Table S1: Fluorescence decay parameters for NCS1 and 1,8-ANS: NCS1 complex in the presence/absence of metal ions.

NCS1	τ_1 (ns)	f_1 (%)	τ_2 (ns)	f_2 (%)	τ_3 (ns)	f_3 (%)	$\langle\tau\rangle$ (ns)	χ^2
Apo	0.66	9	3.20	56	6.30	36	4.11	0.9
Ca^{2+}	0.64	16	2.99	53	5.65	41	3.63	1.5
Tb^{3+}	0.29	5	2.12	36	4.84	59	3.60	2.0
Eu^{3+}	0.47	8	2.45	44	4.99	48	3.54	1.5
NCS1:ANS								
Apo	0.28	8	4.25	15	16.60	77	13.50	1.8
Ca^{2+}	0.28	7	4.47	17	17.20	76	14.05	1.5
Tb^{3+}	0.28	7	5.08	14	17.45	79	14.80	1.1
Eu^{3+}	0.28	7	5.12	14	17.20	79	14.25	1.1

Table S2: CCS values determined for Tb^{3+} and Eu^{3+} ion adducts at 9+ charge state using TIMS-MS.

Tb^{3+} ion		Eu^{3+} ion	
$^{TIMS}CCS_{N2}$ (Å^2)	Ion specie	$^{TIMS}CCS_{N2}$ (Å^2)	Ion specie
2182	$[M-3Ca+3H]^{9+}$	2192	$[M-3Ca+3H]^{9+}$
2204	$[M-Tb+6H]^{9+}$	2201	$[M-3Ca-Na+2H]^{9+}$
2189	$[M-Ca-Tb+4H]^{9+}$	2204	$[M-4Ca+H]^{9+}$
2201	$[M-2Ca-Tb+2H]^{9+}$	2198	$[M-2Na-Eu+4H]^{9+}$
2179	$[M-3Ca-Tb]^{9+}$	2179	$[M-2Ca-Eu+2H]^{9+}$
2195	$[M-2Tb+3H]^{9+}$	2163	$[M-3Ca-Eu]^{9+}$

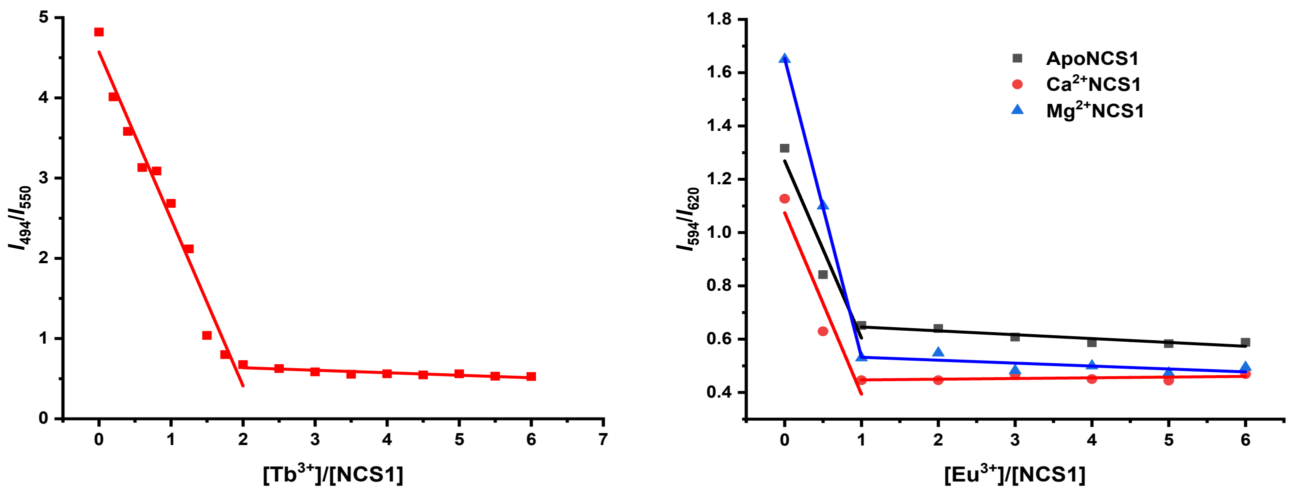

Figure S1: Determination of the stoichiometry of Tb^{3+}/Eu^{3+} bound NCS1.

