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Nickel Binding to Cap43 protein

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INTRODUCTION

Cap43 has been reported to be specifically induced by nickel compounds in a variety of cell lines [1,2]. Although the function of Cap43 protein (MW 43,000) is not clear, it does appear to be induced in response to an increase in intracellular concentration of Ca^{2+}, caused by nickel ion exposure in cultured human cells [2], for this reason is named Cap43: *Ca*lcium *p*rotein *43*,000. Cap43 protein is expressed at low levels in normal tissues however, it is overexpressed in cancer cells. The high level of expression in cancerous status combined with the elevated stability of Cap43 protein makes it an excellent cancer marker.

A possible way to better understand the molecular mechanisms implicated in toxicity and carcinogenicity of nickel compounds is to study the characteristics of the proteins expressed by the genes specifically induced by these carcinogens. For this reason we focused our attention to investigate the interaction ability of nickel to Cap43 protein[3,4].

The peculiarity of Cap43 protein is in its new mono-histidinic motif consisting of ten amino acids ***TRSRSHTSEG*** fragment repeated three times in the C-terminus.

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  1 MSREMQDVDL AEVKPLVEKG ETITGLLQEF DVQEQDIETL HGSVHVTLCG TPKGNRPVIL
 61 TYHDIGMNHK TCYNPLFNYE DMQEITQHFA VCHVDAPGQQ DGAASFPAGY MYPSMDQLAE
121 MLPGVLQQFG LKSIIGMGTG AGAYILTRFA LNNPEMVEGL VLINVNPCAE GWMDWAASKI
181 SGWTQALPDM VVSHLFGKEE MQSNVEVVHT YRQHIVNDMN PGNLHLFINA YNSRRDLEIE
241 RPMPGTHTVT LQCPALLVVG DSSPAVDAVV ECNSKLDPTK TTLLKMADCG GLPQISQPAK
301 LAEAFKYFVQ GMGYMPSASM TRLMRSRTAS GSSVTSLDG
340 TRSRSH345TSEG TRSRSH355TSEG TRSRSH365TSEG
370 AHLDITPNSGA AGNSAGPKSM EVSC
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We have analyzed, for Ni(II) binding, the 30-amino acid C-terminal sequence of the protein, *TRSRSHTSEG-TRSRSHTSEG-TRSRSHTSEG,* by a combined pH-metric and spectroscopic (UV-VIS, CD, NMR) study.

The imidazole nitrogen atom of the histidine residue is the essential binding site for Ni(II) ion. This study was also performed in order to evaluate the binding ability of the fragment to more than one metal ion.

The coordination of the metal ion starts from the imidazole nitrogen atom of the histidine residue and, with increasing the pH, Ni(II) ions are able to deprotonate successive peptide nitrogen atoms till $NiH_{-3}L$ $Ni_2H_{-6}L$ and $Ni_3H_{-9}L$ species above pH 8, are formed.

The formation of stable five membered chelate rings by consecutive deprotonation of nitrogen atoms from the backbone of the peptide is the driving force of the coordination process. At physiological pH and mM concentration of Ni(II), dependently from metal to ligand molar ratio the 30-amino acid fragment forms the NiL (1:1), Ni_2L (2:1) and Ni_3L (3:1) complexes where each metal ion is coordinated by the imidazole nitrogen atom of the histidine residue of each ten-amino acid fragment.

