Exploring endothelial protein c receptor as a therapeutic target: mapping distribution and correlation with nanoparticle targeting

Grace D.M. Eriksen, Martin Bak, David Schultz, Heidi Arps, Doha Ghannam, Anja Brus, Tsinat Berhane, Andrew J.Urquhart

Background

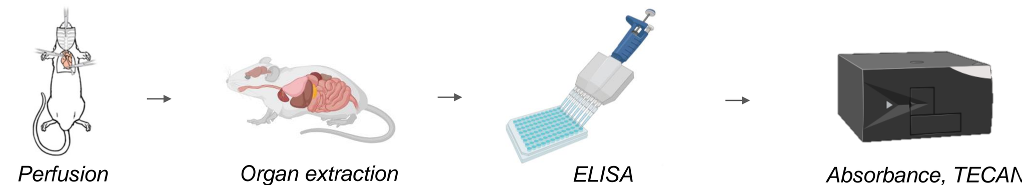
Finding effective therapeutic targets for addressing endothelial dysfunction related to atherosclerosis, diabetes, chronic liver and kidney disease remains challenging.

The endothelial protein C receptor (EPCR) is widely expressed and a keyplayer in endothelial dysfunction. For optimized drug design and to avoid competetive inhibition when targeting EPCR, knowing the distribution is useful.

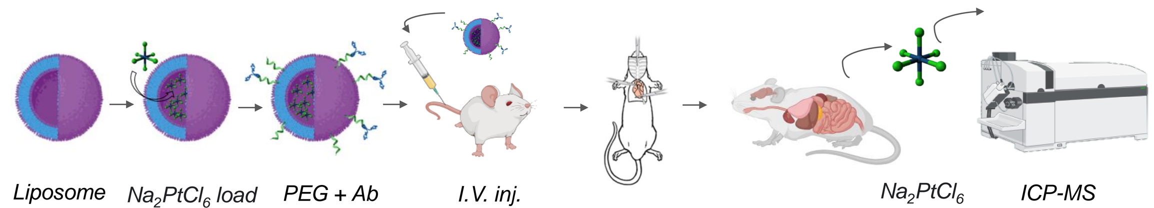
This study investigates EPCR as a potential target by mapping its distribution in a murine model.

Methods

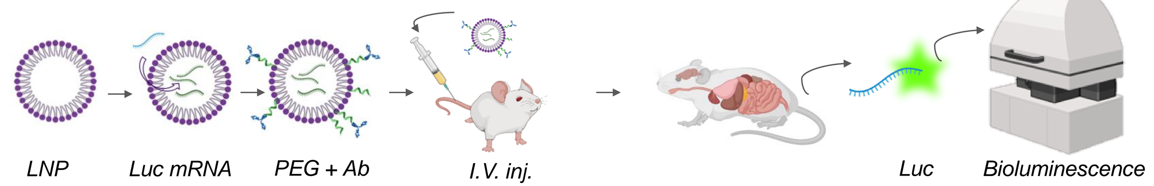
ELISA: quantify **EPCR concentrations** in selected tissues. Perfusion of animals was conducted prior organ extraction.



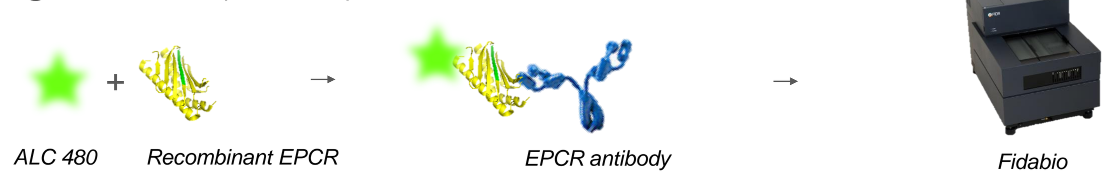
Inductively coupled plasma mass spectrometry (**ICP-MS**): quantify sodium hexachloroplatinate (Pt) accumulation in organs upon IV-injected **Pt-loaded liposomes** with **anti-EPCR monoclonal antibodies** (mAb).

