



HAL
open science

Click-electrochemistry for the rapid labeling of virus, bacteria and cell surfaces

Sébastien Depienne, Mohammed Bouzelha, Emmanuelle Courtois, Karine Pavageau, Pierre-Alban Lalys, Maia Marchand, Dimitri Alvarez-Dorta, Steven Nedellec, Laura Marín-Fernández, Cyrille Grandjean, et al.

► **To cite this version:**

Sébastien Depienne, Mohammed Bouzelha, Emmanuelle Courtois, Karine Pavageau, Pierre-Alban Lalys, et al.. Click-electrochemistry for the rapid labeling of virus, bacteria and cell surfaces. *Nature Communications*, 2023, 14 (1), pp.5122. <10.1038/s41467-023-40534-0>. <hal-04246348>

HAL Id: hal-04246348

<https://hal.science/hal-04246348v1>

Submitted on 17 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Click-electrochemistry for the rapid labeling of virus, bacteria and cell surfaces

Authors: Sébastien Depienne,¹ Mohammed Bouzelha,² Emmanuelle Courtois,³ Karine Pavageau,² Pierre-Alban Lalys,¹ Maia Marchand,¹ Dimitri Alvarez-Dorta,^{1,4} Steven Nedellec,⁵ Laura Marín-Fernández,³ Cyrille Grandjean,³ Mohammed Boujtita,¹ David Deniaud,¹ Mathieu Mével,^{2*} and Sébastien G. Gouin.^{1*}

Affiliations:

¹Nantes Université, CNRS, CEISAM UMR 6230, F-44000 Nantes, France

²Nantes Université, TaRGeT, Translational Research for Gene Therapies, CHU Nantes, INSERM, UMR 1089, F-44000 Nantes, France

³Nantes Université, CNRS, US2B, UMR 6286, F-44000 Nantes, France

⁴Capacités, 26 Bd Vincent Gâche, 44200 Nantes

⁵Nantes Université, CHU Nantes, CNRS, Inserm, BioCore, US16, SFR Bonamy, Nantes, France

Corresponding authors : sebastien.gouin@univ-nantes.fr; mathieu.mével@univ-nantes.fr

Abstract

The remodeling of microorganism surfaces with biomolecules is a powerful tool to study the role of membrane receptors in chemical biology and to develop drug delivery systems in gene therapy using viral vectors and cell-based therapies. Methods for direct covalent ligation of these surfaces remain poorly reported, and mostly based on metabolic engineering for bacteria and cells functionalization. In the latter case, a tagged precursor must first be enzymatically metabolized and delivered to the outer cell membrane to become available for chemo-selective labeling. While effective, a faster method avoiding the bio-incorporation step would be highly complementary. This would also need to be compatible with organisms showing poor levels of precursor assimilation or lacking the metabolic function. Here, we used *N*-methyluminol (NML), a fully tyrosine-selective protein anchoring group after one-electron oxidation, to label the surface of viruses, living bacteria and cells. The functionalization was performed electrochemically and *in situ* by applying a 750 mV vs Ag/AgCl electric potential to aqueous buffered solutions of tagged NML containing the viruses, bacteria or cells. The electro-coupling was performed with NML anchors bearing a bioorthogonal azide, biotin, or carbohydrate (mannose and *N*-acetyl galactosamine) handles. The broad applicability of the click-electrochemistry method was explored on recombinant adeno-associated viruses (rAAV2), *E. coli* (Gram-) and *S. epidermis* (Gram+) bacterial strains, and HEK293 and HeLa eukaryotic cell lines. Surface electro-conjugation was achieved in minutes to yield functionalized rAAV2 that conserved both structural integrity and infectivity properties, and living bacteria and cell lines that were still alive and able to divide. As NML activation immediately stops if there is no current, the method offers reproducible temporal control on the degree of surface functionalization. Thus, click-electrochemistry should significantly expand the scope of bioconjugation methods.

Introduction

Cell surface-associated proteins play a pivotal role in many biological events including intercellular communications and binding, signal transduction, and host-pathogen interactions. A deeper understanding of these biological processes relies on our ability to pattern cell surfaces

with specific probes, drugs, binding proteins or carbohydrates. Genetic or chemical modification of viral capsids, and bacterial and cell membranes has already demonstrated interesting outlooks for a wide range of therapeutic approaches. In the field of gene therapy, genetically or chemically engineered viral capsids with peptides or carbohydrates may improve gene delivery in specific organs and target a wide variety of cell types including cardiomyoblasts, liver, tumor, smooth muscle and endothelium.¹⁻³ The modification of bacterial cell surfaces has also been extensively studied for diagnostic and therapeutic purposes. Research studies using living bacteria to treat solid tumors has seen considerable progress in recent years.⁴ Conventional chemotherapeutics show low penetration and limited accumulation in the poorly vascularized hypoxic areas of tumors. However, anaerobic living bacteria easily colonize and continue to proliferate in these necrotic and deep regions. There, they can initiate antitumor immune response, meaning that they represent complementary avenues for cancer treatment.⁵ Interestingly, this bacterial-based anticancer therapy may be significantly improved by the chemical modification of the bacterial surface with immune checkpoint inhibitors, chemotherapeutic drugs, tumor-specific antigens and photothermal sensitizers.⁶⁻⁸ Last but not least, the chemical reprogramming of eukaryotic cell surfaces is a rapidly evolving research field with strong potential for drug delivery, cell-based therapies and tissue engineering.^{9,10} Cell surface remodeling may be used to: *i*) improve targeting, as illustrated on mesenchymal stem cells carrying a modified sugar coat with E-selectin ligands for enhanced bone tropism;¹¹ and *ii*) mask selective antigens, as shown with the PEGylation of donor red blood cells in chronic transfusions to avoid immune rejections.^{12,13} Thus, the modification of viral, bacterial or cell surface offers highly promising therapeutic applications for a wide range of diseases.

