Making cARMs Sweeter: Expansion of the cARM Platform

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Synthetic immunotherapy uses small molecular tools to adjust one's immune system toward a therapeutic end. Antibody Recruiting Molecules (ARMs) are small synthetic constructs capable of conscripting endogenous serum antibodies to act as cancer killing agents. ARMs function via an antibody binding region capable of reversibly binding specific endogenous serum antibodies (Abs), in combination with a cancer antigen binding region. Collectively, ARMs induce the opsonization of targeted cancer cells (via cell-ARM-Ab ternary complex formation) now recognizable to immune effector cells including NK cells, T cells and macrophages. These effectors respond by inducing cell killing. However, ARMs suffer from rapid in-vivo clearance and dependence on in-system formation of a strong ternary-complex (cell-ARM-Ab). As an alternative, the Rullo Lab has introduced covalent antibody recruiting molecules (cARMs) capable of highly selective covalent tagging of antibodies with a cancer homing moiety [1-2]. The result is a long lived serum stable endogenous Ab capable of forming a simple binary complex (Cell-Ab*) for target cell opsonization and immune-mediated clearance.

Previous work has validated cARMs for labelling various sources of anti-Dinitrophenol (DNP) Ab with a prostate cancer homing moiety [1-2]. The use of DNP as a model hapten provided a synthetically streamlined process to validate the cARM platform and was immunologically justified by the measurable presence of anti-DNP Ab titers in human serum with moderate DNP affinity ($K_d \approx 10^{-7}$-10^{-8}) [1-2]. Building from previous work, the hapten L-Rhamnose (L-Rha) has been substituted for DNP. The titers of anti-Rha Ab in human serum typically exceed those of anti-DNP, however affinity for L-Rha is typically lower (Kd $\approx 10^{-4}$-10^{-6}). Importantly, successful specific Ab labelling with this low affinity hapten will broaden the potential scope of cARM targets and applications. The work herein will review the construction and evaluation of novel L-Rha containing cARMs for specific antibody labelling.

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Protective role of estrogen in pancreatic beta cell health and function

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Sex-dependent differences in the prevalence of diabetes and cardiovascular diseases are well established, with pre-menopausal women being protected, when compared to men or women after menopause. [1] This suggests a protective role for estrogen in the development of diabetes and its associated cardiovascular diseases however, the mechanisms of this protection are still unknown. [1] Our aim is to analyze the molecular mechanisms by which estrogens modulate chronic disease progression. We hypothesize that estrogens protect pancreatic beta cells by activating the adaptive unfolded protein response (UPR) and/or repressing the apoptotic UPR in response to endoplasmic reticulum stress.

We established a mouse model of hyperglycemia-induced atherosclerosis ($ApoE^{-/-}Ins2^{+/Akita}$) which shows sexual dimorphism in terms of glucose regulation. [2] Female $ApoE^{-/-}Ins2^{+/Akita}$ are significantly protected from hyperglycemia and atherosclerosis, relative to males. Male and female $ApoE^{-/-}Ins2^{+/Akita}$ and $ApoE^{-/-}$ controls were fed a standard chow diet ad libitum. Subsets of female $ApoE^{-/-}Ins2^{+/Akita}$ mice were ovariectomized or ovariectomized and given slow releasing estrogen pellets. Subsets of males also received estrogen pellets. Metabolic parameters, pancreatic beta cell mass, markers of adaptive and apoptotic UPR were monitored up to 25 weeks of age. Atherosclerosis was quantified at endpoint. All procedures were pre-approved by the McMaster University AREB.

