“Lanthanide-protein interaction and fluorescence enhancement of transferrin“

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Outline

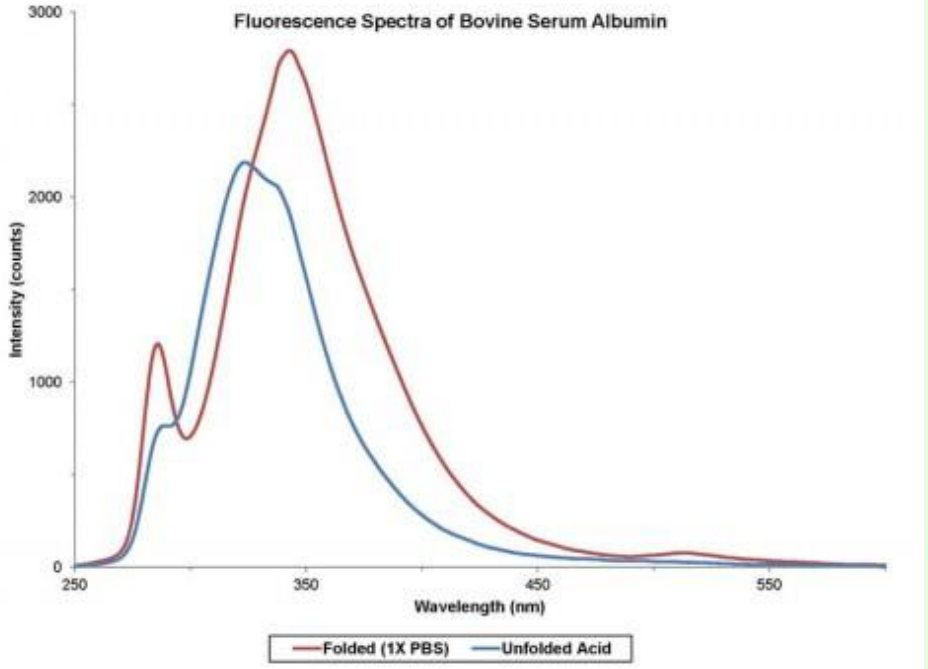
1. Protein (and Tf) intrinsic/native fluorescence
2. Fe 3^{+} coordination on the C-terminal lobe and N-terminal Tf lobe
3. Iron and other metal ions binding Tf
4. Lanthanide luminescence and Tf as “antenna” enhancer and Tb^{3+}
5. Tf intrinsic and Terbium adduct fluorescences
6. pH-dependence luminescence and binding constants of lanthanides with Tf
7. Tf glycoform analysis scheme (HPLC fluorescence and visible detection)
8. Conclusion

Proteins display **intrinsic fluorescence**, by the presence of the three aromatic amino acids — phenylalanine, tyrosine, and tryptophan.

Furthermore, tyrosine and tryptophan display high anisotropies that are often sensitive to protein conformation.

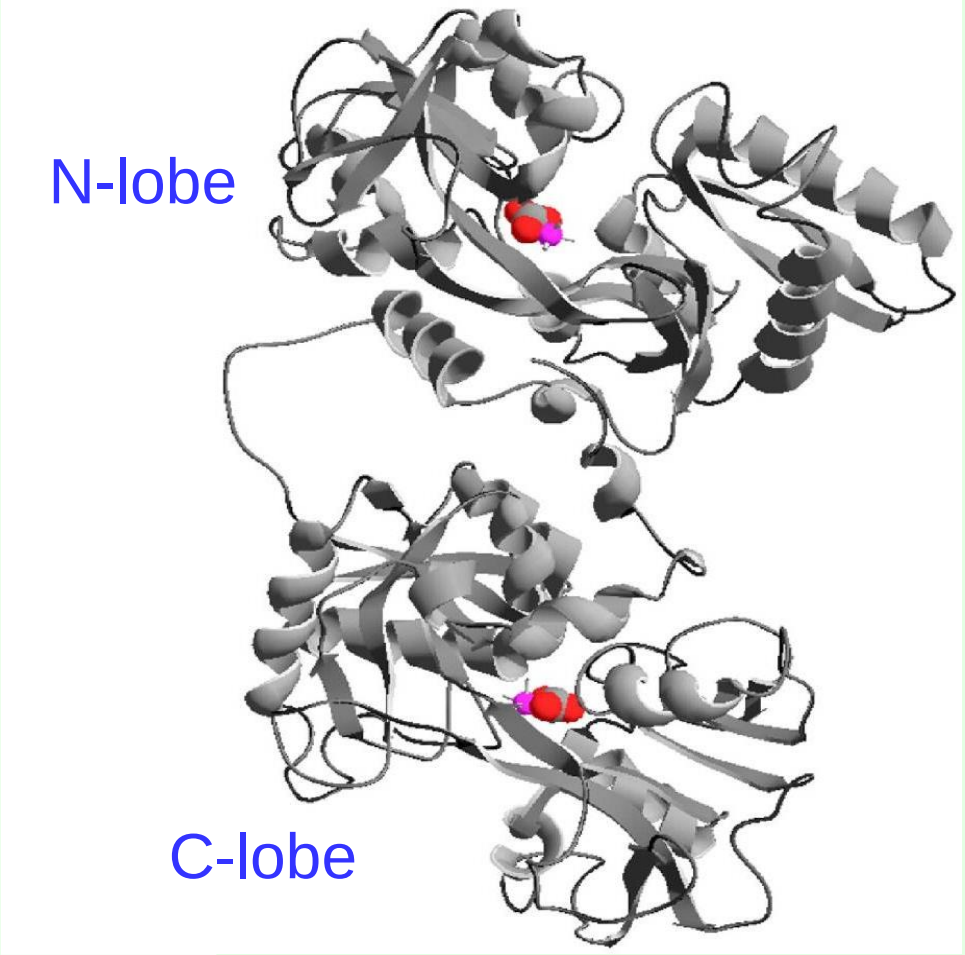
Fluorescence properties of aromatic amino acids in water at neutral pH

	Lifetime (τ)	Absorption		Fluorescence	
	(ns)	λ (nm)	Absorptivity (ϵ)	λ (nm)	Quantum Yield (Φ_F)
Tryptophan	3.1 (mean)	280	5600	348	0.2
Tyrosine	3.6	274	1400	303	0.14
Phenyl alanine	6.4	257	200	282	0.04



ABT Ghisaidoobe et al. Int. J. Mol. Sci. 2014, 15, 22518-22538

Transferrin (Tf), consists of a single polypeptide chain of 679 aminoacids (about 80 kDa) folds into the N-lobe (residues 1-331) and C-lobe (residues 339-679) which either contain an iron binding site.

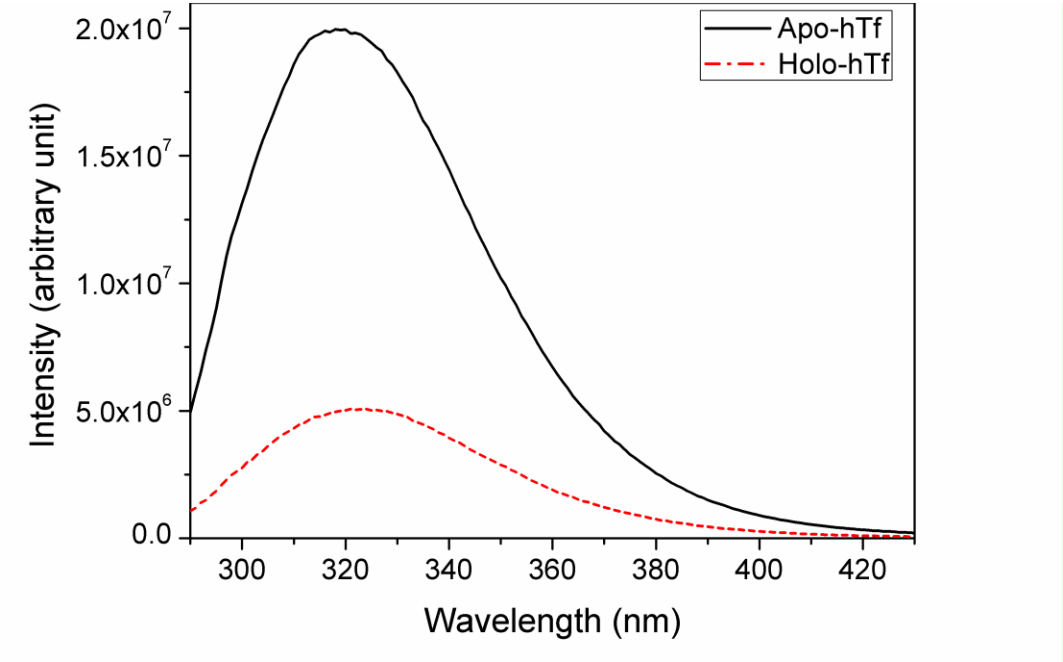


In both of the C- and N-lobe, iron in the binding site is coordinated by an aspartic acid, two tyrosines and a histidine.
The presence of a synergistically bound anion, as carbonate, is essential for iron binding.

