Electronic Supplementary Material (ESI) for Metallomics.

A novel branched TAT_{47-57} peptide for selective Ni^{2+} introduction into the human fibrosarcoma cell nucleus

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Electronic Supplementary Information

Contents:

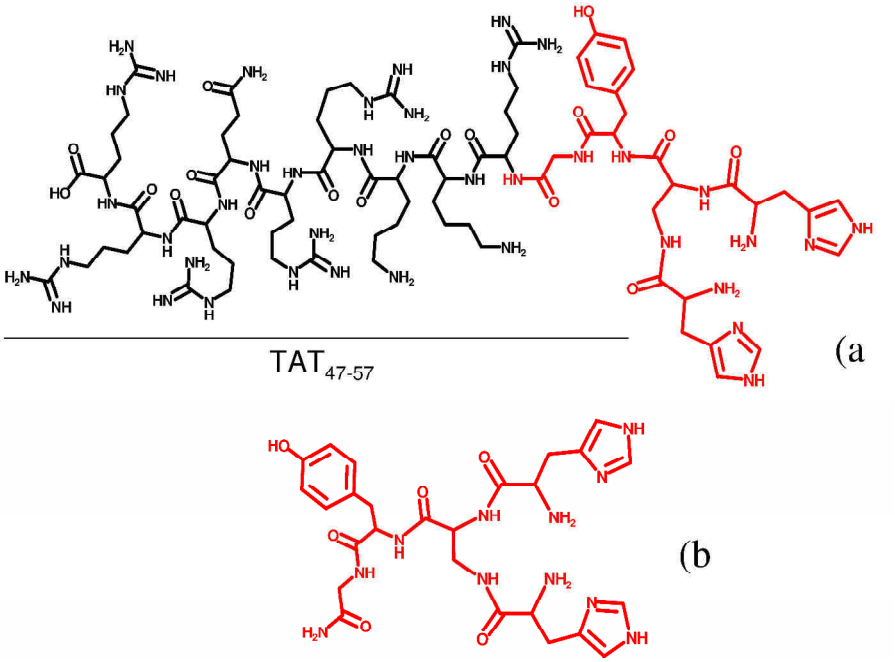
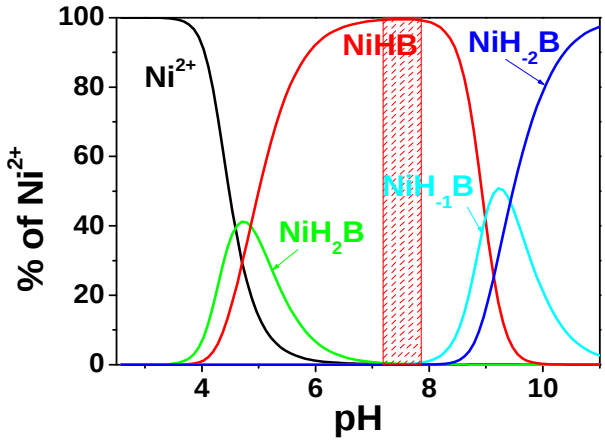


Figure S1. Schematic structures of branch peptides: a) H-His-Dap(H-His)-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-OH (A), b) H-His-Dap(H-His)-Tyr-Gly-NH_2 (B).



a)

	Logβ	log$K$	(λ [nm], ε [$M^{-1}cm^{-1}$])
H_5L	34.02(1)	$4.52(1)_{im}$	
H_4L	29.51(1)	$5.33(1)_{im}$	
H_3L	24.18(1)	$6.63(1)_{NH2}$	
H_2L	17.55(1)	$7.72(1)_{NH2}$	
HL	9.83(1)	$9.83(1)_{Tyr}$	
NiH_2L	24.04(2)	4.86(3)	590, 9
NiHL	19.18(2)		572, 11
$NiH_{-2}L$	1.2(3)	9.47(3)	414, 218
$NiH_{-3}L$	-8.22(3)		415, 221

b)

Figure S2. Speciation diagram for the system containing Ni^{2+} and B peptide, $[Ni^{2+}] = 1\times10^{-3}$ M, 1:1 M: B ratio (a). The physiological pH range is indicated by red dots. The logarithms of the protonation constants ($\log\beta_{HxL}$) and metal complex stability constants ($\log\beta_{NiHxL}$) for Ni^{2+} species with B, UV-Vis data for observed species (b).