Male mice and ovariectomized female $ApoE^{-/-}Ins2^{+/Akita}$ mice were chronically hyperglycemic. Female $ApoE^{-/-}Ins2^{+/Akita}$ and ovariectomized $ApoE^{-/-}Ins2^{+/Akita}$ mice treated with estrogen were transiently hyperglycemic and blood glucose levels normalized by 10 weeks of age. Pancreatic islets from sham operated $ApoE^{-/-}Ins2^{+/Akita}$ female mice and ovariectomized $ApoE^{-/-}Ins2^{+/Akita}$ mice treated with estrogen showed a significant increase in the expression of protective UPR components and a decrease in pro-apoptotic factors, compared to males or ovariectomized females. Atherosclerotic lesions were more advanced in ovariectomized $ApoE^{-/-}Ins2^{+/Akita}$ mice, compared to female sham operated $ApoE^{-/-}Ins2^{+/Akita}$ and female ovariectomized $ApoE^{-/-}Ins2^{+/Akita}$ treated with estrogen. Male $ApoE^{-/-}Ins2^{+/Akita}$ treated with estrogen also showed a significant reduction in atherosclerotic lesion volume.

These results suggest a protective role of estrogens in the maintenance of blood glucose regulation and beta cell health by activating the cell's UPR. This mechanism may explain similar protection observed in premenopausal women.

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Title: Induced Macrophage-Specific Genetic Ablation of GSK3α Accelerates the Atherosclerotic Regression in *Ldlr*$^{-/-}$ Mice

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The molecular mechanisms by which cardiovascular risk factors promote the development of atherosclerosis are poorly understood. Recent evidence from our laboratory suggests that endoplasmic reticulum (ER) stress signaling through glycogen synthase kinase (GSK)-3α/β is involved in the activation of pro-atherosclerotic processes [1]. Targeting the ER stress-GSK3α/β pathway, either genetically or pharmacologically, attenuates the progression and development of atherosclerosis in a mouse model system [2,3,4]. The precise role(s) of GSK-3α/β in atherosclerotic regression is not known. We sought to define the specific roles of GSK3α/β using novel mouse strains that allow for inducible, tissue specific GSK3α or GSK3β deletion.

We created novel strains of LDLR$^{-/-}$ mice in which macrophage-specific GSK3α or GSK3β deletion was inducible upon tamoxifen injection (LiαKO, LiβKO). Five-week-old male and female mice were fed HFD for 16 weeks to promote atherosclerotic lesion formation. Then mice were treated with tamoxifen (5mg/100ul for 3 days) and switched to a chow diet for 12 weeks. All mice were sacrificed at 33 weeks of age. No significant differences in metabolic parameters were observed between experimental groups. Mice with induced macrophage-specific GSK3α deficiency, but not GSK3β deficiency, had reduced plaque volume and necrosis compared to control and baseline mice. Mice with induced macrophage-specific GSK3α deficiency had increased CCR7 and ABCA1 expression in lesional macrophages, which is consistent with regressive lesions.

These studies begin to delineate the specific roles of GSK3α and GSK3β in atherosclerotic regression. The results suggest that GSK3α ablation promotes atherosclerosis plaque regression and therefore identify GSK3α as a target for the development of new therapies to treat existing atherosclerotic lesions in patients with cardiovascular disease.

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Introduction of Cationic Phosphoramidate Linkages into DNA Nanostructures to Increase Cellular Permeability