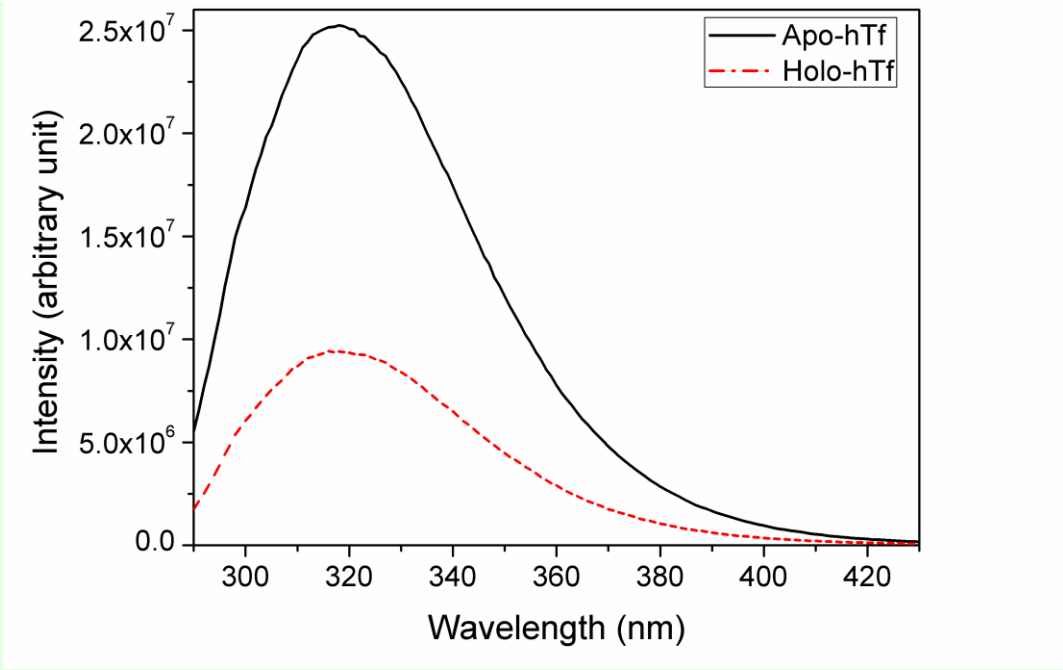
Additionally, upon transitioning from iron-bound (holo-Tf) to iron-free (apo-TF), the fluorescent signal (produced largely by three Trp residues in the N-lobe and five Trp residues in the C-lobe) greatly increases.

Apo- and holo-hTf native fluorescence (λ_{Ex} 280 nm)

Emission spectra of apo-hTf (black line) and holo-hTf (red line)

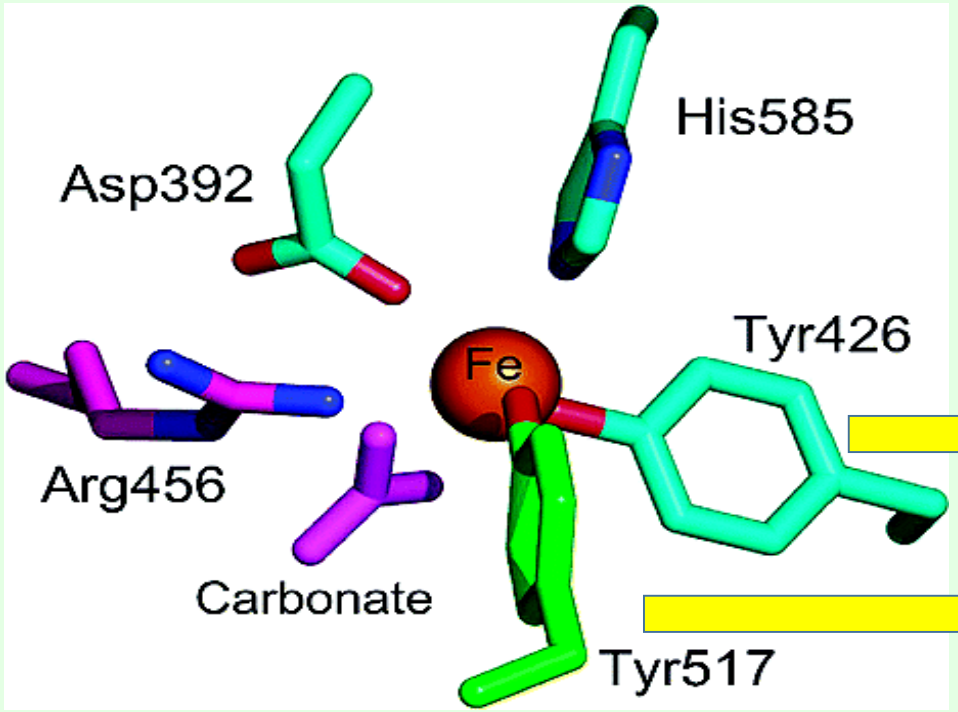


Tris buffer, pH 8



Phosphate buffer, pH 6

Fe 3+ coordination on the C-terminal lobe

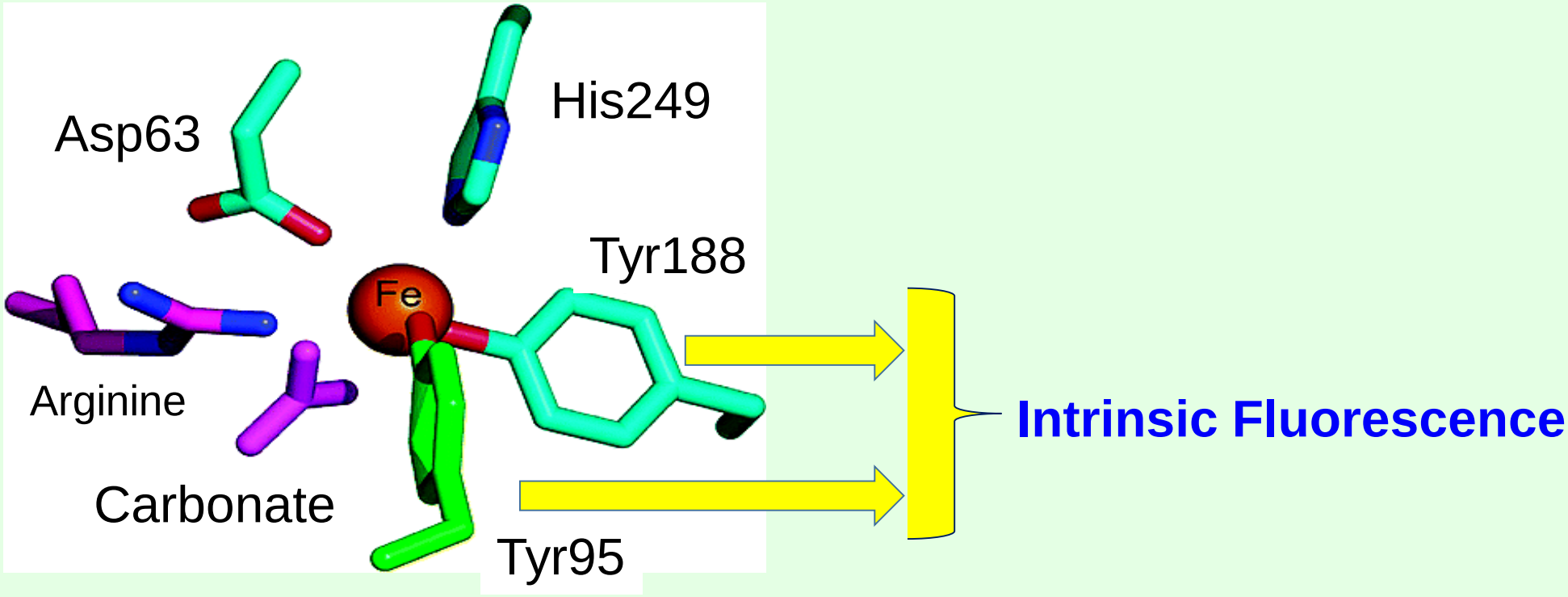


Native Fluorescence
λ_{ex} 280 nm Trp e Tyr

Excitation at both 280 nm (to evaluate the contributions of both Trp and Tyr residues to the signal) and 295 nm (to specifically query the contribution from the Trp residues).

