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Comment on mr-2022-11

Daniel Häussinger (Referee)

Referee comment on "Site-selective generation of lanthanoid binding sites on proteins using 4-fluoro-2,6-dicyanopyridine" by Sreelakshmi Mekkattu Tharayil et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2022-11-RC1, 2022

The manuscript by S. Tharayil et al. reports on a new modular type of tag based on 4-fluoro-2,6-dicyanopyridine for the binding of lanthanoids to proteins. The lanthanoid binding moiety is constructed in two steps and allows for the modification of the final tag, such that different coordination polyhedra of the tag can relatively easily be obtained, resulting in several different tensors with varying orientation and size of the anisotropic components. This strategy is certainly novel and may prove very useful for structural work with a minimum of synthetic effort.

The manuscript is concise and written in a clear way and is definitely suited for publication in Magnetic Resonance.

The major weak points are the poor characterisation of the chelate – lanthanoid complex, the open questions on the dimeric nature of the complex and the lack of information on the affinity of the new tags towards the lanthanoids.

I recommend, therefore, to accept the manuscript with minor changes that address the following points:

1) ^{1}H-DOSY spectra on the titration series of DCP-(L-Cys)$_2$ with YCl_3 should be performed in order to clarify the dimeric nature of the obtained shifts of the complex in slow exchange. (lines 378 ff.)

2) The aforementioned titration experiments have to be carried out with different protein concentrations and should then allow the (at least approximate) determination of an affinity constant. The titration spectra should all be provided in the SI.

3) In the list of crucial properties of an ideal lanthanoid binding site (lines 33ff) a high affinity between tag and metal is mandatory for a generally applicable system for structural work.

4) There is no information provided how the model of the complex for the rotamer libraries (line 166) was obtained (DFT?, force field?) and this holds also true for Figure S11. This should be included in the SI.

5) line 120 should read NMR: spectroscopy

6) In the SI a proper characterization of compounds **2** to **5** (pages S3, S4) needs to be provided (at least NMR, MS)