SOP7-Directional labeling of fVHH with maleimide dye/v3-10-09-2015

The standard protocol for labeling of VHH extended by the cys-tag

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Procedure

1) Reduce VHH sample in PBS (of at least 1mg/ml) by addition of 2.75 Molar excess of TCEP and incubation for 2h at 37° C.
2) Add maleimide label to the reduced VHH + TCEP. Use 6 Molar excess of dye and incubate 2h at room temperature.
3) Separate free label from the VHH sample by 2-passages through a Zeba column (7 kDa)
4) Run a UV-VIS scan of the final sample and calculate concentration and degree of labeling (DOL).

To measure amount of TCEP and Dye to added:

- Use the following formula: To be added (µl) = {[(VHH (µg)/MW)*1000]*X/ stock (nmol/µl)}. Where "X" is the ratio of TCEP (or dye) to VHH.

To measure DOL and VHH concentration after labeling:

- Run a UV-VIS on the nanodrop. Multiply the obtained values by 10 (light length: 1mm in nanodrop, 1cm for the lambert-bear formula!)
- Collect absoption at 280 nm and Amax (789 nm, in case of IRDye)
- To calculate VHH concentration use the formula: [VHH] (M) = (A280 –(Amax * 0.03))/MW of VHH. *[0.03 is correction factor for IRDye CW800]*
- To calculate the DOL=mole of label/mole of VHH, use the formula: DOL= Amax/(240000* [VHH] in M). *[240000 is extinction coefficient of IRDye CW800]*

Analyze 1 µg of the IRDye 800 CW-labeled VHH, next to non-labeled VHH on an SDS-PAGE under reducing and non-reducing sample buffer. Running front should not run out of gel. Scan the gel on an Odyssey.

Quantify the presence of free IRDye 800 CW and presence of labeled dimers and record it in Lab journal. Store data on general computer.