Alternative rapid separation strategy for isolation of no-carrier added ^{90}Nb from Zr target, for application in *immuno*-PET.

Valery Radchenko[1], Dmitry Filosofov[2], Nikolai Lebedev[2], Olga Bochko[2], Frank Roesch[1]

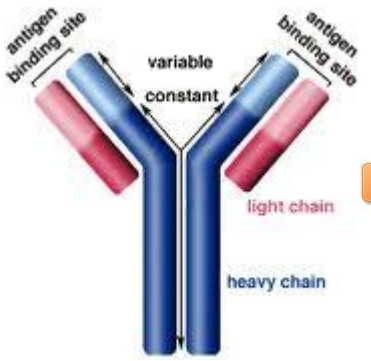
[1] Institute of Nuclear Chemistry, Johannes Gutenberg-University Mainz, Fritz-Strassmann-Weg 2, D-55128 Mainz, Germany;

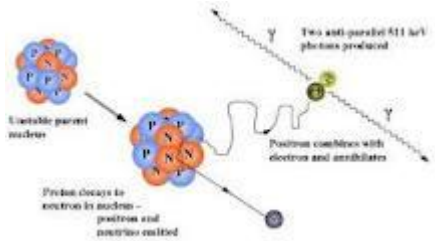
[2] Laboratory of Nuclear Problems, Joint Institute of Nuclear Research, Joliot-Curie 6, 141980, Dubna, Russian Federation.

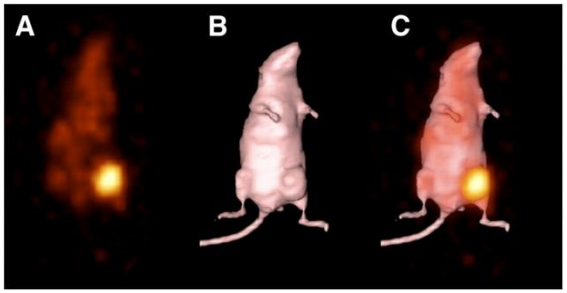
Triskem meeting, Munich, 12.11.2012

Immuno-PET

- Labeling of monoclonal antibody (mAb) with positron emitting radionuclides for tracking, visualization, and measurement the tumor gene expression



