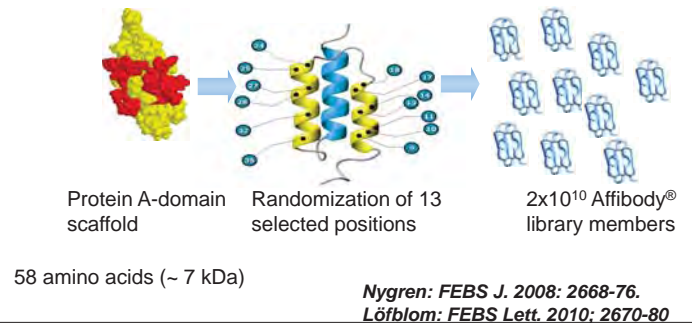


Imaging using Affibody molecules

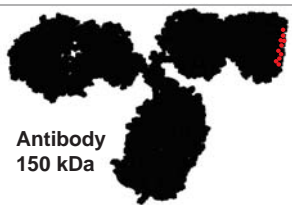
Vladimir Tolmachev

Biomedical Radiation Sciences, Uppsala University

Structure and selection of Affibody molecules



Features of Affibody molecules



Affibody Molecule
6-7 kDa

- High (picomolar) affinity
- Small size
- Robustness
- Both recombinant and synthetic production with site-specific labelling

Targets for Affibody molecules

- **HER2 (0.022 nM)** Orlova: *Cancer Res.* 2006; 4339-48.
- **EGFR (0.9 nM)** Tolmachev: *EJNMMI.* 2010; 613-22.
- **IGF-1R (0.5 nM)** Tolmachev: *J Nucl Med.* 2012; 90-97.
- **HER3 (0.7 nM)** Kronqvist: *PEDS.* 2010; 385-296
- **PDGFRβ (0.4 nM)** Lindborg: *J Mol Biol.* 2011; 298-315.

Imaging of these tyrosine kinase receptors can be used for predicting or monitoring response to specific anti-cancer therapy

Features of Affibody molecules

Robustness:

- cysteine-independent folding;
- re-folding within 3 μs in physiological conditions.

Permits the use of:

- extreme pH (3.0-11.5),
- high temperature (up to 95 °C),
- high concentration of a reductant,
- lipophilic solvents,
- high molarity buffers

Affibody molecules have been labelled for

SPECT

^{99m}Tc
¹¹¹In

¹²⁵I

PET

¹⁸F
⁷⁶Br
¹²⁴I

⁶⁸Ga
⁶⁴Cu
⁵⁷⁽⁵⁵⁾Co

Therapy

¹³¹I
²¹¹At

⁹⁰Y
^{114m}In
¹⁷⁷Lu

¹⁸⁶Re

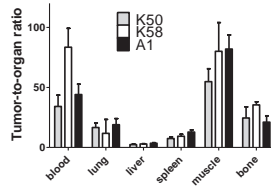
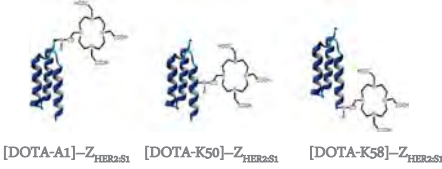


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Site-specific labeling of synthetic Affibody molecules

Site-specific incorporation of chelators during peptide synthesis:

- DOTA, NOTA, NODAGA...
- maGGG, maSSS, maEEE, maESE...



Well-defined conjugates with reproducible biodistribution



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Site-specific labeling of recombinant Affibody molecules

Site-specific coupling of chelators and prosthetic groups to a unique engineered thiol:

- maleimido-derivatives of DOTA, NOTA, NODAGA...
- ¹⁸F-FBEM;
- ⁷⁶Br/¹³¹I-HPBM;
- Mal-OA + ¹⁸F-FBA.