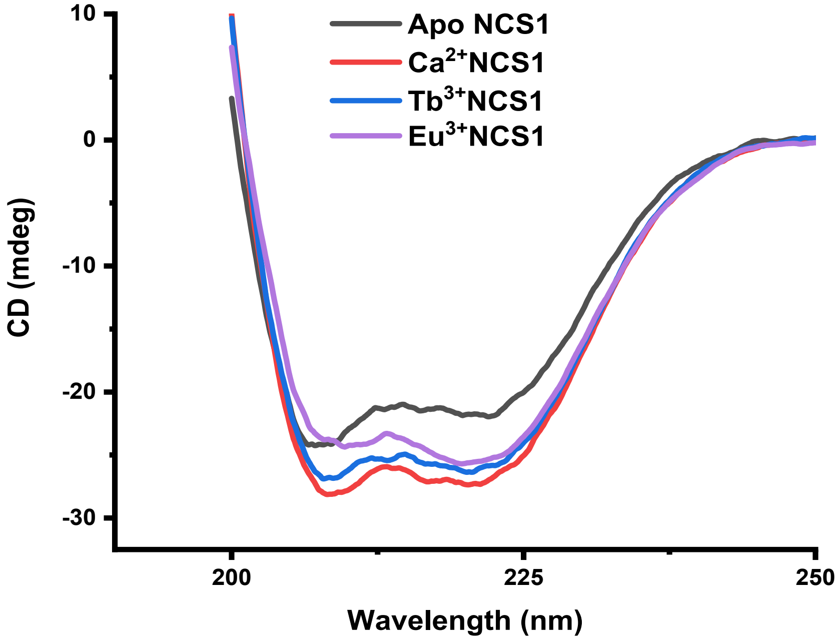

Figure S2: Far-UV circular dichroism spectra of NCS1 in absence and presence of metal ions. Conditions as in the figure 1.