The intrinsic fluorescence (λ_{ex} 295 nm) of the C-lobe is produced mainly by five Trp residues (at positions 344, 358, 441, 460, and 550). Three Trp residues are also present in the N-lobe.

Fe 3+ coordination on the N-terminal lobe



Ferric iron is coordinated by identical ligands in each lobe of hTF.

Byrne SL et al. J Mol Biol 396(2010)130-140

The preferential binding of bicarbonate and iron to the C-site over the N-site of transferrin is due to the faster rate of bicarbonate insertion and higher binding constant.

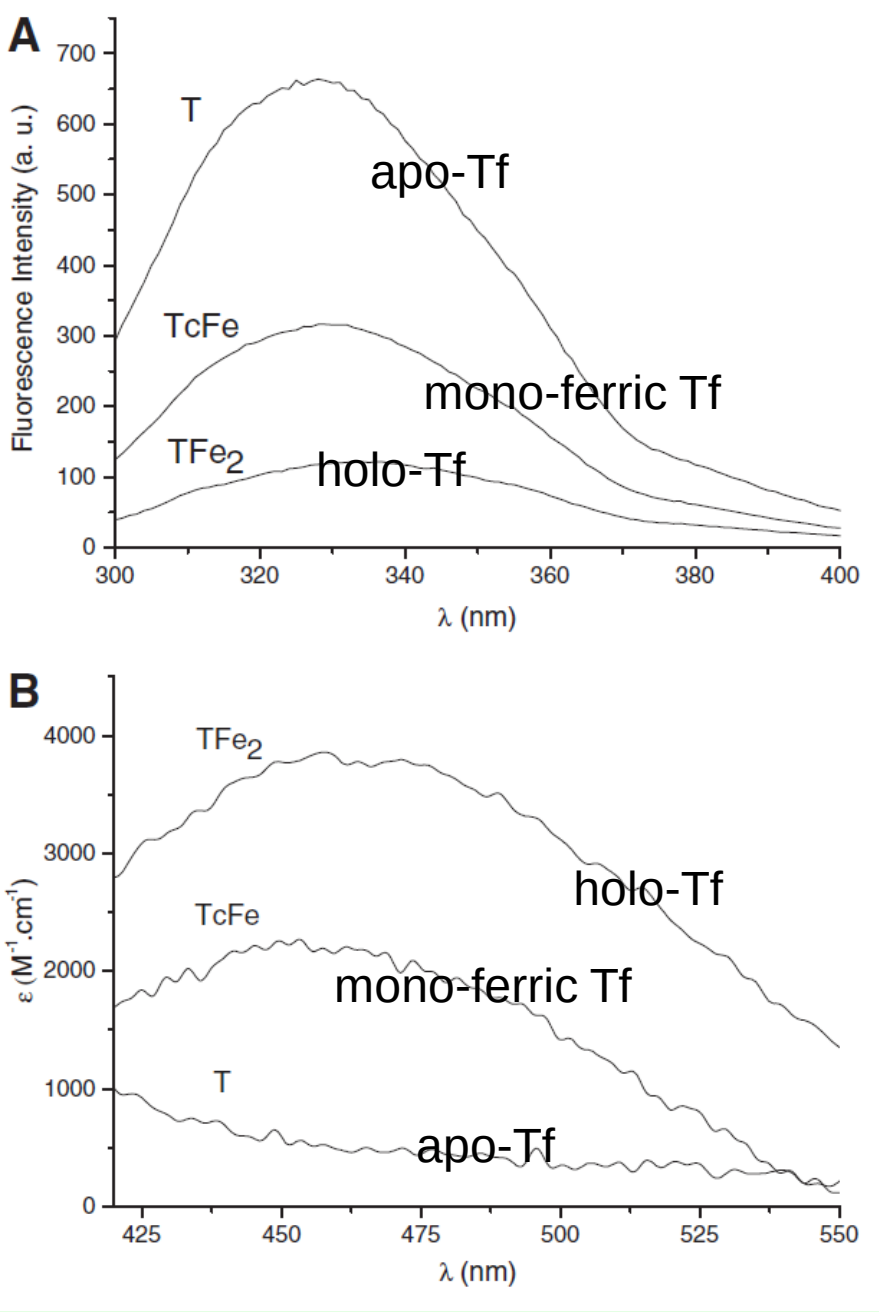
Rapid/Exo for C-site followed by N-site

$K_{1(\text{C-site})} = 3.3 \times 10^{7}\ M^{-1}$; $\Delta H = -8.7$ kcal/mol

$K_{2(\text{N-site})} = 3.9 \times 10^{6}\ M^{-1}$; $\Delta H = -5.6$ kcal/mol

Iron is released from the N-lobe, followed by the C-lobe.

F. Bou-Abdallah, T.R. Terpstra / Biochimica et Biophysica Acta 1820 (2012) 318–325



A.

Fluorescence emission spectra of apo-transferrin (T), mono-ferric transferrin with only an iron-loaded C-lobe (TCFe) and holo-transferrin (TFe_2) with an analytical protein concentration of 2 µM and an excitation maximum (λ_{ex}=280 nm).

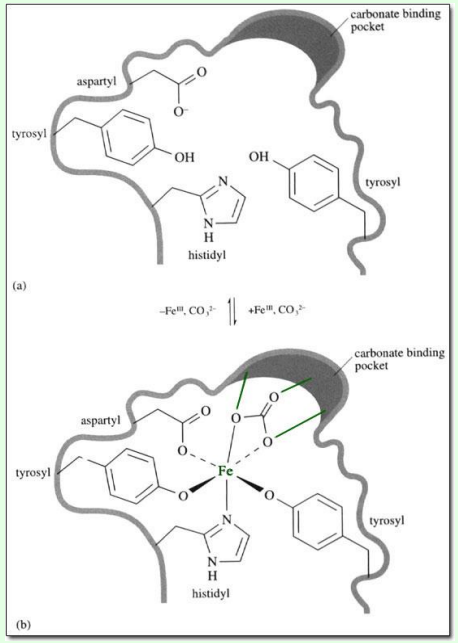
B.

Absorption spectra (400 and 550 nm) of T, TCFe and $TCFe_2$.

