

One repeating unit: One COOH per repeat

MW: 388.327 g/mol

Hyaluronic Acid Maleimide

COOH on HA is the site for MAL addition

Our HA MW: 242 kDa

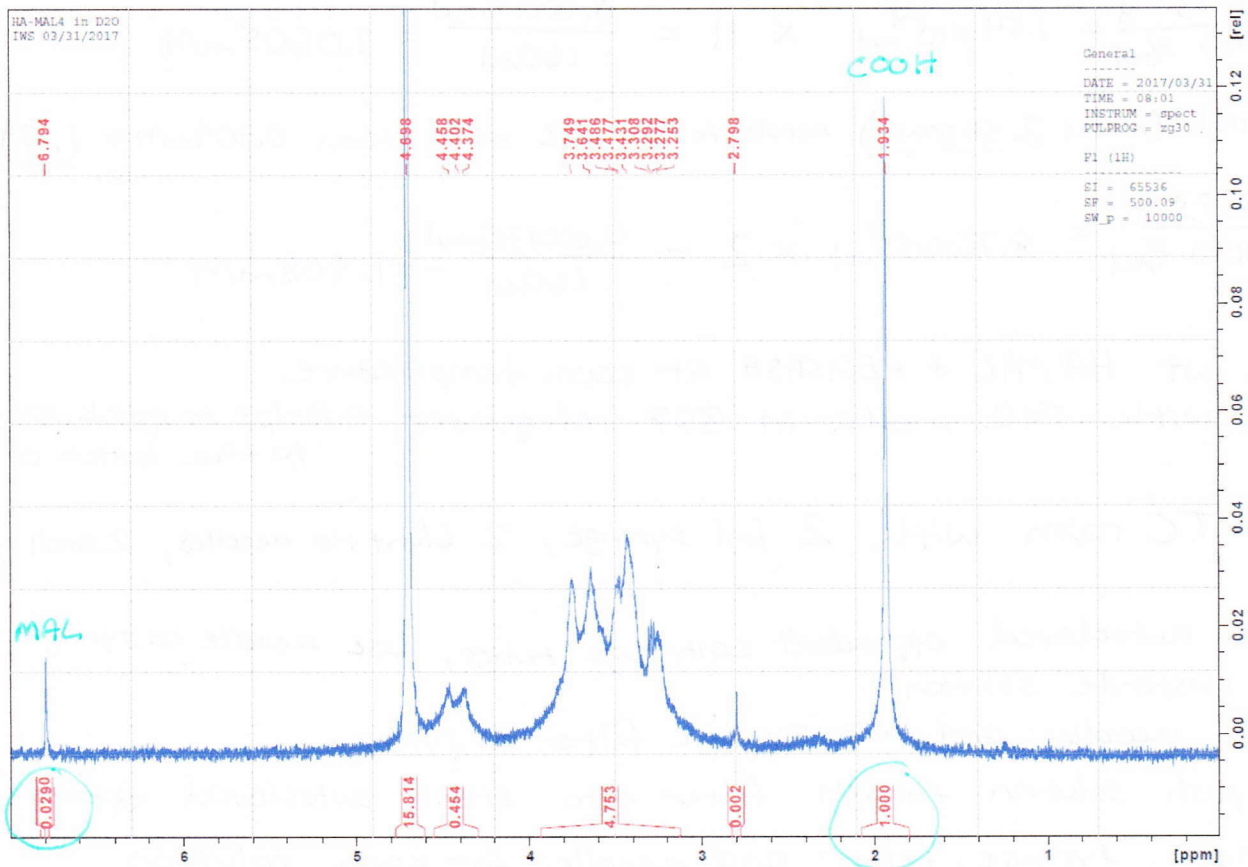
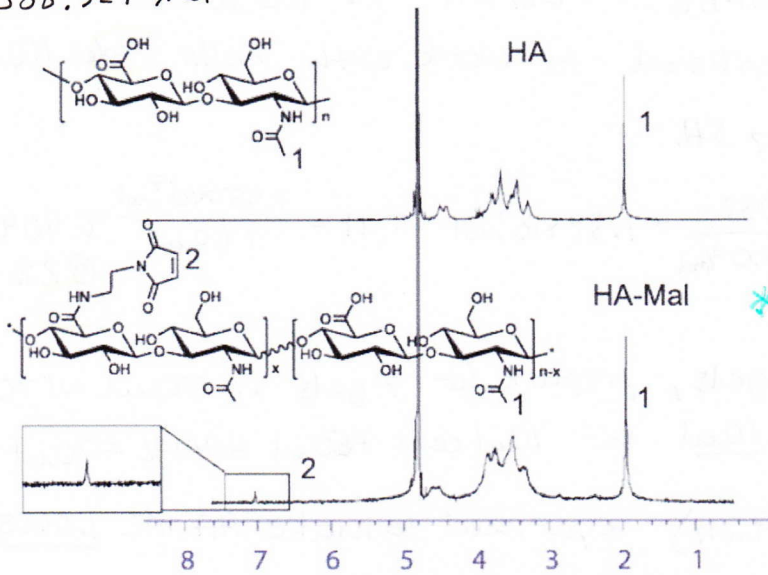
$$\therefore \frac{242000}{388.327} = 623 \text{ COOH on our HA chain}$$

