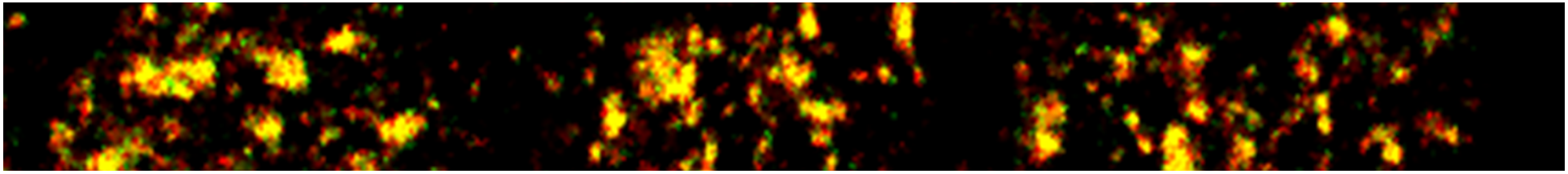

Novel *In Vivo* Imaging Approaches to Measure Target Engagement in Pre-Clinical Research

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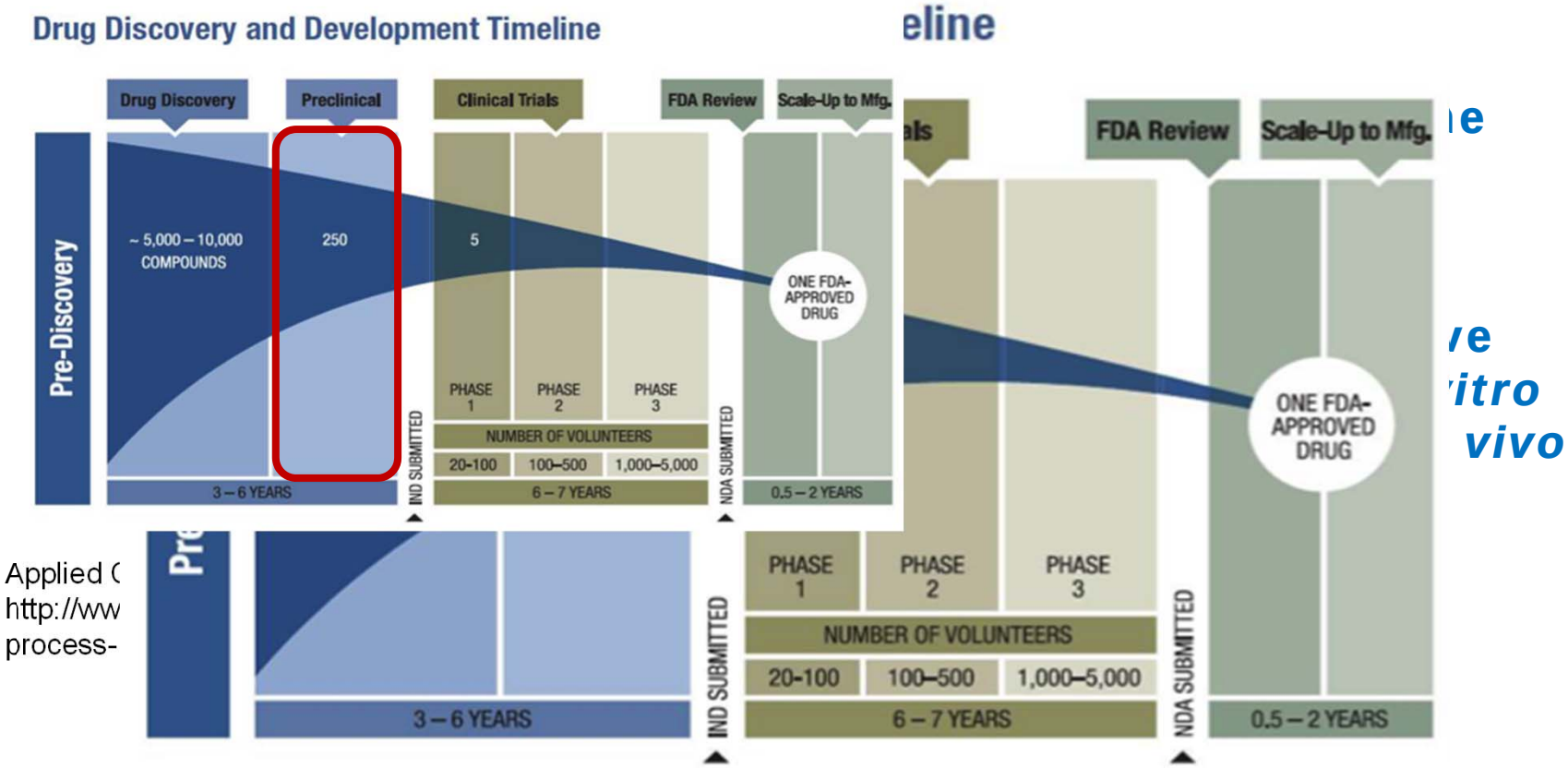
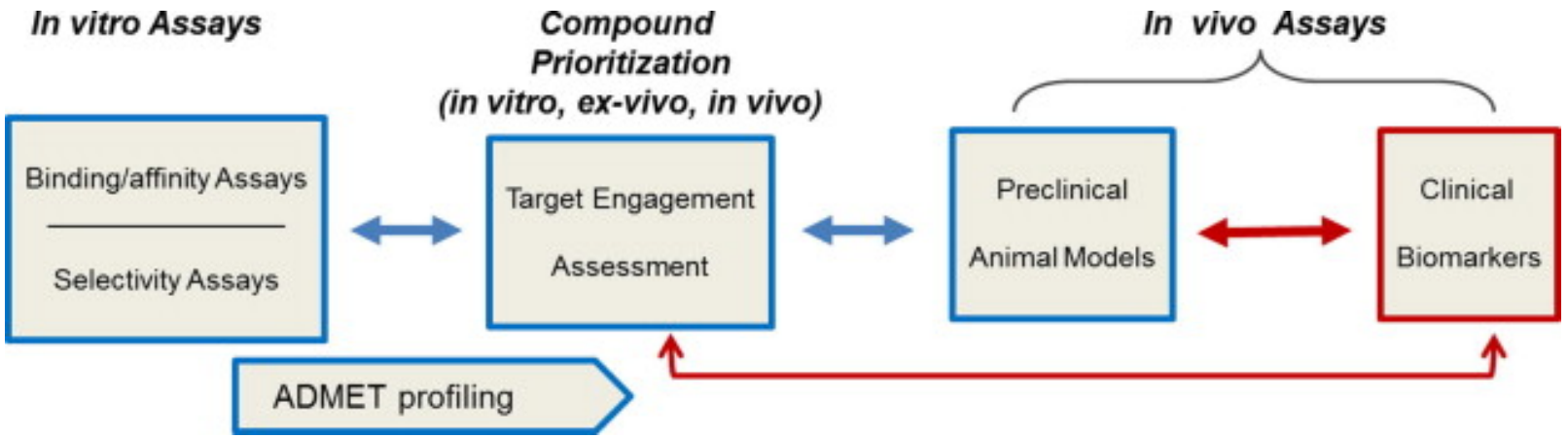
Accelerate pre-clinical drug discovery by developing novel molecular optical imaging assays

Our main focus is to develop imaging assays to quantitate target engagement of anti-cancer agents in pre-clinical research



Target engagement in drug screening



Develop optical imaging whole body assays to perform target engagement assessment in vivo in pre-clinical animal models

Bioorganic & Medicinal Chemistry Letters, Volume 25, Issue 5, 2015, 998–1008

Targeted delivery of anti-cancer drugs

- **Traditional cancer chemotherapy is** currently used when rapid disease control is required or upon the development of tumor resistance to targeted functional therapies.