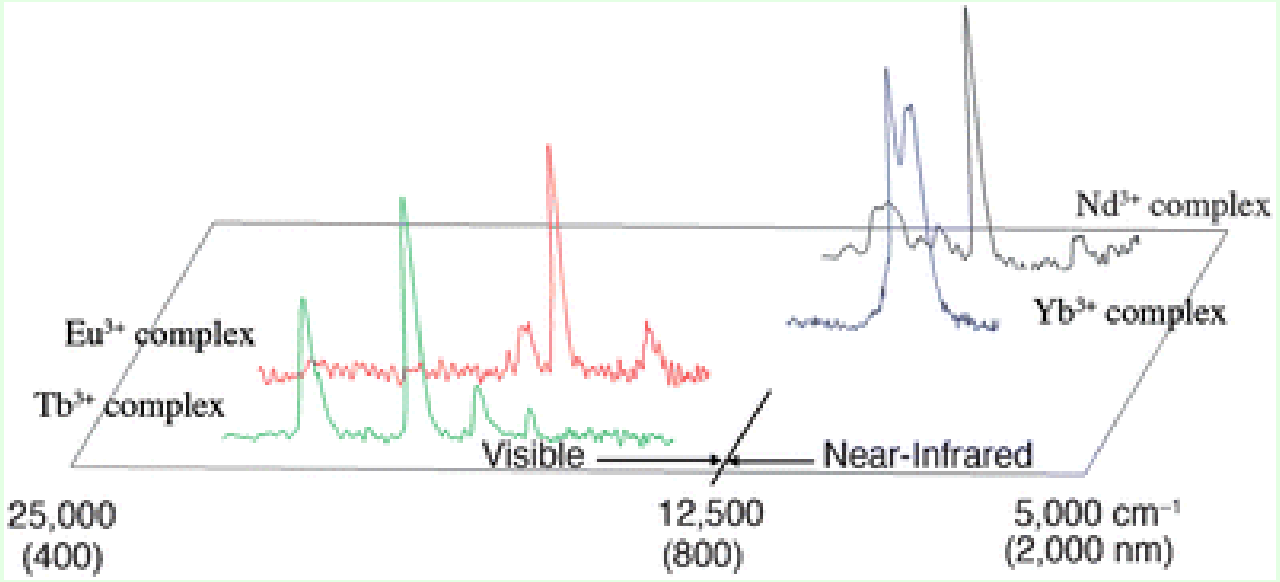
J.M. El Hage Chahine et al. Biochimica et Biophysica Acta 1820 (2012) 334–347 335

While transferrin is primarily an iron-binding and transport protein with a very high affinity for iron (in the range of 10^{21} – 10^{22} M^{-1}), it is only about 30% saturated with iron in normal human serum.

Other metal ions (Al, Cd, Ni, Sm, Bi, Gd, Tb, …) have been shown binding to transferrin.

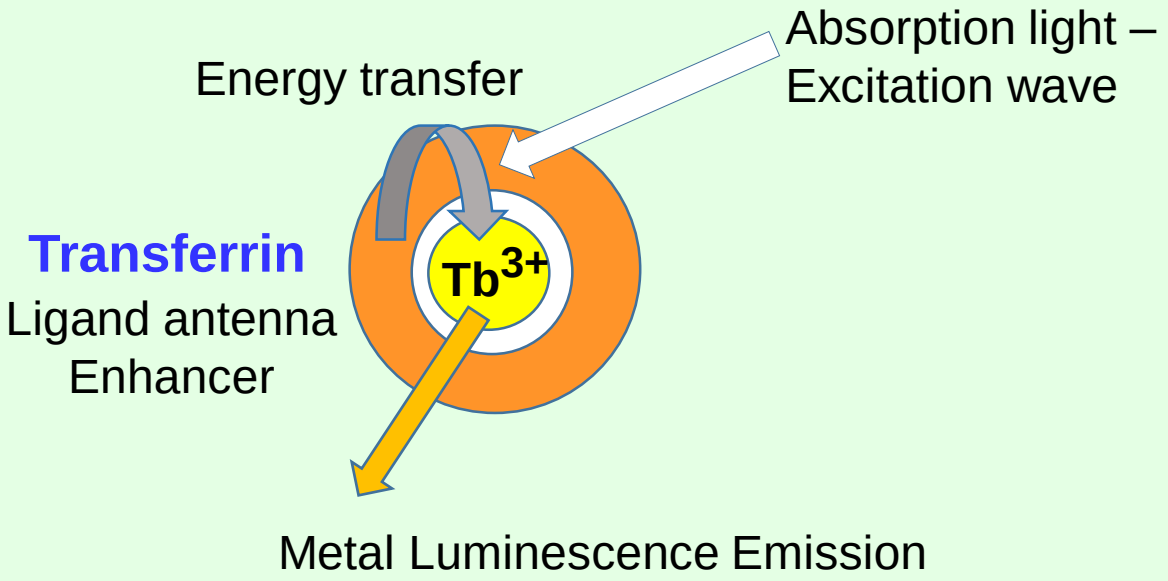


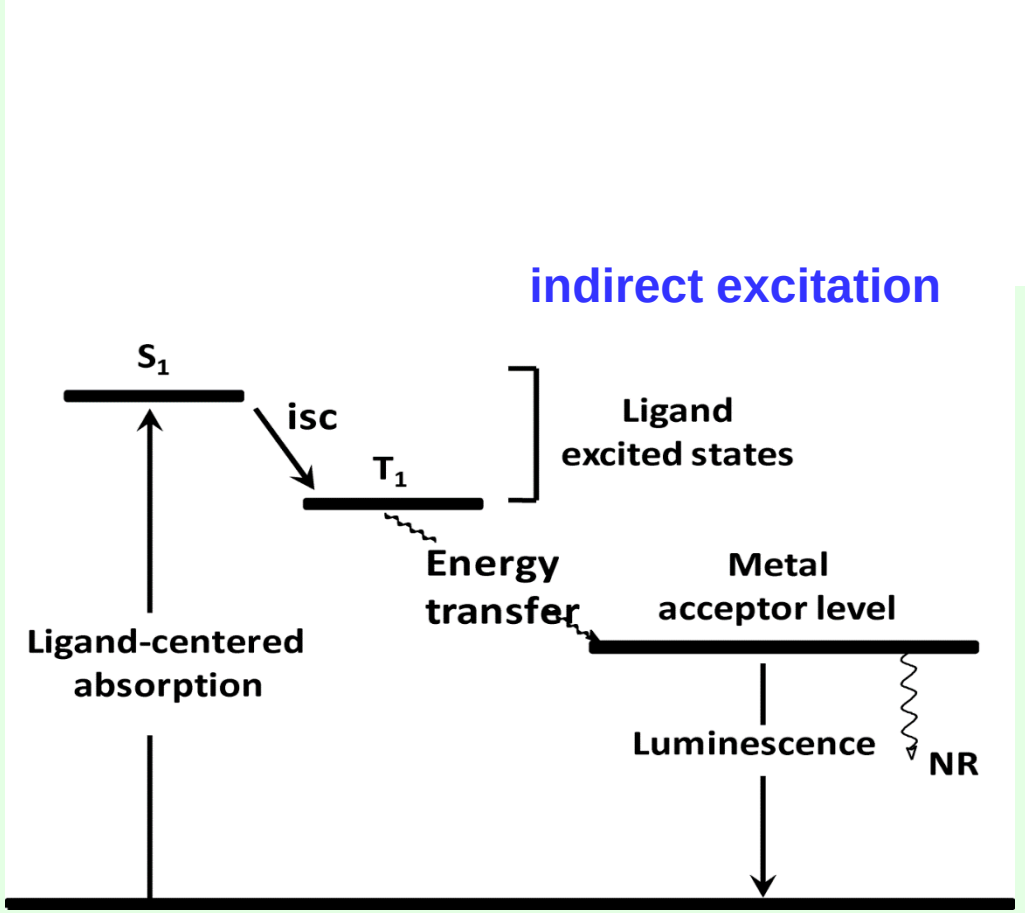
Tf includes two coordination sites for Fe^{3+} ions, composed of one Aspartate, one Histidine, and two Tyrosinate residues as well as one exogenous carbonates completing the near octahedral geometry.



