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Preclinical evaluation of radiolabeled proteins

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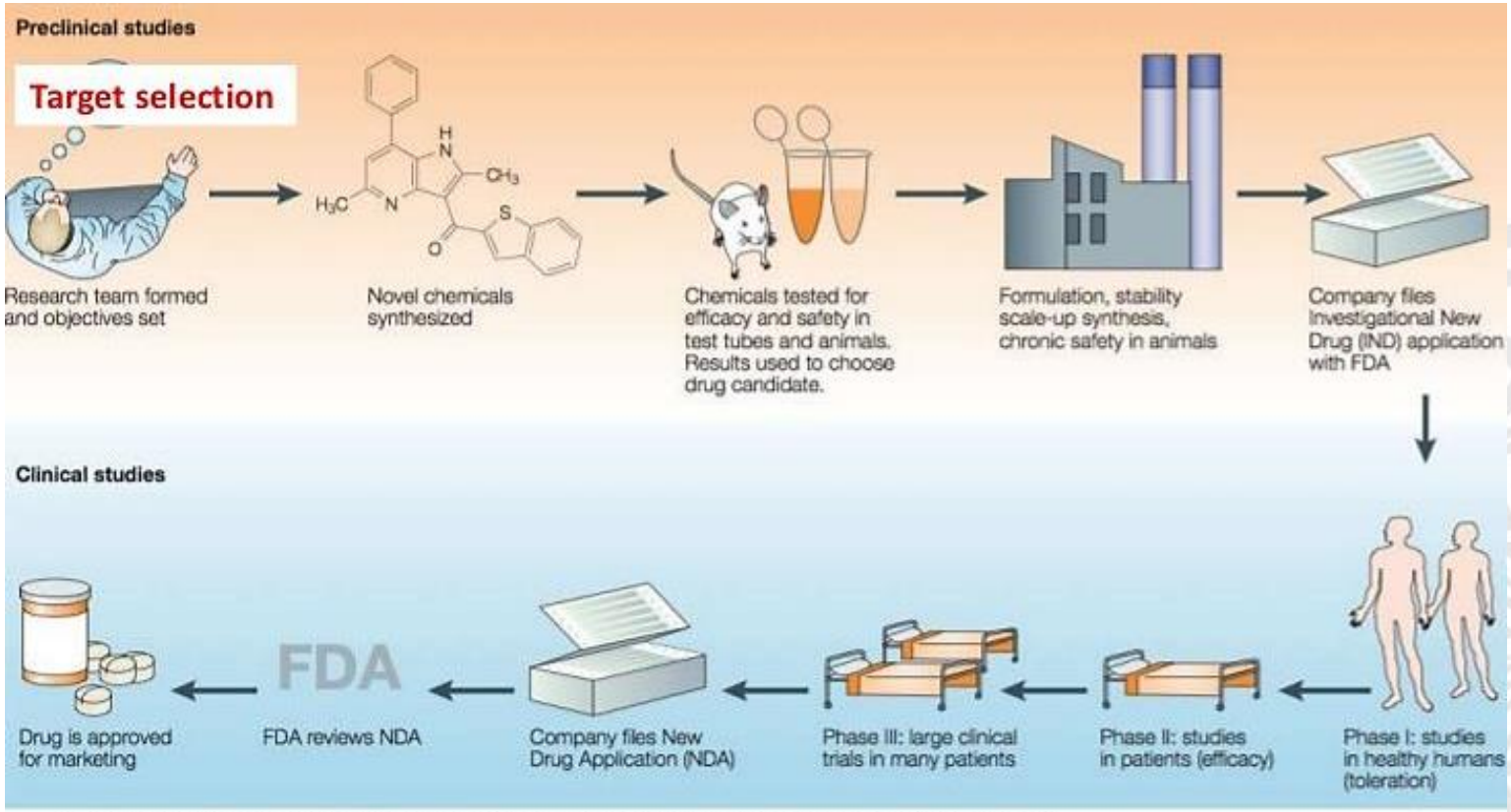
Drug discovery process

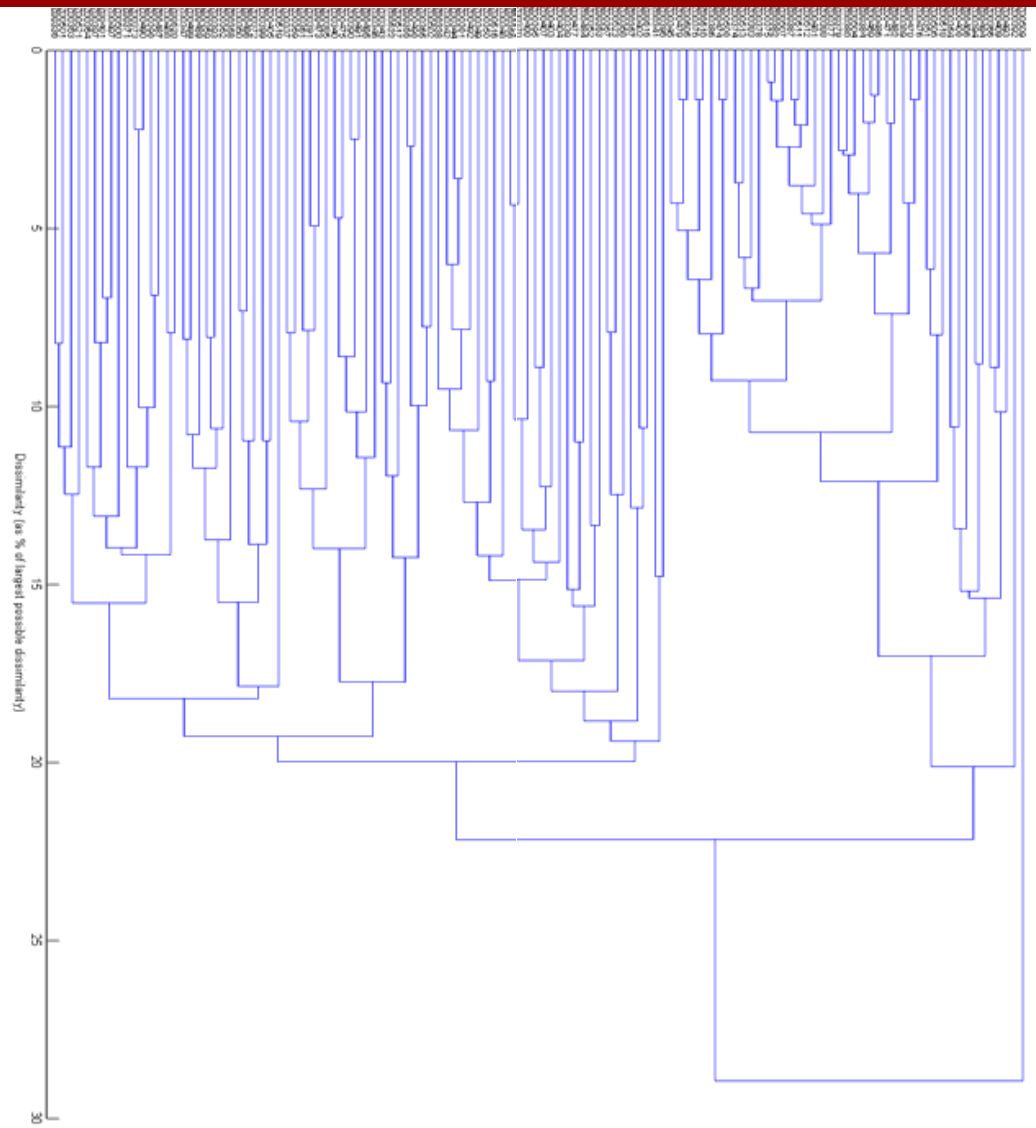


Food and Drug Administration (FDA) in the United States (or comparable agency in other countries)

The development of a new drug can take several decades and billion dollars invested

Lombardino & Lowe. Nature Reviews Drug Discovery (2004) 3, 853-862

Selection of the best candidate



Possible selection criteria:

- Affinity
- Termal stability
- Production
- Labeling radionuclide
- Labeling method
- Label stability
- Protein/target interaction
- Biodistribution profile
- In vivo targeting properties

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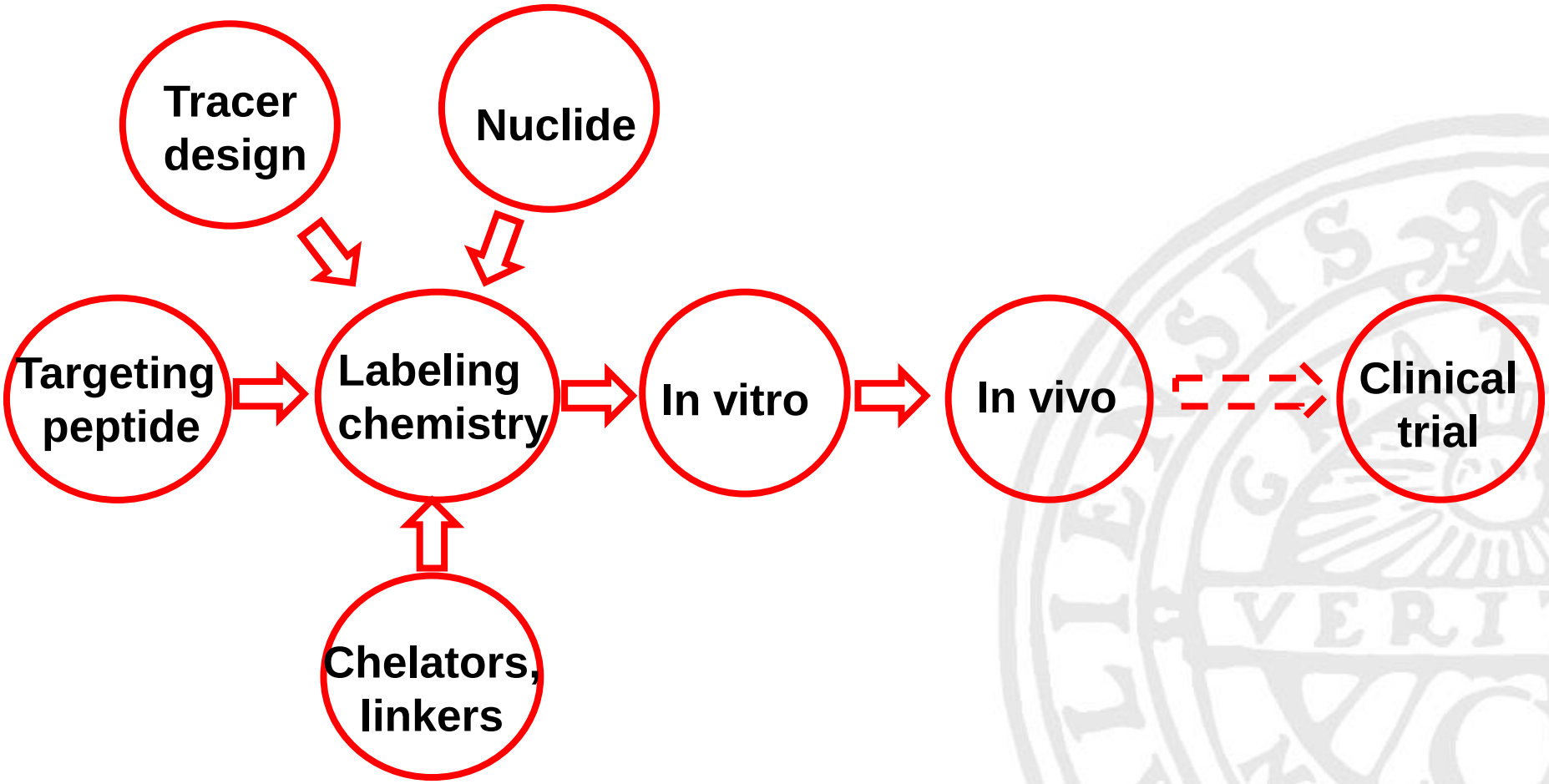
Pre-clinical evaluation of radiolabeled proteins



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Applications of proteins

- **Basic research**
- **Preclinical development**: pharmacokinetic studies, targeting properties, therapeutic dose
- **Clinical diagnostic**: patient stratification, therapy response
- **Treatment planning**: dosimetry estimation

What is the goal? Imaging or therapy?

