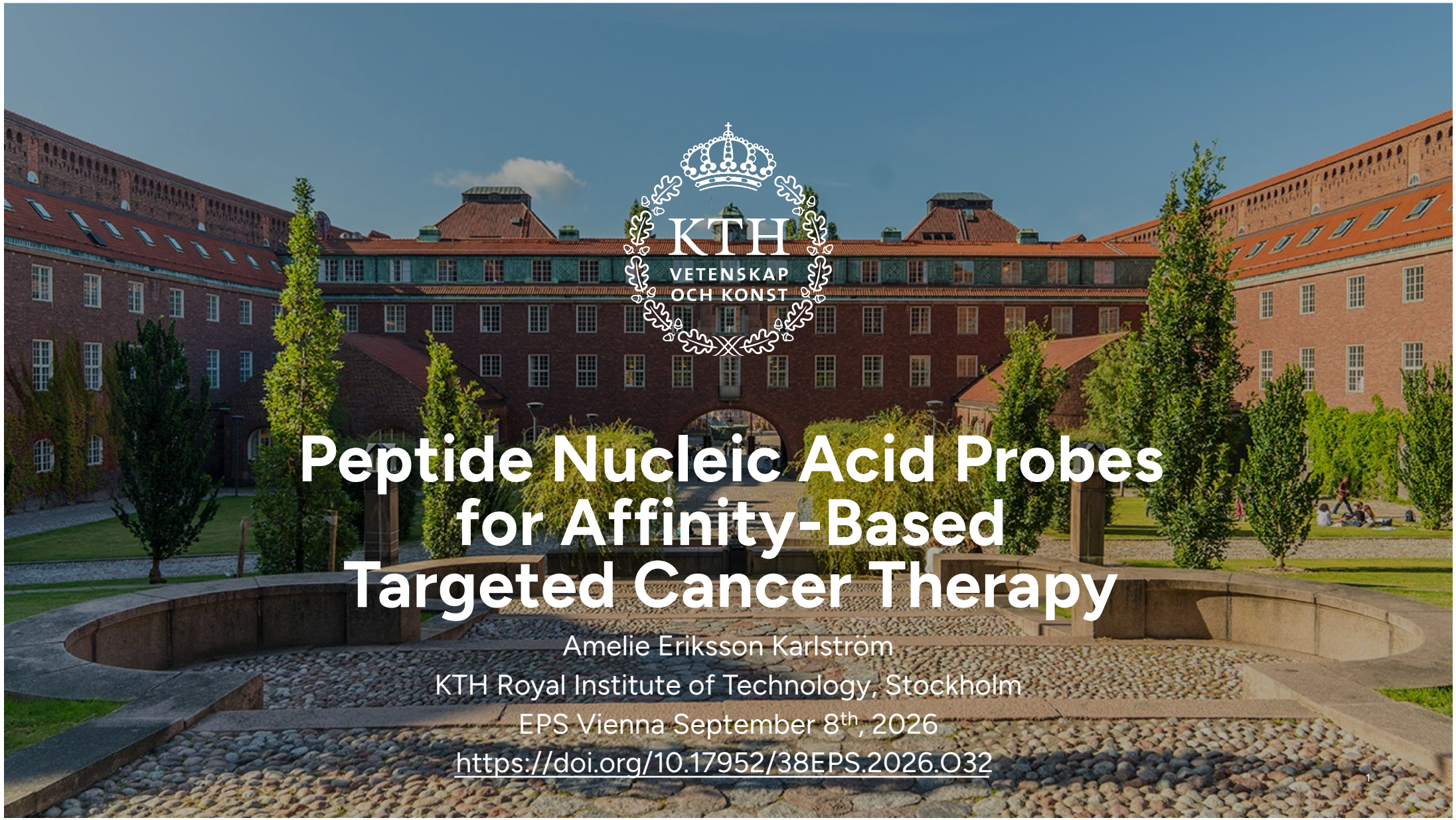


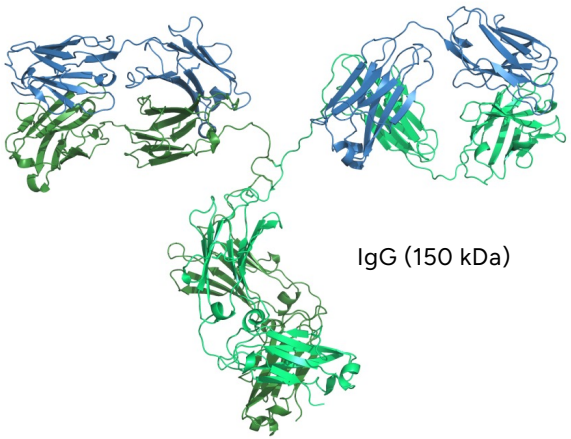
Peptide Nucleic Acid Probes for Affinity-Based Targeted Cancer Therapy

