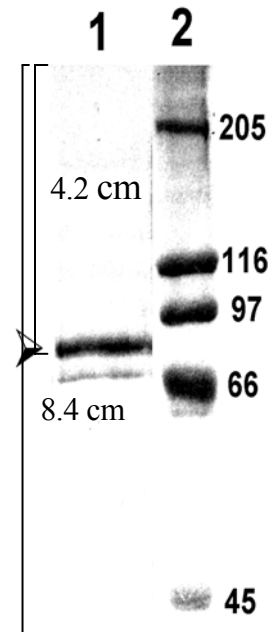


### Calculate size based on SDS-PAGE

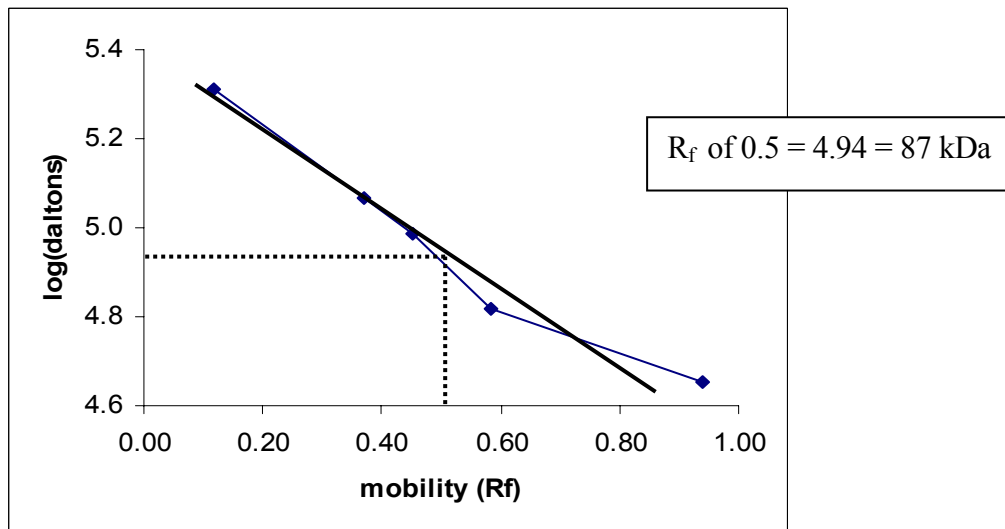
- Determine  $R_f$  value for the standards and unknown

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

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

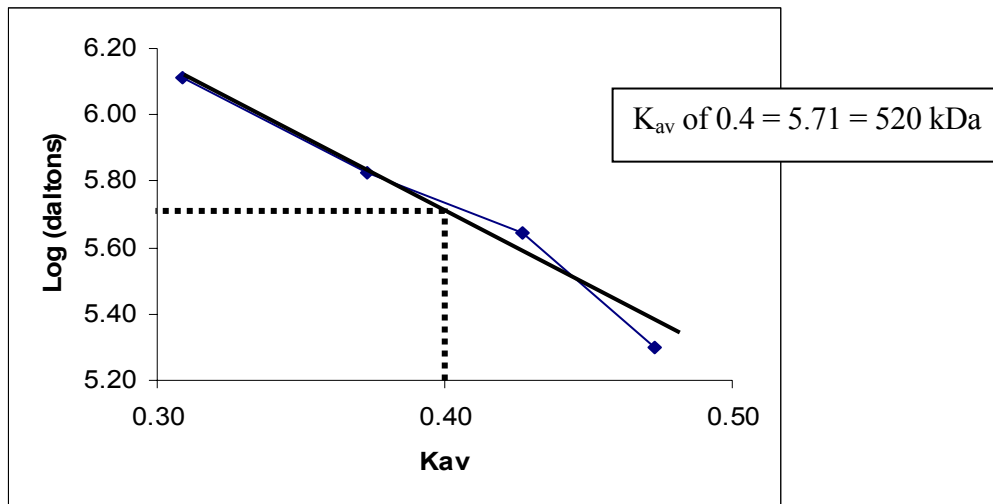
1. Convert elution times into elution volumes and calculate  $K_{av}$  for standards (see table below) and the unknown:

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

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.