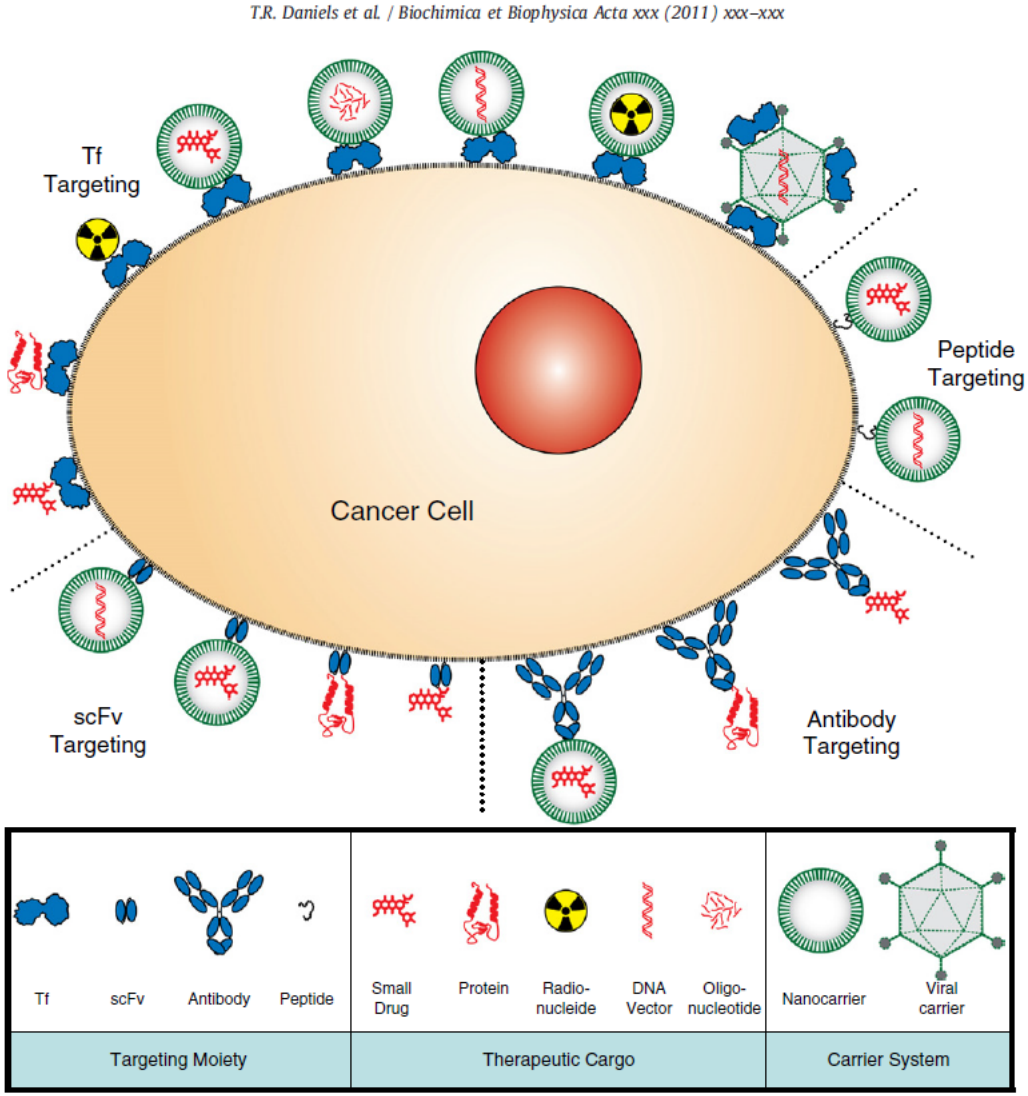
- However, chemotherapy generally leads to harmful side effects and drug resistance, thus warranting the development of **targeted therapy in which drugs or antibodies are specifically delivered to cancer cells.**

- **Targeted therapy** is potentially more effective than radiation or traditional chemotherapy since it:
  - specifically delivers drugs or antibodies to cancer cells
  - keeps drugs away from healthy cells
  - reduced toxic side effects of drugs
  - Better tolerated by cancer patients

Many anti-cancer therapies in development target the transferrin receptor (TfR)

- **The transferrin receptor (TfR)** functions in cellular iron uptake via interaction with its native ligand, the iron-bound **transferrin (Tf)**
- TfR is upregulated and efficiently internalized into in cancer cells
- TfR has been widely used as a **target for molecular imaging**
- **Tf** has been used as a **carrier for anti-cancer drugs** in targeted therapy

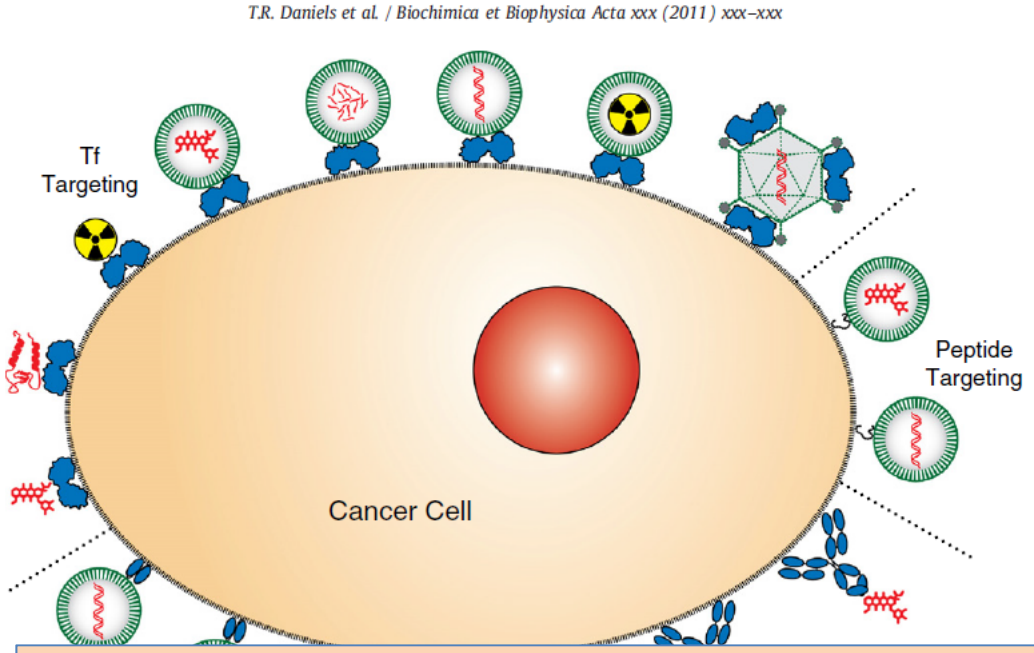
TfR and targeted delivery of imaging or cytotoxic agents



TfR targeting:

- Effective in delivering many therapeutic agents that can cause cytotoxic effects in cancer cells in *vitro* and *in vivo*.

TfR and targeted delivery of imaging or cytotoxic agents



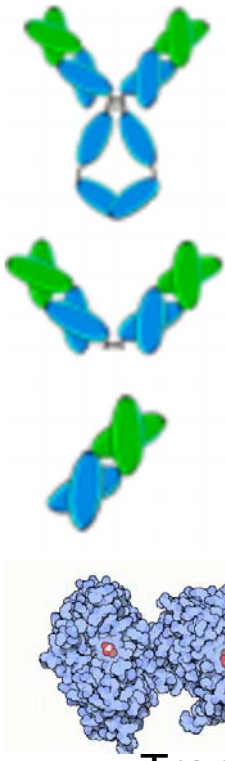
TfR targeting:

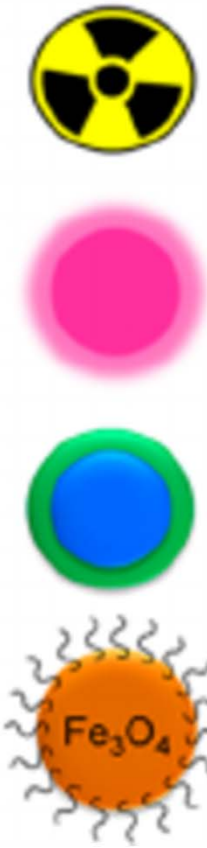
- Effective in delivering many therapeutic agents that can cause cytotoxic effects in cancer cells in *vitro* and *in vivo*.

