# DARPins as Powerful Targeting Agents for Radioligand Therapeutics

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## Introduction

The therapeutic window of radioligand therapeutics (RLTs) is often restricted by suboptimal tumor-to-non-tumor ratios. Antibodies can have high affinity and specificity to tumor targets, but their long systemic half-life frequently results in haematological toxicities. Alternatively, low molecular weight ligands are restricted to a limited number of tumor targets and often exhibit insufficient tumor retention and limiting tissue selectivity. Therefore, alternative molecular platforms are urgently needed to exploit the potential of RLTs in a broader field of indications.

DARPins (Designed Ankyrin Repeat Proteins) are small binding proteins that combine short systemic half-life and ideal binding properties. Due to their rigid-body binding mode, DARPins with very high affinity and specificity can be generated against a broad range of tumor targets, and several DARPin-based products are currently investigated in clinical trials. The simple and robust architecture of DARPins further provides high stability, which is beneficial for labelling with radionuclides under harsh conditions, and which enables engineering approaches that are not compatible with other protein scaffolds. This engineering approach resulted in a strongly reduced kidney accumulation of optimized DARPins, thereby addressing a general problem of protein-based delivery vectors below 60 kDa in size, which are cleared via the renal pathway.

## Affinity-Driven Tumor Uptake of DARPins

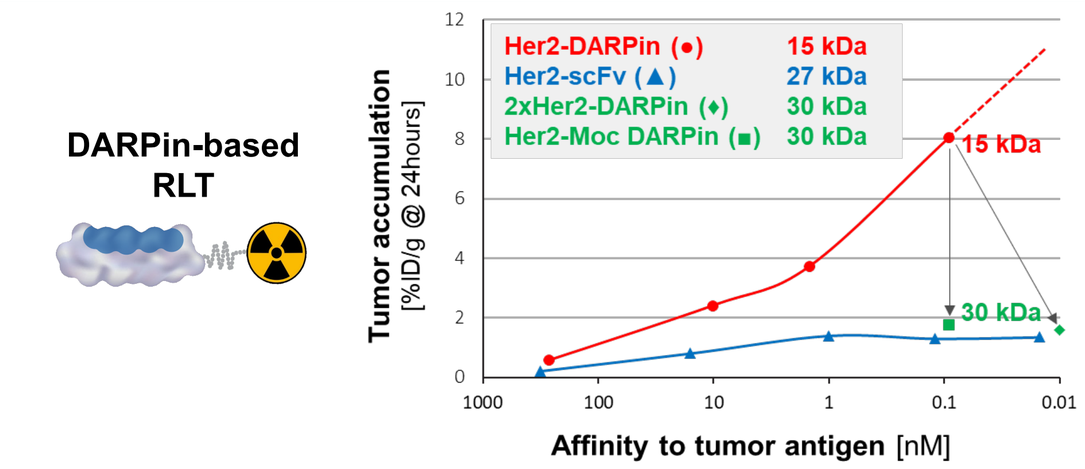
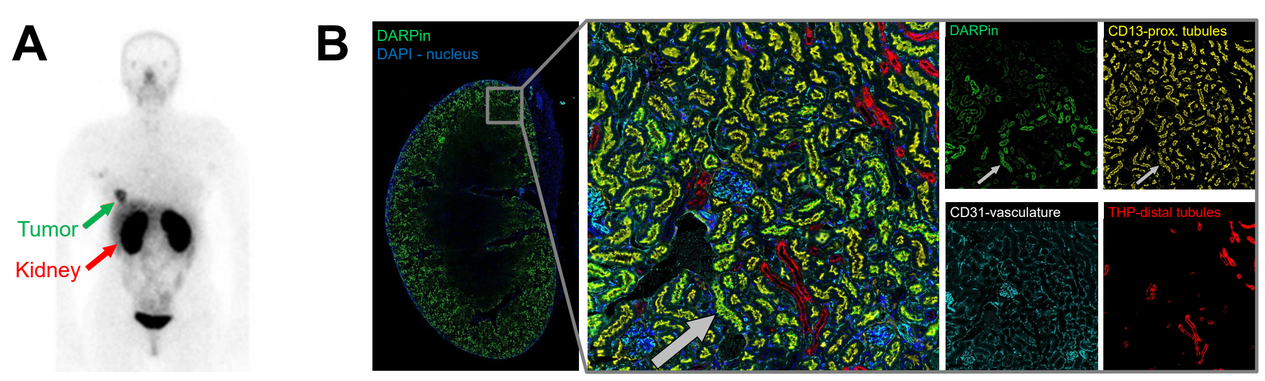



**Figure 1**: For small-sized molecules like mono-DARPins (~15 kDa) an increased affinity to the tumor target correlates with increased tumor uptake [1]. This benefit on tumor uptake is limited for intermediate-sized molecules like scFvs or bivalent DARPin molecules (~27 kDa) despite potential avidity effects [2]. The obtained experimental data are in line with modelling predictions [3].

