What can fluorescence tell you about biomolecular structure and function? Part I

Patricia O'Hara

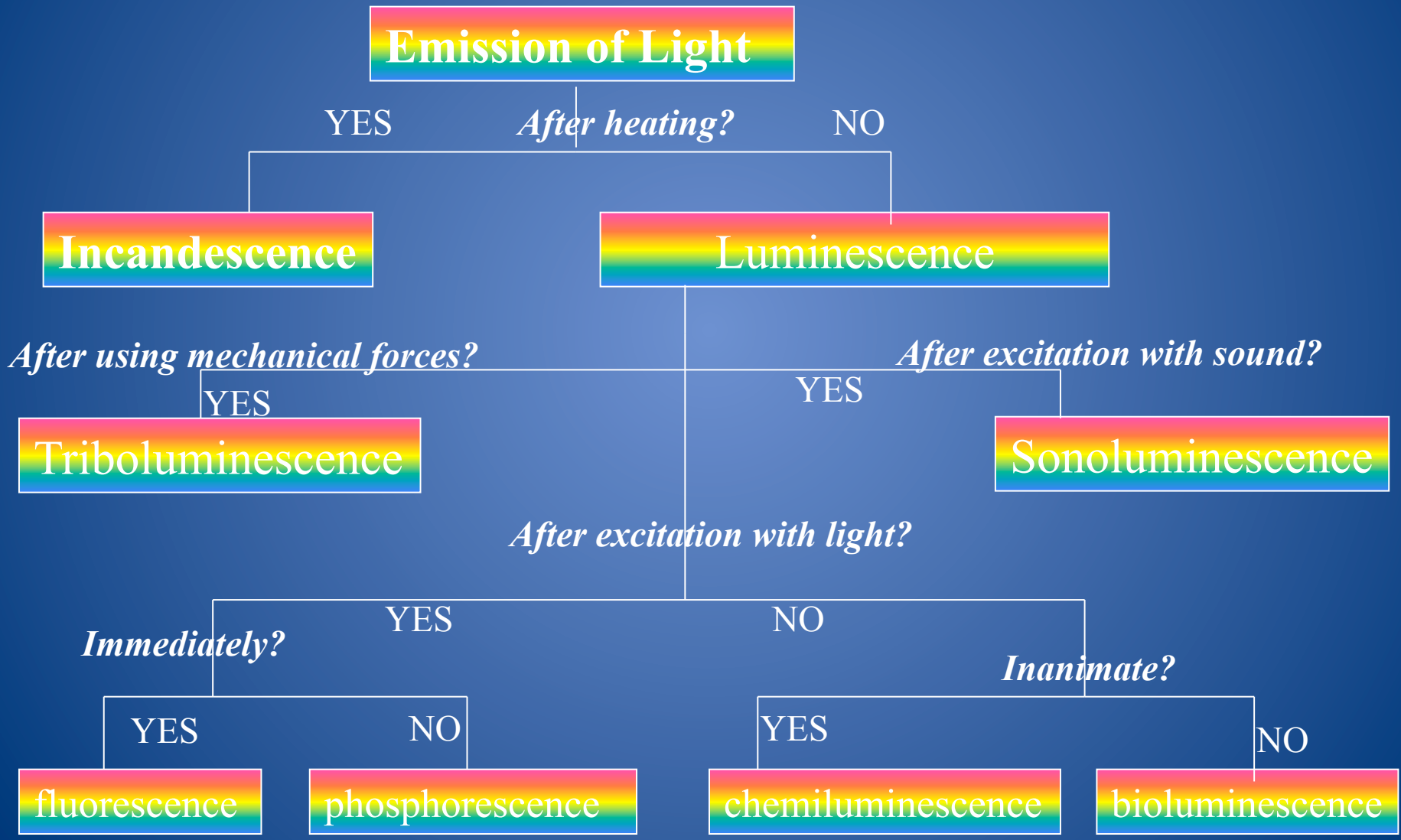
Chem 46B

February 1, 2011

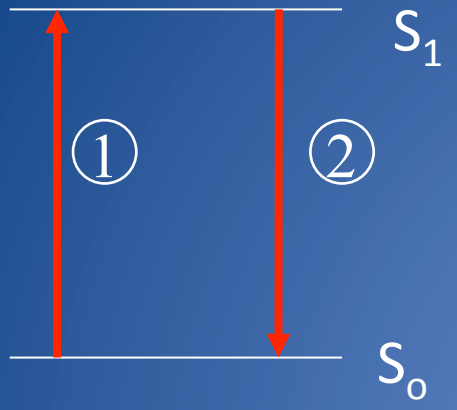
Outline

- What are the ways in which light physically interacts with materials? (i.e. no photochemistry)
- What is fluorescence?
- What can fluorescence tell you about biomolecular structure?
- Example I: Steady State fluorescence – metal binding to transferrin
  - Biological motivation
  - Fluorescence Experiments (intensity quenching, steady state polariztion)

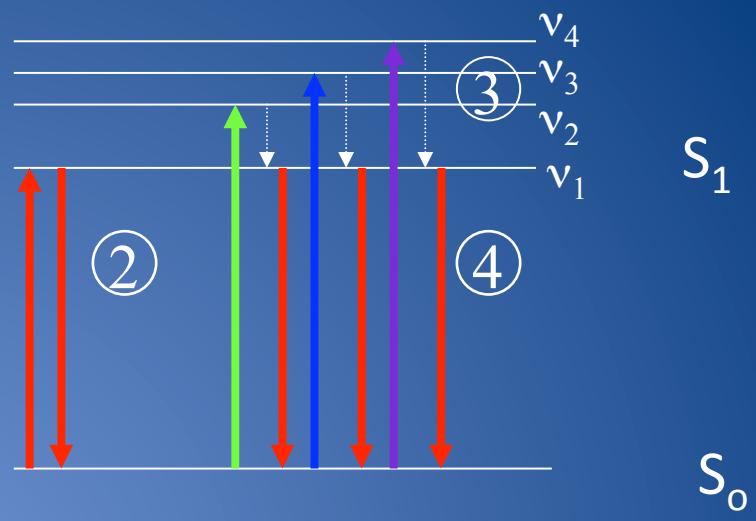
What are the ways in which light physically interacts with materials? (i.e. no photochemistry)



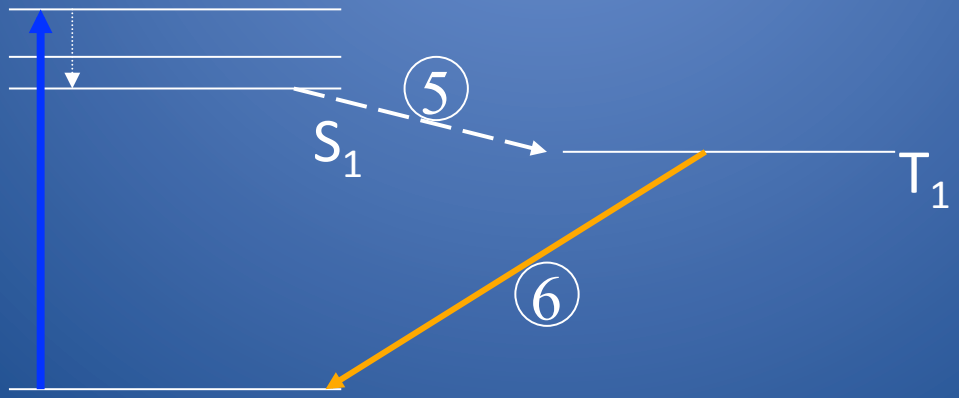
What is fluorescence?