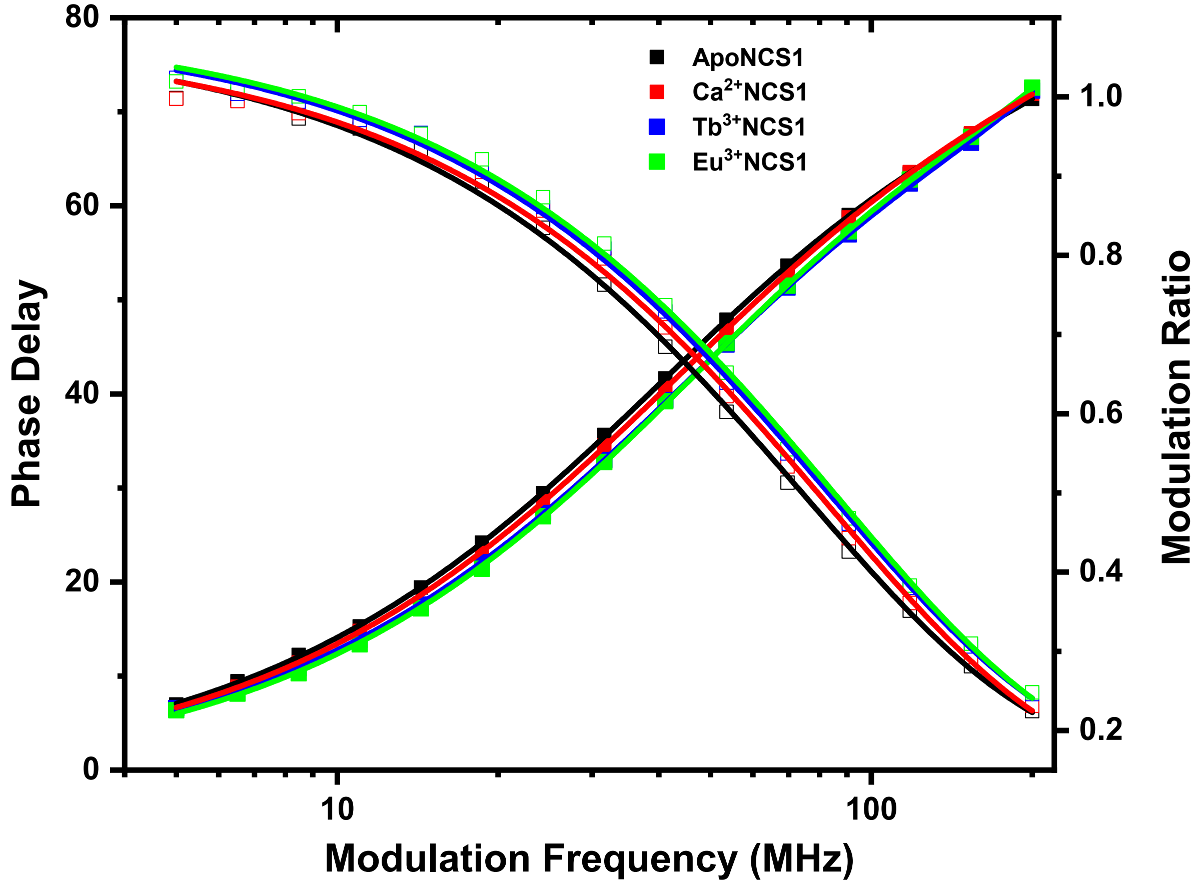

Figure S3: Frequency domain intensity decay of apo- and metal bound NCS1. Solid and open symbols indicate modulation ratio and phase delay data points, respectively. The solid line corresponds to the fit of the experimental data using a sum of three discreet exponential decay model. Conditions: 10 μM NCS1, 20 mM Tris buffer, pH 7.40, and 1 mM EDTA or 1 mM Ca^{2+}, 40 μM Tb^{3+}, 40 μM Eu^{3+}.

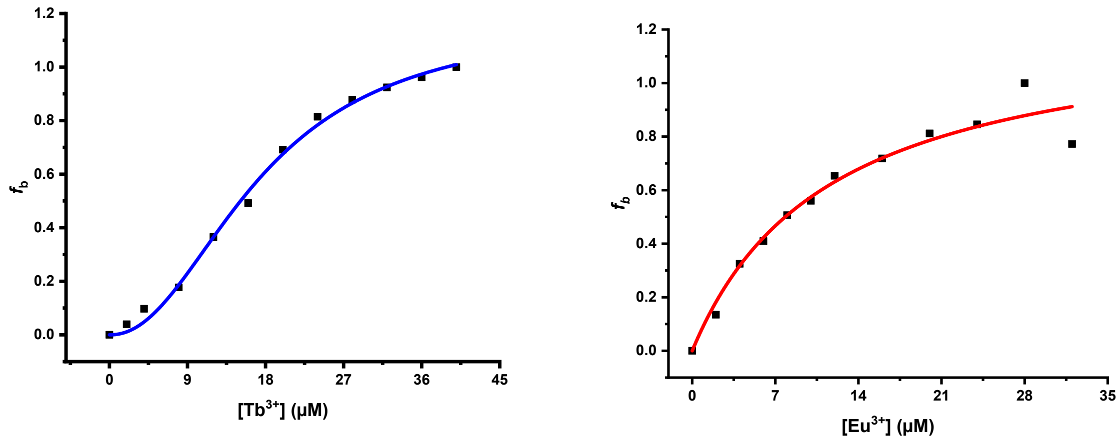

Figure S4: Titration curves of Tb^{3+}/Eu^{3+} binding to Ca^{2+}NCS1:1,8-ANS. Conditions: 10 µM NCS1, 20 µM 1,8-ANS, 1 mM Ca^{2+}, 40 µM Tb^{3+}, 32 µM Eu^{3+} and λ_{exc}=350 nm.

