Electronic Supplementary Material (ESI) for Analyst

Supporting Information

Label-free imaging of drug distribution and metabolism in colon cancer cells by Raman microscopy

Samir F. El-Mashtoly,[a] Dennis Petersen,[a] Hesham K. Yosef,[a] Axel Mosig,[a] Anke Reinacher-Schick,[b] Carsten Kötting,[a] and Klaus Gerwert*[a]

[a]Department of Biophysics, and [b]Department of Hematology and Oncology, St. Josef-Hospital, Ruhr-University Bochum, 44780 Bochum, Germany

General methods

Cytotoxicity assay. Erlotinib obtained from Roche. Working solutions of the drug were prepared in cell culture media. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide] was obtained from Sigma Aldrich. MTT (yellow) is reduced to purple formazan by mitochondrial reductases in living cells, and thus it is commonly used as an indicator of the cell viability and proliferation. Cells were cultured in 24-well plates at a density of 2 x 10^3 cells per well in DMEM medium with all the supplements as listed above. After 24 hours of initial cell attachment, the plates were washed with 100 µl per well PBS and were incubated with different concentrations of erlotinib.

Following a 12 hour exposure, the cells were rinsed with PBS and 100 ml of fresh medium (without supplements) were added to each well. A volume of 10 ml of MTT (5 mg/ml) prepared in PBS was then added to each well and the plates were incubated for 4 hours at 37°C in a 5% CO_2 humidified incubator. After this incubation period, the medium was discarded, the cells were washed with 100 ml of PBS and 100 ml of DMSO was added to each well to extract the dye. The plates were then shaken 240 times per minute for 10 min and absorbance was measured at 570 nm using micro-plate reader (TECAN Sunrise, Austria). Four replicate wells were used for each exposure. The level of the viability in each sample was normalized to that of the control sample.

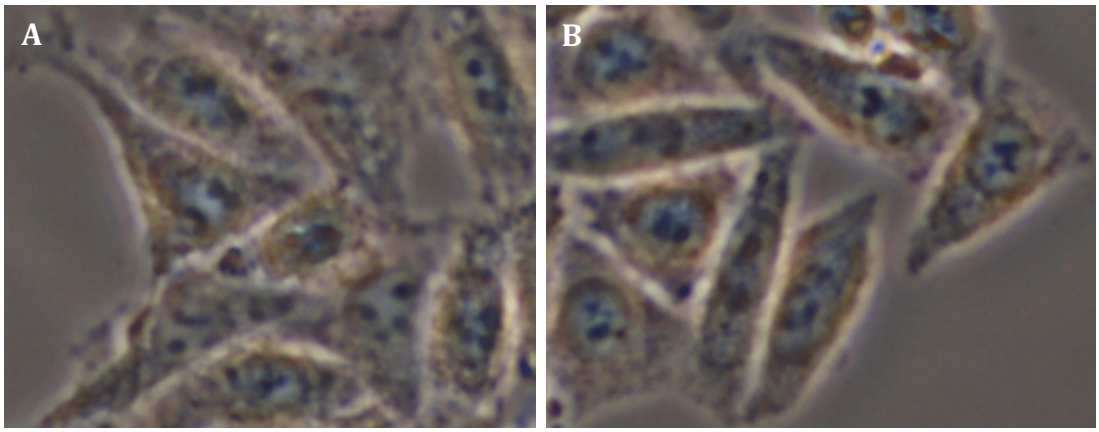

Fig. S1. Bright field images of SW480 cells (control) grown under normal conditions (A) and with serum starvation (B).

