

## Water types distribution in the human skin horny layer in vivo

The stratum corneum (SC) is the uppermost horny layer of the skin and provides an efficient barrier function for regulation of water homeostasis. The water in the SC plays an important role in maintaining the SC's enzyme activity, pH and moisturizing, i.e., for providing the healthy skin physiology. Diseased skin is associated with impairment of the skin barrier function including disturbances in water distribution. There are different binding centers of water in the SC, such as natural moisturizing factor (NMF), intercellular lipids (ICL) and keratin, which are non-homogeneously distributed and are a topic of extensive investigations.

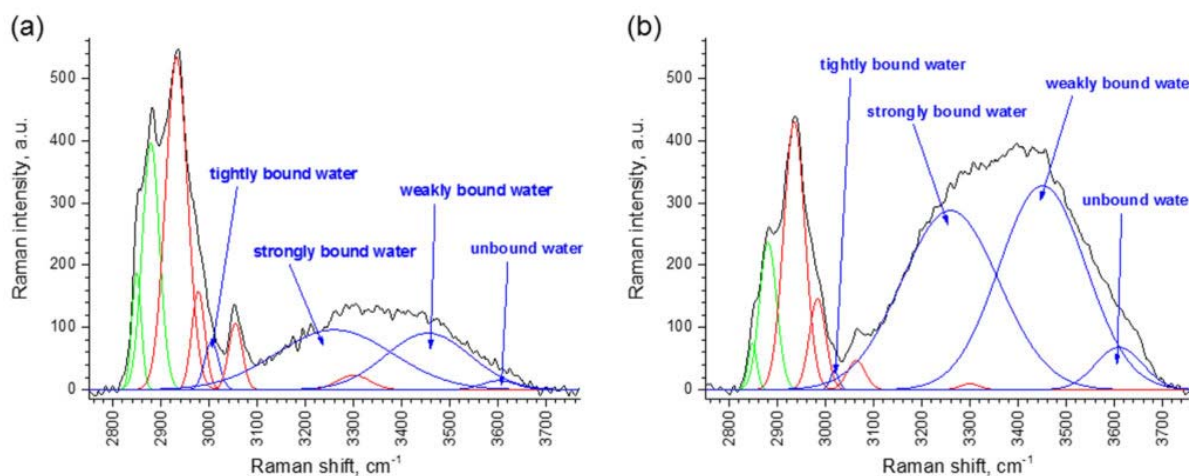


Fig. 1. Raman spectra ( $2750\text{--}3770\text{ cm}^{-1}$ ) of human skin in vivo (black) and corresponding deconvoluted Gaussian functions (green – lipids; red – keratin; blue – water) in exemplary depths of  $2\text{ }\mu\text{m}$  (a) and  $20\text{ }\mu\text{m}$  (b) in the SC.

Raman microscopy is a powerful tool for non-invasive *in vivo* analysis of the depth-dependent chemical composition of the SC. In the  $2800\text{--}3700\text{ cm}^{-1}$  range, the Raman bands of lipids, keratin and water are overlaid and their unique contribution can be determined only after application of the Gaussian function-based deconvolution procedure, as presented in Figure 1.

The corresponding water-related Gaussian peaks (blue lines in Figure 1) characterize the tightly ( $3005\text{ cm}^{-1}$ ), strongly ( $3270\text{ cm}^{-1}$ ), weakly ( $3460\text{ cm}^{-1}$ ) and unbound ( $3600\text{ cm}^{-1}$ ) water.

The calculation of the water-related Raman bands for different SC depths gives information about the distribution of different water types in the SC. Figure 2a shows the mean distribution of tightly, strongly, weakly and unbound water types in the SC, measured *in vivo* on the skin of 6 healthy volunteers. Evidently, the most prevalent water molecule types in the SC are strongly and weakly bound water types, which represent  $>90\%$  of the total SC's water.

The ratio of weakly/strongly bound water molecule types represents the hydrogen bonding states of water, whose depth profile in the SC is shown in Figure 2b. Lower values indicate an increased

amount of hydrogen bonds between the water and the surrounding biomolecules, such as keratin, NMF and ICL, showing the highest water binding ability at the depths of 20–40% of the SC thickness. It should be considered that the  $3460\text{ cm}^{-1}/3270\text{ cm}^{-1}$  ratio is very precise due to the domination of these water types in the SC (>90%).

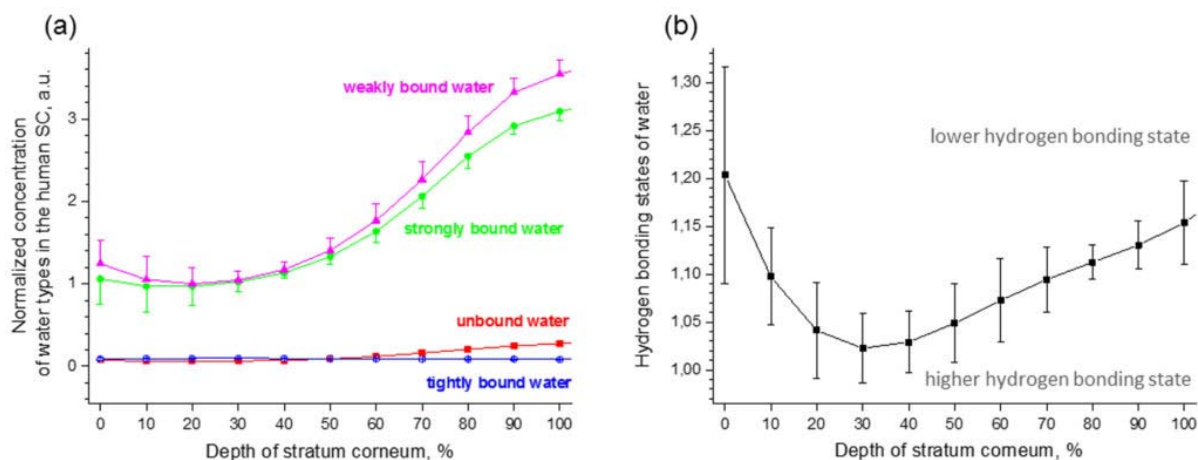


Fig. 2. The depth profile of the tightly ( $3005\text{ cm}^{-1}$ , blue), strongly ( $3270\text{ cm}^{-1}$ , green), weakly ( $3460\text{ cm}^{-1}$ , magenta) and unbound ( $3600\text{ cm}^{-1}$ , red) water molecule types in the human SC (a) and the depth profile of the hydrogen bonding states of water molecules in the SC determined as a  $3460\text{ cm}^{-1}/3270\text{ cm}^{-1}$  ratio (b). Mean  $\pm$  SD of 6 healthy volunteers.

The depth profiles of the hydrogen bonding states of water molecules (Fig. 2b) correlates with the depth profiles of the ICL lateral packing order and NMF, whose obvious maxima are also observed at  $\gg 30\%$  SC depth, but does not sufficiently correlate with the ability of keratin to bind water (keratin folding/unfolding state), whose maximum is observed at  $\gg 60\text{--}70\%$  SC depth.

The obtained results let conclude that NMF and ICL play a basic role in binding the water molecules at least at the uppermost SC layers (0–30% SC depth). In the deeper-located SC layers  $>30\%$  SC depth, keratin could play a comparable role in binding water molecules.

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## **Publication**

[Depth profiles of hydrogen bound water molecule types and their relation to lipid and protein interaction in the human stratum corneum in vivo.](#)

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