After the reaction of MAL to COOH, the NMR spectra is used to determine % modification

$$\frac{0.029}{1.0} = .029 = 2.9\% \text{ modification}$$

How many MAL per HA chain?

$$623 \cdot 0.029 = 18$$



HA gel calculations

• Mix HAMAL with PEG dithiol in a 1:1.2 molar ratio but mix with even volumes.

- Current HA MAL batch is 1.86% modified \therefore 11 MAL per HA chain.
- 2% w/v HAMAL solution w/ cells

Questions to ask before planning:

- How many gels do I need? make 100ul extra for filtering
- What size gels? try to use 10K cells per 20ul of gel
- What volume of cells will I use in the gel? HA MAL concentration will be diluted by cell volume \therefore must start with a more concentrated HAMAL



Say you want 6 gels, 20ul each 10K cells in each.

* $20 \cdot 6 = 120 \text{ul} \rightarrow$ that is HAMAL + PEG SH $\therefore 60 \text{ul}$ of each.

Solutions need to be filter sterilized to work with cells. Add 100ul

• Make 160ul HAMAL and PEG SH.

$$160 \cdot 0.02 = 3.2 = 0.0032 \text{g} \quad \frac{0.0032 \text{g}}{242000 \text{g/mol}} = 1.32 \times 10^{-8} \text{mol} \times 11 = \frac{1.45 \times 10^{-7} \text{mol}}{160 \text{ul}} = 0.909 \text{mM} \quad @ 2\% \text{w/v}$$

You need 60ul HAMAL to make 6 gels, prepare for 7 gels $\therefore 70 \text{ul}$ w/ 70K cells

Choose to make 70K cells in 10ul so 10ul cells + 60ul HAMAL = 70ul

work backwards 60ul (1.0605) = 70ul (0.909mM) * you need to make HAMAL 1.0605mM

$$\text{HAMAL} \quad \frac{0.003733 \text{g}}{242000 \text{g/mol}} = 1.54 \times 10^{-8} \text{mol} \times 11 = \frac{0.00017 \text{mmol}}{160 \text{ul}} = 1.0605 \text{mM}$$

PEG dithiol (means 2 SH groups) needs to be 1.2 more than 0.909mM = 1.0908mM

$$\text{PEG SHSH} \quad \frac{0.000873 \text{g}}{10000 \text{g/mol}} = 8.72 \times 10^{-8} \text{mol} \times 2 = \frac{0.000175 \text{mmol}}{160 \text{ul}} = 1.0908 \text{mM}$$

- Measure wt HAMAL + PEG SHSH at room temperature.
- Dissolve with F12 media in 307 refrigerator. \rightarrow Helps to quick spin powder to the bottom of tube.

Take to TC room with 2 1ml syringe, 2 blunt tip needles, 2 small syringe filters

- Using TC autoclaved eppendorf centrifuge tubes, use needle on syringe to suck up unsterile solution.
 - Remove needle and put syringe filter on syringe.
- Slowly push solution through filter into sterile autoclaved eppendorf tubes.
- Use a new syringe, filter and needle for each solution.
- Mix 10ul of 70K cells into 60ul HAMAL in new sterile tube. vortex
- Mix 10ul HAMAL w/ cells with 10ul PEG SHSH in new sterile tube.
 - \rightarrow very fast reaction. Once HA (10ul) is in the tube, pipette 10ul PEG THEN adjust pipetter to 20ul with PEG still in tip so you can mix and pipette the gel rapidly.