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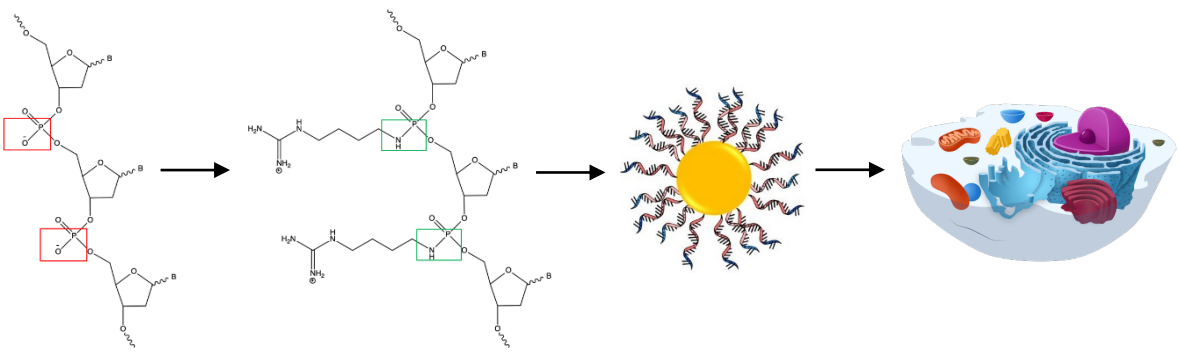
DNA nanotechnology is the design and manufacture of nanoscale nucleic acid structures and has shown great promise as a platform for drug delivery [1]. It takes advantage of the desirable properties of DNA including its biocompatibility, programmability, water solubility, and specific base pairing [2]. By taking DNA out of its biological context, it can be used to assemble DNA nanostructures that exhibit both structural and functional elements that are rigorously controlled at the nanoscale level due to the nature of its base pairing complementarity [3]. However, complications which include enzymatic degradation, and difficulties with cellular internalization due to their negatively charged phosphate backbone and hydrophilicity, lead to sup-optimal performance in drug delivery applications [4]. Specifically, little to no cellular uptake to the cytoplasm and nucleus has been reported when introduced to cells, hindering the ability of spherical nucleic acids to interact with intracellular DNA and RNA, resulting in poor gene knockdown efforts [5]. Additionally, the use of transfecting reagents is often required for nucleic acid-based therapeutics to gain access into cells, which frequently have cytotoxic effects [6] and can induce immunogenic responses [7]. Therefore, these issues need to be addressed to access new biological compartments and to realize the full potential of this technology for biomedical applications. We anticipate that by replacing the intrinsically anionic phosphodiester linkage of DNA with a phosphoramidate linkage, we will be able to incorporate a diverse library of backbone modifications that allows us to modify the surface charge and enzymatic degradation resistance of DNA nanostructures. The biological implications of these modifications will lead to cell penetrating DNA nanostructures while eliminating the need for transfecting reagents and increasing the drug delivery efficacy of these structures. The new functionality provided by the chemical modifications on the backbone of DNA nanostructures, particularly the cationic guanidinium moiety, will contribute to the development of effective DNA nanostructure drug delivery systems that can serve various biomedical applications, especially in the context of gene knockdown [8].



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Chemically Tuning the Immune System using Sulfonyl Fluoride Covalent Antibody Recruiting Molecules

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In the everchanging field of cancer immunotherapy, the use of small synthetic molecules has become a novel and innovative approach to enhance the body's natural function of the immune system. Small molecules that regulate immune responses can be designed and modified in an easier way than antibody- or virus-based immunotherapies and have a substantially lower production cost. To gain insight into antibody-small molecule interactions, the Rullo lab has developed dual-binding compounds known as covalent antibody recruiting molecules (cARMs). These compounds direct antibodies naturally present in human serum to tumor receptors through three general features: an antibody binding domain (ABD), an antibody labelling domain (ALD), and a tumor binding domain (TBD). Proximal to the ABD, the ALD contains an electrophilic group which is attacked by a nucleophilic amino acid residue on the target antibody [1]. In previous designs, cARMs were developed with an acyl-imidazole ALD which selectively recruited anti-DNP IgG in fluorescence SDS-PAGE analysis and facilitated immune recognition and recruitment via streptavidin-beads in a flow cytometry assay [1]. To improve the hydrolytic stability and explore new nucleophilic targets, a second generation of cARMs has been developed with sulfonyl fluoride exchange (SuFEx) chemistry for the ALD. Sulfonyl fluorides have been broadly used in academic and industrial applications due to its very stable high oxidation state which yields strong intermolecular bonds with a variety of nucleophiles.[3] In preliminary data, SuFEx cARMS have a higher hydrolytic stability and different covalent targets in comparison to the acyl-imidazole cARMs. Other biophysical experiments using biolayer interferometry, SDS-PAGE and isothermal calibration are currently being conducted to determine the key binding parameters of the SuFEx-antibody labelling reaction and PSMA-binding.

