

Nano Rome, 20-23 September
2016 Innovation
Conference & Exhibition

A novel ferritin-based nanoconstruct for diagnostic applications

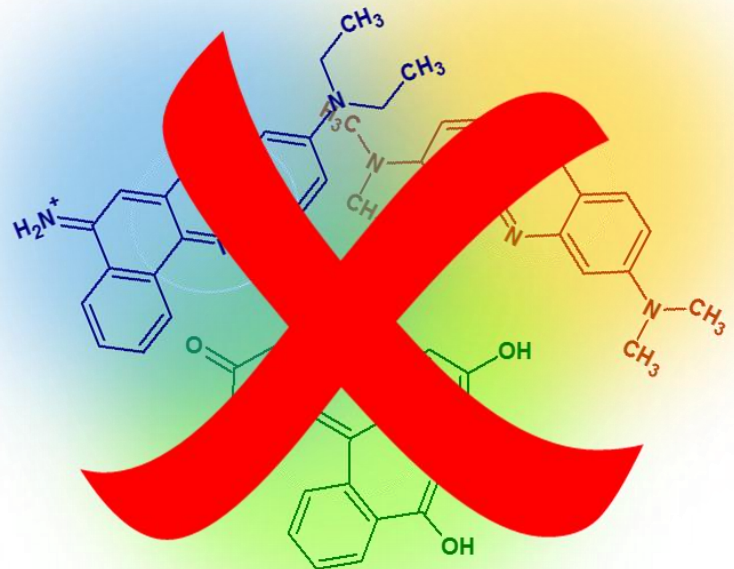


Lorenzo Calisti

Sapienza University of Rome

Lanthanide ions: fascinating tools...

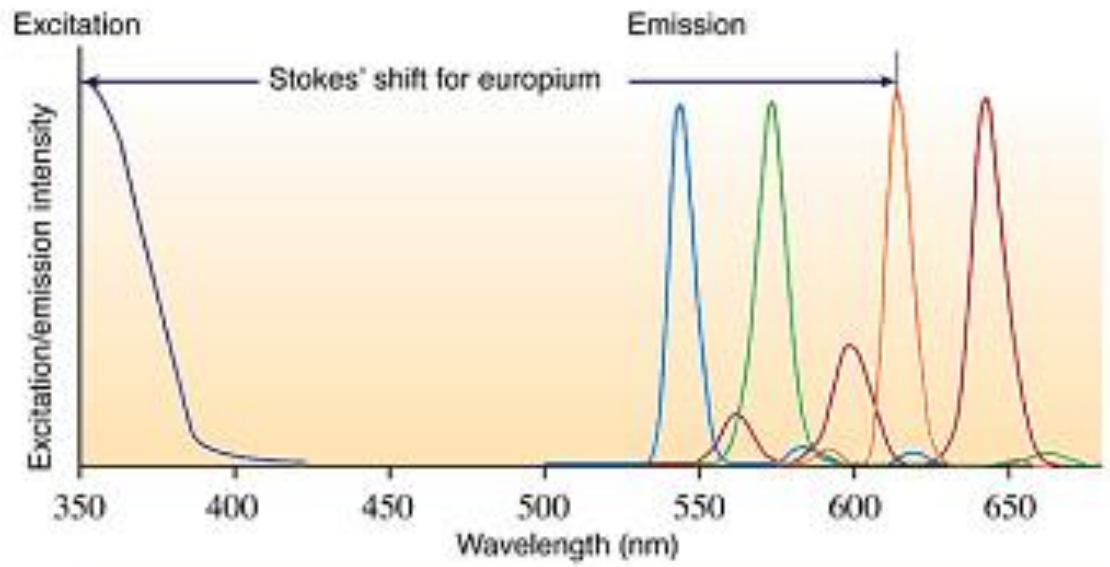
Fluorescent imaging probes



- Long-lived fluorescence
- Not photobleachable

Lanthanide ions: fascinating tools...

Fluorescent imaging probes

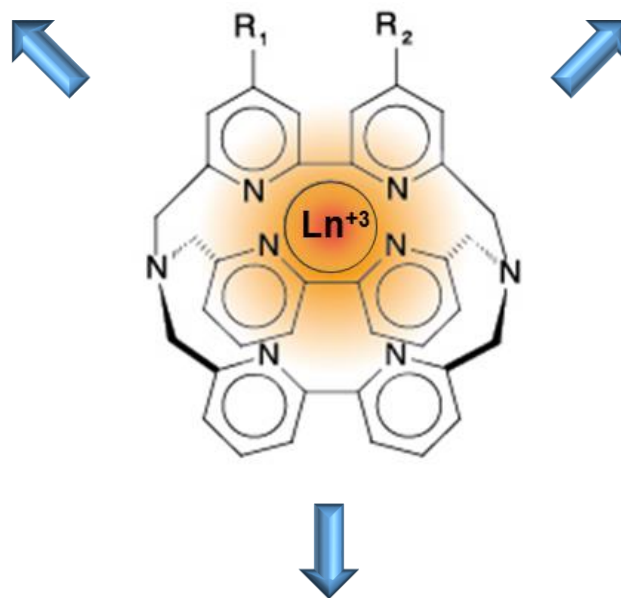


- Large Stokes shift
- Narrow and well-separated bands

...but aromatic chelating agents are required!!!