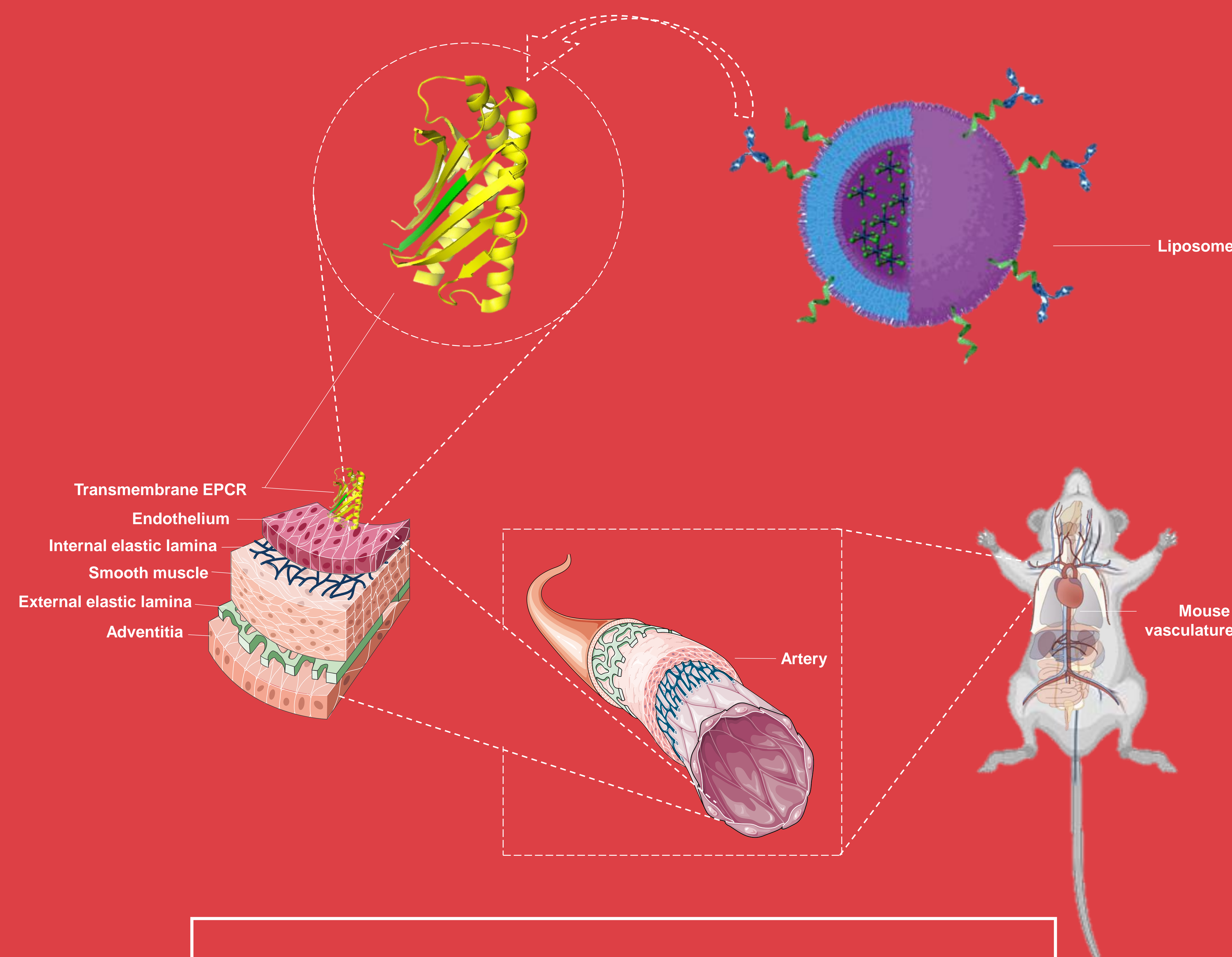


Bioluminescence: image IV-injected **mRNA encoding luciferase (Luc)** encapsulated in **lipid nanoparticles (LNPs)** surface coated with **anti EPCR mAb.**



