

## HANDOUT for *PNAS* 85: 7852-7856

### ➤ **What we have known**

- ✓ The pathway of nonselective bulk-carrier vesicles moving proteins from ER to Golgi have been reconstituted
- ✓ NEM can selectively inactivate Golgi membrane

### ➤ **What are NEM and NSF**

- ✓ N-Ethylmaleimide effect (Nature 1987)
- ✓ A cytosol component is needed during vesicle transport
- ✓ A Golgi-associated protein factor (termed NSF) needs fatty acyl-CoA as a cofactor

### ➤ **Assay of NSF activity to trace NSF during purification**

- ✓ BASIC MECHANISM: adding back untreated “NSF” will restore transport inactivated by NEM
- ✓ Donor and acceptor membranes treated with NEM
- ✓ NSF-free cytosol
- ✓ Fractions from crude ATP-stabilized cytosol
- ✓ Mix, incubate and IP VSV-G-[H3]-GlcNAc

### ➤ **Purification of NSF**

- ✓ PEG precipitation -> DE-52 (anion exchange) flow through -> S Sepharose Fast Flow (cation exchange) flow through -> glycerol gradient velocity sedimentation (protein size) -> Mono S (cation exchange) FPLC

### ➤ **Monoclonal antibodies to NSF**

### ➤ **Conclusion**

- ✓ ATP stabilizes NSF
- ✓ Native NSF is a homo-oligomer of 76 kD polypeptide chains, almost certainly a tetramer