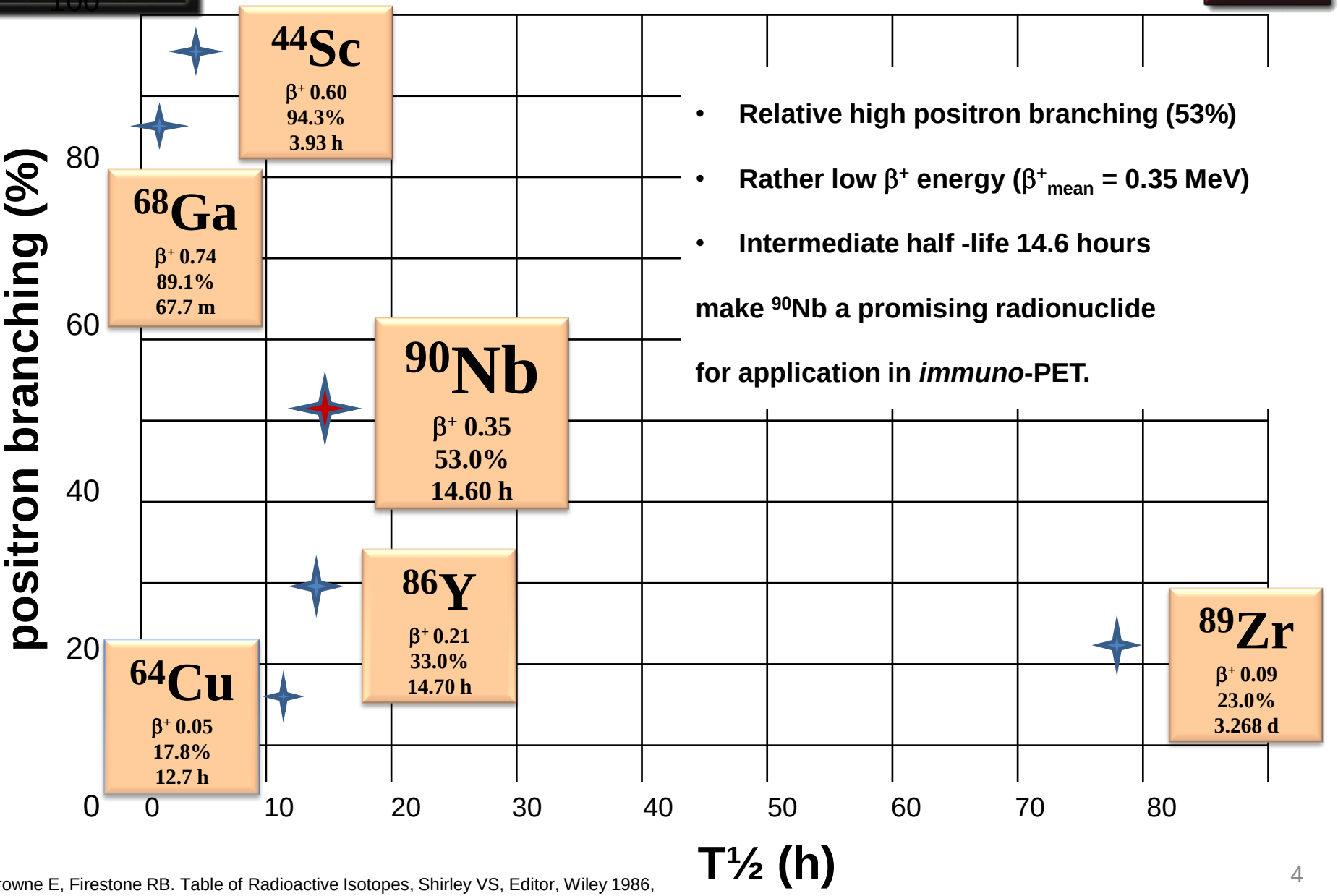




Requirements for *immuno*-PET nuclides

- a physical half-life paralleling the biological half-life of the antibody or antibody fragment
- a preferably low β+ energy to allow high-resolution PET imaging
- a high positron branching with no or weak accompanying irradiation (β^-, γ) to offer high-sensitive PET imaging while reducing the radiation burden of the patient
- the availability of the radionuclide, *i.e.* an efficient production route

Motivation



- **Relative high positron branching (53%)**
- **Rather low β^+ energy (β^+_{mean} = 0.35 MeV)**
- **Intermediate half -life 14.6 hours**

make ^{90}Nb a promising radionuclide

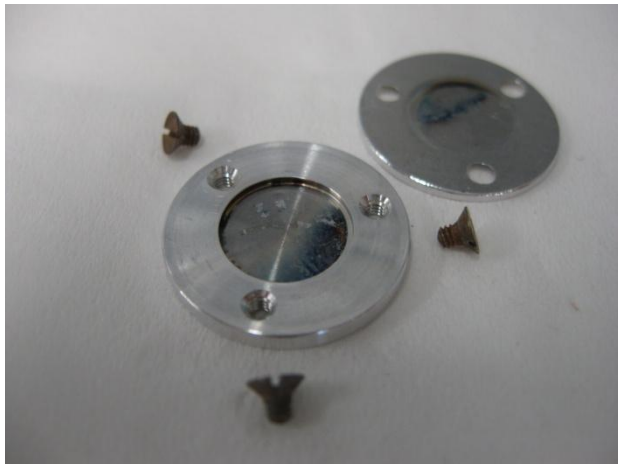
for application in *immuno*-PET.

Browne E, Firestone RB. Table of Radioactive Isotopes, Shirley VS, Editor, Wiley 1986,

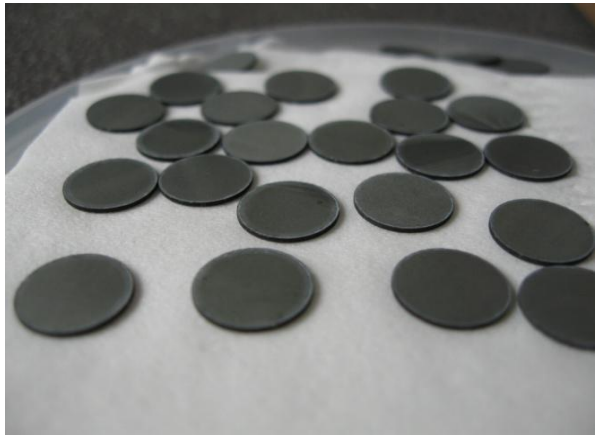
State-of-the-art: ^{90}Nb

- successful labeling of monoclonal antibody (Rituximab) with ^{90}Nb (90% labeling after 1 hour incubation at RT)
- high *in vitro* stability
  (less than 7% degradation after 9 days incubation in FCS at 37°C)
- proved suitability of ^{90}Nb for *immuno*-PET
- **however**: previous separation strategy was too complicated and time consuming (more than 4 hours) for routine *in vivo* application
- **consequently:** fast and more efficient alternative separation strategy was developed