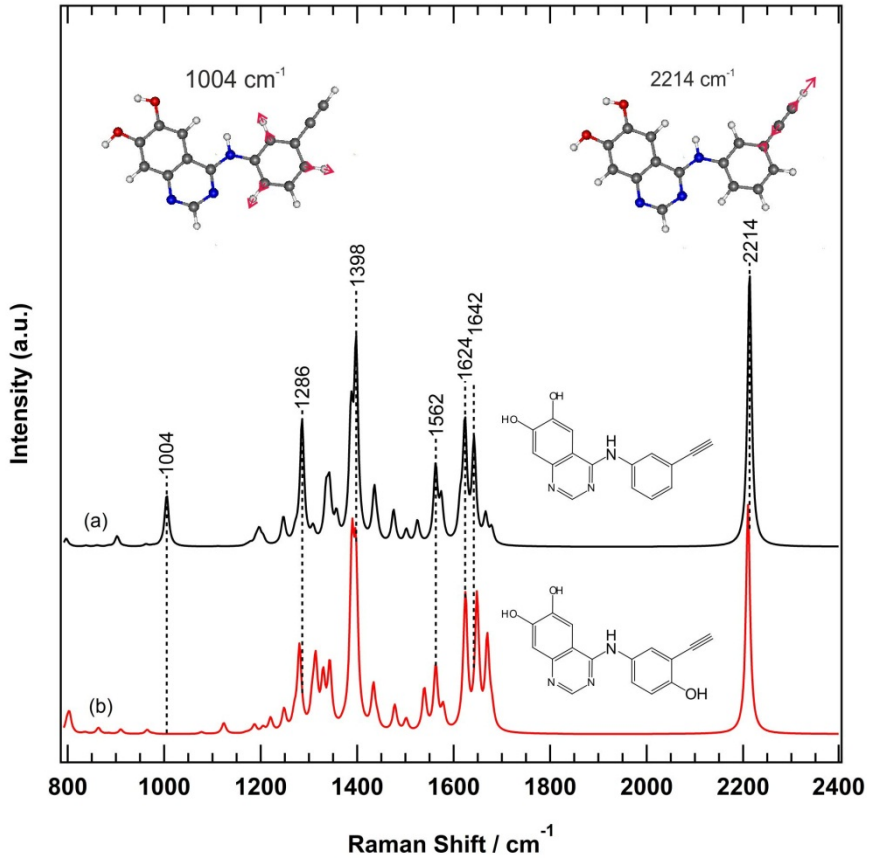

Fig. S2. Calculated Raman spectra of fragments of erlotinib (a) and 4-hydroxylated erlotinib (b).

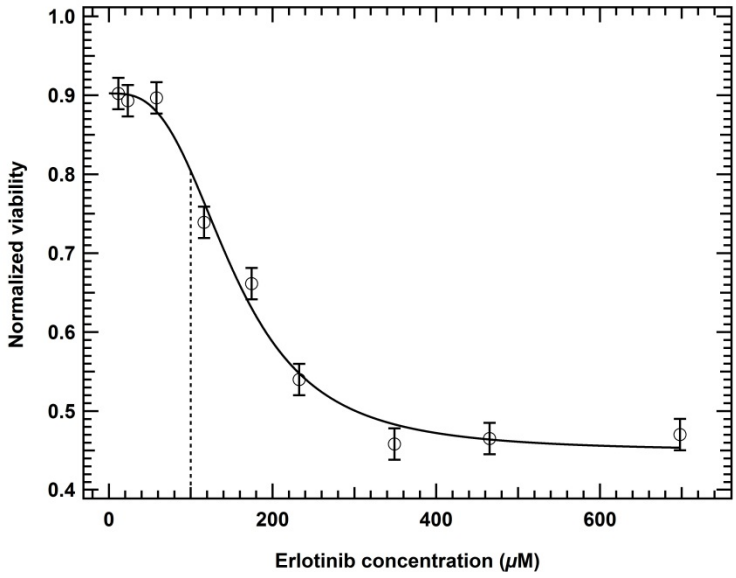

Fig. S3. SW480 cell viability measured by MTT absorbance at 12 h after exposure to erlotinib.

