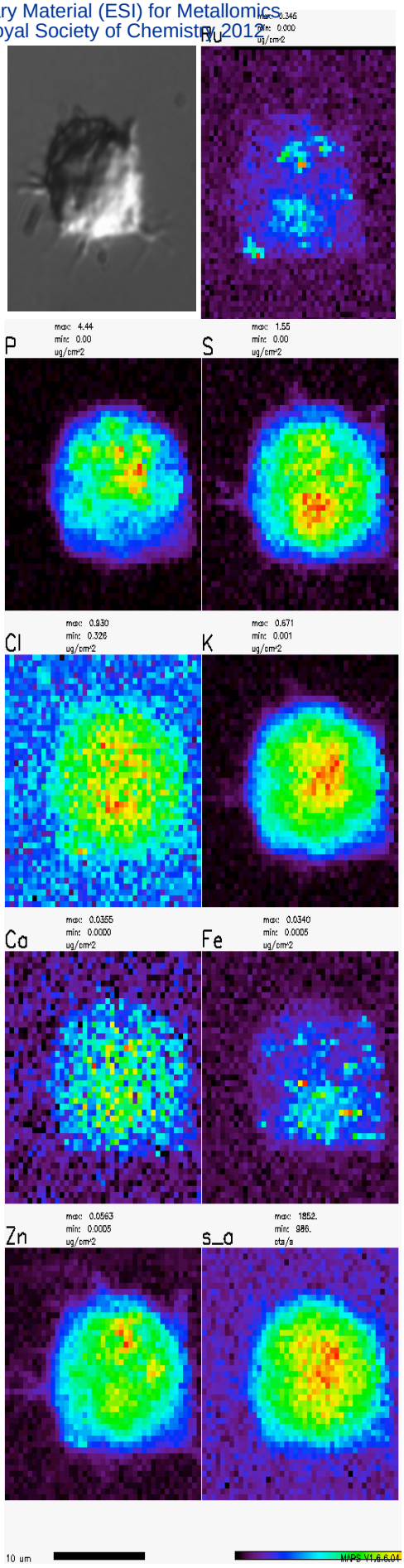

Figure S9: Optical micrograph (top left), scattered X-ray (SA) and XRF elemental distribution maps of P, S, K, Cl, Fe, Cu, Zn, and Ru of an SH-SY5Y cell treated with 200 μM KP1019 for 4 hr. Maximal area densities are given in μg/cm^2 for each.

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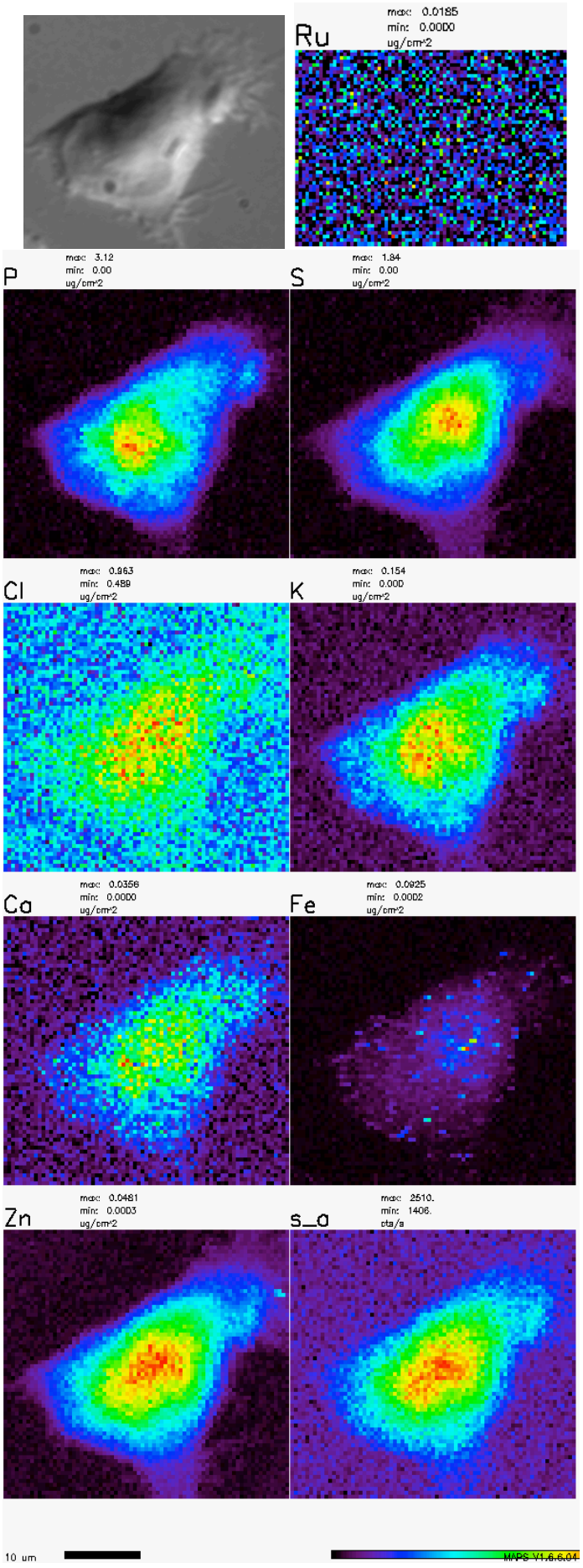

