

Real Time Monitoring of Macromolecular Complex Assembly and Disassembly

We have developed a novel assay using a DynaPro Dynamic Light Scattering (DLS) instrument to study the dynamics of clathrin coated vesicle (CCV) assembly and disassembly.

CCVs move proteins between membranous compartments in all cells. To study the underlying mechanisms, we reconstituted clathrin coat assembly and disassembly from purified components. We found that DLS could be readily adapted to measure clathrin coat assembly, since it can easily distinguish individual clathrin triskelia, which have a diameter of 30 nm, from assembled clathrin coats, which have a diameter of 80 nm.

Clathrin coat assembly reactions were run with different concentrations of AP180 clathrin assembly protein, and subsequently analyzed by the DynaPro or by the conventional sedimentation assay. DynaPro data were fit with Dynamics software and counts corresponding to particles with an average diameter of 80nm were plotted as a function of clathrin concentration (Fig. 1; in blue), and overlaid with results from the sedimentation assay (in black). The fits are very comparable, and the the DynaPro experiment was completed in just a fraction of the time and utilizing just a fraction of the material required by the sedimentation experiment.

We then tested whether we could use the DynaPro to study the kinetics of clathrin coat disassembly. In these experiments we began with clathrin coats and initiated disassembly by adding either wt or mutant Hsc70 proteins (Fig. 2). We monitored the kinetics of disassembly in real time in the DynaPro instrument and fit the data fit to single exponential decays. This allowed us to quantitatively measure the effects of the different mutations on the rate at which Hsc70 disassembles clathrin coats. This information was not available from sedimentation experiments since the latter only permit endpoint measurements.

Our basic approach should be adaptable to real time monitoring of any macromolecular complex assembly/disassembly reaction.

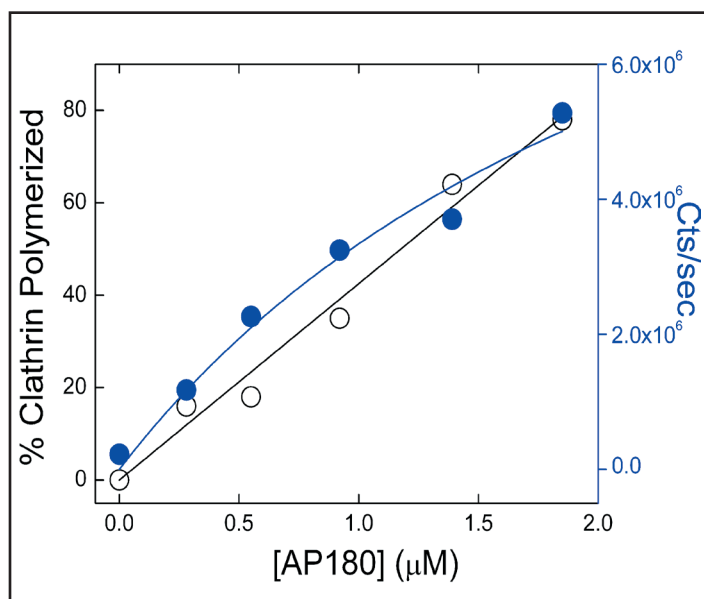


Figure 1: A comparison of AP180 mediated clathrin coat assembly as measured by the conventional sedimentation assay (black open circles, left axis), and the new dynamic light scattering assay (blue closed circles, right axis).

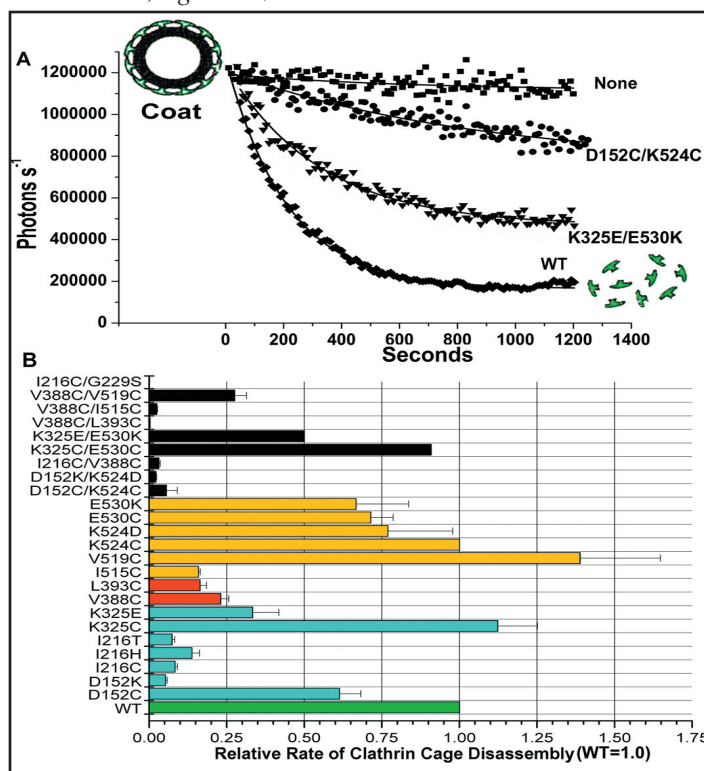


Figure 2: A: DLS monitoring of clathrin coat disassembly initiated at time zero by addition of either wild type or mutant Hsc70 enzymes. B: Relative rates of uncoating for a collection of Hsc70 mutants. (Reproduced from J. Jiang, K. Prasad, E.M. Lafer and R. Sousa. Structural Basis of Interdomain Communication in the Hsc70 Chaperone. *Molecular Cell* 20: 513-524 (2005)).

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