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Title: Synthesis of Ferutinin-derived Anti-Cancer Drug Candidates to Boost Adoptive T Cell Therapy

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Ferutinin is a naturally occurring molecule isolated from roots of the Zallouh plant (*Ferula hermonis*). It has been identified as an anti-cancer therapeutic lead scaffold that can stimulate T cell proliferation for immunotherapy. Adoptive cell therapy (ACT) is a type of immunotherapy in which a patient's own immune cells are harvested, genetically modified, and reinfused to combat tumours. However, in a tumour microenvironment, these modified T cells can be hindered by the lack of costimulatory or cytokine signals necessary for T cell proliferation. Recent work to address this issue has found that supporting signals can be restored by small, biologically active molecules which target pathways involved in T cell anti-tumour activity.[1]

The aim of this project is to synthesize structural analogues of ferutinin and evaluate their ability to promote T cell proliferation. Thus far, we have established an affordable means to obtain multi-gram quantities of ferutinin via isolation from Zallouh root tea that is manufactured in Syria and sold internationally. Using this tea-derived ferutinin as a substrate, we have synthesized more than a dozen structural analogues to date. In parallel, we are developing a total synthesis of ferutinin and its related analogues from commercially available building blocks that do not require the plant-derived ferutinin as a starting material. Evaluation of bioactivity for the synthesized analogues will provide structure-activity relationship insights that can enable the development of new anti-cancer drug-candidates, which could potentially improve the efficacy of existing T cell immunotherapies.

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Title: Evaluation of Anti-Tumour Efficacy and Pharmacokinetics of Covalent Immune Recruiters in an *In Vivo* Model

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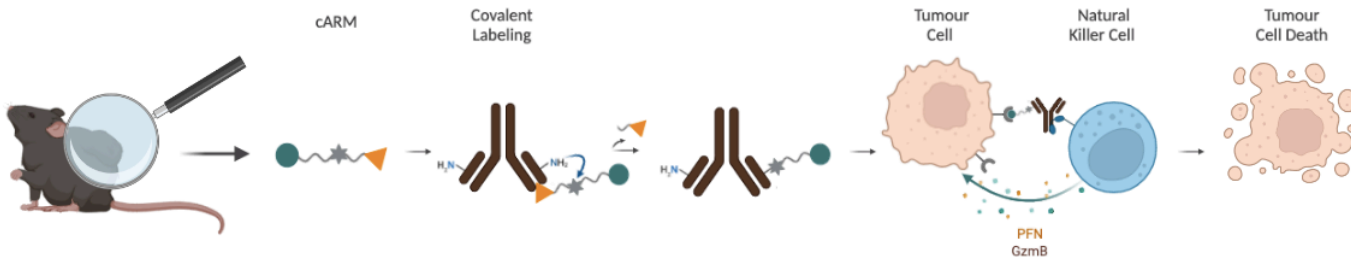
Covalent antibody recruiting molecules (cARMs) are synthetic small molecules that interact with and covalently bind antibodies and redirect them to the tumour cell surface. First generation cARM molecules consist of a human prostate specific membrane antigen (hPSMA) target binding domain, an antibody labelling domain and an anti-dinitrophenyl (DNP) antibody binding domain [1]. cARMS have been proven to recruit antibodies to hPSMA expressing cells and mediate selective antibody-dependent cellular phagocytosis/cytotoxicity (ADCP/ADCC) of cancer cells [1]. The irreversible covalent binding of cARMs to anti-DNP IgG antibodies in serum enables for the generation of a tumour immunotherapeutic antibody directly *in vivo* which can enact natural killer cell function against tumour cells.