The conjugation of proteins may be achieved with an extreme degree of site-selectivity by genetic code expansion techniques, where an unnatural amino acid (UAA) with a bioorthogonal reactive handle is site-specifically incorporated into the protein of interest by the cellular machinery.^{14,15} While UAA-based protein labeling is starting to find numerous applications, this synergistic use of chemistry and biology is conducted in specialized laboratories, and there is still considerable interest for the direct modification of natural proteins. Direct chemical conjugation methods have historically been performed on the two most nucleophilic proteinogenic amino acids, *i.e.* the abundant lysine (K) and rare cysteine (C) residues, with various electrophilic moieties (**Fig. 1a**).¹⁶ Commercial vaccines, PEGylated drugs or antibody-drug conjugates (ADC) are mainly K and C conjugates, but modern strategies targeting less explored AA such as methionine, guanidine, tyrosine or tryptophan are being thoroughly studied in academia.¹⁷⁻²¹ Despite the wide range of reactions now available, the direct AA modification of surface proteins from living bacteria or cells is, however, poorly represented.^{6,8,13,22,23} Difficulties include the high biological complexity and chemical sensitivity of cell surfaces, cell turnover and protease degradations, or the presence of a shielding carbohydrate coat. The paradigm for chemical remodeling of bacterial and cell surfaces remains the two-step metabolic oligosaccharide engineering (MOE) method.^{24,25} In MOE, a carbohydrate precursor with a bioorthogonal handle is biosynthetically processed,²⁶ then attached to cell-surface glycans for further labeling by conventional click chemistry techniques (**Fig. 1b**). MOE is a powerful technique and may be the best for studying cell surface glycans, but the development of direct and faster labeling methods that avoid the bacterial/cell culture step required by the biomachinery (to transform the tagged precursor) would nicely complement the toolbox of bioconjugation reactions. Such methods would particularly suit bacterial/cell lines with low propensity to incorporate synthetic sugar precursors in their glycocalyx, or

organisms lacking metabolic functions, like viruses. In this work, we describe the rapid modification of a therapeutic rAAV, living Gram +/- bacteria and eukaryotic cells using an *in situ* electrochemical activation of a tyrosine-selective anchor (**Fig. 1c**). Although electrochemical bioconjugation methods have flourished for native protein modification,²⁷ with a recent example published by the group of Baran for chemoproteomic-based target identification,²⁸ electrochemistry has never, to our knowledge, been applied for the exterior bioconjugation of viruses, living bacteria or cells.

Results and discussion

A wide range of chemical methods have been developed to selectively target the tyrosine (Y) residues exposed at the surface of native proteins. The groups of Barbas and Nakamura identified cyclic diazodicarboxyamides such as phenylurazole (PhUr)²⁹ or *N*-methyluminol (NML)^{30,31} being among the most promising Y anchors after activation by chemical or enzymatic oxidations.³² Inspired by these previous results, we showed that electrochemistry was a highly versatile approach for the *in situ* oxidative conversion of unreactive PhUr precursors into their highly Y-reactive phenyltriazolinedione equivalents.³³ The scope of this first electro-bioconjugation method, named eY-click, was further expanded with NML derivatives showing improved kinetics, complete Y-chemoselectivity and the interesting possibility of double-tagging the most exposed and reactive Y, as demonstrated on peptides and proteins.^{34,35} For these reasons, we selected the NML anchor in the present study.

