“Trastuzumab radioimmunoconjugates – promising strategy for selective anticancer therapy “

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Introduction

✓ Monoclonal Antibody – targeted therapy

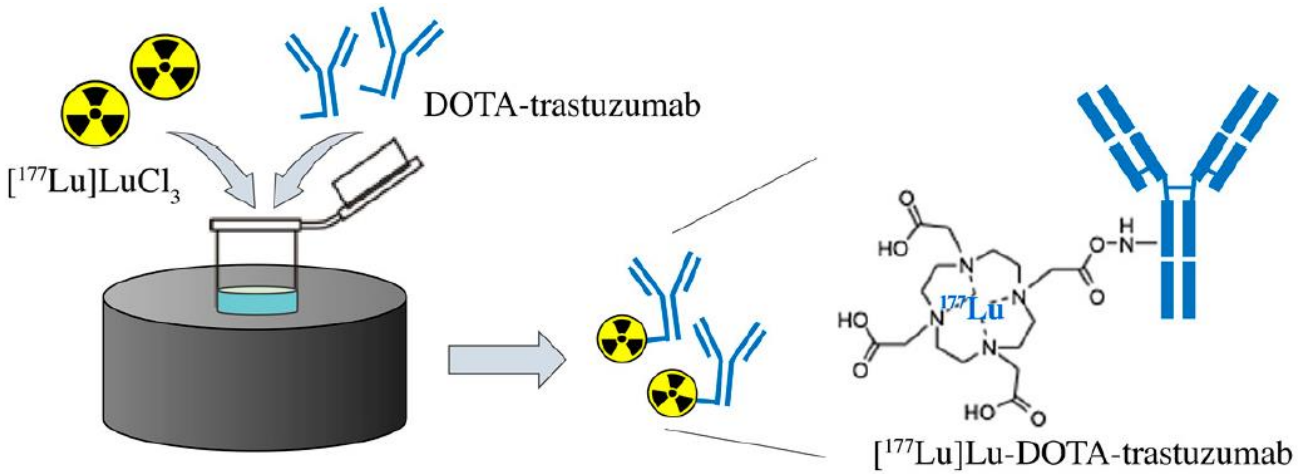
"Monospecific antibodies that are made by identical immune cells that are all clones of a unique parent cell."

✓ Immunoconjugates

Antibody linked either to cytotoxic drugs and toxins or to radioisotopes.

✓ Radioimmunoconjugate

- ^{131}I-tositumomab
- ^{111}In/^{90}Y ibritumomab tiuksetin

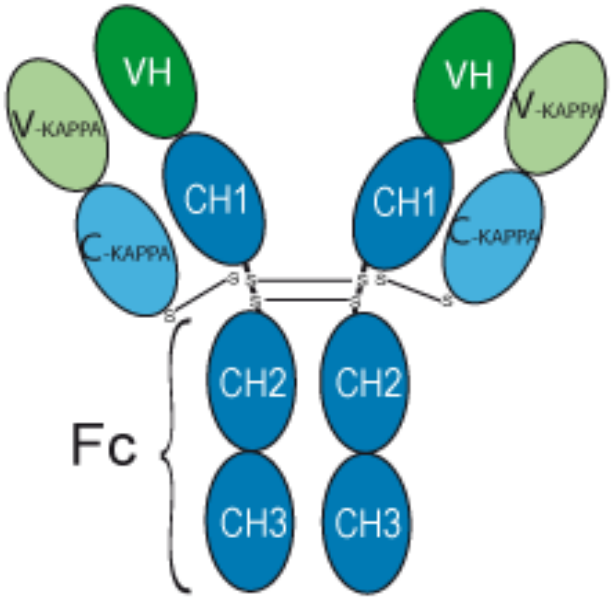


Sample of radioimmunoconjugate

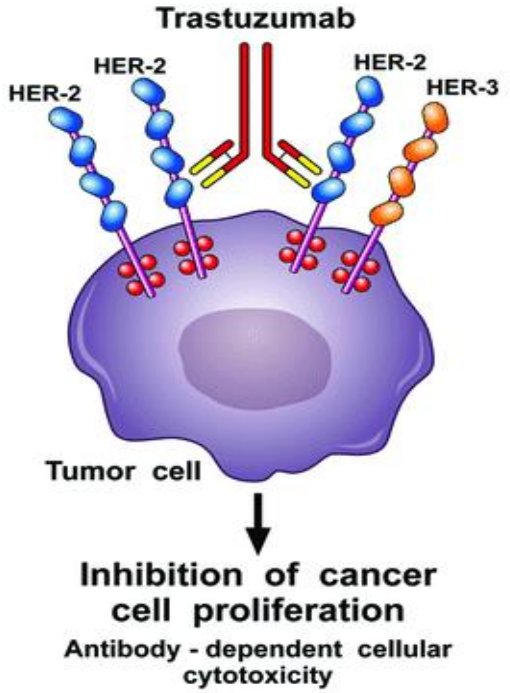
Starting point - mAb

Trastuzumab

- Humanized mAb
- Anti HER2/neu
- Breast cancer, metastatic gastric adenocarcinoma or gastroesophageal junction.