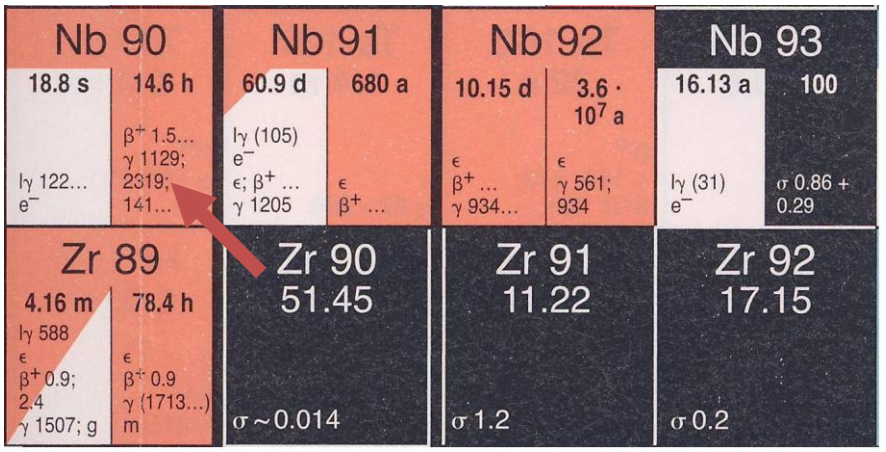
Production of ^{90}Nb

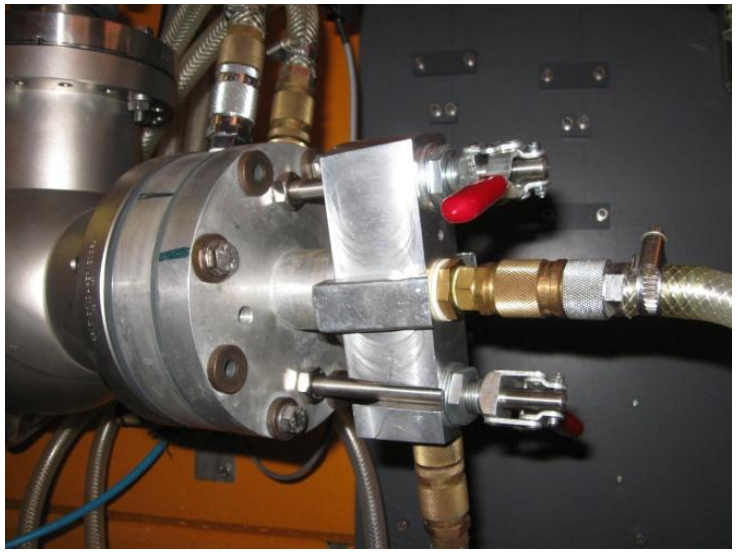


Target holder



Zirconium discs Ø 10 mm



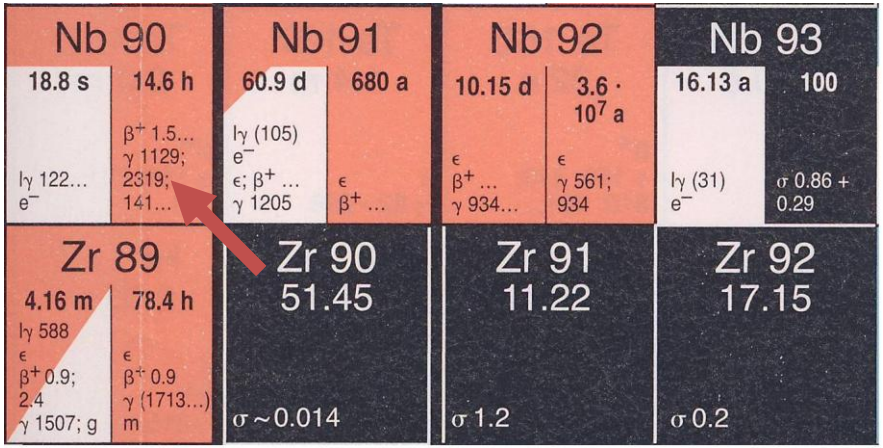


Target station

Production of ^{90}Nb

Irradiation parameters

- E_p : 17.5 MeV (at first foil)
- Current: 5 µA
- Irradiation : 60 min
- Batch : 724.6 MBq
- Production yield 144.9 MBq/ µA·h