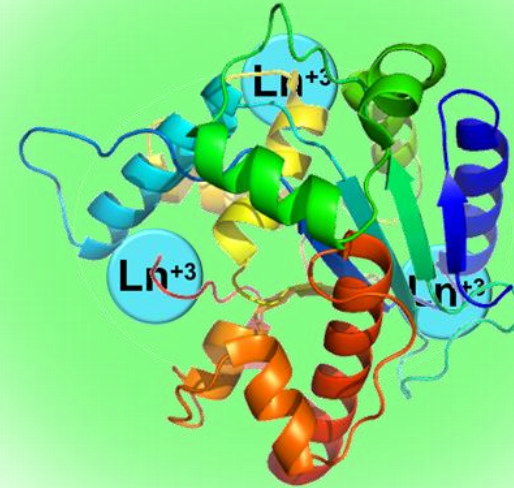
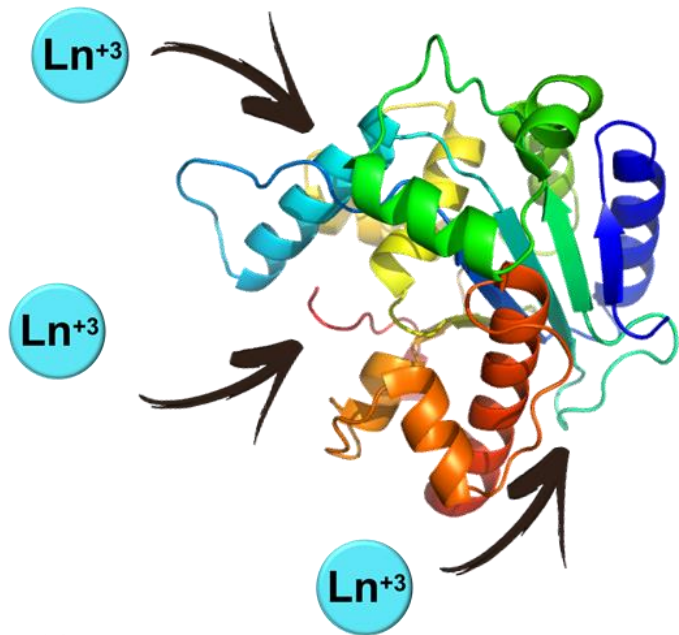
Improvement of **solubility**
and **transport** properties

Improvement of their
toxicological profile



Improvement of their weak fluorescence through
Fluorescence Resonance Energy Transfer (FRET)

Proteins as chelating agents?