- ***However, there is no FDA-approved Tf-based drug delivery system (several in phase I/II trials)***
- ***Optimization of TfR-Tf targeting and delivery is needed***

Construction of protein ligand-based or antibody-based imaging agents

Targeting protein	Label	Imaging Modality
Full Antibody	Radioisotope	PET, SPECT
$F(ab')_2$	Fluorescent Dye	Optical, PA
Fab'	Quantum Dot	Optical, PA
Transferrin (Tf)	Fe_3O_4 Iron Oxide NP	MRI

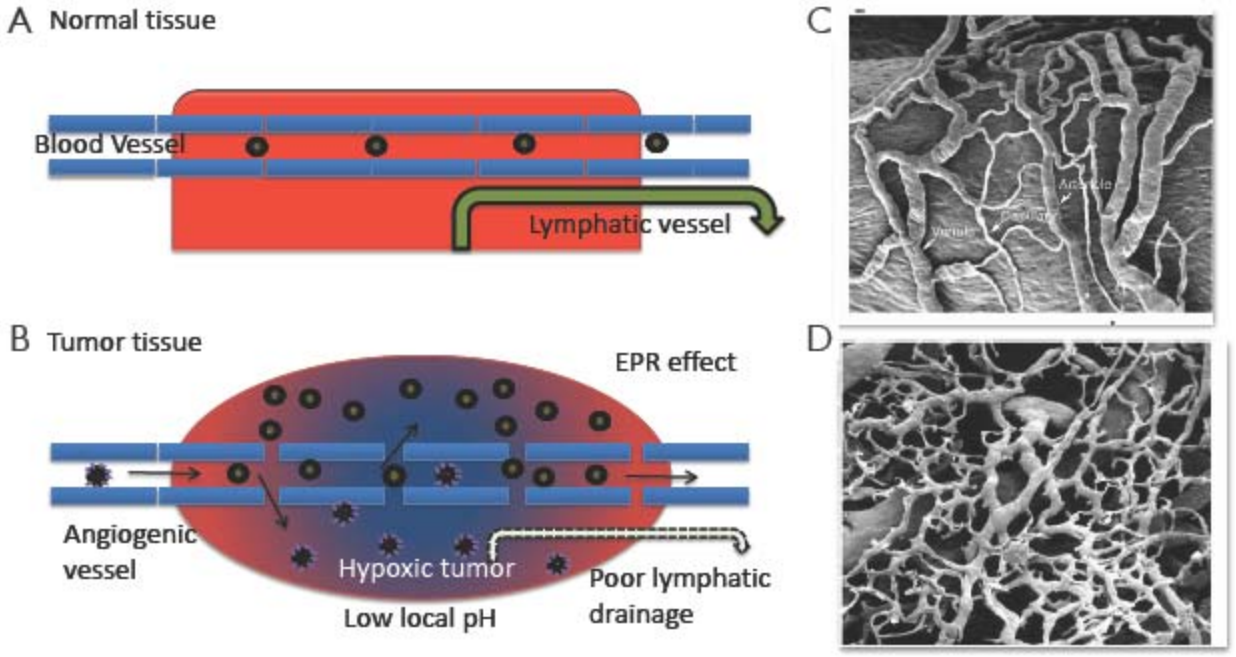




Challenges in imaging targeted therapy

- Due to the enhanced permeability and retention effect **(EPR)**, labeled ligands/drugs accumulate at the tumor region.
- Currently, only invasive biochemical methods can be used to assess ligand or antibody-receptor binding (i.e. **target engagement**) in tumors.
- Thus, **non-invasive imaging methods** are needed to quantitate drug-receptor binding and uptake into tumors in live animals by discriminating between:
    - Soluble ligand, receptor-independent passive tumor accumulation (**EPR effect**)
    - Drug-receptor binding and uptake into tumors (**Target engagement**)

Challenges in imaging targeted therapy



Challenges in imaging targeted therapy

- Ability to non-invasively monitor and **target engagement**, i.e. binding and internalization of drug-ligand or antibody conjugates, into targets within *live subjects*
  - uncertainty due to the **EPR** effect
  - the only way to assess if internalization has occurred is via **invasive**, destructive *ex-vivo* analysis

Fluorescence lifetime Förster Resonance Energy Transfer (FRET)

- Ability to non-invasively **quantify fluorescence signals** through *living tissues* in small animal models
  - high degree of **autofluorescence**
  - poor signal penetration **depth** through biologically heterogeneous tissues

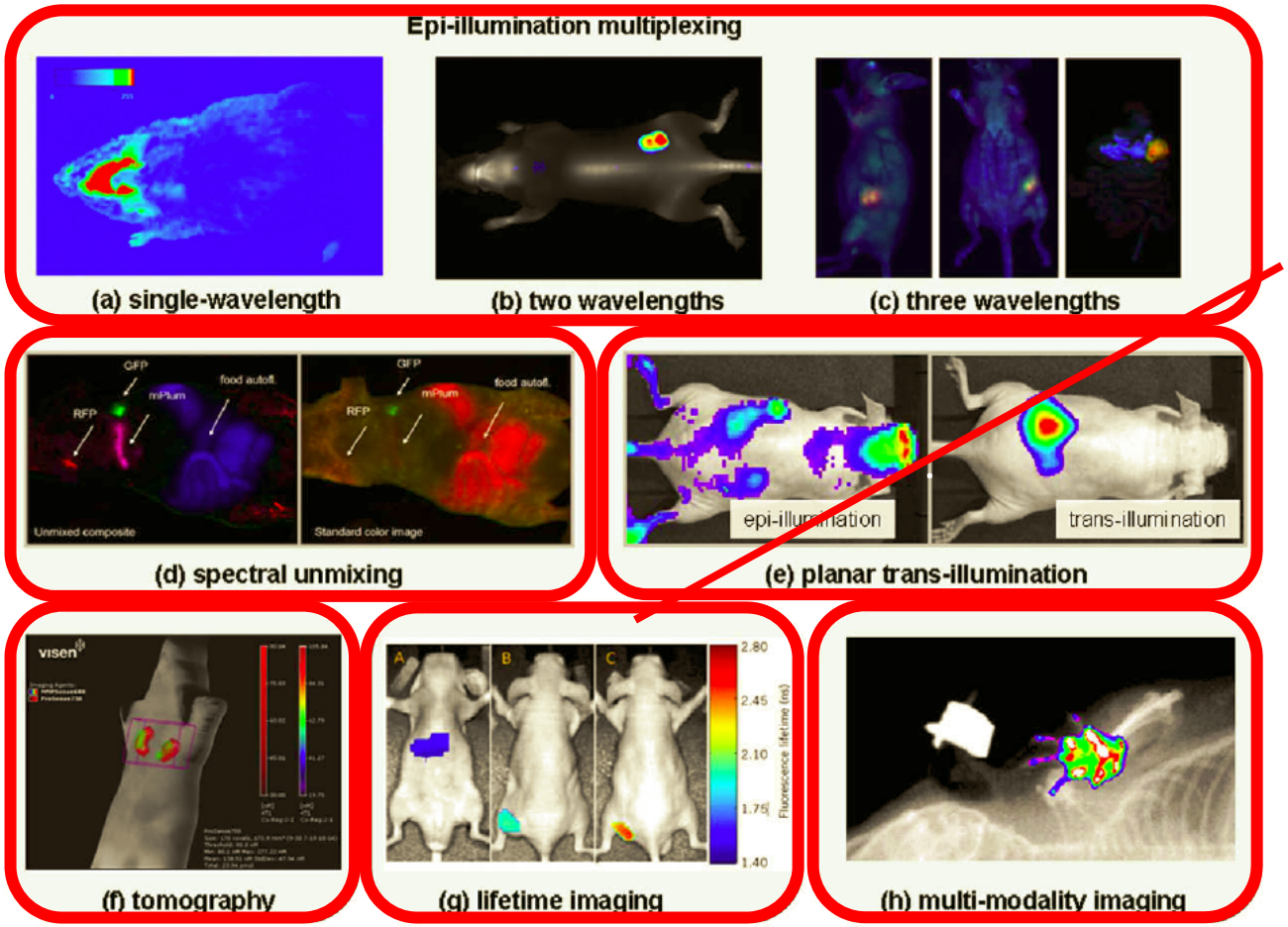
Near infrared (NIR)