Amelie Eriksson Karlström
KTH Royal Institute of Technology, Stockholm

EPS Vienna September 8th, 2026
https://doi.org/10.17952/38EPS.2026.O32

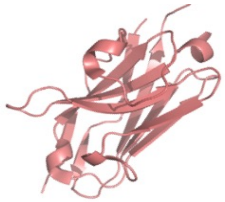
Tumor-targeting affinity proteins for cancer therapy

Monoclonal antibodies

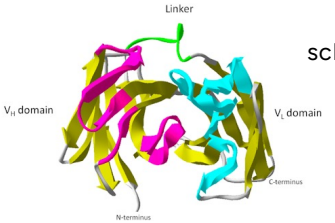


IgG (150 kDa)

Antibody fragments

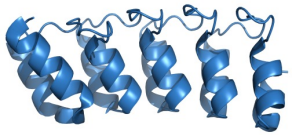


Nanobody/VHH domain (~27 kDa)

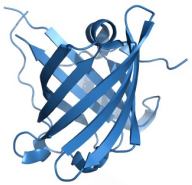


scFv (~27 kDa)

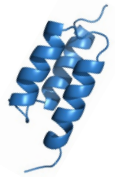
Engineered scaffold proteins



DARPin (14-21 kDa)



Anticalin (20 kDa)



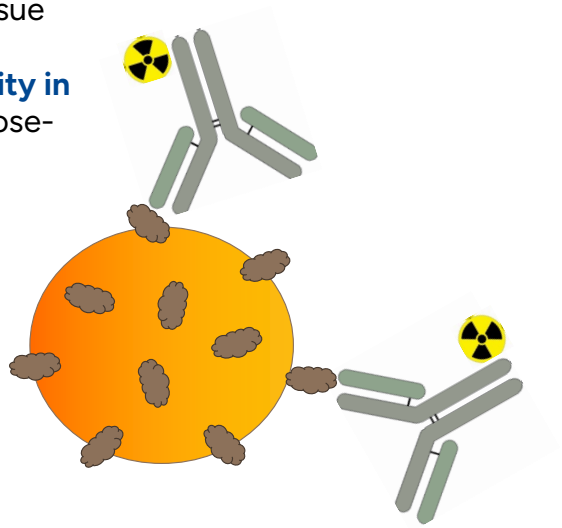
Affibody molecules (6-7 kDa)

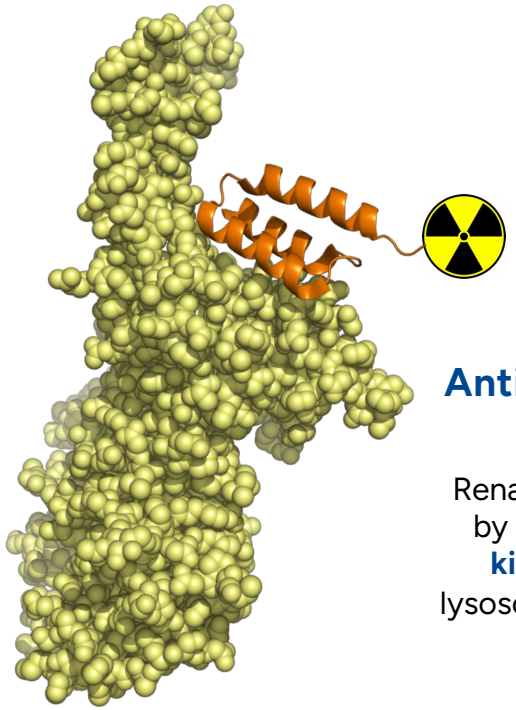
- Proteins binding tumor-associated antigens, typically cell surface receptors
- Can be used for delivery of cytotoxic payloads, e.g. antibody-drug conjugate (ADC) or radioimmunotherapy (RIT)

Targeted radionuclide therapy

Monoclonal antibodies

Long half-life and slow tissue penetration, leading to **accumulation of radioactivity in bone marrow** leading to dose-limiting toxicity