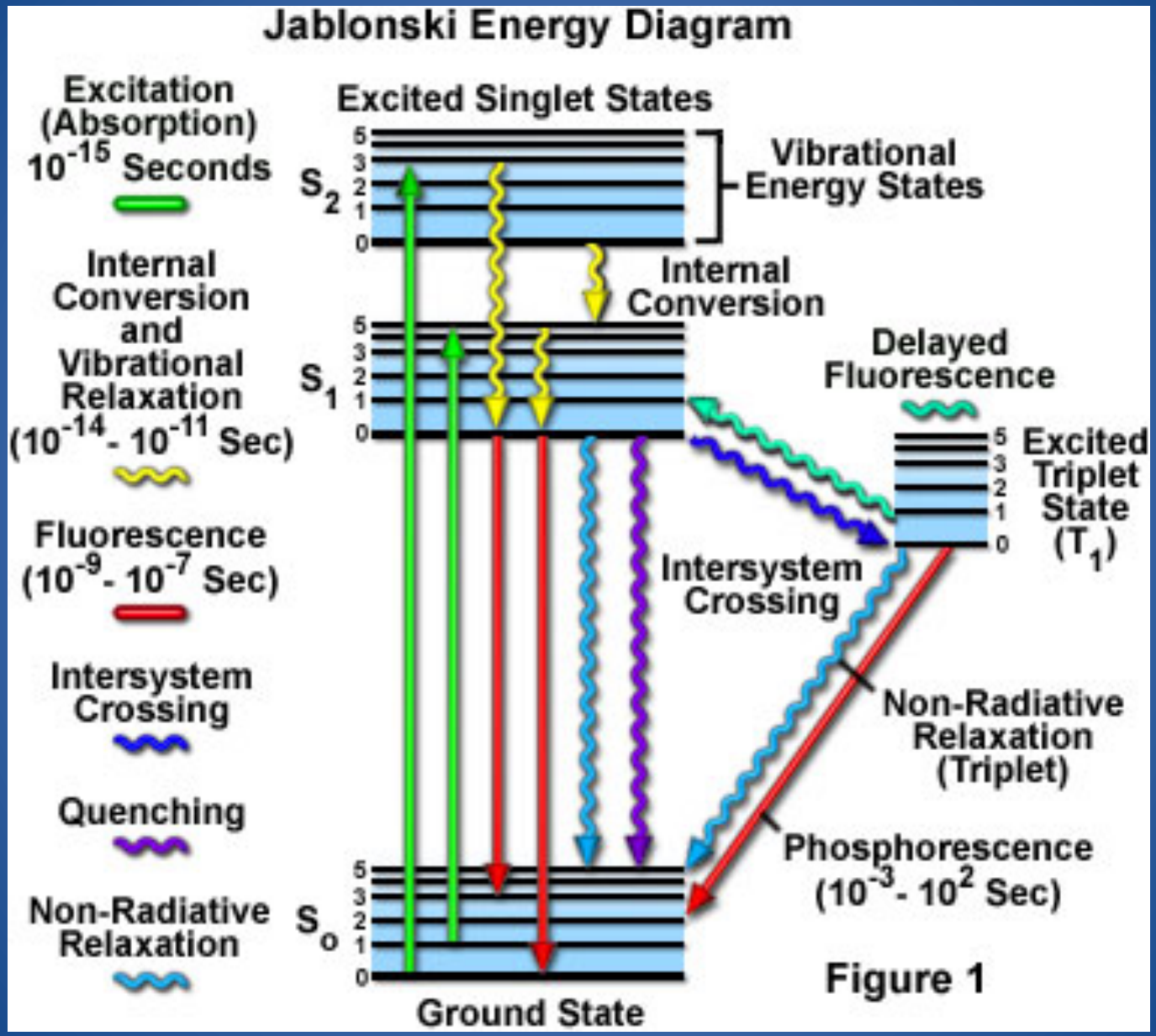
Fluorescent Emission in **Atoms**



Fluorescent Emission in **Molecules**



Phosphorescent Emission from **Molecules**


Jablonski Energy Diagram
Excitation (Absorption) 10^{-15} Seconds
Internal Conversion and Vibrational Relaxation (10^{-14} - 10^{-11} Sec)
Fluorescence (10^{-9} - 10^{-7} Sec)
Intersystem Crossing
Quenching
Non-Radiative Relaxation
Excited Singlet States
S_2
S_1
S_o
5
3
2
1
0
Vibrational Energy States
Internal Conversion
Delayed Fluorescence
Excited Triplet State (T_1)
Intersystem Crossing
Non-Radiative Relaxation (Triplet)
Phosphorescence (10^{-3} - 10^{2} Sec)
Ground State
Figure 1

What you learn depends upon your experimental methodology (bulk)

- Steady state
  - Excitation and emission
    - fingerprints
  - Energies and Intensities
    - Compare w similar molecules
  - Intensity quenching from ligand
    - Kd, Km, Ki
  - Solvent shifts of spectra
    - Calculate energy differences
  - Emission Polarization
    - Bound/Unbound ligands
    - Averaged rotational times
  - FRET
    - Average donor acceptor distances
- Time/Frequency resolved
  - Lifetimes
    - Buried/exposed
    - Unique environments
  - Lifetime distributions
    - Dynamics
    - Local motions
  - Time resolved FRET
    - Accurate donor/acceptor distance
    - Temp dependence to FRET yields info on dynamics
  - Time resolved Polarization
    - Limiting polarization, rotational correlation times, diffusion times.

What you learn depends upon your experimental methodology (single molecule)

- Steady state
  - Excitation and emission
    - fingerprints
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What you learn depends upon your experimental methodology (bulk)

- **Steady state**
  - **Excitation and emission**
    - **fingerprints**
  - Energies and Intensities
    - Compare w similar molecules
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Using fluorescence spectroscopy to probe binding of ruthenium compounds to human serum transferrin

- Funding: John Abele Faculty Development Fund
- Collaborators
  - Renzo Cini (U. Siena, Italy)
  - Lauren Benson ’08 (now chem teacher)
  - Zandra Walton ’09
  - Phoebe Arbogast ’10
- 1981, my first paper measured energy transfer in transferrin

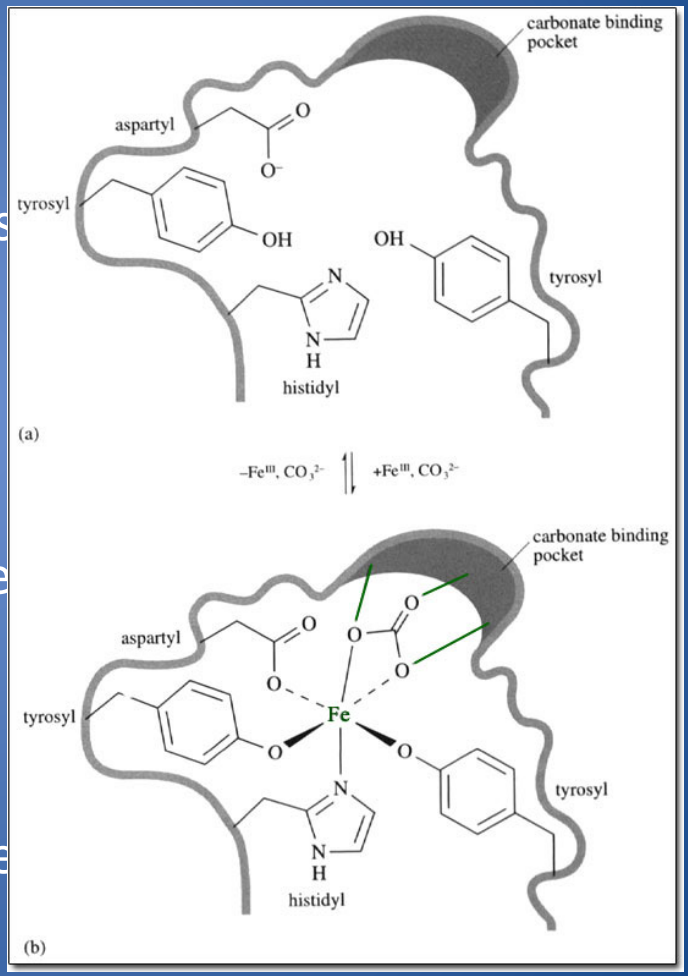
Distance between metal-binding sites in transferrin: energy transfer from bound terbium(III) to iron(III) or manganese(III)

Patricia O'Hara, Simon M. Yeh, Claude F. Meares, and Richard Bersohn

Biochemistry, **1981**, 20 (16), 4704-4708• DOI: 10.1021/bi00519a028 • Publication Date (Web): 01 May 2002

Human Serum Transferrin

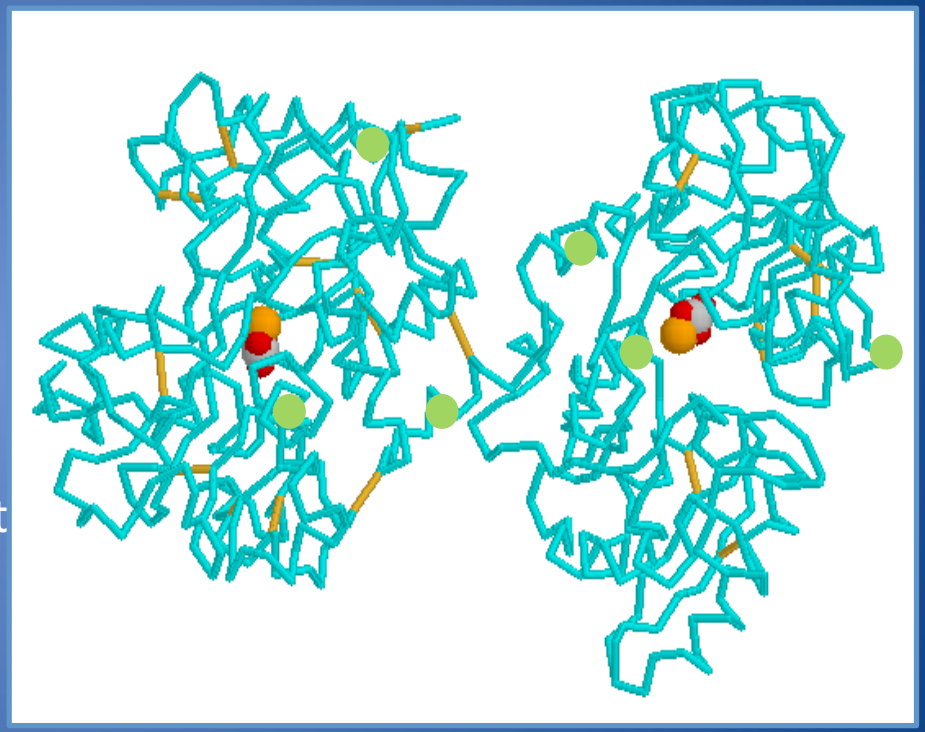
- ***What was known about transferrin then?***
  - a non-heme iron protein that exists in human blood to transport Fe throughout body
  - Fe(III) binding site is shown
  - Fe(III) $K_d < 10^{-27}$ M…pH 7.4
  - Metal is bound at two identical site on two different domains
  - Synergistic anion, typically carbonate necessary
  - Other trivalent metals also bind (i.e Mn(III), Tb(III))



