Reply to Reviewer 2

In this review, the authors classified and described cell membrane transport proteins as a therapeutic target via nanobodies. The paper is well written and interesting and provides important and useful information to the readers. However, there are some points that need to be clarified.

Nanobody has a unique feature as 3 CDR in the VHH domain. Moreover, it might have larger and variable CDRs in order to compensate for the lack of VH-VL combinational diversity in IgG. Small-size and convex paratope might allow it to target cryptic epitope in the cell membrane transport proteins, unlike IgG. However, other platform biotechnology such as a cyclic peptide, DNA/RNA aptamer, or chemically programmed antibody would be applicable to recognize the cryptic structure as well as nanobody. Please explain the advantage of the nanobody strategy for targeting membrane transport proteins compared to others described above.

While we appreciate that other formats than nanobodies can be used for targeting of cryptic epitopes, we feel that elaborating about these different formats is out of the scope of our review.

Moreover, identification of affinity reagents (aptamers, DARPins, monobodies, affibodies, anticalins etc including synthetic single domain antibodies) requires assistance from groups specialized in generating and handling vast synthetic repertoires and/or subsequent affinity maturation. This collaboration will also restrict the freedom to use, when commercial or therapeutic applications are envisaged.
To meet the comment of the reviewer we added following arguments in the text conclusion section (Line 483-491).

"In this regard, aptamers or proteinaceous affinity reagents (DARPins, monobodies, Affibodies, Anticalins, or knob-like structures from cow antibodies) recognizing specifically membrane transport protein-binding can be an interesting alternative. In this review we focused exclusively on nanobodies as they are easily obtainable from immune libraries and free to use subsequently in therapeutic applications. All other formats of affinity reagents are only available from groups specialized in generating good quality, vast and diverse repertoires and handling these libraries and/or possessing the techniques and skills for subsequent affinity maturation. The therapeutic use of such affinity reagents will always somehow be restricted."

The authors highlighted the active molecular targeting of a nanobody. However, unlike research reagent or extracorporeal diagnostic agent use, the information of pharmacokinetic and biodistribution properties are indispensable for therapeutic use in the human body. In addition, safety, including immunogenicity, is also important, e.g., even CDR in some human antibodies can induce an anti-antibody.

Indeed, the reformatting of nanobodies can be useful to adapt their pharmacokinetic and biodistribution properties as was already mentioned in the conclusion and future perspectives-section. This in combination with safety studies is recommended for proceeding with in vivo administration in patients. We now included an extra sentence to cope with the possible immunogenicity (Line 518-520).

"Nanobodies intended for therapeutic use can also be humanized, i.e. mutating camelid-specific amino acid sequences to their human equivalents, to reduce the possible risks of immunogenicity in patients [130]."

Figure 2 shows a structured comparison of a nanobody and IgG. Readers would be grateful if authors outline the difference in functional features between them.

We thank the reviewer for this useful suggestion. Figure 2 has been slightly adapted and the legend to Figure 2 has been updated in the revised manuscript. The latter is now more concise and links the structure of antibodies with their functional features.