Main goal

- To establish **macroscopy fluorescence lifetime FRET (MFLI-FRET)** as the gold standard to **quantitate target engagement in pre-clinical small animal models of breast cancer:**
  - **Across microscopy and macroscopy**
  - **Across visible and NIR ranges**
  - **Across *in vitro* and *in vivo* approaches**

➢ Perform optical imaging of living thick tissue using a whole-body wide-field time-resolved imager to measure **NIR MFLI-FRET *in vivo* (Collaboration with X. Intes, RPI)**

Preclinical molecular optical imaging

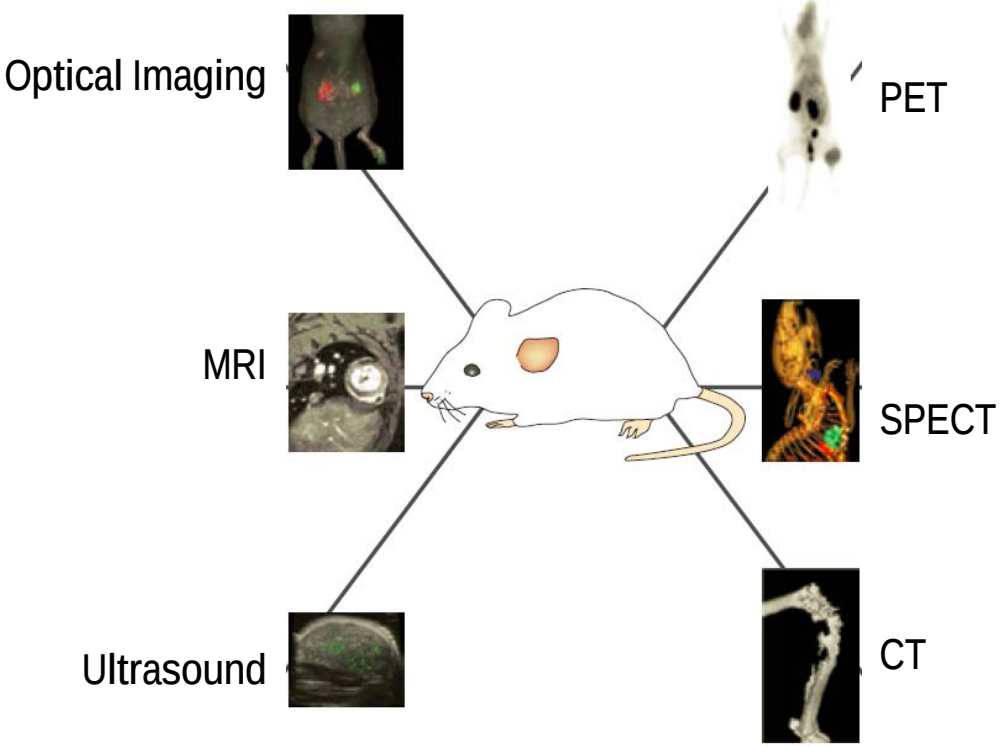


- Microenvironment (O_2, pH, glucose, ions)
- FRET

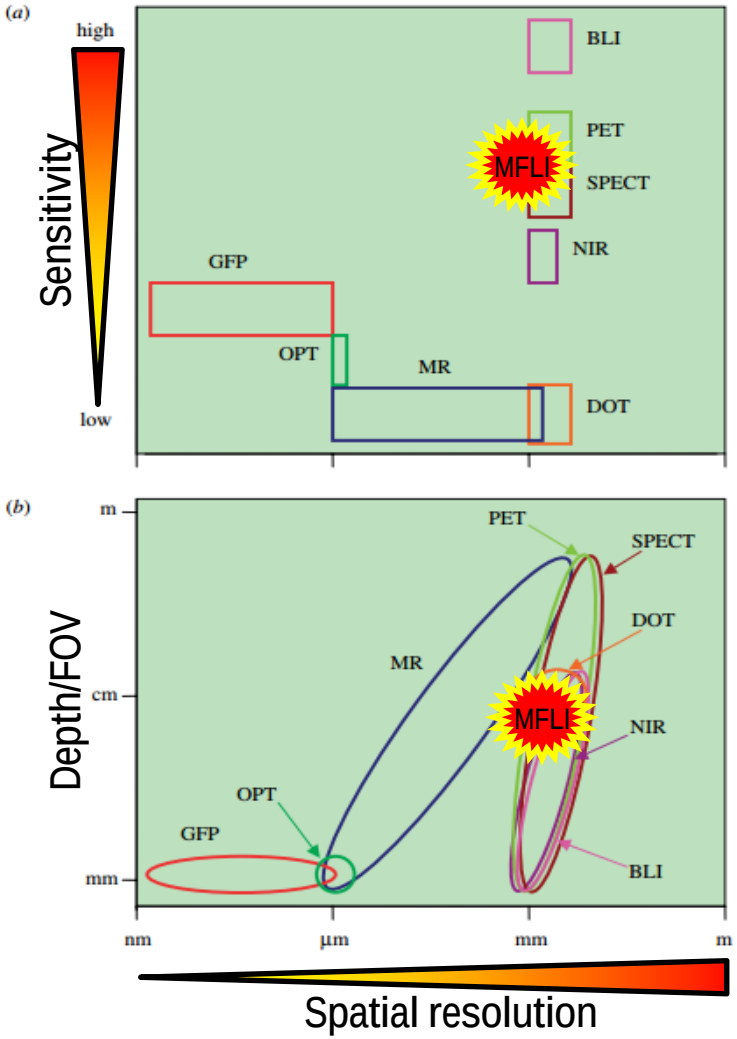
(Leblond *et al*. 2010. J. Photochem. Photobiol. B)

Preclinical applications: optical imaging

Preclinical imaging can early identify potential drug candidate



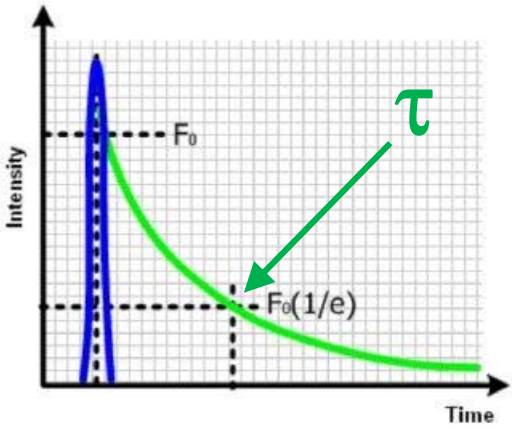
(Gambhir *et al*. 2008. Nat. Rev. Drug Discov.)



(Cassidy. 2005. J. R. Soc. Interface.)