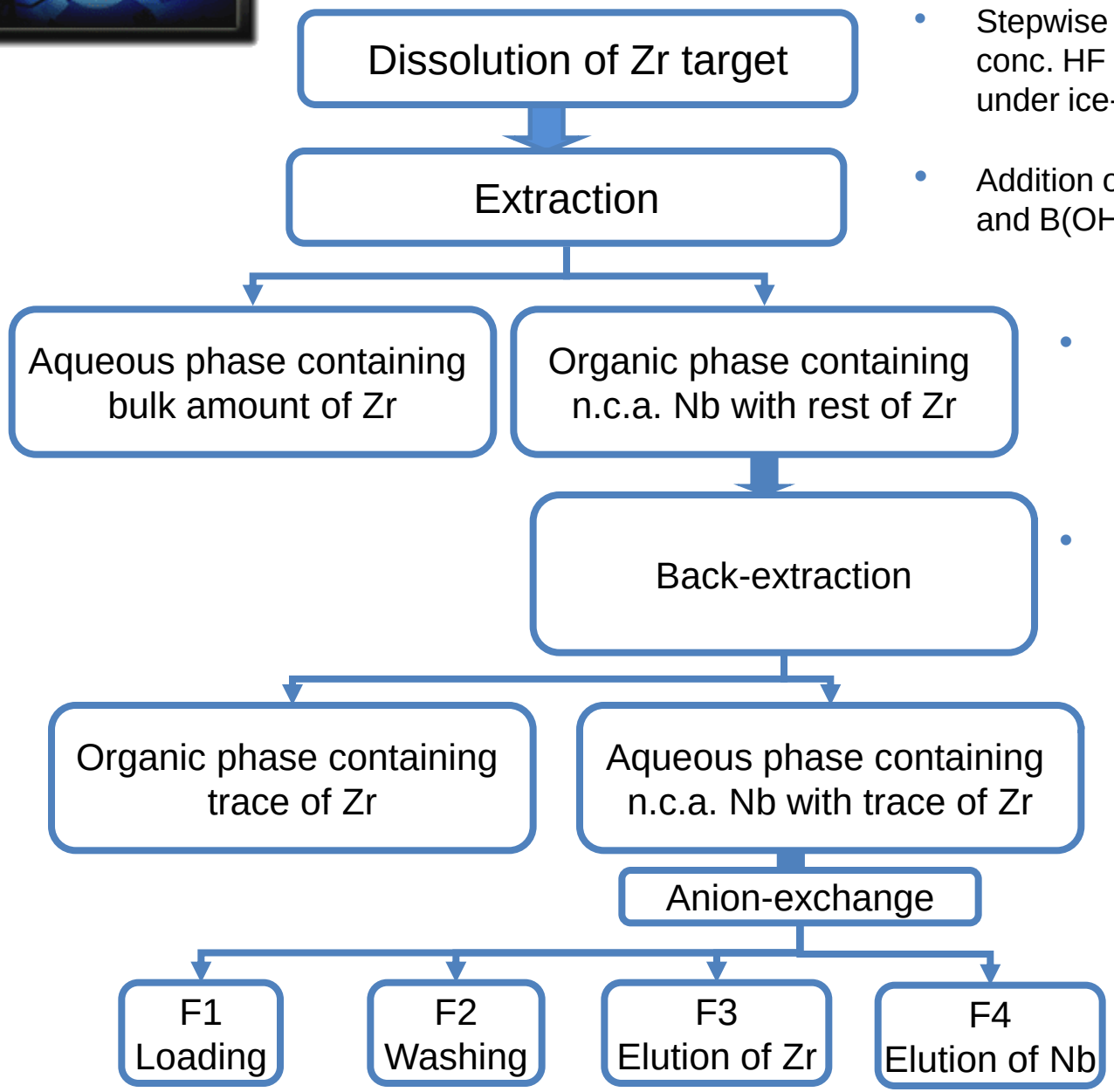
Radionuclide purity EOB

Isotopes	^{90}Nb 14.6 h	^{89}Zr 78.4 h	^{92m}Nb 10.2 d	^{95}Nb 35 d	^{96}Nb 23.4 h
%	96.76	0.29	1.79	0.42	0.74

