1.6 ANALYSIS OF CRYO-EM (CEMOVIS) DATA USING MD SIMULATION AND EM SIMULATION

Atomistic MD simulation combined with EM simulation may be used to analyze CEMOVIS data. Simulated electron micrographs (cf. Rullgård et al., 2011) are then generated at defocuses corresponding to those of the original cryo-EM defocus series data for different atomistic candidate MD models (Lundborg et al., 2018a) (Figure 1.3, lower/front row).

CEMOVIS image analysis is then based on an iterative process where an MD model is modified in a stepwise fashion until optimal correspondence is achieved between the original CEMOVIS data derived from the biological specimen and the simulated EM data derived from the MD model (Figure 1.4). The major advantages of using CEMOVIS for biological structure determination in situ are the near-native preservation of the analyzed biological structure, the high image resolution and information content of cryo-EM images, and the high sensitivity of the cryo-EM image interference patterns to the microscope defocus levels. An advantage of analyzing CEMOVIS data using MD simulation to generate input for EM simulation is that MD simulation allows for investigation of the thermodynamic and other physicochemical properties of the candidate atomic models. Another advantage is that it ensures a realistic model atomic density irrespectively of model topology. EM simulation errors derived from model artifacts, such as atomic overlapping or void spaces, are thereby avoided.

The procedure for MD-simulation/EM-simulation–based CEMOVIS image analysis is as follows (Figure 1.4): (1) collection of a high-resolution cryo-EM image defocus series, (2) construction of a candidate atomic model using model building software, (3) equilibration of the candidate atomic model using MD simulation, (4) generation of a series of simulated EM images at different microscope defocus levels from the equilibrated atomistic MD model using EM simulation, and finally (5) comparison of the original cryo-EM defocus series images and the simulated defocus series images. The procedure is repeated until optimal correspondence is achieved between simulated and original data (Figure 1.3).

1.7 SKIN LIPID FORMATION

In order to appreciate the structure–function relationships of the skin barrier in vivo, it is important to understand skin lipid formation, as the skin's lipid structure may represent a "frozen-in" or "immobilized" open biological system rather than a primary minimum energy equilibrium system. Skin lipid formation is also central from a dermatological standpoint, since barrier malformation may be an etiological factor in barrier-deficient skin conditions such as eczema, psoriasis, and dry skin.

It has been proposed (Norlén, 2001a) that skin lipid formation proceeds via (1) membrane synthesis in the trans-Golgi of a cubic lipid phase, followed by (2) a nonfusion-dependent secretion of the cubic lipid phase into the intercellular space (den Hollander et al., 2016), a subsequent (3) phase transition from cubic to stacked lamellar membrane morphology (Naragifard et al., 2018) with a concomitant (4) dehydration (Wennberg et al., 2018) and (5) condensation (Wennberg et al., 2018) of the stacked lamellar lipid phase, and with a final internal (6) lipid chain rearrangement from a folded (hairpin) to an extended (splayed) chain conformation (Narangifard et al., 2020).

1.8 SKIN LIPID FUNCTION

Current knowledge suggests that a stacked, fully extended (splayed chain) ceramide bilayer arrangement with a high cholesterol content and a heterogeneous, saturated, long-chain lipid composition (Figure 1.1(III), Figure 1.2) may represent an optimized barrier material for skin. This is because it is largely impermeable to water as well as to both hydrophilic and lipophilic substances (Lundborg et al., 2018a,b) because of its condensed lipid chain packing and its alternating lipophilic (alkyl