Novel Molecular Optical Imaging: Lifetime (τ) based imaging

- **Lifetime** is an intrinsic characteristics of fluorophore
- Additional information to fluorescence intensity
- Lifetime is independent on concentration

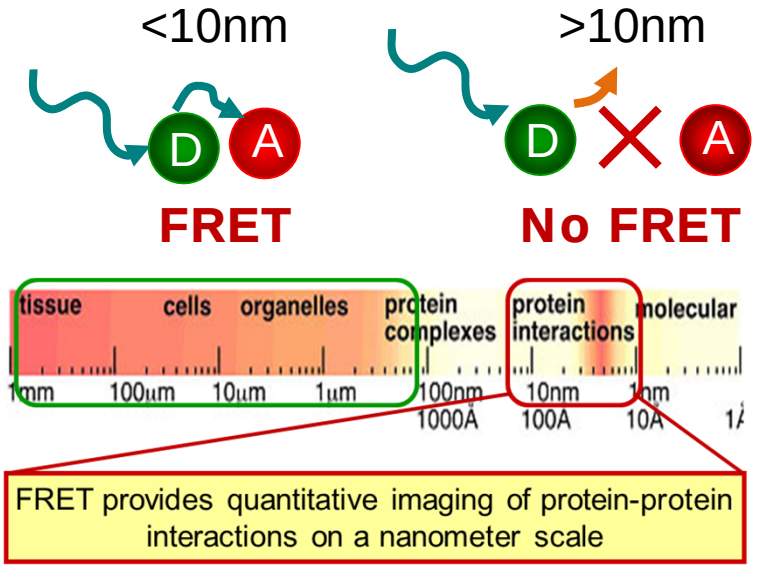


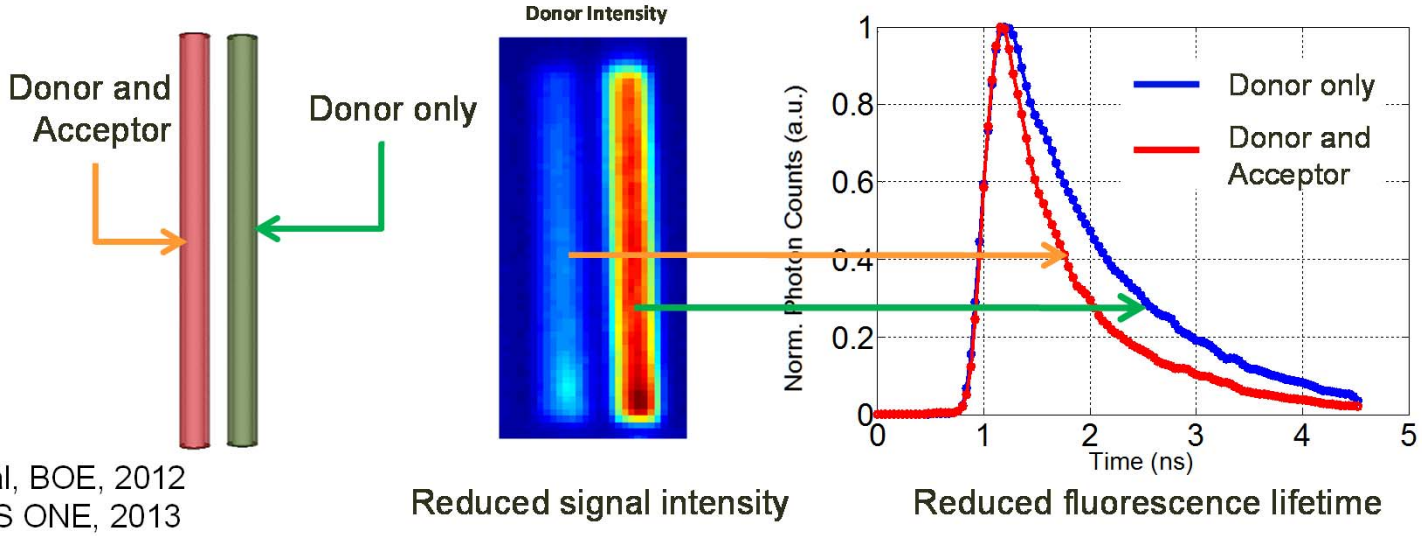
- Lifetime is minimally affected by optical properties
- Mainly used in microscopy to provide:
  - Increased multiplexing power
  - Sense the tissue and cellular microenvironment (pH, temperature, viscosity, analytes concentration, O_2)
  - Nanoscale protein-protein interaction assays (FRET)

Förster Resonance Energy Transfer (FRET) using fluorescence lifetime imaging

FRET :

- Non-radiative energy transfer between **donor (D)** and **acceptor (A)** fluorophores, when distance between donor and acceptor <10nm.
- Fluorescence **lifetime of donor** will be **shortened**
  - **More robust than fluorescence-intensity based FRET**

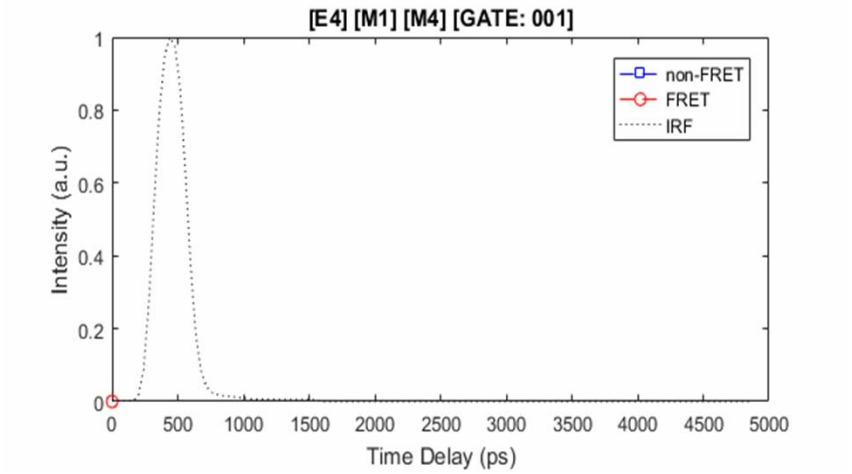




Venugopal et al, BOE, 2012
Abe et al, PLoS ONE, 2013

Wide-field MFLI and FRET

Lifetime-based FRET quantification and FRETing Donor Fraction (FD%)



Bi-exponential decay model:

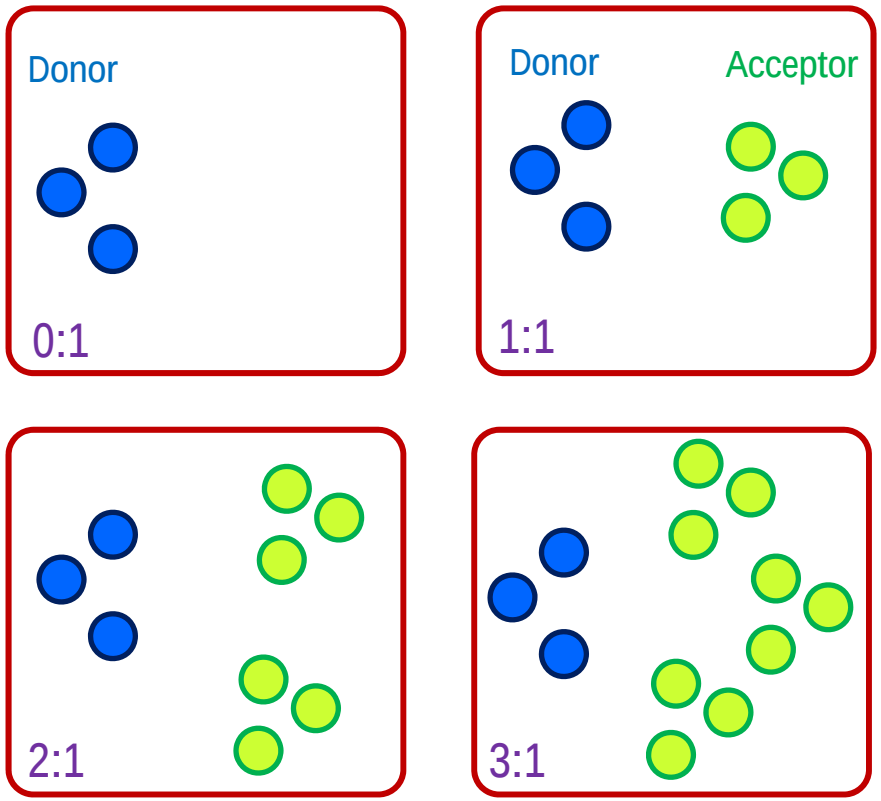
$$I(t) = IRF \otimes (\underbrace{A_1 \cdot e^{-\frac{t}{\tau_1}}}_{\text{FRET}} + \underbrace{A_2 \cdot e^{-\frac{t}{\tau_2}}}_{\text{non-FRET}})$$

- A_1 and A_2 are relative amplitudes ($A_1+A_2 = 1$)
- τ_1 and τ_2 are lifetime values of the two species (known values)
- IRF: instrument response function