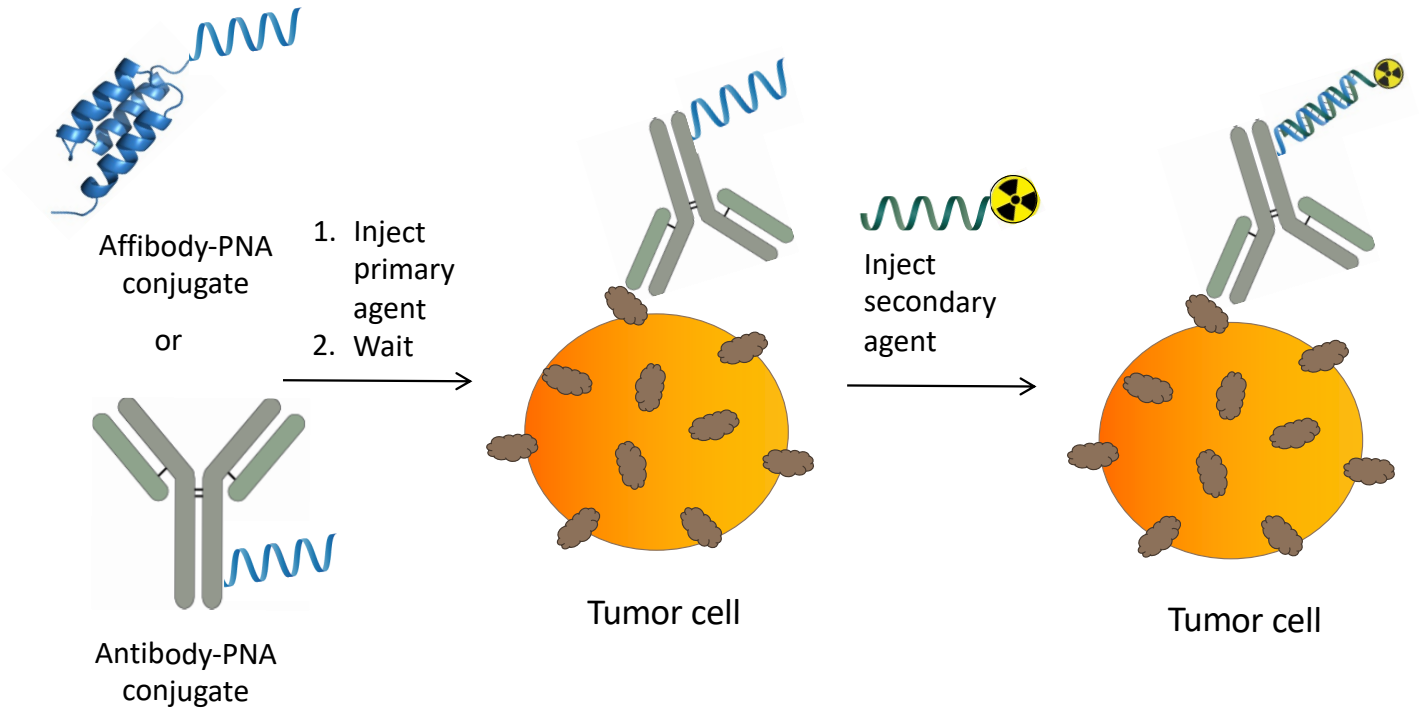


Antibody fragments and small scaffold proteins

Renal clearance typically accompanied by **accumulation of radioactivity in kidneys** because of reabsorption, lysosomal digestion, and retention in the proximal tubule cells

➢ **Difficult to reach therapeutic efficiency because of insufficient tumor-to-nontumor ratio**

Pretargeting strategy for targeted radionuclide therapy



The secondary agent should have:

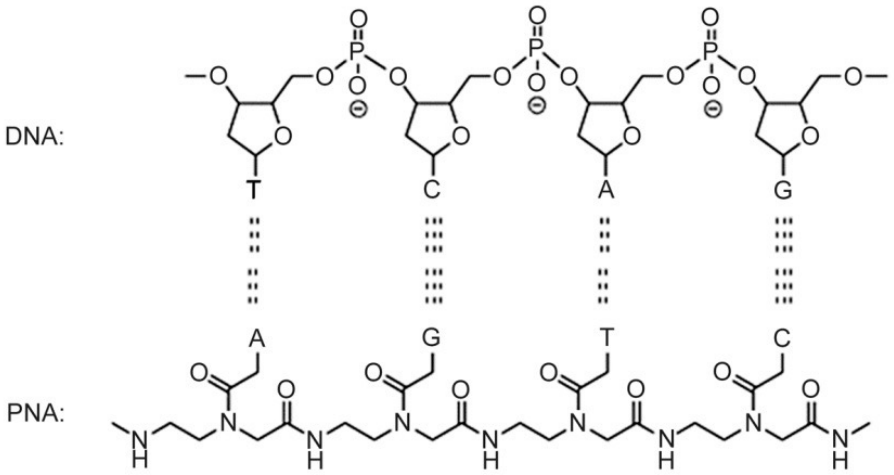
- fast clearance
- low accumulation in nontumor tissue
- high-affinity binding or bioorthogonal covalent reaction with the primary agent

➢ **Delivery of the tumor-targeting protein is separated from delivery of the cytotoxic radionuclide**

Peptide nucleic acid (PNA) probes for pretargeting

Hybridizing PNA probes:

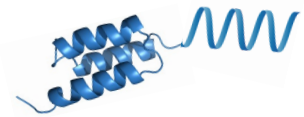
- No reported **immunogenicity** or **toxicity**
- High *in vivo* **stability**
- High specificity
- Easily tunable binding strength
- Synthesis fully compatible with peptide synthesis:
  - standard SPPS protocols
  - possible automation
  - GMP synthesis
  - flexible and modular combination with different chelators and radionuclides



➢ **Pseudo-peptide backbone fully compatible with standard solid phase peptide synthesis (SPPS) protocols**

Proof-of-concept: PNA-based pretargeting with tumor-targeting affibody molecules

Primary agent:

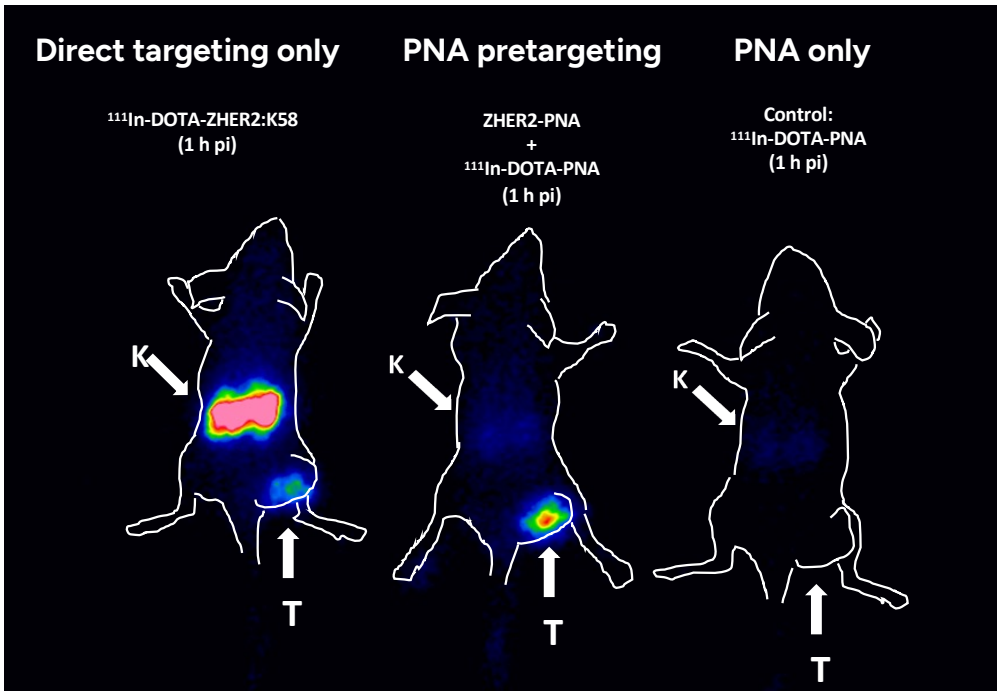


HER2-binding affibody molecule enzymatically conjugated to a synthetic PNA probe

Secondary agent:



Radiolabeled complementary PNA probe



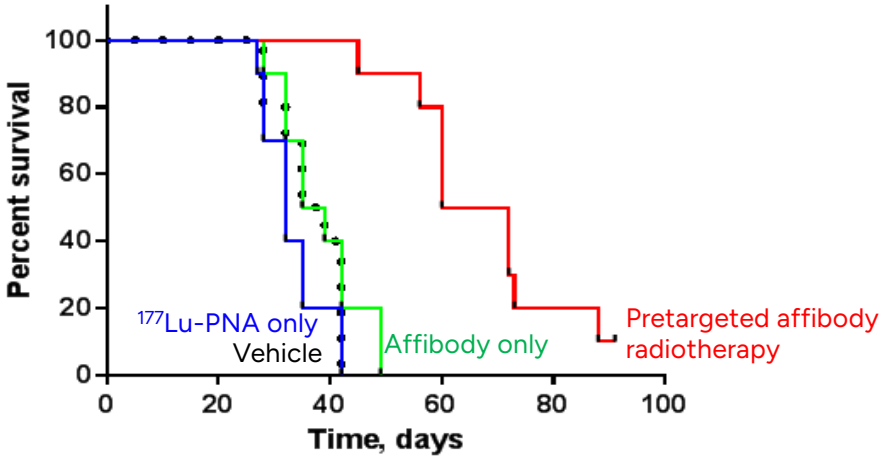
- SKOV3 (HER2+) xenograft tumors in mice
- Anti-HER2 affibody
- Secondary PNA probe labeled with indium-111
- 4 h interval between injections

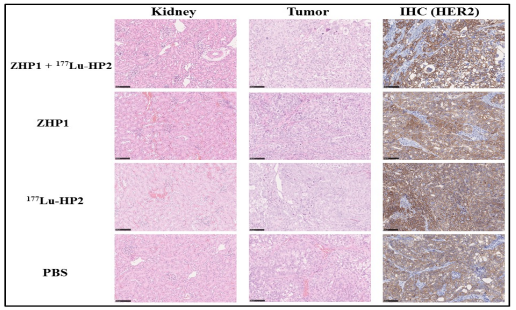
➢ **Affibody-based PNA-mediated pretargeting provides specific delivery of radiometals to tumors with higher radiometal concentration in tumors (T) than in kidneys (K)**

Westerlund *et al, Bioconjug. Chem.*, 2015, 26, 1724; Honarvar *et al, Theranostics,* 2016, 6, 93.