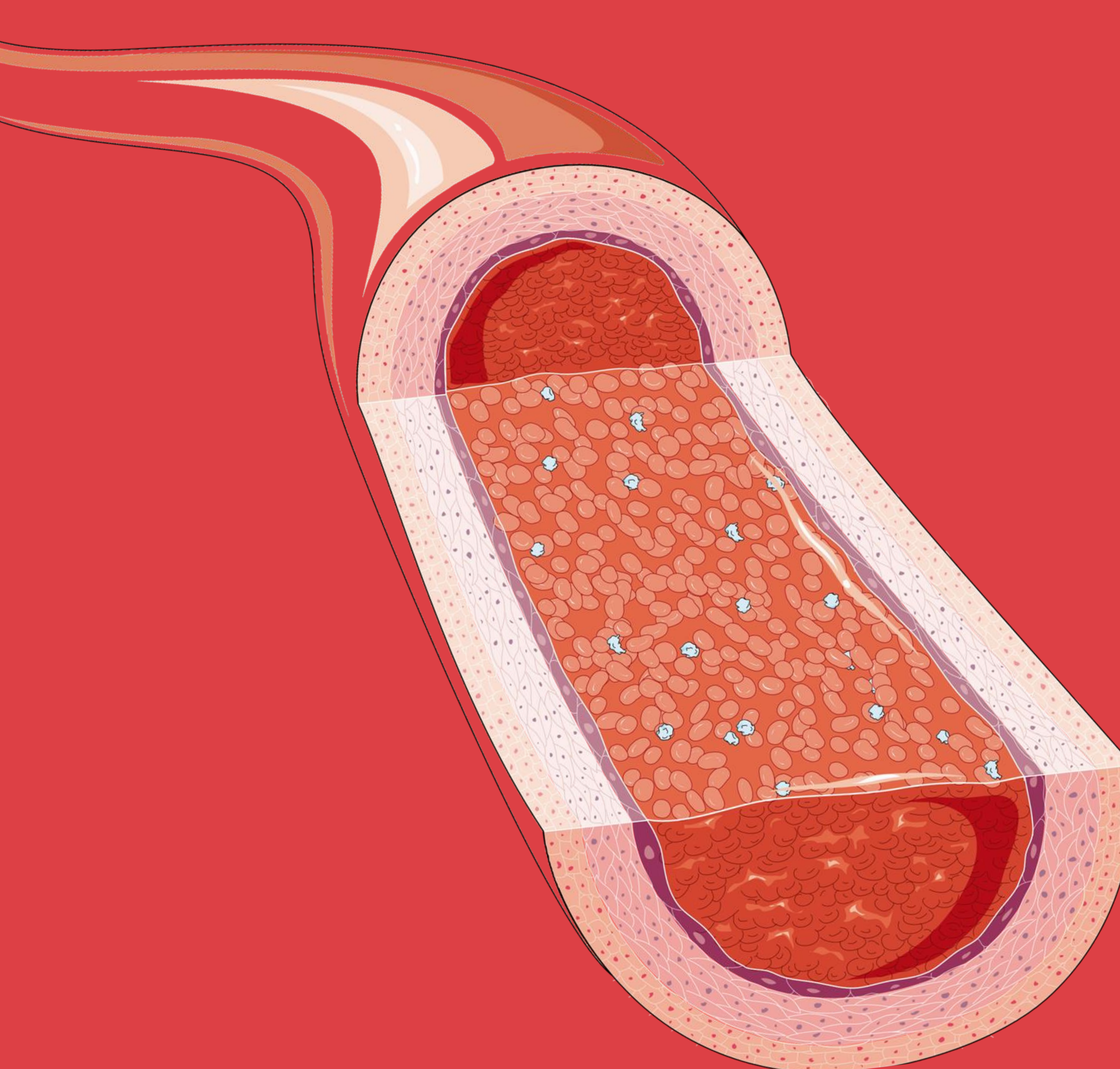
Flow-induced dispersion analysis (FIDA): study **antibody binding affinities** to a **recombinant** and **epitope-mapped** version of the **antigen EPCR** (rEPCR).





Liposome with Pt load as tracer was functionalized with anti-EPCR antibody to target EPCR, a transmembrane bound protein in blood vessel endothelium. Accumulation of Pt was measured in various organs to map the distribution of EPCR.

Mapping EPCR: Unlocking New Avenues in Vascular Therapy



Results

ELISA showed > 10-fold (~ 20.000 pg/mL) and **> 24-fold** (~ 50.000 pg/mL) higher EPCR expression in kidney and liver, respectively, compared to other organs.

ICP-MS depicted varying Pt accumulation. Pt-concentration in liver and spleen ranged from ~ 10-12 ng/mg tissue being 5-20 fold higher, compared to other organs.

Bioluminescence primarily displayed luciferase expression in the liver. Increasing Ab density on LNP surface indicated higher luciferase expression

Flow-induced dispersion analysis (**FIDA**) validated antibody-antigen binding with $\mathbf{K_D}$ values of ~ 750 nM.