Site-specific incorporation cysteine-containing peptide-based chelators for Tc and Re;

Histidine-tag based chelators for Tc and Re;

Well-defined conjugates with reproducible biodistribution



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Features of Affibody molecules

Small size (ca. 7 kDa) in combination with high affinity

- rapid extravasation;
- rapid tissue penetration;
- strong binding to molecular target;
- rapid blood clearance of unbound tracer;
- no EPR effect, highly specific uptake

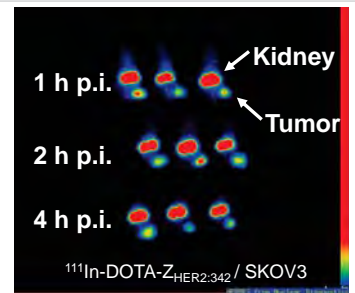
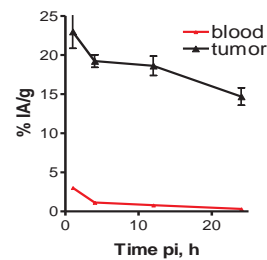
High-contrast imaging shortly after injection



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Rapid Kinetics:

¹¹¹In-DOTA-Z_{HER2:342}



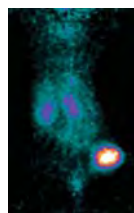
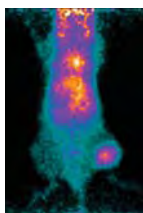
Orlova: Cancer Research, 2007: 2178-2186



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Rapid Kinetics:

¹²⁴I-PIB-Z_{HER2:342} vs ¹²⁴I-trastuzumab



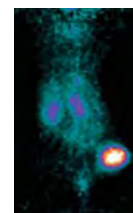
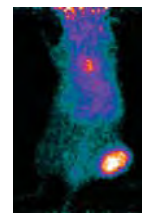
Orlova: Journal of Nuclear Medicine, 2009; 417-425.