## Kidney Uptake as a Key Problem of Polypeptide-Based Delivery Vectors




*SPECT imaging of breast cancer patient with $^{99m}$Tc- Her2 DARPin @ 4 h post injection [4]*

*Her2 DARPin staining of mouse kidney section by IHC @ 30 min post injection*

**Figure 2:** As any protein-based delivery vectors below the renal filtration cut-off (~60 kDa), DARPins are reabsorbed in the kidney leading to high accumulation of attached residualizing radionuclides (**A**). For this class of delivery vectors classical nephroprotectants such as AA cocktails have limited effect [5]. The renal reabsorption of such protein-based delivery vector occurs at the brush border (BB) of proximal tubular cells (co-staining example indicated by ⇗) (**B**)

## Surface Engineering to Reduce Kidney Accumulation of DARPins for RLT

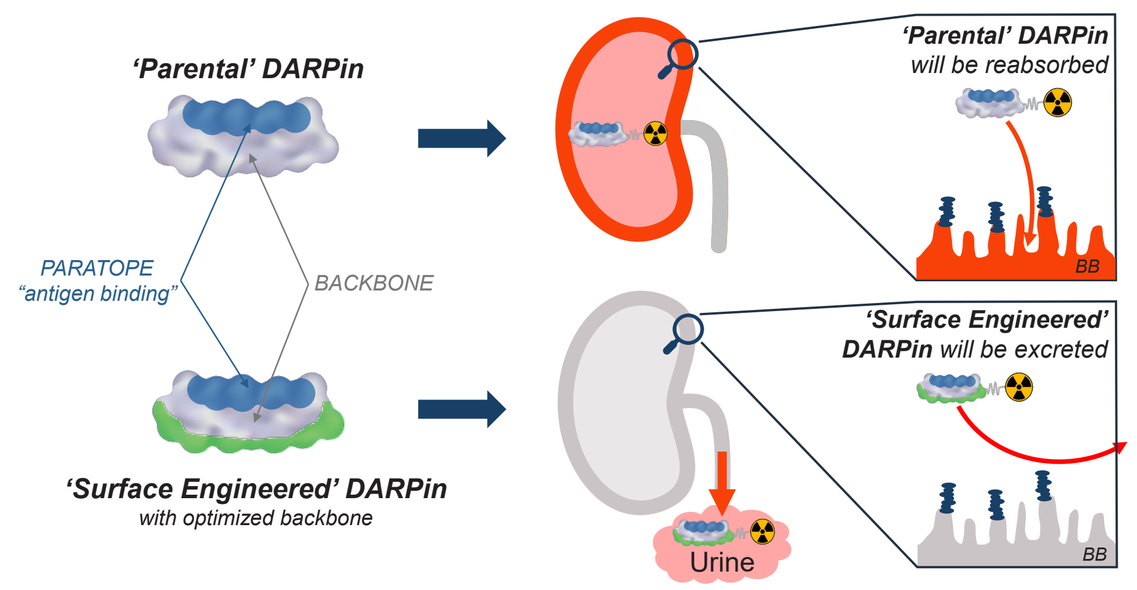



**Figure 3**: Surface optimization of the DARPin backbone to increase radionuclide excretion over reabsorption in the kidney, enabled by the robust architecture of DARPin scaffold. BB; brush border of proximal tubular cells

## Surface Engineered DARPins Show Strongly Reduced Kidney Accumulation, Maintained Tumor Uptake and no Significant Accumulation in Other Healthy Organs