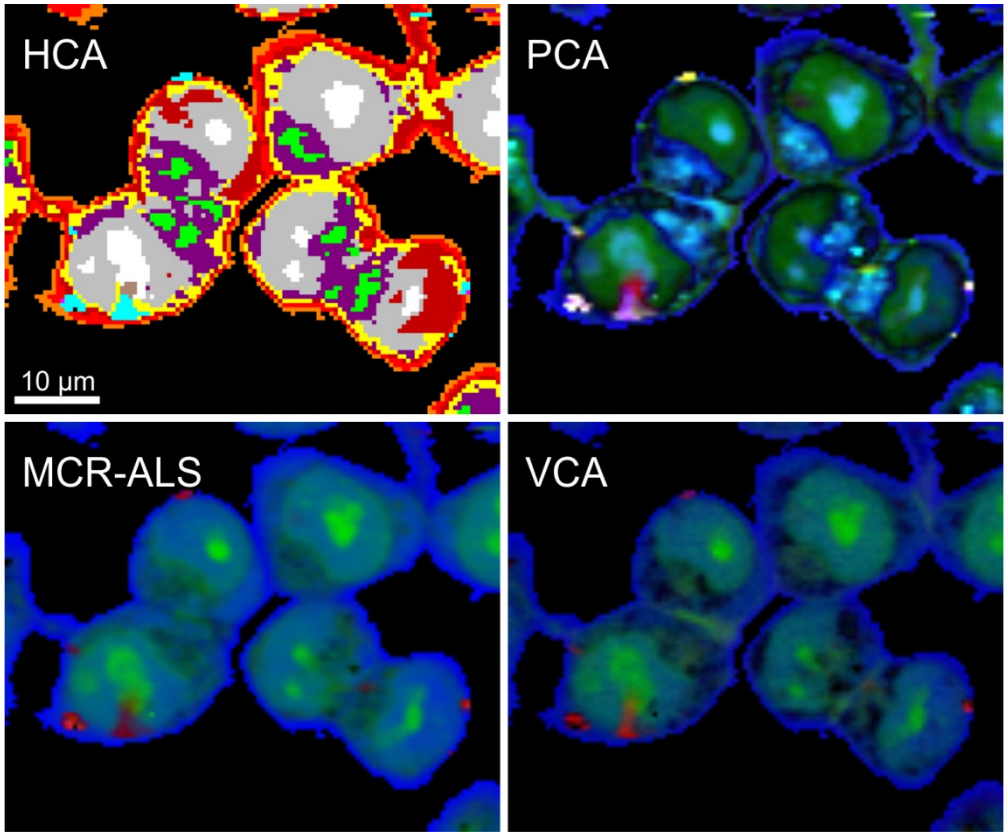

Fig. S4. Hierarchical cluster analysis (HCA), principle component analysis (PCA), multivariate curve resolution-alternating least squares (MCR-ALS), and vertex component analysis (VCA) of Raman dataset (Figure 1) are shown and described in details previously.[1,2]

1. M. Miljković, T. Chernenko, M. J. Romeo, B. Bird, C. Matthäus, and M. Diem, *The Analyst*, 2010, **135**, 2002.
2. I. I. Patel, J. Trevisan, G. Evans, V. Llabjani, P. L. Martin-Hirsch, H. F. Stringfellow, and F. L. Martin, *The Analyst*, 2011, **136**, 4950.