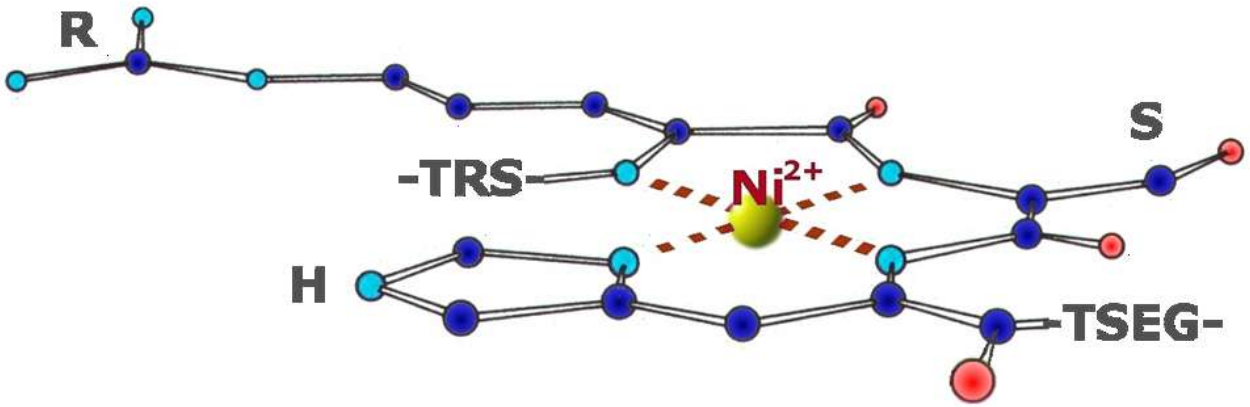


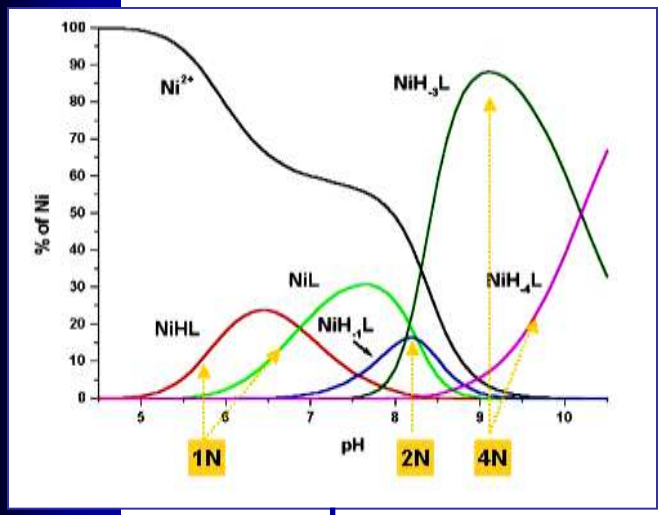
Fig. 1 Scheme of 4N coordination pattern

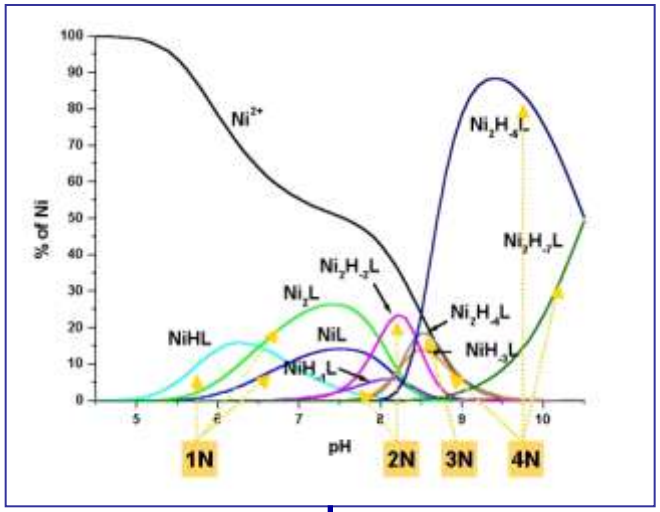
From NMR experiments, the shifts induced by Ni(II) were consistent with the binding of the metal ion in a square-planar site formed by four nitrogen atoms from His (Nd1,N_H) and from N_H of Ser and Arg, of each ten-aminoacid fragment. Strong shifts in the aliphatic proton resonances of arginine suggest an involvement of the side-chain in the complex stability.

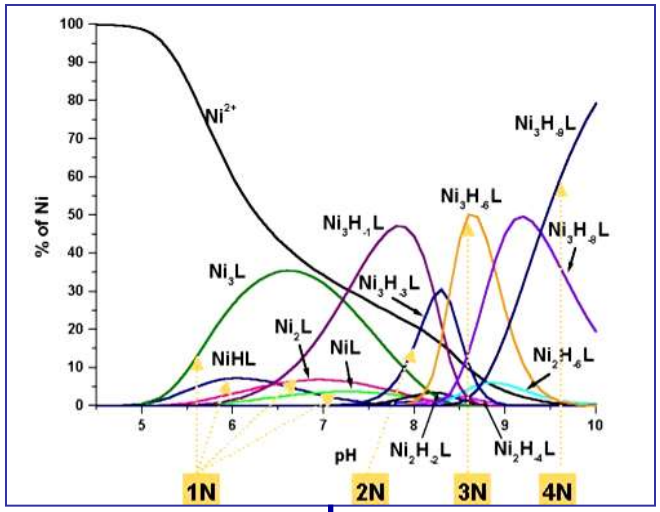
Both spectroscopic and potentiometric studies performed on the 30-amino acids peptide, support the existence of relatively effective metal binding site in the C-terminal region of Cap43 protein.