Luminescence spectra of lanthanide complexes with ligand

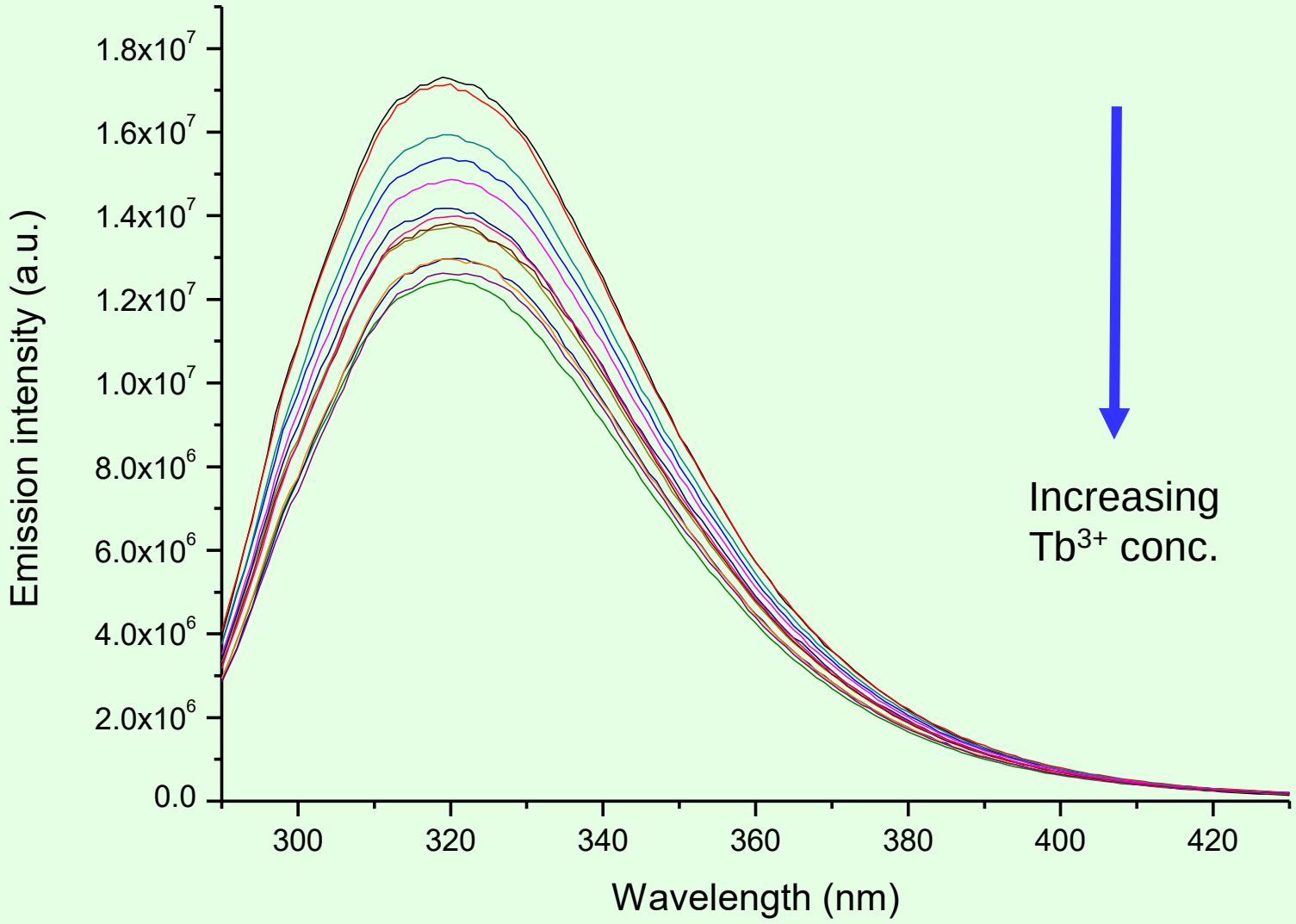
Shinoda S *Analyst*, 2011, **136**, 431-435



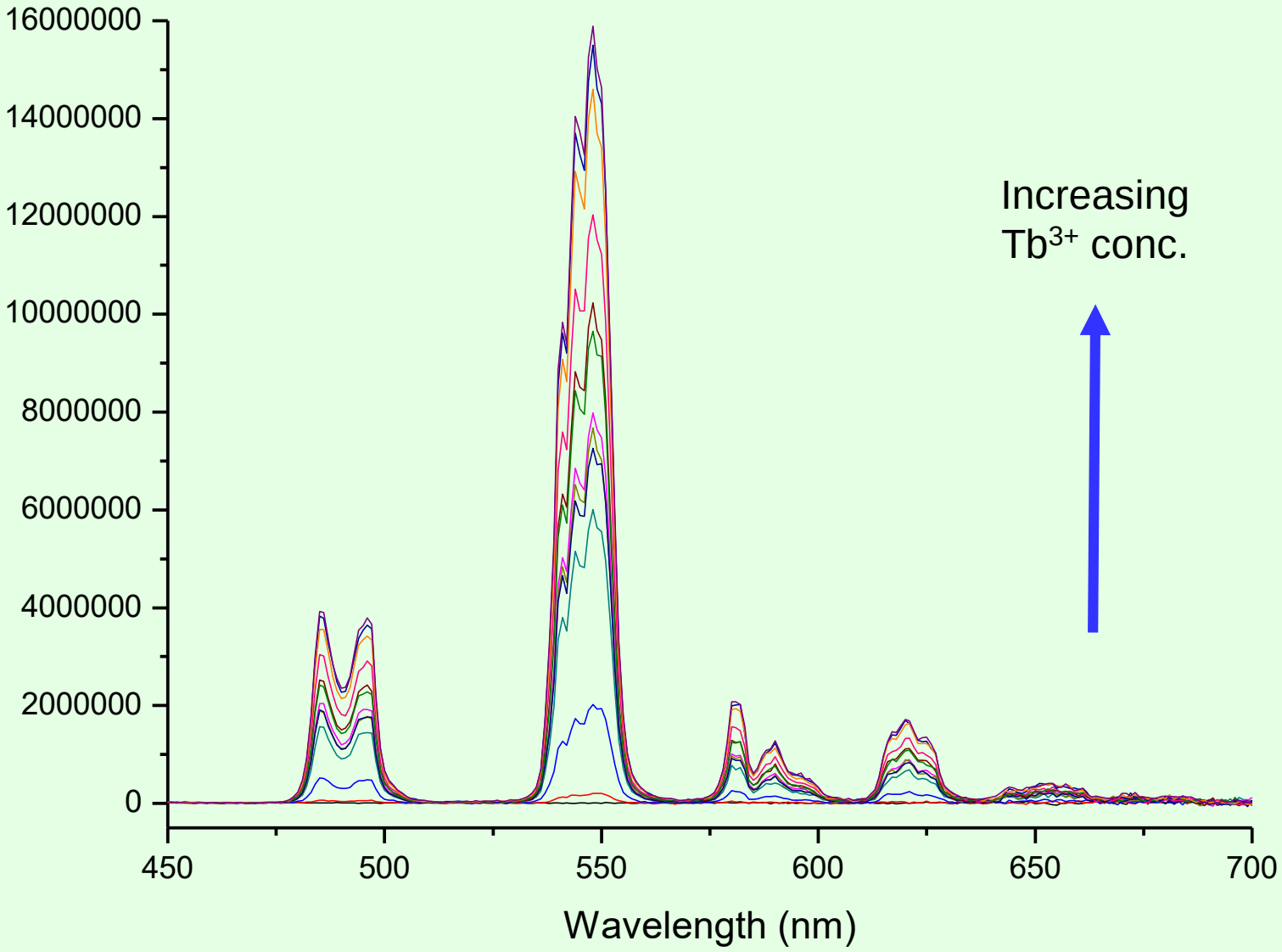


‘antenna’ into the lanthanide-coordinating ligand, allows indirect excitation of the lanthanide ion, usually through an intramolecular energy transfer process.