Affibody-based PNA-mediated pretargeted radionuclide therapy for treatment of HER2-expressing cancer

- SKOV3 (HER2+) xenograft tumors in mice
- Anti-HER2 affibody
- Secondary PNA probe labeled with lutetium-177
- Injections once/week for 6 weeks
- 16 h interval between injections





Hematoxylin and eosin staining of kidneys (left column) and tumor (middle column), IHC of HER2-expression in tumor (right column)

➢ **Pretargeted therapy increases median survival with no signs of toxic impact to kidneys**

Westerlund, et al (2018) *J. Nucl. Med.*, 59, 1092.

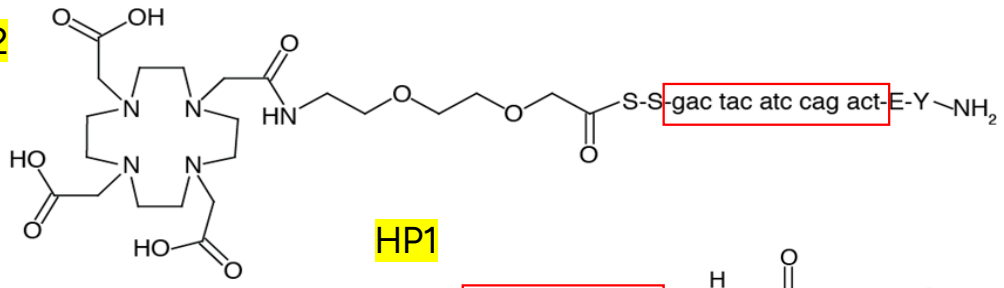
PNA probe engineering

Could shorter PNA probes be used for pretargeting?

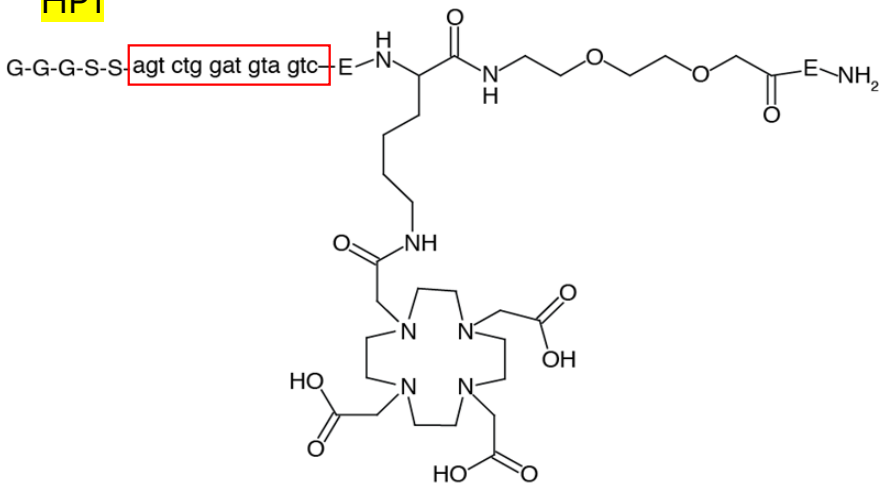
HP1:HP2 hybridization probes

PNA length:	15-mer
MW: approx.	5 kDa
T_m of hybrid:	86 °C

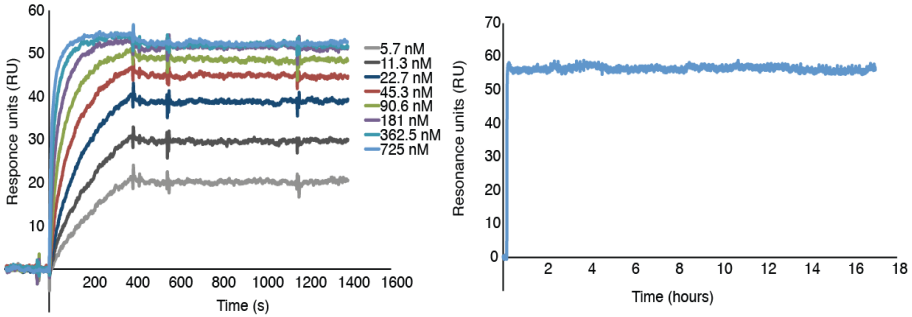
HP2



HP1



SPR sensorgrams of *HP2* binding to immobilized $Z_{HER2:342}$-SR-*HP1* show very slow dissociation:



Evaluation of new shorter secondary PNA probes

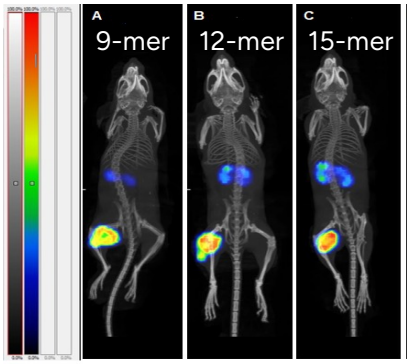
A new primary probe (15-mer):

HP15 GSS-cctggtgttgatgat-EK(DOTA)-AEEA-E-NH_2

Three new secondary probes (15-mer, 12-mer, 9-mer):