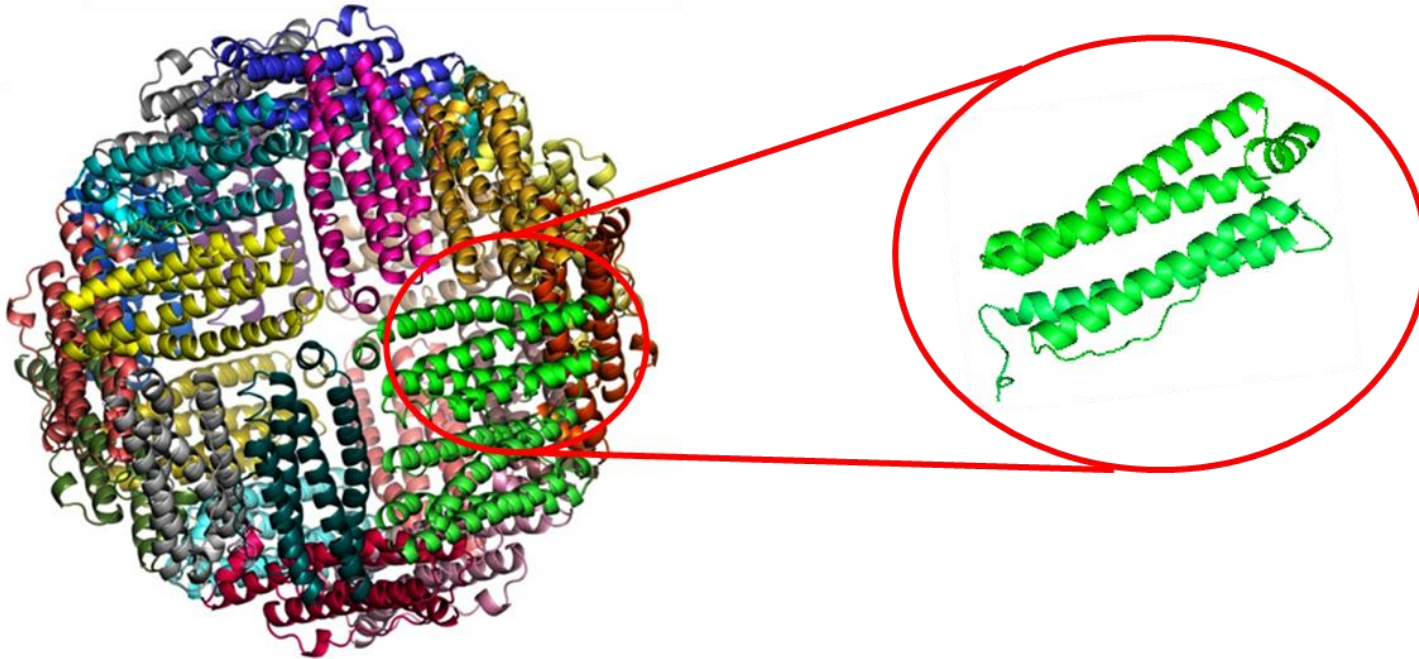


- Biocompatible probes
- FRET sensitization
- Chemical or genetical functionalization
- Targeting properties



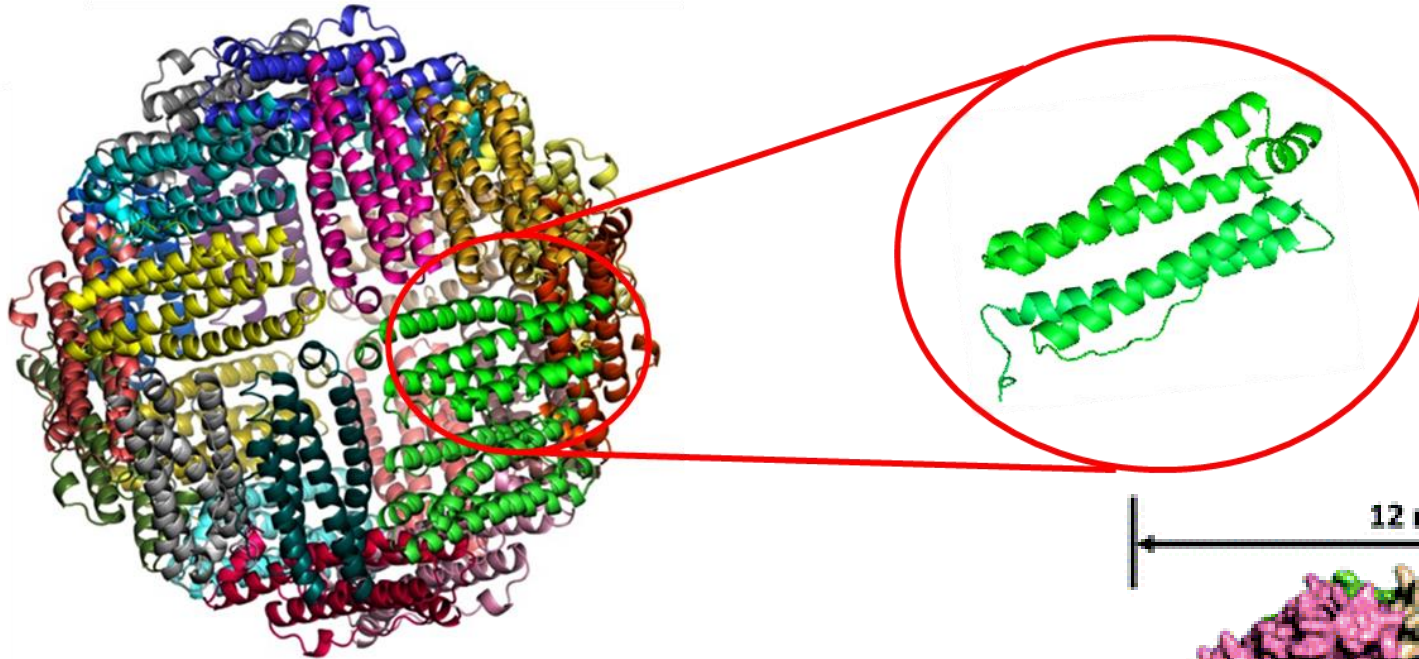
- Chemical labelling with lanthanide chelates
- Introduction of unnatural aminoacids

Ferritin: structure and function

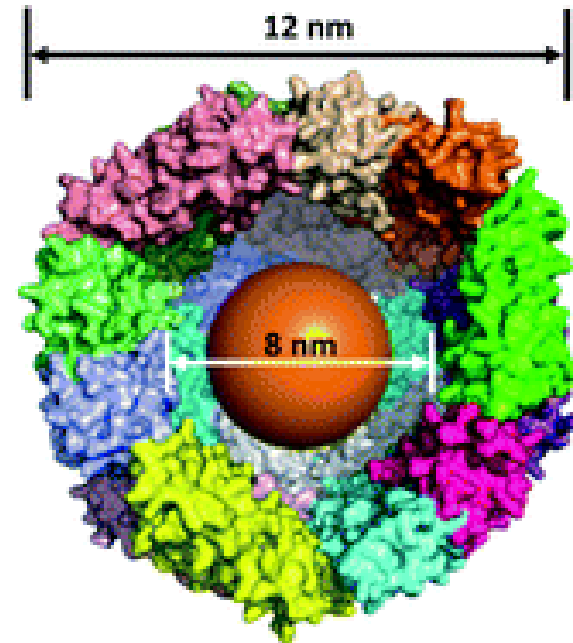


- ubiquitous protein involved in iron-storage, immunity response...
- 24 subunits assembled into a spherical-shape structure
- each subunit is composed of five α -helices