Figure S10: Optical micrograph (top left), scattered X-ray (SA) and XRF elemental distribution maps of P, S, K, Cl, Fe, Cu, Zn, and Ru of an untreated SH-SY5Y cell. Maximal area densities are given in $\mu g/cm^2$ for each element.

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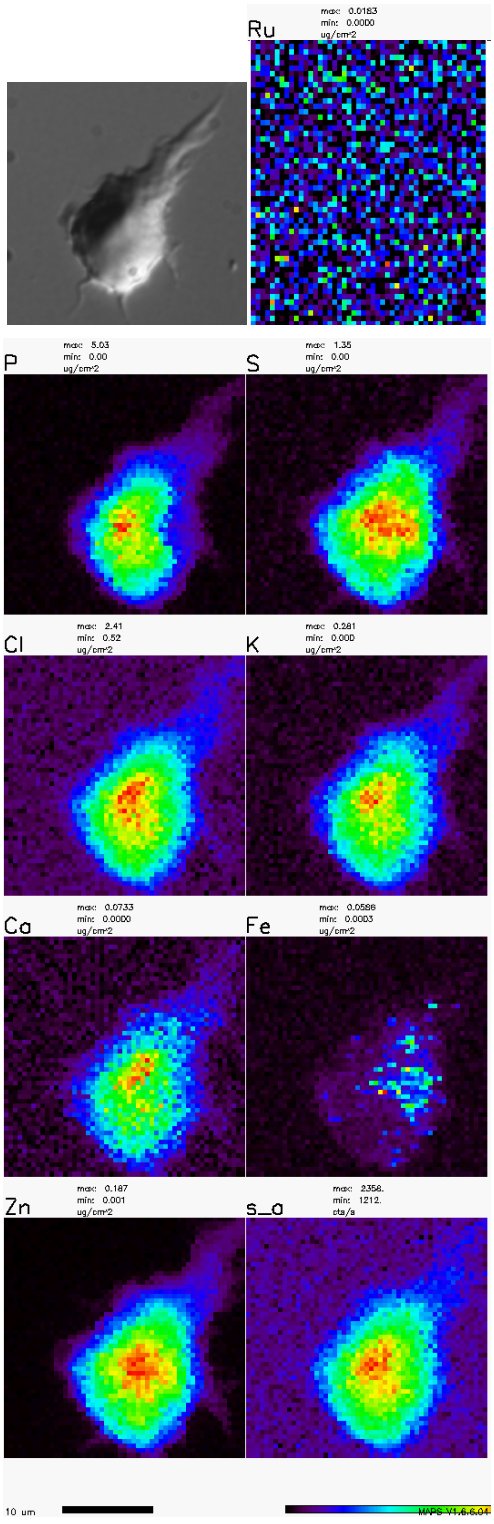

Figure S11: Optical micrograph (top left), scattered X-ray (SA) and XRF elemental distribution maps of P, S, K, Cl, Fe, Cu, Zn, and Ru of an untreated SH-SY5Y cell. Maximal area densities are given in $\mu g/cm^2$ for each element.

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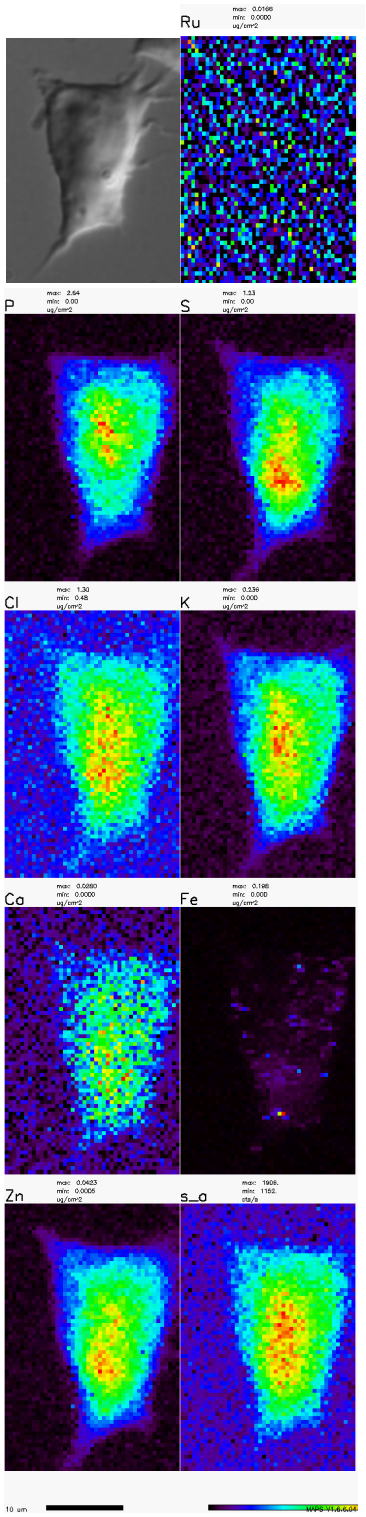