Trastuzumab structure

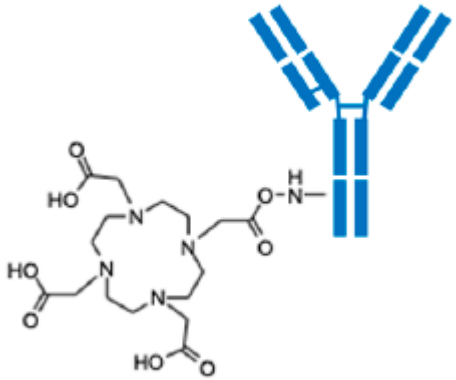


*www.drugbank.com

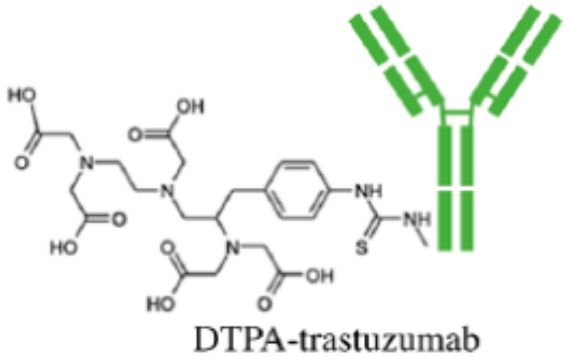
**www.ncbi.nlm.nih.gov/pmc/articles/PMC5846016/#!po=19.3878

Conjugation with chelator (bifunctional group)

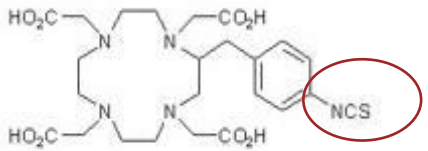
- Acyclic chelator - DTPA (diethylene triamine pentaacetic acid) and 1B4M – DTPA (2-(4-isothi-ocyanatoben-zyl)-6-methyl-diethylene-triaminepenta-acetic acid) (octadentate)
- Macrocyclic - DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) (octadentate)



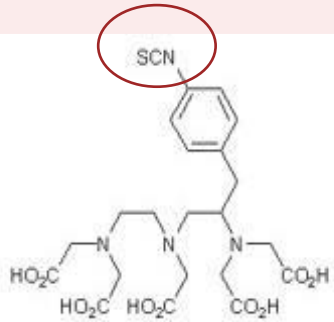
DOTA-trastuzumab



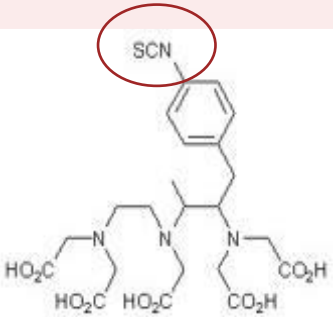
DTPA-trastuzumab



P-SCN-Bn-DOTA

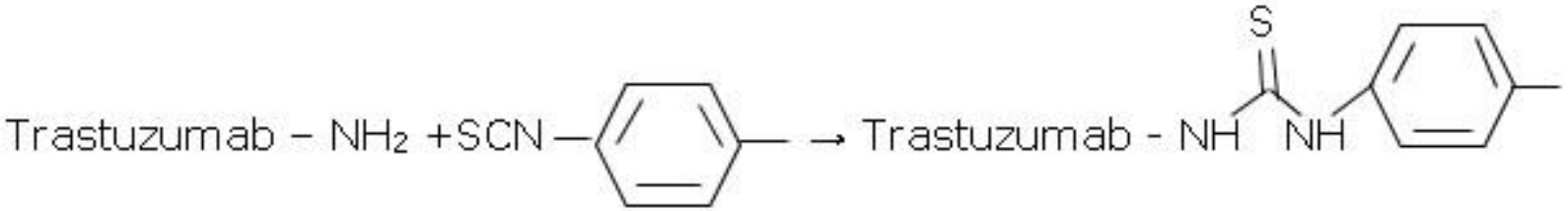


p-SCN-Bn-DTPA



p-SCN-Bn-1B4M-DTPA

4-izothiocyanatobenzyl groups in theirs structures which participate in the process of binding to the antibody



Thiourea linkage is created due to reaction between amino groups of lysine residues of trastuzumab and isothiocyanate groups of chelators.

*M Sterjova, P Apostolova et al. PREPARATION AND INTEGRITY EXAMINATION OF FREEZE DRIED KIT OF TRASTUZUMAB-IMMUNOCONJUGATES AND COLD LABELED IMMUNOCONJUGATES BY APPLYING SDS-PAGE ELECTROPHORESIS. *Acta Medica Medianae 2019;58(3):15-23.*