While promising in *in vitro* assays, the true therapeutic test lies within *in vivo* studies. Following the development of a mouse tumour model expressing hPSMA and sufficient anti-DNP antibody boosting, cARMs and analogous non-covalent ARMs were injected to view anti-cancer effects and efficacy. While dosing and treatment regimens need to be further optimized, initial *in vivo* mouse studies showed significant improved survival in tumour bearing mice that were treated with ARM and cARM compared to PBS negative controls. Results also showed a slight delay in tumour growth as well as reduced tumour volume in the initial days post implantation. *In vivo* labeling studies of anti-DNP antibodies show approximately ~30% of anti-DNP antibodies being labeled in circulation. This work demonstrates the power of using cARMs as an immunotherapeutic. Using cancer immunotherapy in conjunction with chemically synthesized molecules allows for a unique method to form therapeutic antibodies directly *in vivo.* cARMs can be synthesized to target a variety of cancer cell specific antigens, selectively label antibodies and has potential to recruit multi-specific therapeutic antibodies against heterogenic tumours.



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Assessing Tobacco Exposure within the Prospective Urban and Rural Epidemiological (PURE) Study Cohort

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Tobacco use is the second major cause of mortality worldwide, killing more than 8 million people each year.[1] Although tobacco control policies (e.g., pack warnings, ad bans) have been successful in reducing overall smoking prevalence, disparities in tobacco related harms remain an ongoing challenge both within Canada and globally. With the primary addictive substance in tobacco being nicotine, tobacco smoking generates seven major urinary nicotine metabolites and their glucuronide conjugates, collectively known as TNE-7.[1] Overall, nicotine metabolism is mediated by three key enzymes; Cytochrome P450 2A6 (CYP2A6), Flavin monooxygenase and Glucuronosyltransferase 2B10 (UGT2B10) which can be monitored using ratiometric indicators of enzyme activity.[1] Currently large-scale, epidemiological, tobacco studies rely primarily on standardized questionnaires to assess smoking behaviors.[2] While questionnaires are inexpensive to implement in large populations, self-reports of smoking habits to estimate exposures in diverse environments are prone to error due to cultural factors, social desirability and recall biases. Additionally, self-reported data is unable to elucidate differences in metabolism between individuals. [2]

Herein, a nested case-cohort study comprising 1000 participants from the Prospective Urban and Rural Epidemiological Study (PURE), with both urine samples and complete tobacco use history were analyzed by multi-segment injection capillary electrophoresis (MSI-CE-MS) including self-identified never smokers (n=335), light smokers (n=324) and heavy smokers (n=341) from 14 countries of varying income status. Overall, nicotine exposure based on TNE7 in heavy smokers from high income countries are found to be 40% greater than middle- and low-income countries (p-value < 1.0 × 10-8) after adjusting for age, sex, BMI, and cigarettes per day (CPD). TNE-7 also demonstrates a moderate correlation with self-reported CPD in both high-income (r = 0.411, p-value < 0.0001, n=265) and middle-income countries (r = 0.345, p-value < 0.0001, n=268), but not in low-income countries, indicating greater likelihood of misreporting and/or second-hand smoke exposure. Furthermore, partial cluster analysis of a panel of urinary biomarkers relating to tobacco exposure revealed four distinct clusters, including cluster 2 (90% from high-income countries) where higher TNE7, UGT activity, and CYP 2A6 activity was observed compared to other clusters. Cluster 2 also demonstrates higher levels of urinary TMAO, uric acid, cresol sulfate and 1-methyladenosine indicative of greater biological harm. This work highlights the intrinsic value of urinary biomarker assessment that may better distinguish smoking status, compared to self-reports, and help identify and explain variations in smoking hazards between global populations.

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Studying Cellulose Nanostructure Through Labeling and Advanced Microscopy Techniques

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As the major component of the plant cell wall, cellulose is produced by all plant species at an annual rate of over a hundred billion tonnes, making it the most abundant biopolymer on Earth. The hierarchical assembly of cellulose glucan chains into crystalline fibrils, bundles and higher-order networks endows cellulose with its high mechanical strength, but makes it challenging to breakdown and produce cellulose-based nanomaterials and renewable biofuels. In order to fully leverage the potential of cellulose as a sustainable resource, it is important to study the supramolecular structure and hydrolysis of this biomaterial from the nano- to the microscale.