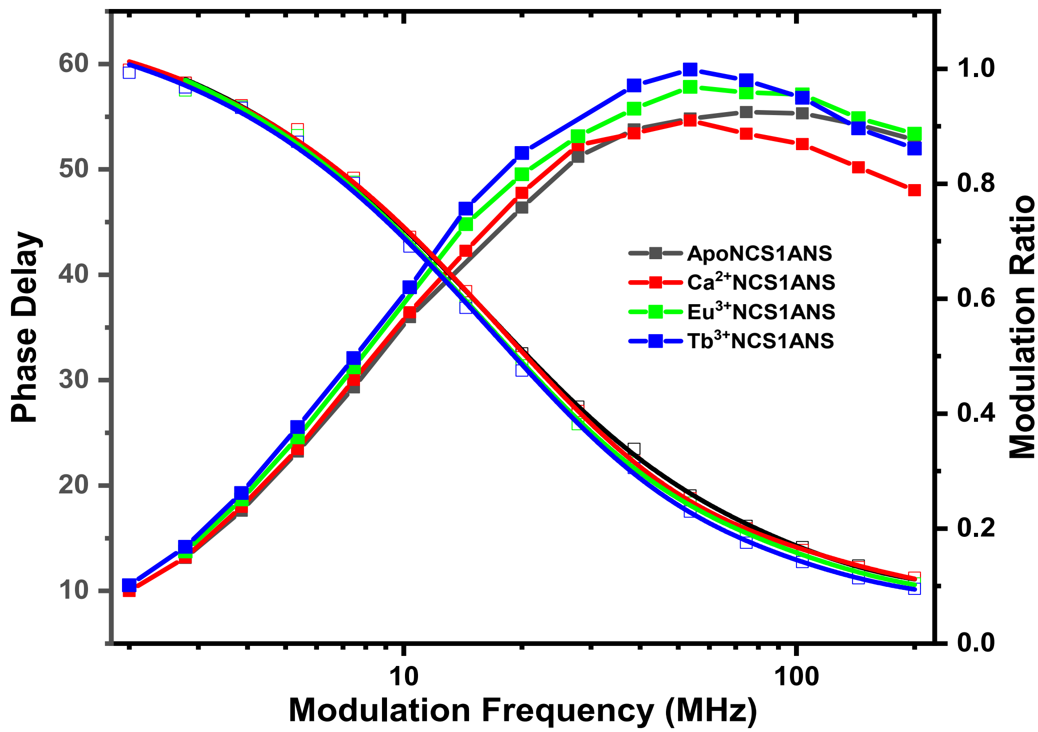

Figure S5: Frequency domain intensity decay of apo- and metal bound NCS1:1,8-ANS complex. Open and solid symbols indicate modulation ratio and phase delay data points, respectively. The solid line corresponds to the fit of the experimental data using a sum of three discreet exponential decay model. Conditions: 10 µM NCS1, 20 µM 1,8-ANS, 20 mM Tris buffer, pH 7.40, and 1 mM EDTA or 1 mM Ca^{2+}, 40 µM Tb^{3+}, 40 µM Eu^{3+}.

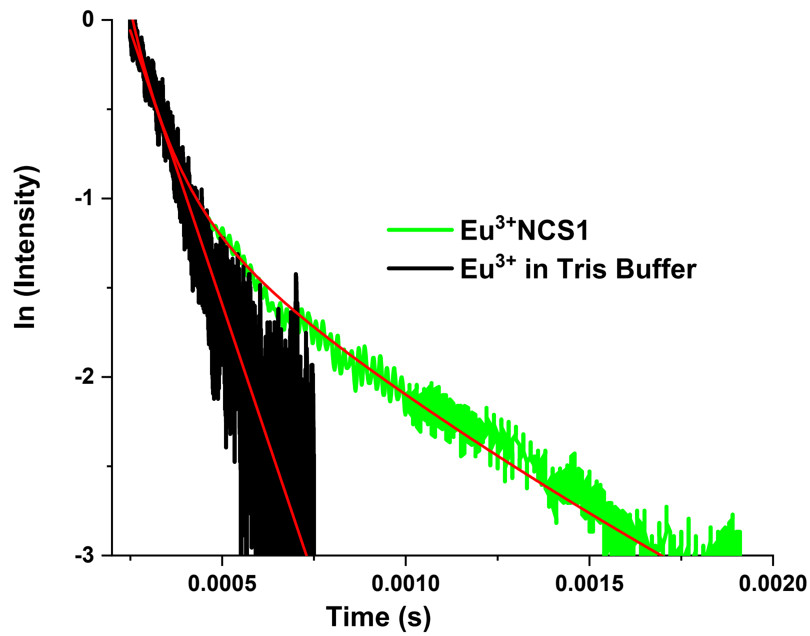

Figure S6: Luminescence decay of Eu^{3+} itself and in presence of NCS1. Conditions: 40 μM NCS1 and 20 μM Eu^{3+}.

