Protocol for Reconstitution of the Hydrophobic Core of Acetyl-LDL
(from Krieger, M. [1986] *Methods Enzymol.* **128,** 608-613)

Reagents:
Acetylated LDL
Dialysis buffer: 0.3mM EDTA, pH 7.0
Potato starch, purified powder
Prosil-28 or equivalent glassware antiwetting agent
Liquid nitrogen
Heptane, reagent grade or better
Compound to be incorporated into Ac-LDL, 30 mg/ml in heptane or benzene
Nitrogen or argon gas
Tricine buffer, 10 mM, pH 8.4, sterile

Preparation of Starch Tubes:
Reconstitution is performed in 13 x 100-mm disposable glass tubes. To prevent Ac-LDL from nonspecifically sticking to the glass, the tubes are first treated with Prosil-28 or an equivalent antiwetting agent. Potato starch (25mg) is added to each tube. The starch must be dried (e.g. By evaporation at room temperature) and the tubes may be stored at room temperature.

Procedure:

1. Lyophilization of Ac-LDL. Add 1.9 mg protein of dialyzed Ac-LDL (~0.3-0.5 ml) to each starch tube, cover with two layers of parafilm, and punch holes in the parafilm with a dissecting needle. Form an even suspension by gently vortexing the mixture and rapidly freeze in a thin shell with dry-ice slurry. Lyophilize for 4-6 h until the sample is completely dry.
2. Extraction of endogenous core lipids. The extraction may be conducted at room temperature. Add 5 ml of heptane to each lyophilized Ac-LDL starch tube, vortex vigorously for 30-60 sec, and pellet the Ac-LDL starch residue by centrifugation at 2000 rpm for 10 min. Discard the yellow supernatant and repeat the extraction two more times. The last supernatant should be colorless. Immediately proceed to the next step after removing the last supernatant. Do not allow the heptane covering the Ac-LDL starch residue to completely evaporate at this stage.
3. Reconstitution with exogenous lipid. Add 6 mg of lipid in 200 µl of apolar solvent to the Ac-LDL starch residue. Heptane, petroleum ether, carbon tetrachloride, and benzene can be used as the solvent. After gently shaking the mixture of Ac-LDL starch residue: lipid, allow to incubate for 5-10 min in standard freezer (-20^{o}C) and then slowly evaporate the solvent under a stream of inert gas (*e.g.*, argon) at room temperature until the sample is completely dry (~30-60 min).
4. Solubilization and isolation. All subsequent steps are performed at 4^{o}C and all liquid transfers are made using either plastic pipet tips. Add 1 ml of sterile buffer (10 mM tricine, pH 8.4) to each tube, vortex gently to resuspend the dry pellet, cover with parafilm and incubate for at least 10 h but no more than 24 h. During

this time, the reconstituted Ac-LDL is released from the starch while any denatured Ac-LDL and excess lipid remain bound to the starch or the glass. Lipids that are liquid at 4°C may form droplets that float on the buffer.

The solubilized reconstituted Ac-LDL is separated from the bulk of the starch and from excess lipid by low-speed centrifugation (2000 rpm). The specimen should be further clarified by at least one or two additional centrifugations (10,000 rpm for 10 min). The success of the reconstitution can be determined by the Lowry method. The reconstituted Ac-LDL should be stored under an inert gas at 4°C.