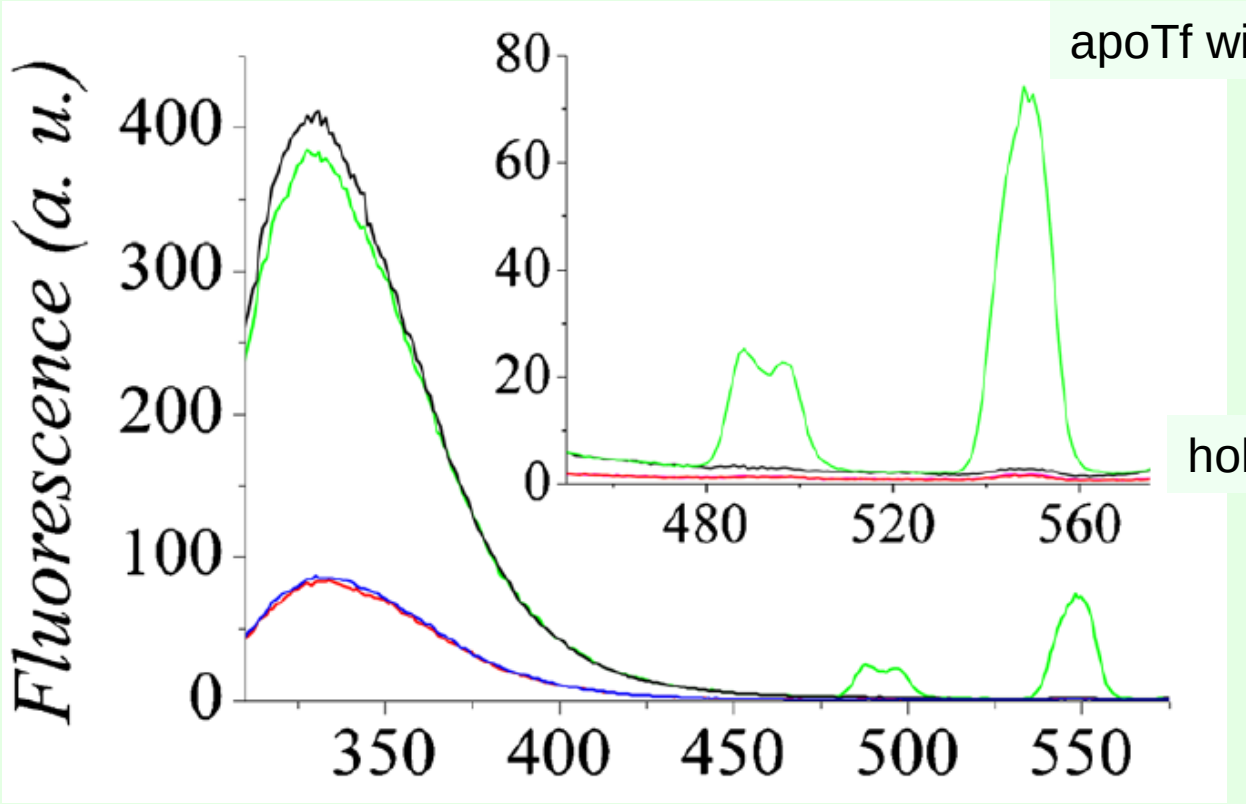
apo-Tf native fluorescence



Fluorescence of apo-Tf Terbium adduct



Fluorescence apo-Tf and holo-Tf emission spectra

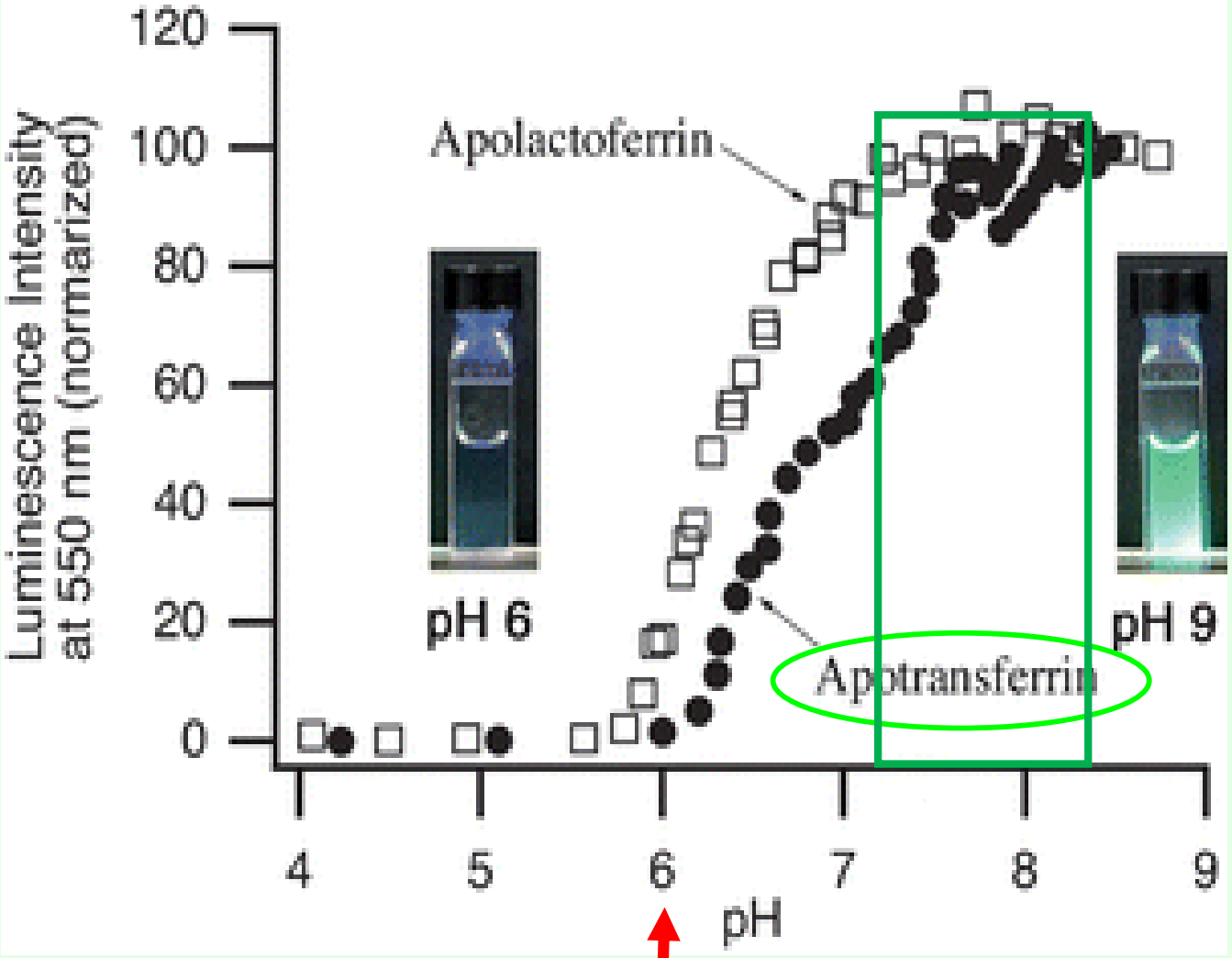


apoTf with $TbCl_3$ (**green**)

holoTf with $TbCl_3$ (**red**)

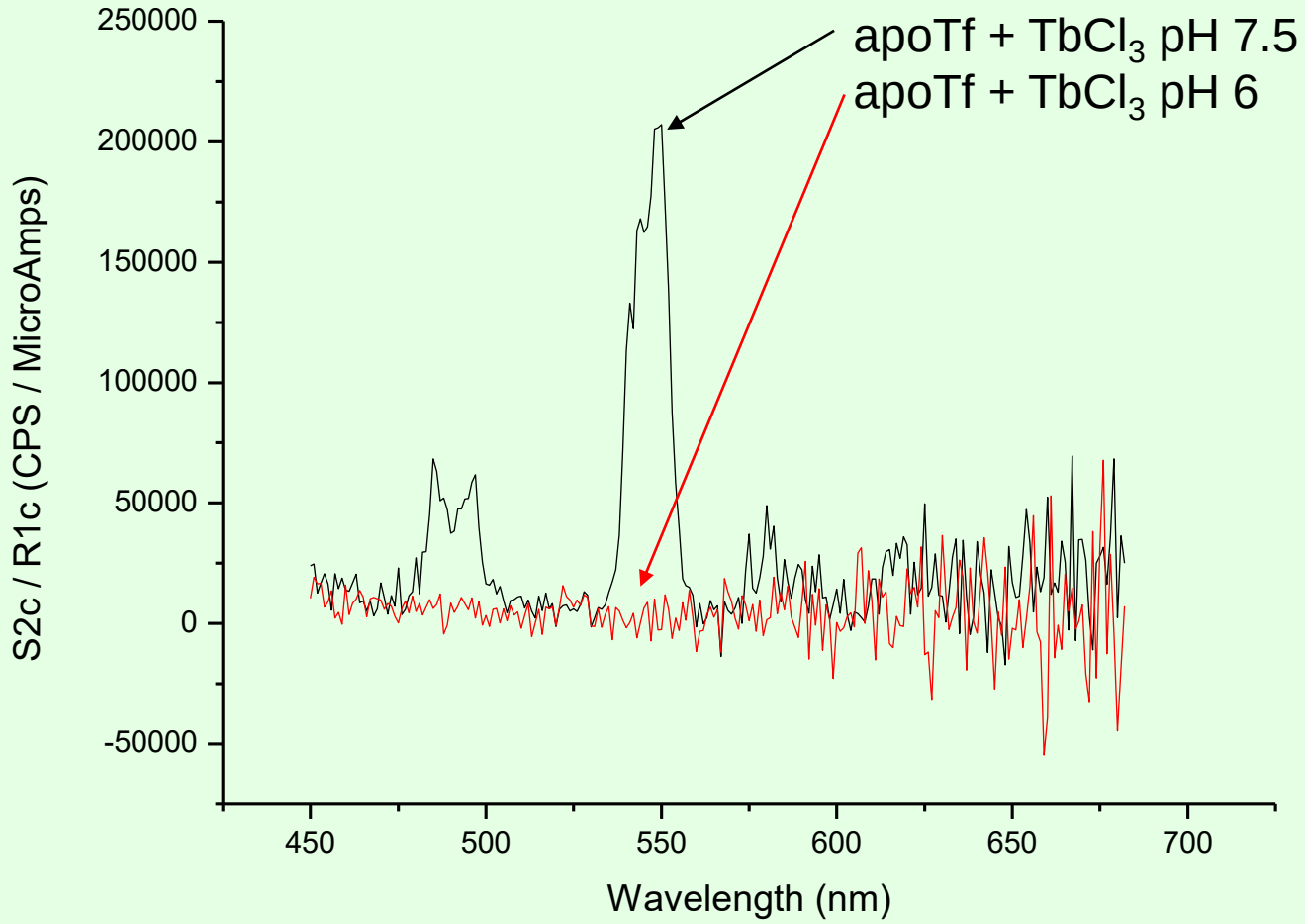
apoTf (black), holoTf (**blue**)
apoTf with $TbCl_3$ (**green**) and holoTf with $TbCl_3$ (**red**).
The inset highlights the emission in the range 450–575 nm.