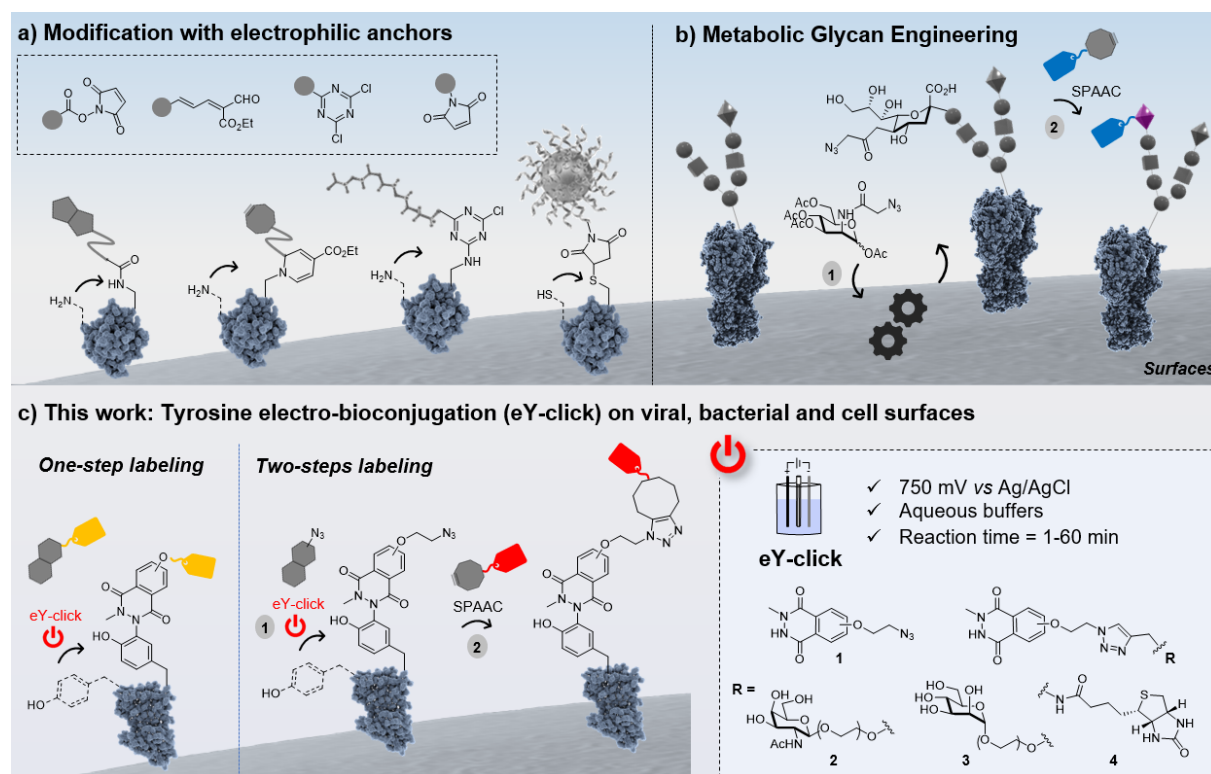


Fig. 1 a) There are only a few reports on direct covalent modification of cell surfaces, with most examples restricted to electrophilic anchors targeting amino and thiol groups from lysine and cysteine. This approach is restricted to a limited number of substrates bearing non-nucleophilic functional groups. b) MOE is a two-step labeling method where a sugar precursor (here a protected azido-tagged *N*-acetylmannosamine) is first internalized, processed by intracellular enzymes and integrated in the host glycoalyx. In a second step, the tagged sugar serves as an anchoring point for the substrate of interest thanks to a bioorthogonal reaction (here the strain-promoted azide-alkyne cycloaddition, SPAAC). c) This work describes the first electrochemical method to modify tyrosines from

viral capsids, and bacterial and cell surfaces. An electro-active anchor (luminol derivative) is activated in the presence of the (living) organisms using a 750 mV vs Ag/AgCl potential difference, applied in an electrochemical cell. This results in a rapid and soft labeling of cell surfaces by one-step or two-step approaches.

eY-Click on recombinant Adeno-Associated Virus rAAV2

Functionalization of free amino groups from viral capsids with carbohydrates was previously reported by our group and others to potentially improve their transduction efficiency in specific cell lines.^{3,36,37} Here, we assessed the potential of electrochemistry to functionalize the Y from viral capsids using rAAV2 as a therapeutically relevant serotype. NML derivative **1** was chemically functionalized beforehand with propargyl derivatives of *N*-acetylgalactosamine (GalNAc) or mannose (Man) to form **2** and **3**, respectively (chemical synthesis in **Fig. S4**). Sugar-coating of rAAV2 capsid was first performed with **2** in a one-step eY-click procedure for 20 sec, 1, 5, 15 or 60 min (**Fig. 2a**). A three-electrodes system (WE = graphite plate, CE = platinum plate, RE = Ag wire in KCl) was immersed in a neutral buffered solution of rAAV2 and **2** and an electrical potential of 750 mV vs. Ag/AgCl was applied, *i.e.* the oxidation potential of NML in these conditions.

The electro-functionalized viruses conserved the integrity of their capsid in all cases, as observed after dot blot staining with the A20 antibody, which detects the assembled capsid (**Fig. 2b**). A similar assay performed with a labeled soybean agglutinin (SBA), a GalNAc binding lectin, unambiguously confirmed the effective electro-conjugations of **2** on the surface of the viral vectors, even after 20sec voltage, with an observable time-dependent increase of staining intensity (**Fig. 2c**). In contrast, when the voltage was applied to a mixture of rAAV2 and a GalNAc derivative with no NML moiety (Ctrl), GalNAc could not be detected by SBA, showing the covalent anchoring of **2** to the capsid during eY-click. Interestingly, **2** labeled the rAAV2 capsid with very fast kinetics (20 sec) presumably thanks to the carbohydrate moiety that drastically increases aqueous solubility of NML, improving both its diffusion rate to the working electrode and the turnover at the anodic diffusion layer for a fast and regular electron transfer. The same observations were made with Man derivative **3**, which could also efficiently label rAAV2 in 20 sec eY-click. All electro-conjugated samples were subjected to capsid denaturation followed by SDS-PAGE separation on gels. The three constitutive envelope proteins (VP1/VP2/VP3 1:1:10) could be clearly identified by silver nitrate staining and western blotting using an anti-VPs polyclonal antibody. A significant mass shift of VP bands was observed for >5 min eY-click conditions for both carbohydrates, implying a high degree of Y functionalization (**Fig. S9**). Labeled lectins SBA and ConA were also used to detect GalNAc and Man, respectively, confirming the covalent conjugation of NML-carbohydrates on the three constitutive proteins of the capsid (**Fig. 2d**).

Next, the transduction efficiency of the electro-conjugated viral particles rAAV2-**2** and rAAV2-**3**, carrying a GFP reporter gene, was evaluated on HEK293 cell line. Cells were incubated at a multiplicity of infection (MOI) of $5 \cdot 10^3$ virus/cell for 48 h and the percentage of GFP+ cells was measured by flow cytometry. Importantly, control experiment of 1 h voltage with GalNAc derivative having no luminol moiety resulted in fully conserved infectivity of the rAAV2 (**Fig. S10**), proving that the applied potential difference does not alter transduction capacity. Satisfyingly, the rAAV2-**2** and rAAV2-**3** samples electro-conjugated during 20 sec and 1 min fully conserved their infectivity (**Fig. 2e**). However, in stark contrast, longer experiments (5, 15 and 60 min eY-click) showed no GFP expression (**Fig. S10**). Too high a proportion of modified

Y seems to impair interactions with cell surface receptors or modify the intra-cellular trafficking of the modified vectors, highlighting the crucial role of this amino acid in AAV infectivity. The level of Y modification on the rAAV2 surface should, therefore, be finely adjusted to conserve transfection and gene expression; this was reproducibly achieved here by time-controlled eY-click for 1 min. It is notable that GalNAc-decorated rAAV2-2 (1 min) showed improved transduction capacity as compared with rAAV2, on human hepatocyte carcinoma-derived HuH-7 cells expressing GalNAc receptors (**Fig. 2f**). This supports the relevance of viral vector functionalization to promote transduction of specific cell lines potentially mediated by ligand-receptor interactions.