Figure S12: Optical micrograph (top left), scattered X-ray (SA) and XRF elemental distribution maps of P, S, K, Cl, Fe, Cu, Zn, and Ru of an untreated SH-SY5Y cell. Maximal area densities are given in $\mu g/cm^2$ for each element.

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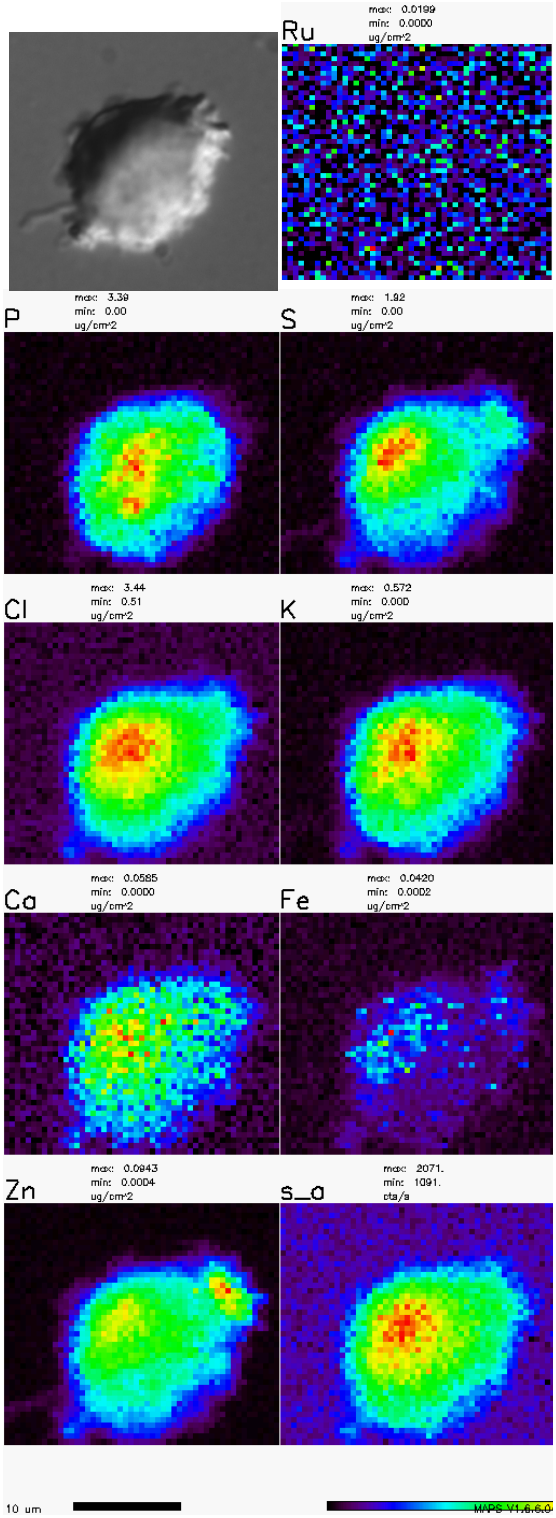

Figure S13: Optical micrograph (top left), scattered X-ray (SA) and XRF elemental distribution maps of P, S, K, Cl, Fe, Cu, Zn, and Ru of an untreated SH-SY5Y cell. Maximal area densities are given in $\mu g/cm^2$ for each element.