Herein, we develop new chemical strategies for fluorescently labeling cellulose and employ advanced imaging techniques to study its supramolecular structure at the single-fibril level. The developed labeling method provides a simple and efficient route for fluorescently tagging cellulose nanomaterials with commercially available dyes, yielding high degrees of labeling without altering the native properties of the nanocelluloses. This allowed the preparation of samples that were optimal for super-resolution fluorescence microscopy (SRFM), which was used to provide for the first time, a direct visualization of periodic disorder along the crystalline structure of individual cellulose fibrils. The alternating disordered and crystalline structure observed in SFRM was corroborated with time-lapsed acid hydrolysis experiments to propose a mechanism for the acid hydrolysis of cellulose fibrils. To gain insight on the ultrastructural origin of these regions, we applied a correlative super-resolution light and electron microscopy (SR-CLEM) workflow and observed that the disordered regions were associated nanostructural defects present along cellulose fibrils. Overall, the findings presented in this work provide significant advancements in our understanding of the hierarchical structure and depolymerization of cellulose, which will be useful for the development of new and efficient ways of breaking down this polymer for the production of renewable nanomaterials and bio-based products like biofuels and bioplastics.

Development of a Bone-Targeted Photoacoustic Imaging Agent

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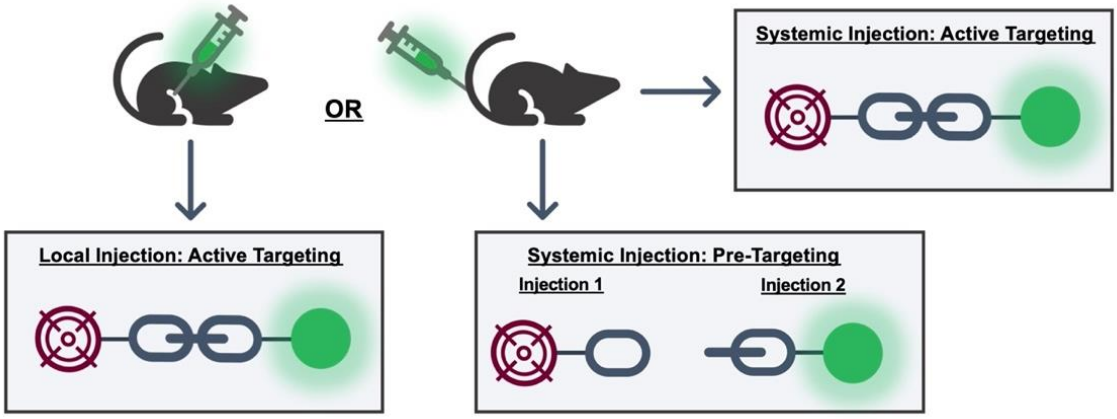
Photoacoustic Imaging (PAI) is an emerging modality that non-invasively captures images by detecting high frequency pressure waves (ultrasound waves) that are emitted during the thermal expansion and contraction of a molecule by pulsed light absorption. [1] Molecularly targeted small molecule dyes present an opportunity to expand photoacoustic imaging applications by allowing for the detection of biological entities that do not possess intrinsic light absorption properties. [2] Here we show the utility of three injection strategies, using a bisphosphonate-derived *trans*-cyclooctene and a 1,2,4,5-tetrazine bound IR-783 dye, employed towards targeted photoacoustic imaging of bone. *In vitro* analysis was used to verify the specificity of the probe to hydroxyapatite, showing bisphosphonate-specific binding using active (62 ± 5.7 %, where $P = 0.005$) and pre-targeting (55 ± 2.7%, where $P < 0.001$) strategies. *In vivo* evaluation was performed to demonstrate the ability of the probe to localize and be retained at bone through intrafemoral injection (0.23 ± 0.11 a.u., where $P \leq 0.03$), actively targeted intravenous injection (0.18 ± 0.02 a.u., where $P < 0.001$), and pre-targeted intravenous injection (0.11 ± 0.03 a.u., where $P < 0.001$), each producing detectable, spectrally verified signal by 4 hours post-injection.



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