Ferritin: structure and function



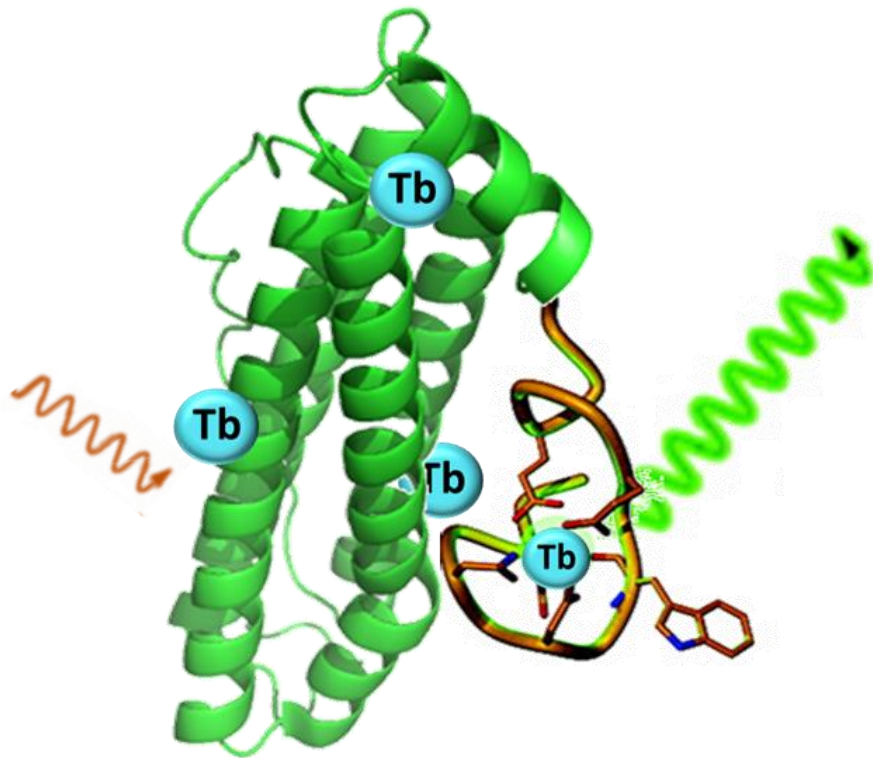
**PROTEIN-CAGE
ARCHITECTURE**



MusFt-LBT



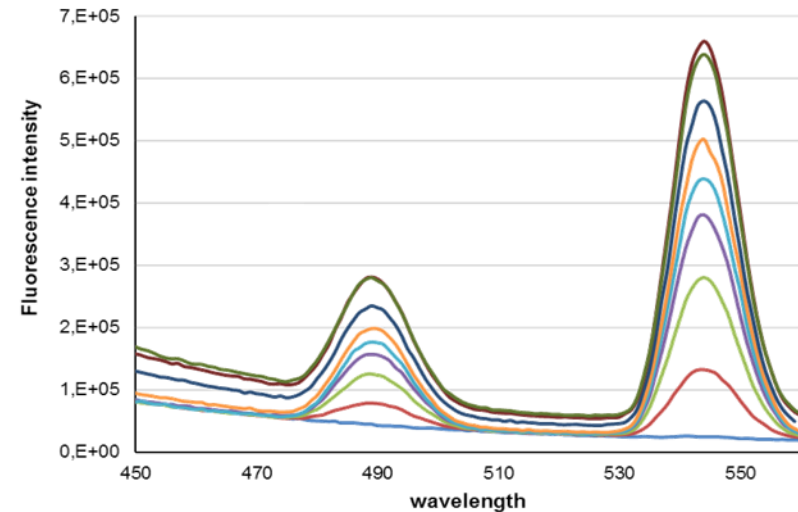
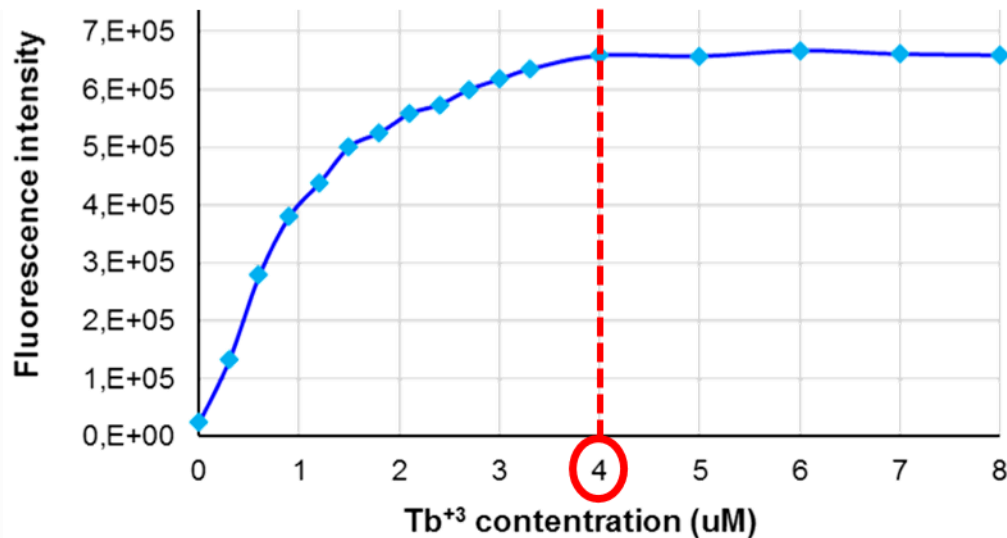
Mouse H-chain Ferritin (**MusFt**) fused with a Lanthanide-Binding Tag (**LBT**)