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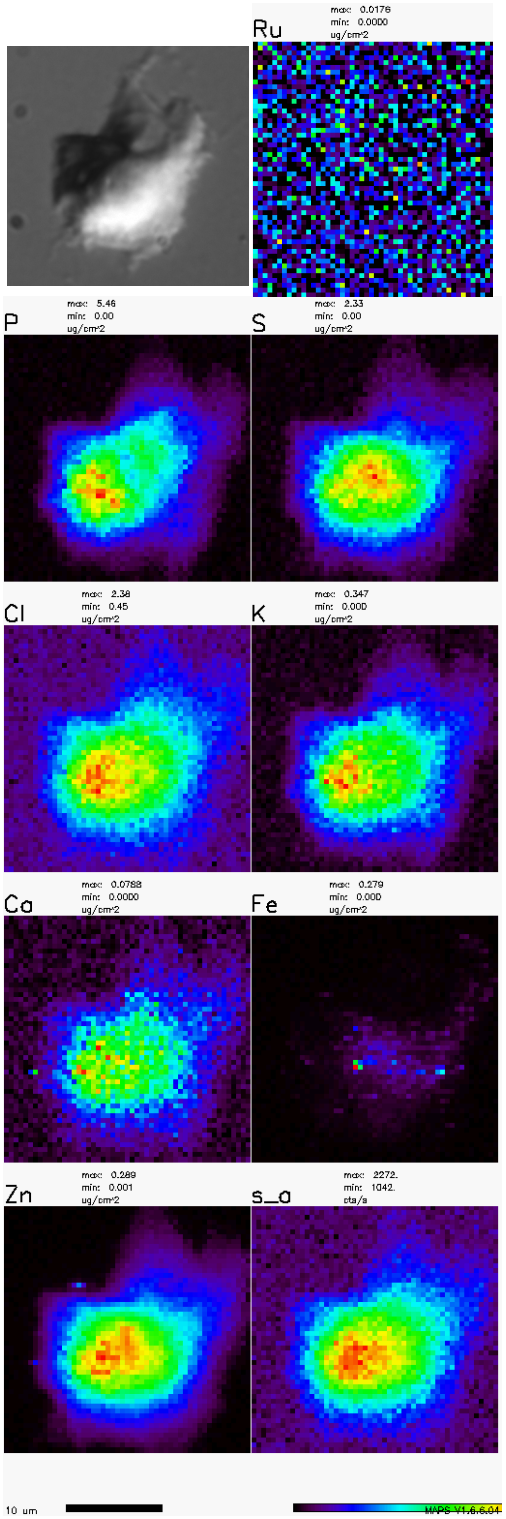

Figure S14: Optical micrograph (top left), scattered X-ray (SA) and XRF elemental distribution maps of P, S, K, Cl, Fe, Cu, Zn, and Ru of an untreated SH-SY5Y cell. Maximal area densities are given in $\mu g/cm^2$ for each element.

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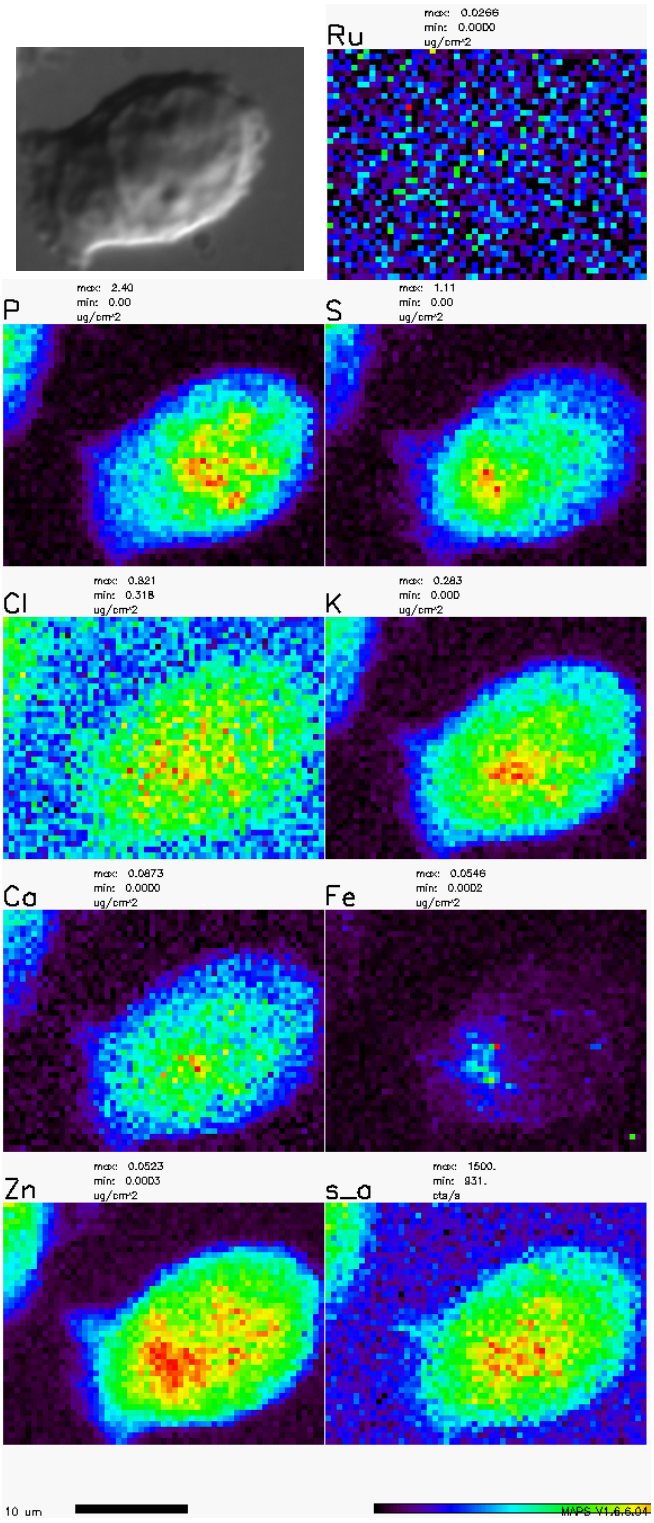

Figure S15: Optical micrograph (top left), scattered X-ray (SA) and XRF elemental distribution maps of P, S, K, Cl, Fe, Cu, Zn, and Ru of an SH-SY5Y cell treated with 200 μM NAMI-A for 4 hr. Maximal area densities are given in $\mu g/cm^2$ for each element.