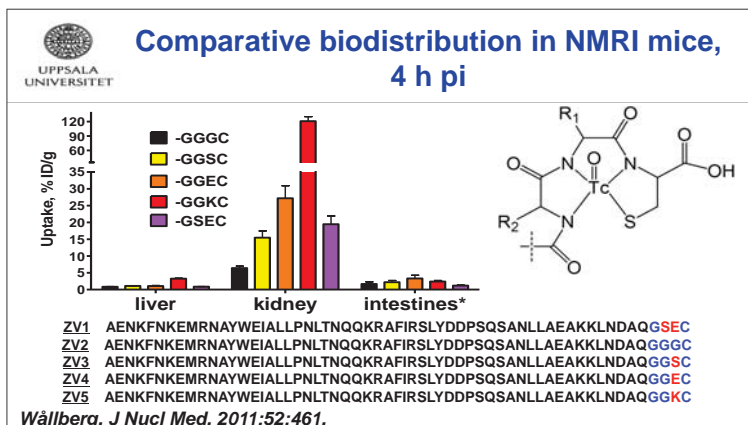
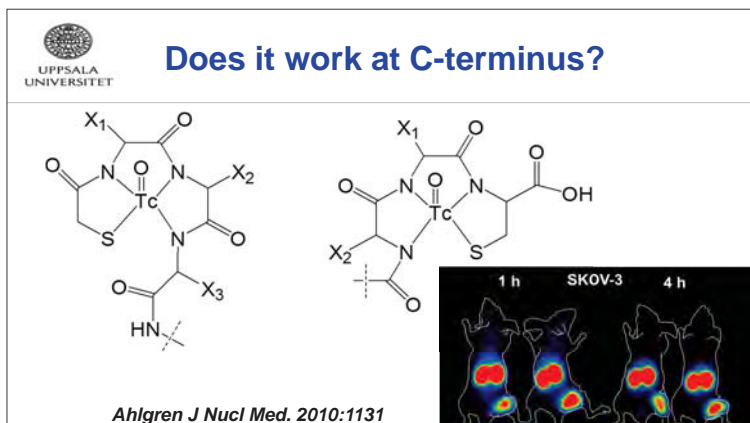
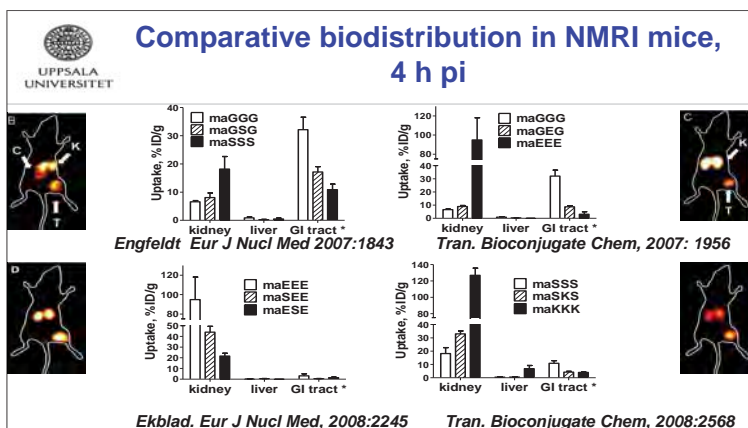
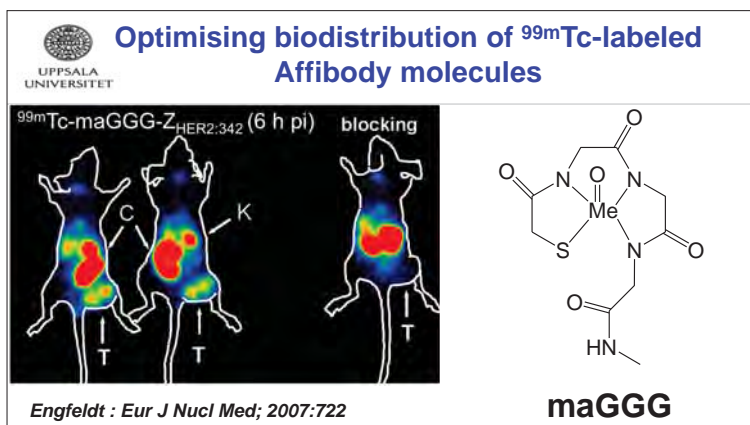
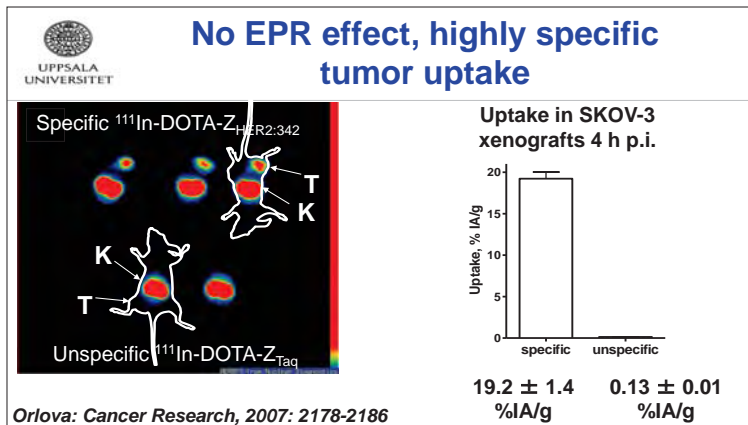
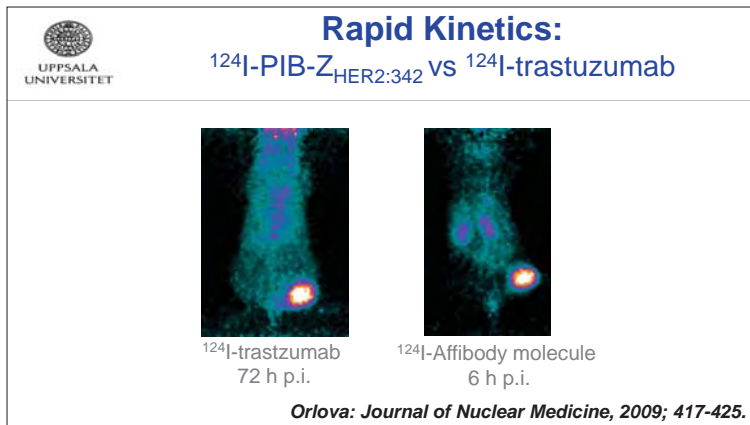
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Rapid Kinetics:

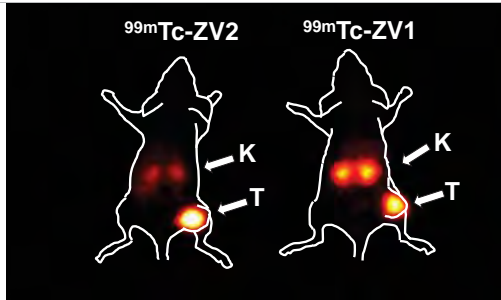
¹²⁴I-PIB-Z_{HER2:342} vs ¹²⁴I-trastuzumab



Orlova: Journal of Nuclear Medicine, 2009; 417-425.

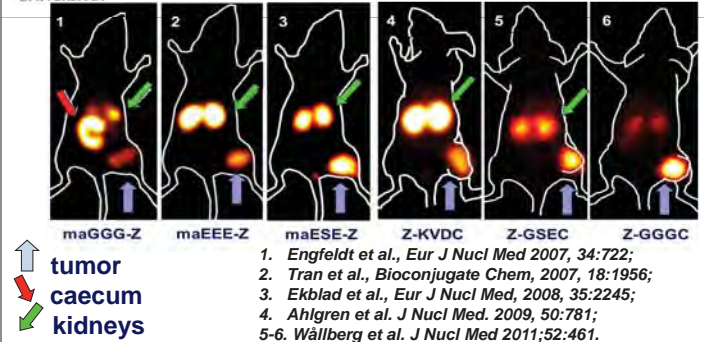


Gamma-camera imaging, 4 h pi (SKOV-3 xenografts)



Wällberg. *J Nucl Med.* 2011;52:461.

Imaging of HER2-expressing SKOV-3 xenografts using ^{99m}Tc-labeled Z_{HER2:342}



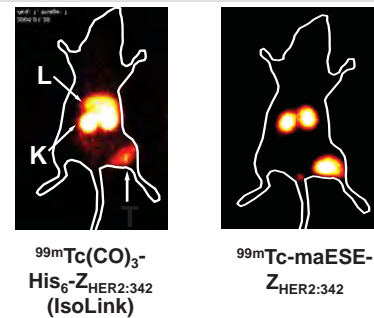
Chemical properties and position of chelator/linker for labelling of Affibody molecules influence:

- Predominant excretion pathway;
- Uptake and retention in excretory organs;
- Blood clearance rate (tracer and radiocatabolites)

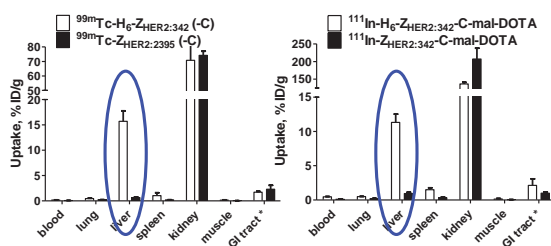
influencing imaging contrast.

Careful studies concerning influence of labeling chemistry on biodistribution and targeting may make a difference between success and failure!

Optimization of purification tags can improve biodistribution



Influence of N-terminal His₆-tag on biodistribution of Affibody molecules site-specifically labelled at C-terminus, 4 h pi



Ahlgren. *J Nucl Med.* 2009 :781.

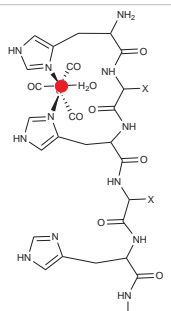
Ahlgren. *Bioconjug Chem.* 2008 :1579.