- ✓ **Tightly and selectively complexes lanthanide ions**
- ✓ **Strongly enhances terbium (Tb^{3+}) luminescence acting as a FRET sensitizer**

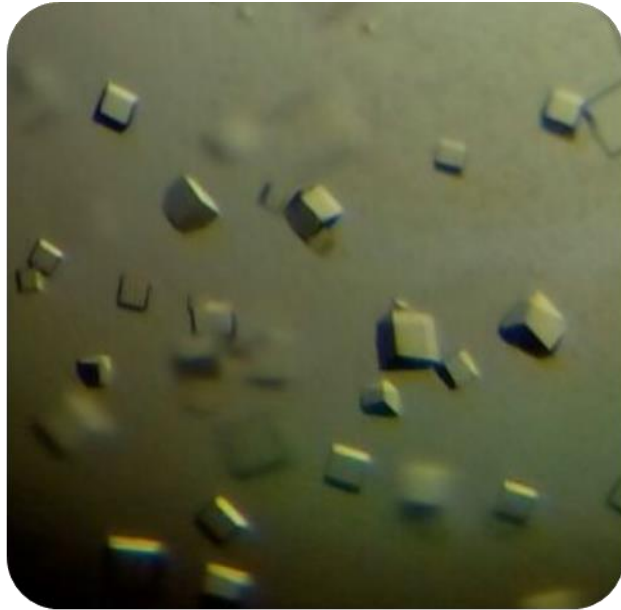
MusFt-LBT: fluorescence measurements

$\lambda_{exc} = 295 \text{ nm}$
 $[\text{FtMus-LBT}] = 1 \text{ } \mu\text{M}$

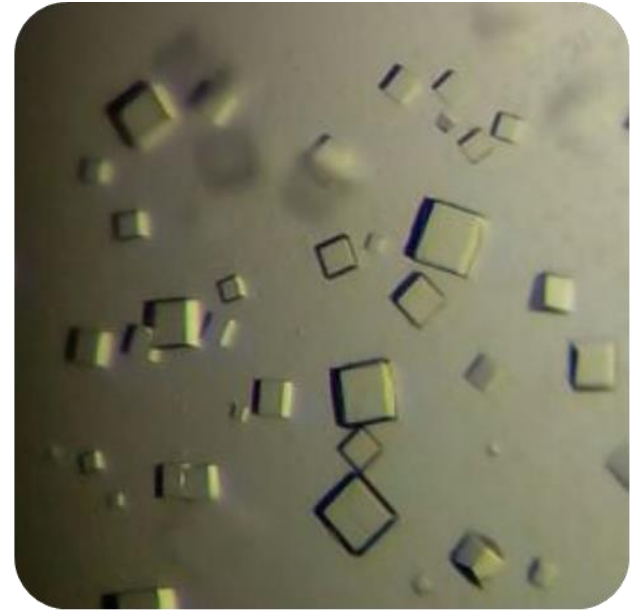


- MusFt-LBT-Tb(III) binds **four Tb(III) ions per subunit**
- MusFt-LBT-Tb(III) had an appreciable fluorescence up to a concentration **10 nM**

MusFt-LBT: crystals



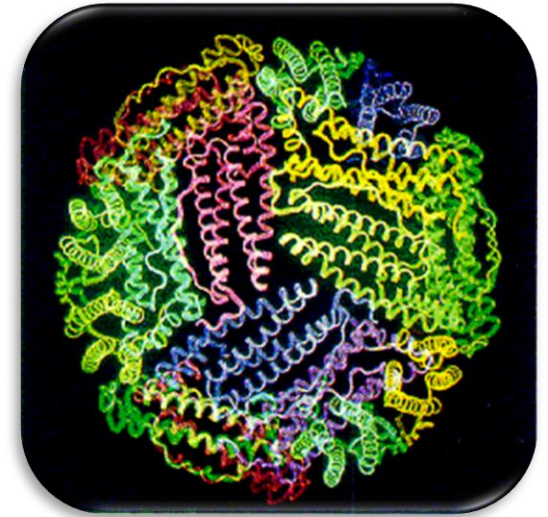
MusFt-LBT



MusFt-LBT-Tb(III)

