

### 3D-Visualization of lipid localization on nanoscale by click chemistry and Transmission Electron Microscopy

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#### Abstract

Because of the lack of adequate and reliable labeling techniques for small lipophilic molecules in the field of electron microscopy, ultra-structural localization of lipids at is currently a challenge. To resolve this problem, we developed a novel approach for three-dimensional (3D) lipid labeling in biological specimens on nanometer-scale.

Our novel labeling technique consists of a click-chemistry based covalent conjugation of colloidal gold particles, functionalized with azide-groups to lipids, which were tagged with alkyne-groups (click-lipids). Previous studies revealed that synthesized click-lipids act virtually native in biological systems [Thiele et al. 2012, Hofmann et al. 2013].

The application of click-lipids is relatively easy. In our approach click-lipids were provided as nutrition for cultured cells. There virtually are no restrictions regarding cell type, species or way of culture. In vivo applications e.g. in rodents are also achievable. The 3D distributions of the up-taken and metabolized click-lipids were evaluated via electron tomography of click-reacted gold particles after plastic embedding and ultra-thin sectioning.

Here we present a procedure using labeling reagents, which are independent of any biological macromolecules (e. g. antibodies, streptavidin, protein A). This approach for the first time allows tracing fatty acid metabolism and subcellular distribution in virtually any biological system with low unspecific background labeling and high sensitivity. With the present novel methodology, we also deliver an ultra-structural visualization of defined lipid species. Furthermore advanced analysis of such visualizations would reveal general aspects of intra- and intercellular lipid trafficking. Evaluation of more specified biomedical relevant but poorly understood processes e.g. uptake of lipophilic lead structures over the blood brain barrier would also be feasible.

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[Thiele et al. 2012] Tracing Fatty Acid Metabolism by Click Chemistry Thiele C, Papan C, Hoelper D, Kusserow K, Gaebler A, Schoene M, Piotrowitz K, Lohmann D, Spandl J, Stevanovic A, Shevchenko A, and Kuerschner L ACS Chemical Biology 2012 7 (12), 2004-2011

[Hofmann et al. 2013] Hofmann K, Thiele C, Schött HF, Gaebler A, Schoene M, Kiver Y, Friedrichs S, Lütjohann D, Kuerschner L. A novel alkyne cholesterol to trace cellular cholesterol metabolism and localization. J Lipid Res. 2014 Mar;55(3):583-91. doi: 10.1194/jlr.D044727. Epub 2013 Dec 12.