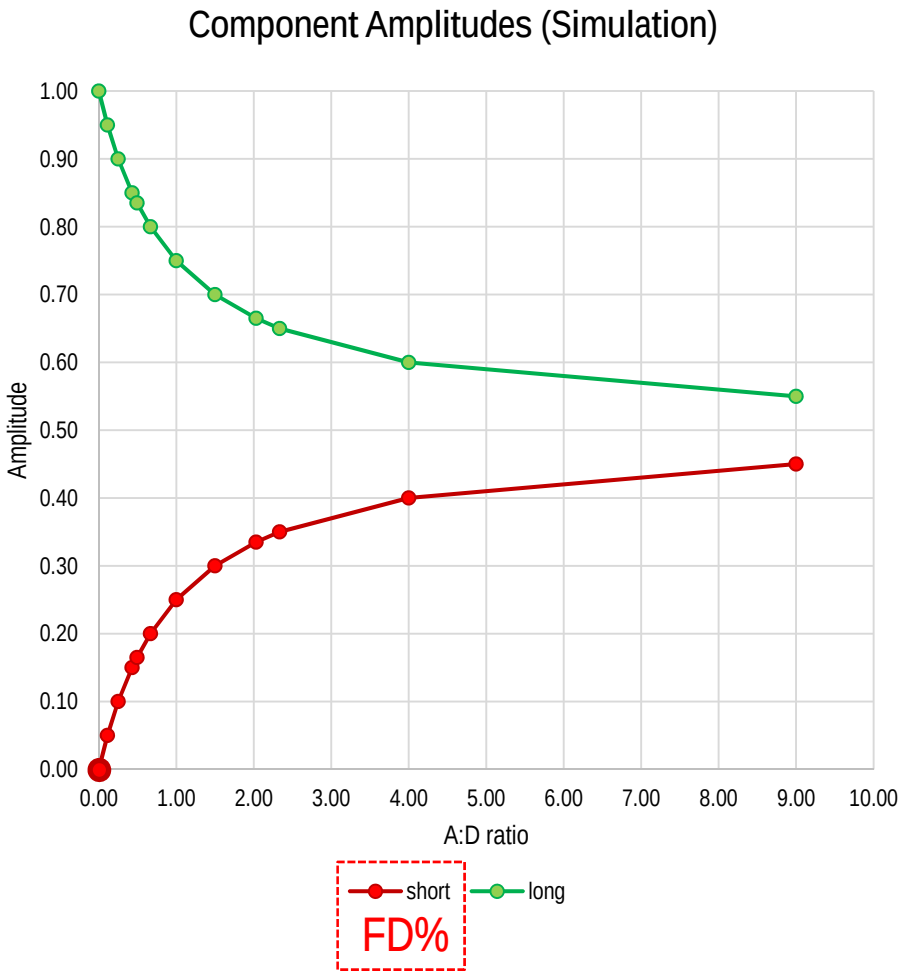
Quantitative FRET parameter = A_1 = FD%

Wide-field MFLI and FRET

Acceptor to Donor ratio (A:D)



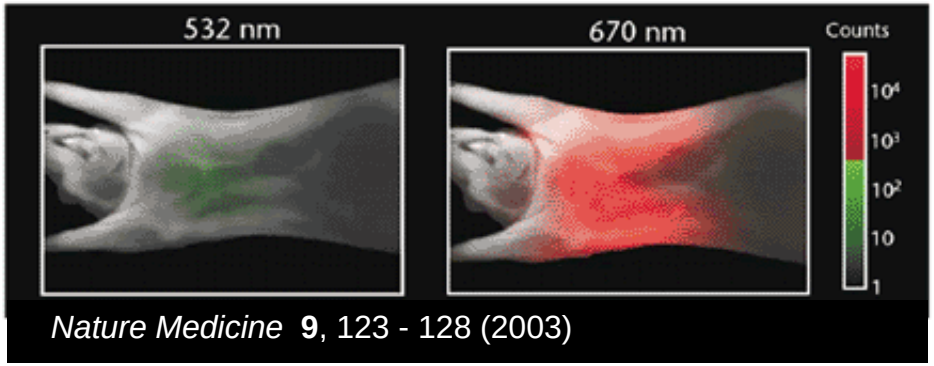
Donor amount is constant.



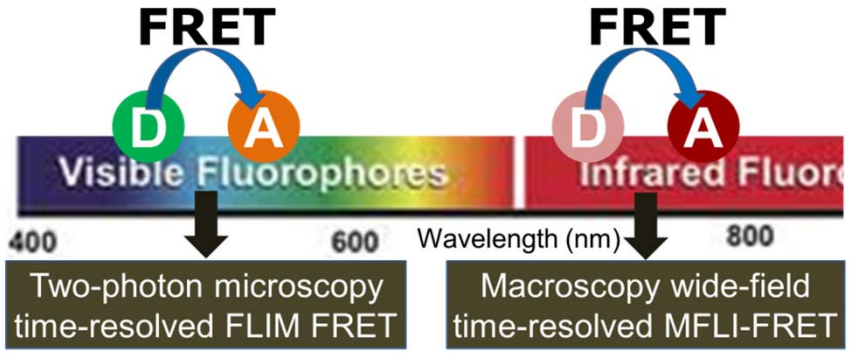
Red-shift FRET to measure target engagement in small animal *in vivo* imaging

Technical considerations

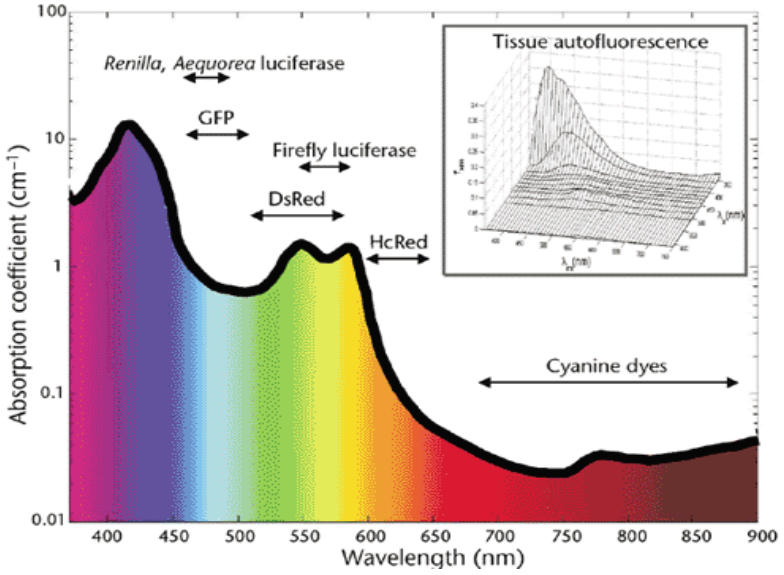
- *In vivo* FRET requires NIR FRET pair
- NIR allows transmission through live animal