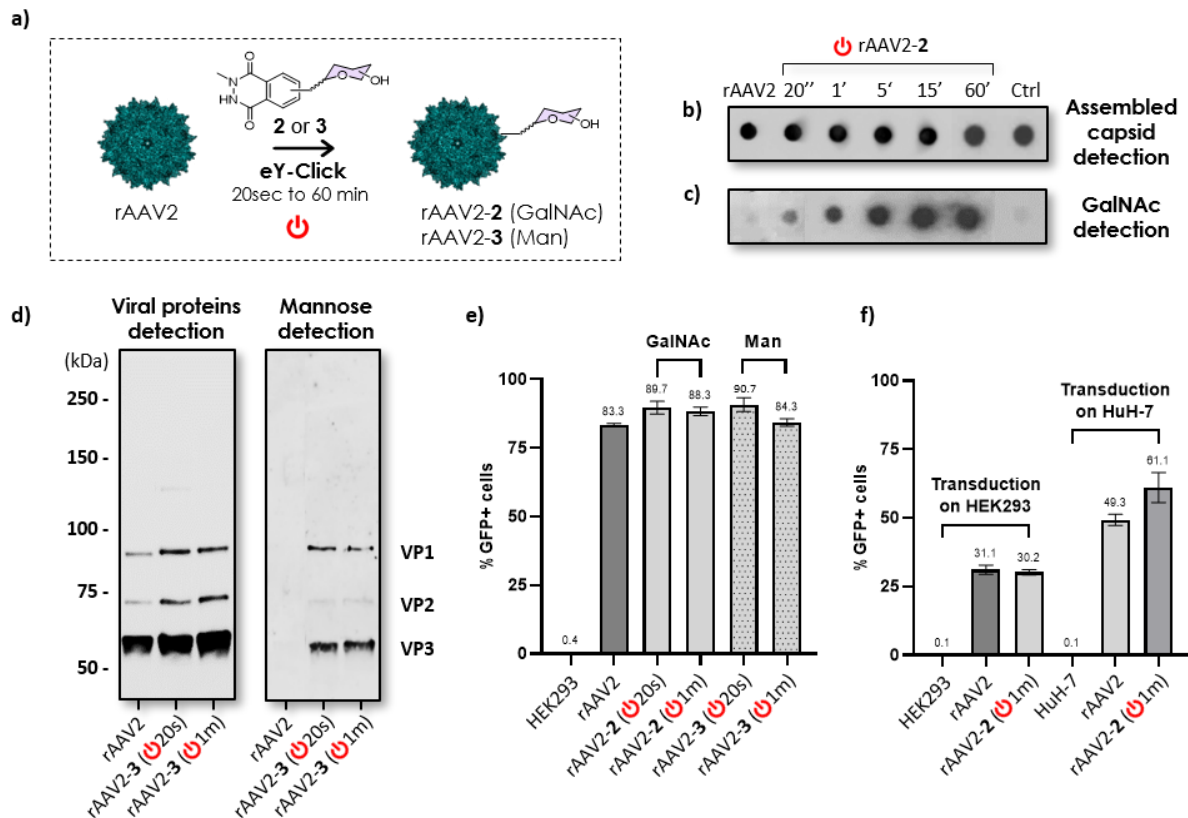


Fig. 2 a) rAAV2 was coated with GalNAc and Man derivatives of NML after *in situ* electro-activation for 20 sec, and 1, 5, 15 and 60 min. b) Dot blot analysis of viral vectors (2.10¹⁰ vg) to detect the assembled capsid using the anti-capsid A20 antibody. c) Dot blot analysis of viral vectors (2.10¹⁰ vg) to detect surface-conjugated GalNAc using a labeled soybean agglutinin (GalNAc-binding lectin). *Note:* 4.10¹⁰ vg of rAAV2-2 (20 sec) were deposited. d) Western blot analysis of viral vectors (2.10¹⁰ vg) after denaturation to detect the three constitutive viral proteins (VP1/VP2/VP3 1:1:10) using a polyclonal anti-VPs antibody (left), or to detect surface-conjugated Man using a labeled concanavalin A (Man-binding lectin). e) %GFP+ cells quantified by flow cytometry after 48 h incubation of viral vectors (carrying a GFP reporter gene) with HEK293 cells at a MOI of 5.10³ virus/cell. f) Comparison of transduction efficiencies of rAAV2 and rAAV2-2 (1 min) on HEK293 and HuH-7 (expressing GalNAc receptors) cell lines at a MOI of 1.10³ virus/cell.

eY-click on living bacteria

Bacteria have an inner phospholipidic cell membrane surrounded by different types of outer cell wall architectures, *i.e.* a thick layer of peptidoglycans and teichoic acid for Gram-positive (g+) bacteria, and a thin layer of peptidoglycans sandwiched by an exterior asymmetric bilayer of lipopolysaccharides and phospholipids for Gram-negative bacteria (g-). We investigated the capacity of eY-click to label membrane proteins from both types of cell envelopes using living *Escherichia coli* (g-) and *Staphylococcus epidermidis* (g+) as models. The bacteria were electrochemically azido-functionalized with **1** during their exponential growth phase (optical density 0.4) in a neutral PBS solution (pH 7.4), followed by fluorescent detection using strain-promoted azide-alkyne cycloaddition (SPAAC) with the rhodamine probe DBCO-PEG₄-CR110 **5a** (**Fig. 3a**). The azido-armed bacteria were washed by repeated centrifugation and rinsing sequences, and SPAAC labeling was performed with **5a** for 1 h, followed by additional washing steps. This two-step sequence allows the preparation of a batch of azide-functionalized bacteria, which can then be modified by a variety of ligands or probes, in analogy with MOE.