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Preclinical evaluation of the targeting molecule

- **Labeling of targeting molecule**: optimisation of labeling yield, confirmation of identity of labeled compound, label stability (shelf-life, stability in blood-plasma)
- **In vitro characterisation**: specificity, binding properties, cellular processing, therapy effect
- **In vivo characterisation**: normal biodistribution, target specificity, biodistribution in tumor bearing mice, confirmatorial image, dosimetry, therapy

Labeling of targeting molecule

Optimization of labeling:

- Simple procedure
- High yield
- Reproducibility

Identity (HPLC analysis, SDS-PAGE)

Stability of labeling:

- Shelf-life (stabiltiy in solution over time)
- Stability under challenge (KI, histidine, EDTA)
- Stability in blood plasma

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Labeling of targeting molecule

- **Stability of tracer in blood**

  transchelation, free radionuclide, colloids

- **Chemical modifications**

  conformation, target recognition, molecular charge, lipophilicity

- **Cellular processing by malignant cells**

  residualising properties of radiocatabolites

- **Retention in healthy tissues**

  residualising properties of radiocatabolites

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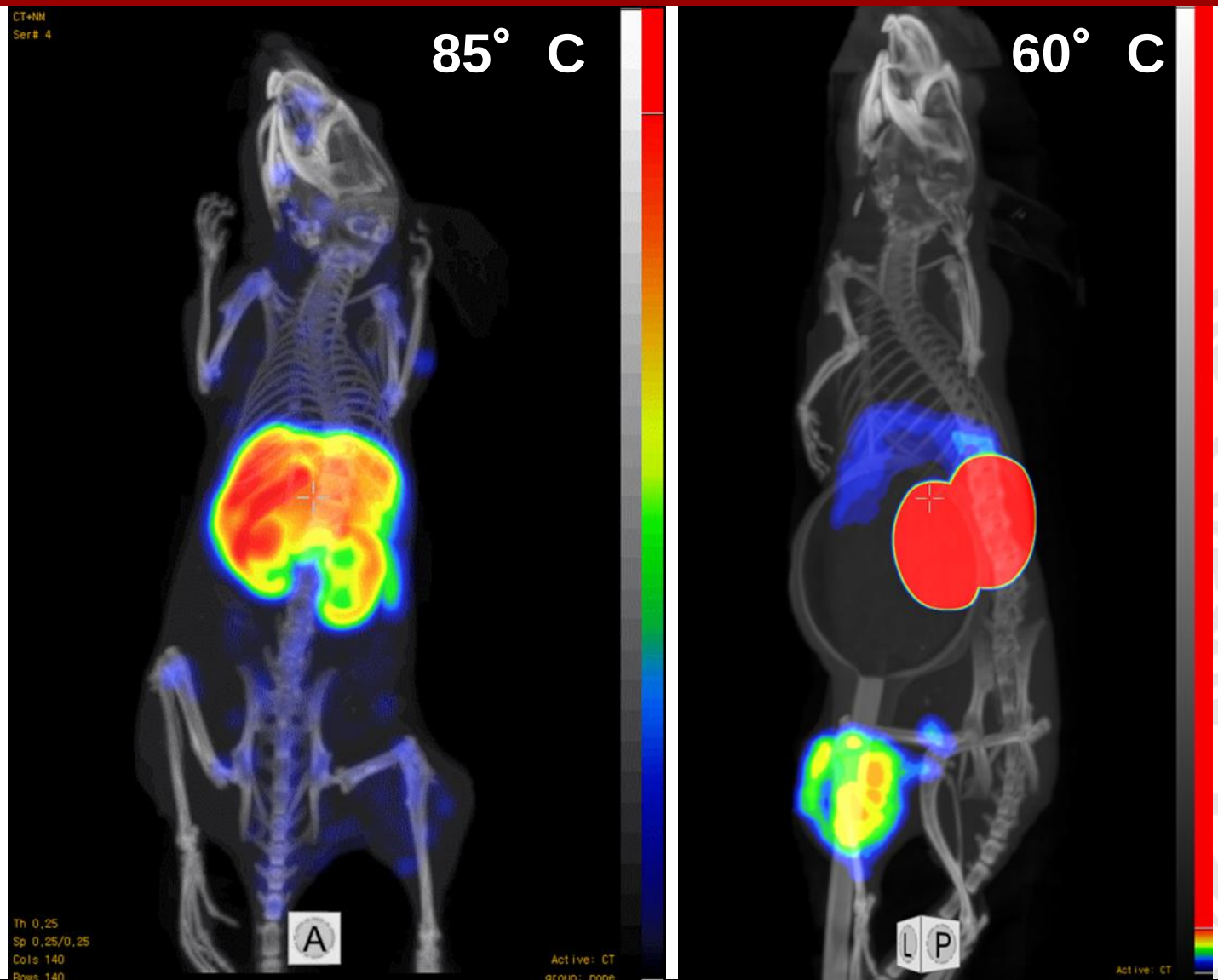
Optimal labeling conditions!



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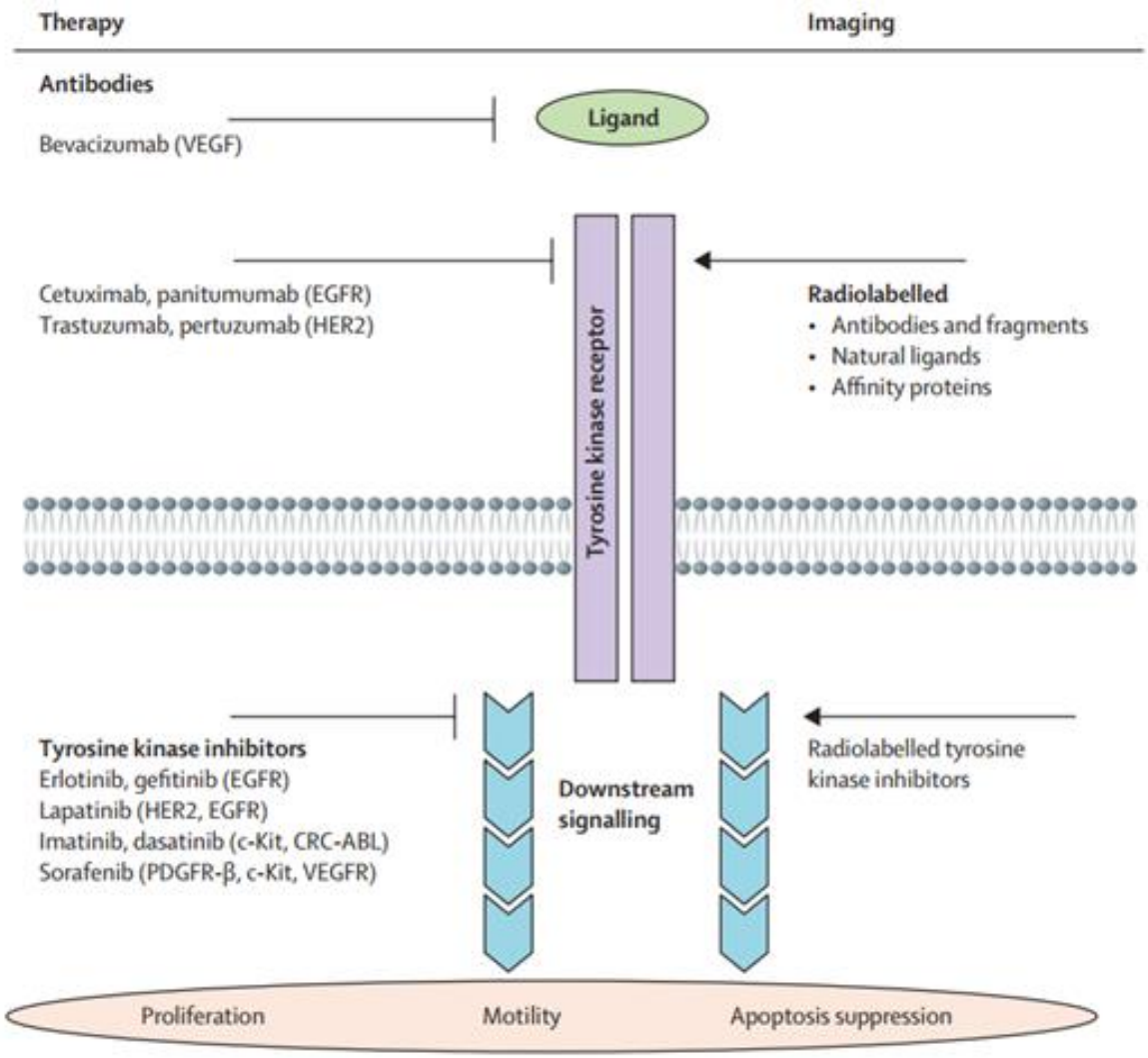
Targeting cell-surface receptors

Example:
Receptor tyrosine kinases

Signalling

- **cell proliferation**
- **suppression of apoptosis**
- **increased motility**
- **recruitment of neovasculature**

RTKs are targets for anticancer drugs



Tolmachev et al. Lancet Oncology, 2010

In vitro characterisation

Specificity:

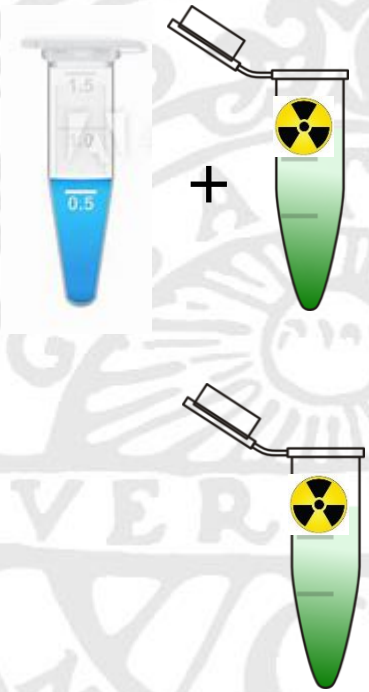
Saturable targeting

No binding to other targets

Cells expressing a target

Saturation by ”cold” + Radiolabeled compound

Radiolabeled compound only



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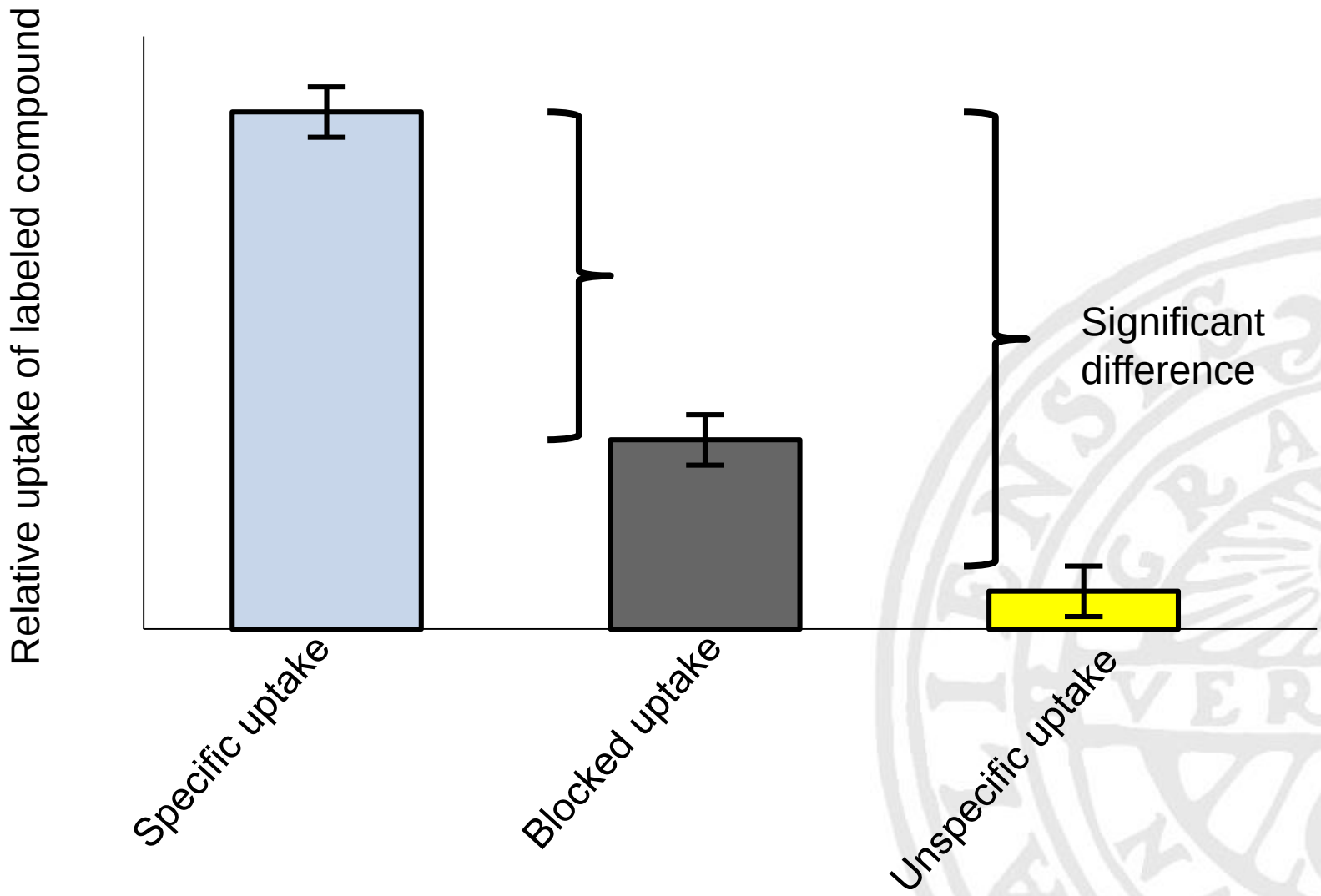
In vitro specificity



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In vitro characterisation

Specificity

Binding properties

- Dissociation constant
- Inhibition
- Binding competent fraction

Cellular processing

Therapy effect

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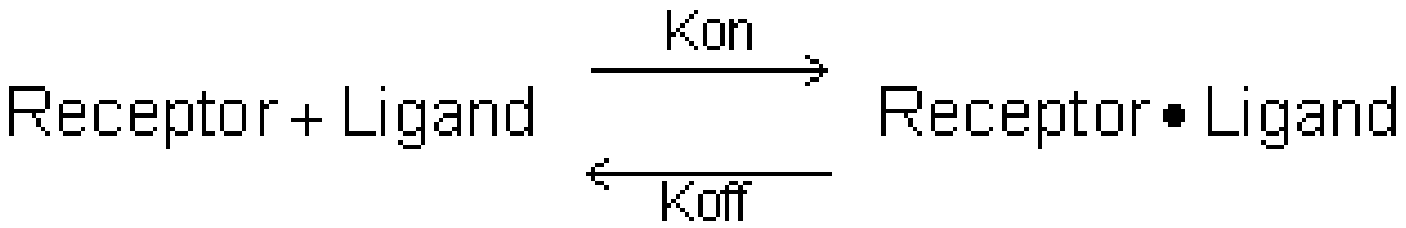
Equilibrium binding studies



Association of a ligand to its receptor and the dissociation of the resulting ligand-receptor complex are reversible processes that occur concomitantly until equilibrium is reached

$$\frac{[\text{Ligand}]\cdot[\text{Receptor}]}{[\text{Ligand}\cdot\text{Receptor}]} = \frac{k_{off}}{k_{on}} = K_d$$

k_{on}	Association rate constant or on-rate constant
k_{off}	Dissociation rate constant or off-rate constant
K_d	Equilibrium dissociation constant

