

Rational design of Lanthanide Binding Peptides

Elaboration rationnelle de ligands peptidiques des ions lanthanides.

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Supplementary Material

Synthesis and characterization of P^{HD'}.

The synthesis of PHD' was performed as previously reported in references^[1-3].

P^{HD'}: Ac-Ser-Ed3a₂-Gly-Trp- Ada₂-Ala-NH₂

Yield of the on-resin synthesis (UV): 83%, Isolated mass: 19.8 mg (isolated yield assuming that the solid is **P^{HD'}**·3TFA: 26.3%).

ES⁺-MS (AcONH₄, pH 7.0): $m/z = 994.4$ [M+H]⁺

ES⁻-MS (AcONH₄, pH 7): $m/z = 992.3$ [M-H]⁻

RP-HPLC: $t_R = 13.6$ min, 97% purity, solvent A=H₂O/TFA (99.925:0.0075, v/v), solvent B CH₃CN/H₂O/TFA (90:10:0.1 v/v/v) eluting gradient from 0% A /100% B to 60% A/40% B in 24 min, flow rate 1 mLmin⁻¹, UV monitoring at 214 nm.

Table S1. ¹H NMR (500 MHz) chemical shifts (δ / ppm) for **P^{HD'}** in H₂O/D₂O v/v 9/1 at 278K, 3.2 mM, pH = 6.8. Signals assigned by COSY, TOCSY and ROESY 2D experiments.

Residue	HN	H α	H β	Others
Ser(1)	8.43	4.38	3.8	Ac: 2.02
Ed3a ₂ (2)	8.6	4.26	2.03, 1.9	H γ : 2.96, 2.8 (m, 2H), H δ , H ϵ : 3.24, 3.14 (m, 2H), H ζ , N η CH ₂ COOH : 3.94, 3.8
Gly(3)	8.9	4.03		
Trp(4)	8.5	4.56	3.2	H ϵ_1 : 10.16, H ϵ_2 : 7.55, H ζ_2 : 7.44, H δ : 7.26, H ζ_1 : 7.10
Ada ₂ (5)	8.3	4.14	1.8, 1.17	H γ : 2.94, 2.49
Ala(6)	7.95	3.89	1.23	CONH ₂ : 7.3, 7
other				Ed3a ₂ (2) and Ada ₂ (5) N δ CH ₂ COOH overlap : 3.5 (m, 6H)

Tb³⁺ complexes

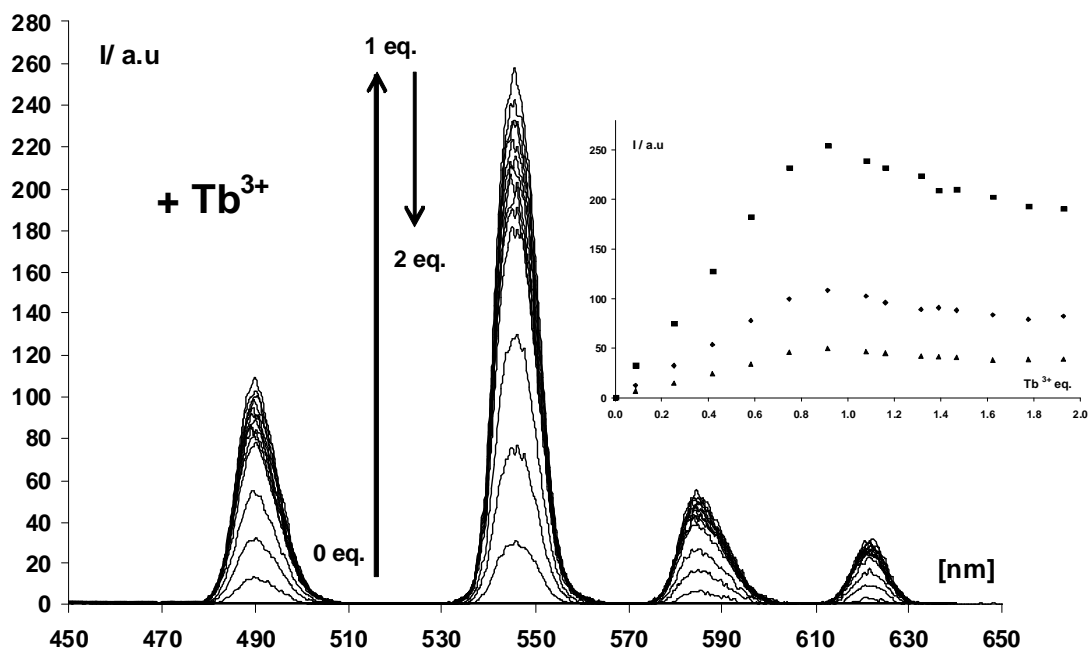


Figure S1. Tb-centered emission during the titration of $P^{HD'}$ (54 μ M) with $TbCl_3$ in HEPES buffer (10 mM, pH 7, 0.1 M KCl). Inset: Variation of the intensities of the peak maxima with Tb (\blacklozenge : 490nm \blacksquare : 545nm \blacktriangle : 587 nm)