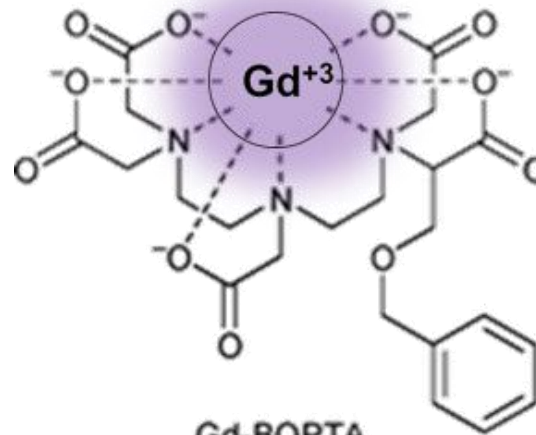
... thus confirming that MusFt-LBT...

- ❖ Efficiently acts as **lanthanide chelating agent** and **FRET sensitizer**
- ❖ Is an highly **biocompatible** and intrinsically fluorescent nanophosphor
- ❖ Can be successfully used for qualitative and **quantitative** diagnostic applications
- ❖ Can be successfully used in **fluorescence cross-correlation spectroscopy** and microscopy by using different lanthanides



Future perspective

MRI contrast agents



Gd-BOPTA
MultiHance®
(Bracco)

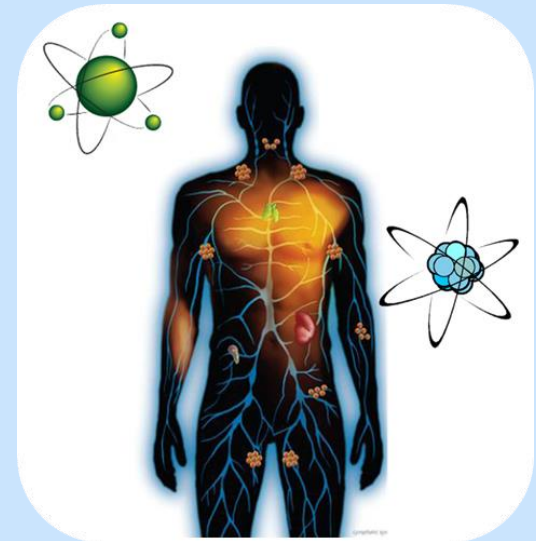
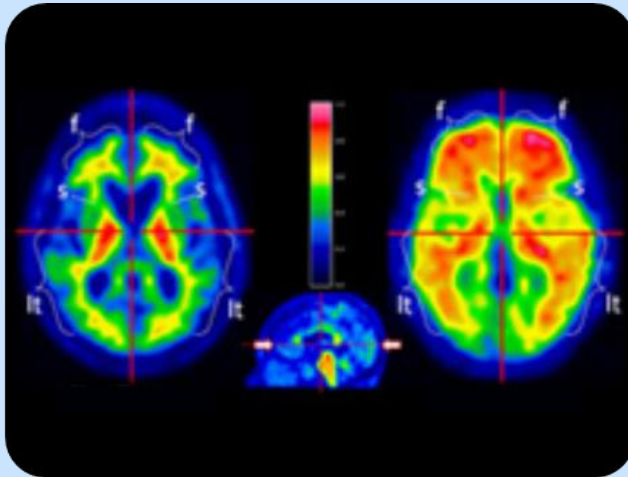
- Lowering Gd toxicity
- Affecting relaxivity

Future perspective

MRI
ag



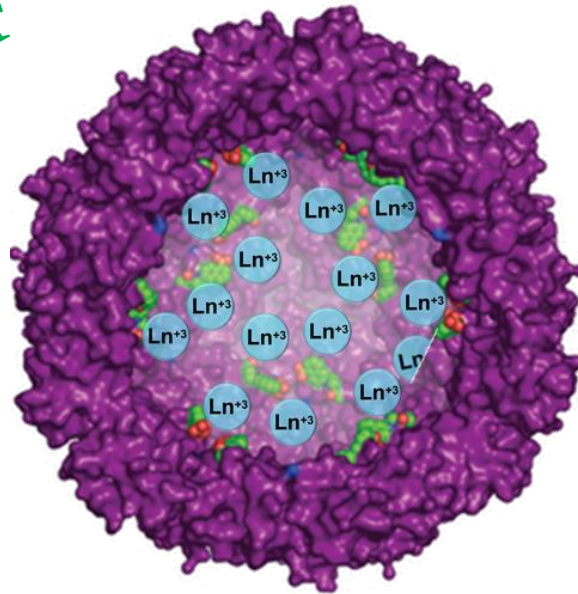
Radionuclides: Cancer diagnosis and treatment