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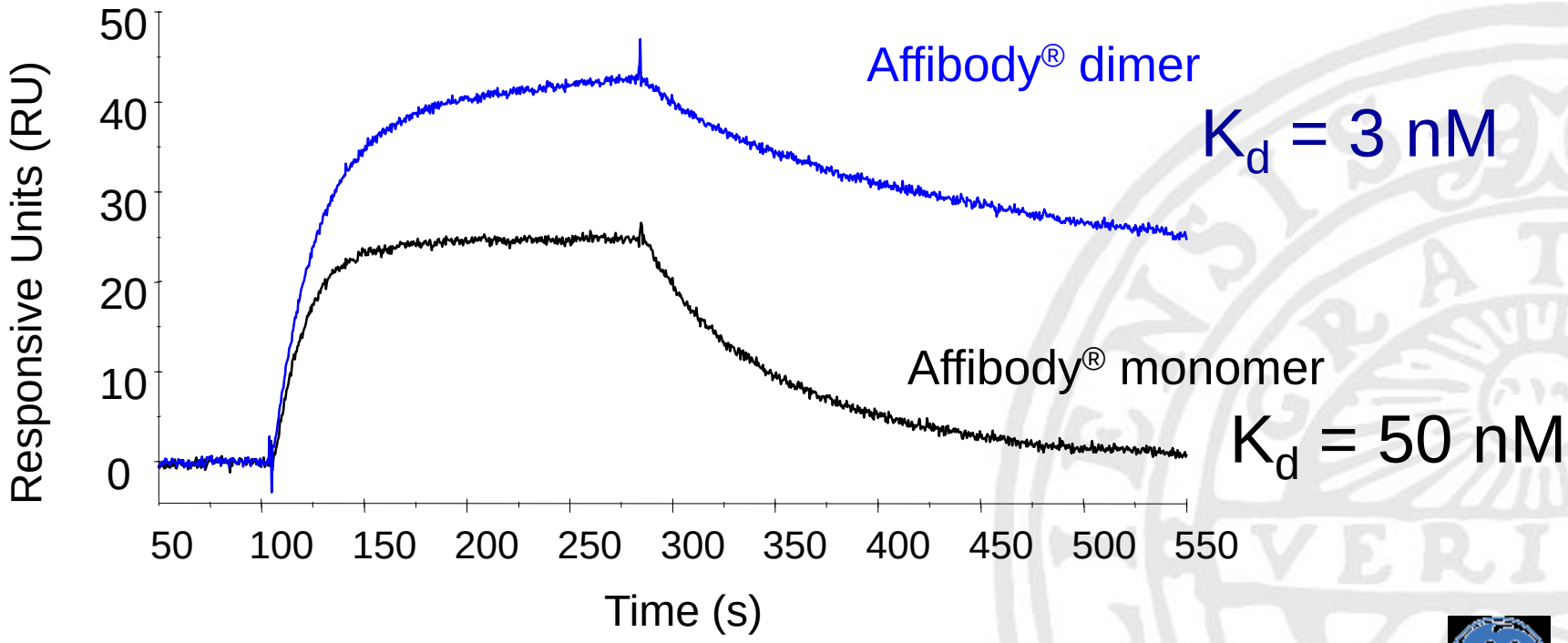
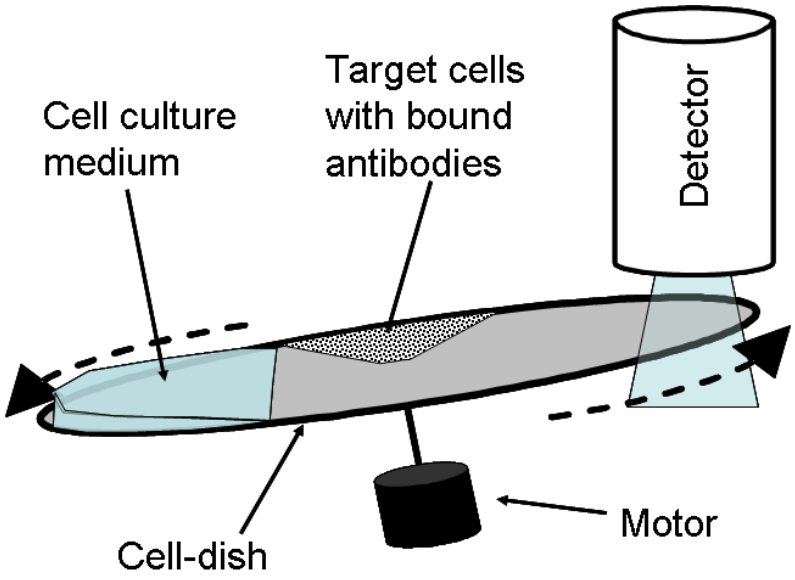
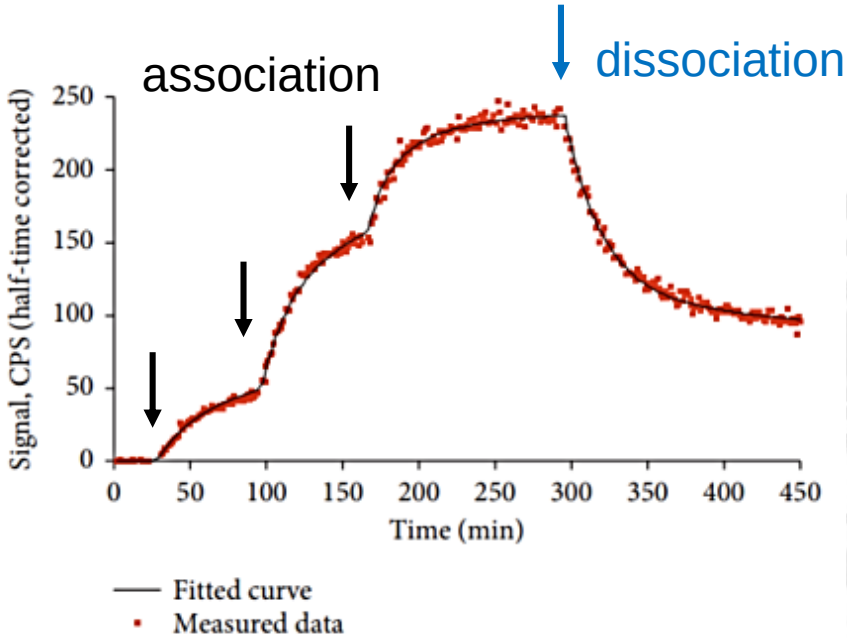
Binding properties:
Dissociation constant, K_D

BiaCore technology (receptors attached on a chip)



Binding properties:
Dissociation constant, K_D





$$K_d = \frac{k_{off}}{k_{on}}$$

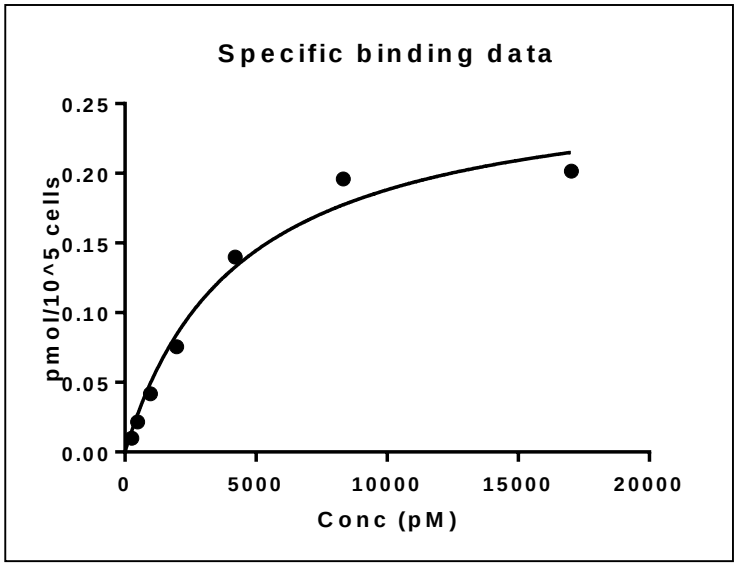
ridgeview instruments ab

Saturation experiment:

Alternative way to measure K_d and B_{max}

1) To determine the affinity of a radioligand for a receptor

2) The density (B_{max}) of a specific receptor or receptor subtype on cells / tissues



Results of the saturation experiment are plotted with pmol/ 100 k cells on the Y axis and concentration of radioactive ligand (pM) on the X axis.
The resulting graph is a hyperbola and is called a saturation curve

Saturation experiment:
Alternative way to measure K_d and B_{max}

- Use several concentrations (up to 10) of radiolabeled ligand

$0.1x\ K_D$ ⟵ K_D ⟶ $10x\ K_D$

- Long incubation (4-8 hours)

- For each concentration 4 dishes (3 non-blocked, 1 blocked) + 1 standard

3 dishes Nonblocked

1 dish blocked

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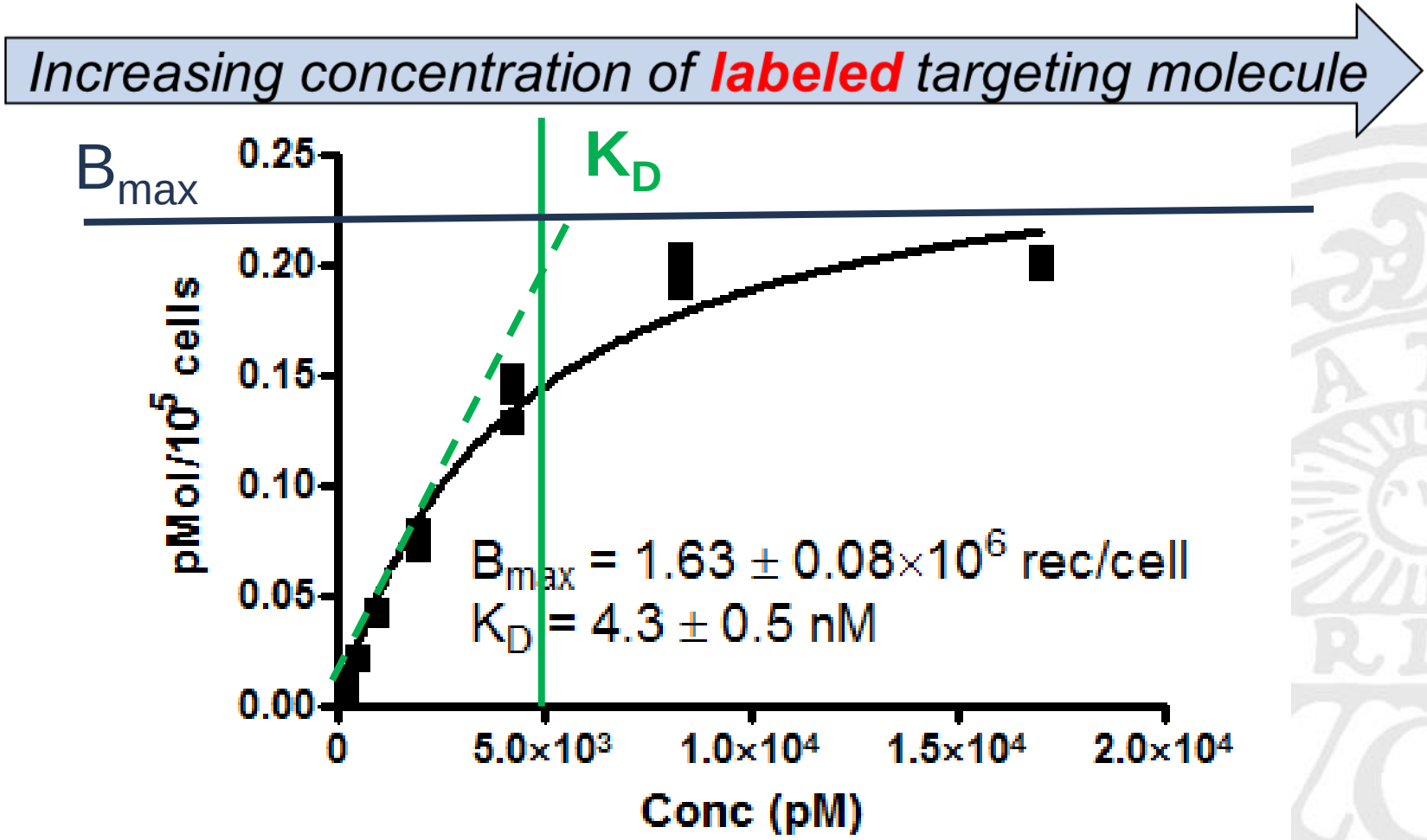
Binding properties:
Dissociation constant, K_D