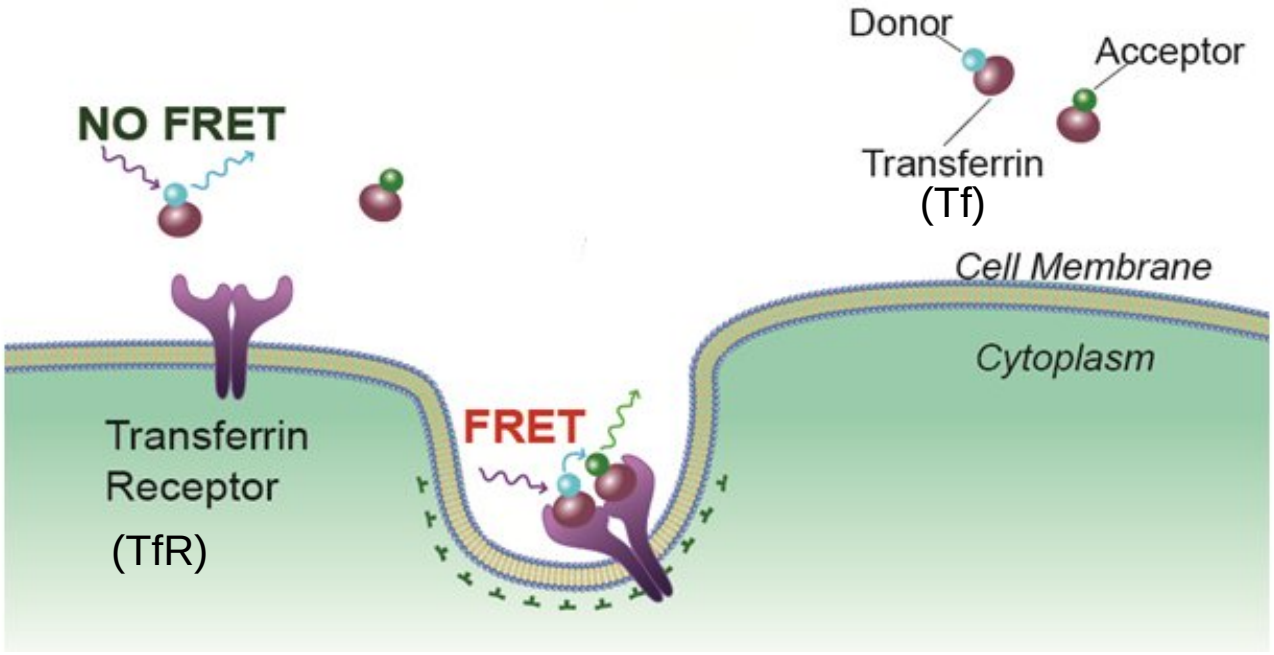
- Shorter lifetimes (300-10500ps)
- Need time-resolved wide-field imager
- Not available commercially



Absorption in the optical window



TfR-Tf Förster Resonance Energy Transfer (FRET) imaging assay



- **FRET** can measure TfR-Tf binding (target engagement), a crucial parameter for optimization of targeted therapy
- **Near infrared (NIR)**-labeled Tf permits deep tissue penetration and the non-invasive longitudinal application of FRET in living mice

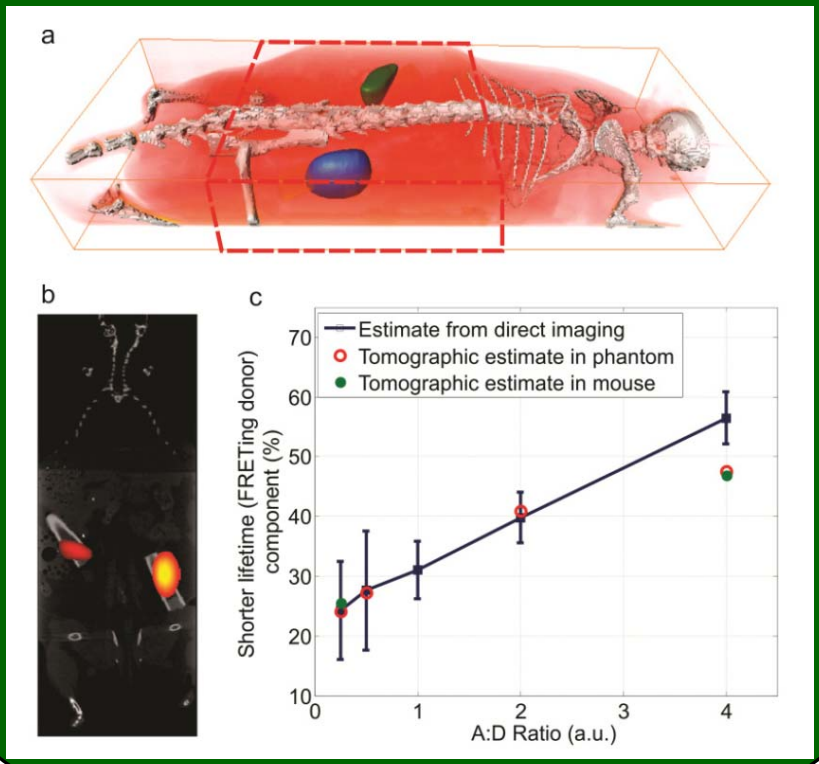
Macroscopic Fluorescence Molecular Tomography

- Whole-body small animal imaging **(~1.5-2cm thick)**
- Lifetime-based sensing
- Resolution **≥1mm**

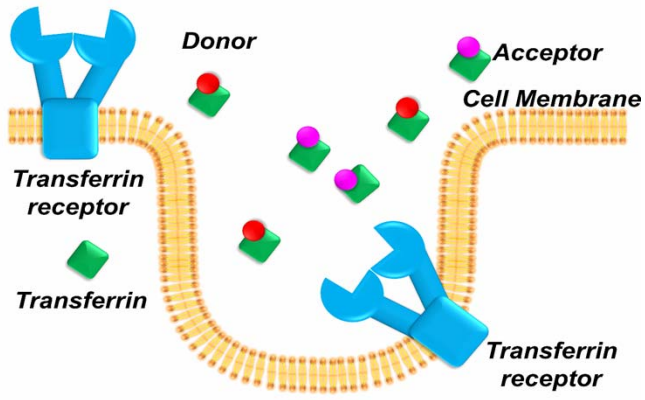
Preclinical imaging

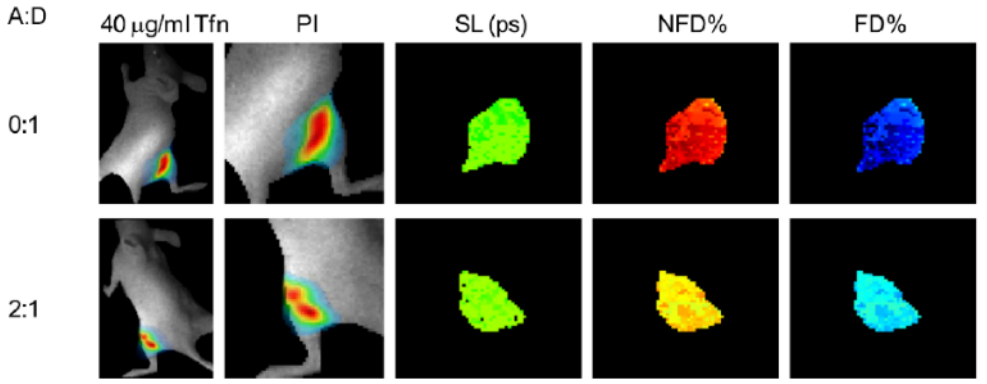
- Nude mice/tumor xenografts
- Molecular probes (NIR-Tf)
- *In vivo* FRET imaging
- Drug delivery assessment

Drug delivery Assessment



FRET: Protein-Protein Interaction





(Abe .et al, Plos one ,2013)

S Rajoria, Current Molecular Imaging, 3, 144-161 (2014).