Freeze drying

10 h
1. Freezing at – 40◦C (rate 0,40 ◦C/min)
2. Annealing step at -15 ◦C

25 h
3. Primary drying at -10 ◦C

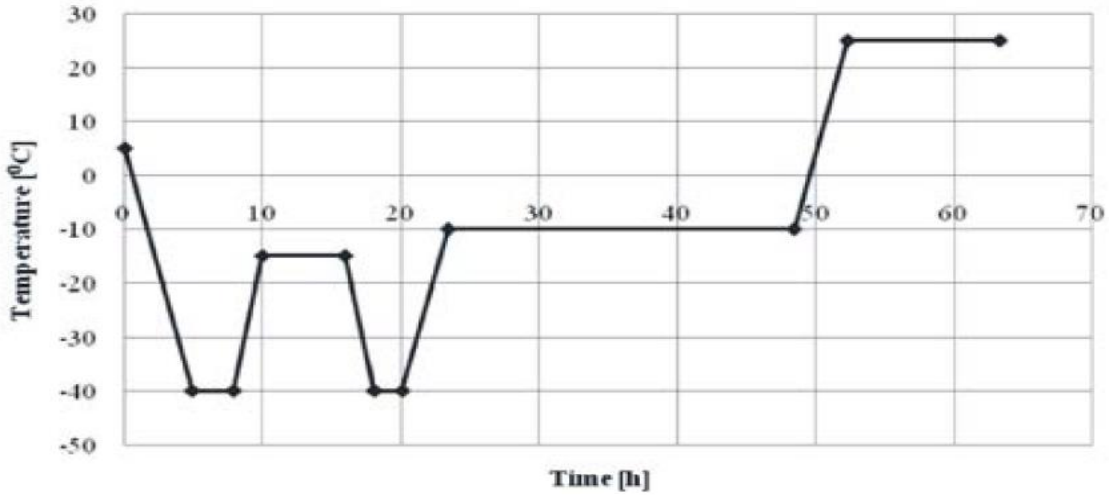
11 h
4. Secondary drying at 25 ◦C

Pressure 0,133 mBar

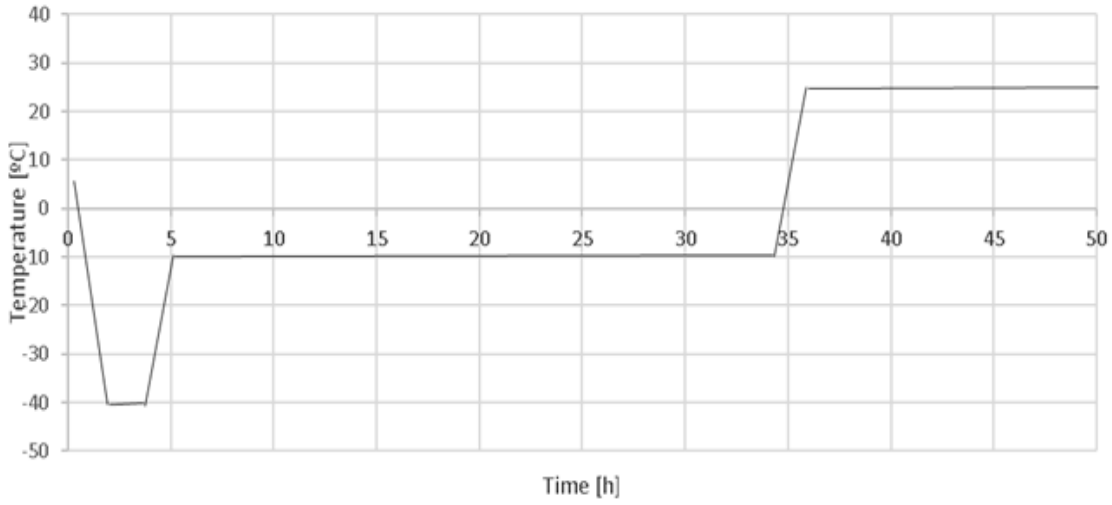
5 h
1. Freezing -40 ◦C (rate 1,0 ◦C /min)

28 h
2. Primary drying at -10 ◦C

14 h
3. Secondary drying at 25 ◦C



Lyophilization protocol for Rituximab



Lyophilization protocol for Trastuzumab

Performed on Labconco Free Zone Stoppering Tray Dryer (Kansas City, Missouri, USA)

