

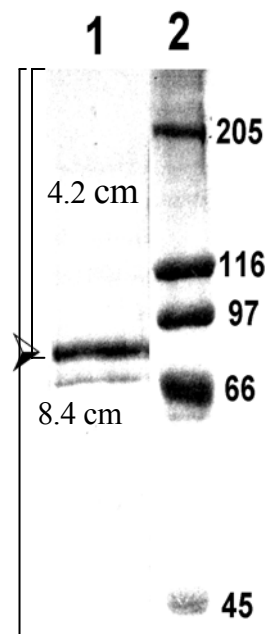
Hw 5 Solution

Calculate size based on SDS-PAGE

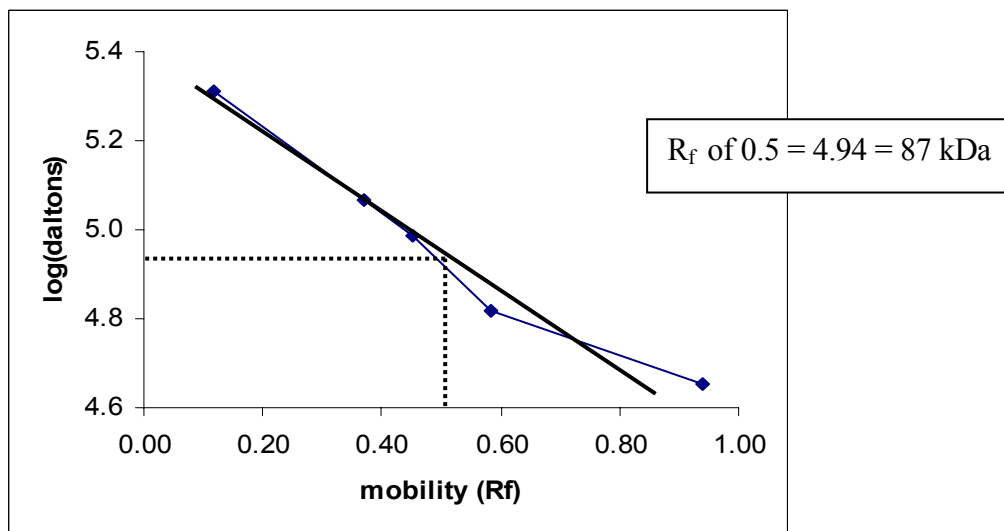
- Determine R_f value for the standards and unknown

$$R_f = \text{distance migrated/gel length} = 4.2/8.4 = 0.5 \text{ for unknown}$$

size	log	cm	R_f
205000	5.31	1.0	0.12
116000	5.06	3.1	0.37
97000	4.99	3.8	0.45
66000	4.82	4.9	0.58
45000	4.65	7.9	0.94



- Plot $\log(\text{mol. wt.})$ against R_f for standards and extrapolate size of unknown.



Calculate size based on gel filtration

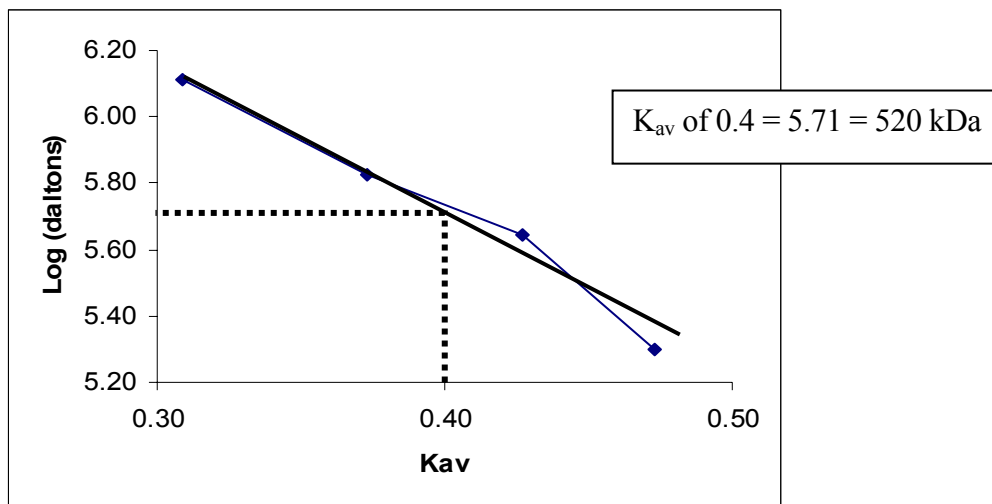
1. Convert elution times into elution volumes and calculate K_{av} for standards (see table below) and the unknown:

$$K_{av} = (V_e - V_o)/(V_t - V_o) = (24.8 - 16)/(38-16) = 0.40$$

Standard (daltons)	Log (mol. wt.)	Elution time (min)	Elution volume (ml)	K_{av} *
1,300,000	6.11	11.4	22.8	0.31
670,000	5.82	12.1	24.2	0.37
440,000	5.64	12.7	25.4	0.43
200,000	5.30	13.2	26.4	0.47

*Could also use $R_f = V_e/V_t$

2. Plot K_{av} vs log(mol. wt.) for the standards and extrapolate the size of the unknown.



Explanation of Discrepancy

SDS disrupts quaternary structure and dissociates proteins into subunits. Therefore the best explanation for the large size calculated by gel filtration is that the protein is a complex of multiple subunits (eg., six 87 kDa subunits).

Protein shape could also affect the calculated size. However, shape affects could not account for the 5-6 fold difference in size. Generally, shape will not affect the calculation by more than two fold.