HP18 DOTA-AEEA-SS-atcatcaacaccagg-EEY-NH_2
HP17 DOTA-AEEA-SS-atcaacaccagg-EEY-NH_2
HP16 DOTA-AEEA-SS-aacaccagg-EEY-NH_2

Estimated dosimetry to tumor and kidney in mice bearing HER2-expressing SKOV3 xenografts:



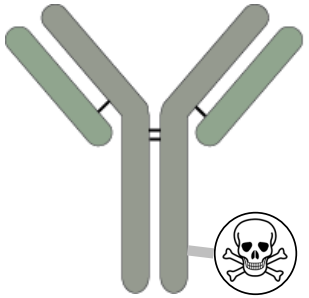
Secondary probe	PNA length	Tumor-to-kidney AUC ratio
[^{177}Lu]Lu-HP16	9-mer	3.8
[^{177}Lu]Lu-HP17	12-mer	2.8
[^{177}Lu]Lu-HP18	15-mer	2.0

The new secondary probes were evaluated in combination with a new 15-mer primary probe

- **The new 9-mer secondary probe HP16 provides the highest tumor-to-kidney AUC ratio**
- **Shorter probes provide better biodistribution, higher solubility and more cost-efficient production**

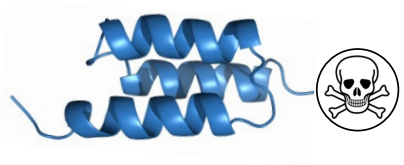
Tano *et al*, *Cancers*, **2021**,

Targeted delivery of cytotoxic drugs



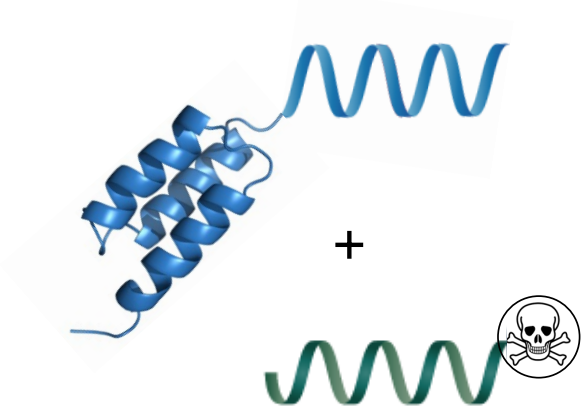
Antibody-drug conjugate (ADC)

- Hydrophobic payloads in ADCs can lead to
  - aggregation
  - rapid clearance
  - hepatotoxicity



Affibody-drug conjugate (AffiDC)

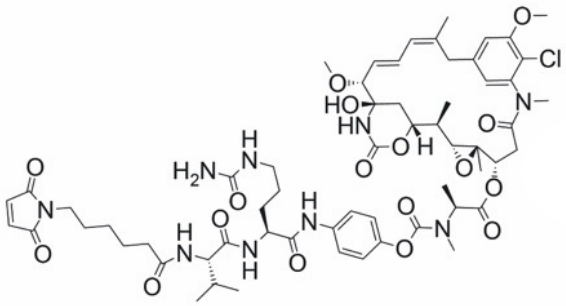
Alternative approach:



PNA-pretargeted delivery of cytotoxic drug

Design of DM1-conjugated secondary PNA probes

SP1	EC(DM1)-K($COCH_3$)-PEG_8-EE-acaccagg-EK(DOTA)-Y-NH_2
SP2	EC(DM1)-K(EE)-EE-acaccagg-EK(DOTA)-Y-NH_2
SP3	EC(DM1)-K($COCH_3$)-EEEE-acaccagg-EK(DOTA)-Y-NH_2
SP4	EC(DM1)-K(EE)-EEEE-acaccagg-EK(DOTA)-Y-NH_2
SP5	EC(DM1)-K(PEG_8)-EE-acaccagg-EK(DOTA)-Y-NH_2
SPc	EC(DM1)-K($COCH_3$)-EE-acaccagg-EK(DOTA)-Y-NH_2