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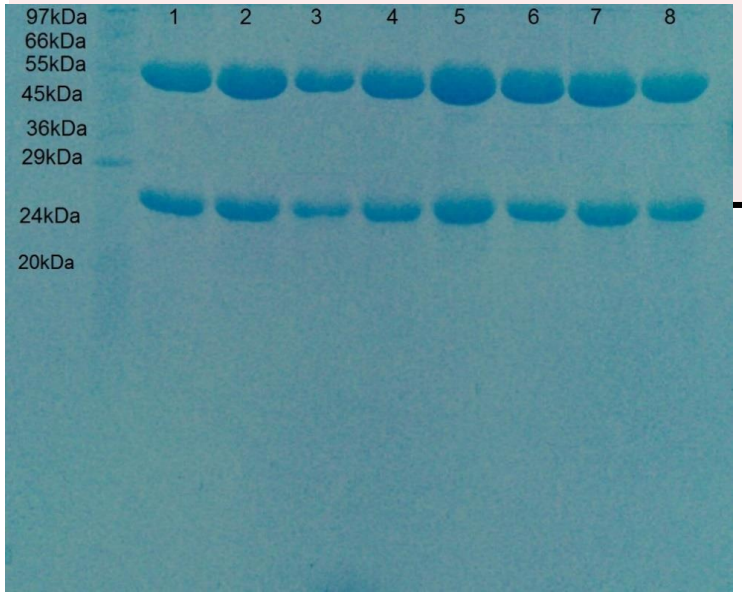
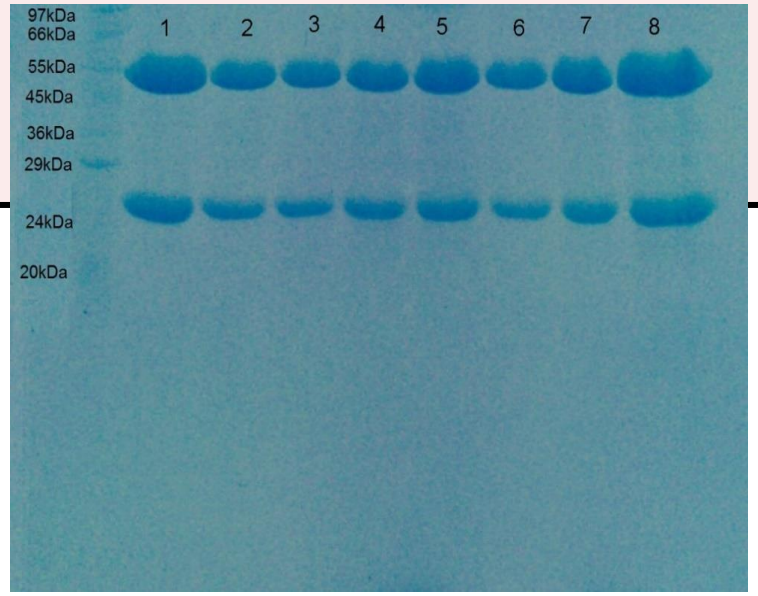
Control of immunoconjugates

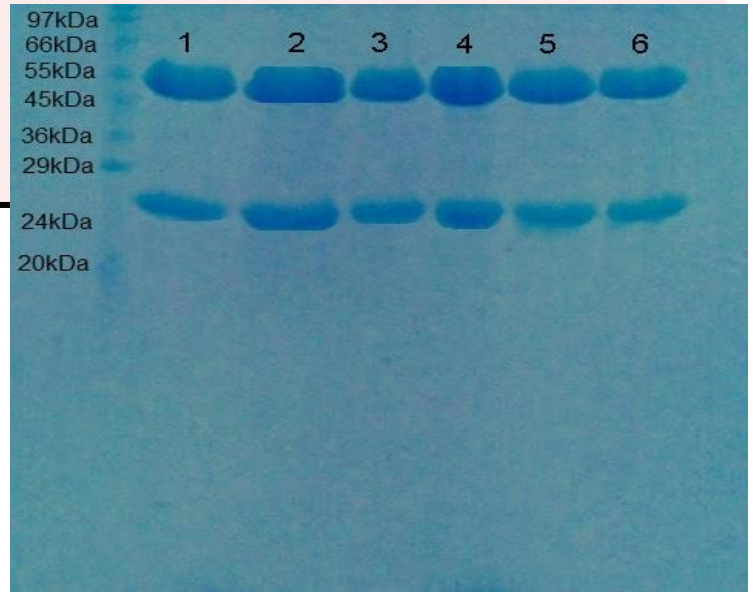
- Electrophoresis (reducing SDS PAGE)



(1) DTPA-trastuzumab (1:10)
(2) DTPA-trastuzumab (1:20)
(3) DTPA-trastuzumab (1:50)
(4) DOTA-trastuzumab (1:20)
(5) 1B4M-DTPA-trastuzumab (1:10)
(6) 1B4M-DTPA-trastuzumab (1:20)
(7) 1B4M-DTPA-trastuzumab (1:50)



(1) Lu-DTPA-trastuzumab (1:10)
(2) Y-DTPA-trastuzumab (1:20)
(3) Lu-DTPA- trastuzumab (1:20)
(4) Y-DTPA- trastuzumab (1:50)
(5) Y-DTPA- trastuzumab (1:50)
(6) Y-DOTA- trastuzumab (1:20)
(7) Lu-DOTA- trastuzumab (1:20)