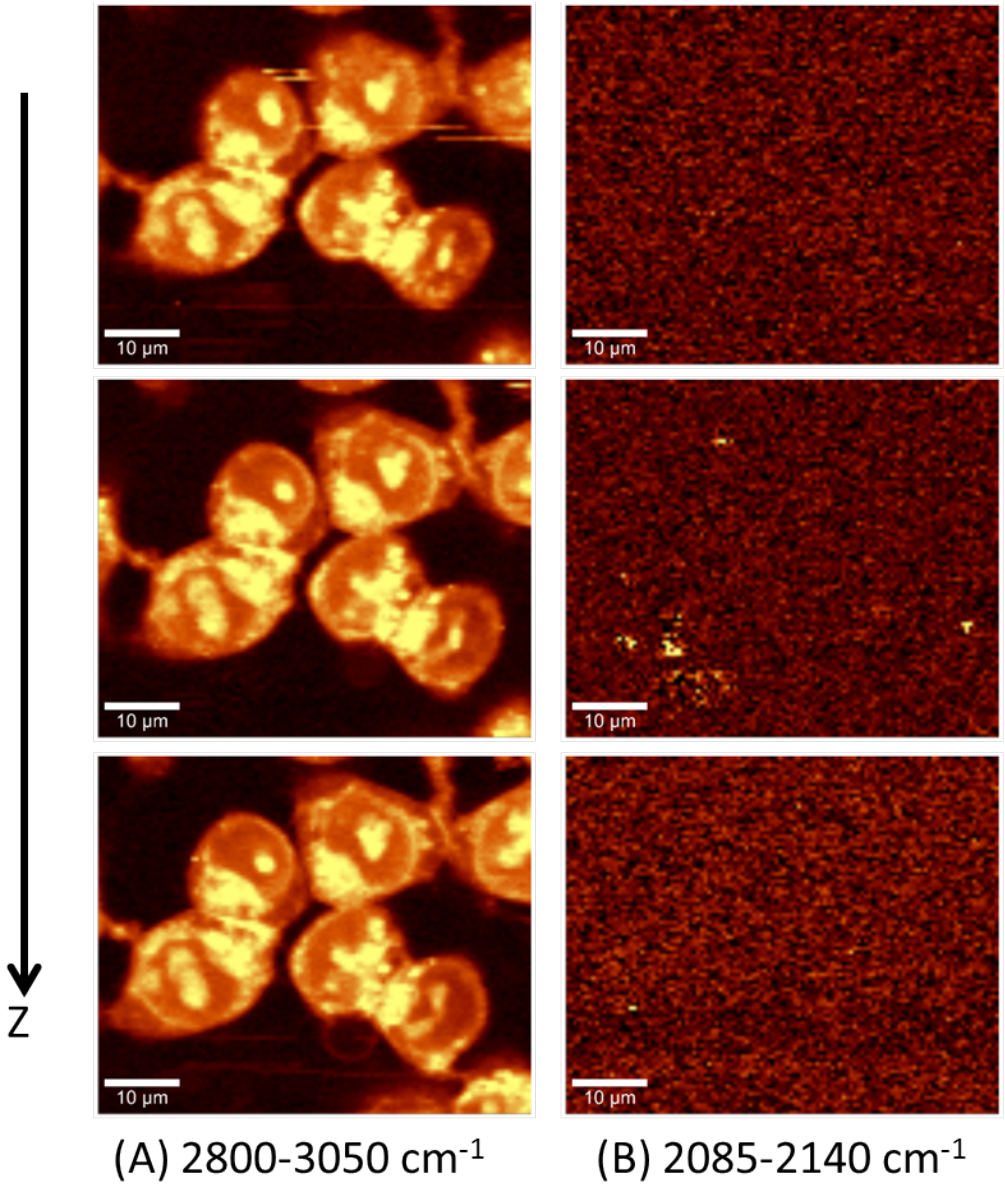

Fig. S5. 3D Raman imaging of SW480 cells treated with ~100 μM erlotinib for 12 h. Raman images reconstructed from the C—H (A) and C≡C (B) stretching intensities. The arrow represents Z direction inside the cells. The confocal depth between each layer is 1μm.

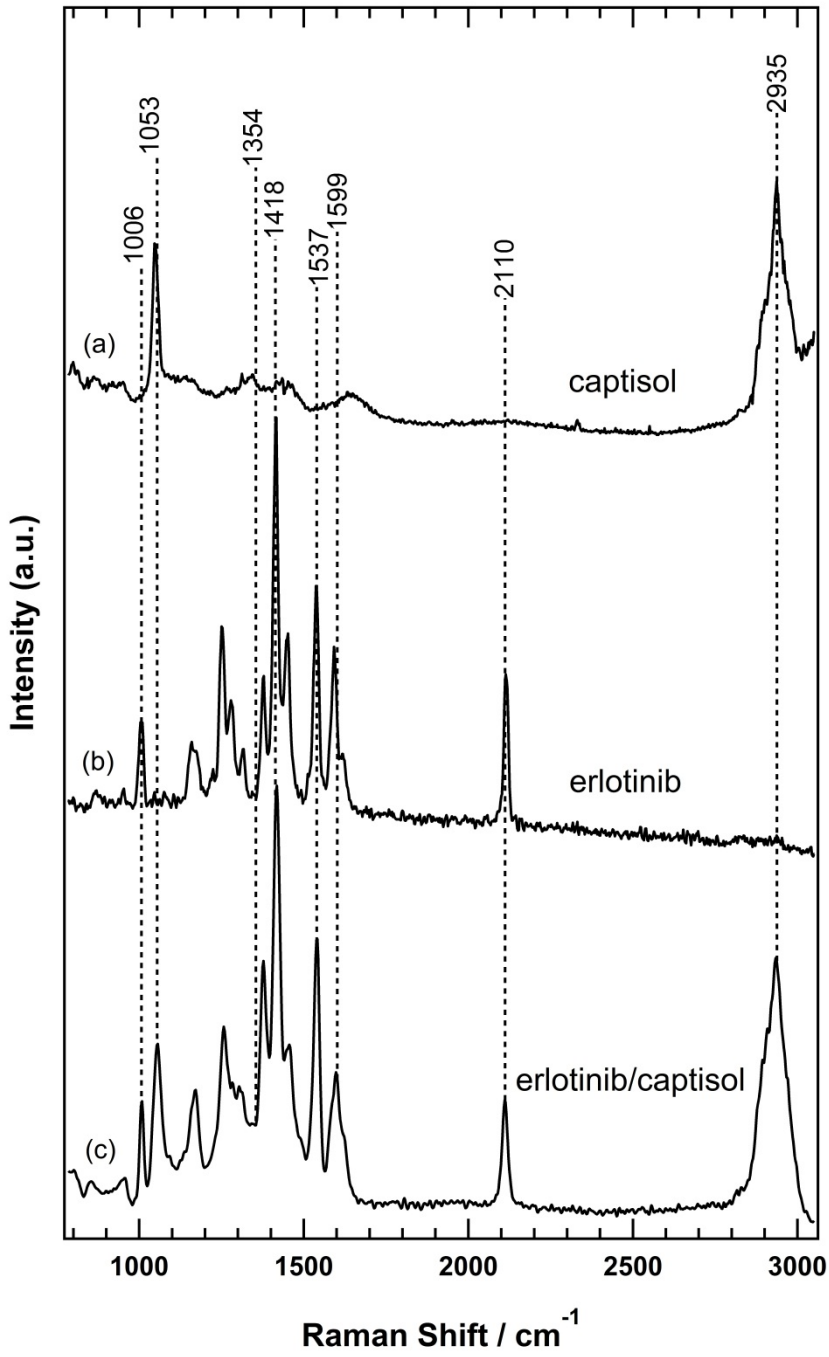

Fig. S6. Raman spectra of captisol (a), erlotinib (b), and erlotinib/captisol mixture (c).