Conclusion

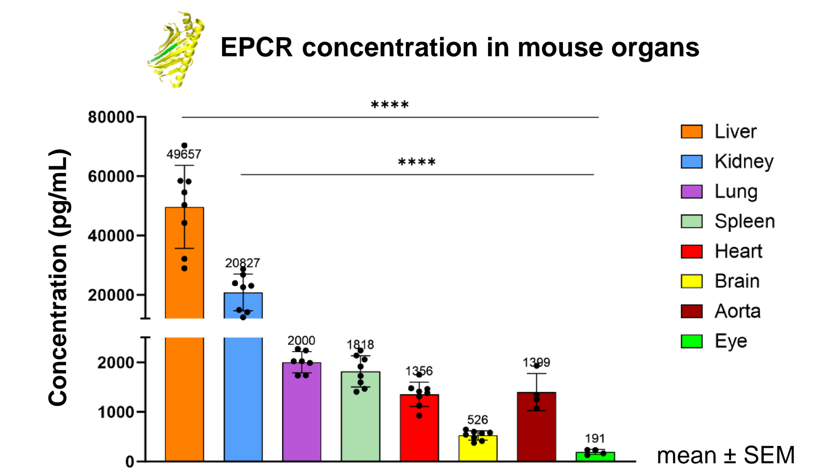
This study suggests **higher EPCR expression** in the mouse **liver and kidney** than **previously reported**, and potential for targeted therapies.

Pt accumulation in **liver and spleen** is likely ascribed metabolic clearance. However, given the observed concentration of EPCR in these organs it could support **nanoparticle-based EPCR targeting**.

These findings propose that due to **widespread EPCR expression** in mice, the design of **effective drug delivery** requires **new strategies** for optimal nanoparticle surface arcitechture.

Mapping (ELISA)

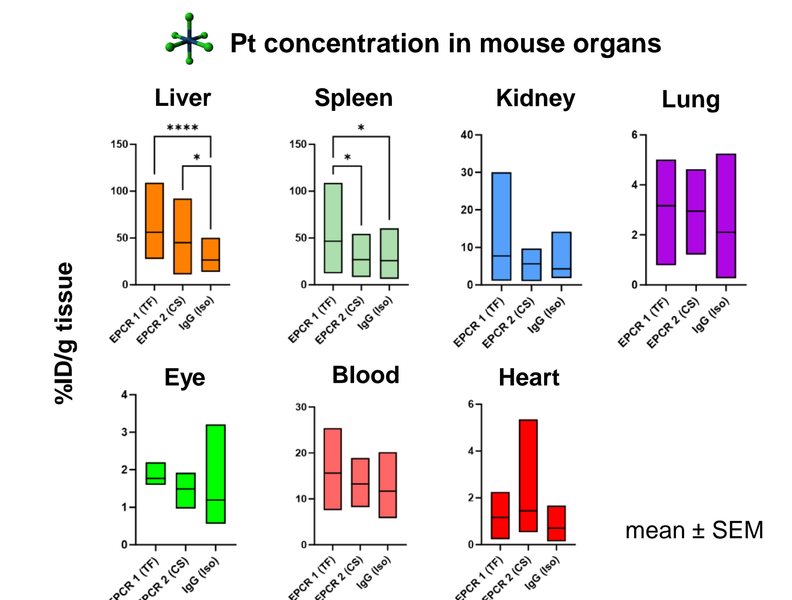
Distribution mapped by quantified EPCR concentration. Liver and kidney exceed >24-fold and >10-fold compared to other organs, respectively.



ELISA immobilized antigen: rEPCR #9068-ER-050. *n* = 8

Biodistribution - Targeting (ICP-MS)