Saturation analysis



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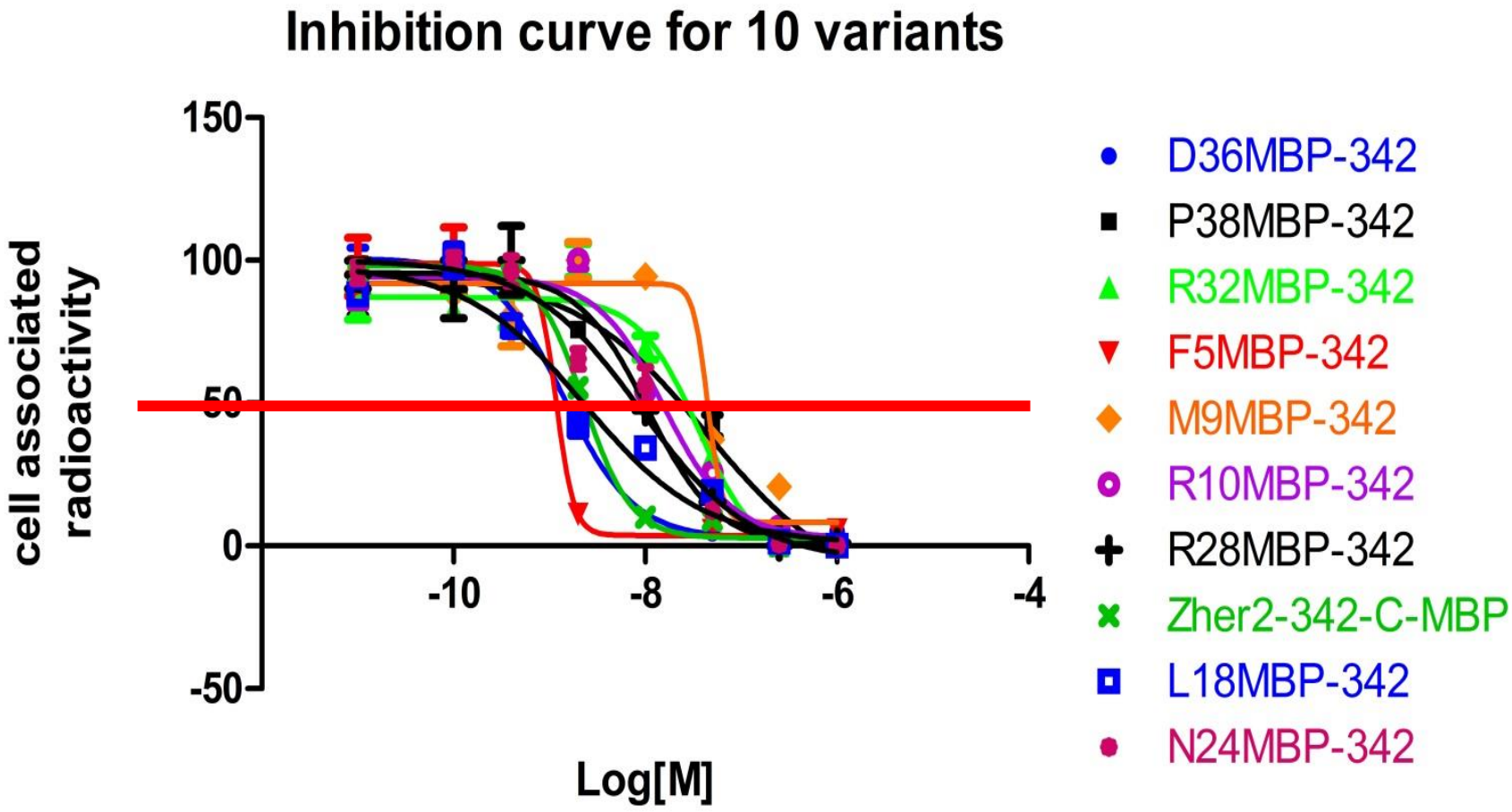
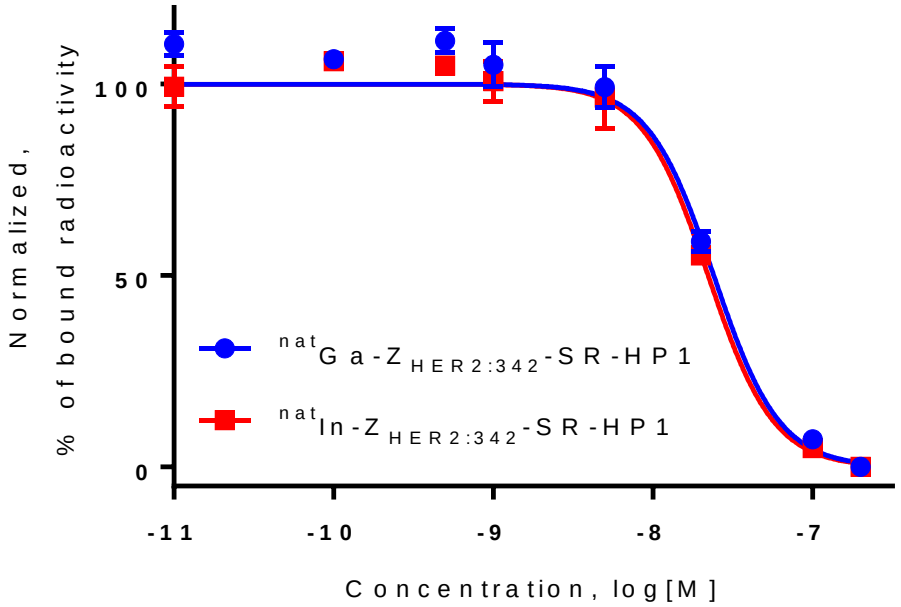
Inhibition, IC_{50}

*Increasing concentration of **non-labeled** targeting molecule*



Competitive binding experiments

measure equilibrium binding of a single concentration of radioligand at various concentrations of an unlabeled competitor



- Single concentration of ^{111}In-labeled affibody
- Range of concentrations of ”cold Ga” labeled affibody analogue
- Range of concentrations of ”cold In” labeled affibody analogue

IC_{50} ^{nat}Ga-ZHP1 = 24.1 ± 2.0 nM

IC_{50} ^{nat}In-ZHP1 = 22.2 ± 1.1 nM

To compare the affinities of several compounds in one assay

Vorobyeva et al. Sci Rep. 2018

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Determination of the immunoreactive fraction of radiolabeled mAbs by linear extrapolation to binding at infinite antigen excess

- Lindmo et al. J Immunol Methods. 1984 Aug 3;72(1):77-89.

Lindmo assay

- For properly performed conjugation procedures, immunoreactive fractions of about 0.9 were obtained, but a prolonged chloramine-T reaction for ^{125}I-labeling resulted in an immunoreactive fraction of 0.6
- Due to its principle of determining binding at infinite antigen excess, the present method is insensitive to variation in the actual amounts of cells and antibody used, as well as the incubation time. We therefore recommend it as a quality control procedure for radiolabeled antibodies

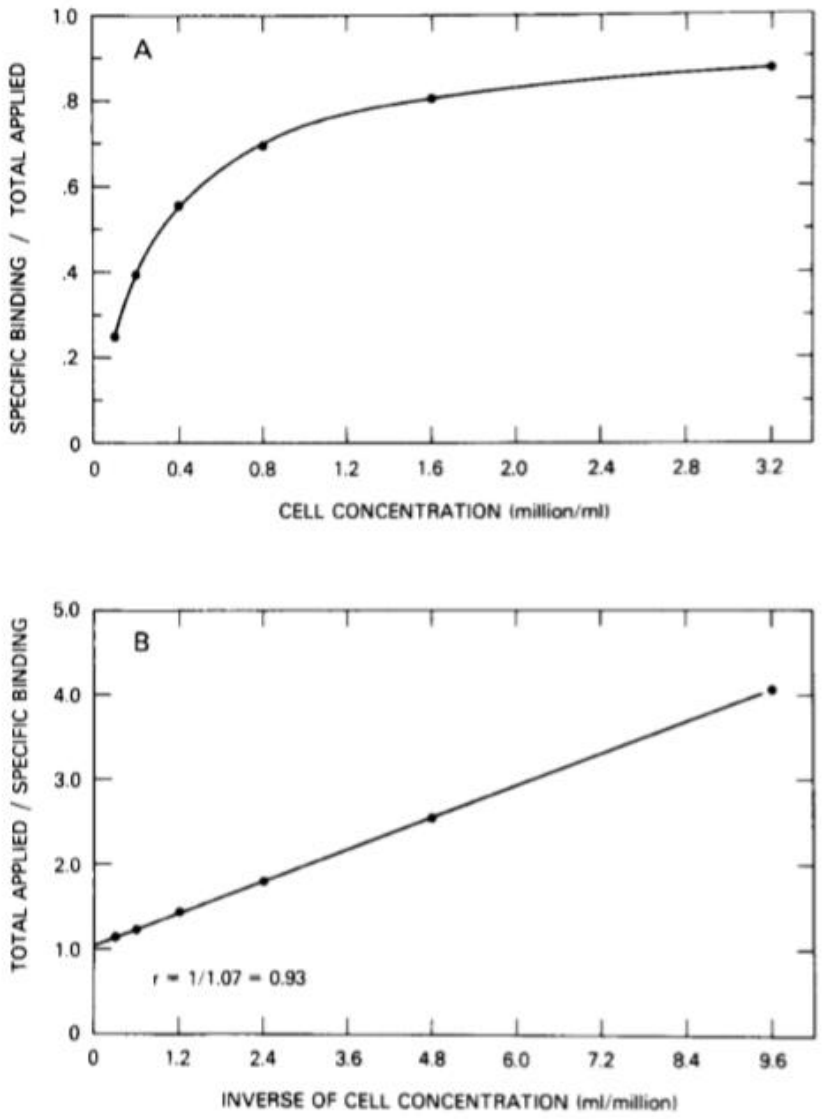

Fig. 1. Binding assay for the determination of the immunoreactive fraction of an ^{111}In-labeled T101 monoclonal antibody. The assay was set up using 6 concentrations of live NCI-H516 cells, in 1:2 dilutions from 3.3 to 0.1 million cells/ml, and the final concentration of ^{111}In-labeled T101 was 13 ng/ml. A shows a conventional plot of specific binding over total applied radioactivity, as a function of increasing cell concentration. B is a double inverse plot according to eq. (3) of the same data as in A, i.e., total applied radioactivity over specific binding, as a function of the inverse cell concentration. By means of linear extrapolation to the ordinate, the immunoreactive fraction can be determined for conditions representing infinite antigen excess.

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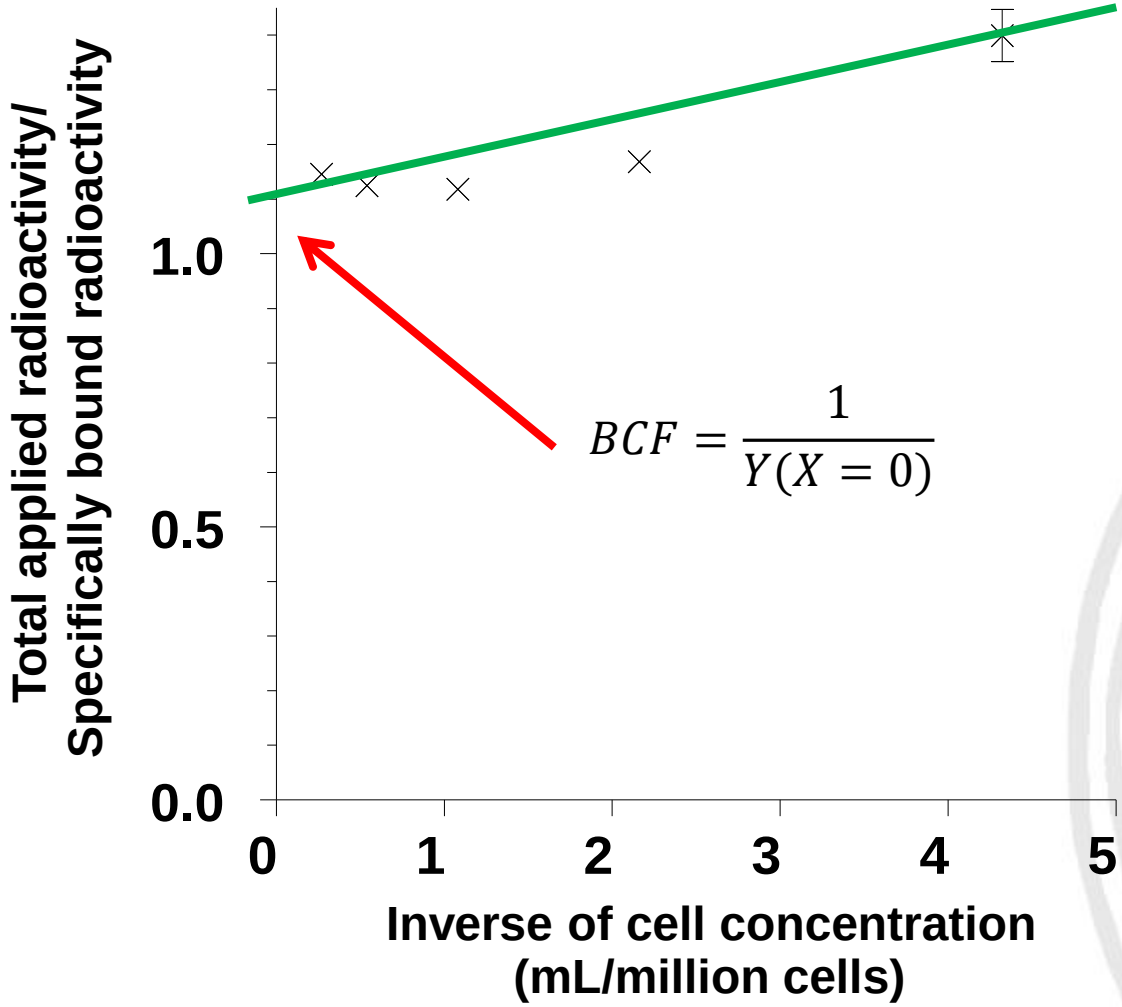
Binding competent fraction

Increasing concentration of targeted ***molecule***



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In vitro characterisation

Specificity:

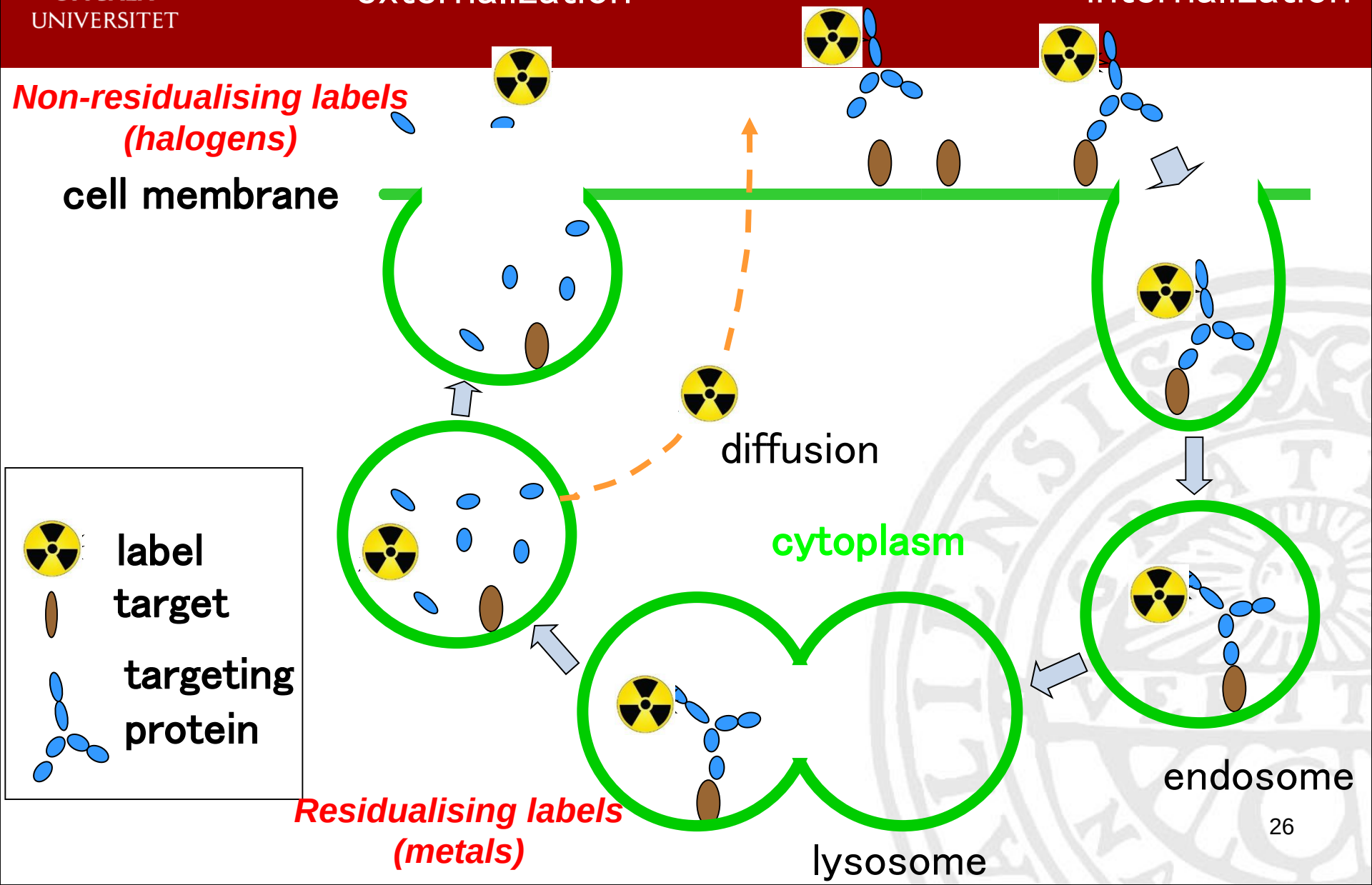
Binding properties:

Cellular processing:

- Binding
- Internalization
- Retention

Therapy effect

Internalization of radiolabeled proteins



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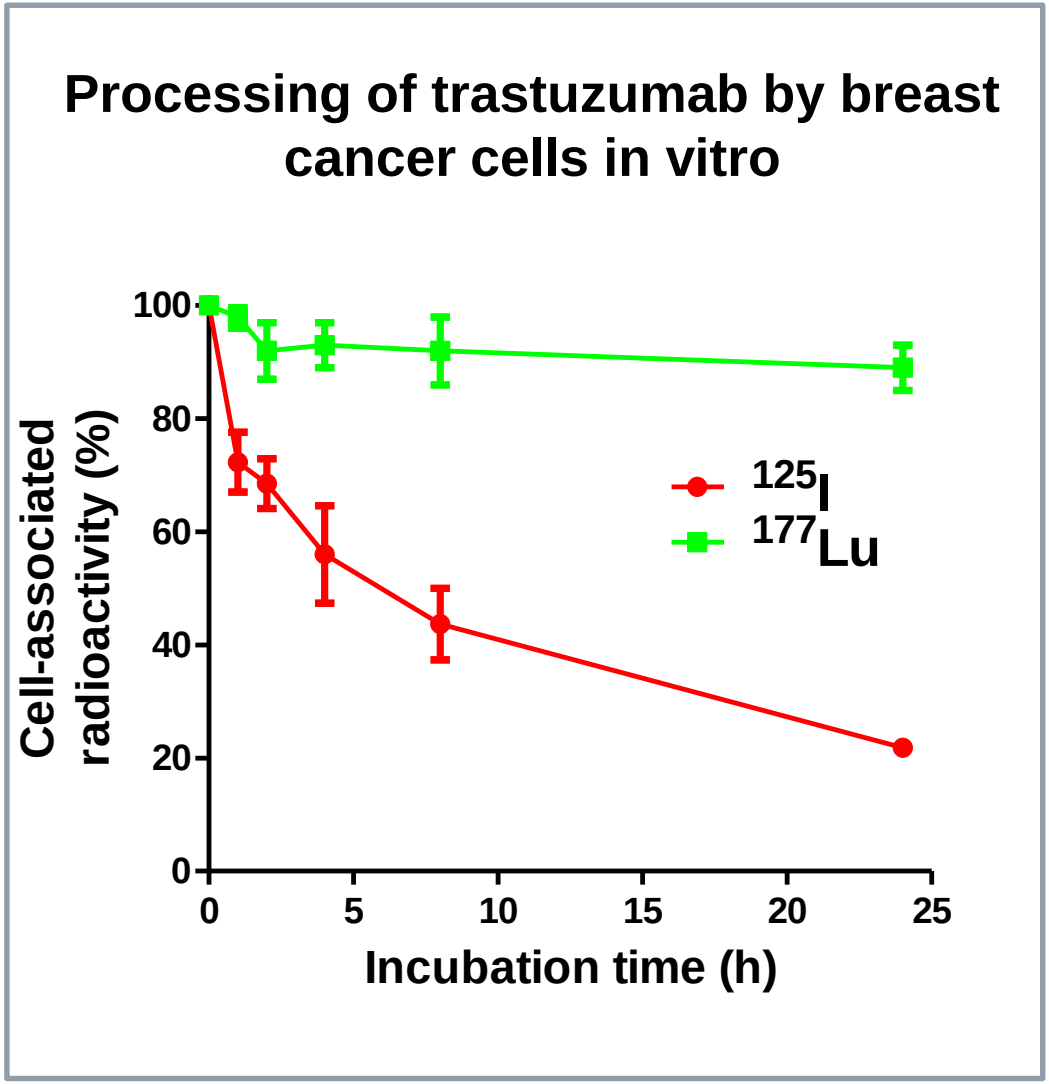
Cellular retention of radionuclides



The use of residualizing labels (metals) improves cellular retention of radionuclides delivered by antibodies because antibodies are internalized rapidly!

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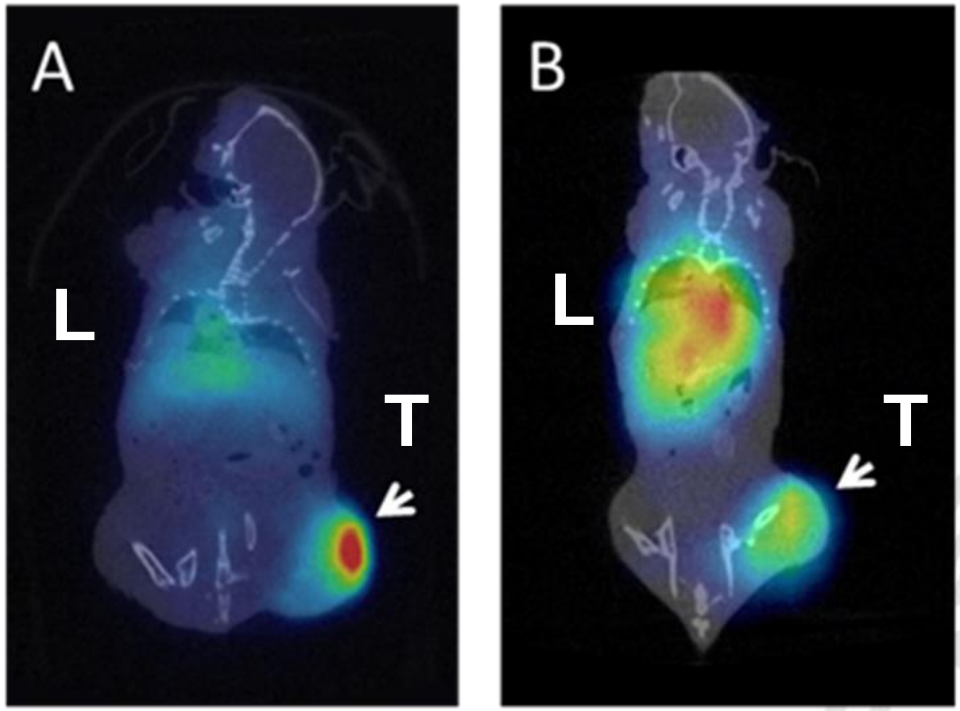
Retention in excretory organs



^{124}I: non-residualizing **^{111}In: residualizing**

Internalization in excretory organs is much faster than in tumors

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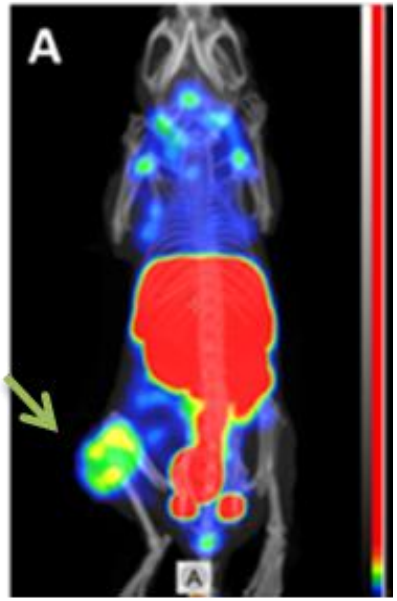
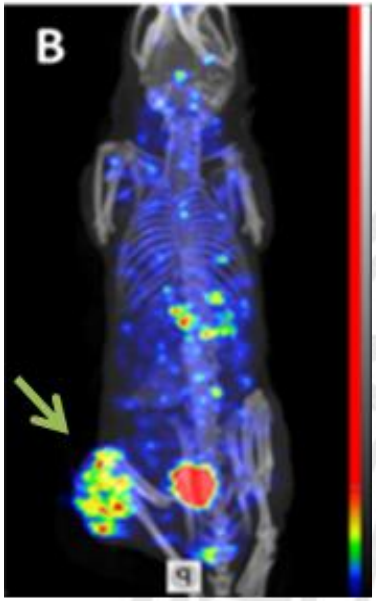
Same protein, different labels

Residualizing label ($^{99m}Tc(CO)_3$)



Retention in tumor,
Retention in normal organs

Non-residualizing label (^{125}I halogen)



Retention in tumor,
Wash-out from normal organs

Scaffold proteins are *usually* internalized slowly by tumor cells

Vorobyeva et al. Contrast Media & Molecular Imaging 2018

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Comparison of labels

A **residualizing label** is trapped inside the cell after internalization and proteolysis of a labeled protein. It shows the location of protein uptake and catabolism that occurred **prior to the studied time point**

A **non-residualizing label**, i.e. its radiocatabolites diffuse from cells, shows the location of a labeled protein in the extracellular space/membrane **at the studied time point**

Comparison of these two types of label **provides information about protein localization** (intracellular space or cell membrane) and speed of internalization *in vivo*

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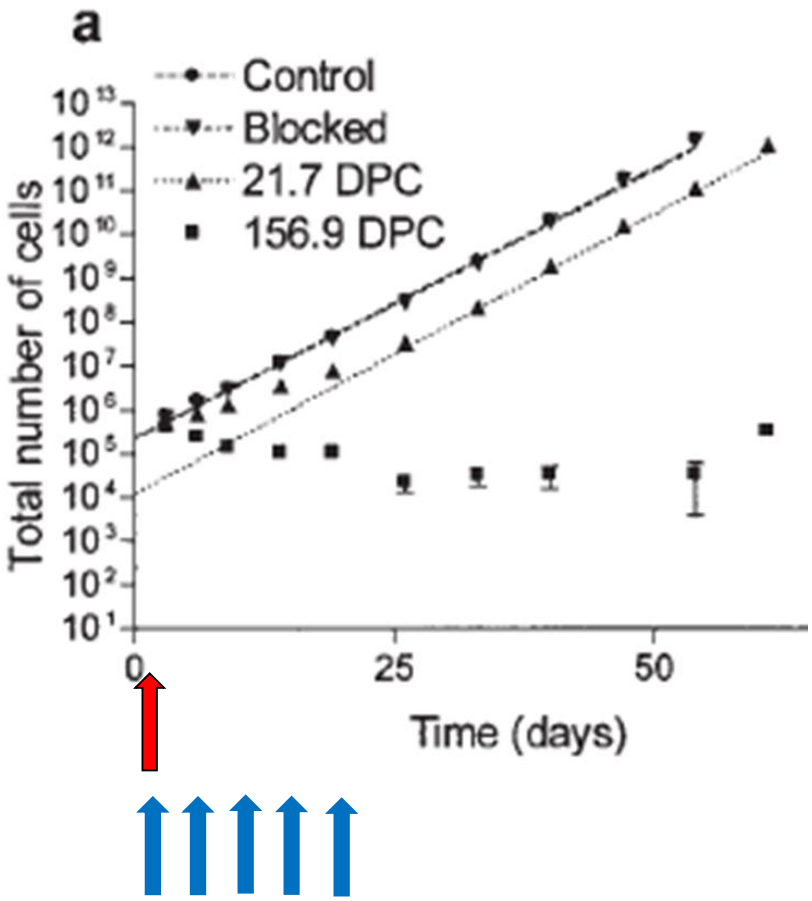
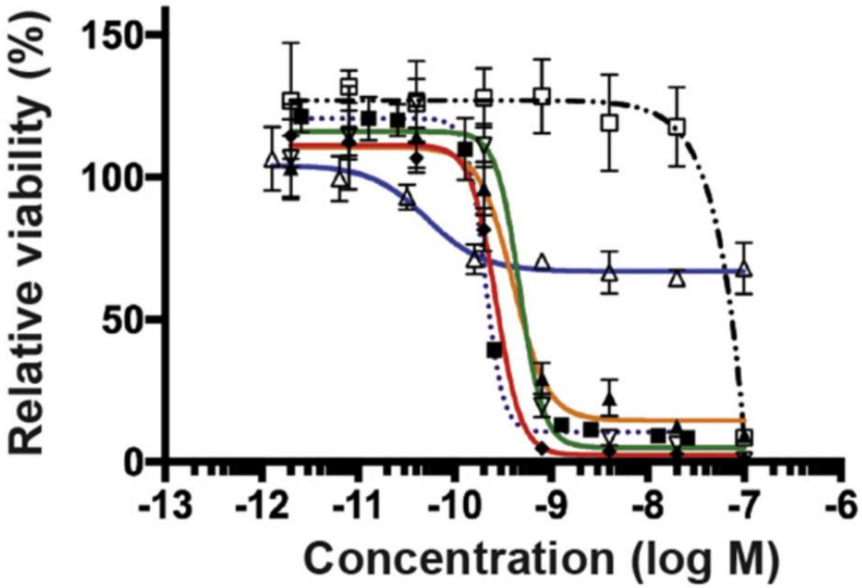
Therapy effect: growth curves