Radchenko et al. Radiochim. Acta (2012) doi: 10.1524/ract.2012.1971

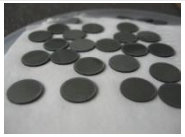
DEUTSCHES KREBSFORSCHUNGSZENTRUM IN DER HELMHOLTZ-GEMEINSCHAFT

Previous separation strategy



- Stepwise addition of 0.63 ml conc. HF to 2 ml of water under ice-cooling
- Addition of conc. HCl (5 ml) and $B(OH)_3$ (3.4 ml)
- Extraction with $2 \cdot 10^{-2}$ M N-benzoyl-N-phenylhydroxylamin (BPHA) (5 ml)
- Back-extraction with 5 ml aqua-regia (5 ml)
- Anion-exchange:
- resin Aminex-27 15 ± 2 µm
- column 20X1.5 mm
- F1 loading
- F2 100 µl 10 M HCl
- F3 200 µl 9 M HCl/0.001 M HF
- F4 200 µl contain Nb in 6 M HCl/0.01 M oxalic acid

Alternative separation strategy

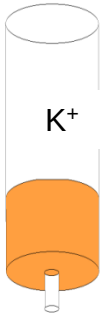


28 M HF

Irradiated Zr target

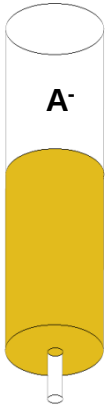
- irradiated zirconium metal dissolved in 2 mL conc. HF

- cation exchange (Dowex® 50X8) column to filtrate unsolved particles and absorb possible contamination of 2+ and 3+ cations



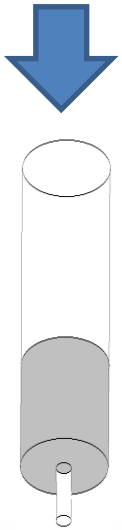
100 mg Dowex® 50X8

- transfer to anion exchange (AG® 1X8) column
- absorb ^{90}Nb from hydrofluoric solution
- zirconium passed trough
- 5 mL of 28 M HF to reduce Zr contamination
- 1 mL 1 M HCl to remove HF traces
- elution of ^{90}Nb with 700 µL 6 M HCl / 1% H_2O_2

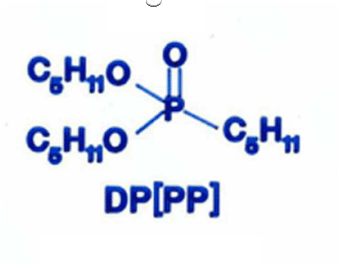


300 mg AG® 1X8

Alternative separation strategy

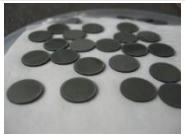


100 mg
UTEVA®



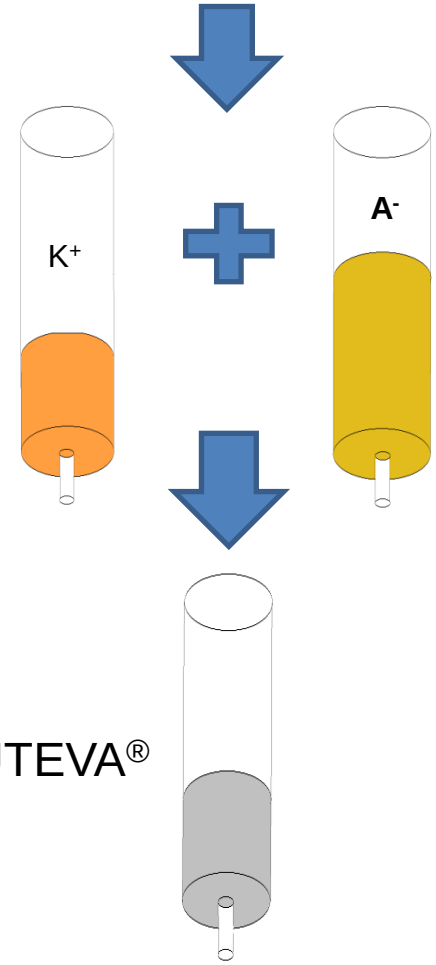
- 700 μL of mixture 6 M HCl / 1% H_2O_2 heated 5 min. at 120 °C
- 700 μL of 12 M HCl added to increase HCl concentration
- mixture loaded on UTEVA® column
- 5 mL 5 M HCl to remove Zr traces
- 200 μL 1 M oxalic acid passed through
- final elution of ^{90}Nb with 200 μL 1 M oxalic acid