S Nicotra et al. Anal Bioanal Chem 409 (2017) 6605–6612



pH-dependent emission profiles of apo-transferrin–Tb^{3+} and apo-lactoferrin–Tb^{3+} complexes.

*Shinoda S Analyst, 2011, **136**, 431-435*



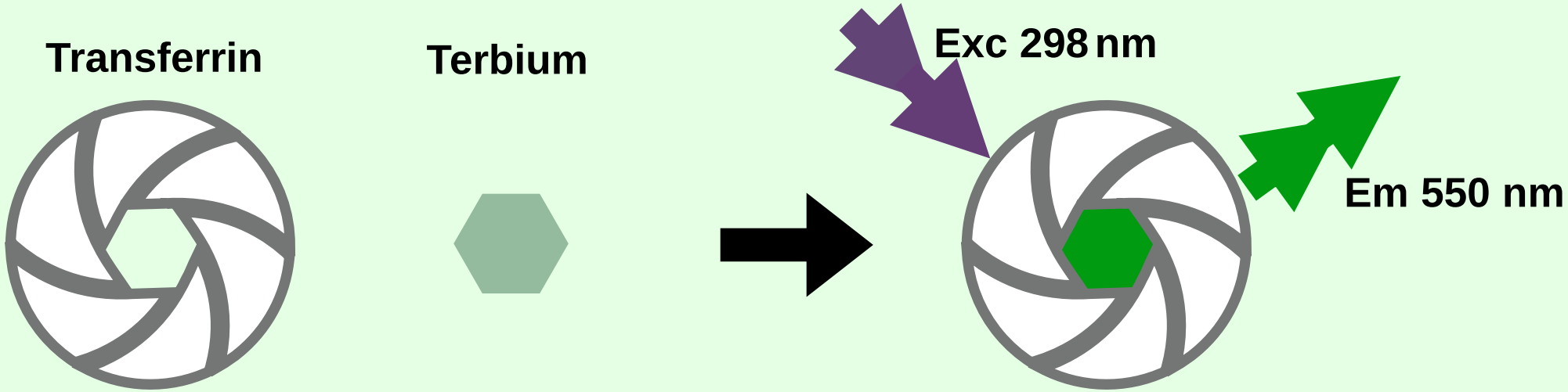
Lowered pH (~5.6) results in protonation of the synergistic carbonate anion and iron/terbium binding residues, which, in turn, loosens the cleft and facilitates iron/terbium release.

Effective binding constants of lanthanides with serum transferrin

Metal ion	Ionic radius (Å) [a]	Log K_{M1}	Log K_{M2}	Δlog K	
Lu^{3+}	0.977	11.08	7.93	3.15	
Tb^{3+}	1.04	11.20	7.61	3.59	Based on absorption
Tb^{3+}	1.04	10.96	8.52	2.44	Based on fluorescence
Gd^{3+}	1.053	9.20	7.18	2.02	
Eu^{3+}	1.066	9.66	7.27	2.39	
Sm^{3+}	1.079	8.37	6.63	1.74	
Nd^{3+}	1.109	7.33	6.28	1.05	

Harris WR et al. Journal of Inorganic Biochemistry 76 *(1999)* 231–242

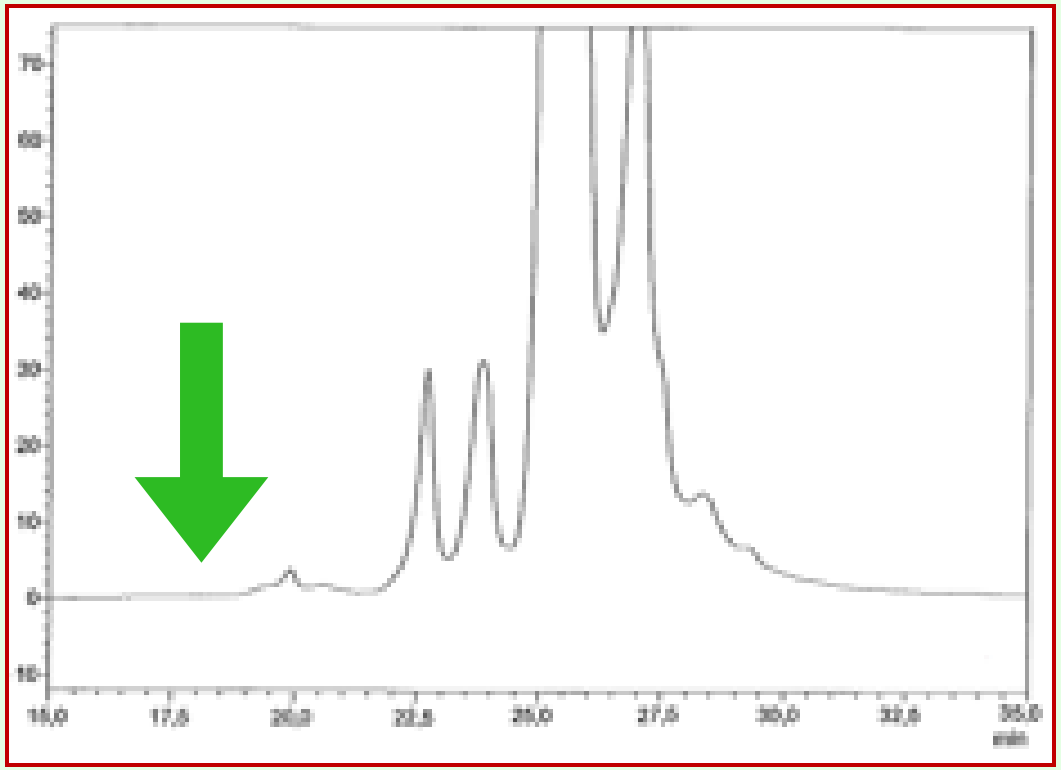
Recently, the formation of fluorescent Tf-Tb3+ adducts has been explored by our research group (Patent No. MI2014A000395 and No. PCT/EP2015/054896.) for the development of new analytical methods to study the different Tf glycoforms (in relation to the number of glycan chains and sialic acid residues) in human body fluids, which have a particular importance in the biomedical context.