Cytotoxicity IC50



Altai et al. JCR 2018

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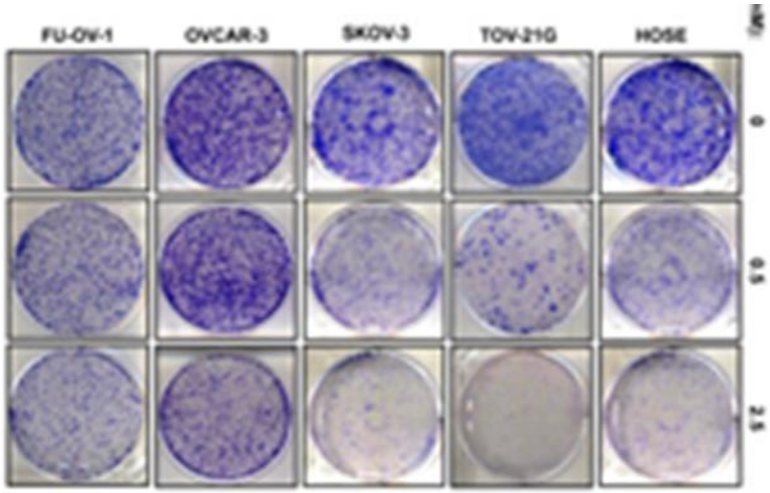
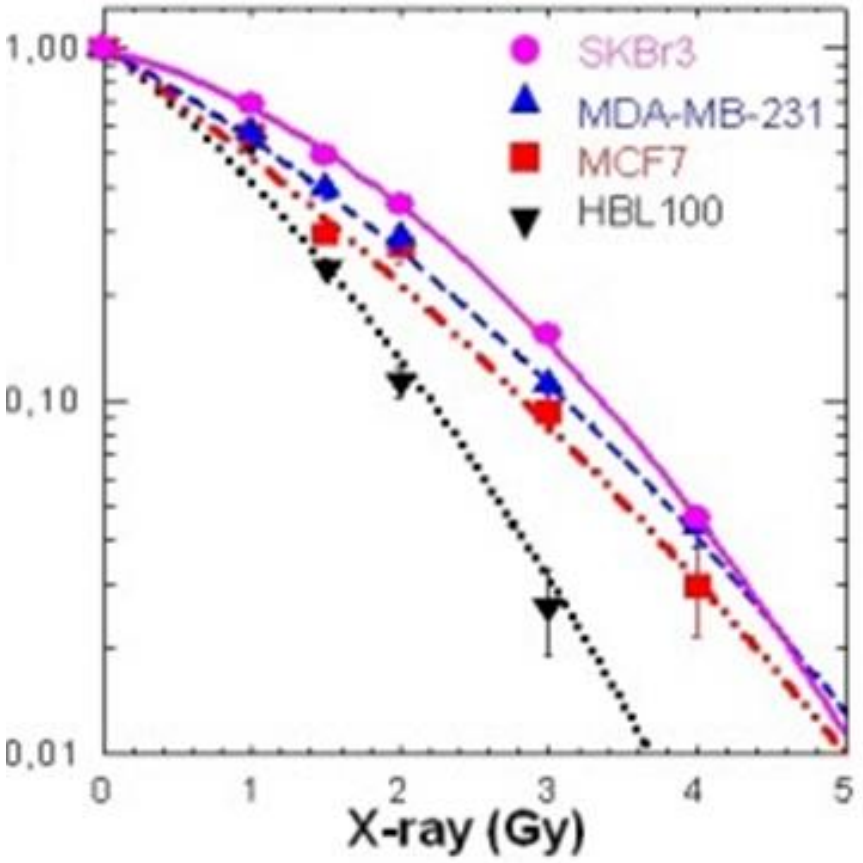
Therapy effect: clonogenic survival assay



External beam radiation therapy



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In vivo characterisation

Normal biodistribution

Target specificity

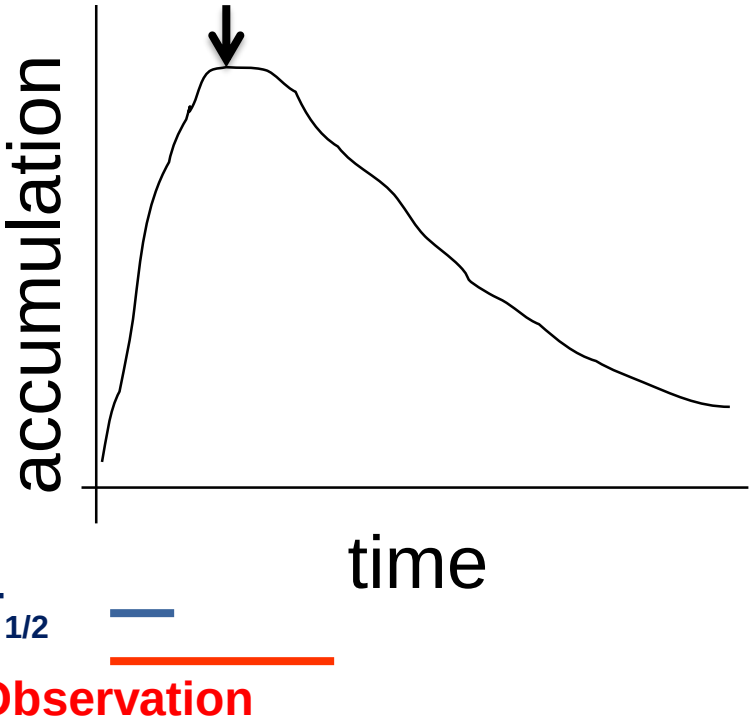
Biodistribution in tumor bearing mice

Imaging (confirmation)

Dosimetry

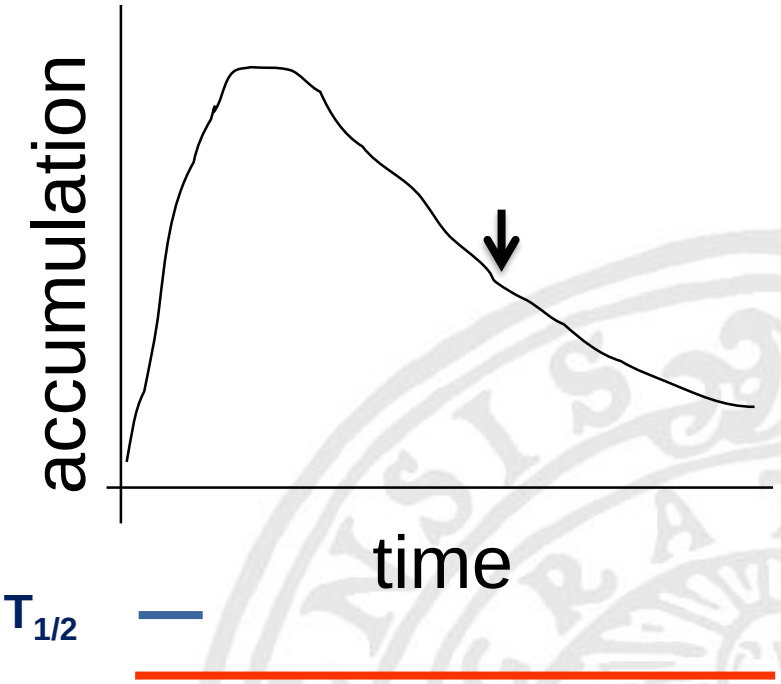
Therapy

Biodistribution: time points



Case 1. Absolute accumulation

Example: small protein, 1 - 4 h



Case 2. Retention and wash out

Example: small protein, 24 - 72 h

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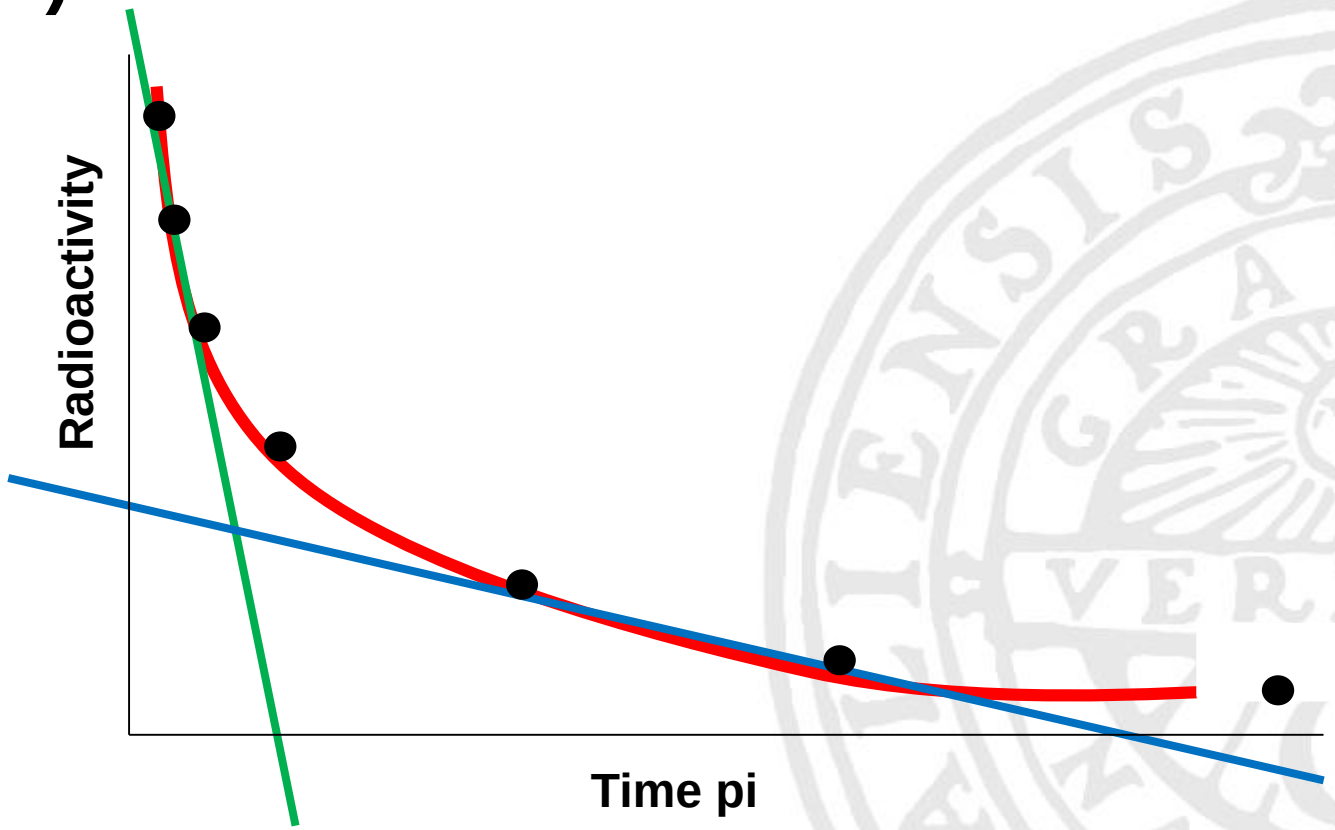
Normal biodistribution

Animals (without tumors)

Several time points (distribution phase + elimination)



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Normal biodistribution

- Animals (without tumors)
- Several time points (distribution phase + elimination)
- Organs should be studied
  - blood
  - excretory organs (liver, kidneys)
  - organs with normal target expression
  - organ with high accumulation of radiocatabolites