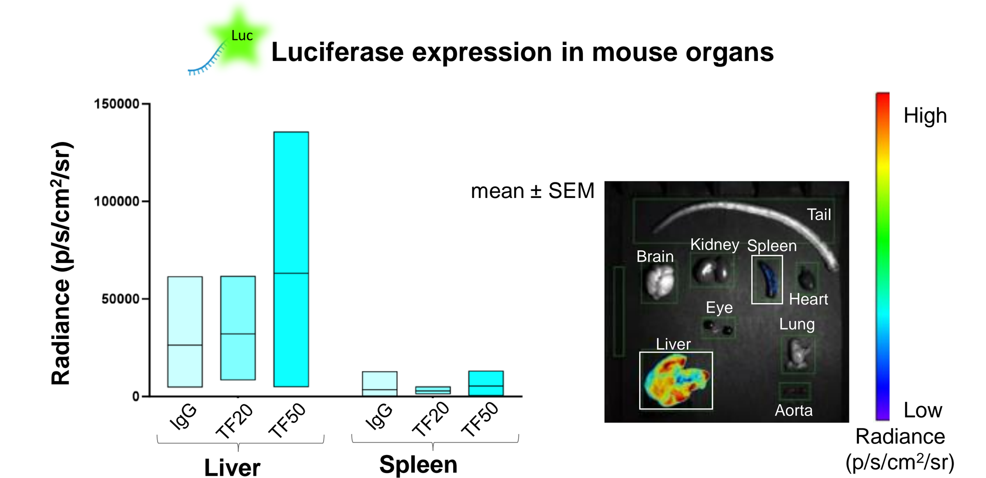
Anti-EPCR Abs and IgG Ab compared for binding specificity. Data suggests EPCR1 (TF) has superior binding than EPCR2 (CS) in liver and spleen.



EPCR1 (TF): # 16-2012-83. EPCR2 (CS): # PAA022Mu01. IgG: # 16-4031-85. *n* = 24

Biodistribution - Targeting (Bioluminescence)

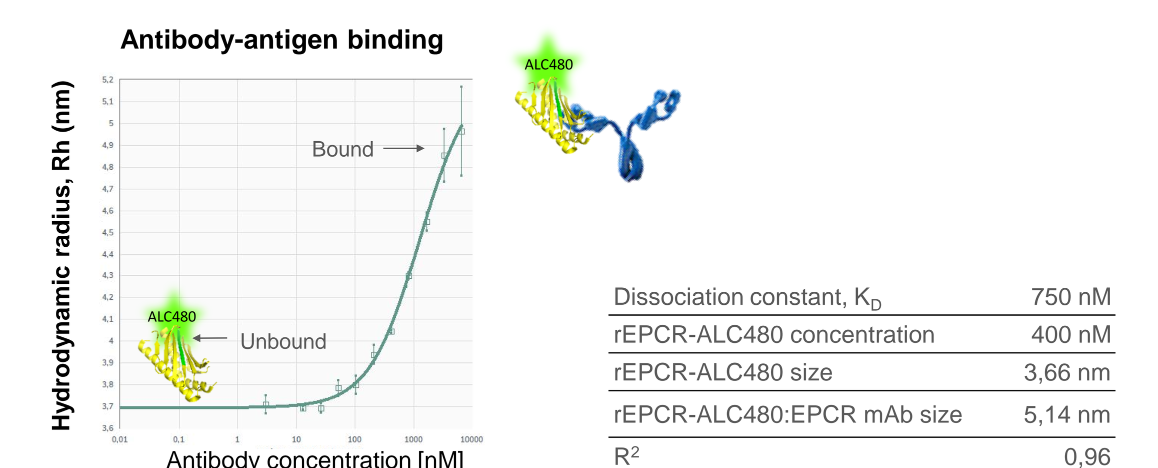
Optimizing nanoparticle design with increased antibody densities in LNP surface might affect targeting of EPCR.



TF: # 16-2012-83. Control, IgG: # 16-4031-85. TF20 or TF50 denotes 20 or 50 Abs/LNP surface. *n* = 30

Binding affinity (FIDA)

Characterization of binding affinity validates that antibody used for in vivo studies binds to recombinant EPCR.



Dissociation constant, K_D	750 nM
rEPCR-ALC480 concentration	400 nM
rEPCR-ALC480 size	3,66 nm
rEPCR-ALC480:EPCR mAb size	5,14 nm
R^2	0,96

rEPCR: #9068-ER-050. EPCR mAB: #16-2012-83.

Notes

All *in vivo* studies were performed in healthy animals.

Figures were created in Biorender.com or contain artwork components of Servier Medical Art. The endothelial protein C receptor protein structure was generated with PyMOL.

gdme@dtu.dk

demalona

About our research, related literature, Fidabio technology and contact info

Colloids and Biological Interfaces, DTU Health Tech, Department of Health Technology, Technical University of Denmark