Live small animal NIR MFLI-FRET imaging

Tumor xenograft creation

Collect ER+ T47D human breast cancer cells

Mix with Cultrex BME 1:1

Inject subcutaneously into mice

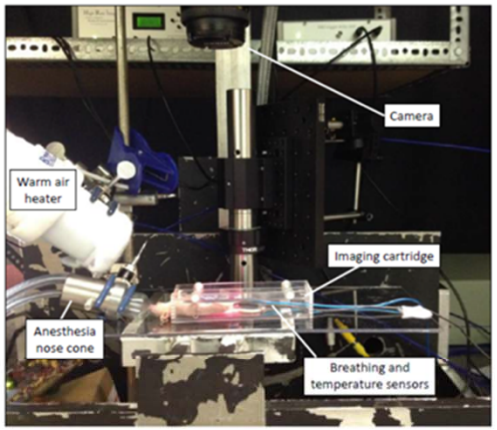
4-6 weeks till tumors reach volume

IV injection of NIR labeled transferrin (Tf)

AF700-Tf

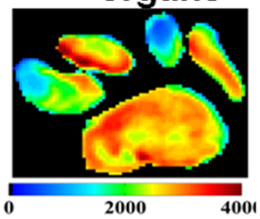
AF750-Tf

Live animal longitudinal imaging

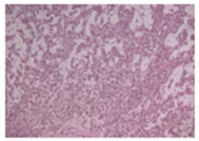


Ex vivo validation

Imaging of excised organs



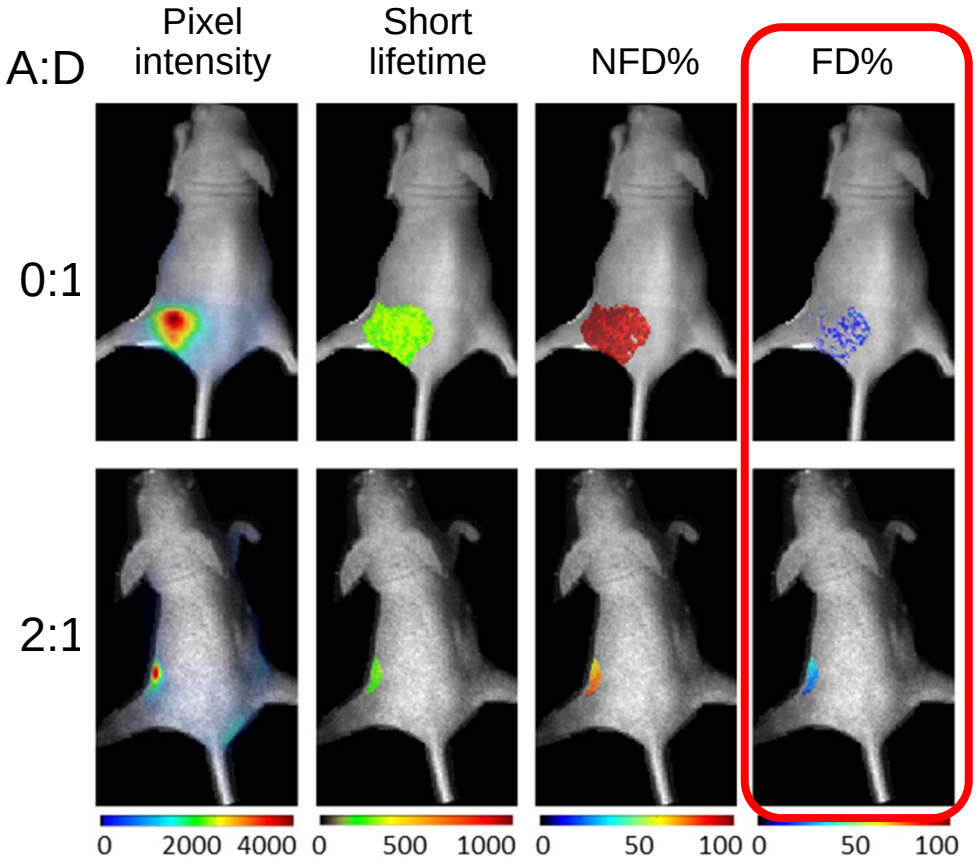
IHC

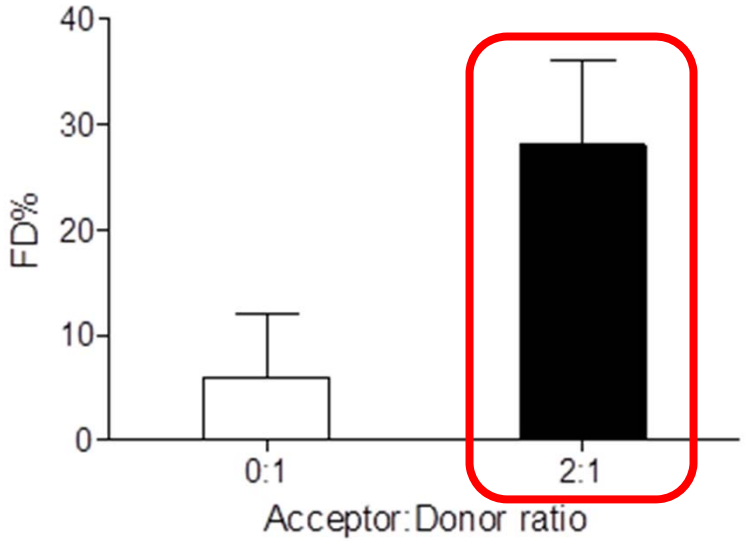


Wide-field time-resolved NIR MFLI-FRET imager

TfR-Tf NIR MFLI-FRET in tumors *in vivo*

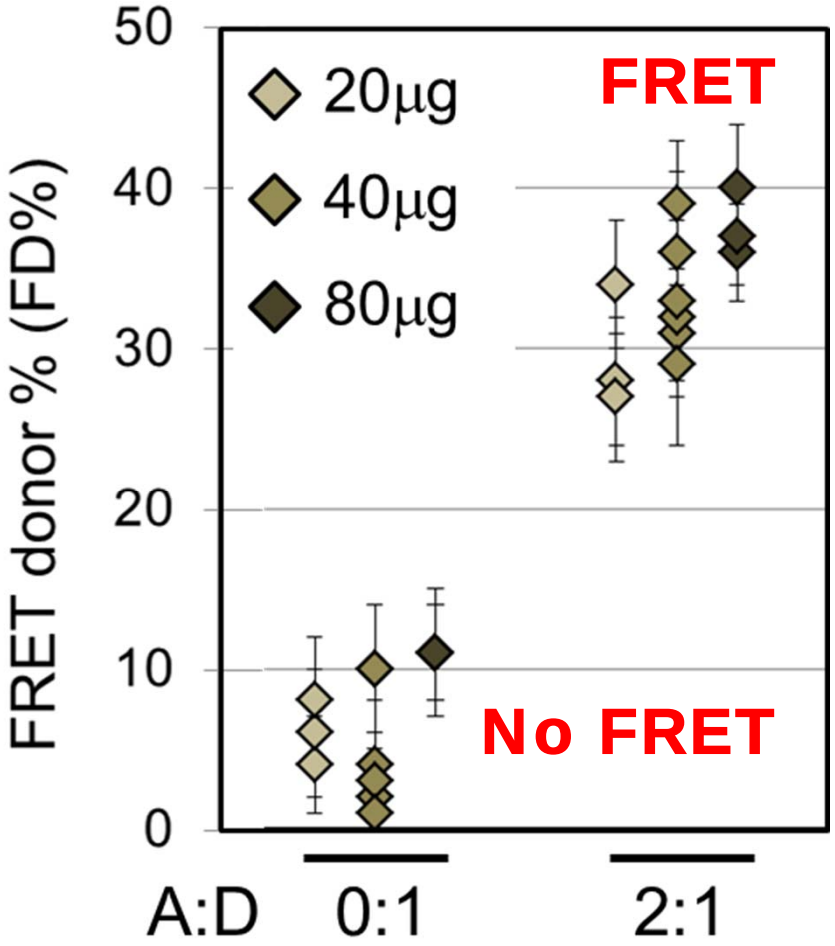
- **Application**: *in vivo* FRET imaging
- **Context:** Drug delivery assessment and optimization
- **Drug Carrier:** NIR-labeled transferrin (NIR-Tf)
- **Established:** Robust, quantitative





Nude mice carrying T47D tumor xenografts are tail injected with donor only or AF700-Tf and AF750-Tf (A:D= 2:1) and imaged 6h later.

NIR MFLI-FRET shows high sensitivity



Results are pooled from 21 imaging sessions of live animals bearing T47D breast cancer tumor xenografts.

- MFLI-FRET measures NIR-labeled Tf uptake into tumor xenografts through living tissues
- Detection is robust across a range of Tf concentrations

FD% = Quantification of donors participating in FRET events in tumor tissue is shown as FD%.

Conclusions

- ***NIR MFLI-FRET imaging correlates with the target engagement of TfR-Tf in tumor cells in vivo.***
- ***NIR MFLI-FRET imaging is a quantitative and non-invasive tool for the optimization of targeted drug delivery systems based on ligand-receptor or antibody-target engagement in tumors in vivo.***