The data showed that increases in pH from 4 to 5 resulted in the formation of minor NiH_2B species, which coexisted in equilibrium with the NiHB form. Unfortunately, the low concentration did not allow for precise determination of spectroscopic parameters for NiH_2B species. Near pH 5, the NiHB form started to be predominant up to pH 8. The involvement of peptide amide (N^-) donors in metal coordination was observed when pH increased to 9. Then, two simultaneous deprotonations were observed, and complex $NiH_{-1}B$ was formed. The double deprotonation was also observed in cases of N,N'-diglycylethylenediamine (DGEN) ligand[30], which is an analog of the Ni-binding domain in the B ligand. Formation of the $NiH_{-1}B$ square planar complex with the $\{2NH_2, 2N^-\}$ donor set was also confirmed based on UV-Vis spectroscopy parameters, which clearly corresponded to those observed in similar Ni^{2+} complexes with the DGEN ligand (λ = 414, ε = 219).[1] Further increases in the pH resulted in deprotonation of non-coordinated tyrosine residue. However, changes of pK = 0.4 logarithmic units were suggestive of minor interactions.

Bibliography

1 K. S. Bai and A. E. Martell, *Journal of the American Chemical Society*, 1969, **91**, 4412–4420.

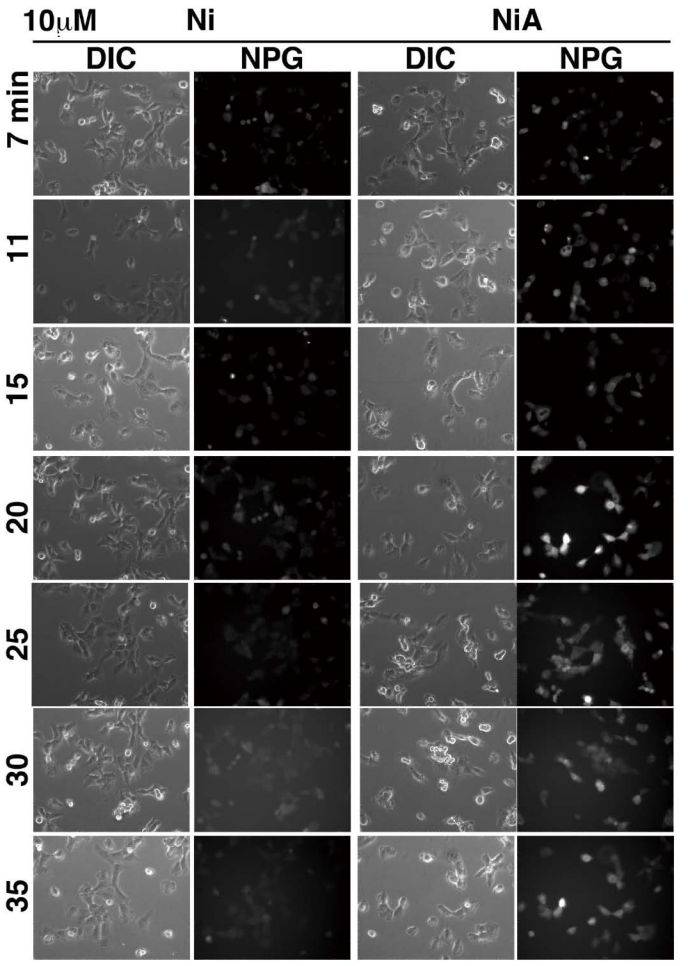


Figure S3. Representative NPG images after treatment with Ni ion and NiA in living cells. Left, DIC image; right, NPG images. It was notable that the NPG signal intensity peaked 20 min after NiA addition. Bar, 50 μm.

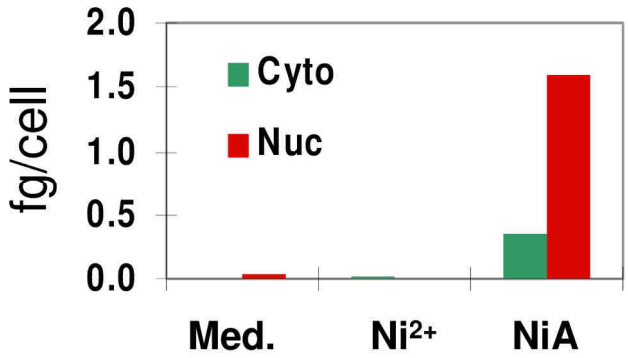


Figure S4. Measured by ICP-MS amount of Ni in cytoplasmic and nuclear fraction of cells incubated in Ni free medium with Ni ions and NiA complex. Cyto, soluble cytoplasmic fraction; Nuc, whole nuclear and insoluble membrane fraction; Med, culture medium.