Angew. Chem. Int. Ed., DOI: 10.1002/anie.200705723

Human SerumTransferrin

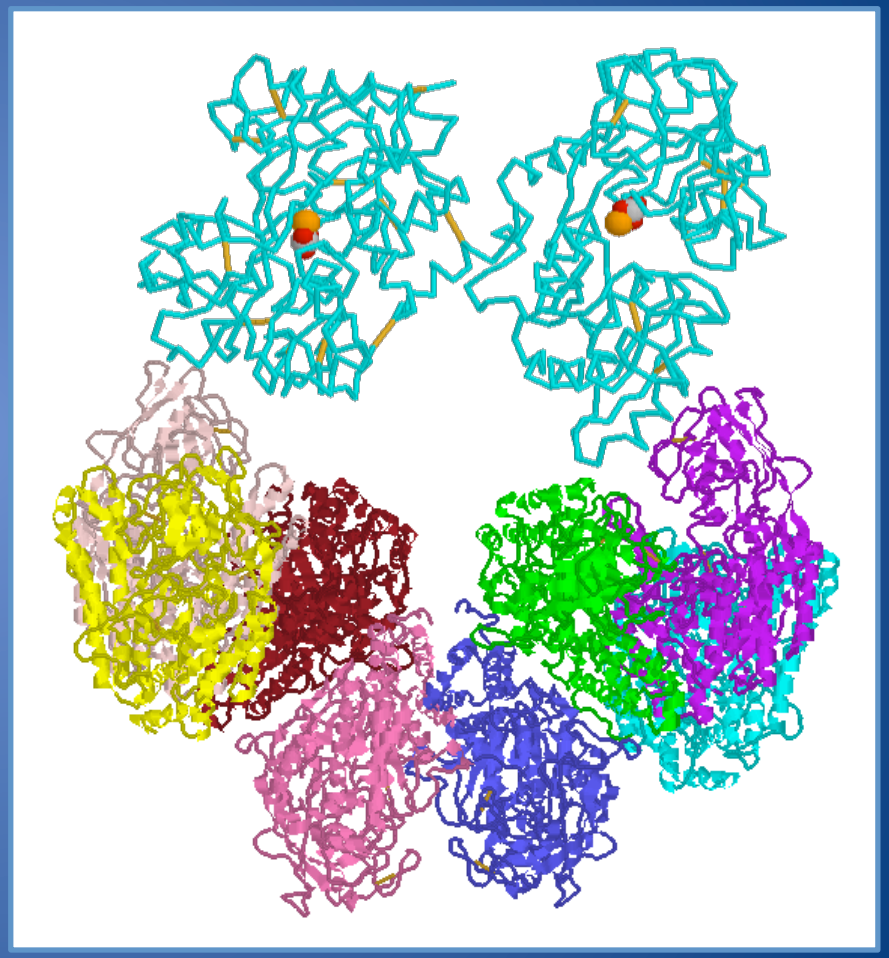
- *What do we know today?*
- Two structurally similar lobes (binding site previous slide)
- Conformational change occurs upon metal binding
  - induces a binding site cleft closure
  - occurs with two domains rotating 63^{o} with respect to each other about hinge
  - MetalloTf shape is more compact
  - Conformational change about Trp in the hinge region between two domains
  - Green circles are fluorescent reporter molecules Trp (W)



Berners-Price, 1996

How does Fe(III) get into the cell?

- Serum Tf in vivo is typically about 30% saturated with iron (pH = 7.4)
- Only Fe-Tf binds to receptor on the cell surface
- Fe-Tf enters the cell in a vesicle
- Inside vesicle (low pH~5.5) Fe(III) is released
- apoTf and receptor are transported back to cell surface, where they dissociate

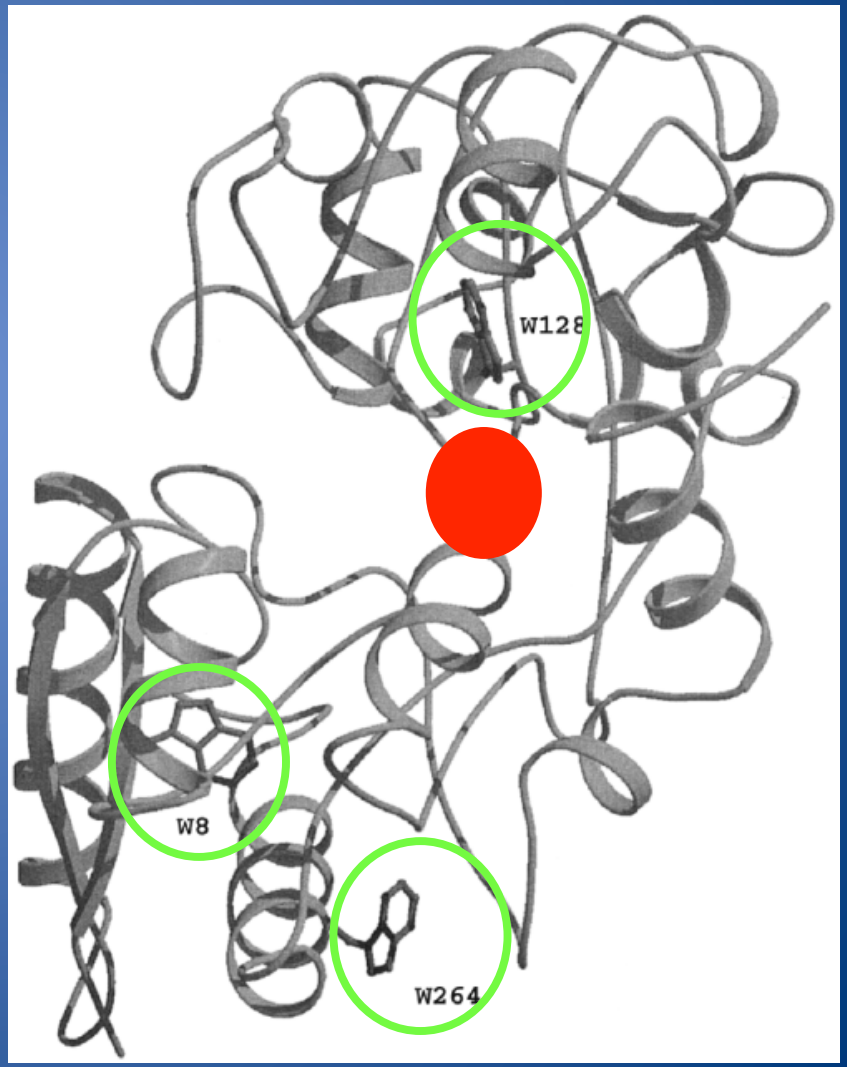


Transferrin and its receptor

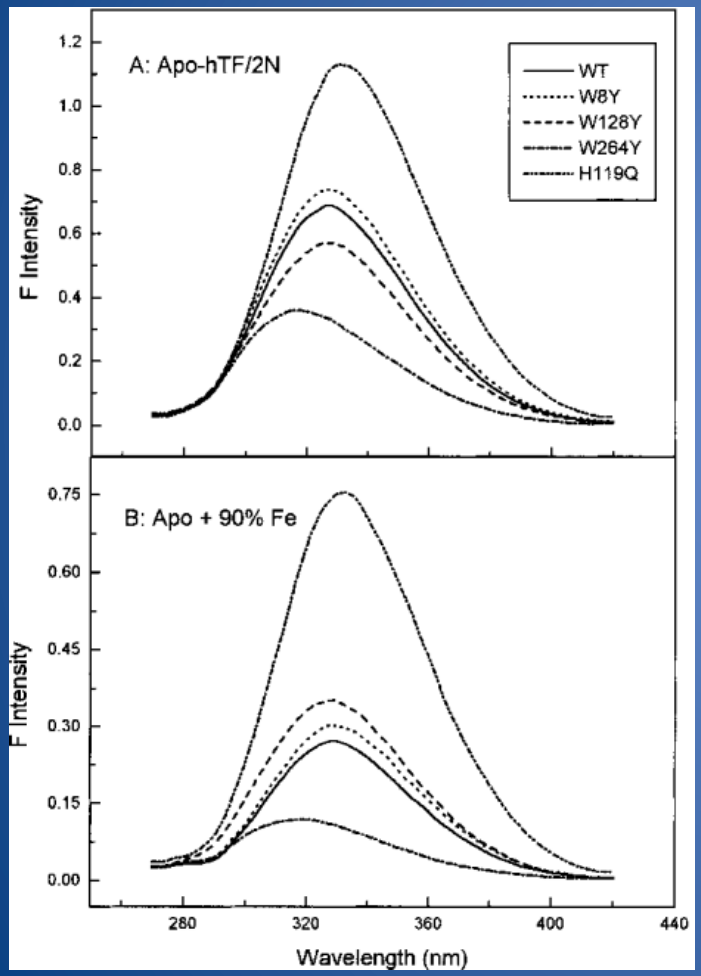
Berners-Price, 1996

Fluorescence properties of Transferrin

- Research Plan is to use fluorescence to follow metal binding of Ru(III) compounds (*why Ru(III)? – more in a moment)*
- Use the protein Trp fluorescence (6 Trp)
- How do 6 Trp contribute to bulk Tf fluorescence?
- One lobe of Fe-Tf shown here- only 3 Trp



