

# Subunit Association (Xinying Shi)

## Gradients

Prepare 11ml sucrose 10%-40% in Buffer C (20mM Mg<sup>2+</sup>)

Needle: 18G1/2

Store at 4°C for at least 2hr before use

10% (w/w, 103.8 mg/ml)    4.2g in 40ml buffer C  
40% (w/w, 470.6mg/ml)    18.8g in 40ml buffer C  
50% (w/w, 614.8mg/ml)    30.8g in 50ml water

	Stock solution	Gradients
50mM Tris-HCl (7.6)	1M	2.0ml
20mM MgCl <sub>2</sub>	1M	0.8ml
100mM NH <sub>4</sub> Cl	5M	0.8ml
6mM BME	14.4M	16ul
water		
Sucrose		4.2g/18.8g
Final volume		40ml

<u>5X Buffer C (20mM Mg<sup>2+</sup>)</u>		1000ul
250mM Tris-HCl, pH7.5	1M	250ul
100mM MgCl <sub>2</sub>	1M	100ul
500mM NH <sub>4</sub> Cl	5M	100ul
30mM BME	14.4M	2ul
water		550ul

## Reaction

30s and 50s subunit were heat activated at 42°C for 20min in buffer C.

To form 70s, 20pmol 30s and 40pmol 50s were associated at 37°C for 10min, then Load onto sucrose gradient

Spin at SW41 rotor, 32000rpm for 15.5hr.

Analyze gradients on ISCO gradient fractionators.

Sensitivity: 0.2 abs, chart speed: 150cm/hr