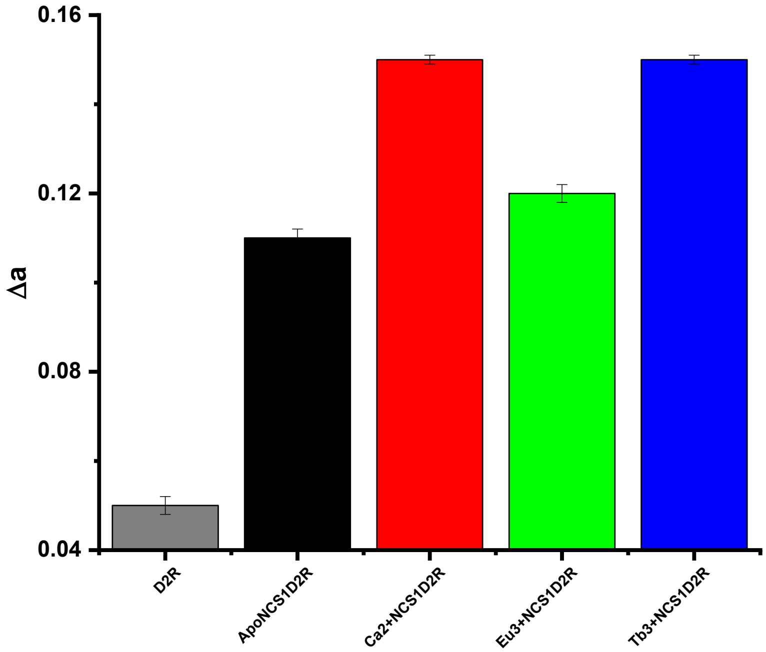

Figure S7: Steady state anisotropy of isolated D2R peptide and NCS1:D2R complex in the absence or presence of metal ions. Conditions: 10 μM NCS1, 500 nM D2R, 1 mM EDTA, 1 mM Ca^{2+}, 40 μM Tb^{3+}, 40 μM Eu^{3+} and λ_{exc}=490 nm.