Single W deletion mutants created in Tf

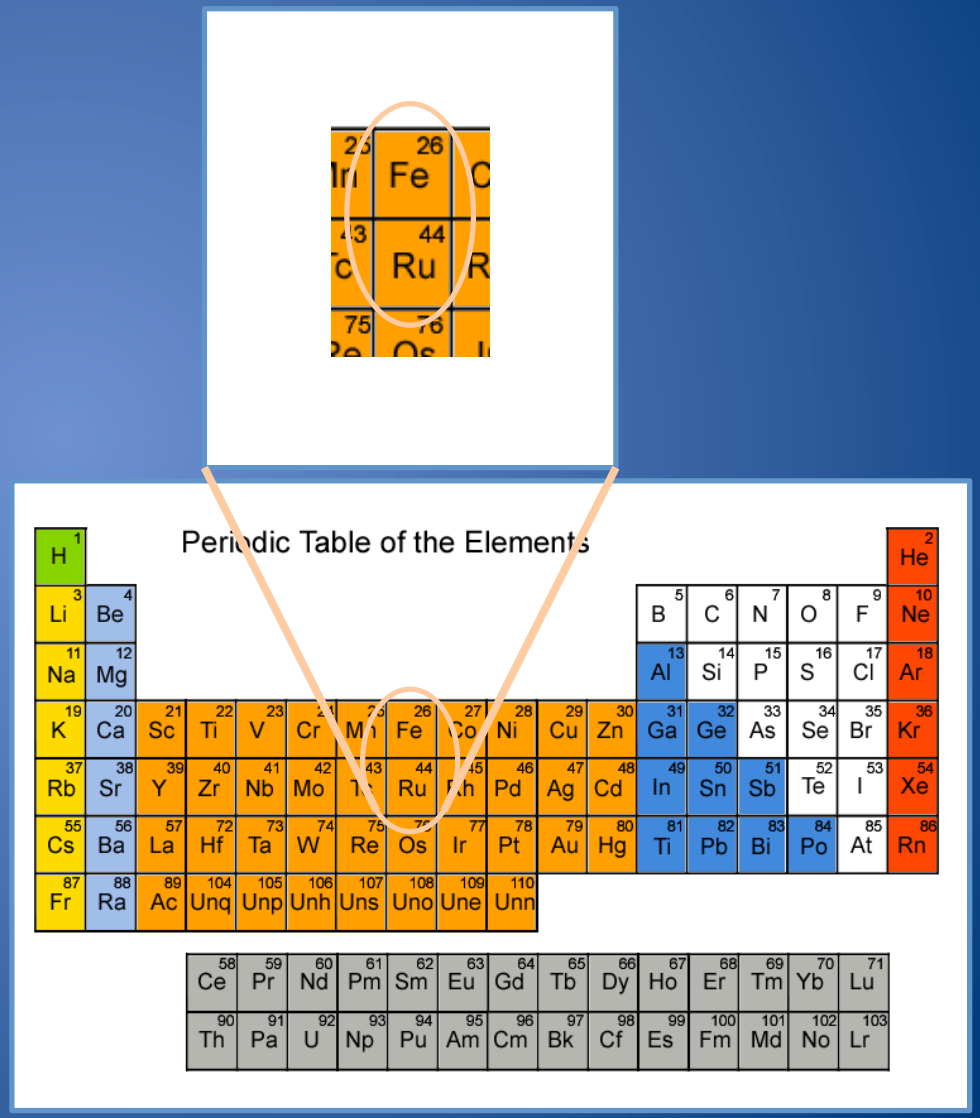


- He *et al* made W mutants to probe their fluorescence contributions
- W8 in hydrophobic box, surrounded by three F, a K residue is nearby, fluorescence totally quenched, independent of metal binding
- W128 fluorescence blue shifted and quenched by metal binding
- W264 fluorescence independent of metal binding, solvent accessible and primary contributor to fluorescence

Biochem. J. (2001) 354 (423-9)

Ruthenium binds to transferrin

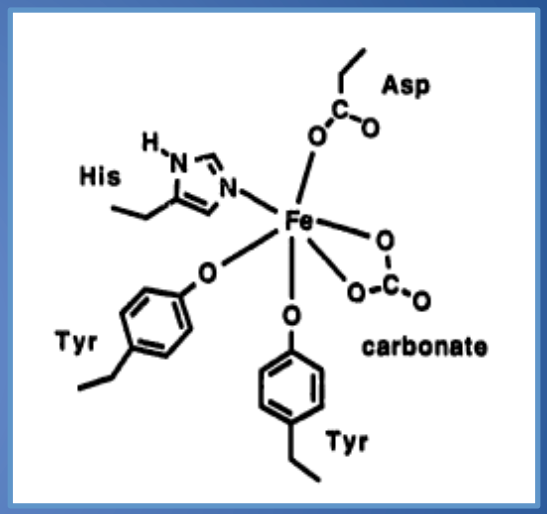
- Other metals can bind to transferrin
- Ruthenium is similar to iron (group VIII of the transition metals)
- Ruthenium can be transported to tissues as a ruthenium-transferrin complex



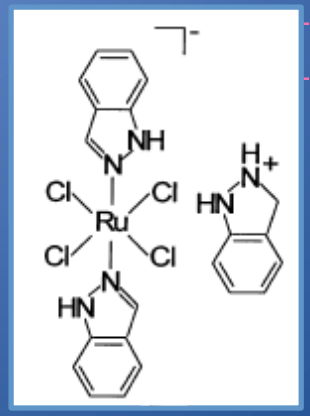
Berners-Price, 1996; Sava, 1990

Ruthenium also binds to transferrin

- x-ray crystallography shows that Ru-In_2 binds to Tf at metal binding site
- Binding occurs through the imidazole ring of the His residue, displacement of Cl^-
- Chemotherapeutic $[RuIn_2Cl_4]^-$ retains its activity against colon cancer cells when bound to transferrin



Hartinger, 2005

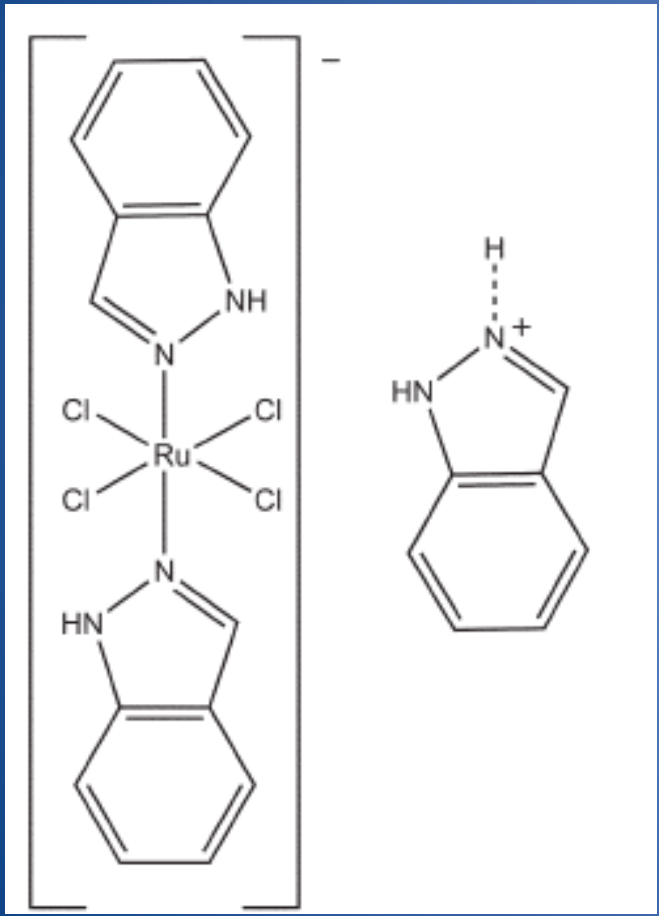


$[RuInd_2Cl_4]^-$

Brabec, 2006

Berners-Price, 1996

Ru-Indazole shown to bind to apoTf



- Structure of trans-indazole
  - tetrachlorobis(indazole thuthenate(III)
  - Kratz et al 1994
- Used against colon cancer trial drug called KP1019
- How does it bind?

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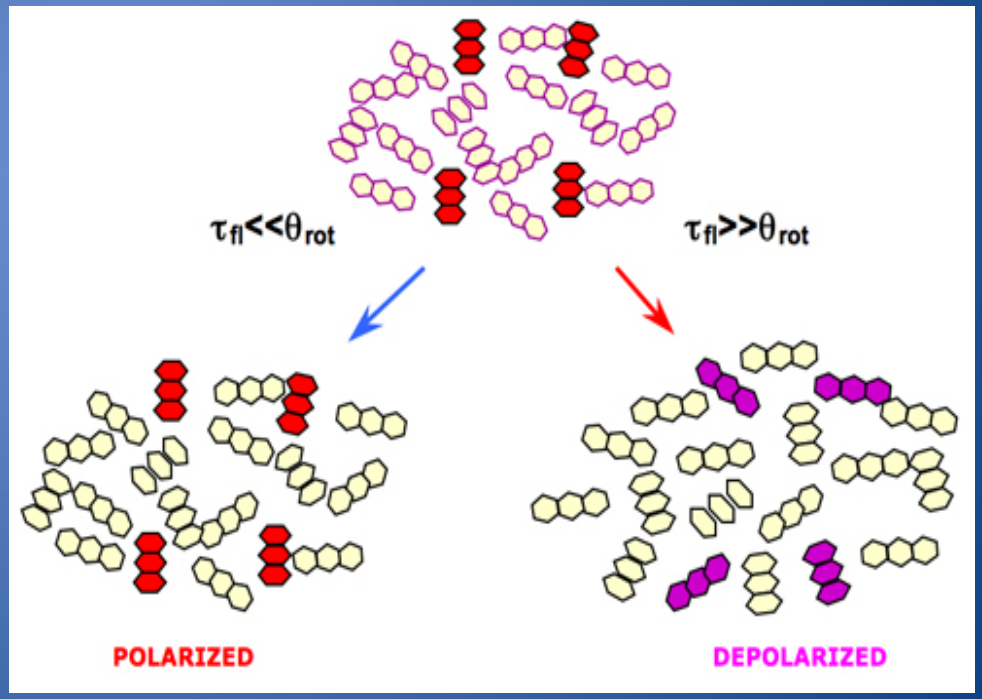
Fluorescence Polarization

Fluorescent Lifetime = τ_{fl}

Rotational correlation time = θ_{rot}

- **If $\tau_{fl} << \theta_{rot}$ (buried W) side group doesn't rotate during excited state lifetime and emitted light will be polarized**
- **If $\tau_{fl} >> \theta_{rot}$ (freely rotating W) side group rotates during excited state lifetime and emitted light will be depolarized**

