



**FIGURE 1.** Flow diagram of the specimen preparation methods for EM tomography described in this chapter. Our preferred route for the study of protein–protein interactions inside large cellular structures is indicated with the thick arrows. Specific steps in lowercase letters are discussed in the overview and procedures. The pre-embedding gold labeling approach for 3D immunolabeling is performed after the chemical fixation step and before the resin-embedding step, as described in the text.