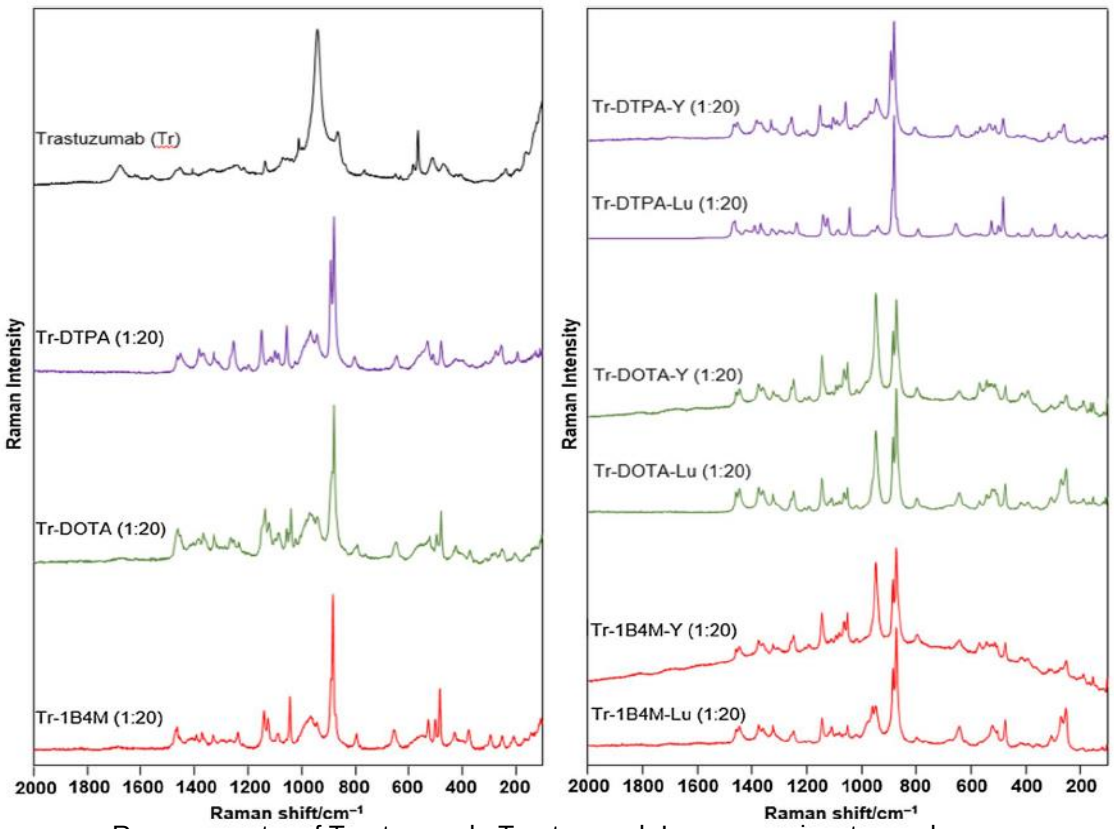


(1) Lu-1B4M-DTPA- trastuzumab (1:10)
(2) Y-1B4M-DTPA- trastuzumab (1:20)
(3) Lu-1B4M-DTPA- trastuzumab (1:20)
(4) Y-1B4M-DTPA- trastuzumab (1:50)
(5) Lu-1B4M-DTPA- trastuzumab (1:50)

*M Sterjova, P Apostolova et al. PREPARATION AND INTEGRITY EXAMINATION OF FREEZE DRIED KIT OF TRASTUZUMAB-IMMUNOCONJUGATES AND COLD LABELED IMMUNOCONJUGATES BY APPLYING SDS-PAGE ELECTROPHORESIS. *Acta Medica Medianae 2019;58(3):15-23.*

Raman spectroscopy

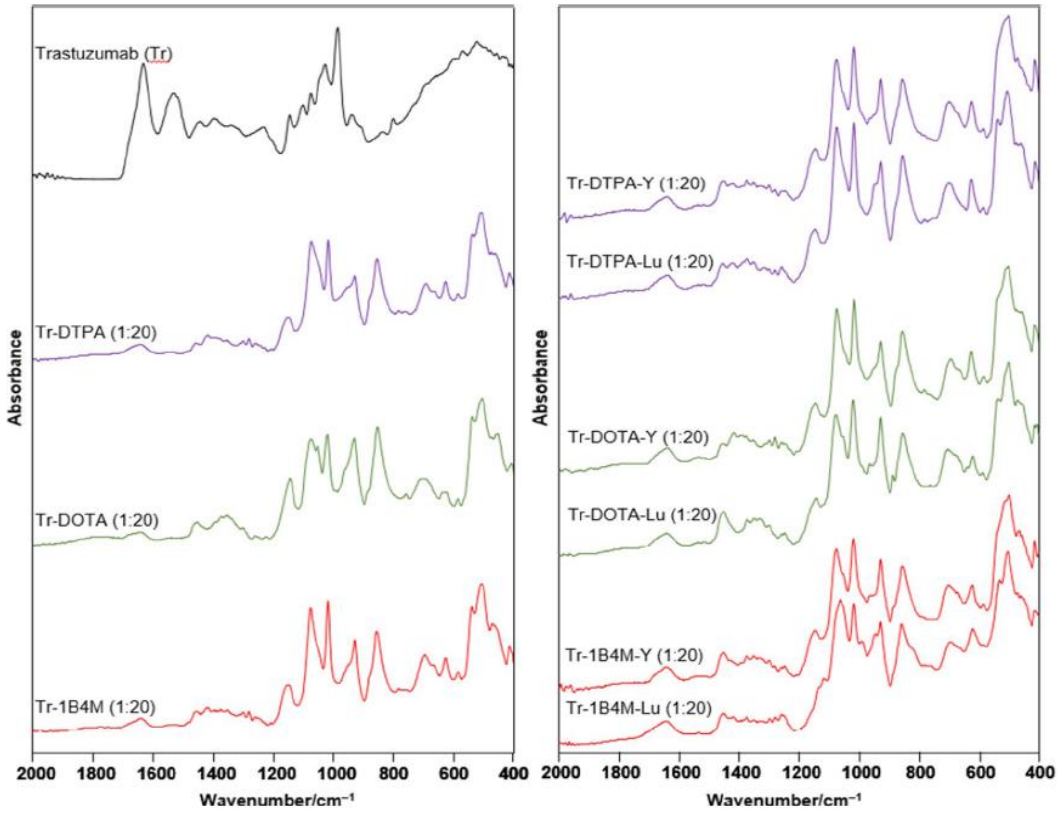
Region	Raman bands
Amide I	~ 1670 cm^{-1}
Amide III	~ 1245 cm^{-1}
S – S	400 – 700 cm^{-1}
- SH	2500 – 2700 cm^{-1}
Absence of characteristic well defined peak at ~ 1634 cm^{-1}	