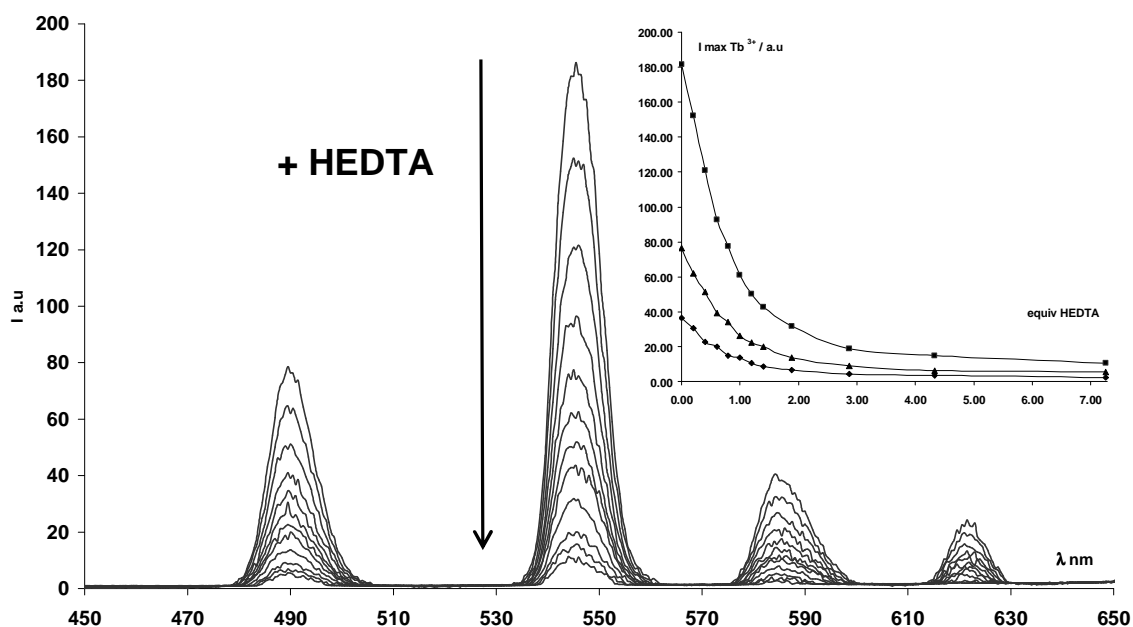


Figure S2. Disappearance of the Tb-centered emission during the titration of $TbP^{HD'}$ (28 μ M) with HEDTA in HEPES buffer (10 mM, 0.1M KCl, pH 7). Inset: Variation of the intensities of the peak maxima (\blacktriangle :490nm \blacksquare :545nm \blacklozenge : 587 nm).

Table S1. Comparison of the properties of the three high density peptides.

Complex	τ_{H_2O} (ms) ^{a)}	τ_{D_2O} (ms) ^{a)}	q ^{b)}	$\log\beta_{11}^{pH=7}$ ^{c)}	$\log\beta_{110}$ ^{d)}	Polymetallic complexes
TbP ^{HD2} [2]	2.35(2)	2.86(2)	0.08(3)	12.7(5)	16.2(5)	no
TbP ^{HD5} [2]	2.30(2)	2.81(2)	0.09(3)	12.7(5)	16.2(5)	yes
TbP ^{HD'}	1.94(2)	2.88(2)	0.5(3)	11.9(4)	15.4(4)	yes

^{a)} τ_{H_2O} and τ_{D_2O} are the luminescence lifetimes of Tb³⁺ in the complexes in HEPES buffer (10 mM, KCl 0.1 M, pH = 7.0) in 100% H₂O and extrapolated to 100% D₂O, respectively. ^{b)} q is the hydration number calculated from the lifetimes with Parker's equation.³⁴ ^{c)} $\beta_{11}^{pH=7}$ is the conditional stability constant of TbP^{HD} at pH 7, 298 K in 0.1 M KCl measured during the competition with HEDTA. ^{d)} β_{110} is the global stability constant of TbP^{HD} calculated from $\beta_{11}^{pH=7}$ and the peptide pKas.

References

- [1] F. Cisnetti, C. Gateau, C. Lebrun, P. Delangle, *Chem. Eur. J.* 15 (2009) 7456.
- [2] A. Niedźwiecka, F. Cisnetti, C. Lebrun, P. Delangle, *Inorg. Chem.* 51 (2012) 5458.
- [3] A. Niedźwiecka, F. Cisnetti, C. Lebrun, C. Gateau, P. Delangle, *Dalton Trans.* 41 (2012) 3239.