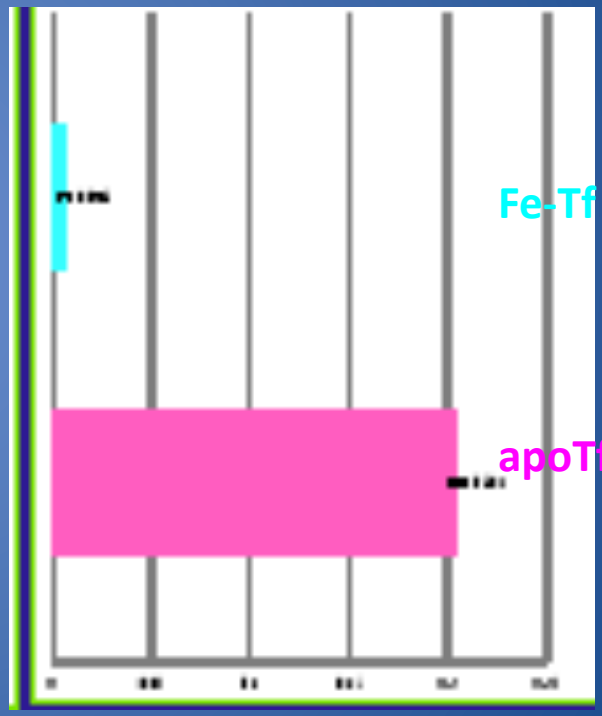


http://www.iss.com/resources/tech2/

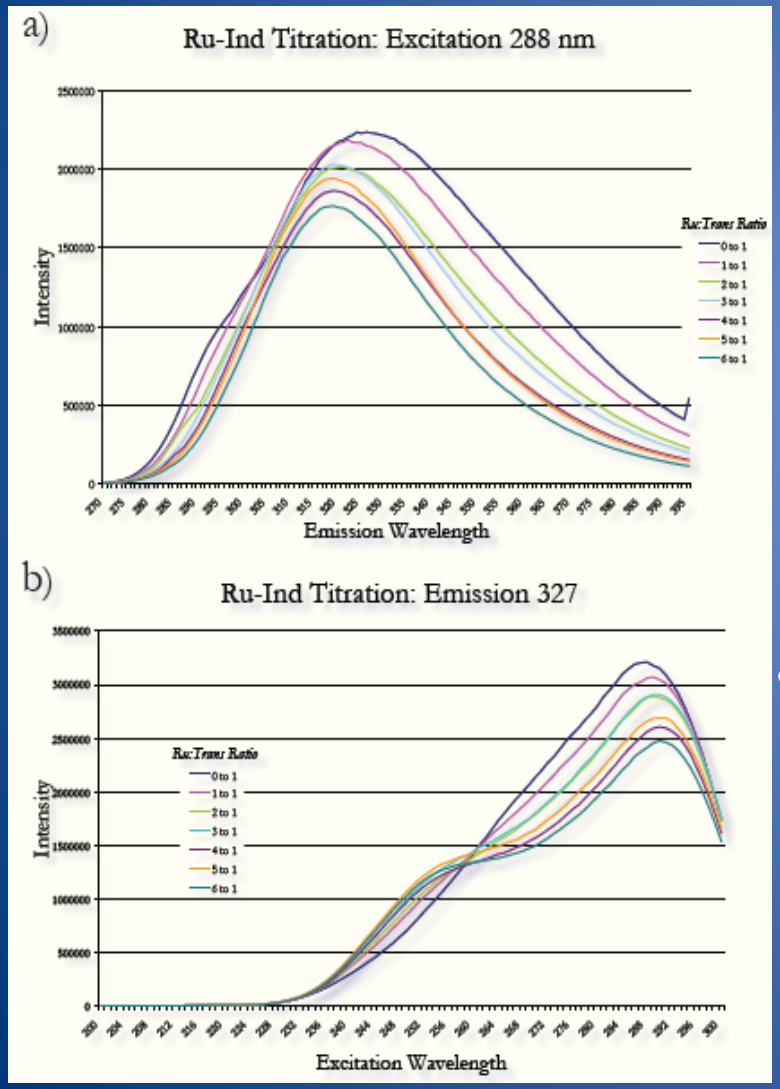
Steady state bulk Trp polarization as a reporter for metal binding

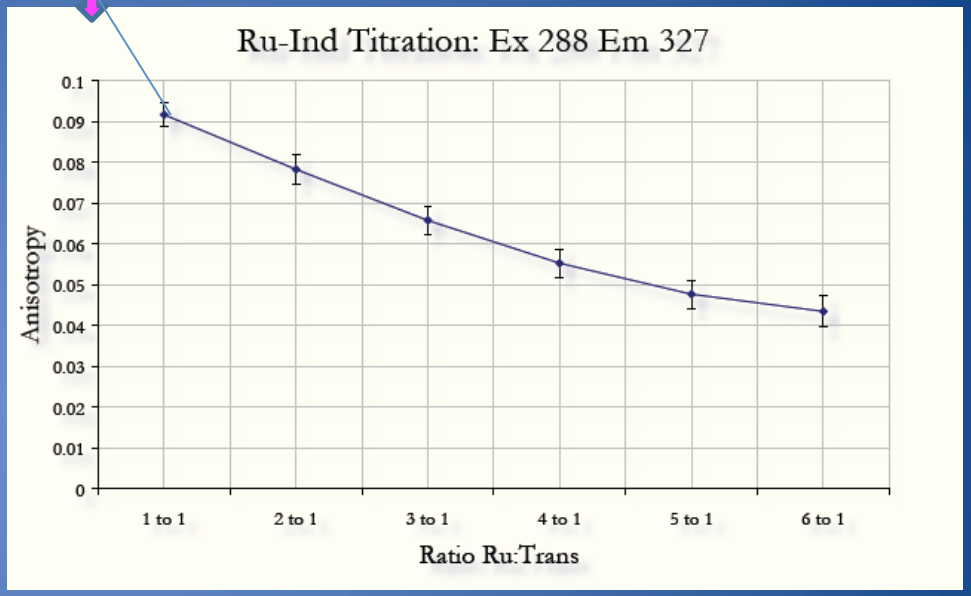
- Fe(III)-Tf shows a very low polarization of W indicating free rotation from dominant fluorescent W248 consistent Fe binding closes metal binding cleft but opens Tf overall conformation

- Removal of Fe changes Tf polarization to much higher value, suggesting burying of W248 in apoTf



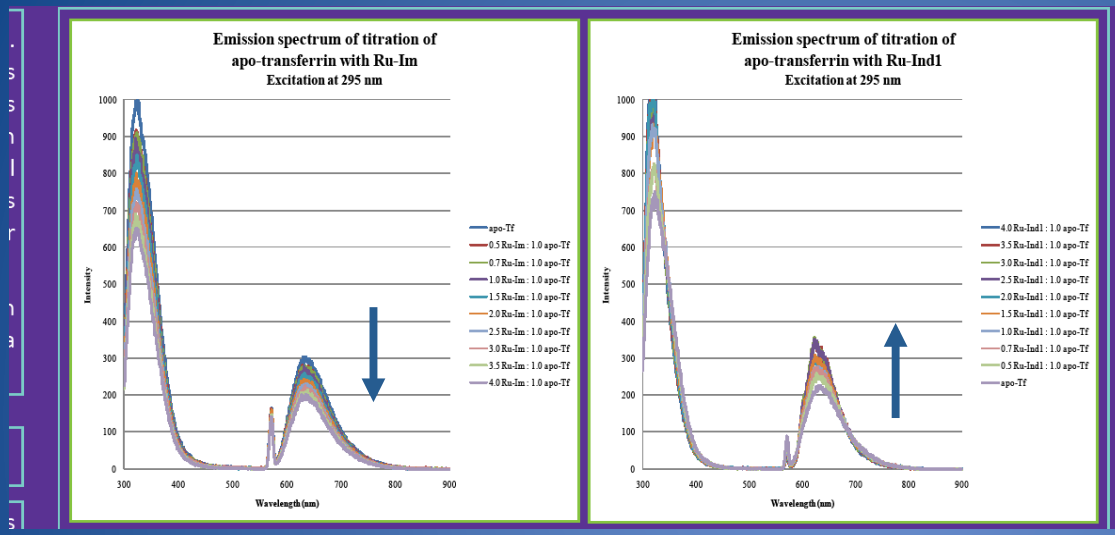
Ru(III) Indazole with apoTf

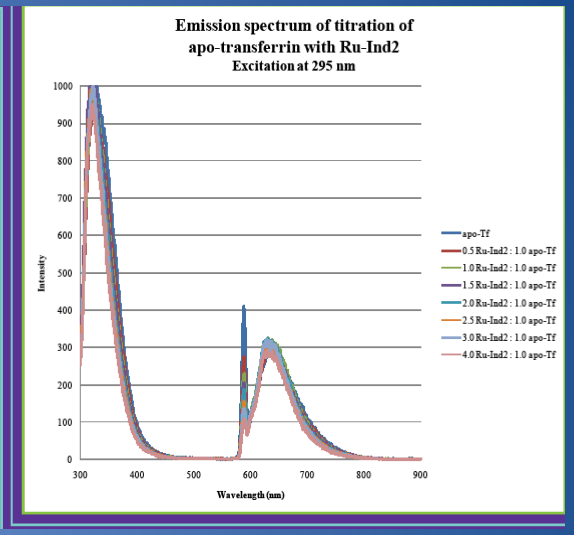


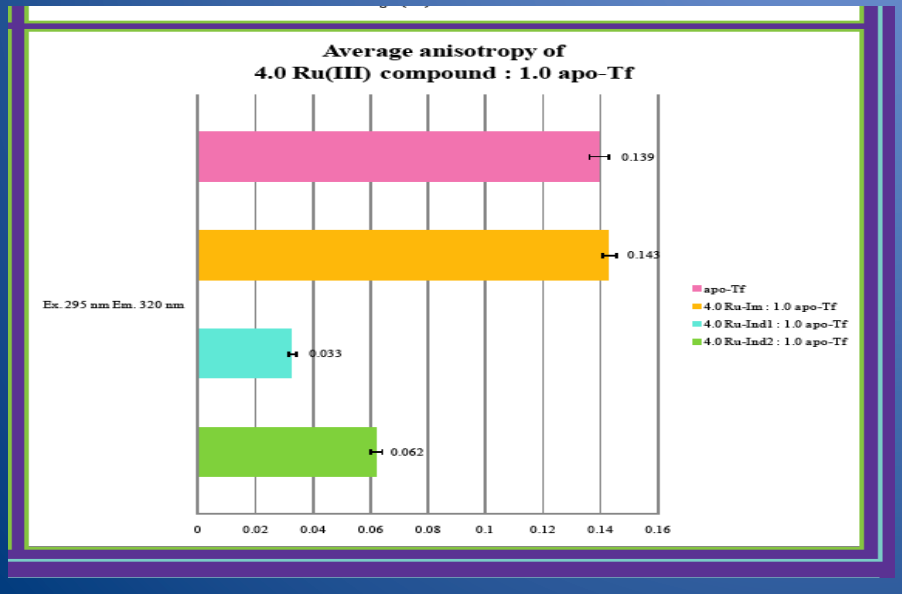


- $[Ru(III)(In)_2Cl_4]^{-1}$ binding to apoTf
  - intensity quenches
  - anisotropy drops
  - binding not as strong as Fe(III)