Raman spectra of Trastuzumab, Trastuzumab Immunoconjugates and Trastuzumab cold labeld

IR

Region	IR bands
Amide I	1700–1600 cm^{-1}
Amide II	1480–1575 cm^{-1}
Amide III	1255–1244 cm^{-1}

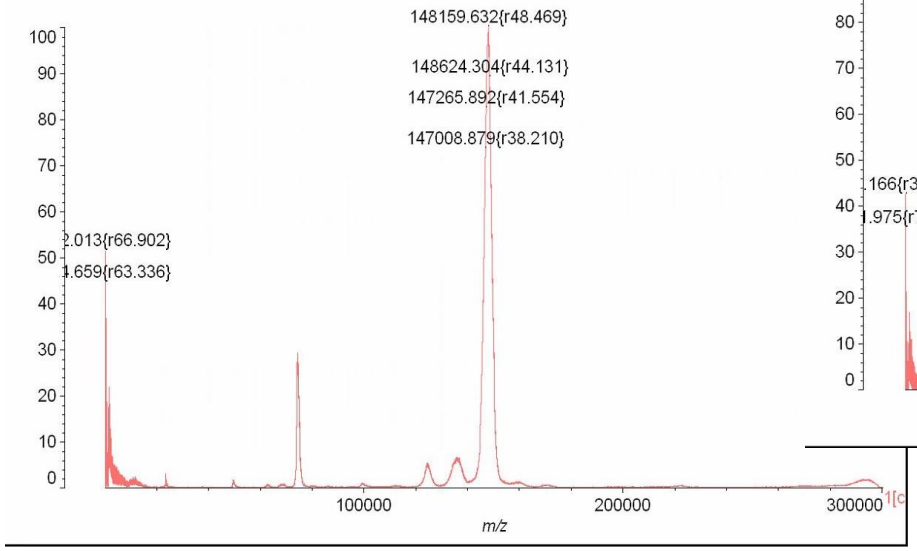


IR spectra of Trastuzumab, Trastuzumab Immunoconjugates and Trastuzumab cold labeled

*Marija Sterjova et al. Vibrational spectroscopy as a tool for examination to the secondary structure of metal-labeled trastuzumab immunoconjugates. J. Radioanal. Nucl. Chem. 320, pages 209–218 (2019)

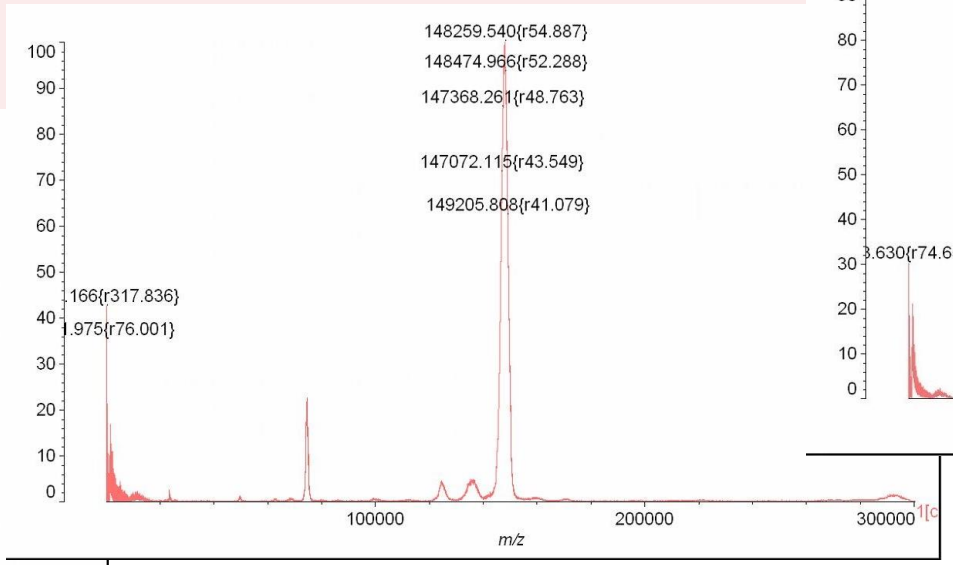
MALDI – TOF MS (*Matrix-assisted laser desorption ionization time-of-flight mass spectrometry*)