We were pleased to see that fluorescence microscopy analysis revealed a strong visual labeling of the bacterial membranes for all *E. coli* and *S. epidermidis* samples subjected to the eY-click + SPAAC sequence (**Fig. 3b**). In contrast, fluorescence was barely detectable in bacteria that only experienced the incubation step with the cyclooctyne **5a**, serving as a control accounting for potential non-specific adsorption of **5a** or alkyne side reactions with cell surface thiols.³⁸ Mean fluorescence was also quantified by flow cytometry and average values from independent triplicates are presented in **Fig. 3c**. A clear time-dependent increase of fluorescence intensity was observed for both bacterial strains after electro-bioconjugation for 15, 30 and 60 min. The levels of labeling observed in less than 1 h of eY-click showed the efficiency of the method to label g(+) and g(-) bacteria harboring highly different cell membrane structures. Importantly, the electrochemical process and the modification of the cell surface with **1** did not alter the viability of the bacteria as they were still able to proliferate at the same rate as unlabeled samples (**Fig. 3d**). Finally, we performed bacterial lysis and extracted membrane proteins (membrane fraction) from cytosolic and periplasmic proteins (cytosolic fraction) in the lysate (total proteins), followed by SDS-PAGE separation on gels. The quantity of proteins migrated in both inner and outer lanes were virtually the same as confirmed with LI-COR imaging system (**Fig. S14**). Coomassie brilliant blue (CBB) staining showed a rich and diverse population of proteins in the non-fractionated (total) sample, as well as in both inner and outer fractions. To our great satisfaction, fluorescence detection exclusively revealed CR110-labeled proteins of various molecular weights in the membrane fractions, while the inner proteins and peptides were not modified (**Fig. 3e**).

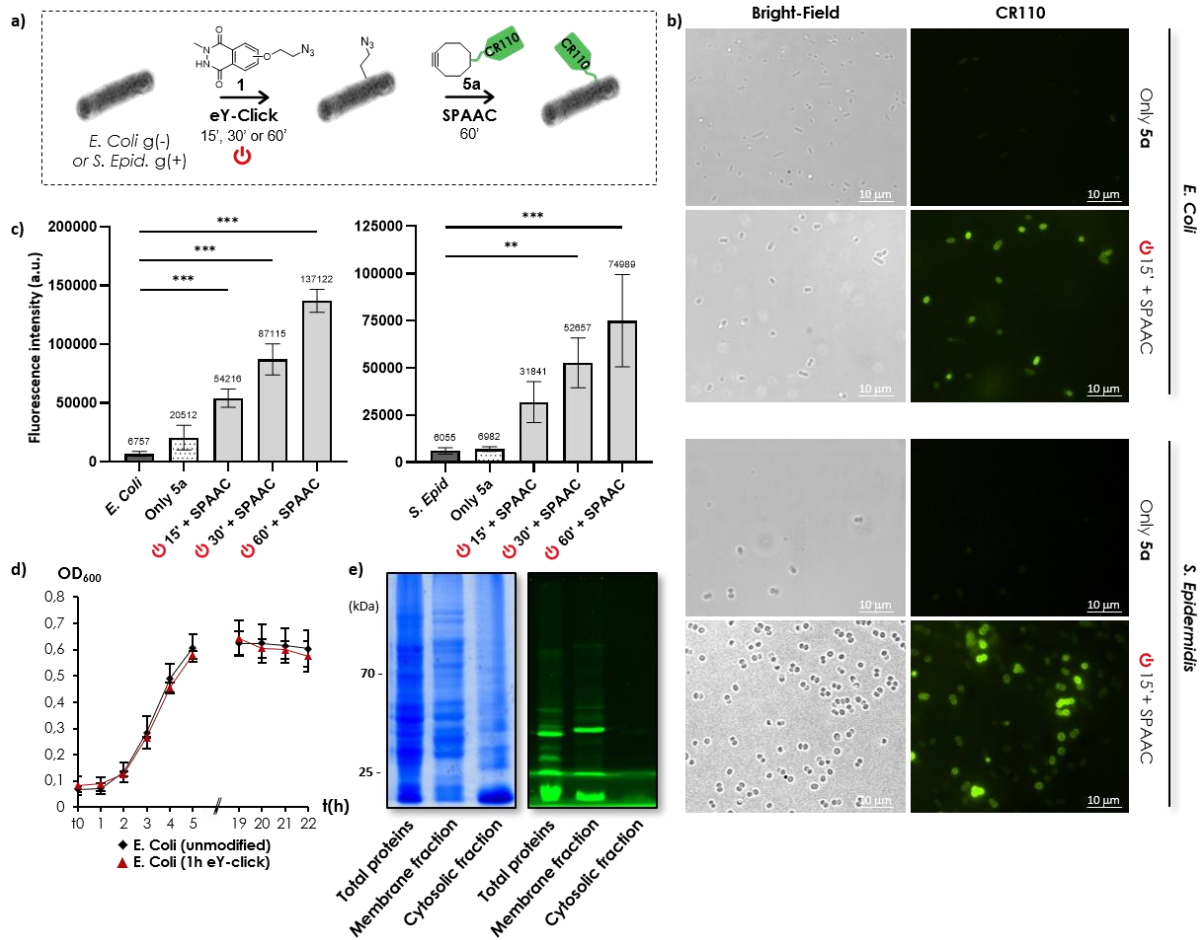


Fig. 3 a) *E. coli* g(-) and *S. epidermidis* g(+) were labeled in a two-step procedure. The bacterial strains were first electrochemically decorated with azido-luminol derivative **1** for 15, 30 or 60 min, and the covalently anchored azido-tag was reacted with the DBCO-PEG₄-CR110 probe **5a** by SPAAC for 1 h. **b)** Optical and fluorescence images of *E. coli* and *S. epidermidis* samples subjected to *i)* only SPAAC (control) and *ii)* eY-click and SPAAC. **c)** Mean fluorescence intensity measured by flow cytometry for unmodified bacteria (*E. coli* or *S. epidermidis*), bacteria incubated with only DBCO-PEG₄-CR110 probe (only **5a**), and conjugation experiments by eY-click with **1** for 15, 30 or 60 min followed by SPAAC with **5a**. Means of three independent experiments. **d)** Bacterial growth curves of **1**-labeled *E. coli* samples after 1 h eY-click compared to untreated strains. Each sample was cultured (n=3) and OD₆₀₀ was regularly measured. **e)** SDS-PAGE electrophoresis on gels of non-fractionated proteins (total proteins), and proteins from membrane and cytosolic fractions after bacterial lysis and fractionation protocol applied to *E. coli* strains after 1 h electro-conjugation with **1** followed by 1 h SPAAC with **5a**. Gels were colored by Coomassie Brilliant Blue (left) to confirm presence of all proteins in the fractions, and fluorescence detection at 490 nm (right) revealed fluorescent proteins in TP and MP lanes only, standing for a membrane electro-labeling.