Future perspectives:

Highly biocompatible
carrier

Targeting
properties



Multiple functionalities onto a
single molecule

Special thanks to:



Consiglio Nazionale
delle Ricerche



- ❖ Prof. Alberto Boffi
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- ❖ Dott. Alberto Macone
- ❖ Dott.ssa Paola Baiocco
- ❖ Dott. Pierpaolo Ceci
- ❖ Dott. Simone de Panfilis
- ❖ Dott.ssa Matilde Trabuco
- ❖ Dott.ssa Irene Benni



Thank you

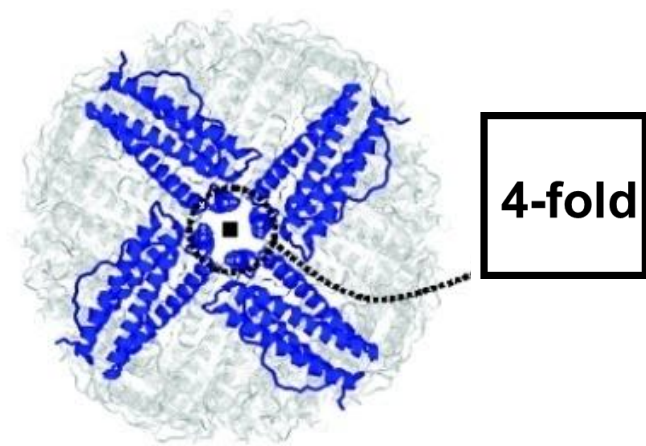
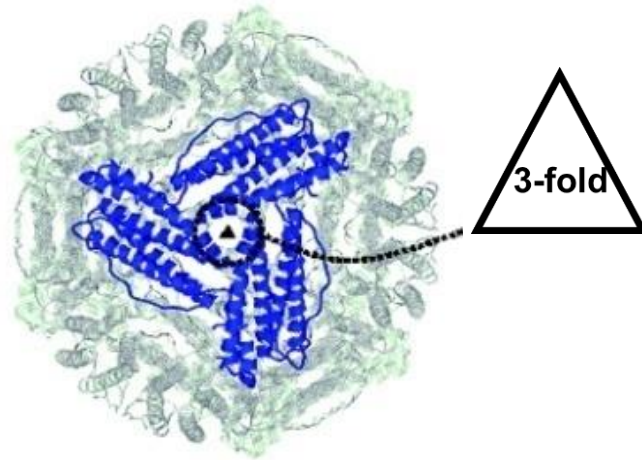
for the

attention!!!

BACK-UP

Ferritin channels and their role...

Channels formed between the intersection of peptide subunits connect the inner cavity to outside



Threefold channels:

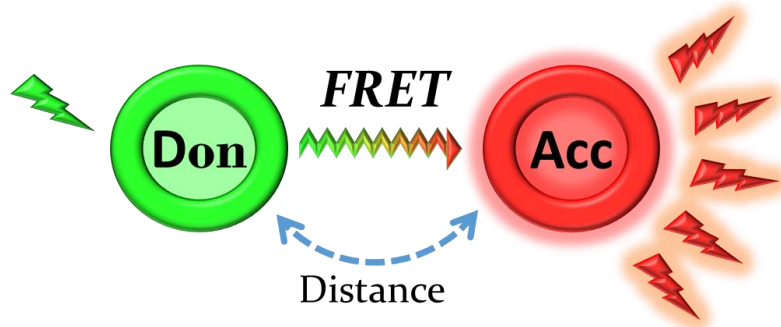
- lined with polar aminoacids
- Allow for the entry and exit of cations

Fourfold channels:

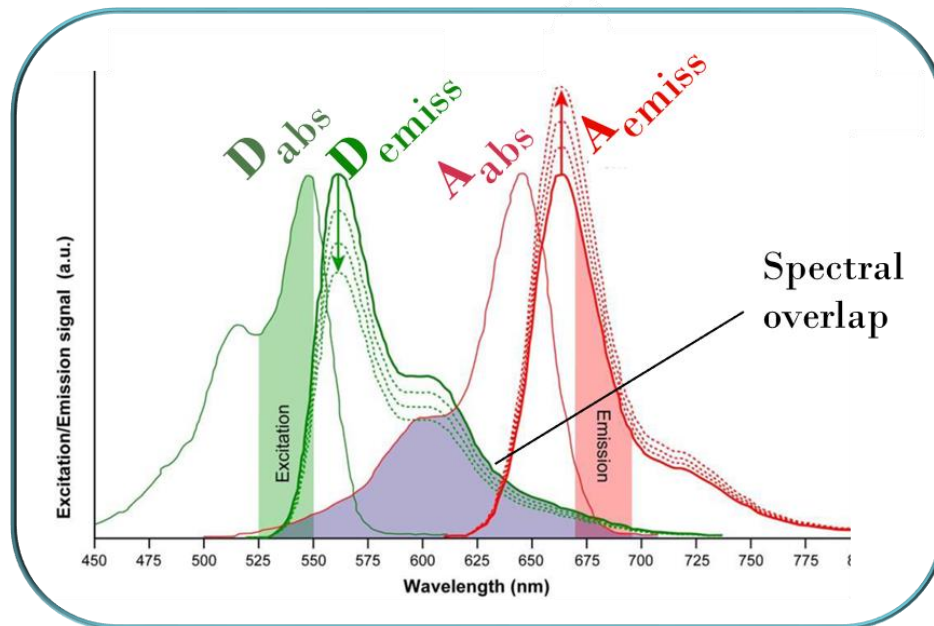
- lined with hydrophobic residues
- Involved in proton transfer for the electroneutrality