Results



28 M HF

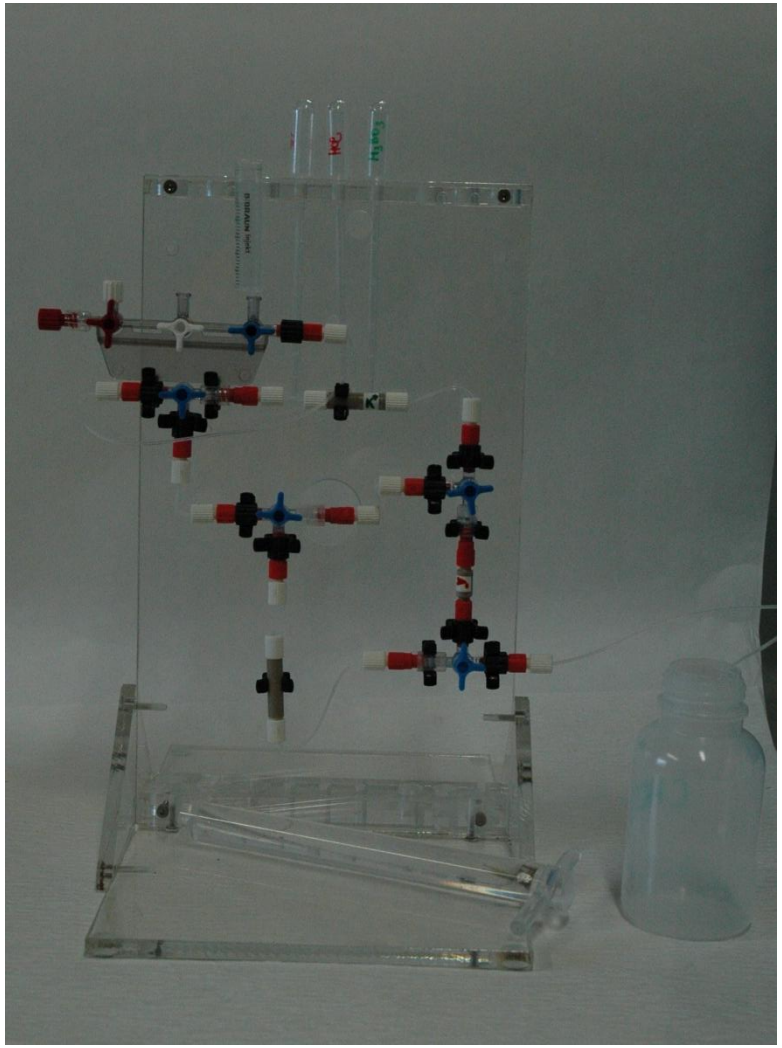
Irradiated Zr target

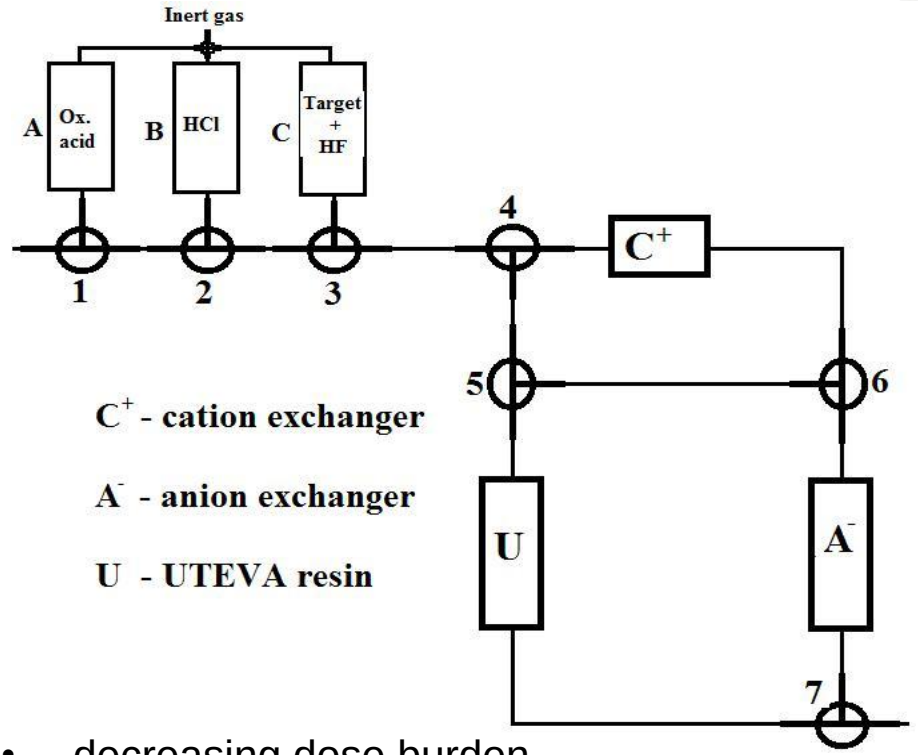


Separation step	Separation yield %	Decontamination factor (ICP-MS)
Dissolution	100	0
Cation+anion	99	$0.97 \cdot 10^5$
UTEVA	95	$3.36 \cdot 10^8$