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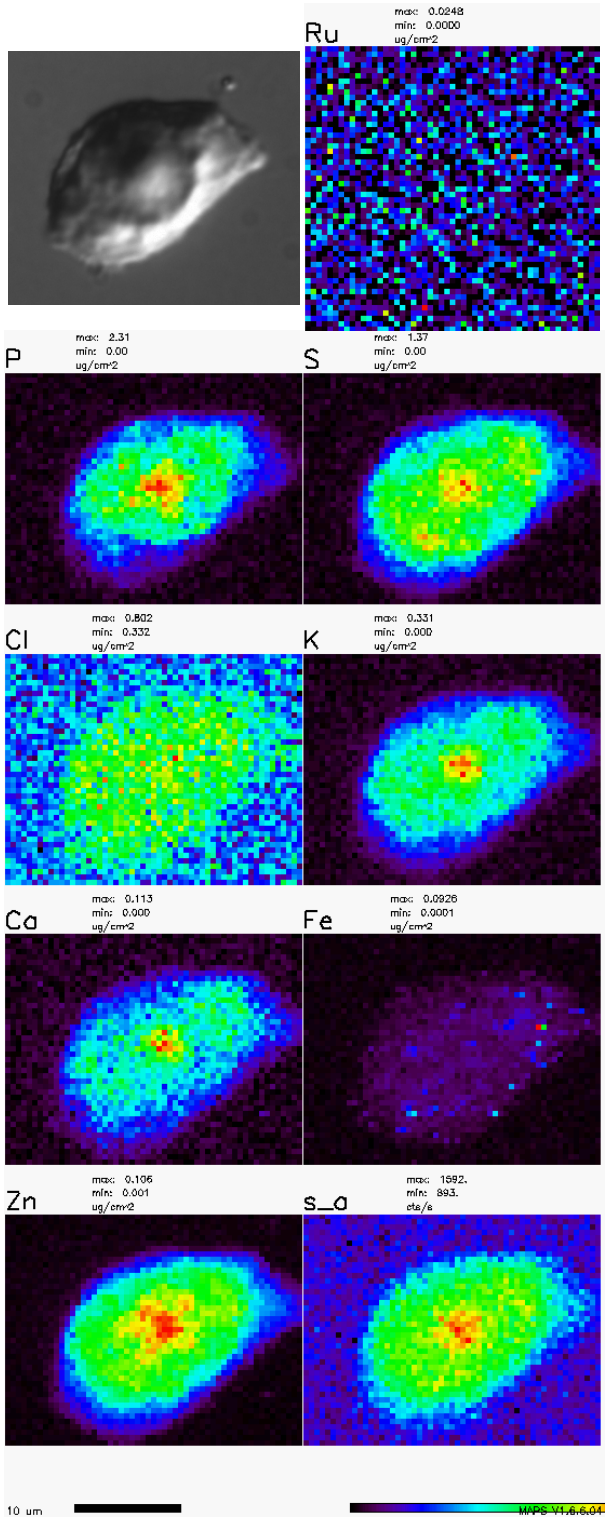

Figure S16: Optical micrograph (top left), scattered X-ray (SA) and XRF elemental distribution maps of P, S, K, Cl, Fe, Cu, Zn, and Ru of an SH-SY5Y cell treated with 200 μM NAMI-A for 4 hr. Maximal area densities are given in $\mu g/cm^2$ for each element.