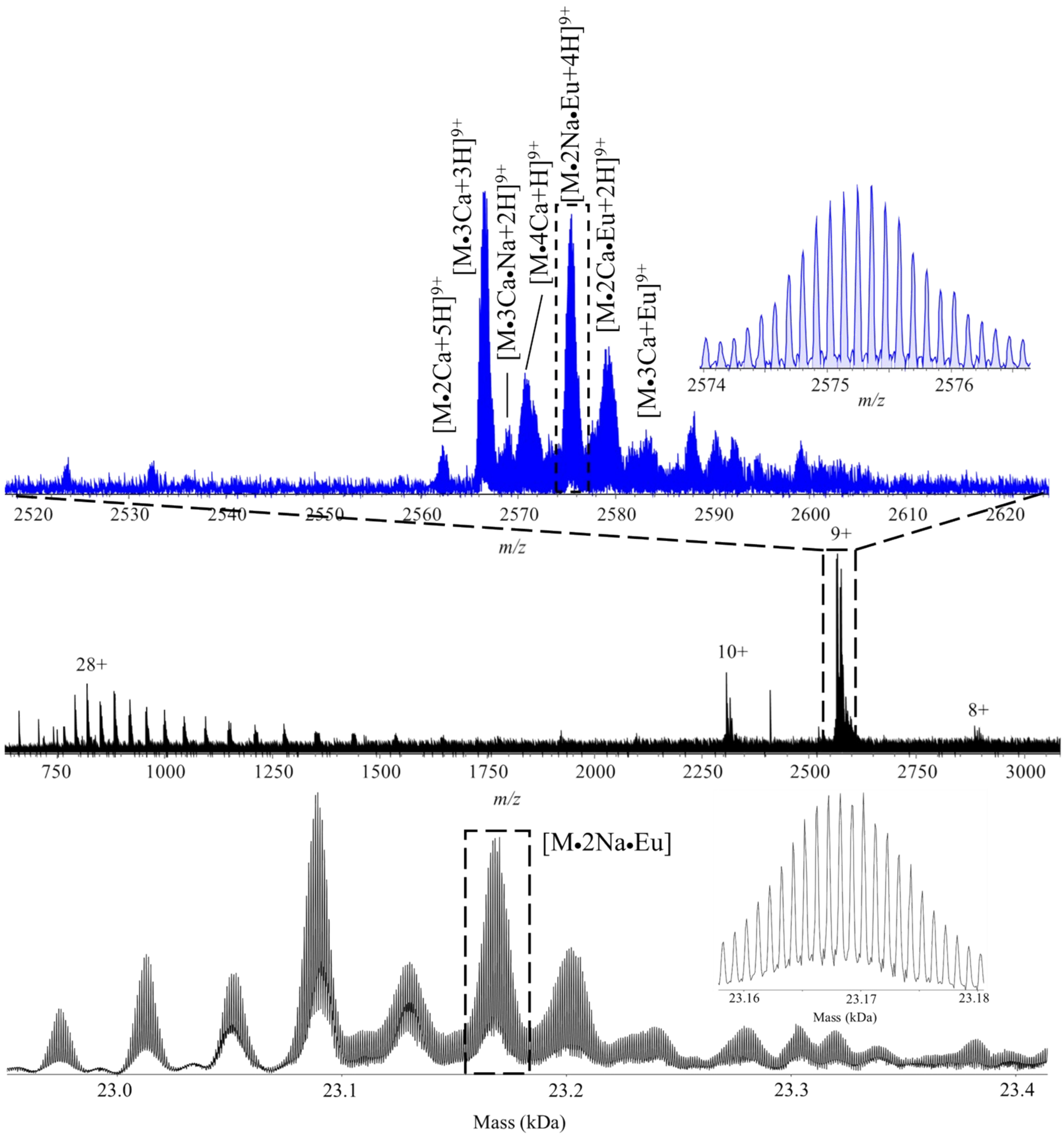

Figure S8: Expanded view of the 9+ charge state with annotated adduct species and the isotopic pattern of the ion $[M•2Na•Eu+4H]^{9+}$ shown in the inset (A). Typical broadband nESI-FT-ICR MS spectrum of NCS1 in the presence of Eu^{3+} (B). Deconvoluted spectrum showing the neutral species. The isotopic pattern shown in the inset confirms the theoretical isotopic average mass of the NCS1 protein 22.976 kDa (C).