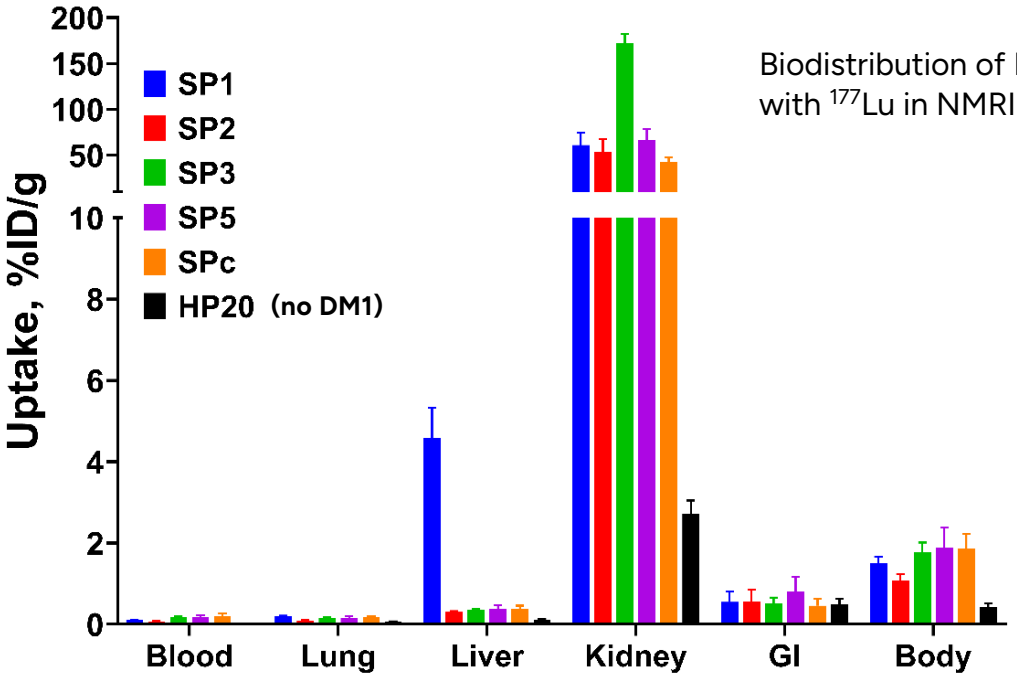


Mal-VC-PAB-DM1

- PNA-drug conjugates based on the 8-mer HP20 probe
- Hydrophilic modifications in backbone or side chain:
  - **Glutamate residues**
  - **PEG8 linkers**
- Maytansine drug DM1 attached via cathepsin-cleavable maleimide-valine-citrulline-*p*-aminobenzylcarbamate (Mal-VC-PAB) linker

Clinton *et al, Bioconjug. Chem, .*, **2026**, 37:1562

Biodistribution of DM1-conjugated PNA probes



Biodistribution of DM1-PNA probes labeled with ^{177}Lu in NMRI mice 4 h after injection.

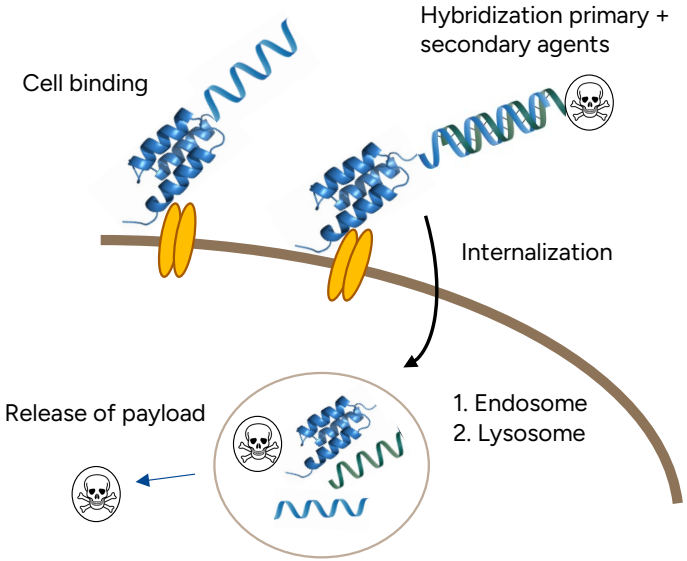
SP1 EC(DM1)-K($COCH_3$)-PEG_8-EE-acaccagg-EK(DOTA)-Y-NH_2
SP2 EC(DM1)-K(EE)-EE-acaccagg-EK(DOTA)-Y-NH_2
SP3 EC(DM1)-K($COCH_3$)-EEEE-acaccagg-EK(DOTA)-Y-NH_2
SP4 EC(DM1)-K(EE)-EEEE-acaccagg-EK(DOTA)-Y-NH_2
SP5 EC(DM1)-K(PEG_8)-EE-acaccagg-EK(DOTA)-Y-NH_2
SPc EC(DM1)-K($COCH_3$)-EE-acaccagg-EK(DOTA)-Y-NH_2