eY-click on living cells

Next, the two-step electro-bioconjugation method was assessed on eukaryotic cell lines with HEK293 cells derived from human embryonic kidney and HeLa cells from cervical cancer. In this study, an additional control experiment was added where **1** was incubated with the cells for 30 min without applying the 750 mV potential difference, and then subjected to incubation with DBCO-PEG₄-FAM probe **5b** (“OFF **1** + SPAAC”). Mean fluorescence intensity of this control was virtually identical to that of unmodified cells only incubated with **5b**. This clearly indicates that compound **1** does not tag the cell surface without electro-oxidative activation. However, when the potential difference was applied, a strong mean fluorescence signal was detected after 30 min for both cell lines (**Fig. 4d**: HEK293, **Fig. 4e**: HeLa). Next, we verified that our labeling sequence effectively occurred at the cell surface and not intracellularly. The electro-conjugated HEK293-FAM ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 490/525$ nm) cells were additionally subjected to intracellular staining with DAPI ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 350/470$ nm) and membrane mapping with a labeled wheatgerm agglutinin (WGA-647, $\lambda_{\text{ex}}/\lambda_{\text{em}} = 650/668$ nm). Individual fluorescence imaging of the probes by confocal microscopy showed unambiguous overlapping signals for WGA-647 and FAM when merged, clearly standing for membrane functionalization during eY-click (**Fig. 4b**). Of note, fitness of both azido-tagged cell lines was not affected, as indicated by their conserved ability to divide in cell culture conditions (**Fig. S15**) and confirmed by trypan blue (**Fig. S16**), zombie yellow and annexin V (**Fig. S17**) viability tests (>95% mean viability, **Fig. 4c**). Finally, we evaluated one-step functional biotinylation of HeLa cells by electro-bioconjugation of NML-biotin derivative **4** (chemical synthesis in **Fig. S7**). After 30 min eY-click, surface biotinylation was probed with a labeled fluorescent streptavidin (SA-FAM) to evaluate efficiency by flow cytometry. In this assay, controls included cells only incubated with SA-FAM, and cells incubated with **4** during 30 min without electrical current followed by SA-FAM probing (“OFF **4** + SA”). We were pleased to reproducibly observe a significantly important population of fluorescent cells in the effective eY-click biotinylation experiment (**Fig. 4f**). As a stark contrast, almost no fluorescent cells were detected for both controls, which confirms the efficient covalent electro-labeling during eY-click.