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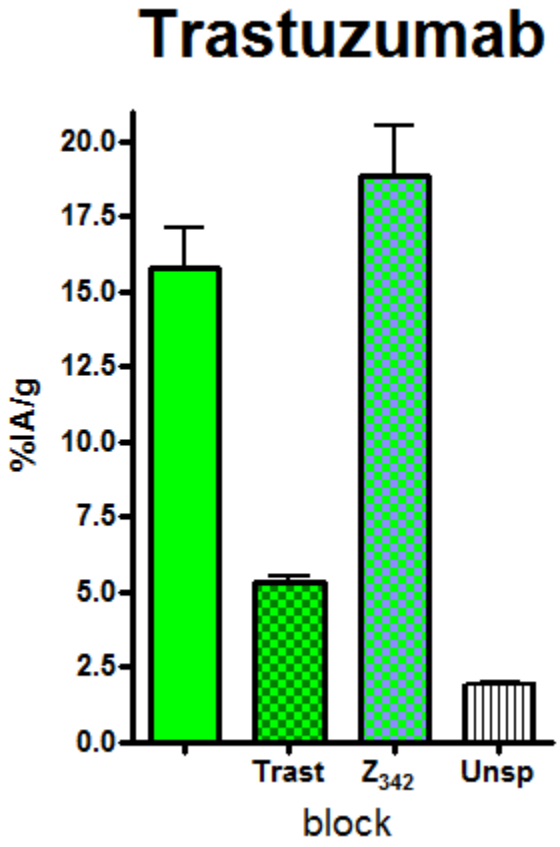
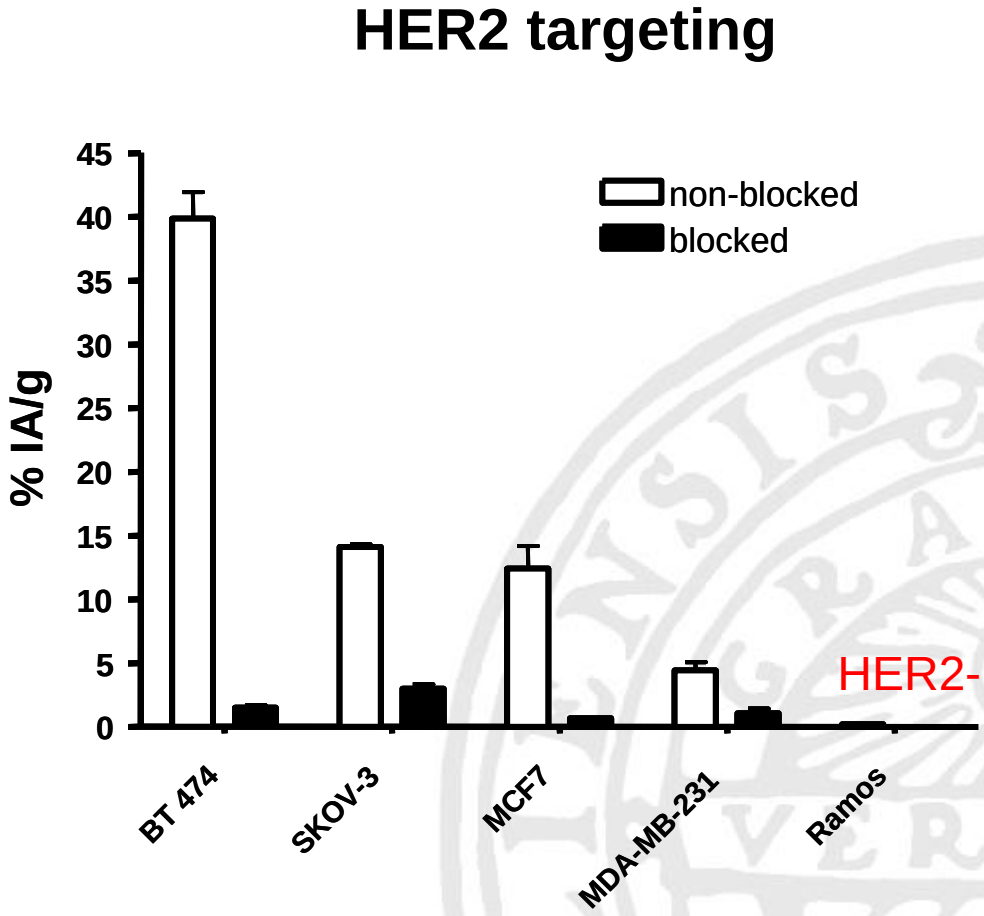
In vivo target specificity





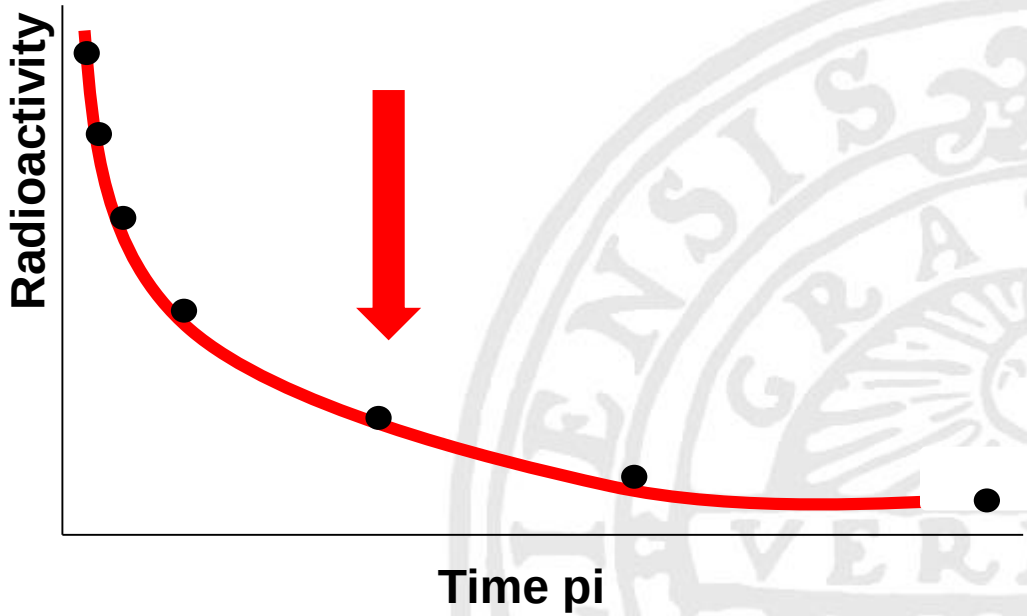
1. Block by itself

2. Use a compound of same size but no specific binding

3. Several HER2+ or HER2- tumor models

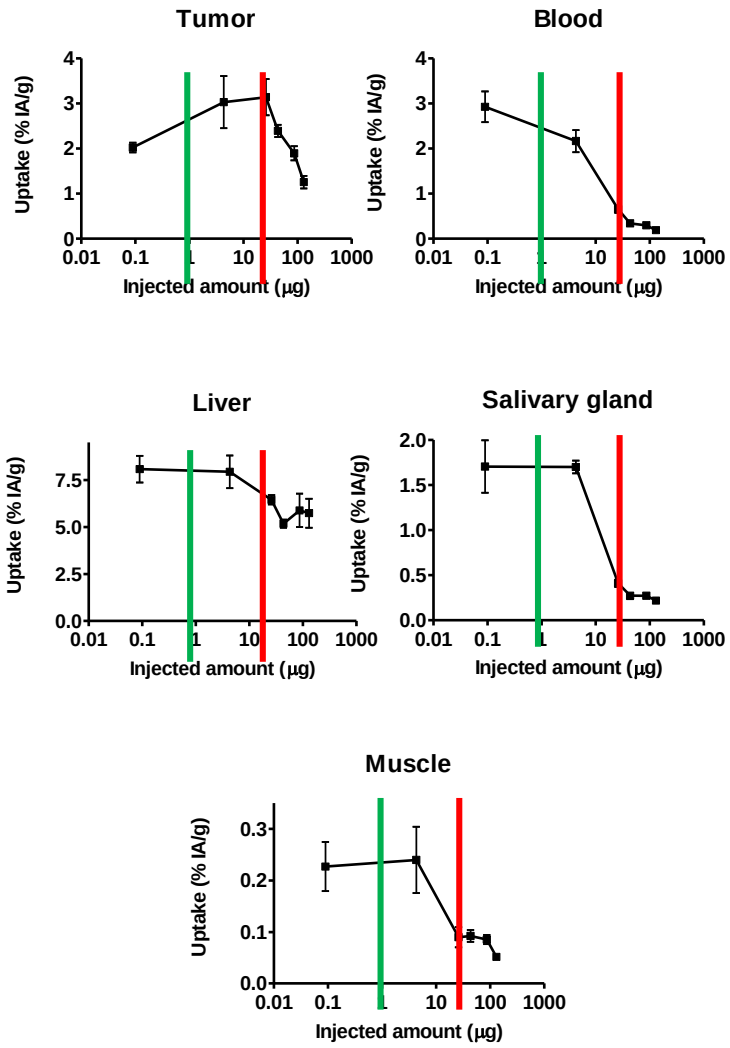
Biodistribution in tumor bearing mice

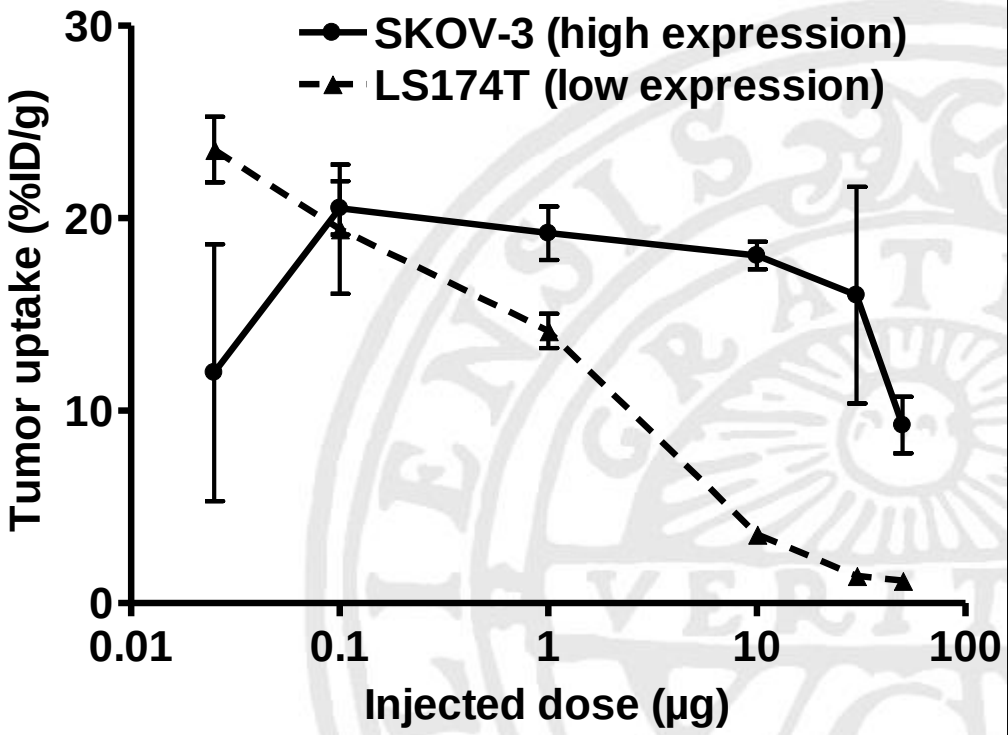
Could be one time point experiment (dedicated from normal biodistribution experiment)