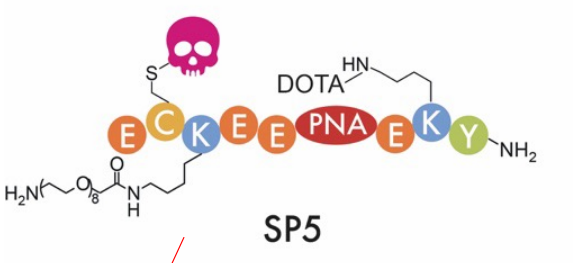
HP20 DOTA-PEG_2-SS-acaccagg-EEY-NH_2

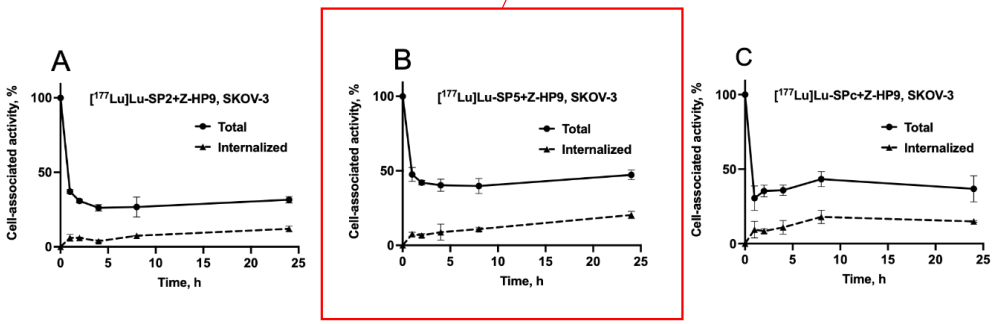
- Significant differences in liver and kidney uptake
- SP2, SP5 and SPc show favorable biodistribution patterns

Clinton *et al, Bioconjug. Chem, .*, **2026**, 37:1562

Cell binding and internalization







Retention and internalization of (**A**) [^{177}Lu]Lu-SP2, (**B**) [^{177}Lu]Lu-SP5, and (**C**) [^{177}Lu]Lu-SPc pretreated with Z-HP9 (1 h) on SKOV-3 cells after interrupted incubation.

- **SP5 shows favorable biodistribution, cell binding, and internalization**
- **SP5 is a promising candidate for *in vivo* tumor targeting**

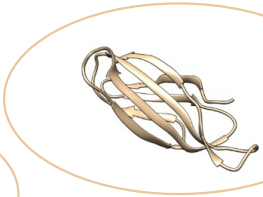
Clinton *et al, Bioconjug. Chem*, ., **2026**, 37:1562

PNA-based pretargeting is a versatile platform technology

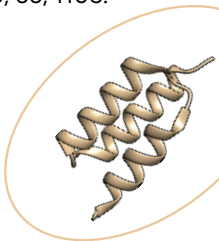


DARPin

- Oroujeni, M., *J. Nucl. Med.*, 2025, 66, 1105.

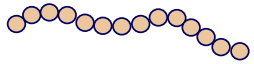


Nanobody



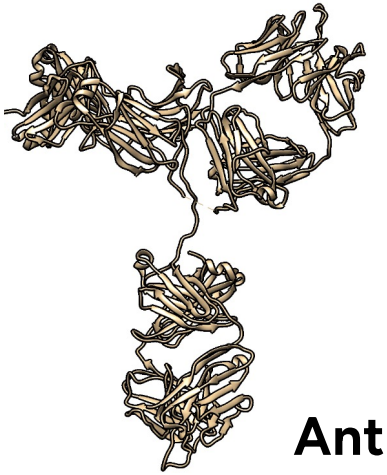
Affibody

- Westerlund, K. *ACS Pharmacol. Transl. Sci.*, 2024, 7, 1595
- Oroujeni, M. *J. Nucl. Med.,* 2022, 63, 1046
- Tano, H. *Cancers,* 2021, 13, 500
- Westerlund, K. *J. Nucl. Med.,* 2018, 59, 1092
- Vorobyeva, A. *Sci. Rep.,* 2019, 8, 9643
- Altai, M. *Nucl. Med. Biol.,* 2017, 54, 1
- Honarvar, H. *Theranostics,* 2016, 6, 93
- Westerlund, K. *Bioconjug. Chem.*, 2015, 26, 1724



Peptide

Tumor-targeting agents



Antibody

- Altai, M. *J. Control. Release,* 2025, 381, 113551
- Myrhammar, A. *Sci. Rep.,* 2020, 10, 20777
- Westerlund, K. *Biomaterials,* 2019, 203, 73

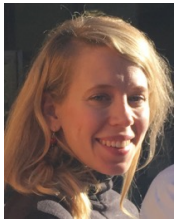
Short half-life
Renal clearance
Kidney toxicity

Long half-life
Slow tissue penetration
Bone marrow toxicity

Acknowledgments

KTH team:


Kristina Westerlund


Hanna Tano


Maxime Gestin


Anders Myrhammar


Ábel Nagy


Jacob Clinton


Pauline Granit


Astrid Haraldsson


Alexander van Deventer


Ebba Nordén


Linnéa Wiklund

Funding:

Vetenskapsrådet

VINNOVA

novo nordisk foundation
Benefitting people and society

CANCERFONDEN

Knut och Alice Wallenbergs Stiftelse

UPPSALA UNIVERSITET

Uppsala University:

- Vladimir Tolmachev
- Anna Orlova
- Maryam Oroujeni
- Anzhelika Vorobyeva
- Hadis Honarvar

LUNDS UNIVERSITET

Lund University:

- Mohamed Altai

POSTERS at 38th EPS:

- **Kristina Westerlund: Poster #443**

"Peptide Nucleic Acid (PNA)-Guided Pretargeting for HER2-Targeted Antibody-Mediated Radionuclide Therapy"

- **Jacob Clinton: Poster #339**

"Affibody-mediated Peptide Nucleic Acid Pretargeting Using Dual Hybridization Sequences for Increased Therapeutic Potential"