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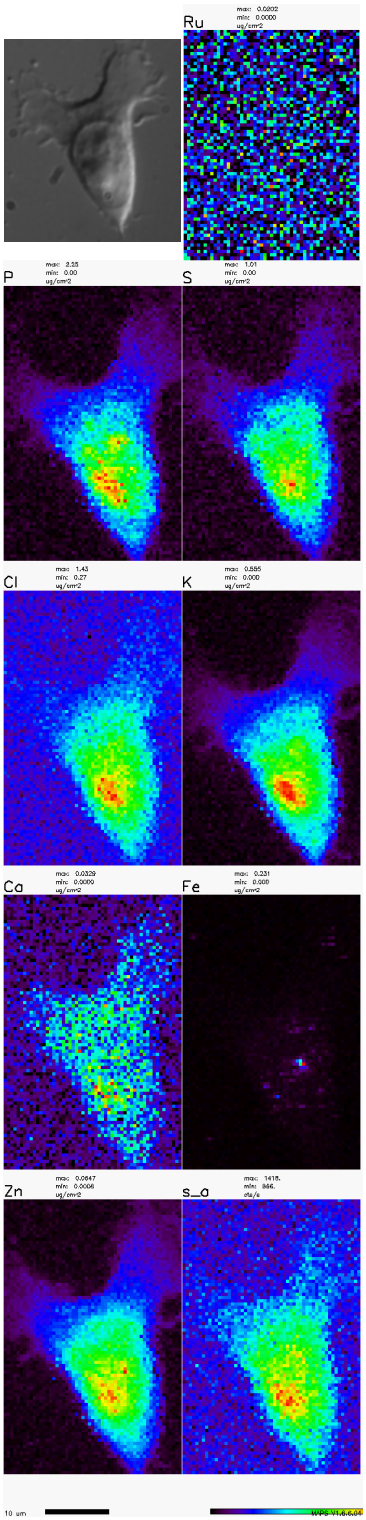

Figure S17: Optical micrograph (top left), scattered X-ray (SA) and XRF elemental distribution maps of P, S, K, Cl, Fe, Cu, Zn, and Ru of an SH-SY5Y cell treated with 200 μM NAMI-A for 4 hr. Maximal area densities are given in $\mu g/cm^2$ for each element.

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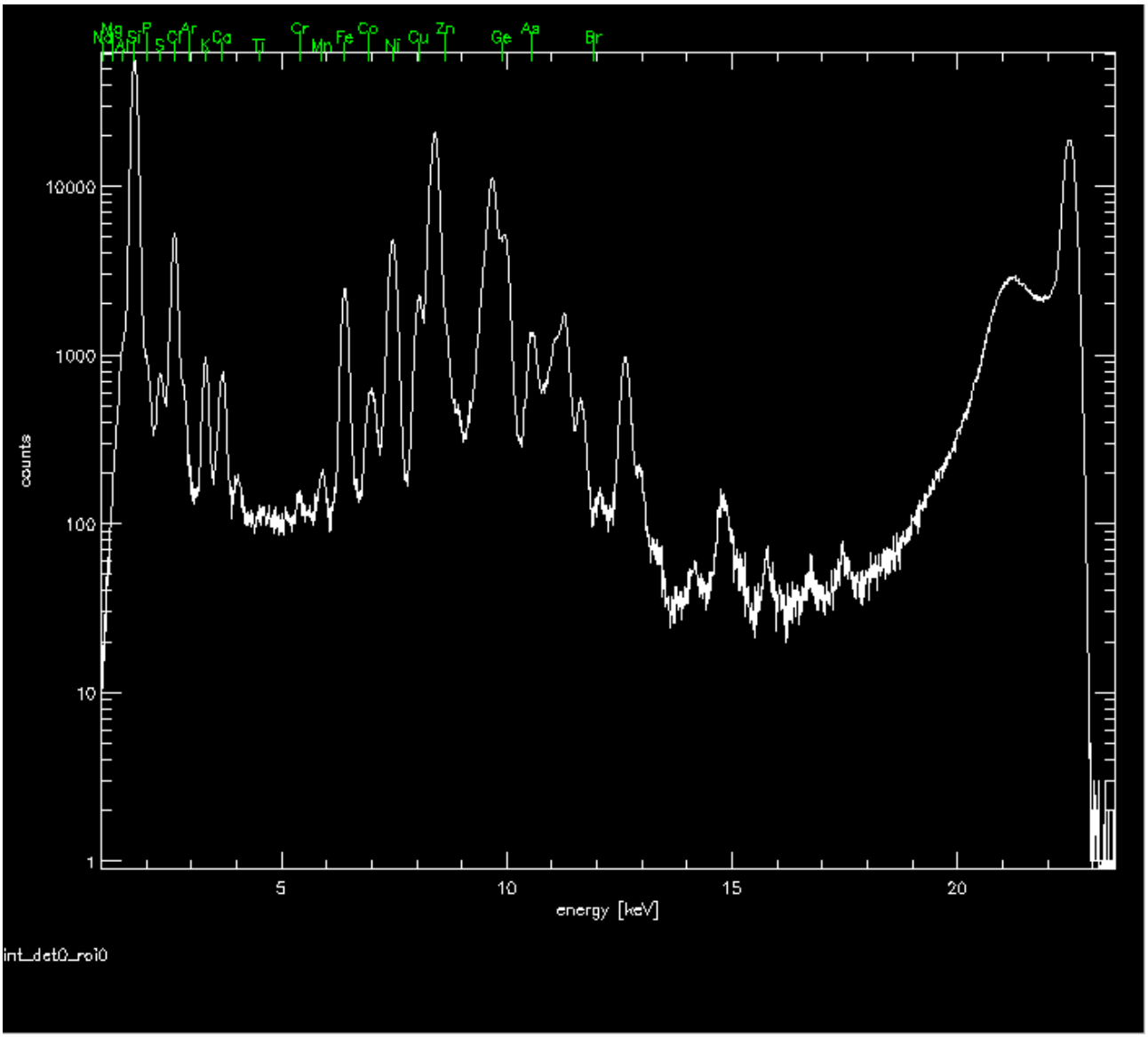