Mr (Trastuzumab) = 145531,50 Da



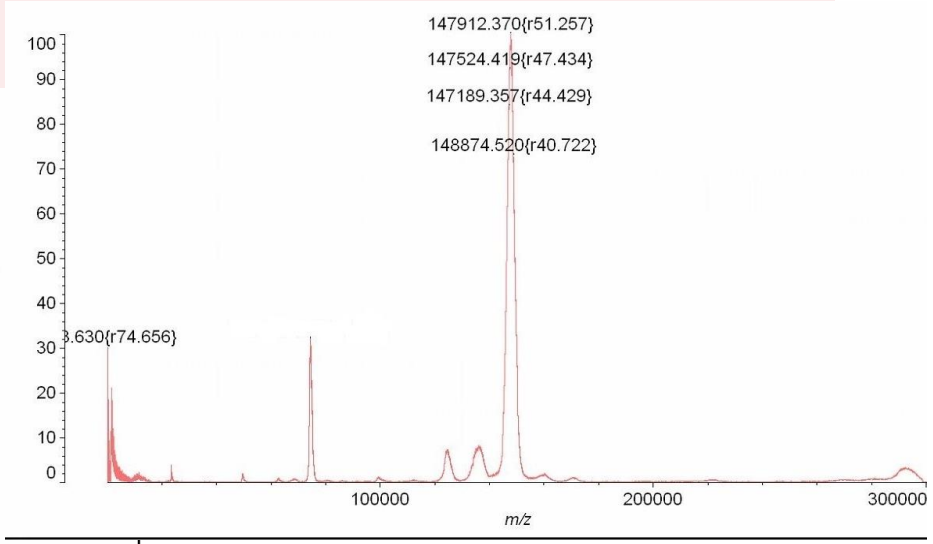
MALDI-TOF MS spectra Tr-DTPA (1:20)

Tr-DTPA (1:20) = 4,8



MALDI-TOF MS spectra Tr-DOTA (1:20)

Tr-DOTA (1:20) = 4,9

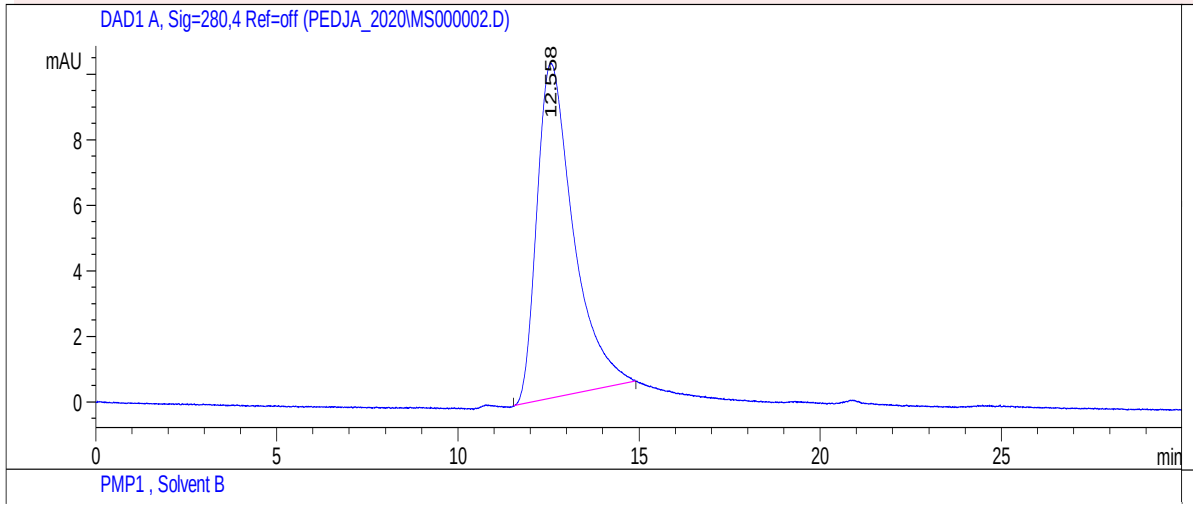


MALDI-TOF MS spectra Tr-1B4M-DTPA (1:20)

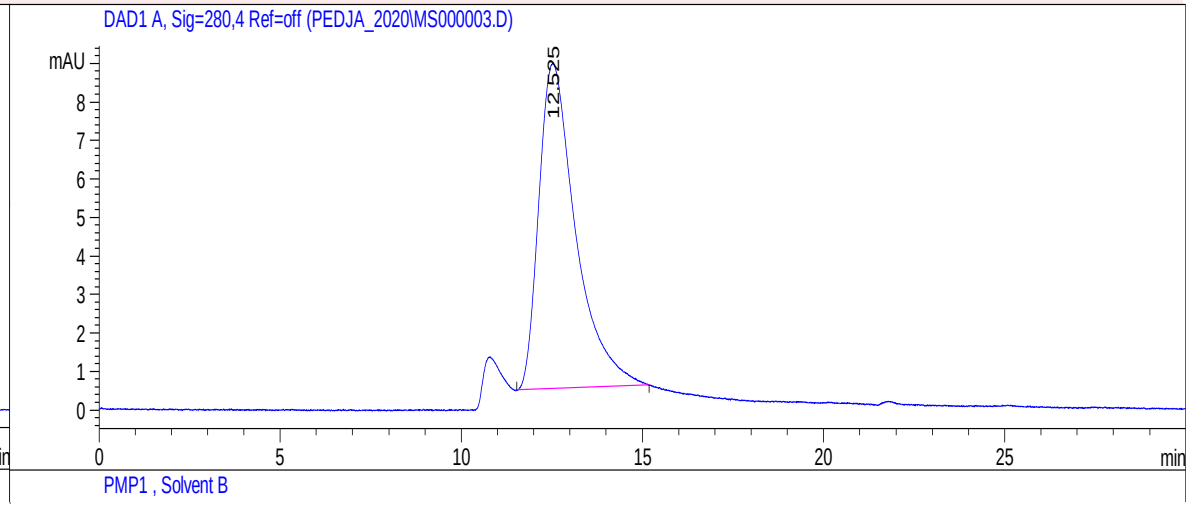
Tr-DTPA (1:20) = 4,3

HPLC

- SE chromatography
- DAD detection



HPLC profile of purified Trastuzumab



HPLC profile of conjugated Trastuzumab-1B4M-DTPA (1:20)

