

opposite tendencies to those observed with CER III and 5. Investigations on the effect of RH on human SC samples showed that the *trans/gauche* conformation [ratio  $(I_{1060} + I_{1130})/I_{1080}$ ] increases with RH = 44% and 60%, then decreased for RH = 75%. The same trend was observed for the conformational state and lateral packing ( $I_{2882}/I_{2852}$ ) (Vyumvuhore et al. 2013). Hence, a more organized SC lipid barrier is present at intermediate RH. A drying experiment on hydrated SC samples by Vyumvuhore et al. (2015) showed that the compactness of the lipid matrix in the SC increases according to drying time at 7% RH, similarly to the relative amount of partially bound water compared to the global water content.

**Protein conformation.** Skin protein conformation is linked to skin water content due to the water present in corneocytes. Vyumvuhore et al. (2013) showed that the decrease in bound water content below 60% RH destabilizes protein conformation. This was evidenced by a decrease in the content of exposed tyrosine ( $830\text{ cm}^{-1}$ ) compared to the buried conformation ( $850\text{ cm}^{-1}$ ) and a shift in the protein conformation ( $2932\text{ cm}^{-1}$  band) towards lower wavenumbers. Additionally, an increase in RH up to 60% yielded an increase in the water sub-band of  $1690\text{ cm}^{-1}$  representing conformational turns and random coils. The interpretation is that the increase in RH caused a swelling of keratin filaments, corresponding to unfolding of long  $\alpha$ -helices and  $\beta$ -sheets and yielding turns and a random coil protein secondary structure. Similar results were obtained by Vyumvuhore and coworkers during the drying of SC from 98% to 7% RH (Vyumvuhore et al. 2015). In highly humidified SC, multilayered water molecules fill spaces between keratin fibers, favoring keratin  $\beta$ -sheet conformation. The drying process removes this water, leaving spaces between the fibers and decreasing  $\beta$ -sheet conformation.

#### 55.4.2.7 Probing the Barrier of Diseased Skin

With its sensitivity to lipid and protein conformation and organization in skin, CRM lends itself well to the characterization of dermatological pathologies that result in a weakened skin barrier and, therefore, a propensity towards increased penetration of compounds compared to healthy skin. Atopic and psoriatic skin in particular have been studied via CRM, with a focus on the same Raman bands and intensity ratios.

Wohlrab et al. (2001) used CRM to highlight the decrease in lipid order in non-lesional skin of patients compared to healthy skin through the study of  $\text{CH}_2$  symmetric stretching mode at  $2848\text{ cm}^{-1}$  and its shift towards higher wavenumbers and the lower intensity of the  $1128\text{ cm}^{-1}$  band. Osada et al. (2004) and Verzeaux et al. (2018) showed a decrease in the lipid matrix structure of psoriatic and mildly atopic skin using the lipid packing order ratio  $v_{\text{asym}}\text{CH}_2 / v_{\text{sym}}\text{CH}_2$  ( $I_{2890}/I_{2850}$  or  $I_{2885}/I_{2850}$ ), also used by Tfayli and coworkers investigating the effects of age and relative humidity on the lipidic aspect of the skin barrier (Sections 55.4.2.5 and 55.4.2.6). Verzeaux et al. (2018) further compared the  $v\text{CC } trans/v\text{CC } gauche$  conformation ratio  $[(I_{1060} + I_{1130})/I_{1080}]$  in mildly atopic vs. healthy skin, also showing a significant decrease in pathological skin.

With regard to protein in the skin, the consensus revealed through CRM is a shift of the amide I band towards higher wavenumbers in the diseased skin compared to healthy skin, indicating a loss in protein conformational structure (Osada et al. 2004; Bernard et al. 2007). Verzeaux et al. (2018) showed a significant increase in the  $\alpha$ -helix and  $\beta$ -sheet structures in atopic skin compared to healthy skin by measuring the ratio between the  $\alpha$ -helix ( $1656\text{ cm}^{-1}$ ) and  $\beta$ -sheet ( $1672\text{ cm}^{-1}$ ) conformations to the overall amide I ( $1600$  to  $1700\text{ cm}^{-1}$ ) band.

Comparing lipid and protein contents, lipid-to-protein ratios are decreased in diseased skin compared to healthy skin (Verzeaux et al. 2018), as well as in non-lesional skin of atopic eczema volunteers compared to their lesional skin (Janssens et al. 2014).

Richters et al. (2017) studied whether indicators of sensitive skin could be reliably detected using CRM. Sensitive skin is defined as “a condition characterized by the perception of skin discomfort following mild stimuli, frequently without objective signs of skin irritation.” They compared water, NMF, and ceramide/fatty acid levels in volunteers’ sensitive skin to those from non-sensitive skin individuals as well as atopic dermatitis (AD) and allergic rhinoconjunctivitis (AR) volunteers.