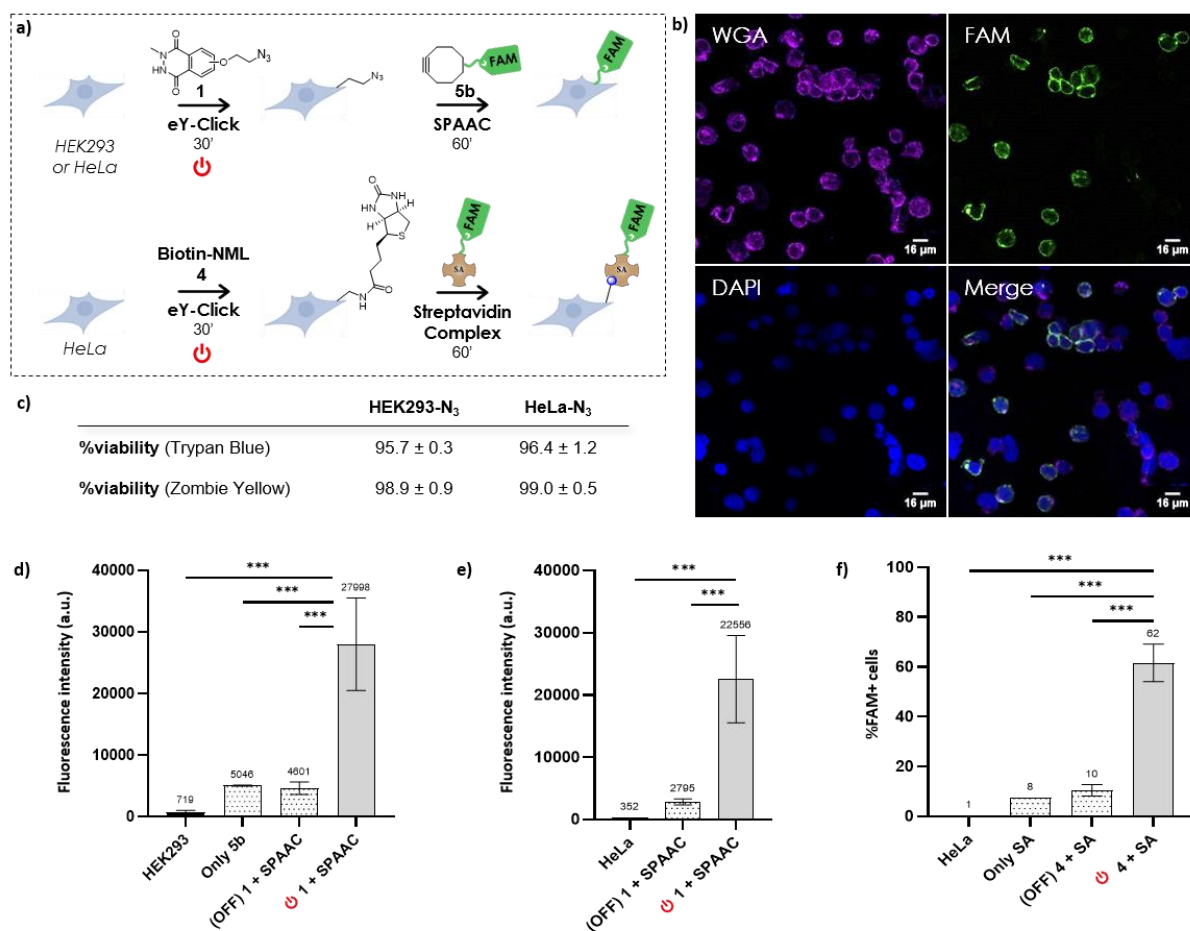


Fig. 4. **a)** HEK293 and HeLa cells were conjugated by 30 min eY-click with **1** or **4**, followed by 1 h SPAAC or streptavidine fluorescent (FAM) probing respectively. **b)** Confocal microscopy images of HEK293 cells treated by 30 min eY-click with **1** and 1 h SPAAC with **5b**. WGA stands for membrane staining, DAPI is nuclei staining, FAM is the fluorescent probe **5b**. Merge image showcases co-localization of WGA and FAM. Scale are given bottom right. **c)** Viability percentage of electro-conjugated samples as evaluated by both automatized trypan blue and zombie yellow assays (n=3). **d)** Mean fluorescence intensity measured by flow cytometry for unmodified HEK293 cells, cells incubated only with DBCO-PEG₄-FAM probe **5b** for 1 h (Only **5b**), cells incubated with **1** (no electro-oxidative activation) for 30 min followed by 1 h SPAAC with **5b**, and cells incubated with **1** under 750 mV activation for 30 min followed by 1 h SPAAC with **5b** (n=3). **e)** Similar analysis as d), *i.e.* mean fluorescence intensity measured by flow cytometry with HeLa cells (n=3). **f)** %FAM positive cells measured by flow cytometry for unmodified HeLa cells, cells incubated only with SA-FAM for 1 h (Only SA), cells incubated with **4** (no electro-oxidative activation) for 30 min followed by 1 h probing with SA-FAM, and cells incubated with **4** under 750 mV activation for 30 min followed by 1 h probing with SA-FAM (n=3).

Conclusion

The direct chemical functionalization of surface-exposed proteins on viral capsids or living bacteria and cell membranes is sparsely reported in the literature, and most approaches for bacteria and cells labeling rely on metabolic engineering methods requiring hours or days for the co-incubation step of the enzymatic transformation and surface exposure of the bioorthogonal reporter. As highlighted here, electrochemistry expands the scope of bioconjugation reactions and provides important advantages as compared with conventional chemical activations. The eY-click method does not require the handling of hazardous chemicals (oxidants) or potentially toxic catalysts and sensitizers for the surface modification of viruses, living bacteria or eukaryotic cells. The low electric potential applied *in situ* for biofunctionalization was shown to i) preserve viral capsid architecture and its ability to transduce cells, and (ii) bacteria and cells viabilities and their capacity to divide. The click-electrochemistry showed rapid kinetics and the level of surface functionalization was reproducibly controlled in a time-dependent manner. We anticipate that click-electrochemistry will be beneficial to the investigation of complex cell surface processes and to viral, bacterial and cell-based therapies.

Acknowledgements

This work was carried out with financial support from the *Centre National de la Recherche Scientifique (CNRS)*, the *Ministère de l'Enseignement Supérieur et de la Recherche* in France and the National Agency for Research (project ECLICK ANR- 19-CE07-0021-01). We thank the vector core of TaRGeT, UMR 1089 (CPV, INSERM and Nantes Université, which is a bioproduction and biotherapy national integrator (ANR-22-AIBB-0001), <http://umr1089.univ-nantes.fr>) for the production of the rAAV vectors used in this study. We also acknowledge the IBISA MicroPICell facility (Biogenouest), member of the national infrastructure France-Bioimaging supported by the French national research agency (ANR-10-INBS-04) for confocal microscopy images.

1. Büning, H. & Srivastava, A. Capsid Modifications for Targeting and Improving the Efficacy of AAV Vectors. *Mol. Ther. - Methods Clin. Dev.* **12**, 248–265 (2019).
2. Smith, M. T., Hawes, A. K. & Bundy, B. C. Reengineering viruses and virus-like particles through chemical functionalization strategies. *Curr. Opin. Biotechnol.* **24**, 620–626 (2013).
3. Bouzelha, M. *et al.* Chemical modification of Adeno-associated virus capsid to improve gene delivery. *Chem. Sci.* (2019) doi:10.1039/C9SC04189C.
4. Zhou, S., Gravekamp, C., Bermudes, D. & Liu, K. Tumour-targeting bacteria engineered to fight cancer. *Nat. Rev. Cancer* **18**, 727–743 (2018).
5. Gupta, K. H., Nowicki, C., Giurini, E. F., Marzo, A. L. & Zloza, A. Bacterial-Based Cancer Therapy (BBCT): Recent Advances, Current Challenges, and Future Prospects for Cancer Immunotherapy. *Vaccines* **9**, 1497 (2021).