Transferrin fluorescence + ruthenium compounds







Tf fluoresces at 320 nm when excited at 295 nm, anisotropy 0.139

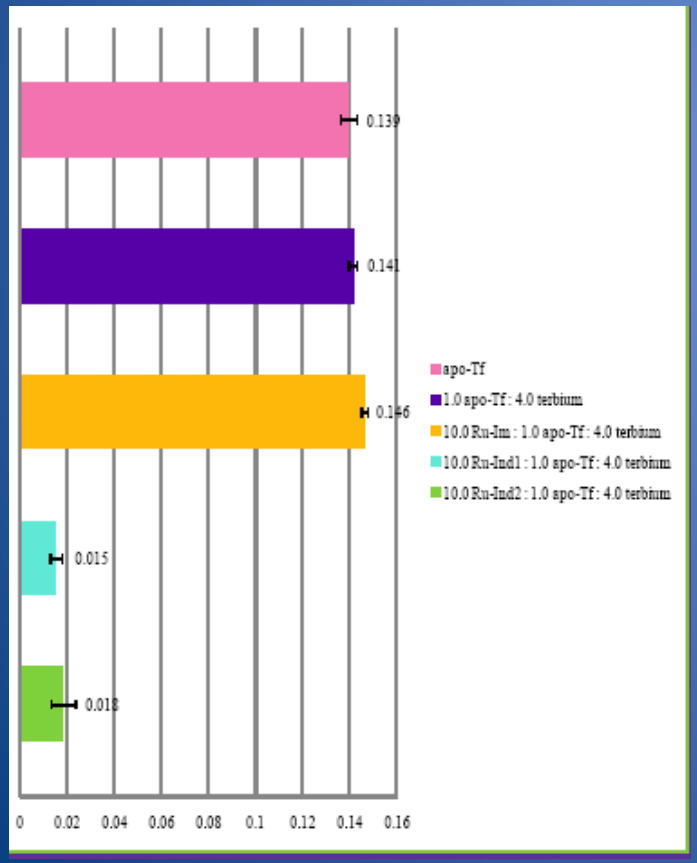
+ $[Ru(im)]^{+3}$, slight intensity quench, anisotropy unchanged (0.143)

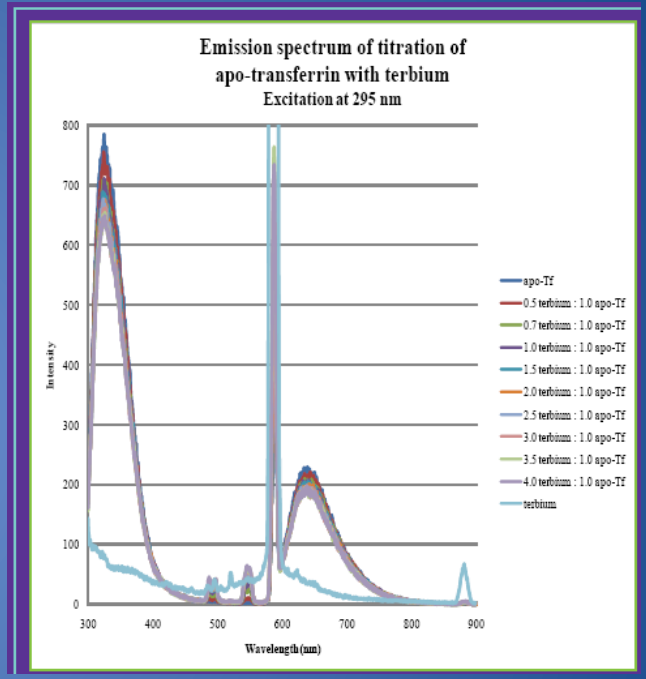
+$[Ru(in1)]^{+3}$, slight intensity increase, anisotropy decreases to 0.033

+$Ru(in2)^{+3}$ intensity, down, then up anisotropy decreases to 0.062

Anisotropy of Terbium-Tf + Ru(III) complexes

Protein Anisotropy 295x/320m

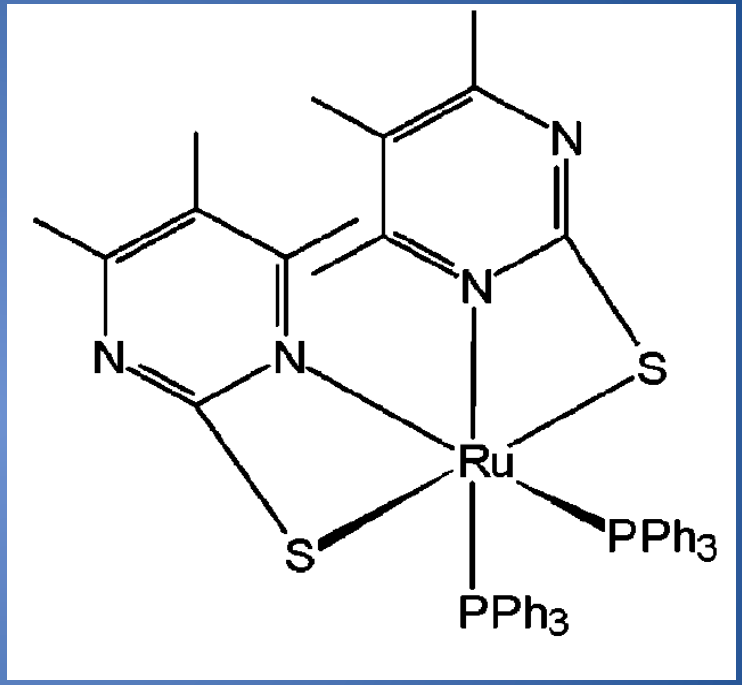




- Tb(III) binding does NOT cause drop anisotropy
- Anisotropy quenched with Fe(III) and Ru(III)$(In)_2$
- Anisotropy NOT quenched with Ru(III)$(Im)_2$
- Ru(III)$(In)_2$ and Tb(III) bind simultaneously to Tf

Cini Ru(III) compounds

- Phosphines show selective cytotoxic and anticancer properties
- Thiopyrimidines possess antiviral and photochemical properties that may prove useful in the design of new photodynamic cancer therapies
- Thiopurines are currently used as antileukemic and antiviral agents



- **Ru-847**

Cis-[$Ru(PPh_3)_2(TPYM)_2$]
PPh_3= triphenylphosphine,
TPYM= 2-thio-1,3, pyrimidine
MW= 847.9

What is the chemo-strategy?

- FIRST, a tangent: Siena, Italy has one of the oldest, craziest horse races in the world
- Each July, over 60,000 people gather to see ten horses and riders each representing a particular neighborhood in Siena run this dirt track.
- Riders don't use saddles or have stirrups
- Horses don't need their riders to win.

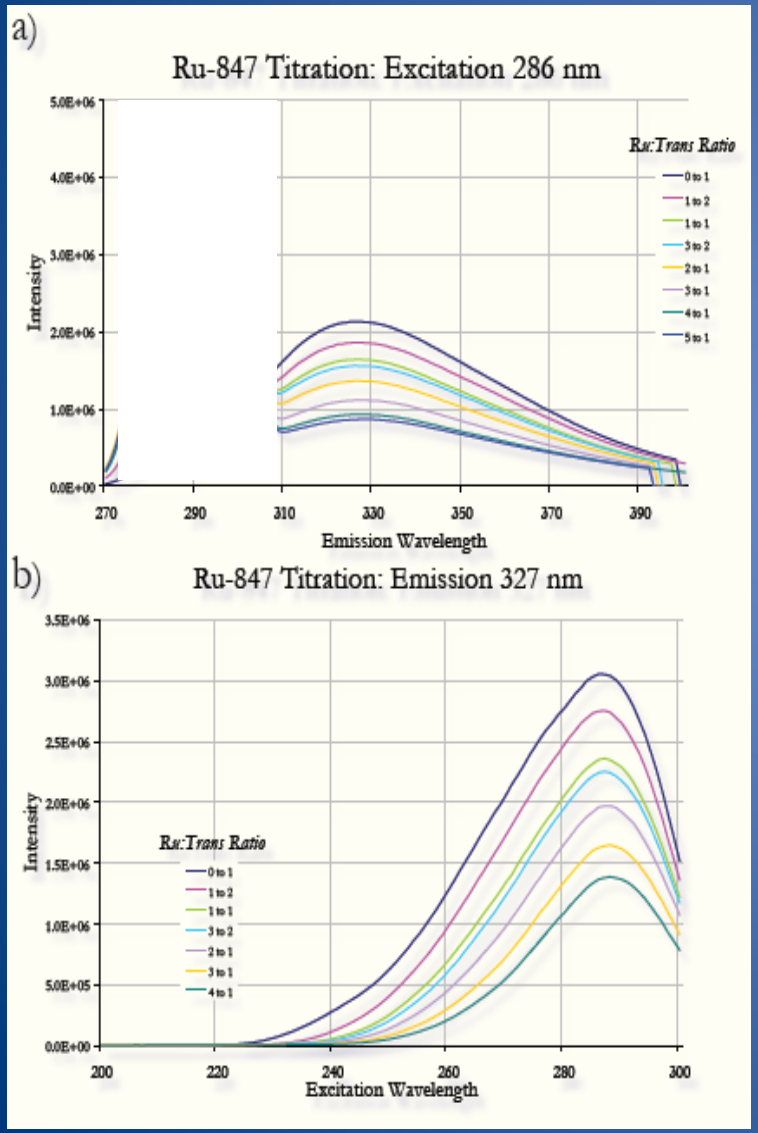
What is the chemo strategy?

