

# Assessment of serum protein binding to predict non-specific uptake in vivo

Created on: 20-07-2022 - Last modified on: 09-08-2022

## Contact person

Sophie Hernot

## Organisation

**Name of the organisation** Vrije Universiteit Brussel (VUB)

**Department** BEFY

**Country** Belgium

## SCOPE OF THE METHOD

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Translational - Applied Research
<b>Type of method</b>	In vitro - Ex vivo
<b>Specify the type of cells/tissues/organs</b>	Serum

## DESCRIPTION

### Method keywords

Serum protein binding  
hepatobiliary clearance  
size-exclusion chromatography

### **Scientific area keywords**

in vivo biodistribution  
non-invasive imaging  
pharmacokinetics

### **Method description**

For therapeutic or reporter molecules to be effective for therapy or imaging applications, proper accumulation of the compounds in the tissue of interest is required, with minimal accumulation in undesired organs to avoid toxic side-effects and increase bioavailability. After initial *in vitro* screening on functionality and potency, a panel of preselected analogues are often evaluated *in vivo* for their pharmacokinetic profile to select a lead compound. This is most often assessed *in vivo* following administration of the compound using chromatography techniques or ELISA on collected tissues/organs, or non-invasive imaging. We propose to screen the compounds beforehand for non-specific protein binding in order to predict undesired background accumulation, in particular in the liver. As such, the number of compounds that need to be tested *in vivo* can be limited to the most promising ones.

### **Lab equipment**

HPLC Fluorescent- or radiolabeled drug-analogue Serum

### **Method status**

History of use

## **PROS, CONS & FUTURE POTENTIAL**

### **Advantages**

By screening beforehand a panel of drugs on their serum protein binding, the number of preselected molecules that need to be evaluated *in vivo* can be reduced as these will exhibit *in vivo* non-specific binding, in particular liver accumulation. Depending on the application, this can lead to undesired toxic side-effects and decrease the bioavailability of the drug.

## Challenges

To differentiate the signal of the drug from the signal of the serum proteins, the drug needs to be labeled either with a fluorophore or a radioisotope. One should be aware that the modification of a drug can also impact the biodistribution profile. In particular, hydrophobic fluorescent dyes are known to exhibit non-specific protein binding. Moreover, one should be reminded that the *in vitro* assay is a static assay that does not necessarily reflect the dynamic process happening *in vivo* and that metabolization, clearance, compartmentalization etc is not taken into account. Moreover, this assay does not predict *in vivo* efficacy of the compounds.

## Modifications

This method could be adapted to screen the binding of drugs to other types of proteins that can predict non-specific accumulation in certain undesired organs/tissues in the body.

## Future & Other applications

This method could be used to screen additional types of drugs (small molecules, peptides, large antibodies) for their non-specific serum protein binding and as such predict their *in vivo* behavior. This will limit the number of compounds tested *in vivo*.

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### References

P. Debie, C. M. Huygen, B. De Sloovere, D.M. van Willigen, ? Mateusiak, N. B. Declerck, J. Bridoux, J. Puttemans, N. Devoogdt, F. W. B. van Leeuwen, S. Hernot. Design and preclinical evaluation of a single-label bimodal nanobody tracer for image-guided surgery. *Biomolecules* (2021) 11, 360. IF2020 4.879 doi.org/10.3390/biom11030360

D.A. Smith, L. Di & E.H. Kerns. The effect of plasma protein binding on in vivo efficacy: misconceptions in drug discovery. *Nature Reviews Drug Discovery* (2010) 9, 929–939

Additional publications upcoming

## Associated documents

[Protocol.docx](#)

*Coordinated by*



*Financed by*



**Vlaanderen**  
verbeelding werkt

