Small Targeting Proteins in Radiopharmaceutics: Strategies for Optimisation and Biodistribution

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Small targeting proteins, including monobodies and single-domain antibodies (sdAbs, aka Nanobodies®), hold immense promise in the field of radiopharmaceutics. Their unique attributes, distinct from conventional monoclonal antibodies (mAbs), make them compelling candidates for selective therapeutic applications[1]. Monobodies and sdAbs exhibit rapid clearance, a critical advantage for alpha-therapy as swift elimination reduces systemic toxicity and enhances patient safety[2]. These proteins also serve as viable alternatives to mAbs in antibody-drug-conjugate (ADC) applications, where despite their reduced molecular size, they maintain precise target specificity. Monobodies and sdAbs are highly stable proteins that can easily be expressed in lower organisms, such as *E. coli* and yeast, heavily reducing biomanufacturing costs compared to mammalian expression systems. Monobodies are synthetic proteins based on the human fibronectin type III (FN3) domain, with a size of around 10 kDa, while sdAbs are derived from camelid heavy chain only antibodies, with a size of approximately 15 kDa. Because of their small size, bioconjugation methods must avoid blocking the binding regions of these proteins, making site-specific conjugation critical to preserve their targeting specificities. In my talk, I will present various strategies for bioconjugation of small targeting proteins and show different approaches carried out in our laboratory. In the context of drug delivery, biodistribution of small targeting proteins is an important factor to take into consideration, not only for radiopharmaceutical applications. sdAbs are known to accumulate in the kidneys[3], which can pose challenges due to potential (off target) organ damage. I will discuss different strategies to modulate the *in vivo* behaviour of these proteins and present how we in the group are investigating some of these approaches to tailor pharmacokinetic profiles.

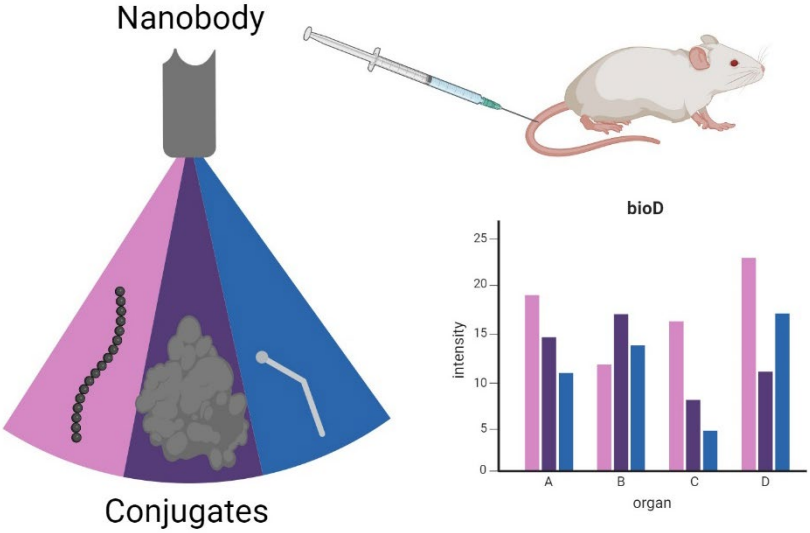

Figure: Biodistribution modulation of nanobodies. Various strategies can be applied for changing the biodistribution of small targeting proteins, such as conjugation of polymers or lipids or generation of fusion proteins.

References:

[1] Bao, G.; et al. , *EJNMMI Res*, **2021**, 11:6
[2] Sgouros, G.; et al., *Nat Rev Drug Discov*., **2020**, Sep;19(9):589-608
[3] D'Huyvetter, M.; et al. *Theranostics* **2014**, 4(7):708-720