FRET: Fluorescence Resonance Energy Transfer

Mechanism of energy transfer between two chromophores

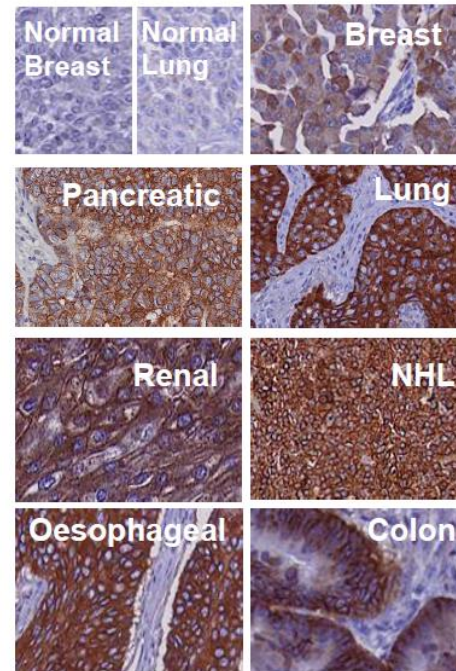
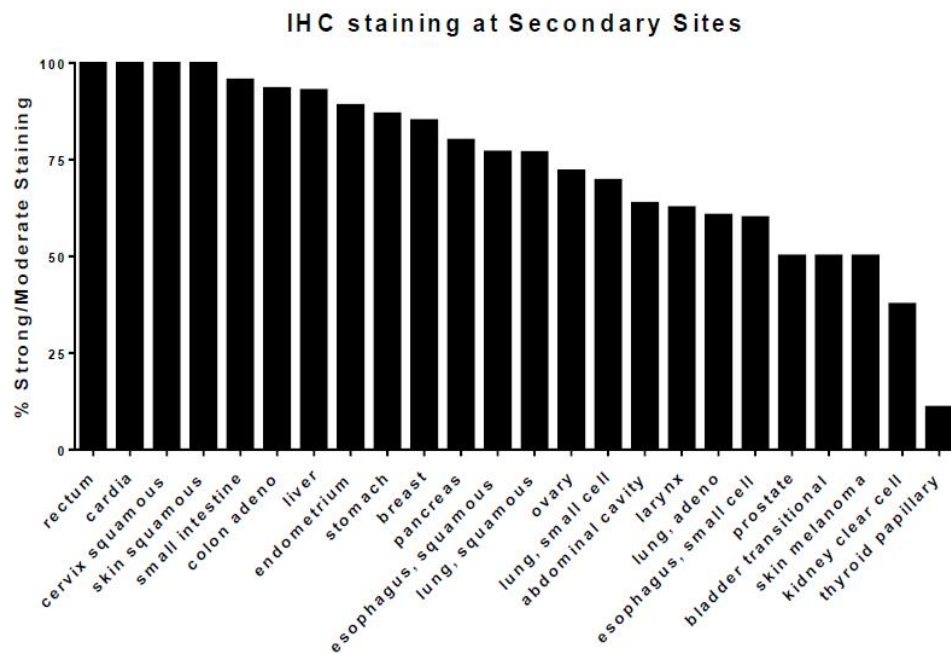


A **donor (D)** chromophore, initially in its excited state, transfers energy to an **acceptor (A)** chromophore and **improves its emission fluorescence**



Requires spectral overlap and **close proximity** between **D emission** and **A absorbance**

CD71 is Highly Expressed in Many Metastatic Cancers



From "Preclinical Development of a Probody TM Drug Conjugate (PDC) Targeting CD71 for the Treatment of Multiple Cancers"
 S Singh, A DuPage, A Weaver, M Krimm, C White, J Sagert, Y Huang, L Diep, S Liu, J Richardson, WM Kavanaugh, JA Terrett, LR Desnoyers

Ferritin

Subcloned into pET 22b vector for recombinant expression in E.coli strain BL21(DE3)

Expression: overnight 37° C with IPTG 1mM

Sonication in HEPES 25 mM pH=7,5 150 mM NaCl (buffer A)

DNasi (0,1 mg/ml) treatment: 1 h 37° C

Heat treatment: 55° C → 80° C

Ammonium sulphate precipitation: 50% → 70%

Overnight dialysis in buffer A of 70% fraction

Gel filtration chromatography

MusFt-LBT: purification