Biodistribution in tumor bearing mice

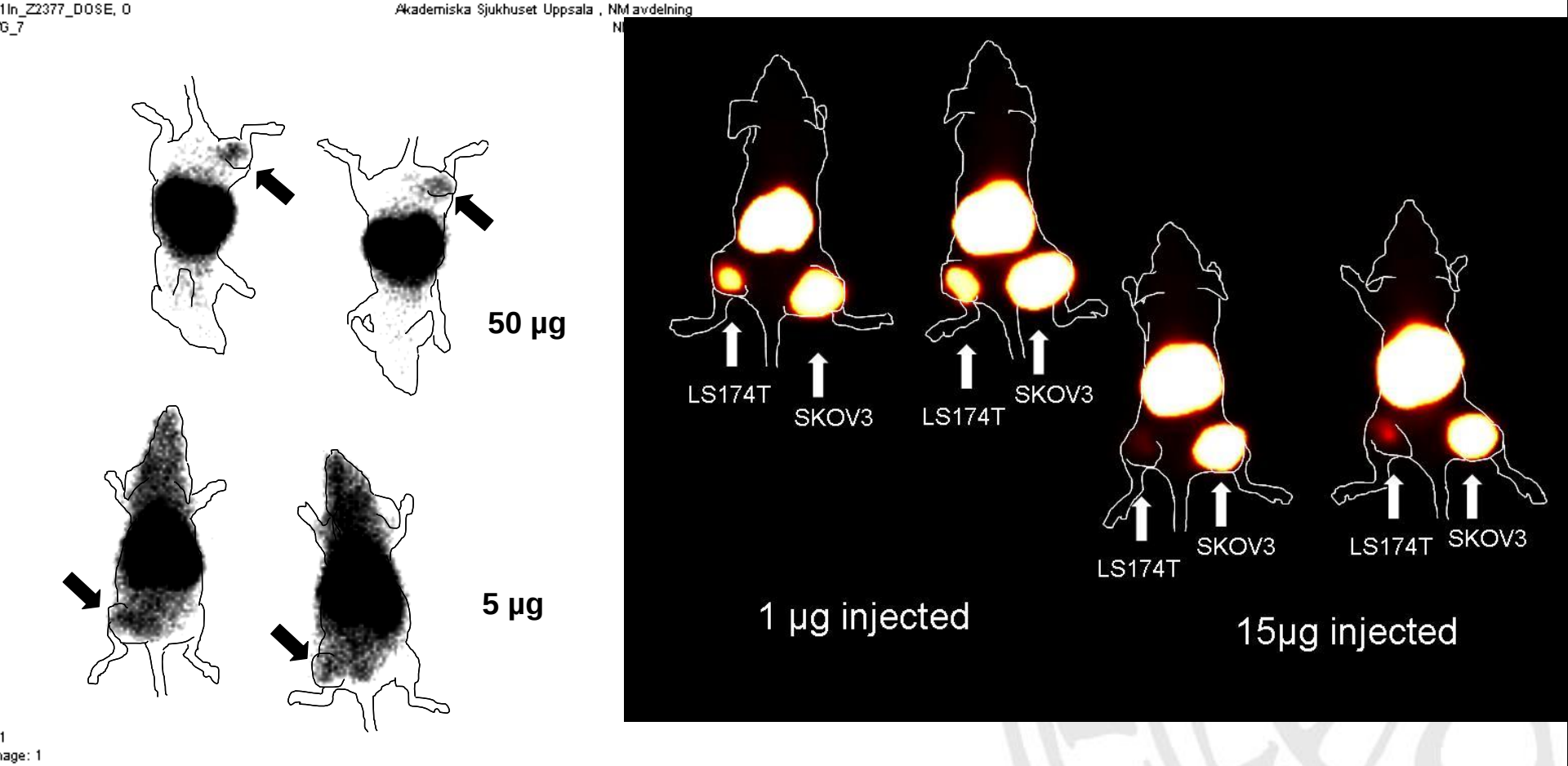
Optimal injected specific radioactivity





Biodistribution in tumor bearing mice

Optimal injected specific radioactivity



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Dosimetry

Imaging targeting molecule

needed if clinical trial is planned

multiple time points full scale biodistribution in normal mice

Nuclide-based therapeutic targeting molecule

needed for planning of therapy experiment

multiple time point full scale biodistribution in tumor bearing mice

Area under curve (AUC) for tumors >> for healthy organs

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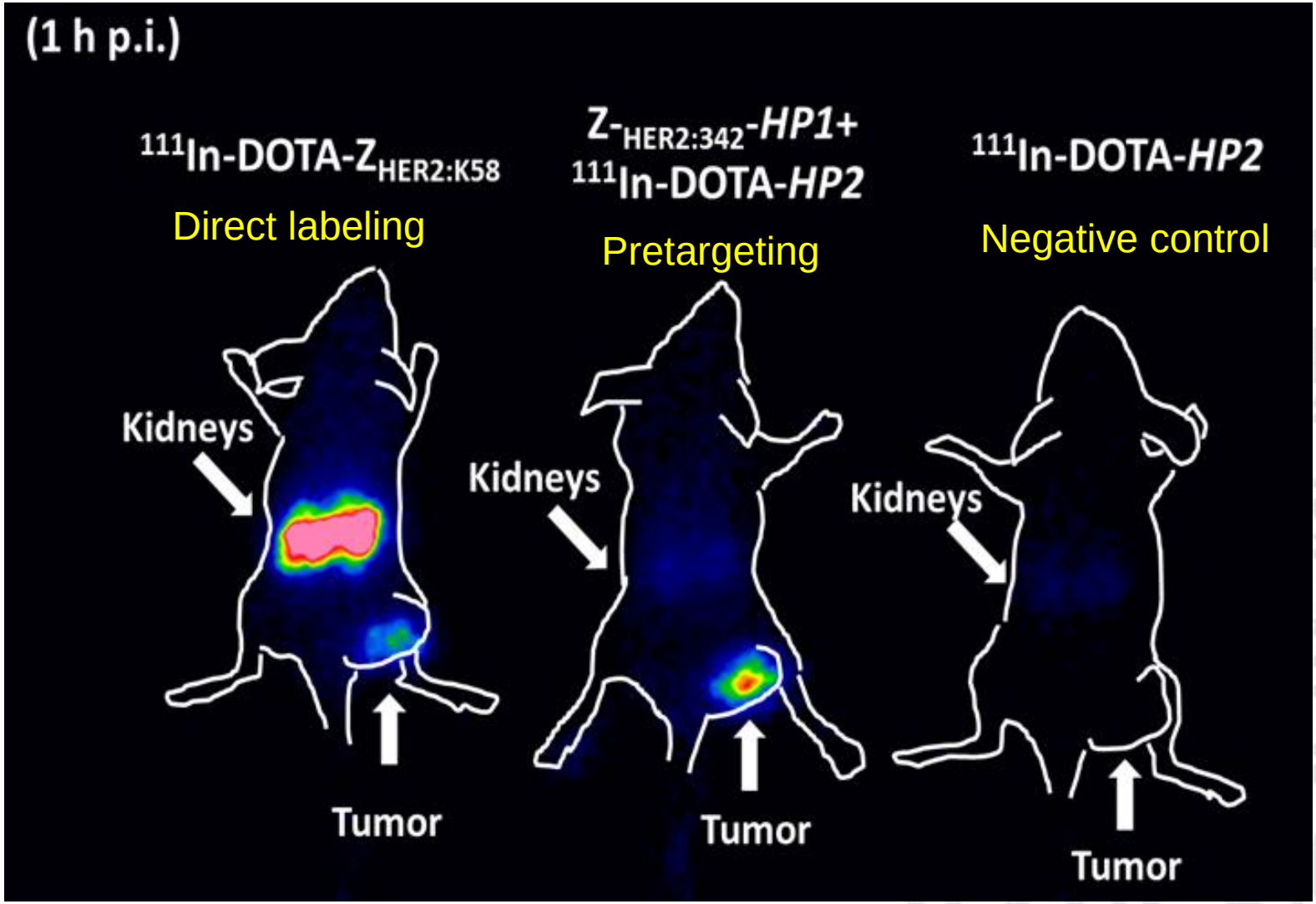
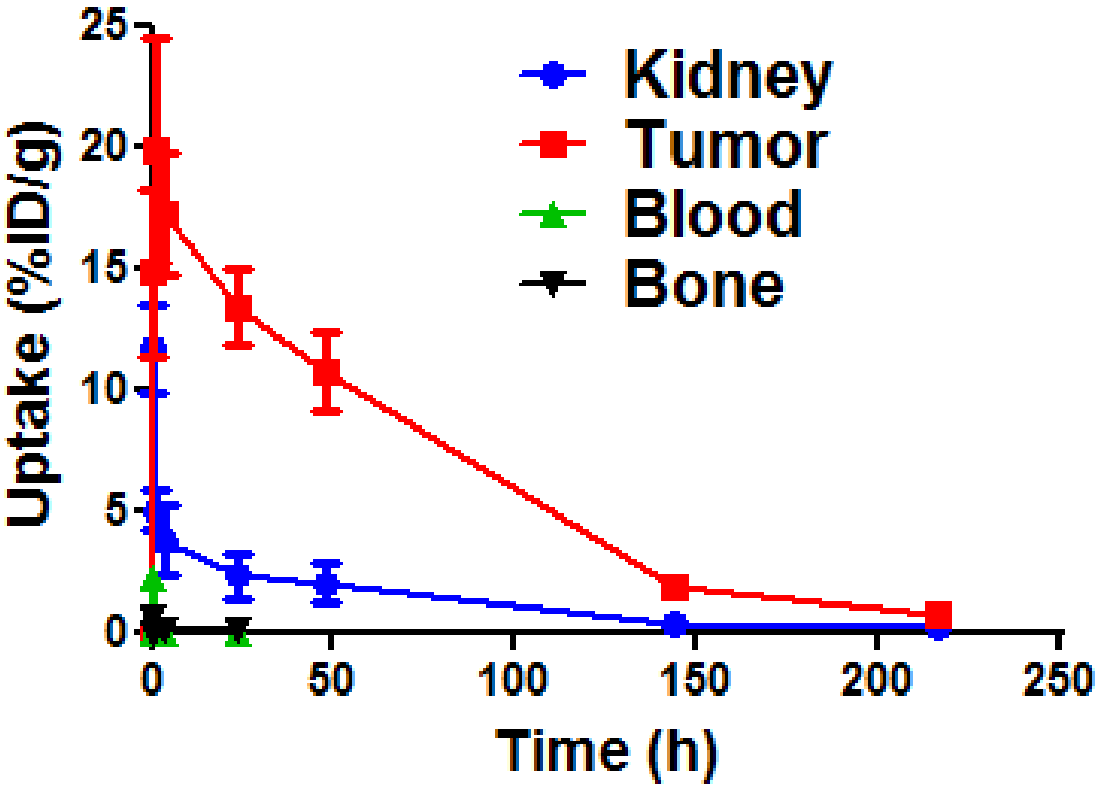
Affibody-Based PNA Pretargeting



Dosimetry
Residence of ^{177}Lu (decay uncorrected)



AUC	ratio
268	5
1362	1
1.9	716
1.2	1048

Calculation of Area Under Curve for Dosimetry Evaluation

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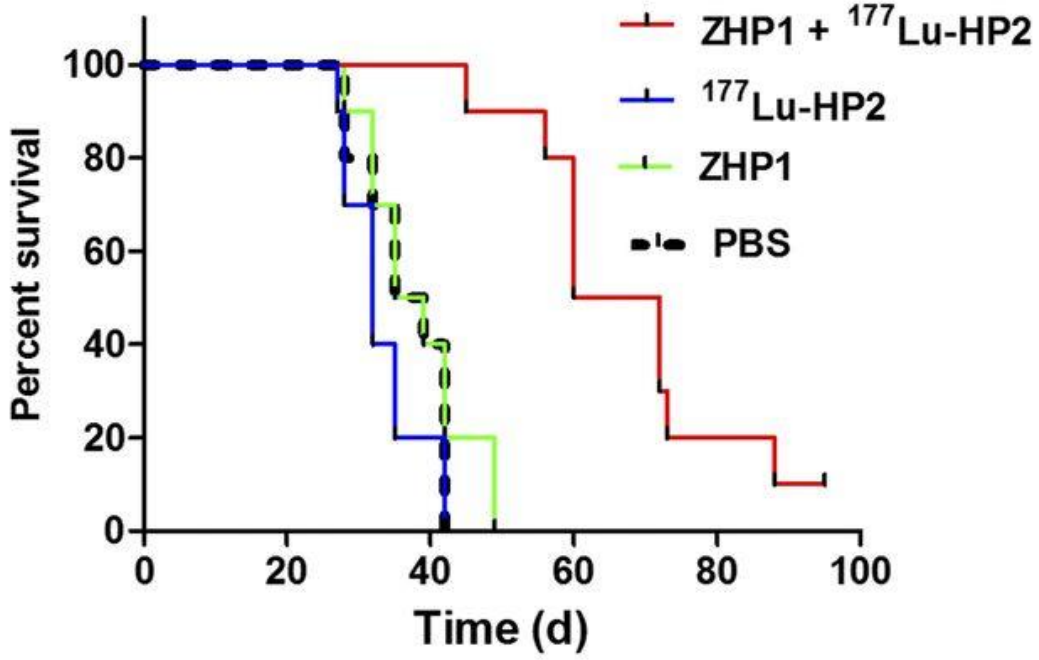
Experimental therapy

Should be carefully planned (expensive, time consuming)

- **appropriate control groups**
  - **vehicle (PBS, saline)**
  - **unspecific molecule with similar kinetics**
  - **un-labeled targeting molecule**
- **number of animals per group**
- **end point**

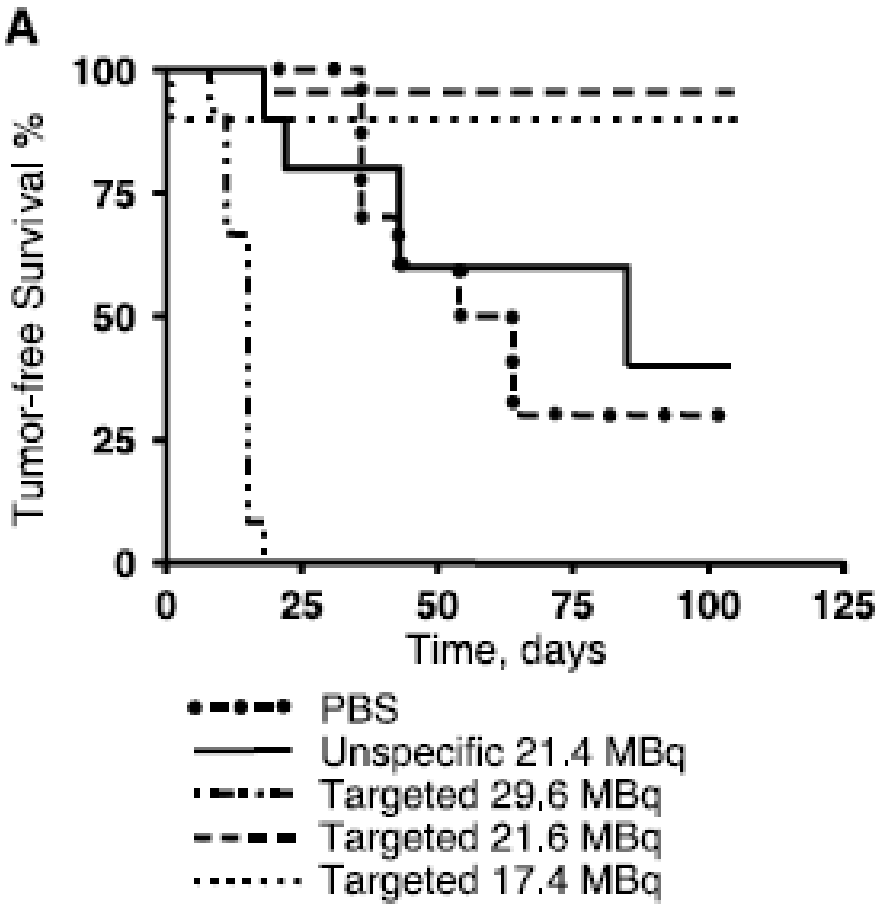
Radionuclide Therapy of HER2+ Xenografts Using Affibody-Based PNA Pretargeting

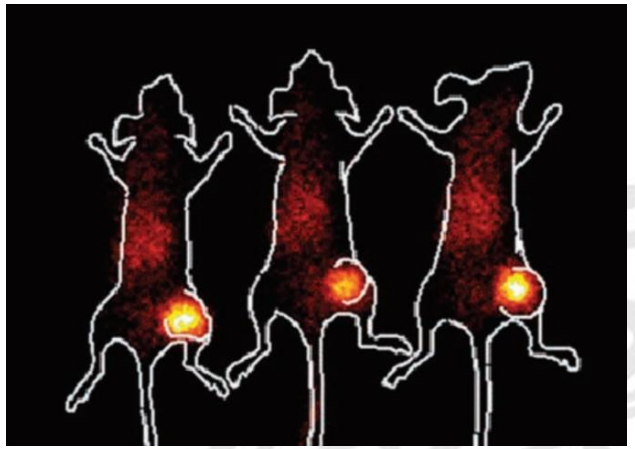


6 cycles of radionuclide therapy with ^{177}Lu-HP2 doubled median survival of mice (66 d. vs 37 d.)

Westerlund, Altai et al. J Nucl Med. 2018 Jul;59(7):1092-1098. doi: 10.2967/jnumed.118.208348.

Radionuclide therapy with ^{177}Lu-affibody-ABD prevented formation of tumors in mice





Tolmachev et al. Cancer Res. 2007; 67(6):2773–82.
Orlova et al. J. Nucl. Med. 2013; 54: 961–968

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Pre-clinical evaluation of radiolabeled proteins