Histone Maleimide Labeling

Materials:

- Atto488 maleimide (Sigma, 28562)
- 5ml HiTrap desalting column (GE, 29-0486-84)
- TCEP
- Lyophilized histone

Labeling Buffer:

	50 mL	Final Concentration
1M Tris, pH7.0	1mL	20mM
GuCl (MW: 95.5g/mol)	33.4g	7M
0.25M EDTA	1mL	5mM

Procedure:

1. Bring up TCEP to 0.5M, adjust pH to 7.5.
2. Bring up histone in labeling buffer to 0.5mM final concentration.

- for H2A 120C, 3.6 mg of histone goes into 516uL labeling buffer
- epsilon = 0.317, MW = 14081g/mol

1. Add TCEP to histone to final concentration of 0.7mM.
2. Incubate at RT for 2 hrs.
3. Dissolve Atto488 maleimide in DMSO to 100mM, vortex and spin down to completely dissolve. Wrap tube in foil and store at -20C.

- for 1mg Atto488, dissolve in 9.37uL DMSO
- Atto488 maleimide MW: 1067g/mol

1. Add Atto488 maleimide to histone:

- for 5ml desalting column, optimal load volume is 100uL-1.5mL; 200uL reaction volumes work well.

- alternative desalting method is to dialyze labeling reaction into labeling buffer in which case you don't need to worry about reaction volumes; dialysis desalting method seems to work well for Atto488 and Atto565

	50 mL	Final Concentration
100mM Atto488	1.2uL	0.6mM
0.5mM histone	40uL	0.1mM
Water	158.8uL	

Incubate 3 hr at RT, then O/N at 4C

1. Equilibrate HiTrap desalting column with labeling buffer at 4C.
    a. Load sample at 1ml/min, collect 200uL fractions.
    b. Observe 2 peaks, 1st narrow and 2nd broad
2. Run SDS-PAGE and visualize with Typhoon imager in Atto488 channel, then coomassie stain.
    a. 1st peak corresponds to protein (labeled and unlabeled elute together)
    b. 2nd peak corresponds to free dye
3. Assess labeling efficiency by NanoDrop (delta between molarity calculated from Atto488 absorbance and molarity calculated from histone absorbance)