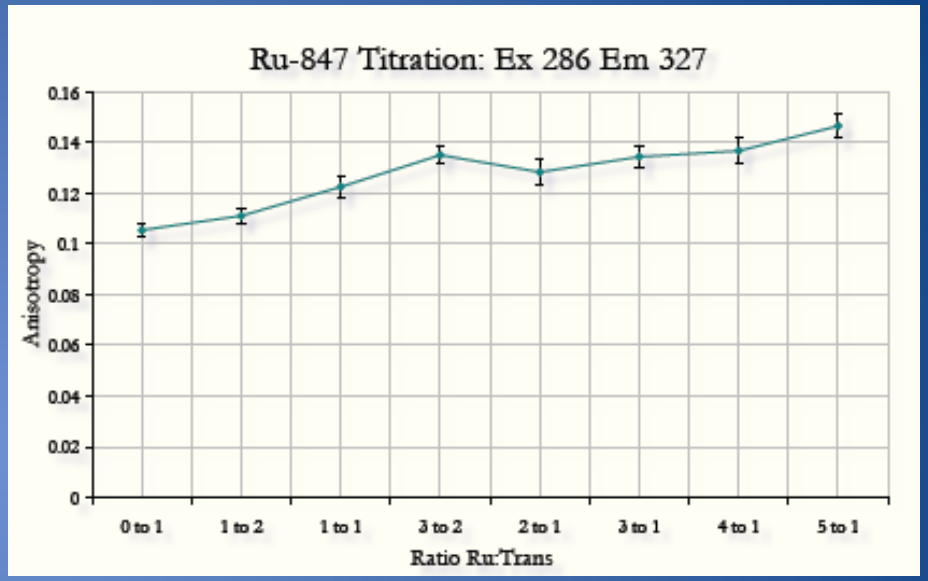
- Upsetting your rival rider from its horse is legal.
- Goal is to have a tight grip on your horse at the start, and an ability to dismount quickly

What is the chemo-strategy?

- This wooden horse stands at the entrance to a church in Siena, Italy
- We can imagine the transferrin, like the horse, might be able to carry a Ru (III) molecule (the rider) to a cancer cell
- Once at the cell, the transferrin releases the Ru (III) which gets reduced to Ru(II) kills the cancer cell.

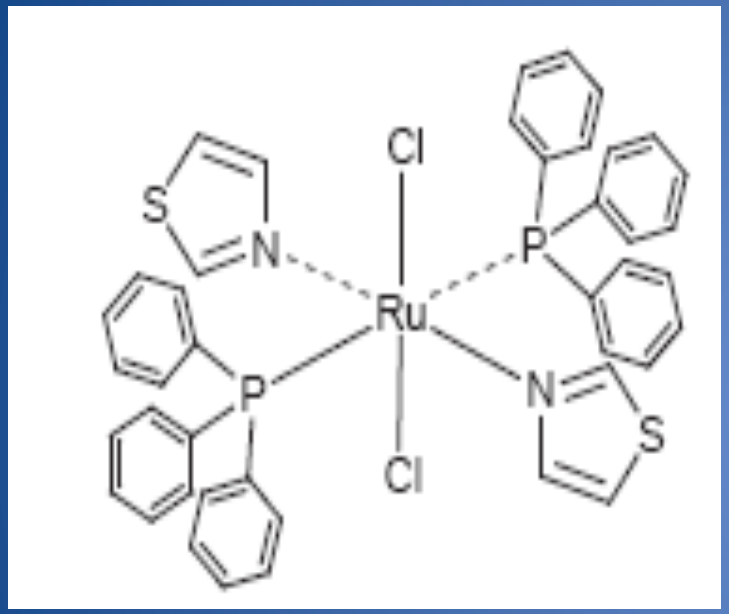
Ru(III)-847 with apoTf

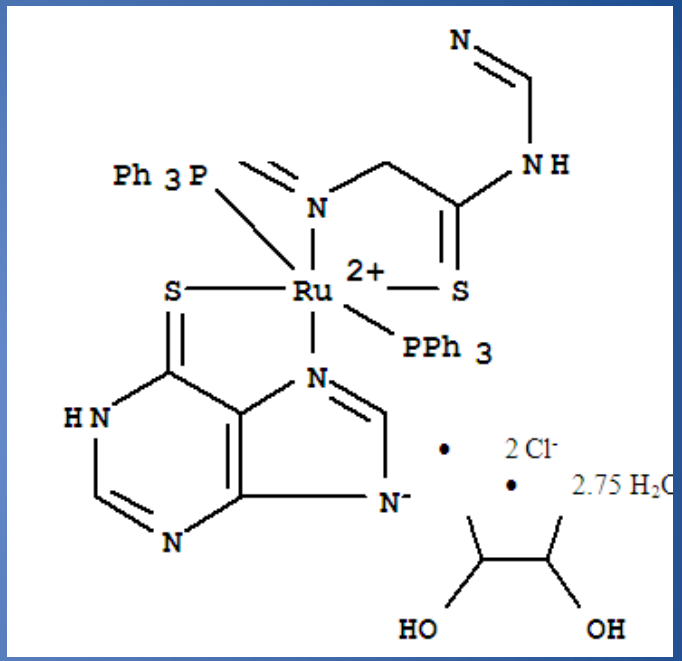




- Ru(III)-847 binding to apoTf
  - intensity quenches (no shift)
  - weak binding, not specific
  - anisotropy does not decrease

More Cini Ru(III) compounds



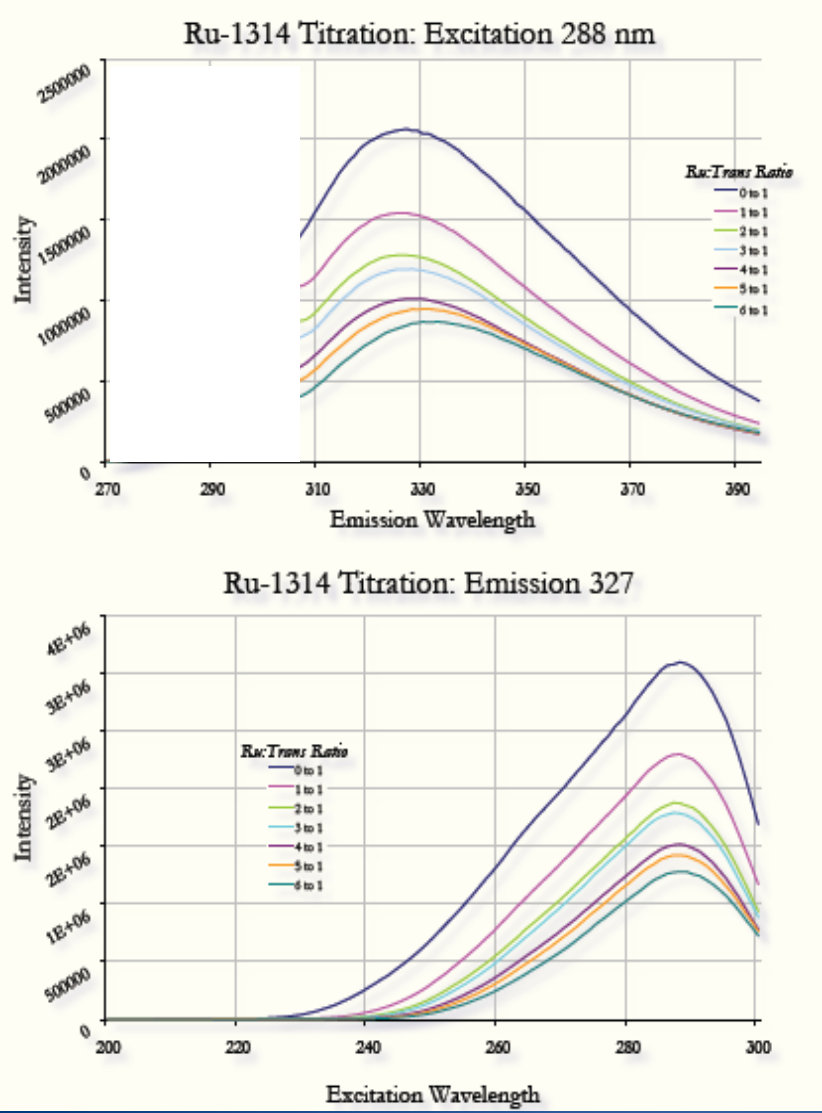


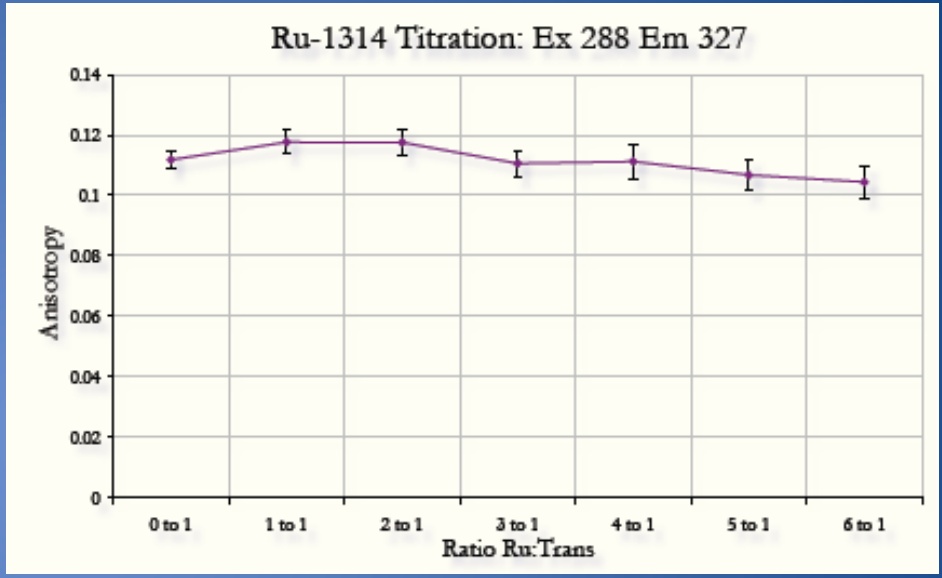
- **Ru-866**

Trans, trans, trans-[$RuCl_2(PPh_3)_2(THZ)_2$]
THZ= thiazole-1,3
MW= 866.8 g/mole

