

# Interaction of Cm(III), Am(III) and Eu(III) with human serum transferrin

Nicole Bauer<sup>1,2</sup>, Daniel R. Fröhlich<sup>1,2</sup>, Petra J. Panak<sup>1,2</sup>

## Introduction

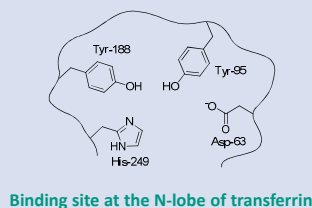
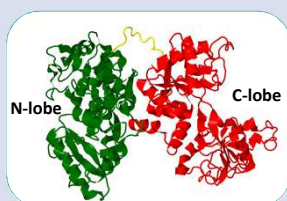
In case of an accidental release of radionuclides to the environment actinides can cause a serious health risk upon incorporation. With regard to the development of potential decontamination therapies, a detailed understanding of the mechanisms of relevant biochemical reactions is required.

Time-Resolved Laser Fluorescence Spectroscopy (TRLFS) studies of Cm(III) and Eu(III) with transferrin

- Multiple information on the number and type of the coordinating ligands can be obtained from the spectroscopic parameters, including shape and position of the emission bands as well as fluorescence lifetimes

## Transferrin

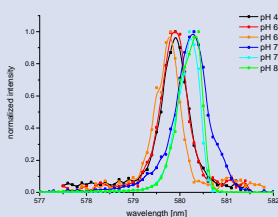
- Single chain glycoprotein with a molecular mass of 79.5 kDa
- Transferrin is folded into two similar but not identical lobes housing the metal binding sites for Fe(III)
- In the normal blood serum, only 30 % of transferrin is saturated with iron, indicating a high capacity for the complexation of other metal ions



## Complexation of Eu(III) with transferrin

- Low-temperature TRLFS measurements ( $T < 20$  K) of Eu(III) transferrin at pH 4.5, 6.0, 7.4 and 8.0

### Excitation spectra



$c(\text{Eu}) = 3.6 \cdot 10^{-4}$  M,  $c(\text{Tf}) = 7.3 \cdot 10^{-3}$  M  
solvent: TRIS buffer 10 mM

Two groups of spectra:

- pH 4.5/6.0:  $\lambda_{\text{max}} = 580.3$  nm
- pH 7.4/8.0:  $\lambda_{\text{max}} = 579.8$  nm

→ Formation of two pH-dependent Eu(III) transferrin species

### Emission spectra recorded by excitation of the emitting state

pH 7.4/8.0:

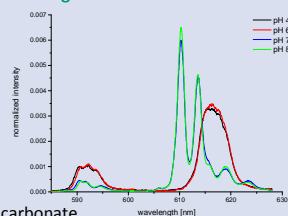
- high fluorescence intensity and maximum splitting of the emission bands → highly unsymmetric coordination environment

- Fluorescence lifetime:  $\tau = 354 \pm 10$   $\mu\text{s}$  → 2.4 H<sub>2</sub>O in the first coordination sphere
- Vibronic sideband indicates complexation of carbonate

pH 4.5/6.0:

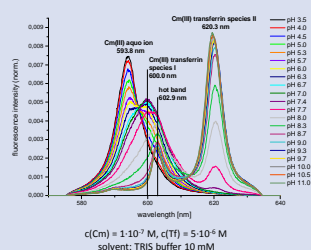
- low fluorescence intensity and without visible splitting of the emission bands

- Formation of two Eu(III) transferrin species
- pH 7.4/8.0: incorporation of Eu(III) at the transferrin binding site (2 H<sub>2</sub>O and 7 other ligands (e.g. amino acids, CO<sub>3</sub><sup>2-</sup>, OH<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>) in the first coordination sphere)
- pH 4.5/6.0: Formation of a Eu(III) transferrin species with partial complexation of Eu(III) by the protein

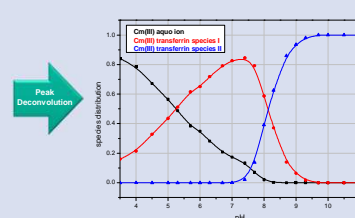


## Complexation of Cm(III) with transferrin

### Cm(III) emission spectra upon increasing pH



### Species distribution



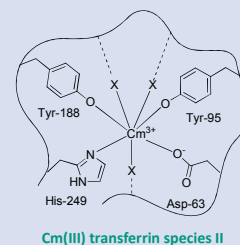
Species	pH	Fluorescence lifetime	n(H <sub>2</sub> O)
Cm(III) transferrin species I	7.4	129 $\mu\text{s}$	4.2
Cm(III) transferrin species II	9.7	218 $\mu\text{s}$	2.1

### Cm(III) transferrin species I

- pH 6.3 – 7.7
- Partial coordination of Cm(III) at the transferrin binding sites
- 4 H<sub>2</sub>O molecules and 5 other ligands (e.g. amino acids, CO<sub>3</sub><sup>2-</sup>, OH<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>) in the first coordination sphere

### Cm(III) transferrin species II

- pH 7.7 – 11.0
- Incorporation of Cm(III) at the transferrin binding site
- 2 H<sub>2</sub>O molecules and 7 additional ligands (4-fold coordination via amino acid groups and 3 other ligands (e.g. CO<sub>3</sub><sup>2-</sup>, OH<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>))



## Complexation of Am(III) with transferrin

EXAFS measurements of Am(III) transferrin at  $T = 77$  K

M	pH	T [K]	1 <sup>st</sup> shell			Red. Er
			N	R / Å	$\sigma/\text{Å}^2$	
Am(III)	7.2	77	8.7 (0.5)	2.47 (0.01)	0.007 (1)	0.41
Am(III)	8.5	77	8.8 (1.0)	2.38 (1)	0.013 (2)	0.67

- pH 7.2: Am(III) aquo ion is formed exclusively
- pH 8.5: Am(III) transferrin species
- 9 neighbors in the first coordination shell
- Average distance  $R = 2.38$  Å → very short → indicates formation of a strong metalorganic complex (Am(III) incorporated at the Fe(III) binding site of transferrin)
- Presence of several shells at higher distances

→ Formation of a Am(III) transferrin complex with Am(III) incorporated at the transferrin binding site (in accordance with Cm(III) results)

## Conclusions

- Formation of two Cm(III) and Eu(III) transferrin species at different pH
- pH > 7: incorporation of the metal ion at the Fe(III) binding site
- EXAFS results of Am(III) transferrin confirm the formation of an incorporated transferrin species at high pH