Figure S18: XRF spectra integrated across the region of interest of untreated SH-SY5Y cell (Cell from Figure 2). The Ru Kα fluorescence peak is at ~19.2 keV

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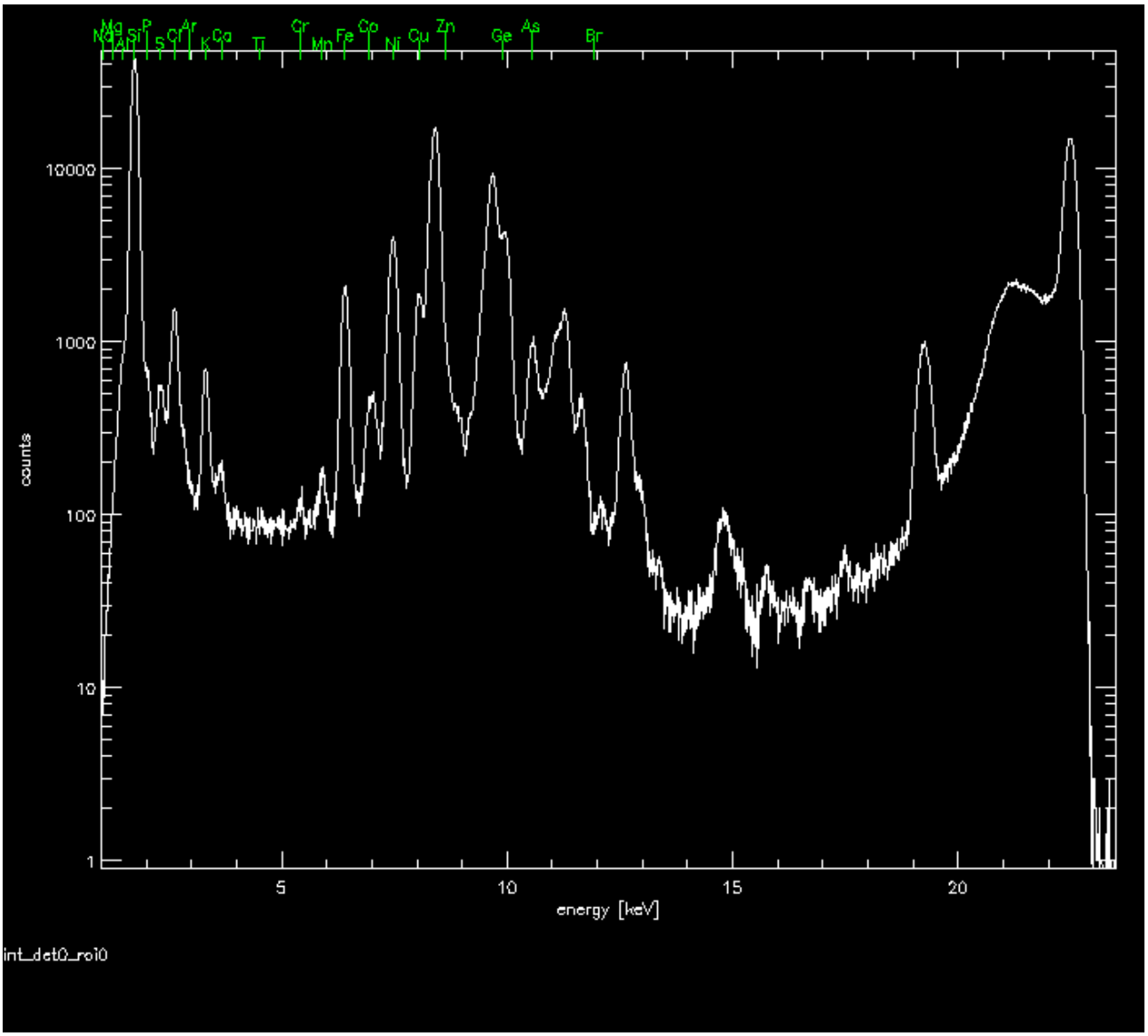

Figure S19: μ-XRF spectra integrated across the region of interest of a SH-SY5Y cell treated with 200 μM KP1019 for 4hr (Cell from Figure 1). The Ru Kα fluorescence peak is at ~19.2keV

