Weekly report

25/09/15 – 01/10/15

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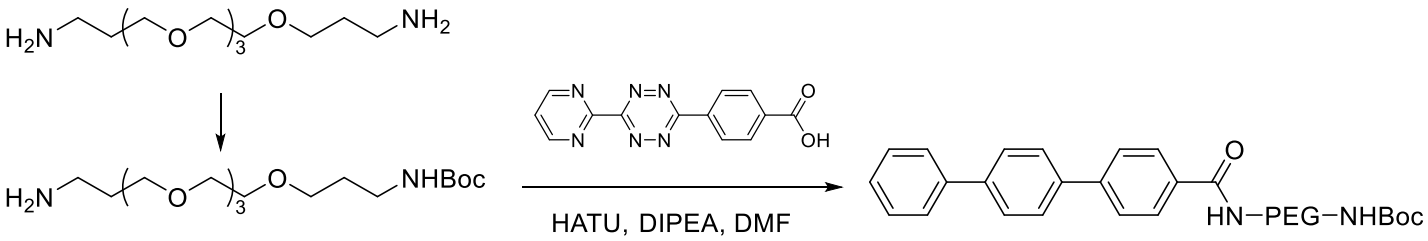
Synthesis

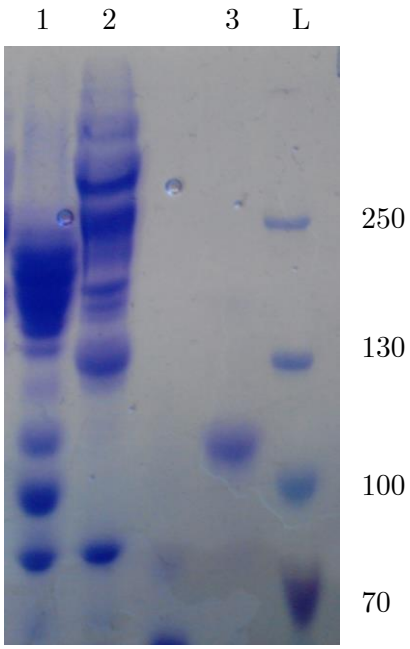
Tetrazine

- Purified Boc-protected PEG-NH_2 and coupled it to previously prepared tetrazine (purification tomorrow)



Conjugation

- Preclicked Astra PD with PEG 5k and PEG 20k overnight at rt then re-bridged Herceptin using sequential (to observe the effect of half-antibody) and *in situ* (which are the conditions used to avoid half-antibody).
- Optimised the gel conditions so that I could see half antibody as well as full antibody + 4 times 20k PEG (theoretical maximum).
- As a control, I also rebridged Fab with preclicked Astra-PEG20k (gel 1 – lane 3).
- Gel 1 – lane 1 = Her+Astra-PEG 5k sequential
- Gel 1 – lane 2 = Her+Astra-PEG 20k sequential
- Gel 2 – lane 1 = Her+Astra-PEG 20k *in situ*
- Gel 2 – lane 2 = Her+Astra-PEG 5k *in situ*



Gel 1

→ The main problem when analysing PEG by SDS-PAGE is that the *protein* ladder is not very useful. For example, Fab+PEG 20k appears at *ca.* 110 kDa instead of the theoretical 70 kDa (gel 1 – lane 3).

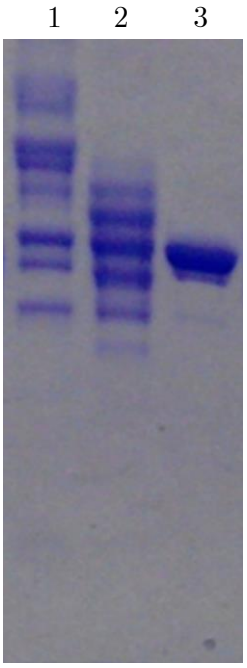
→ Looking at the half antibody of lane 1 and 2, it seems like the preclick wasn't complete (there is still half antibody without PEG attached to it) which results in a mixture of Her with 0–? PEGs attached. Two possibilities:

1. The click is quite slow in DMSO due to the high viscosity and limited solubility of PEG → I increased the number of equivalents in the preclicked mixture from 1.05 to 1.2 and diluted the reaction mixture with H_2O to increase solubility.
2. The strained alkyne part of Astra PD is partially degraded and won't react.

→ A similar trend is observed with the antibody modified *in situ*.

Lane 1 and 2 we can see rebridged but unclicked Her as well as clicked Her with both 20k PEG (lane 1) and 5k PEG (lane 2). Lane 3 is a control Her.

I will do some control to try to have a clearer picture of what I have.



Gel 2