Our results suggest that the entire Cap43 protein could be an interesting target for Ni(II) ions.

pH-METRIC STUDY



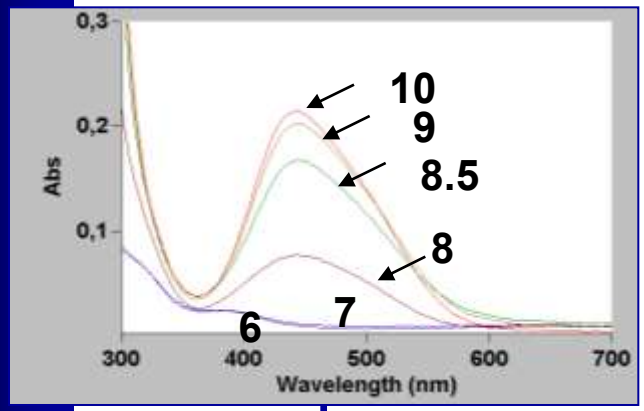


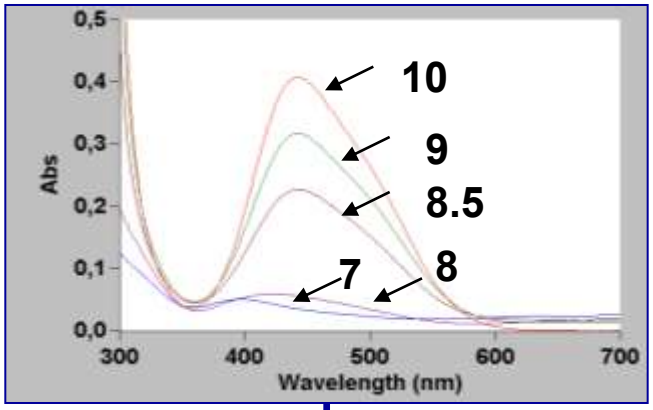


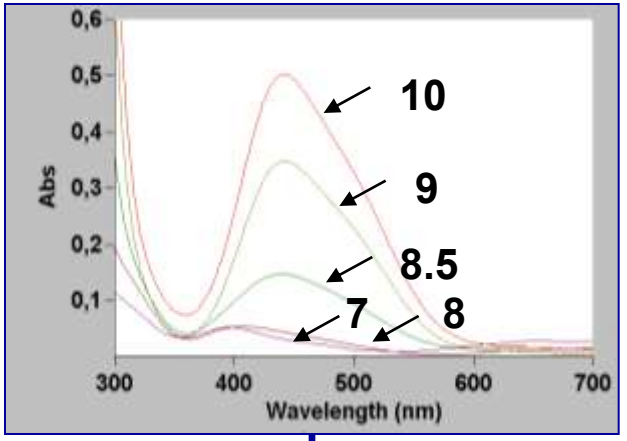
Species distribution curves for 30aa peptide -Ni(II), 1:1, 1:2 and 1:3 molar ratio, respectively