- total separation 1.5 hours
- separation yield 95%
- decontamination factor > 10^8
- < 0.77 ng of Zr
- ^{90}Nb in final fraction appropriate for labeling conditions (200 µL1 M oxalic acid)

Semi-automated separation module





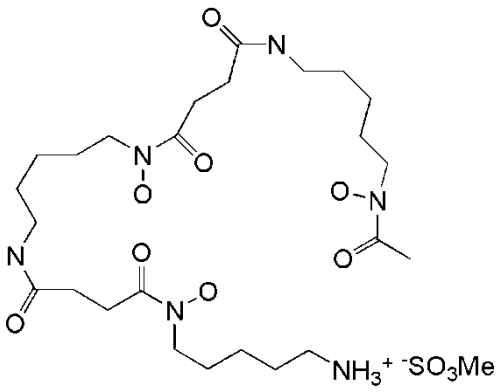
- decreasing dose burden
- separation yield 90%
- faster separation (1 hour)
- similar decontamination factor 10^8

Comparison of both separation strategy

Parameters	New separation strategy	Old separation strategy
Separation time (h)	1-1.5	> 4
Separation yield %	90-95	79-81
Decontamination factor Zr/Nb	10^8	10^7
Automation	available	difficult
Final fraction	200 µL 1 M ox. acid (easy labeling protocol)	200 µL 6M HCl/ 0.01 M ox. acid (complicated neutralization procedure)

Chelator for ^{90}Nb

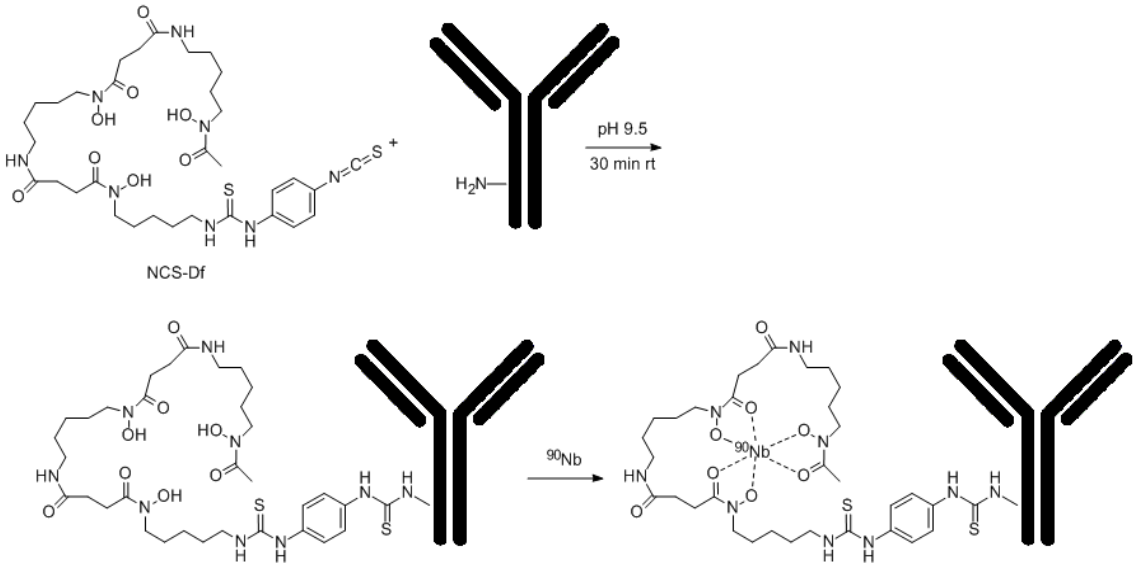
Deferoxamine (Df): chelator of choice



- best complexation with Nb compared to other chelators (DOTA, TETA, DTPA and EDTA)
- successful complexation of Nb at RT
- high stability of *Nb-Df complexes and *Nb-Df-Octreotide
- clinically approved chelator
- well established conjugation chemistry for ^{89}Zr