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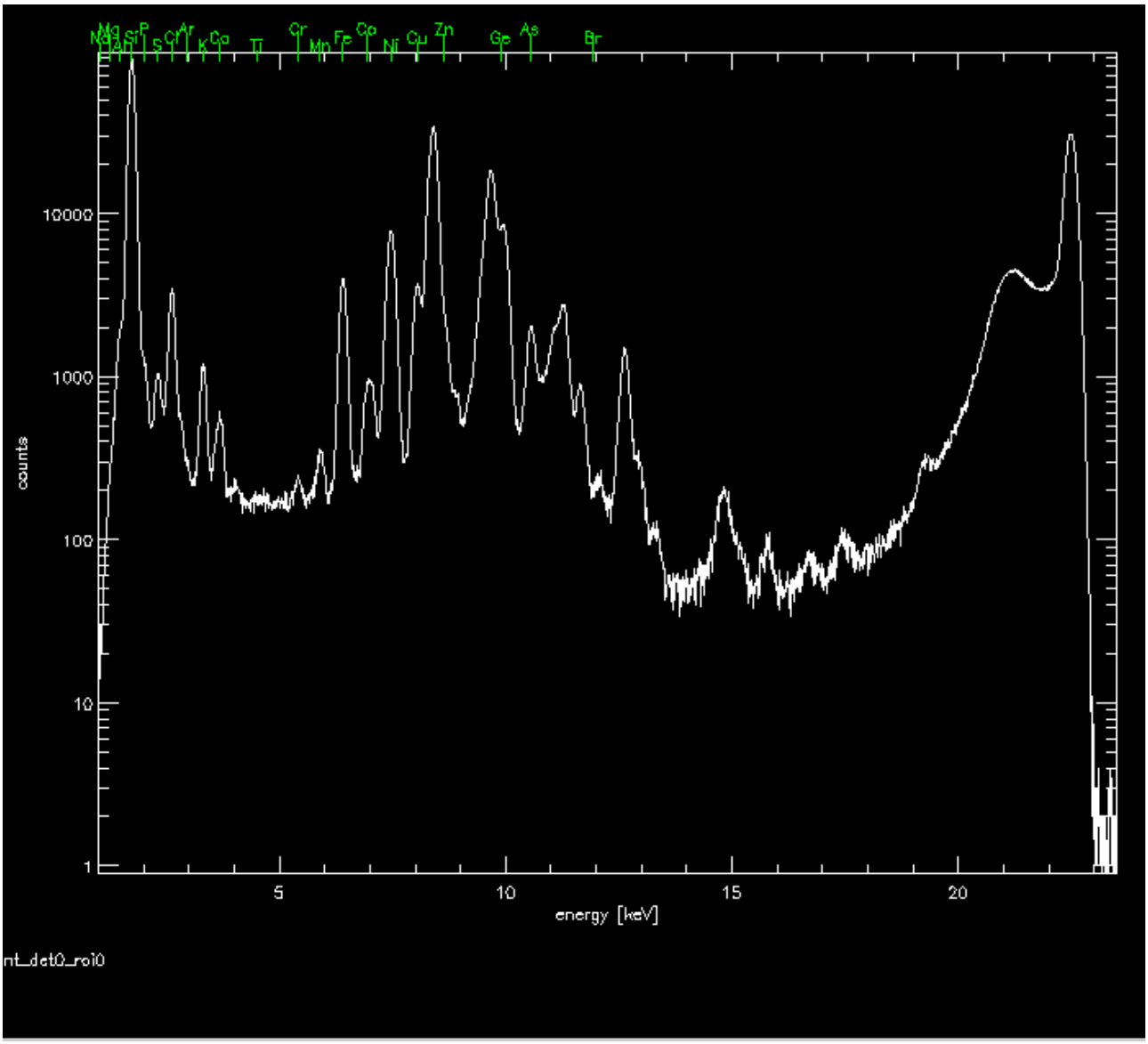

Figure S20: μ-XRF spectra integrated across the region of interest of a SH-SY5Y cell treated with 200 μM NAMI-A for 4hr (Cell from Figure S17). The Ru Kα fluorescence peak is at ~19.2keV

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Table S2: Calculation of net signal for Ru Kα fluorescence counts from integrated signal across cell region of interest.

	Control (N=6)	KP1019 (N=4)	NAMI-A (N=4)
Net Signal[a]	-200(55)	13000 (3210)	500(218)
Net Signal Error[b]	70(10)	150(15)	76(9)

[a] Net signal was calculated by integrating the signal over the Kα fluorescence counts for Ru and subtracting background counts determined by integrating a region of interest on either side of the Ru Kα fluorescence counts. Energy ranges used to determine the background counts (B): 18.92 – 19.10 and 19.47 – 19.65 keV. Energy range used to determine the Kα fluorescence counts for Ru (S): 19.10 – 19.46. The average (Signal – Background) across the cells collected at 22.5 keV are shown with the standard deviation in the last decimal place provided in brackets.
It should be noted that the quantitative values presented in Table S1 are calculated by fitting modified Guassian functions to the fluorescence spectrum rather than simply integrating fluorescence counts across an energy region of interest.
[b] The error was estimated by sqrt(S+B)