SPECTROSCOPIC STUDY

UV-Vis



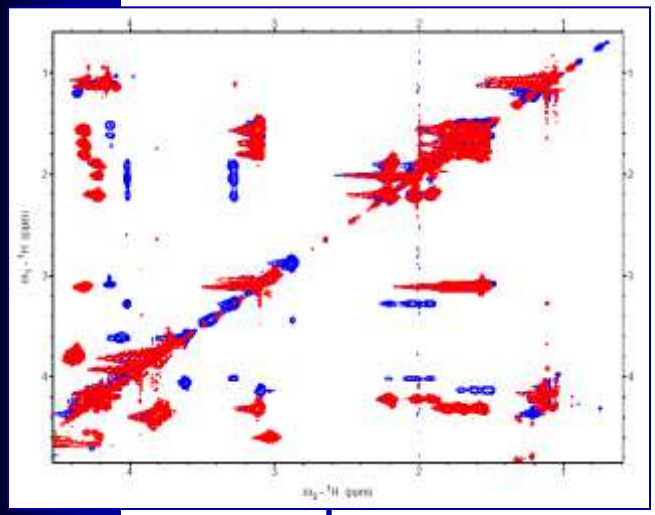




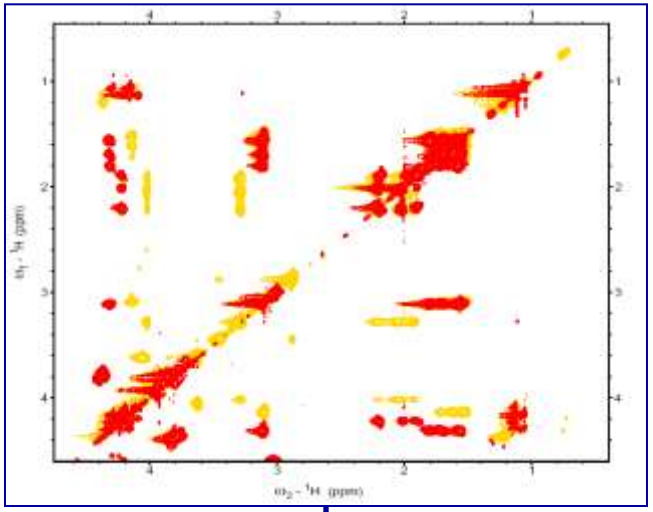
UV-Vis spectra for 30aa peptide - Ni(II), 1:1, 1:2 and 1:3 molar ratio, respectively