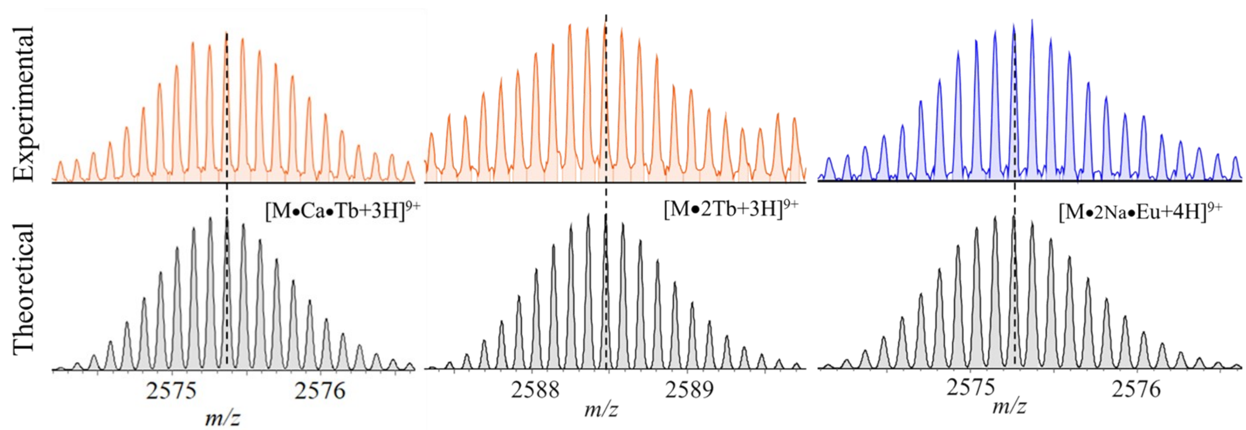

Figure S9: Comparison of experimental isotopic distributions of NCS1:Tb (red) and NCS1:Eu (blue) adduct species obtained from nESI-FT-ICR MS with their theoretical isotope patterns (black). Note the good agreement between profiles indicated by the dashed lines.

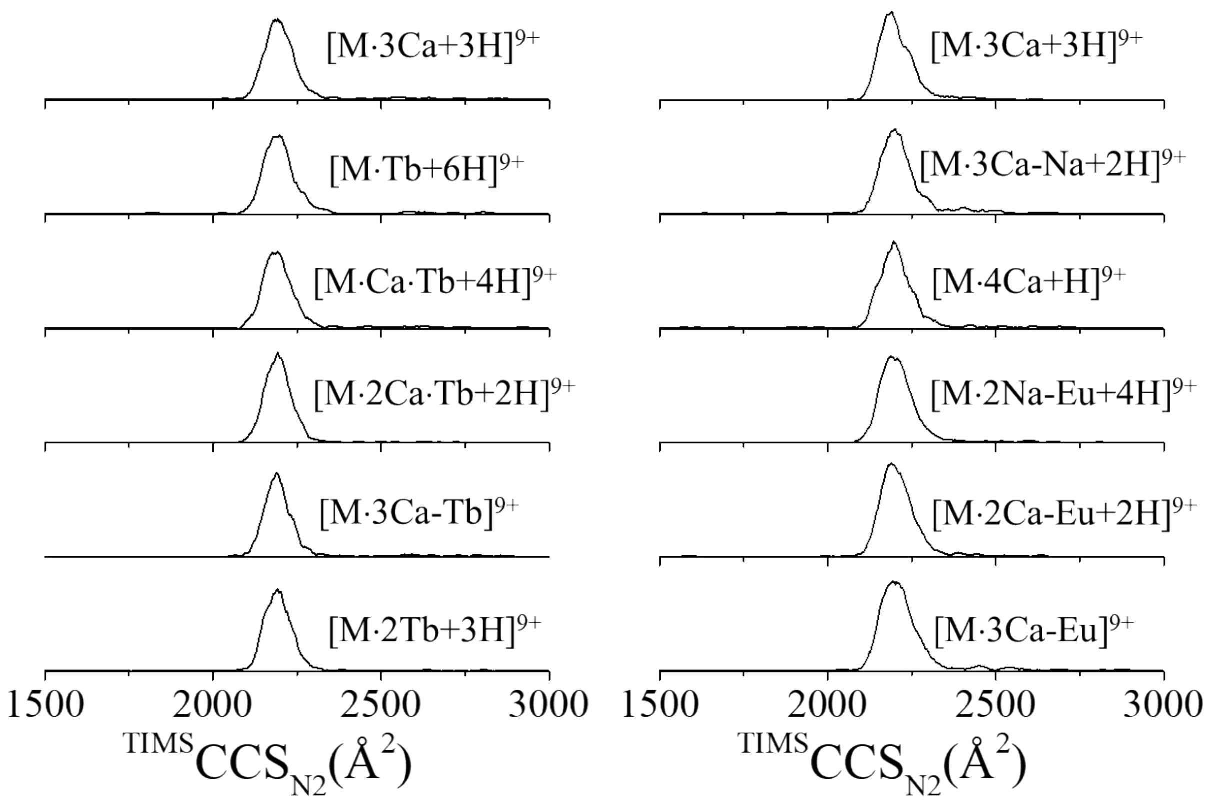

Figure S10: $^{TIMS}CCS_{N2}$ profiles for the charge state 9+ of NCS1 in the presence of Tb^{3+} (left) and Eu^{3+} (right) obtained from nESI-TIMS-TOF MS.