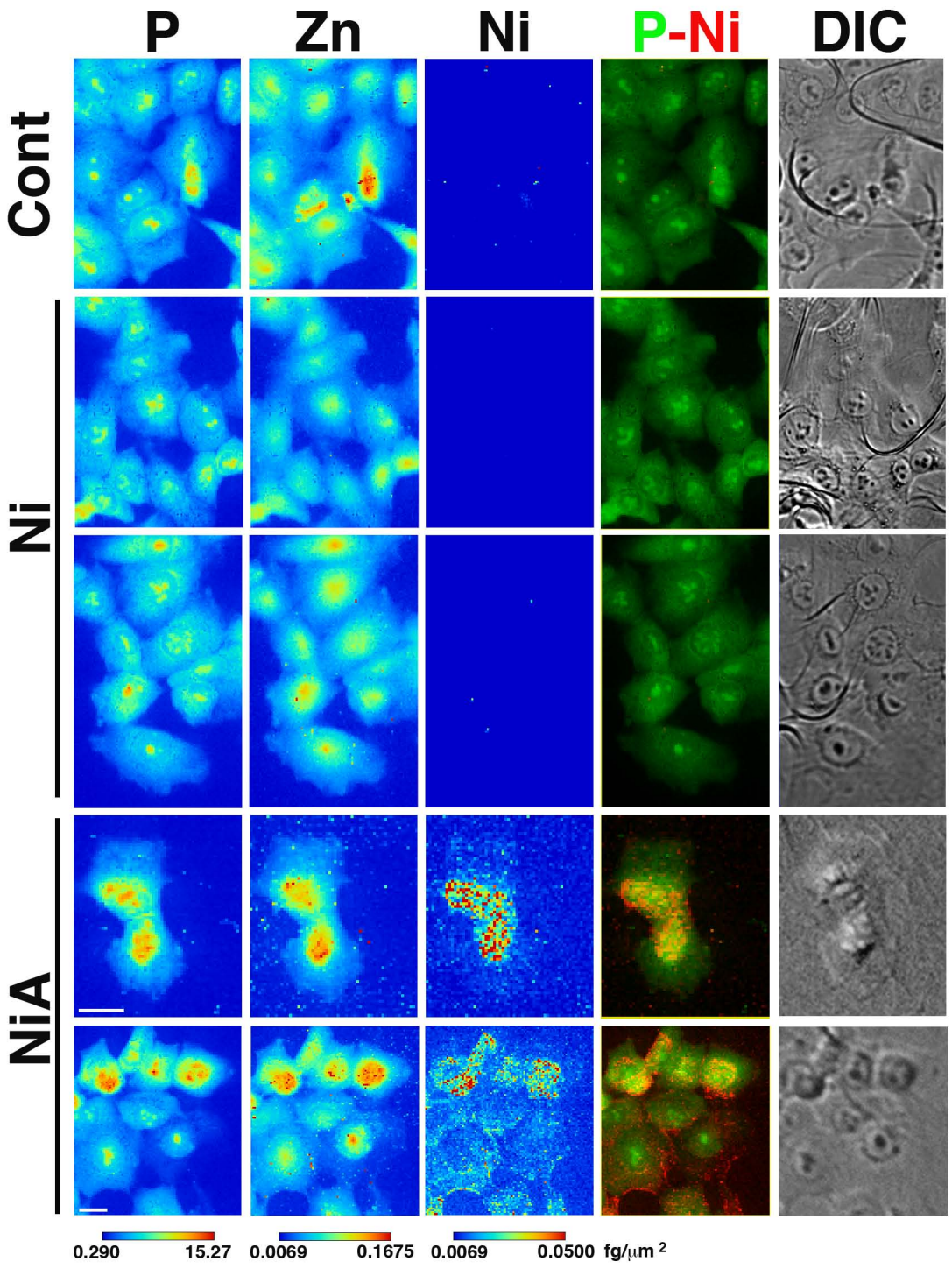


Figure S5. X-ray fluorescence images. From the left, signals of P, Zn, Ni, and DIC images are shown. Each set of panel, control cells (top), and cells treated with 20 μM of Ni ion (middle) and NiA for 20 min (bottom). P, phosphorus; Zn, zinc; Ni, nickel; P-Ni, merged images between phosphorus and nickel; DIC, differential interference contrast images. Brighter colour indicates higher signal intensity. Colour bar, fg/μm2; Bar, 10 μm.