NMR STUDY

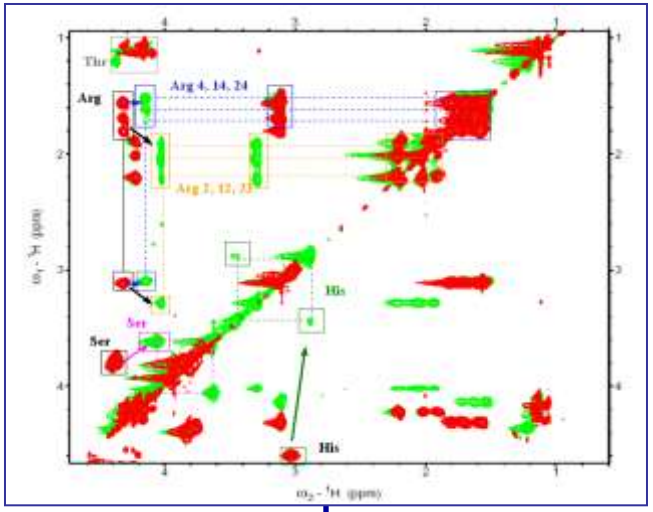
1-1



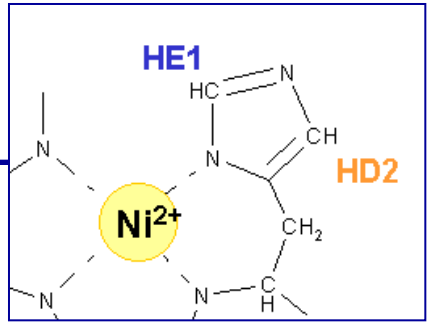
1-2

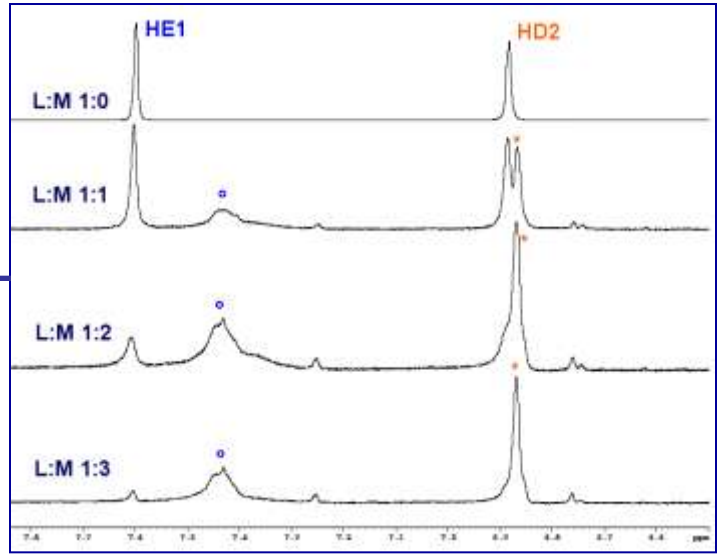


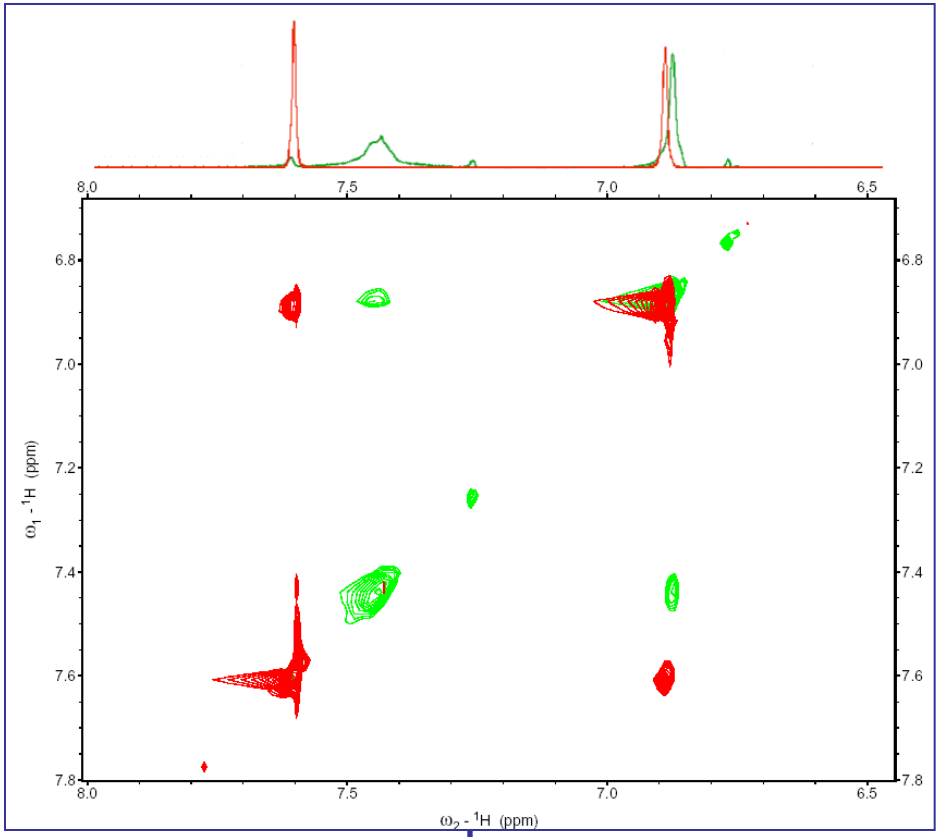
1-3



Superimposition of TOCSY spectra of free 30aa peptide (red) and 30aa peptide-Ni(II) complex at 1:1 (blu), 1:2 (yellow) and 1:3 (green) molar ratio, respectively.