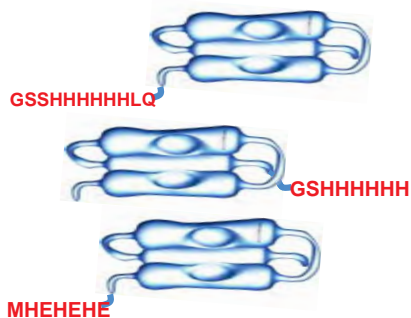
Hypotheses

1. Placement of His-tag on C-terminus could reduce liver uptake of ^{99m}Tc(CO)₃-labelled Affibody molecules;

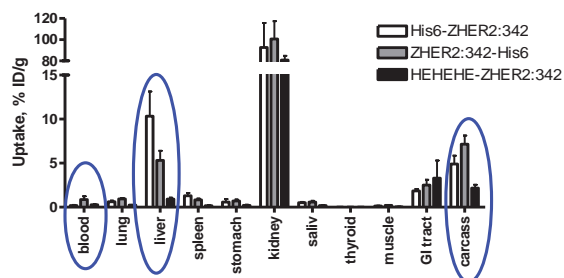
2. Incorporation of negatively charged residues into His-tag could reduce liver uptake even if His-tag is placed at N-terminus.

3. Despite changes in position and composition of histidine tag,
- IMAC purification would be possible
- ^{99m}Tc(CO)₃-labelling would be possible.



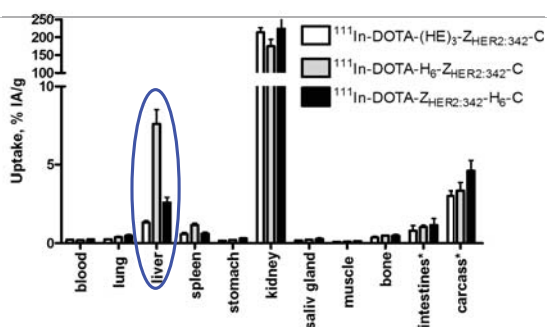


Biodistribution of $^{99m}\text{Tc}(\text{CO})_3$ -labelled Affibody molecules in NMRI mice 4h pi.



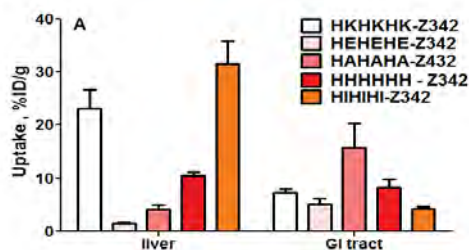
Tolmachev. Bioconjug Chem. 2010:2013.

Biodistribution in NMRI mice 4h pi. Site-specific ^{111}In -labeling using MMA-DOTA



Hofstrom: J Med Chem. 2011:3817.

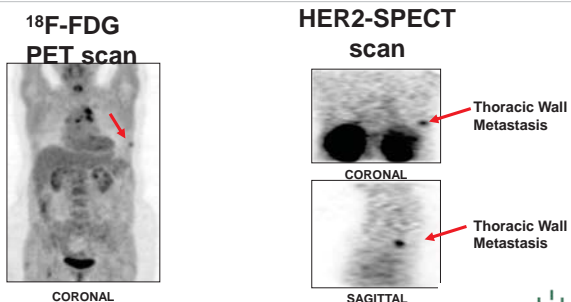
Liver uptake and hepatobiliary excretion of $^{99m}\text{Tc}(\text{CO})_3$ -labeled Affibody molecules in mice



Tolmachev (unpublished data)

Purification tags can be used for modification of biodistribution of targeting radiolabeled scaffold proteins

Clinical proof-of-concept. SPECT using ^{111}In -DOTA-Z_{HER2:342} (3 h p.i)



Baum: J Nucl Med, 2010; 892-7.



Summary

Affibody molecules offer advantages of:

- Fast tumor uptake and rapid clearance from blood
- High affinity
- High specificity
- Robustness
- Defined structure

Affibody molecules is a promising technology platform for development of tracers for molecular imaging.



Acknowledgements



- Amelie E Karlström
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