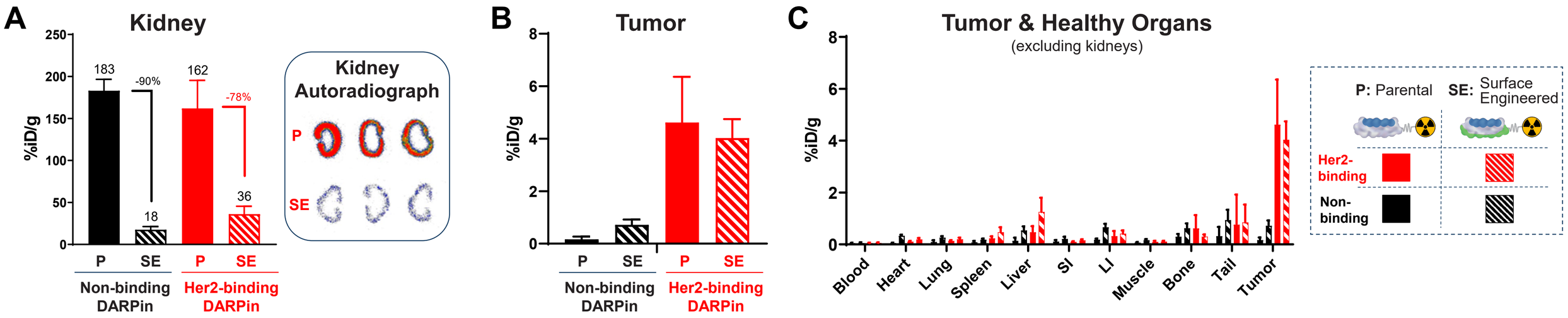
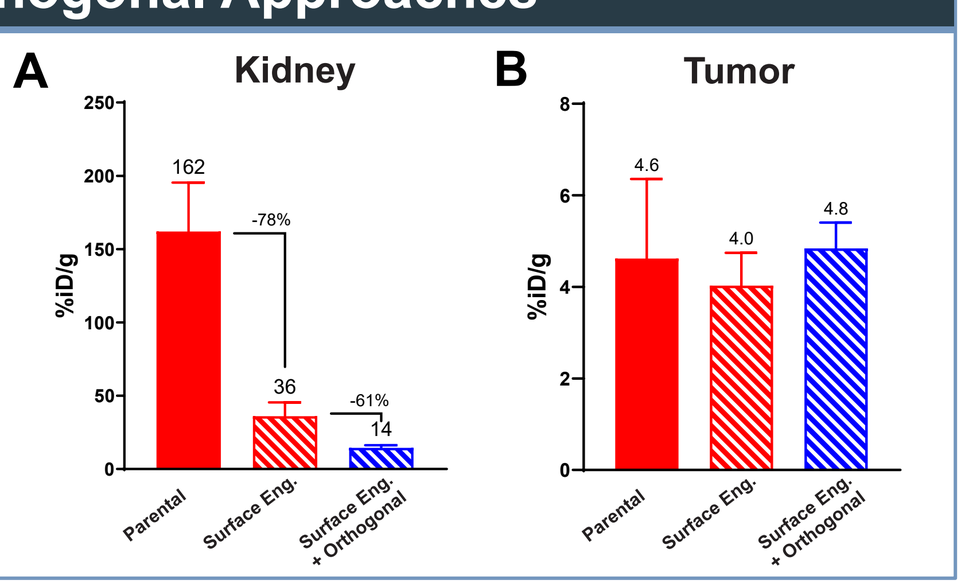



**Figure 4:** Biodistribution of DARPins labelled with 111-Indium in SKOV3 breast cancer mouse model, 4 hours after injection. Surface engineering resulted in reduction of kidney accumulation by up to 90% compared to parental DARPins (**A**), but had no impact on tumor uptake for the Her2-binding DARPin (**B**), and no significant effect on accumulation in healthy organs was observed (**C**).

## Kidney Accumulation is Further Reduced by Orthogonal Approaches

**Figure 5:** The combination of surface engineering with another orthogonal approach for increased radionuclide excretion resulted in a further reduction of kidney accumulation by 61% (**A**) without affecting tumor uptake (**B**). As a result, the tumor to kidney ratio of 1:35 for the parental Her2 DARPin was reduced to 1:9 for the engineered molecule and to 1:3 for the combination approach (same experimental setup as in Figure 4)




## Summary & Conclusions

- Surface engineering is a promising strategy to strongly reduce the kidney accumulation of DARPins without affecting tumor uptake.
- The combination with other orthogonal strategies results in a further reduction of kidney accumulation.
- Our proprietary "Radio DARPin Therapy" platform represents an attractive solution for the development of next-generation RLTs.
- Several programs in indications with high unmet medical need are currently underway (DLL3 as the first disclosed target)

**References**

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2) Adams *et al*., *Cancer Res*, 2001
3) Schmidt & Wittrup, *Mol Cancer Ther*, 2009
4) Bragina *et al, J Nucl Med*, 2022
5) Altai *et al*., *EJNMMI Research*, 2020

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