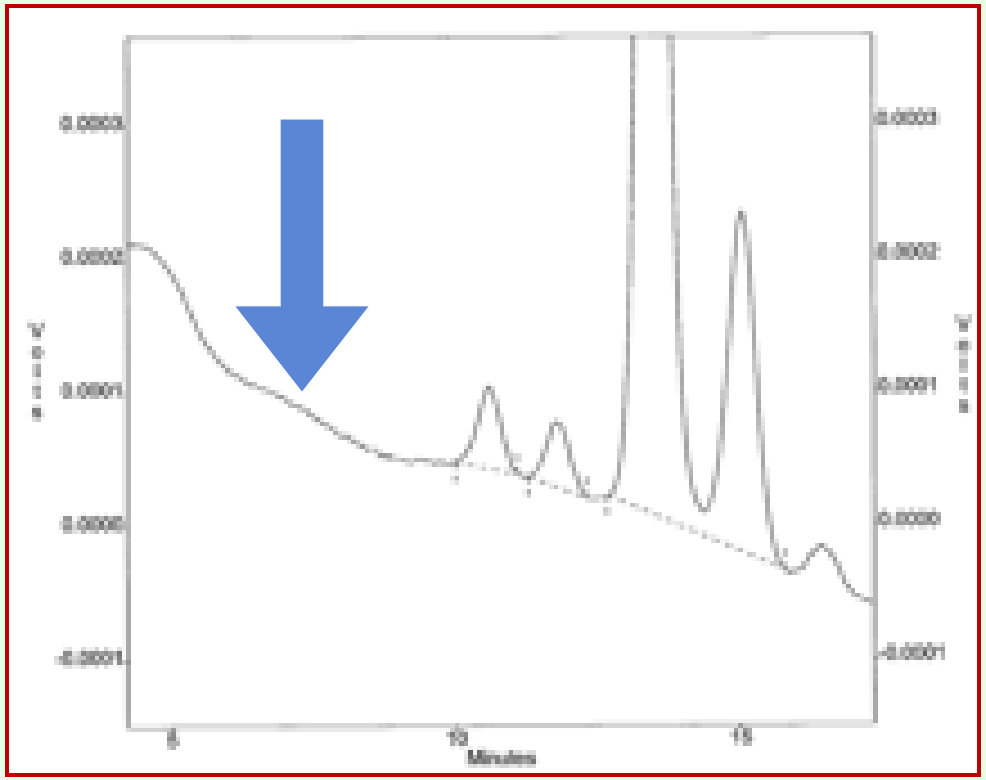
HPLC with Fluorimetric detection

University of Verona Patent No. MI2014A000395 and No. PCT/ EP2015/054896

HPLC-FL



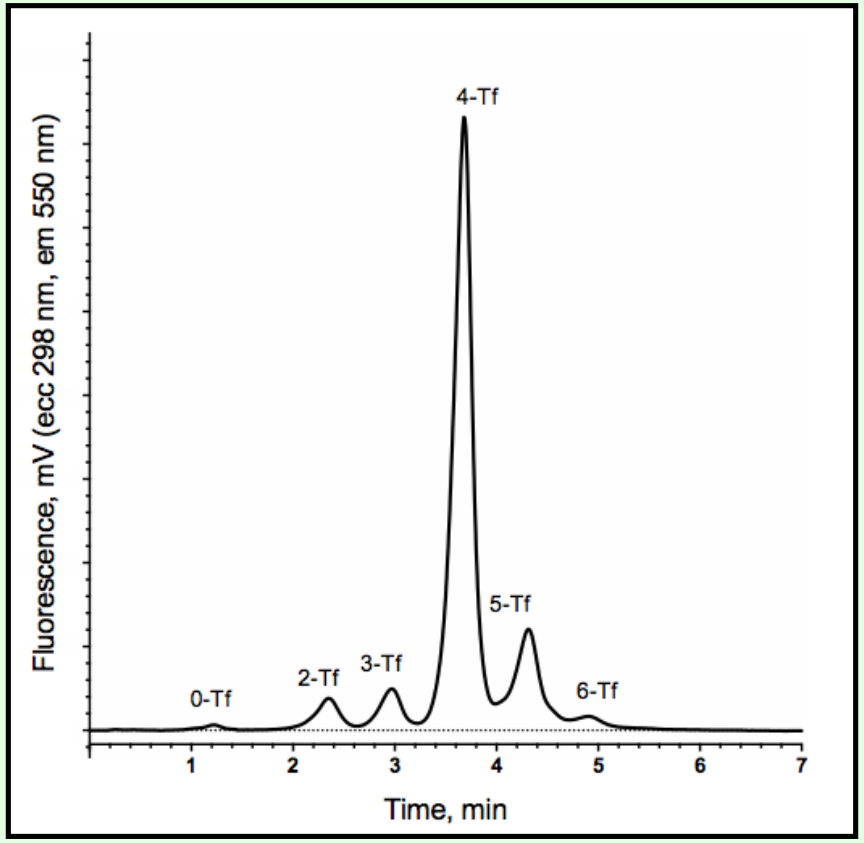
HPLC-Vis



Tf glycan chains can be di- tri- tetra- antennary, numbered by the sialic acid residues)

HPLC-FL

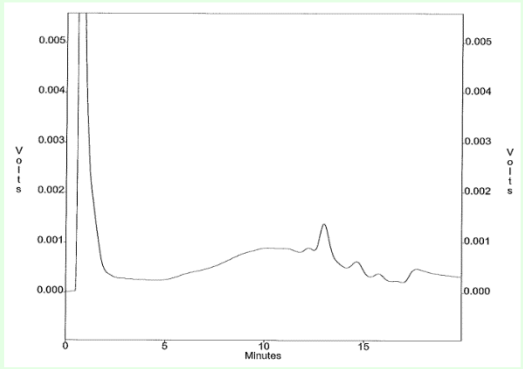
Serum sample – alcohol abuser



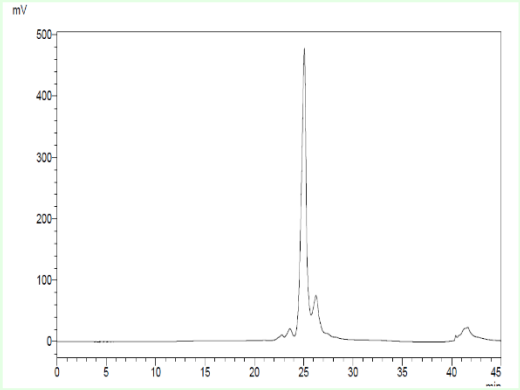
Tf glycan chains can be di- tri- tetra- antennary, numbered by the sialic acid residues)

CDT HPLC

460 nm detection

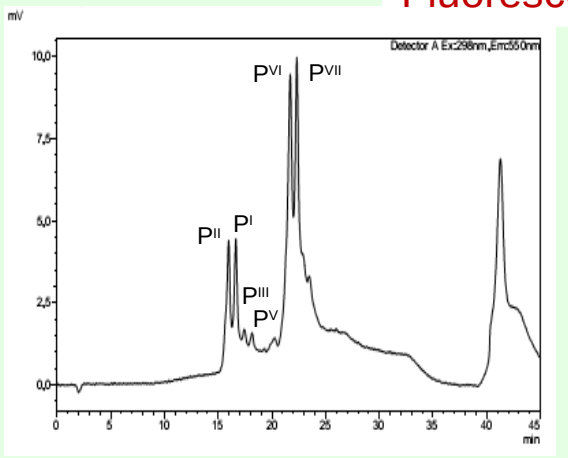


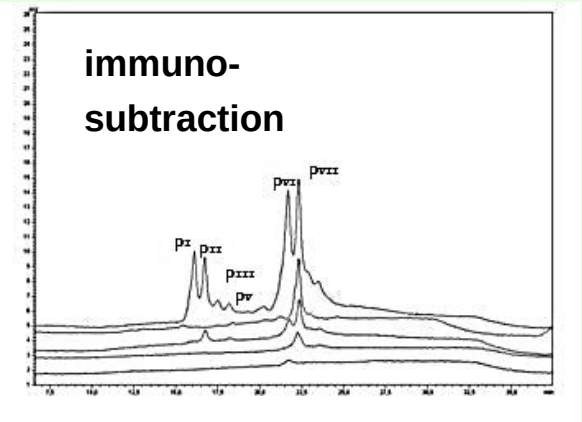
Fluorescence detection



Cadaveric blood

Fluorescence detection





Human cerebro-spinal-fluid (CSF) sample

Thank you