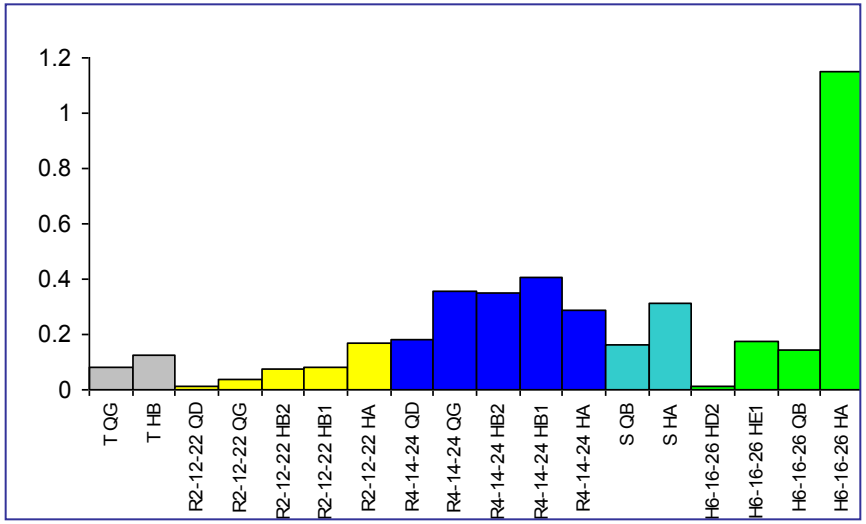






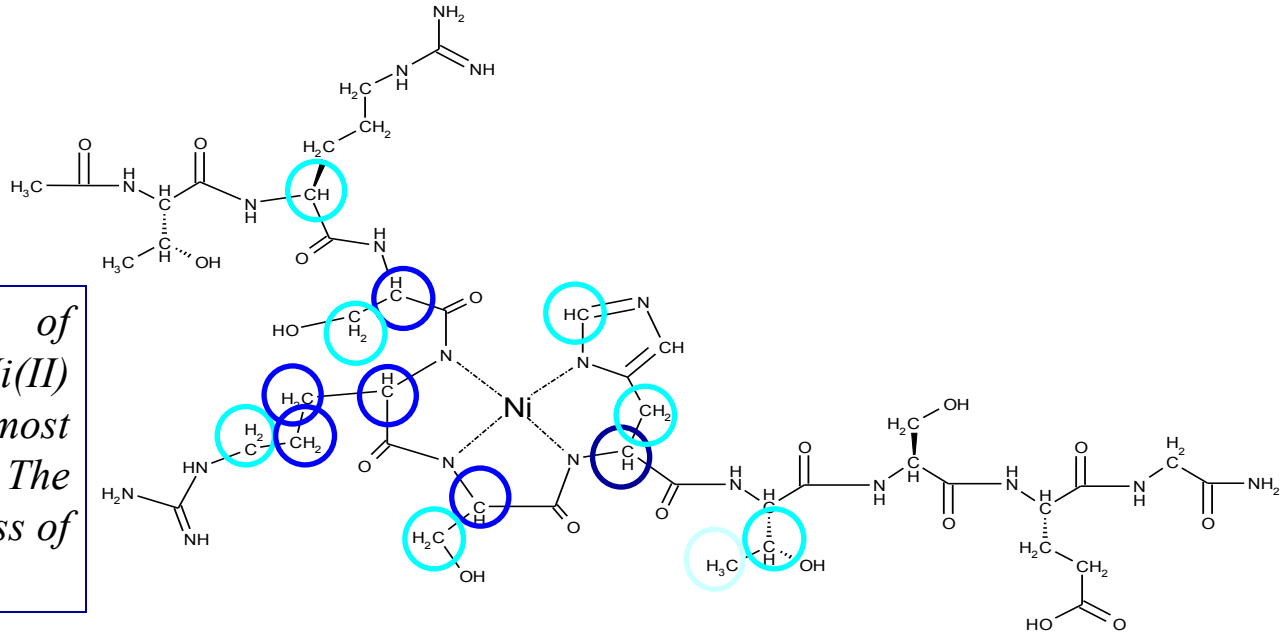
Changes in intensity and chemical shift of histidine aromatic protons (HE1 and HD2) by increasing nickel concentration. Comparison of aromatic region of 1D H NMR spectra of 30aa peptide Cap43-Ni(II), 1:0, 1:1. 1:2, 1:3 molar ratio.

Res-H	Pept Cap43 free Ppm	Pept Cap43-Ni(II) 1-3 ppm	Δδ
T QG	1.125	1.206	-0.081
T HB	4.292	4.168	0.124
R2-12-22 QD	3.111	3.097	0.014
R2-12-22 QG	1.561	1.524	0.037
R2-12-22 HB2	1.694	1.619	0.075
R2-12-22 HB1	1.803	1.719	0.084
R2-12-22 HA	4.313	4.147	0.166
R4-14-24 QD	3.111	3.290	-0.179
R4-14-24 QG	1.561	1.918	-0.357
R4-14-24 HB2	1.694	2.047	-0.353
R4-14-24 HB1	1.803	2.209	-0.406
R4-14-24 HA	4.313	4.024	0.289
S QB	3.793	3.628	0.165
S HA	4.380	4.066	0.314
H6-16-26 HD2	6.888	6.873	0.015
H6-16-26 HE1	7.607	7.435	0.172
H6-16-26 QB	3.029	2.883	0.146
H6-16-26 HA	4.600	3.451	1.149



Chemical shift assegnment for the residues involved in the complex formation, before and after nickel interaction. The differences of chemical shifts are reported in the plot (absolute values).

Scheme of 4N coordination pattern of TRSRSHTSEG Cap43 fragment-Ni(II) complex. The circles indicate the most affected protons after nickel interaction. The hardeness of colors indicate the strongness of changes.



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2. K. Salnikow, D. Zhou , T. Kluz, C. Wang, M. Costa, in : Metal and Genetics, (Sarkar B ed), New York, 1999, 131-144.
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4. M.A. Zoroddu, M. Peana, T. Kowalik-Jankowska, H. Kozlowski, M. Costa, J. Inorg. Biochem., 98(6), 2004, 931-939.

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