Ru-1314 **$Ru(PPh_3)_2(HTPR)_2]Cl_2$**
HTPR=6-thiopurine-riboside
MW = 1314. g/mole

Ru(III) 1314 with apoTf





- Ru(III)-1314 binding to apoTf
  - intensity quenches
  - anisotropy does not change
  - weak non-specific binding

Cellular uptake of Ru(III) into cancer cells

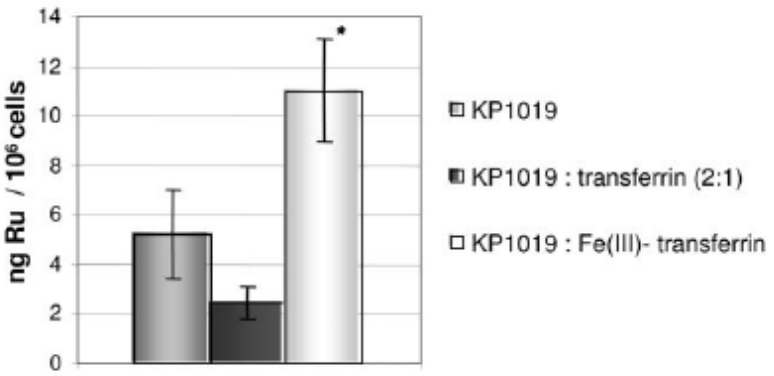


Fig. 4 The cellular uptake of ruthenium into the human colon carcinoma cells SW480 exposed to KP1019, KP1019 bound to human apotransferrin (2:1) and KP1019 bound to Fe(III)-loaded transferrin (1:1). In every case, the concentration of KP1019 was 5 μM. Each data point is the mean ± SD of at least three separate experiments. *Significantly different from KP1019, $p = 0.021$ and from KP1019: transferrin, $p = 0.003$ (unpaired t-test).

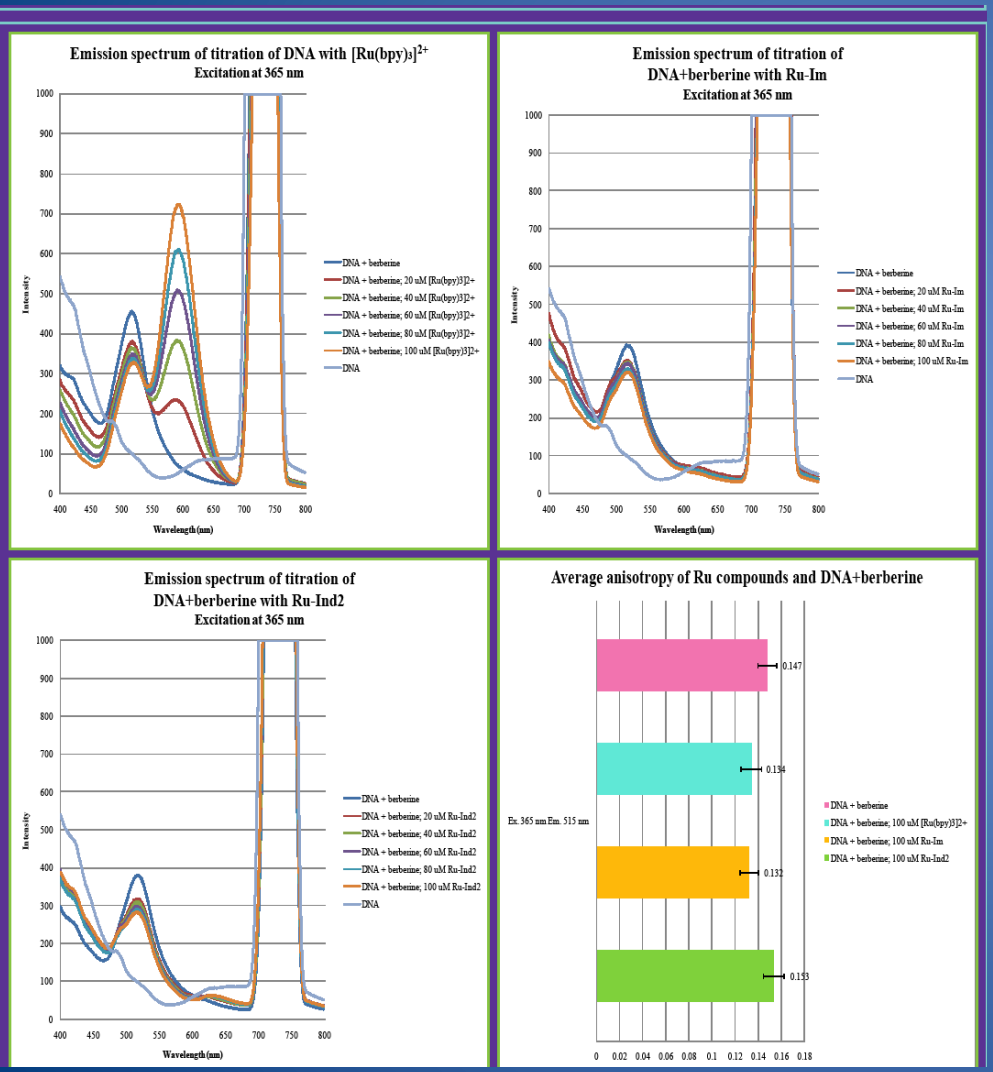
- Ru(III) complexes (KP1019) are better transported into cells when transferrin contains Fe(III)
- Suggests Tf is able to carry BOTH Fe(III) and Ru(III)

Transferrin binding and transferrin-mediated cellular uptake of the ruthenium coordination compound KP1019, studied by means of AAS, ESI-MS and CD spectroscopy†

Martina Pongratz,[a] Petra Schluga,[a] Michael A. Jakupec,[a] Vladimir B. Arion,[a] Christian G. Hartinger,[a] Günter Allmaier[b] and Bernhard K. Keppler*[a]

JAAS
www.rsc.org/jaas

DNA*Berberine + Ru(III)



Berberine is a fluorescent DNA dye that reports on DNA structure

DNA* Berberine emission max 525 nm/365 nm excite, anisotropy 0.147

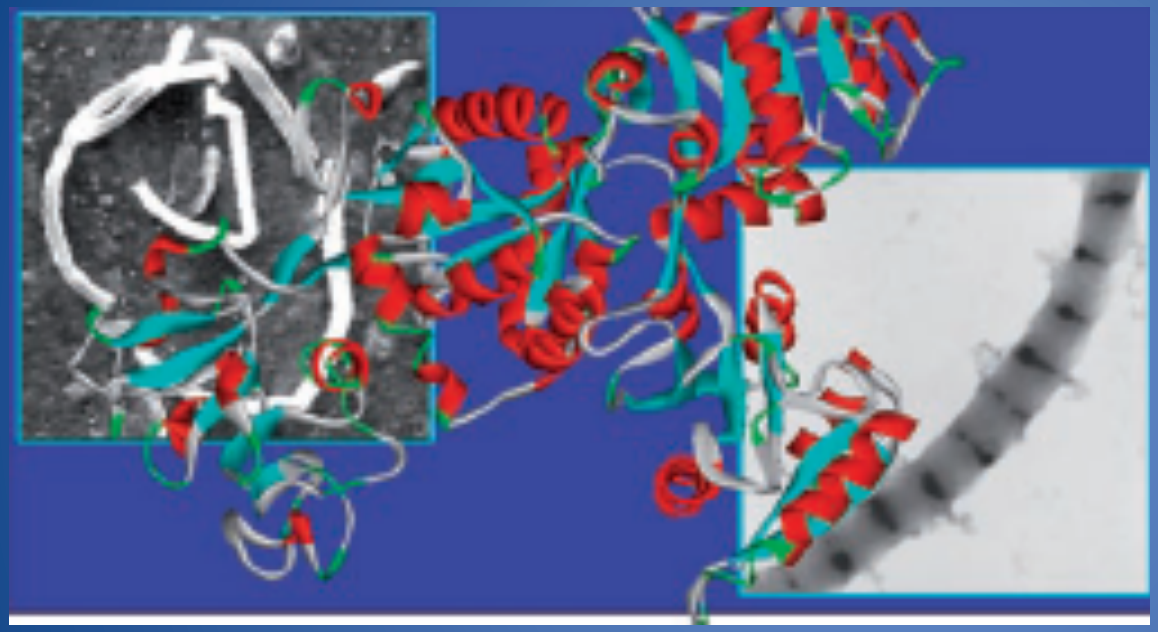
+ $[Ru(bpy)_3]^{+2}$ shows slight berberine quenching, anisotropy 0.134

+ $Ru(Im)_2$ shows slight berberine quenching, anisotropy 0.132

+ $Ru(In)_2$ shows no berberine quenching, anisotropy 0.153

Periodic Iron Nanomineralization in Human Serum Transferrin Fibrils**

Surajit Ghosh, Arindam Mukherjee, Peter J. Sadler, and Sandeep Verma**



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How the Brain Rusts:

Researchers at the University of Warwick and the Indian Institute of Technology Kanpur have discovered that the mechanism that we rely on to transport iron safely through our blood stream can, in certain circumstances, collapse into a state which grows long worm-like "fibrils" banded by lines of iron rust. This process could provide the first insight into how iron gets deposited in the brain to cause some forms of Parkinson's & Alzheimer's and Huntington's diseases.

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