6. Li, J. *et al.* Decorating Bacteria with Triple Immune Nanoactivators Generates Tumor-Resident Living Immunotherapeutics. *Angew. Chem. Int. Ed.* **61**, e202202409 (2022).
7. Cao, Z. & Liu, J. Bacteria and bacterial derivatives as drug carriers for cancer therapy. *J. Controlled Release* **326**, 396–407 (2020).
8. Wang, L., Cao, Z., Zhang, M., Lin, S. & Liu, J. Spatiotemporally Controllable Distribution of Combination Therapeutics in Solid Tumors by Dually Modified Bacteria. *Adv. Mater.* **34**, 2106669 (2022).
9. Bi, X., Yin, J., Chen Guanbang, A. & Liu, C.-F. Chemical and Enzymatic Strategies for Bacterial and Mammalian Cell Surface Engineering. *Chem. – Eur. J.* **24**, 8042–8050 (2018).
10. Kellam, B., Bank, P. A. D. & Shakesheff, K. M. Chemical modification of mammalian cell surfaces. *Chem. Soc. Rev.* **32**, 327–337 (2003).
11. Sackstein, R. *et al.* Ex vivo glycan engineering of CD44 programs human multipotent mesenchymal stromal cell trafficking to bone. *Nat. Med.* **14**, 181–187 (2008).
12. Blackall, D. P., Armstrong, J. K., Meiselman, H. J. & Fisher, T. C. Polyethylene glycol-coated red blood cells fail to bind glycophorin A-specific antibodies and are impervious to invasion by the Plasmodium falciparum malaria parasite. *Blood* **97**, 551–556 (2001).
13. Scott, M. D., Murad, K. L., Koumpouras, F., Talbot, M. & Eaton, J. W. Chemical camouflage of antigenic determinants: Stealth erythrocytes. *Proc. Natl. Acad. Sci.* **94**, 7566–7571 (1997).
14. Wang, L., Brock, A., Herberich, B. & Schultz, P. G. Expanding the Genetic Code of Escherichia coli. *Science* **292**, 498–500 (2001).
15. Lee, K. J., Kang, D. & Park, and H.-S. Site-Specific Labeling of Proteins Using Unnatural Amino Acids. *Mol. Cells* **42**, 386–396 (2019).
16. Chalker, J. M., Bernardes, G. J. L., Lin, Y. A. & Davis, B. G. Chemical Modification of Proteins at Cysteine: Opportunities in Chemistry and Biology. *Chem. - Asian J.* **4**, 630–640 (2009).
17. Boutureira, O. & Bernardes, G. J. L. Advances in Chemical Protein Modification. *Chem. Rev.* **115**, 2174–2195 (2015).
18. Sornay, C., Vaur, V., Wagner, A. & Chaubet, G. An overview of chemo- and site-selectivity aspects in the chemical conjugation of proteins. *R. Soc. Open Sci.* **9**, 211563.
19. Kjærsgaard, N. L., Nielsen, T. B. & Gothelf, K. V. Chemical Conjugation to Less Targeted Proteinogenic Amino Acids. *ChemBioChem* **2022**, e202200245.

20. Seki, Y. *et al.* Transition Metal-Free Tryptophan-Selective Bioconjugation of Proteins. *J. Am. Chem. Soc.* **138**, 10798–10801 (2016).
21. Marmelstein, A. M. *et al.* Tyrosinase-Mediated Oxidative Coupling of Tyrosine Tags on Peptides and Proteins. *J. Am. Chem. Soc.* **142**, 5078–5086 (2020).
22. Ogura, A. & Tanaka, K. Azaelectrocyclization on cell surface: convenient and general approach to chemical biology research. *Tetrahedron* **71**, 4518–4521 (2015).
23. Hsiao, S. C. *et al.* Direct Cell Surface Modification with DNA for the Capture of Primary Cells and the Investigation of Myotube Formation on Defined Patterns. *Langmuir* **25**, 6985–6991 (2009).
24. Prescher, J. A., Dube, D. H. & Bertozzi, C. R. Chemical remodelling of cell surfaces in living animals. *Nature* **430**, 873–877 (2004).
25. Laughlin, S. T., Baskin, J. M., Amacher, S. L. & Bertozzi, C. R. In Vivo Imaging of Membrane-Associated Glycans in Developing Zebrafish. *Science* **320**, 664–667 (2008).
26. Kayser, H. *et al.* Biosynthesis of a nonphysiological sialic acid in different rat organs, using N-propanoyl-D-hexosamines as precursors. *J. Biol. Chem.* **267**, 16934–16938 (1992).
27. Mackay, A. S., Payne, R. J. & Malins, L. R. Electrochemistry for the Chemoselective Modification of Peptides and Proteins. *J. Am. Chem. Soc.* **144**, 23–41 (2022).
28. Kawamata, Y. *et al.* Electroaffinity Labeling: A New Platform for Chemoproteomic-based Target Identification. (2022) doi:10.26434/chemrxiv-2022-s2m8f.
29. Ban, H., Gavriluk, J. & Barbas, Carlos F. Tyrosine Bioconjugation through Aqueous Ene-Type Reactions: A Click-Like Reaction for Tyrosine. *J. Am. Chem. Soc.* **132**, 1523–1525 (2010).
30. Sato, S., Nakamura, K. & Nakamura, H. Tyrosine-Specific Chemical Modification with *in Situ* Hemin-Activated Luminol Derivatives. *ACS Chem. Biol.* **10**, 2633–2640 (2015).
31. Sato, S., Nakamura, K. & Nakamura, H. Horseradish-Peroxidase-Catalyzed Tyrosine Click Reaction. *ChemBioChem* **18**, 475–478 (2017).
32. Dorta, D. A., Deniaud, D., Mével, M. & Gouin, S. G. Tyrosine Conjugation Methods for Protein Labelling. *Chem. – Eur. J.* **26**, 14257–14269 (2020).
33. Alvarez-Dorta, D. *et al.* Electrochemically Promoted Tyrosine-Click-Chemistry for Protein Labeling. *J. Am. Chem. Soc.* **140**, 17120–17126 (2018).
34. Depienne, S. *et al.* Luminol anchors improve the electrochemical-tyrosine-click labelling of proteins. *Chem. Sci.* **12**, 15374–15381 (2021).

35. Sato, S. *et al.* Site-Selective Protein Chemical Modification of Exposed Tyrosine Residues Using Tyrosine Click Reaction. *Bioconjug. Chem.* **31**, 1417–1424 (2020).
36. Neda, H., Wu, C. H. & Wu, G. Y. Chemical modification of an ecotropic murine leukemia virus results in redirection of its target cell specificity. *J. Biol. Chem.* **266**, 14143–14146 (1991).
37. Pearce, O. M. T. *et al.* Glycoviruses: Chemical Glycosylation Retargets Adenoviral Gene Transfer. *Angew. Chem. Int. Ed.* **44**, 1057–1061 (2005).
38. Fairbanks, B. D., Sims, E. A., Anseth, K. S. & Bowman, C. N. Reaction Rates and Mechanisms for Radical, Photoinitiated Addition of Thiols to Alkynes, and Implications for Thiol–Yne Photopolymerizations and Click Reactions. *Macromolecules* **43**, 4113–4119 (2010).