Busse S. PhD thesis, University of Mainz, 2000

Conjugation of Df to mAb



Product identification:

- Macrocyclics Product ID: B-705
- Molecular weight: 752.9 g/mol
- Purity: ≥ 94%
- Desferroxamine-p-SCN
- Molecular Formula: $C_{33}H_{52}N_8O_8S_2$
- Appearance: white solid

Perk et al., Eur. J. Nucl. Med. Mol. Imaging 2010, 37, 250-259 *Vosjan et al., Nature Protocols 2010, 5, 739-743.*

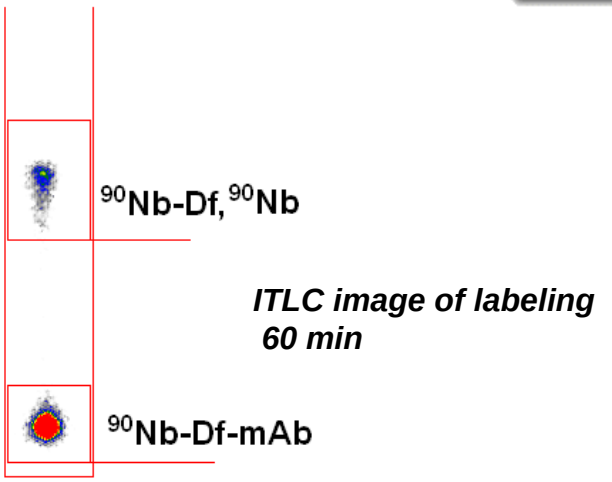
Labeling of Monoclonal Antibody

Monoclonal antibody (IMAB362) as proof-of-principle.

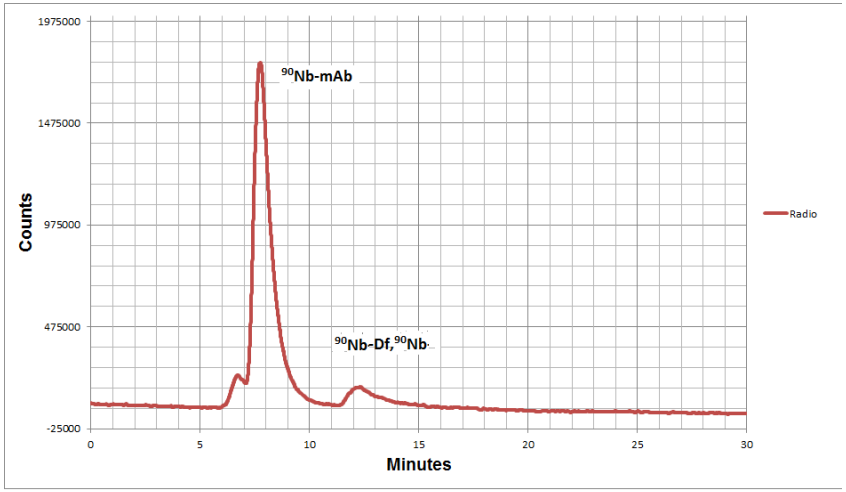
100 μg of mAb (modified with desferrioxamine)
labeled with 10 MBq of ^{90}Nb
1 hour at room temperature and pH 6.8

Results:

- Labeling yield more than 85% (HPLC, ITLC)
- Specific activity > 85 MBq/mg (comparable with ^{89}Zr 180 MBq /mg)
- After purification (PD-10) more than 97% of product
- Product stable (90%) at room temperature and at incubation in FCS at 37°C for 5 days



ITLC image of labeling 60 min



HPLC profile 60 min.

Summary

- aim:
  New separation strategy of ^{90}Nb
  appropriate for *in vivo* evaluation of biomolecules (*immuno*-PET)

- efficient:
  90 - 95% of ^{90}Nb with a decontamination factor of Zr / Nb of > 10^8

- fast:
  1 - 1.5 hours (almost four times faster than with previous separation strategy)

- semi-automated module

- labeling ^{90}Nb-mAb:
  > 85% after 1 h incubation at RT.
  specific activity > 85 MBq/mg

- *stability in vitro*:
  High, <10% of degradation after 5 days of FCS incubation at 37 ºC

Acknowledgments

TRIGA Reactor Mainz

Flerov Laboratory of Nuclear Reaction and Phasotron facility

Prof. Dr. M. Eisenhut
H. Hauser

G. Bukalis
Dr. K. Herbert

Thank you for your attention!