Radioactive immunoconjugate

- ^{90}Y
- ^{177}Lu

ITLC SG

- Yield (> 95%)
- Stability at different time point

Future actions:

- Trastuzumab and alfa emitting radioisotope

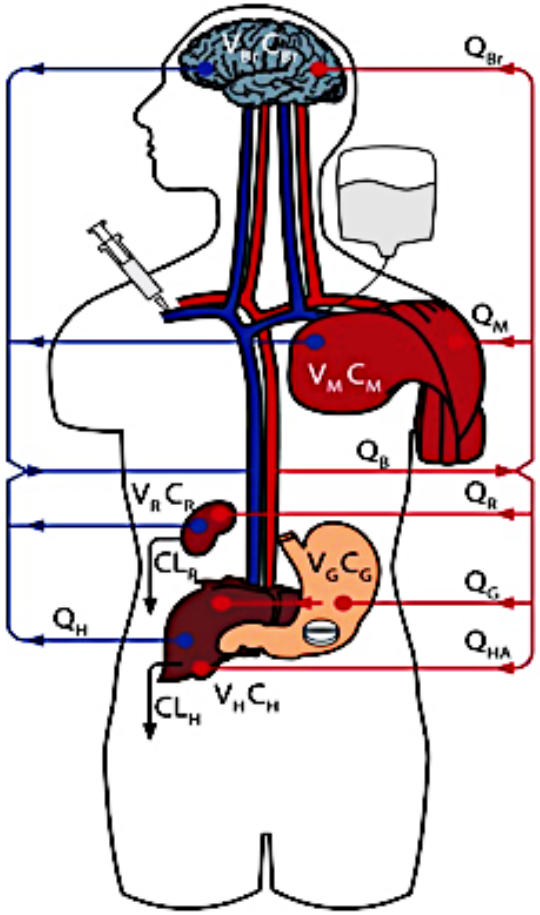
1. Using already available chelators - DTPA, DOTA

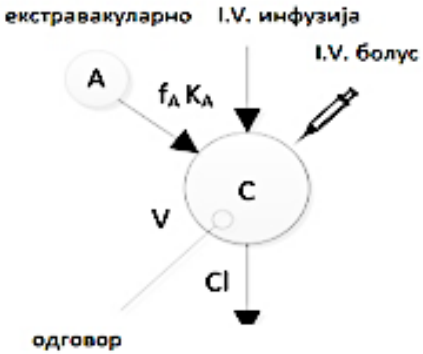
2. Other according to the literature
- m-MeATE - *N*-succinimidyl 3-(trimethylstannyl)benzoate
- Boc_2-SGMTB - *N*-succinimidyl 4-(1,2-bis-*tert*-butoxycarbonyl)guanidinomethyl-3-(trimethylstannyl)benzoate

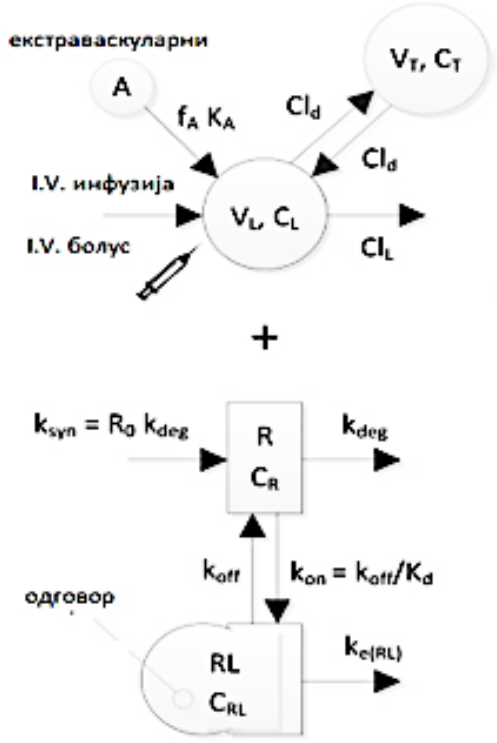
- Prediction of human pharmacokinetics of conjugated monoclonal antibodies by computer modelling using preclinical data

Critical steps in enabling their successful development and use.

Example from our previous work - Physiological model
Distribution Tissue-Blood Drugs - (Human, Rat)







Z, Rustemi, PhD thesis
Maxsim2 - Real-time interactive computer simulation for testing pharmacokinetics and pharmacodynamics
Provided by Mats Jirstrand, Department of Systems and Data Analysis, Fraunhofer-Chalmers Centre, Gothenburg, **Sweden**

Thank you